

Sensory-directed characterisation of distinctive aromas of Sauternes and Viognier wines through semi-preparative liquid chromatography and gas chromatography approaches

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1 Sensory-directed characterisation of distinctive aromas

- 2 of Sauternes and Viognier wines through semi-
- **3 preparative liquid chromatography and gas**
- 4 chromatography approaches

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6	Tracey E. Siebert ^{a,b,c,1,*} , Panagiotis Stamatopoulos ^{c,1,2} , I. Leigh Francis ^{a,b} , Philippe
7	Darriet ^c
8	^a The Australian Wine Research Institute, PO Box 197, Glen Osmond, Adelaide SA 5064, Australia
9	^b School of Pharmacy and Medical Science, University of South Australia, G.P.O Box 2471, Adelaide, SA 5001,
10	Australia
11	^c Universty of Bordeaux, Unité de recherche Œnologie, EA 4577, USC 1366 INRAE, F-33140 Villenave
12	d'Ornon, France
13 14	*Corresponding author: tracey.siebert@awri.com.au
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¹ These authors contributed equally to this work.

² Hennessy, Rue de la Richonne – CS 20020, F-16101 Cognac Cedex, France

21 ABSTRACT

Gas chromatography-olfactometry-mass spectrometry (GC-O-MS) has been very 22 useful in identifying aroma compounds from within the complex matrix of wine. 23 Supplementary separation can be required to overcome co-elution of volatiles or other 24 sensory-directed chromatographic strategies are needed, including multidimensional 25 26 chromatography and preparative fraction collection coupled to GC. Studies investigating 'overripe orange' aroma in sweet Sauternes wine and the similar 'apricot' aroma in Viognier 27 wine were conducted. Wines with the targeted aroma attributes were selected and 28 concentrated wine extracts prepared. GC-O found no individual aroma compounds with the 29 targeted aroma attribute. Semi-preparative HPLC was used to obtain less complex fractions of 30 the wine extracts. The fractions were eluted in water/ethanol and, therefore, could be smelled 31 directly. Fractions with the targeted aroma character were further resolved by GC-preparative 32 fraction collection (GC-PFC). Recombinational GC-PFC demonstrated the importance of the 33 34 components within a 4 min preparative GC fraction to the 'overripe orange' aroma of typical Bordeaux dessert wine. In Viognier wine, monoterpenes linalool, α-terpineol and geraniol as 35 well as benzaldehyde were found to be associated with the 'apricot' character. Thus, several 36 wine aroma compounds interact for these specific aromas to be perceived. This sensory-led 37 combination of separation techniques is a powerful tool for the identification of key 38 39 compounds responsible for specific aromas across the wine and beverage industries.

41

1. Introduction

42 Wine is a beverage enjoyed by many consumers across the world and there is a large world-wide industry producing many varieties and different styles of wine. In recent years, 43 new markets have emerged, especially in Asia, because of changing tastes and higher incomes 44 [1]. Research to understand the distinct flavours important to specific varieties and styles is 45 highly valuable to the wine industry as it provides them with information on how viticulture 46 and winemaking practices can be managed and improved to consistently produce specific 47 wine styles. While several flavour properties of white wines are relatively well understood, 48 there remain certain important characteristics where the causative volatile compounds are not 49 known. 50

Wine is a very complex matrix. Gas chromatography coupled to olfactometry and 51 mass spectrometry (GC-O-MS) has often been very useful in identifying aroma-active 52 53 compounds in this beverage [2-4]. However, there can be occasions when the separation power of GC is not enough, and further chromatographic resolution is required to overcome 54 55 co-elution of volatiles or masking of aromas at the sniff-port. Multidimensional gas chromatography techniques coupled to olfactometry and mass spectrometry detection are thus 56 efficient alternatives[5]. Furthermore, the aromatic component of wines perceived by the 57 58 tasters, does not result from an algebraic sum of individual odorous compounds but has been shown to be the result of the presence of both a particular mixture of volatiles and the 59 response from cognitive processing. To recognize a particular aroma character requires the 60 integration of complex mechanisms at the brain level, including central and peripheral 61 processes [6]. Consequently, with a combination of aroma compounds being required for a 62 particular aroma to be perceived, alternative strategies are needed to progress in the 63 characterisation of wine aroma component. 64

Among the techniques implemented, semi-preparative chromatographic approaches, 65 including LC and GC approaches coupled with olfactory detection, have proved to be relevant 66 [7]. They allow the fractionation of wine extracts to obtain a lower complexity. Interest in 67 these approaches is related to combining with sensory reconstitution and omission tests to 68 confirm the relevance of the odorous compounds for the matrix. Among these techniques, a 69 specific methodology involving liquid-liquid extraction of wine followed by fractionation of 70 the extracts by semi-preparative reversed-phase HPLC using water and ethanol as solvents, 71 permitted assessment of odorous fractions and gave the possibility for identification of 72 volatile compounds. This technique, initially developed by Ferreira, Hernández-Orte, 73 74 Escudero, López and Cacho [8] and adapted by Barbe, Pineau and Ferreira [9], played a crucial role in the identification of several odorous compounds [10-12] among them ethyl 2-75 hydroxy-4-methylpentanoate, a compound involved in blackberry aroma in red wines [13]. 76 77 Notably, the elution order from the reversed-phase HPLC column was quite different to that observed from a GC column and in this technique the HPLC fractions collected can be 78 79 directly assessed for aroma characteristics, either individually or in combination. In addition, studies using reconstitution and omission methodologies involving semi-80 preparative GC, GC-recomposition-O (GC-R-O), have been successfully implemented to 81 reconstitute the perception of several extracts from natural sources [14-16]. This was recently 82 illustrated through the study of 'lavender' aroma and in the direct and indirect evaluation 83 effects of selected compounds on characteristic aroma attributes of Angostura bitters [17, 18]. 84 In these works, headspace-solid-phase microextraction (HS-SPME) was used to extract the 85 volatiles. The volatiles were separated by GC, recombined selectively in-line utilising a 86 switching device and cryogenic trap and then released for olfactometry evaluation. Also, GC-87 preparative fraction collection (GC-PFC) has been used, in combination with olfactometry, to 88 identify the compound responsible for a 'minty' aroma, p-menth-1-en-3-one, in some red 89

wines [19]. This method was also useful for selectively collecting larger quantities of several
compounds from within several repeat GC runs [20]. Thus, this approach lends itself to
collecting several compounds from a GC elution zone in one trap or, potentially, recombining
several compounds from different retention times in one trap. Thus, rather than assessing the
olfactive behaviour of mixtures prepared from reference standards, samples can be prepared
directly from wine fractions, preserving the targeted wine aroma and isolating the aroma
compounds involved.

