

# Demonstration of cork stoppers impact in chemical and sensorial quality of sparkling wines

Joana Marques de Almeida

M  
2021



**Demonstration of cork stoppers impact in chemical and sensorial quality of sparkling wines**

Joana Marques de Almeida



FACULDADE DE FARMÁCIA DA UNIVERSIDADE DO PORTO

# **Demonstration of cork stoppers impact in chemical and sensorial quality of sparkling wines**

**Joana Marques de Almeida**



Dissertation thesis for the master's degree in Quality Control from the Faculty of Pharmacy, University of Porto.

Work elaborated under the supervision of Doctor Joana Isabel Monteiro Pinto and co-supervision of Doctor Maria Paula Guedes de Pinho and Professor Maria de Lourdes Bastos

July, 2021

É AUTORIZADA A REPRODUÇÃO PARCIAL DESTA TESE, APENAS PARA EFEITOS  
DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A  
TAL SE COMPROMETE

# Abstract

Over the last few years, the importance of sparkling wines in the global wine market has been growing. However, there is still a lack of information regarding alterations in the aroma of this beverage after second fermentation and *dégorgement*. Hence, the influence of aging and type of closure on the volatile profile of sparkling wines was evaluated in this thesis. In addition, the effect of different closures on the volatile composition of a white wine was also studied. The volatile profile was measured by headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME/GC-MS), following a targeted approach focused on the identification and quantification of volatile compounds (esters, aldehydes, ketones, terpenes, among others) with largest impact on the sensory properties of sparkling and white wines.

In the first part of the work, the evolution of aroma compounds during ageing in bottle was evaluated in two Italian sparkling wines, A and B after 12, 24 and 36 months post-bottling and one Spanish sparkling wine after 24 and 80 months post-bottling. The Italian sparkling wine A showed significant differences in the levels of eighteen volatile compounds over time. A great majority of compounds demonstrated an increase in their concentration during ageing, namely, isoamyl alcohol, 1-octanol, phenylacetaldehyde, ethyl butanoate, ethyl isovalerate, diethyl succinate, furfural, 2-heptanone, 2-undecanone,  $\beta$ -damascenone and  $\alpha$ -ionone. Contrarily, the levels of  $\beta$ -cyclocitral, hexyl acetate, phenylethyl acetate, ethyl decanoate, limonene,  $\beta$ -linalool and 1,4-cineole decreased over time.

In the Italian sparkling wine B, sixteen volatile compounds were found with significant differences between timepoints, from which isoamyl alcohol, phenylethyl alcohol, 1-octanol, ethyl butanoate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, diethyl succinate, furfural, 2-heptanone, 1,4-cineole and  $\alpha$ -ionone demonstrated an increase in their levels over time. On the other hand, significant lower levels of limonene, eucalyptol, phenylethyl acetate, and 5-methyl-2-furfural were found during ageing. Moreover, eucalyptol and limonene were not detected at 36 months post-bottling.

Similar to the Italian sparkling wines, in the Spanish sparkling wine, almost all compounds were found in significant higher amounts at the last timepoint evaluated, such as isoamyl alcohol, 1-hexanol, phenylethyl alcohol, 1-octanol, phenylacetaldehyde, decanal, ethyl butanoate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, ethyl heptanoate, diethyl succinate, furfural, linalool oxide and  $\alpha$ -ionone. Only four volatile compounds, namely, 1-decanol, octanol, ethyl decanoate and 2-nonanone were found in significant lower concentrations at 80 months after bottling. Furthermore, 1-hexanol, 1-decanol, decanal, and 2-nonanone were only found significantly different in this sparkling

wine when compared with both Italian wines.

Overall, some indicators of long ageing were identified among the three sparkling wines, namely diethyl succinate, ethyl isovalerate, furfural, isoamyl alcohol,  $\alpha$ -ionone and ethyl butonate. In addition, ethyl decanoate was identified as a possible indicator of young sparkling wines.

In the second part of the work, the impact of various stoppers in the volatile composition of Spanish sparkling wine and one French sparkling wine was evaluated at distinct ageing periods. The Spanish sparkling wine bottles were sealed with a 2D cork stopper and a crown cap, whereas the French sparkling wine was closed with micro CSP (agglomerated cork stopper), and STO (is a technical stopper with a body of agglomerated cork and a disc of natural cork in the extremity in contact with wine). Spanish sparkling wine revealed differences in the levels of five compounds between stoppers, namely, camphor, limonene,  $\alpha$ -ionone and 5-methyl-2-furfural. Sparkling wine sealed with cork stoppers demonstrated lower levels of  $\alpha$ -ionone and 5-methyl-2-furfural. Moreover, camphor was not identified, and limonene was detected below the limit of quantification in sparkling wine sealed with crown cap. The French sparkling wine sealed with STO and micro CSP stoppers uncovered differences in the levels of 8 compounds. The volatile profile of the sparkling wine sealed with STO stoppers showed significant amounts of 1-decanol, ethyl butanoate, ethyl isobutyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-nonanone and  $\beta$ -damascenone. These results revealed that cork stoppers have a better capacity to preserve the esters content, and as result maintain the fruity aromas of the wine.

In the last part of this study, the volatile profile of an Italian still white wine sealed with three types of cork stoppers with different densities and finishing procedures (with and without wax) was studied. Major differences were obtained between cork stoppers A (density of 150-170 kg/m<sup>3</sup> with wax) and C (density of 150-170 kg/m<sup>3</sup> without wax), with 20 compounds found significantly increased in wines sealed with A compared with C stoppers, namely, four alcohols (isoamyl alcohol, 3-hexen-1-ol, 1-hexanol, 1-octanol), one aldehyde (benzaldehyde), nine esters (ethyl isobutyrate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl isovalerate, isoamyl acetate, diethyl succinate, ethyl octanoate, phenylethyl acetate and ethyl decanoate), three ketones (2-nonanone,  $\beta$ -damascenone and  $\alpha$ -ionone) and one terpene (linalool oxide). Wines sealed with C stoppers in comparison to wines sealed with B stoppers (density of 180-200 kg/m<sup>3</sup> without wax) unveiled significant higher levels of six volatile compounds namely benzaldehyde, ethyl isovalerate,  $\beta$ -damascenone, linalool oxide,  $\alpha$ -ionone and furfural.

These results highlighted the impact of ageing and the choice of closure on the quality of sparkling and still wines. It contributed to a better understanding of changes in esters, terpenes, alcohols, furans and aldehydes content that take place during ageing and

their possible effect on sensory properties of sparkling wines. Furthermore, it also demonstrated the importance of different closures on the volatile profile of these beverages and how they can contribute positively or negatively to the quality of still and sparkling wines. More studies should be realized to provide a more holistic information on the alterations occurring during ageing. Additionally, different sealing systems should be tested to select the more efficient one.

**Keywords:** sparkling wine, white wine, volatile composition, headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry, ageing, cork stoppers



# Resumo

Nos últimos anos, a importância dos vinhos espumantes no mercado mundial do vinho tem vindo a aumentar. No entanto, ainda há falta de informação sobre as alterações no aroma desta bebida após a segunda fermentação e *dégorgement*. Assim, a influência do envelhecimento e do tipo de vedante no perfil volátil dos vinhos espumantes foi avaliado nesta tese. Além disso, o impacto de diferentes vedantes na composição volátil de um vinho branco foi também alvo de estudo. O perfil volátil foi medido por microextração de fase sólida de *headspace* acoplada a cromatografia gasosa por espectrometria de massa (HS-SPME/GC-MS), seguindo uma abordagem centrada na identificação e quantificação de alguns compostos voláteis (ésteres, aldeídos, cetonas, terpenos, entre outros) que apresentam um maior impacto nas propriedades sensoriais dos vinhos tranquilos e espumantes brancos.

Na primeira parte do trabalho, a evolução dos compostos aromáticos durante o envelhecimento em garrafa foi avaliada em dois espumantes italianos, A e B (após 12, 24 e 36 meses em garrafa) e um espumante espanhol (após 24 e 80 meses em garrafa). O vinho espumante italiano A mostrou diferenças significativas nos níveis de dezoito compostos ao longo do tempo. A grande maioria dos compostos demonstrou um aumento na sua concentração durante o envelhecimento, nomeadamente, álcool isoamílico, 1-octanol, fenilacetaldéido, butanoato de etilo, isovalerato de etilo, succinato de etilo, furfural, 2-heptanona, 2-undecanona,  $\beta$ -damascenona e  $\alpha$ -ionona. Por outro lado, a concentração de  $\beta$ -ciclocitral, acetato de hexilo, acetato de feniletilo, decanoato de etilo, limoneno,  $\beta$ -linalool e 1,4-cineole diminuiu ao longo do tempo.

No vinho espumante italiano B, foram encontrados dezasseis compostos com diferenças estatisticamente significativas entre *timepoints*, dos quais onze, nomeadamente, álcool isoamílico, álcool feniletilico, 1-octanol, butanoato de etilo, isovalerato de etilo, acetato de isoamilo, hexanoato de etilo, succinato de dietilo, furfural, 2-heptanona, 1,4-cineole e  $\alpha$ -ionona demonstraram um aumento do seu teor, enquanto que, limoneno, eucaliptol, acetato de feniletilo, e 5-metil-2-furfural demonstraram uma diminuição da sua concentração, em alguns casos mesmo, dois compostos voláteis (eucaliptol e limoneno) não foram detetados aos 36 meses.

À semelhança dos espumantes italianos, no espumante espanhol, quase todos os compostos apresentaram quantidades significativamente mais elevadas no último tempo avaliado (80 meses), como é o caso do álcool isoamílico, 1-hexanol, álcool feniletilico, 1-octanol, fenilacetaldéido, decanal, butanoato de etilo, isovalerato de etilo, acetato de isoamilo, hexanoato de etilo, heptanoato de etilo, succinato de dietilo, furfural, óxido de

linalool e  $\alpha$ -ionona. Apenas quatro compostos voláteis, nomeadamente, 1-decanol, octanol, decanoato de etilo e 2-nonanona, foram encontrados em concentrações inferiores aos 80 meses. Além disso, 1-hexanol, 1-decanol, decanoato de etilo, e 2-nonanona apenas demonstraram diferenças estatisticamente significativas neste espumante em comparação com os espumantes italianos.

Globalmente, entre os três espumantes foram identificados alguns indicadores de envelhecimento, nomeadamente succinato de dietilo, isovalerato de etilo, furfural, álcool isoamílico,  $\alpha$ -ionona e butanoato de etilo. O decanoato de etilo foi identificado como um possível indicador de vinhos espumantes jovens.

Na segunda parte do trabalho, o impacto de vários tipos de rolhas na composição volátil de um espumante espanhol e de um espumante francês foi avaliado em períodos de envelhecimento distintos. As garrafas de espumante espanhol foram vedadas com uma rolha micro aglomerada 2D ou com *crown cap* e o vinho espumante francês foi fechado com micro CSP (rolha aglomerada de cortiça), e STO (rolha técnica com um corpo de cortiça aglomerada e um disco de cortiça natural numa das extremidades). O espumante espanhol revelou diferenças nos níveis de cinco compostos entre as rolhas, nomeadamente, cânfora, limoneno,  $\alpha$ -ionona e 5-metil-2-furfural. O espumante que se encontrava vedado com rolhas de cortiça demonstrou níveis inferiores de  $\alpha$ -ionona e 5-metil-2-furfural. Além disso, nos espumantes vedados com *crown cap* a cânfora não foi identificada e o limoneno foi detetado abaixo do limite de quantificação. O vinho espumante francês vedado com rolhas STO e micro CSP apresentou diferenças nos níveis de oito compostos. No perfil volátil do espumante vedado com rolhas STO foi possível detectar quantidades superiores de 1-decanol, butanoato de etilo, isobutirato de etilo, hexanoato de etilo, octanoato de etilo, decanoato de etilo, 2-nonanona e  $\beta$ -damascenona. Estes resultados revelaram que as rolhas de cortiça têm uma melhor capacidade de preservar o conteúdo de ésteres, e como resultado mantêm os aromas frutados.

Na última parte deste trabalho, foi estudado o perfil volátil de um vinho branco italiano vedado com três tipos de rolhas de cortiça com diferentes densidades e processos de acabamento (com e sem cera). Foram obtidas maiores diferenças entre as rolhas de cortiça A (densidade de 150-170 kg/m<sup>3</sup> com cera) e C (densidade de 150-170 kg/m<sup>3</sup> sem cera), dos quais vinte compostos apresentaram alterações significativas, nomeadamente quatro álcoois (álcool isoamílico, 3-hexen-1-ol, 1-hexanol, 1-octanol), um aldeído (benzaldeído), nove ésteres (isobutirato de etilo, butanoato de etilo, 2-metilbutanoato de etilo, isovalerato de etilo, acetato de isoamilo, succinato de dietilo, octanoato de etilo, acetato de feniletilo e decanoato de etilo), três cetonas (2-nonanona,  $\beta$ -damascenona e  $\alpha$ -ionona) e um terpeno (óxido de linalool), estando todos presentes em maior quantidade nos vinhos selados com rolhas C. Os vinhos vedados com rolhas C em comparação com

as rolhas B (densidade de 180-200 kg/m<sup>3</sup> sem cera) demonstraram menos alterações significativas, apenas seis compostos voláteis (benzaldeído, isovalerato de etilo,  $\beta$ -damascenona, óxido de linalool,  $\alpha$ -ionona e furfural). Estes seis compostos estavam presentes em maior quantidade nos vinhos selados com rolhas C.

Estes resultados evidenciam o impacto do envelhecimento e a escolha do vedante na qualidade dos espumantes e vinhos tranquilos. Assim, este estudo contribuiu para uma melhor compreensão das alterações nos ésteres, terpenos, álcoois, furanos e aldeídos que ocorrem durante o envelhecimento e o seu possível efeito nas propriedades sensoriais dos vinhos espumantes. Além disso, também demonstrou a importância de diferentes vedantes no perfil volátil destas bebidas e de que forma podem contribuir positiva ou negativamente para a qualidade dos vinhos tranquilos e espumantes. Conclui-se que devem ser realizados mais estudos para fornecer uma informação mais detalhada sobre as alterações no perfil volátil que ocorrem durante o envelhecimento. Adicionalmente, devem ser testados diferentes vedantes por forma a seleccionar o mais eficiente.

**Palavras-chave:** vinho espumante, composição volátil, cromatografia gasosa acoplada a espectrometria de massa através de microextração em fase sólida por *headspace*, envelhecimento, rolhas de cortiça



# Agradecimentos

Esta dissertação representa o culminar de uma longa caminhada que não teria sido possível sem todas as pessoas com que tive oportunidade de me cruzar e que de forma direta ou indireta ajudaram a que este objetivo se concretizasse. Desta forma, gostaria de dedicar um especial agradecimento a todos vós.

À Doutora Joana Pinto, expresso o meu profundo agradecimento por todo o apoio, ensinamentos e disponibilidade mostrada ao longo deste ano.

À Professora Doutora Paula Guedes de Pinho e à Professora Doutora Maria de Lourdes Bastos pela simpatia, disponibilidade e comentários valiosos na revisão do texto.

À Mestre Filipa Amaro, obrigada pela forma com que me recebeste no laboratório, por todo o tempo e paciência que tiveste para me explicar os métodos e responder a todas as minhas dúvidas, mas acima de tudo, obrigada pela tua amizade e os momentos que partilhamos ao longo deste ano.

À empresa Amorim Cork S.A. por me ter cedido as amostras para realizar este trabalho.

A todos os elementos do Laboratório de Toxicologia por me terem feito sentir integrada desde o primeiro dia e a forma com que sempre se mostraram disponíveis para me auxiliar no que fosse preciso.

Aos meus pais, um agradecimento muito especial por todo o esforço que fizeram para eu conseguir chegar aqui, por me terem sempre acompanhado em cada uma das etapas.

Aos meus irmãos, Patrícia e Tiago que me motivaram sempre a continuar e foram os meus “assistentes informáticos” quando o word não queria colaborar.

Aos meus amigos, um obrigada por todos os momentos de convívio, todos os cafés e passeios para espairecer, por me animarem e ouvirem os meus desabafos.

Aos escuteiros, que são uma parte essencial da minha vida, que me ensinaram a ser resiliente e a dar o meu melhor em qualquer situação.

Porque trilhar este caminho não teria sido possível sem vocês ao meu lado, um obrigada a todos!



*“Success consists of going from failure to failure,  
without loss of enthusiasm”*

Winston Churchill



# Table of Contents

|                                                                                                                                             |           |
|---------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>Chapter 1. Introduction .....</b>                                                                                                        | <b>21</b> |
| 1.1. Historical Context .....                                                                                                               | 21        |
| 1.2. Production of Sparkling wine .....                                                                                                     | 22        |
| 1.2.1. Base wine .....                                                                                                                      | 22        |
| 1.2.2. Second Fermentation .....                                                                                                            | 23        |
| 1.3. Types of closures for sparkling wines .....                                                                                            | 26        |
| 1.4. Methods for the analysis of volatile compounds .....                                                                                   | 26        |
| 1.4.1. Volatile extraction .....                                                                                                            | 28        |
| 1.4.2. Analysis of volatile compounds by gas chromatography-mass spectrometry (GC-MS) .....                                                 | 28        |
| 1.5. Volatile composition of sparkling wines .....                                                                                          | 30        |
| 1.6. Context of the research topics in sparkling wines .....                                                                                | 39        |
| 1.7. Impact of ageing in the volatile composition of sparkling wines .....                                                                  | 41        |
| 1.8. Impact of stoppers in the volatile composition of wine .....                                                                           | 42        |
| 1.9. Aims of this thesis .....                                                                                                              | 49        |
| <b>Chapter 2. Materials and methods .....</b>                                                                                               | <b>50</b> |
| 2.1. Chemicals .....                                                                                                                        | 50        |
| 2.2. Sparkling and white wine samples .....                                                                                                 | 50        |
| 2.3. Analysis of volatile compounds by HS-SPME/GC-MS .....                                                                                  | 51        |
| 2.4. Statistical analysis .....                                                                                                             | 53        |
| <b>Chapter 3. Results and Discussion on the evolution of volatile compounds in sparkling wines during bottle ageing .....</b>               | <b>55</b> |
| 3.1. Identification of volatile and semi volatile compounds in sparkling wines .....                                                        | 55        |
| 3.2. Evolution of the volatile composition of sparkling wines over time .....                                                               | 61        |
| <b>Chapter 4. Results and Discussion on the impact of different stoppers on the volatile composition of sparkling and white wines .....</b> | <b>70</b> |
| 4.1. Identification of volatile and semi volatile compounds in French sparkling wine and Italian white wine .....                           | 70        |
| 4.2. Impact of different stoppers on the volatile composition of sparkling wines .....                                                      | 80        |
| <b>Chapter 5. Conclusions .....</b>                                                                                                         | <b>91</b> |
| <b>References .....</b>                                                                                                                     | <b>93</b> |
| <b>Appendix .....</b>                                                                                                                       | <b>99</b> |

# List of Figures

|                                                                                                                                                                                                                                                                                                                                                                                            |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 1. Scheme of sparkling wines production by traditional method (Torresi, Frangipane, and Anelli 2011; Buxaderas and López-Tamames 2012) .....                                                                                                                                                                                                                                        | 24 |
| Figure 2. Scheme of sparkling wines production using the Charmat method (Buxaderas and López-Tamames 2012) .....                                                                                                                                                                                                                                                                           | 25 |
| Figure 3. Example of 1+1 technical cork stopper [reprinted from (APCOR-2)]. .....                                                                                                                                                                                                                                                                                                          | 26 |
| Figure 4. Diagram of the steps of extraction by SPME [reprinted from (Coelho 2010)] ....                                                                                                                                                                                                                                                                                                   | 28 |
| Figure 5. Representation of the constitution of a GC system [Adapted from (Chromacademy)].....                                                                                                                                                                                                                                                                                             | 29 |
| Figure 6. Representative HS-SPME-GC/MS chromatogram of volatile compounds identified in the Italian sparkling wine A at 36 months. Compounds: 1- isoamyl alcohol; 2- ethyl lactate; 3- 1-hexanol; 4- hexanoic acid; 5- ethyl hexanoate; 6- phenylethyl alcohol; 7- diethyl succinate; 8- ethyl octanoate; 9- nonanoic acid; 10-ethyl decanoate. ....                                       | 55 |
| Figure 7. A- PLS-DA scores scatter plot of i) Italian sparkling wine A, ii) Italian sparkling wine B and iii) Spanish sparkling wine; B- Important features of i) Italian sparkling wine A, ii) Italian sparkling wine B and iii) Spanish sparkling wine.....                                                                                                                              | 62 |
| Figure 8. Heatmap illustrating the levels of volatile compounds changing over time in Italian sparkling wine A ( <b>A</b> ), Italian sparkling wine B ( <b>B</b> ) and Spanish sparkling wine ( <b>C</b> ). Columns correspond to each sample while rows correspond to the concentration of each discriminant volatile coloured from minimum (−1, dark blue) to maximum (1, dark red)..... | 65 |
| Figure 9. Boxplots of statistically significant compounds found altered in Italian sparkling wine A at different timepoints (12, 24 and 36 months). Statistical significance assessed using the Mann-Whitney test (* - $p < 0.05$ ; ** - $p < 0.01$ ; *** - $p < 0.001$ ; **** - $p < 0.0001$ ). ND-not detected. ....                                                                     | 68 |
| Figure 10. Representative HS-SPME-GC/MS chromatogram of VOCs identified in an Italian white wine sealed with A cork stopper. Compound Identification: 1- isoamyl alcohol; 2- 2,3-butanediol; 3- 1-hexanol; 4- ethyl hexanoate; 5- phenylethyl alcohol; 6- diethyl succinate; 7- ethyl octanoate; 8- decanoic acid; 9- ethyl decanoate. ....                                                | 70 |
| Figure 11. Representative HS-SPME-GC/MS chromatogram of VCCs identified in an Italian white wine sealed with A cork stoppers. Compound Identification: 1- isobutanol; 2- (1E)-3-methylpentanal; 3- (E)-3-heptanone; 4- methylglyoxal.....                                                                                                                                                  | 71 |
| Figure 12. A- Spanish sparkling wine at 24 months sealed with 2D and crown cap stoppers: i) PCA scores scatter plot; ii) PLS-DA scores scatter plot; iii)- Important features; B- Spanish sparkling wine at 80 months sealed with 2D and crown cap stoppers: i) PCA scores scatter                                                                                                         |    |

|                                                                                                                                                                                                                                                                                                                                                  |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| plot; ii) PLS-DA scores scatter plot; iii) - Important features. ....                                                                                                                                                                                                                                                                            | 81 |
| Figure 13. A - Boxplots of compounds found significantly altered in Spanish sparkling wine sealed with 2D and crown cap stoppers at 24 months; B- Boxplots of compounds found significantly altered in Spanish sparkling wine sealed with 2D and crown cap stoppers at 80 months. ....                                                           | 82 |
| Figure 14.A – French sparkling wine at 18 months post-bottling: i) PCA scores scatter plot; ii) PLS-DA scores scatter plot; ii) Important features. ....                                                                                                                                                                                         | 83 |
| Figure 15. Boxplots of statistically significant compounds found altered between French sparkling wine sealed with Micro CSP and STO stoppers. Statistical significance assessed using the t-test (*- p value $\leq 0.05$ ; ** - p value $\leq 0.01$ ; *** - p value $\leq 0.001$ ) ....                                                         | 85 |
| Figure 16.A- PCA of an Italian white wine sealed with A, B and C stoppers; B- Italian white wine sealed with A and C stoppers and their respective i) PLS-DA scores scatter plot, ii) Important features; C- Italian white wine sealed with A and B stoppers and their respective i) PLS-DA scores scatter plot and ii) Important features. .... | 87 |
| Figure 17. Boxplots of statistically significant compounds found altered between Italian white wine sealed with different cork stoppers (A, B and C). Statistical significance assessed using the t-test (* - p < 0.05; ** - p < 0.01; *** - p < 0.001; **** - p < 0.0001). ....                                                                 | 89 |
| Figure 18.A- Heatmap of the Italian white wine sealed with A and C stoppers; B- Heatmap of the Italian white wine sealed with B and C stoppers. Columns correspond to each sample while rows correspond to the concentration of each discriminant volatile coloured from minimum (-1, dark blue) to maximum (1, dark red). ....                  | 90 |

