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Article

Characterization of the Volatilome of *Tuber canaliculatum* Harvested in Quebec, Canada

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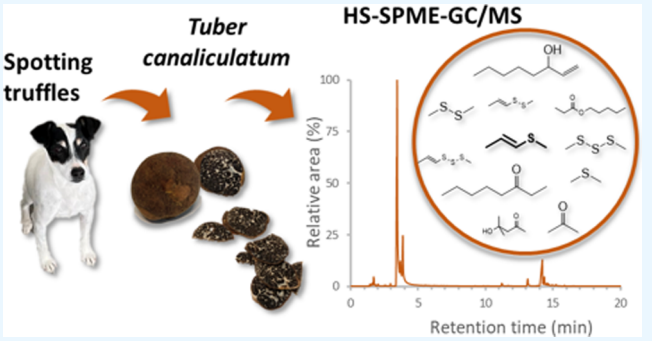
ABSTRACT: The first detailed characterization of volatile compounds from *Tuber canaliculatum*, a truffle newly grown in Quebec, Canada, was performed with headspace solid phase microextraction (HS-SPME) coupled with gas chromatography−mass spectrometry (GC/MS). A total of 30 compounds were identified, making up more than 98% of the volatile extract. The volatilome of *T. canaliculatum* is dominated by (*E*)-1-methylthio-1-propene, (*Z*)-1-methylthio-1-propene, dimethyl disulfide, and 1-octen-3-ol. It also includes six compounds identified for the first time in truffles, namely, 4-hydroxy-4-methyl-2-pentanone, pentyl propanoate, (*Z*)-1-methyl-2-(prop-1-en-1-yl)disulfide, (*E*)-1-methyl-2-(prop-1-en-1-yl)disulfide, (*Z*)-1-methyl-3-(prop-1-en-1-yl)-trisulfide, and (*E*)-1-methyl-3-(prop-1-en-1-yl)trisulfide. With the growing interest in gastronomy in truffles in North America, it is becoming important to gather knowledge for identification purposes and to delineate the key volatile compounds responsible for the aroma of North American truffles, especially the newly harvested *T. canaliculatum*.



INTRODUCTION

Truffles (*Tuber* sp.) are a type of fungus appreciated worldwide for their complex and unique aroma. Their limited seasonal availability, short shelf life, undersupplied market, and, most importantly, unique gastronomic qualities rank them amongst the most expensive luxury food products.

Truffles are underground ectomycorrhizal fungi that develop in symbiosis with the roots of certain tree species and microorganisms. The cultivation of truffles requires a significant investment of time, money, and technical expertize.[1] Obtaining the first truffles can take more than 10 years from the time of initial planting.

More than 180 species of truffles have been identified worldwide, yet only about 20 are commercialized.[2] Fraud and misidentification are widespread. For example, *Tuber indicum* is very similar to *T. melanosporum* in its macroscopic characteristics but is of much less commercial value due to its very faint aroma.[3] However, *T. indicum* is sometimes described under the name of *T. melanosporum* in China and therefore sold at higher prices.[4] Each species of truffle has its own unique and distinctive aroma; therefore, analysis of their volatilome is a powerful tool to distinguish different species.

Appalachian truffle *T. canaliculatum* Gilkey, also called the Michigan truffle, belongs to the *T. macrosporum* clade[5] and has attracted increasing interest in North America in the last decade. New advances in truffle cultivation in Quebec have made it possible to cultivate the *T. canaliculatum* Gilkey species.[6] This truffle is common in the eastern region of the United States and Canada.[7] Its culinary values have been recognized by several chefs, and it is a truffle of significant commercial interest.[8−11] The aroma has been described as strongly pungent but pleasing.[8] *T. canaliculatum* grows in association with a wide range of host trees but particularly members of the Pinaceae and Fagacea.[7] Its peridium has a brown-to-cinnamon-colored, warty surface. The spores are dark brown, ellipsoid, and covered with alveoli. The gleba is firm, dark brown with white meandering veins at maturity.[8,11,12] The overall morphology of *T. canaliculatum*, including the peridium and gleba, is shown in Figure 3. Despite its growing fame, no scientific study has focused on the molecular composition of its aroma.