Viognier wine is often characterised as having a distinct varietal 'apricot' aroma
attribute [21, 22]. A GC-O-MS study of Viognier wine indicated 'stone fruit' aroma was
caused by a mixture of aroma compounds, including monoterpenes [23]. Sweet botrytised
dessert wines, such as Sauternes, are produced from ripe grapes affected by the *Botrytis cinerea* fungus. Some typical aroma descriptors of Sauternes wines include 'honey', 'apricot',
'peach', 'butterscotch', 'coconut', 'spice', 'pineapple', 'tropical fruit' and particularly
'marmalade' and 'orange peel' notes [24, 25].

104 Building upon the knowledge from the GC-O-MS detailed in Siebert, Barter, de 105 Barros Lopes, Herderich and Francis [23] and the protocols of Falcao, Lytra, Darriet and Barbe [13] and Pons, Lavigne, Darriet and Dubourdieu [19], the aim of this work was to 106 identify two examples of typical white wine aroma nuances, i.e., the 'overripe 107 orange/marmalade' character and the related 'apricot' character in Sauternes wine and in 108 Viognier wine respectively using HPLC fractionation and GC-PFC methodologies, applying a 109 novel sensory directed approach with semi-preparative LC and GC in order to progress in the 110 evidence of key odorous compounds in wines. 111

112

113 **2.** Materials and Methods

114 2.1. Chemicals and reference compounds

115	Dichloromethane (99.99%) was supplied by Fischer Scientific (Illkirch, France) and
116	absolute ethanol (99.9%) by Merck (Semoy, France). The ethanol (Merck) was redistilled in-
117	house for use in sensory evaluations and for HPLC mobile phase. Water was obtained from a
118	Milli-Q purification system (Millipore, Millipore, Bedford, MA, USA). The reference
119	compounds 2-phenylacetaldehyde (90%), 6-heptyloxan-2-one (δ-dodecalactone; 96%), 2-
120	ethyl-4-hydroxy-5-methylfuran-3-one (homofuraneol; 97%), and 3-sulfanylhexan-1-ol (96%)
121	were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and 3,7-dimethylocta-
122	1,6-dien-3-ol (linalool; 97%) from Lancaster Synthesis (Bischheim, France). Standard
123	solutions of the reference compounds were prepared in dichloromethane (10 mg/L).
124	
125	2.2. Wine Samples
126	All wines were commercially produced with the basic chemical composition shown in
127	Table S1. Wines were evaluated by experienced and trained wine tasters from within the
128	oenology research laboratory staff at the Institut des Sciences de la Vigne et du Vin (ISVV),
129	University of Bordeaux.
130	The four botrytised-style sweet white dessert wines selected consisted of three typical
131	Sauternes AOC (Denomination of Appellation Origin) wines from four to six years old, with
132	one similar style sweet dessert wine, AOC Loupiac, (two years old) plus one dry white wine,
133	AOC Entre-deux Mers (four years old), all from the Bordeaux region. All wines were made
134	from the same three grape varieties: Sauvignon Blanc, Sémillon, and Muscadelle. As part of

- the expected overall wine aroma, the three typical Sauternes dessert wines (TD1 3) were
- 136 sensory orthonasally evaluated to have 'over-ripe orange' character by a group of experienced
- 137 wine tasters (n = 3) whereas the non-typical Loupiac dessert wine (NTD) and dry white wine
- 138 (DW) did not, and these two wines were treated as negative controls. Wine selection is further

discussed in Section 3. The wines were purchased directly from wineries except the Loupiacdessert wine which was donated by the winery.

Dearomatized wine was prepared according to Lytra, Tempere, de Revel and Barbe 141 [26] by removing the volatiles of the typical dessert wine TD3 using a rotary evaporator (20 142 °C; Laborota 4010 Heidolph, Germany), reconstituting the dearomatized wine to its original 143 volume and alcohol concentration with ethanol and water, and a final treatment with a direct 144 145 addition of LiChrolut EN resin (40–120 µm; Sigma-Aldrich) then stirred (12 hrs) and filtered. French Viognier wines of respected wine brands from the Rhône Valley, including 146 AOC Condrieu and Vin de Pays des Collines Rhodaniennes, together with one 147 148 Rousanne/Marsanne wine, AOC Saint-Joseph, were purchased from several wine retail outlets or directly from wineries. The wines were sourced from wineries considered to regularly 149 produce wines described as having varietal 'apricot' character. The wines were assessed 150 151 independently under blind conditions in a dedicated sensory laboratory by a group of experienced wine tasters (n = 7). The tasters were asked to describe any 'stone fruit' attribute 152 and rate its intensity as none, low, medium or high. Following independent assessment using 153 free choice notes, the samples were discussed. Five wines with an obvious 'apricot' aroma 154 attribute, i.e. moderate to high intensity, were selected initially, with a sixth wine included 155 156 subsequently (V1 - 6). The wines were up to three years old.

157

158 *2.3*.

Reversed-phase HPLC fractionation

159 2.3.1. Crude wine extracts for HPLC fractionation

Samples were prepared by liquid-liquid extraction (LLE) as described previously [27].
For the dessert wines sample set, a 750 mL wine sample was progressively extracted using 60,
60 and 40 mL of dichloromethane. The combined organic phases were dried (anhydrous
sodium sulfate), concentrated using a rotary evaporator (20 °C; R-114 from Buchi, Rungis,

France) to approximately 2 mL, and then were further concentrated under nitrogen flow (100 mL/min) in order to obtain 750 μ L of crude wine extract. The same protocol was utilized for the Viognier wines except 700 mL of wine was used for the LLE and the crude wine extract was subsequently reduced to approximately 1000 μ L.

168 2.3.2. Semi-preparative HPLC

Fractionation of the crude wine extracts was achieved utilizing an Ultimate 3000 semipreparative HPLC system (Dionex, Courtaboeuf, France) according to a published procedure [27]. In summary, after injection of a wine extract (250 μ L) onto a Novapak C18 column (300 mm × 7.8 mm, 6 μ m; Waters, Saint Quentin, France) plus guard column, fifty individual fractions of 1 mL were collected by using gradient elution of water to ethanol, 0–100%.

