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Biotransformation of 1,8-cineole by solid-state fermentation of *Eucalyptus* waste from the essential oil industry using *Pleurotus ostreatus* and *Favolus tenuiculus*

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Abstract Biotechnological conversion of low-cost agro-industrial by-products, such as industrial waste or terpenes from the distillation of essential oils from plants into more valuable oxygenated derivatives, can be achieved by using microbial cells or enzymes. In Argentina, the essential oil industry produces several tons of waste each year that could be used as raw materials in the production of industrially relevant and value-added compounds. In this study, 1,8-cineole, one of the components remaining in the spent leaves of the *Eucalyptus cinerea* waste, was transformed by solid-state fermentation (SSF) using the two edible mushrooms *Pleurotus ostreatus* and *Favolus tenuiculus*. As a result, two new oxygenated derivatives of 1,8-cineole were identified: 1,3,3-trimethyl-2-oxabicyclo [2.2.2]octan-6-ol and 1,3,3-trimethyl-2-oxabicyclo [2.2.2]octan-6-one. Additionally, changes in the relative percentages of other aroma compounds present in the substrate were observed during SSF. Both fungal strains have the ability to produce aroma compounds with potential applications in the food and pharmaceutical industries.

Introduction

The agro-food and forest industries generate millions of tons of waste annually, which causes serious disposal issues and, consequently, results in considerable costs to industry and the environment (Laufenberg et al. 2003). Argentina is estimated to have about 28,000 cultivated hectares of aromatic and essence-containing plants that are use to obtain spices, condiments and essential oils, resulting in several tons of spent waste after steam distillation (Elechosa and Juarez 2003). The *Eucalyptus* species accounts for 24 % of the forested area in Argentina of which 39.100 ha are located in the province of Buenos Aires (Beale and Ortiz 2013). The principal related industry is focused on the production of wood or pulp for the paper industry, but without any general recovery of waste, with leaves and branches being left on the ground. Environmental legislation, however, is becoming more rigid, and this is forcing the industry to find solutions and alternative destinations for the spent biomass or waste. One possibility is to use them as raw materials in the production of industrially relevant and value-added compounds through an integrated management according to the concept "reduce, reuse and recycle" (Laufenberg et al. 2003; Bicas et al. 2010). In this context, solid-state fermentation (SSF) using microorganisms has been subject to increasing attention. This bioprocess has successfully converted inexpensive agro-industrial residues (spent leaves of *Eucalyptus cinerea* or

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Laurus nobilis, cassava bagasse, sugar cane bagasse, citric pulp, coffee husk and pomace of carrots or apples) into a great variety of products, such as edible mushrooms, flavourings, bioactive compounds, enzymes, organic acids and bioethanol, among others, which have the advantage of being recognized as "natural" (Berger 2009; Christen et al. 1997; Laufenberg et al. 2003; Omarini et al. 2010a; Pandey et al. 2000a; Pandey et al. 2000b; Rossi et al. 2009; Shimoni et al. 2000; Wilkins 2009; Zheng and Shetty 1998).

Eucalyptus leaves are rich in essential oils that can be obtained by steam distillation (FAO 1995). However, during the distillation process leading to essential oil (EO) recovery is not 100 % efficient, as the water vapour drag technique cannot remove it completely and some oil remains trapped in the plant tissues. Nevertheless, spent industrial waste could be an interesting low-cost raw material to obtain high-quality and value-added products (e.g. aroma and fragrances) through SSF using microorganisms (Dudai et al. 2001; Shimoni et al. 2000; Weinberg et al. 1999). Related to this, it is important to point out that not all fungal species are able to grow in the presence of a high concentration of essential oils or their main terpene constituents because of the cytotoxicity present in these non-polar compounds, which produce an increase in the fluidity of the fungal membranes (Koroch et al. 2007; Weber and de Bont 1996). Wilkins (2009) reported that citrus peel waste could be used as raw materials for biofuel production by fermentation, but requires the previous removal of D-limonene, due to it being extremely toxic to biological activity and the fact that it inhibits anaerobic digestion processes. In another study, Onken and Berger (1999) demonstrated the capability of the white rot fungus *Pleurotus sapidus* to grow in a liquid medium supplemented with limonene and to biotransform the terpene into *cis:trans*-carveol and carvone as the main products. The natural habitats of some microorganisms, including *Pleurotus*, are terpene-containing plant materials, e.g. wood or soil, which contain rotting leaves or wood and implies that they possess adaptation mechanisms to counteract the membrane damage produced by toxic substances, such as limonene (Onken and Berger 1999). This fact permitted, Omarini et al. (2010a) to use spent leaves waste of *Eucalyptus cinerea* (EW) and *Laurus nobilis* (LW) to cultivate by SSF the two edible mushrooms, *Pleurotus ostreatus* (Jacq.) P. Kumm. and *Favolus tenuiculus* (a species also known as *Polyporus tenuiculus* (P. Beauv.) Fr. or *Favolus brasiliensis* (Fr.) in the region). Both these fungal species were able to grow and produce good quality fruiting bodies, with appreciable differences in their sensory characteristics, compared with those grown on wheat straw (Omarini et al. 2010a). Both strains belong to white rot fungi and produce different wood-degrading enzymes which enable them to use the EW and LW as carbon sources (Bajwa and Arora 2009; Dudai et al. 2001; Moredo et al. 2003; Weinberg et al. 1999). Hence, during mushroom cultivation by SSF, many

compounds may be released from the plant tissues or transformed by the action of their specific enzymes.

