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Volatile Organic Compounds Determination from Intestinal Polyps and in Exhaled Breath by Gas Chromatography–Mass Spectrometry

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Abstract: In this paper, a new protocol is described, based on solid phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC-MS), to monitor ex vivo changes in endogenous volatile organic compounds (VOCs) released by surgically resected colonic tissues (normal colonic mucosa and adenomatous polyps) from seven patients undergoing operative colonoscopy to identify their molecular pattern. The exhalated volatile organic molecules from these patients were sampled by the ReCIVA® breath sampler, shortly before surgery, and analyzed by GC-MS. Comparing VOC patterns identified in the tissues and in the breath of the same patients, a possible correlation can be found between the levels of methylbenzene and benzaldehyde exhaled and the presence of colonic adenomatous polypoid lesions.

Keywords: adenomatous colonic polyp; colonic mucosa; VOCs; exhaled breath; SPME-GC-MS; ReCIVA®-GC-MS

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1. Introduction

SPME is an efficient, sensitive, rapid, and solvent-free sample preparation technique that merges, in one step, sampling, isolation, and enrichment of analytes. It is commonly joined with GC or liquid chromatography (LC) to separate and detect the extracted analytes [1–5]. The simplicity of sampling and the design flexibility of the probe make SPME a useful extraction technique for analyzing biological samples of different natures and sizes. SPME is a useful rapid diagnostic tool for in vitro/ex vivo and in vivo studies [2–6]. The development of minimally invasive SPME microfibers has made it possible to monitor target analytes in vivo or changes in the metabolome/lipidome of a cell or living organ [7,8].

Colorectal cancer (CRC) is one of the most common tumors, both in Europe and USA, and is associated with high mortality [9–11]. Following a multistep process involving genetic, histological, and morphological changes, CRC generally originates from adenomatous polyps. Early detection and endoscopic treatment of polypoid lesions are the primary CRC prevention and can significantly reduce CRC incidence and mortality [12,13].

Recently, GC-MS analysis of exhaled VOCs has been demonstrated to be able to differentiate between CRC patients and healthy people (negative colonoscopy) [14]. A correspondence between VOCs exhaled and released by surgically resected cancer tissue of the same CRC patient was found using the SPME-GC-MS technique [15].

In this study, we applied the same headspace (HS)- and direct immersion (DI)-SPME-GC-MS protocols, optimized in our previous work [15], to determine and monitor ex vivo, for one week, the endogenous VOCs produced from tissues (healthy mucosa and colorectal adenomatous polyps) obtained from patients undergoing operative colonoscopy and compared them with their exhaled VOCs, sample by ReCIVA® device and investigated by GC-MS, to search any possible correlation.

2. Materials and Methods

2.1. Patients and Polyp Characteristics

After obtaining written informed consent, the exhaled breath and fresh specimens of healthy colonic mucosa and resected adenomatous polyps of seven patients, enlisted to undergo endoscopic treatment, were analyzed. Table 1 resumes the demographic and co-morbidities of patients and polypoid lesions characteristics.

Table 1. Demographics and co-morbidities of adenomatous colonic polypoid lesion-affected patients (n = 7) and polyp characteristics.

Demographics and Co-Morbidities of Adenomatous Colonic Polypoid Lesion-Affected Patients (n = 7)	
Mean Age (years)	63
Sex ratio (M:F)	5:2
Hypertension	2
Diabetes	0
Hypothyroidism	0
Smoker	1
Polyp characteristics	
Polyp size	2.0 ± 0.5 cm
Hystology	7 adenomatous polyps
Grading	2 moderate and 7 severe dysplasia

2.2. Tissues (Colonic Adenomatous Polypoid Lesion and Normal Mucosa) Analysis

2.2.1. Chemicals and SPME Sampling Device

Reference standards of benzaldehyde, ethylbenzene, indole, methylbenzene, phenol, and octanal (analytical grade; purity > 98%) were purchased from Sigma-Aldrich (Milano, Italy) to confirm the chemical structure of the molecules recognized by the National Institute of Standards and Technology (NIST) library. Stock solutions (10 mg/mL) of each compound were prepared in methanol (Sigma-Aldrich), stored at 8 °C, and daily diluted in fresh culture medium (Dulbecco's Modified Eagle Medium, Euroclone, Milano, Italy) to prepare working standard mixtures. The SPME (Supelco, Milano, Italy) mounting kit included a manual holder and a 50/30 mm CAR/DVB/PDMS (carboxen, divinylbenzene, polydimethylsiloxane) fiber of 1 cm in length. Before use, the fiber was conditioned in the GC injector according to the supplier's instructions.

2.2.2. SPME VOCs Extraction

Excised tissue was cut in biopsies weighing 0.020 ± 0.005 g and placed in a 1.7 mL screw top amber glass vial, hermetically sealed with a PTFE (polytetrafluoroethylene)/silicone septum (Sigma-Aldrich, St. Louis, MO, USA), containing 0.2 mL of sterile culture medium. Within 30 min from resection, the vials were incubated at 37 °C and analyzed immediately (time zero) and after 1, 4, and 7 days by HS- and DI-SPME. A "blank" vial containing 0.2 mL of culture medium was simultaneously subjected to the same protocol. A volume of 0.01 mL of standard working mixtures at appropriate concentrations was then added to a 1.7 mL amber glass vial equipped with PTFE /silicone septum and screw cap (Sigma-

Aldrich, St. Louis, MO, USA), containing 0.2 mL of sterile culture medium. These samples were subjected to the same analytical procedures.

