Food Chemistry 126 (2011) 849–854

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Utilisation of orange peel in the production of α-terpineol by *Penicillium digitatum* (NRRL 1202)

A.Z.M. Badee *, Shahinaz A. Helmy, Nashwa F.S. Morsy

Food Science and Technology Dept., Fac. of Agric., Cairo University, Giza, Egypt

ARTICLE INFO

Article history:
Received 17 November 2009
Received in revised form 13 July 2010
Accepted 10 November 2010
Available online 19 November 2010

Keywords:
Biotransformation
Penicillium digitatum
Bioconversion
D-Limonene
α-Terpineol

ABSTRACT

The use of orange peel oil in the biotransformation of D-limonene was investigated. The physicochemical properties of cold-pressed orange peel oil, used in this investigation were determined to define its identity. The chemical composition of orange peel oil was determined by using GC/MS. Monoterpene compounds amounted to 98.0%, followed by aldehdyde components 1.09%. The main component of orange peel oil was D-limonene, which represented 96.1%, of the total content. A strain of *Penicillium digitatum* NRRL 1202 was used to carry out the biotransformation of D-limonene to α-terpineol. Two different media, malt yeast broth (MYB) and malt extract broth (MEB) were used. It was found that the highest bioconversion of D-limonene into α-terpineol was obtained by using MYB medium (pH 6.1).

The α-terpineol selectivity was recorded as 67.7%, by using MYB medium at 25–27 °C, 31 h after the second substrate addition. Meanwhile, the selectivity was 47.1% by using MEB medium (pH 5.4) under the same conditions. The bioconversion, in MYB medium, increased with increasing time, where it was recorded 79% and 95.5% 3 h and 7 h after the second substrate addition on the first day, respectively. Meanwhile, bioconversion of D-limonene on the second day reached 91.0% 7 h after the fourth addition of orange peel oil. By contrast, the bioconversion of D-limonene on using MEB medium was 87.8% 7 h after the second substrate addition on the first day. Meanwhile, bioconversion of D-limonene in the same media reached 100% 7 h after the fourth addition of orange peel oil on the second day.

1. Introduction

Flavours and fragrances are widely used in the food, beverage, and cosmetic industries. Most of the flavours in the world market today are obtained by chemical synthesis. In recent years, according to the FDA and European legislation, products obtained by biotechnological methods can also be considered natural, if the substrate for the process is of natural origin (Serra, Fuganti, & Brenna, 2005), so many studies have focused on biotechnological production of flavours and fragrances (Xu, Hua, & Ma, 2007).

Terpenes and especially their oxygenated derivatives, are extensively used in the flavour industry. Via transformation, monoterpene precursors are converted into their more valuable oxygenated derivatives (Van der Werf, De Bont, & Leak, 1997).

D-Limonene (4-isopropenyl-1-methylcyclohexene), a monocyclic monoterpene, is the most widespread terpene in the world and is the major constituent of citrus peel essential oils, in which it is usually found at concentrations between 90% and 96%, as a by-product of the fruit juice industry. Annually, approximately 36 million kilograms of D-limonene are recovered as a by-product of the citrus industry (Nonino, 1997). Due to its low price, D-limonene is an attractive starting compound for industrially relevant fine chemicals and flavour compounds with identical carbon skeletons, such as carveol, carvone, and perrillyl alcohol. Microbial strains found, so far, to be capable of bioconversion of D-limonene, generally yielded a mixture of oxidation products (Rozenbaum, Patitucci, Antunes, & Pereira, 2006). Microorganisms and their enzymes have proven to be versatile biocatalysts and are extensively used for biotransformation of various terpenoids. Unlike traditional chemical processes, which require extreme temperatures and pressures, microbial conversions take place under mild conditions and, in some instances, the products are formed stereoselectively. Various enzymes occur in several microorganisms (such as bacteria, yeast, and fungi) which are effective in biotransformations of various terpenoids and can be used *in vivo* (Chatterjee & Bhattacharyya, 2001). Recent examples are the conversion of D-limonene to iso-piperitenone, limonene-1,2 trans-diol, cis-carveol, perrillyl alcohol, isopiperitenol, and α-terpineol by *Aspergillus cellulosae* (Noma, Yamasaki, & Asakawa, 1992), or to carveol , α-terpineol, perillyl alcohol, and perrillyl aldehyde by *Bacillus stearothermophilus* BR388 (Chang & Oriel, 1994), and the same conversions by an *Escherichia coli* construct containing a plasmid with chromosomal inserts from this strain (Chang, Gage, & Oriel, 1995). In most of

* Corresponding author.
E-mail address: prof.adelbadee@yahoo.com (A.Z.M. Badee).