In the present work, *P. ostreatus* and *F. tenuiculus* were used to evaluate for their biotechnological applications in aroma release and biotransformations using SSF of spent leaves waste from the essential oil industry (*E. cinerea*). Thus goal of this work was to investigate the capacity of these two edible mushrooms to transform the terpene compounds present in the spent *Eucalyptus* essential oil industry waste by SSF. The volatile compounds present in the essential oil extracted from the substrates during two stages of the crop cycle were identified by gas chromatography mass spectrometry (GC/MS).

Material and methods

Microorganisms and spawn preparation

The fungi used in this study are conserved in the IIB-INTECH Collection of Fungal Cultures (ICFC) and referenced in the WDCM database as WDCM 826: *Favolus tenuiculus* ICFC 383/00, Brazil, Rio Grande do Sul, Porto Alegre and *Pleurotus ostreatus* ICFC 153/99, commercial strain. They were both grown and maintained on Potato Dextrose Agar (PDA, Britania™, 39 g/L) and stored at 4 °C.

Spawn (SP) was prepared from boiled wheat seeds (*Triticum* sp.) supplemented with 1 % (w/w) $CaCO_3$, placed in polypropylene bags and then sterilized at 121 °C for 2 h (Omarini et al. 2009). Once cooled, each bag was inoculated with mycelia, which was grown on PDA, and then incubated in darkness at 25 °C for 3 weeks until the mycelia had completely covered the wheat seeds.

Substrate preparation and culture conditions

Leaves of *E. cinerea* were collected from natural plantations located at Chascomús (Buenos Aires, Argentina). In order to simulate the industrial steam distillation process and to obtain the spent leaf waste, each sample was put in a cooker with distilled water and boiled for an hour without a cover to evaporate the essential oils (Omarini et al. 2010b). After this process, the water from each sample was drained to obtain a final moisture level of 70 % (wet basis).

Solid-state fermentation procedure

Polypropylene bags (20*45 cm) containing 350 g of wet spent leaf waste of *E. cinerea* (100 g dry weight; EW) and 2 % $CaCO_3$ were autoclaved twice at 121 °C for 2.5 h. After cooling, the bags were inoculated with 5 % (w/w) spawn of *F. tenuiculus* or *P. ostreatus* and incubated in darkness at 25 °C. The time periods used for the spawn runs were 45 days for *F. tenuiculus* and 30 days for *P. ostreatus*. The samples (production blocks) were

removed from the polypropylene bags and then transferred to the production room. Ten replicates per fungus strain were used, with the cultivation conditions for fruiting body production being (i) temperature 20±2 °C, (ii) photoperiod with 9 h light/15 h dark, and (iii) relative humidity between 86–90 %, being automatically performed by spray every 8 h for 5 min (fog type). Finally, SSF was carried out for 50–60 days.

Biotransformation experiments by SSF

The samples, the complete production block (100 g wet weight) of each strain were randomly taken (in triplicate) at two stages of the cultivation cycle: (i) incubation period (25 days after inoculation) and (ii) fructification period (after the first production flush, 50–60 days after inoculation). This sampling design was chosen based on previous studies, where the fungal enzyme production and activities were reported to show differences according to (i) the cultivation time, (ii) the fungal species or (iii) the nature of the substrate used (Chen et al. 2003; Rodrigues da Luz et al. 2012; Shashirekha and Rajarathnam 2007; Singh et al. 2011). Substrates not inoculated with mushroom (EW) and the spawn (SP) used to inoculate substrate bags (wheat seeds inoculated with each strain) were used as control samples.

The essential oil of each sample was extracted by hydrodistillation in a Clevenger-type apparatus for 2 h with a separated extraction chamber (Clevenger 1928). The stem oils were recovered and mixed with methylene chloride (40 % *v/v*) in a separating funnel, shaken vigorously and left standing for a few minutes to separate the organic phase (CH_2Cl_2), which contained the EO. The resulting EO was dried over anhydrous-sodium sulphate and stored at −20 °C in darkness until analysis (López et al. 2004). Prior to analysis, the organic phase was separated from EO by evaporation in a rotary evaporator at 37 °C and the EO content was expressed as a percentage (g per 100 g of wet substrate biomass; Table 1). Volatile compounds were analysed by gas chromatography, as described below.

