Black tea volatiles fingerprinting by comprehensive two-dimensional gas chromatography – Mass spectrometry combined with high concentration capacity sample preparation techniques: Toward a fully automated sensomic assessment

Black tea volatiles fingerprinting by two-dimensional comprehensive gas chromatography - mass spectrometry combined with high concentration capacity sample preparation techniques: toward a fully automated sensomic assessment

Federico Magagna[1], Chiara Cordero[1]*, Cecilia Cagliero[1], Erica Liberto[1], Patrizia Rubiolo[1], Barbara Sgorbini[1] and Carlo Bicchi[1]

[1]Authors affiliation:

Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Pietro Giuria 9, I-10125 Turin, Italy

* Address for correspondence:

Dr. Chiara Cordero - Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Pietro Giuria 9, I-10125 Torino, Italy – e-mail: chiara.cordero@unito.it ; phone: +39 011 6707662; fax: +39 011 2367662

Abstract

The present research implements the principles of *sensomics* into advanced and integrated multidimensional platforms based on comprehensive two-dimensional gas chromatography (GC×GC) coupled with Mass Spectrometry (MS) and High Concentration Capacity (HCC) sample preparation (Head Space Solid Phase Microextraction, Headspace Sorptive Extraction, Dynamic-Headspace, Stir Bar Sorptive Extraction and In-solution SPME). The focus is on black tea volatiles and their informative role as key-indicators of tea aroma profile; insights on post-harvesting practices, climate variations and technological manipulations are also tackled due to: (a) high information power of the approach; (b) possibility of apply advanced fingerprinting methodologies on 2D patterns and (c) effective scripting functions on MS signatures.

The approach demonstrates to be effective and reliable covering up to 95% of key-aroma compounds described for black-teas suggesting that a fully automated and highly informative screening of the volatiles could be possible by combining all the analytical dimensions in a single platform.

Keywords

black tea volatiles; advanced fingerprinting; comprehensive two-dimensional gas chromatography; high concentration capacity sampling techniques; head space solid phase microextraction; Stir bar sorptive extraction; Dynamic Headspace; sensomics

1. Introduction

Tea, prepared by infusion of dried leaves of *Camellia sinensis* (L.) Kuntze is the second world's most popular beverage, after water. Its consumption may be associated with potential health benefits because of the relatively high amount of polyphenols (Del Rio et al 2004) made bio-available by colonic gut microflora (Del Rio, Calani, Scazzina, Jechiu, Cordero and Brighenti, 2010; Del Rio, Calani, Cordero, Salvatore, Pellegrini and Brighenti, 2010). Tea chemical composition is thus an important attribute, related not only to its sensory quality and consumption pleasure, but also to nutritional facts thus influencing quality, market value and consumer preferences.

While phenolic compounds and xantines condition tea taste and color, volatiles are not only fundamental to define its peculiar aroma (Hofmann and Schieberle, 2011; Yang, Baldermann and Watanabe, 2013) but are also informative of several other characteristics such as cultivar, geographical origin, storage and processing (i.e., withering, rolling, fermentation and firing).

Tea volatiles belong to different chemical classes such as hydrocarbons, alcohols, aldehydes, ketones, acids, esters, lactones, aromatic derivatives, sulfur compounds and many others (Yang et al, 2013). Volatiles origin and formation pathways have been the objective of extensive researches since early thirties (Coggon, Romanczyk and Sanderson 1977; Gohain et al, 2012; Mick and Schreier, 1984; Ravichandran and Parthiban, 1998; Sanderson and Graham, 1973; Selvendran, Reynolds and Galliard, 1978; Takeo, 1981; Yang et al, 2013) and nowadays their peculiar quali-quantitative distribution is considered as a distinctive chemical signature encrypting a number of information about botanical/geographical origin, post-harvest treatments, technological manipulations and aroma quality.

Advanced multidimensional analytical platforms based on GC×GC-ToFMS combined with multivariate data analysis have recently been applied to characterize the volatile fraction of tea extracts (Zhang, Zeng, Zhao, Kong, Lu and Xu, 2013). In this study, green, oolong and black tea extracts (obtained with Simultaneous Distillation Extraction SDE) were compared and classified as a function of fermentation/oxidation degree on the basis of volatile distribution.

In general, the potentials of such informative platforms in revealing subtle (compositional) differences within more homogeneous samples is of interest also for commercial purposes and industrial production of ready-to-drink products, where the overall quality of the herbal infusion has to be kept constant and coherent/comparable to a reference standard over time.

Therefore, the focus of the present research, that implements the principles of sensomics (Schieberle and Hofmann, 2011) to advanced and integrated multidimensional platforms, is a deep and meaningful investigation on the different chemical signatures within the volatile fraction of black tea. In particular, extraction-separation-identification of analytes is obtained combining the separation power of two dimensional comprehensive gas chromatography coupled with mass spectrometry (GC×GC-MS) with miniaturized and/or automated sample preparation techniques (Cordero et al, 2013). This last step is crucial to provide a consistent, representative and meaningful picture of informative analytes (sensory or technologically-related) in a fully automated work-flow.

Over the past years, many conventional extraction techniques, including SDE (Chaintreau, 2001), steam distillation under reduced pressure (SDR) (Kumazawa and Masuda, 2002), direct organic solvent extraction, solvent-assisted flavor evaporation (SAFE) (Hofmann and Schieberle, 2011; Schieberle and Hofmann, 2011) etc., have been used to characterize the tea volatile fraction. These conventional methodologies afford to obtain reliable overview of the components of a sample although some drawbacks cannot be avoided. In particular, the use of solvents as extraction media may modify sample characteristics by producing artifacts or causing analytes degradation (Chaintreau, 2001); moreover, oxidation (oxygen effect) and temperature triggered reactions may occur. Furthermore, solvent properties (boiling-point, solvation properties etc.) can affect extraction efficiency and selectivity causing losses of highly volatile compounds or exerting discriminations related to analytes physico-chemical properties.

Miniaturized-high concentration capacity (HCC) sampling techniques (Solid Phase Microextraction, SPME, Stir Bar Sorptive Extraction, SBSE, Head Space Sorptive Extraction, HSSE and Dynamic Headspace technique) may be of great help (Bicchi, Cordero and Rubiolo, 2004) to limit artifacts occurring in traditional extraction methods and to match the recent analytical requirements of automation.

HS-SPME and Headspace Sorptive Extraction are the most widely-used high concentration capacity-headspace static (HCC-HS) techniques, where analytes are recovered through multiple partition (or adsorption) equilibria between sample, headspace and extraction polymer or adsorbent. Dynamic Headspace technique, designed to trap volatiles and semi-volatiles by a stream of inert gas through or above a solid or liquid media, can either be a valid alternative to static approaches or a complement when a medium-to-low volatility compounds have to be effectively extracted from sample. HS Stir Bar Sorptive Extraction (SBSE) and in-solution-SPME are also of potential interest for liquid samples since they produce a sample profile complementary to HS and bring further information about analytes distribution between infusion (liquid phase) and its headspace.

This study aimed at obtaining a chemical fingerprint with a high information potential having as a reference benchmark key-analytes considered as markers of tea production chain and "cup" sensory quality. In this perspective, commercial grade blends of black tea from Sri-Lanka (Ceylon) representative of the production of two years (2012 and 2013) were submitted to different extraction approaches known for their selective and extensive capabilities. Volatiles were extracted from the headspace of dry plant material (as it is or after addition of water to improve volatile distribution in the headspace) or directly from infusions (standard and strong infusions).

Sampling parameters (time/temperature/phase ratio β) were varied depending on the approach (static or dynamic sampling, headspace or in-solution etc..) and set to obtain the highest information content in terms of both number of detected compounds and absolute amount transferred to the analytical system. The aroma blueprint was defined within the investigated analytes that contribute to obtain a "comprehensive" chemical signature, and method(s) effectiveness critically evaluated in a perspective of aroma quality classification/characterization.

2. Experimental

2.1 Reference compounds and samples

Pure reference standards for identity confirmation (key-aroma compounds and informative volatiles) of acetone, (*E*)-2-heptenal, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal, (*E*)-2-nonenal, (*E,E*)-2,4-decadienal, (E,E)-2,4-nonadienal, (*E,Z*)-2,6-nonadienal, (*Z*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*Z*)-4-heptenal, 1-butanol, 1-heptanol, 1-hexanol, 1-octen-3-ol, 1-pentanol, 2-heptanone, 2-methyl butanal, 2-methyl propanal, 2-phenyl ethanol, 3-methyl butanal, 6-methyl-5-hepten-2-one, acetic acid, benzaldehyde, benzyl alcohol, butanal, butanoic acid, caffeine, decanal, ethyl acetate, furfural, geranial, geraniol, heptanal, hexanal, hexanoic acid, limonene, linalool, methyl salicylate, nonanal, octanal, pentanal, pentanoic acid, phenyl acetaldehyde, propanoic acid, vanillin, α-ionone, β-damascenone, β-ionone and n-alkanes (*n*-C9 to *n*-C25) for Linear Retention Index (I^T_S) determination were from Sigma-Aldrich (Milan, Italy).

Internal Standards for analytes response normalization and method validation were α- and β-thujone from Fluka (Milan, Italy); a standard stock solution of ISTDs at 1 µg/L was prepared in dibuthylphtalate (Sigma-Aldrich, Milan, Italy) and stored in a sealed vial at -18°C.

Premium quality fermented black tea leaves of homogeneous particle size from Ceylon (Flowery Orange Pekoe) were kindly supplied by Soremartec Italia srl (Alba, CN, Italy).

2.2 Sample Preparation

2.2.1 Preparation of tea samples for headspace analysis and in-solution sampling

Dried plant material were exactly weighted (1.500 g) in headspace glass vials (20 mL) and submitted to headspace extraction following the different approaches listed in **Table 1**.

Some experiments were also run by adding 2.000 mL of ultrapure water to the dry material to test the resulting headspace sensitivity.

Tea infusions were prepared according to European Medicine Agency – EMA (EMA, 2010) indications by suspending 3.00 g of dried plant material in 300 mL of ultrapure boiling water. Static extraction was run for

5 minutes, particulate was thus removed through cellulose paper filtration and the resulting solution immediately transferred to headspace vials (20 mL) for in-solution sampling.

"Strong" infusions were also prepared (9.00 g of dried plant material in 50 mL of ultrapure boiling water - extraction time 5 minutes) to evaluate the effects of solute/solvent proportions on quali-quantitative distribution of volatiles. Concentrates, based on water extraction, are commonly used to prepare ready-to-drink teas (Cordero, Canale, Del Rio and Bicchi 2009).

