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Production of flavor compounds by hydroperoxide lyase from enzymatic extracts of *Penicillium* sp.

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Abstract

Crude enzymatic extracts obtained from *Penicillium camemberti* and *Penicillium roqueforti* were incubated with the 9-, 10-, 12- and 13-hydroperoxide isomers of linoleic acid (HPODs), used as substrates. The 10-HPOD isomer was the only substrate to be enzymatically converted into selected aldehydes, alcohols and acids by both enzymatic extracts. Gas–liquid chromatography/mass spectrometry (GC/MS) analyses showed the production of volatile C8-compounds, mainly 1-octen-3-ol, as well as non-volatile C10-compounds, including 10-oxo-8-decenoic acid, 10-oxodecanoic acid and 10-hydroxydecanoic acid from the 10-HPOD. The chromatographic analyses also indicated that the concentration of 1-octen-3-ol increased 2.8 and 3.1 times after incubation of the 10-HPOD isomer with the enzymatic extracts from *P. camemberti* and *P. roqueforti*, respectively. In addition, quantitative GC/MS analyses revealed that volatile C8- and non-volatile C10-compounds were produced at a molar ratio of about 1:1. A mild thermal treatment (70 °C, 5 min) of the crude enzymatic extracts, prior to their incubation with the 10-HPOD, inhibited 50% of the production of 1-octen-3-ol.

Keywords: *Penicillium* sp.; Hydroperoxide lyase; Characterization

1. Introduction

The bioconversion of polyunsaturated fatty acids, containing a 1(*Z*),4(*Z*)-pentadiene moiety, into regio-specific hydroperoxides by lipoxygenase (LOX; EC 1.13.11.12), is a key step in the production of flavor compounds. The hydroperoxides undergo secondary enzymatic reactions, such as that catalyzed by hydroperoxide lyase (HPLS), to produce short-chain volatile compounds and ω-oxoacid fragments. Depending on the origin and the specificity of both LOX and HPLS, the volatile compounds, including aldehydes, alcohols, alkanes and alkenes, may exhibit flavor properties [1].

In plants, the HPLS activity has been reported to produce C6- and C9-aldehydes and their corresponding C12- and C9-oxoacids, upon cleavage of the 13- and 9-hydroperoxides of linoleic acid (HPODs) and linolenic acid (HPOTs) generated by LOX activity [2]. In edible mushrooms [3–8] as well as in fungi, such as *Penicillium* sp. [9,10], the combined activities of LOX and HPLS are involved in the bioconversion of linoleic acid and linolenic acid into volatile C8- and non-volatile C10-compounds. Using a crude enzymatic extract from *Agaricus bisporus*, Grosch and Wurzenberger [4] suggested that linoleic acid was

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first oxidized by a LOX to a 10-HPOD, which in turn was cleaved by a HPLS into 1-octen-3-ol and 10-oxo-8-decenoic acid. The 10-HPOD was partially identified as a LOX product in *A. bisporus* [8], and the production of the 10-HPOD by the LOX activity of extracts from *Penicillium* sp. was also reported [11]. In addition, HPLS activities from algae [12,13] were reported to produce C5-alcohols, C5-hydrocarbons and C13-oxoacids from the 13-HPODs and 13-HPOTs.

The specific objective of this study was to investigate the presence of HPLS-cleaving activity in the fungi *Penicillium camemberti* and *Penicillium roqueforti* as well as to characterize the HPLS-cleaving activity with respect to substrate and end product specificities.

2. Materials and methods

2.1. Culture growth and harvesting conditions

The biomasses of *P. camemberti* and *P. roqueforti* were grown on media, containing glucose (10.0 g/l), $NaNO_3$ (3.0 g/l), KH_2PO_4 (1.0 g/l), KCl (0.5 g/l), $MgSO_4{\cdot}7H_2O$ (0.5 g/l), and $FeSO_4{\cdot}7H_2O$ (10.0 mg/l). The culture of *P. roqueforti* contained K_2HPO_4 (1.0 g/l) in the place of KH_2PO_4. The culture media were adjusted to pH 6.0 and 4.0 with 1 M NaOH before sterilization at 120 °C for 15 min for *P. camemberti* and *P. roqueforti*, respectively. After inoculation (10^7 spores/ml) in 2 l Erlenmeyer flasks containing 1 l of medium, the cultures of *P. camemberti* and *P. roqueforti* were incubated on a rotary shaker (100 rpm) at 20 and 28 °C, respectively for 10 days. Biomass yields of approximately 0.6 g/l for both strains were the harvested and recovered by filtration, lyophilized and stored at −80 °C [11].