The extraction of the VOCs by HS-SPME was carried out by placing the fiber at 37 °C for 30 min.

DI-SPME was carried out by drawing a volume of 15 µL from HS vials with a microliter syringe from Hamilton (Sigma-Aldrich, St. Louis, MO, USA) and placed into a 1.7 mL vial containing 1.5 mL of 15% NaCl sterile solution and a magnetic stirrer bar (Sigma-Aldrich, St. Louis, MO, USA). After sealing with a cap equipped with PTFE/silicone septa, the fiber was immersed in the solution under agitation (700 rpm) for 60 min at room temperature. The volume subtracted from each HS vial was restored with sterile fresh culture medium before storing the vial in the incubator at 37 °C.

2.2.3. GC-MS Apparatus and Analysis Experimental Conditions

For this study, a TRACE GC Ultra (Thermo Scientific, Waltham, MA, USA) with an ion-trap mass spectrometer (Polaris Q, Thermo Scientific, Waltham, MA, USA) was used. A TRACE TR-5 MS-fused silica capillary column (30 m × 0.25 mm i.d., 0.25 mm film thickness; Thermo Scientific) was used for chromatographic separation, and helium (Rivoira, Torino, Italy) with a constant flow rate of 1.0 mL/min was used as carrier gas.

The chromatographic conditions were those optimized in our previous work [15]. The mass spectrometer was operated in the electron impact (EI+) mode with a source temperature of 200 °C, ionizing voltage of 70 eV, and transfer line temperature of 240 °C. Full scan mode (SCAN; 40–250 m/z with a total scan time of 0.34 s) was used for the mass analysis. Total ion count (TIC) chromatograms were acquired and extracted ion chromatograms (XIC) were obtained for individual analytes by selecting their characteristic m/z values. VOC identification was carried out using the MS database of the NIST.

After extraction, fibers were transferred into the injection port of the GC for 2 min in splitless mode. In ex vivo experiments, fibers were exposed for an additional 5 min in the GC injector port at 250 °C before a new sampling to avoid memory effects.

2.2.4. Linear Regression Analysis, Limits of Detection (LOD), and Quantification (LOQ)

The HS- and DI-SPME conditions employed were evaluated with linear regression analysis of peak area versus analyte concentration in culture medium, utilizing standard solutions of suitable concentration. For the selected compounds (benzaldehyde, ethylbenzene, indole, methylbenzene, octanal, and phenol), the linear ranges, calibration curve equations, as well as LOD and LOQ were determined with LOD ≅ (3·sda)/b and LOQ ≅ (10·sda)/b, where sda is the standard deviation of the y-intercept and b is the slope of the regression line.

2.3. Analysis of the Exhaled Breath VOCs

2.3.1. Breath Sampling and Characterization

The breath of seven patients with adenomatous colonic polyps was sampled with a ReCIVA® breath sampler (Owlstone Medical, Cambridge, UK). The breath-sampling kit consists of a mask and thermal desorption (TD) tubes ensuring reproducible collection of VOCs during real-time monitoring of the patient's breathing. A mask made in medical-grade silicone, including a low-resistance bacterial filter, was fixed to the device before each sampling. A medical air canister was then connected via a plastic pressure reducer, set to 15 L·min^{-1}. The exhaled breath was captured in four TD tubes (Markes International, Llantrisant, UK; biomonitoring sorbent tubes) capable of retaining carbon compounds from C4 to C30. The alveolar fraction of the exhaled breath was selected through infrared carbon dioxide detection. The ReCIVA® breath sampler was connected to a laptop equipped with breath-sampling software (Owlstone Medical, Cambridge, UK), designed to ensure the accurate monitoring of breathing air pressure (partial pressure of carbon dioxide). All subjects fasted for at least 4 h before breath sampling.

Sampling was always performed in the same room, aerated for 30 min before each procedure.

The mask was securely adhered to the face and patients were instructed to breathe the air released by the medical air canister. After a 60-s ReCIVA® device washout with pure air (purity 99.99%; SOL Group, Monza, Italy), patient breath was collected for 10 min under PC-dedicated program control.

Straight after sampling, the TD tubes were removed, covered with a plastic cap, and delivered to the chemistry department within 24 h for GC-MS analysis.

Three TD tubes containing room air were sample-tested before commencement of the breath sampling on each sampling day to exclude environmental contaminations.

VOCs collected in stainless steel TD tubes were desorbed with a thermal desorber (Unity-xr, Markes International), directly connected to the gas chromatograph with a heated transfer line. After heating each tube for 10 min at 220 °C, the desorbed VOCs were directly transferred into the gas chromatograph injector at 200 °C, operating in split mode (50% in and 50% out), utilizing helium as carrier gas, at a linear velocity of 0.5 $cm \cdot s^{-1}$. A gas chromatograph (Clarus 680, PerkinElmer, Waltham, MA, USA) coupled with a quadrupole mass spectrometer (Clarus SQ 8T, PerkinElmer) was used for separation and quantification of desorbed VOCs. A 60 m × 0.25 mm i.d., 1.4 µm film thickness, capillary column Rtx®-VMS (Restek, Bellefonte, PA, USA) was utilized at 50 °C for 5 min, then ramped 10 $°C \cdot min^{-1}$ to 160 °C, 5 min at 160 °C, ramped 10 $°C \cdot min^{-1}$ to 220 °C, and 5 min at 220 °C. The temperature of the transfer line was 280 °C, whereas the ion source of quadrupole was 220 °C. The MS was performed at 70 eV electron impact ionization energy, in full-scan mode (SCAN) with scan range of 40–250 amu. For compound identification and quantification SCAN monitoring mode was used. The Clarus SQ8 GC-MS software (PerkinElmer) was used for acquisition and elaboration of the data.