2.2.2 Automated Solid Phase Microextraction and Headspace Solid Phase Microextraction

Automated SPME for in-solution sampling and HS-SPME for headspace analysis were performed using a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) installed on the GC×GC-MS system. SPME fibers, Divinylbenzene/Carboxen/ Polydimethyl siloxane (DVB/CAR/PDMS) d_f 50/30 µm - 2 cm were from Supelco (Bellefonte, PA, USA). Fibers were conditioned before use as recommended by the manufacturer. Sampling conditions and parameters are summarized in **Table 1**.

ISTDs (α- and β-thujone) used for peak response normalization were pre-loaded into SPME fibers. ISTDs loading procedure was run before samples' extraction by exposing the SPME fiber to 5 µL of ISTDs standard stock solution for 20 minutes at 50°C.

2.2.3 Stir Bar Sorptive Extraction and Headspace Sorptive Extraction

SBSE for in solution-sampling and HSSE for headspace sampling were performed with commercial Twister™ devices. 100% PDMS d_f 500 µm - 2 cm twisters were supplied by Gerstel (Mülheim a/d Ruhr, Germany). Sampling was carried out in a thermostatic bath with constant stirring; HSSE twisters were suspended in the vapour phase with a stainless steel wire (Sgorbini et al, 2012), volatiles were thus transferred to GC×GC-MS by a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) equipped with a Thermo Desorption Unit (TDU) and a CIS-4 PTV injector (Gerstel, Mülheim a/d Ruhr, Germany). Sampling conditions and parameters are reported in **Table 1**.

ISTDs loading procedure was run before tea samples' extraction by exposing Twister™ devices to 2 µL of ISTDs standard stock solution for 10 minutes at 50°C.

2.2.4 Dynamic Headspace sampling

Dynamic headspace sampling was performed with trapping devices assembled in the authors laboratory with characteristics suitable to obtain comparable data, a combination head-to-tail of 100% PDMS foams (15 mm length – 30 mg ± 2) and 20 mg (±2) 100% PDMS particles supplied by Gerstel (Mülheim a/d Ruhr, Germany). Extractants were packed on inert, single taper, glass liners to be directly desorbed into the TDU unit.

During sampling, traps were gas-tight connected to the outlet of a 20 mL sampling vial kept at 50°C, analytes were trapped with a nitrogen flow-rate of 10 mL/min for an extraction time of 50 min (200 mL of total volume). Traps were maintained at room temperature during sampling to increase extraction efficiency, sampling time/volume were extended to improve the extraction of medium-to-low volatility analytes known to be discriminated by static sampling (e.g. HS-SPME). Detailed conditions and parameters are given in **Table 1**.

2.3 GC×GC-MS instrument set-up and analytical conditions

GC×GC analyses were performed on an Agilent 6890 GC unit coupled with an Agilent 5975C MS inert detector operating in the EI mode at 70 eV (Agilent, Little Falls, DE, USA). The transfer line was set at 270°C. An *Auto Tune* option was used and the scan range was set at *m/z* 35-250 with a scan rate of 12,500 amu/s to obtain a 30Hz of sampling frequency. The system was equipped with a two-stage KT 2004 loop thermal modulator (Zoex Corporation, Houston, TX) cooled with liquid nitrogen controlled by Optimode™ V.2 (SRA Instruments, Cernusco sul Naviglio, MI, Italy). Hot jet pulse time was set at 250 ms, modulation time was 4 s and cold-jet total flow progressively reduced with a linear function from 40% of Mass Flow Controller (MFC) at initial conditions to 8% at the end of the run. A deactivated fused silica capillary loop (1 m × 0.1 mm d_c) was used. The column set was configured as follows: ^{1}D SE52 column (95% polydimethylsiloxane, 5%

193 phenyl) (30 m × 0.25 mm d_c, 0.25 µm d_f) coupled with a ^{2}D OV1701 column (86% polydimethylsiloxane, 7%
194 phenyl, 7% cyanopropyl) (1 m × 0.1 mm d_c, 0.10 µm d_f). Columns were from Mega (Legnano, Milan, Italy).
195 One microliter of the *n*-alkane sample solution for Linear Retention Index (I^T_S) determination was
196 automatically injected with an Agilent ALS 7683B injection system under the following conditions:
197 split/splitless injector, split mode, split ratio 1:50, injector temperature 280°C.
198 Volatiles extracted by in-solution or headspace sampling were injected as reported in **Table 1**. For all
199 experiments, carrier gas was helium kept at a constant flow with an initial head pressure 298 kPa. The
200 temperature program was 50°C (1 min) to 280°C (10 min) at 2.5°C/min.
201
202 **2.4 Data acquisition and 2D data automatic processing**
203 Data were acquired by Agilent MSD ChemStation *ver* D.02.00.275 and processed by GC Image® GC×GC
204 Edition Software, Release 2.5 (GC Image, LLC Lincoln NE, USA).
205 Statistical analysis was performed with SPSS 14.0 (SPSS Inc. Chicago, Illinois, USA) and heat map
206 visualization by GENE-E v 3.0.77 (Broad Institute, Inc. Cambridge, MA, USA).
207
208 **2.5 Method performance parameters**
209 To establish method performance in terms of precision for quantitative descriptors (i.e. 2D Normalized
210 Peak Volumes measured on analytes Target Ion (*Ti*), a simple validation protocol was designed, including
211 experiments on HS-SPME with DVB/CAR/PDMS, HSSE and SBSE with 100% PDMS Twister™ and D-HS
212 sampling with PDMS foam/particles. Precision data on retention times and 2D Peak Volumes (response
213 referred to Target Ions *Ti*) on a selection of key-odorants and informative analytes were evaluated by
214 replicating analyses (three replicates) during a period of one month. Results on analytes 2D Normalized
215 Peak Volumes referred of an acceptable precision and CVr% never exceeded 20%. Detailed information is
216 provided as Supplementary information - **Supplementary Table 1** - **ST1**-
217

218 **2.6 Analytes identification**

219 Analytes were identified on the basis of their linear retention indices (I^T_S) and EI-MS spectra compared to
220 those of authentic standards (see paragraph 2.1) or tentatively identified through their EI-MS
221 fragmentation patterns and database available retention indices (see Table 2 for details).

222

223 **3. Results and Discussion**

224 This study investigates the informative potential of tea volatiles fingerprints obtained by combining
225 headspace and in-solution high-concentration capacity (HCC) extraction techniques and dynamic headspace
226 (D-HS) with GC×GC-MS in a unified analytical platform. The informative role of samples' fingerprint is
227 evaluated through a selection of key-analytes for which is known: (*a*) the biosynthetic pathway
228 (monoterpenoids, phenylpropanoids, carotenoid and fatty acid derivatives), (*b*) their relationship with
229 technological treatments (as for example products of glycosides hydrolysis) or, (*c*) because of their relevant
230 sensory impact to define the aroma blueprint (Christlbauer and Schieberle, 2009).
231 The following paragraphs illustrate experimental results and discuss investigation strategies peculiar of
232 GC×GC data set resulting in a productive exploitation of the information collected by combining multiple
233 analytical dimensions in a single analysis.

234

235 **3.1 Effectiveness and informative potential of sampling approaches**

236 The volatiles detected and identified with authentic standard confirmation and or by combination
237 of linear retention index (I^T_S) and EI-MS spectrum are listed in **Table 2** together with their absolute
238 retention times (^{1}D min and ^{2}D sec), experimental I^T_S and those reported in commercial databases (Adams,
239 2007), percent of normalized 2D Peak Volumes resulting from three analytical replicates and referred to
240 two commercial lots. Key-odorants in black teas from different origin (Darjeeling from India (Kawakami,
241 Ganguly, Banerjee and Kobayash1, 1995; Schuh and Schieberle, 2006), Chinese Keemun and Sri-Lanka
242 clones DT-1 and 2025 (Wang, Lee, Chung, Baik, So and Park, 2008)) are indicated and reported together

with odor quality and odor threshold (mg/Kg in water) as reported in reference papers (Schieberle and Hofmann, 2011; Wang et al, 2008; Kawakami et al, 1995).

A group of 123 target analytes was defined and matched through the sample set by the *comprehensive template matching* approach (Reichenbach, Carr, Stoll and Tao, 2009). The approach is classified as *peak feature* methodology and enables establishing reliable correspondences between 2D peaks from the same chemical entity across multiple chromatograms (Kiefl, Cordero, Nicolotti, Schieberle, Reichenbach and Bicchi, 2012). **Figure 1A** shows the 2D chromatogram from volatiles sampled by HS-SPME on dry leaves and water addition of a black tea sample from Lot#A. Analytes quali-quantitative distribution across samples is visualized as heat-map in the **Supplementary Figure 1** - **SF1**.

Insert here Figure 1

Within headspace approaches, the multi-polymer SPME fiber, combining polar adsorption phases (DVB and Carboxen) with sorptive apolar material (PDMS), gave satisfactory results in term of number of detected analytes. A 78/73 over 123 targets were detected above method Limit of Detection (LOD) from the headspace of dry leaves (from Lot #A and Lot #B respectively). The number of analytes increased to 88/78 when 2.00 mL of water were added to the plant material, since it promotes the headspace vaporization of analytes with lower water solubility. Interestingly, HSSE and D-HS with higher amounts of sorptive material (100% PDMS), although effective in terms of absolute amount of analytes extracted, showed complementary sampling attitudes when compared to HS-SPME. Medium-to-low volatility analytes were better recovered from sample headspace by HSSE and D-HS, in terms of both number of detected analytes and absolute abundance (predominance of dark brown spots in the region of high I^T_S values in **Supplementary Figure 1**). The information capabilities of sorptive extraction towards medium-to-low volatility/low polarity analytes were confirmed by SBSE sampling results. In-solution sampling enables to characterize directly the infusion chemical signature matching the objective of a fingerprinting methodology directed to a product ready for consumption or as intermediate at the basis of industrial production of ready-to-drink teas. Based on the experiment set-up, complementary information for a

269 comprehensive chemical signature of the sample volatiles was expected by combining the results from the
270 different approaches and sample types.
271 Unsupervised approach, i.e. Principle Component Analysis - PCA, was applied to map the natural
272 conformation of sample groups (dry leaves, leaves to which ultrapure water was added and tea infusion)
273 and to confirm the information provided by each sampling technique.
274 **Figure 2A** shows the scores plot on the first and the third principal components (F3-F1 plane). The variance
275 explained from the first principal component (F1) was 38.79% while that of the third principal component
276 (F3) was 13.78%. In this case, the second component (F2) was not enough informative in terms of
277 discrimination potential of target analytes as a function of the different sampling approaches. Autoscaling
278 and mean centering were applied as pre-processing steps, baseline correction was already applied for 2D
279 data elaboration by GC Image. The corresponding loadings plot is shown in **Figure 2B**.
280
281 **Insert Figure 2 here**
282 The PCA carried out on experimental data (e.g., 123 target analytes), shows a fairly good clustering
283 between in-solution sampling of tea infusions (normal and strong infusion - upper part of the Cartesian
284 plane) and headspace sampling of dry plant material (lower section of the Cartesian plane). Watered dried
285 leaves are located in the right intermediate part of the plane. Samples distribution clearly indicate that the
286 addition of water impacts on volatiles partition and consequently influences the information potential of
287 each 2D plot. Water enhances the headspace sensitivity for less polar analytes: saturated and unsatured
288 aldehydes from C3/C4 (2-methyl propanal, 2 and 3-methyl butanal) to C9 ((E)-2-nonenal, (E,Z)-2,6-
289 nonadienal) and short chain alcohols are better recovered by HS-SPME on wet plant material. Loading plot
290 (**Fig. 2B**) indicates the compounds responsible for this discrimination. Interestingly, all these compounds
291 are connoted by a relatively low water solubility accompanied by an incremental vapor pressure as a
292 function of the molecular weight.

