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Monitoring and fast detection of mycotoxin-producing fungi based on headspace solid-phase microextraction and headspace sorptive extraction of the volatile metabolites

Jan C.R. Demyttenaere*, Rosa M. Moriña, Pat Sandra
Department of Organic Chemistry, Faculty of Sciences, Ghent University, Krijgslaan 281 (S4), B-9000 Ghent, Belgium

Abstract

Solid phase microextraction in combination with capillary GC–MS was used as monitoring technique for the collection and detection of the fungal volatile metabolite (+)-aristolochene by sporulated surface cultures of *Penicillium roqueforti*. A comparison was made between different toxigenic and nontoxigenic strains of *P. roqueforti*. Different growth conditions and media, such as malt extract agar, potato dextrose agar and sabouraud dextrose agar were compared. Whereas toxigenic strains produced large amounts of (+)-aristolochene, β-elemene, valencene and germacrene A, nontoxigenic *P. roqueforti* strains showed a remarkably different headspace profile, in which ethyl-2-hexenoate, *E*-β-caryophyllene, aromadendrene and β-patchoulene were the predominant volatiles, apart from other sesquiterpene hydrocarbons present at lower concentrations. Stir bar sorptive extraction, was also applied in the headspace sampling mode, i.e. headspace sorptive extraction (HSSE) for the enrichment of fungal volatiles from sporulated surface cultures to differentiate between toxigenic and nontoxigenic fungi. Hence, it can be concluded that headspace analysis of volatile fungal metabolites by SPME and HSSE in combination with capillary GC–MS is a suitable monitoring technique for the fast detection of mycotoxin producing fungi.

Keywords: *Penicillium roqueforti*; Solid-phase microextraction; Headspace sorptive extraction; Mycotoxins; (+)-Aristolochene

1. Introduction

Fungal infection in stored cereals decreases their nutritional value and can pose health hazards because of the formation of mycotoxins and potentially allergenic spores. Hence, there is a need for methods that can accurately quantify the degree of fungal infection in grains at an early stage of mould growth [1]. Only few techniques are suited for this purpose and the common available ones suffer some severe drawbacks. Microbial methods giving a direct answer on fungal presence and abundance such as conventional plate techniques are laborious and time consuming to specify qualitatively and quantitatively fungal contamination on cereal products. On the other hand, indirect methods based on detection of specific chemical markers such as ergosterol, ATP, or chitin often require an intensive sample preparation procedure [2]. The aim of the current study was to develop a method to detect mycotoxin producing fungi based on the analysis of the headspace profile of sporulated surface cultures.

Fungi are known to produce a wide range of

*Corresponding author. Tel.: +32-9-264-4459; fax: +32-9-264-4998.
E-mail address: jan.demyttenaere@rug.ac.be (J.C.R. Demyttenaere).

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secondary metabolites, some of which are volatile [3]. Fungal volatiles have been studied for several reasons, like detection of undesired fungal growth, mostly on cereals [4], detection of off-flavours caused by fungi [5], and the possible relation between fungal volatiles and the ''sick building syndrome'' [6]. In all these studies, compounds like ethanol, isobutanol, isopentanol, ketones and terpenes seem to be produced by many species under different conditions. Important volatile metabolites are 1-octen-3-ol, 3-octanone, 3-methylfuran, and the malodorous 2-methylisoborneol and geosmin [7]. Volatile sesquiterpenes have been used for taxonomic classification and species identification in *Penicillium*, as well as to indicate mycotoxin formation in *Fusarium* and *Aspergillus* [8]. The correlation between biosynthesis of sesquiterpenes and production of mycotoxins has also been described by other groups [9,10] and was recently reviewed [2].

Different methods have been used for the collection of fungal volatiles [11]. Steam distillation extraction (SDE) under a nitrogen atmosphere, with or without vacuum, followed by liquid–liquid extraction and concentration of the organic extract is a very common extraction technique. Steam distillation–solvent extraction (SDSE), using a Likens-Nickerson apparatus was also performed to collect volatiles from large amounts of fungal biomass. Purging and trapping of headspace gases is another widely used method for collection of fungal volatiles. This technique has the advantage of being nondestructive and very sensitive. The headspace sampling can be coupled to gas chromatography–mass spectrometry (GC–MS) analysis. In the case of conventional headspace sampling an inert gas is purged through or above the sample and consequently through an adsorbing material like activated charcoal or porous polymers such as Tenax, that traps the volatile metabolites. An alternative and simple method for collection of volatile fungal metabolites is diffusive sampling from Petri dishes [12]. The volatiles are collected by diffusive sampling onto tubes containing either carbon black or Tenax TA. This method was used to collect and to compare profiles of volatiles of two taxonomically closely related *Penicillium* species. More recently headspace solid-phase microextraction (HS-SPME) was used to collect the volatile organic compounds (VOCs) emitted from fungi of the genus *Penicillium* [13]. This technique, in combination with GC–MS enables characterisation and analysis of the profile of volatiles, characteristic for every fungal species. Low molecular volatile markers as well as sesquiterpenes can be selectively enriched on the SPME fiber.

