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ABSTRACT

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

1. Introduction

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, new markets have emerged, especially in Asia, because of changing tastes and higher incomes [1]. Research to understand the distinct flavours important to specific varieties and styles is highly valuable to the wine industry as it provides them with information on how viticulture and winemaking practices can be managed and improved to consistently produce specific wine styles. While several flavour properties of white wines are relatively well understood, there remain certain important characteristics where the causative volatile compounds are not known.

Wine is a very complex matrix. Gas chromatography coupled to olfactometry and mass spectrometry (GC-O-MS) has often been very useful in identifying aroma-active 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 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 efficient alternatives[5]. Furthermore, the aromatic component of wines perceived by the 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 response from cognitive processing. To recognize a particular aroma character requires the integration of complex mechanisms at the brain level, including central and peripheral processes [6]. Consequently, with a combination of aroma compounds being required for a particular aroma to be perceived, alternative strategies are needed to progress in the characterisation of wine aroma component.

Among the techniques implemented, semi-preparative chromatographic approaches, including LC and GC approaches coupled with olfactory detection, have proved to be relevant [7]. They allow the fractionation of wine extracts to obtain a lower complexity. Interest in these approaches is related to combining with sensory reconstitution and omission tests to confirm the relevance of the odorous compounds for the matrix. Among these techniques, a specific methodology involving liquid-liquid extraction of wine followed by fractionation of the extracts by semi-preparative reversed-phase HPLC using water and ethanol as solvents, permitted assessment of odorous fractions and gave the possibility for identification of volatile compounds. This technique, initially developed by Ferreira, Hernández-Orte, 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-hydroxy-4-methylpentanoate, a compound involved in blackberry aroma in red wines [13]. 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 directly assessed for aroma characteristics, either individually or in combination.

In addition, studies using reconstitution and omission methodologies involving semi-preparative GC, GC-recomposition-O (GC-R-O), have been successfully implemented to reconstitute the perception of several extracts from natural sources [14-16]. This was recently illustrated through the study of ‘lavender’ aroma and in the direct and indirect evaluation effects of selected compounds on characteristic aroma attributes of Angostura bitters [17, 18]. In these works, headspace-solid-phase microextraction (HS-SPME) was used to extract the volatiles. The volatiles were separated by GC, recombined selectively in-line utilising a switching device and cryogenic trap and then released for olfactometry evaluation. Also, GC-preparative fraction collection (GC-PFC) has been used, in combination with olfactometry, to identify the compound responsible for a ‘minty’ aroma, *p*-menth-1-en-3-one, in some red

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].

Building upon the knowledge from the GC-O-MS detailed in Siebert, Barter, de 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 identify two examples of typical white wine aroma nuances, i.e., the 'overripe orange/marmalade' character and the related 'apricot' character in Sauternes wine and in Viognier wine respectively using HPLC fractionation and GC-PFC methodologies, applying a novel sensory directed approach with semi-preparative LC and GC in order to progress in the evidence of key odorous compounds in wines.

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
135 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 Loupiac dessert wine which was donated by the winery.

Dearomatized wine was prepared according to Lytra, Tempere, de Revel and Barbe [26] by removing the volatiles of the typical dessert wine TD3 using a rotary evaporator (20 °C; Laborota 4010 Heidolph, Germany), reconstituting the dearomatized wine to its original volume and alcohol concentration with ethanol and water, and a final treatment with a direct 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 AOC Condrieu and Vin de Pays des Collines Rhodaniennes, together with one 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 produce wines described as having varietal 'apricot' character. The wines were assessed 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 and rate its intensity as none, low, medium or high. Following independent assessment using free choice notes, the samples were discussed. Five wines with an obvious 'apricot' aroma attribute, i.e. moderate to high intensity, were selected initially, with a sixth wine included subsequently (V1 – 6). The wines were up to three years old.

2.3. Reversed-phase HPLC fractionation

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,

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

168 *2.3.2. Semi-preparative HPLC*

169 Fractionation of the crude wine extracts was achieved utilizing an Ultimate 3000 semi-
170 preparative HPLC system (Dionex, Courtaboeuf, France) according to a published procedure
171 [27]. In summary, after injection of a wine extract (250 µL) onto a Novapak C18 column (300
172 mm × 7.8 mm, 6 µm; Waters, Saint Quentin, France) plus guard column, fifty individual
173 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*

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

184 Difference testing was performed as triangle tests, described by Martin and de Revel
185 [28]. For dessert wine reconstitutions, the panel consisted of 15 panellists, 5 males and 10
186 females of 30.5 ± 4.6 (mean ± SD) years of age. For Viognier wine reconstitutions, the panel
187 consisted of 11 panellists, 5 males and 6 females of 32.9 ± 7.9 (mean ± SD) years of age.
188 Only the aroma of the reconstituted wines was evaluated by the panels. All panellists