293 On the other hand, D-HS and HSSE are characterized by a good recovery of less polar and low volatility
294 analytes such as C10-C18 saturated aldehydes, C11 to C15 methyl ketones and some medium chain
295 alcohols (1-dodecanol, 1-tetradecanol, 1-hexadecanol).
296 In-solution sampling better recovers jasmonic acid esters (methyl-(Z)-jasmonate, cis-methyl dihydro
297 jasmonate and trans-methyl dihydro jasmonate) and carotenoid derivatives. This class of secondary
298 metabolites class also include some potent flavour components that characterize the aroma of black tea
299 infusions: β-ionone with *violet-like* odour and β-damascenone, which contributes with its *fruity* note to the
300 overall perception.
301
302 **3.2 Insights in the tea volatile fraction and its chemical signatures**
303 Within the identified compounds, it is worth mentioning the class of plant secondary metabolites
304 from both the shikimate pathway (benzaldehyde, benzyl alcohol, phenyl acetaldehyde and 2-phenyl
305 ethanol) and mevalonate - MVA/ methylerythritol phosphate - MEP pathways (limonene, geraniol,
306 hotrienol, linalool and its oxidized derivatives *cis*- and *trans*-linalool-3,6-oxide, *cis*- and *trans*-linalool-3,7-
307 oxide). Their quali-quantitative distribution can define botanical/geographical origin as well as seasonal
308 variations and harvest periods (Yang et al, 2013).
309 Linalool, the most abundant monoterpenoid in black tea leaves, mainly occurs in its free form, whereas
310 linalool oxides are present as glycosides (Sakata, Mizutani, Cho, JKinoshita and Shimizu, 2008) (β-
311 primeverosides) in young and old tea leaves and stems, they are liberated by specific enzymes
312 (primeverosidases) during harvest and post-harvest treatments (Mizutani et al 2002). The non-enzymatic
313 hydrolysis due to hot water during the infusion process is also well documented (Schuh and Schieberle,
314 2006). The glycosidic precursors of damascenone, a nor-isoprenoid derived from enzyme-catalyzed
315 cleavage of carotenoids (Carotenoid Cleavage Enzymes CCDx) (Kinoshita et al, 2010), undergoes to
316 hydrolysis during pasteurization producing an unpleasant off-flavor (Kumazawa and Masuda, 2001).
317 **Figure 3A** shows the distribution (Normalized 2D Peak Volumes) of glycoside aglycones in the headspace of
318 dry leaves with and without water addition.

Insert Figures 3A-B here

The effect of water on the headspace sensitivity with these analytes is evident, although it cannot be excluded that other concurrent effects (e.g. water solubility and osmotic pressure on plant cells and stems) may promote their release and, as a consequence, higher headspace concentration. In particular, β-damascenone was detected only in presence of water, being its headspace concentration below method LOD when dry leaves were directly sampled without treatment. The increase for the other compounds ranges from about 4 (+385%) of benzyl alcohol and *cis*-linalool-3,7-oxide, to a maximum of about 90 folds (+9125%) of methyl salicylate. Results based on absolute abundances are corroborated by the relative distribution counterparts based on Normalized 2D peak Volume % (data not shown) with the only exception of benzyl alcohol whose percent distribution does not markedly change.

Another group of informative volatiles for black tea qualification, mainly because of their intense odor, is that of carotenoids derivatives. As mentioned, this group of volatiles is formed from enzymatic cleavage by CCDs, a superfamily of polyene chain oxigenases. In particular, 6-methyl-5-hepten-2-one and geranial are formed by 5,6 or 7,8 double bond cleavage of lycopene (Zhang et al, 2013; Vogel, Tan, McCarty and Klee, 2008) while β-ionone and β-damascenone (C-13 apocarotenoids) have β-carotene and neoxanthin respectively as precursors. These components may reflect seasonal variations; the phosphorylation status of oxigenase enzymes increases from spring to autumn impacting on the chemical fingerprint of this volatiles (Vogel et al, 2008). Their distribution between samples will be discussed in the next paragraph (Section *3.3*) focused on odor active compounds.

The group of volatiles derived from saturated and unsaturated fatty acids oxidation is the most represented and counts 55 analytes belonging to different chemical classes: alcohols, carbonyl derivatives, acids and esters including some lactones. They are formed through a primary reaction catalyzed by region-selective lipoxygenases (9-LOX and 13-LOX) that forms dioxygenated intermediates (hydroperoxides) from unsaturated fatty acids. The hydroperoxides are thus cleaved by hydroperoxide lyases (HPLs) to oxo-acids (precursors of cyclic esters - lactones) and C6-aldehydes. The picture is thus completed by β-, γ-unsaturated

carbonyls isomerization, enzymatically or non-enzymatically mediated, and reduction to the corresponding alcohols catalyzed by alcohol dehydrogenases (ADHs).

This complex formation pathway is highly sensitive to enzymatic activity changes mostly induced by shifts in environmental temperatures than by maturity of the leaves (Sekiya, Kajiwara and Hatanaka, 1984); the chemical fingerprint of these volatile derivatives can in consequence be adopted as informative signature of climate variations. It is therefore interesting to observe hydroperoxide derivatives distribution from dried tea leaves as a function of the different HS approaches investigated.

Figure 3B shows the distribution (Normalized 2D Peak Volume) of saturated and unsaturated aldehydes, sorted by molecular weight, as they were recovered from sample headspace by: HS-SPME (dark colorization), HSSE (medium intensity colorization) and D-HS (light colorization).

As expected, HS-SPME reached the highest sensitivity with highly-volatile aldehydes (from C5 to C7) while D-HS, settled-up to enrich the medium-to-low volatility fraction, provides information about aldehydes with a carbon skeleton above 10/11 C units. These analytes are connoted by quite low odor thresholds, especially if unsaturated, making their contribution to the overall sensory perception of tea infusions not negligible. The extraction capability of HSSE has to be stressed: it provides a rather complete picture of this analyte group and, compared to D-HS, its optimization is easier.

The transferability of the method to routine and high-throughput controls takes advantages by the fingerprinting capabilities of HCC-HS techniques to sample directly dry leaves and might also be speeded up at the data elaboration step by applying suitable scripting functions. Scripting on 2D metadata enables to isolate and visualize the response of certain analytes' group or classes as a function of the spectral signature and/or relative retention (position) on the 2D separation space. This operation is usual in the petrochemical field for the "group-type" analysis (Jennerwein, Eschner, Gröger, Wilharm and Zimmermann, 2014), and was also successfully adopted in a previous study on milk volatiles (Cordero et al, 2013), although limited to a few chemical groups (i.e. saturated aldehydes and lactones) because of the high sample chemical dimensionality (Giddings, 1995) and limited presence of homologue series.

Figures 1B and **1C** show the resulting 2D images after scripting: *normal* and *iso*-alkanes (Fig.**1B)** and saturated and unsaturated aldehydes (**Fig. 1C)**.

The function was implemented by the Computer Language for Identifying Chemicals (CLIC - GC-Image™) and adopts functions, arithmetic operators, logical operators, relational operators combined in specific expressions that can be applied to single image pixels to produce an image in which pixels where the expression is verified (evaluated to true) are unchanged and pixels that are evaluated as false are set to zero (i.e. masked).

The expression for *normal* and *iso*-alkanes was built on the basis of the fragmentation pattern characteristics of this chemical class and corresponds to:

`(Relative(43)>0.98)&(Relative(57)>0.78)&(Relative(71)>0.68)&(Relative(85)>0.50)`

Saturated aldehydes are characterized by some common fragments (41, 44 and 56/57 *m/z*) while the unsaturated ones by 41, 55, 68/69/70 and 83 *m/z*. The expression was built to include both series and was as follows:

```
AND((Relative(41)>0.70)&(Relative(44)>0.70)&(Relative(57)>0.50)),((Relative(41)>
0.90)&(Relative(55)>0.70)&(Relative(69)>0.40)&(Relative(83)>0.40)).
```

The resulting filtered 2D plot is shown in **Figure 1C**.

Normal and *iso*-alkanes deserve to be considered because of their informing role about the impact of both fermentation on long chain fatty acids and mineral oil saturated hydrocarbons (MOSH) contamination during the processing chain. The latter is of increasing interest for food safety, and has found in GC×GC-FID/MS one of the most performing platforms for reliable characterization and quantitation (Biedermann and Grob, 2015).

3.3 Definition of black teas Chemical Odor Code

The complex phenomenon of aroma perception is triggered by volatile molecules, mostly hydrophobic, interacting with Odor Receptors (ORs) expressed in the olfactory epithelium (Fleischer, Breer and Strotmann, 2009). The perception, activated by multiple and simultaneous ligand-receptor interactions, is the result of a complex pattern of signals (i.e., the Receptor Code) that is integrated by

peripheral and central nervous system (Dunkel et al, 2014). The comprehensive chemical characterization of the mixture of potential ligands (i.e., the Chemical Odor Code) is fundamental to understand and objectify food aroma perception (Schieberle and Hofmann, 2011).

The investigation approach adopted in this study can therefore help for an accurate and rational characterization of sensory active compounds within samples providing also the analyst of an effective tool for their classification.

Apart from quality control aspects, changes of the chemical fingerprint of aroma active compounds may be of interest also for post-harvesting treatments and processing practices optimization. Therefore, ideally, the entire set of key odorants has to be measured without any discrimination between the highly abundant and chromatographically well-resolved peaks. GC×GC has proven to be a valuable tool to perform quickly a comprehensive assessment of odorants (Cordero, Kiefl, Schieberle, Reichenbach and Bicchi, 2015) as it is clearly confirmed with tea aroma active compounds.