Recently, we reported on the use of SPME for headspace analysis of *P. roqueforti* [14]. This paper presents an analytical method to detect mycotoxin producing fungi based on the headspace analysis of sporulated surface cultures by both solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) in the headspace mode, i.e. headspace sorptive extraction (HSSE) [15].

SBSE is a recent technique developed by Baltussen et al. [16]. The principle of this method is the same as for SPME but it uses a magnetic stir bar covered with a polydimethylsiloxane (PDMS) film instead of the fiber used in SPME. The bars are incorporated in a glass tube giving an outer diameter of 1.2 mm and are coated with a layer of 1 mm PDMS which represents a total thickness of the stir bar of 3.2 mm. The main advantage of this technique is the higher amount of polymer that covers the bar. For a bar with a length of 10 mm the amount of PDMS is 55 μl against ~0.6 μl for a typical 100-μm PDMS fiber [15,17]. Whereas SPME is ideally suited for routine analysis of the headspace profile of fungi, and for detection of compounds present at higher concentration, SBSE is more sensitive and can be used for trace and ultratrace analysis [16,17]. In the current paper its use in the headspace sampling mode, i.e. HSSE, is reported.

2. Experimental

2.1. Microorganisms and cultivation

Five *Penicillium roqueforti* strains were used in this study, three strains of *P. roqueforti* aggr. (marked CCV, CDG and CZW) isolated from contaminated culture media [14], a toxigenic strain of *P. roqueforti* var. roqueforti, kindly offered by Henryk Jeleń (Agricultural University of Poznań, Poland) (marked PRR), and a nontoxigenic *P. roqueforti* var. roqueforti, isolated from fresh edible Roquefort cheese (marked ROQ). The fungi were cultivated

and conserved on malt extract agar (MEA: malt extract 2%, bacteriological peptone 0.1%, glucose 2% and agar 2%, pH 5.4±0.2). They were also cultivated on potato dextrose agar (PDA: potato extract 0.4%, glucose 2% and agar 1.5%, pH 5.6±0.2) and sabouraud dextrose agar (SAB: mycological peptone 1%, glucose 4% and agar 1.5%, pH 5.6±0.2). All culture media were obtained from Fluka (Bornem, Belgium).

2.2. Headspace analysis of surface cultures by SPME and SBSE

The fungi were cultivated as small sporulated surface cultures in 22-ml SPME vials (Supelco, Bornem, Belgium) and the volatile metabolites were extracted by headspace SPME for 30 min at 25 °C with a 100-μm PDMS fiber as described previously [18]. SPME fibers were obtained from Supelco (Bornem, Belgium). For SBSE extraction, a PDMS stir bar (Gerstel, Mülheim a/d Ruhr, Germany) was placed in the headspace of a 22-ml vial in which a fungal surface culture had sporulated, by piercing a metal needle (40 mm length×0.8 mm diameter) through the septum of the vial, turning the end of the needle into an eye, and placing the magnetic stir bar into the eye of the needle, to which it was attached by simple magnetic force. The stir bar was then exposed to the headspace of the surface culture for 1 h at 25 °C [15].

2.3. Analysis of the extracts by GC–MS

GC–MS analyses of the SPME extracts were performed with an Agilent 6890 GC Plus coupled to a quadrupole mass spectrometer 5973 MSD (mass selective detector, Agilent), and equipped with a HP5-MS capillary column (30 m×0.25 mm I.D.; 0.25 μm df). Working conditions were: injector 250 °C, transfer line to MSD 250 °C, oven temperature: start 40 °C, hold 2 min, programmed from 40 to 200 °C at 10 °C min^{-1}, from 200 to 250 °C at 15 °C min^{-1}, hold 5 min; carrier gas (He) 1.0 ml min^{-1}; SPME desorption was carried out using a CIS-4 PTV injector (Gerstel) in split mode (1:50); ionisation: EI 70 eV; acquisition parameters: scanned m/z: 40–200 (2–10 min), 40–300 (>10 min).

