

Characterization of Volatile Compounds of Indian Cress Absolute by GC-Olfactometry/VIDEO-Sniff and Comprehensive Two-Dimensional Gas Chromatography

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Indian cress (*Tropaeolum majus* L.) has been consumed in salad or soup for decades, but its odor's detailed molecular composition is still unknown. Here we report on the study of the odorant profile and odor-impact compounds of Indian cress absolute by GC-olfactometry/vocabulary–intensity–duration of elementary odors by sniffing (GC-O/VIDEO-Sniff) on an eight-way multiport system, combined with GC×GC-MS analyses for the identification of odorant trace constituents. Odor impact compounds of Indian cress absolute were determined by GC-O, and the overall influence of sulfury and fruity notes stood out. Forty-four odorant compounds were identified among which 22 (50% of the identified odorant molecules) were identified by using comprehensive two-dimensional GC coupled to a time-of-flight-mass spectrometer (TOFMS). These trace compounds were not detected by 1D-qMS or could only be found with specific searches once they were detected by comprehensive 2D-GC, although they were well perceived by the judges in GC-O. This was amongst others the case for the two molecules having the highest odor impact, (*E*)-hex-2-enal (fruity) and diethyl trisulfide (alliaceous, sulfury, cabbage). A powerful sulfur-containing odor compound whose first identification in cress was recently reported by the authors was detected by GC-O: *O,S*-diethyl thiocarbonate (fruity/red fruit and sulfury odor).

KEYWORDS: GC-olfactometry; VIDEO-Sniff; 8W-GC-O; comprehensive two-dimensional GC; cress

INTRODUCTION

In GC-olfactometry (GC-O), the human nose serves as detector, generally parallel to a physical detector. So-called “odorant zones” in a chromatogram are thus rapidly determined (1). Information on the olfactory impact of compounds in a sample can be gained by applying GC-O methodologies. These methodologies are generally divided into three groups and are widely discussed in the literature: detection frequency methods, direct time-intensity methods, and dilution to threshold methods (1). The French National Agronomy Research Institute (INRA) has developed a hybrid method that combines intensity and detection frequency methods and also includes the vocabulary given by the judges in the data processing: the VIDEO-Sniff method (vocabulary-intensity-duration of elementary odors by sniffing). By sorting the vocabulary in olfactory classes, an aromagram is obtained where each olfactory signal is assigned to at least one odor class having a specific color. Hence, visual interpretation of the aromagram becomes possible (2). As a result of its hybrid character, this methodology combines the properties of detection frequency and intensity methods. For example, whereas intensity

methods are known to be precise, intense training for the panelists is required. Detection frequency, on the other hand, might be less precise than intensity methods, but is simpler and quicker. The VIDEO-Sniff method combines both and is hence more precise than, for example, a simple detection frequency. The intensity scale used (from 1 to 5) might be subject to discussion, since some analysts prefer larger scales up to 10, and others smaller scales from 1 to 3. However, a minimum of eight judges is required for VIDEO-Sniff, which can be time-consuming. To limit analysis time, the INRA has developed a fully computerized eight-way GC-O system (8W-GC-O) that allows for individual aromagrams of a panel of eight judges to be obtained simultaneously and in one analysis (Figure 1) (3, 4).

The identification of odorant trace compounds is crucial in most cases and can be difficult because of coelutions and also because these compounds can be present in quantities below the qMS system's detection limit. One possible solution for their identification is the use of comprehensive two-dimensional GC. GC×GC uses two columns with different (mostly orthogonal) stationary phases that are connected in series. This allows resolution of coelutions that occur in a conventional 1DGC system but also increases sensitivity. The combination of GC×GC with a TOFMS detector provides a powerful and

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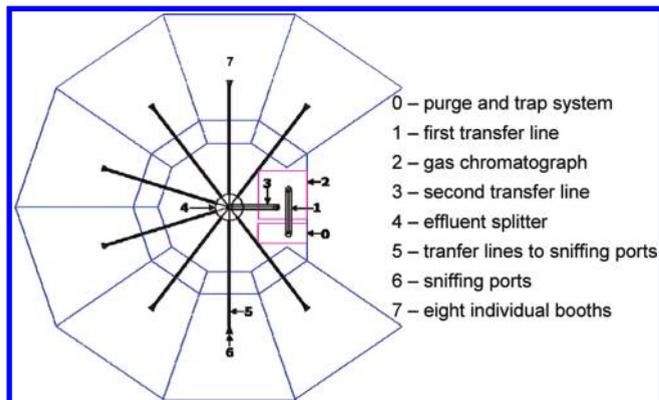


Figure 1. Architecture and overview of the 8W-GC-O device. The configuration presented is coupled to a system of extraction-concentration of volatile components of the purge-and-trap type. The setup consists of eight individual booths each with their own lighting, soundproofing, and laminar flow conditioned air (deodorized by filtering through active carbon and acclimatized at 22 °C). The air circulates from top to bottom so as to isolate the sniffing zone as much as possible from olfactory interference by ambient air or the sniffers' body odor. The diameter of the entire system is 4.5 m.

sensitive tool for the detection of trace compounds. The increase of both resolution and peak capacity leads to the detection of thousands of compounds in odorant matrices, and the identification of all constituents would be too time-consuming and not useful. The data should, in order to obtain pertinent outcome, be coupled to other information, such as GC-O results. Hence, the combination of GC-O with GC×GC, even when conducted on separate instruments, is a powerful tool for the identification of odorant trace compounds. Two-dimensional comprehensive GC and its use in flavor and fragrance analytics are extensively discussed in the literature (5–17).

