



The role of microalgae-based systems in the dynamics of odors compounds in the meat processing industry

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ABSTRACT

The aim of this work was to evaluate the dynamics of odors compounds in the meat processing industry through microalgae-based processes. The study focused on the characterization of odorant profile from raw wastewater, on the deodorization of the compounds and the formation of the volatile organic compounds as co-products of the process. The results showed that emissions from the wastewater treatment plant are composed of 4 sulfur, 7 aldehydes, 1 furan, 2 hydrocarbon, 10 terpenes, 7 alcohols, 2 ketones, 3 amines, and 4 phenolic compounds. The levels of these volatile organic compounds from wastewater, regardless of polarity range, decreased with concomitant formation of other compounds, usually with desirable odor description, as residence time increased. A total of 15 compounds of various chemical structures (such as aldehydes, alcohols, ketones, esters, terpenes, acids, and nitrogen compounds) were formed. Regardless of these organic classes, three main odor categories (fruity, spicy, and resinous) emerged. Based on these results, we found the potential of the microalgae-based processes for odor abatement of the meat industry in parallel to production of desirable compounds.

Keywords: Algae/cyanobacteria; Agro-industrial wastes; Volatile organic compounds; Deodorization; Bioproducts

1. Introduction

Two typical human behaviors are meat eating and food processing. Evidence indicates that people began to increase meat consumption at least 2.6 million years ago, contributing for the growth of the meat product industry to the point of making it become one of the largest in the food sector [1,2]. Thus, the meat supply chain is a complex operation, with global sourcing strategies to secure supply. However, managing this segment can be difficult and can expose vulnerabilities which include environmental issues [2,3].

Thus, some facts have affected the food industry; for example, complaints from people living near meat processing

facilities have prompted regulatory agencies to address public concerns [4] officially. The production of animal protein, in particular, is a substantial and growing driver of odor pollution, accounting for approximately half of all food production-related emissions [5].

The volatile profile from meat processing plants includes specific groups of odorants such as alcohols, volatile fatty acids, aldehydes, and ketones, which are products derived from decomposition of carbohydrates, proteins, and lipids. Meat spoilage can contribute to emissions of amine compounds, indole, and skatole, which poses challenges to the deodorization process. The technological challenge

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of removing indole and skatole is posed by the simultaneous presence of hydrophobic and hydrophilic structural components; benzene, and pyrrole rings, together with CH bonds are hydrophobic surfaces while the center is hydrophilic because of the N heteroatom [6,7]. Also, these compounds are malodorous and have very low odor thresholds, potentially resulting in an impact of odors on nearby populations [8,9].

The simultaneous presence of hydrophobic and hydrophilic chemical moieties in odor pollution and the significant contribution of these compounds to unpleasant odors from industrial facilities pose technological challenges for odor removal in waste treatment. In this sense, the use of microalgae-based processes can be an innovative technology for deodorization in the meat processing industry because it is a cost-effective, environmental friendly alternative, whose metabolic plasticity is an advantage, as it converts polar and nonpolar molecules of wastewater.

Microalgae are considered to be a potentially new and valuable source of biologically active compounds for applications in several biotechnology sectors [10]. Moreover, the use of microalgae plays a vital role in conversion of waste to a multitude of products, e.g., biofuels, nutraceuticals, polymers, pigments, and varieties of chemicals. Algae inherently have the potential to transform industrial greenhouse gases as well as wastewater into useful products, thus serving as an effective carbon capture and utilization platform [11].

Although, a number of commercial uses have been found for microalgae, not much is known on the application of microalgae-based systems for odor pollution removal, and the potential bioconversion of value-added products using odor waste substrates. At times when people perceive waste as wealth, this hypothesis should be investigated. Thus, the objective of this study was to evaluate the dynamics of odorous compounds in the meat processing industry in microalgae-based processes. The study focused on the (i) characterization of the odorant profile of raw wastewater, (ii) deodorization of compounds, and (iii) formation of volatile organic compounds as co-products of the process. In addition, to the best of our knowledge, it is the first time that a heterotrophic microalgal bioreactor, using *Phormidium autumnale*, was simultaneously applied for deodorization in meat industry facilities and production of desirable industrial compounds.

2. Material and methods

2.1. Standards

The standards benzyl alcohol, 2-heptanone, butanal, toluene, α -terpineol, hexanol, linalool, limonene, α -terpinene, p -cresol, and 6-methyl-5-hepten-2-one, as well as 3-octanol (which were used as an internal standard) were purchased from Sigma-Aldrich (Bellefonte-PA, USA). The paraffin homologues were obtained from Polyscience (Chicago-IL, USA). The identities of volatile compounds were confirmed with retention indices and comparison with the MS spectral database.

2.2. Microalgae and culture media

Axenic cultures of *Phormidium autumnale* were used in the experiments. Stock cultures were propagated and

maintained in solidified agar-agar (20 g.L⁻¹) containing synthetic BG11 medium [12]. The incubation conditions were 25°C, photon flux density was 15 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ and the photoperiod was 12 h. To obtain the inoculums in liquid form, 1 mL of sterile synthetic medium was transferred to slants; the colonies were scraped and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

2.3. Food processing wastewater

Slaughterhouse wastewater used in the experiments was obtained from an industry located in Santa Catarina, Brazil (27°14'02" S, 52°01'40" W). It was collected at the discharge point of an equalization tank over a period of one year, and analyzed for pH, chemical oxygen demand (COD), total nitrogen (N-TKN), total phosphorus (P-PO₄⁻³), total solids (TS), suspended solids (SS), volatile solids (VS), and fixed solids (FS) following the Standard Methods for the Examination of Water and Wastewater [13]. This is the average composition of the wastewater: pH of 5.9 \pm 0.05, COD of 4.100 \pm 874 (mg.L⁻¹), NTK-N of 128.5 \pm 12.1 (mg.L⁻¹), P-PO₄⁻³ of 2.84 \pm 0.2 (mg.L⁻¹), TS of 3.8 \pm 2.7 (mg.L⁻¹), SS of 1.9 \pm 0.8 (mg.L⁻¹), VS of 2.9 \pm 0.4 (mg.L⁻¹), and FS of 0.9 \pm 0.3 (mg.L⁻¹).

2.4. Heterotrophic microalgal bioreactor

Measurements were made in a batch bubble column bioreactor [14], fed on 2.0 L of wastewater. The bioreactor, which included filtering units, was previously autoclaved at 121°C for 30 min. The experimental conditions were determined as follows: initial concentration of inoculum 100 mg.L⁻¹, temperature 25°C, pH adjusted to 7.6, and aeration of 1.0 VVM (volume of air per volume of culture per minute), absence of light and residence time of 144 h. To confirm the dynamics of formation and degradation of volatile organic compounds by microalgae, an experiment control (without inoculum addition) was used. The wastewater was pneumatically aerated in the bubble column bioreactor at a rate of 1.0 VVM. The experiments were performed twice and in duplicate. Therefore, data refer to the mean value of four repetitions.