189 belonged to the oenology research laboratory staff at the ISVV. The panellists were selected
190 for their experience in assessing fruity aromas.
191 For sensory reconstitutions studies utilising the HPLC fractions, relevant fractions
192 were combined then diluted with ethanol and water to obtain an ethanol level of 14% (v/v)
193 and to reproduce the initial concentrations in the original wines. For the dessert wine samples,
194 a second set was prepared in dearomatized wine. Samples (50 mL) were evaluated at
195 controlled room temperature (20 °C) in individual booths using covered black AFNOR
196 glasses that were coded with random three-digit numbers, except 20 mL was used for
197 reconstitutions of Viognier wines. Sessions lasted approximately 10 min.
198 For triangle tests, three samples were presented in random order. Two samples were
199 identical and the third one was different. Each panellist was asked to select the sample in the
200 set that was different from the other two, even if they were not sure. Data analysis to
201 determine statistical significance was carried out using the binomial model as in the
202 prescribed tables [28].

203 *2.3.4. Back-extraction of HPLC fractions*

204 Each fraction (1 mL) assessed as having an aroma of interest was diluted with water to
205 obtain approximately 12% ethanol (v/v) and back-extracted with dichloromethane (3 × 1 mL).
206 The organic phases were combined, dried (anhydrous sodium sulfate) and concentrated to 250
207 µL under nitrogen for GC analysis analysis.
208

209 *2.4. Gas chromatography*

210 *2.4.1. GC-O analysis*

211 Gas chromatography-olfactometry (GC-O) was performed utilizing a HP5890 series II
212 GC (Agilent Technologies, Palo Alto, CA, USA), fitted with a standard split/splitless inlet,
213 flame ionization detector (FID), and sniffing port (ODO-1; SGE, Ringwood, Australia). A

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

225 *2.4.2. Preparative gas chromatography*

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

233 Capillaries of deactivated fused silica tubing (0.32 mm i.d.) were fitted from the PFC
234 switching device to all PFC traps with seven 100 µL glass U-tube traps (six sample traps and
235 one waste trap) installed. The traps were cryogenically cooled with liquid nitrogen at
236 controlled temperature. The compound separations were achieved using a HP-5 ‘megabore’
237 column (30 m × 0.53 mm × 1.5 µm film thickness; Agilent) connected to a 0.87 m × 0.32 mm
238 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 × 250 µL), concentrated under nitrogen flow (100 mL/min) to obtain 100 µL. 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 × 2 µL) 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 × 250 µL), the washings collected and concentrated to 20 µL 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 µm 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 µL under nitrogen. Manual liquid injections (6 × 2 µL), of the three-fraction 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.

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

2.4.3. Sensory evaluation of GC-PFC fractions

For aroma assessment of GC-PFC fractionation, concentrated trap washings (20 µL) 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).

2.4.4. GC-MS analysis

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

For the PFC-GC trap fractions, a 5977 GC-MS system (Agilent) was used in simultaneous selected ion monitoring (SIM) and scan modes to allow for more sensitive screening of the samples for γ- and δ-lactones. MS data was recorded for scan mode in the range of m/z 35−350 and for SIM mode the ions monitored were m/z 85, 96, 99, 136 and 196. Helium was used as carrier gas (Air Liquide) and column head-pressure set at 183.4 kPa (1 mL/min nominal flow rate). Automated liquid injections (2 μL) were performed in splitless mode, the inlet was fitted with a deactivated glass liner (glass wool inserted, 4mm i.d.; Agilent) and held at 240 °C, and the splitter was opened after 2 min (purge flow, 50 mL/min). The oven temperature of 45 °C was held for 1 min, then raised to 230 °C at 3 °C/min and then held for 20 min.

Linear retention indices (LRI) were obtained by reverse calculation from the RIs of known compounds in the crude wine extract.

Data analysis was performed using the MassHunter Qualitative Analysis software (Agilent, version B.07.00). Aroma compound identity was achieved by chromatogram deconvolution and comparison to mass spectral libraries (NIST11, Wiley275) then comparing each compound's calculated linear retention index (LRI) to that found in the literature.

3. Results and Discussion

At the outset, it was essential for suitable sample sets of wines to be chosen for study. A subset of Bordeaux wines were selected for the dessert wine study from within a larger set of wines utilised by Stamatopoulos, Frérot, Tempère, Pons and Darriet [27], Stamatopoulos [29], comprising wines with 'overripe orange/marmalade' sensory properties as well as examples without this character. A set of Viognier wines was specifically selected regarding 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.