174 2.3.3. Sensory evaluation of HPLC fractions

The aroma of every HPLC fraction from each crude wine extract was evaluated 175 directly from the collection vial (screw cap HPLC vial, 2 mL; Agilent) by experienced wine 176 tasters (assessors; n = 3 for dessert wines; n = 2 for Viognier wines). For Viognier wines, 177 several fractions perceived as the most intense in 'fruity' aroma were then included in a subset 178 of 10 sequential fractions (33-42) for further sensory assessment. The selected 10 fractions 179 were transferred into standard black wine tasting glasses, Association Française de Normes 180 (AFNOR), and assessed by a larger group of panellists (n = 5) under blind conditions. The 181 panellists provided free choice notes and also noted any 'stone fruit', 'apricot' or 'peach' 182 attributes. 183

Difference testing was performed as triangle tests, described by Martin and de Revel [28]. For dessert wine reconstitutions, the panel consisted of 15 panellists, 5 males and 10 females of 30.5 ± 4.6 (mean \pm SD) years of age. For Viognier wine reconstitutions, the panel consisted of 11 panellists, 5 males and 6 females of 32.9 ± 7.9 (mean \pm SD) years of age. Only the aroma of the reconstituted wines was evaluated by the panels. All panellists belonged to the oenology research laboratory staff at the ISVV. The panellists were selectedfor their experience in assessing fruity aromas.

For sensory reconstitutions studies utilising the HPLC fractions, relevant fractions 191 were combined then diluted with ethanol and water to obtain an ethanol level of 14% (v/v) 192 and to reproduce the initial concentrations in the original wines. For the dessert wine samples, 193 a second set was prepared in dearomatized wine. Samples (50 mL) were evaluated at 194 controlled room temperature (20 °C) in individual booths using covered black AFNOR 195 glasses that were coded with random three-digit numbers, except 20 mL was used for 196 reconstitutions of Viognier wines. Sessions lasted approximately 10 min. 197 198 For triangle tests, three samples were presented in random order. Two samples were identical and the third one was different. Each panellist was asked to select the sample in the 199 set that was different from the other two, even if they were not sure. Data analysis to 200

determine statistical significance was carried out using the binomial model as in the
prescribed tables [28].

203 2.3.4.

Back-extraction of HPLC fractions

Each fraction (1 mL) assessed as having an aroma of interest was diluted with water to obtain approximately 12% ethanol (v/v) and back-extracted with dichloromethane (3 × 1 mL). The organic phases were combined, dried (anhydrous sodium sulfate) and concentrated to 250 μ L under nitrogen for GC analysis analysis.

- 208
- 209 2.4. Gas chromatography

210 2.4.1. GC-O analysis

Gas chromatography-olfactometry (GC-O) was performed utilizing a HP5890 series II
 GC (Agilent Technologies, Palo Alto, CA, USA), fitted with a standard split/splitless inlet,

flame ionization detector (FID), and sniffing port (ODO-1; SGE, Ringwood, Australia). A

BP-20 (50 m \times 0.22 mm i.d \times 0.25 µm film thickness; SGE) or a HP-5 capillary column (30 214 215 $m \times 0.25$ mm i.d. $\times 0.25$ µm film thickness; Agilent) were used with hydrogen as carrier gas (Air Liquide, Floirac, France) and column head-pressure was set to obtain a 1 mL/min 216 nominal flow rate (100 kPa or 82 kPa respectively). Manual liquid injections (2 µL) were 217 performed in splitless mode, the inlet was fitted with a deactivated glass liner (glass wool 218 inserted, 4mm i.d.; Agilent) and held at 230 °C, and the splitter was opened after 1 min (purge 219 flow, 50 mL/min). The oven temperature of 45 °C was held for 1 min, then raised to 230 °C at 220 3 °C/min and then held for 20 min. Dessert wine extracts were assessed by experienced wine 221 tasters (n = 3) and Viognier wine extracts were assessed by one panellist, from after the 222 223 solvent front up to 60 min. Calculated linear retention indices (LRI) were obtained by injection of a series of alkanes (C7–C23) with the relevant GC column installed. 224

225 2.4.2. Preparative gas chromatography

For the trapping temperature optimization, recovery tests and analysis of dessert wine fractions, the GC-PFC system used is shown in Fig. 1. The system consisted of a HP5890 Series II GC (Agilent Technologies, Palo Alto, United States) equipped with a FID, sniffing port (ODO-1; SGE) and preparative fraction collector (PFC; Gerstel, Mülheim an der Ruhr, Germany) connected via a heated (230°C) transfer line. The PFC consisted of an eight-port zero-dead volume valve in a heated interface and was connected to a Gerstel 505 controller to establish the trapping zones.

Capillaries of deactivated fused silica tubing (0.32 mm i.d.) were fitted from the PFC switching device to all PFC traps with seven 100 μ L glass U-tube traps (six sample traps and one waste trap) installed. The traps were cryogenically cooled with liquid nitrogen at controlled temperature. The compound separations were achieved using a HP-5 'megabore' column (30 m × 0.53 mm × 1.5 μ m film thickness; Agilent) connected to a 0.87 m × 0.32 mm i.d. segment of deactivated fused silica tubing, which was threaded through the transfer line from the GC oven directly to the PFC. Manual liquid injections (2 µL) were performed in
splitless mode (230 °C, purge time: 1 min, purge flow: 50 mL/min). The GC oven
temperature was programmed from 45 °C for 1 min and then raised to 230 °C at a rate of
3 °C/min and held for 20 min. Hydrogen was used as carrier gas (Air Liquide) with a constant
head pressure of 22 kPa (1.2 mL/min nominal flow rate). Column effluent was transferred
automatically from the main column to the traps via the switching device at defined cut times
and trapped by the PFC.

During optimization of PFC trapping temperature and recovery tests, the U-tubes containing the trapped compounds were rinsed with dichloromethane ($4 \times 250 \mu$ L), concentrated under nitrogen flow (100 mL/min) to obtain 100 μ L. Different trapping temperatures were assessed (-10, -20, -30, -40, -50 and -100 °C).

The retention time of the aroma zone of interest was confirmed with the HP-5 megabore GC column installed into the sniffing port (Fig. 1). Subsequently with the system reconfigured for GC-PFC (Fig. 1), five successive injections ($5 \times 2 \mu L$) of crude wine extract or back-extracted HPLC fractions were fractionated and trapped (-40 °C) with defined PFC cut times: 0 - 36 min; 36 - 40 min; and 40 - 82.66 min. The traps were individually rinsed with dichloromethane ($4 \times 250 \mu L$), the washings collected and concentrated to $20 \mu L$ under nitrogen.