2.5. Analytical methods

2.5.1. Isolation of the volatile organic compounds

The volatile compounds were isolated from the matrix by using headspace solid-phase micro-extraction (HS-SPME) with divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (50/30 μm film thickness \times 20 mm; Supelco, Bellefonte, PA). A wastewater sample of 20 mL was collected and equally separated into two portions. Each portion was placed in a vial containing 3 g of NaCl and 10 μL of a 3-octanol internal standard solution. The SPME fiber was exposed into the headspace of the vial containing the sample for 45 min at 40°C, under constant stirring (400 rpm) with a magnetic stir bar. After this period, the fiber was removed from the vial and submitted to chromatographic analysis. The analytical procedure was performed twice and in duplicate. Therefore,

data refer to the mean value of four repetitions. HS-SPME was coupled with GC/MS for the quantitative determination of the volatile compounds [15].

2.5.2. GC/MS analysis

The volatile compounds were analyzed in a Shimadzu QP 2010 Plus gas chromatograph coupled to a mass spectrometer (Shimadzu, Kyoto, Japan). The fiber was thermally desorbed for 10 min in a split/splitless injector, operating on the splitless mode (1.0 min splitter off) at 250°C. Helium was used as a carrier gas at a constant flow rate of 1.6 mL.min⁻¹. Analytes were separated on a DB-Wax fused silica capillary column, 60 m in length, 0.25 mm id, and 0.25 µm film thickness (Chrompack Wax 52-CB). The initial column temperature was set at 35°C for 5 min, followed by a linear increase of 5°C.min⁻¹ to 250°C, and this temperature was held for 5 min. The MS detector was operated on electron impact ionization mode +70 eV and mass spectra obtained by scan range from *m/z* 35 to 350. The volatile compounds were identified by a comparison of experimental MS spectra with those provided by the computerized library (NIST MS Search). Also, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a standard mixture of homologous series of paraffins (C₆–C₂₄) to aid identification [16]. The sample and the standard mixture were injected both separately and together to obtain the experimental LRI and mass spectra values for the purpose of compound identification by directed comparison. Analytes were quantified by internal standard calibration. The relative concentration of the investigated compounds was determined by relating the standard internal area with a known concentration (0.082 µg.mL⁻¹) to the area of the compound of interest. The response factor between internal standard and analytes was assumed as one.

3. Results and discussion

A characterization of odorant composition and profile of raw wastewater is the first step to improve the understanding of the mechanism of odor formation and degradation as well as to optimize treatment technology with high deodorization performance. The initial data analysis (Table 1) shows the volatile profile from agro-industrial wastewater. A total of 40 different compounds were separated in the raw wastewater, *p*-cresol, peak 52, was the major volatile compound (19.1%), followed by benzaldehyde, peak 35, (11.9%), limonene, peak 17, (10.8%), linalool, peak 36, (7.5%) and hexanol, peak 27, (6.2%).

The odors and air pollutants from wastewater treatment plants are a complex mixture of chemical compounds, including a range of volatile organic compounds that contribute to malodor. As shown in Table 1, the emissions from the wastewater treatment plant are composed of about 4 sulfur compounds (peaks 1, 2, 10, and 28), 7 aldehydes (peaks 3, 6, 7, 8, 11, 35, and 43), 1 furan (peak 4), 2 hydrocarbons (peaks 9 and 34), 10 terpenes (peaks 15, 17, 18, 21, 22, 36, 37, 38, 45, and 46), 7 alcohols (peaks 19, 24, 27, 33, 39, 42, and 47), 2 ketones (peaks 23 and 44), 3 amines (peaks 25, 54, and 55) and 4 phenolic compounds (peaks 48, 50, 51, and 52). A large number of the compounds detected in this study show low

concentrations and have very low odor thresholds, and agree with data available in the literature [4,8,28–30].

Odors compounds have a threshold value (odor unit), in which an odor is not detectable below a given concentration. Most volatile malodors present trace level concentration and potent odor. Weber's law (1834) and Steven's Law (1970) mathematically confirm that odor perception relates psychological interpretation to physiological reception. Thus, the minor compounds found in the wastewater, mainly peaks 1, 2, 10, 28, 50, 51, 54, and 55, are indispensable for the complex evaluation of odor released from wastewater facilities.

Table 1 also includes each volatile odor intensity (ranging from 2.0 × 10⁸ µg.m⁻³ for benzyl alcohol to 5.6 × 10⁻⁴ µg.m⁻³ for skatole) and odor description. As shown Table 1, important minor malodors compounds such as dimethyl disulfide (1.1%), indole (1.3%) and skatole (0.3%), and compounds with higher contents, *p*-cresol (92.0 µg.m⁻³) and benzaldehyde (11.9 µg.m⁻³) show the lowest threshold, which makes deodorization of this wastewater a challenging task. In this context, odor removal utilizing microalgal heterotrophic bioreactor is an interesting biotechnology that should be taken into consideration.

Fig. 1 and Table 2 show the impact of residence time on the performance of the bioreactor in the treatment of the volatile organic compounds of wastewater. As expected, similar qualitative and quantitative volatile organic compounds profiles were found in the raw wastewater (40 peaks) and in the microalgal heterotrophic bioreactor at time 0 h (44 peaks), although, 4 compounds (peaks 26, 40, 49, and 53), not previously detected in the raw wastewater, were detected at time 0 h. In fact, detection of 6-methyl-5-hepten-2-one, menthol, benzothiazole and 1-penten-3-ol at the initial residence time is not surprising, considering that these volatile components were present in the microalgae biomass utilized in the experiment. The natural biosynthesis of volatile from microalgae is derived from the carotenoid cleavage (6-methyl-5-hepten-2-one), carbon-rearranged monoterpenes (menthol), amino acid (benzothiazole), and fatty acid (1-penten-3-ol) pathways [25,31]. As previously reported [15], off-flavors were not identified in *Phormidium autumnale* biomass; this is a technological advantage when compared with other microalgae that are capable of releasing a range of malodorous compounds into surface waters. The levels of volatile organic compounds from wastewater decreased with concomitant formation of the others compounds (in general with desirable odor description) as time of cultivation increased. A combined total of 55 compounds were identified (Fig. 1 and Table 2). At the initial residence time (0 h), 97.5% of volatile organic compounds from raw wastewater along with 4 compounds in small amounts (2.5%) were found (Table 2). As a consequence of residence time in the microalgal bioreactor, the ratio values were found for volatile organic compounds from wastewater in comparison to the volatile organic compounds formed (VOC_w/VOC_c), changing from 98:2 to 10:90 after 72 h of residence time.