## List of Tables

|                                                                                                                                                                      |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 1. Summary of the main advantages and drawbacks of the extraction methods applied in wine volatile analysis [Adapted from (Marín-San Román et al. 2020)] ..... | 27 |
| Table 2. List of volatile and semi-volatile organic compounds identified in sparkling wines. ....                                                                    | 32 |
| Table 3. Studies focused on the impact of different cork closures on the volatile composition of white wine and wine model solution.....                             | 44 |
| Table 4. Summary of the studies of ageing and impact of closures. ....                                                                                               | 51 |
| Table 5. Compounds identified in Italian sparkling wines A and B and in the Spanish sparkling wine. ....                                                             | 56 |
| Table 6. Volatile compounds found altered in Italian sparkling A and B and Spanish sparkling wine over time. ....                                                    | 63 |
| Table 7. Compounds detected in an Italian white wine sealed with A, B and C stoppers and in a French Sparkling wine .....                                            | 72 |
| Table 8. VCCs identified in the Italian white wine sealed with A, B and C stoppers .....                                                                             | 78 |
| Table 9. Compounds found altered in the Spanish sparkling wine sealed with 2D stoppers compared with crown cap.....                                                  | 82 |
| Table 10. Compounds found altered in the French sparkling wine sealed with micro CSP stoppers compared to STO stoppers. ....                                         | 84 |
| Table 11. Table of compounds found statistically significant between Italian white wine sealed with different stoppers (A, B and C). ....                            | 88 |

## List of Annexes

|                                                                                                                                             |     |
|---------------------------------------------------------------------------------------------------------------------------------------------|-----|
| A 1. Quantification of volatile and semi volatile compounds in Italian sparkling wine A at different timepoints (12,24 and 36 months). .... | 100 |
| A 2. Quantification of volatile and semi volatile compounds in Italian sparkling wine B at different timepoints (12,24 and 36 months). .... | 102 |
| A 3. Quantification of volatile and semi volatile compounds in Spanish sparkling wine at different timepoints (24 and 80 months). ....      | 105 |
| A 4. Quantification of volatile and semi volatile compounds in Spanish sparkling wine sealed with 2D and crown cap. ....                    | 107 |
| A 5. Quantification of volatile and semi-volatile compounds in Italian white wine sealed with different stoppers (A, B and C). ....         | 110 |

|                                                                                                                          |     |
|--------------------------------------------------------------------------------------------------------------------------|-----|
| A 6. Quantification of volatile and semi-volatile compounds in French sparkling wines sealed with Micro CSP and STO..... | 113 |
|--------------------------------------------------------------------------------------------------------------------------|-----|

# Abbreviations

|                       |                                                   |
|-----------------------|---------------------------------------------------|
| <b>Atm</b>            | Atmosphere                                        |
| <b>CO<sub>2</sub></b> | Carbon dioxide                                    |
| <b>EEFAs</b>          | Ethyl esters of fatty acids                       |
| <b>4-EG</b>           | 4-Ethylguaiaicol                                  |
| <b>4-EP</b>           | 4-Ethylphenol                                     |
| <b>FID</b>            | Flame ionization detector                         |
| <b>GC-MS</b>          | Gas chromatography coupled with mass spectrometry |
| <b>GC-O</b>           | GC-olfactometry                                   |
| <b>HAAs</b>           | Higher alcohol acetates                           |
| <b>HS</b>             | Headspace                                         |
| <b>IBMP</b>           | 3-Isobutyl-2-methoxypyrazine                      |
| <b>IPMP</b>           | Isopropyl-2-methoxypyrazine                       |
| <b>LDPE</b>           | Low density polyethylene                          |
| <b>MEFAs</b>          | Methyl esters of fatty acid                       |
| <b>OTR</b>            | Oxygen transfer rate                              |
| <b>PCA</b>            | Principal component analysis                      |
| <b>PLS-DA</b>         | Partial least square discriminant analysis        |
| <b>PDMS</b>           | Polydimethyl siloxane                             |
| <b>ROTE</b>           | Roll-on tamper evident                            |
| <b>SBSE</b>           | Stir-bar-sorptive-extraction                      |
| <b>SO<sub>2</sub></b> | Sulfur dioxide                                    |
| <b>SPE</b>            | Solid-phase extraction                            |
| <b>SPME</b>           | Solid-phase microextraction                       |
| <b>TCA</b>            | 2,4,6-Trichloroanisole                            |
| <b>TDN</b>            | 1,2-Dihydro-1,1,6-trimethylnaphthalene            |
| <b>VCCs</b>           | Volatile carbonyl compounds                       |
| <b>VIP</b>            | Variable importance to the projection             |
| <b>VOCs</b>           | Volatile organic compounds                        |

# Chapter 1. Introduction

## 1.1. Historical Context

The Limoux region in France was a great producer of white ones, namely those obtained with the *Mauzac* grape variety. By the year of 1530, Abbey of St. Hillaire bottled and sealed with cork all of the wine production of that year. Later, that wine presented a different characteristic, it had effervescence and as a result the first sparkling wine created with the name of *Vin de Blanquette* (CASCO 2014).

In the seventeenth century, the same phenomenon occurred in the region of Champagne, France. This happened because wine suffered two fermentations. The first fermentation was interrupted due to the lower temperatures of the winter season and restarted when the temperatures increased (during Spring) leading to a second fermentation. This wine was stored in bottles using wood as stoppers and canvas. At that time, these materials were not capable of withstand the pressure and consequently the bottle exploded (Buxaderas and López-Tamames 2012). Dom Pérignon came up with the idea of using cork as a stopper to seal his wine before the second fermentation occurred, as a result, the gas was then possible to be contained inside the bottle. So, Dom Pérignon unravelled the secret of Champagne (Bullock, Wang, and Bullock 1998).

Another important milestone to the development of sparkling wine was achieved in the nineteenth century by Barbe-Nicole Clicquot-Ponsardin. Her company created a new technique with the aim of eliminating the cloudiness of sparkling wine. This method was called riddling and consisted of riddling racks with supports that enable the bottles to be arranged at diverse angles. Initially, bottles were placed in a horizontal position and endured a rotation of one-eight daily. These turns and the fact that the bottles were held upside down resulted in the accumulation of the sediment near the stopper. Then, the wine closely to the stopper was removed and the bottles refilled, causing a sparkling wine free from cloudiness but keeping the effervescence (Buxaderas and López-Tamames 2012).

Sparkling wine production widened to other European countries, namely Spain, Italy and Germany. In Spain, the first sparkling wine produced was from the region of Penedés, more properly Cava. Cava sparkling wine is one of the most well-known Spanish sparkling wines (Buxaderas and López-Tamames 2012). In Italy, sparkling wines are denominated “spumante” and the most famous are Prosecco, Asti, Franciacorta and Lambrusco provenient from Venetto, Piedmont, Lombardy and Emilia regions, respectively. In Germany, it is possible to find two main types of sparkling wines: Schaumwein and Qualitäts-schaumwein (Sekt) (Buxaderas and López-Tamames 2012).

During the twentieth century, more developments occurred in this field with particular

relevance for a more profound understanding of the microorganisms responsible for fermentation. Furthermore, the *liqueur de tirage* and the second fermentation were considered essential steps to produce sparkling wines (Buxaderas and López-Tamames 2012).

According to the International Organisation of Vine and Wine (OIV), sparkling wines are “special wines produced from grapes, musts or wines processed according to the techniques accepted by OIV, characterised on uncorking by the production of a more or less persistent effervescence resulting from the release of carbon dioxide of exclusively endogenous origin” (OIV-1). Thus, the designation sparkling wine is only applied for wines in which the amount of carbon dioxide is a product of the fermentation process. Hence, Champagne is a sparkling wine with *Appellation d’Origine Contrôlée* (AOC) (this designation refers to a product with a specific origin and that follow particular rules of production and manufacturing) (Champagne-1). Therefore, Champagne denomination is only applied to sparkling wine produced in the region of Champagne, France (Champagne-2).

Sparkling wines are characterized for having pressures of carbon dioxide in bottle at least of 3.5 bars at a temperature of 20 °C (Buxaderas and López-Tamames 2012). They can be distinguished in (OIV 2020):

- Brut: maximum of 12 g/L of sugar with a tolerance of + 3 g/L;
- Extra-dry: the sugar contents vary between 12 g/L and 17 g/L with a tolerance of +3 g/L;
- Dry: sugar content is comprised between 17 g/L minimum to a maximum of 32 g/L with a tolerance of +3 g/L;
- Demi-sec: the sugar content is between 32 to 50 g/L;
- Sweet: the concentration of sugar is above 50 g/L.

## **1.2. Production of Sparkling wine**

The production of sparkling wines englobes two main phases. The first phase refers to the production of a base wine and the second phase includes a fermentation of the base wine in a closed recipient by adding sugar and ageing in contact with lees (Buxaderas and López-Tamames 2012).

### **1.2.1. Base wine**

According to OIV, base wine is a wine intended for the production of a sparkling wine (OIV-2).

The production of the base wine is similar to the process of production of table white wines with the exception of sulfur dioxide (SO<sub>2</sub>) addition once this wine will suffer a second fermentation step (Coelho 2010). Moreover, base wines do not contain an alcohol concentration weight more than 10% v/v once the alcohol content will increase in the second fermentation (Coelho 2010; Buxaderas and López-Tamames 2012).

It is essential to take into consideration that grapes should be in the best condition when harvesting. The maceration step should be performed carefully in order to avoid a bitter taste. The juice obtained from the first pressing, denominated *cuvée*, has a superior quality being only used for making sparkling wines with higher quality. After the first pressing, juices will have a decreascent quality and are utilised for lower quality sparkling wines or table wines (Buxaderas and López-Tamames 2012).

The base wine should bring together a set of characteristics, such as: bright colour, a fruity aroma, few residual sugar, low volatile acidity and be tartaric steadied (Torresi, Frangipane, and Anelli 2011).

### **1.2.2. Second Fermentation**

Refermentation is a key step for making sparkling wine since it will provide carbon dioxide and, consequently, the effervescence and foam that are characteristics of this wine. At this stage, sugars need to be added to the base wine to serve as food for the yeast since sugars of grapes were already metabolized in the first fermentation. Yeasts used at this stage must be capable of fermenting in an alcohol concentration of 9-10 % v/v, underneath the carbon dioxide pressure and to flocculate (Buxaderas and López-Tamames 2012). The main methods to perform the second fermentation are Champenoise, classic or traditional method and Charmat or Granvas method (or bulk method).

#### *Champenoise, classic or traditional method*

Figure 1 summarises the steps necessary to the production of sparkling wines recurring to the traditional method. Making a sparkling wine recurring to this method requires filling glass bottles with the base wine. Then, it is added a *tirage* solution that is composed of yeasts, saccharose, and some agents (e.g., bentonite, ammonium phosphate, among others) to promote yeast growth. The *tirage* solution must be made in adequate proportions, considering the alcoholic content as well as the amount of carbon dioxide pressure desired. Theoretically, each bottle of 750 mL receives about 40-42 mL of *tirage* solution from which around 21 to 24 grams is sucrose that is sufficient to produce between 5-6 atm pressure, the goal for the final content of CO<sub>2</sub> (Buxaderas and López-Tamames 2012). Then, the bottles are hermetically closed with a *bidule* (is a polyethylene cup that averts the leak of

sparkling wine) and a crown cap, followed by storage in the horizontal position (Torresi, Frangipane, and Anelli 2011). At this point, the second fermentation takes place and is usually completed after 0.5-1.5 months (Buxaderas and López-Tamames 2012).

When the second fermentation is finished, the ageing process in contact with lees begins. At this stage, it occurs the autolysis of yeasts, as a result some compounds are transferred into the sparkling wine, namely amino acids, peptides, proteins, polysaccharides, nucleic acid derivatives and lipids. Sparkling wine will mature and develop essential aromas (Torresi, Frangipane, and Anelli 2011).

The next step, called riddling, consists of separating the deposit of yeasts from the sparkling wine. Initially, riddling was a manual process but, currently, there are automated systems that rotate all bottles at the same time. As soon as the deposit of yeasts is completed in the bottleneck area closer to the stopper, it is then possible to remove it, a step called *dégorgement*. In this stage, the neck of the bottle is inserted in a solution of calcium chloride or glycol, freezing both the sparkling wine in the bottleneck area as well as the sediments. Then the bottle is placed upwards, uncorked and the existing pressure expels the deposit (Torresi, Frangipane, and Anelli 2011).

The bottles are then filled with the shipping liquor composed of wine, sugar, brandy, SO<sub>2</sub>, citric acid and copper sulphate. Lastly, they are corked, wired, foiled, labelled and the final product is ready to be distributed (Torresi, Frangipane, and Anelli 2011).

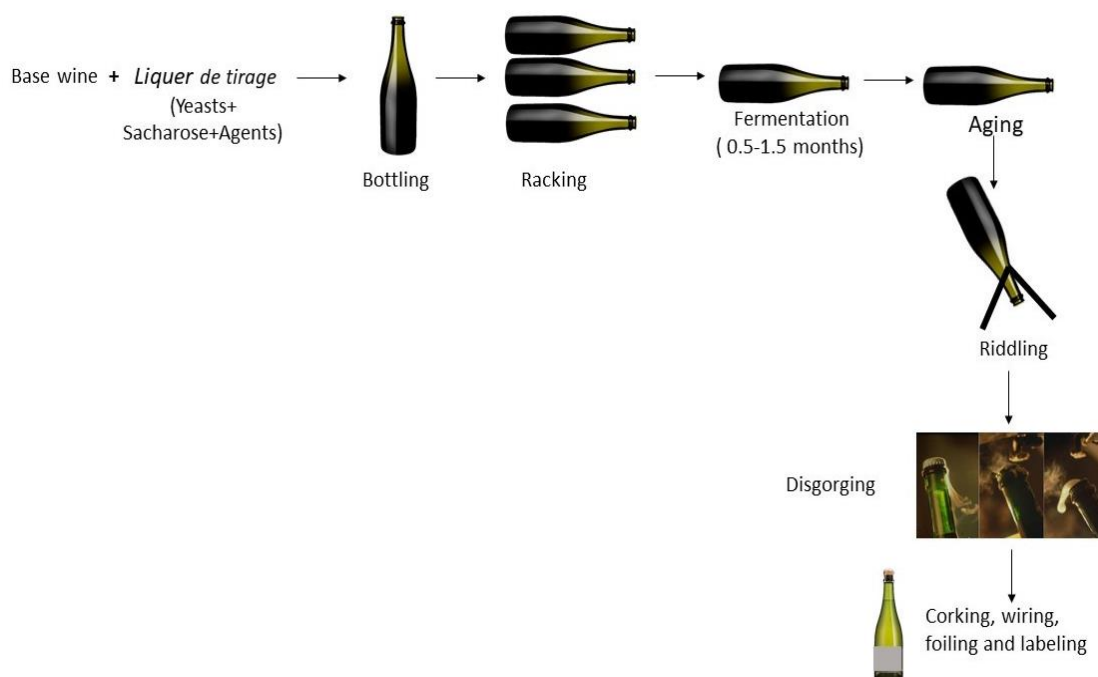


Figure 1. Scheme of sparkling wines production by traditional method (Torresi, Frangipane, and Anelli 2011; Buxaderas and López-Tamames 2012)

### *Charmat or Granvas method*

The Charmat method (Figure 2) is an alternative option to produce sparkling wine, in which the second fermentation occurs in a tank denominated charmat tank (Buxaderas and López-Tamames 2012). Similarly, to the traditional method, a base wine is first prepared from which the *liqueur the tirage* is added. The wine is transferred to a tank that contains agitators and controls the temperature, assuring a complete fermentation (Buxaderas and López-Tamames 2012). This stage ends when a 4 atm pressure of carbon dioxide is achieved, then the temperature automatically decreases to 8 °C in order to stop the fermentation. According to the amount of time that wine is leave in contact with lees, it is possible to separate two Charmat methods, short and long. In the first one, the contact with lees does not last more than 3 months and the sparkling wine produced has a young, fresh, fruity aroma. In the second one, the time in contact with lees is longer, about 6 months and results in sparkling wines with a sensory aroma more in common with the ones created using the traditional method (Buxaderas and López-Tamames 2012).

The next step is clearing the wine from dead yeasts. Wine will be transferred to another tank and endure a filtration or centrifugation under isobaric conditions (Buxaderas and López-Tamames 2012). The final steps include adding the dosage, which is a liqueur characteristic of each company and gives specific sensory properties, and bottling is executed under isobaric conditions to prevent the loss of CO<sub>2</sub> (OIV 2020). Ultimately, sparkling wine is sealed and labelled. In Figure 2 is represented a scheme of all the steps mentioned above to produce sparkling wines using the Charmat method.

To conclude, the Charmat method when compared to the traditional one brings some economical vantages, namely the control of pressure and temperature and the absence of two steps, riddling and disgorging (Buxaderas and López-Tamames 2012).

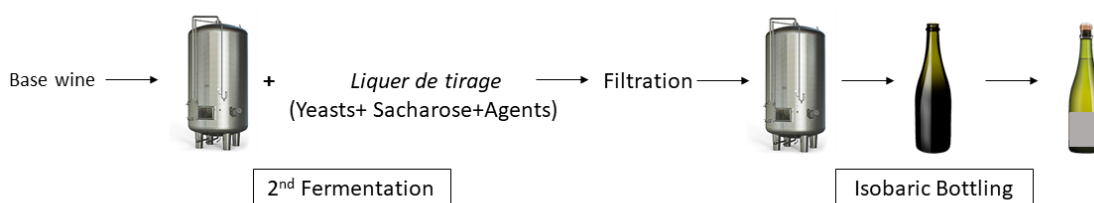


Figure 2. Scheme of sparkling wines production using the Charmat method (Buxaderas and López-Tamames 2012)

### 1.3. Types of closures for sparkling wines

Champagne and sparkling wine cork stoppers are a category of stoppers that belongs to the family of technical stoppers (APCOR-1). Technical cork stoppers were created with the intent to seal wines to be consumed in a period no longer than two to three years. These types of stoppers are constituted of an agglomerate cork body with cork discs attached on one or both extremities. Stoppers with just one disc at each extremity are called 1+1 technical cork stoppers, whereas stoppers with 2 discs at both ends are denominated 2+2 technical cork stoppers. An important point to be considered is the fact that the glue applied in these types of closures must fulfil the requirements to be used in food matrices according to Food and Drug Administration (FDA). They have a bigger diameter than natural cork stoppers in order to maintain the high pressures of sparkling wines (APCOR-1). Champagne and sparkling wine stoppers present the advantages of being chemically steady and very resistant (APCOR-1). After the insertion of these stoppers into the bottle, a natural expansion of the outside part of the stopper will occur leading to a shape similar to a mushroom (APCOR-1).



Figure 3. Example of champagne cork stopper [reprinted from (APCOR-1)].

There are other available options in the market to seal sparkling wines, such as microagglomerated stoppers. Microagglomerated stoppers are made of granules of cork with a specific calibre united by a food safe glue approved by FDA (APCOR-3). These stoppers have a great structural stability and are recommended for wine with a short period of storage (AMORIM CORK).

### 1.4. Methods for the analysis of volatile compounds

The aroma of wine is originated due to various volatile compounds present at smaller concentrations. In order to determine its volatile composition, it is necessary a previous step of sample preparation before the chromatographic analysis (Sánchez-Palomo, Diaz-Maroto, and Perez-Coello 2005). This first step is critical once a great quantity of analyte can be lost which can cause significant errors. Therefore, the right technique chosen for sample preparation affects the analysis reliability and precision. Methods to

extract these compounds take into consideration their physicochemical properties, such as volatility, solubility, and polarity. Hence, they also should be very sensitive, selective and relatively simple to operate (Callejón et al. 2016). There are several techniques to perform volatile extraction, namely liquid-liquid extraction, purge and trap, stir bar sorptive extraction, headspace trap, solid phase extraction and solid phase microextraction. Generally, the preferred technique to realise volatile extraction in wine is headspace-solid phase microextraction. Table 1 presents a summary of the main advantages and drawbacks of the extraction methods mentioned before.

Table 1. Summary of the main advantages and drawbacks of the extraction methods applied in wine volatile analysis [Adapted from (Marín-San Román et al. 2020)]

| Method                       | Advantages                                                                                                                                                                                                                        | Drawbacks                                                                                                                                                                                                      |
|------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Solid-phase microextraction  | High reproducibility<br>Low cost<br>Does not need solvents and previous sample preparation<br>Simple<br>More selective fibers coating<br>Reduced volume of sample used<br>Less expensive than LLE and SPE<br>High detection limit | Fibers are easy to break<br>Precision and accuracy are dependent on the quality of the fiber                                                                                                                   |
| Liquid-Liquid extraction     | Extensively used<br>Enables the extraction of compounds with several volatilities and polarities<br>Requires a small volume of sample                                                                                             | Requires high solvent volume<br>Time consuming<br>Low reproducibility<br>Low selectivity<br>Source of environmental pollution<br>Expensive<br>Analyte loss<br>Harmful for the operator                         |
| Purge-and-trap               | Lower detection limits<br>Direct thermal volatilization                                                                                                                                                                           | Requires more maintenance<br>Affected by the foaming of the sample, content of alcohol and water                                                                                                               |
| Stir bar sorptive extraction | Higher sensitivity than SPME<br>Can be used with great amounts of water or other solutes<br>One step extraction and concentration<br>No need of organic solvents<br>Rapid and simple                                              | Desorption process is slower than SPME<br>Only PDMS and EG stir bars are commercially available<br>Requires <i>in situ</i> derivatization to retain polar solutes<br>The system is prone to carry-over effects |
| Head-space trap              | Good repeatability without carry-over effects<br>Adequate for extraction of volatile compounds of almost any sample matrix<br>Requires small sample amounts and minimal preparation                                               | Trap enrichment might be necessary for minor volatile compounds<br>Too high trap temperatures can cause analyte loss                                                                                           |
| Solid-phase extraction       | Faster<br>Low solvent consumption<br>Safe to work<br>Allows a wide variety of analytes to be extracted                                                                                                                            | Small reproducibility<br>Small selectivity<br>Analyte loss<br>Automatization is complex<br>Requires a great volume of sample                                                                                   |

#### 1.4.1. Volatile extraction

In this thesis, the analytical technique employed for the extraction of volatile compounds in still wine and sparkling wine was HS-SPME. Therefore, HS-SPME will be presented in further detail in this section.

##### *Headspace solid-phase microextraction*

Headspace solid-phase microextraction (HS-SPME), is an extraction technique, created by Janus Pawliszyn in the nineties (Zhang and Pawliszyn 1993). Initially, this approach was focused on identifying contaminants in water, only later and recurring to different fibers was applied to analyse the wine bouquet (Barros et al. 2012).

HS-SPME approach is based in sorption using a silica fiber coated with an extracting phase, that can be liquid or solid. Therefore, the extraction is performed by entering the fiber into contact with the headspace, which allows the extraction of the volatile compounds released to the space above the sample (Chromacademy).

This methodology consists in an equilibrium of a three-phase system, namely the sample, the headspace directly above the sample and the fiber. Succinctly, the fiber is inserted in a closed vial where the sample is (Figure 8), then the analytes are adsorbed into the fiber coating (e.g., polydimethylsiloxane (PDMS)) until the system reaches an equilibrium (Figure 8), allowing the quantification of the analyte because the concentration of the analytes in the headspace is proportional to the original amount in the sample. Afterwards, the fiber is removed from the vial (Figure 4) , rapidly injected into the GC and all the analytes will be withdraw onto the GC column (Pawliszyn 1997).

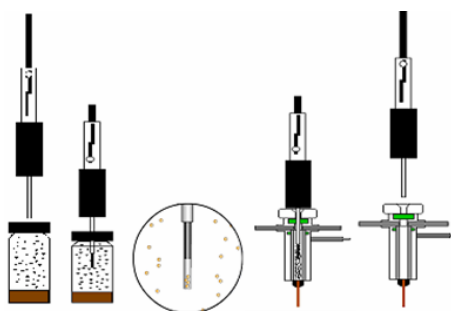


Figure 4. Diagram of the steps of extraction by SPME [reprinted from (Coelho 2010)]

#### 1.4.2. Analysis of volatile compounds by gas chromatography-mass spectrometry (GC-MS)

GC-MS is an analytical technique that combines the capacity of separation of gas chromatography with the detection capacity characteristic of mass spectrometry. This way, it allows the separation and identification of numerous compounds present in a sample (Chauhan, Goyal, and Chauhan 2014). The GC system is constituted by several parts (Figure 5):

- 1) Injector, where the sample is introduced and will be volatilized.
- 2) Chromatographic column, which is made of fused silica and coated with polyimide. The stationary phase is attached in the interior wall of the column, where the analytes of the sample will interact.
- 3) Detector, which transforms a physicochemical property into an electrical signal.
- 4) Data system, which creates a chromatogram (Shellie 2013).