Within the group of volatiles identified and listed in **Table 2**, volatile fatty acids derivates (above all hexanol, (*Z*)-2-hexenol, (*Z*)-3-hexenol, hexanal, (*E*)-2-hexenal) significantly contribute to the typical tea aroma with their *fresh-green* odors; in particular these compounds are associated with different sensory descriptors, i.e. *flowery*, *violet-like*, *grassy*, *fresh*, *sweet*. The same pathway also originate higher molecular weight derivatives, such as saturated and unsaturated aldehydes above 7/8 carbon units that are responsible for *fatty*, *fishy* and *oat-flake*-like notes ((*Z*)-4-heptenal, (*E,E*)-2,4-heptadienal, (*E,E*)-2,4-heptadienal, (*E*)-2-nonenal, (*E,E*)-2,4-octadienal, (*E,E*)-2,4-nonadienal, (*E,E*)-2,4-decadienal) (Schuh and Schieberle, 2006).

Strecker aldehydes (2-methylpropanal, 2-methylbutanal and 3-methylbutanal), formed during fermentation, impress *malty* and *buttery* notes while phenylacetaldehyde, derived from L-phenylalanine (L-Phe), is responsible the pleasant *honey*-like note. L-Phe is also precursor of benzylalcohol and phenylethanol, major contributors to the *fruity*, *floral* smells as well as benzaldehyde, benzylalcohol and coumarin with *sweet*, *fruity* and *almond*-like notes. Carotenoid derivatives (β-ionone and damascenone)

422 and terpenoids (linalool, linalool-oxides and geraniol) complete the *floral* bouquet with their characteristic
423 notes.
424 Looking at the chemical odor code encrypted in the 2D patterns and assuming as reference benchmark, the
425 list of potent odorants identified by Kawakami et al (1995) and by Schuh and Schieberle (2006), who also
426 considered their impact relative to the odor threshold (OT), the most meaningful sampling approach results
427 HS-SPME of the watered dry leaves. HS-SPME detects 39 compounds from Lot#A with the exception of
428 methyl jasmonate that was below method LOD; and 34 from Lot#B over forty key-aroma compounds. In
429 Lot#B six compounds were lost: (E,E)-2,4-nonadienal, methyl-(Z)-jasmonate, β-damascenone, decanoic
430 acid, 3-methyl butanoic acid and vanillin.
431
432 **Insert Figure 4 here**
433 **Figure 4** visualizes as heat-map the combination of chemical data (analyte quantitative distribution across
434 samples/sampling approaches) and sensory descriptors. General descriptors (first column) were based on
435 the aroma profile delineated by descriptive sensory analysis (DSA) reported by Schuh and Schieberle
436 (2006). For potent odorants (third column, right side) most common odor descriptor(s) are also reported
437 (second column, central). The heat-map quantitative descriptors (normalized 2D volumes) are reported in
438 logarithmic scale to give a quick and effective indication on the potential of each single sampling technique
439 and its informative role. With HS-SPME on watered leaves dark colored spots prevail for all classes of
440 odorants. Interestingly, the two tea Lots shows a different distribution of key-odorants indicating that,
441 although their overall sensory profile was evaluated from a panel as compliant with a reference
442 benchmark, harvest year influences the relative distribution of some key-flavors.
443
444

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Figure Captions:

Figures 1A-C: (**1A**) Pseudocolorized GC×GC chromatogram of volatiles sampled by HS-SPME on dry leaves and water addition of a black tea sample from Lot#A. (**1B**) pink-colored circles highlight 2D peaks where the scripting function : [Relative(43)>0.98)&(Relative(57)>0.78)&(Relative(71)>0.68)&(Relative(85)>0.50] was verified - *normal* and *iso*-alkanes. (**1C**) green and cyano colored circles highlight 2D peaks where the scripting function : [AND((Relative(41)>0.70)&(Relative(44)>0.70)&(Relative(57)>0.50)),((Relative(41)>0.90)&(Relative(55)>0.70)&(Relative(69)>0.40)&(Relative(83)>0.40))] was verified - *saturated* and *unsaturated*-aldehydes.

Figures 2A-B: PCA results: (**2A**) scores plot on the first and the third principal components (F3-F1 plane) based on volatiles distribution across all samplings/samples (14 × 123 matrix - samples × analytes). (**2B**) corresponding loadings plot.

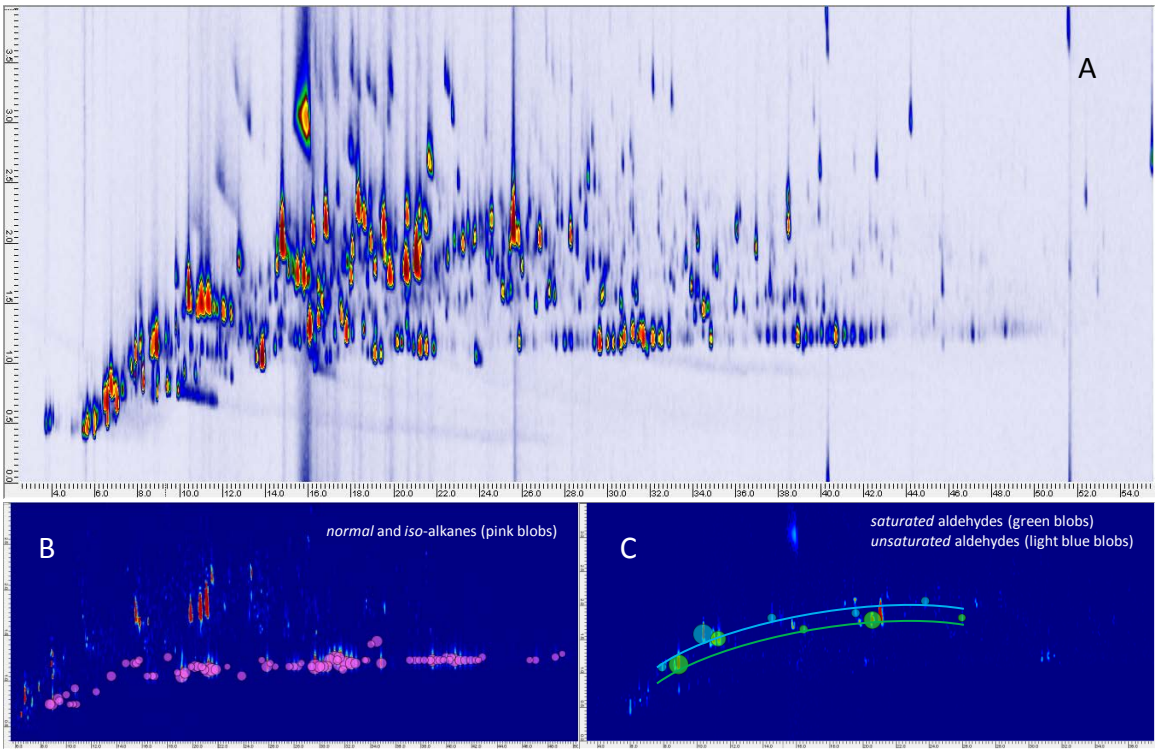
Figures 3A-B: (**3A**) histogram illustrating the effect of water addition on a selection of volatiles known to be present in black tea leaves as both free and glycosidically bounded forms. Percentages indicate the effect of water on HS-SPME recovery estimated on Normalized 2D Peak Volumes. (**3B**) graphical rendering showing the distribution (Normalized 2D Peak Volumes) of f saturated and unsaturated aldehydes, sorted by molecular weight, as they were recovered from sample headspace by: HS-SPME (dark colorization), HSSE (medium intensity colorization) and D-HS (light colorization)

Figure 4: heat-map showing the combination of chemical data (analyte quantitative distribution across samples/sampling approaches) and sensory descriptors. General descriptors (first column) are based on the aroma profile delineated by descriptive sensory analysis (DSA) reported by Schuh and Schieberle (2006). Potent odorants (third column, right side) are listed together with most common odor descriptor(s) (second column, central). The heat-map quantitative descriptors (Normalized 2D Peak Volumes) are reported in logarithmic scale.

Table Captions:

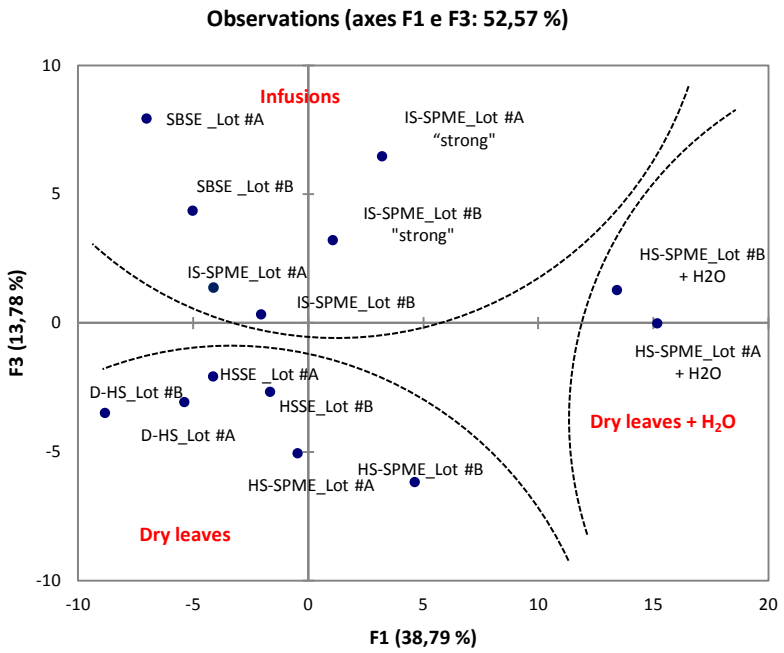
Table 1: List of analyzed samples and sampling conditions.

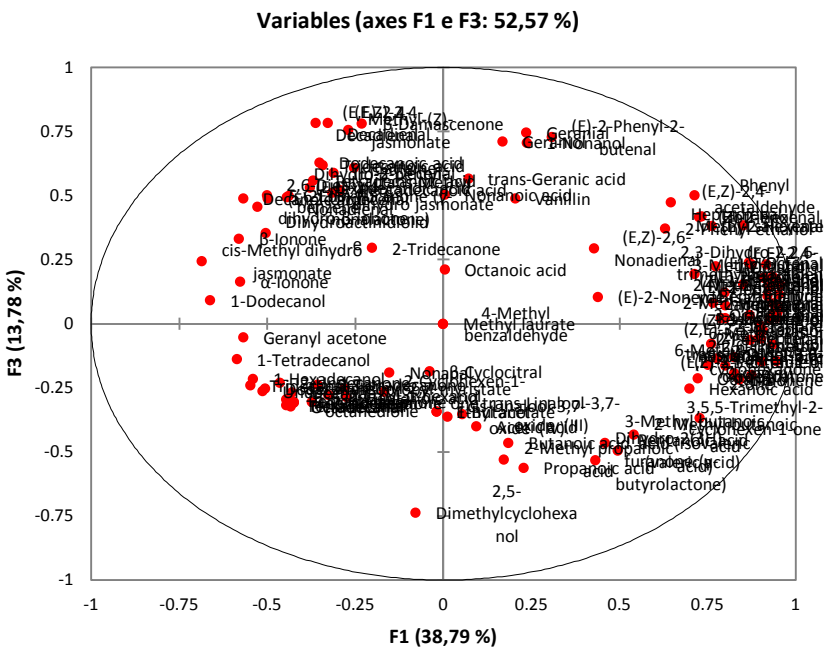
Table 2: List of the target analytes together with ^{1}D and ^{2}D retention times, I^{T}_{S} (experimental, reference form authentic standards analysis ($) or reported in literature (£)), and sensory descriptors as reported in reference literature. The 2D Peak Volume data is provided for all sampling approaches and Lots#. Values are means of two analytical replicates. Analytes that were confirmed by the analysis of reference standards are reported in italics.