For the analysis of the SBSE extracts, the stir bar was placed into a glass tube (Gerstel) (178 mm×6.0 mm O.D.) and then thermodesorption was carried out using a Gerstel thermo desorption system (TDS2) (Gerstel), coupled to a CIS-4 PTV injector (Gerstel). The TDS2 oven was programmed from 50 °C (hold 1 min) to 280 °C at 60 °C min^{-1} (hold 10 min at 280 °C) and the volatile analytes were thermally desorbed at a flow of 100 ml min^{-1} and cryo-focused in a CIS-4 PTV-injector (Gerstel) at −150 °C; injection of the analytes was done by fast heating the injector from −150 to +300 °C at 12 °C s^{-1}. The analysis was carried out on a HP5-MS capillary column (25 m×0.25 mm I.D.; 0.25 μm df) using the following temperature program: start 40 °C, hold 2 min, programmed from 40 to 160 °C at 8 °C min^{-1}, from 160 to 250 °C at 15 °C min^{-1}, hold 3 min; carrier gas (He) 0.9 ml min^{-1}.

The identification of the main fungal metabolite from the toxigenic cultures, (+)-aristolochene was based on co-injection and comparison of its mass spectrum and retention index (I) with an authentic sample of aristolochene, kindly provided by Professor W. König (University of Hamburg, Germany) [14]. The Kóvats I value of (+)-aristolochene on the HP-5 MS stationary phase is 1482.

Mass spectrum of (+)-aristolochene (EI-MS): m/z (rel. int.): 204 $[M]^+$ (12), 189 (100), 105 (76), 91 (40), 121 (39), 107 (38), 93 (34), 133 (31), 161 (25), 119 (23), 80 (22).

The other fungal metabolites were identified by comparison of their mass spectra and retention indexes (Kováts indexes) with those of reference substances (where possible—see below) and by comparison with the NIST mass spectral library (Version 1.6d, 1998) and with literature data [19,20]. The following reference substances were obtained from Fluka: 1-octen-3-ol (98%), α-terpinene (97%), (R)-(+)-limonene (>99%), ocimene (97%), terpinolene (90%), (E)-β-caryophyllene (99%), β-chamigrene (90%, sum of enantiomers), (+)-valencene (>90%). Other reference substances were purchased from Across (Geel, Belgium): β-myrcene (90%), γ-terpinene (>98%), (E)-cinnamaldehyde (99%). Retention indexes were measured by co-injection of a series of n-alkanes (n-octane–n-hexadecane) into the gas chromatograph by using a linear temperature program according to the literature [19]:

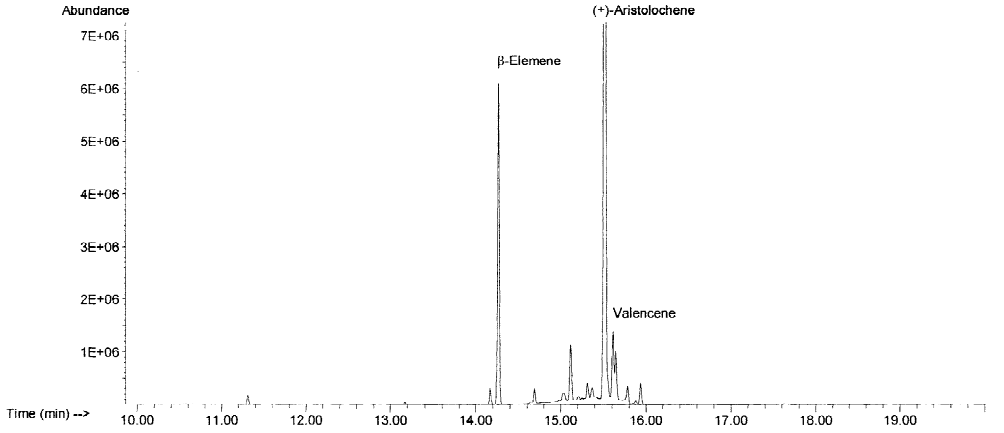

Fig. 1. Chromatogram of SPME extract of a surface culture of *P. roqueforti* aggr. (strain CDG)—the chromatograms of the other strains, CCV and CZW were completely alike.

start 60 °C, programmed from 60 to 200 °C at 3 °C min^{-1}, from 200 to 250 °C at 15 °C min^{-1}, hold 5 min. Carrier gas (He) 1 ml min^{-1}.