2.2. Preparation of enzymatic extracts

The fresh biomasses (8 g) of *Penicillium* sp. were suspended in 33 ml of phosphate buffer solution (0.1 m, pH 6.5) and homogenized (5 × 15 s, 22,500 rpm) using a Virtis homogenizer (Virtis Company, Gardiner, NY), followed by glass bead homogenization (2 × 2 min) using a MSK cell homogenizer (Braun, Melsungen, Germany) [11]. The homogenized cell suspensions of *P. camemberti* and *P. roqueforti* were centrifuged (12,000 × *g*, 15 min) and the supernatants, considered to be the enzymatic extracts, FI and FI′, respectively, were subjected to further characterization.

2.3. Protein determination

The protein content of the enzymatic fractions was determined according to a modification of the Lowry method [14]. Bovine serum albumin (Sigma, St. Louis, MO) was used as a standard for calibration.

2.4. Preparation of hydroperoxide isomers

2.4.1. Photooxidation

The 9-, 10-, 12- and 13-HPODs were prepared according to the procedure described by Schieberle et al. [15]. The reaction medium was composed of 6 ml of linoleic acid (9(*Z*),12(*Z*)-octadecadienoic acid) (Nu-Check-Prep, Elysian, MN), 44 ml of benzene and 6.23 mg of meso-tetraphenyl porphine (Sigma) as a sensitizer. The photooxidation was performed at 15 °C by bubbling O_2 into the reaction medium; the stirred solution was irradiated with a 500 W halogen light through a 1 cm layer of deionized water to filter IR radiation.

2.4.2. Purification of hydroperoxide isomers

The removal of non-oxidized linoleic acid from the polar oxygenated products was carried out with the use of a SPE silica column (Supelclean LC-Si, Supelco Inc., Bellefonte, PA) according to the procedure described by Toschi et al. [16]. The purified HPODs were quantified using the ferrous thiocyanate assay, according to the procedure described by Wurzenberger and Grosch [17].

2.4.3. Separation of hydroperoxide isomers by high-performance liquid chromatography

The separation of HPOD isomers was performed by normal phase high-performance liquid chromatography (NP-HPLC), using a Beckman Gold system (Beckman Instruments Inc., San Ramon, CA) equipped with a UV diode-array detector (DAD) and a laser light-scattering detector (LLSD) assembled in series and fitted with a computerized data handling integrated system (Beckman model 126) [11]. A Beckman analog interface Model 406 was used to

transfer data from the LLSD to the HPLC system. Scanning between 190 and 270 nm was done each second for the entire run; in addition, UV detection was performed specifically at 234 nm. The analyses using the LLSD were performed at 75 °C in the presence of an inert gas (N_2) with a flow rate of 40 ml/min. Injection was carried out with an automatic injector (Varian, Autosampler 9095, Varian Associates Inc., Walnut Creek, CA) fitted with a 20 μl loop.

A 50 μmol concentration of HPODs, obtained from the polar oxygenated SPE fraction, was separated on an Alphabond silica column (300 × 3.9 mm i.d., 5 μm; Altech Associates Inc., Deerfield, IL); the eluant system was a mixture of hexane/2-propanol/acetic acid (995:5:1, v/v/v) at a flow rate of 0.75 ml/min.

2.5. Substrate preparation

The substrates were prepared according to the procedure described by Wurzenberger and Grosch [17]. The collected HPLC effluent was washed with an equal volume of deionized water and the HPOD isomer was extracted from the organic layer with one-tenth volume of methanol. After determination of the concentration of the HPOD isomer in the methanolic solution by the ferrous thiocyanate assay, the appropriate amount of Tween 80 (0.3 μl for 0.9 μmol of HPOD isomer) was added. An appropriate volume of methanolic solution containing 0.9 μmol of HPOD isomer was introduced into a 4 ml vial and the methanol was evaporated under a gentle stream of nitrogen.

2.6. Enzymatic reaction

Each of the 9-, 10-, 12- and 13-HPOD isomers (0.9 μmol) was prepared in a 4 ml vial and diluted with 0.25 ml of phosphate buffer solution (0.01 m, pH 7.0) and sonicated for 2 min. The enzymatic reaction was initiated by the addition of 3.75 ml of the crude enzymatic extract FI (4.50 mg protein) or FI′ (7.88 mg protein) at 25 °C.

2.7. Recovery of enzymatic end products

2.7.1. Extraction of volatile compounds

Standards of alcohols and carbonyl compounds, including 1-octen-3-ol, 3-octanone, 1-nonanol, hexanal and 2-octenal, were purchased from Aldrich (Milwaukee, WI). The standard of 1-octen-3-one was prepared by chromic acid oxidation of 1-octen-3-ol as described by Brown and Garg [18]. The extraction of volatile compounds was carried out by vortexing the enzymatic reaction medium with 2 ml of pentane for 1 min. After centrifugation (2500 × *g*, 5 min), the upper pentane layer was dried over Na_2SO_4 and subjected to gas–liquid chromatography/mass spectrometry (GC/MS) analysis.