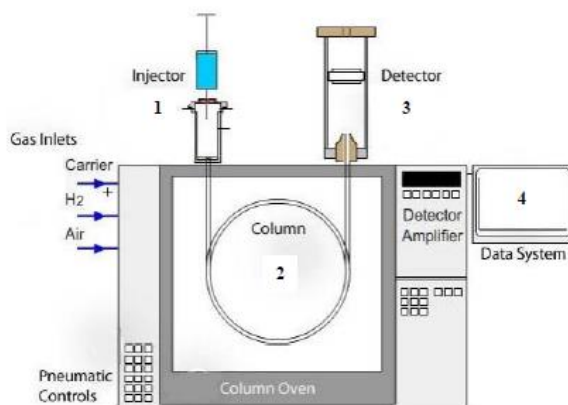


Figure 5. Representation of the constitution of a GC system [Adapted from (Chromacademy)].

During the chromatographic analysis, the volatile fraction is transported by the mobile phase (carrier gas such as helium or hydrogen) that interacts with the stationary phase, causing the components to elute at different times, therefore each compound will have a different retention time. Once the compounds are eluted from the column, they are heated and bombarded with electrons, with the goal of breaking down the molecules and consequently forming ions. The ions are then directed to a mass analyser (e.g., quadrupole or ion trap) where they are separated according to their mass to charge ratio ( $m/z$ ). After their separation, the ions pass through a detector that will register a signal as a function of time, leading to a peak for each compound in a chromatogram (Boudaoud and Eveleigh 2003). To identify the volatile compounds present in a sample, the retention time and mass spectra obtained is compared with those of commercial standards or, when standard compounds are not commercially available, the identification is done by comparing the mass spectra with those of a database (e.g., National Institute of Standards and Technology (NIST)) (Oliveira et al. 2020).

## 1.5. Volatile composition of sparkling wines

Sparkling wine is a complex matrix with a great percentage of alcohol content and a lower quantity of aroma-active components. The final content of volatile compounds in sparkling wine is the result of various factors, namely the type of grape and its degree of maturation, the technology used for production of sparkling wine and the methods chosen for fermentation, yeast strains, temperature, duration of maturation period, ingredients that constitute the *liqueur d'expedition*, type of stoppers, amount of SO<sub>2</sub> and CO<sub>2</sub>, among others (Jagatić Korenika et al. 2020).

Despite having a great importance in wines, volatile organic compounds are present in a small concentration, approximately 0.8-1.2 g/L, which only represents 1% of the amount of ethanol (Rapp 1998). Hence, it is possible to distinguish aroma compounds in sparkling wines accordingly to their origin in:

- Primary or varietal aromas: aroma obtained due to compounds found in grapes that are related with variety of grape, environmental conditions like soil, climate, rootstock. These compounds are majority terpenes, C13-norisoprenoides, benzenoids, pyrazines, ketones and aldehydes.
- Secondary grape aromas: compounds that are originated when transforming the grapes, for example crushing, and by chemical and biochemical reactions, as the example of fermentation.
- Tertiary aromas: aroma compounds created during ageing, in bottle or not, second fermentation corresponding to oxidation-reduction reactions (Forleo et al. 2020).

The volatile and semi-volatile fraction of sparkling wine comprehends compounds from several groups, namely alcohols, esters, ketones, C-13 norisoprenoids, fatty acids, phenols, amides and others (Rapp 1998). Table 2 presents a list of volatile and semi volatile organic compounds usually identified in sparkling wine and their corresponding organoleptic characteristics.

Higher alcohols (also known as fusel oils) are products formed during the alcoholic fermentation reaction by yeast with either amino acids or sugars. There are several elements of this family already described, for instance, principal higher alcohols found in wine are 3-methylbutanol, 2-methylbutanol, 2-methylpropanol and 1-propanol (Blazquez 2004; Panighel and Flamini 2014).

Esters are one of the most important families of volatile compounds, usually they are responsible for giving pleasant aromas to wine, such as fruity or floral. This group of compounds is present in small concentrations, frequently not beyond 100 mg/L. Esters can be divided into two categories: 1) the ones that are originated due to enzymatic reactions;

and 2) esters that are produced over time, during wine ageing. In this class of volatiles, it is worth to mention the acetate esters which have positive aroma descriptors, including isoamyl acetate (*banana*), ethyl hexanoate (*brandy*), 2-phenylethylacetate (*roses*), ethyl 2-methylbutanoate (*pineapple* and *strawberry*), ethyl butanoate and ethyl decanoate (*fruity* and *floral*) (Sumbly, Grbin, and Jiranek 2010).

Ketones and aldehydes exist in a smaller quantity and are created as a product of fermentation and oxidation (Waterhouse, Sacks, and Jeffery 2016). Some examples of aldehydes found in sparkling wines are propanal, acetaldehyde, phenylacetaldehyde, among others. They can be originated in the presence of alcohols and other precursors due to reactions of oxidation. In spite of the fact that various aldehydes have already been described as normal constituents of wine, a great part of the negative alterations found in wine aroma when exposed to oxygen were justified by the formation of acetaldehyde and its acetals (Culleré, Cacho, and Ferreira 2007).

Norisoprenoids are originated from carotenoids present in grapes (Waterhouse, Sacks, and Jeffery 2016). They are found in wine at lower levels, but it is thought that their sensory perception threshold is between 2-10 µg/L. Some examples of norisoprenoids are  $\beta$ -damascenone, vitispirane and 1,6-trimethyl-1,2-dihydronaphthalene (TDN) (Waterhouse, Sacks, and Jeffery 2016; Henschen 2015).

Fatty acids correspond to a small amount of the total volatile composition (Sumbly, Grbin, and Jiranek 2010). There are several groups already identified but mid-chain fatty acids and their esters have the most impact in wine organoleptic properties because of their high solubility and volatility. However, mid chain fatty acids and ethyl esters in higher concentrations can result in sluggish fermentations (Waterhouse, Sacks, and Jeffery 2016). The smaller fatty acids include isobutyric, isovaleric and propanoic acids that are usually associated with *sweat* and *cheese* aromas. Medium-chain fatty acids comprise hexanoic, octanoic and decanoic acids described as having *sweat*, *cheese* and *rancid fat* aromas. So, in general, fatty acids are responsible for unpleasant aromas in wine (Robinson et al. 2014).

Volatile phenols are produced by microbiological or chemical reactions from precursors in grapes or from toasted oak wood, once most of wine is storage in barrels made of oak. This family of compounds has lower olfactory perception thresholds which could negatively affect the sensory properties of wine (Waterhouse, Sacks, and Jeffery 2016). Some examples of volatile phenols found in wines are 4-ethylguaiacol, 4-ethylphenol, 4-methylguaiacol, vinylphenols, guaiacol, eugenol, and vanillin (Ward 2015).

Monoterpenes and sesquiterpenes are biosynthesised in grapes from isopentyl pyrophosphate and dimethylallyl pyrophosphate, conferring floral and citrus notes to wine (Robinson et al. 2014). This group has an important function in discriminating wine varieties (Rapp 1998).

Table 2. List of volatile and semi-volatile organic compounds identified in sparkling wines.

| Compound                    | CAS number | Odour descriptor                             | Reference                                                                    |
|-----------------------------|------------|----------------------------------------------|------------------------------------------------------------------------------|
| <b>Alcohols</b>             |            |                                              |                                                                              |
| Propanol                    | 71-23-8    | Alcohol, pungent <sup>b</sup>                | (Bosch-Fusté et al. 2007)                                                    |
| Isobutanol                  | 78-83-1    | Alcohol, nail polish <sup>a</sup>            | (Jagatić Korenika et al. 2020; Torrens et al. 2010)                          |
| 1-Butanol                   | 71-36-3    | Medicinal <sup>a</sup>                       | (Jagatić Korenika et al. 2020)                                               |
| 1,4-Butanediol              | 110-63-4   | –                                            | (Jagatić Korenika et al. 2020)                                               |
| 2,3-Butanediol              | 513-85-9   | Buttery, creamy <sup>a</sup>                 | (Jagatić Korenika et al. 2020)                                               |
| 1-Methoxy-2-propanol        | 107-98-2   | –                                            | (Jagatić Korenika et al. 2020)                                               |
| Methionol                   | 505-10-2   | Sweet, potato <sup>b</sup>                   | (Torrens et al. 2010)                                                        |
| 3-(Methylthio)-1-propanol   | 505-10-2   | Cooked potato <sup>a</sup>                   | (Jagatić Korenika et al. 2020)                                               |
| Isoamyl alcohol             | 123-51-3   | Alcohol, nail polish <sup>a</sup>            | (Jagatić Korenika et al. 2020; Torrens et al. 2010; Bosch-Fusté et al. 2007) |
| Furfuryl alcohol            | 98-00-0    | Sweet, nutty <sup>a</sup>                    | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| 3-Methyl-3-buten-1-ol       | 763-32-6   | –                                            | (Jagatić Korenika et al. 2020)                                               |
| 1-Pentene-3-ol              | 918-85-4   | –                                            | (Bosch-Fusté et al. 2007)                                                    |
| 2-Pentene-1-ol              | 20273-24-9 | –                                            | (Jagatić Korenika et al. 2020)                                               |
| 1-Pentanol                  | 71-41-0    | Bitter, almond, balsamic <sup>a</sup>        | (Jagatić Korenika et al. 2020; Bosch-Fusté et al. 2007)                      |
| 2-Methyl-1-butanol          | 137-32-6   | Whiskey, burnt, nail polish <sup>a</sup>     | (Jagatić Korenika et al. 2020)                                               |
| 4-(Methylthio)-1-butanol    | 20582-85-8 | Metallic-bitter, garlic, earthy <sup>a</sup> | (Jagatić Korenika et al. 2020)                                               |
| 2-Pentadecanol              | 1653-34-5  | –                                            | (Jagatić Korenika et al. 2020)                                               |
| Cyclohexanol                | 108-93-0   | Camphor, menthol <sup>c</sup>                | (Jagatić Korenika et al. 2020)                                               |
| <i>cis</i> -2-Hexene-1-ol   | 928-94-9   | Green <sup>a</sup>                           | (Jagatić Korenika et al. 2020)                                               |
| <i>cis</i> -3-Hexene-1-ol   | 928-96-1   | Grass, green <sup>a</sup>                    | (Jagatić Korenika et al. 2020)                                               |
| <i>trans</i> -2-Hexen-1-ol  | 928-95-0   | Herbaceous, green <sup>a</sup>               | (Jagatić Korenika et al. 2020)                                               |
| <i>trans</i> -3-Hexene-1-ol | 928-97-2   | Grass, resinous, cream <sup>b</sup>          | (Jagatić Korenika et al. 2020)                                               |
| 2-Methyl-1-pentanol         | 105-30-6   | –                                            | (Bosch-Fusté et al. 2007)                                                    |
| 3-Methyl-1-pentanol         | 589-35-5   | –                                            | (Bosch-Fusté et al.                                                          |

| Compound                | CAS number | Odour descriptor                             | Reference                                                                    |
|-------------------------|------------|----------------------------------------------|------------------------------------------------------------------------------|
|                         |            |                                              | 2007; Jagatić Korenika et al. 2020)                                          |
| Isohexanol              | 626-89-1   | Almond, toasted <sup>a</sup>                 | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| 1-Hexanol               | 111-27-3   | Grass just cut <sup>a</sup>                  | (Jagatić Korenika et al. 2020; Torrens et al. 2010; Bosch-Fusté et al. 2007) |
| 2-Butoxy-ethanol        | 111-76-2   | –                                            | (Jagatić Korenika et al. 2020)                                               |
| Benzyl alcohol          | 100-51-6   | Roasted, toasted, sweet, fruity <sup>a</sup> | (Jagatić Korenika et al. 2020)                                               |
| 1-Heptanol              | 111-70-6   | Oily <sup>a</sup>                            | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| 2-Heptanol              | 543-49-7   | Fruity, herbaceous <sup>a</sup>              | (Jagatić Korenika et al. 2020)                                               |
| Phenylethyl alcohol     | 60-12-8    | Floral, rose, honey <sup>a</sup>             | (Jagatić Korenika et al. 2020)                                               |
| 1-Octanol               | 111-87-5   | Chemical <sup>a</sup>                        | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| 3-Octanol               | 589-98-0   | Moss, nut, mushroom <sup>b</sup>             | (Jagatić Korenika et al. 2020)                                               |
| 4-Ethylcyclohexanol     | 19781-62-5 | –                                            | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| 3,4-Dimethyl-2-hexanol  | 19550-05-1 | –                                            | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| 2,6-Dimethyl-4-heptanol | 108-82-7   | Ether <sup>c</sup>                           | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| 1-Decanol               | 112-30-1   | Pear, waxy, violet <sup>a</sup>              | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| <b>Aldehydes</b>        |            |                                              |                                                                              |
| Acetaldehyde            | 75-07-0    | Pungent, ether <sup>b</sup>                  | (Torrens et al. 2010)                                                        |
| Hexanal                 | 66-25-1    | Herbaceous <sup>a</sup>                      | (Jagatić Korenika et al. 2020; Bosch-Fusté et al. 2007)                      |
| Heptanal                | 111-71-7   | Fat, citrus, rancid <sup>b</sup>             | (Jagatić Korenika et al. 2020)                                               |
| Octanal                 | 124-13-0   | Fat, soap, lemon, green <sup>b</sup>         | (Bosch-Fusté et al. 2007)                                                    |
| Nonanal                 | 124-19-6   | Fat, citrus, green <sup>b</sup>              | (Jagatić Korenika et al. 2020)                                               |
| Decanal                 | 112-31-2   | Soap, orange peel, tallow <sup>b</sup>       | (Jagatić Korenika et al. 2020)                                               |
| Undecanal               | 112-44-7   | Oil, pungent, sweet <sup>b</sup>             | (Bosch-Fusté et al. 2007)                                                    |
| 2-Heptenal              | 18829-55-5 | Green <sup>a</sup>                           | (Jagatić Korenika et al. 2020)                                               |

| Compound                      | CAS number | Odour descriptor                     | Reference                                                                    |
|-------------------------------|------------|--------------------------------------|------------------------------------------------------------------------------|
| (E, E)-2,4-Heptadienal        | 4313-03-5  | Nut, fat <sup>b</sup>                | (Jagatić Korenika et al. 2020)                                               |
| (Z, Z)-2,4-Heptadienal        | 4313-02-4  | –                                    | (Jagatić Korenika et al. 2020)                                               |
| 2,4-Nonadienal                | 5910-87-2  | Cucumber <sup>a</sup>                | (Jagatić Korenika et al. 2020)                                               |
| 2,4-Decadienal                | 25152-84-5 | Floral <sup>a</sup>                  | (Jagatić Korenika et al. 2020)                                               |
| $\alpha$ -Amyl-cinnamaldehyde | 122-40-7   | –                                    | (Bosch-Fusté et al. 2007)                                                    |
| Benzaldehyde                  | 100-52-7   | Bitter, almond <sup>a</sup>          | (Jagatić Korenika et al. 2020)                                               |
| Phenylacetaldehyde            | 122-78-1   | Honey, sweet <sup>a</sup>            | (Jagatić Korenika et al. 2020)                                               |
| 1,1-Diethoxybutane            | 3658-95-5  | –                                    | (Bosch-Fusté et al. 2007)                                                    |
| 1,1-Diethoxy-3-methylbutane   | 3842-03-3  | –                                    | (Bosch-Fusté et al. 2007)                                                    |
| 1,1-Diethoxypentane           | 3658-79-5  | –                                    | (Bosch-Fusté et al. 2007)                                                    |
| 1,1-Diethoxyheptane           | 688-82-4   | –                                    | (Bosch-Fusté et al. 2007)                                                    |
| 1,1-Diethoxynonane            | 54815-13-3 | –                                    | (Bosch-Fusté et al. 2007)                                                    |
| <b>Esters</b>                 |            |                                      |                                                                              |
| Ethyl propanoate              | 105-37-3   | Fruit <sup>b</sup>                   | (Bosch-Fusté et al. 2007; Torrens et al. 2010)                               |
| Ethyl lactate                 | 97-64-3    | Butter <sup>a</sup>                  | (Bosch-Fusté et al. 2007; Torrens et al. 2010; Jagatić Korenika et al. 2020) |
| Methyl-2-furoate              | 611-13-2   | –                                    | (Jagatić Korenika et al. 2020)                                               |
| Ethyl-2-oxopropanoate         | 617-35-6   | –                                    | (Jagatić Korenika et al. 2020)                                               |
| Diethyl oxalate               | 95-92-1    | –                                    | (Jagatić Korenika et al. 2020)                                               |
| Dimethyl malate               | 1587-15-1  | –                                    | (Jagatić Korenika et al. 2020)                                               |
| Ethyl isobutanoate            | 97-62-1    | Sweet, rubber <sup>b</sup>           | (Bosch-Fusté et al. 2007; Torrens et al. 2010)                               |
| Ethyl butanoate               | 105-54-4   | Pineapple, apple, peach <sup>a</sup> | (Jagatić Korenika et al. 2020; Torrens et al. 2010; Bosch-Fusté et al. 2007) |
| Isobutyl acetate              | 110-19-0   | Apple, banana <sup>a</sup>           | (Jagatić Korenika et al. 2020; Torrens et al. 2010)                          |
| Ethyl-2-hydroxybutanoate      | 52089-54-0 | –                                    | (Jagatić Korenika et al. 2020)                                               |

| Compound                          | CAS number | Odour descriptor                             | Reference                                                                    |
|-----------------------------------|------------|----------------------------------------------|------------------------------------------------------------------------------|
| Ethyl-3-hydroxybutanoate          | 5405-41-4  | Grape, fruity, caramel, toasted <sup>a</sup> | (Jagatić Korenika et al. 2020)                                               |
| Isopropyl disulfide               | 4253-89-8  | –                                            | (Bosch-Fusté et al. 2007)                                                    |
| Diethyl malonate                  | 105-53-3   | Sweet, fruity, apple <sup>a</sup>            | (Jagatić Korenika et al. 2020; Bosch-Fusté et al. 2007)                      |
| Ethyl methyl succinate            | 627-73-6   | –                                            | (Jagatić Korenika et al. 2020)                                               |
| Ethyl-2-methylbutanoate           | 7452-79-1  | Apple, strawberry <sup>a</sup>               | (Jagatić Korenika et al. 2020)                                               |
| Ethyl isovalerate                 | 108-64-5   | Fruit, pineapple <sup>a</sup>                | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| Isoamyl acetate                   | 123-92-2   | Banana <sup>a</sup>                          | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| Methyl hexanoate                  | 106-70-7   | Fruit, fresh, sweet <sup>b</sup>             | (Jagatić Korenika et al. 2020; Bosch-Fusté et al. 2007)                      |
| Ethyl-2-hydroxy-3-methylbutanoate | 2441-06-7  | –                                            | (Jagatić Korenika et al. 2020)                                               |
| Isobutyl lactate                  | 585-24-0   | –                                            | (Torrens et al. 2010; Jagatić Korenika et al. 2020)                          |
| Ethyl-3-ethoxypropanoate          | 763-69-9   | –                                            | (Bosch-Fusté et al. 2007)                                                    |
| Ethyl hexanoate                   | 123-66-0   | Fruity, green apple, banana <sup>a</sup>     | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| Hexyl acetate                     | 142-92-7   | Fruity, green, sweet <sup>a</sup>            | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| Ethyl 2-furancarboxylate          | 614-99-3   | –                                            | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| Ethyl octanoate                   | 106-32-1   | Sweet, floral, fruity, pear <sup>a</sup>     |                                                                              |
| Methyl octanoate                  | 111-11-5   | Orange <sup>b</sup>                          | (Bosch-Fusté et al. 2007)                                                    |
| Diethyl succinate                 | 123-25-1   | Overripe, aged <sup>a</sup>                  | (Jagatić Korenika et al. 2020; Bosch-Fusté et al. 2007; Torrens et al. 2010) |
| Phenyl acetate                    | 122-79-2   | –                                            | (Jagatić Korenika et al. 2020)                                               |
| Ethyl 2-hexenoate                 | 1552-67-6  | –                                            | (Bosch-Fusté et al. 2007)                                                    |
| Ethyl 3-hexenoate                 | 2396-83-0  | –                                            | (Bosch-Fusté et al. 2007)                                                    |

| Compound                    | CAS number | Odour descriptor                        | Reference                                                                    |
|-----------------------------|------------|-----------------------------------------|------------------------------------------------------------------------------|
| Methyl 3-hydroxyisovalerate | 6149-45-7  | –                                       | (Jagatić Korenika et al. 2020)                                               |
| Diethyl hydroxysuccinate    | 7554-12-3  | Brown sugar, sweet <sup>b</sup>         | (Jagatić Korenika et al. 2020)                                               |
| Isoamyl lactate             | 97-64-3    | Fruity, apple, banana <sup>a</sup>      | (Jagatić Korenika et al. 2020)                                               |
| Ethyl-hydroxyhexanoate      | –          | –                                       | (Jagatić Korenika et al. 2020)                                               |
| Ethyl-3-hydroxyhexanoate    | 2305-25-1  | Rubber <sup>a</sup>                     | (Jagatić Korenika et al. 2020)                                               |
| Methyl-3-hydroxyoctanoate   | 7367-87-5  | –                                       | (Jagatić Korenika et al. 2020)                                               |
| Diethyl glutarate           | 818-38-2   | –                                       | (Jagatić Korenika et al. 2020)                                               |
| 2-Phenylethyl acetate       | 93-92-5    | Rose, honey, tobacco <sup>a</sup>       | (Jagatić Korenika et al. 2020; Torrens et al. 2010; Bosch-Fusté et al. 2007) |
| o-Methylbenzyl acetate      | 17373-93-2 | –                                       | (Jagatić Korenika et al. 2020)                                               |
| Ethyl vanillate             | 617-05-0   | Creamy, vanilla <sup>a</sup>            | (Jagatić Korenika et al. 2020)                                               |
| Ethyl phenylacetate         | 101-97-3   | Fruit, sweet <sup>a</sup>               | (Jagatić Korenika et al. 2020)                                               |
| 1,1-Diethoxyhexane          | 3658-93-3  | –                                       | (Bosch-Fusté et al. 2007)                                                    |
| Methyl geranate             | 1189-09-9  | Wax, green, fruity, flower <sup>c</sup> | (Jagatić Korenika et al. 2020)                                               |
| Methyl decanoate            | 110-42-9   | Wine <sup>b</sup>                       | (Bosch-Fusté et al. 2007)                                                    |
| Ethyl propionate            | 123-29-5   | –                                       | (Bosch-Fusté et al. 2007)                                                    |
| Ethyl 9-decenoate           | 67233-91-4 | –                                       | (Bosch-Fusté et al. 2007)                                                    |
| Ethyl decanoate             | 110-38-3   | Floral, grape, fruity <sup>a</sup>      | (Jagatić Korenika et al. 2020; Bosch-Fusté et al. 2007; Torrens et al. 2010) |
| Isoamyl octanoate           | 2035-99-6  | –                                       | (Bosch-Fusté et al. 2007)                                                    |
| Ethyl dodecanoate           | 106-33-2   | Leaf <sup>b</sup>                       | (Bosch-Fusté et al. 2007)                                                    |
| Methyl tetradecanoate       | 124-10-7   | Orris <sup>b</sup>                      | (Bosch-Fusté et al. 2007)                                                    |
| Ethyl tetradecanoate        | 124-06-1   | Ether <sup>b</sup>                      | (Bosch-Fusté et al. 2007)                                                    |
| Methyl hexadecanoate        | 112-39-0   | –                                       | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020)                      |
| Isopropyl myristate         | 110-27-0   | –                                       | (Bosch-Fusté et al. 2007)                                                    |
| Ethyl hexadecanoate         | 628-97-7   | Wax <sup>b</sup>                        | (Jagatić Korenika et al. 2020; Bosch-Fusté et al. 2007)                      |
| Methyl octadecanoate        | 27234-05-5 | –                                       | (Jagatić Korenika et                                                         |