3. Results and discussion

3.1. SPME extraction of the fungal cultures

Four different fungal strains, namely the nontoxigenic *Penicillium roqueforti* var. roqueforti isolated from Roquefort cheese (ROQ), and three strains of *P. roqueforti* aggr. (CCV, CDG and CZW) were grown as sporulated surface cultures in SPME vials on different growth media, and the production of fungal volatiles was monitored by headspace SPME sampling. From the headspace SPME extracts a very clear difference could be noted between *P. roqueforti* aggr. (chromatogram of strain CDG on MEA shown) and *P. roqueforti* var. roqueforti (Figs. 1 and 2).

Whereas the first strain produced significant amounts of (+)-aristolochene and β-elemene as main metabolites (on all media), the headspace of the latter one was more complex, in which two unidentified sesquiterpene hydrocarbons (marked X and Y) were the predominating volatiles, apart from other sesquiterpene hydrocarbons, such as (*E*)-β-caryophyllene present at lower concentration (Fig.

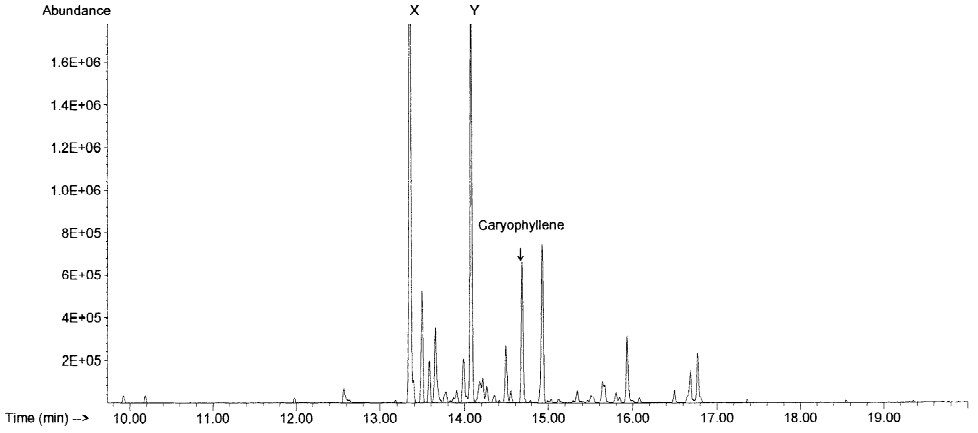

Fig. 2. Chromatogram of SPME extract of a surface culture of *P. roqueforti* (nontoxigenic strain ROQ).

2). No (+)-aristolochene was observed in the headspace of the nontoxigenic strain (ROQ).

3.2. HSSE extraction of the fungal cultures

From the previous results obtained by SPME, it was assumed that the *P. roqueforti* aggr. strains were toxigenic. To check the correlation between toxigenicity and (+)-aristolochene production, the headspace profile of a known toxigenic strain of *P. roqueforti* var. roqueforti (marked PRR) [21] was compared with that of the nontoxigenic ROQ and the three *P. roqueforti* aggr. strains (CDG, CCV and CZW), using SBSE in the headspace mode (HSSE) as sampling technique (see Experimental). The headspace of the *P. roqueforti* aggr. strains consisted mainly of (+)-aristolochene (66–75%), β-myrcene (10–14%), limonene (2–3%), β-elemene (2–3%), valencene and α-terpinene (Table 1 and Fig. 3—chromatogram of strain CZW). The main compounds contributing to the headspace profile of the toxigenic strain PRR were also (+)-aristolochene (53%), β-elemene (17%), germacrene A (9%), valencene (8%) and β-myrcene (2%) (Fig. 4), confirming the correlation of (+)-aristolochene production and PR toxin production [21].

The headspace of the nontoxigenic strain *P. roqueforti* var. roqueforti (ROQ) was again more complex, containing mainly ethyl 2-hexenoate and the two unknown terpene hydrocarbons (marked X and Y) as predominating peaks, which were also detected in the headspace SPME extracts of these cultures (Fig. 5). The mass spectra of the two unidentified sesquiterpenes X and Y are shown in Figs. 6 and 7, respectively.

A scheme with the main de novo produced fungal volatiles obtained from the headspace extracts of the toxigenic *P. roqueforti* strains is depicted in Fig. 8.

