Towards a Molecular Understanding of the Typicality of Chardonnay Wines: Identification of Powerful Aromatic Compounds Reminiscent of Hazelnut

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ABSTRACT

Chardonnay wines impart a unique complex aroma characterized by its buttery, yellow stone fruit, melon, bready and woody notes. Among the terms used in the sensory analysis of these wines, this study investigated hazelnut-like attributes. Multi-dimensional gas-chromatography coupled to olfactometry identified five pyrroles reminiscent of hazelnut: 1-ethylpyrrole-2-carboxaldehyde, 1*H*-pyrrole, 2-acetyl-1*H*-pyrrole (first identification in wine), 1-methylpyrrole-2-carboxaldehyde, and 1*H*-pyrrole-2-carboxaldehyde. Quantitative analyses demonstrated their significantly higher abundance in Chardonnay wines. However, they proved irrelevant in sensory terms, given the low amounts measured in wine compared to their olfactory detection threshold. Nevertheless, the presence of methanethiol-derivatives from these pyrroles was investigated in wine. 1-Methylpyrrole-2-methanethiol and 1-ethylpyrrole-2-methanethiol were identified and exhibited hazelnut-like aroma. These compounds, which have not been observed in natural products to date, are potent volatile compounds with detection thresholds of 0.7 and 1.4 ng/L in model wine. These findings open up promising perspectives concerning the interpretation of the typical aromatic nuances of some Chardonnay wines.

KEYWORDS

Aroma, Chardonnay wine, typicality, hazelnut-like notes, pyrrole, pyrrolemethanethiols

INTRODUCTION

When tasters smell a glass of wine, they first describe their emotions and try to associate the perception with their sensory memories. Beyond the fermentative or aging notes resulting from winemaking, some aromas are related to grape variety. Thus, the ability of a blind taster to recognize a variety is strongly associated with the specificity of these odors. Whereas many varieties can be easily identified by the sensory properties of the wines they provide, only a few of them have been elucidated from a chemical point of view. For instance, the catty-like and grapefruit-like notes of Sauvignon Blanc wines are associated with polyfunctional thiols.[1,2] The specific notes of Muscat[3] and Gewürztraminer[4,5] are linked to monoterpenes while the kerosene-like notes of Riesling are due to the emergence of 1,1,6-trimethyl-1,2-dihydronaphthalene.[6] All these compounds have a varietal origin and their concentrations in wine can be modulated by maturation conditions and grape growing region.[7]

Chardonnay is the world's most planted white grape variety and the wines it produces are easily recognizable by experts.[8] Several authors have reported the most widely encountered nuances of this variety as "tropical/green fruits", "butter/caramel", "honey", "ash", "woody", and "citrus".[9] The characterization of volatile compounds has mainly evidenced the contribution of non-varietal markers such as diketones, acetate esters,[8,10,11] ethyl esters,[8,11] fusel alcohols,[10] volatile phenols,[8,12,13] and lactones.[10,12] Compounds coming from grape have also been identified but their concentrations were similar to those obtained in other grape varieties[14] so that they cannot be considered as responsible for the aromatic typicality of Chardonnay wines.

Nevertheless, the finest Chardonnay wines present a complex bouquet described by experts as having "hazelnut", "flint", "oatmeal" and "grilled bread" nuances.[15,16] This aspect

has received little attention and, to our knowledge, only one scientific article by Sauvageot and Vivier[17] has associated hazelnut notes with Chardonnay wines. This character is to be differentiated from the nutty, curry-like off-flavors generally associated with the presence of sotolon in prematurely aged dry white wines.[18,19] Historically, the Chardonnay wines of Burgundy, which have been regarded as models by most of Chardonnay producers worldwide, have been aged on lees in oak barrels, thereby limiting the organoleptic occurrence of sotolon.[19] Malolactic fermentation, a frequent practice on Chardonnay wines and in particular in Burgundy, enhances these hazelnut notes and advantageously reveals the typicality of these wines.[17] However, despite the recurrent citation of hazelnut (fresh and roasted) descriptor in Chardonnay wines, there has been no specific investigation on it and still no chemical explanation for it. While comprehensive two-dimensional gas chromatography (GCxGC) can provide untargeted insights into the quantitative variability of certain compounds and relate them to the grape variety, [20] GC-Olfactometry (GC-O) is usually used to detect the odorous volatile compounds reminiscent of a specific character perceived by tasters in wines. [21]

Thus, the present work aimed at identifying the molecular determinants reminiscent of the characteristic hazelnut notes in Chardonnay wines through a sensory-guided approach. After having confirmed the relevance of the hazelnut attribute, various analytical techniques such as GC-O and multi-dimensional gas chromatography - mass spectrometry (MDGC-MS) were implemented to search for compounds exhibiting this aroma. Their sensory properties were also assessed.

MATERIAL AND METHODS

Chemicals. Dichloromethane (99.99%) was supplied by Fisher Scientific (Illkirch, France), Lichrolut EN SPE cartridges, absolute ethanol (99.9%) and methanol HPLC grade by Merck (Semoy, France). Ultrapure water (Milli-Q, resistivity = 18.2 MΩ cm, Millipore, Saint-Quentin-en-Yvelines, France) was used. Anhydrous sodium sulfate, octan-3-ol, 2-acetyl-1*H*-pyrrole, 1*H*-pyrrole, 1-methylpyrrole-2-carboxaldehyde, (*Z*) and (*E*) oak lactones and L-cysteine were purchased from Sigma Aldrich (Steinheim, Germany). 1*H*-Pyrrole-2-carboxaldehyde was provided by Acros Organics (Geel, Belgium), and 1-ethylpyrrole-2-carboxaldehyde by Fluorochem (Derbyshire, United-Kingdom). 1-Methylpyrrole-2-methanethiol (CAS: 59303-06-9) and 1-ethylpyrrole-2-methanethiol (CAS: 1420967-06-1) were provided by Amber MolTech (Chester, PA, USA). Those compounds constitute reference standards (> 97% purity). Dry active *Saccharomyces cerevisiae* yeast (8% moisture content, 2.10^{10} living cells SADY CFU/g, Zymaflore X5) was provided by Biolaffort (Bordeaux, France).

Samples. Selected wines listed in Table 1 were used for sensory and analytical studies, and sequential distillation. Oak wood dust scraped off a *Quercus petraea* stave was provided by Seguin-Moreau cooperage (Merpins, France). The stave was previously air-dried for two years and toasted according to the cooperage process. The species has been identified using the method described by Marchal *et al.*.[22] The oak wood was macerated (20 g/L) during 96 h in wine model media (12% EtOH (v/v); 5 g/L tartaric acid; pH adjusted at 3.4 with NaOH).

Sensory Analyses. Sensory analyses were carried out as described by Martin and de Revel.[23] Samples (about 50 mL) were poured into black INAO wine glasses (NF V09-110, 1971) labeled with random three-digit codes and covered with half of a plastic Petri dish. Evaluations were performed in a dedicated room (ISO 8589:2007) equipped with individual booths to prevent communication between assessors, under normal daylight and at room

temperature. In all cases, wine glasses were simultaneously presented to each judge in random order.

Sensory Profiling. Five wine consultants having a good knowledge of the diversity and the typicality of Chardonnay wines short listed four wines out of thirty one, as representative single Chardonnay grape variety. The aroma profiling of these four wines of various vintages and origins were assayed. The panel was composed of 24 experienced tasters (researchers in wine science, teachers, and enologists) and was not trained specifically for this study. However, the tasters have followed a general training in wine tasting and are in particular trained to recognize and describe wine aromas in a naturalist way by comparison with fruits, flowers, spices or other natural products. These 24 panelists were asked to provide the descriptors corresponding to their orthonasal appreciation of the four Chardonnay wines. Then, descriptors were clustered on the basis of the same aromatic family.[24] The main odorant attributes cited were collected and grouped together in a contingency table displaying the frequency of citation for each term.

Determination of Olfactory Thresholds. The olfactory detection threshold corresponds to the lowest concentration perceived by 50% of tasters. Olfactory thresholds of the pyrroles were determined by presenting a three-alternative forced choice in model wine (12% EtOH (v/v); 5 g/L tartaric acid; pH adjusted at 3.4 with NaOH) or white wine (MUS31). The panel of 24 experienced tasters was used. Among the three glasses, one contained a supplemented sample with stepwise increasing concentrations (factor 2) of the compound to be evaluated. Best estimate individual thresholds were obtained by calculating the geometric mean between the last concentration missed and the first concentration detected. Perception threshold was defined by mean evaluation of the geometric mean of the best individual estimates.