Various methods have been used to analyze the olfactory profile of truffles. The most widely used sampling method is headspace solid phase microextraction (HS-SPME).[13] It offers many advantages over other traditional extraction methods. It is simple and solvent-free, requires small quantities of the sample,[13] is particularly fast and sensitive, and provides linear results for a wide concentration of an analyte.[13−15] In the case

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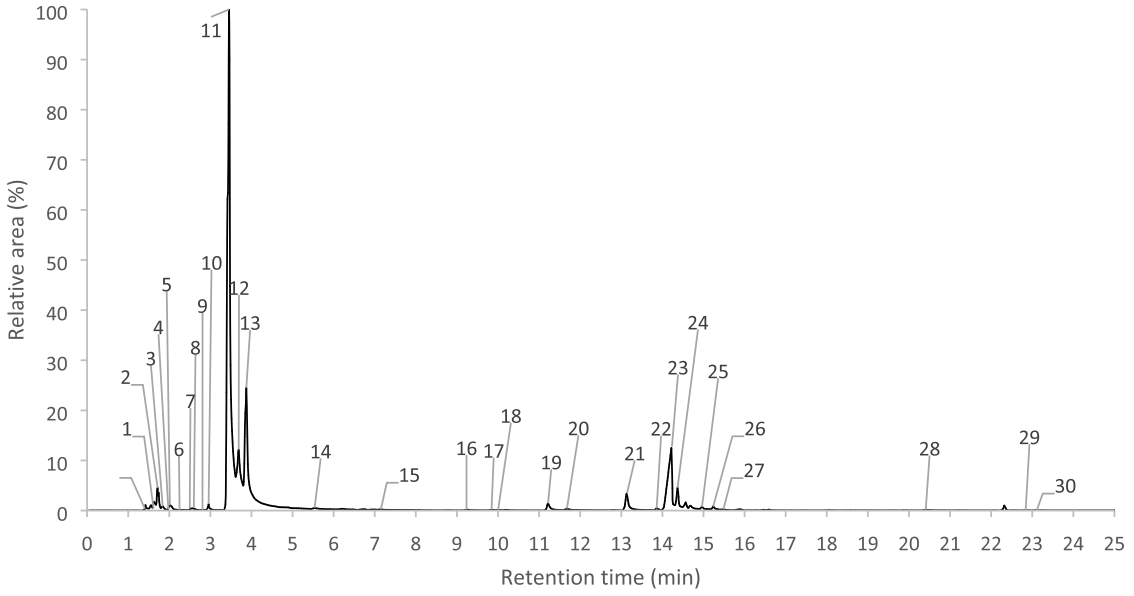

Figure 1. HS-SPME−GC/MS chromatogram of a fresh *T. canaliculatum* truffle (sample II) from St-Denis-de-Brompton, Québec, Canada. The labels on the signals correspond to the compound number in Table 1.

of truffles, it is particularly useful considering their short shelf life,[16] the difficulty of supply, and their high cost, which therefore generally requires a rapid analysis of a small quantity of material. It is often combined with gas chromatography−mass spectrometry (GC/MS) for the separation and identification of the analyte.

Herein, we describe the first detailed characterization of the volatilome of fresh *T. canaliculatum* harvested in Quebec, Canada, by HS-SPME coupled with GC/MS.

RESULTS AND DISCUSSION

The very first characterization of the volatilome of fresh specimens of *T. canaliculatum* has been performed. Volatile compounds were extracted by HS-SPME and analyzed by GC/MS using conditions adapted from those reported for the characterization of other truffle species.[17] Macroscopic evaluation and genomic analysis confirmed the identification of *Tuber canaliculatum* truffle. A typical GC/MS chromatogram for the volatilome of *T. canaliculatum* is shown in Figure 1, and peak assignments are presented in Table 1. Notably, samples I, II, and III of *T. canaliculatum* led to very similar chromatograms (Figures S2 and S3 in the Supporting Information).

Thirty compounds that were identified in the chromatograms are shown in Table 1. Results are expressed as the relative percentage obtained from the peak area and represent the mean and standard deviation of three replicates. Our results for each of the three truffles illustrate the variability from one specimen to another. The compounds we identified represent between 97.9 and 98.5% of the extracts (in relative proportions to the total integrated peak areas).Major and novel compounds are illustrated in Figure 2.