The sesquiterpene hydrocarbon (+)-aristolochene was recently isolated for the first time as a fungal metabolite of *Aspergillus terreus*, together with (−)-γ-cadinene [22]. The absolute configuration of the

Table 1
Relative composition (%) of HSSE extracts of *P. roqueforti* cultures

t_R (min)	*I*	Compound	Culture			
			CDG	CCV	CZW	PRR
4.08		Isoamyl acetate	0.41	1.51	3.80	0.00
5.97	978	1-Octen-3-ol	0.95	4.07	1.48	0.00
6.15	989	β-Myrcene	12.05	10.49	14.41	2.36
6.59	1017	α-Terpinene	1.86	1.49	1.96	0.32
6.82	1031	D-Limonene	2.38	3.05	2.30	0.47
7.24	1053	(*E*)-β-Ocimene	0.23	0.26	0.39	0.10
7.41	1062	γ-Terpinene	0.14	0.22	0.23	0.07
7.96	1091	Terpinolene	0.15	0.20	0.38	0.06
9.33	1163	Unknown (main *m*/*z* 73)	0.17	1.46	1.06	0.98
11.29	1266	(*E*)-Cinnamaldehyde	0.09	0.30	0.41	0.81
12.29	1330	Unknown	0.51	1.63	0.87	1.06
13.12	1383	β-Elemene enantiomer	0.15	0.24	0.17	1.24
13.23	1390	β-Elemene	1.95	2.81	2.24	16.73
13.65	1417	(*E*)-β-Caryophyllene	0.17	0.21	0.16	0.52
14.12	1447	Unknown sesquiterpene HC	0.45	0.55	0.41	2.03
14.40	1465	9-epi-(*E*)-Caryophyllene	0.12	0.18	0.14	0.54
14.53	1473	β-Chamigrene	0.12	0.16	0.13	0.61
14.69	1482	(+)-Aristolochene	74.66	66.83	65.97	52.57
14.80	1490	Valencene	2.12	2.46	2.03	8.25
14.98	1502	Germacrene A	1.02	1.73	1.27	9.42
15.09	1509	Unknown (mol. ion 202)	0.32	0.17	0.20	0.13
		Other compounds				1.76
Total			100	100	100	100

t_R, Retention time; *I*, retention index (see Experimental); CDG, CCV, CZW = *Penicillium roqueforti* aggr.; PRR, toxigenic *P. roqueforti* var. roqueforti.

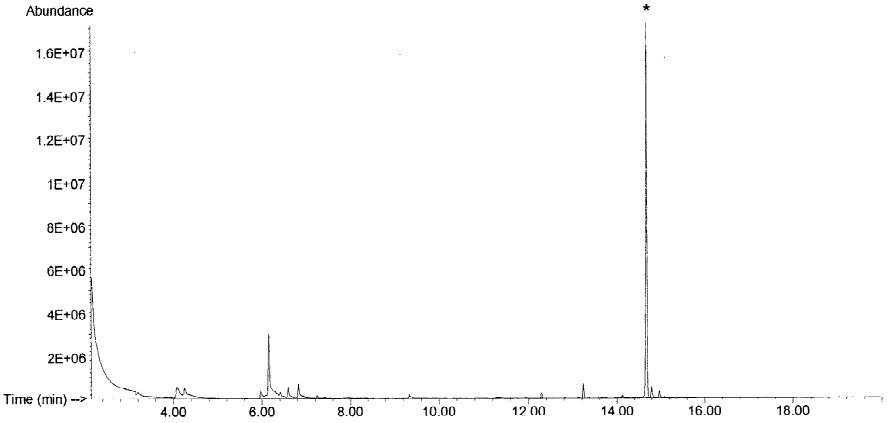

Fig. 3. Chromatogram of HSSE extract of a surface culture of *P. roqueforti* aggr. (strain CZW) Assigned peak: *=(+)-Aristolochene—the chromatograms of the extracts of the other strains, CCV and CDG were comparable.

fungal aristolochene was later confirmed by comparison with semisynthetic (−)-aristolochene, prepared from (+)-valencene [23].

Biogenetically, aristolochene seems to be a very important compound. The biosynthesis of (+)-aristolochene takes place via the cyclisation of *E*,*E*-farnesyldiphosphate (FPP), the universal precursor of the sesquiterpenes [24]. The mechanism of the cyclisation has been studied extensively [25] and takes place through the monocyclic intermediate germacrene A, which is formed by allylic diphosphate ionisation of FPP and electrophilic attack of the resulting cation at C-10 of the distal double bond, followed by loss of a proton from one of the two adjacent methyl groups [26].

The presence of germacrene A in the headspace extracts of the toxigenic cultures of *Penicillium roqueforti* (Fig. 4) is interesting to note in this respect.

Both steps in the cyclisation are catalysed by one enzyme, aristolochene synthase. Two distinct aristolochene synthases, acting by identical mechanisms have been isolated. A first aristolochene synthase has been isolated from *Penicillium roqueforti*, purified and studied [27]. A second aristolochene synthase was later isolated from *Aspergillus terreus* [24].