| Compound                 | CAS number | Odour descriptor                   | Reference                                                                    |
|--------------------------|------------|------------------------------------|------------------------------------------------------------------------------|
|                          |            |                                    | al. 2020)                                                                    |
| Ethyl linoleate          | 544-35-4   | –                                  | (Jagatić Korenika et al. 2020)                                               |
| <b>Fatty acids</b>       |            |                                    |                                                                              |
| Propanoic acid           | 79-09-4    | Rancid, oily <sup>a</sup>          | (Jagatić Korenika et al. 2020)                                               |
| Butanoic acid            | 107-92-6   | Rancid, cheese <sup>a</sup>        | (Jagatić Korenika et al. 2020)                                               |
| 2-Methylpropionic acid   | 79-31-2    | Rancid, cheesy <sup>a</sup>        | (Jagatić Korenika et al. 2020)                                               |
| Isovaleric acid          | 503-74-2   | Sweat, rancid <sup>a</sup>         | (Jagatić Korenika et al. 2020; Torrens et al. 2010)                          |
| Hexanoic acid            | 142-62-1   | Cheese, oily <sup>a</sup>          | (Jagatić Korenika et al. 2020; Torrens et al. 2010)                          |
| Hexadecanoic acid        | 57-10-3    | –                                  | (Bosch-Fusté et al. 2007)                                                    |
| Heptanoic acid           | 111-14-8   | Rancid, cheesy <sup>a</sup>        | (Jagatić Korenika et al. 2020)                                               |
| Octanoic acid            | 124-07-2   | Rancid, oily <sup>a</sup>          | (Jagatić Korenika et al. 2020; Bosch-Fusté et al. 2007; Torrens et al. 2010) |
| Decanoic acid            | 334-48-5   | Rancid, waxy <sup>a</sup>          | (Jagatić Korenika et al. 2020; Torrens et al. 2010; Bosch-Fusté et al. 2007) |
| Tetradecanoic acid       | 544-63-8   | –                                  | (Bosch-Fusté et al. 2007)                                                    |
| <b>Furans</b>            |            |                                    |                                                                              |
| Furfural                 | 98-01-1    | Burn, almond, yeast <sup>a</sup>   | (Bosch-Fusté et al. 2007; Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| 5-Methyl-furfural        | 620-02-0   | Bitter, almond, spice <sup>a</sup> | (Jagatić Korenika et al. 2020)                                               |
| 5- Hydroxymethylfurfural | 67-47-0    | Almond <sup>a</sup>                | (Jagatić Korenika et al. 2020)                                               |
| <b>Ketones</b>           |            |                                    |                                                                              |
| Methylthiophen-3-one     | 13679-85-1 | Cabbage, onion, must <sup>b</sup>  | (Bosch-Fusté et al. 2007)                                                    |
| 2-Octanone               | 111-13-7   | Soap, gasoline <sup>b</sup>        | (Bosch-Fusté et al. 2007)                                                    |
| $\alpha$ -Ionone         | 127-41-3   | Floral <sup>a</sup>                | (Jagatić Korenika et al. 2020)                                               |
| <b>Lactones</b>          |            |                                    |                                                                              |
| $\gamma$ -butyrolactone  | 96-48-0    | Coconut, caramel <sup>a</sup>      | (Jagatić Korenika et al. 2020; Torrens et al. 2010)                          |
| $\gamma$ -Hexalactone    | 823-22-3   | Sweet, cake, peach <sup>a</sup>    | (Jagatić Korenika et al. 2020)                                               |
| $\gamma$ -Octalactone    | 104-50-7   | Coconut <sup>a</sup>               | (Jagatić Korenika et                                                         |

| Compound                      | CAS number | Odour descriptor                          | Reference                                           |
|-------------------------------|------------|-------------------------------------------|-----------------------------------------------------|
|                               |            |                                           | al. 2020)                                           |
| <i>cis</i> -Whiskey lactone   | 80041-00-5 | Nutty, coconut <sup>a</sup>               | (Jagatić Korenika et al. 2020)                      |
| <i>trans</i> -Whiskey lactone | 39638-67-0 | Nutty, coconut <sup>a</sup>               | (Jagatić Korenika et al. 2020)                      |
| $\gamma$ -Nonalactone         | 104-61-0   | Coconut, peach <sup>a</sup>               | (Jagatić Korenika et al. 2020)                      |
| $\gamma$ -Decalactone         | 706-14-9   | Peach, fruity <sup>a</sup>                | (Jagatić Korenika et al. 2020)                      |
| $\gamma$ -Undecalactone       | 104-67-6   | Apricot, peach <sup>a</sup>               | (Jagatić Korenika et al. 2020)                      |
| <b>Norisoprenoids</b>         |            |                                           |                                                     |
| Vitispirane                   | 65416-59-3 | –                                         | (Bosch-Fusté et al. 2007; Torrens et al. 2010)      |
| $\beta$ -Damascenone          | 23696-85-7 | Sweet, fruity, floral, honey <sup>a</sup> | (Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| <b>Phenols</b>                |            |                                           |                                                     |
| Guaiacol                      | 90-05-1    | Smoky, hospital <sup>a</sup>              | (Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| 4-Vinylphenol                 | 2628-17-3  | Phenolic, medicinal <sup>a</sup>          | (Jagatić Korenika et al. 2020)                      |
| Vanillin                      | 121-33-5   | Vanilla <sup>a</sup>                      | (Jagatić Korenika et al. 2020)                      |
| 4-Ethylphenol                 | 123-07-9   | Phenol, stable <sup>a</sup>               | (Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| Tyrosol                       | 501-94-0   | Toasted bread, smoky, clove <sup>a</sup>  | (Jagatić Korenika et al. 2020)                      |
| 4-Vinylguaiacol               | 7786-61-0  | Clove, curry <sup>a</sup>                 | (Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| 4-Ethylguaiacol               | 2785-89-9  | Toasted bread, smoky, clove <sup>a</sup>  | (Jagatić Korenika et al. 2020; Torrens et al. 2010) |
| Homovanillyl alcohol          | 2380-78-1  | –                                         | (Jagatić Korenika et al. 2020)                      |
| Eugenol                       | 97-53-0    | Cinnamon, clove <sup>a</sup>              | (Jagatić Korenika et al. 2020)                      |
| <b>Terpenes</b>               |            |                                           |                                                     |
| Limonene                      | 138-86-3   | Lemon, orange <sup>b</sup>                | (Ubeda et al. 2016)                                 |
| $\alpha$ -Terpineol           | 10482-56-1 | Lilac, floral, sweet <sup>a</sup>         | (Jagatić Korenika et al. 2020)                      |
| <i>cis</i> -Linalool oxide    | 1365-19-1  | Flower <sup>a</sup>                       | (Jagatić Korenika et al. 2020)                      |
| <i>trans</i> -Linalool oxide  | 34995-77-2 | Flower <sup>b</sup>                       | (Ubeda et al. 2016)                                 |
| Citronellol                   | 6812-78-8  | Rose <sup>a</sup>                         | (Jagatić Korenika et al. 2020)                      |
| Geraniol                      | 106-24-1   | Citrus, citric fruit <sup>a</sup>         | (Jagatić Korenika et al. 2020)                      |

| Compound                 | CAS number | Odour descriptor                             | Reference                      |
|--------------------------|------------|----------------------------------------------|--------------------------------|
| Geranyl acetate          | 105-87-3   | Flower <sup>a</sup>                          | (Jagatić Korenika et al. 2020) |
| Hotrienol                | 20053-88-7 | Fresh, floral, fruity <sup>a</sup>           | (Jagatić Korenika et al. 2020) |
| Nerol                    | 106-25-2   | Rose, fruity, floral <sup>a</sup>            | (Jagatić Korenika et al. 2020) |
| Nerolidol                | 40716-66-3 | Rose, apple, green, waxy, woody <sup>a</sup> | (Jagatić Korenika et al. 2020) |
| <i>trans</i> -Rose oxide | 876-18-6   | Floral, green <sup>a</sup>                   | (Jagatić Korenika et al. 2020) |

<sup>a</sup> Extracted from (Jagatić Korenika et al. 2020)

<sup>b</sup> Extracted from (Flavornet)

<sup>c</sup> Extracted from (The Good Scents Company)

Campo et al. 2008 described the aromatic profile of Cava sparkling wines between young dry wines and the rest of wines with higher amounts of some volatile compounds, such as, acetaldehyde, methional, phenylacetaldehyde, 3-methylbutanal,  $\beta$ -damascenone and sotolon (Campo, Cacho, and Ferreira 2008).

Welke et al. 2014 reported that the major groups of volatiles identified in base and sparkling wines were esters, alcohols, and other families, such as aldehydes, C-13 norisoprenoids, ketones, among others (Welke et al. 2014). The results showed that among the 119 volatile compounds identified, C-13 norisoprenoids, such as, TDN, vitispirane and  $\beta$ -damascenone; some esters, namely ethyl laurate, ethyl 2-hydroxybutanoate, ethyl decanoate, ethyl 2-hydroxypropanoate, ethyl pentanoate; alcohols, 4-butoxy butanol, 1-propanol and methionol; aldehydes, 3-phenyl-2-propenal, nonanal and undecanal; acids, acetic acid, 2-ethylhexanoic acid, butanoic acid; ketones, acetoin and 2,3-butanedione and phenols, 4-vinylguaiacol, 4-ethylphenol were the main responsible to differentiate base wines from sparkling wines (Welke et al. 2014).

## 1.6. Context of the research topics in sparkling wines

Over the last two decades, the global wine market has experienced an increase in the demand for sparkling wines. In 2017, the production of sparkling wine achieved its peak, corresponding to 8% of the total wine production (OIV 2020).

Despite the economic importance of sparkling wine, the scientific data existent is still limited in comparison with other alcoholic beverages (Ruiz-Moreno et al. 2017). Most of the studies performed in sparkling wine have been focused on factors that affect the quality, i.e., the foam characteristics, colour, acidity and aroma. Among these, aroma is thought to be one of the most important markers of the quality of a sparkling wine (Ubeda et al. 2019).

Volatile composition of sparkling wine is correlated with grape variety, the maturity of the grape, the production method (traditional or charmat), first and second fermentation,

choice of yeast, and time of ageing on lees (Ubeda et al. 2019). Among the points mentioned, second fermentation and ageing in contact with lees have the most impact on the volatile composition of sparkling wines (Ubeda et al. 2019).

First, the viticultural process impacts some important properties of sparkling wine, namely, flavour, aroma and foaming. Therefore, studies have focused principally on the grape variety and their level of maturation (Kemp et al. 2015). The choice of grape variety is made according to environmental factors like climate, foaming capacity of the juice, base wine, ageing, among others. It has been reported that the grape variety affects the aroma of sparkling wine, for example, sparkling wines produced with Fernão-Pires grapes showed a superior aroma potential in comparison to sparkling wines produced with Baga grapes (Coelho et al. 2009). Additionally, some researchers were dedicated to the use of alternatives to the traditional grapes varieties to produce sparkling wines. Some examples are reported by Martínez et al. 2013 that studied Albarino, Verdejo and Godello grape varieties (Martínez-Lapuente et al. 2013). Coelho et al. 2011 studied Baga and Fernão-Pires varieties (Coelho et al. 2011) and Song et al. 2013 researched the use of Mieli grape variety to produce Spanish, Portuguese and Chinese sparkling wines, respectively. Regarding grape maturity, Coelho et al. 2009 studied the impact of different ripening stages and soil composition on the volatiles of sparkling wines (Coelho et al. 2009). In addition, Esteruelas et al. 2015 aimed to study the impact of grape maturity in the foaming properties of Cava sparkling wine (Esteruelas et al. 2015).

Second, one of the key steps involved in the production of sparkling wine is the first and second fermentation. During alcoholic fermentation, sugars are converted into ethanol and CO<sub>2</sub> by yeasts (Kemp et al. 2015). It is also at this stage that a great number of flavor compounds are produced. The formation of these compounds is dependent on yeast species as well as on the yeast strain (Torrens et al. 2008). Regarding the first fermentation, Torrens et al. 2008 studied the volatile profile of Macabeo/Viura, Xarel·lo, and Parellada base wines obtained with six commercial yeast strains. The results unveiled that yeast strain impacts the chemical and volatile composition of those base wines. The differences between yeast strains were attributed mainly to some esters, glycerol, phenylethanol, terpenes, fatty acids and alcohols (Torrens et al. 2008). Concerning the second fermentation, research is more recently focusing on the effect in sparkling wine aroma of adopting non-conventional wild yeasts (Kemp et al. 2015)

Third, when secondary fermentation is completed, the period of ageing in contact with lees begins. At this point, yeast associated compounds and various molecules are released. The type and number of compounds released is dependent on yeast strain. Several authors, such as Hidalgo et al. 2004, Buxaderas et al. 2012, Riu-Aumatell et al. 2006, and Martínez-Garcia et al. 2021, had been studying changes in the volatile profile of

sparkling during the period of ageing in contact with lees (Hidalgo et al. 2004; Buxaderas and López-Tamames 2012; Riu-Aumatell et al. 2006; Martínez-García, Moreno, et al. 2021). Furthermore, lees are also capable of adsorbing volatile compounds during this period, so Gallardo-Chacon et al. 2010, investigated how the degree of sorption of volatile compounds varies during ageing and which compounds are mostly retained (Gallardo-Chacon et al. 2010). Additionally, other works by Martínez-Rodríguez et al. 2002 and Perpetuini et al. 2016 have been performed with the aim of evaluating the autolytic capacity of yeasts. The yeast autolysis generally only starts from 2 to 4 months after the secondary fermentation is finished so, the selection of yeast strains with a fast autolytic capacity could bring economic advantages since it could decrease the time of production (Di Gianvito et al. 2018).

Lastly, the flocculation ability of yeast strains is also a target of study. Flocculation is the inherent aptitude of yeast strains to form clumps that fall to the bottom and consequently facilitate the elimination of sediments at disgorging (Di Gianvito et al. 2018). A recent study by Di Gianvito et al. 2018 evaluated the flocculation degree of 6 flocculent *S. cerevisiae* strains concluding that selected yeast strains and commercial strains had a similar behaviour (Di Gianvito et al. 2018).

According to the data found at this point research is mainly focused on some stages of sparkling wine production, such as, the viticultural process, first and second fermentation, ageing in contact with lees and how they affect sparkling aroma quality.

### **1.7. Impact of ageing in the volatile composition of sparkling wines**

The ageing time defines the type and number of volatile compounds found in sparkling wines (Pérez-Magariño et al. 2015). However, most of the studies are focused on the evolution of the volatile profile of sparkling wines during the ageing period in contact with lees, only one study was found regarding the evaluation of the volatile composition of sparkling wines in bottle after disgorging.

Pérez-Magariño et al. 2015 evaluated the volatile composition of eight *rosé* and white sparkling wines after 12 months of disgorging (without lees) (Pérez-Magariño et al. 2015). Volatile compounds were analysed by LLE-GC/MS. Sparkling wine samples that underwent 12 months of ageing in addition to the 9 and 18 months in contact with lees demonstrated a reduction in the levels of ethyl esters of straight-chain fatty acids (ethyl hexanoate, ethyl octanoate, isoamyl acetate, and 2-phenylethyl acetate), alcohols (1-hexanol, benzyl alcohol, and *trans*-3-hexen-1-ol), fatty acids (hexanoic acid, octanoic acid, and decanoic acid), and some terpenes (linalool and citronellol). In addition, ethyl esters of

branched-chain fatty acids, such as ethyl 2-methylbutyrate and ethyl isovalerate, and 1 phenol namely vanillin increased with ageing time (Pérez-Magariño et al. 2015). These results agreed with data already reported for the evolution of the volatile profile of still wines in bottle during ageing (Pérez-Coello et al. 2003; Díaz-Maroto, Schneider, and Baumes 2005). Ethyl esters of straight-chain fatty acids characterized by floral and fruity odours were present in higher concentration in young wines. Contrarily, branched-chain fatty acid ethyl esters tended to increase with ageing, being responsible for unpleasant aromas (Díaz-Maroto, Schneider, and Baumes 2005). Furthermore, the results also showed that the varietal characteristics of each sparkling wine were maintained during the ageing on lees and afterwards (Pérez-Magariño et al. 2015).

## **1.8. Impact of stoppers in the volatile composition of wine**

The evolution of wine after bottling is closely related to its initial composition as well as the storage conditions, namely light, humidity, and temperature. Moreover, the choice of package is also a determining factor since the type of stopper can modulate the organoleptic characteristics of wine over time (Oliveira et al. 2020). Nowadays, there are several stoppers available in the market, so a large part of the studies carried out in this field focus on comprehending the effect of different closures on chemical and sensory properties of wine during ageing in bottle (Silva et al. 2011). Some of the factors evaluated in these studies are related to the desorption of compounds from the stopper to wine, scalping (adsorption) and oxidation. A great number of studies related to the effect of different closures on the volatile composition of still wines can be found, but there is still a gap concerning sparkling wines. Table 3 lists several studies performed in white wines (Cabernet, Semillon and Chardonnay) and wine model solution. The data collected has a publication date ranging from 2003 to 2020. Regarding to the type of closure, natural cork stoppers and cork-based stoppers, such as 1+1 technical cork, microagglomerated cork and colmated cork stoppers were the main closures evaluated. Besides that, granulates of cork were part of two studies. Lastly, the studies were performed in a wide range of the ageing period in bottle (from 5 days to 14 years).

Capone et al. 2003 set a trial with the aim of studying the adsorptive capacity of various closures, roll-on tamper evident (ROTE), natural cork stoppers, technical cork stoppers and synthetic stoppers in Semillon white wines for a period of 2 years (Capone et al. 2003). Wine was spiked with known concentrations of esters, monoterpenes, naphthalene and TDN. Firstly, of the 6 esters studied (ethyl isobutyrate, ethyl butyrate, ethyl isovalerate, ethyl hexanoate, ethyl octanoate and ethyl decanoate) only ethyl hexanoate, ethyl octanoate and ethyl decanoate were affected by the different closures. Ethyl

hexanoate was not adsorbed by either natural or technical closures. Ethyl octanoate and ethyl decanoate were adsorbed in 5 and 20% by natural cork stoppers and 10 and 35% by technical cork stoppers, respectively. The group of monoterpenes was not affected by natural or technical cork stoppers. Thirdly, naphthalene showed a significant decrease in its levels, it was the compound most affected by the type of closure. In wines sealed with natural and technical cork stoppers its concentration decreased 50% and 70%, respectively. This work showed that natural cork stoppers scalped the least. Technical cork stoppers had a similar behaviour to natural cork stoppers just with a higher capacity to adsorb compounds. Overall, natural cork stoppers demonstrated to preserve better wine volatile composition (Capone et al. 2003).

Table 3. Studies focused on the impact of different cork closures on the volatile composition of white wine and wine model solution.

| Wine<br>(time of<br>analysis)                | Types and number<br>of samples                                                                              | Analyses                 | Compounds<br>measured                                                                                       | Main Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Reference                           |
|----------------------------------------------|-------------------------------------------------------------------------------------------------------------|--------------------------|-------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| <i>Semillon</i><br>(2 years)                 | ROTE screw cap,<br>Natural cork (grade 2<br>and 3), technical<br>cork, seven synthetic<br>closures<br>(n=5) | GC-MS                    | 6 Esters, 4<br>monoterpenes,<br>naphthalene and<br>1,1,6-trimethyl-<br>1,2-<br>dihydronaphthale<br>ne (TDN) | <p>The capacity of adsorbing volatile compounds of the closures was natural cork stopper &lt; technical stopper &lt; synthetic stopper</p> <p>Ethyl hexanoate, ethyl octanoate, ethyl decanoate and TDN were affected by the closure: ↓ their concentration in wine</p> <p><b>Natural cork stoppers vs. technical cork stoppers vs. synthetic stoppers:</b><br/>           ↓ ethyl hexanoate of 5-15% only in synthetic stoppers<br/>           ↓ ethyl octanoate of 5% vs. 10 % vs. 30-50%<br/>           ↓ ethyl decanoate of 20 % vs. 35 % vs. 50-70%<br/>           ↓ TDN of 50% vs. 70% vs. 96-98% (was the compound most adsorbed)</p> | (Capone et al. 2003)                |
| <i>Wine model solution</i><br>(10 days)      | Five synthetic stoppers (S1, S2, S3, S4, S5) and three natural cork stoppers                                | PT-GC-O<br>GC-O<br>GC-MS | Esters, phenolics, terpenes, <i>m</i> -cresol and 1-hepten-3-one                                            | <p><b>Macerates of natural cork stoppers:</b> No negative aromas were detected, and various pleasant descriptors were reported: sweet/fruity, toasted, sweet wood and floral/muscat attributed to esters, terpenes, and others compound present in cork</p> <p>Discriminant compounds in <b>natural cork</b> vs. <b>synthetic stoppers</b>:<br/>           ↑ ethyl isobutyrate, octanal, borneol, and guaiacol<br/>           ↓ 1-hepten-3-one and an unknown compound</p>                                                                                                                                                                   | (Culleré, Cacho, and Ferreira 2009) |
| <i>Bordeaux Sauvignon Blanc</i><br>(2 years) | Screw cap (Saran-tin and Saranex), natural cork, colmated, technical cork, agglomerated,                    | GC-MS                    | 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH),                                         | <p><b>Colmated cork stoppers vs. other closures:</b> ↑ concentration of 4MMP</p> <p><b>Screw cap Saran-tin and agglomerated cork stoppers vs. other closures:</b> ↑ concentration of 3 MH</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                | (Lopes et al. 2009)                 |

| Wine<br>(time of<br>analysis)                      | Types and number<br>of samples                                                                                                            | Analyses                  | Compounds<br>measured                                                                                      | Main Results                                                                                                                                                                                                                                                                                                                                                                                       | Reference                |
|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
|                                                    | microagglomerated<br>and synthetic<br>(n=10)                                                                                              |                           | hydrogen sulfide<br>(H <sub>2</sub> S) and sotolon                                                         | <p><b>Microagglomerated cork stoppers and synthetic stopper</b><br/>vs. <b>other closures</b>: ↑ concentration of Sotolon</p> <p><b>Screw-cap</b> vs. <b>other closures</b>: ↑ concentration of H<sub>2</sub>S</p> <p>↓ concentration of thiols due to oxidative reactions</p> <p><b>Sensory Analysis:</b><br/>Colmated and natural cork stoppers vs. other<br/>stoppers: ↑ fruity characters.</p> |                          |
| <i>Chardonnay</i><br>(4 years)                     | Three synthetic<br>closures (Nomacorc<br>Light, Nomacorc<br>classic and<br>Nomacorc Premium),<br>natural cork and<br>technical cork (n=3) | HS-<br>SPME/G<br>C-MS     | 31 volatile<br>compounds: 5<br>aldehydes and<br>phenol, 11<br>alcohols, 11 esters<br>and 4 fatty acids     | <p><b>Natural cork stoppers</b> vs. <b>synthetic stoppers</b>:<br/>↑ 1-pentanol and ethyl decanoate<br/>↓ acetoin, 1-butanol, 2-phenylethanol, (Z)-3-hexen-1-ol and<br/>2-nonanol</p> <p><b>Sensory analysis:</b><br/>Cork stoppers vs. synthetic stoppers: ↑ fruity and flowery<br/>descriptors.</p>                                                                                              | (Liu et al.<br>2015)     |
| <i>Semillon</i><br><i>white wine</i><br>(14 years) | Screw cap (ROTE),<br>two natural cork<br>stoppers (1 and 2)<br>and two synthetic<br>stoppers (1 and 2)<br>(n= 4)                          | GC-<br>MS/MS              | 18 compounds: 4<br>(E)-alkenals, 4<br>strecker<br>aldehydes, 8<br>aldehydes, 3<br>furans and 3<br>alcohols | <p>↑ 2-phenylacetaldehyde, 3-methylbutanal, and methional for<br/>wines sealed with synthetic stoppers &gt; natural cork stoppers<br/>&gt; screw caps</p> <p>↓ (E)-2-hexenal, (E)-2-nonenal and furans in control wines &lt;<br/>cork closures &lt; synthetic closures</p> <p>Sotolon was not found in wines sealed with cork closures</p>                                                         | (Mayr et al.<br>2015)    |
| <i>Vintage</i><br><i>White wines</i>               | Natural cork,<br>neutrocork                                                                                                               | HS-SPME<br>/ GC-<br>IT/MS | Esters, terpenes,<br>norisoprenoids<br>and alcohol                                                         | <p><b>Natural cork stoppers</b> vs. <b>Neutrocork stoppers</b>:<br/>↑ 2-phenylethanol, 2-phenylethyl acetate, ethyl octanoate,<br/>α-terpineol and β-damascenone</p>                                                                                                                                                                                                                               | (Moreira et<br>al. 2018) |

| Wine<br>(time of<br>analysis)                            | Types and number<br>of samples                                                                                                                                      | Analyses              | Compounds<br>measured                                                                                                                                  | Main Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Reference              |
|----------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|
| <i>from the<br/>Borba region<br/>(12 months)</i>         | (microagglomerated)<br>and BIB<br>(n=15)                                                                                                                            |                       |                                                                                                                                                        | <b>Sensorial analysis:</b><br>- Bottled wines vs BIB presented ↑ scores for aroma intensity, freshness, and fruit attributes                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                        |
| <i>Chardonnay<br/>dry white<br/>wine<br/>(18 months)</i> | Three natural cork stoppers (N1, N2 and N3), one 1+1 technical cork (TC), one agglomerated cork (AC), one polymer synthetic plug (SP) and one screw cap (SC) (n=30) | HS-<br>SPME/G<br>C-MS | 72 volatile compounds: 15 alcohols, 32 esters, 5 aliphatic acids, 7 terpenes, 3 norisoprenoids and 10 other volatile compounds                         | <b>Natural cork vs. agglomerated cork stoppers and 1+1 technical cork stoppers:</b><br>↓ α-ionone and TDN<br><br><b>Natural cork stoppers vs. screw cap:</b><br>↓ 2-ethylhexanol, 1-decanol, 2-hydroxy-5-methylacetophenone, β-farsene, α-ionone and styrene                                                                                                                                                                                                                                                                                                                                                                                              | (Ling et al. 2019)     |
| <i>Vintage white<br/>wine of 2013<br/>(48 months)</i>    | Two different 1+1 cork (A and B), microagglomerated cork (A and B) and co-extruded synthetic closures (A and B) (n= 3)                                              | HS-<br>SPME-<br>GC/MS | 69 volatile compounds: 10 alcohols, 8 aldehydes, 32 esters, 4 organic acids, 2 ketones, 3 lactones, 3 norisoprenoids, 6 terpenes, 1 phenol derivative. | <b>1+1 cork stoppers vs. synthetic stoppers:</b><br>↑ ethyl hexanoate, octanoate and decanoate, 2-phenylethyl acetate, an ionone like compound, and TDN<br><i>L</i> -camphor was only identified in wine sealed with 1+1 cork stoppers<br><br><b>Microagglomerated cork A stoppers vs. Synthetic stoppers:</b><br>↑ ionone like compound and TDN<br><i>trans</i> -4- <i>tert</i> -butylcyclohexanol was only detected in wines sealed with microagglomerated cork A stoppers<br><br><b>Sensory analysis:</b><br>- <b>1+1 cork stoppers and microagglomerated cork stoppers vs. synthetic stoppers:</b> ↑ aroma intensity, aroma balance and aroma quality | (Oliveira et al. 2020) |

Lopes et al. 2009 studied the effect of oxygen intake through different closures on the content of 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and sotolon in a Sauvignon Blanc wine after 24 months of storage (Lopes et al. 2009). Among cork closures, 4MMP and 3MH were found in greater amounts in wines sealed with colmated cork stoppers and agglomerated cork stoppers, respectively. These volatile thiols are responsible for grapefruit and passion fruit aromas. In the sensory analysis, samples with higher concentrations of these compounds, such as wines sealed with colmated and natural cork stoppers presented the highest scores for the fruit character. Sotolon was found in higher amounts in wines sealed with microagglomerated cork closures. In conclusion, volatile thiols suffer degradation by oxygen since wines that were less exposed to it, such as wines sealed with colmated and natural cork stoppers, had the highest concentrations (Lopes et al. 2009).