The same GC-PFC instrumentation set up was used for the Viognier wine samples except the GC was fitted with a DB-Wax column (60 m × 0.25 mm i.d. with 0.25 μ m film thickness; Agilent). Column-head pressure was set at 103.4 kPa (1.4 mL/min nominal flow rate). The organic phases from the three most interesting fractions were combined, dried, and concentrated to 200 μ L under nitrogen. Manual liquid injections (6 × 2 μ L), of the threefraction extracts, were performed and the oven temperature commenced at 50 °C and was held for 1 min, then raised to 250 °C at 5 °C/min. The switching device and transfer line were

held at 250 °C with defined PFC cut times: 0 - 10 min to waste; 10 - 20 min to trap 1 (T1); 20
- 35 min to trap 2 (T2); 35 - 40 min to trap 3 (T3); and 40 - 51 min to waste.
Calculated linear retention indices (LRI) were obtained by injection of a series of
alkanes (C7–C23) with the relevant GC column installed into a FID under the same GC
conditions as the samples.

269 The concentrated trap washings were assessed for their aromas and by GC-MS270 analysis.

271 2.4.3. Sensory evaluation of GC-PFC fractions

For aroma assessment of GC-PFC fractionation, concentrated trap washings (20 μ L) were applied to perfume blotter test strips. The assessments were performed promptly after the dichloromethane was allowed to evaporate from the strip, using the same sensory panels and under the same environmental conditions as described above. The panellists provided free choice notes to describe any aroma attributes and, for dessert wine samples, a triangle test was performed as outlined above (2.3.3).

278 2.4.4. GC-MS analysis

For the Viognier sample set, back-extractions of HPLC fractions using GC-MS 279 analysis was performed on a 6890 GC coupled to a 5973N mass selective detector (Agilent) 280 and equipped with a MPS2 multipurpose sampler (Gerstel). The instrument was fitted with 281 the same BP-20 column as for the GC-O analysis and the same parameters were used except 282 the carrier gas was helium (Air Liquide), at a constant flow rate of 1.2 mL/min (initial 283 nominal pressure 171 kPa), and injections were performed automatically. The MS quadrupole 284 temperature was set at 150 °C, and the source was set at 230 °C. The MS transfer line was 285 held at 240 °C. Positive ion electron impact spectra at 70 eV were recorded in the range of 286 m/z 35–350. 287

288	For the PFC-GC trap fractions, a 5977 GC-MS system (Agilent) was used in
289	simultaneous selected ion monitoring (SIM) and scan modes to allow for more sensitive
290	screening of the samples for γ - and δ -lactones. MS data was recorded for scan mode in the
291	range of m/z 35–350 and for SIM mode the ions monitored were m/z 85, 96, 99, 136 and 196.
292	Helium was used as carrier gas (Air Liquide) and column head-pressure set at 183.4 kPa (1
293	mL/min nominal flow rate). Automated liquid injections (2 μ L) were performed in splitless
294	mode, the inlet was fitted with a deactivated glass liner (glass wool inserted, 4mm i.d.;
295	Agilent) and held at 240 °C, and the splitter was opened after 2 min (purge flow, 50 mL/min).
296	The oven temperature of 45 °C was held for 1 min, then raised to 230 °C at 3 °C/min and then
297	held for 20 min.
298	Linear retention indices (LRI) were obtained by reverse calculation from the RIs of
299	known compounds in the crude wine extract.
300	Data analysis was performed using the MassHunter Qualitative Analysis software
301	(Agilent, version B.07.00). Aroma compound identity was achieved by chromatogram
302	deconvolution and comparison to mass spectral libraries (NIST11, Wiley275) then comparing
303	each compound's calculated linear retention index (LRI) to that found in the literature.
304	
305	3. Results and Discussion
306	At the outset, it was essential for suitable sample sets of wines to be chosen for study.
307	A subset of Bordeaux wines were selected for the dessert wine study from within a larger set
308	of wines utilised by Stamatopoulos, Frérot, Tempère, Pons and Darriet [27], Stamatopoulos
309	[29], comprising wines with 'overripe orange/marmalade' sensory properties as well as
310	examples without this character. A set of Viognier wines was specifically selected regarding
311	their 'apricot' attribute by a group of experienced wine tasters.

As presented below, a common approach for both target wine aromas was followed, using reverse phase HPLC, GC-O, and GC with preparative fraction collection, with sensory assessment as each stage.

315

316 *3.1. Reversed phase HPLC fractionation*

317 3.1.1. Sensory evaluation of HPLC fractions

Aromatic reconstitution and sensory evaluation of HPLC fractions was initially 318 conducted to identify fractions of sensory interest. Assuming 100% extraction efficiency, a 319 320 solvent extract from the equivalent of up to 250 mL of wine was injected onto the HPLC column. Compounds were eluted with a water/ethanol solvent system and collected in 50 321 individual 1 mL fractions. This allowed for the fractions to be smelled directly from the vial 322 323 or transferred to a wine glass for sensory assessment without toxic or malodorous solvent. Successful fractionation of crude aroma extracts was achieved by semi-preparative HPLC as 324 evidenced by the differing descriptors of the fractions. Table S2 (Supplementary material) 325 details the aromas noted (n = 2 assessors) in the 50 vials containing the HPLC fractions of 326 three Viognier wines (wines V1, V2 and V3) with 'apricot' attributes. 'Apricot' and/or 327 328 'peach' aroma was perceived in three sequential fractions, 38 - 40, across the three wines assessed (Table S2). Similarly, the 50 HPLC fractions of three Sauternes wines (wines TD1 -329 3) were assessed (n = 3 assessors) and 'overripe orange' aroma was perceived in two 330 331 sequential fractions, 37 – 38, across the three wines (Table S3).

Subsequently, the sensory panel (n = 5 assessors) evaluated the subset of ten sequential fractions, 33-42, across five Viognier wines (V1 – 5): the three fractions identified above; the five preceding 'fruity' fractions; and the following two. Table 1 summarises the descriptors provided by the sensory panel for the subset of 10 HPLC fractions. In agreement with the preliminary assessment of the HPLC fractions, 'apricot' aroma was detected,

together with other fruity notes, in the same fraction 39 from each of the five wines (V1 - 5)evaluated and also in fractions 38 and 40 for most of the wines (Table 1). From the initial wine bench tastings, wine V2 was considered to have the highest level of 'apricot' aroma and wine V3 the lowest.