0308-8146/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2010.11.046

the biotransformation studies performed previously, the researchers used microorganisms which do not grow on D-limonene as a sole source of carbon and energy, and many of the strains appeared to have more than one biotransformation pathway. However, limonene is a relatively unstable compound, and some of the products identified in culture media are also the major auto-oxidation products of D-limonene (Duetz, Fjallman, Ren, Jourdat, & Witholt, 2001; Van der Werf, Swarts, & De Bont, 1999).

An interesting end-product, resulting from the bioconversion of D-limonene, is the monoterpene alcohol, α-terpineol. Bioconversion of D-limonene to R-(+)-α-terpineol has been described, using microorganisms as catalysts: *Penicillium digitatum* DSM 62840 (Abraham, Stumpf, & Kieslich, 1986; Adams, et al., 2003), *Pseudomonas gladioli* (Cadwallader, Braddock, Parish, & Higgins, 1989); *A. cellulosae* M-77 (Noma et al., 1992); *B. stearothermophilus* BR388 (Chang & Oriel, 1994).

R-(+)-α-terpineol has a floral, typically lilac odour, while (S)-(−)-α-terpineol has a coniferous odour characteristic. α-Terpineol is considered as one of the most commonly used fragrance compounds. It is mostly produced chemically, starting from pinene or crude turpentine oil by acid hydration to terpine, followed by partial dehydration. A great advantage of enzymatic processes, as compared to chemical synthesis, is enantiospecificity (Boelens, Boelens, & Van Gemert, 1993).

To increase the commercial value of the orange peel oil it would be of interest to be able to convert its major constituent, D-limonene, into more valuable compounds, such as α-terpineol. Thus, this study spotlights the impact of several parameters on the biosynthesis of α-terpineol from D-limonene by using *P. digitatum* and its optimisation.

2. Materials and methods

2.1. Materials

One hundred kilograms of orange fruits (*Citrus sinensis*), balady variety, at mature stage, was obtained from Abdel-Hady farm, Benha, Kalubia Governorate, Egypt. Authentic hydrocarbons (C_6—C_{24} *n*-alkanes) were obtained from Merck, Germany. In addition, D-limonene, α-terpineol, α-pinene, myrcene, camphene, linalool, nonanal, decanal, β-pinene, geranial, ethyl butyrate, hexanal, and E-2-hexenal (GC-grade) were obtained from Merck, Germany. *P. digitatum* NRRL 1202 strain was obtained from Microbial Genomics and Bioprocessing Res., Peoria, IL, United States Department of Agriculture (USDA), USA.

2.2. Extraction of orange peel oil

The oil was extracted by applying the cold-pressing method. Citrus fruit was peeled manually and the peels were then shredded to a size of 2.0 × 0.3 cm, by using a citrus peel shredder. Cold-expressing of peels was done at ambient temperature. The extraction mixture obtained was centrifuged for 45 min at 28–30 °C and at 3000 rpm, using a Hettich Universal centrifuge. After centrifugation, the oil was separated by using separating funnel, and dried over anhydrous sodium sulphate. The oil was stored at 4 °C in opaque glass bottles.

2.3. Physicochemical properties

Specific gravity at 20 °C, solubility in 95% ethyl alcohol, evaporation residue, refractive index at 20 °C, acid number, and aldehydes (%) were determined according to (Guenther, 1948).

2.4. Microorganism and cultivation

A strain of *P. digitatum* NRRL 1202 was maintained on malt extract agar (MEA: malt extract 2%, bacteriological peptone 0.1%, glucose 2%, and agar 2% – pH 5.4), according to Adams et al. (2003).

2.5. Broth media

Malt Yeast Broth (MYB): 2% malt extract, 1% glucose, 1% bacteriological peptone, 0.3% yeast extract, and the pH was 6.1 (Tan, Day, & Cadwallader, 1998). Malt Extract Broth (MEB): 2% malt extract, 2% glucose, 0.1% bacteriological peptone, and the pH was 5.4 (Demyttenaere & Willemen, 1998).

2.6. Bioconversion by liquid cultures

Biotransformation experiments by rotary liquid cultures of *P. digitatum* were run during 4 days according to the method of Adams et al. (2003) with some modifications concerning substrate and sampling time. The mold was cultivated in 500 ml conical flasks, filled with 100 ml of liquid medium (Malt Yeast Broth, MYB, or Malt Extract Broth, MEB). Inoculation was performed with a 1 ml spore suspension of 1.3×10^7 spores/ml (Demyttenaere, Van Belleghem, & De Kimpe, 2001). The test substrate orange peel oil was added as a solution of a 20% (v/v) orange peel oil/absolute ethanol. Two additions of 250 μl and two of 125 μl of a 20% (v/v) orange peel oil/absolute ethanol were made (= 750 μl solution). The first and second substrate additions took place 40 and 42 h after inoculation, respectively. The third and fourth substrate additions took place 64 and 66 h after inoculation, respectively. The final alcohol concentration in the liquid broth was 0.6% (v/v).

