JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Subscriber access provided by University of Sussex Library

Chemistry and Biology of Aroma and Taste

Quantitation, organoleptic contribution and potential origin of diethyl acetals formed from various aldehydes in Cognac.

Fannie Thibaud, Svitlana Shinkaruk, and Philippe Darriet

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.9b01084 • Publication Date (Web): 19 Feb 2019

Downloaded from http://pubs.acs.org on February 25, 2019

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Quantitation, organoleptic contribution and potential origin of diethyl acetals formed from various aldehydes in Cognac.

Fannie Thibaud,† Svitlana Shinkaruk,‡ † and Philippe Darriet†

† Univ. Bordeaux, Unité de recherche Œnologie, EA 4577, USC 1366 INRA, ISVV, 33882 Villenave d'Ornon cedex, France

‡ Univ. Bordeaux, ISM, Institut des Sciences Moléculaires, UMR 5255 CNRS, 33405 Talence, France

Abstract

Cognac Wine Distillate (WD), especially during their aging, are marked by complex and elegant aroma. This work aimed at expanding the knowledge on the Cognac WD aroma by a sensory-guided approach, involving fractional distillation technique and gas chromatography coupled to olfactometry and mass spectrometry (GC-O-MS). In doing so, a fruity odorous zone was highlighted in WD extracts which was attributed to the diethyl acetal family. Ten additional diethyl acetals were detected by GC-MS. Next, an assay method was developed and validated for seven of these diethyl acetals. Their detection thresholds were evaluated in a model solution of water/ethanol (v/v 60/40). 1,1-Diethoxy-3-methylbutane was shown to present a significant organoleptic impact because its olfactory detection threshold (323 μg/L) is lower than its range of concentrations in WD (461 to 3 337 μg/L). Given that, diethyl acetals result from the reaction between ethanol and aldehydes, quantitative correlations between diethyl acetals and corresponding aldehydes were considered.

Keywords :

Aroma, Cognac spirit, diethyl acetals, sensory impact

Introduction

Cognac spirits are traditional products from the Nouvelle Aquitaine region (France) elaborated in a context of denomination of appellation origin (AOC Cognac). They originate initially from fermented grape juices mainly from *Vitis Vinifera* Ugni blanc (i.e. Trebbiano bianco). This grape variety produces wines with a low ABV content (Alcohol By Volume less than 10% vol.) and a high level of acidity. In the Cognac process, before march 31th, the wines should be distilled through a traditional double distillation in a copper still known as *Methode Charentaise*. Young WD at 70% ABV are obtained and placed in oak barrels for sometimes a very long aging period (several decades). At the end, depending on the Cognac quality, the cellar master proceeds to blend several WD from different vintages in order to create the final Cognac spirit.[1]

During the aging process, the young WD, usually marked by a component of fermentative aromas, is enriched by the wood components, including odorous and taste compounds.[2,3] WD is the place of multiple chemical reactions (oxidation, hydrolysis, esterification, rearrangement, etc) which induce changes in its composition of volatile compounds throughout aging.[4–6] The process of ethanol and water evaporation leads to an increase of volatile compounds concentration. These phenomenon help to limit the hotness sensation, as well as heavy aromatic notes with green character.[7] Also, the aging phenomenon contributes to enhancing particular aromatic notes (wood, vanilla, dried and fresh fruits, balsamic, spicy) depending on the age of the WD[8], grouped under the term "*rancio charentais*"[4,8]. After this long process, WD present a great complexity and finesse of their aromatic components, complemented by a strong harmony of taste and flavor which is emphasized in the old commercial Cognac spirits by the blend of numerous WD.

The complexity of Cognac spirit aromas related to the presence of a wide diversity of volatile odorous compounds associated with production and aging process.[1,9] Nowadays, nearly 500 of them from different chemical families have been identified, including alcohols, acids, esters, ethers, aldehydes, ketones, hydrocarbons, terpenes, C13-norisoprenoids, lactones, phenols, sulfur and nitrogen compounds.[10–18] Many of these compounds have been identified and assayed in freshly distilled wine spirit. During aging, the balance between these molecules is much modified.[6,19]

Consequently, the complexity of Cognac matrix, makes analyze of odorous compounds, particularly the more odor-active, very difficult.[18] Various sample preparation methods can be considered to make this matrix less complex with dedicated analytical strategies, such as selective extraction of odorous compounds of interest with Solid Phase Extraction (SPE),[20,21] fractionation with High Performance Liquid Chromatography (HPLC)[22,23] or vacuum distillation previous to gas chromatography analysis.[24,25] Among various approaches, the one focused on a specific odor is pertinent to identify compounds using GC-Olfactometry (GC-O).[26] This strategy has been considered by several authors for the characterization of Cognac aroma.[9,16,27–29] But in such complex matrix, the strategy developed for the characterization of odorous volatiles should implicate to develop not only one, but several fractionation methods, each of them associating at each stage analytical/sensorial approach.

While numerous publications have studied young Cognac WD spirits, the purpose of this work was to make progress in the identification of odorous volatile compounds present in aged WD Cognac spirit via a sensory-guided approach. This research first used fractionated distillation of WD before applying a GC-O/GC-MS analysis. In doing so, the chemical family of diethyl acetals was highlighted, some of which have already been identified in Cognac,[16,28–31] Chinese liquors,[32] Tequila,[33] Grappas[34] and other brandies.[35] Their synthesis, their implication in the aromas of aged Cognac spirit, and the ways in which they are formed during aging, were also considered in the present study.

Material and methods

Chemicals

Dichloromethane (99%) was supplied by Fisher Scientific (Illkirch, France). Absolute ethanol (EtOH) (99%), Silica gel and methanol HPLC grade were from Merck (Semoy, France). 4-Methylpentan-2-ol (98%), 1,1-diethoxybutane (97%), 1,1-diethoxy-2-methylpropane (97%), 1,1,3-triethoxypropane (97%), 2-(diethoxymethyl)furan (97%), 1,1-diethoxypentane (97%), 1,1-diethoxyhexane (97%), undecanal (97%), heptanal (97%), benzenacetaldehyde (95%), 3-methylbutanal (97%), pentanal (97%), hexanal (98%), anhydrous sodium sulfate (99%), Supelclean™ LC-18 SPE tube, *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) (99%), 4-fluorobenzaldehyde (98%), *p*-toluenesulfonic acid monohydrate (>98.5%), diethyl ether, pentane (99%) and molecular sieves 3Å were purchased from Sigma-Aldrich Chemicals (Saint Quentin Fallavier, France). 2,2-Diethoxyethylbenzene (97%) was purchased from Life Chemical, Canada. 1,1-diethoxy-3-methylbutane (97%) was obtained from Matrix Scientific (Elgin, USA). Ultrapure water (Milli-Q, resistivity = 18.2 MΩ cm, Millipore, Saint-Quentin-en-Yvelines, France) was used.

Samples

All WD samples were provided by E.Rémy Martin & C°. They were not commercial products but selected WD from different vintages that were not blended or diluted. A WD is the product of several distillations that have been made during the same vintage. All of these WD were then raised in oak barrels within the company. Depending on the vintage, ABV vary between 55% and 69%. The percentage of alcohol is presented in the supporting information (Table S1). The samples were first selected in-house by an internal expert panel as being representative of premium spirits. All the WD were removed from barrels in the same year (2016) so that their age count stopped at bottling. Ten WD were selected in total (2015, 2006, 2002, 1999, 1998, 1997, 1996, 1983, 1982, 1972). For the quantitative measurement of diethyl acetals and aldehydes WD of 2009 was used for calibration.