3.1. Reversed phase HPLC fractionation

3.1.1. Sensory evaluation of HPLC fractions

Aromatic reconstitution and sensory evaluation of HPLC fractions was initially conducted to identify fractions of sensory interest. Assuming 100% extraction efficiency, a 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 individual 1 mL fractions. This allowed for the fractions to be smelled directly from the vial 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 evidenced by the differing descriptors of the fractions. Table S2 (Supplementary material) details the aromas noted (n = 2 assessors) in the 50 vials containing the HPLC fractions of three Viognier wines (wines V1, V2 and V3) with 'apricot' attributes. 'Apricot' and/or '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 – 3) were assessed (n = 3 assessors) and 'overripe orange' aroma was perceived in two 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,

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

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

350 It was fortunate for both studies that the aroma compounds needed to give the targeted
351 aroma attributes eluted in just two or three sequential HPLC fractions. However, having the
352 HPLC fractions in water/ethanol solvent system allows for easy blending of fractions and
353 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
355 perception of overall wine aroma, reconstitution and omission sensory experiments were
356 conducted for both the dessert wines and Viognier wines. Full aromatic reconstitutions of the
357 wines were prepared by combining all 50 HPLC fractions together and partial aromatic
358 reconstitutions were prepared by omitting the fractions of interest (dessert wine TD3,
359 fractions 37 – 38; Viognier wines V1 and V6, fractions 38 – 40). For the typical dessert wine,
360 triangle tests showed significant differences ($P < 0.01$) between the full aromatic
361 reconstitution and the partial aromatic reconstitutions sample in an aqueous ethanol solution

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

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

382

3.2. Gas chromatography

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 both polar phase (BP-20) and non-polar phase (HP-5) capillary GC columns. As with dessert wine extracts in a related study [27], no 'overripe orange' aroma zone was evident using the polar phase but with the non-polar phase an 'overripe orange' aroma was noted across an unusually wide 1 min time period (LRI 1414 – 1443) (Table 4). Usually in GC-O with a suitably set up instrument, an aroma can be smelled for around the same length of time as it 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' 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. An option explored was GC-PFC.

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

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).

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

3.2.2. Method development of GC-PFC

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

(47 – 66%), except for the extreme temperature of −100 °C where the recovery was only about 10% (Fig. 3). For analyses of wine fraction extracts, −40 °C was selected because of 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 3-sulfanylhexanol the lowest (34%) but the reproducibility for individual compounds was consistent (80%) (Fig. 4).

3.2.3. Application of GC-PFC

Even though GC-PFC appeared to be a useful next-step to assist identifying the 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' 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 compounds eluting in retention time window of the 'overripe orange' aroma active zone could further decrease the number of possible candidate aroma compounds involved in the targeted aroma by removing some non-essential volatile compounds from the sample. For the Viognier wine, no 'apricot' aroma active zone was detected by GC-O, but 'apricot' aroma was evident in several sequential HPLC fractions. Therefore, the compounds required for 'apricot' aroma might elute sporadically across the entire GC-PFC chromatogram. Thus, gradually adjusting the GF-PFC cut times to trap smaller retention time windows, by omitting cuts or combining them whilst still collecting 'apricot' aroma in one trap could, again, reduce the complexity of the fraction.

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

For further separation using GC-PFC, the organic phases from the back-extracts of the three most interesting Viognier HPLC fractions (38 – 40) were combined, dried and concentrated to 200 µL under nitrogen (F×3). Post GC-PFC, most of the concentrated washings (15 µL of 20 µL) of individual cryogenically cooled traps, T1, T2 and T3, were spotted onto perfume blotter paper strips and their aromas were compared to the triple fraction extract (F×3) (6 µL). F×3 smelled very fruity, with a floral Viognier-like character, T1 had no 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 (BP-20), geraniol co-eluted with hexanoic acid. However, the deconvolution software could identify both compounds in the F×3 extract. In the trapped fractions, geraniol was compared using the fragment ion m/z 69, which is the most abundant ion in a geraniol mass spectrum but a minor ion in hexanoic acid. Due to the very low concentrations of lactones in the samples the GC-MS data was recorded in SIM mode. Therefore, the relative amounts of the lactones were compared using the respective SIM spectra of the three traps T1, T2 and T3.

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 account for the dilution of the traps' contents. Several compounds were overloaded in the F×3 chromatogram: 3-methylbutyl acetate; ethyl hexanoate; α-terpineol; and octanoic acid. Octanoic acid had saturated the MS detector, therefore, the peak area for a low abundance ion, i.e. its molecular ion (m/z 144), was used to calculate the relative response. The components that were contained within each trap are shown in Fig. 5. Any compounds eluting below LRI 1100 were directed to the waste, compounds eluting between LRI 1100 and 1515 were

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

496 When using the HP-5 megabore GC column installed in the GC-PFC system, the
497 retention time span for the 'over-ripe orange' aroma active zone was determined to be 36 – 40
498 min by GC-O for both the crude extract of TD3 and the back-extracted fraction 37.
499 Subsequently, preparative GC fractions were collected at defined PFC cut times: 0 – 36 min;
500 36 – 40 min; 40 – 82.66 min; or in combinations of those cut times. This technique enabled
501 volatile compounds to be cryogenically trapped, using liquid nitrogen, after their
502 chromatographic separation to study their sensory contribution. Omitting or not trapping
503 certain volatile compounds or groups of compounds is useful to study their impact on the
504 overall aroma of the sample [33].