The first series of samples was taken 3, 5, and 7 h after the second substrate addition. The second series of samples was taken 3, 5, and 7 h after the fourth substrate addition. Five millilitre samples was taken and extracted with 2 ml of diethyl ether, twice. After an addition of 1 ml of a standard solution of 0.1% (v/v) *n*-decane in Et_2O, the samples were directly analysed by GC. Experiments were also run with control flasks, which contained sterile culture broth that was not inoculated with mold strain, and to which the substrate was added. The culture flasks were stirred at 120 rpm at 25 °C.

The reduction in the limonene area (at each sampling time) between blank and sample experiments was used in the calculation of limonene bioconversion (%) according to Demyttenaere et al. (2001) as follows:

$$\text{Limonene bioconversion } (\%) = \frac{(\text{area of limonene in a blank} - \text{area of limonene in a sample})}{\text{area of limonene in a blank}} \times 100$$

On the other hand, production of α-terpineol was followed by GC analysis of the same culture ether extract samples, as mentioned above, in calculation of D-limonene bioconversion. Selectivity of α-terpineol was calculated according to Yadav et al. (2009) as follows:

$$\text{Selectivity of product} = \frac{\text{GC peak area of } \alpha\text{-terpineol}}{\sum \text{GC peak area of resulted product}} \times 100$$

2.7. Analysis of the samples with GC

Gas chromatography (GC) analyses were done for samples extracted from cultures (inoculated and uninoculated ones), as described elsewhere by Marostica and Pastore (2007). The GC

analysis was done using a Shimadzu gas chromatograph equipped with a split/splitless-injector, an FID-detector and a WCOT fused silica column. The stationary phase was a DB-5 column (0.25 mm I.D. × 30 m; coating thickness of 0.25 μm), and the working conditions included injector and detector temperatures of 275 °C and 340 °C, respectively, with He (1 ml/min) as the make-up gas. The oven temperature was programmed from 70 °C to 250 °C at 30 °C/min with an initial holding time of 1 min and from 250 °C to 310 °C at 20 °C/min with a final holding time of 2 min. Identification was done by comparison with the internal standard (*n*-decane). Specific components were identified by matching their retention indices (RI) with those of standards analysed under identical conditions and/or literature data (Anon., 2009).

2.8. Mass spectrometry

A 0.2 μl sample of undiluted orange essence oil was injected (splitless) into a Finnigan GCQ (Sanjose, CA, USA) with a capillary column (DB-5, 30 m × 0.25 mm I.D., 0.25 μm film thickness; J & W Scientific). Oven temperature was programmed from 40 °C to 250 °C at 7 °C/min with an initial hold time of 0.5 min and final hold time of 10 min. The MS (electron impact ionisation) conditions were: ionisation energy, 70 eV; mass range, 40–300 u; scan rate, 2 scans/s; electron multiplier voltage, 1050 V. Injector temperature was 200 °C and transfer line temperature 250 °C.

Substances were identified by comparison of their mass spectra and retention indices (Kovats Indices) with those of reference substances (where possible) and with literature according to Hognadottir and Rouseff (2003) and by comparison with the NIST Mass Spectral Library.

3. Results and discussion

3.1. Physicochemical properties

The physicochemical properties of orange peel oil obtained by cold-pressing method are tabulated in Table 1. Results indicated that the yield (%) of orange peel oil obtained by cold pressing was 0.2%. This result is in agreement with that obtained by Matthews and Braddock (1987), who reported that the yield of cold-pressed orange peel oil ranged between 0.15% and 0.44%.

From the results in Table 1 it could be noted that specific gravity, refractive index, and aldehydes (as decyl aldehyde %) of the isolated orange peel oil were in accordance with those of the Egyptian Standard for citrus oils (2006). The same results indicate that evaporation residue (%) was 3.49%. Guenther (1949) reported that the evaporation residue % of cold-pressed orange peel oil ranged from 2.76% to 3.9%. Concerning the solubility of the investigated orange peel oil in ethyl alcohol (95%), it was 5 ml, and up to 10 ml, while acid number reached 1.35 mg KOH/g oil. These results are in harmony with those reported by Guenther (1949).

Table 1
Physico-chemical properties of orange peel oil (mean ± SD).

Properties	Value
Specific gravity at 20 °C/20 °C	0.8506 ± 0.001
Refractive index at 20 °C	1.4741 ± 0.0002
Evaporation residue (%)	3.49 ± 0.05
Solubility in ethyl alcohol (95%)	5 ml and up to 10 ml
Acid number (mg KOH/g oil)	1.35 ± 0.04
Aldehyde as decyl aldehyde %	1.515 ± 0.003
Yield (g/100 g)[a]	0.2 ± 0.01

[a] The yield (%) of cold-pressed orange peel oil.