2.7.2. Extraction of non-volatile compounds

The capric acid standard was purchased from Nu-Check-Prep. After extraction of the volatile compounds from the reaction medium was carried out using pentane, a sufficient volume of 1 m HCl was added to the 4 ml reaction medium to adjust the pH to 3.0. A 100 μl volume of methanol containing 10 μg of capric acid as internal standard was also added. The extraction of non-volatile compounds, including oxoacids, was performed by vortexing the reaction medium with 5 ml of diethyl ether for 1 min. After centrifugation (2500 × *g*, 5 min), the diethyl ether phase was successively dried over Na_2SO_4, concentrated to 3 ml by evaporation under a gentle stream of nitrogen, and subjected to methylation with diazomethane [19]. After evaporation of the diethyl ether under nitrogen, the methylated residue was dissolved in 500 μl of dichloromethane and subjected to GC/MS analysis.

2.8. Derivatization of linoleic acid hydroperoxides

The HPODs were derivatized into their corresponding methyl trimethylsilyloxy (MTMS) stearate derivatives by successive reduction with sodium borohydride, methylation with diazomethane, hydrogenation with platinum oxide and trimethylsilylation with *N*,*N*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), as described by Bisakowski et al. [19].

2.9. Gas–liquid chromatography

The gas–liquid chromatography (GC) analysis was performed with a HP 6890 Series GC System (Hewlett Packard Co., Palo Alto, CA) equipped with computerized integration and data handling (MS ChemStation G1701BA, Version B.01.00, HP) software and

a flame ionization detector. On-column injection was carried out through an automatic liquid sampler (HP 6890 Series Injector, HP) and the volume of the analyzed sample was 1 μl. The separation of different compounds was performed on a fused silica capillary HP-5M column (30 m × 0.25 mm × 0.25 μm film thickness, HP) connected to an uncoated pre-column (1 m × 0.53 mm, HP) with a flow rate of carrier gas (He) of 1.2 ml/min. For the analysis of volatile compounds, the initial column temperature was 35 °C for 5 min and increased at a rate of 2 °C/min to 60 °C, followed by a rate of 4 °C/min to a maximum of 150 °C where it was held for 10 min. For the analysis of the methylated non-volatile compounds, the initial column temperature was 50 °C and increased at a rate of 4 °C/min to 200 °C, followed by a rate of 10 °C/min to a maximum of 270 °C where it was held for 10 min. Flow rates for the hydrogen and air were set at 30 and 300 ml/min, respectively. The detector temperature was at 280 °C.

2.10. Gas–liquid chromatography/mass spectrometry

The GC/MS analysis was performed with the HP 6890 GC system equipped with a 5973 Mass Selective Detector (HP). The injection (1 μl) was performed in a pulsed splitless mode, using an inlet pressure of 25 psi for 1.50 min after each injection. The separation was carried out using the same conditions as for the GC analysis, except the fused silica capillary column had a smaller inside diameter (HP-5MS, 30 m × 0.25 mm × 0.25 μm film thickness), the pre-column was absent and the flow rate of carrier gas (He) was at 25 psi. The mass spectra were obtained by electron impact (EI) ionization at 70 eV. Six characteristic MS fragments (*m*/*z*) were used to investigate for the presence of selected methylated C10- and C11-oxoacids and hydroxyacids, including methyl 10-oxo-8-decenoate (74, 98, 83, 138, 121, 166), methyl 10-hydroxy-8-decenoate (73, 129, 225, 150, 257, 271), methyl 10-oxodecanoate (74, 87, 125, 157, 169, 172), methyl 10-hydroxydecanoate (74, 98, 172, 110, 152, 129), methyl 10-oxoundecanoate (125, 58, 97, 157, 183, 214), and methyl 10-hydroxyundecanoate (117, 73, 159, 241, 146, 273) [20], using the "Extract Ion Chromatograms (EIC)" menu item displayed in the MS ChemStation software.

3. Results and discussion

3.1. Preparation of hydroperoxide isomers

A racemic mixture of the 9-, 10-, 12- and 13-HPODs was produced by the photooxidation of linoleic acid in the presence of a sensitizer producing singlet oxygen [17]. The HPOD isomers, obtained from the sensitized oxidation, were separated by NP-HPLC (Fig. 1). The LLSD (Fig. 1B) showed the presence of four major peaks, 1, 2, 4 and 5, with elution times at 33.8, 36.1, 42.4 and 47.8 min, respectively, and a minor one, 3, with an elution time at 39.5 min. The UV-DAD analysis shows that the compounds corresponding to peaks 1 and 5 (Fig. 1A) had similar elution times and maximum wavelengths of absorbance (λ_{max}) at 233 nm compared to those of authentic (*Z*),(*E*) isomers of 13- and 9-HPODs standards (not shown), respectively. The compound corresponding to peak 3 showed a lower λ_{max}, hypsochromically shifted to 229 nm, which is characteristic of conjugated (*E*),(*E*)-diene systems [16]. The compounds