The aromatic profile of *T. canaliculatum* is dominated by (*E*)-1-methylthio-1-propene (57.2−59.0%) and its cis isomer, (*Z*)-1-methylthio-1-propene (8.5−10.2%). These compounds are said to have an acrid, strong, garliclike odor.[21] Their di- and trisulfide analogues were also identified, namely, (*Z*) and (*E*)-1-methyl-2-(prop-1-en-1-yl)disulfide and (*Z*) and (*E*)-1-methyl-3-(prop-1-en-1-yl)trisulfide. Overall, the three specimens presented very similar volatilome chemical compositions. Identification of the major compound (*E*)-1-methylthio-1-propene was carried out from the mass spectrum (Figure S4 in the Supporting Information) and by comparing its LRI with values from the literature.[20,22,23] Indeed, the LRI reported in the NIST database was slightly different.

Several typical volatile eight-carbon compounds have been identified, namely, 1-octen-3-ol (7.8−17.2%), octan-3-one (1.2−2.0%), 3-octanol (0.3−0.5%), octanal (0.2−0.5%), and 1-octen-3-one (0.1−0.2%). These compounds are typically responsible for the so-called fungal smell. They are ubiquitous in fungi of all species and would have key roles in biological processes, from mushroom initiation to response to pathogens and biotic interactions.[24,25] A high variability of the 1-octen-3-ol concentration was observed in the three specimens of *T. canaliculatum* studied. Indeed, it was more than twice as large in specimen II as in the other two. This variability in four- and eight-carbon volatile compounds (2-butanone and 2-butanol, 1-octen-3-one, 1-octen-3-ol, and trans-2-octenal) is known for *T. aestivum*, and these variations could be attributed to genetic variations.[26,27]

Several other sulfur compounds have been identified in *T. canaliculatum*, including dimethyl sulfide (1.2−1.7%), dimethyl disulfide (5.8−12.4%), and dimethyl trisulfide (0.8−2.0%). Significant variability in the relative concentration of these compounds has been observed from one specimen to another. Dimethyl sulfide and dimethyl disulfide have been described as having a cabbagelike, rotten smell and dimethyl trisulfide as having a rotten seaweedlike odor.[28] Despite their questionable description, these compounds constitute an important contribution to the aroma of various fruits and cooked vegetables[29] and they are found in many truffles species.[13] Furthermore, the analysis of altered and older specimens showed a greater proportion of these compounds, which gave them a less pleasant odor than the fresh specimens (data not shown). 2,4-Dithiapentane has been found in small amounts (0.1%) in *T. canaliculatum*. This compound is considered a key compound responsible for the unique aroma of *T. magnatum*.[30−33] It has also been identified in traces amount

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Table 1. Volatile Compounds Identified in the Three *T. canaliculatum* Specimens