Indian cress, *Tropaeolum majus* L., belongs to the botanical order *Brassicales* and to the *Tropaeolaceae* family (18). The plant (flowers, leaves, seeds) is edible and is also called *Nasturtium* as an allusion to its spicy flavor (in Latin: *nasum* = nose and *torquere* = to grimace) (19). Its absolute is obtained by preparation of the concrete by extraction of the plant material with a nonpolar solvent (hexane, petroleum ether), followed by dissolution of the concrete in ethanol, icing, and vacuum distillation of the alcohol (20). Its analysis has already led to the identification of two new families of odorant compounds, thiocarbamates and thiocarbonates (21, 22). However, solvent extracts such as absolutes also contain nonvolatile molecules. Further sample preparation is hence necessary prior to GC-olfactometry in order to avoid introduction of nonvolatile, inodorous matter into the GC system. Numerous sample preparation techniques are available and are extensively discussed in the literature (20, 23–27). Headspace techniques are particularly suitable for the study of odorant compound of a perfumery extract because they exclusively capture volatile molecules. Dynamic headspace (D-HS) was chosen as sampling method because it (1) imitates the actual conditions of the odorant perception of the oil by nasal inhalation at body temperature (sample and trap were kept at 40 °C) and (2) is a suitable method for the concentration of volatile compounds (20, 28–30). As on a multiport 8W-GC-O system the effluents are split between eight sniffers, a powerful enrichment step is needed in order to make sure that the sniffers perceive a sufficient amount of matter.

Here we report the study of the odorant profile and odor-impact compounds of Indian cress absolute headspace by GC-O/VIDEO-Sniff on a multiport system, combined with GC×GC-MS analyses for the identification of odorant trace constituents.

To date, to the authors' knowledge, there is no publication on a complete GC-O study of Indian cress.

MATERIALS AND METHODS

Chemicals and Extracts. *Chemicals.* All chemical reference compounds were either purchased from Sigma-Aldrich/Fluka (St. Quentin Fallavier, France) [butenone (Aldrich 269549), butanone (Aldrich W217018), (*E*)-pent-2-enal (Aldrich W321818), ethyl thiocyanate (Aldrich 308951), hexan-2-one (Fluka 02473), hexanal (Aldrich W255718), (*E*)-hex-2-enal (Aldrich W256005), (*Z*)-hex-3-enol (Aldrich W256307), 2-ethylthiophene (Aldrich E49207), heptan-2-one (Aldrich W254401), heptanal (Aldrich W254010), 2-propylthiophene (Aldrich P54345), (*E*)-hept-2-enal (Aldrich W316504), benzaldehyde (Aldrich W212717), oct-1-en-3-one (Aldrich W351504 50% w/w in oct-1-en-3-ol), oct-1-en-3-ol (Aldrich W280518), dimethyl trisulfide (Aldrich W327506), 6-methylhept-5-en-2-one (Aldrich W270733), 2-thiophenecarboxaldehyde (Aldrich T32409), 3-thiophenecarboxaldehyde (Aldrich 196282), ethyl hexanoate (Aldrich W243914), octanal (Aldrich W279714), (*Z*)-hex-3-enyl acetate (Aldrich W317101), (*E,E*)-hepta-2,4-dienal (Aldrich W316407), limonene (Aldrich W524905), benzyl alcohol (Aldrich W213705), 1,8-cineole (Aldrich W246506), 2-hydroxybenzaldehyde (Aldrich W300403), nonan-2-one (Aldrich), nonanal (Aldrich W278203), isophorone (Aldrich W355305), benzyl acetate (Aldrich W213500), 2-methoxy-3-isobutylpyrazine (Aldrich W313203), decanal (Aldrich W236209)] or Frutarom (Dijon, France) [diethyl trisulfide (E01289)] or synthesized in our facilities according to a previous publication [*O,S*-diethyl thiocarbonate (22)].

Botanical Source and Plant Material. Commercial Indian cress absolute oil was provided by Robertet S.A. (Grasse, France). It had been produced for Cavallier Frères, a subsidiary of Robertet, by Rolan Essential Oils in Rustenburg, South Africa, by extraction of the fresh plant with *n*-hexane followed by treatment with ethanol.

Analytical Studies. *8W-GC-O.* The 8W-GC-O device used is described in Berdagué *et al.* (2–4), and its architecture is shown in **Figure 1**.

Extraction, Concentration, and Injection. The volatile components of Indian cress absolute were extracted by D-HS (Tekmar, Cincinnati, OH). A 4.5 mg portion of absolute was placed on glass wool at the bottom on the glass frit of a Pyrex glass extraction cartridge (reference M3, Mallières Frères, Aubière, France). Extraction conditions were optimized as follows: the extractor was maintained at 40 °C and purged for 10 min with a helium stream at a flow rate of 10 mL/min (He/U purity of 99.995%, Messer, Puteaux, France). The trap (working length 180 mm, i. d. 1/4 in. (1 in. = 2.54 cm), packed with Tenax TA 60–80 mesh adsorbent (Supelco, Bellefonte, PA) was operated at 40 °C. The dry purge step was set at 3 min to eliminate as much water as possible while minimizing loss of the volatile fraction (31). The volatile components were then desorbed from the trap at 180 °C for 10 min using helium (He/N55 purity 99.9995%, Messer, Puteaux, France) and sent through the first transfer line (**Part 1, Figure 1**) into the cryo-focalization zone (cooled at –150 °C with liquid nitrogen). The introduction system is described in Berdagué's paper (2). Splitless injection was used by heating the cryo-concentration zone to 220 °C in 10 s.