Biotransformation experiments in liquid medium culture

The bioconversion products yielded by the two fungal species using SSF were confirmed by liquid culture supplemented with the precursor compound 1,8-cineole. The liquid culture medium (SNL) was prepared according to Onken and Berger (1999). The standard medium (1 L) contained 30 g D-glucose monohydrate, 4.5 g L-asparagine monohydrate, 3 g yeast extract, 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot H_2O$ and 1 mL trace elements solution (80 mg/L $FeCl_3 \cdot 6H_2O$, 90 mg/L $ZnSO_4 \cdot 7H_2O$, 30 mg/L $MnSO_4 \cdot H_2O$, 5 mg/L $CuSO_4 \cdot 5H_2O$ and 0.4 g/L EDTA). The pH was adjusted to 6.0 with 1 M KOH prior to autoclaving.

Experimental cultures of *F. tenuiculus* and *P. ostreatus* were prepared as described by Onken and Berger (1999). A 30-mL culture volume (SNL) was inoculated with 1.5 mL of 4-day-old precultures which had been grown in the same medium and homogenized using a blender prior to inoculation. The precultures of each strain were prepared by adding 1 cm^2 of agar plugs to 90 mL of SNL. Cultivation was performed on a rotary shaker at 180 rpm and 24 °C in darkness.

Biotransformations were started 2.5 days after inoculation by adding 0.1 % (*v/v*) 1,8-cineole (Fluka, Switzerland) directly into the culture flasks. A chemical blank was performed in the same way, but without mycelium, to ensure the absence of chemical transformation reactions. Cultivation was performed on an orbital shaker at 180 rpm and 24 °C for 12 days; after which, the cultures were centrifuged and the supernatants were extracted with CH_2Cl_2, as previously described for the SSF substrates. The organic phase (2 μL) was analysed using gas chromatography.

Analytical procedures

The terpene fractions present in the EO samples, derived from (i) SSF and (ii) liquid media, were analysed by gas chromatography with a flame ionization detector (Perkin Elmer Clarus 500) fitted with a non-polar DB-5 column (25 m length×0.25 mm id×0.25 μm film thickness) and a polar Supelcowax 10 capillary glass column (25 m length× 0.25 mm id×0.25 μm film thickness). The GC operating conditions were oven temperature 50 °C (2 min) to 200 °C at 3 °C/min; injector and detector temperatures 200 and 270 °C, respectively; detector FID; and carrier gas helium at a constant flow of 0.9 mL/min.

GC/MS analyses were performed using gas chromatography equipped (HP 5890II-HP 5971 MSD, Hewlett-Packard) with a DB-5 column (25 m length×0.25 mm id×0.25 μm film thickness) and a mass spectrometer detector. Analytical conditions were as follows: oven temperature programmed from 50 °C (2 min) to 200 °C at 3 °C/min; injector and detector temperatures 200 and 270 °C, respectively; carrier gas helium at a constant flow of 0.9 mL/min; and source 70 eV.

All analyses were performed in triplicate, and the volatile compounds were identified by comparing their retention indices (RIs; determined on the basis of homologous *n*-alkane hydrocarbons under the same conditions) and mass spectra with those of authentic standards when possible or with commercial mass spectral databases of the Wiley library (Wiley 275; J. Wiley & Sons Ltd, Wet Sussex, UK) and NIST 98 MS Library (Rev. D. 01.00; HP, Ringoes, NJ, USA).

Statistical analysis

Results were analysed by the software SigmaStat® programme for Windows, version 3.1 (Systat software Inc., Point Richmond, CA, USA). The relative percentage of each volatile compound was calculated from the average of three

Table 1 Percentage of essential oils (g 100 g^{-1} wet substrate biomass) obtained by hydrodestilation of control and samples of *F. tenuiculus* and *P. ostreatus* treatments (incubation and fructification stages)

Samples	Cultivation time	EO yields (%) (g per 100 g of wet substrate)
Control*	0	0.017±0.001
P. ostreatus	I	0.015±0.001
F.tenuiculus	I	0.016±0.001
P. ostreatus	F	0.025±0.001
F.tenuiculus	F	0.028±0.0008

I incubation time, *F* after the first fructification

Table 2 Relative percentage concentrations of the volatile compounds present in the EO extracts derived from samples treated with *F. tenuiculus* and *P. ostreatus* (incubation and after fruiting stages) and from control substrate (*E. cinerea*)