3.2. GC–MS analysis of orange peel oil

The orange peel oil was analysed by GC–MS and the identified compounds and their percentages are shown in Table 2. Sixteen compounds, representing 99.8% of the GC profile, were identified. The quantitatively most important components in orange peel oil were monoterpenes followed by aldehydes. The monoterpene contents of orange peel oil were 98.0%, D-limonene, and sabinene representing 96.1% and 1.57%, respectively, of them, which was in agreement with the range (93–96%) reported by E.S. (2006). The identified volatile aldehydes and alcohols, nonanal, octanal, decanal, geranial, linalool, and α-terpineol, are important for the flavour and aroma of citrus cold-pressed oils, as reported by Moshonas and Shaw (1979) and Shaw (1979). The same table indicates that the investigated orange peel oil contained octanal, geranial, valencene, and α-sinensal with the same levels outlined in the E.S. (2006). In the oxygenated fraction, aldehydes were the major components (1.09%), while alcohols represented 0.31% and esters 0.04%. Among them, nonanal (0.58%), octanal (0.26%), geranial (0.13%), decanal (0.08%), and α-sinensal (0.04%) were the most relevant. Sesquiterpenes were found in low quantities (0.4%). In general, orange peel oil had 1.44% oxygenated compounds, while the non-oxygenated compounds represented 98.4% of the oil content. These results are in agreement with those reported by De Rodriguez, Ysambertt, de Ferrer, and Cabrera (2003).

3.3. Bioconversion

A strain of *P. digitatum* NRRL 1202 was used to carry out the bioconversion of D-limonene present in orange peel oil (≈96.1% from

Table 2
Chemical composition of orange peel oil (means ± SD).

Compounds	Kovats(RI)[a]DB-5	%	ID[b]
1-Monoterpenes			
α-Pinene	922	0.15 ± 0.02	A,B
Camphene	962	0.13 ± 0.02	A,B
Sabinene	971	1.57 ± 0.05	B
Myrcene	997	0.04 ± 5.77	A,B
D-limonene	1032	96.1 ± 0.87	A,B
Total		98.0 ± 0.82	
2-Sesquiterpenes			
Valencene	1513	0.04 ± 0	B
7-Epi-α-selinene	1542	0.04 ± 0.01	B
Germacrene B	1552	0.32 ± 0.04	B
Total		0.40 ± 0.03	
3-Alcohols			
Linalool	1146	0.09 ± 0.01	A,B
α-Terpineol	1201	0.22 ± 0.02	A,B
Total		0.31 ± 0.01	
4-Aldehydes			
Octanal	1004	0.26 ± 0.05	A,B
Nonanal	1089	0.58 ± 0.04	A,B
Decanal	1211	0.08 ± 0.01	A,B
Geranial	1317	0.13 ± 0.02	A,B
α-Sinensal	1756	0.04 ± 0	B
Total		1.09 ± 0.04	
5-Esters			
p-Ment-1-en-9-ol acetate	1427	0.04 ± 0	B
Total		0.04 ± 0	
Total unknown		0.19	
Total known		99.81	
Total oxygenated compounds (%)		1.44	
Total non-oxygenated compounds (%)		98.4	

[a] Kovats retention indices calculated for DB-5 capillary column in GC–MS in reference to C_6—C_{24} *n*-alkane.

[b] ID: (A) Both mass spectrum and retention time identical with an authentic compound; (B) Mass spectrum and Kovats index from literature in accordance.

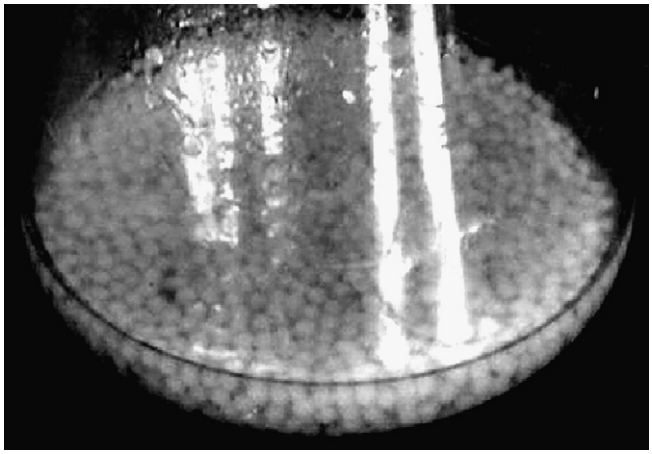

Photo 1. Growth behaviour of *Penicillium digitatum* inoculated in MYB medium.