Separation. Volatile components were separated in an Agilent 4890D chromatograph (Agilent, Massy, France) on an RTX-5 fused-silica capillary column (5% diphenyl-, 95% dimethylpolysiloxane, length 60 m × i.d. 0.53 mm × film thickness 1.50 μm; Restek, Evry, France). Temperature was programmed as follows: 5 min isothermal at 40 °C, 4 °C/min rise to 205 °C, and 5 min isothermal at 205 °C. Carrier gas: helium. Constant flow: 8 mL/min. A second transfer line heated to 210 °C (**Part 3, Figure 1**) connected the column to the effluent divider. Division and transfer to the eight sniffing ports was done according to the literature (2).

Identification of Odor-Active Compounds. An independent single GC-O-MS setup composed of an Agilent chromatograph 6890 (Agilent), a mass detector MSD5973 (Agilent), and a sniffing port were used according to the literature (2). The GC-O data from the multiport system were synchronized with those from the single port GC-O-MS setup by using the AcquiSniff software (32) and the mass spectrometry software MSDChem Agilent C.00.00 (2, 33). Volatile components were identified by computer matching against spectral databases (34–36), LRI (36, 37), and odors (homemade database, LCBMA, Université de Nice-Sophia

Table 1. Distribution of Vocabulary Items Used by the Eight Sniffers in the Nine Olfactory Classes Defined for Indian Cress Absolute

olfactory class (class color)	vocabulary items
balsamic-spicy-pharmaceutical (mint)	almond bitter, almonds, benzaldehyde, almond marzipan, marzipan, anise, annica, carrot, chlorine, clove, chlorophylle-minty, minty, mint, menthol, coriander, spicy-green, marzipancake, licorice, pharmaceutical, cinnamon apple
empyreumatic (black)	broth, burnt, peanut, roasted peanut, rancid peanut, cacao, coffee, hot chocolate, torrefied coffee, caramel, caramelized pop-corn, chocolate, black chocolate, leather, leather-animal, empyreumatic, roasted cereals, grilled, roasted, roasted chestnut, toasted, toasted bread, fire, smoked, hot oil, hot sunflower oil, malt, breadcrumb, nut, grilled hazelnut, hazelnut, hot bread, phenolic, pyrazine, hot, cooked meat
fruity-floral (pink)	citrus fruit, grapefruit, pineapple candy, chewing-gum, strawberry candy, strawberry, fruit candy, citrus, citrus-orange, citrusy, quince, orange peel, passion fruit, orange, fruity, fruity-alcohol, fruity-glue, fruity-floral, red fruit, fruity-plastic, apple, fruity-sugary-honey, Mirabelle, cut grass-fruity and hexanal, pear, green apple, fruity garbage, prune, sugary pear, terpeny-limonene, strawberry yogurt, alcoholic, perfume, soap, cosmetics, hand cream, cologne water, flower, rose, flowery, gorse, floral, floral-bitter, marguerite, floral artificial candy, floral-chemical, lily, flora-sugary, tulip, poppy seed, ground poppy seed, gentian, violet
lactic-cheesy (yellow)	butter, melted butter, buttery, lactic-butter, dirty socks, fermented, cheese, bad cream, feet, rancid, roasted butter, lactic, vomit, rotten, rancid, wet mop, acerb, musty, moldy, clabber, St. Nectaire cheese, vanilla, vanilla cream, yogurt
plastic-chemical-solvent (light gray)	Acid, alcohol, alcoholic, rubber, burnt rubber, chemical, chemical-acerb, pungent, chemical-burnt, solvent, chemical-floral, chemical-smoked, chemical-sulphury, chemical-mains gas, chemical-cement, modeling clay, chemical-orange, chemical-plastic, chemical-resinous, chlorine, chlorine-plastic, glue, paper glue, varnish, dish water, gasoline, alcoholic gasoline, iodine-salty and chemical, liquor, plastic, burnt plastic, plastic mushroom, hot plastic, plastic-chemical, new plastic, plastic-solvent, solvent chemical, ether, wine, red wine, vinegar
sulfury (orange)	garlic, garlic cheese, garlicky, garlic-rotten, alliaceous, animal-sulphury, green cheese, mushroom-ail, cabbage, rotten cabbage, cabbage-sulphury, cauliflower, broccoli, cheesy-garlic, smoked-sulphury, mains gas, moss-sulphury, pungent, mustard, sulphury, rotten-sulphury, sulfur, sulfur rubber, sulphury rubber boots, sulphury-fruity, sulphury-feet, sulphury-potato, sulphury-rotten and faded flower, sulphury then orange, old cheese, rotten-sulphury wood, dry wood, wood wax, wood mushroom, woody, cut dry wood, moldy cellar, mushroom, moss, mushroom and gorse, bad mushroom, mushroom metallic, mushroom moss wood, rotten mushroom, earthy mushroom, pencil, chanterelle, metallic, copper oxide, moldy, rotten, moss cork, undergrowth, nut-earthy, composting vegetables, rotten-earthy, dusty, aldehydes, nonanal, rancid and faded flower, earth, black earth, compost earth, mud, potting soil, earthy, earthy-roasted potato, earthy-rotten, green- mushroom, smashed insect, bug
green-vegetable (green)	bitter, green, chrysanthemum, oat, wood-grass, salad, green wood, woody-green, grilled cereals and baked potato, potato, geranium, green beans, herbaceous, herbaceous-peppery, cut grass, smashed grass, cooked vegetable, olive tree, perfume chrysanthemum-vegetiver, perfume vegetable green bitter, peas, peppery, potato, baked potato, roasted potato-amine, cooked white rice, soup, vegetable soup, earthy potato, vegetable, bitter, dry grass, pungent vegetable, green vegetable, hay, green-bitter, vegetable, green gorse, green-vegetable marguerite chrysanthemum, fresh green, green geranium cress absolute, cress absolute earthy, green nut, vegetable fresh, sunchoke
not classified (white)	?, pleasant, amine-urine, ammoniac, animal, hairy beast, buttery-fruity, hot, horse, chocolate orange, cooked, fruity-empyreumatic, smoked-powdery-pungent-vegetable, ham, cooked ham, heavy, fish, rotten-animal, salty, sausage, salmon, cold tobacco, earth grass, earth ashes, smoked vegetable, grilled wine

Antipolis, France, unpublished). Injection of reference compounds was done where possible in order to confirm identification.