The perception threshold of 1-methylpyrrole-2-methanethiol and 1-ethylpyrrole-2-methanethiol was determined by using an adaptation of the ASTM-E1432 method (AFNOR 2002). The panel was composed of 44 tasters (24 experienced panelists and 20 students of Diplôme National d'Œnologue) that had never smelled the pyrrolemethanethiols prior to this experiment. The concentration/response function is a psychometric function and fits a sigmoid curve ($y = 1/ (1 + e(-\lambda x))$). Detection probability was corrected by using the chance factor (one-third for 3-AFC: proportion corrected by the chance effect, 1/3 for 3-AFC = $(3 \cdot p - 1)/2$, where p = proportion of correct responses for each concentration and P = proportion corrected by the chance effect, 1/3 for 3-AFC). Sigma Plot 8 (SYSTAT) software was used for graphic resolution and nonlinear regression by ANOVA transform (SYSTAT, San Jose, CA, USA).[25]

Preparation of Representative Extract by Sequential Vacuum Distillation.

Ten wines were used for this experiment: four typical Chardonnay wines (already selected for the sensory profiling), two Chardonnay wines presenting low typicality and four non-Chardonnay wines (Sauvignon Blanc, Semillon, Viognier, and Riesling wines). Five hundred milliliters of each wine were poured into a flask of a rotary evaporator steeped in a bath at room temperature. Volatiles were trapped with a condenser containing glycol recirculating through a cooled system down to -2 °C. Sequential distillation parameters were determined after assays combining vacuum levels and durations in a window from 1 to 90 min and 120 to 5 mbar, respectively. Final diagram was set as follows: 70 mbar for 1 h (Fraction F1), 50 mbar for 15 min (Fraction F2), and 30 mbar for 5 min (Fraction F3). Each distillate collected from the receiving flask was diluted with ultrapure water according to the alcohol by volume content measured (F1 generally 70%, F2 around 50% and F3 around 30% (v/v) to reach 12% EtOH (v/v). Thus, 30 fractions were obtained and randomly presented to five wine experts. The tasters were not informed of the origin of the fractions and were asked to shortly describe

143 their main olfactory characteristics. An attribute was associated to a fraction when it was
144 cited by at least three experts. The most interesting fractions, *i.e.* the fractions presenting the
145 attributes frequently described in typical wines, were selected for GC analysis. Liquid/liquid
146 extraction was applied to these selected fractions (3 times with 5 mL dichloromethane,
147 stirring 5 min each time). The organic phases were combined and dried on anhydrous sodium
148 sulfate. The supernatant was transferred by use of Pasteur pipette to a concentration tube to
149 be evaporated to 0.25 mL under nitrogen flow.

150 **Single Dimension Gas Chromatography – Olfactometry Analysis.** GC-O analysis
151 was carried out on a Hewlett-Packard 5890 gas chromatograph (Agilent Technologies, Palo
152 Alto, CA, USA) equipped with a split/splitless injector (230 °C; purge time, 1 min; purge
153 flow, 50 mL/min), a flame ionization detector (FID), and a sniffing port (ODO-1 from SGE,
154 Ringwood, Australia). Separation was achieved on a Carbowax-type capillary column (BP
155 20, 50 m length, 0.22 mm i.d., 0.25 µm film thickness, SGE, Pflugerville, Texas, USA). One
156 µL of the distillate extract was injected. Hydrogen 5.0 was used as carrier gas at constant
157 pressure set at 100 kPa. The initial GC oven temperature was set at 45 °C for 1 min, before
158 rising to 230 °C at 3 °C/min, and was then maintained at 230 °C for 20 min.

159 Data from GC-O aromagrams was processed with Acquisniff® software.[26] Three
160 panelists experienced in sensory analysis sniffed every extract by 4 experimental sessions of
161 15 min interrupted by 15 min rest (full run done in 2 sessions – 3 replicates by operator). The
162 olfaction started 5 min after the beginning of the GC run with nose humidification (20
163 mL/min) provided all along. Odorant sensory information (odorous zone descriptors, relative
164 intensities and durations) were monitored by recording the voice of the sniffer as start and
165 stop signals. The three panelists, who are accustomed to making GC-O, were asked to give a
166 qualitative description when an odorant was perceived. Use of Acquisniff® software allowed
167 individual aromagrams to be compiled from sessions performed by different operators. An

OZ was considered as perceived when at least two out of three operators detected it at the sniffing port.

Multidimensional Gas Chromatography coupled with Olfactometry and Mass Spectrometry (MDGC-O-MS) . Multidimensional separations were achieved on a system consisting of two independent gas chromatographs (Agilent 6890; Agilent Technologies, Santa Clara, USA) interconnected by means of a thermoregulated transfer line kept at 230 °C (West 4400, West Instruments, Gurnee, IL, USA). Two µL of F1 extract were injected in a split/splitless injector (230 °C; purge time, 1 min; purge flow, 50 mL/min). The 1D separation device was a HP 6890 chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a polar BP20 (30 m length, 0.25 mm i.d., 0.5 µm film thickness, SGE, USA). Helium N55 was used as carrier gas at a constant flow of 1.2 mL/min. On the 2D, a ramp pressure program was set in to ensure constant flow in the 2D column (224 kPa for 1 min, then increased by 1.4 kPa/min to 310 kPa, and maintained at this pressure for 30 min). Initial temperature of the 1D-GC oven was set at 45 °C, increased by 3 °C/min up to 220 °C and held for 10 min. The 1D column outlet was connected to the 2D system by means of the multi column switching device (MCS, Gerstel, Germany). Ten percent of the flow from the 1D column was constantly directed through a deactivated fused silica column to an FID or Olfactometric port; the rest was transferred (counter current flow off in the cross piece) and trapped at the head of the 2D column by means of a cryogenic trap system (CTS, Gerstel). The counter-current flow was switched off during the transfer of the “heart-cut” eluate in the 2D system. The 2D system was equipped with a non-polar HP5 column (30 m length, 0.32 mm i.d., 0.5 µm film thickness, Agilent J&W, USA) or BP1 column (30 m length, 0.25 mm i.d., 0.5 µm film thickness, SGE, USA). 2D column outflow was split 2:1 between an olfactometric detection port (transfer line regulated at 250 °C; ODP2, Gerstel, Germany) and the mass spectrometric detector (5973 inert; Agilent Technologies, Santa Clara, USA). The

MS transfer line was set at 150 °C, ion source at 230 °C and electron ionization (EI) voltage at 70 eV.

Constitution of Wine and Grape Juice Extracts for Quantitative Assays. Extraction was performed in 2015 by solid-phase extraction (SPE) according to the method of Culleré *et al.*.[27] A Lichrolut-EN cartridge (500 mg) containing divinylbenzene copolymer was first conditioned (10 mL CH_2Cl_2 then 5 mL MeOH finally 10 mL 10% EtOH in water). Then fifty milliliters of wine or two hundred milliliters of diluted juice (juice/water; 25/75; v/v) were spiked with 50 µL octan-3-ol (5 mg/L in EtOH) and poured through the cartridge. The solid phase was rinsed with ultrapure water, dried with air and elution was performed with 5 mL dichloromethane. The eluate was dried with anhydrous sodium sulfate, the supernatant was transferred by use of Pasteur pipette to a concentration tube to be evaporated to 500 µL under nitrogen stream (flow close to 100 mL/min) prior to analysis.

Identification and Quantitation of Pyrroles and Lactones by GC-MS. Identification was conducted by assessing on one hand the coincidence of retention time with pure standard injected in the same chromatographic conditions and, on the second hand, the increase of the peaks corresponding to targeted compounds in the extract spiked with standard solutions. Relative ion intensities within the ± 20% authenticated the identification. (*Z*) and (*E*) oak lactones were quantitated according to the methodology described by Ferreira et al.[28] For quantitation of pyrroles, pure analytes were used to determine calibration curves and the limits of quantitation (LOQ) and detection (LOD). A stock solution at 5 mg/L in ethanol was prepared for every standard and multi-reference standard solutions were constituted and diluted stepwise with ethanol to obtain individual concentrations of 250 µg/L, 25 µg/L and 2.5 µg/L. Wine (50 mL) with trace amounts of pyrroles was supplemented with concentrations ranging from 5 to 5 000 ng/L for 1-methylpyrrole-2-carboxaldehyde, 1-

ethylpyrrole-2-carboxaldehyde, 1*H*-pyrrole, and 2-acetyl-1*H*-pyrrole, and from 25 to 25 000 ng/L in the case of 1*H*-pyrrole-2-carboxaldehyde.