Preparation of Extracts by sequential vacuum distillation

Two WD (2006 and 1997) were used at beginning for this experiment. One hundred milliliters of each WD and 400 mL of water, to obtain an ABV of around 14%, were placed in the flask of a rotary evaporator steeped in a bath at room temperature.[24] Under vacuum volatiles were trapped with a condenser equipped with a cooling system (-5°C). The following sequential distillation parameters were used: 50 mbar for 15 min which permitted to collect fraction F1, then 30 mbar for 15 min (collection of fraction F2), and 20 mbar for 15 min (fraction F3). At the end, the distilled volumes obtained corresponded to 10 mL for F1, 25 mL for F2 and 40 mL for F3. Each fraction collected presented a high level of ethanol (F1 ≈ 65%, F2 ≈ 40%, F3 ≈ 20% (v/v)), and they were diluted with ultrapure water to reach 12% EtOH (v/v). After this, the three fractions obtained were subjected to sensory evaluation by a panel of five experts who were asked to describe the main olfactory notes of the distillates. The fraction(s) selected by the sensory panel were then extracted twice by dichloromethane (magnetic stirring: 10 min each time; 750 rpm). Then the organic phases were combined, dried on anhydrous sodium sulfate and concentrated to 0.25 mL under nitrogen flow close to 100 mL/min at room temperature before GC analysis.

Quantitation of ethyl esters

Ethyl acetate, ethyl butyrate, ethyl caproate (ethyl hexanoate), ethyl caprylate (ethyl octanoate), ethyl caprate (ethyl decanoate), ethyl lactate and ethyl laurate (ethyl dodecanoate) (total of ethyl ester) were quantified using direct injection of the distillate by GC-FID. Initially, ten milliliters of each WD were supplemented with 100 µL of internal standard (4-methylpentan-2-ol at 30 g/L in ethanol). Then, one microliter of each sample was directly injected in split mode (1/30). The capillary column used was a CP WAX 57 CB (Agilent Technologies, Santa Clara, CA, USA) (50 m length; 0.32 mm internal diameter (i.d.); 0.20 µm film thickness). The injector was at 260 °C and the flow was constant (1.6 mL/min). The oven was programmed as follows: 35 °C for 6.5 min, then increasing by 5 °C/min to 190 °C and maintained at this temperature for 18 min. The FID detector was maintained at 300 °C.

Gas chromatography coupled with olfactometry and mass spectrometry analysis (GC-O-MS)

GC-O-MS analysis was carried out on an Agilent 7890 B LTM (Low Thermal Mass) (Agilent Technologies, Santa Clara, CA, USA) gas chromatograph coupled to an olfactometric port (ODP-3 (Gerstel, Germany). Two microliters of the concentrated organic extract were injected using a splitless injector (240 °C; splitless time 1 min; purge flow 50 mL/min). Separation was achieved on a DB-WAX (30 m length; 0.25 mm i.d.; 0.25 µm film thickness). Helium N55 was used as a carrier gas at a constant flow of 1.5 mL/min. The initial GC oven temperature was set at 45 °C for 1 min, before rising to 230 °C at 5 °C/min, and was maintained at 230 °C for 15 min. A two-way splitter (Agilent Technologies, Santa Clara, CA, USA) at the column exit permitted to transfer 50% of the effluent to a MS 5977 A (Agilent Technologies, Santa Clara, CA, USA) mass spectrometer (transfer line at 250 °C, ion source at 250 °C, electron impact (EI) voltage at 70 eV, ions acquisition within a range of *m/z* 40-250), while 50% of the effluent was constantly directed through a deactivated fused silica column to a sniffing port. Olfactory data collecting during the GC-O analysis were processed with the Olfactory Recorder Software Dragon (Nuance Communications, Newton, MA, USA). During a GC-O analysis, the operator started 7 min after the beginning of the GC run and continued up to 40 min. Odorous zone descriptors and their durations were monitored by recording the voice of the operator doing sniffing as a start and stop signal. For each extract, GC-O analysis was done by three experts. Instrument setting, data acquisition and processing were controlled by ChemStation (B.04.01) software. Chemical identification was obtained with the help of the NIST 2004 library (U.S. National Institute of Standards and Technology, Gaithersburg, MD, USA). Then, each volatile identification was confirmed by injection of pure standard in the same chromatographic conditions.

Synthesis of 1,1-diethoxyheptane, 1,1-diethoxyundecane and 1,1-diethoxy-2-methylbutane

A solution of *p*-toluenesulfonic acid monohydrate (1.90 g, 10 mmol), the corresponding aldehyde (50 mmol), and molecular sieves 3Å (6.5 g) in anhydrous ethanol (50 mL) was placed under argon and under gentle oscillating stirring for 18 h. After this, the reaction mixture was filtered to remove the molecular sieves and then carefully evaporated at 40 °C under reduced pressure at 150 mbar to remove the ethanol. The crude was diluted with diethyl ether and filtered again. The organic phase was concentrated at 30 °C under reduced pressure at 600 mbar, yielding make the crude product as a colorless oil. The ^{1}H NMR analysis of crude product showed that at least 75% of the aldehyde was converted to the corresponding acetal. The residue was then purified by silica gel column chromatography using diethyl ether and pentane as an eluent in a volume ratio of 2:98.

*NMR***:** Nuclear Magnetic Resonance Spectroscopy (NMR): ^{1}H, and ^{13}C NMR spectra were recorded on a Bruker Avance I (^{1}H: 300 MHz, ^{13}C: 75 MHz), and the spectra referenced using the lock frequency of deuterated solvent. Chemical shifts (δ) and coupling constants (*J*) are expressed in ppm and Hz, respectively. Merck silica gel 60 (70–230 mesh and 0.063–0.200 mm) was used for flash chromatography. Spots were revealed with potassium permanganate stain.

1,1-diethoxyheptane: colorless liquid (75% conversion, 64% isolated yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, *J* = 6.9 Hz, 3H, $C\underline{H}_3$-7), 1.18 (t, *J* = 7.1 Hz, 6H, $OCH_2C\underline{H}_3$), 1.38 – 1.22 (m, 8H, $C\underline{H}_2$-3-6), 1.65 – 1.50 (m, 2H, $C\underline{H}_2$-2), 3.47 (dq, *J* = 9.4, 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 3.62 (dq, *J* = 9.4, 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.46 (t, *J* = 5.8 Hz, 1H, $C\underline{H}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.97 (C-7), 15.26 ($OCH_2\underline{C}H_3$), 22.51 (C-6), 24.65 (C-3), 29.09(C-4), 31.72 (C-5), 33.56 (C-2), 60.76 ($O\underline{C}H_2CH_3$), 102.92 (C-1). The NMR data correspond to those already reported in the literature.[36]

1,1-diethoxyundecane: colorless liquid (85% conversion, 78% isolated yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, *J* = 6.9 Hz, 3H, $C\underline{H}_3$-11), 1.20 (t, *J* = 7.1 Hz, 6H, $OCH_2C\underline{H}_3$), 1.38 – 1.23 (m, 16H, $C\underline{H}_2$-3-10), 1.68 – 1.54 (m, 2H, $C\underline{H}_2$-2), 3.48 (dq, *J* = 9.4, 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 3.63 (dq, *J* = 9.4, 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.47 (t, *J* = 5.8 Hz, 1H, $C\underline{H}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.11 (C-11), 15.36 ($OCH_2\underline{C}H_3$), 22.69 (C-10), 24.77 (C-3), 29.33, 29.50, 29.55, 29.58, 29.61 (from C-4 to C-8), 31.91 (C-9), 33.61 (C-2), 60.80 ($O\underline{C}H_2CH_3$), 102.98 (C-1).