To prevent memory effects, after each analysis, two empty TD tubes (without adsorbent phase) were analyzed to remove eventual residues of the previous sample from the thermal desorber and analysis apparatus.

After each analysis, TD tubes were conditioned at 340 °C for 3 h, as recommended by the producer, capped, sealed with parafilm, and stored at 8 °C.

2.3.2. Linear Regression Analysis, LOD and LOQ

VOCs produced by normal colonic mucosa and adenomatous polyps, recognized also in the breath of the patients by MS NIST database, were tested by linear regression analysis. Stock solutions (1 $mg \cdot mL^{-1}$) of each chosen volatile molecule (purity ≥ 97%; Sigma-Aldrich) were prepared in methanol (purity ≥ 98%; Sigma-Aldrich), stored at 8 °C, and diluted to prepare working solutions. Working solution (1 µL) containing authentic standards (5, 10, 15, 25, 50, and 100 $ng \cdot mL^{-1}$) was added into a TD tube which was then analyzed following the procedure described above. The identification of VOCs was performed with the MS database of the NIST.

Linear regression analysis was performed plotting the peak area against the amount (ng) of each analyte in TD tube.

As previously described (Section 2.2.4), LOD and LOQ values were determined by LOD ≅ (3·sda)/b and LOQ ≅ (10·sda)/b, where, also in this case, sda is the standard deviation of the y-intercept and b is the slope of the regression line.

3. Experimental Results

VOCs produced by normal colonic mucosa and adenomatous polyp specimens were analyzed and monitored by HS- and DI-SPME-GC-MS procedures for seven days under ex vivo conditions. The analyses showed that both tissues of the same patient produced a similar VOC pattern but with different fingerprints. Figure 1 shows two typical HS-SPME-GC-MS chromatograms obtained analyzing the tissues of the same patient at time zero.

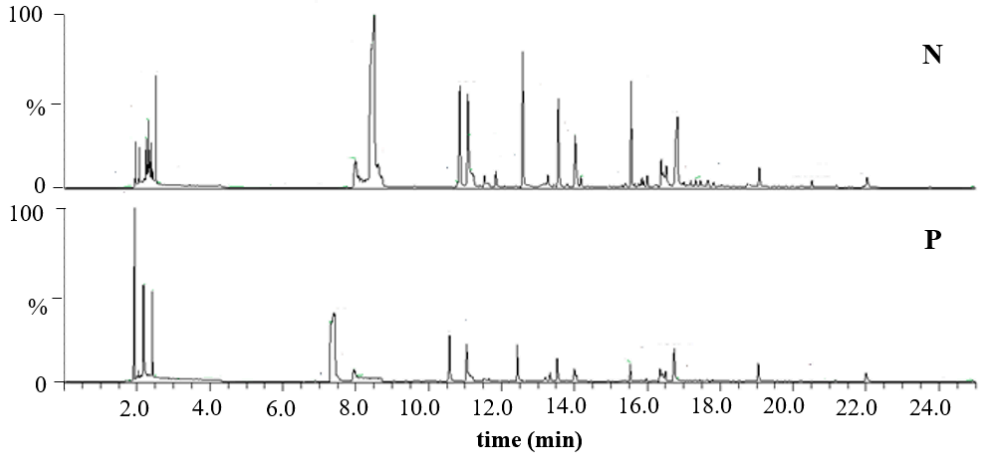

Figure 1. HS-SPME-GC-MS chromatograms of VOCs released by colon normal mucosa (N) and adenomatous polyp (P) tissues from the same patient (n° 2) at time zero.

Thirty-two different VOCs were identified by inspecting the acquired chromatograms with those reported in the library of the NIST. The list of the compounds identified with their retention times (RT), the characteristic ions (m/z) used to recall the XIC, the numbers for each of them in the hit list (i.e., match factor and probability) created by NIST library for the identification of their chemical structures, the confirmation of identity by standards, and the frequency of appearance in the acquired HS- and DI-SPME-GC/MS chromatograms are reported in Table 2, which clearly shows that benzaldehyde, ethylbenzene, indole, methylbenzene, phenol, octanal, and oxime methoxy-phenyl are the most frequently detected compounds in chromatograms (Frequency > 50%). Benzaldehyde and oxime methoxy-phenyl were also found in chromatograms obtained from the culture medium (control) and their basal values were subtracted from those of tissues, indicating that all tissues exhale and/or metabolize these compounds.

Table 2. Lists of compounds, RT, characteristic ions (m/z), match factor, probability, standard identity confirmation, and frequency of appearance in the acquired HS- and DI-SPME-GC-MS chromatograms related to secreted compounds by normal colonic mucosa and adenomatous polypoid lesion.