The dessert wine samples were examined in a similar manner. A subset of the results 341 is highlighted in Table 2 providing a direct comparison of the aromas detected in seven 342 sequential HPLC fractions (35 - 41) from the three typical dessert wines, the non-typical 343 dessert wine, and the dry white wine. Clear aroma differences were noted between the wine 344 fractions by the sensory panel (n = 3), particularly fractions 37 and 38. 'Ripe orange' aroma 345 346 was smelled in the typical dessert wine fractions 37 and 38 whereas the same fractions in the non-typical dessert wine and the dry white wine were described as 'citrus' and 'floral'. Other 347 fractions had an aroma description common to both wine styles, for example 'banana' in 348 349 fraction 41.

It was fortunate for both studies that the aroma compounds needed to give the targeted aroma attributes eluted in just two or three sequential HPLC fractions. However, having the HPLC fractions in water/ethanol solvent system allows for easy blending of fractions and sensory assessment to find a particular aroma attribute, if needed.

354 To confirm the importance of the fractions with the aroma attributes of interest to the perception of overall wine aroma, reconstitution and omission sensory experiments were 355 conducted for both the dessert wines and Viognier wines. Full aromatic reconstitutions of the 356 wines were prepared by combining all 50 HPLC fractions together and partial aromatic 357 reconstitutions were prepared by omitting the fractions of interest (dessert wine TD3, 358 fractions 37 – 38; Viognier wines V1 and V6, fractions 38 – 40). For the typical dessert wine, 359 triangle tests showed significant differences ($P \le 0.01$) between the full aromatic 360 reconstitution and the partial aromatic reconstitutions sample in an aqueous ethanol solution 361

(Table 3). A similar result, almost significant (P < 0.1), was found when using dearomatized 362 wine as the matrix (Table 3). In contrast, no significant difference was found between the 363 same full and partial aromatic reconstitutions of the dry white wine (Table 3). The results 364 showed a clear effect of those specific HPLC fractions on the overall aroma of the 365 reconstituted dessert wines, and provided good evidence that the identified fractions contain 366 aroma compounds necessary for the expression of typical 'over-ripe orange' aroma in 367 Bordeaux dessert wines. Significant effects were seen for the Viognier wines assessed (Table 368 3), with the aroma compounds in HPLC fractions 38 - 40 contributing 'apricot' character. 369 Therefore, further investigation into the aroma compound composition of the fractions was 370 needed. 371

Surprisingly, the fractions of interest for both the dessert wines and the Viognier wines 372 were similar in elution number. However, it should be noted that the dessert wine study and 373 374 the Viognier wine study were conducted at different times. Thus, even though the same HPLC column was utilized, differences in the separation of aroma compounds would be expected. 375 Therefore, this similarity in fraction number was likely to be just coincidence. However, 376 'apricot' aroma was detected in fraction 39 from two dessert wines, TD2 and NTD. In 377 previous studies, higher concentrations of alkyl lactones, commonly described as having 378 379 'apricot' and 'coconut' aroma have been reported in sweet wines, such as botrytised wines and ice wines. [30, 31]. Fraction 39 from TD2 and NTD was found to contain several alkyl 380 lactones (data not shown). 381

382

383 *3.2. Gas chromatography*

384 3.2.1. GC-O analysis

Care was taken to consistently produce the crude LLEs and the HPLC fractions of the wines in the same manner, but no internal standard was added to avoid any exogenous aromas.

The back-extracted fraction 37 from dessert wine TD-3 was assessed by GC-O using 388 both polar phase (BP-20) and non-polar phase (HP-5) capillary GC columns. As with dessert 389 wine extracts in a related study [27], no 'overripe orange' aroma zone was evident using the 390 polar phase but with the non-polar phase an 'overripe orange' aroma was noted across an 391 unusually wide 1 min time period (LRI 1414 – 1443) (Table 4). Usually in GC-O with a 392 393 suitably set up instrument, an aroma can be smelled for around the same length of time as it 394 takes for the compound to elute off the column, that is the peak width, say 5 to 10 seconds. Therefore, it was not possible to identify compound(s) responsible for the 'overripe orange' 395 396 aroma by LRI. Further refining of the HPLC fraction was required to hone in on this aroma of interest. Therefore, a different strategy was needed to further fractionate the HPLC fraction. 397 An option explored was GC-PFC. 398

For the Viognier wine samples, the aromas of fractions 38-40 were further assessed by 399 GC-O and GC-MS for wines V2, V5 and V6. Both HS-SPME, using parameters similar to 400 that for wines previously [23], and also liquid injection of the LLE of the HPLC fractions 401 were compared for this set. As the HS-SPME-GC-O technique is solvent free, the aroma of 402 any early eluting compounds in the selected fractions could be evaluated, but no aromas of 403 interest were noted in the early part of the chromatogram. Stronger aromas were found for 404 LLE than with HS-SPME and separation of aromas was better when using a polar GC column 405 than the non-polar column. Therefore, the favoured option for the Viognier study was liquid 406

injection of the LLEs onto a polar (BP-20) column and the effluent was evaluated from 8 min
to 60 min (LRI 1050 to 2340).

409 Fig. 2 highlights the difference observed between the three fractions (38-40) in their 410 volatile profiles by GC-MS and their major aroma-active zones. 3-Methylbutyl acetate, 411 corresponding to 'banana candy' aroma, was present but decreased in concentration across the 412 three fractions, whereas ethyl hexanoate, giving a 'pineapple' aroma, increased. The 'citrus' 413 and 'floral' smelling monoterpenes, linalool and α-terpineol, were present as well as β-414 damascenone, with a 'jam' aroma.

Table 5 summarises the aroma compounds frequently detected by GC-O and identified by GC-MS in LLE of HPLC fractions (38-40), providing aroma descriptors, GC retention indices and compound identity. However, no 'apricot' aromas were consistently detected by GC-O. These findings continue to support the hypothesis from a previous GC-O study that a combination of compounds was responsible for 'apricot' aroma in Viognier wines [23]. To investigate which combination of compounds were required, GC-PFC was considered to be a useful option.