There was a clear change in the volatile profile of the heterotrophic microalgal bioreactor at residence time between 0–24 h; 25 compounds disappeared, and all of the 15 compounds were formed in this period. However, following 24 h of treatment, removal of the volatile organic

Table 1
Quantification of volatile compounds ($\mu\text{g}\cdot\text{m}^{-3} \pm \sigma$) of wastewater and their corresponding threshold values and odor descriptors

Peak	Compound	Chemical formula	Molecular weight ($\text{g}\cdot\text{mol}^{-1}$)	Concentration ^a ($\mu\text{g}\cdot\text{m}^{-3}$)	Odor threshold ^b ($\mu\text{g}\cdot\text{m}^{-3}$)	Odor description ^b
1	Carbon disulfide	CS_2	76.1	1.1 ± 0.1	3×10^2	Disagreeable, sweet
2	Dimethyl sulfide	$\text{C}_2\text{H}_6\text{S}$	62.1	0.6 ± 0.2	2×10^4	Decayed cabbage, sulfurous
3	2-propenal	$\text{C}_3\text{H}_4\text{O}$	56.1	6.0 ± 0.4	7×10^2	Burnt, sweet
4	2-methylfuran	$\text{C}_5\text{H}_6\text{O}$	82.1	7.1 ± 1.9	3.5×10^3	Roasted meat, chocolate
6	Butanal	$\text{C}_4\text{H}_8\text{O}$	72.1	4.9 ± 0.1	1.5×10^4	Sweet
7	2-methylbutanal	$\text{C}_5\text{H}_{10}\text{O}$	86.1	4.0 ± 0.3	1×10^3	Cocoa, almond
8	3-methylbutanal	$\text{C}_5\text{H}_{10}\text{O}$	86.1	5.2 ± 0.3	2×10^2	Malt, smell of oil
9	Toluene	C_7H_8	92.1	23.8 ± 1.4	5.95×10^5	Rubbery, tarry, mothballs
10	Dimethyl disulfide	$\text{C}_2\text{H}_6\text{S}_2$	94.2	5.2 ± 1.9	3.5×10^3	Rotten cabbage, putrefaction
11	Hexanal	$\text{C}_6\text{H}_{12}\text{O}$	100.1	18.1 ± 3.4	2×10^2	Grass, tallow, fat
15	1,4-cineole	$\text{C}_{10}\text{H}_{18}\text{O}$	154.3	2.0 ± 0.1	na ^c	Spice
17	Limonene	$\text{C}_{10}\text{H}_{16}$	136.2	51.9 ± 2.9	1.7×10^3	Lemon
18	1,8-cineole	$\text{C}_{10}\text{H}_{18}\text{O}$	154.3	4.5 ± 0.5	1.3×10^3	Spice
19	1-pentanol	$\text{C}_5\text{H}_{12}\text{O}$	88.1	6.2 ± 0.1	5×10^2	Balsamic, fruity
21	α -terpinene	$\text{C}_{10}\text{H}_{16}$	136.2	3.9 ± 0.3	na	Lemon
22	ρ -cymene	$\text{C}_{10}\text{H}_{14}$	134.2	6.7 ± 0.1	7.1×10^3	Lemon, fruity, fuel like
23	Cyclohexanone	$\text{C}_6\text{H}_{10}\text{O}$	98.1	4.3 ± 1.6	3×10^2	Pepper, acetone
24	2-heptanol	$\text{C}_7\text{H}_{16}\text{O}$	116.2	1.6 ± 0.1	1×10^5	Herb
25	Pyrrolidine-2,4-dione	$\text{C}_4\text{H}_5\text{NO}_2$	99.1	2.1 ± 0.1	na	na
27	Hexanol	$\text{C}_6\text{H}_{14}\text{O}$	102.2	29.7 ± 1.1	1×10^1	Flower, green
28	Dimethyl trisulfide	$\text{C}_2\text{H}_6\text{S}_3$	126.3	1.0 ± 0.1	1×10^2	Rotten, vegetables
33	1-heptanol	$\text{C}_7\text{H}_{16}\text{O}$	116.2	24.7 ± 1.1	2.5×10^6	Chemical, green
34	3-propylcyclopentene	C_8H_{14}	110.2	4.5 ± 0.9	na	na
35	Benzaldehyde	$\text{C}_7\text{H}_6\text{O}$	106.1	57.5 ± 3.9	1×10^1	Burnt, sweet
36	Linalool	$\text{C}_{10}\text{H}_{18}\text{O}$	154.2	36.0 ± 0.1	1.4×10^2	Flower, lavender
37	Fenchol	$\text{C}_{10}\text{H}_{18}\text{O}$	154.2	4.8 ± 0.7	5×10^4	Camphor
38	4-terpineol	$\text{C}_{10}\text{H}_{18}\text{O}$	154.2	4.1 ± 0.9	3.4×10^{-1}	Turpentine, nutmeg, must
39	2-octen-1-ol	$\text{C}_8\text{H}_{16}\text{O}$	128.2	7.8 ± 0.9	5×10^4	Soap, plastic
42	1-nonanol	$\text{C}_9\text{H}_{20}\text{O}$	144.3	6.5 ± 0.6	5×10^1	Fat, green
43	Phenylacetaldehyde	$\text{C}_8\text{H}_8\text{O}$	120.1	9.4 ± 2.2	4×10^3	Honey, sweet
44	Acetophenone	$\text{C}_8\text{H}_8\text{O}$	120.1	6.4 ± 1.1	6.5×10^{-1}	Must, flower, almond
45	Limonen-4-ol	$\text{C}_{10}\text{H}_{16}\text{O}$	152.2	4.7 ± 1.6	na	Fresh, mint
46	α -terpineol	$\text{C}_{10}\text{H}_{18}\text{O}$	154.2	15.6 ± 1.4	2.5×10^5	Oil, anise, mint
47	Benzyl alcohol	$\text{C}_7\text{H}_8\text{O}$	108.1	4.3 ± 0.4	2×10^8	Sweet, flower
48	2-phenylethanol	$\text{C}_8\text{H}_{10}\text{O}$	122.1	1.9 ± 0.2	8.6×10^4	Rosy
50	o-cresol	$\text{C}_7\text{H}_8\text{O}$	108.1	0.4 ± 0.1	2×10^1	Medicinal, phenolic
51	Phenol	$\text{C}_6\text{H}_6\text{O}$	94.1	2.9 ± 0.1	2×10^4	Medicinal, phenolic plastic rubber
52	ρ -cresol	$\text{C}_7\text{H}_8\text{O}$	108.1	92.0 ± 2.9	2×10^1	Fecal, horse stable-like
54	Indole	$\text{C}_8\text{H}_7\text{N}$	117.1	6.5 ± 0.5	3×10^{-1}	Manure, fecal, nauseating
55	Skatole	$\text{C}_9\text{H}_9\text{N}$	131.2	1.6 ± 0.7	5.6×10^{-4}	Fecal, nauseating

^aMean and standard deviation often independent experiments.