#	RT (min)	Compound	Characteristic Ions (*m*/*z*)	Match	Prob. (%)	Standard Identity Confirmation	Frequency (%)	
							HS	DI
1	2.02 ± 0.04	Dimethyl chloroacetal	47	782	75.5		3.1	3.1
2	2.21 ± 0.09	Acetaldehyde oxime	14, 59	962	37.3		12.5	28.1
3	3.03 ± 0.02	2-Butanone,4-hydroxy	43, 61	674	39.1		3.1	0
4	3.30 ± 0.08	1-Butanol	41, 56	827	40.0		5.6	3.1
5	5.54 ± 0.05	Acetal	73, 103	849	77.0		12.5	6.3
6	6.06 ± 0.07	1-Butanol,3-methyl	41, 55	867	20.5		0	3.1
7	6.19 ± 0.09	Disulfide, dimethyl	45, 79	939	97.1		9.4	12.5
8	6.84 ± 0.07	Methylbenzene	91	979	60	yes	93.8	96.9
9	8.82 ± 0.08	Ethylbutanoate	43, 71	877	90.0		3.1	3.1
10	8.98 ± 0.07	Ethyl 2-methyl butanoate	57, 102	851	76.8		18.8	3.1
11	9.10 ± 0.08	Ethyl 3-methyl butanoate	88, 115	824	88.5		12.5	3.1
12	9.29 ± 0.09	Ethylbenzene	91, 106	954	71.6	yes	68.8	31.3
13	9.53 ± 0.10	xylene	91, 106	908	61.5	yes	46.3	3.1
14	10.07 ± 0.09	xylene	91, 106	788	30.2	yes	46.9	9.4

Table 2. *Cont.*

#	RT (min)	Compound	Characteristic Ions (*m*/*z*)	Match	Prob. (%)	Standard Identity Confirmation	Frequency (%)	
							HS	DI
15	10.22 ± 0.09	Pentanoic acid, ethylester	57, 101	812	89.2		0	3.1
16	10.71 ± 0.09	Oxime, methoxy-phenyl	133, 151	813	84.2		68.8	96.9
17	11.78 ± 0.09	Dimethyl trisulfide	79, 126	849	96.9		12.5.	6.3
18	11.93 ± 0,09	Benzaldehyde	77, 106	930	92	yes	18.8	65.6
19	12.12 ± 0.09	Phenol	66, 94	984	88	yes	31.3	62.5
20	12.29 ± 0.08	Octanal	43, 56	888	60	yes	9.4	59.4
21	12.52 ± 0.08	1-Hexanol, 2-ethyl-	57	829	13.0		21.9	31.3
22	12.74 ± 0.09	Isooctanol	55, 112	800	20.3		21.9	12.5
23	13.47 ± 0.09	Nonanol	57, 69	827	11.1	yes	25.0	25.0
24	14.05 ± 0.09	Nonanal	41			yes	3.1	3.1
25	15.11 ± 0.08	Octanoic acid	60, 144	781	30.9	yes	12.5	9.4
26	15.31 ± 0.08	Decanal	43, 138			yes	31.3	15.6
27	15.63 ± 0.08	Dodecane	43, 170	956	49	yes	25.0	9.4
28	16.11 ± 0.09	Benzenepropanol	117, 136	909	60.0		6.3	18.8
29	16.61 ± 0.09	Triethanolamine	118	900	67.0		0	6.3
30	16.86 ± 0.09	Undecano	57, 156	908	44	yes	21.9	3.1
31	17.07 ± 0.08	Indolo	117	982	70	yes	40.6	62.5
32	18.39 ± 0.09	Tetradecane	57, 198	934	53	yes	25.0	12.5

For benzaldehyde, ethylbenzene, indole, and methylbenzene, clear differences were found between healthy and adenomatous tissue of the same patient when evolution over time was followed, ex vivo, by SPME-GC-MS. Figure 2 shows the typical time-dependent monitoring (0, 1, 4, and 7 days) of the seven selected compounds by DI-SPME-GC-MS in tissue samples from one of the patients (n° 2).