422 3.2.2. Method development of GC-PFC

Previous studies have shown the effect of altering various parameters of preparative 423 GC systems on the recovery of a range of volatile compounds [20, 32]. The GC-PFC system 424 was initially evaluated with five wine aroma compounds with different functional groups 425 (phenylacetaldehyde, δ -dodecalactone, homofuraneol and 3-sulfanylhexanol) to optimize 426 experimental conditions. Recovery tests with different trap temperatures were tested (-10, -10)427 -20, -30, -40, -50 and -100 °C). The recovery (%) was calculated by comparing the peak 428 heights of the reference compounds from the GC-MS scan runs of the concentrated trap 429 washings to a direct injection of the standard solutions, corrected for dilution. The trapping 430 temperature showed no major differences between the recovery of the reference compounds 431

432 (47 - 66%), except for the extreme temperature of -100 °C where the recovery was only 433 about 10% (Fig. 3). For analyses of wine fraction extracts, -40 °C was selected because of 434 smaller recovery deviations observed between injection series (Fig. 3).

The choice of trap temperature was also in agreement with another wine aroma study [19]. The reference compounds selected were wine aroma compounds, covered the LRI span of the GC-O aroma zone (Table 4) and a range of chemistries. Different recoveries were noticed between the reference compounds with δ -dodecalactone the highest (61%) and 3sulfanylhexanol the lowest (34%) but the reproducibility for individual compounds was consistent (80%) (Fig. 4).

441 3.2.3. Application of GC-PFC

442 Even though GC-PFC appeared to be a useful next-step to assist identifying the 443 important aroma compounds for the target aromas for the two wine studies, different approaches were needed for each target aroma. For the dessert wine, the 'overripe orange' 444 445 aroma active zone was detected by GC-O but it was over a wide retention time with no discrete peaks in the aromagram. Therefore, selecting precise cut times to capture just the 446 compounds eluting in retention time window of the 'overripe orange' aroma active zone could 447 further decrease the number of possible candidate aroma compounds involved in the targeted 448 aroma by removing some non-essential volatile compounds from the sample. For the Viognier 449 wine, no 'apricot' aroma active zone was detected by GC-O, but 'apricot' aroma was evident 450 in several sequential HPLC fractions. Therefore, the compounds required for 'apricot' aroma 451 might elute sporadically across the entire GC-PFC chromatogram. Thus, gradually adjusting 452 the GF-PFC cut times to trap smaller retention time windows, by omitting cuts or combining 453 them whilst still collecting 'apricot' aroma in one trap could, again, reduce the complexity of 454 the fraction. 455

456 Previously, studies utilised single injections of the sample solutions [20, 32] but, to 457 enable the compounds to be enriched within the traps, multiple injections ($5 \times 2 \mu L$ or 6×2 458 μL) were utilised for the present studies.

For further separation using GC-PFC, the organic phases from the back-extracts of the 459 three most interesting Viognier HPLC fractions (38 - 40) were combined, dried and 460 concentrated to 200 µL under nitrogen (F×3). Post GC-PFC, most of the concentrated 461 washings (15 μ L of 20 μ L) of individual cryogenically cooled traps, T1, T2 and T3, were 462 spotted onto perfume blotter paper strips and their aromas were compared to the triple fraction 463 extract (F×3) (6 µL). F×3 smelled very fruity, with a floral Viognier-like character, T1 had no 464 465 detectable aroma, T2 was 'fruity', 'apricot' and T3 was 'smoky', 'char'. The remaining content of each trap was utilised for GC-MS analysis. With the particular GC column utilised 466 (BP-20), geraniol co-eluted with hexanoic acid. However, the deconvolution software could 467 identify both compounds in the F×3 extract. In the trapped fractions, geraniol was compared 468 using the fragment ion m/z 69, which is the most abundant ion in a geraniol mass spectrum 469 but a minor ion in hexanoic acid. Due to the very low concentrations of lactones in the 470 samples the GC-MS data was recorded in SIM mode. Therefore, the relative amounts of the 471 lactones were compared using the respective SIM spectra of the three traps T1, T2 and T3. 472 473 Fig. 5 shows the relative amounts of volatile compounds (%) identified in the three traps compared to that in the LLE of F×3. The peak areas from the F×3 were adjusted to 474 account for the dilution of the traps' contents. Several compounds were overloaded in the Fx3 475 chromatogram: 3-methylbutyl acetate; ethyl hexanoate; α -terpineol; and octanoic acid. 476 Octanoic acid had saturated the MS detector, therefore, the peak area for a low abundance ion, 477 i.e. its molecular ion (m/z 144), was used to calculate the relative response. The components 478

that were contained within each trap are shown in Fig. 5. Any compounds eluting below LRI

480 1100 were directed to the waste, compounds eluting between LRI 1100 and 1515 were

directed to T1, compounds eluting between LRI 1515 and 2130 were directed to T2, 481 482 compounds eluting between LRI 2130 and 2330 were directed to T3, and any compounds eluting after LRI 2330 were directed to waste. Small amounts of compounds eluting around 483 trap switching time were found in two traps, e.g. furfural and benzaldehyde in T1 and T2, 484 (Fig. 5). Seventeen aroma compounds including the monoterpenes linalool, α -terpineol and 485 geraniol, benzaldehyde and γ -nonalactone were present in T2, which was described as having 486 an 'apricot' aroma on the perfume strip. The monoterpenes and benzaldehyde were also found 487 by Siebert, Barter, de Barros Lopes, Herderich and Francis [23] as being associated with 488 Viognier wines high in 'stone fruit' aroma and, in the same study, γ -nonalactone was 489 490 significantly higher in high 'stone fruit' Chardonnay and Viognier wines. γ -Nonalactone is described in literature as 'peach' and 'coconut'. However, the aroma was not detected 491 consistently at the corresponding RI (1368 on DB-5, 2065 on wax) in the GC-O evaluations 492 493 of the HPLC fractions. In the present study, the monoterpenes linalool and α -terpineol were described as 'citrus', 'floral' and 'fruity' but not 'apricot'. Geraniol and benzaldehyde were 494 495 not consistently detected by GC-O. When using the HP-5 megabore GC column installed in the GC-PFC system, the 496

retention time span for the 'over-ripe orange' aroma active zone was determined to be 36-40497 498 min by GC-O for both the crude extract of TD3 and the back-extracted fraction 37. Subsequently, preparative GC fractions were collected at defined PFC cut times: 0 - 36 min; 499 36 - 40 min; 40 - 82.66 min; or in combinations of those cut times. This technique enabled 500 volatile compounds to be cryogenically trapped, using liquid nitrogen, after their 501 chromatographic separation to study their sensory contribution. Omitting or not trapping 502 certain volatile compounds or groups of compounds is useful to study their impact on the 503 overall aroma of the sample [33]. 504