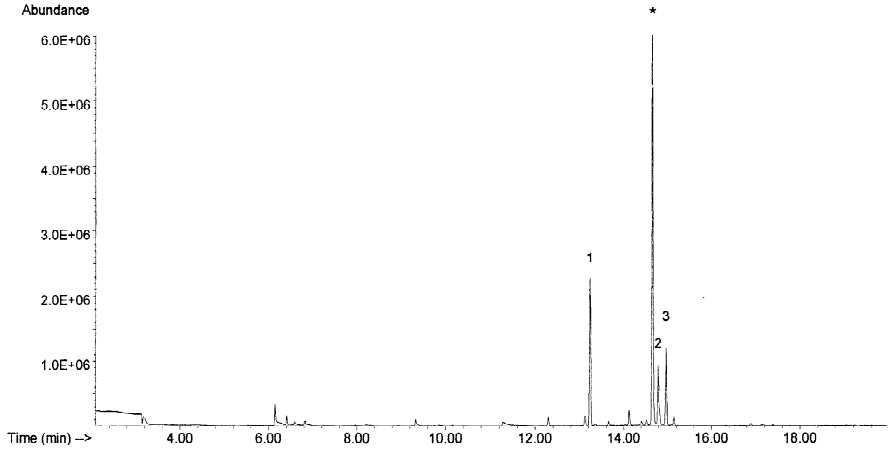

Fig. 4. Chromatogram of HSSE extract of a surface culture of *P. roqueforti* var. roqueforti (toxigenic strain PRR) Assigned peaks: 1=β-elemene, *=(+)-aristolochene, 2=valencene, 3=germacrene A.

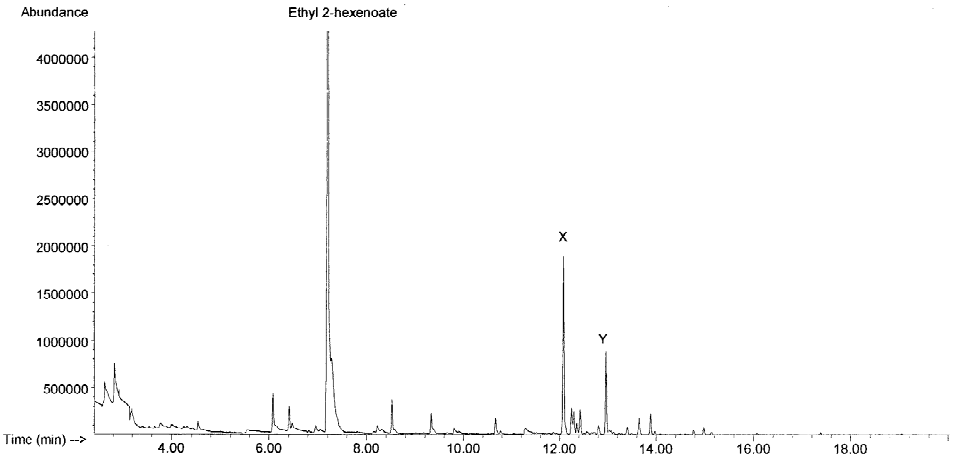

Fig. 5. Chromatogram of HSSE extract of a surface culture of *P. roqueforti* (nontoxigenic strain ROQ) Assigned peaks X and Y are two unidentified sesquiterpene hydrocarbons (see mass spectra below).

The formation of (+)-aristolochene is believed to be the first step in the biosynthesis of a number of fungal toxins. The most important of these toxins are the PR-toxin of *Penicillium roqueforti* and sporogen-AO1 of *Aspergillus oryzae* [28,29] (Fig. 9).

4. Conclusion

In this paper the correlation between (+)-aristolochene production and mycotoxin production by toxigenic *P. roqueforti* strains was confirmed.

Both SPME and HSSE are fast and sensitive techniques to differentiate between toxigenic and nontoxigenic *P. roqueforti* strains, based on the headspace profile of the sporulated surface cultures. However, since HSSE is more often used for trace and ultratrace analysis [15], and the fungal metabolites described here (aristolochene, valencene, β-elemene, ...) are present at detectable amounts (up to 40 ppm [14]), and since SPME is faster, simpler and does not require a thermal desorption device, it can be concluded that SPME is the method of choice for fast routine screening of toxigenic fungi. Moreover, SPME can easily be automated and used for fast detection of fungal contamination of food sam-