^bAccording to: [4, 7, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27].

^cna: not available in the literature.

Table 2

Dynamics of conversion and production of volatile compounds ($\mu\text{g}\cdot\text{m}^{-3} \pm \sigma$) and removal efficiency in the heterotrophic microalgal bioreactor

Peak	Compound	Chemical formula	LRI DB-Wax ^a	Residence time ^b				Removal efficiency (%)
				0 h	24 h	48 h	72 h	
1	Carbon disulfide	CS ₂	762	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	nd ^c	100
2	Dimethyl sulfide	C ₂ H ₆ S	771	0.4 ± 0.1	nd	nd	nd	100
3	2-propenal	C ₃ H ₄ O	856	5.7 ± 0.1	nd	nd	nd	100
4	2-methylfuran	C ₅ H ₆ O	872	7.9 ± 1.1	5.5 ± 1.5	5.0 ± 1.9	nd	100
5	Acetaldehyde	C ₆ H ₁₄ O ₂	890	nd	2.6 ± 0.3	nd	nd	na ^d
6	Butanal	C ₄ H ₈ O	883	5.5 ± 1.2	nd	nd	nd	100
7	2-methylbutanal	C ₅ H ₁₀ O	917	3.8 ± 0.8	nd	nd	nd	100
8	3-methylbutanal	C ₅ H ₁₀ O	921	4.5 ± 0.1	nd	nd	nd	100
9	Toluene	C ₇ H ₈	1049	22.8 ± 0.1	nd	nd	nd	100
10	Dimethyl disulfide	C ₂ H ₆ S ₂	1080	5.8 ± 0.1	2.3 ± 0.4	1.9 ± 0.2	1.8 ± 0.4	69.0
11	Hexanal	C ₆ H ₁₂ O	1092	15.7 ± 0.1	nd	nd	nd	100
12	2-methylpentanol	C ₆ H ₁₄ O	1099	nd	0.5 ± 0.1	nd	nd	na
13	2-methyl-3-hexanone	C ₇ H ₁₄ O	1140	nd	4.2 ± 0.5	nd	nd	na
14	Acetyl valeryl	C ₇ H ₁₂ O ₂	1153	nd	2.5 ± 0.6	nd	nd	na
15	1,4-cineole	C ₁₀ H ₁₈ O	1168	3.6 ± 0.1	nd	nd	nd	100
16	2-heptanone	C ₇ H ₁₄ O	1181	nd	1.2 ± 0.4	5.0 ± 0.5	5.0 ± 0.7	na
17	Limonene	C ₁₀ H ₁₆	1182	49.9 ± 0.1	20.4 ± 0.4	13.4 ± 0.5	nd	100
18	1,8-cineole	C ₁₀ H ₁₈ O	1193	4.9 ± 0.7	1.1 ± 0.1	nd	nd	100
19	1-pentanol	C ₅ H ₁₂ O	1203	6.3 ± 0.7	nd	nd	nd	100
20	3-methylbutanol	C ₅ H ₁₂ O	1221	nd	0.4 ± 0.1	nd	nd	na
21	α-terpinene	C ₁₀ H ₁₆	1226	3.7 ± 0.5	nd	nd	nd	100
22	ρ-cymene	C ₁₀ H ₁₄	1253	6.8 ± 1.4	nd	nd	nd	100
23	Cyclohexanone	C ₆ H ₁₀ O	1285	5.4 ± 0.4	nd	nd	nd	100
24	2-heptanol	C ₇ H ₁₆ O	1301	1.1 ± 0.8	nd	nd	nd	100
25	Pyrrolidine-2,4-dione	C ₄ H ₅ NO ₂	1311	2.2 ± 0.9	nd	nd	nd	100
26	6-methyl-5-hepten-2-one	C ₈ H ₁₄ O	1327	3.8 ± 0.4	3.8 ± 0.4	2.0 ± 0.6	nd	na
27	Hexanol	C ₆ H ₁₄ O	1338	30.3 ± 0.7	nd	nd	nd	100
28	Dimethyl trisulfide	C ₂ H ₆ S ₃	1363	1.2 ± 0.2	0.9 ± 0.2	nd	nd	100
29	2-nonanone	C ₉ H ₁₈ O	1382	nd	1.1 ± 1.6	1.4 ± 0.8	2.3 ± 0.8	na
30	Methyl 3-methyl 2-hydroxybutanoate	C ₆ H ₁₂ O ₃	1390	nd	2.4 ± 0.4	nd	nd	na
31	Cyclohexanol	C ₆ H ₁₂ O	1395	nd	6.5 ± 1.0	nd	nd	na
32	5-ethyl-2-nonanol	C ₁₁ H ₂₄ O	1399	nd	2.3 ± 0.2	nd	nd	na
33	1-heptanol	C ₇ H ₁₆ O	1447	25.5 ± 0.1	nd	nd	nd	100
34	3-propylcyclopentene	C ₈ H ₁₄	1510	3.9 ± 1.0	3.0 ± 1.2	nd	nd	100
35	Benzaldehyde	C ₇ H ₆ O	1545	55.4 ± 0.1	nd	nd	nd	100
36	Linalool	C ₁₀ H ₁₈ O	1552	36.0 ± 2.3	nd	nd	nd	100
37	Fenchol	C ₁₀ H ₁₈ O	1574	4.3 ± 1.2	nd	nd	nd	100
38	4-terpineol	C ₁₀ H ₁₈ O	1605	4.8 ± 0.8	3.7 ± 0.5	nd	nd	100
39	2-octen-1-ol	C ₈ H ₁₆ O	1611	8.5 ± 0.9	nd	nd	nd	100
40	Menthol	C ₁₀ H ₂₀ O	1642	4.4 ± 0.2	5.7 ± 0.4	7.3 ± 0.8	7.6 ± 0.6	na
41	3-methylpentanoic acid	C ₆ H ₁₂ O ₂	1655	nd	0.7 ± 0.2	nd	nd	na
42	1-nonanol	C ₉ H ₂₀ O	1655	6.0 ± 0.1	nd	nd	nd	100
43	Phenylacetaldehyde	C ₈ H ₈ O	1662	7.9 ± 0.1	nd	nd	nd	100
44	Acetophenone	C ₈ H ₈ O	1679	7.2 ± 0.4	3.2 ± 0.4	nd	nd	100
45	Linomen-4-ol	C ₁₀ H ₁₆ O	1687	5.3 ± 0.9	4.1 ± 0.7	nd	nd	100
46	α-terpineol	C ₁₀ H ₁₈ O	1697	17.3 ± 1.9	14.6 ± 1.4	nd	nd	100