Compounds [3]	RI [1]	Relative percentage [2]				
		P. ostreatus	*F. tenuiculus*	*P. ostreatus*	*F. tenuiculus*	*E. cinerea*
		Incubation	Incubation	Fruiting	Fruiting	Control
p-Cymene	1025	0.52 (0.09)b	0.10 (0.02)a	0.48 (0.19)b	0.25 (0.05)a	0.60 (0.04)b
Limonene	1029	1.29 (0.39)a	0.72 (0.23)a	1.18 (0.46)a	0.76 (0.22)a	0.90 (0.02)a
1,8-Cineole	1031	7.55 (1.14)b	7.46 (1.38)b	6.28 (1.06)b	7.72 (0.88)b	1.83 (0.02)a
cis-Linalool oxide	1087	0.44 (0.19)a	0.10 (0.01)a	0.49 (0.15)a	0.30 (0.01)a	0.30 (0.02)a
Linalool	1097	0.10 (0.01)a	0.10 (0.02)a	0.15 (0.05)a	0.13 (0.01)a	0.10 (0.02)a
Sabinene hydrate	1098	0.99 (0.12)c	0.10 (0.01)a	0.64 (0.14)b	0.10 (0.01)a	0.00 a
Myrcenol	1123	0.35 (0.28)a	0.10 (0.01)a	0.44 (0.24)a	0.13 (0.06)a	0.10 (0.01)a
Camphor	1146	0.10 (0.01)a	0.60 (0.31)ab	0.37 (0.16)a	0.60 (0.14)ab	1.10 (0.01)b
Isoborneol	1162	1.37 (0.46)a	2.16 (0.05)b	0.98 (0.10)a	2.27 (0.52)b	2.00 (0.02)b
Pinocarvone	1165	0.10 (0.01)a	2.67 (0.31)c	0.73 (0.21)b	2.85 (0.48)c	3.40 (0.02)d
4-Terpineol	1177	2.83 (1.50)a	3.00 (0.26)a	1.95 (0.60)a	3.03 (0.25)a	4.83 (0.02)b
cis-Pinocarveol	1184	0.10 (0.01)a	2.19 (0.76)b	0.10 (0.01)a	1.52 (0.60)b	2.20 (0.02)b
α-Terpineol	1189	36.81 (0.97)a	43.09 (1.83)c	36.20 (1.04)a	42.61 (1.88)c	38.30 (0.05)b
1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol	1192	1.87 (0.34)b	2.23 (0.20)b	1.75 (0.60)b	2.38 (0.64)b	0.00a
1,3,3-Trimethyl-2-oxabicyclo (2.2.2)octan-6-one	1197	4.94 (0.27)b	6.11 (0.45)b	5.11 (1.05)b	5.93 (0.77)b	0.00a
Verbenone	1205	1.77 (0.55)b	0.59 (0.27)a	0.67 (0.28)a	0.30 (0.10)a	0.55 (0.03)a
trans-Carveol	1217	0.00a	0.00a	0.00a	0.00a	0.70 (0.03)b
Piperitone	1253	0.00a	0.00a	0.00a	0.00a	0.56 (0.05)b
Methyl nerolate	1283	1.20 (0.75)b	0.10 (0.01)a	0.94 (0.33)b	0.15 (0.05)a	2.27 (0.05)c
α-Terpenyl acetate	1349	17.41 (0.96)b	14.38 (2.85)ab	16.99 (1.50)ab	13.95 (3.14)ab	13.56 (0.05)a
Eugenol	1359	0.00a	0.00a	0.00a	0.00a	0.33 (0.05)b
Longifolene	1408	2.47 (1.10)b	1.00 (0.01)a	2.88 (0.12)b	1.26 (0.65)a	4.24 (0.04)c
β-Caryophyllene	1419	1.01 (0.09)b	1.41 (0.36)b	1.05 (0.13)b	0.84 (0.34)b	0.33 (0.06)a
Alloaromadendrene	1460	1.21 (0.70)a	0.84 (0.07)a	1.29 (0.39)a	0.87 (0.12)a	1.02 (0.12)a
γ-Gurjunene	1477	0.68 (0.29)a	0.85 (0.37)a	1.02 (0.61)ab	0.93 (0.27)a	2.10 (0.10)b
Valencene	1496	2.20 (0.74)a	2.03 (0.63)a	2,18 (0.72)a	1.72 (0.10)a	1.72 (0.03)a
Viridiflorene	1497	1.23 (0.59)a	1.16 (0.58)a	1.80 (0.50)ab	1.01 (0.29)a	2.53 (0.02)b
Globulol	1585	1.65 (0.08)b	0.34 (0.19)a	1.83 (0.21)b	0.34 (0.20)a	5.92 (0.02)c
α-Cadinol	1654	0.77 (0.22)b	0.34 (0.20)a	0.81 (0.25)b	0.35 (0.20)a	2.91 (0.02)c
Drimenol	1767	1.50 (0.62)b	0.35 (0.18)a	1.70 (0.52)b	0.43 (0.13)a	0.30 (0.02)a

[1] Retention index

[2] Relative percentages of the volatile compounds are based on the peak areas obtained, without MS detector response factor correction. Values with different letters are significantly different from each other according to Duncan's multiple range test at $p \leq 0.05$ (*n*=3). (Comparing the percentage of each component with respect to each treatment and control)

[3] Each cell in the table is formatted according to the scheme mean (standard error of the mean)

replicates. Means and standard deviations were also calculated, and a one-way analysis of variance was performed. Significance (at $p \leq 0.05$) was assessed using Duncan's multiple range test.