Acquisition and Analysis of Olfactometric Data. Data were acquired using the AcquiSniff software at a rate of 1 scan/s according to Berdagué's paper (2). During the sniffing sessions, the sniffers were instructed (a) to signal each odor perceived by pressing a push button for as long as the odor lasted, (b) to describe the odors orally, and (c) to quantify their intensity on a five-point scale (2). Sniffing sessions lasted 30 min, and the analysis was conducted twice with the same eight judges, giving $2 \cdot 8 = 16$ individual aromagrams. All sniffers were trained to use the equipment and were selected as nonsmokers without any known pathology for their sensitivity and ability to detect and consistently describe a wide range of odors during GC-O tests. To describe the odors, the sniffers could use a free choice of vocabulary that was to remain as simple and precise as possible. The sniffers had no information on the nature of the sample. A numerical recording of vocabulary items, intensity, and persistence of odors was launched at the "start of injection" of the volatile compounds into the chromatographic column. Thus were obtained eight aromagrams and eight individual digital audio recordings that were read after each session in order to incorporate their content into the aromagrams using the AcquiSniff software. The data were analyzed using the VIDEO-Sniff method by breaking the total olfactory signal (TOS, sum of the eight individual aromagrams) up into olfactory classes that were defined during data processing in order to highlight the key-odor zones of an aromagram (Table 1) (2, 4, 32).

Headspace Solid-Phase Micro Extraction-GC×GC-Time-of-Flight Mass Spectrometry (HS-SPME-GC×GC-TOFMS). A SPME 75 μm fiber (carboxen/polydimethylsiloxane) purchased from Supelco (Bellefonte, PA) was used for the extraction of volatile compounds from the absolute headspace. The fiber was conditioned according to manufacturer recommendations prior to analysis. A 8.6 mg portion of absolute oil was placed in a sealed 20 mL SPME vial. After headspace equilibrium procedure (30 min), the SPME needle was picked through the vial, and the fiber was exposed to the headspace for 2 min at 21 °C (2 cm; without agitation of the sample). After sampling, the fiber was thermally

desorbed in the glass SPME liner of the GC injection port during 2 min at 280 °C. Split injection (split ratio 1:20) was performed with a SPME Combipal autosampler (CTC Analytics AG, Switzerland) on a 6890N chromatograph (Agilent) equipped with two separate ovens, a time-of-flight mass spectrometer LECO Pegasus 4D (LECO Corporation, St. Joseph, MI), and a cryogenic modulator (LECO Quad Jet Modulator). The two separate columns and the modulator are placed in the oven of the 6890N chromatograph. The first dimension chromatographic separation (column 1, oven 1) was performed on a SPB-5 capillary column (5% diphenyl-, 95% dimethylpolysiloxane, length 30 m, i.d. 0.32 mm, film thickness 1 μm ; Supelco, St-Germain-en-Laye, France). The second dimension chromatographic separation (column 2, oven 2) was performed on a DB-17 capillary column (50% dimethyl-, 50% diphenylpolysiloxane, length 2.50 m, i.d. 0.178 mm, film thickness 0.30 μm ; J&W, Folsom). The column 1 oven was held at 40 °C for 5 min, then ramped at 3 °C/min to 230 °C and held for 10 min. The column 2 oven was constantly set 15 °C higher than the column 1 oven. Ultra high purity helium (Air Liquide U quality 99.9995%; flow 1 mL/min) was used as carrier flow. The transfer line was heated at 250 °C, and the ion source set point was 200 °C. The detector voltage was 1600 V. The modulator sequences were modulation period 7 s and hot pulse 0.8 s. Mass spectra (EI) were collected from m/z 33 to 230 u at a scan rate of 200 spectra/s. Chromatograms were processed using the automated data processing software ChromTOF with a signal-to-noise ratio of 50 (meaning that for the software to recognize a signal as a peak, the S/N-ratio had to be at least 50). The NIST/EPA/NIH mass spectral library (NIST05) (NIST, Gaithersburg, MD) was used for peak identification.

RESULTS AND DISCUSSION

Forty-seven odorant zones were detected by the eight sniffers in two separate analyses (total amount of $2 \cdot 8 = 16$ individual aromagrams), and an example of the individual aromagrams for one analysis with eight judges is given in Figure 2. A total amount of 44 odorant compounds could be identified (Table 2). Twenty-two compounds could be easily identified by combining odor,