1,1-diethoxy-2-methylbutane: colorless liquid (32% isolated yield of 95% purity due to high volatility and instability). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, *J* = 7.4 Hz, 3H, $C\underline{H}_3$-4), 0.88 (d, *J* = 6.8 Hz, 3H, $CHC\underline{H}_3$), 1.02-1.16 (m, 1H, $C\underline{H}_2$-3a), 1.17 (t, *J* = 7.1 Hz, 6H, $OCH_2C\underline{H}_3$), 1.18 (t, *J* = 7.1 Hz, 6H, $OCH_2C\underline{H}_3$), 1.47-1.71 (m, 2H, $C\underline{H}_2$-3b, $C\underline{H}_2$-2), 3.70 – 3.56 (m, 2H, $OC\underline{H}_2CH_3$), 3.53 – 3.40 (m, 2H, $OC\underline{H}_2CH_3$), 4.15 (d, *J* = 6.4 Hz, 1H, $C\underline{H}$-1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.32 (C-4), 13.91 ($CH\underline{C}H_3$), 15.29 ($OCH_2\underline{C}H_3$), 24.56 (C-3), 37.90 (C-2), 61.82, 61.97 ($O\underline{C}H_2CH_3$), 106.73 (C-1).

GC on-column analysis

Manual injection of the organic extracts of diethyl acetal standards which has been done by splitless injection at 240 °C on GC Thermo trace GC ultra (Thermo Electron SAS, Courtabœuf, France) with Flame Ionisation Detection (FID) was repeated on the same chromatograph by on-column injection at (30 °C) using a fused-silica needle (10 µL, SGE, Australia). The analysis was carried out on a capillary BP20 (30 m x 1 µm x 0.53 mm). The ramp GC oven temperature was set at 45 °C for 1 min, before rising to 230 °C at 5 °C/min, and was maintained at 230 °C for 15 min. Detection was carried out by Flame Ionization (FID, Thermo).

Quantitation of diethyl acetals in WD by GC-MS

SPE Procedure. Extraction of diethyl acetals in WD was performed through solid-phase extraction (SPE) with a Supelclean™ LC-18 cartridge (500 mg) using a robot GX-27X Large-Volume SPE (Gilson, USA). The cartridge was first conditioned with 7 mL of methanol, then 3 mL of a solution of EtOH/H_2O (90/10; v/v). Twenty-five milliliters of samples (10 mL of WD supplemented by 15 mL of ultrapure water) were spiked with dodecanol (100 µL of a solution at 10mg/L) as an internal standard (IS) and poured through the cartridge. The solid phase was rinsed with 9 mL of ultrapure water and dried with pulsed air. The elution was performed with 5 mL of dichloromethane. The eluate was dried with anhydrous sodium sulfate, and the supernatant was transferred to a concentration tube to be concentrated to 250 µL under nitrogen stream.

GC-MS analysis. Two milliliters of the concentrated organic extract were injected, each time in triplicate, into a GC system (GC 6890N, Agilent Technologies, Santa Clara, USA) using splitless mode and separated on a BP20 capillary (50 m length; 0.25 mm i.d.; 0.22 µm film thickness, SGE, USA). Ion detection was done in Selected Ion Monitoring (SIM) mode on an Agilent 5973N mass spectrometer (Agilent Technologies, Santa Clara, USA). The initial GC oven temperature was set at 45 °C for 1 min, before rising to 230 °C at 5 °C/min, and was maintained at 230 °C for 15 min. A stock solution in ethanol at 1 mg/L was prepared for each standard. The reference WD (2009 vintage) was supplemented with concentrations ranging from 2 to 32 µg/L for 1,1-diethoxybutane, 1,1-diethoxyheptane and 1,1-diethoxyundecane; 4 to

64 µg/L for 1,1-diethoxypentane; 10 to 160 µg/L for 1,1-diethoxyhexane and 2,2-diethoxyethylbenzene; 200 to 3200 µg/L for 1,1-diethoxy-3-methylbutane. The ratio between the peak area of each targeted analyte and the peak of the IS was plotted against the spiked concentration.

Method Validation. Repeatability assays were carried out with the same WD (2009 vintage) spiked at the lowest and the highest concentration of calibration for each diethyl acetal then extracted and analyzed 3 times. Reproducibility assays were also carried out at 2-day intervals for 17 days. Limit of quantitation (LOQ = 10S/N) and limit of detection (LOD = 3S/N) were also determined for each diethyl acetal.

Quantitation of aldehydes (SPME-GC/MS)

Concentrations of the corresponding aldehydes were measured by solid-phase micro-extraction coupled to gas chromatography and mass spectrometry (SPME-GC-MS) using a method adapted from López-Vázquez *et al*.[37]

SPME. A Combi PAL sampler (CTC Analytics, Zwigen, Switzerland) and an Agilent 6890N gas chromatograph (Agilent, Palo Alto, CA, USA), coupled to an Agilent HP 5973N mass spectrometer (EI mode at 70 eV) was used. Three milliliters of WD were put in a 20 mL headspace amber vial and diluted with 7 mL of deionized water. Twenty microliters of IS solution of 4-fluorobenzaldehyde at 1 mg/L and 200 µL of a solution of PFBHA at 50 g/L were put into the vial before closure with a PTFE-faced silicone septum/aluminum crimp cap and homogenized manually. A 1 cm, 50/30 µm divinylbenzene-polydimethylsiloxane (DVB-PDMS) SPME fiber purchased from Supelco Inc. (Bellefonte, PA, USA) was used. The sample was preincubated for 5 min at 60 °C. Adsorption lasted 30 min, at the same temperature, with stirring at 500 r.p.m. (3 sec on and 2 sec off). Then, desorption took place in the injector in splitless mode for 5 min at 240 °C. The fiber was reconditioned for 10 min at 250 °C.

GC-MS analysis. The carrier gas was helium N60 (Air Liquide) with a flow rate of 1 mL/min. A BPX5 column was used (50 m; 0.25 mm, 0.22 µm) (SGE, Ringwood, Australia). Temperatures were programmed as follows: 50 °C for 5 min, increasing by 3 °C/min to 240 °C with an isotherm at the final temperature for 15 min. The mass spectrometer, operating in EI mode (70 eV), was connected to the GC with a heated transfer line at 230 °C. The compounds were quantified using the SIM method on MSD ChemStation software (B.03.01) from Agilent. The selected ion for IS (4-fluorobenzaldehyde) was *m/z* 319. For the 3-methylbutyraldehyde, hexanal, heptanal, valeraldehyde and undecanal the quantifier and qualifier ion was *m/z* 239, and it was *m/z* 297 for phenylacetaldehyde.

Method validation. The linearity of the method was determined by analysis of WD (2009 vintage) sample extract spiked with five increasing concentration levels of the abovementioned aldehydes. Repeatability was estimated by 5 injections of the lowest and the highest concentrations of the ranges for each aldehyde. Reproducibility assays were carried out on spiked WD (2009 vintage), at 2-day intervals for 17 days.

Determination of olfactory detection thresholds

Sensory analyses took place in a temperature-controlled room (ISO 8589:2007) maintained at 20 ± 1 °C, equipped with individual boxes. The olfactory detection threshold corresponds to the lowest concentration perceived by 50% of tasters. Olfactory thresholds of the diethyl acetals were determined by presenting a three-alternative forced choice (3-AFC) in a Water / Ethanol 60/40 (v/v) matrix (ISO 13301:2002). A panel of 23 experienced tasters was considered. Among three glasses, one contained a supplemented sample with stepwise increasing concentrations (factor 2) of the compound to be evaluated. 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 ($P = (3p - 1)/2$, where p = the proportion of correct responses for each concentration and P = the 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)[38].

Sensory profiling

The individual diethyl acetals were submitted for sensory evaluation by a panel of 25 experts with previous experience in sensory analysis of spirits. The panelists were asked to provide descriptors in the context of an orthonasal perception. The odorant attributes cited were collected and their occurrence frequencies were calculated by using the ratio number of occurrences of descriptor/total number of descriptors (ISO 11035:1994).

Statistical analysis

Statistical calculations were performed 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 for fruity odorous zone determined by GC-O in Cognac spirits distillates and identification of the related compounds.