Results and discussion

The two edible mushrooms *P. ostreatus* and *F. tenuiculus* were cultivated by SSF using spent leaves of *E. cinerea* waste with the aim of evaluating their ability for aroma compound release and subsequent transformation. For this purpose, the EO (Table 1) extracted from the control (EW and SP of each strain) and the treatment samples (production blocks of *F. tenuiculus* and *P. ostreatus* incubation and fructification stages) were analysed by GC and GC/MS. The relative concentration percentages of the volatile compounds present in the EO extracts derived from samples treated with *F. tenuiculus* and *P. ostreatus* (incubation and after fruiting stages) as well as from the control substrate (EW) are shown in Table 2.

The chromatograms revealed two new product peaks produced by *P. ostreatus* (incubation, I) transformation (Fig. 1b), with the same product peaks being obtained with EO samples of *P. ostreatus* during the fructification stage (F) and in samples of *F. tenuiculus* (I and F). GC/MS results of the EO extracts from treatments and control substrates (EW) showed a total of 30 different compounds corresponding to the terpene fraction (Table 2). The major volatile compounds found were oxygenated monoterpenes (17) and sesquiterpene hydrocarbons (6) and to a lesser extent oxygenated sesquiterpenes (3), monoterpene hydrocarbons (2) and esters (2). Of all the terpenes present in the EO extracted from each treatment, only the two fungal strains were able to biotransform 1,8-cineole. These two new biotransformation products were not found in the EW control substrate (Fig. 1a) or in the spawn (SP) of the *F. tenuiculus* or *P. ostreatus* used to inoculate the production blocks (Online resource 1). The mass spectral comparison with the Wiley and Nist libraries allowed these two new compounds to be identified as 1,3,3-trimethyl-2-oxabicyclo [2.2.2]octan-6-ol and 1,3,3-trimethyl-2-oxabicyclo [2.2.2]octan-6-one (Figs. 2 and 3, respectively). The relative