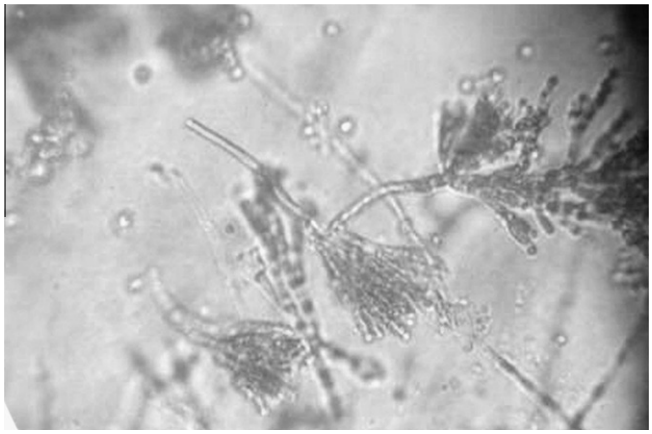

Photo 2. Appearance of *Penicillium digitatum* under microscope (×40).

the oil). The fungal strain was inoculated in two media (MYB and MEB) by a rate of 1 ml spore suspension (1.3×10^7 spores/ml) in 100 ml broth medium. Photo 1 shows the growth behaviour of *P. digitatum* NRRL 1202. Photo 2, illustrates the appearance of *P. digitatum*.

Results in Figs. 1 and 2 demonstrate that the bioconversion of D-limonene by *P. digitatum* reached ⩽80% 3 h after the 2nd addition of D-limonene (100 μl) during the 1st day, when MYB culture was used instead of 62% in the presence MEB culture. This trend was reversed on the 2nd day of bioconversion between the two cultures. Extending D-limonene bioconversion time on each day (after 2nd addition) from 3 to 5 h was accompanied by a gradual increase in bioconversion activity. Tan et al. (1998) observed that accumulation of α-terpineol inhibited bioconversion of D-limonene in the reaction mixture. Bowen (1975) and Chatterjee and Bhattacharyya (2001) reported that increasing limonene concentration >0.2% had an inhibitory effect on various microorganisms and decreased the bioconversion activity.

Results in Fig. 3 indicates that α-terpineol selectivity(%), resulting from the bioconversion of D-limonene by *P.* digitatum in MYB liquid culture, was clearly higher than that obtained with MEB liquid culture throughout the course of bioconversion. According to GC–Mass analysis of the ether extract of non-inoculated medium (blank), no α-terpineol was detected. This result is in line with that obtained by Bicas, Barros, Wagner, Godoy, and Pastore (2008), who reported that α-terpineol produced in the biotransformation process did not originate from the auto-oxidation of D-limonene .

Also, results in Fig. 3 shows that extending D-limonene bioconversion by *P.* digitatum (using MYB liquid culture) to 31 h increased α-terpineol selectivity to >65% instead of <50% when bioconversion process was carried out in MEB liquid culture. According to the equations extrapolated from trend lines representing α-terpineol selectivity in Fig. 3 the production rate of α-terpineol in MYB culture was higher than that obtained with MEB culture.

Tan et al. (1998) found that the highest production of α-terpineol was obtained by a sequential substrate induction.

The higher bioconversion capacity of MYB than that of MEB medium could be explained by the stability of D-limonene (the substrate) under the acidic conditions, as reported by Adams et al. (2003). It is well known that the pH of MEB is 5.4, but it is 6.1 in MYB, so the acidity is higher in MEB than MYB medium, which negatively affected the bioconversion of D-limonene in MEB medium. Another point is the higher glucose content in MEB(2%) than MYB(1%). Bowen (1975) and Duetz et al. (2001) reported that additional glucose decreases the transformation of D-limonene to α-terpineol, and, the microorganism (*P. digitatum* NRRL 1202) will use the glucose as a carbon and energy sources instead of D-limonene. Our finding is in harmony with that reported by Adams et al. (2003) who found that, after the completion of bioconversion of D-limonene (amount of remaining nonconverted D-limonene was 0%) using the *P. digitatum* isolated from spoiled mandarin, the α-terpineol reached 65.3%. They also proved that MYB was the best medium for bioconversion. By contrast, medium MEB gave lower bioconversion yields. Also, Tan et al. (1998) found that the highest production of α-terpineol