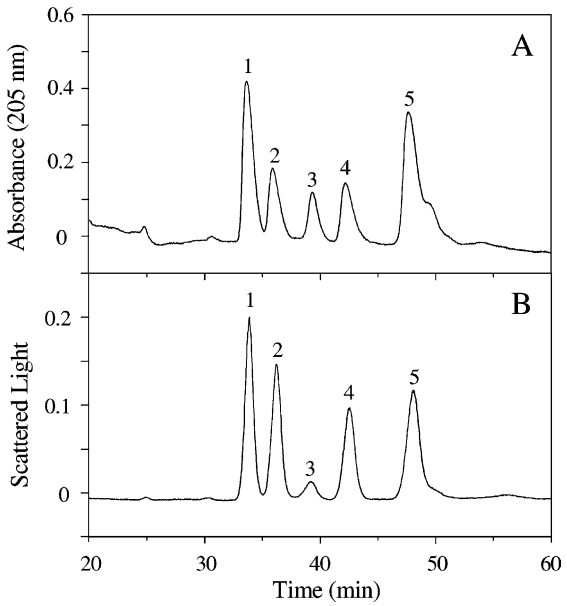

Fig. 1. Normal phase high-performance liquid chromatography (NP-HPLC) elution profile of the hydroperoxide (HPOD) isomers produced by the photooxidation of linoleic acid: (1) (*Z*),(*E*) isomer of 13-HPOD; (2) 12-HPOD; (3) (*E*),(*E*) isomer of 13-HPOD; (4) 10-HPOD; (5) (*Z*),(*E*) isomer of 9-HPOD.

corresponding to peaks 2 and 4 did not show any absorbance at 234 nm, thereby indicating the absence of a conjugated diene system.

In order to be identified, each HPOD isomer was individually collected and derivatized into its corresponding MTMS stearate derivative and analyzed by GC/MS. The experimental results (not shown) indicated that the MTMS stearate derivatives of the compounds that corresponded to peaks 1 and 3, and 5 possessed the same mass spectra as those of the 13- and 9-HPODs, respectively, as reported by Perraud et al. [21], whereas the mass spectra of the MTMS stearate derivatives of compounds 2 and 4 possessed the characteristic fragmentation patterns of those of the 12- and 10-HPODs, respectively, as reported by Bisakowski et al. [19].

The overall HPLC and GC/MS analyses suggest that the compounds corresponding to peaks 1, 2, 3, 4 and 5 are the (*Z*),(*E*) isomer of 13-HPOD, the 12-HPOD, the (*E*),(*E*) isomer of 13-HPOD, the 10-HPOD and the (*Z*),(*E*) isomer of 9-HPOD, respectively. The main HPOD isomers (corresponding to peaks 1, 2, 4 and 5) were collected from the HPLC column and used as substrates for further experimental work.

3.2. Substrate specificity

The individual HPOD isomers of linoleic acid, including the (*Z*),(*E*) isomer of 9-HPOD, the 10-HPOD, the 12-HPOD and the (*Z*),(*E*) isomer of 13-HPOD, were incubated separately with the crude enzymatic extracts of *P. camemberti* (FI) and *P. roqueforti* (FI′). Table 1 indicates an important decrease, 47 and 14%, in the initial concentration of 10-HPOD when it was incubated with the crude enzymatic extracts of *P. camemberti* and *P. roqueforti*, respectively. The results obtained for *P. camemberti* are similar to those reported by Wurzenberger and Grosch [17], when the 10-HPOD was incubated with a mushroom protein fraction; Wurzenberger and Grosch [22] indicated that the incompleteness of the reaction was due to the stereoselectivity of a HPLS which would cleave only one enantiomer of the racemic 10-HPOD mixture that was produced during the photooxidation of linoleic acid.

Table 1
Substrate specificity of linoleic acid hydroperoxide (HPOD)-consuming activity from *Penicillium* sp.

HPOD isomer	HPOD consumption[a]	
	P. camemberti	*P. roqueforti*
9-HPOD	0.016 (±0.004)[b]	0.012 (±0.003)[b]
10-HPOD	0.423 (±0.018)[b]	0.127 (±0.010)[b]
12-HPOD	0.019 (±0.004)[b]	0.013 (±0.007)[b]
13-HPOD	0.003 (±0.001)[b]	0.003 (±0.001)[b]

[a] Difference of the amount of HPOD (μmol) between the beginning of the reaction (0.9 μmol) and that after 20 min of reaction.