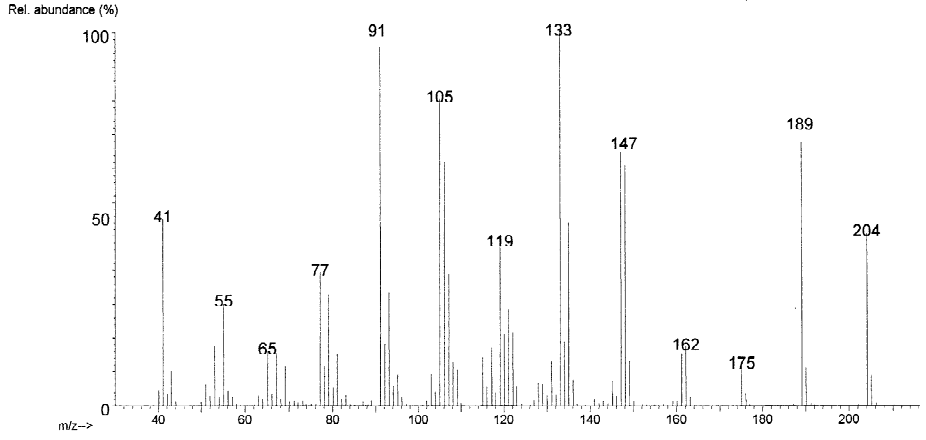

Fig. 6. Mass spectrum of compound X (t_R 12.08 min) produced by *P. roqueforti* ROQ.

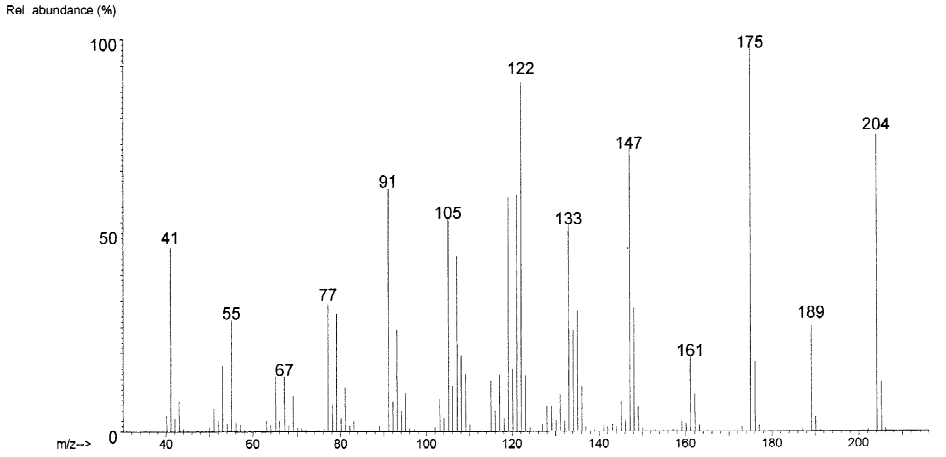

Fig. 7. Mass spectrum of compound Y (t_R 12.96 min) produced by *P. roqueforti* ROQ.

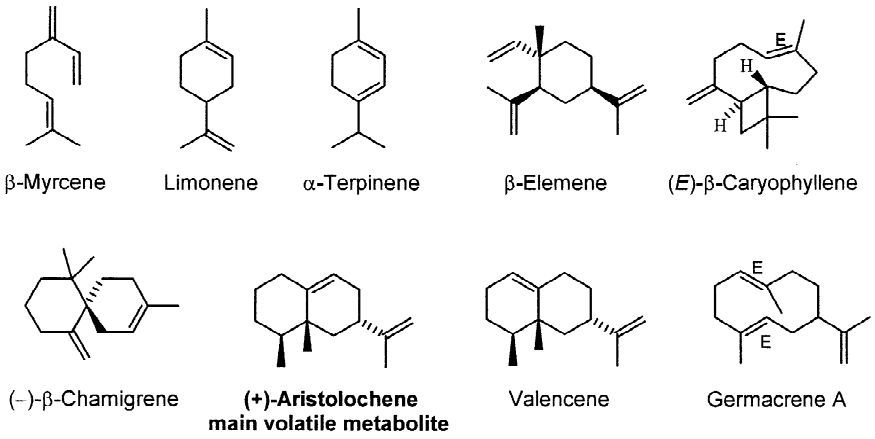

Fig. 8. Main fungal volatiles obtained from the headspace extracts of toxigenic *P. roqueforti*.

ples, such as cereals, cheeses and other dairy products. On the other hand SBSE is more easily applicable for off-line sampling of stored foods.