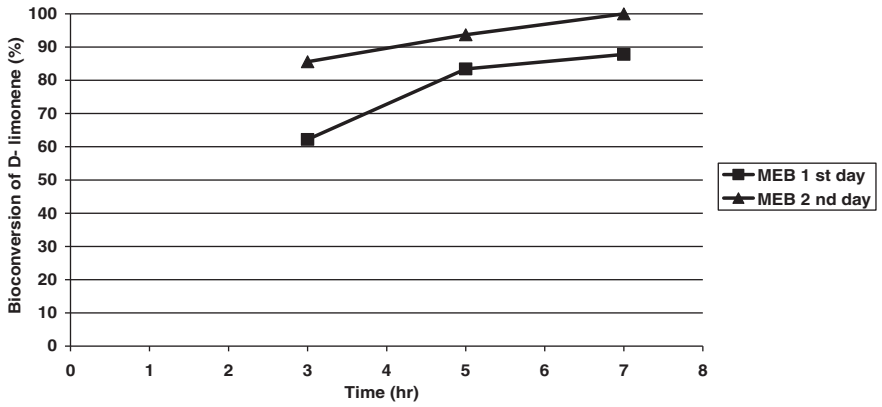

Fig. 1. Effect of time on the bioconversion of D-limonene from orange peel oil by *Penicillium digitatum* NRRL 1202 using MEB medium.

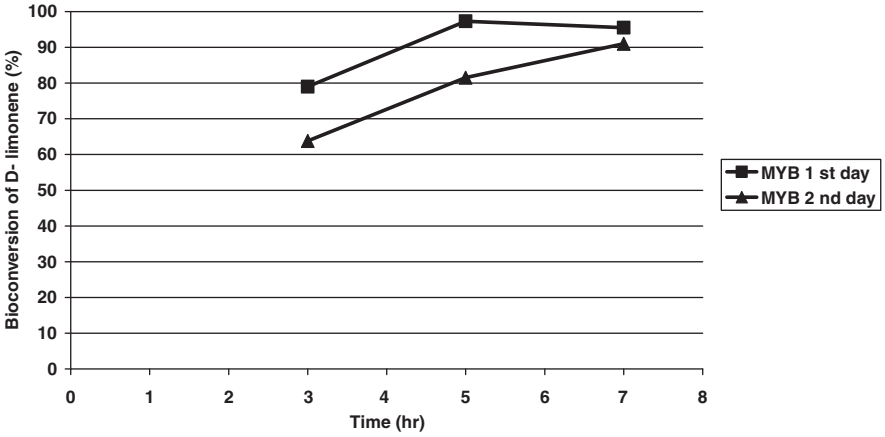

Fig. 2. Effect of time on the bioconversion of D-limonene from orange peel oil by *Penicillium digitatum* NRRL 1202 using MYB medium.

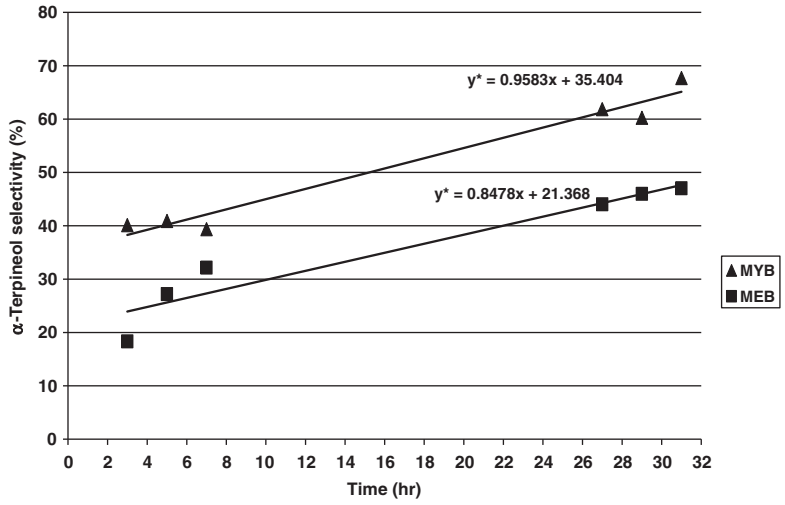

Fig. 3. Effect of limonene bioconversion time and type of medium on the α-terpineol selectivity (%) (*$y = bx + a$ (y = ordinate, b = slope, x = abscissa, a = intercept).

was obtained by a sequential substrate induction. They also reported that the bioconversion of D-limonene by *P. digitatum* was *via* epoxidation, followed by reductive cleavage of the epoxide. Epoxidation reactions are often catalysed by cytochrome P450-dependent mono oxygenases. Van der Werf, Keijzer, and Van der Schaft (2000) reported that the bioconversion of D-limonene involves attack at the 8,9-double bond, forming limonene-8,9-epoxide. Limonene-8,9-epoxide has, however, been implicated as an intermediate in the formation of α-terpineol from D-limonene by *P. digitatum*.

Cadwallader et al. (1989) bioconverted D-limonene to α-terpineol and perillic acid using *P. gladioli*. They found that the concentration of α-terpineol in the fermentation flasks increased to a maximum of 702 ppm at day 4 and decreased by about 12% at day 10. They attributed this decrement to evaporation of α-terpineol.