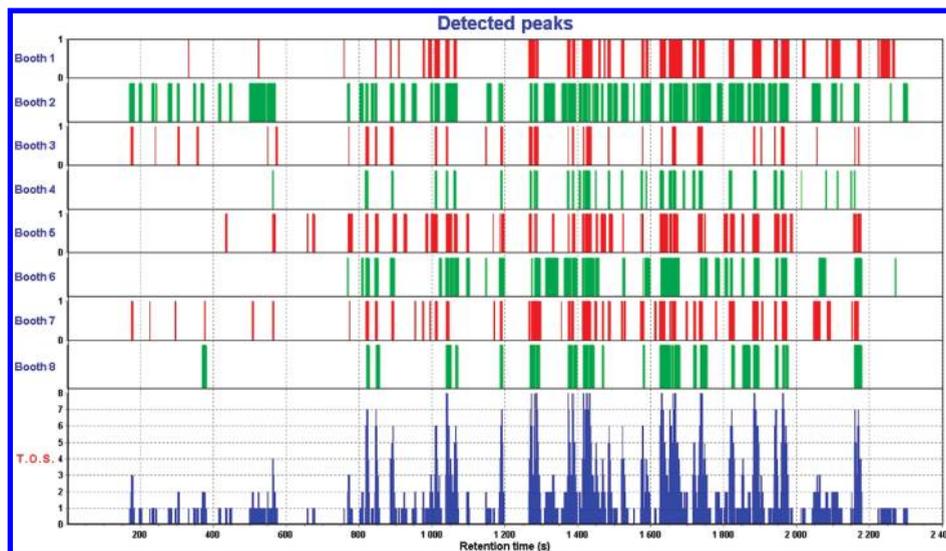


Figure 2. Raw data from individual detection of the eight sniffers (booths 1–8) and total olfactory signal (TOS) expressed in number of detections.

LRI, and qMS information. Among those, the main compounds according to detection frequency are: ethyl thiocyanate (intensity 2.9, detected by 88% of the panel), hexanal (2.3, 75%), (*Z*)-hex-3-enol (2.9, 69%), heptanal (3.4, 75%), diethyl disulfide (3.4, 81%), *O,S*-diethyl thiocarbonate (4.3, 94%), benzaldehyde (3.3, 88%), dimethyl trisulfide (3.9, 94%), octanal (3.1, 69%), limonene (2.2, 63%), and ethyl benzoate (3.1, 75%). The other 22 identified molecules are trace compounds that were instrumentally detected and identified by using comprehensive two-dimensional GC. A total of 30 compounds were identified with certainty, e.g., by LRI, MS, odor comparison, and injection of the reference compound. In addition to the compounds listed above, the main trace compounds identified are (*E*)-hex-2-enal (intensity 3.3, perceived by 94% of the panel), oct-1-en-3-ol and -one (both 2.9, 94%), 6-methylhept-5-en-2-one (3.5, 94%), benzylic alcohol (2.3, 75%), diethyl trisulfide (3.9, 94%), and 2-methoxy-3-isobutylpyrazine (3.3, 100%). Eleven molecules have to be denoted as “tentatively identified” because either the reference compounds were not available for injection, or there was a slight difference between the odor description of the analyte and the description of the reference compound, although the LRI and MS were identical (marked as odor^r in the identification column in **Table 2**). Three odor zones remain not identified.

When looking at the mean olfactory signal by classes ($\overline{\text{OSC}}_{\text{Int} \times \text{Det}}$) where the descriptive vocabulary was attributed to nine olfactory classes according to **Table 1**, **Figure 3** shows that the “fruity-floral” class (pink) and the “sulfury” class (orange) are dominant, followed by the “earthy-undergrowth” (brown) and “green-vegetable” (green) class. The highest olfactory signals are obtained for peaks 7 (fruity-floral) and 38 (sulfury).

All compounds belonging to the “fruity-floral” class could be identified with certainty (peaks 2, 4, 5, 7, 8, 11, 16, 23, 24, 25, 28, and 29). The most influent ones are hexan-2-one that was detected by 63% of the panel with a consensus of its odor descriptors of 94% (peak 4; fruity), hexanal with a detection frequency of 75% and 75% consensual descriptors (peak 5; green, apple, cut grass; this compound also belongs to the “green-vegetable” class with 25% descriptive consensus), (*E*)-hex-2-enal with a detection frequency of 94% and 100% consensus (peak 7; fruity), (*Z*)-hex-3-enol with a detection frequency of 69% and a descriptive consensus of 45% “fruity”, 36% “green-vegetable”, and 19% other descriptions (peak 8; apple, cut grass). Octanal was detected by 69% of the panel and consensually described as “fruity” (64% consensus; peak 24; citrus fruit, green), and limonene was detected

by 63% of the sniffers, but no real descriptive consensus was obtained (peak 28). This might be due to its “terpeny-citrus-fruity” note, which was perceived and described differently by the judges. Among those 12 compounds, six were detected by GC×GC-TOFMS and not by 1D-qMS in the given conditions (peaks 2, 4, 7, 16, 25, and 29). The identification of odor peak 7, (*E*)-hex-2-enal, which is together with odor peak 38 the most important odorant compound, was easily possible due to GC×GC. Once the compound identified by comprehensive 2D-GC, we were able to find traces of it in the 1D-chromatogram as well.

Compound 15 belongs to the “fruity-floral” and to the “sulfury” class and is detected by 94% of the panel with a high intensity (4.3 on a scale from 1 to 5) and a “fruity-sulfury” note. This previously unknown compound is present at only 0.1% (FID area) in the absolute, and after isolation by preparative GC and NMR studies, it was determined to be *O,S*-diethyl thiocarbonate. The detailed identification pathway, spectral data, synthesis of its homologues, and their odor evaluation by GC-Sniffing are presented in a separate publication (22).

Four of the six impact compounds belonging to the “sulfury” class (peaks 6, 14, 20, 22, 33, and 38) were identified with certainty: diethyl disulfide was detected by 81% of the judges and the descriptors belonged to both the “lactic-cheesy” and the “sulfury” class (peak 14; sulfur, cabbage), dimethyl trisulfide with a detection frequency of 94% and 53% consensus (peak 20; 7 times described as sulfury, 7 times described as rotten cabbage), and diethyl trisulfide, detected by 94% with a very good consensus of 87% (peak 38; alliaceous, sulfury, cabbage, pungent, mustard). 2/3-Thiophenecarboxaldehyde was detected by only 50% of the judges. Both reference compounds were available, but whether the substitution is in position 2 or 3 could not be determined. We hence decided to attribute the olfactory stimulus to a mixture of both compounds.