Olfactory test of Cognac WD extracts. Cognac spirit is known to be a very complex matrix comprising a wide diversity of volatile odorous compounds. In order to achieve progress in the characterization of volatile compounds associated with fruity odors, two Cognac WD (1997 and 2006) were subjected to a fractionation by vacuum distillation. These two WD were chosen because they were far enough in age to have organoleptic differences, their volume in stock being quite substantial and the experts having selected them for their organoleptic qualities corresponding to their age. In doing so, three fractions were obtained per WD. Then five experts were asked to freely describe the odor nuances detected of each fraction of the two WD. All the fractions presented fruity notes but the F3, particularly from 1997 WD presented soapy and complex notes. The experts recognized the aromatic nuances of F3 of 1997 WD as being representative of a high-quality Cognac. Moreover, the total quantity of ethyl esters (ethyl acetate, ethyl butyrate, ethyl caprate, ethyl caproate, ethyl caprylate, ethyl lactate and ethyl laurate) in F3 from 1997 WD (8 mg/L) was very low in comparison to that determined in F1 and F2, 702 mg/L and 71 mg/L respectively. It could thus be considered that the fruity notes described in F3 were not entirely due to the presence of ethyl esters. That is why fraction F3 was selected and subjected to a liquid/liquid extraction prior to GC-O analysis.

GC-O of F3. An inductive approach using GC-O was adopted. Organic extracts of F3 distillate fractions from the WD 1997 were subjected to GC-O by single-dimension chromatography. Fruity odorous zones were detected and their linear retention indices (LRI) were established according to the Van den Dool and Kratz equation[39] (aromagram in supporting information; Table S2). One odorous zone was particularly emphasized as it presented peach notes, at LRI of 1065 on DB-WAX and 958 on BPX5 column. The mass spectrum matched with that of the 1,1-diethoxy-3-methylbutane in the mass spectral database (NIST, 2004). This compound was previously identified in Cognac[30] but without mention of its olfactory descriptors. We took the opportunity presented by the interesting nuances related to this compound to search for other representatives of the diethyl acetal family. Next, considering the olfactory properties of this diethyl acetal, the presence of compounds belonging to the same family was investigated directly in the WD 1997 extracts. The ion *m/z* 103 is characteristic of the diethyl acetals family, corresponding to the fragment $C_5H_{11}O_2$.[40] This ion was targeted in the GC-MS chromatograms. Screening of the GC-MS chromatograms led to the emergence of eleven peaks which corresponded to other diethyl acetals (figure 1). Then, before studying their organoleptic impact, the pure standards of diethyl acetals were both injected in splitless and on-column mode under the same chromatographic conditions. These molecules are possibly thermosensitive. Indeed, the diethyl acetals could have turned into aldehyde in the high temperature of a splitless injector for the analysis of the eleven diethyl acetals. The abundance of all the peaks remained the same for both modes of injection (chromatogram in supporting information Figure S1). This indicated that the molecules were not degraded during chromatographic analysis and did not undergo any effect of heat during their injection.

Validation of the presence of diethyl acetals in Cognac. To confirm these molecules were present in various Cognac, pure standards were injected by GC-MS. 1,1-Diethoxyheptane, 1,1-diethoxyhexane, and 1,1-diehtoxy-2-methybutane had to be synthesized, whereas 1,1-diethoxybutane, 1,1,3-triethoxypropane, 2-(diethoxymethyl)furan, 1,1-diethoxy-2-methylpropane, 1,1-diethoxyundecane, 1,1-diethoxy-3-methylbutane, 1,1-diethoxypentane and 2,2-diethoxyethylbenzene were commercially available. After injection and co-injection the presence of these eleven diethyl acetals was validated.

Quantitation of diethyl acetals in wine distillate of Cognac

Standard addition of pure compounds in the WD 2009 of Cognac prior to extraction by SPE allowed us to determine the quantitation slopes for each compound investigated (table 1). Regarding the validation method, the LOQ and LOD were estimated between 0.06 and 1.15 µg/L and 0.01 and 0.34 µg/L respectively. Repeatability and reproducibility were under 20%. However, 1,1,3-triethoxypropane, 1,1-diehtoxy-2-methylpropane, 2-(diethoxymethyl)furan and 1,1-diethoxy-2-mehtylbutane could not be quantified because their repeatability and their reproducibility were above 20%.

A GC-MS analysis targeting specific *m/z* ions was conducted on the extracts (figure 2). In detail, 1,1-diethoxyundecane was more abundant in aged WD (concentration ranges 1 - 10 µg/L). Conversely, 1,1-diethoxybutane (< LOQ - 18 µg/L), 1,1-diethoxy-3-methylbutane (461 - 3337 µg/L), 2,2-diethoxyethylbenzene (0 µg/L - 53 µg/L) and 1,1-diethoxypentane (27 µg/L -108 µg/L) have a tendency to be present in lower concentrations in relation to the age of the WD. 1,1-diethoxyhexane and 1,1-diethoxyheptane respectively have concentration ranges from 48 to 159 µg/L and from 5 to 13 µg/L respectively, and seem to be unrelated to the age of the WD.

The quantity of these compounds was different from that reported in the literature except for the 2,2-diethoxyethylbenzene (table 2). These differences could be explained by the fact that the Cognacs studied by Schreier[41] were end products corresponding to blends of WD from different vintages. Vinification, fermentation, distillation and maturation modalities could also be very different and all of these factors have an impact on the concentrations of diethyl acetals.

Olfactory detection threshold of diethyl acetals, descriptors and sensory impact

Detection threshold. To assess the role of these compounds on aroma, their detection thresholds were estimated in the EtOH 40% matrix (table 3). All the diethyl acetals presented a detection threshold higher than the ranges of concentrations determined in spirits, except 1,1-diethoxy-3-methylbutane. This compound was the most represented diethyl acetal with concentrations from 461 µg/L to 3337 µg/L over its olfactory threshold which is at 323 µg/L. Thus, the content/threshold ratio defining the odor activity value (OAV) index was higher than 1 and we postulated that this molecule had an impact on the aroma of Cognac WD.

Organoleptic impact of a mix of diethyl acetals. A mixture of all these compounds prepared at average concentrations in the WD of Cognac was subjected to an olfactory differentiation through an orthanasal triangular test. The aim was to find out if an olfactory difference was perceivable between a mix of H_2O/EtOH (v:v 60:40) and that with the added diethyl acetals. Out of 25 judges, 23 found an olfactory difference, the test was significant with a *p*-value of less than 0.05 and the olfactory impact of diethyl acetals mix being validated. Next, a sensory profile was carried out with a panel of 25 experts who were not informed of the aim of the study, i.e. to describe the olfactory descriptors of each molecule in matrix H_2O/EtOH 40% at double their detection threshold concentration. A total of 21 descriptors were collected for all the molecules, gathered in main categories and listed according to the number of citations (results presented in supporting information Table S3). Only the descriptors with occurrence frequencies > 15% were retained (table 3). Even though these molecules belong to the same chemistry family, their odor perception was different. Two main olfactory notes were highlighted: fruity and green. 1,1-diethoxybutane, 1,1-diethoxy-3-methylbutane, 1,1-diethoxypentane and 1,1-diethoxyheptane were mainly described as having fruity notes but they had nuances of alcohol, vanilla, floral and green respectively. 1,1-diethoxyhexane, 2,2-diethoxybenzene and 1,1-diethoxyundecane had green notes with earthy, fruity and spicy nuances respectively.