[b] Standard deviation of duplicate samplings.

3.3. Characterization of end products

3.3.1. Volatile compounds

Fig. 2 shows a sample of the GC/MS analysis of the volatile compounds, extracted after incubation of the crude enzymatic extract of *P. camemberti* with the 10-HPOD. Table 2 indicates that the overall results obtained from the GC/MS analysis of the volatile compounds endogenously present in the crude enzymatic extracts of *Penicillium* sp., as well as the volatile compounds extracted after incubation of the enzymatic extracts with 10-HPOD. The results show that 1-octen-3-ol was the only endogenous volatile compound present in the crude enzymatic extracts; however, its concentration increased by 2.8 and 3.1 times after incubation of the enzymatic extracts of *P. camem-*

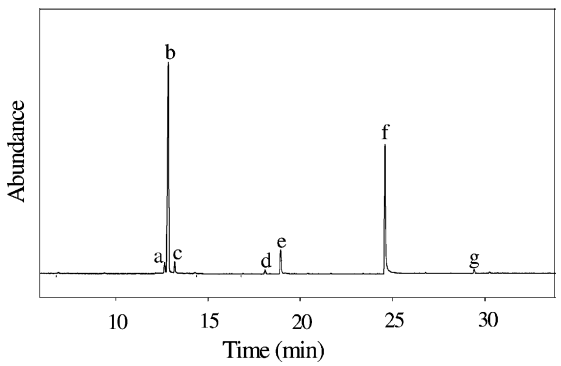

Fig. 2. Gas chromatography (GC) elution profile of the volatile compounds produced by the incubation of the 10-hydroperoxide of linoleic acid (10-HPOD) with the enzymatic extract from *P. camemberti*: (a) 1-octen-3-one; (b) 1-octen-3-ol; (c) 3-octanone; (d) 2-octenal; (e) 2-octen-1-ol; (f) 1-nonanol (internal standard); (g) 2,4-decadienal.

Table 2
Gas–liquid chromatography/mass spectrometry (GC/MS) analyses of the volatile compounds that are endogenously present in the enzymatic extracts from *Penicillium* sp. and that are extracted after the incubation of the 10-hydroperoxide isomer of linoleic acid (10-HPOD) with these enzymatic extracts

Compound	Retention time (min)	Mean of identification	Relative concentration (μg/ml)[a]			
			P. camemberti		*P. roqueforti*	
			Endogenous[b]	10-HPOD[c]	Endogenous[b]	10-HPOD[c]
Hexanal[d]	10.02	RT[e]	n.d.[f]	0.07 (±0.00)[g]	n.d.[f]	0.05 (±0.01)[g]
1-Octen-3-one	12.65	RT[e]; MS[h]	n.d.[f]	0.18 (±0.04)[g]	n.d.[f]	0.23 (±0.03)[g]
1-Octen-3-ol	12.86	RT[e]; MS[h]	5.83 (±0.46)[g]	16.53 (±1.74)[g]	0.78 (±0.03)[g]	2.40 (±0.07)[g]
3-Octanone	13.21	RT[e]; MS[h]	n.d.[f]	0.22 (±0.06)[g]	n.d.[f]	n.d.[f]
2-Octenal	18.07	RT[e]; MS[h]	n.d.[f]	0.07 (±0.01)[g]	n.d.[f]	0.07 (±0.01)[g]
2-Octen-1-ol	18.93	MS[h]	n.d.[f]	0.53 (±0.09)[g]	n.d.[f]	0.39 (±0.06)[g]
1-Nonanol	24.51	IS[i]	IS[i]	IS[i]	IS[i]	IS[i]
2,4-Decadienal	29.39	MS[h]	0.05 (±0.01)[g]	0.07 (±0.01)[g]	0.03 (±0.01)[g]	0.04 (±0.01)[g]

[a] Relative peak area of the volatile compound to that obtained with 10 μg of internal standard (IS) of 1-nonanol per ml of reaction medium.
[b] Volatile compounds obtained after the incubation of the enzymatic extract without the 10-HPOD.
[c] Volatile compounds obtained after the incubation of the enzymatic extract with the 10-HPOD.
[d] Detected by gas–liquid chromatography/flame ionization detector (GC/FID).
[e] Identification by retention time (RT).
[f] Not detected.
[g] Standard deviation of triplicate samplings.
[h] Identification by mass spectrometry (MS).
[i] Internal standard consists of 10 μg of 1-nonanol per 1 ml of reaction medium.

berti and *P. roqueforti*, respectively, with 10-HPOD. The production of other volatile C8-compounds, including 2-octen-1-ol, 1-octen-3-one, 3-octanone and 2-octenal at lower concentrations as well as hexanal and 2,4-decadienal was also demonstrated when 10-HPOD isomer was incubated with the enzymatic extracts. These experimental findings are in agreement with those reported in literature with regard to the production of 1-octen-3-ol and other associated volatile C8-compounds by several fungi, including *P. camemberti*, *P. caseicolum* [9,10,23,24] and other *Penicillium* sp., *Aspergillus* sp., and *Fusarium* sp. [25,26]. In addition, it is well known that most species of edible mushrooms generate volatile C8-compounds, particularly 1-octen-3-ol which is considered to be the major contributor to the typical flavor of mushroom [27–29].