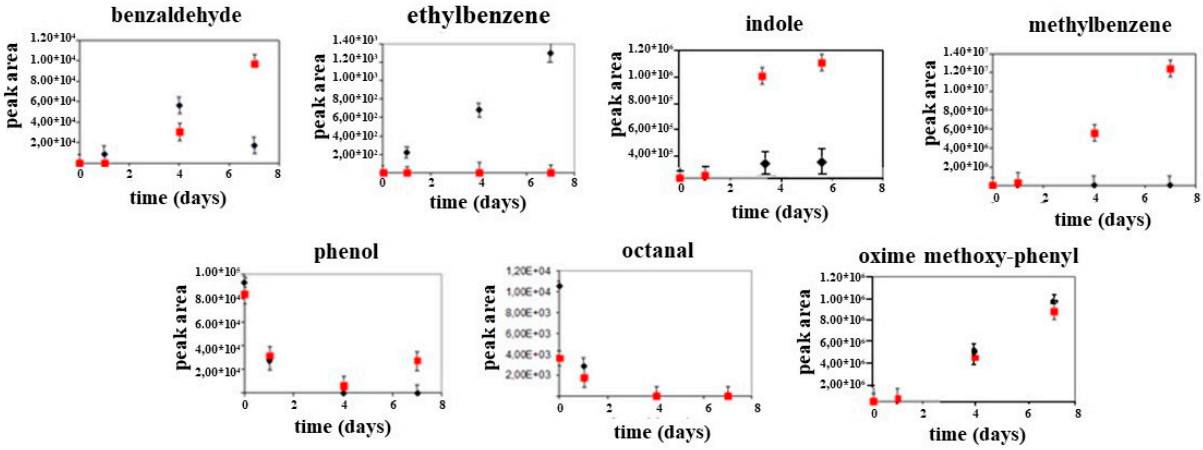

Figure 2. Time-dependent monitoring (0, 1, 4, and 7 days) peak area of benzaldehyde, ethylbenzene, indole, methylbenzene, phenol, octanal, and oxime methoxy-phenyl by DI-SPME-GC-MS in tissue samples (normal colonic mucosa and adenomatous polyp) from the same patient (n° 2), before undergoing curative surgery.

Comparative analysis of the kinetic profiles obtained showed that phenol and octanal were always present at time zero in both healthy tissues and polypoid lesions. In addition, for these two VOCs, a gradual reduction over time was constantly observed until their

disappearance in all samples was analyzed. Oxime methoxy-phenyl also appeared in control samples, and its levels increased over time in all the cases. Benzaldehyde, ethylbenzene, indole, and methylbenzene were observed, for all samples, only after 24 h of incubation. Their levels, in general, increased progressively over the seven days but were significantly higher in adenomatous tissue than in healthy mucosa, whereas the opposite happens for ethylbenzene.

Table 3 shows the linear regression analysis results and the concentration ranges, estimated in this study, for standardly identified VOCs (i.e., benzaldehyde, ethylbenzene, indole, methylbenzene, phenol, and octanal) in culture media containing tissues (normal colonic mucosa and adenomatous polyps). No standard was available for oxime methoxy-phenyl. The F-test results showed that only for the concentration levels of benzaldehyde, phenol, and octanal there was no statistically significant difference ($p < 0.05$) between healthy mucosal and adenomatous polypoid lesion tissues.

Table 3. Linear regression analysis and concentration ranges of VOCs in culture media with colon tissues (normal mucosa and adenomatous polypoid lesion) from seven selected patients.

Compounds	Equation	R^2	Normal Mucosa Range (mg/mL)	Adenomatous Polypoid Lesion Range (mg/mL)
Benzaldehyde	Y = 1912X + 456	0.9995	nd-1.43 ± 0.62	nd-1.60 ± 0.70
Ethylbenzene	Y = 2358X + 2998	0.9988	nd-1.52 ± 0.23	nd-0.96 ± 0.12
Indole	Y = 807X + 408	0.9995	nd-7.03 ± 1.05	nd-13.58 ± 1.12
Methylbenzene	Y = 17,243X + 1807	0.9990	nd-1.29 ± 0.16	nd-4.95 ± 0.11
Phenol	Y = 861X + 734	0.9902	nd-3.45 ± 0.05	nd-3.11 ± 0.04
Octanal	Y = 2090X + 597	0.9998	nd-0.78 ± 0.30	nd-0.27 ± 0.13

With reference to the exhaled breath, Figure 3 shows an example of the chromatogram of the same patient (n° 2; acquired in SCAN mode), before endoscopic treatment.

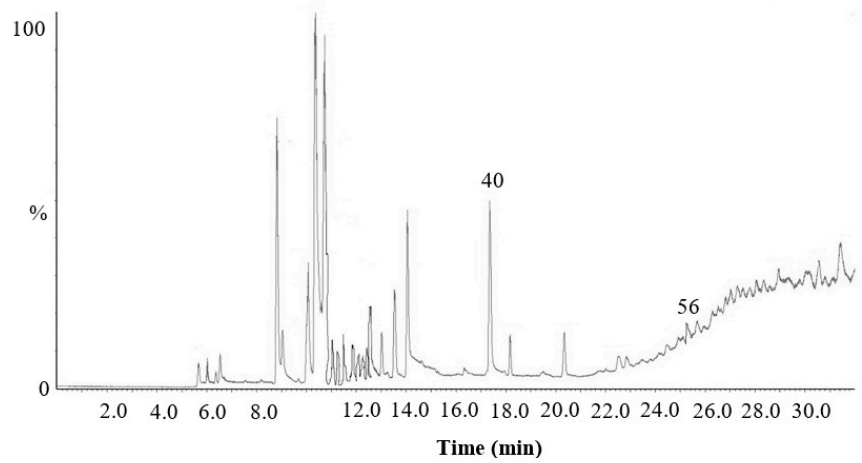

Figure 3. Chromatographic profile (SCAN mode) of the exhaled breath of patient (n° 2) affected by an adenomatous colonic polypoid lesion before endoscopic treatment.

Sixty-six VOCs were detected ($S/N \geq 3$) overall and sixty-one were identified and reported in Table 4. Benzaldehyde and methylbenzene, already evidenced in the colonic polyps and normal mucosa tissues, were also detected in the breaths of the same patients and, therefore, a possible correlation between their quantity in the breath and that released from tissues was hypothesized.

Table 4. Lists of compounds, RT, characteristic ions (m/z), match factor, probability, and standard identity confirmation detected (S/N $\geq$ 3) in the breath of the seven patients with adenomatous colonic polypoid lesion, before endoscopic treatment.