(continued)

Table 2 (continued)

Peak	Compound	Chemical formula	LRI DB-Wax ^a	Residence time ^b				Removal efficiency (%)
				0 h	24 h	48 h	72 h	
47	Benzyl alcohol	C ₇ H ₈ O	1848	4.0 ± 0.7	nd	nd	nd	100
48	2-phenylethanol	C ₈ H ₁₀ O	1865	1.6 ± 0.6	nd	nd	nd	100
49	Benzothiazole	C ₇ H ₅ NS	1896	3.3 ± 0.7	2.2 ± 0.7	3.8 ± 1.0	5.0 ± 0.1	na
50	o-cresol	C ₇ H ₈ O	1909	0.8 ± 0.1	0.3 ± 0.1	0.2 ± 0.4	nd	100
51	Phenol	C ₆ H ₆ O	1915	3.0 ± 0.3	0.6 ± 0.9	0.6 ± 0.7	nd	100
52	p-cresol	C ₇ H ₈ O	1991	90.0 ± 0.7	47.3 ± 1.0	nd	nd	100
53	1-penten-3-ol	C ₅ H ₁₀ O	2041	0.6 ± 0.3	5.0 ± 0.9	1.0 ± 0.7	0.3 ± 0.5	na
54	Indole	C ₈ H ₇ N	2390	7.3 ± 1.2	3.0 ± 0.9	1.0 ± 0.7	0.3 ± 0.5	95.9
55	Skatole	C ₉ H ₉ N	2437	1.2 ± 0.6	nd	nd	nd	100

^aLinear retention indices in the DB-Wax column.

^bMean and standard deviation of the independent experiments.

nd: not detected.

na: not applicable.

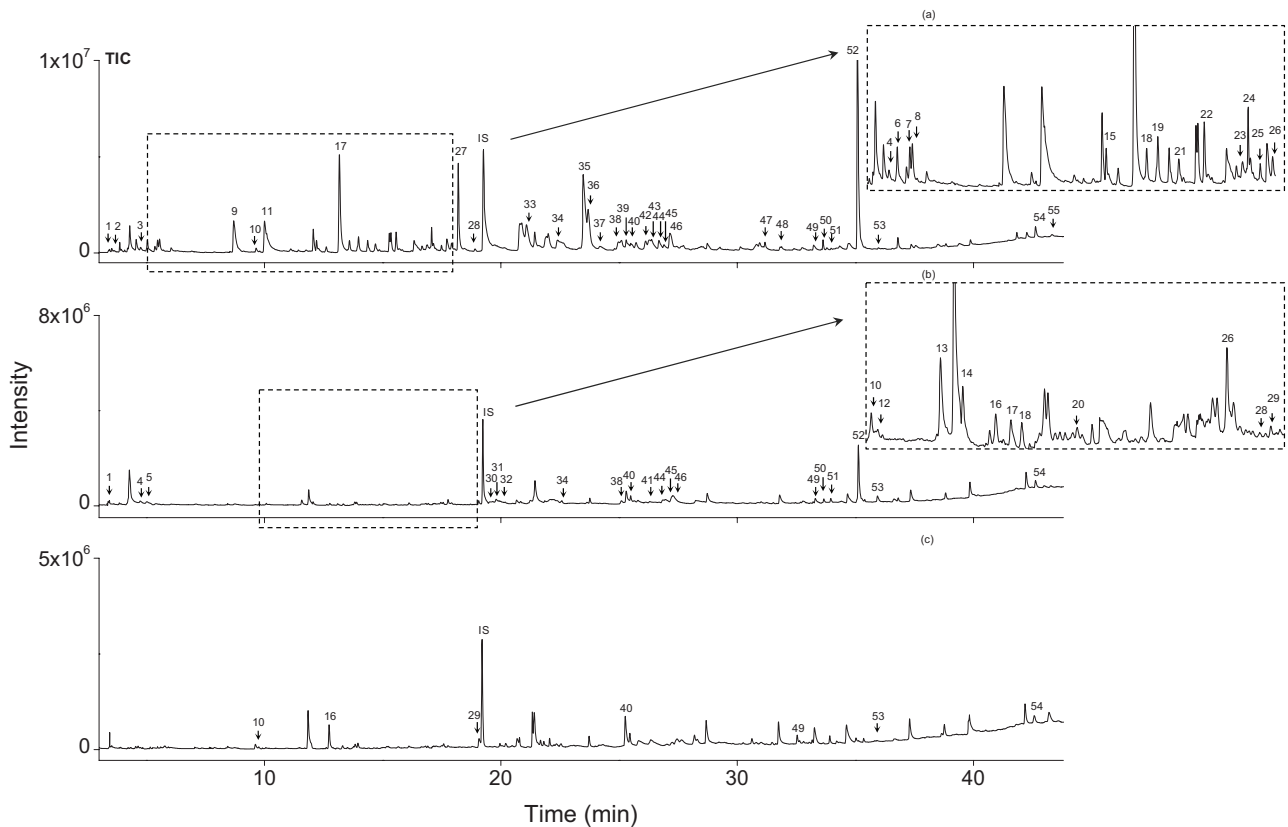


Fig. 1. Chromatogram (total ion current) of the volatile organic compounds from the heterotrophic microalgal bioreactor. The letters correspond to the residence times with which the chromatograms were obtained: (a) 0 h, (b) 24 h, (c) 72 h.

compounds did not exceed 76.8%. Between 24 and 72 h of residence time, just 8 compounds disappeared with odor abatement efficiencies of 95.1%, and in the complete cycle of treatment (48 h), more 5 compounds from wastewater disappeared, and total odorant concentration was reduced by 99.6%.

Studies about 7 usual odor treatment technologies in wastewater treatment plants, e.g., those carried out by

Estrada [32], reported that odor removal ranged from 70% to 95%. According to the Logan [33], total residence time of 260 h was necessary to reduce 99.7% of odor emission from swine wastewater by using microbial fuel cells.