no.	LRI exp[a]	LRI ref	compound name	cas no.	identification	relative proportion [% ± SD][b] I			II			III		
1		486[c]	acetone[e]	67-64-1	MS	1.3	±	0.6	0.8	±	0.2	0.7	±	0.2
2		520[c]	dimethyl sulfide[e]	75-18-3	MS	1.3	±	0.8	1.2	±	0.4	1.7	±	0.4
3		552[c]	2-methylpropanal[e]	78-84-2	MS	0.5	±	0.2	0.4	±	0.2	0.2	±	0.1
4	599	595[c]	2,3-butanedione	431-03-8	MS, LRI	0.1	±	0.1	0.1	±	0.0		tr	
5	603	598[c]	2-butanone	78-93-3	MS, LRI	0.5	±	0.2	0.7	±	0.2	0.5	±	0.1
6	624	625[c]	2-methyl-1-propanol	78-83-1	MS, LRI		tr			tr			tr	
7	649	652[c]	3-methylbutanal	590-86-3	MS, LRI	0.2	±	0.0	0.4	±	0.2	0.1	±	0.0
8	658	662[d]	2-methylbutanal	96-17-3	MS, LRI	0.2	±	0.0	0.3	±	0.1	0.1	±	0.1
9	680	674[c]	3-methyl-2-butanol	598-75-4	MS, LRI		tr			tr			tr	
10	699	698[c]	2,3-pentanedione	600-14-6	MS, LRI	0.1	±	0.0	0.1	±	0.1		tr	
11	717	720[f]	(*E*)-1-methylthio-1-propene	42848-06-6	MS, LRI	59.0	±	9.7	57.2	±	0.6	58.0	±	0.8
12	728	728[c]	(*Z*)-1-methylthio-1-propene	52195-40-1	MS, LRI	10.2	±	1.9	10.1	±	0.6	8.5	±	0.2
13	736	746[c]	dimethyl disulfide	624-92-0	MS, LRI	12.4	±	6.7	5.8	±	0.6	12.4	±	2.0
14	803	800[c]	hexanal	66-25-1	MS, LRI	0.1	±	0.0	0.2	±	0.1	0.2	±	0.0
15	839	838[c]	4-hydroxy-4-methyl-2-pentanone[g]	123-42-2	MS, LRI		tr			tr		0.1	±	0.0
16	885	889[c]	2,4-dithiapentane	1618-26-4	MS, LRI	0.1	±	0.0	0.1	±	0.0	0.1	±	0.0
17	898		2-nitropentane[e]	4609-89-6	MS	0.1	±	0.0	0.1	±	0.0	0.1	±	0.0
18	902	901[c]	heptanal	111-71-7	MS, LRI	0.1	±	0.0	0.1	±	0.0	0.1	±	0.0
19	925	932[c]	(*Z*)-1-methyl-2-(prop-1-en-1-yl) disulfide[g]	23838-18-8	MS, LRI	0.4	±	0.2	0.2	±	0.1	0.9	±	0.1
20	934	940[c]	(*E*)-1-methyl-2-(prop-1-en-1-yl) disulfide[g]	23838-19-9	MS, LRI	0.1	±	0.1	0.1	±	0.0	0.3	±	0.1
21	962	970[c]	dimethyl trisulfide	3658-80-8	MS, LRI	1.5	±	0.8	0.4	±	0.1	2.0	±	0.3
22	976	973[d]	1-octen-3-one	4312-99-6	MS, LRI	0.1	±	0.1	0.1	±	0.0	0.2	±	0.0
23	983	980[c]	1-octen-3-ol	3391-86-4	MS, LRI	7.8	±	0.8	17.2	±	1.9	9.4	±	2.4
24	986	986[d]	octan-3-one	106-68-3	MS, LRI	1.2	±	0.3	1.5	±	0.2	2.0	±	0.2
25	997	994[c]	3-octanol	589-98-0	MS, LRI	0.3	±	0.3	0.4	±	0.0	0.5	±	0.1
26	1002	1003[c]	octanal	124-13-0	MS, LRI	0.2	±	0.3	0.5	±	0.2	0.3	±	0.1
27	1007	1006[d]	pentyl propanoate[g]	624-54-4	MS, LRI	0.1	±	0.0	0.1	±	0.0	0.1	±	0.1
28	1103	1104[d]	nonanal	124-19-6	MS, LRI		tr		0.1	±	0.0	0.1	±	0.0
29	1152	1164[c]	(*Z*)-1-methyl-3-(prop-1-en-1-yl) trisulfide[g]	23838-24-6	MS, LRI		tr			tr			tr	
30	1157	1169[c]	(*E*)-1-methyl-3-(prop-1-en-1-yl) trisulfide[g]	23838-25-7	MS, LRI		tr			tr			tr	

[a]LRI shown in Table 1 for nonpolar column DB-5MS are obtained according to standards of *n*-alkanes (C6−C30). [b]Relative areas are represented by the average of triplicate runs for three specimens of truffles (I, II, and III). tr: Relative proportion is marked as a trace for values <0.1%. [c]LRI in NIST 14 database Mainlib and Replib.[18] [d]LRI in FFNSC 3 database.[19] [e]Tentative identification is made solely from mass spectrum evaluation. [f]Based on the value reported in ref 20. [g]First occurrence of the compound in truffles.

in several truffles including *T. sinensis*, *T. sinoalbidum*, and *T. sinoexcavatum*.[34] Because of its very high odor activity value, low cost, and low toxicity, it is often added to truffle oil and various flavored food products to artificially strengthen the truffle aroma.[35]