#	RT (min) [a]	Common Compound Name	Match (%)	Probability (%)	Standard Identity Confirmation [b]
1	5.71 ± 0.05	Carbon dioxide	891	90	
2	6.04 ± 0.02	Unidentified			
3	6.45 ± 0.06	2,4-Dimethyl pentane	930	91	
4	6.52 ± 0.07	Hexene	879	89	
5	6.66 ± 0.08	Sulfur dioxide	878	87	
6	6.78 ± 0.09	Difluoro methyl-silane	801	52	
7	6.82 ± 0.06	Trimethyl silylanol	773	55	
8	6.93 ± 0.06	Ethane, 1,2-diethoxy	801	61	
9	7.01 ± 0.03	1-Pentene-4-methyl	822	54	
10	7.20 ± 0.09	2-Propane	833	60	yes
11	7.61 ± 0.08	1,1,1,1-Trifluoro trimethyl-silylanol	828	56	
12	7.94 ± 0.05	Cyclobutanol	903	78	
13	8.37 ± 0.05	Trichloro-monofluoro-methane	822	57	
14	8.95 ± 0.06	1,3-Pentadiene	954	75	
15	9.12 ± 0.06	2-Propanol-1-methoxy	930	80	
16	9.77 ± 0.02	Unidentified			
17	10.11 ± 0.04	2-Pentene	915	85	
18	10.24 ± 0.05	2-Butanol-3-methyl	907	84	
19	10.31 ± 0.06	2-Methyl pentanal	839	58	
20	10.54 ± 0.05	Cyclopentane	903	88	
21	10.83 ± 0.05	2,3-Dimethyl pentane		66	
22	10.91 ± 0.03	Hexane	913	92	yes
23	11.00 ± 0.03	4-Methyl-2-pentyne	877		
24	11.44 ± 0.06	Acetonitrile	920	90	yes
25	11.52 ± 0.02	Unidentified			
26	11.63 ± 0.08	Benzene	938	89	yes
27	12.42 ± 0.05	Unidentified			
28	12.91 ± 0.05	1,3,5-Trifluoro benzene	852	57	
29	13.27 ± 0.03	Dichloromethane	931	93	yes
30	13.55 ± 0.06	Hexamethyl disiloxane	828	81	
31	13.82 ± 0.04	2-Butanone	948	96	yes
32	14.13 ± 0.07	Heptene	899	88	yes
33	14.33 ± 0.02	3-Hexanol	866	77	
34	14.96 ± 0.04	Acetic acid	915	67	
35	15.90 ± 0.05	2-Propanol-1-methoxy	838	52	
36	16.49 ± 0.03	1,4-Dioxane	828	51	

Table 4. *Cont.*

#	RT (min) [a]	Common Compound Name	Match (%)	Probability (%)	Standard Identity Confirmation [b]
37	16.61 ± 0.05	2-Pentanone	903	89	
38	16.70 ± 0.03	Butanoic acid	933	97	yes
39	17.42 ± 0.06	Cyclotrisiloxane hexamethyl	807	58	
40	17.51 ± 0.04	Methyl benzene	938	97	yes
41	18.27 ± 0.06	Octine	907	70	yes
42	18.40 ± 0.05	2-Hexanone	881	68	
43	19.66 ± 0.05	Hexanal			
44	19.80 ± 0.05	Methyl isobutyl ketone	902	76	
45	20.00 ± 0.07	Hexanoic acid, methyl ester	874	83	
46	20.16 ± 0.04	Nonane	934	54	yes
47	20.32 ± 0.08	Pentanoic acid, methyl ester	879	79	
48	20.53 ± 0.05	Pentanoic acid	809	54	yes
49	22.00 ± 0.07	Di(isobutyl)acetone	815	58	
50	22.49 ± 0.07	Hexanoic acid	879	79	yes
51	22.95 ± 0.04	3-Heptanone	918	82	yes
52	23.02 ± 0.06	Heptanoic acid, methyl ester	988	83	
53	23.55 ± 0.03	Eptane, 2,2,4,6,6-pentamethyl	888	55	
54	23.99 ± 0.06	Tetrasiloxane, decamethyl	848	51	
55	25.10 ± 0.03	Butanoic acid, dimethyl ester	855	74	
56	25.52 ± 0.05	Benzaldehyde	933	95	yes
57	25.93 ± 0.06	Octanoic acid, methyl ester	832	68	
58	26.26 ± 0.07	Decane	932	55	yes
59	26.88 ± 0.06	Benzoic acid, methyl ester	815	54	
60	27.54 ± 0.08	1-Decanol-2-esil	877	53	
61	28.33 ± 0.06	Dodecane	928	54	yes
62	29.00 ± 0.08	Unidentified			
63	29.65 ± 0.06	Silane, ethyl-dimethyl-phenyl	813	62	
64	29.83 ± 0.07	4-Phenyl benzofurane	822	56	
65	30.51 ± 0.06	Tri-tetra-contane	812	56	
66	31.53 ± 0.04	Pentacosane	811	58	

[a] Values expressed as mean (s.d.); [b] authenticated using the NIST library and standard injection.

Table 5 shows the concentration ranges, estimated in this study, for these two standardly identified VOCs.

Table 5. Concentration ranges of VOCs exhaled by seven selected patients affected by adenomatous colonic polypoid lesion, before curative surgery.