Wine spiked with these standards and with Internal Standard (IS: octan-3-ol, 5 µg/L) were extracted using SPE technique and analyzed by GC-MS in order of increasing concentration (8 points covering the concentration range, analysis conducted in duplicate). The ratio between the peak area of every targeted analyte and the peak area of the IS was plotted against the spiked concentration. Linear regression using least-squares estimation was performed to establish the individual linear equation of the calibration curve ($R^2 \geq 0.995$; Table 2; Microsoft® Excel® 2010, Microsoft® Office 2010 Proofing Tools, © 2010 Microsoft Corporation). Repeatability below 8% and recoveries between 94 and 106% were obtained for the five analytes as indicated in Table 2.

LOQ and LOD were determined by analyzing samples of wine spiked at 5, 10, 20, 30 and 50 ng/L with standard solutions of each compound. Repeated GC-MS analyses (n = 3) were performed and the individual LOQ were expressed as concentrations giving a signal-to-noise ratio > 10 at the peak apex (RSD ≤ 20%). The same procedure was used for the LOD with signal-to-noise > 3.

Generation of Methanethiol Derivatives from Pyrrolecarboxaldehydes. Generation of Pyrrole-Cysteine Adducts. The procedure was conducted according to the method of Schubert[29] adapted by Huynh-Ba *et al.*.[30] Ten millimoles of 1-methylpyrrole-2-carboxaldehyde and 1-ethylpyrrole-2-carboxaldehyde dissolved in 4 mL HPLC grade ethanol were added to 30 mL of an aqueous solution of cysteine (600 mM) and stirred for one hour. The resulting precipitate was 0.45 µm-sucked filtered on a cellulose disk (Merck Millipore, Molsheim, France), washed with ethanol and freeze-dried. The reaction mixture was then suspended in ultrapure water for analysis by ultra-high performance liquid chromatography – high resolution mass spectrometry (UHPLC-HRMS) to control the presence of adducts (2-

cysteine-1-ethylpyrrole-2-carboxyaldehyde and 2-cysteine-1-methylpyrrole-2-carboxyaldehyde).

Liquid Chromatography coupled to High Resolution Mass Spectrometry. UHPLC system was coupled with an Exactive Orbitrap mass spectrometer equipped with a heated electrospray ionization (HESI) probe (both from Thermo Fisher Scientific, Bremen, Germany). Mass acquisitions were carried out for 6 min in negative HRMS ionization mode at 3 kV. The vaporizer temperature of the source was set at 320 °C, the capillary temperature at 350 °C, the nitrogen sheath gas at 75, the auxiliary gas at 18, and the sweep gas at 0 (arbitrary units). The capillary voltage, the tube lens voltage offset, and the skimmer voltage were set at −95, −190, and −46 V, respectively. A mass range of *m/z* 100−500 was acquired in full scan MS mode with a mass resolution of 25 000 (m/Δm, fwhm at *m/z* 200).

Incubation of Pyrrole-cysteine Adducts with Yeast. Dry active *S. cerevisiae* yeast (Zymaflore X5, Biolaffort, Bordeaux, France) was hydrated in water with 5 g/L glucose for 1 h at room temperature. The suspension was then centrifuged and the pellet was suspended in 0.1 M phosphate buffer and pH set at 6.9 by means of sodium hydroxide solution. The incubation of yeast with 1 g of the cysteine conjugate precipitate was carried out under inert atmosphere (flush of N_2) at 30 °C (water bath thermoregulated) as recommended by Huynh-Ba *et al.*[30] and stirred for 24 h. Fifty mL of the mixture were sampled and adjusted to pH 4.0 (2 M hydrochloric acid) and liquid/liquid-extracted 3 times with 5 mL dichloromethane. The extracts were dried over anhydrous sodium sulfate; the supernatant was transferred by use of Pasteur pipette to a concentration tube prior being evaporated to 0.5 mL under nitrogen flow stream before analysis by GC-MS and GC-O. The resulting compounds were identified by GC-MS analysis as follow: 1-methylpyrrole-2-methanethiol (**6**) at 31.0 min (LRI_{BP20} 1787) [*m/z* (relative intensity): 127(20)–94(100)-

95(17)-82(9)-67(7)-53(5)] and 1-ethylpyrrole-2-methanethiol (**7**) at 31.3 min (LRI_{BP20} 1813) [*m/z* (relative intensity) 141(22)–108(100)-80(32)-67(20)-93(14)].

Detection of Pyrrolemethanethiol by Gas Chromatography–Tandem Mass Spectrometry (GC-MS/MS) . GC-MS/MS separation was performed on a ZB-1MS capillary column (60 m length, 0.25 mm i.d., 1 µm, Phenomenex, Le Pecq, France) connected to a BP-20 pre-column (polyethylene glycol, 2 m length, 0.22 mm i.d., 0.25 µm, SGE Analytical Science, Victoria, Australia). Helium N55 (Linde Gas, Saint-Priest, France) was used as carrier gas at a constant flow rate of 1 mL/min. A 1 µL extract was injected into a split/splitless programmable temperature injector (valve closure: 1 min, split flow 30 mL/min) and set as follows: 0.3 min at 200 °C, then raised to 230 °C at 14 °C/min, maintained for 1 min, and then raised to 250 °C at 14 °C/min and kept at that temperature for 10 min. Oven temperature was initially set at 45 °C, held for 1 min, then raised to 176 °C at 3 °C/min, raised to 250 °C at 50 °C/min, and finally kept at that temperature for 5 min. The MS transfer line was maintained at 250 °C. The chromatographic system included a Trace GC Ultra gas chromatograph (Thermo Electron SAS, Courtaboeuf, France) coupled to a triple quadrupole mass spectrometer TSQ Quantum XLS operated in EI mode. The GC system was equipped with a TriplusRSH auto-sampler.

The Mass Spectrometer source temperature was set at 230 °C, electron energy at 25 eV, emission current 30 µA, and electron lens set at 100 V. Argon was used as collision gas at a pressure of 1 mTorr. Selected reaction monitoring (SRM) conditions and collision energy and gas pressure values applied to the precursor ion were dependent on the transition. Resolution was set to 0.7 Da full width at half maximum, scan width: *m/z* 0.7, and scan time: 0.1 s. Instrument setting, data acquisition, and processing were performed using Xcalibur software (version 2.1.0). PFTBA (perfluorotri-n-butylamine) was used for mass calibration.

Statistical Analysis. Statistical calculations of homoscedasticity, normality and discriminative power of compounds (non-parametric study of variance by Kruskal-Wallis test) of the values were performed by using R i386 3.1.3 version (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org).

RESULTS AND DISCUSSION

Evidence of Hazelnut Nuances in Chardonnay Wines and Isolation of Associated Compounds by Vacuum Distillation. Before searching for determinants of the "hazelnut-like" sensory attribute in Chardonnay wines, the relevance of this descriptor had to be confirmed. Thus, four Chardonnay wines from the Burgundy region (St Aubin 1er Cru: CHSA5, Chassagne Montrachet: CHCM6, CHCM7, Pernand Vergelesses: CHPV2; Table 1) that had previously been selected by wine experts for their typical aromatic character were subjected to sensory analysis with a 24-panelist jury who were not informed of the objectives of the study. Once sensory analysis had been done, 35 descriptors were collected and listed in descending order of the number of citations (Figure 1). Besides the recurrent descriptors such as "butter", "creamy", "gunflint" and "yellow stone fruit", the terms "hazelnut", "almond", "bergamot", "jasmine", "honeysuckle" and "verbena" emerged as important descriptors (frequency of occurrence > 5; Figure 1). In particular, hazelnut was the 5th most elicited descriptor (frequency of occurrence: 17; Figure 1). This confirmed the relevance of the hazelnut-like character in the selected typical Chardonnay wines.

Then, ten wines underwent vacuum distillation: four typical Chardonnay (mentioned above), two Chardonnay wines presenting low typicality (no hazelnut note perceived) and four non-Chardonnay wines. Once several assays with different vacuum levels and durations

315 had been conducted, a sequential distillation diagram could be designed to give three
316 fractions as described in Materials and Methods section. The fractions were randomly
317 presented to five wine experts in order to compare their olfactory properties.

318 For the four typical Chardonnay wines, the first fraction F1 (70 mbar) was described
319 as imparting hazelnut, woody and verbena notes. These aromas were not perceived in the F1
320 obtained from non-Chardonnay wines and from Chardonnay with low typicality. The fraction
321 F2 (50 mbar) from Chardonnay wines revealed strong butter-like notes and a slight almond
322 aroma, particularly for the typical wines. These notes were not perceived in fractions F2
323 obtained from non-Chardonnay wines. Finally, the fraction F3 (30 mbar) had more common
324 white wine characters (dry apricot, brioche notes) and was not discriminative for Chardonnay
325 and non-Chardonnay wines.