Several other volatile compounds that are common to several truffle species were identified in small amounts in *T. canaliculatum*: acetone, 2-methylpropanal, 2-butanone, 2-methyl-1-propanol, 3-methyl-butanal, 2-methyl-butanal, hexanal, heptanal, octanal, and nonanal.[13,17,36] Finally, we have also identified in *T. canaliculatum* 3-methyl-2-butanol, 2,3-butanedione, 2,3-pentanedione, and 2-nitropentane. 2-Nitropentane has so far only been identified in *Tuber rufum*.[21] 2- and 3-methylbutanal are known to have a malty smell and a high odor activity value.[37] They have an important role in the distinctive odor of *T. melanosporum*, in which they are found in significant proportion.[21,38] 2,3-Butanedione also has a high odor activity value and is known to have a buttery smell.[37,39]

On the other hand, 4-hydroxy-4-methyl-2-pentanone, pentyl propanoate, the respective di- and trisufide of the main compounds, (*Z*)-1-methyl-2-(prop-1-en-1-yl)disulfide, (*E*)-1-methyl-2-(prop-1-en-1-yl)disulfide, (*Z*)-1-methyl-3-(prop-1-en-1-yl)trisulfide, and (*E*)-1-methyl-3-(prop-1-en-1-yl)-trisulfide were identified from *T. canaliculatum*. All of these compounds were observed in small quantities in every sample. Interestingly, to the best of our knowledge, this is the first report of these compounds in a species of the *Tuber* genus.

Considering that 1-(methylthio)-1-propene is a compound present in significant proportions in *T. canaliculatum* as well as *T. macrosporum* and *T. excavatum*, comparing these species using identical conditions would allow us to highlight greater differences or similarities. For comparison, the key volatile compounds of *T. macrosporum* and *T. excavatum* are 1-(methylthio)-propane and 1-(methylthio)-1-propene.[21,36] The authors reported that these two *Tuber* species have similar volatile compounds but in different ratios. Interestingly, as a distinction, 1-methylthio-propane was not identified in *T. canaliculatum* and the proportion of 1-(methylthio)-1-propene was about twice as much in *T. canaliculatum* than what was reported for the two other species. Other compounds

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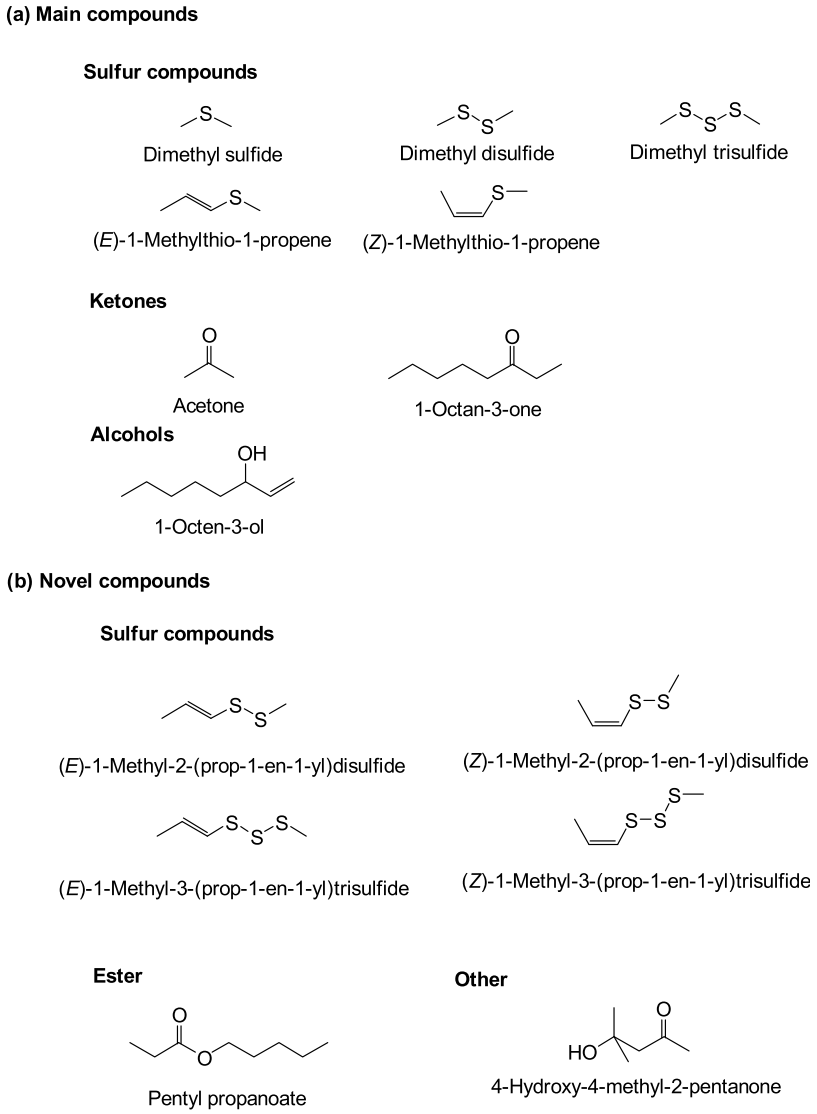