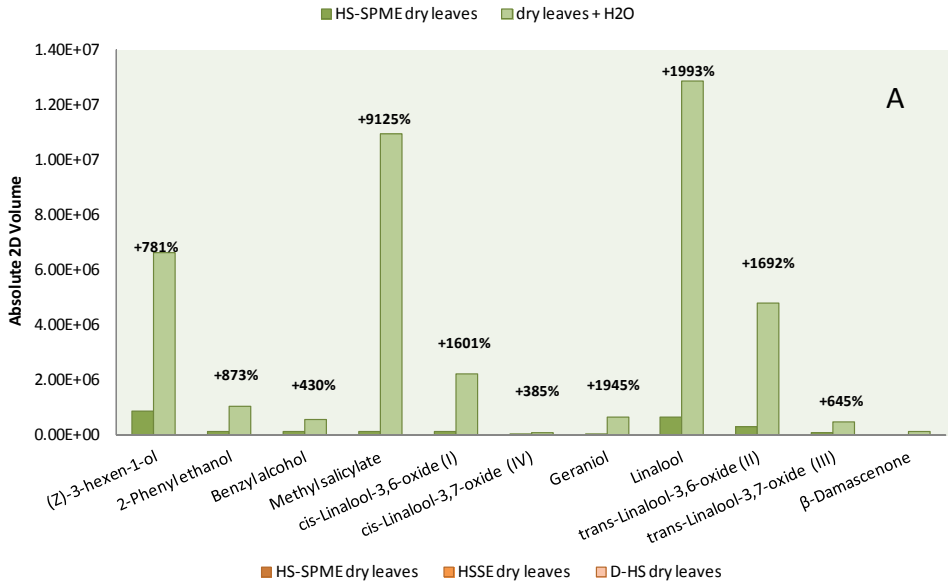

A

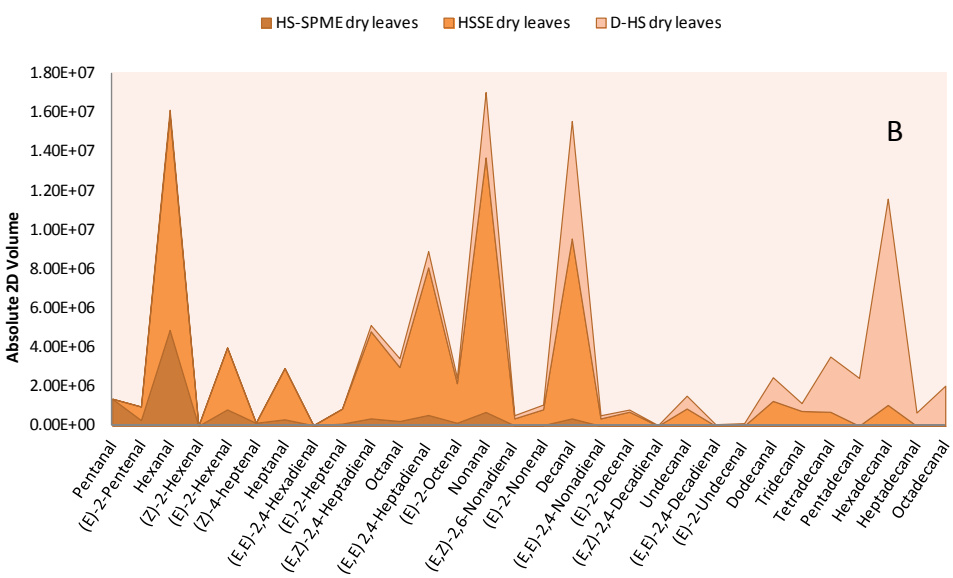
B
normal and *iso*-alkanes (pink blobs)

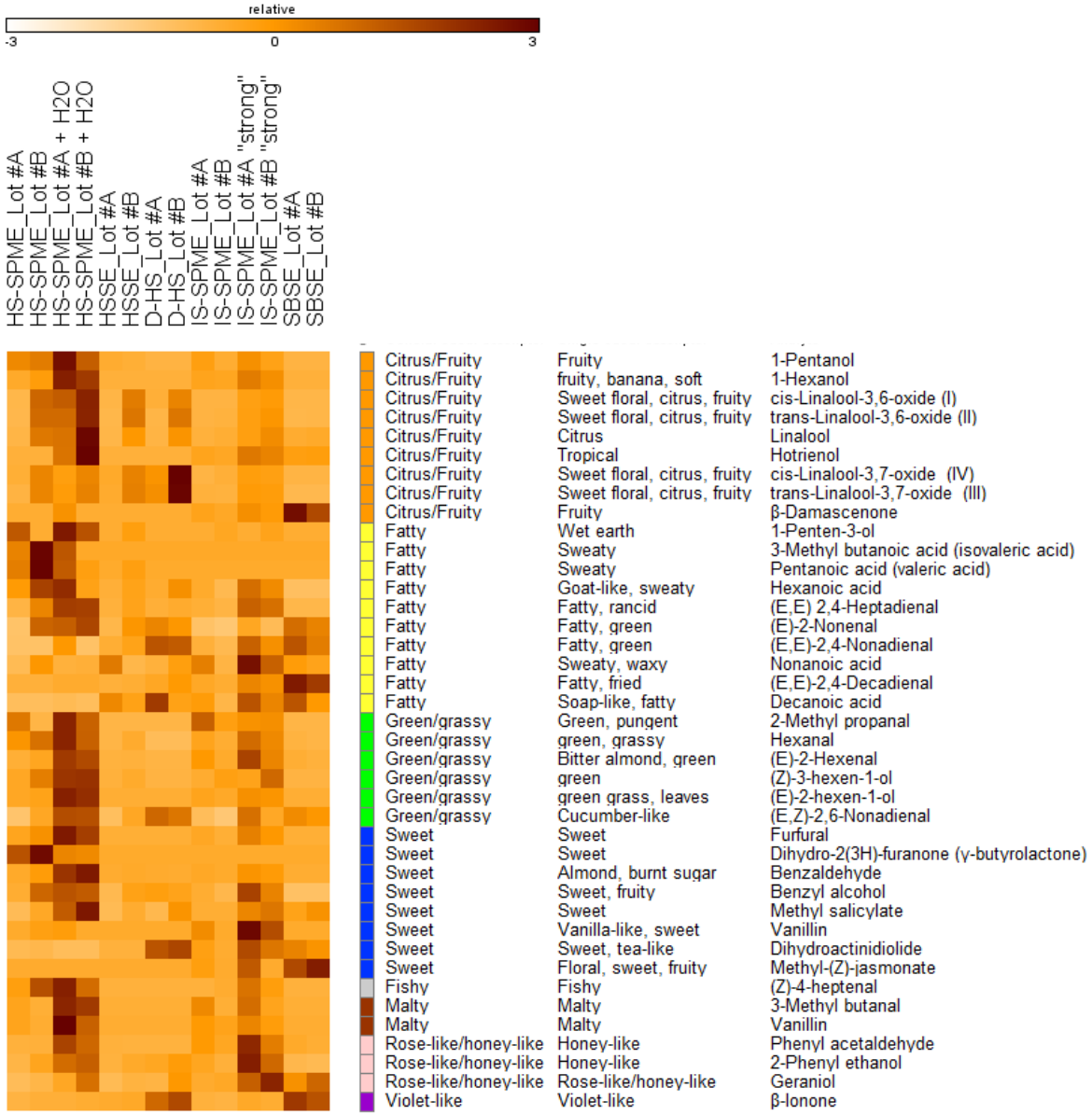
C
saturated aldehydes (green blobs)
unsaturated aldehydes (light blue blobs)


Observations (axes F1 e F3: 52,57 %)
Infusions
SBSE _Lot #A
IS-SPME_Lot #A
"strong"
SBSE _Lot #B
IS-SPME_Lot #B
"strong"
IS-SPME_Lot #A
IS-SPME_Lot #B
HS-SPME_Lot #B
+ H2O
HS-SPME_Lot #A
+ H2O
HSSE _Lot #A
D-HS_Lot #B
HSSE_Lot #B
D-HS_Lot #A
HS-SPME_Lot #B
HS-SPME_Lot #A
Dry leaves + H2O
Dry leaves
F3 (13,78 %)
F1 (38,79 %)
10
5
0
-5
-10
-10
-5
0
5
10
15
20


Variables (axes F1 e F3: 52,57 %)
(E)-2-Phenyl-2-butenal
trans-Geranic acid
vanillin
Phenyl acetaldehyde
(E,Z)-2,6-
cis-Methyl dihydro
jasmonate
α-Ionone
2-Tridecanone
Octanoic acid
1-Dodecanol
4-Methyl benzaldehyde
Methyl laurate
Geranyl acetone
1-Tetradecanol
Hexanoic acid
3,5,5-Trimethyl-2-
Propanoic acid
butyrolactone)
2,5-
Dimethylcyclohexa
nol
F3 (13,78 %)
F1 (38,79 %)
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
-1
-0.75
-0.5
-0.25
0
0.25
0.5
0.75
1


HS-SPME dry leaves
dry leaves + H2O
A
Absolute 2D Volume
1.40E+07
1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
+781%
+873%
+430%
+9125%
+1601%
+385%
+1945%
+1993%
+1692%
+645%
(Z)-3-hexen-1-ol
2-Phenylethanol
Benzyl alcohol
Methyl salicylate
cis-Linalool-3,6-oxide (I)
cis-Linalool-3,7-oxide (IV)
Geraniol
Linalool
trans-Linalool-3,6-oxide (II)
trans-Linalool-3,7-oxide (III)
β-Damascenone