Fig. 1 GC-FID chromatograms, corresponding to the EO extracted from the different samples. **a** EO of the control substrate (EW): no fungal action (before mushroom inoculation); **b** EO of substrate plus mushroom mycelium: after treatment with *P. ostreatus* (incubation). *Arrows* plus letter "*a*" correspond to the 1,8-cineole, and "*b*" and "*c*" correspond to the two new products produced by fungal transformation
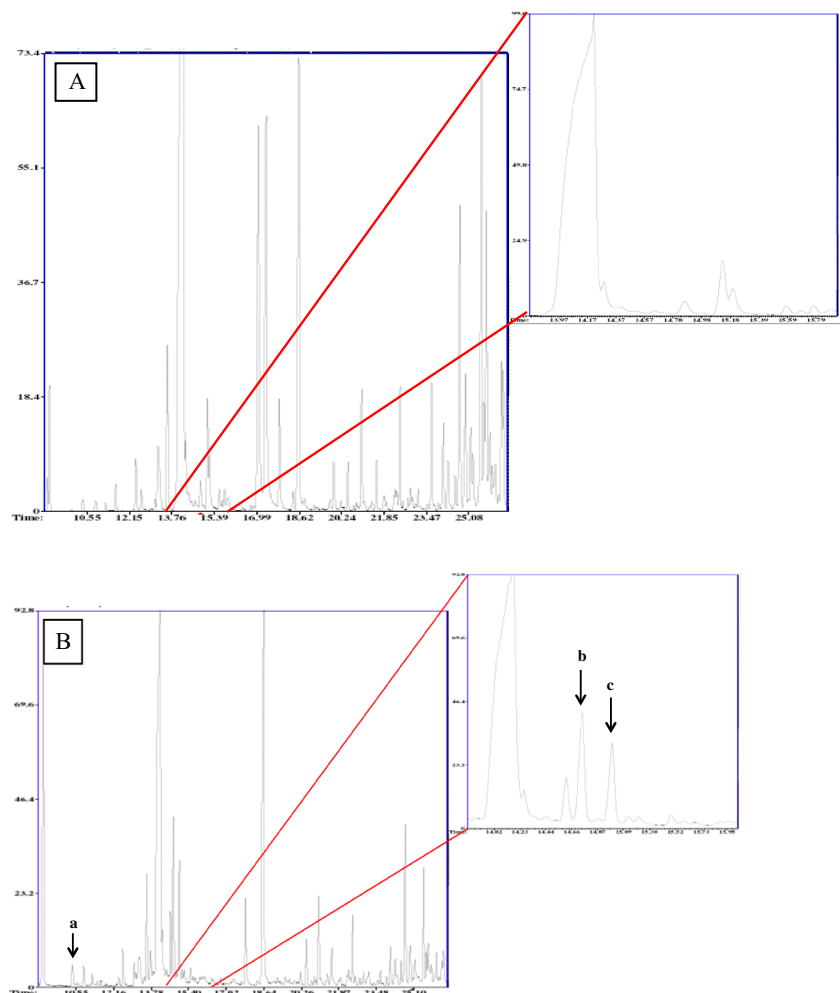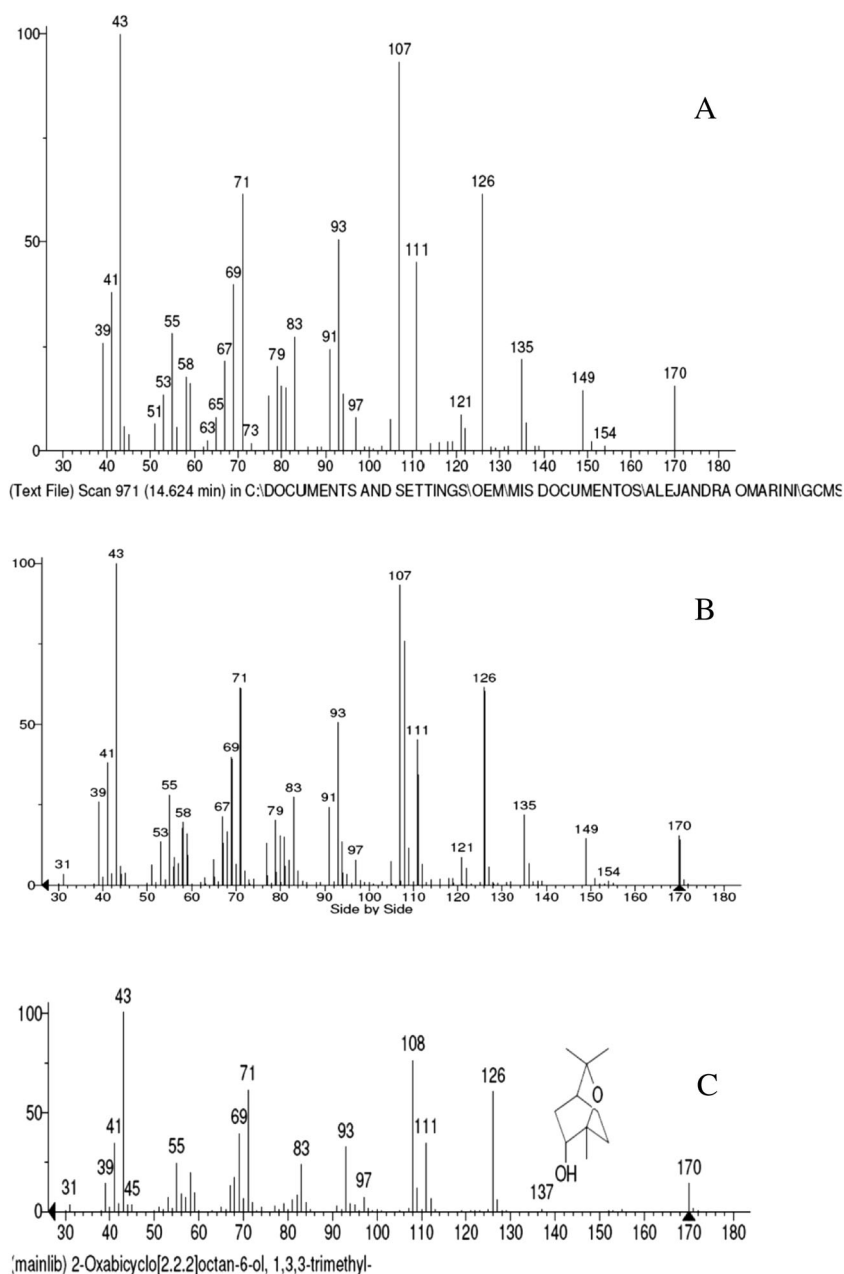
Fig. 2 Mass spectrum of 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ol obtained by GC/MS. **a** Spectrum of the compound obtained by *F. tenuiculus* and *P. ostreatus* biotransformation. **b** Mass spectrum of GC/MS obtained from the NIST library. **c** Mass spectrum of GC/MS obtained from the Wiley library


percentages of each new biotransformation product were similar in both fungal strains and treatments (Table 2). However, 1,3,3-trimethyl-2-oxabicyclo [2.2.2]octan-6-one revealed the highest relative percentages in all the samples of both fungal strains and treatments (at the I and F stages).