Two sulfur compounds are only tentatively identified because the reference compounds were not available for injection: 2-butylthiophene, detected by 94% with a consensual description of 60% (peak 33; fermented, cheese, sulfury, alliaceous; empyreumatic descriptors are also given, but no coelution was found by GC×GC-TOFMS) and ethyl methyl disulfide with a detection frequency of 69%. Here, descriptor consensus was not satisfying: 45% of the vocabulary belongs to the “sulfury” class (peak 6; sulfury, plastic, cabbage, rancid). Comprehensive two-dimensional GC was used for the MS detection and identification of the sulfur compounds 2/3-thiophenecarboxaldehyde, 2-butylthiophene,

Table 2. Potent Odorants Identified in Indian Cress Absolute by D-HS-8W-GC-O, GC-O-MS, and Comprehensive 2D-GC

no. ^a	LRI ^b		compound ^c	odor description ^d	mean intensity ^e	detection frequency ^f		identification ^g
	RTX-5	HP-20M				over 16 judges	%	
1	597		butenone	buttery	3.0	1	6	LRI, odor, TOFMS, std
			butanone					LRI, odor, MS, std
2	758	1106	(<i>E</i>)-pent-2-enal	fruity	2.0	8	50	LRI, odor, TOFMS, std
3	776	1147	ethyl thiocyanate	plastic, mushroom, earthy, peanut	2.9	14	88	LRI, MS, std
4	784		hexan-2-one	fruity	2.5	10	63	LRI, odor, TOFMS, std
5	798	1089	hexanal	green, apple, cut grass	2.3	12	75	LRI, odor, MS, std
6	839	1120	ethyl methyl disulfide ^f	sulfury, plastic, cabbage, rancid ^f	3.5	11	69	LRI, MS
7	849		(<i>E</i>)-hex-2-enal	fruity	3.3	15	94	LRI, odor, TOFMS, std
8	856	1345	(<i>Z</i>)-hex-3-enol	apple, cut grass	2.9	11	69	LRI, odor, MS, std
10	870	1149	2-ethylthiophene	coffee, toasted bread, peanut	2.7	3	19	LRI, MS, std
11	889	1155	heptan-2-one	fruity	2.0	1	6	LRI, odor, MS, std
12	893		2,3-dimethylthiophene ^f	earthy, ashes, vegetable ^f	1.0	2	13	TOFMS
13	900	1180	heptanal	chemical, smoked, limonene	3.4	12	75	LRI, odor, MS, std
14	926	1188	diethyl disulfide	sulfur, cabbage	3.4	13	81	LRI, odor, MS, std
15	931	1244	<i>O,S</i> -diethyl thiocarbonate	fruity-sulfury	4.3	15	94	LRI, odor, MS, std
16	963		2-propylthiophene	fruity, floral, chemical, earth	2.4	13	81	LRI, TOFMS, std
	966	1291	(<i>E</i>)-hept-2-enal	almonds	3.3	14	88	LRI, odor, TOFMS, std
17		1473	benzaldehyde					LRI, odor, MS, std
18	977		oct-1-en-3-one	mushroom, metallic	2.9	15	94	LRI, odor, TOFMS, std
19	977	1415	oct-1-en-3-ol	mushroom, metallic	2.9	15	94	LRI, odor, TOFMS, std
20	978	1337	dimethyl trisulfide	cheese, sulfury, cabbage, rotten	3.9	15	94	LRI, odor, MS, std
21	984	1323	6-methylhept-5-en-2-one ^f	geranium, undergrowth, moss, earthy	3.5	15	94	LRI, odor, ^f TOFMS, std
22	990		2/3-thiophenecarboxaldehyde	potato, sulfury	2.4	8	50	LRI, odor, TOFMS, std
23	994	1212	ethyl hexanoate	solvent, fruity	1.8	9	56	LRI, odor, MS, std
24	1000		octanal	citrus fruit, green	3.1	11	69	LRI, odor, MS, std
25	1002		(<i>Z</i>)-hex-3-enyl acetate	citrus fruit, green	3.6	7	44	LRI, odor, TOFMS, std
27	1016		(<i>E,E</i>)-hepta-2,4-dienal ^f	green, geranium, moss, plastic, earthy	2.2	10	63	LRI, odor, ^f TOFMS, std
28	1034	1174	limonene	fruity-citrus fruit, chemical, plastic	2.2	10	63	LRI, odor, MS, std
29	1037	1810	benzyl alcohol	fruity, floral, chemical, minty, plastic	2.3	12	75	LRI, odor, TOFMS, std
30	1039	1186	1,8-cineole	floral, minty, fruity	2.6	7	44	LRI, odor, MS, std
31	1054		ethyl hex-2-enoate ^f	empyreumatic, chemical, resinous, earth, herbaceous, peppery, roasted potato ^f	4.1	15	94	LRI, odor, ^f TOFMS
			2-hydroxybenzaldehyde ^f					LRI, odor, ^f TOFMS, std
32	1062		heptanoic acid ^f	floral, chemical	2.9	10	63	LRI, odor, ^f MS, std
33	1066		2-butylthiophene ^f	fermented, mushroom, cabbage, sulfury, alliaceous ^f	4.3	15	94	LRI, TOFMS
34	1091		non-1-en-3-ol ^f	plastic, metallic, garlic, mushroom ^f	3.3	4	25	LRI, odor, TOFMS
35	1094		nonan-2-one	green, fruity, earth, potato, rancid	4.0	14	88	LRI, odor, TOFMS, std
			unknown					
36	1100	1375	nonanal	potato, earth, glue, varnish, green	3.8	8	50	LRI, odor, MS, std
37	1129		isophorone ^f	chemical, burnt, earthy, mushroom, flowery	3.3	13	81	LRI, odor, ^f MS, std
38	1153	1464	diethyl trisulfide	alliaceous, cabbage, sulfury, pungent, mustard	3.9	15	94	LRI, odor, TOFMS, std
40	1163	1674	benzyl acetate	green, plastic	2.7	6	38	LRI, odor, MS, std
41	1165	1406	2-pentylthiophene ^f	sulfury, cabbage, caramelized pop-corn ^f	4.5	2	13	LRI, TOFMS
42	1173	1607	ethyl benzoate	green, chrysanthemum, geranium, earthy	3.1	12	75	LRI, odor, MS, std
43	1185		2-methoxy-3-isobutylpyrazine	floral, green-bitter, peanut	3.3	16	100	LRI, odor, TOFMS, std
44	1200	1470	decanal	grilled	4.0	1	6	LRI, odor, MS, std
46	1275		a pyrazine	roasted nut/peanut, cacao, chocolate	2.7	13	81	odor
47	1279		unknown	menthol, licorice, peanut, almonds	2.7	11	69	