Figure 2. Compounds identified in *T. canaliculatum*. (a) Main volatile compounds and (b) compounds identified for the first time in truffle species.

identified in *T. canaliculatum* and *T. macrosporum* included dimethyl sulfide, 1-octen-3-ol, 3-octanone, 3-methyl-butanal, 2-butanone, dimethyl disulfide, and 2-methylpropanal. In contrast to compounds identified in *T. macrosporum* by Strojnik et al.,[21] no aromatic compounds were identified in *T. canaliculatum*. Also, the relative proportions of the compounds identified in the two truffles were very different. Even if some similarities could be highlighted between *T. macrosporum* and *T. canaliculatum*, our results showed that the latter has a volatilome that differs notably from what is currently known for other truffles. Indeed, it comprises an unusually high concentration of 1-(methylthio)-1-propene, a unique proportion of major volatile compounds, and the presence of six compounds not identified until now in truffles. Although it was not possible to assign an olfactive value or a culinary value from the volatilome composition, it was reasonable to presume that *T. canaliculatum* has a distinctive aroma associated with its unique chemical composition of volatile compounds.

The local sampling done for this study hints at the natural variability of the volatilome of *T. canaliculatum* truffles; despite the limited number of specimens, we still do not know much about how the volatilome of *T. canaliculatum* varies according to the geographical position of the samples or their maturity. As well, the olfactory profile of truffles has a direct dependence on the mode of preservation used,[40] and the preservation time of truffles varies from one species to another.[16] The study of the variation of the volatile molecules of a specimen as a function of the age and mode of preservation would make it possible to identify the key molecules involved in the degradation process of *T. canaliculatum* to establish the duration of its preservation and potentially improve the best preservation practices. Such studies have already been carried out on various species of truffles,[16,33,41−43] but this would be the first for *T. canaliculatum*.

Despite its advantages, extraction by HS-SPME also has its shortcomings, particularly that it is highly selective for the volatile compounds with high affinity with the fiber coating, which results in partial extraction of the olfactory profile.[13−15] In particular, HS-SPME is known to be more selective toward highly volatile compounds.[44] Other methods such as direct solvent extraction/solvent-assisted flavor evaporation (DSE-SAFE)[45] and simultaneous distillation-extraction (SDE)[44] require solvents, larger samples (20 g), and are more time-