After GC-PFC, the concentrated washings (20 µL) of cryogenically cooled traps were spotted onto perfume blotter paper strips and their aromas were assessed. Fig. 6 shows the aroma profiles of the trapped fractions from the crude extract of a typical dessert wine (TD3). The contents of the trap corresponding to the whole extract (0 – 82.66 min) was described as 'overripe fruits', 'citrus', 'floral', 'honey' and 'baked sugar' by the sensory panel. Whereas the trap corresponding to the 0 – 36 min plus 40 – 82.66 min, but omitting 36 – 40 min, was described as 'honey', 'creamy', 'yeasty' and 'spicy'. In a triangle test, the overall aroma of the omission trap was found to be significantly different to the whole extract trap ($P < 0.01$). As expected, the trap containing only the cut of 36-40 min was described as 'overripe orange', but also 'dried apricot'.

HPLC fraction 37 from the typical Bordeaux dessert wine TD3 produced the best example of 'overripe orange' aroma. Thus, GC-PFC analysis was repeated using the back-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 – 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

530 that the ‘overripe orange’ aroma was not present in non-typical dessert wine fractions nor in
531 dry white wine fractions, noting all were Bordeaux wines made with the same grape varieties.
532 The results highlight the importance of the aroma compounds in the 36 – 40 min
533 fraction to the overall aroma of typical Bordeaux dessert wines aroma. The HPLC fractions
534 presented a less complex composition than the crude wine extract and the use of GC-PFC
535 analysis of the HPLC fractions was a further step towards ascertaining the aroma compounds
536 in the mixture perceived ‘overripe orange’.
537 In a complementary study, the aroma compounds in the 36 – 40 min trap were
538 identified using multidimensional GC-MS-O [27, 29]. In brief, two interconnected GC-Os
539 (Agilent 6890) were utilised with GC-O#1 fitted with a BP-5 capillary column and FID, and
540 GC-O#2 fitted with a BP-20 and MS. After determining the target ‘overripe orange’ aroma
541 active zone on GC-O#1, a 3 min heart-cut was transferred to GC-O#2 via a pressure-driven
542 switching valve (MCS; Gerstel). Aroma active zones described as ‘coconut’, ‘spicy clove’,
543 ‘ripe/fruity’ and ‘minty/fruity’ were identified as *cis*-oak lactone, eugenol, γ-nonalactone and
544 2-nonen-4-olide respectively [27, 29]. Thus, a mixture of four aroma compounds were found
545 to interact for ‘overripe orange’ aroma to be perceived.
546

547 **4. Conclusions**

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

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

564 The combination of separation techniques with a strong, simple and straightforward
565 sensory-directed approach was inspired by the need for a different methodology for
566 identifying aroma compounds that are responsible for specific wine aroma attributes,
567 especially when the perception of an aroma is due to a mixture of aroma compounds within
568 the complex matrix of wine. The use of small panel sensory methods, rather than time
569 consuming conventional procedures requiring large volumes of sample, allowed sufficient
570 rigour to make progress in tracking the target aroma attributes, while avoiding the use of a
571 single individual such as might have been used in the past. Exacting quantification of aroma
572 compounds is not required, and reconstitution and omission sensory experiments can be
573 conducted easily with HPLC fractions and GC-PFC fractions. The described protocol is a
574 powerful tool for the identification of key aroma compounds responsible for specific aromas
575 across the wine and beverage industries as well as other food industries.
576