It is essential to note that the major differences between the data reported in this study and those previously reported by Chang and Oriel (1994) and Noma et al. (1992) who produced α-terpineol as a minor component in the bioconversion of D-limonene. The major components were perillyl alcohol, (+)-iso-piperitenone, (+)-limonene-1,2-trans-diol, and (+)-cis-carveol, While α-terpineol was the major component in our bioconversion products. This phenomenon could be attributed to enantiospecificity.

It is known, in most enzymatic reactions, that the reaction product concentration affects the velocity of reaction, hence affecting the final product; so the bioconversion rate is not stable over all the reaction time.

Results in Table 3 indicate that yields of α-terpineol were 47.2%, 48.1%, and 46.3% after 3, 5, and 7 h of bioconversion on the first day, respectively, using MYB medium. Meanwhile yields of α-terpineol reached 48.5%, 47.2%, and 53.1% after 3, 5, and 7 h of bioconversion on the second day, respectively in the same medium. Results in the same table indicate that the yields of α-terpineol were 19.4%, 28.8%, and 34.1% after 3, 5, and 7 h of bioconversion on the first day, respectively, using MEB medium. Meanwhile yields of α-terpineol reached 31.1%, 32.5%, and 33.2%, after 3, 5, and 7 h of bioconversion on the second day, respectively, in the same medium.

Table 3
Effect of medium and bioconversion time on the yield % (g/100 g) of α-terpineol.

Parameter	1st day			2nd day		
	3	5	7	3	5	7
MYB medium	47.2	48.1	46.3	48.5	47.2	53.1
MEB medium	19.4	28.8	34.1	31.1	32.5	33.2

Table 4
Physicochemical properties of α-terpineol-rich product (mean ± SD).

Properties	Value
Specific gravity at 20 °C/20 °C	0.932 ± 0.003
Refractive index at 20 °C	1.480 ± 0.001
Solubility in ethyl alcohol (70%)	1 ml and up to 10 ml
Acid number (mg KOH/g oil)	0.02 ± 0.004

3.4. Physicochemical properties of α-terpineol-rich product

Physicochemical properties of α-terpineol, obtained by bioconversion of D-limonene using *P. digitatum* (NRRL 1202), were determined to confirm its identity; thus specific gravity, refractive index, solubility in ethyl alcohol and acid number were estimated and the obtained data are shown in Table 4.

Table 4 shows that specific gravity, refractive index and solubility in ethyl alcohol (70%) and acid number of the α-terpineol-rich product were 0.932, 1.484, 1, and up to 10 ml and 0.02 mg KOH/g oil, respectively. These results are in agreement with those reported by Burdock (2009) and prove that the obtained substance was α-terpineol in high purity form.

References

Abraham, W. R., Stumpf, B., & Kieslich, K. (1986). Microbial transformations of terpenoids with 1-ρ-menthene skeleton. *Applied Microbiology and Biotechnology, 24*, 24–30.

Adams, A., Demyttenaere, J. C. R., & De Kimpe, N. (2003). Biotransformation of (R)-(+) and (S)-(−) limonene to α-terpineol by *Penicillium digitatum* – investigation of the culture conditions. *Food Chemistry, 80*, 525–534.

Anonymous (2009). The essential chromatography and spectroscopy catalogue. <http://www.agilent.com/chem/supplies>. Accessed on 10/10/2009.

Bicas, J. L., Barros, F. F. C., Wagner, R., Godoy, H. T., & Pastore, G. M. (2008). Optimisation of R-(+)-α-terpineol production by the biotransformation of R-(+)-limonene. *Journal of Industrial Microbiology and Biotechnology, 35*, 1061–1070.

Boelens, M. H., Boelens, H., & Van Gemert, L. J. (1993). Sensory properties of optical isomers. *Perfumer and Flavourist, 18*(6), 1–16.

Bowen, E. R. (1975). Potential by-products from the microbial transformation of D-limonene. *Florida State Horticultural Society, 88*, 304–308.

Burdock, G. A. (2009). *Fenaroli's handbook of flavour ingredients* (6th ed., 2162pp.). New York: CRC Press Taylor & Francis Group.

Cadwallader, K. R., Braddock, R. J., Parish, M. E., & Higgins, D. P. (1989). Bioconversion of (+)–limonene by *Pseudomonas gladioli*. *Journal of Food Science, 54*(5), 1241–1245.

Chang, H. C., Gage, D. A., & Oriel, P. J. (1995). Cloning and expression of a limonene degradation pathway from *Bacillus stearothermophillus* in *Escherichia coli*. *Journal of Food Science, 60*(3), 551–553.

Chang, H. C., & Oriel, P. J. (1994). Bioproduction of perrillyl alcohol and related monoterpenes by isolates of *Bacillus stearothermophilus*. *Journal Food Science, 59*(3), 660–662.

Chatterjee, T., & Bhattacharyya, D. K. (2001). Biotransformation of limonene by *Pseudomonas putida*. *Applied Microbiology and Biotechnology, 55*, 541–546.