^a Peak number according to Figure 3. Detection frequency less than 50% is generally considered as noise (38) except for compounds easily identified by LRI, MS, and odor.

^b LRI on nonpolar (RTX-5 of the 8W-GC-O device) and polar column (HP-20M) determined with a series of *n*-alkanes. ^c Compounds are listed in elution order on RTX-5 column.

^d Main odor descriptors given by the judges. ^e Mean intensity given by the 16 judges (on a scale from 1 to 5). ^f Detection frequency over 16 judges. ^g Identification methods: LRI = linear retention index, odor = comparison of odor descriptors to our home-made GC-O data base and to the reference compound if injected; MS = mass spectrum obtained by qMS; TOFMS = mass spectrum obtained by GC×GC-TOFMS; std = reference compound injection. ^h Tentatively identified (reference compounds not injected or odor descriptor of reference compound not exactly matching and/or no detection by MS; see column with identification method).

and diethyl trisulfide (peak 38; one of the two most important odor compounds of the absolute).

Generally, the consensual description of an odor by the panel agrees with the information obtained by MS, LRI, and GC-O databank research. Variations within panel description can among other reasons indicate coelutions or different perceptions of certain molecules by the sniffers: peak 5, hexanal, belongs to two olfactory classes, the “fruity-floral” and the “green-vegetable” class. This is because some people smell it as “green apple,

fruity”, and others more like “freshly cut grass”. The same can be noted for peak 8, (*Z*)-hex-3-enol. However, as stated earlier, the use of olfactory classes and vocabulary descriptions can also point out coelutions: odor peak 31 is a coelution between ethyl hex-2-enoate and 2-hydroxybenzaldehyde. Discrepancy within the odor descriptions of the panel led to that discovery, which was then confirmed by GC×GC. On the other hand, comprehensive 2D-GC indicated that peak 43 was a coelution between three molecules: α -terpineol, ethyl octanoate, and

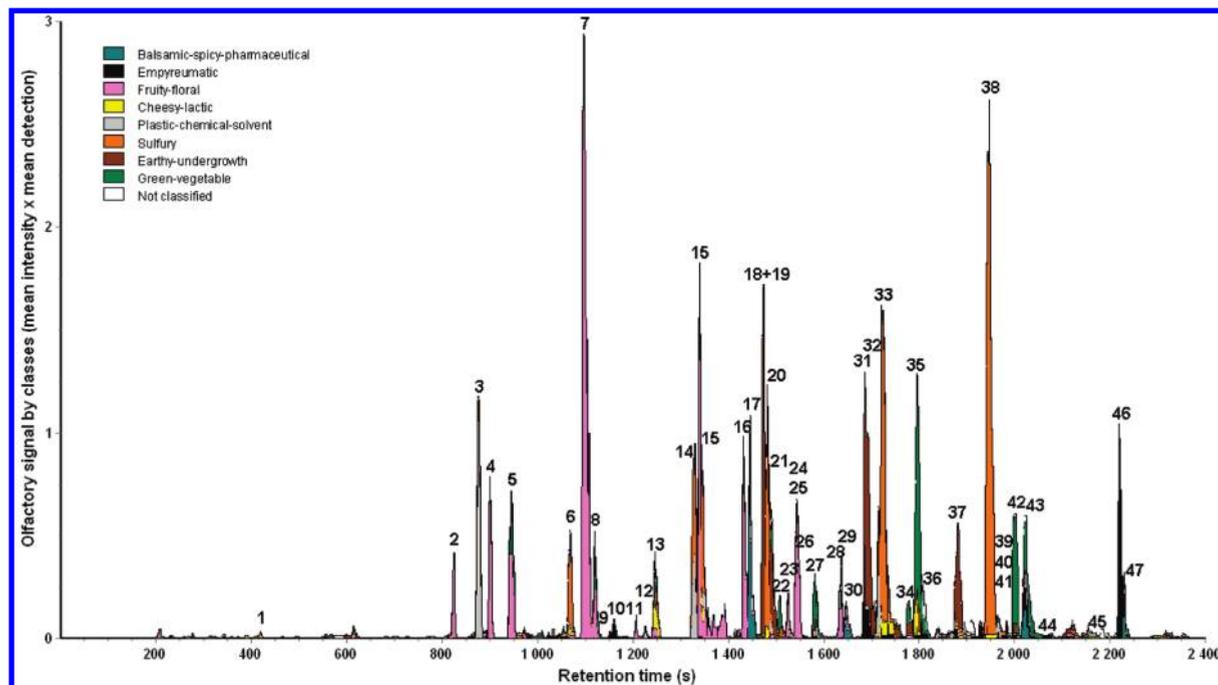


Figure 3. Mean olfactory signal by classes ($\overline{OSC}_{Int \times Det}$) of Indian cress absolute. The breakdown of the mean total olfactory signal ($\overline{TOS}_{Int \times Det}$) into nine classes shows the odorant zones belonging to a given olfactory class. Labels corresponding to the peak numbers are given in **Table 2**.