Moreover, the results (Table 2) suggest that the 10-HPOD is the precursor for the biogeneration of 1-octen-3-ol and other volatile compounds; these findings are in agreement with those reported for the homogenate of *A. bisporus* [17], suggesting a HPLS activity in the enzymatic extracts from *Penicillium* sp., which catalyzes the cleavage of 10-HPOD thereby producing the 1-octen-3-ol.

In order to investigate the HPLS activity, which has been reported to be heat sensitive [8,13], the enzymatic extracts were subjected to a thermal treatment (70 °C, 5 min) prior to their incubation with the 10-HPOD. The experimental data indicated approximately 50% inhibition of the production of 1-octen-3-ol by the enzymatic extracts of *Penicillium* sp. upon thermal treatment. These results are in agreement with those obtained previously, which showed an inhibition of the HPLS activity upon thermal treatment in the enzymatic extracts of *A. bisporus* [8] and *Chlorella pyrenoidosa* [13].

3.3.2. Non-volatile compounds

Since the formation of volatile C8-compounds was suggested to result from the cleavage of the 10-HPOD by a HPLS activity, the presence of corresponding C10-non-volatile metabolites was investigated in the reaction medium [17,20]. The methylated non-volatile compounds generated by the enzymatic extracts from *Penicillium* sp. were analyzed by GC/MS, with the

Table 3
Gas–liquid chromatography/mass spectrometry (GC/MS) analyses of the oxoacids and hydroxyacids that are endogenously present in the enzymatic extracts from *Penicillium* sp., as well as that are extracted after the incubation of the 10-hydroperoxide of linoleic acid (10-HPOD) with these enzymatic extracts

Compound	Retention time (min)	MS fragments (*m*/*z*)[b]	Relative concentration (μg/ml)[a]			
			P. camemberti		*P. roqueforti*	
			Endogenous[c]	10-HPOD[d]	Endogenous[c]	10-HPOD[d]
Methyl caprate	16.08	n.a.[e]	IS[f]	IS[f]	IS[f]	IS[f]
Methyl 10-oxodecanoate	22.79	74, 87, 125, 157, 169, 172	1.14 (±0.14)[g]	3.62 (±1.56)[g]	n.d.[h]	0.12 (±0.11)[g]
Methyl 10-hydroxydecanoate	24.70	74, 98, 172, 110, 152, 129	0.31 (±0.03)[g]	3.49 (±2.04)[g]	n.d.[h]	n.d.[h]
Methyl 10-oxo-8-decenoate	24.52	74, 98, 83, 138, 121, 166	0.07 (±0.04)[g]	11.01 (±2.45)[g]	0.23 (±0.21)[g]	3.81 (±0.69)[g]
Methyl 10-oxoundecanoate	25.35	125, 58, 97, 157, 183, 214	n.d.[h]	1.00 (±0.44)[g]	n.d.[h]	0.12 (±0.03)[g]

[a] Relative peak area of the selected compound to that obtained with 10 μg of internal standard (IS) of capric acid per ml of reaction medium.
[b] The characteristic MS fragments [20] used to investigate the presence of the corresponding oxoacids and hydroxyacids using the "Extracting Ions Chromatogram" menu item displayed in the MS ChemStation software.
[c] Selected compound obtained after the incubation of the enzymatic extract without the 10-HPOD.
[d] Selected compound obtained after the incubation of the enzymatic extract with the 10-HPOD.
[e] Not applicable.
[f] Internal standard consisted of 10 μg of capric acid per 1 ml of reaction medium.
[g] Standard deviation of triplicate samplings.
[h] Not detected.

instrument on full-scan mode, from 50 to 360 amu, to obtain a total ion chromatogram (TIC). Using the MS ChemStation software, the investigation of the presence of selected methylated oxo- and hydroxyacids was further performed by extracting from the TIC six ions (*m*/*z*) characteristic of the mass spectrum of each compound (Table 3) to obtain the extracted ion chromatograms (IECs).