Liu et al. 2015 focus on the effect of distinct closures, namely natural and technical cork stoppers and synthetic stoppers, on the volatile composition and sensory properties of Chardonnay white wine after 4 years of bottling (Liu et al. 2015). Overall, 31 volatile compounds (5 aldehydes, 1 phenol, 11 alcohols, 11 esters, and 4 fatty acids) were quantified by HS-SPME/GC-MS. Statistically significant differences were detected for the concentration of 7 compounds between all closures in the study, such as acetoin, 1-butanol, 2-phenylethanol, 1-pentanol, (*Z*)-3-hexen-1-ol, 2-nonanol, and ethyl decanoate. Premium synthetic stoppers demonstrated high levels of acetoin, 1-butanol and 2-nonanol in wines, while 2-phenylethanol and (*Z*)-3-hexen-1-ol were found in higher amounts in wines sealed with classic synthetic stoppers. Wines under natural cork stoppers presented high levels of 1-pentanol and ethyl decanoate and the lowest concentration of hexanal, furfural, and benzaldehyde. In the sensorial analysis, cork closures were characterized in general by fruity and flowery descriptors (Liu et al. 2015). Ethyl decanoate is hydrolysed when wines have a higher level of oxidation, therefore it was found in greater amounts in wines with a lower content of oxygen (wines sealed with cork closures). Likewise, wines bottled sealed with cork stoppers preserved better the esters content.

Mayr et al. 2015 studied 18 compounds characterized as oxidative off-flavours in Semillon wines under different closures (screw cap, 2 natural corks and 2 synthetic closures) after 14 years (Mayr et al. 2015). Among Strecker aldehydes, 3-methylbutanal, methional and 2-phenylacetaldehyde had the highest concentration in wines sealed with synthetic closures followed by natural cork sealed wines. Among the four (*E*)-2-alkenals quantified, only (*E*)-2-hexenal and (*E*)-2-nonenal showed significant differences. The concentration of (*E*)-2-hexenal was 1.5 times higher in wines sealed with natural cork stoppers than in control wines and (*E*)-2-nonenal was found in highest concentration in

wines sealed with natural cork stoppers. In the sensory analysis, wines sealed with natural cork stoppers presented intermediate scores, which corroborates the results obtained in the volatile quantification where wines sealed with natural cork stoppers demonstrated median values for almost all volatile compounds linked with oxidized aromas (Mayr et al. 2015).

Moreira et al. 2018 carried out a study to assess the effect on the volatile composition and sensorial properties of a white wine under different packaging configurations (BIB, glass bottles sealed with natural cork stoppers and Neutrocork) after 12 months of aging (Moreira et al. 2018). Volatile compounds, esters (ethyl butanoate, ethyl hexanoate, ethyl octanoate, 2-phenylethyl acetate, hexyl acetate), terpenes (linalool,  $\alpha$ -terpineol), norisoprenoids ( $\beta$ -damascenone) and 2-phenylethanol were quantified by HS-SPME/GC-IT/MS. Comparing both wines in bottle, at the end of the study wines sealed with natural cork stoppers showed higher levels of 2-phenylethanol, 2-phenylethyl acetate, ethyl octanoate,  $\alpha$ -terpineol and  $\beta$ -damascenone (Moreira et al. 2018).

Ling et al. 2019 compared the effects of natural, technical, and agglomerated cork stoppers, polymer synthetic plug and screw cap on the volatile profile of white wine after 18 months of bottling (Ling et al. 2019). Overall, 72 volatile compounds were quantified by HS-SPME/GC-MS, namely 15 alcohols, 32 esters, 5 aliphatic acids, 7 terpenes, 3 norisoprenoids and 10 other volatile compounds. The results showed that  $\alpha$ -ionone and TDN presented lower levels in wines sealed with natural cork stoppers related with the capacity of cork to scalp TDN (Ling et al. 2019).

Recently, Oliveira et al. 2020 analysed the effect of different closures (1+1 cork, microagglomerated cork, and synthetic closures) on the volatile profile of white wine at 48 months post-bottling (Oliveira et al. 2020). It was identified 69 volatile compounds, including 10 alcohols, 8 aldehydes, 32 esters, 4 organic acids, 2 ketones, 3 lactones, 3 norisoprenoids, 1 phenol derivative, and 6 terpenes. Wines under 1+1 cork stoppers demonstrated high values of ethyl hexanoate, ethyl octanoate, and ethyl decanoate, 2-phenylethyl acetate, an ionone like compound, and TDN. Camphor was only identified in wines sealed with 1+1 cork stoppers. Additionally, wines under microagglomerated cork showed great levels of an ionone like compound and TDN. Furthermore, wine sealed with cork stoppers showed a slower development towards oxidation. According to the sensory analysis, wines bottled with cork stoppers were evaluated with the best aroma intensity, quality and balance (Oliveira et al. 2020).

From the studies mentioned it is possible to conclude that cork stoppers can interact with wine, they are able of transmitting or adsorbing compounds, besides, different stoppers can be more or less permeable to oxygen. All these different

behaviours of cork stoppers lead to changes in the volatile composition of wine and consequently affecting its aroma.

## **1.9. Aims of this thesis**

Aromatic compounds play an essential role in the quality of sparkling wines and consequently in their organoleptic properties. In the ageing phase chemical reactions will occur that develop the tertiary aromas, thus the duration of aging as well as packaging, more specifically the type of closure has a major impact on the volatile composition.

Although the range of options available in the market for sealing sparkling wines is less than for table wines, cork remains the primary choice for closure. So far, several studies have been conducted in order to understand the effect of different types of closures on the volatile fraction of wines, whether they are white, red or *rosé*, however the same is not true for sparkling wines. Besides that, there is also a lack of information about alterations in the volatile composition of sparkling wines after disgorging.

Taking this into consideration, this thesis has the following main objectives:

- To describe the volatile profile of sparkling wines with different ageing times.
- To determine the impact of different closures on the organoleptic properties of sparkling and white wines.

In order to achieve these objectives, this work was divided into the following tasks:

- To apply HS-SPME/GC-MS to study the VOC and VCC profiles of sparkling wines with different types of closures and at several ageing periods;
- To quantify the volatile compounds for which standards were commercially available;
- To perform multivariate and univariate statistical analyses to better recognise the compounds changing over time or in the presence of different closures.

## Chapter 2. Materials and methods

### 2.1. Chemicals

The following chemicals 2-heptanone (99%),  $\alpha$ -pinene (98%), octanal (99%), 1,4-cineole (98.5%), limonene (97%), eucalyptol (99%), camphor (95%), 2-undecanone (98%),  $\alpha$ -ionone (90%), ethyl isobutyrate (99%), ethyl 2-methylbutanoate (99%), 3-hexen-1-ol (98%), benzaldehyde (99.5%), hexyl acetate (99%), linalool oxide (97%),  $\beta$ -linalool (97%), decanal (98%), phenylethyl acetate (99%), 1-decanol (98%), ethyl decanoate (98%), ethyl butanoate (98%), ethyl isovalerate (98%), isoamyl acetate (95%), ethyl hexanoate (98%), phenylacetaldehyde (90%), 1-octanol (99%), ethyl heptanoate (99%), 5-methyl-2-furfural (99%), vinyl decanoate (99%), 1-hexanol (99.5%), furfural (99%), isoamyl acetate (97%), ethyl hexanoate (99%), diethyl succinate (99%), isoamyl alcohol (98%), phenylethyl alcohol (99%), 3-methyl-butanone, 2-pentanone (99.5%), 3-methyl-1-butanol, methional, propanal, 2-methyl-1-butanol (90%), 3-penten-2-one (70%) and PFBHA (99%) were purchased from Sigma-Aldrich (Madrid, Spain). Ethanol (98.8%) and hydrochloric acid (37%) were supplied by Fischer Scientific (U.S.A) and Merck (Germany), respectively. Ultrapure water was obtained from a Milli-Q Millipore purification system (Millipore, Billerica, Massachusetts, USA).

### 2.2. Sparkling and white wine samples

Sparkling wine and white wine samples were gathered at *Comissão de Viticultura da Região dos Vinhos Verdes*, Porto and they were kindly provided by Amorim Cork. All bottles of sparkling and white wines were open at that moment. Then, 5 ml of each sample was collected to a vial for analysis.

For the study of ageing Italian sparkling wines, A and B, of the same batch were collected at three timepoints, namely 12, 24 and 36 months after bottling. Regarding the Spanish sparkling wine, it was collected at 24 and 80 months after bottling, however this sparkling wine was in the period of ageing in contact with lees since 06/03/2014, the *dégorgement* was performed at the time of the sampling in order to clear the wine. Italian sparkling wine A is a Classic vintage of 2011 from the Piedmont region while Italian sparkling wine B is a Reserve Brut Vintage of 2005. Both Italian sparkling wines were produced recurring to the traditional method and the *dégorgement* was made in 2017. In addition, the variety of grapes used in Italian sparkling wines were Chardonnay and Pinot noir, whereas in Spanish sparkling wine were Xarel.lo, Macabeu and Parellada.

Regarding the study of the impact of closures, it was gathered samples of (i) a

French sparkling wine (Melon de Bourgogne), 18 months post-bottling, sealed with two different stoppers: 1) micro CSP, which consists of an agglomerated body of cork; and 2) STO, which is a technical stopper of an agglomerated body of cork with a disc of natural cork in one extremity; (ii) a Spanish sparkling wine (Xarel.lo, Macabeu and Parellada), 24 and 80 months post-bottling, sealed with 2D cork stoppers and crown cap; and (iii) an Italian white wine, 44 months post-bottling, under three different types of cork closures, namely A, B and C that differed in the density of cork and presence or absence of wax. Cork stoppers of type A presented a density of 150-170 kg/m<sup>3</sup> with wax, while B cork stoppers presented a density of 180-200 kg/m<sup>3</sup>, and C cork stoppers had a density of 150-170 kg/m<sup>3</sup>.

Table 4 presents a brief description of the wine samples used in both studies.

Table 4. Summary of the studies of ageing and impact of closures.

| Study              | Wine description         | Time points post-bottling | Groups in study (number of samples) |
|--------------------|--------------------------|---------------------------|-------------------------------------|
| Ageing             | Italian sparkling wine A | 12, 24 and 36 months      | 12 months (n=6)                     |
|                    |                          |                           | 24 months (n=8)                     |
|                    |                          |                           | 36 months (n=6)                     |
|                    | Italian sparkling wine B | 12, 24 and 36 months      | 12 months (n=10)                    |
|                    |                          |                           | 24 months (n=8)                     |
|                    |                          |                           | 36 months (n=6)                     |
| Impact of closures | Spanish sparkling wine   | 24 and 80 months          | 24 months (n=10)                    |
|                    |                          |                           | 80 months (n=10)                    |
|                    | Spanish sparkling wine   | 24 and 80 months          | 2D cork (n=5)                       |
|                    |                          |                           | Crown cap (n=5)                     |
|                    | French sparkling wine    | 18 months                 | Micro CSP (n=3)                     |
|                    |                          |                           | STO (n=3)                           |
|                    | Italian white wine       | 44 months                 | A (n=8)                             |
|                    |                          |                           | B (n=8)                             |
|                    |                          |                           | C (n=8)                             |

### 2.3. Analysis of volatile compounds by HS-SPME/GC-MS

All samples studied in this work were analysed by the volatile organic compounds (VOCs) methodology. However, only samples of Italian white wine were also analysed with the volatile carbonyl compounds (VCCs) technique.

For analysis of VOCs, 250 µL of sparkling or white wine were placed in a vial of 20 mL and analysed by HS-SPME-GC/MS (method adapted from (Barros et al. 2012)).

Volatile compounds were extracted by a 50/30  $\mu\text{m}$  divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco Inc., Bellefonte, Pennsylvania, USA), using a Combi-PAL autosampler (Varian Pal Autosampler, Switzerland). First, all wine samples were incubated during 5 min at 45 °C, then extracted for 30 min at 45 °C, with a stirring speed of 250 rpm. Afterwards, a thermal desorption of analytes into a GC injector was performed throughout 6 min at 250 °C. All samples were randomly injected.

The GC analysis was performed in a Scion-436 gas chromatograph coupled to a Bruker single quadrupole (SQ) equipped with a SCION SQ ion trap mass detector and a Bruker Daltonics MS workstation version 8.2.1. A fused silica (Rxi-5Sil MS) capillary column (30 m  $\times$  0.25 mm internal diameter  $\times$  0.25 mm; Restek Corporation, U.S., Bellefonte, Pennsylvania) was used in GC system. The carrier gas was high purity helium (Gasin, Porto, Portugal) at a flow rate of 1 mL/min. The oven temperature was fixed at 40 °C for 1 min, followed by an increase from 40 °C to 250 °C (5 °C/min) and from 250 to 300 °C (0 min). The transfer line temperature was 250 °C, the manifold temperature was 41 °C and the energy level of electron ionization was 70 eV. Data acquisition was performed in full scan mode with a mass range of 40-400  $m/z$  and a scan time of 500 ms.

For the analysis of VCCs, 250  $\mu\text{L}$  of wine was placed in a vial of 20 mL with 11.3  $\mu\text{L}$  of the derivatizing agent PFBHA (method adapted from (Moreira et al. 2019)). The extraction VCCs was made by HS-SPME with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco Inc., Bellefonte, Pennsylvania), using a Bruker CTC PALxt autosampler (Bruker Daltonics). Primarily all samples were incubated at 38 °C for 10 min, then extracted during 30 min maintaining the same temperature (38 °C), under a stirring speed of 250 rpm, followed by desorption into the GC injector throughout 6 min at 250 °C. All samples were randomly injected.

The GC analysis was performed in a 436-GC model (Bruker Daltonics) coupled to an EVOQ triple quadrupole (TQ) mass spectrometer (Bruker Daltonics). The GC system was equipped with a fused silica capillary column (30 m  $\times$  0.25 mm internal diameter  $\times$  0.25  $\mu\text{m}$ ; from Restek Corporation, U.S., Belfonte, Pennsylvania) and helium C-60 with high purity (Gasin, Portugal) at a flow rate of 1.0 mL/min as carrier gas. The oven temperature was held at 40 °C for 1 min, then increase to 250 °C (5.0 °C/min) and from 250 to 300 °C (0 min). The transfer line temperature was 260 °C, the manifold temperature was 41 °C and the energy level of electron ionization was 70 eV. Data acquisition was performed in full scan mode with a mass range of 35-600  $m/z$  with a scan time of 250 ms.

The identification of volatile compounds was accomplished by matching the

retention indices (RI), obtained using a commercial hydrocarbon mixture (C6-C20) and the mass spectra fragmentation pattern of every compound with the RI and the mass spectra found in the National Institute of Standards and Technology (NIST) (NIST v2.2, 2014) database spectral library. The tolerance among the experimental and theoretical RI obtained was  $\pm 20$ . When commercially available, the identification was confirmed by the analysis of standard compounds injected in the same conditions.

For the quantification of volatile compounds, commercial standards were dissolved in a wine model solution constituted of 12% ethanol and 5 g/L of tartaric acid at pH 3.2 and analysed under the same conditions by HS-SPME/GC-MS. Thus, calibrations curves were achieved by injecting a range of known concentrations of each compound and computed by the respective area of the peak versus concentration.

## 2.4. Statistical analysis

The absolute quantification of volatile compounds was uploaded to MetaboAnalyst version 5.0 (MetaboAnalyst) for multivariate analysis, such as principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA). The robustness of the model was evaluated by visual separation of groups in the first and second components and through the parameters obtained in cross validation, namely the sum of squares captured by the model ( $R^2$ ) and an estimative of the predictive capacity of the model ( $Q^2$ ). Higher  $R^2$  and  $Q^2$  values (near 1) are associated with a better discrimination between two classes.

Compounds with a Variable Importance to the Projection (VIP) higher than 1 in PLS-DA models were considered important for the separation between the sample groups. These compounds were then subjected to a univariate analysis in GraphPad Prism version 9. To ascertain if the data follows a normal distribution, the Shapiro-Wilk test was performed, then unpaired Student t-test was used for groups with a normal distribution, and the Mann-Whitney test was applied in the groups that followed a non-normal distribution. Parameters like percentage (%) of variation and uncertainty were calculated by employing mathematical formulas (Equation 1 and 2) where 1 and 2 corresponds to group 1 and group 2, respectively,  $n$  represents the number of samples,  $S$  denotes the standard deviation and  $\bar{x}$  is the mean.

Equation 1                      
$$\% \text{ Variation} = \frac{\bar{x}_1 - \bar{x}_2}{\bar{x}_2} \times 100$$

Equation 2

$$\text{Uncertainty} = \frac{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}{\frac{(n_1 \times \bar{x}_1 + n_2 \times \bar{x}_2)}{n_1 + n_2}}$$

## Chapter 3. Results and Discussion on the evolution of volatile compounds in sparkling wines during bottle ageing

This chapter presents the volatile compounds identified in sparkling wines by HS-SPME-GC-MS (subchapter 3.1), as well as the results obtained on the evolution of the volatile composition of sparkling wines during bottle ageing (subchapter 3.2).

### 3.1. Identification of volatile and semi volatile compounds in sparkling wines

A representative chromatogram of the volatile profile of the Italian sparkling wine A at 36 months post-bottling is shown in Figure 6. Table 5 lists all volatile compounds identified in Italian sparkling wines A and B, and one Spanish sparkling wine over time. Overall, 94 compounds were identified, including 1 anisole, 19 alcohols, 10 aldehydes, 36 esters, 5 fatty acids, 2 furans, 9 ketones, 1 naphthalene derivative, 6 terpenes, 1 lactone, 2 phenols, 1 organic acid and 1 other compound. Esters and alcohols were the group with more compounds found in both Italian and Spanish sparkling wines.

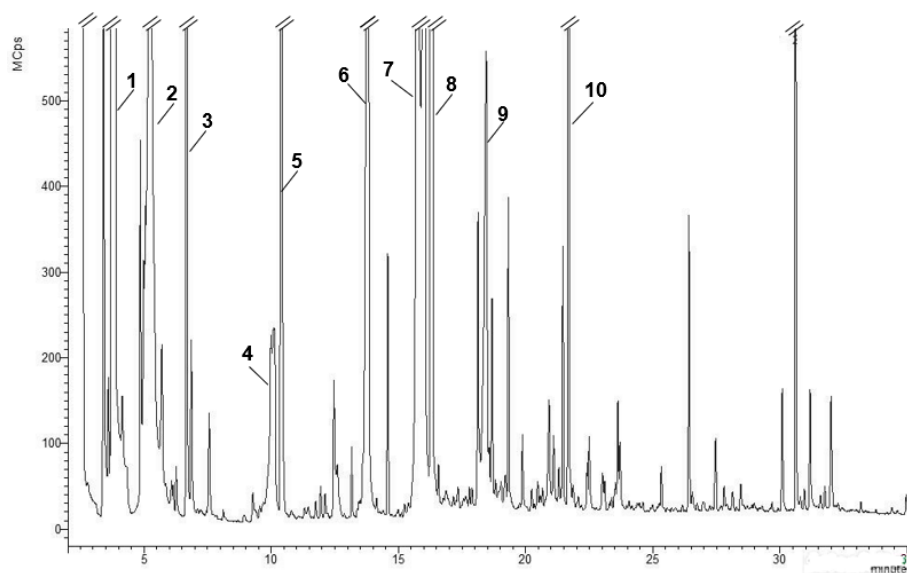


Figure 6. Representative HS-SPME-GC/MS chromatogram of volatile compounds identified in the Italian sparkling wine A at 36 months. Compounds: 1- isoamyl alcohol; 2- ethyl lactate; 3- 1-hexanol; 4- hexanoic acid; 5- ethyl hexanoate; 6- phenylethyl alcohol; 7- diethyl succinate; 8- ethyl octanoate; 9- nonanoic acid; 10-ethyl decanoate.