Compounds	pg*mL^{-1} in Exhaled Breath
Benzaldehyde	n.d.-LOD
Methylbenzene	>50

4. Discussion

In this study, seven volatile compounds, namely benzaldehyde, ethylbenzene, indole, methylbenzene, phenol, octanal, and oxime methoxy-phenyl were the most significant VOCs detected by SPME and CG-MS, for the different levels and kinetic profiles, in normal colonic mucosa and adenomatous polypoid lesion tissue of the same patient in ex vivo condition. Furthermore, benzaldehyde and methylbenzene, which are produced by both tissues, can also be detected in the expired breath of the same patients. High levels of methylbenzene (concentration range: >50 pg*mL^{-1}) and absence/traces of benzaldehyde (concentration range: n.d.-LOD) were found in the breath of patients affected by adenomatous polypoid lesions (see Table 4). On the other hand, ethylbenzene, indole, phenol, octanal, and oxime methoxy-phenyl were not found in the corresponding expired breath samples, suggesting that they do not cross the capillary–alveolar barrier to be exhaled or that different metabolic pathways involving the intestinal microbiota could contribute to their production.

Comparing these data with those reported in another paper by our group, and performed with the same methodology, on CRC specimens, some of the VOCs highlighted in this study, such as benzaldehyde, ethylbenzene, methylbenzene, and octanal were identified in ex vivo CRC tissue samples [15]. When the kinetic profiles of these compounds are compared with those of CRC patients [15], it is evident that benzaldehyde, ethylbenzene, and methylbenzene levels are different, whereas the octanal profile looks the same. In addition, differences were also found between the concentration ranges of these compounds in polyps compared with colonic adenocarcinoma. Specifically, ethylbenzene and octanal concentrations were lower in adenomatous polypoid lesions compared to CRC, whereas methylbenzene values were comparable.

Moreover, the kinetic analysis by DI-SPME has highlighted several differences between the VOCs produced by colonic polyps and CRC cancer tissues, probably correlated to differences in the metabolic pathways involved in tumor progression. A decrease in ethylbenzene production may be related to increased consumption or binding of this compound with protein or lipid [16]. In fact, as in the literature reported, ethylbenzene undergoes α- and ω-oxidation by microorganisms, whereas the octanal metabolism could be linked to oxidative stress [17]. Klemenz et al. [16] showed that aldehydes, under different conditions, were consumed and/or bound by cultured human adipose tissue cells. In the case of benzaldehyde, Zimmermann et al. [18] described different metabolic pathways, such as tryptophan metabolism, in which this compound could be involved. Thanks to intestinal microbiota, tryptophan produces indole and a lot of its metabolites [19] precursors or signal molecules for many biologically active substances that play an important role in the "gut-brain axis" [20].

Phenol was considered for the first time in this study and showed comparable levels between normal mucosa and adenomatous polypoid lesion tissues. Phenol and its derivate, in general, because of their peroxidative capacity, could result in hematotoxic and hepatotoxic effects, facilitating mutagenesis and carcinogenesis in humans and other living organisms. Phenol is also produced during the natural decomposition of organic materials [21].

Furthermore, Oxime methoxy-phenyl is a typical chemical compound in body odor [22].

Finally, a recent paper analyzing the VOC pattern exhaled by CRC patients suggests that low levels of ethylbenzene and tetradecane, coupled with high levels of methylbenzene, could be predictive of a CRC [15]. A similar VOC pattern was also found in patients affected by colonic polyps; therefore, it could be speculated that this breath analysis might play a role as a mass screening tool since high levels of methylbenzene with no or very low traces of benzaldehyde and tetradecane seem to suggest the presence of colon neoplasia (polyp or cancer).

5. Conclusions

In this paper, an SPME-GC-MS-optimized protocol was advantageously employed to monitor ex vivo changes in endogenous VOCs produced by surgically resected colonic tissues (normal colonic mucosa and adenomatous polyp) from seven patients undergoing operative colonoscopy, to identify their secreted molecules.

To find a possible correlation between the pattern of VOCs exhaled by a patient affected by a polypoid lesion and the substances released by its colonic tissues, the breath of each patient was sampled by the ReCIVA® breath sampler, shortly before surgery, and analyzed by GC-MS.

Methylbenzene and benzaldehyde levels in the breath seemed to be connected to the presence of adenomatous colonic polypoid lesions.