326 This experiment showed that F1, exhibiting hazelnut aromas in high typical Chardonnay
327 wines, was the most distinctive fraction. Although less distinctive, fraction F2, revealing
328 almond aroma, was also perceived as specific. So, fractions F1 and F2 of the ten wines were
329 selected and submitted to liquid/liquid extraction prior to GC-O analysis.

330 **Evidence of Hazelnut Odorous Zones by GC-O Analysis of Distillates and**
331 **Identification of Related Compounds by MDGC-O-MS.** In order to investigate the
332 molecular determinants of the hazelnut-like notes, an inductive approach using GC-O was
333 implemented. Liquid/liquid extracts of the above mentioned distillate fractions from the
334 Chardonnay wines were subjected to single dimension GC-O analysis (three operators).
335 Given their aromatic characteristics, only F1 and F2 fractions of the ten selected wines were
336 used. The individual aromagrams were compiled and resulted in an exhaustive aromagram
337 exhibiting the consensually perceived odoriferous zones (OZ). The panelists were not
338 informed of the nature of the injected sample prior to each experiment. For a given time of
339 analysis, more numerous OZ were perceived in the extracts from Chardonnay wines than in

the corresponding Non-Chardonnay wines (80 to 90 OZ monitored for Chardonnay wines while below 70 OZ for Sémillon, Viognier, Sauvignon Blanc, or Riesling wine extracts). Pairwise comparison of the aromagrams obtained from Chardonnay and Non-Chardonnay wine analyses evidenced sixteen hazelnut-like OZ in the Chardonnay wine aromagrams (Table 3). Codes were attributed to each odorant zone and their Linear Retention Index (LRI) was established according to Van den Dool and Kratz equation.[31] Fifteen of the sixteen zones were detected in fraction F1 and six in fraction F2 (Table 3). Although the sequential distillation enabled the partition of most of the hazelnut reminiscent OZ between F1 and F2, the OZ "C", "K", "L" and "O" were not properly resolved and perceived in both F1 or F2 (Table 3).

Then, MDGC-O-MS analysis with specific heart-cuts was performed on the organic extracts previously analyzed by GC-O in order to identify the related compounds associated with the hazelnut odoriferous zones. Thus, at the retention time of the most intense OZ reminiscent of "roasted hazelnut-like" (OZ "F") perceived in F1 extract, a cut was performed between 30 and 33 min (RT 32.5 min, LRI_{BP20} 1617) and the so-eluted compounds were transferred to a second capillary column. The same odor was perceived at RT 31.1 min at the outlet of the second capillary (apolar phase BP1, LRI_{BP1} 1026). Considering the MS at the same retention time, a major peak, which was tentatively identified as ethyl 4-oxopentanoate, partially overlapped the peak of interest (Figure 2A). After subtracting the *m/z* associated with ethyl-4-oxopentanoate, the spectrum matched with the 1-ethylpyrrole-2-carboxaldehyde (CAS 2167-14-8, (**1**), Figure 3) in the mass spectral database (NIST, 2004). Injection and co-injection of the pure standard showed the coincidence of RT, odor and ion fragments. 1-Ethylpyrrole-2-carboxaldehyde is also called tea pyrrole because found in Oolong tea and Hojicha green tea,[32] but also in lotus flower[32] and coffee.[33] Although this compound was

tentatively identified in Merlot wine,[20] to our knowledge this is the first time it has been identified in dry white wine.

The sweet hazelnut-like OZ "G" (1D LRI_{BP20} 1641), at a RT very close to OZ "F" (LRI_{BP20} 1617), was also heart-cut and separated by using another set of separative columns ($1D_{BP20}$ - $2D_{HP5}$). The second dimension allowed the resolution of a major peak at 50.5 min (HP5 column) that was synchronous with the hazelnut-like odor and yielded good spectrum purity (Figure 2B). The ion fragments matched with the 1-methylpyrrole-2-carboxaldehyde spectrum (CAS 1192-58-1, (**2**), Figure 3). A solution of pure standard injected and co-injected with the wine extract confirmed the identification and could be positioned on the first set of column $1D_{BP20}$ - $2D_{BP1}$ with the LRI assessed LRI_{BP1} 975. This compound was also previously tentatively identified in a Brazilian Merlot wine by Welke *et al.*,[20] and in a Semillon wine by Schmidtke *et al.*[34] who both used a GC-comprehensive technique (HS-SPME-GC×GC/TOFMS and SPE-GC×GC-MS, respectively).

Then, considering the olfactory properties of these two pyrroles, the presence of compounds belonging to the same family was investigated directly in Chardonnay wine extracts. The unsaturated 5-member ring heterocycle cation was characteristic of pyrrole moiety in EI source[35], so the corresponding ions were targeted in the GC-MS chromatograms. Thus, the ionization of heterocycle yielded *m/z* 66, 67, as well as *m/z* 80, 94 or 108 when N-substituted. Screening of the GC-MS chromatograms led to the emergence of a peak at LRI_{BP20} 1950 with the main ion fragments *m/z* 66, 94, 109 and a peak at LRI_{BP20} 1994 with *m/z* 66, 94, 95. The peaks corresponded respectively to the OZ "M" (LRI_{BP20} 1946) and "N" (LRI_{BP20} 2010) described as "smoked hazelnut-like" and "hazelnut", "coffee". The mass spectra were tentatively associated with 2-acetyl-1*H*-pyrrole (CAS: 1072-83-9, (**3**), Figure 3) and 1*H*-pyrrole-2-carboxaldehyde (CAS: 1003-29-8, (**4**), Figure 3), respectively. Identification was confirmed by injection of the pure standard solution and co-injection with

the extract on polar and apolar columns (LRI_{BP1} 1020 and 990, for compounds (**3**) and (**4**), respectively). 2-Acetyl-1*H*-pyrrole was previously identified in rice wine.[36] It has now been identified in grape wine. In dark chocolate, 2-acetyl-1*H*-pyrrole is believed to play a role in praline aroma and be partially formed during conching.[37] 1*H*-Pyrrole-2-carboxaldehyde was recently tentatively identified in Semillon and Chardonnay wines by GC×GC analytical approaches.[34,38]

1*H*-Pyrrole (CAS: 109-97-7, (**5**), Figure 3) was identified with simultaneous *m/z* 52 and 67 signals generating a peak at LRI_{BP20} 1505. Validation with the pure standard allowed us to associate this compound with "grilled nut" OZ "E" (RI_{BP20} 1508). It was previously evidenced in Merlot wine.[20]

While these five pyrroles have been evidenced and associated with hazelnut OZ in Chardonnay wines extracts, four of them have been also identified in hazelnut extracts: 1-methylpyrrole-2-carboxaldehyde (**2**), 1*H*-pyrrole (**5**), 1*H*-pyrrole-2-carboxaldehyde (**4**), and 2-acetyl-1*H*-pyrrole (**3**). Some of them have also been proposed as markers of roasting of hazelnut.[39–42] Nevertheless, little is known about their sensory impact on hazelnut aroma. In this work, no clear identification of compounds associated to the OZs A, B, C, D, H, I, L, O and P has been elucidated: low signal and co-elutions are probably the limiting factors for identifications of compounds associated. Higher levels of purification and enrichment would help achievement of extending the list of compound identified.

As hazelnut is perceived in typical Chardonnay wines, we set out to analyze and compare the individual amounts of these five pyrroles in Chardonnay (n = 14) and Non-Chardonnay wines (n = 14).

Quantitation of Pyrroles in Wine Extracts and Assessment of their Sensory Impact. Volatile organic compounds were extracted by using the SPE according to the method developed by Culleré *et al.*.[27] Standard addition of pure compounds in wine prior to

414 extraction allowed the determination of the quantitation slopes for every compound
415 investigated. GC-MS analysis targeting specific *m/z* in the dedicated time windows was
416 conducted on the SPE extracts (Table 2).