HS-SPME dry leaves
HSSE dry leaves
D-HS dry leaves
B
Absolute 2D Volume
1.80E+07
1.60E+07
1.40E+07
1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
Pentanal
(E)-2-Pentenal
Hexanal
(Z)-2-Hexenal
(E)-2-Hexenal
(Z)-4-heptenal
Heptanal
(E,E)-2,4-Hexadienal
(E)-2-Heptenal
(E,Z)-2,4-Heptadienal
Octanal
(E,E)-2,4-Heptadienal
(E)-2-Octenal
Nonanal
(E,Z)-2,6-Nonadienal
(E)-2-Nonenal
Decanal
(E,E)-2,4-Nonadienal
(E)-2-Decenal
(E,Z)-2,4-Decadienal
Undecanal
(E,E)-2,4-Decadienal
(E)-2-Undecenal
Dodecanal
Tridecanal
Tetradecanal
Pentadecanal
Hexadecanal
Heptadecanal
Octadecanal


relative
-3
0
3
HS-SPME_Lot #A
HS-SPME_Lot #B
HS-SPME_Lot #A + H2O
HS-SPME_Lot #B + H2O
HSSE_Lot #A
HSSE_Lot #B
D-HS_Lot #A
D-HS_Lot #B
IS-SPME_Lot #A
IS-SPME_Lot #B
IS-SPME_Lot #A "strong"
IS-SPME_Lot #B "strong"
SBSE_Lot #A
SBSE_Lot #B
Citrus/Fruity Fruity 1-Pentanol
Citrus/Fruity fruity, banana, soft 1-Hexanol
Citrus/Fruity Sweet floral, citrus, fruity cis-Linalool-3,6-oxide (I)
Citrus/Fruity Sweet floral, citrus, fruity trans-Linalool-3,6-oxide (II)
Citrus/Fruity Citrus Linalool
Citrus/Fruity Tropical Hotrienol
Citrus/Fruity Sweet floral, citrus, fruity cis-Linalool-3,7-oxide (IV)
Citrus/Fruity Sweet floral, citrus, fruity trans-Linalool-3,7-oxide (III)
Citrus/Fruity Fruity β-Damascenone
Fatty Wet earth 1-Penten-3-ol
Fatty Sweaty 3-Methyl butanoic acid (isovaleric acid)
Fatty Sweaty Pentanoic acid (valeric acid)
Fatty Goat-like, sweaty Hexanoic acid
Fatty Fatty, rancid (E,E) 2,4-Heptadienal
Fatty Fatty, green (E)-2-Nonenal
Fatty Fatty, green (E,E)-2,4-Nonadienal
Fatty Sweaty, waxy Nonanoic acid
Fatty Fatty, fried (E,E)-2,4-Decadienal
Fatty Soap-like, fatty Decanoic acid
Green/grassy Green, pungent 2-Methyl propanal
Green/grassy green, grassy Hexanal
Green/grassy Bitter almond, green (E)-2-Hexenal
Green/grassy green (Z)-3-hexen-1-ol
Green/grassy green grass, leaves (E)-2-hexen-1-ol
Green/grassy Cucumber-like (E,Z)-2,6-Nonadienal
Sweet Sweet Furfural
Sweet Sweet Dihydro-2(3H)-furanone (γ-butyrolactone)
Sweet Almond, burnt sugar Benzaldehyde
Sweet Sweet, fruity Benzyl alcohol
Sweet Sweet Methyl salicylate
Sweet Vanilla-like, sweet Vanillin
Sweet Sweet, tea-like Dihydroactinidiolide
Sweet Floral, sweet, fruity Methyl-(Z)-jasmonate
Fishy Fishy (Z)-4-heptenal
Malty Malty 3-Methyl butanal
Malty Malty Vanillin
Rose-like/honey-like Honey-like Phenyl acetaldehyde
Rose-like/honey-like Honey-like 2-Phenyl ethanol
Rose-like/honey-like Rose-like/honey-like Geraniol
Violet-like Violet-like β-Ionone

Samples	Sampling approach	Sample weight/volume	Temperature and time	Other	Replicates	Acronym
Dry plant material Lot #A and Lot #B	HS-SPME - DVB/CAR/PDMS	1.500g dry leaves	Temperature: 50°C Sampling time: 50 min	Constant stirring -Desorption time: 5 (min) S/SL injector: 270 °C -Split ratio 1:10	3 +3	HS-SPME_Lot#
		1.500g dry leaves 2.000 mL of water	Temperature: 50°C Sampling time: 50 min		3 +3	HS-SPME_Lot# +H2O
	HSSE -Twister™ 100% PDMS	1.500g dry leaves	Temperature: 50°C Sampling time: 50 min Sample incubation: 50°C	TDU conditions: from 30°C to 270°C (5 min) at 60°C/min; flow mode: splitless -Transfer line: 270°C.	3 +3	HSSE_Lot#
	D-HS 100% PDMS*	1.500g dry leaves	Trap: room temperature Carrier: nitrogen Sampling flow: 10 mL/min Sampling time: 50 min	CIS-4 PTV injector temp: -50°C -coolant: liquid CO_2; Injection temp program: from -50°C to 270°C (10 min) at 12°C/s. Inlet operated in split mode: split ratio 1:10.	3 +3	D-HS_Lot#
Tea infusion Lot #A and Lot #B	SPME - DVB/CAR/PDMS	Sample volume 20 mL	Temperature: 50°C Sampling time: 50 min	Constant stirring -Desorption time: 5 (min) S/SL injector: 270 °C -Split ratio 1:10	3 +3	IS-SPME_Lot#
	SBSE -Twister™ 100% PDMS	Sample volume 20 mL	Temperature: 50°C Sampling time: 50 min	TDU conditions: from 30°C to 270°C (5 min) at 60°C/min; flow mode: splitless -Transfer line: 270°C. CIS-4 PTV injector temp: -50°C -coolant: liquid CO_2; Injection temp program: from -50°C to 270°C (10 min) at 12°C/s. Inlet operated in split mode: split ratio 1:10.	3 +3	SBSE_Lot#