Aldehydes, and the potential origin of the diethyl acetals in Cognac spirit

Diethyl acetals derive from the reaction between the corresponding aldehydes and ethanol, probably during the distillation process[42] and/or aging as an chemical equilibrium.[43,44] So, the aldehydes corresponding to the diethyl acetals under study were quantified. Indeed, isobutanal has been described as having green and slightly unpleasant aromatic notes and correlations have been cited of its decrease during aging and formation of the corresponding diethyl acetal.[45] Thus, we could postulate that if their quantity were lower, then these aromas might disappear and make the WD more pleasant.[46] The quantitation method for aldehydes was validated with a repeatability and a reproducibility inferior to 20% for all of them (table 4). Several trends were observed concerning the aldehyde concentrations in relation with WD age (figure 3). Benzenacetaldehyde concentrations are lower in WD in relation with aging. This could be probably due to its evaporation or acetalization reactions.[45] For other aldehydes, differences were observed depending on WD but without correlation with age. Presumably, enological parameters could be taken into account in these fluctuations. These differences could originate from different levels of oxidation mechanisms and Strecker degradation.[47] Nevertheless, no clear correlation could be observed between aldehyde concentrations profiles in WD and age or concentrations of corresponding diethyl acetals.

In conclusion, the fractionation of WD of Cognac by sequential vacuum distillation analyzed by GC-O-MS, evidenced a fruity odorous belonging to diethyl acetal family. Eleven diethyl acetals were detected in WD by GC-MS analysis as 1,1,3-triethoxypropane; 1,1-diethoxy-2-methylpropane; 2(diethoxymethyl)furan; 1,1-diethoxy-2-methylbutane; 1,1-diethoxy-3-methylbutane; 1,1-diethoxybutane; 1,1-diethoxypentane; 1,1-diethoxyheptane; 1,1-diethoxyhexane; 2,2-diethoxyethylbenzene and 1,1-diethoxyundecane. Seven of these compounds were quantified by GC-MS. To clarify their sensorial impact in Cognac WD, their detection thresholds were established thanks to a 3-AFC method. After all, only 1,1-diethoxy-3-methylbutane had an olfactory impact in WD. Diethyl acetals being the results of chemical reactions between corresponding aldehydes and ethanol, concentrations of aldehydes were determined with GC-MS in various WD but without significant correlations. More efforts will be aimed at the monitoring of the kinetics of concentration of diethyl acetals and aldehydes in the same WD during aging.

Acknowledgements

We thank Dr. Sophie Tempère for her support in sensory analysis.

Supporting information

Table S1: Alcohol by volume (%) of the WD

Table S2: Aromagram of F3 1997 on DB-WAX. Linear retention index (LRI) established according to the Van den Dool and Kratz equation.

Figure S1: Overlay of chromatogram obtained with GC-FID in split-splitless injection mode (regular line) and in on-column injection mode (dotted line) for pure standards of diethyl acetals.

Table S3: Descriptors and occurrence frequencies of diethyl acetals.

Figure S2: Spearman test for correlations between concentrations in diethyl acetals and concentrations of aldehydes. The age of WD are put in each graphic.

References

(1) Cantagrel, R.; Lurton, L.; Vidal, J. P.; Galy, B. From Vine to Cognac. In *Fermented beverage production*; Lea A.G.H., Piggott J.R. Springer: Boston, MA, 1995; pp 208–228.

(2) Singleton, L. Maturation of Wines and Spirits: Comparisons, Facts, and Hypotheses. *Am. J. Enol. Vitic.* **1995**, *46* (1), 98–115.

(3) Fiches, G.; Saint Eve, A.; Jourdren, S.; Déléris, I.; Brunerie, P.; Souchon, I. Temporality of Perception during the Consumption of French Grape Brandies with Different Aging Times in Relation with Aroma Compound Release: Perception and Aroma Compound Releases during Brandy Consumption. *Flavour Fragr. J.* **2016**, *31* (1), 31–40.

(4) Marche, M.; Joseph, E.; Groizet, A.; Audebert, J. Etude Théorique Sur Le Cognac, Sa Composition et Son Vieillissement Naturel En Fûts de Chêne. *Rev Fr. Oenol* **1975**, *57*, 1–106.

(5) Onishi, M.; Guymon, J. F.; Crowell, E. A. Changes in Some Volatile Constituents of Brandy during Aging. *Am. J. Enol. Vitic.* **1977**, *28*, 152–158.

(6) Watts, V. A.; Butzke, C. E.; Boulton, R. B. Study of Aged Cognac Using Solid-Phase Microextraction and Partial Least-Squares Regression. *J. Agric. Food Chem.* **2003**, *51* (26), 7738–7742.

(7) Guymon, J. F. Influence of Warehouse Temperatures on the Aging of California Brandy. *Wines Vines* **1973**, 36–38.

(8) Léauté, R.; Mosedale, J. R.; Mourgues, J.; Puech, J. L. Barrique et Vieillissement Des Eaux-de-Vie. In *Oenologie fondements scientifiques et technologiques*; Flanzy, C.; Tec&Doc: Paris, France, 1998; pp 1085–1142.

(9) Ferrari, G.; Lablanquie, O.; Cantagrel, R.; Ledauphin, J.; Payot, T.; Fournier, N.; Guichard, E. Determination of Key Odorant Compounds in Freshly Distilled Cognac Using GC-O, GC-MS, and Sensory Evaluation. *J. Agric. Food Chem.* **2004**, *52* (18), 5670–5676.

(10) Schaefer, J.; Timmer, R. Flavor Components in Cognac. *J Food Sci* **1970**, *35* (1), 10–12.

(11) Caumeil, M. Le Cognac. *Pour la Science.* **1983**, No. 74, 48–56.

(12) De Rijke, D.; Ter Heide, R. Flavour Compounds in Rum, Cognac and Whisky. In *Flavour distilled beverage, origin and development*; Piggott J.R. Ellis Horwood: Chichester, UK, 1983; pp 192–202.

(13) Lurton, L.; Mazerolles, G.; Galy, B.; Cantagrel, R.; Vidal, J. P. Influence de La Technologie de Vinification Sur La Qualité Des Eaux-de-Vie de Cognac, Exemple Des Norisoprenoides et Des Alcools Supérieurs. In *1er Symposium international : Les eaux-de-vie traditionnelles d'origine viticole*; Bertrand, A. Tec&Doc: Paris, France, 1990; pp 127–136.

(14) Lurton, L.; Ferrari, G.; Snakkers, G. Cognac: Production and Aromatic Characteristics. In *Alcoholic Beverages*; Piggott J.R.: New York, NY, 2012; pp 242–266.

(15) Slaghenaufi, D.; Perello, M.-C.; Marchand, S.; de Revel, G. Quantification of Megastigmatrienone, a Potential Contributor to Tobacco Aroma in Spirits. *Food Chem.* **2016**, *203*, 41–48.

(16) Ledauphin, J.; Le Milbeau, C.; Barillier, D.; Hennequin, D. Differences in the Volatile Compositions of French Labeled Brandies (Armagnac, Calvados, Cognac, and Mirabelle) Using GC-MS and PLS-DA. *J. Agric. Food Chem.* **2010**, *58* (13), 7782–7793.

(17) Uselmann, V.; Schieberle, P. Decoding the Combinatorial Aroma Code of a Commercial Cognac by Application of the Sensomics Concept and First Insights into Differences from a German Brandy. *J. Agric. Food Chem.* **2015**, *63* (7), 1948–1956.

(18) Malfondet, N.; Gourrat, K.; Brunerie, P.; Le Quéré, J.-L. Aroma Characterization of Freshly-Distilled French Brandies; Their Specificity and Variability within a Limited Geographic Area: Aroma Characterization of Freshly-Distilled French Brandies. *Flavour Fragr. J.* **2016**, *31* (5), 361–376.

(19) Puech, J. L.; Léauté, R.; Clot, G.; Nomdedeu, L.; Mondiés, H. Evolution de Divers Constituants Volatils et Phénoliques Des Eaux-de-Vie de Cognac Au Cours de Leur Vieillissement. *Sci Aliments* **1984**, *4*, 65–80.