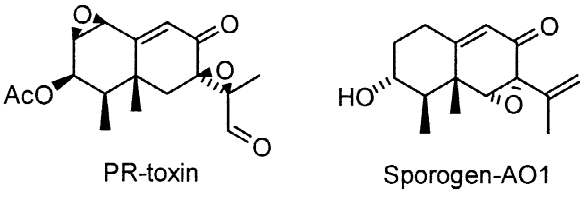

Fig. 9. Aristolochene derived mycotoxins.

Acknowledgements

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References

[1] T. Börjesson, U. Stöllman, J. Schnürer, Appl. Environ. Microbiol. 58 (1992) 2599.
[2] H. Jeleń, E. Wasowicz, Food Rev. Int. 14 (1998) 391.
[3] T.O. Larsen, J.C. Frisvad, Mycol. Res. 99 (1995) 1153.
[4] T. Börjesson, U. Stöllman, P. Adamek, Cereal Chem. 66 (1989) 300.
[5] T.S. Börjesson, U.M. Stöllman, J.L. Schnürer, J. Agric. Food Chem. 41 (1993) 2104.
[6] K.F. Nielsen, M. Hansen, T.O. Larsen, U. Thrane, Int. Biodeterior. Biodegrad. 42 (1998) 1.
[7] J. Schnürer, J. Olsson, T. Börjesson, Fungal Genet. Biol. 27 (1999) 209.
[8] H.H. Jeleń, C.J. Mirocha, E. Wasowicz, E. Kamiński, Appl. Environ. Microbiol. 61 (1995) 3815.
[9] A.L. Pasanen, S. Lappalainen, P. Pasanen, Analyst 121 (1996) 1949.
[10] H.J. Zeringue Jr., D. Bhatnagar, T.E. Cleveland, Appl. Environ. Microbiol. 59 (1993) 2264.
[11] T.O. Larsen, J.C. Frisvad, J. Microbiol. Meth. 24 (1995) 135.
[12] T.O. Larsen, J.C. Frisvad, J. Microbiol. Meth. 19 (1994) 297.
[13] T. Nilsson, T.O. Larsen, L. Montanarella, J.Ø. Madsen, J. Microbiol. Meth. 25 (1996) 245.
[14] J.C.R. Demyttenaere, A. Adams, K. Van Belleghem, N. De Kimpe, W.A. König, A.V. Tkachev, Phytochemistry 59 (2002) 597.
[15] C. Bicchi, C. Cordero, C. Iori, P. Rubiolo, P. Sandra, J. High Resolut. Chromatogr. 23 (2000) 539.
[16] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcolumn Separations 11 (1999) 737.
[17] E. Baltussen, C.A. Cramers, P.J.F. Sandra, Anal. Bioanal. Chem. 373 (2002) 3.
[18] J.C.R. Demyttenaere, M. del C. Herrera, N. De Kimpe, Phytochemistry 55 (2000) 363.
[19] R.P. Adams, Identification of Essential Oil Components By Gas Chromatography/mass Spectrometry, Allured, Carol Stream, IL, 1995.
[20] D. Joulain, W.A. König, in: The Atlas of Spectral Data of Sesquiterpene Hydrocarbons, E.B, Hamburg, 1998, p. 99.
[21] H.H. Jeleń, in: Abstracts of 32nd International Symposium on Essential Oils, Wrocław, Poland, 9–12 September, 2001, p. L17.
[22] D.E. Cane, B.J. Rawlings, C. Yang, J. Antibiot. 15 (1987) 1331.
[23] D.E. Cane, E.J. Salaski, P.C. Prabhakaran, Tetrahedron Lett. 31 (1990) 1943.
[24] D.E. Cane, P.C. Prabhakaran, E.J. Salaski, P.H.M. Harrison, H. Noguchi, B. Rawlings, J. Am. Chem. Soc. 111 (1989) 8914.
[25] D.E. Cane, P.C. Prabhakaran, J.S. Oliver, D.B. McIlwaine, J. Am. Chem. Soc. 112 (1990) 3209.
[26] D.E. Cane, Z. Wu, R.H. Proctor, T.M. Hohn, Arch. Biochem. Biophys. 304 (1993) 415.
[27] T.M. Hohn, R.D. Plattner, Arch. Biochem. Biophys. 272 (1989) 137.
[28] T.M. Hohn, R.H. Proctor, A.E. Desjardins, Molecular biology of filamentous fungi, in: Proceedings EMBO-Workshop, Berlin, 1991, p. 99.
[29] R.H. Proctor, T.M. Hohn, J. Biol. Chem. 268 (1993) 4543.