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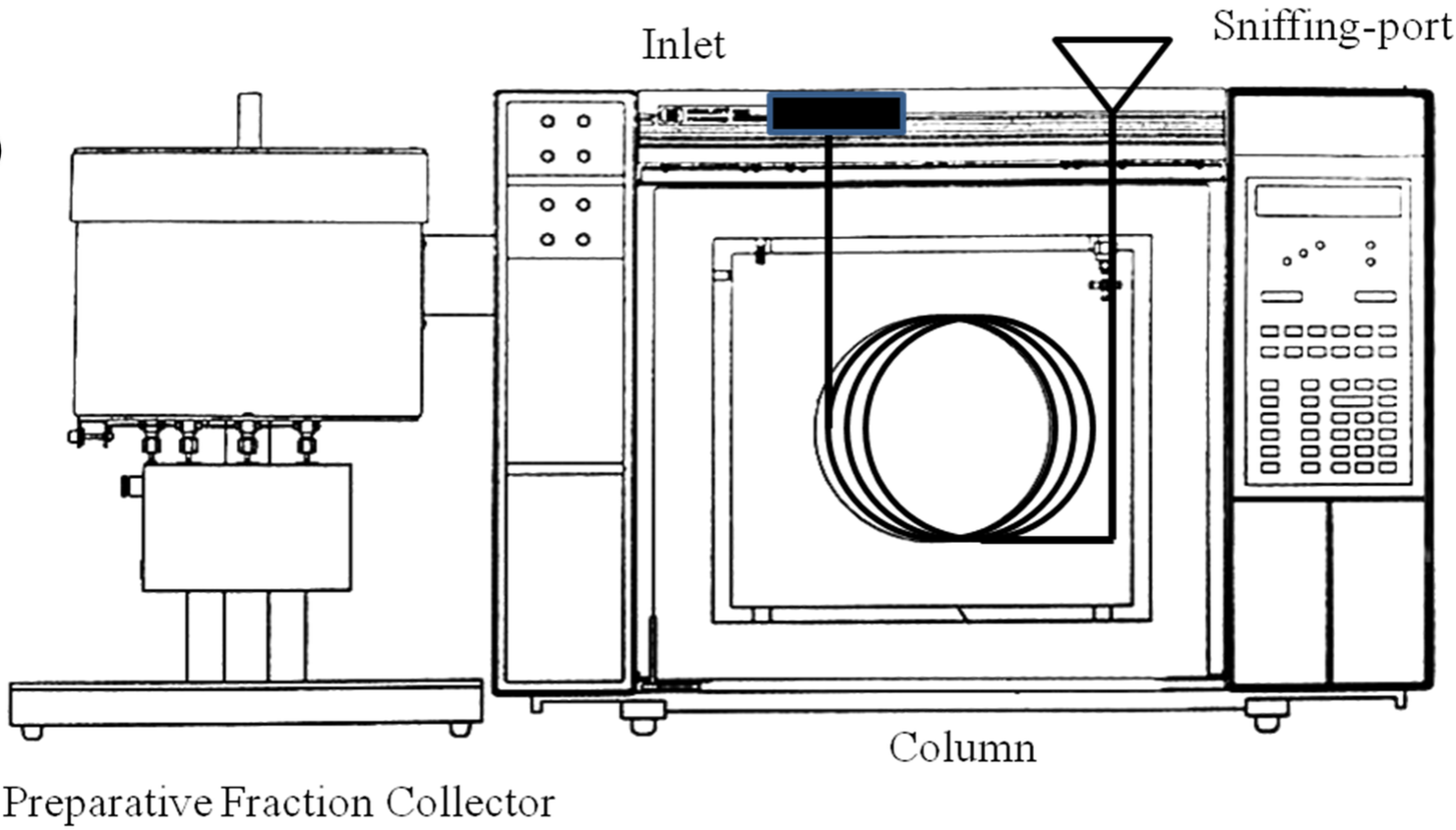
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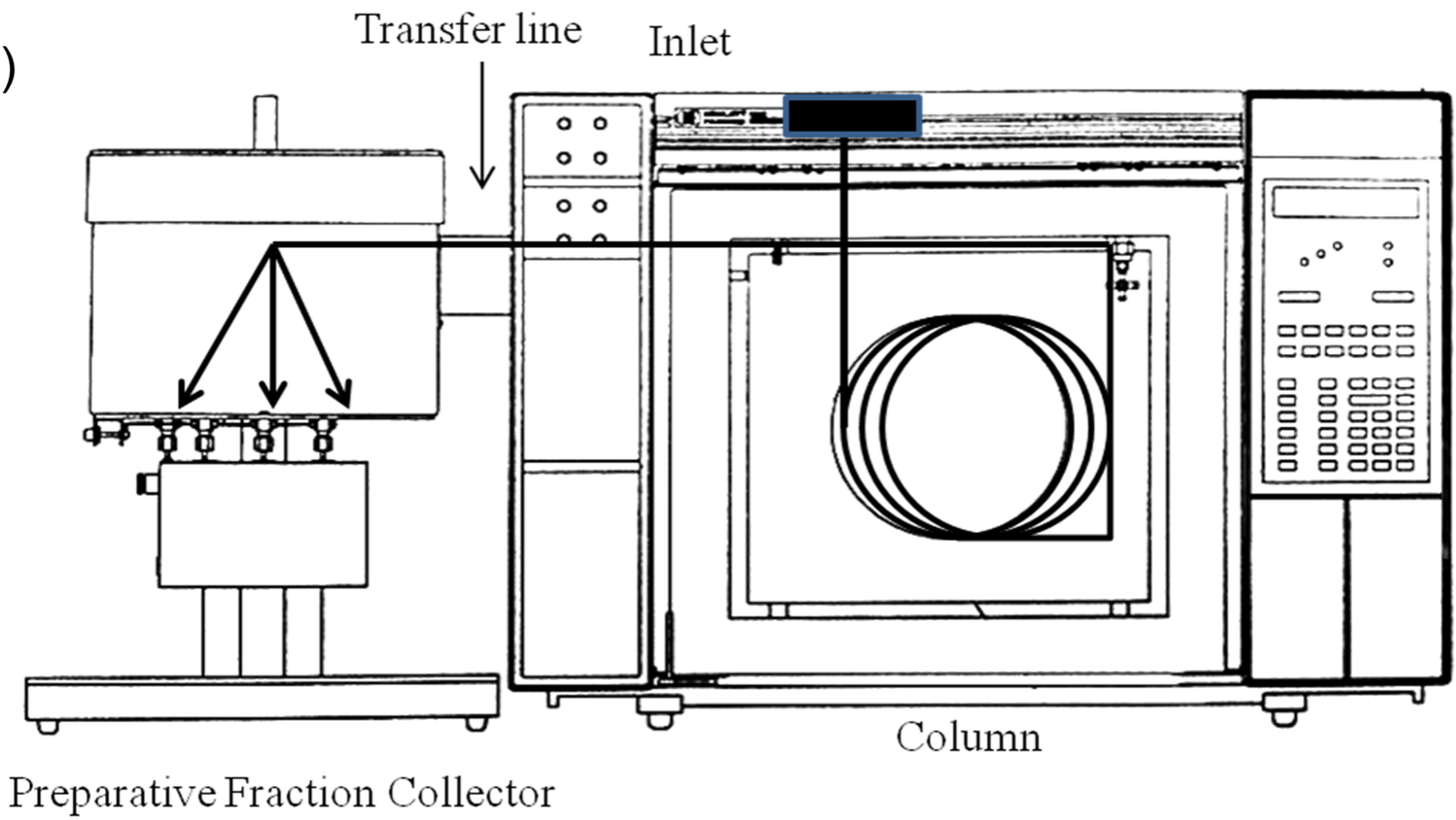
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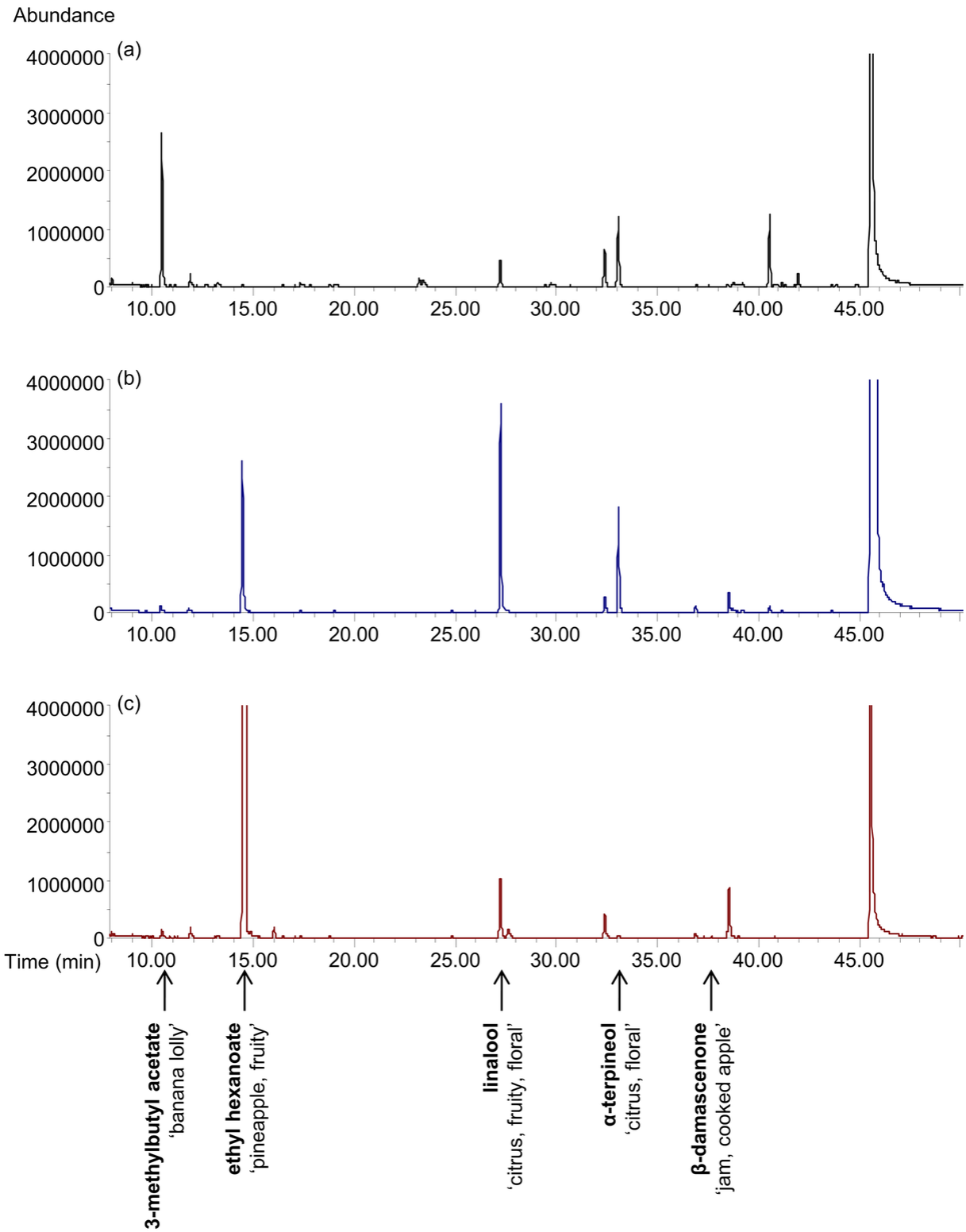
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25.00
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40.00
45.00
(b)
(c)
Time (min)
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'banana lolly'