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References

1. Zambonin, C.; Aresta, A. Review: Recent applications of solid phase microextraction coupled to liquid chromatography. *Separations* **2021**, *8*, 34. [CrossRef]
2. Boiko, B. Solid-phase microextraction: A fit-for-purpose technique in biomedical analysis. *Anal. Bioanal. Chem.* **2022**, *414*, 7005–7013.
3. Aresta, A.; Cotugno, P.; De Vietro, N.; Massari, F.; Zambonin, C. Determination of polyphenols and vitamins in wine-making by-products by supercritical fluid extraction (SFE). *Anal. Lett.* **2020**, *53*, 2585–2595. [CrossRef]
4. Aresta, A.; Di Grumo, F.; Zambonin, C. Determination of major isoflavones in soy drinks by solid-phase micro extraction coupled to liquid chromatography. *Food Anal. Methods* **2016**, *9*, 925–933. [CrossRef]
5. De Vietro, N.; Aresta, A.; Rotelli, M.T.; Zambonin, C.; Lippolis, C.; Picciariello, A.; Altomare, D.F. Relationship between cancer tissue derived and exhaled volatile organic compound from colorectal cancer patients. Preliminary results. *J. Pharm. Biomed. Anal.* **2020**, *180*, 113055–113065. [CrossRef] [PubMed]
6. Musteata, F.; Musteata, M.; Pawliszyn, J. Fast in vivo microextraction: A new tool for clinical analysis. *Clin. Chem.* **2006**, *52*, 708–715. [CrossRef] [PubMed]
7. Piri-Moghadam, H.; Ahmadi, F.; Gómez-Ríos, G.A.; Boyacı, E.; Reyes-Garcés, N.; Aghakhani, A.; Bojko, B.; Pawliszyn, J. Fast quantitation of target analytes in small volumes of complex samples by matrix-compatible solid-phase microextraction devices. *Angew. Chem. Int. Ed. Engl.* **2016**, *55*, 7510–7514. [CrossRef]
8. Jaroch, K.; Taczyńska, P.; Czechowska, M.; Bogusiewicz, J.; Łuczykowski, K.; Burlikowska, K.; Bojko, B. One extraction tool for in vitro-in vivo extrapolation? SPME-based metabolomics of in vitro 2D, 3D, and in vivo mouse melanoma models. *J. Pharm. Anal.* **2021**, *11*, 667–674. [CrossRef]
9. Arnold, M.; Sierra, M.S.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* **2017**, *66*, 683–691. [CrossRef]
10. Siegel, R.L.; Miller, K.D.; Goding Sauer, A.; Fedewa, S.A.; Butterly, L.F.; Anderson, J.C.; Cercek, A.; Smith, R.A.; Jemal, A. Colorectal cancer statistics. *Cancer J. Clin.* **2020**, *70*, 145–164. [CrossRef]
11. Armstrong, H.; Bording-Jorgensen, M.; Wine, E. Review: The multifaceted roles of diet, microbes, and metabolites in cancer. *Cancers* **2021**, *13*, 767. [CrossRef] [PubMed]

12. Aarons, C.B.; Shanmugan, S.; Bleier, J.I.S. Management of malignant colon polyps: Current status and controversies. *World J. Gastroenterol.* **2014**, *20*, 16178–16183. [CrossRef] [PubMed]
13. Altomare, D.F.; Porcelli, F.; Picciariello, A.; Pinto, M.; Di Lena, M.; Caputi Iambrenghi, O.; Ugenti, I.; Guglielmi, A.; Vincenti, L.; De Gennaro, G. The use of the PEN3 e-nose in the screening of colorectal cancer and polyps. *Tech. Coloproctol.* **2016**, *20*, 405–409. [CrossRef] [PubMed]
14. Altomare, D.F.; Picciariello, A.; Rotelli, M.T.; De Fazio, M.; Aresta, A.M.; Zambonin, C.G.; Vincenti, L.; Trerotoli, P.; De Vietro, N. Chemical signature of colorectal cancer: Case–control study for profiling the breath print. *BJS Open* **2020**, *4*, 1189–1199. [CrossRef] [PubMed]
15. De Vietro, N.; Aresta, A.M.; Picciariello, A.; Rotelli, M.T.; Zambonin, C. Determination of vocs in surgical resected tissues from colorectal cancer patients by solid phase microextraction coupled to gas chromatography–mass spectrometry. *Appl. Sci.* **2021**, *11*, 6910. [CrossRef]
16. Klemenz, A.C.; Meyer, J.; Ekat, K.; Bartels, J.; Traxler, S.; Schubert, J.K.; Kamp, G.; Miekisch, W.; Peters, K. Differences in the emission of volatile organic compounds (vocs) between non-differentiating and adipogenically differentiating mesenchymal stromal/stem cells from human adipose tissue. *Cells* **2019**, *8*, 697. [CrossRef]
17. Berthe-Corti, L.; Fetzner, S. Bacterial metabolism of n-alkanes and ammonia under oxic, suboxic and anoxic conditions. *Acta Biotechnol.* **2002**, *22*, 299–336. [CrossRef]
18. Zimmermann, D.; Hartmann, M.; Moyer, M.P.; Nolte, J.; Baumbach, J.I. Determination of volatile products of human colon cell line metabolism by GC/MS analysis. *Metabolomics* **2007**, *3*, 13–17. [CrossRef]
19. Zhang, Q.F.; Xiao, H.M.; Zhan, J.T.; Yuan, B.F.; Feng, Y.Q. Simultaneous determination of indole metabolites of tryptophan in rat feces by chemical labeling assisted liquid chromatography-tandem mass spectrometry. *Chin. Chem. Lett.* **2022**, *33*, 4746–4749. [CrossRef]
20. Morais, L.H.; Schreiber, H.L.T.; Mazmanian, S.K. The gut microbiota–brain axis in behaviour and brain disorders. *Nat. Rev. Microbiol.* **2021**, *19*, 241–255. [CrossRef]
21. Michałowicz, J.; Duda, W. Phenols—Sources and Toxicity. *Pol. J. Environ. Stud.* **2007**, *16*, 347–362.
22. Jh, S.K.; Hayashi, K. Molecular structural discrimination of chemical compounds in body odor using their GC–MS chromatogram and clustering methods. *Int. J. Mass Spectrom.* **2017**, *423*, 1–14. [CrossRef]