After GC-PFC, the concentrated washings (20 µL) of cryogenically cooled traps were 505 spotted onto perfume blotter paper strips and their aromas were assessed. Fig. 6 shows the 506 aroma profiles of the trapped fractions from the crude extract of a typical dessert wine (TD3). 507 The contents of the trap corresponding to the whole extract (0 - 82.66 min) was described as 508 'overripe fruits', 'citrus', 'floral', 'honey' and 'baked sugar' by the sensory panel. Whereas 509 the trap corresponding to the 0 - 36 min plus 40 - 82.66 min, but omitting 36 - 40 min, was 510 described as 'honey', 'creamy', 'yeasty' and 'spicy'. In a triangle test, the overall aroma of 511 the omission trap was found to be significantly different to the whole extract trap (P < 0.01). 512 As expected, the trap containing only the cut of 36-40 min was described as 'overripe orange', 513 but also 'dried apricot'. 514 HPLC fraction 37 from the typical Bordeaux dessert wine TD3 produced the best 515 example of 'overripe orange' aroma. Thus, GC-PFC analysis was repeated using the back-516 517 extracted HPLC fraction 37. Various combinations of trap cut times were utilised (Fig. 6) and the traps were assessed by the sensory panel (Fig. 6): (a) 0 - 82.66 min; (b) 0 - 36 min plus 40 518

519 - 82.66 min; (c) 36 - 40 min; (d) 0 - 36 min; and (e) 40 - 82.66 min. 'Overripe orange'

aroma was clearly evident in trap (c) together with 'dried apricot' aroma. It was also
interesting to note that the 'almond paste' aroma described in trap (b) was not described in
either trap (d) and (e). Therefore, the perception of 'almond paste' aroma must be due to a
combination of components within traps (d) and (e).

Furthermore, a triangle test showed a significant difference between the aromas of the 36 – 40 min trap contents from TD3 and NTD fraction 37 extracts (P < 0.01). 'Overripe orange' aroma was described in 36 – 40 min trap contents of the typical dessert wine (TD3) but not in the non-typical dessert wine (NTD), noted as more fresh fruit and lemon. There was also a difference noted in the intensity of the samples by the assessors was reported. Confirming the results found in the initial HPLC fraction sensory assessment (Table 2), such

that the 'overripe orange' aroma was not present in non-typical dessert wine fractions nor in
dry white wine fractions, noting all were Bordeaux wines made with the same grape varieties.
The results highlight the importance of the aroma compounds in the 36 – 40 min
fraction to the overall aroma of typical Bordeaux dessert wines aroma. The HPLC fractions
presented a less complex composition than the crude wine extract and the use of GC-PFC
analysis of the HPLC fractions was a further step towards ascertaining the aroma compounds
in the mixture perceived 'overripe orange'.

In a complementary study, the aroma compounds in the 36 - 40 min trap were 537 identified using multidimensional GC-MS-O [27, 29]. In brief, two interconnected GC-Os 538 539 (Agilent 6890) were utilised with GC-O#1 fitted with a BP-5 capillary column and FID, and GC-O#2 fitted with a BP-20 and MS. After determining the target 'overripe orange' aroma 540 active zone on GC-O#1, a 3 min heart-cut was transferred to GC-O#2 via a pressure-driven 541 542 switching valve (MCS; Gerstel). Aroma active zones described as 'coconut', 'spicy clove', 'ripe/fruity' and 'minty/fruity' were identified as *cis*-oak lactone, eugenol, γ -nonalactone and 543 2-nonen-4-olide respectively [27, 29]. Thus, a mixture of four aroma compounds were found 544 to interact for 'overripe orange' aroma to be perceived. 545

546

547 **4.** Conclusions

From extracts of typical Bordeaux dessert wines, from the Sauternes region, GC-O had revealed a 1 min wide aroma active zone with the targeted 'overripe orange' aroma attribute. Using an approach of sensory assessment of semi-preparative HPLC isolates, fractions presenting clear 'overripe orange' fruity aroma were obtained. Subsequent GC-O analysis of the HPLC fractions directed further fractionation using preparative GC with cryogenic trapping. Reconstitution and omission sensory experiments demonstrated the impact of 'overripe orange' nuances on the overall aroma of typical Bordeaux dessert wine.

The HPLC fractionation approach was also successfully applied to a set of Viognier 555 556 wines to isolate fractions with the related 'apricot' aroma. No individual aroma compounds could be found to be causal for this aroma attribute. However, GC-O and GC-PFC techniques 557 highlighted several compounds, notably linalool, α -terpineol, geraniol and benzaldehyde, that 558 are likely to contribute to 'apricot'. This further strengthened the concept that several aroma 559 compounds were likely to interact to produce the perception of 'apricot' aroma in wine, 560 561 specifically Viognier wine. Several alkyl lactones were also present, previously suggested to be involved in this aroma, but they appeared to have minimal impact regarding the perception 562 of 'apricot' aroma in Viognier wine. 563

564 The combination of separation techniques with a strong, simple and straightforward sensory-directed approach was inspired by the need for a different methodology for 565 identifying aroma compounds that are responsible for specific wine aroma attributes, 566 567 especially when the perception of an aroma is due to a mixture of aroma compounds within the complex matrix of wine. The use of small panel sensory methods, rather than time 568 consuming conventional procedures requiring large volumes of sample, allowed sufficient 569 rigour to make progress in tracking the target aroma attributes, while avoiding the use of a 570 single individual such as might have been used in the past. Exacting quantification of aroma 571 572 compounds is not required, and reconstitution and omission sensory experiments can be conducted easily with HPLC fractions and GC-PFC fractions. The described protocol is a 573 powerful tool for the identification of key aroma compounds responsible for specific aromas 574 across the wine and beverage industries as well as other food industries. 575

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Preparative Fraction Collector



Preparative Fraction Collector













Summary of aromas detected in a subset of HPLC fractions of five Viognier wines (n = 5 assessors).