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linalool
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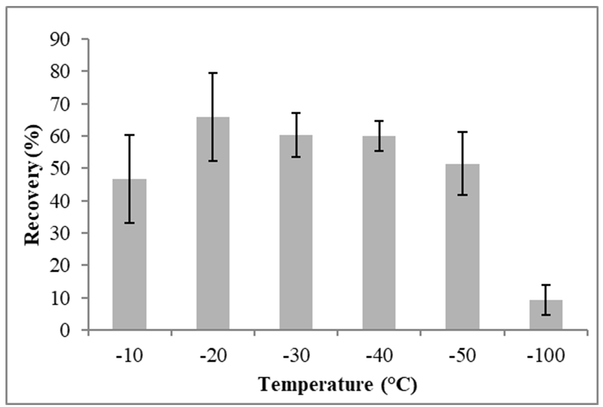
α-terpineol
'citrus, floral'
β-damascenone
'jam, cooked apple'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-30
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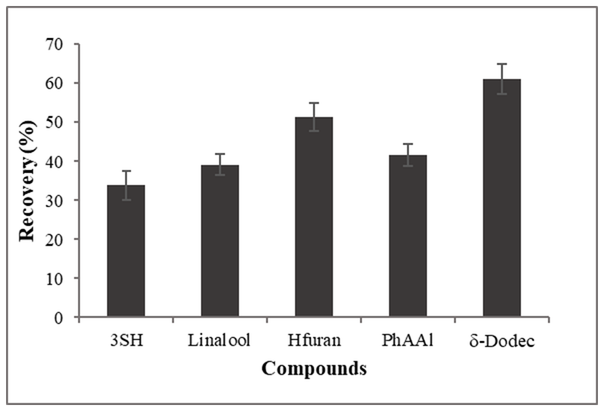
Temperature (°C)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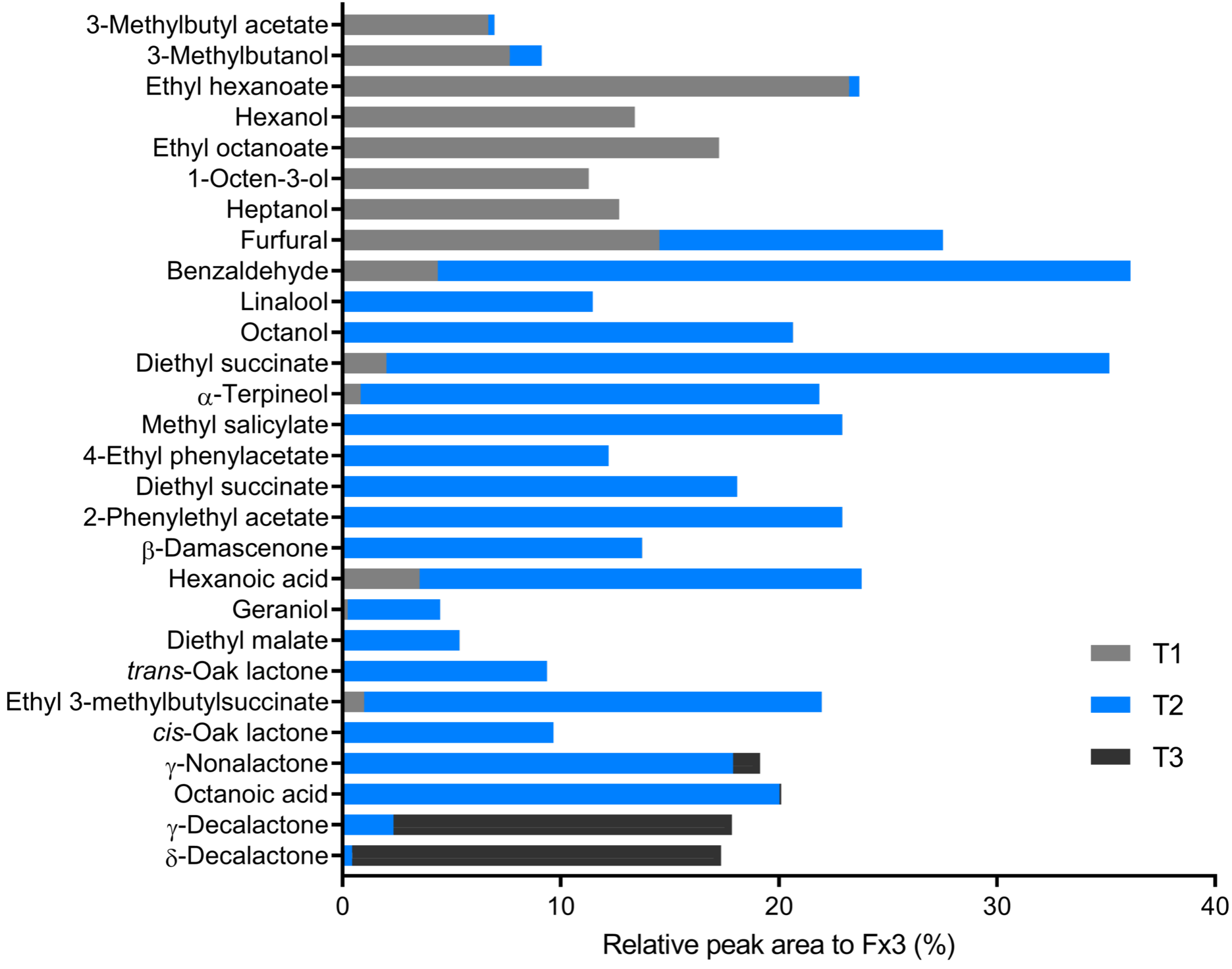
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Octanol
Diethyl succinate
α-Terpineol
Methyl salicylate
4-Ethyl phenylacetate
Diethyl succinate
2-Phenylethyl acetate
β-Damascenone
Hexanoic acid
Geraniol
Diethyl malate
trans-Oak lactone
Ethyl 3-methylbutylsuccinate
cis-Oak lactone
γ-Nonalactone
Octanoic acid
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T1
T2
T3
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10
20
30
40
Relative peak area to Fx3 (%)