A few studies have already been carried out on the oxygenation of 1,8-cineole by chemical (Asakawa et al. 1988) and biological processes (Garcia et al. 2009; MacRae et al. 1979; Nishimura et al. 1982; Rodriguez et al. 2006; Trudgill 1990). Garcia et al. (2009) have described the biotransformation of 1, 8-cineole in liquid medium using a native *Aspergillus terreus* isolated from *Eucalyptus* leaves. As a result, four oxygenated derivative compounds were produced, namely 2-*exo*-hydroxy-1,8-cineole, 2-*endo*-hydroxy-1,8-cineole, 3-*exo*-hydroxy-1,8-cineole and 3-*endo*-hydroxy-1,8-cineole. MacRae et al. (1979) reported that *Pseudomonas flava* cultivated in a mineral salts medium containing 1,8-cineole produced 2-α and 2-β-1,8-cineole as the transformation products.

Even though the two new products we obtained by the action of *F. tenuiculus* and *P. ostreatus* partially agreed with previous reports, the novelty of the present study is the use of two edible mushrooms as biotransforming agents and spent *E. cinerea* leaves waste as the substrate. Thus, through SSF of low-cost raw materials, it was possible to obtain simultaneously two valuable products in the form of fruiting bodies and aroma compounds. Additionally, the 1,8-cineole

Fig. 3 Mass spectrum of 1,3,3-
trimethyl-2-oxabicyclo
[2.2.2]octan-6-one obtained by
GC/MS. **a** Spectrum of the
compound obtained by
F. tenuiculus and *P. ostreatus*
biotransformations. **b** Mass
spectrum of GC/MS obtained
from the NIST library. **c** Mass
spectrum of GC/MS obtained
from the Wiley library
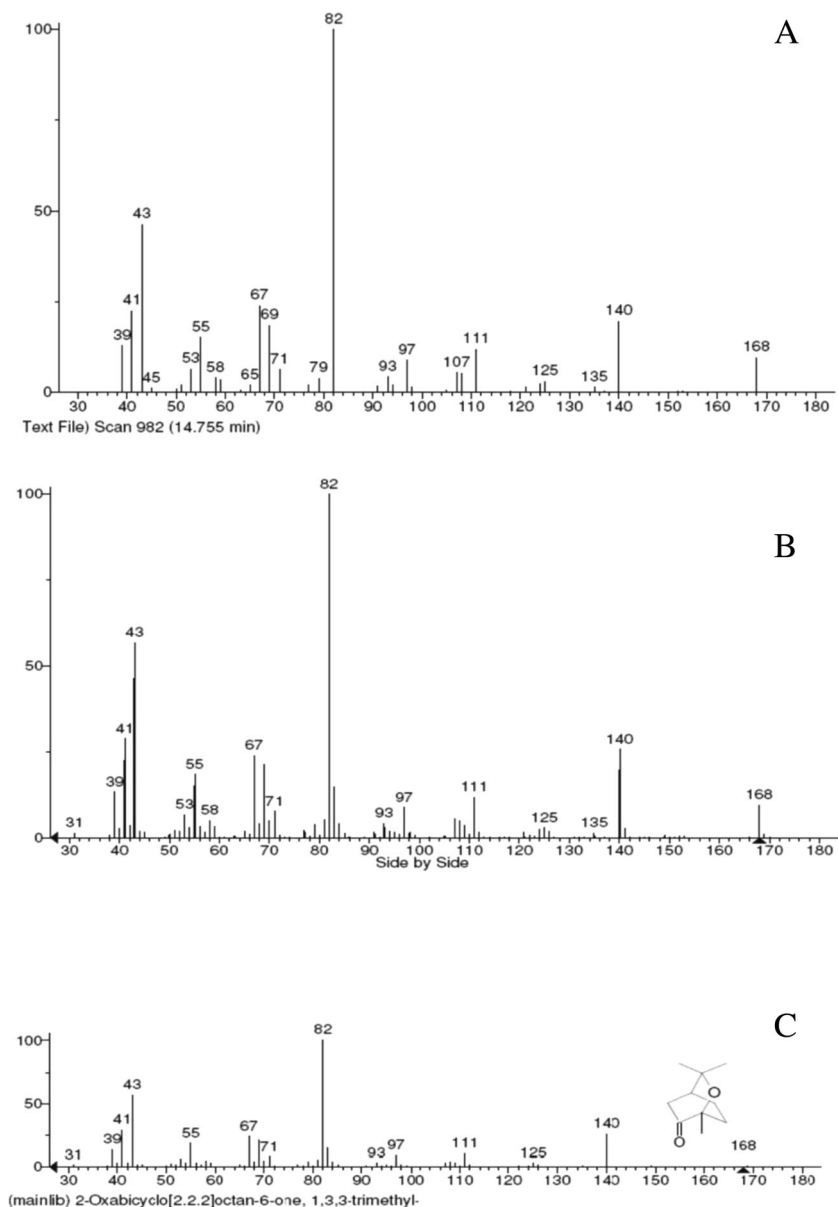
biotransformation was validated by experiments carried out in liquid culture medium (Online Resource 2). After 12 days of culture (stationary growth phase), *F. tenuiculus* and *P. ostreatus* transformed 1,8-cineole into 1,3,3-trimethyl-2-oxabiciclo [2.2.2]octan-6-ol, 1,3,3-trimethyl-2-oxabiciclo [2.2.2]octan-6-one and a third unknown compound whose structure could not be determined. This unidentified compound may have been an oxygenated monoterpene, considering that the molecular ion (M^+) corresponded to the mass spectrum peak 170, whose structure might be associated with the biotransformed compound: 1,3,3-trimethyl-2-oxabiciclo [2.2.2]octan-6-ol. A blank experiment without the inoculum demonstrated that these two new compounds did not originate from autoxidation of 1,8-cineole. Therefore, it is conceivable that both white rot basidiomycetes have the capability to