Considering that compounds with low odor threshold values play an important role in the negative effects on odor release from wastewater treatment plants, the indolic, phenolic, and sulfur compounds are a key group

in malodors of agro-industrial wastewaters. In this work, these categories contain carbon disulfide (peak 1), dimethylsulfide (peak 2), dimethyl disulfide (peak 10), dimethyl trisulfide (peak 28), o-cresol (peak 50), phenol (peak 51), p-cresol (peak 52), indole (peak 54), and skatole (peak 55). All these compounds were totally degraded as a function of residence time, with exception of dimethyl disulfide and indole. However, these compounds showed 69.0% and 95.9% removal efficiency respectively.

The major compounds p-cresol and benzaldehyde were totally removed at 48 and 24 h of residence time (Fig. 2) while other important compounds for nuisance odor from raw wastewater had the following results: skatole and dimethyl sulfide were totally removed at 24 h; dimethyl trisulfide at 48 h; and carbon disulfide, o-cresol, and phenol at 72 h.

In the present study, there was removal of apolar compounds. A total of 10 terpenes were completely removed: limonene (peak 17) and its derivatives (peaks 21, 22, 45), α -terpineol (peak 46) and its derivatives (peaks 15, 18, 38), linalool (peak 36) and fenchol (peak 37). Limonene ($49.9 \mu\text{g}\cdot\text{m}^{-3}$), was totally removed at 72 h; the isomers of limonene, α -terpinene, and p-cymene disappeared at 24 h of residence time and the same occurred with 1,4-cineole, linalool, and fenchol. Moreover, α -terpineol, 4-terpineol, and 1,8-cineole were degraded at 48 h of residence time.

This fact is interesting, considering that the best available techniques for odor abatement show severe mass transfer limitations when treating hydrophobic odorants [34].

The results reported by previous studies in literature for removal limonene from wastewater did not exceed 90% [35]. In this work, terpenes were completely removed. Volatile organic compounds are usually resistant to biodegradation, thereby limiting the performance of traditional biotechnology dealing with waste gas containing such pollutants, especially in the mixture [36]. Therefore, a unique process of odor abatement that shows good performance for removal of a functional group of hydrophobic and hydrophilic character, sometimes in the same molecule (mainly peaks 48, 50, 51, 52, 54, and 55), is one of the main challenges of bioprocess engineering for degradation of malodors gases.

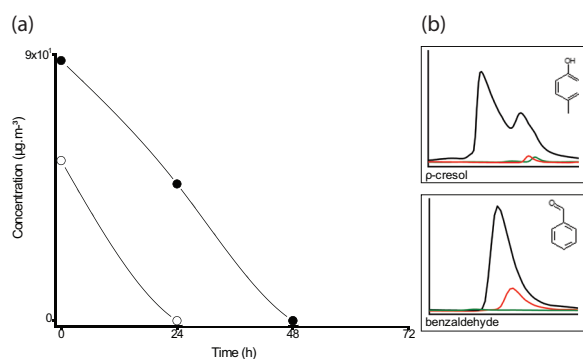


Fig. 2. Changes in the volatile organic compounds observed during residence time of the bioreactor, (a) dynamics of degradation of p-cresol (○) and benzaldehyde (●), (b) Chromatogram detail with degradation of the peak during residence time of the heterotrophic microalgal bioreactor 0 h (black line), 24 h (red line), 48 h (green line).

In this context, in the present study, there was a high removal performance of volatile organic compounds from meat process wastewater, hence one of the main technological advantages of the microalgal heterotrophic bioreactor was the polarity range of odor compounds removed from raw wastewater. This fact was not a surprise, and it can be explained by the metabolic diversity of microalgae. The dominant growth physiology of the diverse cyanobacteria is phototrophic. However, these organisms also display other metabolic capabilities. One of them is of particular importance to cyanobacteria: the maintenance of the structure in the dark [37]. Under heterotrophic conditions, the growth of microalgae is dependent on exogenous organic compounds; in this case, organic compounds provide the organism with a source of carbon and energy. In this particular culture condition, the microalgae show a very different ability from those commonly found in the phototrophic environment, for example, the removal of odorous compounds from water, despite the information reported in the literature that microalgae produced unpleasant odors mainly in the form of geosmin and 2-methylisoborneol in drinking water [38–40]. Based on the results of our previous works [41,42], which show carotenoids and volatile profile from different microalgae cultivated under heterotrophic and phototrophic conditions, it can be suggested that the hypothesis for total degradation of terpenes found in this study is related to carotenoid production in the dark.

Cyanobacteria produce a wide variety of carotenoids, and for many years it was believed that carotenoid production depends on high light irradiance under photosynthetic conditions [43,44]. However, more recent studies have focused on carotenoid production in the heterotrophic microalgal bioreactor and identified pigments with very different structural characteristics, such as a greater number of carbon atoms, conjugated double bonds, and hydroxyl groups, all of which contribute to their great antioxidant capacity [42,45].

Taking into account the structures of terpenes identified in this work and of the tetraterpenes detected in previous works [41,42], the mechanism for degradation of terpenes and production of microalgal carotenoids in the dark was proposed (Fig. 3). Limonene and other terpenes (Table 2 and Fig. 1) were metabolized in the heterotrophic growth via an oxidative pentose-phosphate cycle. These catabolic routes are yield precursors in the methylerythritol phosphate pathway (MEP). Synthesized by this pathway, geranyl pyrophosphate (GPP) is produced, and a head to head condensation of the two GPP C₂₀ compounds formed the first carotene, the phytoene (C₄₀) precursor of keto and acetylated microalgae carotenoid.

Also, the volatile organic compounds formed by *Phormidium autumnale* cultivated in the heterotrophic bioreactor were found in this work (Fig. 1, Table 2). A total of 15 compounds were formed, 14 of which had odor description of various chemical structures such as aldehyde (peak 5), alcohols (peaks 12, 20, 31, and 53), ketones (peaks 13, 14, 16, 26, and 29), ester (peak 30), terpene (peak 40), acid (peak 41), and nitrogen compound (peak 49).