Table 3 summarizes the GC/MS analysis of the oxo- and hydroxyacids endogenously present in the crude enzymatic extracts from *Penicillium* sp. as well as those extracted after incubation of the enzymatic extracts with 10-HPOD. Among the six C10- and C11-oxoacids and hydroxyacids investigated, a total of four were detected by the GC/MS analysis. The mass spectra (Fig. 3) obtained for the selected methylated oxo- and hydroxyacids corresponded to those reported by Tressl et al. [20]. The results (Table 3) indicate a net generation of important quantities of 10-oxo-8-decenoic acid (10.94 μg/ml), 10-hydroxydecanoic acid (3.58 μg/ml) and 10-oxodecanoic acid (2.48 μg/ml) by the enzymatic extract from *P. camemberti*, using 10-HPOD as substrate; however, the 10-oxo-8-decenoic acid (3.25 μg/ml) was the main product generated by the extract of *P. roqueforti*. In addition, small quantities

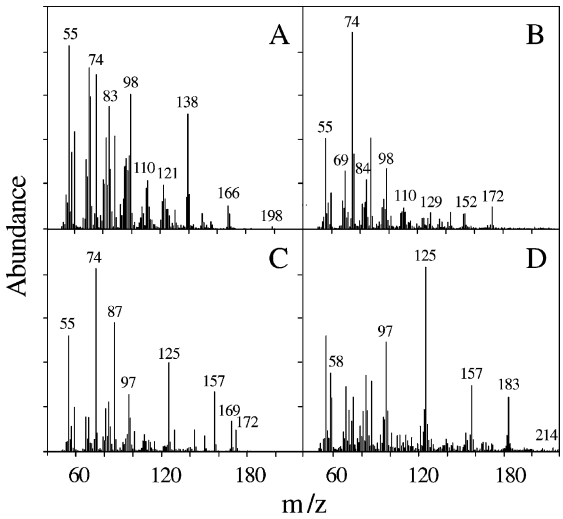

Fig. 3. Mass spectra of methylated C10- and C11-oxoacids and hydroxyacids produced upon incubation of the 10-hydroperoxide of linoleic acid (10-HPOD) with the enzymatic extracts from *Penicillium* sp.: (A) methyl 10-oxo-8-decenoate; (B) methyl 10-oxodecanoate; (C) methyl 10-hydroxydecanoate; (D) methyl 10-oxoundecanoate.

of a C11-oxoacid and 10-oxoundecanoic acid were produced by both enzymatic extracts.

Using 10-HPOD as substrate, the biogeneration of the 10-oxo-8-decenoic acid by the HPLS activity of *Penicillium* sp. is similar to that reported in mushroom homogenates where linoleic acid and 10-HPOD were used as substrates [8,17,30]. Moreover, Tressl et al. [20] also reported the production of the 10-hydroxydecanoic acid as well as other C10- and C11-oxoacids, including 10-oxodecanoic and 10-oxoundecanoic acids. As suggested by Tressl et al. [20], the formation of the 10-hydroxydecanoic acid might be due to an alcohol dehydrogenase activity acting on 10-oxo-8-decenoic acid; similarly, the 10-oxodecanoic could result from the reduction of the 10-oxo-8-decenoic by a double bond reducing enzymatic system. The relatively poor reproducibility of the data (Table 3), shown by the high standard deviations values (± 0.03 to $\pm 2.45\,\mu g/ml$), may be due to the presence of different enzymatic systems that could reduce the amount of 10-oxo-8-decenoic produced during the incubation of the 10-HPOD with the crude enzymatic extracts of *Penicillium* sp.

The volatile C8- and the non-volatile C10-compounds were formed at very similar concentrations, with molar ratios of 1:1.04 and 1:1.14, after incubation of the 10-HPOD with the enzymatic extracts from *P. camemberti* and *P. roqueforti*, respectively; these findings strongly support the presence of a HPLS activity in the enzymatic extracts from *Penicillium* sp., since the cleavage of the 10-HPOD by this enzymatic activity produces an equimolar ratio of 1-octen-3-ol and 10-oxoacid, as reported previously for the mushrooms *A. bisporus* [30] and *Pleurotus pulmonarius* [8], using linoleic acid as substrate.

The results (Tables 2 and 3) show that the reaction products generated by the HPLS activity from *Penicillium* sp. are similar to those described for the soluble homolytic-type HPLS reaction in mushrooms and algae [2]; this author suggested that this HPLS cleaved the HPODs at the carbon–carbon bond distal to the carboxylate and adjacent to the HPOD function, which resulted in the formation of an alcohol or an hydrocarbon (alkane or alkene) and an ω-oxoacid. The mechanism of the homolytic-type HPLS is different to that of the membrane-bound heterolytic-type HPLS, found in higher plants, which catalyzes the cleavage of fatty acid HPODs at the carbon–carbon bond adjacent to the HPOD function and proximal to the carboxyl carbon, producing an aldehyde and an ω-oxoacid [2].