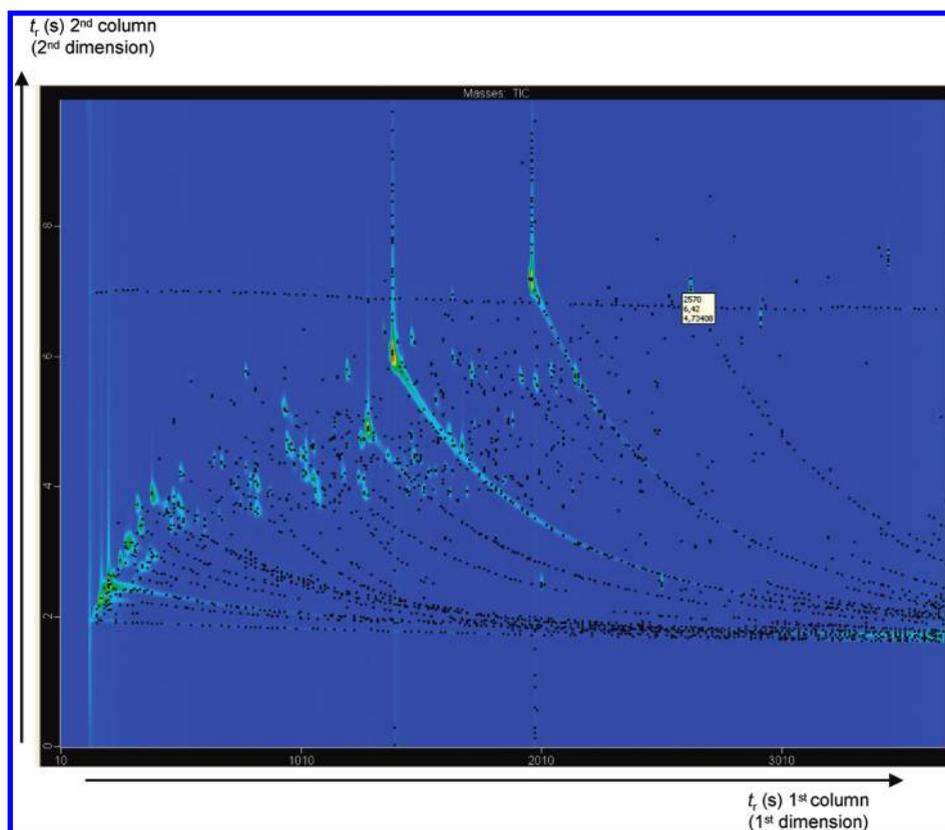


Figure 4. HS-SPME-GC \times GC-TOFMS chromatogram of Indian cress absolute headspace.

2-methoxy-3-isobutylpyrazine. Here, injection of all three reference compounds in GC-O allowed for the olfactory stimulus to be attributed to the pyrazine only.

Twenty-two odorant compounds were perceived by the panel but could not be directly detected by the 1D-qMS system because they were present in trace quantities only or subject to coelutions. The use of GC \times GC-TOFMS, combined with the

odor information obtained by GC-O, allowed for these molecules to be identified or at least tentatively identified (nine compounds). GC \times GC-TOFMS leads to the detection of thousands of peaks due to its increased peak capacity and resolution, and the data processing of the whole amount of information would be too time-consuming and its use questionable. Coupling this powerful technique to GC-O information seems an efficient way of rapidly

identifying trace odor impact compounds. The 2D-plot of Indian cress absolute obtained by HS-SPME-sampling is given in **Figure 4**. Over 2,000 peaks were automatically integrated by the software. This huge amount of information was analyzed by searching for compounds likely to provoke the odorant stimuli found during GC-O. More specifically, we particularly focused on odor zones that were well perceived by the judges, but to which we could not attribute any clear signals in the 1D-chromatogram. The first dimension of the 2D-plot was searched by comparing the LRI of the odorant stimuli perceived on the 1D-GC-O system. Combined with GC-O database searches, we were in most cases able to find candidate compounds with satisfying match percentages of over 74% with the MS libraries that were subsequently confirmed by reference compound injection: according to our results, the two main odor compounds of Indian cress absolute are (*E*)-hex-2-enal and diethyl trisulfide. Both were easily and unequivocally identified by comprehensive 2D-GC with good spectra match percentages of 89.2% for the aldehyde and 87% for the sulfide. Once their identity was known, we were also able to find these compounds in the 1D-chromatogram. However, without comprehensive 2D-GC, the identification process would have been longer and more fastidious. This shows the power of coupling comprehensive two-dimensional gas chromatography to GC-O data: out of a total number of 44 identified odorant compounds, 22 (50%) were well perceived by the panel but could not be directly and clearly identified by 1D-qMS. The use of GC×GC-TOFMS was highly useful for their detection by MS and avoided time-consuming enrichment steps that might result in artifact formation.

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