Regardless of the organic class of the compounds formed, three odor categories (fruity, spicy, and resinous) emerged. The literature [16] reported that peaks 12, 13, 30, and 53

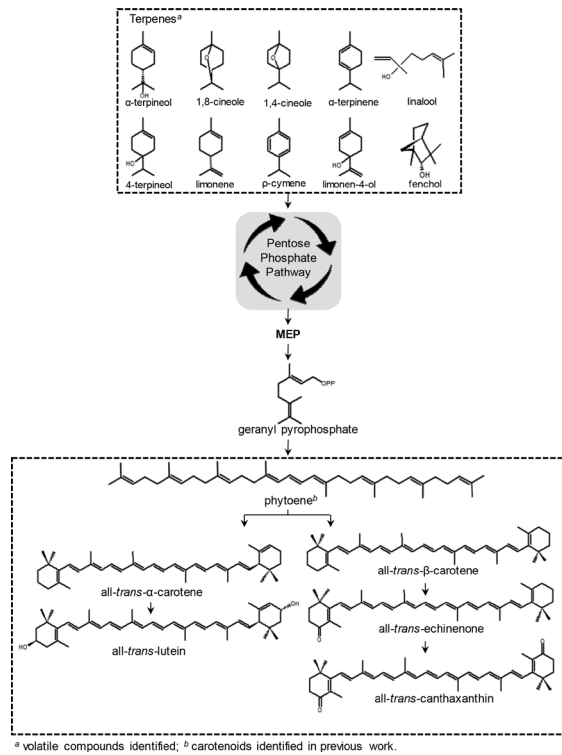


Fig. 3. Overview of proposed the mechanism for degradation of terpenes and production of microalgal carotenoids in the dark.

show an odor descriptor that may be classified as fruity. The compounds (peaks 5, 14, 16, 29, 41, and 49) were classified with a resinous odor, peaks 20, 40 were classified with a burnt odor, and peaks 26 and 31 showed a spicy odor (Fig. 4). Among the chemical compounds identified, menthol (peak 40) showed $7.6 \mu\text{g}\cdot\text{m}^{-3}$ at 72 h, followed by cyclohexanol (peak 31), with $6.5 \mu\text{g}\cdot\text{m}^{-3}$ at 24 h of residence time.

The predominant volatile compound was formed as time of cultivation increased: menthol (peak 40), an isomer of limonene (Fig. 4). Altogether, this result supports the hypothesis of the present research that the terpene compounds was the main volatile organic compound to be removal from meat processing wastewater and metabolized for production of microalgae-based products.

These compounds could, therefore, be a source of useful chemicals products, based on a nonconventional technological route. Thirteen compounds produced by *Phormidium autumnale* in the heterotrophic microalgal bioreactor are commercially available from other biotechnological routes. The flavor biotechnology will be the next generation of the industrial biotechnology. The chemicals obtained from biobased technologies are sold at prices up to 1,000 times higher than synthetic chemicals, hence there is great potential for exploitation of such processes [15,30].

Finally, to confirm whether the volatile organic compounds had been removed from raw wastewater by biological mechanisms, a parallel experiment containing only wastewater and pneumatic aeration was conducted (Table 1, Supplementary data). In this experiment with the

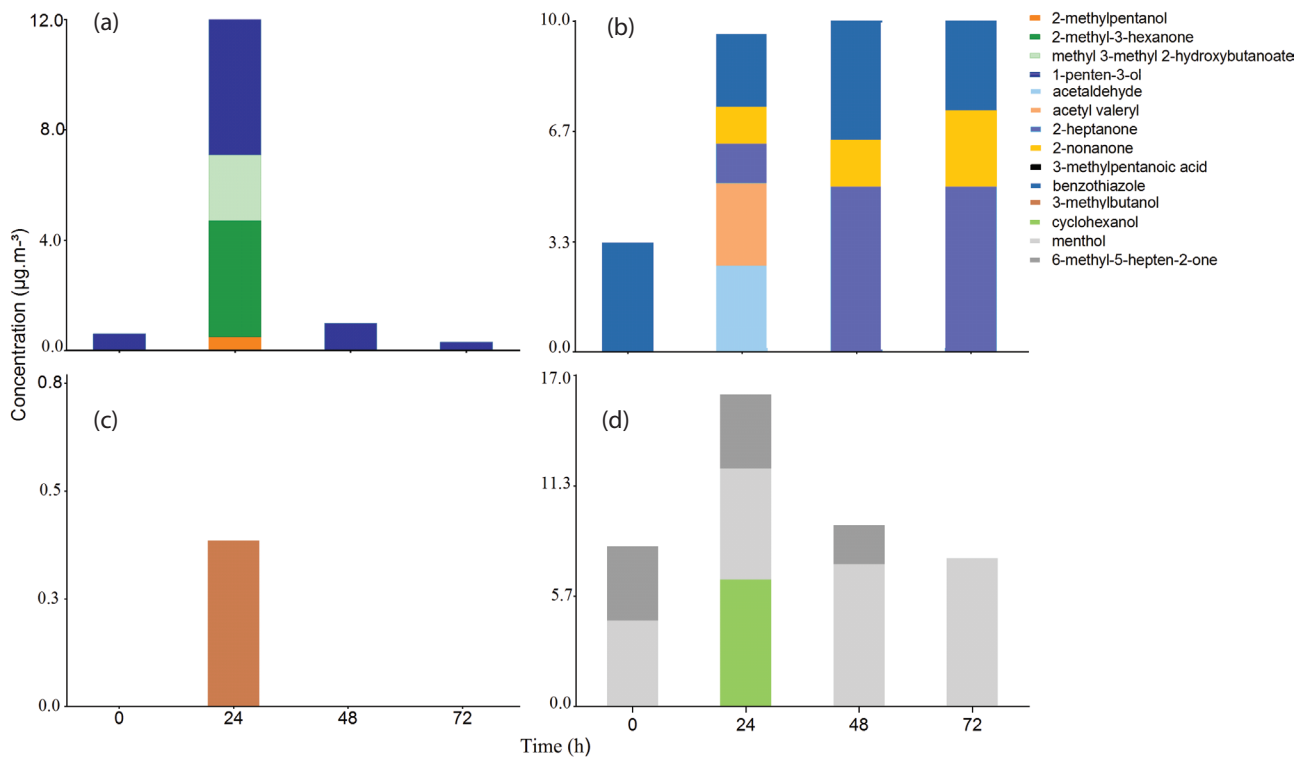


Fig. 4. Dynamics of production of the volatile organic compounds in the bioreactor: (a) fruity, (b) resinous, (c) burnt and (d) spice.

absence of microalgae, only 26 compounds were totally removed – in general, with substantially increased residence time of the bioreactor. Between the more recalcitrant compounds, terpenes (limonene, 1,8-cineole, and linalool) practically were not removed. This result shows the potential of the microalgal heterotrophic bioreactor in odor emission abatement in meat processing wastewater, particularly in the terpene family.