Table 5. Compounds identified in Italian sparkling wines A and B and in the Spanish sparkling wine.

| Compound                    | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions ( <i>m/z</i> ) | Identification<br>method | Italian<br>A | Italian<br>B | Spanish |
|-----------------------------|------------|-------------|----------------|------|--------------------------------------|--------------------------|--------------|--------------|---------|
| <b>Anisoles</b>             |            |             |                |      |                                      |                          |              |              |         |
| Anethole                    | 104-46-1   | 18.8        | 1286           | 1284 | 147/148                              | NIST 14                  | ✓            | ✓            | ✓       |
| <b>Alcohols</b>             |            |             |                |      |                                      |                          |              |              |         |
| 4-Penten-2-ol               | 625-31-0   | 2.46        | 647            | -    | 41/43/45                             | NIST 14                  | ✓            | ✓            | ✗       |
| Isoamyl alcohol             | 123-51-3   | 3.82        | 736            | -    | 41/42/55/70                          | STD                      | ✓            | ✓            | ✓       |
| 2-Methyl-1-butanol          | 1565-80-6  | 3.86        | -              | -    | 41/56/57/70                          | NIST 14                  | ✓            | ✗            | ✗       |
| 1-Pentanol                  | 71-41-0    | 4.33        | 765            | -    | 42/55/70                             | NIST 14                  | ✓            | ✓            | ✓       |
| 2,3-Butanediol              | 513-85-9   | 4.86        | 788            | -    | 43/45                                | NIST 14                  | ✓            | ✓            | ✓       |
| 3-Methyl-1-pentanol         | 589-35-5   | 6.09        | 844            | 838  | 55/56/69/84                          | NIST 14                  | ✓            | ✗            | ✓       |
| 3-Hexen-1-ol                | 928-96-1   | 6.33        | 854            | 857  | 55/67/82/104                         | STD                      | ✓            | ✓            | ✓       |
| 1-Hexanol                   | 111-27-3   | 6.67        | 868            | 867  | 56/69/84                             | STD                      | ✓            | ✓            | ✓       |
| 2-Butanol                   | 14898-79-4 | 7.39        | -              | 895  | 45/59                                | NIST 14                  | ✗            | ✗            | ✓       |
| 3-Ethyl-4-methyl-1-pentanol | -          | 11.1        | 1023           | 1019 | 41/55/69/84                          | NIST 14                  | ✗            | ✓            | ✗       |
| 2-Ethylhexanol              | 104-76-7   | 11.3        | 1027           | 1030 | 57/70/83/108                         | NIST 14                  | ✓            | ✓            | ✗       |
| Benzyl alcohol              | 100-51-6   | 11.4        | 1036           | 1033 | 51/79/108                            | NIST 14                  | ✓            | ✗            | ✗       |
| 1-Octanol                   | 111-87-5   | 12.6        | 1071           | 1071 | 56/70/84                             | STD                      | ✓            | ✓            | ✓       |
| 2-Nonanol                   | 628-99-9   | 13.5        | 1101           | 1100 | 45/55/68                             | NIST 14                  | ✓            | ✓            | ✗       |
| Phenylethyl Alcohol         | 60-12-8    | 13.8        | 1116           | 1110 | 65/91/92/122                         | STD                      | ✓            | ✓            | ✓       |
| 1-Decanol                   | 112-30-1   | 18.5        | 1273           | 1273 | 55/70/83/97                          | STD                      | ✓            | ✓            | ✓       |
| 1-Dodecanol                 | 112-53-8   | 23.7        | 1473           | 1472 | 55/69/83/97/111                      | NIST 14                  | ✓            | ✓            | ✓       |
| 2-Methyl-1-hexadecanol      | 2490-48-4  | 29.7        | -              | 1729 | 43/57/69/83                          | NIST 14                  | ✓            | ✓            | ✗       |
| <b>Aldehydes</b>            |            |             |                |      |                                      |                          |              |              |         |
| Benzaldehyde                | 100-52-7   | 9.28        | 962            | 960  | 51/77/105/106                        | STD                      | ✓            | ✓            | ✓       |

| Compound                           | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions (m/z) | Identification<br>method | Italian<br>A | Italian<br>B | Spanish |
|------------------------------------|------------|-------------|----------------|------|-----------------------------|--------------------------|--------------|--------------|---------|
| Octanal                            | 124-13-0   | 10.5        | 1001           | 1003 | 56/69/82/104                | STD                      | ✓            | ✓            | ✓       |
| Phenylacetaldehyde                 | 122-78-1   | 11.7        | 1041           | 1048 | 55/65/91/97                 | STD                      | ✓            | ✓            | ✓       |
| Nonanal                            | 124-19-6   | 13.6        | 1104           | 1104 | 57/70/82/98                 | STD                      | ✓            | ✓            | ✓       |
| Decanal                            | 112-31-2   | 16.6        | 1206           | 1205 | 57/70/82/95                 | STD                      | ✓            | ✓            | ✓       |
| 2,5-Dimethylbenzaldehyde           | 5779-94-2  | 16.8        | 1208           | 1214 | 77/91/105/133               | NIST 14                  | X            | X            | ✓       |
| β-Cyclocitral                      | 432-25-7   | 17.0        | 1218           | 1220 | 55/67/81/137                | STD                      | ✓            | X            | ✓       |
| Dodecanal                          | 112-54-9   | 22.1        | 1409           | 1407 | 55/69/82/95                 | NIST 14                  | ✓            | ✓            | X       |
| Tridecanal                         | 10486-19-8 | 24.6        | 1508           | 1512 | 57/67/82/82/96              | NIST 14                  | ✓            | X            | X       |
| Tetradecanal                       | 124-25-4   | 26.6        | 1613           | 1611 | 41/57/67/82                 | NIST 14                  | X            | X            | X       |
| <b>Esters</b>                      |            |             |                |      |                             |                          |              |              |         |
| Ethyl acetate                      | 141-78-6   | 2.33        | 612            | -    | 43/45/61/70                 | NIST 14                  | ✓            | ✓            | X       |
| Ethyl propanoate                   | 105-37-3   | 3.34        | 709            | -    | 57/75/102                   | NIST 14                  | ✓            | X            | ✓       |
| Ethyl isobutyrate                  | 97-62-1    | 4.14        | 755            | -    | 43/71/116                   | STD                      | ✓            | ✓            | ✓       |
| Ethyl butanoate                    | 105-54-4   | 4.99        | 802            | 802  | 60/71/88/101                | STD                      | ✓            | ✓            | ✓       |
| Ethyl lactate                      | 97-64-3    | 5.25        | 815            | 812  | 45                          | NIST 14                  | ✓            | ✓            | ✓       |
| Ethyl 2-methylbutanoate            | 7452-79-1  | 6.16        | 849            | 847  | 57/74/85/102                | STD                      | ✓            | ✓            | ✓       |
| Ethyl isovalerate                  | 108-64-5   | 6.27        | 854            | 852  | 57/70/88/115                | STD                      | ✓            | ✓            | ✓       |
| Isoamyl acetate                    | 123-92-2   | 6.86        | 876            | 874  | 55/61/70/87                 | STD                      | ✓            | ✓            | ✓       |
| Ethyl hexanoate                    | 123-66-0   | 10.4        | 1000           | 997  | 43/60/88/99                 | STD                      | ✓            | ✓            | ✓       |
| Hexyl acetate                      | 142-92-7   | 10.8        | 1011           | 1010 | 56/84/101                   | STD                      | ✓            | ✓            | ✓       |
| Ethyl hex-2-enoate                 | 1552-67-6  | 11.7        | 1037           | 1042 | 41/55/73/97                 | NIST 14                  | ✓            | X            | X       |
| Ethyl 2-furoate                    | 614-99-3   | 12.0        | 1047           | 1049 | 95/112/140                  | NIST 14                  | ✓            | ✓            | ✓       |
| Ethyl 2-hydroxy-4-methylpentanoate | 10348-47-7 | 12.1        | 1060           | 1054 | 43/69/87                    | NIST 14                  | ✓            | ✓            | ✓       |
| Ethyl heptanoate                   | 106-30-9   | 13.4        | 1095           | 1097 | 60/88/113                   | STD                      | ✓            | ✓            | ✓       |
| Methyl octanoate                   | 111-11-5   | 14.1        | 1126           | 1122 | 43/55/74/87                 | NIST 14                  | ✓            | X            | X       |

| Compound                     | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions (m/z) | Identification<br>method | Italian<br>A | Italian<br>B | Spanish |
|------------------------------|------------|-------------|----------------|------|-----------------------------|--------------------------|--------------|--------------|---------|
| Diethyl succinate            | 123-25-1   | 15.8        | 1182           | 1178 | 55/73/101/129               | STD                      | ✓            | ✓            | ✓       |
| Ethyl octanoate              | 106-32-1   | 16.3        | 1196           | 1196 | 70/88/101/127               | STD                      | ✓            | ✓            | ✓       |
| Diethyl methylsuccinate      | 4676-51-1  | 16.5        | 1201           | 1205 | 73/87/115/143               | NIST 14                  | ✓            | X            | X       |
| Ethyl phenylacetate          | 101-97-3   | 17.6        | 1246           | 1240 | 65/91/164                   | NIST 14                  | ✓            | ✓            | ✓       |
| Isoamyl hexanoate            | 2198-61-0  | 17.8        | 1252           | 1248 | 55/70/99                    | NIST 14                  | ✓            | X            | ✓       |
| 2-Phenylethyl acetate        | 103-45-7   | 17.9        | 1258           | 1252 | 43/91/104                   | STD                      | ✓            | ✓            | ✓       |
| Diethyl malate               | 7754-12-3  | 18.1        | 1271           | 1260 | 43/71/89/117                | NIST 14                  | ✓            | ✓            | ✓       |
| Diethyl glutarate            | 818-38-2   | 18.6        | 1275           | 1283 | 55/87/115/143               | NIST 14                  | ✓            | ✓            | X       |
| Ethyl nonanoate              | 123-29-5   | 19.0        | 1296           | 1293 | 60/88/101/115               | STD                      | ✓            | ✓            | ✓       |
| Ethyl 9-decenoate            | 67233-91-4 | 21.5        | 1383           | 1387 | 55/69/88/101                | NIST 14                  | ✓            | ✓            | ✓       |
| Ethyl decanoate              | 110-38-3   | 21.7        | 1396           | 1392 | 55/70/88/101                | STD                      | ✓            | ✓            | ✓       |
| Ethyl isopentyl succinate    | 28024-16-0 | 22.5        | 1436           | 1424 | 55/71/101/129               | NIST 14                  | ✓            | ✓            | ✓       |
| Ethyl dodecanoate            | 106-33-2   | 26.5        | 1589           | 1595 | 57/69/88/101                | NIST 14                  | ✓            | ✓            | X       |
| Octyl octanoate              | 2306-88-9  | 30.6        | 1779           | 1772 | 41/57/83/145                | NIST 14                  | ✓            | ✓            | X       |
| Ethyl tetradecanoate         | 124-06-1   | 30.9        | 1794           | 1787 | 88/101                      | NIST 14                  | ✓            | ✓            | X       |
| Isopropyl myristate          | 110-27-0   | 31.6        | 1827           | 1819 | 43/60/102/228               | STD                      | ✓            | ✓            | X       |
| Isoamyl laurate              | 6309-51-9  | 32.0        | 1846           | 1840 | 43/55/70                    | NIST 14                  | ✓            | ✓            | X       |
| Ethyl pentadecanoate         | 41114-00-5 | 35.0        | 1894           | 1991 | 88/101                      | NIST 14                  | ✓            | ✓            | X       |
| Ethyl hexadecanoate          | 628-97-7   | 35.0        | 1993           | 1991 | 55/73/88/101                | NIST 14                  | ✓            | ✓            | X       |
| Isopropyl palmitate          | 142-91-6   | 35.5        | 2023           | 2020 | 60/73/102/149               | NIST 14                  | ✓            | ✓            | X       |
| Isopropyl palmitate          | 142-91-6   | 35.5        | 2023           | 2020 | 43/60/102/239               | NIST 14                  | ✓            | ✓            | X       |
| <b>Fatty acids</b>           |            |             |                |      |                             |                          |              |              |         |
| (2S)-2-Hydroxypropanoic acid | 79-33-4    | 2.63        | -              | -    | 43/45                       | NIST 14                  | ✓            | ✓            | X       |
| Hexanoic acid                | 142-62-1   | 10.1        | 990            | 987  | 55/60/73/87                 | NIST 14                  | ✓            | ✓            | ✓       |
| Octanoic acid                | 124-07-2   | 16.1        | 1186           | 1180 | 55/60/73/101                | NIST 14                  | ✓            | ✓            | ✓       |

| Compound                                     | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions (m/z) | Identification<br>method | Italian<br>A | Italian<br>B | Spanish |
|----------------------------------------------|------------|-------------|----------------|------|-----------------------------|--------------------------|--------------|--------------|---------|
| Nonanoic acid                                | 112-05-0   | 18.5        | 1273           | 1272 | 60/73/115/129               | NIST 14                  | ✓            | ✓            | ✓       |
| Decanoic acid                                | 334-48-5   | 20.9        | 1373           | 1363 | 41/60/73/129                | NIST 14                  | ✓            | ✓            | ✓       |
| <b>Furans</b>                                |            |             |                |      |                             |                          |              |              |         |
| Furfural                                     | 98-01-1    | 5.70        | 833            | 829  | 67/95/96                    | STD                      | ✓            | ✓            | ✓       |
| 5-Methyl-2-furfural                          | 620-02-0   | 9.23        | 958            | 938  | 53/110                      | STD                      | ✓            | ✓            | ✓       |
| <b>Ketones</b>                               |            |             |                |      |                             |                          |              |              |         |
| 2-Heptanone                                  | 110-43-0   | 7.20        | 891            | 888  | 58/71/114                   | NIST 14                  | ✓            | ✓            | ✓       |
| Oxolan-2-one                                 | 96-48-0    | 8.02        | 915            | 917  | 42/56/86                    | STD                      | ✓            | ✓            | ✗       |
| 2-Nonanone                                   | 821-55-6   | 13.2        | 1092           | 1089 | 43/58/71                    | STD                      | ✓            | ✓            | ✓       |
| Menthone                                     | 491-07-6   | 15.4        | 1164           | 1164 | 41/55/69/112                | NIST 14                  | ✓            | ✓            | ✗       |
| L-Carvone                                    | 6485-40-1  | 17.6        | 1246           | 1243 | 54/82/93/108                | NIST 14                  | ✓            | ✓            | ✗       |
| $\alpha$ -Ionone                             | 127-41-3   | 18.7        | -              | 1294 | 93/121/136                  | STD                      | ✓            | ✓            | ✓       |
| 2-Undecanone                                 | 112-12-9   | 19.0        | 1290           | 1305 | 58/73/85/111                | STD                      | ✓            | ✓            | ✓       |
| $\beta$ -Damascenone                         | 23726-93-4 | 21.3        | 1386           | 1377 | 69/77/105/121               | STD                      | ✓            | ✓            | ✓       |
| Geranyl Acetone                              | 3796-70-1  | 23.0        | 1453           | 1445 | 69/107/136                  | NIST 14                  | ✓            | ✓            | ✗       |
| <b>Naphtalene derivatives</b>                |            |             |                |      |                             |                          |              |              |         |
| 1,1,5-Trimethyl-1,2-dihydronaphthalene (TDN) | 30364-38-6 | 20.6        | 354            | 1353 | 142/157/172                 | NIST 14                  | ✗            | ✗            | ✓       |
| <b>Terpenes</b>                              |            |             |                |      |                             |                          |              |              |         |
| Limonene                                     | 138-86-3   | 11.3        | 1030           | 1028 | 67/68/93                    | STD                      | ✓            | ✓            | ✓       |
| Eucalyptol                                   | 470-82-6   | 11.4        | 1031           | 1032 | 71/81/108                   | STD                      | ✓            | ✓            | ✓       |
| Linalool oxide                               | 5989-33-3  | 12.6        | 1069           | 1074 | 59/68/94/111                | STD                      | ✓            | ✓            | ✓       |
| $\beta$ -Linalool                            | 78-70-6    | 13.5        | 1099           | 1099 | 55/71/93                    | STD                      | ✓            | ✓            | ✓       |
| Nerol oxide                                  | 1786-08-9  | 15.0        | 1150           | 1153 | 53/67/83/119                | NIST 14                  | ✓            | ✓            | ✗       |
| Camphor                                      | 76-22-2    | 15.2        | 1145           | 1158 | 55/81/95/108                | STD                      | ✓            | ✓            | ✓       |
| <b>Lactone</b>                               |            |             |                |      |                             |                          |              |              |         |

| Compound                | CAS      | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions (m/z) | Identification<br>method | Italian<br>A | Italian<br>B | Spanish |
|-------------------------|----------|-------------|----------------|------|-----------------------------|--------------------------|--------------|--------------|---------|
| Butyrolactone           | 96-48-0  | 7.94        | 915            | 914  | 42/56/86                    | NIST 14                  | ✓            | ✓            | ✓       |
| <b>Phenol</b>           |          |             |                |      |                             |                          |              |              |         |
| 3-Ethylphenol           | 620-17-7 | 15.4        | 1168           | 1165 | 77/107/122                  | NIST 14                  | ✓            | ✓            | X       |
| 2,4-Di-tert-butylphenol | 96-76-4  | 24.5        | 1519           | 1519 | 57/191/206                  | STD                      | ✓            | ✓            | X       |
| <b>Organic acids</b>    |          |             |                |      |                             |                          |              |              |         |
| Acetic acid             | 64-19-7  | 3.32        | 610            | -    | 43/45/60                    | NIST 14                  | ✓            | ✓            | ✓       |
| <b>Others</b>           |          |             |                |      |                             |                          |              |              |         |
| Tetradecane             | 629-59-4 | 21.9        | 1400           | 1398 | 43/57/71/85                 | NIST 14                  | X            | X            | ✓       |

### 3.2. Evolution of the volatile composition of sparkling wines over time

To understand the evolution on the volatile profile of Italian and Spanish sparkling wines, only the volatile compounds with a commercially available standard were quantified, namely 38 volatile compounds. The absolute quantification of each compound is presented in Annex 1, 2 and 3 for Italian sparkling wine A, Italian sparkling wine B and Spanish sparkling wine, respectively. The evolution of the levels of these volatile compounds present in Italian and Spanish sparkling wines was evaluated over time through multivariate analysis methods. A clear distinction between 12, 24 and 36 months and 24 and 80 months was obtained for Italian and Spanish sparkling wines in PCA and PLS-DA models, but only the PLS-DA models were used for interpretation of the changes occurring in the levels of volatile compounds over time.

In the PLS-DA models, two components were able to discriminate the volatile composition of the Italian sparkling wine A (Figure 7.A-i)), Italian sparkling wine B (Figure 7.A-ii)) and Spanish sparkling wine (Figure 7.A-iii)) over time, respectively. The values of the  $R^2$  were closer to 1, which means that the model fits the data. Concerning the  $Q^2$ , the three PLS-DA models demonstrated high values (above 0.8), which mean that the model has a good prediction ability.

The compounds responsible for group separation were interpreted in the PLS-DA VIP scores plot and only compounds with  $VIP > 1$  were considered relevant to differentiate the groups. The statistical significance of the levels of these compounds was then investigated through univariate analysis. Table 6 shows the compounds that changed significantly over time for each sparkling wine, as well as their percentage of variation. In addition, the levels of those compounds were represented in a heatmap (Figure 8) to have a more holistic view of their evolution over time.

For the Italian sparkling wine A, from all the compounds quantified, eighteen revealed a VIP score higher than 1 and statistically significant differences between the time points evaluated. These altered volatile compounds comprised several families (Figure 7.B-i), Figure 8.A, Table 6), such as esters (ethyl butanoate, ethyl isovalerate, hexyl acetate, diethyl succinate, phenylethyl acetate and ethyl decanoate), terpenes (limonene,  $\beta$ -linalool and 1,4-cineole), alcohols (isoamyl alcohol and 1-octanol), aldehydes (phenylacetaldehyde and  $\beta$ -cyclocitral), furans (furfural) and ketones ( $\alpha$ -ionone).

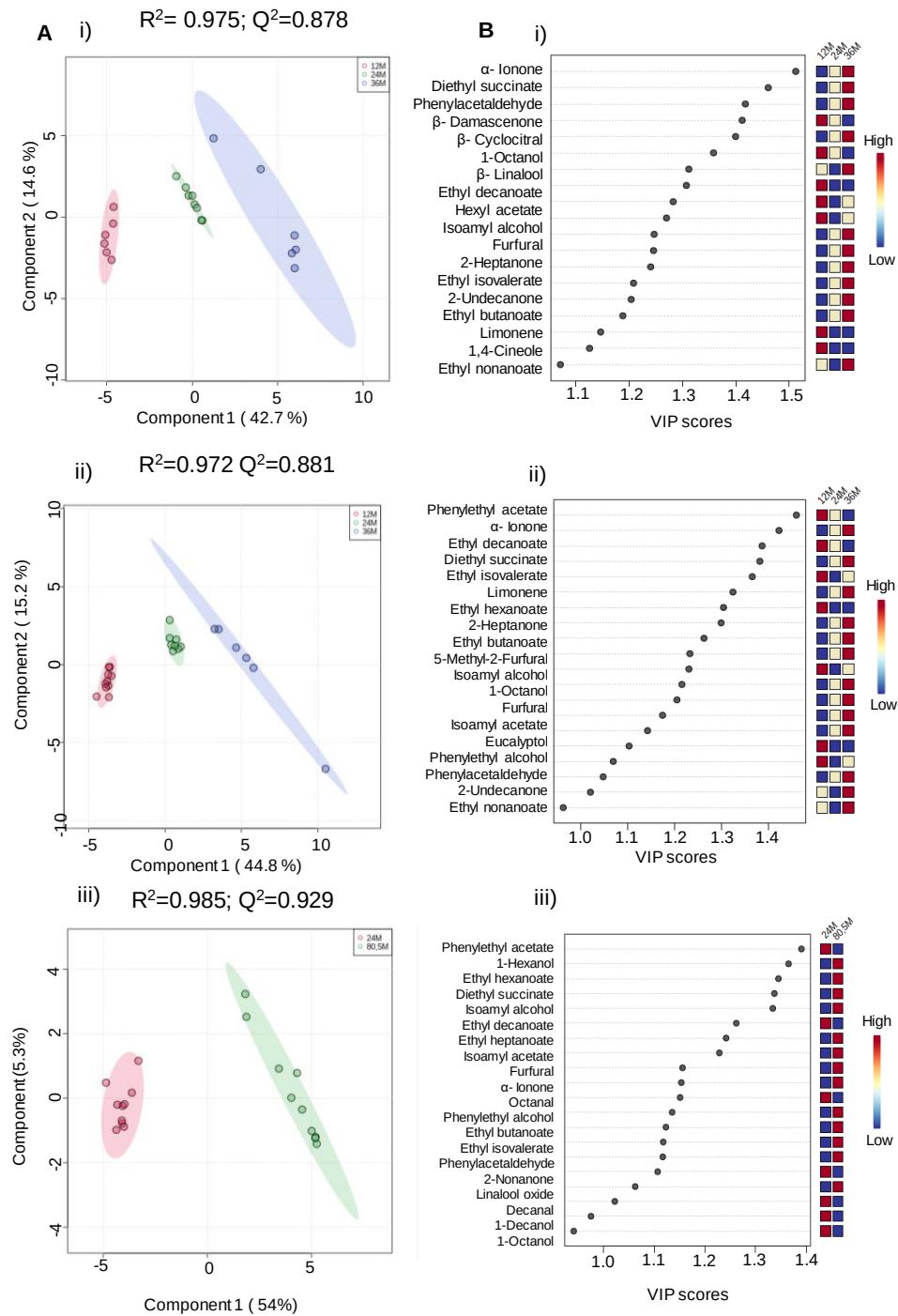


Figure 7. A- PLS-DA scores scatter plot of i) Italian sparkling wine A, ii) Italian sparkling wine B and iii) Spanish sparkling wine; B- Important features of i) Italian sparkling wine A, ii) Italian sparkling wine B and iii) Spanish sparkling wine.