(20) Culleré, L.; Escudero, A.; Cacho, J.; Ferreira, V. Gas Chromatography−Olfactometry and Chemical Quantitative Study of the Aroma of Six Premium Quality Spanish Aged Red Wines. *J Agric Food Chem* **2004**, *52* (6), 1653–1660.

(21) Campo, E.; Cacho, J.; Ferreira, V. Solid Phase Extraction, Multidimensional Gas Chromatography Mass Spectrometry Determination of Four Novel Aroma Powerful Ethyl Esters. *J. Chromatogr. A* **2007**, *1140* (1–2), 180–188.

(22) Stamatopoulos, P.; Frérot, E.; Tempère, S.; Pons, A.; Darriet, P. Identification of a New Lactone Contributing to Overripe Orange Aroma in Bordeaux Dessert Wines via Perceptual Interaction Phenomena. *J Agric Food Chem* **2014**, *62* (12), 2469–2478.

(23) Ferreira, V.; Hernández-Orte, P.; Escudero, A.; López, R.; Cacho, J. Semipreparative Reversed-Phase Liquid Chromatographic Fractionation of Aroma Extracts from Wine and Other Alcoholic Beverages. *J. Chromatogr. A* **1999**, *864* (1), 77–88.

(24) Gros, J.; Lavigne, V.; Thibaud, F.; Gammacurta, M.; Moine, V.; Dubourdieu, D.; Darriet, P.; Marchal, A. Toward a Molecular Understanding of the Typicality of Chardonnay Wines: Identification of Powerful Aromatic Compounds Reminiscent of Hazelnut. *J Agric Food Chem* **2017**, *65* (5), 1058–1069.

(25) MacNamara, K.; van Wyk, C. J.; Augustyn, O. P. H.; Rapp, A. Flavour Components of Whiskey. I. Distribution and Recovery of Compounds by Fractional Vacuum Distillation. *South Afr. J. Enol. Vitic.* **2017**, *22* (2), 69–74.

(26) Van Ruth, S. M. Methods for Gas Chromatography-Olfactometry: A Review. *Biomol. Eng.* **2001**, *17* (4–5), 121–128.

(27) Leclaire, E.; Cantagrel, R.; Maignial, L.; Snakkers, G.; Ferrari, G. Contribution to characterisation of young Cognac aroma. *OENO One* **1999**, *33* (3), 133.

(28) Ledauphin, J.; Basset, B.; Cohen, S.; Payot, T.; Barillier, D. Identification of Trace Volatile Compounds in Freshly Distilled Calvados and Cognac: Carbonyl and Sulphur Compounds. *J Food Compos Anal* **2006**, *19* (1), 28–40.

(29) Nykänen, L.; Suomalainen, H. *Aroma of Beer, Wine and Distilled Alcoholic Beverages*; D. Reidel Publishing Company: Dordrecht, 1983.

(30) Ledauphin, J.; Saint-Clair, J.-F.; Lablanquie, O.; Guichard, H.; Founier, N.; Guichard, E.; Barillier, D. Identification of Trace Volatile Compounds in Freshly Distilled Calvados and Cognac Using Preparative Separations Coupled with Gas Chromatography−Mass Spectrometry. *J Agric Food Chem* **2004**, *52* (16), 5124–5134.

(31) Nykanen, L. Formation and Occurrence of Flavor Compounds in Wine and Distilled Alcoholic Beverages. *Am. J. Enol. Vitic.* **1986**, *37* (1), 84–96.

(32) Fan, W.; Qian, M. C. Characterization of Aroma Compounds of Chinese “Wuliangye” and “Jiannanchun” Liquors by Aroma Extract Dilution Analysis. *J Agric Food Chem* **2006**, *54* (7), 2695–2704.

(33) Benn, S. M.; Peppard, T. L. Characterization of Tequila Flavor by Instrumental and Sensory Analysis. *J Agric Food Chem* **1996**, *44* (2), 557–566.

(34) Masino, F.; Montevecchi, G.; Riponi, C.; Antonelli, A. Composition of Some Commercial Grappas (Grape Marc Spirit): The Anomalous Presence of 1,1-Diethoxy-3-Methylbutane: A Case Study. *Eur Food Res Technol* **2009**, *228* (4), 565–569.

(35) Mihajilov-Krstev, T. M.; Denić, M. S.; Zlatković, B. K.; Stankov-Jovanović, V. P.; Mitić, V. D.; Stojanović, G. S.; Radulović, N. S. Inferring the Origin of Rare Fruit Distillates from Compositional Data Using Multivariate Statistical Analyses and the Identification of New Flavour Constituents. *J Sci Food Agr* **2015**, *95* (6), 1217–1235.

(36) Pérez-Mayoral, E.; Martín-Aranda, R. M.; López-Peinado, A. J.; Ballesteros, P.; Zukal, A.; Čejka, J. Green Synthesis of Acetals/Ketals: Efficient Solvent-Free Process for the Carbonyl/Hydroxyl Group Protection Catalyzed by SBA-15 Materials. *Top. Catal.* **2009**, *52* (1–2), 148–152.

(37) López-Vázquez, C.; Orriols, I.; Perelló, M.-C.; de Revel, G. Determination of Aldehydes as Pentafluorobenzyl Derivatives in Grape Pomace Distillates by HS-SPME-GC/MS. *Food Chem.* **2012**, *130* (4), 1127–1133.

(38) Cometto-Muñiz, J. E.; Abraham, M. H. Human Olfactory Detection of Homologous N-Alcohols Measured via Concentration-Response Functions. *Pharmacol. Biochem. Behav.* **2008**, *89* (3), 279–291.

(39) Van den Dool, H. A Generalization of the Retention Index System Including Linear Temperature Programmed Gas-Liquid Partition Chromatography. *J Chromatogr A* **1963**, *11*, 463–471.

(40) Ruelle, H. D. L.; Klok, J.; Rinken, M.; Felix, M. Characterization of Acetals by Means of Capillary Gas Chromatography/Mass Spectrometry. *Rapid Commun Mass Sp* **1995**, *9* (15), 1507–1511.

(41) Schreier, P.; Drawert, F.; Winkler, F. Composition of Neutral Volatile Constituents in Grape Brandies. *J Agric Food Chem* **1979**, *27* (2), 365–372.

(42) Awad, P.; Athès, V.; Decloux, M. E.; Ferrari, G.; Snakkers, G.; Raguenaud, P.; Giampaoli, P. Evolution of Volatile Compounds during the Distillation of Cognac Spirit. *J Agric Food Chem* **2017**, *65* (35), 7736–7748.

(43) Madrera, R. R.; Gomis, D. B.; Alonso, J. J. M. Influence of Distillation System, Oak Wood Type, and Aging Time on Volatile Compounds of Cider Brandy. *J Agric Food Chem* **2003**, *51* (19), 5709–5714.

(44) Vidal, J. P.; Mazerolles, G.; Estreguil, S.; Cantagrel, R. Analyse Quantitative de La Fraction Carbonylée Volatile Des Eaux-de-Vie de Cognac. In *Proceedings of the 1er Symposium Scientifique International du Cognac, “Elaboration et connaissance des spiritueux”;* Cantagrel, R ; Tec&Doc: Paris, France, 1993; pp 11–15.

(45) Galy, B.; Loizeau, A.; Giraud, N.; Lurton, L. Mécanismes de formation et conséquences qualitatives de l’isobutanal présent dans les Eaux-de-vie de Cognac. In *2ème Symposium sur les eaux-de-vie traditionnelles d’origine viticole*; Bertrand, A. Tec&Doc: Paris, France, 2007; pp 111–115.

(46) Escudero, A.; Asensio, E.; Cacho, J.; Ferreira, V. Sensory and Chemical Changes of Young White Wines Stored under Oxygen. An Assessment of the Role Played by Aldehydes and Some Other Important Odorants. *Food Chem.* **2002**, *77* (3), 325–331.