* 100% PDMS particles + foam

Analyte*	^{1}D (min)	^{2}D (sec)	Exp. I^{T}_{S}	Ref. I^{T}_{S} [$,£]	Odour descriptor	Odour threshold (mg/Kg)	Key-aroma	HS-SPME Lot #A	HS-SPME Lot #A + H_2O	HSSE Lot #A	D-HS Lot #A	IS-SPME Lot #A	IS-SPME Lot #A "strong"	SBSE Lot #A	HS-SPME Lot #B	HS-SPME Lot #B + H_2O	HSSE Lot #B	D-HS Lot #B	IS-SPME Lot #B	IS-SPME Lot #B "strong"	SBSE Lot #B
Acetone	3.42	0.56	746	750[$]	pungent	500		1.52	0.41	0.00	0.00	0.00	0.11	0.00	0.61	0.48	0.00	0.00	0.00	0.00	0.00
2-Methyl propanal	3.62	0.10	750	751[$]	green, pungent	0.0023	x	0.54	0.21	0.00	0.00	0.40	0.08	0.00	0.00	0.12	0.00	0.00	0.12	0.09	0.00
Acetic acid	3.89	0.49	755	754[$]	sour, vinegary	50		15.80	0.48	1.29	0.00	0.00	0.00	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00
Butanal	3.89	0.21	755	756[$]	pungent, green	0.018		0.40	0.32	0.00	0.00	0.00	0.05	0.00	0.00	0.24	0.00	0.00	0.00	0.00	0.00
Ethyl acetate	4.02	0.21	758	757[$]	pineapple	0.94		1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1-Butanol	4.49	0.42	768	769[$]	winey	0.15		0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3-Methyl butanal	4.55	0.31	769	770[$]	malty	0.013	x	0.63	1.18	0.00	0.00	1.17	0.76	0.00	0.00	0.97	0.00	0.00	0.00	0.15	0.00
2-Methyl butanal	4.62	0.31	770	771[$]	malty	0.01	x	1.15	2.93	0.00	0.00	1.37	0.99	0.00	0.00	1.31	0.00	0.00	0.59	0.48	0.00
1-Penten-3-ol	4.82	0.45	774	775[$]	wet earth	0.4	y	4.58	1.39	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.68	0.00	0.24	0.16	0.00
Propanoic acid	4.89	1.15	776	776[$]	fruity, pungent	20		1.80	0.08	0.00	0.00	0.00	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.00	0.00
Pentanal	5.09	0.42	780	781[$]	pungent, almond-like	0.04		4.29	1.44	0.00	0.00	0.86	0.70	0.00	0.00	0.67	0.00	0.00	0.39	0.50	0.00
2-Methyl propanoic acid	6.02	1.43	799	793[£]	sweaty	8.10		0.12	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
(E)-2-Pentenal	6.15	0.70	801	-	green, apple, tomato, pungent	0.3		0.91	1.10	0.30	0.00	0.48	0.87	0.00	0.95	0.85	0.16	0.00	0.22	0.51	0.00
1-Pentanol	6.42	0.77	807	807[$]	fruity	3	y	2.09	1.24	0.56	0.00	0.64	0.43	0.00	1.03	0.67	0.19	0.00	0.18	0.23	0.00
(Z)-2-Penten-1-ol	6.49	0.87	808	783[£]	-	-		2.58	1.45	0.79	0.00	0.00	0.43	0.00	1.99	1.19	0.35	0.00	0.00	0.35	0.00
Butanoic acid	6.75	1.74	813	813[$]	sweaty, rancid	0.24		0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00	0.00	0.00	0.00	0.00	0.00
Hexanal	7.29	0.77	824	823[$]	green apple, grassy	0.08	xy	15.20	8.14	4.72	0.29	5.47	5.99	1.51	9.52	4.98	4.53	0.29	3.20	4.28	1.55
Furfural	8.42	1.33	847	848[$]	sweet	3	y	0.36	0.73	0.00	0.00	0.15	0.33	0.00	0.39	0.54	0.00	0.00	0.00	0.19	0.00
3-Methyl butanoic acid	8.62	2.13	851	834[£]	sweaty	0.7	y	0.15	0.05	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.00	0.00
(Z)-2-Hexenal	8.89	1.05	857	855[$]	-	-		0.00	0.18	0.00	0.00	0.20	0.26	0.00	0.00	0.11	0.00	0.00	0.11	0.07	0.00
2-Methyl butanoic acid	8.95	2.09	858	832[£]	sweaty, sweet	0.7		0.17	0.07	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00
(E)-2-Hexenal	9.15	1.12	862	863[$]	bitter almond, green	0.42	y	2.60	8.46	1.36	0.00	7.53	9.85	1.09	2.75	7.06	0.90	0.00	2.42	4.74	0.68
(Z)-3-hexen-1-ol	9.22	1.22	864	864[$]	green	1.5	x	2.62	3.81	0.84	0.00	0.86	0.82	0.00	3.85	3.72	1.27	0.00	2.17	3.03	0.00
(E)-2-hexen-1-ol	9.62	1.22	872	872[$]	green grass, leaves	5	xy	0.58	1.71	0.00	0.00	0.49	0.57	0.00	0.38	1.50	0.00	0.00	0.22	0.51	0.00
1-Hexanol	9.75	1.19	874	873[$]	fruity, banana, soft	0.4	y	0.66	1.90	0.00	0.00	0.66	0.97	0.00	0.74	1.51	0.00	0.00	0.51	0.69	0.00
Pentanoic acid	10.35	2.55	887	888[$]	sweaty	3	y	0.76	0.23	0.00	0.00	0.00	0.00	0.00	0.97	0.04	0.15	0.00	0.00	0.00	0.00
2-Heptanone	10.35	1.12	887	888[$]	sweet, fruity	0.3		0.41	1.07	0.00	0.00	0.22	0.28	0.07	0.48	0.77	0.26	0.00	0.10	0.21	0.09
(Z)-4-heptenal	10.95	1.15	899	898[$]	fishy	0.00006	x	0.48	0.50	0.00	0.00	0.00	0.30	0.00	0.75	0.25	0.00	0.00	0.06	0.12	0.00
Heptanal	11.09	1.08	901	901[$]	oily, fatty, woody	0.5		1.02	1.43	1.12	0.00	0.56	0.63	0.24	1.29	0.85	0.96	0.00	0.23	0.35	0.32
Dihydro-2(3H)-furanone (*γ-butyrolactone*)	11.49	2.97	909	908[$]	-	-	y	0.63	0.06	0.00	0.00	0.00	0.00	0.00	0.43	0.05	0.00	0.00	0.00	0.00	0.00
(E,E)-2,4-Hexadienal	11.55	1.53	911	907[£]	green	0.010		0.09	0.75	0.00	0.00	0.37	0.49	0.00	0.24	0.51	0.00	0.00	0.11	0.25	0.00
(E)-2-Heptenal	13.75	1.47	955	955[$]	fatty, almond-like	0.005		0.34	0.45	0.33	0.00	0.15	0.27	0.07	0.21	0.28	0.11	0.00	0.08	0.16	0.00
Benzaldehyde	14.09	1.64	962	962[$]	almond, burnt sugar	0.35	y	1.97	7.40	1.58	0.45	1.64	2.61	0.21	3.23	8.46	1.42	0.40	0.90	1.95	0.26
1-Heptanol	14.35	1.43	968	968[$]	herb	0.003		0.11	0.14	0.00	0.00	0.00	0.12	0.00	0.29	0.13	0.16	0.00	0.00	0.08	0.00
1-Octen-3-ol	14.89	1.40	978	977[$]	mould, earthy	0.05		0.40	0.96	0.00	0.11	0.23	0.37	0.00	0.90	1.04	0.35	0.18	0.14	0.32	0.06
6-Methyl-5-hepten-2-one	15.09	1.43	983	984[$]	pungent, green	1		1.51	2.28	2.46	1.03	0.40	0.68	0.00	2.67	2.23	2.56	0.84	0.29	0.65	0.44
2-Octanone	15.22	1.36	985	988[£]	mould, green	0.51		0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.20	0.29	0.00	0.00	0.00	0.05	0.00
Hexanoic acid	15.35	2.90	988	987[$]	goat-like, sweaty	3	y	4.62	2.60	2.74	0.86	1.43	1.95	0.00	4.81	0.65	2.35	2.05	0.40	1.40	0.00
2-Pentyl furan	15.42	0.98	989	984[£]	buttery, green bean-like	0.006		1.06	3.01	0.65	0.00	0.00	0.00	0.00	1.83	2.74	0.93	0.00	0.07	0.09	0.00
(E,Z)-2,4-Heptadienal	15.82	1.78	997	996[£]	fatty, rancid	10		1.17	1.48	1.91	0.36	1.75	3.33	0.59	1.44	1.32	1.07	0.34	0.99	1.91	0.60
(Z)-3-Hexen-1-ol acetate	15.95	1.19	1000	1004[£]	sweet	0.008		0.00	0.38	0.00	0.00	0.00	0.05	0.00	0.09	0.78	0.00	0.00	0.00	0.11	0.12
Octanal	16.09	1.33	1003	1002[$]	fatty, sharp	0.32		0.75	0.59	1.18	0.51	0.20	0.13	0.28	0.62	0.39	0.56	0.36	0.07	0.08	0.21
(E,E) 2,4-Heptadienal	16.55	1.81	1011	1005[£]	fatty, rancid	0.36	y	1.72	3.39	3.24	0.95	1.53	3.22	0.45	3.66	3.18	3.17	1.24	0.87	2.81	0.56
2-Ethyl-1-hexanol	17.49	1.53	1028	-	-	-		0.23	0.29	0.00	3.26	0.10	0.27	0.00	0.07	0.00	0.00	1.97	0.03	0.17	0.00
Limonene	17.55	0.94	1030	1030[$]	citrus, mint	0.01		1.62	0.76	1.26	0.26	0.00	0.00	0.00	0.56	0.74	0.32	0.00	0.00	0.00	0.00
3-Octen-2-one	17.82	1.64	1035	1030[£]	-	-		0.23	0.59	0.35	0.13	0.10	0.20	0.00	0.36	0.51	0.30	0.00	0.05	0.13	0.08
2,2,6-Trimethyl cyclohexanone	17.89	1.43	1036	-	-	-		0.40	0.48	0.00	0.00	0.10	0.16	0.18	0.82	0.45	1.06	0.39	0.07	0.13	0.26
Benzyl alcohol	17.89	2.48	1036	1036[$]	sweet, fruity	10	y	0.39	0.31	0.23	0.25	0.22	0.44	0.00	0.57	0.28	0.40	0.21	0.09	0.25	0.00
Phenyl acetaldehyde	18.35	1.99	1045	1046[$]	honey-like	0.0063	x	0.44	3.66	0.74	0.09	3.37	5.64	0.80	0.25	2.50	0.19	0.10	1.47	2.52	0.56
(E)-2-Octenal	19.15	1.71	1059	1049[£]	green, nut, fat	0.004		0.47	0.79	0.87	0.33	0.29	0.47	0.50	0.64	0.64	0.59	0.28	0.14	0.27	0.51
3,5,5-Trimethyl-2-cyclohexen-1-one	19.22	1.50	1061	-	-	-		0.26	0.30	0.51	0.15	0.00	0.11	0.00	0.74	0.30	0.88	0.30	0.05	0.11	0.13