Table 6. Volatile compounds found altered in Italian sparkling A and B and Spanish sparkling wine over time.

| Compound               | Italian sparkling wine A |                |                | Italian sparkling wine B |                 |               | Spanish sparkling wine<br>80 M vs 24M |
|------------------------|--------------------------|----------------|----------------|--------------------------|-----------------|---------------|---------------------------------------|
|                        | 24M vs 12M               | 36M vs 12M     | 36M vs 24M     | 24M vs 12M               | 36M vs 12M      | 36M vs 24M    |                                       |
| <b>Alcohols (n=5)</b>  |                          |                |                |                          |                 |               |                                       |
| Isoamyl alcohol        | 43.7±9.7***)             | 288±27.3(**)   | 170±24.7(***)  | 41.6±5.7(****)           | 192±27.9(***)   | 106±25.1(***) | 266±9.7(****)                         |
| 1-Hexanol              | -                        | -              | -              | -                        | -               | -             | 942±12.1(****)                        |
| Phenylethyl alcohol    | -                        | -              | -              | -71.8±11.8(****)         | -50.9±16.4(***) | 74.3±26.1(**) | 81.1±11.1(****)                       |
| 1-Octanol              | -8.23±4.0(*)             | 625±12.8(**)   | 690±13.0(***)  | -                        | 441±29.0(***)   | 421±28.9(***) | 394±15.9(****)                        |
| 1-Decanol              | -                        | -              | -              | -                        | -               | -             | BLOQ at 80 M                          |
| <b>Aldehydes (n=4)</b> |                          |                |                |                          |                 |               |                                       |
| Octanal                | -                        | -              | -              | -                        | -               | -             | -                                     |
| Phenylacetaldehyde     | 142±5.53(***)            | 455±21.9(**)   | 129±18.1(***)  | -                        | -               | -             | 181±8.7(****)                         |
| Decanal                | -                        | -              | -              | -                        | -               | -             | -82.0±74.5(****)                      |
| β-Cyclocitral          | -98.5±2.45 (***)         | ND at 36M      | -              | -                        | -               | -             | -                                     |
| <b>Esters (n=9)</b>    |                          |                |                |                          |                 |               |                                       |
| Ethyl butanoate        | -                        | 874±38.6(**)   | 577±37.2(***)  | 36.9±14.2(*)             | 423±30.5(***)   | 282±29.0(***) | 1043±13.2(****)                       |
| Ethyl isovalerate      | 363±39.7(**)             | 3425±45.6(**)  | 661±41.8(***)  | 272±20.7(****)           | 1388±35.0(***)  | 300±30.3(***) | 967±13.7(****)                        |
| Isoamyl acetate        | -                        | -              | -              | 27.7±14.6(*)             | 272±34.0(***)   | 192±32.5(***) | 83.7±10.7(****)                       |
| Ethyl hexanoate        | -                        | -              | -              | -                        | 242±17.6(***)   | 169±16.6(***) | 538±13.7(****)                        |
| Hexyl acetate          | BLOQ at 24M              | -92.4±12.8(**) | BLOQ at 24M    | -                        | -               | -             | -                                     |
| Ethyl heptanoate       | -                        | -              | -              | -                        | -               | -             | 820±9.46(****)                        |
| Diethyl succinate      | 96.9±5.0(***)            | 513±9.8(**)    | 211±8.6(***)   | 40.1±13.2(***)           | 359±13.0(***)   | 228±11.4(***) | 309±11.3(****)                        |
| Phenylethyl acetate    | -75.5±3.7(***)           | -82.8±4.2(**)  | -29.9±5.6(***) | -69.7±4.5(****)          | -79.3±5.5(***)  | -31.8±8.2(**) | -                                     |
| Ethyl decanoate        | -91.3±9.0(***)           | -85.9±10.2(**) | -              | -85.8±16.8(****)         | -93.4±17.9(***) | -53.4±33.5(*) | -77.2±19.9(****)                      |
| <b>Furans (n=2)</b>    |                          |                |                |                          |                 |               |                                       |
| Furfural               | 91.9±7.3(***)            | 321±31.9(**)   | 119±27.3(***)  | 136±5.56(****)           | 147±27.6(***)   | -             | 104±16.2(****)                        |
| 5-Methyl-2-fufural     | -                        | -              | -              | -50.0±10.7(****)         | -50.0±14.3(***) | -             | -                                     |
| <b>Ketones (n=5)</b>   |                          |                |                |                          |                 |               |                                       |
| 2-Heptanone            | 921±34.1(***)            | 1871±45.9(**)  | -              | 397±15.9(****)           | 687±45.1(***)   | -             | -                                     |
| 2-Nonanone             | -                        | -              | -              | -                        | -               | -             | -55.3±19.7(***)                       |
| 2-Undecanone           | -                        | 3635±46.4(**)  | 512±42.4(**)   | -                        | -               | -             | -                                     |
| β- Damascenone         | 35.6±5.64(***)           | 153±12.4(**)   | 86.8±11.5(***) | -                        | -               | -             | -                                     |
| α- Ionone              | ND at 12M                | ND at 12M      | 81.0±13.5(***) | ND at 12M                | ND at 12M       | -             | 763±23.5(****)                        |
| <b>Terpenes (n=5)</b>  |                          |                |                |                          |                 |               |                                       |

| Compound       | Italian sparkling wine A |            |            | Italian sparkling wine B |            |            | Spanish sparkling wine |
|----------------|--------------------------|------------|------------|--------------------------|------------|------------|------------------------|
|                | 24M vs 12M               | 36M vs 12M | 36M vs 24M | 24M vs 12M               | 36M vs 12M | 36M vs 24M | 80 M vs 24M            |
| Limonene       | ND at 24M                | ND at 36M  | -          | ND at 24M                | ND at 36M  | -          | -                      |
| Eucalyptol     | -                        | -          | -          | BLOQ at 24M              | ND at 36M  | -          | -                      |
| Linalool oxide | -                        | -          | -          | -                        | -          | -          | 5221±41.4(****)        |
| β- Linalool    | ND at 24M                | ND at 36M  | -          | -                        | -          | -          | -                      |
| 1,4-Cineole    | BLOQ at 24M              | ND at 36M  | -          | -                        | -          | 27.3±16.3  | -                      |

Statistical significance assessed using the Mann-Whitney test (\* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; \*\*\*\* -  $p < 0.0001$ ); BLOQ- Below limit of quantification; ND-Not detected; n-number



Figure 8. Heatmap illustrating the levels of volatile compounds changing over time in Italian sparkling wine A (**A**), Italian sparkling wine B (**B**) and Spanish sparkling wine (**C**). Columns correspond to each sample while rows correspond to the concentration of each discriminant volatile coloured from minimum (−1, dark blue) to maximum (1, dark red).

For Italian sparkling wine B, eighteen discriminant compounds were found with VIP score higher than 1. However, statistically significant differences were only obtained for sixteen volatile compounds comprising several families (Figure 7.B-ii), Figure 8.B,

Table 6), such as esters (ethyl butanoate, ethyl isovalerate, isoamyl acetate, diethyl succinate, phenylethyl acetate and ethyl decanoate), furans (furfural and 5-methyl-2-furfural), terpenes (limonene, eucalyptol and 1,4-cineole), ketones (2-heptanone and  $\alpha$ -ionone) and alcohols (isoamyl alcohol, phenylethyl alcohol and 1-octanol).

Lastly, the Spanish sparkling wine also presented eighteen compounds with a VIP score higher than 1. In the univariate analysis, the levels of all discriminant compounds demonstrated statistically significant differences (Figure 7.B-iii), Figure 8.C Table 6), namely esters (ethyl hexanoate, diethyl succinate, ethyl decanoate, ethyl heptanoate, isoamyl acetate, ethyl butanoate and ethyl isovalerate), alcohols (1-hexanol, isoamyl alcohol, phenylethyl alcohol, 1-octanol and 1-decanol), aldehydes (phenylacetaldehyde and decanal), ketones (2-nonanone and  $\alpha$ -ionone), terpenes (linalool oxide) and furans (furfural).

Comparing the results obtained for the three sparkling wines, alterations in seven volatile compounds were found in common, namely in diethyl succinate, ethyl decanoate, isoamyl alcohol, furfural, ethyl butanoate,  $\alpha$ -ionone and ethyl isovalerate. In addition, an increased tendency over time was found for almost all compounds, except for ethyl decanoate, which decreased.

Esters were the family of volatiles that gathered the highest number of compounds with significant changes, namely, diethyl succinate, ethyl decanoate, ethyl butanoate, and ethyl isovalerate. Ethyl isovalerate, ethyl butanoate and diethyl succinate showed higher concentrations for aged sparkling wines while ethyl decanoate decreased with ageing time. Ethyl decanoate is formed during yeast fermentation (Liu et al. 2015). It has been described that its concentration declines over time because it can hydrolyse to produce decanoic acid and ethanol in the presence of high amounts of oxygen. As a result, it can become undetectable after an extended period of storage (Liu et al. 2015).

On the other hand, diethyl succinate is a volatile that is originated during ageing of sparkling wine in contact with lees from the second fermentation. Usually, its concentration increases over time and it has been considered an indicator of the evolution of Cava sparkling wines (Riu-Aumatell et al. 2006; Torrens et al. 2010). Thus, the consistently increase of diethyl succinate levels over time in Italian and Spanish sparkling wines agrees with the literature. In addition, an increase in the concentration of ethyl isovalerate might be explained by a shifting in the equilibrium of acid-esters because of the development of branched fatty acids (Díaz-Maroto, Schneider, and Baumes 2005). Lastly, ethyl butanoate is produced from the lipid metabolism of yeast and generally its concentration decreases during ageing (Díaz-Maroto, Schneider, and Baumes 2005). However, in this work this volatile compound was found in higher amounts in older sparkling wines. Torrens et al. 2010 concluded that some esters, could

demonstrate an increase in their concentration due to the autolysis of lees and release of lipids and other molecules and the time of contact, which might explain the results obtained (Torrens et al. 2010).

Furfural belongs to the family of compounds denominated as furans obtained from sugar degradation (Torrens et al. 2010). Studies with Cava sparkling wines revealed that the concentration of this volatile increases during the ageing process of Cava (Torrens et al. 2010). Additionally, other works even pointed out furfural as an oxidation marker for still wines (Pinto et al. 2018). In the present work, furfural was present in higher amounts in the older groups of sparkling wines which is in accordance with the literature.

Isoamyl alcohol is a fusel alcohol that when present in concentrations higher than 300 mg/L causes a pungent smell and taste, whereas at concentrations below 300 mg/L contributes positively for the fruity and floral notes (Garofalo et al. 2018). In this work, its concentration also increased during ageing. However, other works observed a reduction in higher alcohols after a long period of ageing (Martínez-García, Mauricio, et al. 2021).

$\alpha$ -Ionone is responsible for *wood*, *violet* aromas in wine (Flavornet) and it was found to change during ageing in this study, for the first time to our knowledge. This ketone was present in higher amounts at the last timepoint evaluated. These alterations might be explained by the degradation of some carotenoids that are precursors of this compound, as has already been reported for some of the main aromatic compounds produced by carotenoid degradation during ageing for Port wines (Ferreira et al. 2008), or by hydrolysis of glycoside molecules (Riu-Aumatell et al. 2006).

In general, the evolution of the three sparkling wines in bottle after disgorging showed a similar behaviour to ageing in still wines. Besides that, some compounds, such as diethyl succinate, ethyl isovalerate, furfural, isoamyl alcohol,  $\alpha$ -ionone and ethyl butanoate are possible markers of long aged sparkling wines, contrarily, ethyl decanoate could be a marker of young sparkling wines.

Furthermore, the evolution of the volatile profile of each sparkling wine under study showed unique differences. Overall, the Italian sparkling wine A demonstrated a tendency for an increase in the concentration of alcohols, aldehydes, ketones and furans and a decrease in the concentration of esters and terpenes over time. Thus, some compounds, mainly terpenes, such as limonene,  $\beta$ -linalool and 1,4-cineole and one aldehyde,  $\beta$ -cyclocitral, were not detected at 36 months post-bottling. These compounds are described as having *floral*, *citrus*, *herbal*, *lemon and orange* aromas and, therefore, a reduction in their amounts contributes to the loss of freshness and the fruity character of older sparkling wines compared to younger ones. In Figure 9 are represented boxplots

of some compounds found altered in the Italian sparkling wine A between 12, 24 and 36 months.

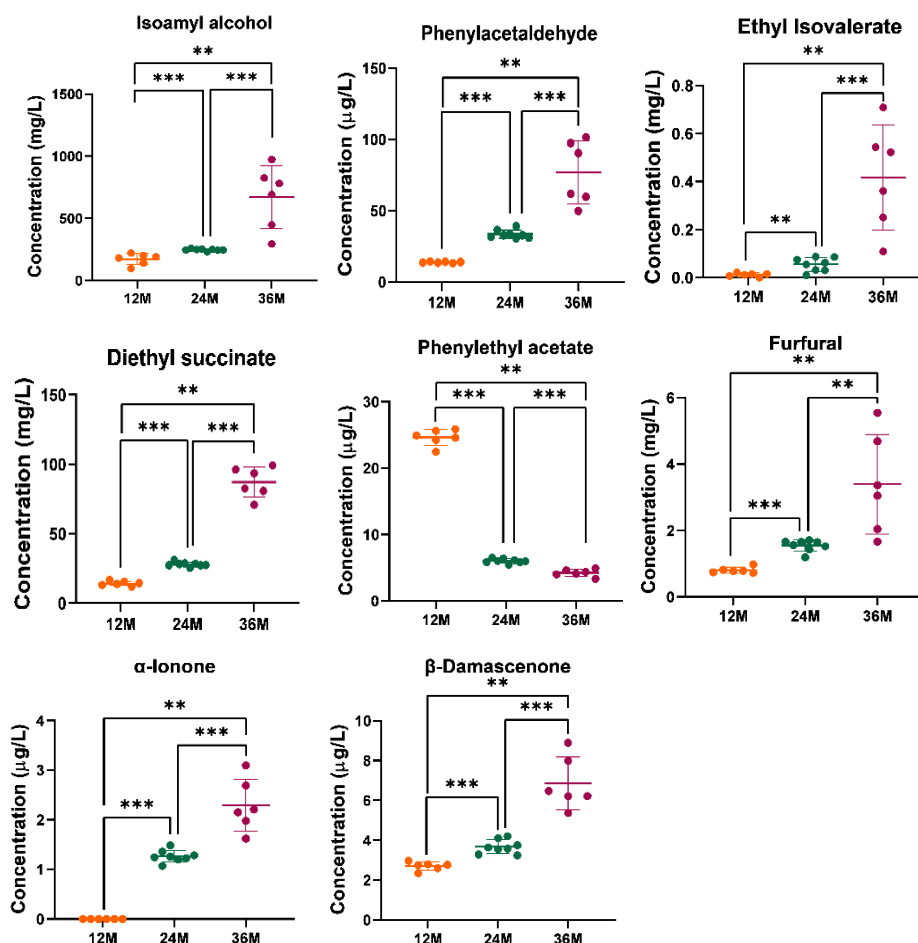


Figure 9. Boxplots of statistically significant compounds found altered in Italian sparkling wine A at different timepoints (12, 24 and 36 months). Statistical significance assessed using the Mann-Whitney test (\* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; \*\*\*\* -  $p < 0.0001$ ). ND-not detected.

The Italian sparkling wine B was the only one with statistically significant differences in the levels of 5-methyl-2-furfural and eucalyptol between timepoints. The 5-methyl-2-furfural concentration differed between timepoints, specifically it showed a decrease from 12 to 24 months and then an increase to 36 months. Additionally, eucalyptol constantly decreased with ageing and it was not detected at 36 months.

Concerning the Spanish sparkling wine, some specific significant differences in the evolution of its volatile composition during ageing were also found, namely in the levels of 1-hexanol, ethyl heptanoate, linalool oxide, 1-decanol, decanal, and 2-nonanone. Three volatile compounds (1-hexanol, ethyl heptanoate and linalool oxide) increased after 80 months of storage. On the other hand, the levels of 1-decanol, 2-nonanone and decanal decreased.

In conclusion, these results highlighted the alterations occurring on the aromatic composition of sparkling wines during ageing. In general, these results unveiled the loss of freshness and fruity notes, mainly due to a decrease in the esters content, and the development of new aromas due to an increase in the content of some volatile compounds.

## Chapter 4. Results and Discussion on the impact of different stoppers on the volatile composition of sparkling and white wines

This chapter presents the volatile compounds identified in French sparkling wine and Italian white wine by HS-SPME-GC-MS (subchapter 4.1), as well as the results obtained on the impact of different stoppers on the volatile composition of a French sparkling wine (subchapter 4.2) and an Italian white wine (subchapter 4.3).

### 4.1. Identification of volatile and semi volatile compounds in French sparkling wine and Italian white wine

The volatile composition of an Italian white wine sealed with three types of stoppers, A, B and C at 44 months post-bottling was assessed by HS-SPME/GC-MS analysis. A representative chromatogram of VOCs and VCCs present in the Italian white wine sealed with A stoppers is shown in Figure 10 and 11, respectively. A list of all compounds identified is presented in Table 7 and 8.

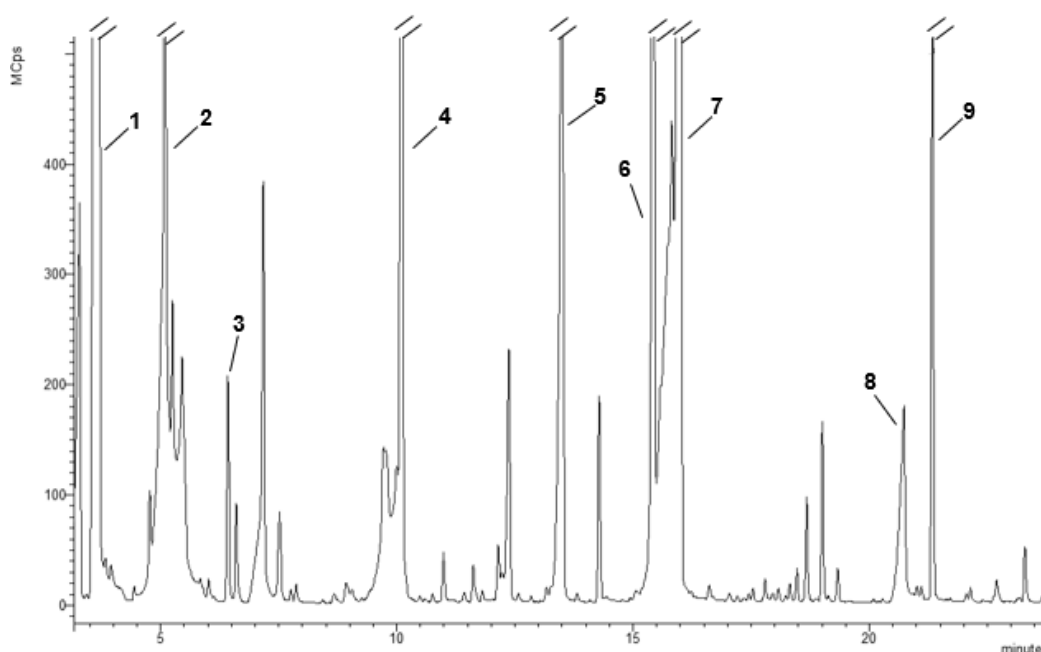


Figure 10. Representative HS-SPME-GC/MS chromatogram of VOCs identified in an Italian white wine sealed with A cork stopper. Compound Identification: 1- isoamyl alcohol; 2- 2,3-butanediol; 3- 1-hexanol; 4- ethyl hexanoate; 5- phenylethyl alcohol; 6- diethyl succinate; 7-ethyl octanoate; 8- decanoic acid; 9- ethyl decanoate.

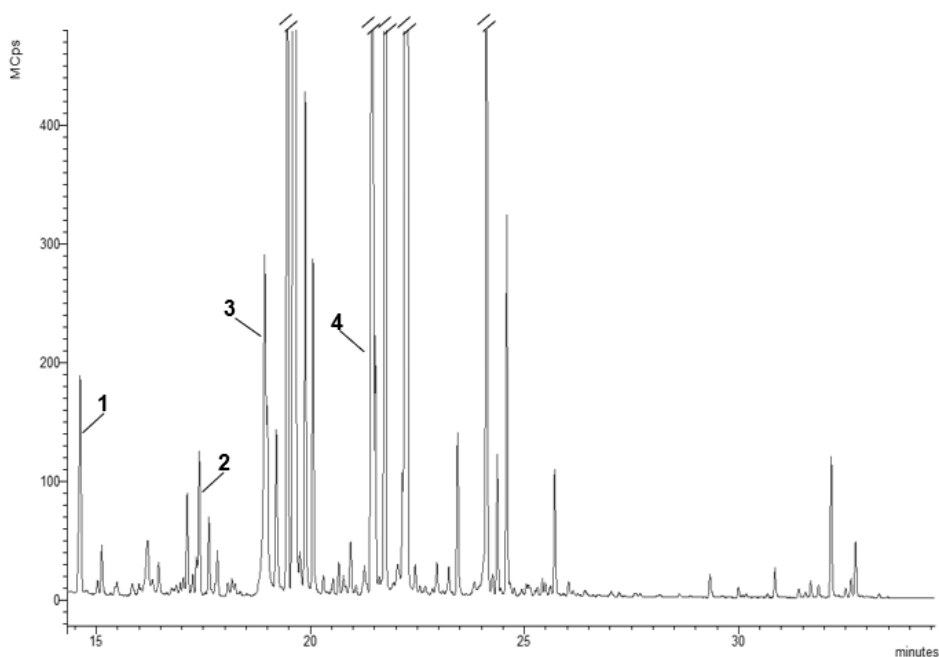


Figure 11. Representative HS-SPME-GC/MS chromatogram of VOCs identified in an Italian white wine sealed with A cork stoppers. Compound Identification: 1- isobutanal; 2- (1*E*)-3-methylpentanal; 3- (*E*)-3-heptanone; 4- methylglyoxal.

A total of 99 compounds were found in the VOCs composition of the Italian white wine (Table 7), including 41 esters, 22 alcohols, 7 aldehydes, 7 fatty acids, 6 ketones, 3 terpenes, 3 furans, 3 glycols, 3 phenols, 1 naphthalene derivate and 1 organic acid. Regarding the VOCs composition of the French sparkling wine, a total of 67 compounds were identified, comprising 29 esters, 13 alcohols, 8 aldehydes, 6 ketones, 4 fatty acids, 4 terpenes, 1 anisole, 1 furan, 1 lactone, 1 phenol and 1 organic acid. Esters and alcohols were the families with more compounds present in the Italian white wine and the French sparkling wine. A great part of these compounds have already been reported as part of the normal composition of wines, impacting their sensory characteristics (Lengyel and Sikolya 2015). Though, the presence of camphor and 2,4-*di-tert*-butylphenol were identified for the first time, according to the data found, in the volatile composition of sparkling wines.

Only the Italian white wine was analysed concerning its VCCs profile, enabling the identification of a total of 38 compounds (Table 8). The major classes were ketones and aldehydes with minor classes being esters, lactones and naphthalene derivatives. Only the identification of eight compounds was confirmed with a commercial standard (Table 8).

Table 7. Compounds detected in an Italian white wine sealed with A, B and C stoppers and in a French Sparkling wine

| Compound                    | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions ( <i>m/z</i> ) | Identification<br>method | A | B | C | French |
|-----------------------------|------------|-------------|----------------|------|--------------------------------------|--------------------------|---|---|---|--------|
| <b>Anisoles</b>             |            |             |                |      |                                      |                          |   |   |   |        |
| Anethole                    | 104-46-1   | 18.80       | 1286           | 1284 | 147/148                              | NIST 14                  | X | X | X | ✓      |
| <b>Alcohols</b>             |            |             |                |      |                                      |                          |   |   |   |        |
| 1-Butanol                   | 71-36-3    | 2.67        | 659            | -    | 41/43/56                             | NIST 14                  | ✓ | X | X | X      |
| Isobutyl alcohol            | 78-83-1    | 2.35        | 625            | -    | 41/42/43                             | NIST 14                  | X | ✓ | ✓ | X      |
| 4-Penten-2-ol               | 625-31-0   | 2.47        | 647            | -    | 41/43/45                             | NIST 14                  | X | ✓ | X | X      |
| Isoamyl alcohol             | 123-51-3   | 3.65        | 736            | -    | 42/55/70                             | STD                      | ✓ | ✓ | ✓ | ✓      |
| 1-Pentanol                  | 71-41-0    | 4.17        | 765            | -    | 42/55/70                             | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| 3-Ethyl-2-pentanol          | 609-27-8   | 4.18        | -              | -    | 45/55/70                             | NIST 14                  | X | X | X | ✓      |
| 2,3-Butanediol              | 513-85-9   | 4.92        | 788            | -    | 43/45/57                             | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| 3-Methyl-1-pentanol         | 589-35-5   | 5.82        | 838            | 834  | 41/56/69                             | NIST 14                  | X | X | ✓ | ✓      |
| 3-Hexen-1-ol                | 928-96-1   | 6.33        | 854            | 857  | 55/67/82/104                         | STD                      | ✓ | ✓ | ✓ | ✓      |
| 1-Hexanol                   | 111-27-3   | 6.43        | 868            | 858  | 41/56/69                             | STD                      | ✓ | ✓ | ✓ | ✓      |
| 2-Butanol                   | 14898-79-4 | 7.06        | -              | 882  | 45                                   | NIST 14                  | X | ✓ | X | X      |
| 1-Heptanol                  | 111-70-6   | 9.26        | 970            | 971  | 41/56/70                             | NIST 14                  | ✓ | X | X | ✓      |
| 3-Ethyl-4-methyl-1-pentanol | 38514-13-5 | 10.8        | 1023           | 1022 | 41/55/69/84                          | NIST 14                  | ✓ | ✓ | ✓ | X      |
| 2-Ethylhexanol              | 104-76-7   | 11.0        | 1030           | 1029 | 41/55/70/83                          | NIST 14                  | ✓ | ✓ | ✓ | X      |
| Benzyl alcohol              | 100-51-6   | 11.2        | 1036           | 1036 | 77/79/108                            | NIST 14                  | ✓ | X | ✓ | ✓      |
| 1-Octanol                   | 111-87-5   | 12.2        | 1071           | 1058 | 41/56/69/84                          | STD                      | ✓ | ✓ | ✓ | ✓      |
| 2-Nonanol                   | 628-99-9   | 13.2        | 1101           | 1102 | 45/69                                | NIST 14                  | ✓ | X | ✓ | X      |
| Phenylethyl Alcohol         | 60-12-8    | 13.5        | 1116           | 1112 | 65/91/92/122                         | STD                      | ✓ | ✓ | ✓ | ✓      |