417 The N-substituted pyrrole, 1-methylpyrrole-2-carboxaldehyde (**2**) was quantitated at
418 916 ± 213 ng/L in Chardonnay wines, an eight-fold increase compared to Non-Chardonnay
419 wines (115 ± 54 ng/L; Figure 3), which was found to be a significant difference in the non-
420 parametric Kruskal-Wallis test (p-value < 0.005). Similarly, with an amount measured at 300
421 ± 94 ng/L for Chardonnay wines, the homologue 1-ethylpyrrole-2-carboxaldehyde (**1**) was on
422 average four times more abundant in Chardonnay wines than in Non-Chardonnay wines (74 ±
423 27 ng/L; Figure 3). The Kruskal-Wallis test showed that this difference was significant (p-
424 value < 0.001). 2-Acetyl-1*H*-pyrrole (**3**) showed the lowest levels of all five pyrroles, with
425 average concentrations of 223 ± 46 ng/L in the Chardonnay wines and significantly lower
426 levels in Non-Chardonnay wines (60 ± 23 ng/L) (p-value < 0.001 in the Kruskal-Wallis test).
427 The average concentrations of 1*H*-pyrrole-2-carboxaldehyde (**4**) were assayed at 5,060 ±
428 2,423 ng/L in the Chardonnay wines investigated here, which was over five times higher than
429 that in Non-Chardonnay wines (1,015 ± 598 ng/L – distribution shown in Figure 3). So, this
430 compound, which was previously quantitated at 16,800 ng/L in a Chardonnay wine,[38] was
431 significantly more present in Chardonnay wines (p-value < 0.005). 1*H*-Pyrrole (**5**) was found
432 at levels of 2,018 ± 610 ng/L in the assessed Chardonnay wines and 1,166 ± 602 ng/L in
433 Non-Chardonnay wines, and these differences were not found to be significant (p-value >
434 0.05). The large overlap between the concentrations of Chardonnay and Non-Chardonnay
435 wines meant that this compound is not discriminant.

436 In order to assess the role of those compounds on aroma, their individual detection
437 thresholds were estimated in model wine and in a dry white wine. The roasted-like tea pyrrole
438 (**1**) was the most perceivable compound in dry white wine and model wine with values

around 1 mg/L (Table 4). 1-Methylpyrrole-2-carboxaldehyde was perceived around 20 mg/L in wine. The two most abundant pyrroles, (**4**) and (**5**), were perceived in wine at 8 and 26 mg/L, respectively (Table 4). 2-Acetyl-1*H*-pyrrole (**3**), the least represented pyrrole, also proved to be the least odor-active compound with a perception threshold above 120 mg/L (Table 4). Thus, the content/threshold ratio defining the Odor Activity Value (OAV) index was below 10^{-3} for all compounds, suggesting that individually these pyrroles have no sensory effect on Chardonnay wine aroma. Regarding to their common moiety, synergistic effects could occur between these five-membered ring heterocycles.[43] Nevertheless, the addition of a mixture containing compounds (**1**) to (**5**) at concentrations similar to those observed in wine did not modify the aroma of a white wine. Consequently, despite their higher amounts in Chardonnay wines and their almond/hazelnut notes, these pyrroles do not have any direct impact on the flavor of the Chardonnay wines studied here.

Assessment of Enological Parameters. Despite occurring at levels below their individual odor threshold and therefore likely not having any impact, pyrroles (**1**-**4**) seemed to chemically discriminate Chardonnay and Non-Chardonnay wines. Interestingly, Rizzi *et al*. have reported that *N*-alkyl-2-acylpyrroles can be produced by reaction between α-amino-acids and furfural, a volatile aldehyde released by oak wood.[44] Moreover, a recent study dealing with the adsorption of wood volatiles on yeast cell-walls showed the release of 1*H*-pyrrole-2-carboxaldehyde from lees previously macerated in an alcoholic extract of oak wood.[45] Jointly, these observations seemed to indicate that some pyrroles could be released by oak wood. Therefore, owing to the more frequent storage in oak barrels of Chardonnay but not Non-Chardonnay wines, the presence of pyrroles was assessed in an hydro-alcoholic extract of oak wood (*Quercus petrae*) collected from a barrel stave. After GC-MS analysis, pyrroles (**2**-**4**) were detected in oak wood extracts while 1-ethylpyrrole-2-carboxaldehyde (**1**) was not observed (data not shown). These results suggested that the presence of pyrroles (**2**-

464 **4**) in wine could be partly due to their release from oak wood. Moreover, Chardonnay wine s
465 are more often fermented and aged in contact with oak than other white wines which supports
466 this hypothesis. However, some of the Chardonnay wines investigated contained only traces
467 or even no detectable presence of oak wood markers ((*E*)- and (*Z*)-oak lactones below LOQ 1
468 µg/L, data not shown) and paradoxically had significant levels of pyrroles (682 and 348 ng/L
469 detected in CHAUS9 respectively for 1-ethylpyrrole-2-carboxaldehyde and 2-acetyl-1*H*-
470 pyrrole). In particular, the wine CHCHAB11 has been fermented and aged exclusively in
471 stainless steel tank and presented the highest amount of 1*H*-pyrrole-2-carboxaldehyde
472 (18,860 ng/L) and significant levels of the other pyrroles. On the other hand, Non-
473 Chardonnay wines aged in oak barrels such as SBS21 and MCB29 (containing over 30 and
474 70 µg/L of (*Z*)- and (*E*)-oak lactones, respectively) exhibited lower contents of 1-
475 ethylpyrrole-2-carboxaldehyde, 1-methylpyrrole-2-carboxaldehyde, and 2-acetyl-1*H*-pyrrole
476 when compared to Chardonnay wines (below 162 ng/L, 308 ng/L and 31 ng/L, respectively).
477 The same analysis applied on Chardonnay grape juice from the Languedoc region prior to
478 any contact with oak wood allowed the detection of 1-methylpyrrole-2-carboxaldehyde and
479 1*H*-pyrrole-2-carboxaldehyde (data not shown). Consequently, the high levels of pyrroles
480 observed in non-oaked Chardonnay wines, their low levels in oaked Non-Chardonnay wines
481 and their presence in Chardonnay grape juice before contact with oak suggest that pyrroles
482 are not only provided by oak wood but also originate from grape juice and wines. Further
483 studies are required to clearly establish the relative contribution of the varietal origin and the
484 aging conditions on pyrrole levels in wine.

485 **Investigation of the Presence of Thiol-derived Pyrroles.** Furfural and 5-
486 methylfurfural are among the most abundant compounds released from oak wood into wine
487 during aging, and were found at contents up to 6 mg/L and 0.8 mg/L in oak-aged wines.[46]
488 Those two heterocyclic compounds never reach their individual odor threshold (ranging from

20 to 65 mg/L in wine).[46,47] However, their transformation products 2-furanmethanethiol and 5-methyl-2-furanmethanethiol have been identified in wine.[48] These thiol derivatives exhibit very low odor thresholds (0.4 and 50 ng/L, respectively) and significantly impact wine aroma, contributing to roasted coffee and toasted notes.[49] Furthermore, Floch *et al.*[49] recently showed that the vanillin transferred to wine during oak aging was partly transformed into vanillylthiol, lowering the detection threshold from 65 to 3.8 µg/L.[49] Regarding the common structure of pyrrole carboxaldehydes and the potent reactivity of the aldehyde group, it appeared relevant to investigate the occurrence of thiol derivatives of pyrroles in Chardonnay wines. Derivatives of pyrroles (**1**) and (**2**) were particularly targeted. As the corresponding pyrrolemethanethiols had never been observed in natural products and were not easily available, we first sought to obtain them through a one-pot reaction in order to investigate their potential presence in wine. Thiol can be generated from an aldehyde via the conjugation to cysteine and it can be further biotransformed by yeast activity.[30,50] 1-Methylpyrrole-2-carboxaldehyde and 1-ethylpyrrole-2-carboxaldehyde were mixed with cysteine according to the procedure described by Huynh-Ba *et al.*[30] adapted from Schubert.[29] The resulting conjugates were tentatively characterized by UHPLC–HRMS. The analysis revealed the presence of one peak associated with the cysteine-methylpyrrole conjugate protonated ion ($[M + H]^+$, *m/z* 213.277 and another peak associated with the cysteine-ethylpyrrole conjugate protonated ion ($[M + H]^+$, *m/z* 227.303).

The precipitate was bioprocessed in the presence of yeast for the expected *ß*-lyase activity.[30] GC-MS analysis of the extracted medium allowed the detection of two peaks responding to *m/z* 127 and 141 for the two expected products (Figure 4). The spectra were tentatively attributed to 1-methylpyrrole-2-methanethiol (**6**) at 31.0 min (LRI_{BP20} 1787, Figure 4A) and 1-ethylpyrrole-2-methanethiol (**7**) at 31.3 min (LRI_{BP20} 1813, Figure 4B). Identification was confirmed by the injection of pure standard compounds (**6**) and (**7**),

514 indicating also that compounds (**6**) and (**7**) were actually present in the reaction mixture
515 described above.

516 GC-O analysis of the extract of the bioprocessed reaction mixture led to the
517 perception at the specified LRI of a strong grilled roasted almond-like odor at 38.6 min
518 (LRI_{BP20} 1783) and a grilled hazelnut-like odor at 39.4 min (LRI_{BP20} 1813). These two
519 odorant zones corresponded to the RT and odor of the OZ "J" and "K" that are specific to F1
520 and F2 in Chardonnay wine extracts (Table 3). Moreover, the co-injection of standards (**6**)
521 and (**7**) by GC-O analysis on a polar capillary (BP20) confirmed the coincidence of the RT
522 with OZ "J" and "K".