4. Conclusion

The results gathered in this study demonstrated the presence of a HPLS activity, in the enzymatic extracts from *Penicillium* sp., that cleaved the 10-HPOD to produce 1-octen-3-ol and 10-oxo-8-decenoic acid. In addition, the presence of secondary reducing enzymatic reactions that acted on the 10-oxo-8-decenoic acid was suggested.

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References

[1] R.J. Hsieh, Contribution of lipoxygenase pathway to food flavors, in: C.-T. Ho, T.G. Hartman (Eds.), Lipids in Food Flavors, ACS Symposium Series, Washington, DC, 1994, pp. 30–48.
[2] H.W. Gardner, Biochim. Biophys. Acta 1084 (1991) 221–239.
[3] R. Tressl, D. Bahri, K.H. Engel, Lipid oxidation in fruits and vegetables, in: R. Teranishi, H. Barrera-Benitez (Eds.), Quality of Selected Fruits and Vegetables of North America, ACS Symposium Series, Washington, DC, 1981, pp. 213–232.
[4] W. Grosch, M. Wurzenberger, Enzymic formation of 1-octen-3-ol in mushrooms, in: J. Adda (Ed.), Progress in Flavour Research 1984, Elsevier, Amsterdam, 1985, pp. 253–259.
[5] J.L. Mau, R.B. Beelman, G.R. Ziegler, J. Food Sci. 57 (1992) 704–706.
[6] P.A. Belinky, S. Masaphy, D. Levanon, Y. Hadar, C.G. Dosoretz, Appl. Microbiol. Biotechnol. 40 (1994) 629–633.
[7] S. Assaf, Y. Hadar, C.G. Dosoretz, J. Agric. Food Chem. 43 (1995) 2173–2178.
[8] S. Assaf, Y. Hadar, C.G. Dosoretz, Enzyme Microbiol. Technol. 21 (1997) 484–490.
[9] C. Karahadian, D.B. Josephson, R.C. Lindsay, J. Dairy Sci. 68 (1985) 1865–1877.
[10] H.E. Spinnler, O. Grosjean, I. Bouvier, J. Dairy Res. 59 (1992) 533–541.

[11] X. Perraud, S. Kermasha, J. Am. Oil Chem. Soc. 77 (2000) 335–342.
[12] R.H. Andrianarison, J.L. Beneytout, M. Tixier, Plant Physiol. 91 (1989) 1280–1287.
[13] B.A. Vick, D.C. Zimmerman, Plant Physiol. 90 (1989) 125–132.
[14] E.P. Hartree, Anal. Biochem. 48 (1972) 422–427.
[15] P. Schieberle, F. Haslbeck, G. Laskawy, W. Grosch, Z. Lebensm. Unters. Forsch. 179 (1984) 93–98.
[16] T.G. Toschi, F. Stante, P. Capella, G. Lercker, J. High. Resol. Chromatogr. 18 (1995) 764–766.
[17] M. Wurzenberger, W. Grosch, Biochim. Biophys. Acta 794 (1984) 25–30.
[18] H.C. Brown, C.P. Garg, J. Am. Chem. Soc. 83 (1961) 2952–2953.
[19] B. Bisakowski, X. Perrauld, S. Kermasha, Biosci. Biochem. Biotech. 61 (1997) 1262–1269.
[20] R. Tressl, D. Bahri, K.H. Engel, J. Agric. Food Chem. 30 (1982) 89–93.
[21] B. Bisakowski, X. Perrauld, S. Kermasha, Process Biochem. 34 (1999) 819–827.
[22] M. Wurzenberger, W. Grosch, Biochim. Biophys. Acta 795 (1984) 163–165.
[23] C. Karahadian, D.B. Josephson, R.C. Lindsay, J. Agric. Food Chem. 33 (1985) 339–343.
[24] N. Jollivet, J.M. Belin, Y. Vayssier, J. Dairy Sci. 76 (1993) 1837–1844.
[25] E. Kaminski, S. Stawicki, E. Wasowicz, Appl. Microbiol. 27 (1974) 1001–1004.
[26] A.L. Pasanen, S. Lappalainen, P. Pasanen, Analyst 121 (1996) 1949–1953.
[27] D.A. Cronin, M.K. Ward, J. Sci. Food Agric. 22 (1971) 477–479.
[28] J.L. Mau, R.B. Beelman, G.R. Ziegler, Aroma and flavor components of cultivated mushrooms, in: G. Charalambous (Ed.), Spices, Herbs, and Edible Fungi, Elsevier, Amesterdam, 1994, pp. 657–684.
[29] H. Pyysalo, Acta Chem. Scand. B 30 (1976) 235–244.
[30] M. Wurzenberger, W. Grosch, Z. Lebensm. Unters. Forsch. 175 (1982) 186–190.