(E,E)-3,5-Octadien-2-one	19.75	1.88	1070	1068[£]	geranium-like	0.0005		2.04	2.35	3.68	1.38	0.68	1.47	0.39	4.44	2.64	4.60	2.74	0.45	1.33	0.53
1-Octanol	19.82	1.60	1072	1063[£]	moss, nut, mushroom	0.11		0.30	0.41	0.24	0.32	0.20	0.27	0.26	0.26	0.41	0.19	0.38	0.10	0.21	0.24
cis-Linalool-3,6-oxide (I)	19.89	1.43	1073	1067[£]	sweet floral, citrus, fruity	-	y	0.42	1.26	0.83	0.40	0.39	0.57	0.19	2.44	1.83	3.19	1.61	0.36	0.67	0.23
Heptanoic acid	20.15	2.86	1078	1083[£]	rancid	3		0.08	0.04	0.00	0.86	0.12	0.15	0.00	0.23	0.01	0.35	0.19	0.04	0.10	0.00
2-Cyclohexen-1-one	20.29	2.44	1080	-	-	-		0.08	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Methyl benzaldehyde	20.55	1.88	1085	1077[£]	-	-		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
trans-Linalool-3,6-oxide (II)	20.75	1.47	1089	1084[£]	sweet floral, citrus, fruity	-	y	0.88	2.76	1.57	0.55	1.01	1.48	0.41	5.88	4.47	8.48	4.83	1.09	1.92	0.61
(Z,E)-3,5-octadien-2-one	21.09	1.92	1095	1096[£]	-	-		0.41	0.65	0.64	0.37	0.19	0.42	0.11	0.85	0.63	0.68	0.59	0.09	0.30	0.11
Linalool	21.42	1.53	1101	1100[$]	citrus	0.0006	xy	2.00	7.42	2.12	1.01	2.89	3.99	2.89	14.90	16.39	14.65	9.15	3.76	6.60	4.63
6-Methyl-3,5-heptadien-2-one	21.55	1.92	1104	-	-	0.38		0.12	0.15	0.00	0.00	0.06	0.13	0.00	0.27	0.18	0.31	0.30	0.04	0.11	0.06
Hotrienol	21.55	1.60	1104	1101[£]	tropical	-	y	0.00	0.14	0.00	0.00	0.11	0.17	0.09	0.00	0.35	0.00	0.00	0.07	0.14	0.13
Nonanal	21.69	1.47	1106	1105[$]	fatty, waxy, pungent	0.15		2.19	2.19	5.58	3.83	0.39	0.22	1.95	0.97	0.39	6.77	5.58	0.16	0.20	4.04
α-Thujone (ISTD)	21.95	1.67	1111	1102[£]	-	-		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,5-Dimethylcyclohexanol	22.09	1.88	1113	1099[£]	-	-		3.26	0.53	6.42	2.36	0.00	0.00	0.00	3.57	0.47	6.03	3.80	0.13	0.22	0.00
2-Phenyl ethanol	22.29	2.37	1117	1116[$]	honey-like	1	xy	0.36	0.58	0.71	0.40	0.62	1.31	0.10	0.46	0.64	0.59	0.58	0.31	0.77	0.07
β-Thujone (ISTD isomer)	22.55	1.60	1121	1112[£]	-	-		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
(E,Z)-2,6-Nonadienal	24.42	1.85	1154	1153[$]	cucumber-like	0.000003	x	0.08	0.11	0.15	0.19	0.07	0.11	0.13	0.09	0.10	0.16	0.17	0.03	0.05	0.15
(E)-2-Nonenal	24.82	1.74	1161	1160[$]	paper-like, fatty	0.9	x	0.11	0.20	0.35	0.28	0.07	0.10	0.34	0.40	0.22	0.40	0.22	0.03	0.04	0.35
1-Nonanol	25.22	1.64	1168	1165[£]	-	0.050		0.00	0.08	0.00	0.16	0.08	0.12	0.19	0.00	0.12	0.00	0.12	0.05	0.09	0.23
cis-Linalool-3,7-oxide (IV)	25.49	1.88	1173	1170[£]	sweet floral, citrus, fruity	-	y	0.07	0.05	0.18	0.14	0.00	0.06	0.00	0.20	0.09	0.35	0.60	0.03	0.06	0.00
Octanoic acid	25.62	2.72	1175	1167[£]	sweaty	3		0.08	0.04	0.57	0.35	0.24	0.57	0.00	0.18	0.02	0.67	0.23	0.07	0.25	0.00
trans-Linalool-3,7-oxide (III)	25.75	1.92	1178	1173[£]	sweet floral, citrus, fruity	-	y	0.22	0.26	0.91	0.72	0.21	0.34	0.00	0.90	0.43	1.61	2.48	0.16	0.33	0.00
Methyl salicylate	26.75	1.78	1195	1194[$]	-	-	y	0.37	6.31	0.82	0.60	3.50	4.97	4.06	3.51	9.67	4.66	6.37	3.23	6.11	7.98
2,3-Dihydro-2,2,6-trimethylbenzaldehyde (safranal)	27.15	1.78	1202	1196[£]	saffron	-		0.09	0.27	0.00	0.21	0.14	0.18	0.22	0.26	0.37	0.40	0.36	0.05	0.13	0.28
Decanal	27.42	1.50	1207	1207[$]	penetrating, sweet, waxy	0.65		1.17	0.41	3.95	6.89	0.61	0.13	0.88	0.37	0.24	1.09	2.59	0.15	0.35	0.92
(E,E)-2,4-Nonadienal	27.82	1.99	1215	1215[$]	fatty, green	0.00016	x	0.00	0.04	0.16	0.19	0.06	0.10	0.18	0.00	0.00	0.12	0.17	0.03	0.06	0.16
β-Cyclocitral	28.29	1.78	1223	1217[£]	fruity	0.005		0.68	0.53	1.85	1.19	0.00	0.27	1.06	1.46	0.74	2.81	3.34	0.14	0.31	1.64
Geraniol	29.89	1.81	1252	1250[$]	rose-like	0.0032	xy	0.10	0.36	0.34	0.30	0.90	1.58	1.35	0.26	1.03	0.60	1.22	0.96	2.42	2.90
(E)-2-Decenal	30.42	1.81	1262	1260[£]	painty, fishy, fatty	0.01		0.00	0.03	0.31	0.12	0.02	0.03	0.18	0.00	0.03	0.07	0.00	0.00	0.02	0.22
Geranial	30.75	1.92	1268	1267[$]	citrus	0.032		0.00	0.05	0.00	0.00	0.08	0.12	0.21	0.00	0.15	0.00	0.16	0.04	0.10	0.34
(E)-2-Phenyl-2-butenal	30.89	2.30	1271	1273[£]	-	-		0.00	0.09	0.00	0.00	0.18	0.41	0.16	0.00	0.07	0.00	0.00	0.07	0.20	0.13
Nonanoic acid	30.89	2.58	1271	1267[£]	sweaty, waxy	3	y	0.05	0.04	1.20	0.07	0.45	0.44	0.18	0.23	0.04	0.07	0.17	0.07	0.26	0.18
3,3-Dimethyl-2,7-octanedione	31.15	2.69	1276		-	-		0.00	0.00	0.00	0.94	0.00	0.00	0.12	0.19	0.00	0.00	0.86	0.00	0.11	0.08
2-Undecanone	32.09	1.57	1293	1293[£]	fruity	0.007		0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.05
(E,Z)-2,4-Decadienal	32.29	1.92	1296	1292[£]	deep-fried	0.01		0.00	0.01	0.00	0.04	0.09	0.15	0.81	0.00	0.01	0.00	0.00	0.04	0.07	0.68
Undecanal	32.89	1.50	1308	1305[£]	sweet, fatty, waxy-floral-citrus	0.005		0.00	0.00	0.38	0.75	0.05	0.00	0.08	0.00	0.00	0.00	0.41	0.00	0.00	0.08
(E,E)-2,4-Decadienal	33.49	2.02	1319	1319[$]	deep-fried	0.18	x	0.00	0.03	0.00	0.09	0.12	0.22	1.01	0.00	0.02	0.05	0.21	0.04	0.17	1.03
trans-Geranic acid	35.35	2.62	1355		-	-	y	0.00	0.00	0.00	0.00	0.53	1.37	0.24	0.00	0.00	0.00	0.00	0.28	1.38	0.30
Dihydro-5-pentyl-2(3H)-Furanone (γ-nonalactone)	35.69	3.00	1362		fruity, peach-like	0.030		0.00	0.02	0.43	0.26	0.35	0.21	0.18	0.00	0.00	0.00	0.22	0.06	0.14	0.16
(E)-2-Undecenal	35.89	1.78	1365	1357[£]	-	-		0.00	0.00	0.00	0.15	0.00	0.00	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.18
Decanoic acid	36.09	2.48	1369	1364[£]	soap-like, fatty	10	y	0.00	0.01	0.94	0.52	0.26	0.27	0.41	0.00	0.00	0.25	0.14	0.06	0.16	0.23
ϐ-Damascenone	36.75	1.78	1382	1383[$]	fruity	0.000004	x	0.00	0.06	0.00	0.00	0.07	0.08	0.50	0.00	0.00	0.00	0.00	0.03	0.05	0.41
2-Dodecanone	37.42	1.54	1395	1388[£]	-	-		0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00	0.00
Vanillin	37.69	3.38	1400	1400[$]	vanilla-like	0.020	x	0.00	0.03	0.00	0.00	0.17	0.32	0.00	0.05	0.00	0.00	0.00	0.00	0.19	0.00
Dodecanal	38.22	1.54	1411	1408[£]	fatty, citrus-like	0.002		0.00	0.00	0.55	1.39	0.00	0.00	0.16	0.00	0.00	0.16	0.56	0.00	0.00	0.13
α-Ionone	38.95	1.92	1425	1426[$]	violet-like	0.005		0.15	0.06	0.78	1.11	0.09	0.12	1.17	0.14	0.08	0.49	1.44	0.04	0.07	1.05
Geranyl acetone	40.15	1.71	1449	1453[£]	magnolia, green	0.060		0.27	0.16	3.15	3.76	0.13	0.14	2.16	0.33	0.24	1.85	4.39	0.12	0.13	2.54
1-Dodecanol	41.49	1.60	1476	1469[£]	-	-		0.00	0.00	0.62	0.49	0.00	0.00	0.47	0.00	0.00	0.36	0.30	0.00	0.00	0.17
ϐ-Ionone	41.82	1.95	1483	1484[$]	violet-like	0.0002	xy	0.44	0.22	2.76	4.52	0.51	0.59	6.62	0.48	0.30	1.65	5.97	0.23	0.40	6.86
5,6-Epoxy-5,6-dihydro-β-ionone	41.95	2.20	1485		fruity, floral, woody	-		0.26	0.08	1.25	2.15	0.68	1.27	2.46	0.21	0.19	0.81	2.99	0.34	0.82	2.39
2-Tridecanone	42.42	1.54	1495	1495[£]	-	-		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Tridecanal	43.22	1.50	1511	1509[£]	waxy, citrus	-		0.00	0.00	0.32	0.46	0.00	0.00	0.10	0.00	0.00	0.11	0.21	0.00	0.00	0.03
Methyl laurate	43.82	1.33	1524		-	-		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Dihydroactinidiolide	44.29	3.52	1534	1539[£]	sweet, tea-like	-	y	1.37	0.09	5.89	10.16	5.56	6.88	6.14	0.84	0.08	2.76	11.25	3.03	4.67	6.18
Dodecanoic acid	45.82	2.27	1567	1565[£]	fatty	10		0.00	0.00	0.00	0.00	0.00	0.00	1.87	0.00	0.00	0.00	0.00	0.00	0.00	1.00
2-Tetradecanone	47.22	1.60	1597		-	-		0.00	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tetradecanal	48.02	1.54	1615	1611[£]	fatty, waxy, citrus odor	0.060		0.00	0.00	0.31	3.24	0.00	0.00	0.07	0.00	0.00	0.11	1.78	0.00	0.00	0.00
Methyl-(Z)-jasmonate	49.35	2.37	1645	1648[£]	floral, sweet, fruity	-	y	0.00	0.00	0.00	0.00	0.06	0.12	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.40
cis-Methyl dihydro jasmonate	49.69	2.30	1652	1654[£]	-	-		0.00	0.00	0.50	0.28	0.05	0.06	0.30	0.00	0.00	0.19	0.21	0.00	0.00	0.15
Tridecanoic acid	50.15	2.13	1663	1678[£]	-	-		0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.13
trans-Methyl dihydro jasmonate	50.89	2.23	1679	1682[£]	-	-		0.00	0.00	0.11	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1-Tetradecanol	50.89	1.54	1679	1671[£]	-	-		0.00	0.00	0.60	0.88	0.00	0.00	0.33	0.00	0.00	0.29	0.35	0.00	0.00	0.09
2-Pentadecanone	51.75	1.57	1698	1697[£]	-	-		0.00	0.00	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00
Pentadecanal	52.49	1.54	1716	1710[£]	-	-		0.00	0.00	0.00	2.81	0.00	0.00	0.00	0.00	0.00	0.00	1.28	0.00	0.00	0.19
Tetradecanoic acid	54.62	2.16	1767	1768[£]	fatty	10		0.00	0.00	0.00	0.69	0.00	0.00	7.04	0.00	0.00	1.49	0.00	0.00	0.00	1.80
Hexadecanal	56.82	1.57	1820	1819[£]	-	-		0.00	0.00	0.46	12.10	0.00	0.00	0.00	0.00	0.00	0.00	6.17	0.00	0.00	0.00
Isopropyl myristate	57.02	1.33	1825	1825[$]	-	-		0.00	0.00	1.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00
Caffeine	58.02	3.70	1849	1848[$]	-	-		0.60	0.25	17.12	13.94	45.00	23.37	28.12	2.94	1.14	3.85	0.53	67.40	38.12	38.61
Pentadecanoic acid	58.69	2.06	1866	1866[£]	-	-		0.00	0.00	0.00	0.00	0.00	0.00	2.68	0.00	0.00	0.50	0.00	0.00	0.00	0.00
1-Hexadecanol	59.22	1.54	1879	1874[£]	-	-		0.00	0.00	0.57	0.87	0.00	0.00	0.19	0.00	0.00	0.17	0.31	0.00	0.00	0.06
Heptadecanal	60.69	1.50	1916	1897[£]	-	-		0.00	0.00	0.00	0.78	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00
Hexadecanoic acid	62.69	2.09	1968	1959[£]	-	-		0.00	0.00	0.00	2.21	0.00	0.00	14.06	0.00	0.00	0.00	0.77	0.00	0.00	2.18
Octadecanal	64.55	1.50	2018	2021[£]	-	-		0.00	0.00	0.00	2.35	0.00	0.00	0.00	0.00	0.00	0.00	1.38	0.00	0.00	0.00

* Analytes confirmed by reference standards analysis are reported in italics

$: experimental index from standard reference compound analyzed with the current column configuration

£: R. P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th ed. (Allured Publ., Carol Stream, IL,2007).

Rows are ordered according with $^{1}D\ I^{T}_{S}$ (apolar-medium polarity column combination) from left to right the retention index increases, and 2D Peak Volumes are normalized by dividing by column standard deviation; headspace (HS) and in-solution (IS) sampling are clustered together to make easier their comparison.