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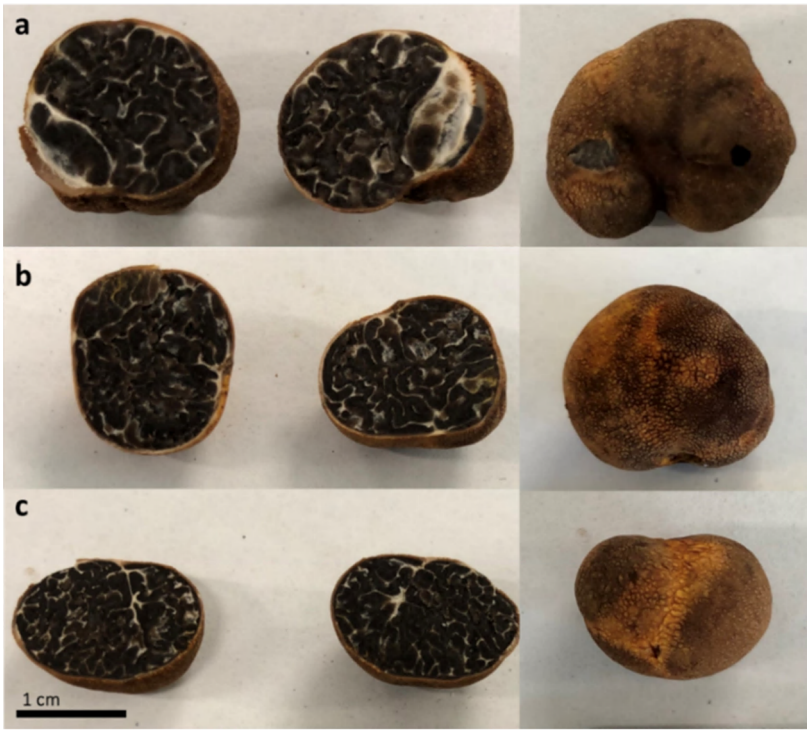

Figure 3. Specimens I (a), II (b), and III (c) of *T. canaliculatum* used in the present study showing gleba, peridium, and size.

consuming than HS-SPME. Nevertheless, they offer the advantage of extracting less volatile, higher-molecular-weight compounds. For this reason, they are considered complementary techniques and should be considered in further studies.

It is also worth noting that the relative proportions presented here were obtained by MS, which is not considered a good detection mode for semiquantitative analysis due to (1) its lack of repeatability over time, (2) the fact that the number of ions produced is specific to each analyte, and (3) the number of ions is often not linearly related to the concentration of the compound.[46,47] Therefore, these proportions are presented only to give an idea of the order of magnitude and cannot be considered as exact values.

Although this study gives a fingerprint of the volatilome of *T. canaliculatum*, it does not make it possible to determine the real contribution of each individual chemical to the human-sensed aroma. For that purpose, other detection methods will need to be used such as olfactometry[28,34,48] and electronic nose.[33,39,45] These methods are particularly useful to identify the distinctive smell and contribution of each molecule to the overall aroma of the truffle.

Nonetheless, the detailed characterization of the volatilome of *T. canaliculatum* highlights the main volatile compounds responsible for its characteristic odor. The diversity of truffles in Quebec is still little known. A study on micromammal stools in Quebec has identified at least 12 species of truffles by DNA analysis, including species unknown and/or never listed in the territory.[49] These results suggest that there are many more species yet to be discovered. The present studies will be very useful in comparing the volatilome of other species and demonstrating the richness of fungal diversity in the Quebec region. With the growing interest in truffles worldwide, the current results are significant both scientifically and economically.

EXPERIMENTAL SECTION

Samples. *T. canaliculatum* specimens were harvested on October 7, 2021 at Arborinnov truffle farm, in St-Denis-de-Brompton, Québec, Canada (N 45°26′49″ W 72°2′54″; altitude 270 m). The truffles were found with the help of a Jack Russel dog at a location near an American hazel, *Corylus americana*, and a northern red oak, *Quercus rubra* (Figure S1 in the Supporting Information). Specimens are shown in Figure 3. Freshly found truffles were placed in a paper bag in a cooler filled with ice packs covered with cardboard to avoid direct contact with the ice. The specimens were then stored in a refrigerator at 8 °C and analyzed within 24 h. Three mature specimens of 1.8, 2.9, and 3.7 g, free from alteration, were used. The three truffles (named specimens I to III) are illustrated in Figure 3.

Identification of Truffles. Identification of specimens was done by macroscopic evaluation by Mr. Jérôme Quirion, co-owner of Arborinnov, and by genomic analysis.