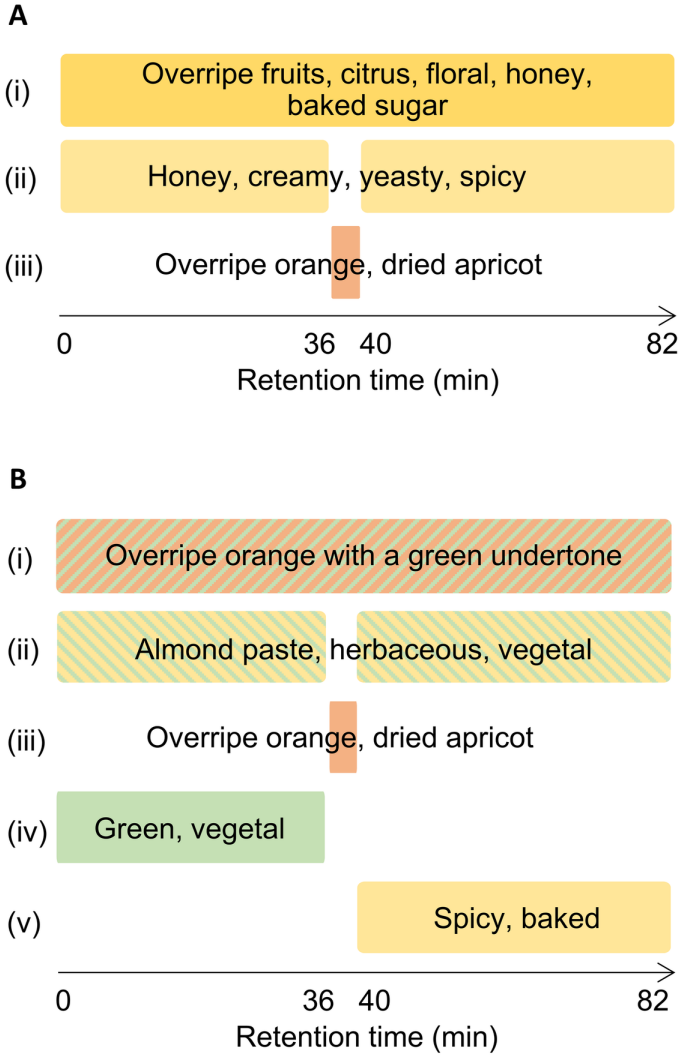
A
(i)
Overripe fruits, citrus, floral, honey, baked sugar
(ii)
Honey, creamy, yeasty, spicy
(iii)
Overripe orange, dried apricot
0
36
40
82
Retention time (min)
B
(i)
Overripe orange with a green undertone
(ii)
Almond paste, herbaceous, vegetal
(iii)
Overripe orange, dried apricot
(iv)
Green, vegetal
(v)
Spicy, baked
0
36
40
82
Retention time (min)

Table 1

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

Fraction	Wine				
	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

Table 2

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	Dessert wines				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

Table 3

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; * $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$

Table 4

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].

Table 5

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	✓			1167	Methyl hexanoate	106-70-7	A, MS, RI
Cheesy, savoury biscuit	✓	✓		1175	3-Methylbutanol	123-51-3	A, MS, RI
Canned pineapple, fruity	✓	✓	✓	1220	Ethyl hexanoate	123-66-0	A, MS, RI
Fresh pineapple			✓	1240	Hexyl acetate	142-92-7	A, MS, RI
Chicken biscuit, savoury		✓	✓	1312	2-Methyl-3-furanthiol	28588-74-1	A, RI
Fruity, pineapple			✓	1350	Ethyl heptanoate	106-30-9	A, MS, RI
Floral		✓	✓	1380	*cis*-Rose oxide	876-17-5	A, RI
Green leaf	✓	✓	✓	1421	1-Octen-3-ol	3391-86-4	A, MS, RI
Floral, citrus leaf, fruity	✓	✓	✓	1548	Linalool	78-70-6	A, MS, RI
Fruity, soapy, floral	✓	✓		1697	α-Terpineol	98-55-5	A, MS, RI
Red fruit syrup		✓	✓	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.