transform 1,8-cineole into oxyfunctionalized compounds, as was reported by the experiments cited above carried out by SSF of *E. cinerea*. Related to this, Onken and Berger (1999) reported the ability of *Pleurotus sapidus* to biotransform limonene into *cis*:*trans*-carveol and carvone as the main products.

In the present investigation, it was also observed that the relative percentages of several compounds present in the EW changed their values after fungal treatments (I and F stages; Table 2). In some cases, the relative percentages increased significantly after fungal treatments (I and F), as in the case of 1,8-cineole ($p < 0.05$). Other compounds revealed an increase in their relative values after the I and F treatments for one of the strains, such as in the case of (i) sabinene hydrate and drimenol in the EO samples of *P. ostreatus*, (ii) α-terpineol in EO samples of *F. tenuiculus*, and (iii) β-

caryophyllene in EO samples of *P. ostreatus* and *F. tenuiculus*. Finally, verbenone only increased in the EO samples of the *P. ostreatus* incubation.

In general, the observed release of compounds, especially in *P. ostreatus*, may be explained as follows: (i) by the action of different enzymes, mainly the ligninolytic enzymatic system, which plays an important role in the mobilization of compounds during SSF (Bajwa and Arora 2009; Dudai et al. 2001; Moredo et al. 2003; Weinberg, et al. 1999) and (ii) by the ability of fungi to perform de novo synthesis of compounds from the precursor compounds present in the substrate (Berger 2009; Lapadatescu et al. 2000; Lesage-Meessen et al. 1999; Longo and Sanromán 2006). In addition, the heterogeneity of the substrates used (EW) in terms of particle size, or differences in the availability of carbon and nitrogen sources, may contribute to the differences in the patterns of aroma compounds obtained by the strains (Laufenberg et al. 2003; Marostica and Pastore 2007; Rossi et al. 2009). It was reported previously that the addition of selected precursors or different carbon/nitrogen sources as supplements in coffee husk culture substrates influenced the nature and intensity of the aromas produced by *Ceratocystis fimbriata* and at the same time enhanced the production rate (Bramorski et al. 1998; Christen et al. 1997; Christen et al. 1994). Hence, the employment of spent industrial waste (e.g. EW) represents a good option to obtain edible mushrooms by SSF, and once the fruiting bodies are harvested, the spent substrates can then be used to obtain aroma compounds with potential industrial applications. For example, Barton et al. (2010) reported an improvement in herbicidal activity of hydroxy derivates of 1,8-cineole against ryegrass (*Lolium rigidum*) and radish (*Raphanus* sativus) compared to 1,8-cineole or *Eucalyptus* oil. Moreover, 2-*endo*-hydroxy-1,8-cineole and its derivative esters showed antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Villecco et al. 2008). In this way, phytotoxic compounds derived from natural products represent a potential source of new natural pesticides, which exhibit novel modes of action to reduce the development of pesticide resistance (Duke et al. 2000). Furthermore, in the current study, it was possible to recover a mix of interesting compounds and also two new ones as a result of fungal biotransformation. In fact, the biotechnological conversion of low-cost agro-industrial by-products offers a number of advantages, such as the following: the conversion takes place under mild conditions, good regio- and enantioselectivity, no production of toxic waste and generation of "natural" products.

In conclusion, this exploratory study demonstrates that the use of microorganisms to carry out biotransformation reactions by SSF is a great biotechnological advance over existing traditional organic synthesis processes. The two edible mushrooms, *F. tenuiculus* and *P. ostreatus*, were able to transform 1,8-cineole into two new compounds 1,3,3-trimethyl-2-oxabiciclo [2.2.2]octan-6-ol and 1,3,3-trimethyl-2-oxabiciclo [2.2.2]octan-6-one by SSF. It is worth adding that it was possible to identify the other compounds present in the substrates, whose relative percentages were altered through the enzymatic action of each strain. The biotransformation capability of each strain can be used for new aroma compound production with potential industrial applications.

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