DNA Extraction and PCR Amplification. DNA was extracted from 100 mg (wet weight) of each truffle's sample using the DNeasy plant mini kit (Qiagen, Mississauga, Ontario). The internal transcribed spacer (ITS) of the nuclear ribosomal DNA (rDNA) was amplified using universal primers ITS-1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′)[50] and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′).[51]

Polymerase chain reaction (PCR) was performed to amplify DNA. A quantity of 0.1 ng of DNA was added to a mix containing 20 mmol·L^{-1} Tris−HCL, 50 mmol·L^{-1} KCl, 1.5 mmol·L^{-1} $MgCl_2$, 0.2 mM dNTP, 0.1 mg·mL^{-1} BSA (Roche, Basel, Switzerland), 0.2 μmol·L^{-1} each primer (Sigma-Aldrich, St-Louis, Missouri), and 0.025 U·L^{-1} Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, California) for a total reaction volume of 20 μL. PCR amplifications were performed with a PTS-225 thermocycler (MJ Research, Waltham, Massachusetts) under the following cycling parameters: initial

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denaturation at 95 °C for 2.5 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 55 s, and extension at 72 °C for either 45 s (cycles 1−13), 2 min (cycles 14−26), or 3 min (cycles 27−30), and a final extension at 72 °C for 10 min. PCR products were visualized by gel electrophoresis with 1.5% agarose stained with ethidium bromide. We included a negative control in each PCR batch to confirm the absence of contamination.

ITS DNA Sequencing and Analyses. Sanger sequencing was performed on a 3500 Genetic Analyzer (ThermoFisher) at the Plateforme d'Analyses Génomiques of the Institut de Biologie Intégrative et des Systèmes (PAG-IBIS, Université Laval, Québec, Canada). Sequences were submitted to a BLASTN search[52] against GenBank, and alignment results with ≥99% homology were considered a match for species identification. Alignments with <99% allowed the identification of genus or family groups. The unique sequences generated and used in this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/nuccore/OM948973).

Headspace Solid Phase Microextraction (HS-SPME). Analyses were performed using conditions adapted from Díaz et al.[17] SPME fibers coated with a layer of 50/30 μm divinylbenzene/carboxen/poly(dimethylsiloxane) (DVB/CAR/PDMS) (Supelco) were used for the analysis. Immediately before the analyses, the specimens were brushed gently and washed with demineralized water. Then, ∼0.5 g of fresh truffles was finely cut using a sharp knife and introduced into a 20 mL SPME vial sealed with a PTFE (silicon/polytetrafluoroethylene) septum cap. The SPME fiber was conditioned before analysis according to the supplier's instructions. The vial was immersed in a water bath at 53 °C for 5 min. Once equilibrium was reached, the fiber was then introduced to the vial and exposed to the headspace for 13.6 min. Each truffle was subjected to three analyses to consider potential instrumental variability.

Volatilome Analysis by Gas Chromatography/Mass Spectrometry (GC/MS). Sample volatile compounds were identified using a Thermo Scientific GC/MS (Trace GC Ultra with DSQ II detector). The analyses were carried out using a nonpolar phase column (DB-5MS 30 m × 0.25 mm × 0.25 μm). The carrier gas was helium, at a flow rate of 1.0 mL·min^{-1}, in constant flow mode. Thermal desorption of the compounds was carried out in the GC injector at 230 °C for 5 min in splitless mode. The temperature program is set as follows: 40 °C for 5 min, then increasing at 3 °C·min^{-1} to 140 °C, then to 220 °C at 12 °C·min^{-1}, and holding at this final temperature for 5 min. The mass range was 40−450 Da. The ionization energy was 70 eV. Thermo Scientific software Excalibur was used for instrument control and acquisition, and Thermo Scientific QualBrowser was used for processing. Identification of volatile compounds was carried out by comparing their mass spectra and linear retention indices to GC/MS commercial spectral libraries (FFNSC 3 (Wiley)[19] and NIST 14[18]). The alkane standard for the linear retention index determination was prepared from a C7-C30 saturated alkane solution (Millipore Sigma, 49451-U). For the C6 standard for retention time determination, HPLC-grade hexanes were used. Linear retention indices were calculated using the equation from van Den Dool and Kratz.[53] The relative area of each compound in the total extracts is expressed as the percentage from the total chromatogram integration recorded and was calculated with the data obtained from GC/MS analyses and manual peak integration without correction factors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c02877.

Figure S1, harvesting site of Arborinnov including *Corylus americana* and *Quercus rubra*; Figures S2 and S3, GC/MS chromatograms of specimens I and III of *Tuber canaliculatum*; and Figure S4, mass spectrum of (*E*)-1-methylthio-1-propene (PDF)

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Notes

The authors declare no competing financial interest.

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