523 Analysis of the wine fraction by GC-MS using Selected Ion Monitoring Mode (SIM)
524 (30-32 min, *m/z* 127, 94 and 95 for 1-methylpyrrole-2-methanethiol and *m/z* 141, 108, 80 for
525 1-ethylpyrrole-2-methanethiol) evidenced only a noise threshold in the chromatograms and
526 did not allow the detection of either of the two compounds. Given the lack of specificity of
527 MS detection, a method using specific MS/MS transitions was developed. GC-triple
528 quadrupole analysis has been shown to be a powerful technique for the detection and
529 quantitation of trace level compounds involved in wine aroma.[51] The main ions obtained from
530 EI ionization were filtered and fragmented and the most responsive transitions were used in
531 the method.

532 GC-MS/MS analysis of the reaction mixture (Figure 5A) exhibited a peak at 38.40
533 min ($LRI_{ZB\text{-}1}$ 1111) for the transitions 127→94 and 94→53. The analysis of pure standard
534 compounds showed that these transitions and retention times were characteristic of (**6**). After
535 injection of several blank samples to ensure the absence of any carry-over effect (data not
536 shown), the analysis of a Chardonnay wine extract (CHSA5) showed a peak for each of these
537 two transitions at the same retention time (Figure 5B). Similarly, GC-MS/MS chromatograms
538 of the reaction mixture (Figure 5A) and the SPE Chardonnay wine extract (Figure 5B)

539 evidenced a peak at 41.6 min (LRI_{ZB-1} 1172) for the transitions 141→108 and 108→53.
540 These transitions and retention times were characteristic of (**7**). The relative retention time of
541 both pyrrolemethanethiols in the sample and the calibration solution varied less than ± 5%.
542 Five identification points were verified: two precursor ions, each with one daughter; relative
543 ion intensities less than ± 20%.[52] Co-injection of the synthetic extract with the wine extract
544 generated a single sharp peak for every of the two compounds (at 38.4 min and 41.6 min).
545 GC-O analysis revealed the elution of this compound in the OZ "J" and "K". To our
546 knowledge, this is the first identification of pyrrolemethanethiols (**6**) and (**7**) in wine and
547 more generally in a natural product.

548 Olfactory analysis of the pure standards showed that compounds (**6**) and (**7**) exhibited
549 strong aromas of grilled hazelnut and roasted almond consistent with some notes often
550 perceived in typical Chardonnay wines. The olfactory detection thresholds of these two new
551 compounds were determined by a panel of 44 tasters. In model wine, the odorant thresholds
552 of (**6**) and (**7**) were 0.7 ng/L and 1.4 ng/L, respectively (Table 5). These extremely odorant
553 compounds were perceived at amounts 10^7 and 10^6 lower than the corresponding pyrrole
554 carboxaldehydes.

555 The discovery of such powerful odoriferous compounds in wine opens up promising
556 perspectives. Further investigations will aim at determining the concentrations and the
557 sensory role of these two highly odoriferous grilled hazelnut-like compounds in Chardonnay
558 wines. The chemical mechanisms involved in the formation and evolution of these
559 compounds also need to be elucidated as well as the enological parameters modulating their
560 concentrations in wine. Such results would provide new insights into the molecular origin of
561 the volatiles contributing to the identity of typical Chardonnay wines in order to improve
562 their winemaking and aging techniques.

For the first time, the presence of volatile markers sharing a common structure associated with Chardonnay wines is proposed. Despite their irrelevant contribution to sensory analysis since they are below their sensory threshold, the presence of 1-ethylpyrrole-2-carboxaldehyde, 1-methylpyrrole-2-carboxaldehyde, 1*H*-pyrrole-2-carboxaldehyde and 2-acetyl-1*H*-pyrrole were found at significantly higher concentrations in Chardonnay wines. Methanethiol derivatives of 1-ethylpyrrole-2-carboxaldehyde and 1-methylpyrrole-2-carboxaldehyde were identified here for the first time and their odorant power drastically increases (10^6 factor) in comparison with the corresponding pyrroles. Their impact on Chardonnay wine aroma now needs to be investigated.

Abbreviations Used

LRI, Linear Retention Index; LOQ, Limit of Quantitation; LOD, Limit of Detection; IS, Internal Standard; RT, Retention Time.

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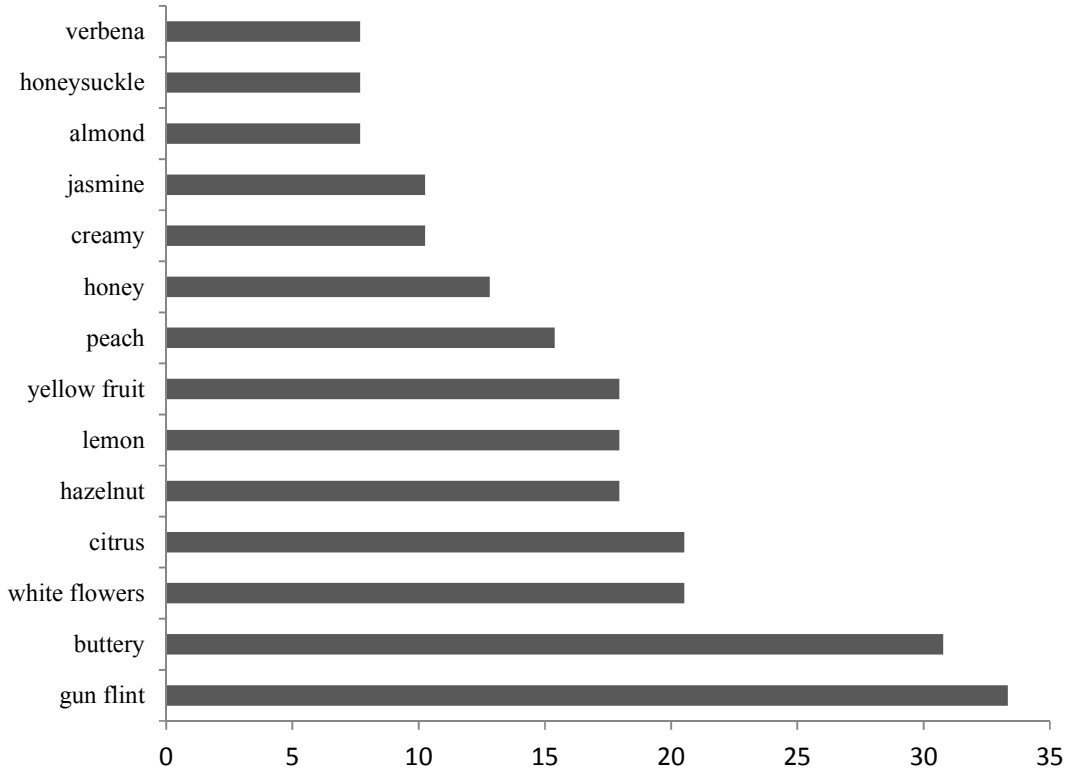

Figure 1. Emergence of the 14 most elicited descriptors from sensory analysis of four Chardonnay wines by 24 panelists (descriptor occurrence frequencies = number of occurrences of descriptor/total number of descriptors).