Demyttenaere, J. C. R., Van Belleghem, K., & De Kimpe, N. (2001). Biotransformation of (R)-(+)- and (S)-(−)-limonene by fungi and the use of solid phase microextraction for screening. *Phytochemistry, 57*(2), 199–208.

Demyttenaere, J. C. R., & Willemen, H. M. (1998). Biotransformation of linalool to furanoid and pyranoid linalool oxides by *Aspergillus niger*. *Phytochemistry, 47*(6), 1029–1036.

De Rodriguez, G. O., Ysambertt, F., de Ferrer, B. S., & Cabrera, L. (2003). Volatile fraction composition of Venezuelan sweet orange essential oil (*Citrus sinensis* L. osbeck). *CIENCIA, 11*(1), 55–60.

Duetz, W. A., Fjallman, A. H. M., Ren, S., Jourdat, C., & Witholt, B. (2001). Biotransformation of D-limonene to (+) trans-carveol by toluene-grown *Rhodococcus opacus* PWD4 cells. *Applied and Environmental Microbiology, 67*(6), 2829–2832.

E.S., Egyptian Standard. (2006). The Egyptian Organisation for Standardization A.R.E. Citrus oils. Part:3. Oil of sweet orange. Egyptian Standards (No. 689-3).

Guenther, E. S. (1948). *The essential oils* (Vol. 1). New York: Van Nostrand Comp. Inc. pp. 376–379.

Guenther, E. S. (1949). *The essential oils* (Vol. 3). Princeton, New Jersy, Toronto, New York, London: Van Nostrand Company Inc.

Hognadottir, A., & Rouseff, R. L. (2003). Identification of aroma active compounds in orange essence oil using gas chromatography–olfactometry and gas chromatography–mass spectrometry. *Journal of Chromatography A, 998*, 201–211.

Marostica, M. R., Jr., & Pastore, G. M. (2007). Production of R-(+)-α-terpineol by the biotransformation of limonene from orange essential oil, using cassava waste water as medium. *Food Chemistry, 101*, 345–350.

Matthews, R. F., & Braddock, R. J. (1987). Recovery and application of essential oils from oranges. *Food Technology, 41*(1), 57–61.

Moshonas, M. G., & Shaw, P. E. (1979). Composition of essence oil from overripe oranges. *Journal of Agriculture Food Chemistry, 27*(6), 1337–1339.

Noma, Y., Yamasaki, S., & Asakawa, Y. (1992). Biotransformation of limonene and related compounds by *Aspergillus cellulosae*. *Phytochemistry, 31*(8), 2725–2727.

Nonino, E. A. (1997). Where is the citrus industry going? *Perfumer and Flavourist, 22*, 53–58.

Rozenbaum, H. F., Patitucci, M. L., Antunes, O. A. C., Jr., & Pereira, N. (2006). Production of aromas and fragrances through microbial oxidation of monoterpenes. *Brazilian Journal of Chemical Engineering, 23*(3), 273–279.

Serra, S., Fuganti, C., & Brenna, E. (2005). Biocatalytic preparation of natural flavours and fragrances. *Trends in Biotechnology, 23*(4), 193–198.

Shaw, P. E. (1979). Review of quantitative analyses of citrus essential oils. *Journal of Agriculture Food Chemistry, 27*(2), 246–257.

Tan, Q., Day, D. F., & Cadwallader, K. R. (1998). Bioconversion of (R)-(+)-limonene by *P. digitatum* (NRRL 1202). *Process Biochemistry, 33*(1), 29–37.

Van der Werf, M. J., De Bont, J. A. M., & Leak, D. J. (1997). Opportunities in microbial biotransformation of monoterpenes. In T. Scheper (Ed.), *Advances in Biochemical Engineering/Biotechnology* (pp. 147–176). Berlin: Springer-Verlag.

Van der Werf, M. J., Keijzer, P. M., & Van der Schaft, P. H. (2000). *Xanthobacter* sp.C20 contains a novel bioconversion pathway for limonene. *Journal of Biotechnology, 84*, 133–143.

Van der Werf, M. J., Swarts, H. J., & De Bont, J. A. M. (1999). *Rhodococcus erythropolis* DCL14 contains a novel degradation pathway for limonene. *Applied and Environmental Microbiology, 65*(5), 2092–2102.

Xu, P., Hua, D., & Ma, C. (2007). Microbial transformation of propenylbenzenes for natural flavour production. *Trends in Biotechnology, 25*(12), 571–576.

Yadav, M. K., Patil, M. V., & Jasra, P. R. V. (2009). Acetoxylation and hydration of limonene and α-pinene using cation-exchanged zeolite beta. *Journal of Molecular Catalysis A, 297*, 101–109.