Ensetien	Wine						
Fraction	V1	V2	V3	V4	V5		
33	Fruity, fresh, floral, spice, reduced, burnt, solvent	Fruity, reduced, chewing gum	Burnt, confectionary	Exotic fruit, confectionary reduced, off-flavour	Fruity, liquorice, spicy, reduced, Confectionary		
34	Fruit, spicy, floral, reduced, chewing gum	Green, cooked vegetable, fruity, spicy	Oak, mint, spice, reduced, solvent	Oak, spicy, vanilla, chewing gum	Vanilla, oak, spice		
35	Bread, spice, solvent	Green, spicy, oak, floral	Oak, green, spicy, mint, low peach	Green, cooked vegetable, floral	Floral, oak, spice, rubber		
36	Green, confectionary, pear	Fresh, fruity, spicy, confectionary	Green, oak, spice	Strawberry, oak, fresh, confectionary	Liquorice, confectionary, strawberry		
37 (2)	Green, fruit, banana lolly, solvent, pear	Fresh, chemical, fruit, banana lolly, pear	Fresh, minty, fruit, banana lolly	Peach, pear, green, blueberry, banana lolly,	Ripe peach, oak, green, fruity, banana lolly, chewing gum		
38 (6)	Apricot, green, fruit, Burnt	Apricot, green, confectionary, fruit	Green, fruity, vegetal, spice, burnt	Apricot, floral, green, confectionary, lactate, burnt	Apricot, confectionary, marshmallow, strawberry		
39 (19)	Apricot, peach, exotic fruit, Muscat, mint	Apricot, exotic fruit, jam	Apricot, Muscat, fruity	Apricot, peach, exotic fruit, wine, Muscat	Apricot, Muscat, fruity, wine		
40 (7)	Peach, Muscat, fruit, minty, apple, pear	Peach, apricot, fruity	Apricot, peach, exotic fruit, Muscat, floral	Fresh, spicy, exotic fruit, Muscat, mint	Apricot, exotic fruit, Muscat, mint		
41	Fruity, apple	Floral	Spicy, fruit	Fresh fruit, spice	Floral violet, fruity, fresh, mint		
42	Spicy, fruit, solvent	Weak	Weak	Weak	Off-flavour, animal, weak		

Numbers in parentheses denotes the total number of times that 'apricot' or 'peach' descriptor was used

Summary of aromas detected in a subset of HPLC fractions of three typical and one non-typical Bordeaux dessert wines and one dry white wine (n = 3 assessors).

Fractions		Dry white wine			
	TD1	TD2	TD3	NTD	DW
35	Solvent	Fruity, exotic fruit	Floral, rose, artificial fruity	Floral, rose, spicy	Citrus, lemon, floral
36	Mouldy, hazelnut	Odourless	Citrus, minty, hazelnut	Citrus, thiols, spicy	Thiols, spicy, herbaceous
37	Ripe orange	Ripe orange	Ripe orange	Citrus, spicy	Citrus
38	Ripe orange, mouldy	Ripe orange	Ripe orange, woody	Fresh fruit	Floral, fruity, green
39	Mushroom	Apricot, floral, Muscat	Mouldy, mushroom	Apricot, floral, thyme	Green, vegetables, herbaceous
40	Cherry, red fruits, spicy	Odourless	Spicy, resin	Spicy, resin, medicinal	Solvent
41	Banana	Banana	Banana	Banana	Banana, fruity

TD, typical dessert wine; NTD, non-typical dessert wine; DW, dry white wine

Results of aroma triangle tests, comparing samples with all 50 HPLC fractions added to a aqueous ethanol solution or dearomatized white wine, to the same sample with fractions omitted (assessors: dessert n = 15; Viognier n = 11).

Wines	Matrix	Fraction omitted	Significance [†]			
Dessert TD3	Aqueous ethanol solution	37	***			
Dessert TD3	Dearomatized wine	37	*			
Dry white DW	Aqueous ethanol solution	37	ns			
Viognier V1	Aqueous ethanol solution	38 - 40	***			
Viognier V6	Aqueous ethanol solution	38 - 40	**			
[†] Where: ns, not significant; $*P < 0.1$; $**P < 0.05$; $***P < 0.01$						

Aroma active zones found in fraction 37 of a typical Bordeaux dessert wine (TD3) extract analysed by GC-O using a HP-5 GC column

LRI	Retention time (min)	Descriptor
1263	31.4	Fruity
1346	34.7	Floral
1360	35.3	Citrus
1414 - 1443	38.3 - 38.9	Overripe orange
1602	41.4	Plastic
1791	48.8	Citrus

LRI, calculated linear retention index.

Adapted from [34].

Aroma compounds detected by GC-O and GC-MS in liquid-liquid extracts of HPLC fractions of Viognier wines with 'apricot' attribute; aroma descriptors, GC retention indices, compound identity liquid and CAS number.

Aroma Descriptors	37	38	39	LRI ^a	Compound	CAS No.	Identity ^b
Fruity apple	✓	✓		1064	Ethyl butanoate	105-54-4	A, MS, RI
Stinky, cabbage		✓		1100	Dimethyl disulfide	624-92-0	A, MS, RI
Confectionary - banana	✓	✓		1114	3-Methylbutyl acetate	123-92-2	A, MS, RI
Cheesy, fusel	✓			1126	2-Methylpropanol	78-83-1	A, MS, RI
Pineapple, fruity	\checkmark			1167	Methyl hexanoate	106-70-7	A, MS, RI
Cheesy, savoury biscuit	\checkmark	✓		1175	3-Methylbutanol	123-51-3	A, MS, RI
Canned pineapple, fruity	✓	\checkmark	\checkmark	1220	Ethyl hexanoate	123-66-0	A, MS, RI
Fresh pineapple			\checkmark	1240	Hexyl acetate	142-92-7	A, MS, RI
Chicken biscuit, savoury		\checkmark	\checkmark	1312	2-Methyl-3-furanthiol	28588-74-1	A, RI
Fruity, pineapple			\checkmark	1350	Ethyl heptanoate	106-30-9	A, MS, RI
Floral		\checkmark	\checkmark	1380	cis-Rose oxide	876-17-5	A, RI
Green leaf	✓	\checkmark	\checkmark	1421	1-Octen-3-ol	3391-86-4	A, MS, RI
Floral, citrus leaf, fruity	\checkmark	✓	\checkmark	1548	Linalool	78-70-6	A, MS, RI
Fruity, soapy, floral	✓	\checkmark		1697	α-Terpineol	98-55-5	A, MS, RI
Red fruit syrup		✓	\checkmark	1748	Diethyl pentanedioate	818-38-2	A, RI
Jam, tobacco		✓	✓	1840	β-Damascenone	2306-91-4	A, MS, RI

^a Calculated linear retention index (LRI); GC wax phase column

^b Method of identification: A, aroma match with literature; MS, data in agreement with NIST11/Wiley275 libraries; RI, data in agreement with those of authentic compound and/or literature.