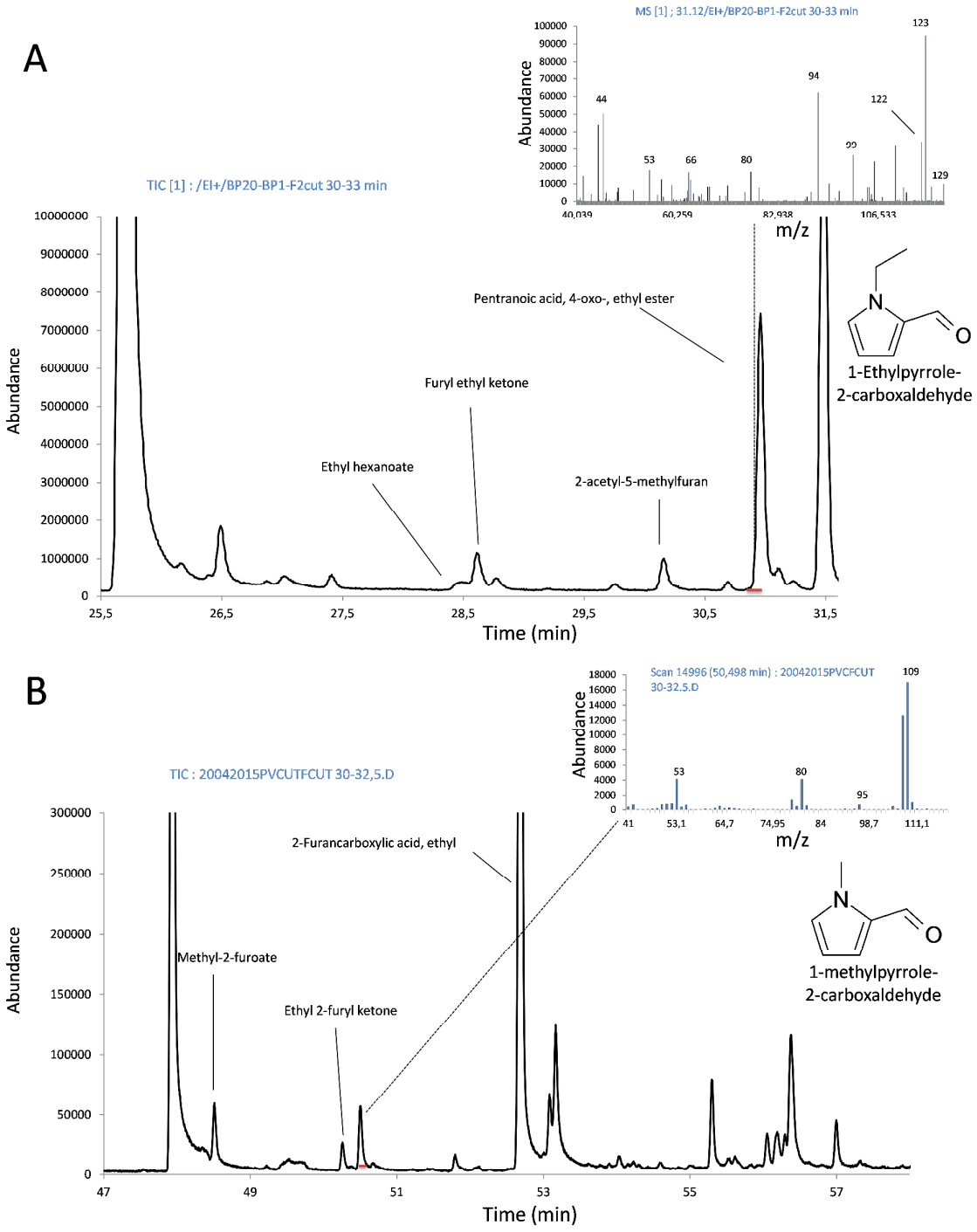

Figure 2. (A) MS chromatogram main run (BP1) of Pernand Vergelesses 2011 (CHPV3) wine extract analysis obtained from heart cut between 30-33 min on pre-run (BP20). (B) MS chromatogram of main run (HP5) of Pernand Vergelesses 2011 (CHPV3) wine extract analysis obtained from heart cut between 30.5-32.5 min on pre-run (BP20).

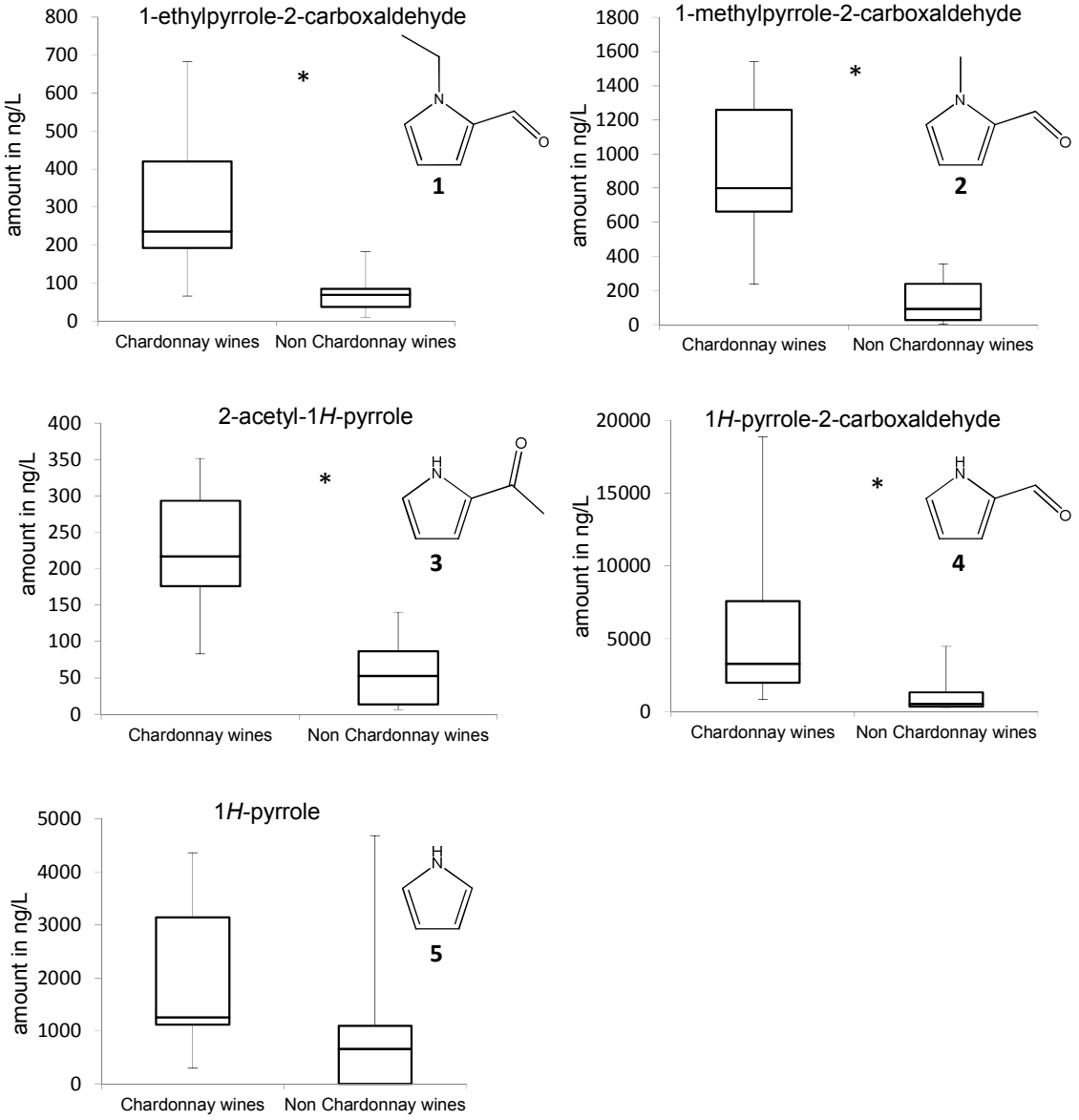

Figure 3. Quantitation of 1-ethylpyrrole-2-carboxaldehyde, 1-methylpyrrole-2-carboxaldehyde, 2-acetyl-1*H*-pyrrole, 1*H*-pyrrole-2-carboxaldehyde, and 1*H*-pyrrole in Chardonnay (n = 14) and non-chardonnay wines (n = 14). *Significant difference assessed by Kruskal-Wallis test (p value < 0.05).

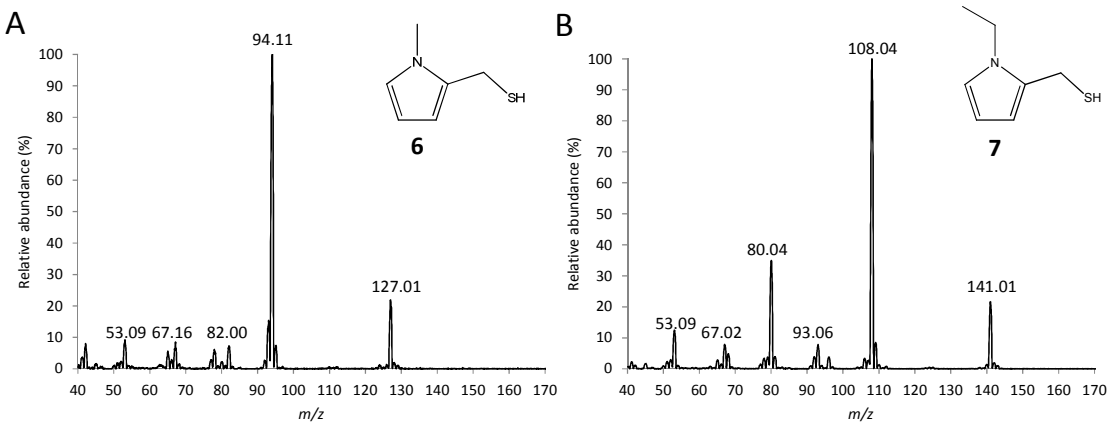

Figure 4. FullScan MS spectra recorded at 38.4 min on 1-methylpyrrole-2-methanethiol (A) and at 41.6 min on 1-ethylpyrrole-2-methanethiol (B).