4. Conclusions

The meat processing wastewater presents a total of 40 odor compounds, with a wide range of odor thresholds. The microalgal heterotrophic bioreactor was able to totally remove 38 volatile organic compounds. Dimethyl disulfide and indole were the most recalcitrant compounds, with removal efficiencies in the order of 69.0% and 95.9%, respectively.

In parallel to this odor abatement, 13 industrially interesting volatile compounds were produced (menthol, 25.0 $\mu\text{g}\cdot\text{m}^{-3}$; benzothiazole, 14.3 $\mu\text{g}\cdot\text{m}^{-3}$; 2-heptanone, 11.2 $\mu\text{g}\cdot\text{m}^{-3}$; 6-methyl-5-hepten-2-one, 9.6 $\mu\text{g}\cdot\text{m}^{-3}$; 1-penten-3-ol, 6.9 $\mu\text{g}\cdot\text{m}^{-3}$; cyclohexanol, 6.5 $\mu\text{g}\cdot\text{m}^{-3}$; 2-nonanone, 4.8 $\mu\text{g}\cdot\text{m}^{-3}$; 2-methyl-3-hexanone, 4.2 $\mu\text{g}\cdot\text{m}^{-3}$; acetaldehyde, 2.6 $\mu\text{g}\cdot\text{m}^{-3}$; acetyl valeryl, 2.5 $\mu\text{g}\cdot\text{m}^{-3}$; 3-methylpentanoic acid, 0.7 $\mu\text{g}\cdot\text{m}^{-3}$; 2-methylpentanol, 0.5 $\mu\text{g}\cdot\text{m}^{-3}$ and 3-methylbutanol, 0.4 $\mu\text{g}\cdot\text{m}^{-3}$), thus potentializing the application of these biobased feedstocks for both food and non-food industries.

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Symbols

COD	–	Chemical oxygen demand, $\text{mg}\cdot\text{L}^{-1}$
N-TKN	–	Total nitrogen, $\text{mg}\cdot\text{L}^{-1}$
P- PO_4^{-3}	–	Total phosphorus, $\text{mg}\cdot\text{L}^{-1}$
TS	–	Total solids, $\text{mg}\cdot\text{L}^{-1}$
SS	–	Suspended solids, $\text{mg}\cdot\text{L}^{-1}$
VS	–	Volatile solids, $\text{mg}\cdot\text{L}^{-1}$
FS	–	Fixed solids, $\text{mg}\cdot\text{L}^{-1}$
VVM	–	Volume of air per volume of wastewater per minute
HS-SPME	–	Headspace solid-phase microextraction
DVB/Car/PDMS	–	Divinylbenzene/carboxen/polydimethylsiloxane
GC/MS	–	Gas chromatography-mass spectrometry
LRI	–	Linear retention index
VOC_w	–	Volatile organic compounds from wastewater
VOC_f	–	Volatile organic compounds formed
MEP	–	Methylerythritol phosphate pathway
GPP	–	Geranyl pyrophosphate pathway

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Supporting information

Table S1

Odor concentration ($\mu\text{g}\cdot\text{m}^{-3}\pm\sigma$) in the wastewater using aeration (1.0 volume of air per volume of wastewater per minute) in the heterotrophic bioreactor.

Peak	Compound	Residence time ^a			
		0 h	24 h	48 h	72 h
1	Carbon disulfide	1.1±0.1	0.1±0.7	nd ^b	nd
2	Dimethyl sulfide	0.6±0.2	0.4±0.2	0.4±0.2	0.5±0.2
3	2-propenal	6.0±0.4	nd	nd	nd
4	2-methylfuran	7.1±1.9	4.9±0.4	4.8±0.1	4.3±0.4
6	Butanal	4.9±0.1	nd	nd	nd
7	2-methylbutanal	4.0±0.3	nd	nd	nd
8	3-methylbutanal	5.2±0.3	1.0±0.2	nd	nd
9	Toluene	23.8±1.4	16.4±0.5	12.8±1.1	7.1±2.1
10	Dimethyl disulfide	5.2±1.9	5.4±2.2	5.5±2.5	5.4±2.5
11	Hexanal	18.1±3.4	nd	nd	nd
15	1,4-cineole	2.0±0.1	nd	nd	nd
17	Limonene	51.9±2.9	50.0±3.1	51.0±1.8	51.0±1.4
18	1,8-cineole	4.5±0.5	2.7±1.1	2.3±1.6	2.9±2.1
19	1-pentanol	6.2±0.1	6.4±0.1	6.0±0.1	1.0±0.1
21	α -terpinene	3.9±0.3	nd	nd	nd
22	ρ -cymene	6.7±0.1	nd	nd	nd
23	Cyclohexanone	4.3±1.6	3.2±2.3	1.0±0.3	nd
24	2-heptanol	1.6±0.1	nd	nd	nd
25	Pyrrrolidine-2,4-dione	2.1±0.1	nd	nd	nd
27	Hexanol	29.7±1.1	8.7±0.6	3.5±0.5	3.3±0.3
28	Dimethyl trisulfide	1.0±0.1	1.1±0.1	1.2±0.8	nd
33	1-heptanol	24.7±1.1	1.1±0.2	nd	nd
34	3-propylcyclopentene	4.5±0.9	4.0±0.7	nd	nd
35	Benzaldehyde	57.5±3.9	nd	nd	nd
36	Linalool	36.0±0.1	32.8±1.7	37.4±3.4	33.4±2.2
37	Fenchol	4.8±0.7	0.9±0.2	0.6±0.4	nd
38	4-terpineol	4.1±0.9	4.4±0.5	nd	nd
39	2-octen-1-ol	7.8±0.9	nd	nd	nd
42	1-nonanol	6.5±0.6	nd	nd	nd
43	Phenylacetaldehyde	9.4±2.2	nd	nd	nd
44	Acetophenone	6.4±1.1	5.8±1.8	3.2±0.9	nd
45	Limonen-4-ol	4.7±1.6	5.8±1.2	3.4±0.9	nd
46	α -terpineol	15.6±1.4	14.0±0.4	nd	nd
47	Benzyl alcohol	4.3±0.4	5.7±0.4	2.4±0.8	nd
48	2-phenylethanol	1.9±0.2	nd	nd	nd
50	o-cresol	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.1
51	Phenol	2.9±0.1	2.8±0.6	1.7±0.8	0.4±0.3
52	ρ -cresol	92.0±2.9	71.0±2.6	74.9±2.7	63.8±2.0
54	Indole	6.5±0.5	6.1±2.2	6.5±1.7	6.3±1.3
55	Skatole	1.6±0.7	1.6±2.4	1.6±1.1	1.5±1.1

^a Mean and standard deviation often independent experiments.

^b nd: not detected.