(47) Oliveira, C. M.; Ferreira, A. C. S.; De Freitas, V.; Silva, A. M. S. Oxidation Mechanisms Occurring in Wines. *Food Res. Int.* **2011**, *44* (5), 1115–1126.

Figure Captions

Figure 1: Diethyl acetal compounds evidenced in Cognac spirit (identification confirmed by LRI, MS and standard injection).

Figure 2: Concentrations (µg/L) of diethyl acetals (mean ± standard deviation; n=3) in the 10 WD analyzed by GC-MS.

Figure 3: Concentrations (µg/L) of aldehydes (mean ± standard deviation; n=3) in the 10 WD analyzed by SPME-GC-MS.

Table 1: Validation data of GC-MS method for diethyl acetals analyses. Repeatability (n=3). Reproducibility (2-day intervals for 17 days). LOD = 3S/N. LOQ = 10S/N.

Name	*m/z* qualifier (quantifier)	R²	Repeatability (%)	Reproducibility (%)	LOD[a] (µg/L)	LOQ[b] (µg/L)
1,1-diethoxybutane	103 (101)	0.9964	8	17	0.02	0.08
1,1-diethoxy-3-methylbutane	103 (115)	0.9899	14	17	0.33	1.11
1,1-diethoxypentane	103 (115)	0.9936	14	19	0.04	0.17
1,1-diethoxyhexane	103 (129)	0.9974	11	16	0.34	1.15
1,1-diethoxyheptane	103 (143)	0.9918	10	14	0.03	0.09
2,2-diethoxyethylbenzene	103 (121, 149)	0.9964	6	10	0.08	0.26
1,1-diethoxyundecane	103 (199)	0.9957	18	9	0.01	0.06

[a] Limit of detection. [b] Limit of quantitation

Table 2: Comparison of concentrations of diethyl acetals previously cited in the literature and this study.

Name	**Range of diethyl acetals concentrations in Cognac spirits (cited from literature) (µg/L)**[41]	**Range of diethyl acetals concentrations in Cognac spirits (determined in this study) (µg/L)**
1,1-diethoxybutane	nd	0 – 18
1,1-diethoxy-3-methylbutane	28 – 300	461 – 3337
1,1-diethoxypentane	2 – 11	27 – 108
1,1-diethoxyhexane	14 - 61	48 – 159
1,1-diethoxyheptane	nd	5 – 13
2,2-diethoxyethylbenzene	1 – 39	0 – 53
1,1-diethoxyundecane	nd	1 – 10

nd: not determined

Table 3: Olfactory detection thresholds and descriptors of the diethyl acetals.

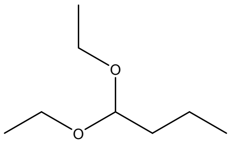
Name	Olfactory detection threshold (µg/L)[a]	Odor descriptors[b]	OAV[c]
1,1-diethoxybutane	616	Fruity, alcohol	<1
1,1-diethoxy-3-methylbutane	323	Fruity, vanilla	>1
1,1-diethoxypentane	501	Fruity, floral	<1
1,1-diethoxyhexane	1995	Green, earthy	<1
1,1-diethoxyheptane	512	Fruity, green	<1
2,2-diethoxyethylbenzene	741	Green, fruity	<1
1,1-diethoxyundecane	1253	Green, spicy	<1

[a] Determined in a H_2O/EtOH 60:40 (v:v); [b] Descriptors mainly mentioned by the panel with occurrence frequencies > 15%; [c] Odor Activity Value (ratio between the concentration of the diethyl acetal in WD and its detection threshold)

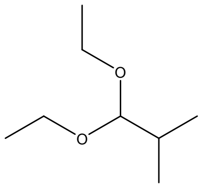
Table 4: Validation data of GC-MS method for various aldehydes. Repeatability (n=3). Reproducibility (2-day intervals for 17 days)

Name	**CAS**	***m/z* quantifier after derivatization**	**R²**	**Repeatability (%)**	**Reproducibility (%)**
butanal	123-72-8	239	0.9740	4	11
3-methylbutanal	590-86-3	239	0.9932	4	10
pentanal	110-62-3	239	0.9921	4	9
hexanal	66-25-1	239	0.9925	6	16
heptanal	111-71-7	239	0.9922	5	12
benzeneacetaldehyde	122-78-1	297	0.9903	2	7
undecanal	112-44-7	239	0.9961	5	14

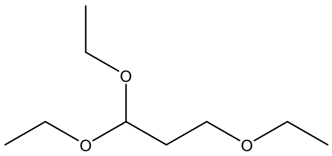
Figure 1:



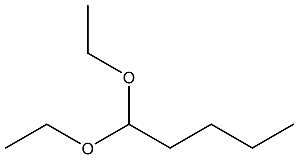
1,1-diethoxybutane



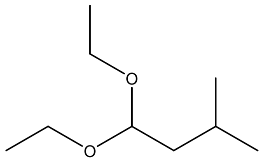
1,1-diethoxy-2-methylpropane



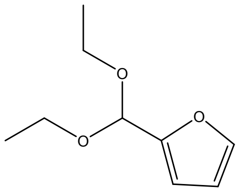
1,1,3-triethoxypropane



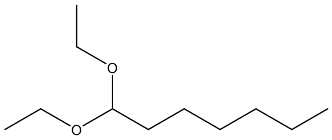
1,1-diethoxypentane



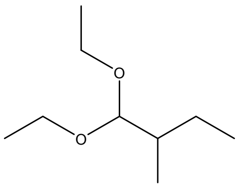
1,1-diethoxy-3-methylbutane



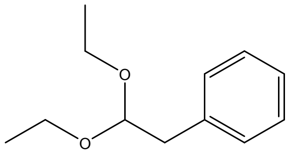
2-(diethoxymethyl)furan



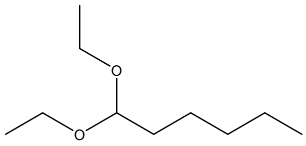
1,1-diethoxyheptane



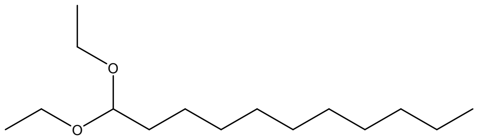
1,1-diethoxy-2-methylbutane



2,2-diethoxyethylbenzene



1,1-diethoxyhexane



1,1-diethoxyundecane

Figure 2:

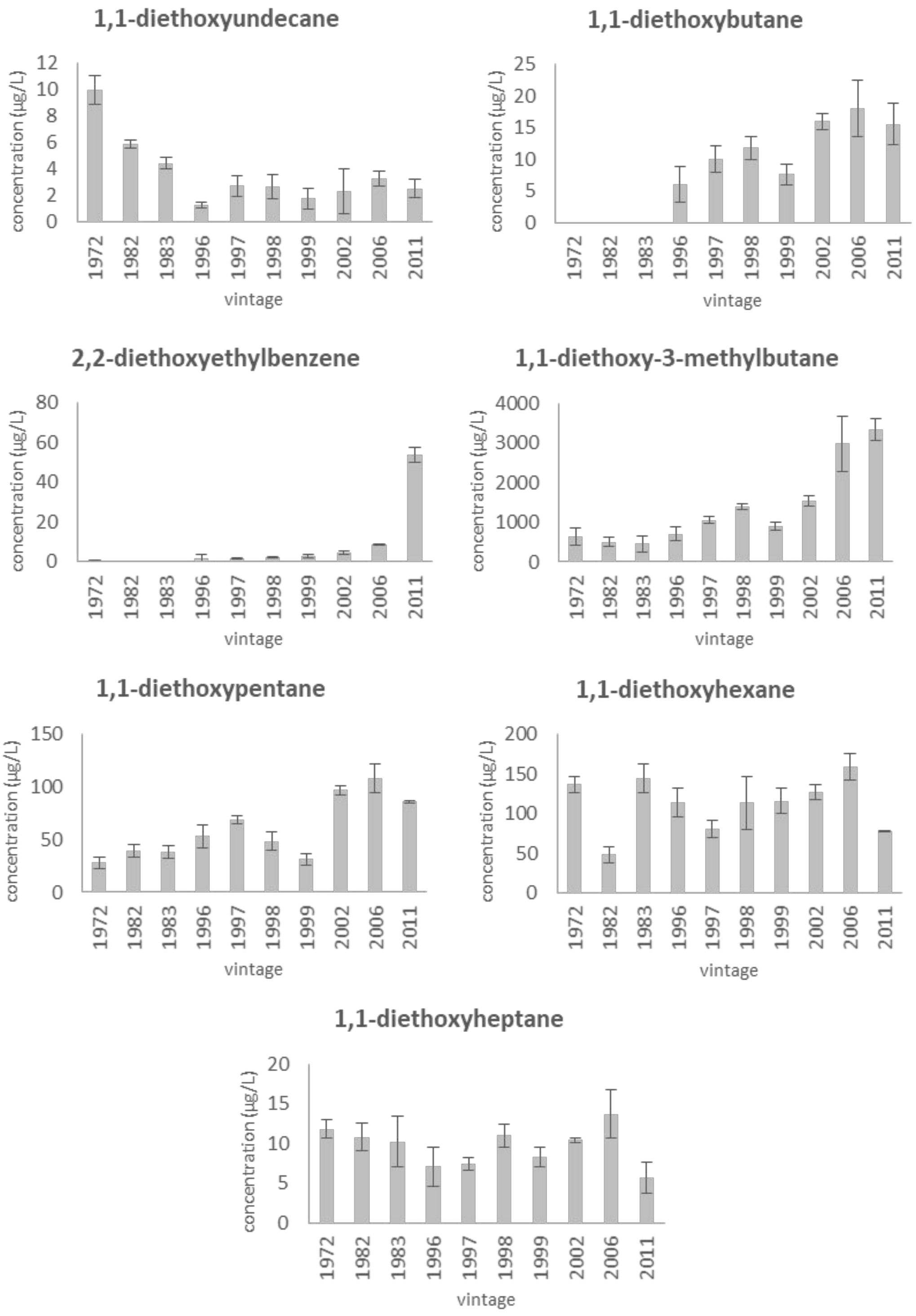
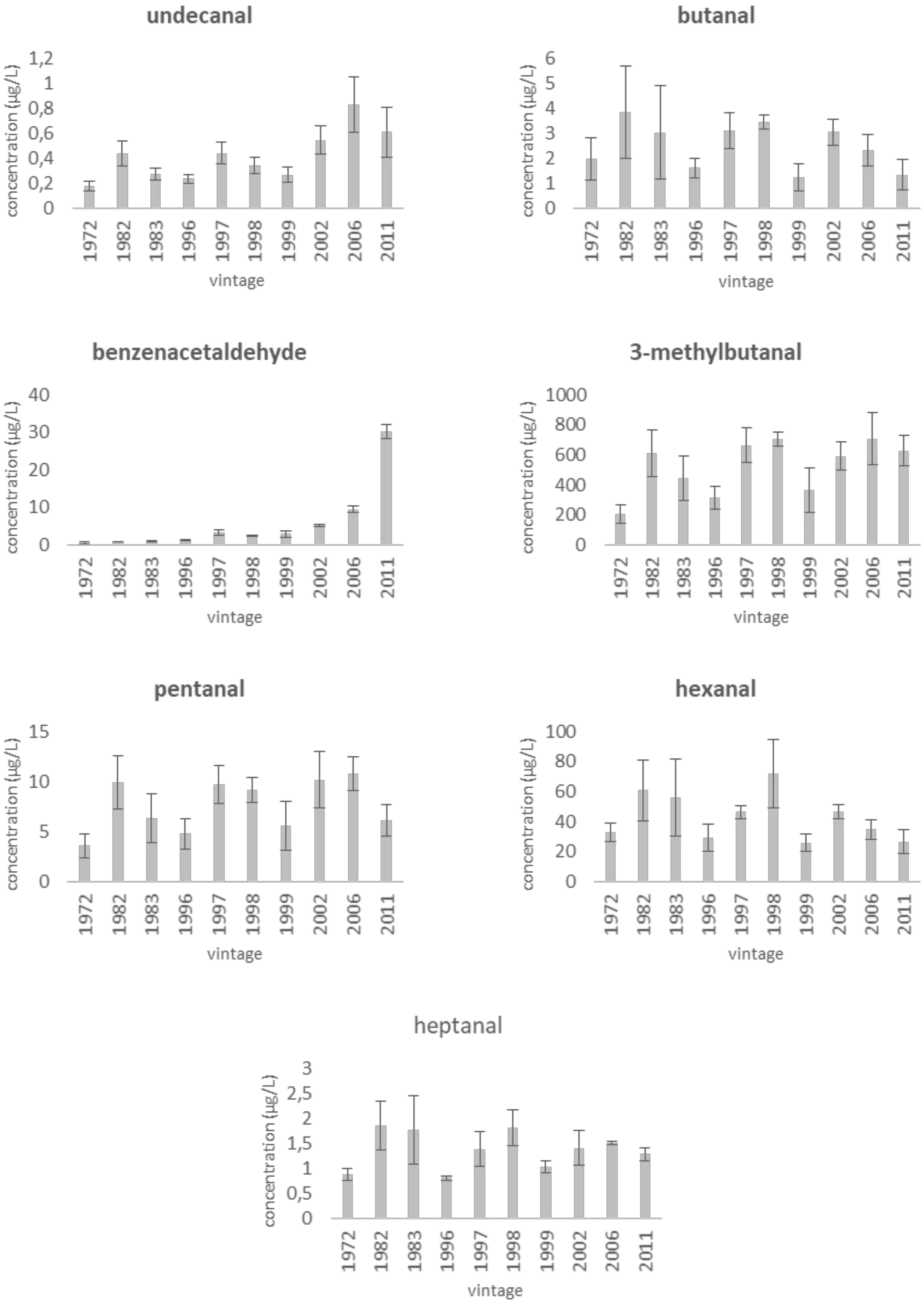

1,1-diethoxyundecane
concentration (µg/L)
0
2
4
6
8
10
12
1972
1982
1983
1996
1997
1998
1999
2002
2006
2011
vintage
1,1-diethoxybutane
concentration (µg/L)
0
5
10
15
20
25
vintage
2,2-diethoxyethylbenzene
concentration (µg/L)
0
20
40
60
80
vintage
1,1-diethoxy-3-methylbutane
concentration (µg/L)
0
1000
2000
3000
4000
vintage
1,1-diethoxypentane
concentration (µg/L)
0
50
100
150
vintage
1,1-diethoxyhexane
concentration (µg/L)
0
50
100
150
200
vintage
1,1-diethoxyheptane
concentration (µg/L)
0
5
10
15
20
vintage

Figure 3:


undecanal
concentration (µg/L)
1,2
1
0,8
0,6
0,4
0,2
0
1972
1982
1983
1996
1997
1998
1999
2002
2006
2011
vintage
butanal
concentration (µg/L)
6
5
4
3
2
1
0
vintage
benzenacetaldehyde
concentration (µg/L)
40
30
20
10
0
vintage
3-methylbutanal
concentration (µg/L)
1000
800
600
400
200
0
vintage
pentanal
concentration (µg/L)
15
10
5
0
vintage
hexanal
concentration (µg/L)
100
80
60
40
20
0
vintage
heptanal
concentration (µg/L)
3
2,5
2
1,5
1
0,5
0
vintage

TOC graphic

For table of contents only