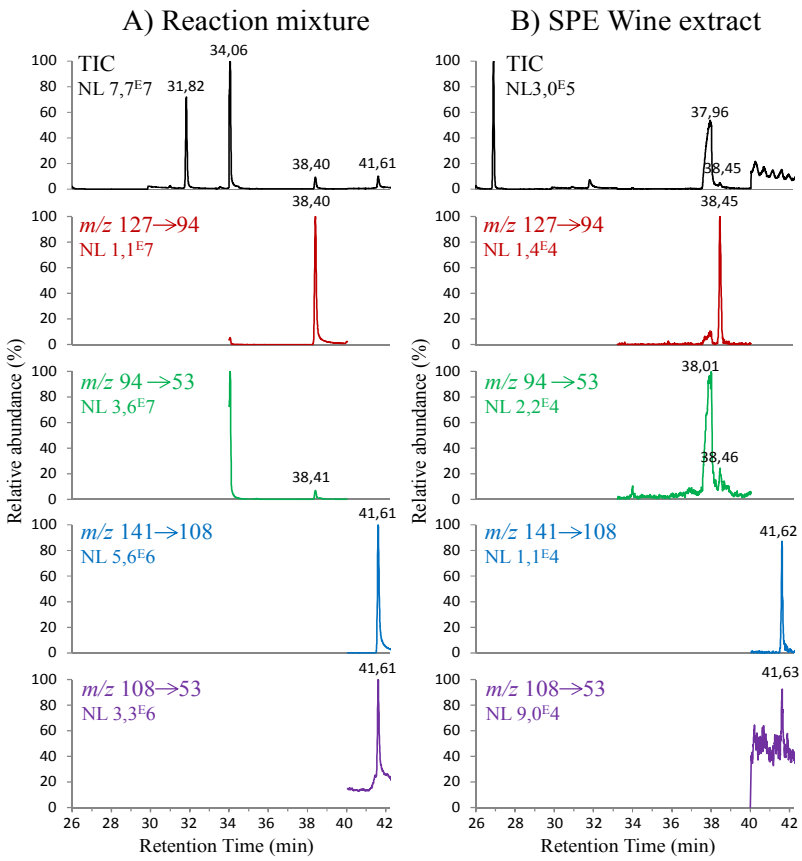

Figure 5. Comparison of GC-MS/MS chromatograms recorded for pyrrole reaction mixture (left) and a Chardonnay wine SPE extract (right). From top to bottom: Total ion chromatogram, chromatograms in SRM mode corresponding to transitions for 1-methylpyrrole-2-methanethiol (**6**) (127 → 94; 94 → 54) and 1-ethylpyrrole-2-methanethiol (**7**) (141 → 108; 108 → 54). NL: normalized intensity level

TABLES

Table 1. Grape Variety, Identification, Origin and Vintage of Wine Samples.

Num.	Grape Variety	Identifier	Origin	Vintage
1	Chardonnay	CHPV1	Pernand Vergelesses 1er cru - France	2007
2	Chardonnay	CHPV2	Pernand Vergelesses 1er cru - France	2011
3	Chardonnay	CHPV3	Pernand Vergelesses - France	2011
4	Chardonnay	CHCM4	Chassagne Montrachet - France	2010
5	Chardonnay	CHSA5	St Aubin 1er Cru - France	2011
6	Chardonnay	CHCM6	Chassagne Montrachet - France	2008
7	Chardonnay	CHCM7	Chassagne Montrachet - France	2011
8	Chardonnay	CHAUS8	Margaret River - Australia	2012
9	Chardonnay	CHAUS9	Victoria – Australia	2007
10	Chardonnay	CHCHAB10	Chablis - France	2009
11	Chardonnay	CHCHAB11	Chablis - France	2007
12	Chardonnay	CHCHAB12	Chablis - France	2011
13	Chardonnay	CHMEUR13	Meursault - France	2011
14	Chardonnay	CHPUL14	Puligny Montrachet - France	2010
15	Chardonnay	CHPUL15	Puligny Montrachet - France	1997
16	Chardonnay	CHBEA16	Beaune 1er Cru - France	1996
17	Riesling	RIES17	Alsace - France	2009
18	Sauvignon Blanc	SB18	Pessac-Léognan - France	2009
19	Sauvignon Blanc	SB19	Pays d'Oc - France	2013
20	Sauvignon Blanc	SB20	Sancerre - France	2012
21	Sauv. Blanc - Semillon	SBS21	Bordeaux - France	2012
22	Sauv. Blanc - Semillon	SBS22	Bordeaux - France	2010
23	Aligoté	Ali23	Bourgogne - France	1998
24	Aligoté	Ali24	Bouzeron - France	2007
25	Viognier	VIOA25	Tumbarumba - Australia	2013
26	Viognier	VIOA26	Trentham- Australia	2010
27	Viognier	VIOR27	Collines Rhodaniennes - France	2010
28	C.-Sauvignon, Merlot	MCB28	Graves - France	2013
29	C.-Sauvignon, Merlot	MCB29	Saint Julien - Medoc - France	2010
30	Grenache	GRE30	Vallée du Rhône - France	2013
31	Melon B	MUS31	Muscadet Sèvre-et-Maine - France	2013

Table 2. Validation Data for GC-MS Method.

Name	*m/z* quantifier (qualifier)	R^2	slope	linear range	recovery at 150 ng/L	LOD (ng/L)[a]	LOQ (ng/L)[b]
1*H*-pyrrole	67 (52; 41)	0.995	130539	10^3	102	10	25
1-ethylpyrrole-2-carboxaldehyde	123 (108; 94)	0.998	76472	5.10^2	101	13	32
1-methylpyrrole-2-carboxaldehyde	109 (108; 80)	0.996	59540	10^2	97	12	25
2-acetyl-1*H*-pyrrole	94 (109; 66)	0.997	11522	10^2	94	8	14
1*H*-pyrrole-2-carboxaldehyde	95 (94; 66)	0.999	127303	10^3	106	15	37

[a] LOD, Limit of Detection; [b] LOQ, Limit of Quantitation.

Table 3. Hazelnut-like Odoriferous Zones Evidenced by GC-Olfactometry in Distillate Fractions (Analysis on Carbowax-type Capillary).

odorant zone	descriptor	LRI[a] BP20	perception in distillate fractions analyzed in GC-O[b]	
			F1	F2
A	almond, sweet	1378	-	+
B	fresh hazelnut	1415	+	-
C	dry hazelnut	1438	+	+
D	grilled toasted	1467	+	-
E	almond, sweet hazelnut	1505	+	-
F	roasted hazelnut	1617	+	-
G	hazelnut	1641	+	-
H	hazelnut	1708	+	-
I	almond, flowery	1751	+	-
J	roasted almond	1783	-	+
K	grilled hazelnut	1813	+	+
L	raw hazelnut	1910	+	+
M	smoked hazelnut, sweet	1946	+	-
N	hazelnut, coffee	2010	+	-
O	hazelnut, praline	2078	+	+
P	hazelnut, almond, nougat	2250	+	-

[a] LRI, Linear Retention Index; [b] +, perceived; -, not perceived

Table 4. Analytical and Sensory Characteristics of Pyrroles Identified in this Study.

LRI[a] BP20	LRI[a] BP1	compound	threshold (mg/L)	
			model wine[b]	white wine
1505	-	1*H*-pyrrole (**5**)	21.3	26.1
1617	1026	1-ethylpyrrole-2-carboxaldehyde (**1**)	0.7	1.2
1641	975	1-methylpyrrole-2-carboxaldehyde (**2**)	13.6	19.6
1946	1020	2-acetyl-1*H*-pyrrole (**3**)	94.1	126
2010	990	1*H*-pyrrole-2-carboxaldehyde (**4**)	3.2	7.9

[a] LRI, Linear Retention Index; [b] Model media, 12% EtOH/H_2O (v/v); pH 3.4; 5 g/L tartaric acid.

Table 5. Analytical and Sensory characteristics of Pyrrolemethanethiols Identified in this Study.

LRI[a] $_{BP20}$	LRI[a] $_{ZB1}$	compound	threshold (ng/L)[b]
1783	1111	1-methylpyrrole-2-methanethiol (**6**)	0.7
1813	1172	1-ethylpyrrole-2-methanethiol (**7**)	1.4

[a] LRI, Linear [a] Linear Retention Index; [b] threshold determined in model media, 12% EtOH/H_2O (v/v); pH 3.4; 5 g/L tartaric acid.

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