| Compound                       | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions ( <i>m/z</i> ) | Identification<br>method | A | B | C | French |
|--------------------------------|------------|-------------|----------------|------|--------------------------------------|--------------------------|---|---|---|--------|
| <i>p</i> -Coumaryl alcohol     | 3690-05-9  | 15.0        | -              | 1150 | 107/150                              | NIST 14                  | ✓ | X | X | X      |
| 1-Decanol                      | 112-30-1   | 18.5        | 1273           | 1273 | 55/70/83/97                          | STD                      | ✓ | ✓ | ✓ | ✓      |
| 2-Methyl-1-hexadecanol         | 2490-48-4  | 23.8        | -              | 1477 | 43/57/69                             | NIST 14                  | ✓ | X | ✓ | ✓      |
| ( <i>E</i> )9-Hexadecen-1-ol   | 64437-47-4 | 28.1        | -              | 1657 | 43/55/69/83/97                       | NIST 14                  | ✓ | ✓ | ✓ | X      |
| 1-Hexadecanol                  | 36653-82-4 | 32.4        | 1880           | 1881 | 43/55/83/97                          | NIST 14                  | X | ✓ | ✓ | X      |
| <b>Aldehydes</b>               |            |             |                |      |                                      |                          |   |   |   |        |
| Heptanal                       | 111-71-7   | 7.26        | 901            | 903  | 41/55/70/91                          | NIST 14                  | X | X | X | ✓      |
| Benzaldehyde                   | 100-52-7   | 9.28        | 962            | 960  | 51/77/105/106                        | STD                      | ✓ | ✓ | ✓ | ✓      |
| Octanal                        | 124-13-0   | 10.5        | 1001           | 1003 | 56/69/82/104                         | STD                      | ✓ | ✓ | ✓ | ✓      |
| Phenylacetaldehyde             | 122-78-1   | 11.7        | 1041           | 1048 | 55/65/91/97                          | STD                      | ✓ | ✓ | ✓ | ✓      |
| Nonanal                        | 124-19-6   | 13.2        | 1104           | 1102 | 57/70/82/98                          | STD                      | ✓ | ✓ | ✓ | ✓      |
| Decanal                        | 112-31-2   | 16.2        | 1206           | 1204 | 41/55/70/82                          | STD                      | ✓ | ✓ | ✓ | ✓      |
| $\beta$ -Cyclocitral           | 432-25-7   | 17.0        | 1218           | 1220 | 55/67/81/137                         | STD                      | ✓ | ✓ | ✓ | X      |
| Dodecanal                      | 112-54-9   | 21.7        | 1409           | 1407 | 41/57/67/82                          | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Tetradecanal                   | 124-25-4   | 26.6        | 1613           | 1611 | 41/57/67/82                          | NIST 14                  | X | X | X | ✓      |
| <b>Esters</b>                  |            |             |                |      |                                      |                          |   |   |   |        |
| Ethyl acetate                  | 141-78-6   | 2.20        | 612            | -    | 43/61/70                             | NIST 14                  | ✓ | ✓ | ✓ | X      |
| Ethyl propanoate               | 105-37-3   | 3.18        | 709            | -    | 57/75/102                            | NIST 14                  | ✓ | X | X | X      |
| Ethyl isobutyrate              | 97-62-1    | 3.95        | 755            | -    | 43/71/88/116                         | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl butanoate                | 105-54-4   | 4.78        | 802            | 806  | 43/71/88                             | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl ( <i>S</i> )-(-)-Lactate | -          | 5.03        | -              | 816  | 45                                   | NIST 14                  | X | ✓ | X | X      |
| Ethyl lactate                  | 97-64-3    | 5.25        | 815            | 824  | 45                                   | NIST 14                  | ✓ | X | X | ✓      |

| Compound                                               | CAS         | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions ( <i>m/z</i> ) | Identification<br>method | A | B | C | French |
|--------------------------------------------------------|-------------|-------------|----------------|------|--------------------------------------|--------------------------|---|---|---|--------|
| Ethyl 2-methylbutanoate                                | 7452-79-1   | 5.91        | 849            | 850  | 41/57/102                            | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl Isovalerate                                      | 108-64-5    | 6.02        | 854            | 855  | 57/60/85/88                          | STD                      | ✓ | ✓ | ✓ | ✓      |
| Isoamyl acetate                                        | 123-92-2    | 6.60        | 876            | 877  | 43/55/70                             | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl 3-hydroxybutanoate                               | 5405-41-4   | 8.27        | 944            | 937  | 43/71/87/117                         | NIST 14                  | ✗ | ✗ | ✓ | ✗      |
| 2-Ethylcyclohexyl 4-ethylbenzoate                      | -           | 9.05        | -              | 964  | 110/133/151                          | NIST 14                  | ✓ | ✗ | ✗ | ✗      |
| 4-Methylhexyl acetate                                  | 91367-59-8  | 9.25        | -              | 970  | 43/55/70                             | NIST 14                  | ✗ | ✗ | ✓ | ✗      |
| Ethyl hexanoate                                        | 123-66-0    | 10.1        | 1000           | 999  | 88/99                                | STD                      | ✓ | ✓ | ✓ | ✓      |
| Hexyl acetate                                          | 142-92-7    | 10.5        | 1011           | 1012 | 43/56/61                             | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl Hex-2-enoate                                     | 1552-67-6   | 11.4        | 1037           | 1042 | 55/73/97                             | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Ethyl 2-furoate                                        | 614-99-3    | 11.6        | 1047           | 1049 | 95/112/140                           | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Ethyl <i>DL</i> -Leucate                               | 10348-47-7  | 11.8        | 1060           | 1055 | 43/69/87                             | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Isopentyl methoxyacetate                               | -           | 12.1        | -              | 1065 | 43/45/70                             | NIST 14                  | ✗ | ✗ | ✓ | ✗      |
| Pentyl methoxyacetate                                  | 168920-35-2 | 12.1        | -              | 1065 | 45/55/70                             | NIST 14                  | ✓ | ✓ | ✗ | ✓      |
| 10-Undecenyl phenylacetate                             | -           | 13.3        | -              | 1105 | 41/55/91                             | NIST 14                  | ✓ | ✗ | ✗ | ✗      |
| Ethyl heptanoate                                       | 106-30-9    | 13.4        | 1095           | 1109 | 60/88/113                            | STD                      | ✓ | ✓ | ✓ | ✓      |
| Methyl octanoate                                       | 111-11-5    | 13.8        | 1126           | 1122 | 74/87                                | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Diethyl succinate                                      | 123-25-1    | 15.4        | 1182           | 1177 | 101/129                              | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl octanoate                                        | 106-32-1    | 16.0        | 1196           | 1197 | 88/101/127                           | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl 2-phenylacetate                                  | 101-97-3    | 17.2        | 1246           | 1240 | 65/91/164                            | STD                      | ✓ | ✓ | ✓ | ✗      |
| Diethyl 2-(1-methoxy-1-methylethoxy)-3-methylsuccinate | 88481-58-7  | 17.3        | -              | 1244 | 85/131                               | NIST 14                  | ✗ | ✗ | ✓ | ✗      |
| Isoamyl hexanoate                                      | 2198-61-0   | 17.5        | 1252           | 1251 | 43/55/70/99                          | NIST 14                  | ✓ | ✓ | ✓ | ✗      |

| Compound                      | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions ( <i>m/z</i> ) | Identification<br>method | A | B | C | French |
|-------------------------------|------------|-------------|----------------|------|--------------------------------------|--------------------------|---|---|---|--------|
| 2-Phenylethyl acetate         | 103-45-7   | 17.5        | 1258           | 1251 | 43/91/104                            | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Diethyl malate                | 7554-12-3  | 17.8        | 1271           | 1262 | 43/71/117                            | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Diethyl glutarate             | 818-38-2   | 18.2        | 1283           | 1276 | 87/115/143                           | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Ethyl nonanoate               | 123-29-5   | 19.0        | 1296           | 1305 | 55/60/88/101/115                     | STD                      | ✓ | ✓ | ✓ | ✓      |
| Methyl decanoate              | 110-42-9   | 19.4        | 1325           | 1320 | 74/87                                | NIST 14                  | ✗ | ✓ | ✗ | ✗      |
| Diethyl <i>L</i> -tartrate    | 87-91-2    | 20.0        | -              | 1343 | 76/104/133                           | NIST 14                  | ✓ | ✗ | ✓ | ✗      |
| 1-Methyldodecyl pentanoate    | 55193-13-0 | 20.8        | -              | 1373 | 85                                   | NIST 14                  | ✗ | ✗ | ✓ | ✗      |
| Vinyl decanoate               | 4704-31-8  | 21.0        | -              | 1391 | 43/155                               | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl 9-decenoate             | 67233-91-4 | 21.1        | 1387           | 1384 | 55/88/101/135                        | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Ethyl decanoate               | 110-38-3   | 21.3        | 1396           | 1392 | 88/101                               | STD                      | ✓ | ✓ | ✓ | ✓      |
| Ethyl isopentyl succinate     | 28024-16-0 | 22.1        | 1436           | 1423 | 101/129                              | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Isoamyl octanoate             | 2035-99-6  | 22.6        | 1446           | 1443 | 43/70/127                            | NIST 14                  | ✗ | ✗ | ✓ | ✓      |
| Ethyl dodecanoate             | 106-33-2   | 26.5        | 1589           | 1595 | 57/69/88/101                         | NIST 14                  | ✗ | ✗ | ✗ | ✓      |
| Ethyl tetradecanoate          | 124-06-1   | 30.6        | 1794           | 1793 | 88/101                               | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Isopropyl myristate           | 110-27-0   | 31.2        | 1827           | 1822 | 43/60/102/228                        | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Ethyl hexadecanoate           | 628-97-7   | 35.0        | 1993           | 1991 | 88/101                               | NIST 14                  | ✗ | ✗ | ✗ | ✓      |
| <b>Fatty acids</b>            |            |             |                |      |                                      |                          |   |   |   |        |
| <i>L</i> -Lactic acid         | 79-33-4    | 6.91        | -              | 889  | 43/45                                | NIST 14                  | ✓ | ✗ | ✗ | ✗      |
| Hexanoic acid                 | 142-62-1   | 9.45        | 990            | 977  | 41/60/73                             | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| Octanoic acid                 | 124-07-2   | 15.6        | 1180           | 1184 | 60/73/101                            | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| 11-Cyclopentylundecanoic acid | 6053-49-2  | 16.2        | -              | 1204 | 41/55/67                             | NIST 14                  | ✓ | ✗ | ✗ | ✗      |
| 3-Nonenoic acid               | 4124-88-3  | 16.5        | -              | 1215 | 41/55/96/138                         | NIST 14                  | ✗ | ✗ | ✓ | ✗      |
| Nonanoic acid                 | 112-05-0   | 18.0        | 1273           | 1269 | 41/60/73                             | NIST 14                  | ✓ | ✓ | ✓ | ✓      |

| Compound                        | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions ( <i>m/z</i> ) | Identification<br>method | A | B | C | French |
|---------------------------------|------------|-------------|----------------|------|--------------------------------------|--------------------------|---|---|---|--------|
| Decanoic acid                   | 334-48-5   | 20.7        | 1373           | 1369 | 60/73/129                            | NIST 14                  | ✓ | ✓ | ✓ | ✓      |
| <b><i>Furans</i></b>            |            |             |                |      |                                      |                          |   |   |   |        |
| Furfural                        | 98-01-1    | 5.45        | 833            | 832  | 95/96                                | STD                      | ✓ | ✓ | ✓ | ✓      |
| Furfuryl methanoate             | 13493-97-5 | 7.18        | 902            | 900  | 53/81/126                            | NIST 14                  | ✓ | ✓ | ✓ | ✗      |
| 5-Methyl-2-furfural             | 620-02-0   | 8.93        | 965            | 959  | 53/109/110                           | STD                      | ✓ | ✓ | ✓ | ✗      |
| <b><i>Glycols</i></b>           |            |             |                |      |                                      |                          |   |   |   |        |
| Propylene glycol                | 4254-14-2  | 4.24        | -              | -    | 43/45                                | NIST 14                  | ✓ | ✗ | ✓ | ✗      |
| <i>Di</i> -1,2-propylene glycol | 110-98-5   | 10.9        | 1038           | 1026 | 45/59/89                             | NIST 14                  | ✓ | ✗ | ✗ | ✗      |
| <i>Di</i> -1,3-propylene glycol | 2396-61-4  | 11.5        | -              | 1046 | 45/59/103                            | NIST 14                  | ✓ | ✗ | ✗ | ✗      |
| <b><i>Ketones</i></b>           |            |             |                |      |                                      |                          |   |   |   |        |
| 2-Heptanone                     | 110-43-0   | 7.20        | 891            | 901  | 58                                   | STD                      | ✓ | ✓ | ✓ | ✓      |
| 2-Furyl methyl ketone           | 1192-62-7  | 7.44        | 911            | 909  | 95/110                               | NIST 14                  | ✗ | ✗ | ✓ | ✗      |
| 2-Nonanone                      | 821-55-6   | 12.8        | 1092           | 1089 | 43/58                                | STD                      | ✓ | ✓ | ✓ | ✓      |
| 2-Undecanone                    | 112-12-9   | 19.0        | 1294           | 1305 | 58/73/85/111                         | STD                      | ✓ | ✓ | ✓ | ✓      |
| $\beta$ -Damascenone            | 23726-93-4 | 20.9        | 1386           | 1377 | 69/121                               | STD                      | ✓ | ✓ | ✓ | ✓      |
| $\alpha$ -Ionone                | 127-41-3   | 22.4        | 1420           | 1435 | 93/121/136                           | STD                      | ✓ | ✓ | ✓ | ✓      |
| <b><i>Terpenes</i></b>          |            |             |                |      |                                      |                          |   |   |   |        |
| Limonene                        | 138-86-3   | 11.3        | -              | 1028 | 67/68/93                             | STD                      | ✗ | ✗ | ✗ | ✓      |
| Eucalyptol                      | 470-82-6   | 11.4        | 1031           | 1042 | 71/81/108                            | STD                      | ✗ | ✓ | ✗ | ✗      |
| Linalool oxide                  | 5989-33-3  | 12.6        | 1069           | 1082 | 59/68/94/111                         | STD                      | ✓ | ✓ | ✓ | ✓      |
| Camphor                         | 76-22-2    | 15.2        | 1145           | 1158 | 55/81/95/108                         | STD                      | ✓ | ✓ | ✓ | ✓      |
| <b><i>Lactones</i></b>          |            |             |                |      |                                      |                          |   |   |   |        |

| Compound                              | CAS        | RT<br>(min) | Reported<br>RI | RI   | Most abundant<br>ions (m/z) | Identification<br>method | A | B | C | French |
|---------------------------------------|------------|-------------|----------------|------|-----------------------------|--------------------------|---|---|---|--------|
| Butyrolactone                         | 96-48-0    | 7.94        | 915            | 926  | 42/56/86                    | NIST 14                  | ✓ | ✗ | ✓ | ✓      |
| Whiskey lactone                       | 39212-23-2 | 19.3        | 1302           | 1316 | 41/69/87/99                 | NIST 14                  | ✓ | ✓ | ✓ | ✗      |
| <b><i>Naphatalene derivatives</i></b> |            |             |                |      |                             |                          |   |   |   |        |
| TDN                                   | -          | 20.3        | -              | 1354 | 142/157/172                 | NIST 14                  | ✓ | ✓ | ✓ | ✗      |
| <b><i>Organic acids</i></b>           |            |             |                |      |                             |                          |   |   |   |        |
| Acetic acid                           | 64-19-7    | 2.86        | 610            | -    | 43/45/60                    | NIST 14                  | ✗ | ✗ | ✓ | ✓      |
| <b><i>Phenols</i></b>                 |            |             |                |      |                             |                          |   |   |   |        |
| 3-Ethylphenol                         | 620-17-7   | 15.1        | 1169           | 1167 | 107/122                     | NIST 14                  | ✓ | ✗ | ✗ | ✗      |
| 2,4-Di-tert-butyl-5-methylphenol      | 497-39-2   | 24.0        | -              | 1500 | 57/205/220                  | NIST 14                  | ✗ | ✓ | ✗ | ✗      |
| 2,4-Di-tert-butylphenol               | 96-76-4    | 26.5        | 1519           | 1519 | 57/191/206                  | STD                      | ✗ | ✗ | ✗ | ✓      |
| 2,4-Di-tert-4-butylphenol             | 17540-75-9 | 26.9        | -              | 1623 | 57/233/247                  | NIST 14                  | ✓ | ✓ | ✓ | ✗      |

A - Cork stopper density 150-170 kg/m<sup>3</sup> with wax; B - Cork stopper density 180-200 kg/m<sup>3</sup>; C- Cork stopper density 150-170 kg/m<sup>3</sup>

Table 8. VCCs identified in the Italian white wine sealed with A, B and C stoppers

| Compound                           | CAS         | RT (min)  | Reported RI | RI   | Most abundant ions (m/z) | Identification method | A | B | C |
|------------------------------------|-------------|-----------|-------------|------|--------------------------|-----------------------|---|---|---|
| <b>Aldehydes</b>                   |             |           |             |      |                          |                       |   |   |   |
| Isobutanal                         | -           | 14.6      | -           | 1162 | 181                      | NIST 14               | ✓ | ✓ | ✓ |
| (1E)-2-Methylbutanal               | -           | 17.0      | -           | 1241 | 181/239                  | NIST 14               | ✓ | ✓ | ✓ |
| (1E)-3-Methylpentanal              | -           | 17.4      | -           | 1253 | 181/239                  | NIST 14               | ✓ | ✓ | ✓ |
| 2-Methyl-1-butanal                 | -           | 17.4+17.5 | -           | 1253 | 57/181                   | STD                   | ✓ | ✓ | ✓ |
| (1E)-4-Methylpentanal              | -           | 17.6      | -           | 1260 | 181/239                  | NIST 14               | ✓ | ✓ | ✓ |
| 3-methyl-1-butanal                 | -           | 18.9+19.1 | -           | 1300 | 181/239                  | STD                   | ✓ | ✓ | ✓ |
| Methylglyoxal                      | 618858-55-2 | 20.1      | -           | 1344 | 181                      | NIST 14               | ✓ | ✓ | ✓ |
| (1E)-4-Methylpentanal              | -           | 21.1      | -           | 1382 | 181/239                  | NIST 14               | ✓ | ✓ | ✓ |
| (1E)-3-Methylpentanal              | -           | 25.4      | -           | 1558 | 181                      | NIST 14               | ✓ | ✓ | ✓ |
| Phenylacetaldehyde                 | -           | 27.0+27.2 | -           | 1626 | 91/117/181               | NIST 14               | ✓ | ✓ | ✓ |
| (1E)-Nonanal                       | -           | 27.6      | -           | 1650 | 181/239                  | NIST 14               | ✓ | ✓ | ✓ |
| <b>Esters</b>                      |             |           |             |      |                          |                       |   |   |   |
| Ethyl 3-hydroxy-4-methylpentanoate | 40309-42-0  | 15.8      | -           | 1203 | 43/71/117                | NIST 14               | ✓ | ✓ | ✓ |
| Ethyl 5-methylnonanoate            | 116530-40-6 | 19.5      | -           | 1322 | 70/88/101                | NIST 14               | ✓ | ✓ | ✓ |
| Ethyl hexyl Butanedioate           | -           | 20.3      | -           | 1353 | 101/129                  | NIST 14               | ✓ | ✓ | ✓ |
| <b>Ketones</b>                     |             |           |             |      |                          |                       |   |   |   |
| (2E) -2-Butanone                   | -           | 15.0+15.1 | -           | 1175 | 56/181/250               | NIST 14               | ✓ | ✓ | ✓ |
| 5-Phenyl-2-pentanone               | 2235-83-8   | 15.5      | -           | 1191 | 43/104                   | NIST 14               | ✓ | ✓ | ✓ |
| trans-3-Methyl-4-octanolide        | 39638-67-0  | 16.5      | -           | 1223 | 43/71/99                 | NIST 14               | ✓ | ✓ | ✓ |
| 3-Pentanone oxime                  | 1188-11-0   | 17.0      | -           | 1239 | 181/264                  | NIST 14               | ✓ | ✓ | ✓ |
| (2E) -2-Pentanone                  | -           | 17.3      | -           | 1248 | 181/253/281              | NIST 14               | ✓ | ✓ | ✓ |
| 1,3-Dihydroxyacetone               | 934591-63-6 | 17.8+18.1 | -           | 1265 | 43/181                   | NIST 14               | ✓ | ✓ | ✓ |

| Compound                                              | CAS         | RT (min)  | Reported RI | RI   | Most abundant ions ( <i>m/z</i> ) | Identification method | A | B | C |
|-------------------------------------------------------|-------------|-----------|-------------|------|-----------------------------------|-----------------------|---|---|---|
| 2-Pentanone                                           | -           | 18.6+18.8 | -           | 1291 | 181/253                           | STD                   | ✓ | ✓ | ✓ |
| 3-Heptanone                                           | -           | 18.9      | -           | 1302 | 86/181/267                        | NIST 14               | ✓ | ✓ | ✓ |
| 3-Penten-2-one                                        | -           | 20.8+20.9 | -           | 1372 | 56/181                            | STD                   | ✓ | ✓ | ✓ |
| Cyclopentanone                                        | -           | 20.9      | -           | 1377 | 68/98/181                         | NIST 14               | ✓ | ✓ | ✓ |
| (3 <i>E</i> )-2-Methyldihydro-3(2 <i>H</i> )-furanone | -           | 21.3      | -           | 1389 | 84/98/181                         | NIST 14               | ✓ | ✓ | ✓ |
| 1,3-Dihydroxyacetone                                  | 934591-63-6 | 22.0      | -           | 1420 | 43/181                            | NIST 14               | ✓ | ✓ | ✓ |
| Cyclohexanone                                         | -           | 22.7      | -           | 1445 | 67/181                            | NIST 14               | ✓ | ✓ | ✓ |
| (2 <i>Z</i> )-4-Hydroxy-2-butanone                    | -           | 23.8      | -           | 1491 | 43/181                            | NIST 14               | ✓ | ✓ | ✓ |
| <b>Lactones</b>                                       |             |           |             |      |                                   |                       |   |   |   |
| (-)-trans-Whisky lactone                              | 39638-67-0  | 17.3      | -           | 1251 | 41/71/99                          | NIST 14               | ✓ | ✓ | ✓ |
| <b>Naphtalene derivatives</b>                         |             |           |             |      |                                   |                       |   |   |   |
| TDN                                                   | -           | 18.2      | -           | 1279 | 157/172                           | NIST 14               | ✓ | ✓ | ✓ |

A - Cork stopper density 150-170 kg/m<sup>3</sup> with wax; B - Cork stopper density 180-200 kg/m<sup>3</sup>; C- Cork stopper density 150-170 kg/m<sup>3</sup>

## 4.2. Impact of different stoppers on the volatile composition of sparkling wines

To understand the impact of different closures on the volatile profile of sparkling wine and white wines, only the 38 volatile compounds with a commercially available standard were quantified (Annex 5 and 6).

In order to study the effect of different closures on the volatile composition of sparkling wine, a Spanish sparkling wine was sealed with 2D stoppers and crown cap and analysed by HS-SPME/GC-MS at 24 months and 80 months post-bottling. At 24 months, no separation was observed between sparkling wine samples sealed with 2D stoppers from crown cap in PCA model (Figure 12. A-i). On the other hand, the PLS-DA model (Figure 16. A-ii) showed a clear distinction between the two groups, with a  $Q^2$  of 0.35. Fourteen compounds were found as potentially relevant for the discrimination (VIP score > 1) (Figure 12.A-iii), and the ones that demonstrated statistically significant differences were camphor and limonene (Table 9 and Figure 13.A). Camphor, a monoterpene characterized by *fresh*, *minty* and *camphor* descriptors, was only present in samples sealed with 2D stoppers, endorsing previous works where this volatile was only found in wines sealed with natural cork stoppers or discs (Oliveira et al. 2020). Limonene was also only detected in the Spanish sparkling wine sealed with 2D stoppers. Furthermore, the results unveiled a tendency for higher levels of esters in samples sealed with 2D stoppers, without statistical relevance.

At 80 months, a clear separation was also observed between the Spanish sparkling wine sealed with 2D stoppers and crown cap in the PLS-DA model (Figure 12.B-ii), From the 32 volatile and semi-volatile compounds quantified, nine were determined as potentially relevant for the discrimination (Figure 12.B-iii), but statistically significant differences were only found in the levels of 5-methyl-2-furfural,  $\alpha$ -ionone and camphor (Table 9 and Figure 13.B). Camphor was once again only found in wines sealed with 2D stoppers. Moreover, sparkling wines sealed with 2D stoppers demonstrated significantly lower levels of 5-methyl-2-furfural and  $\alpha$ -ionone. Besides that, the volatile profile of sparkling wine sealed with crown cap closures showed higher levels of ketones and aldehydes at this timepoint.

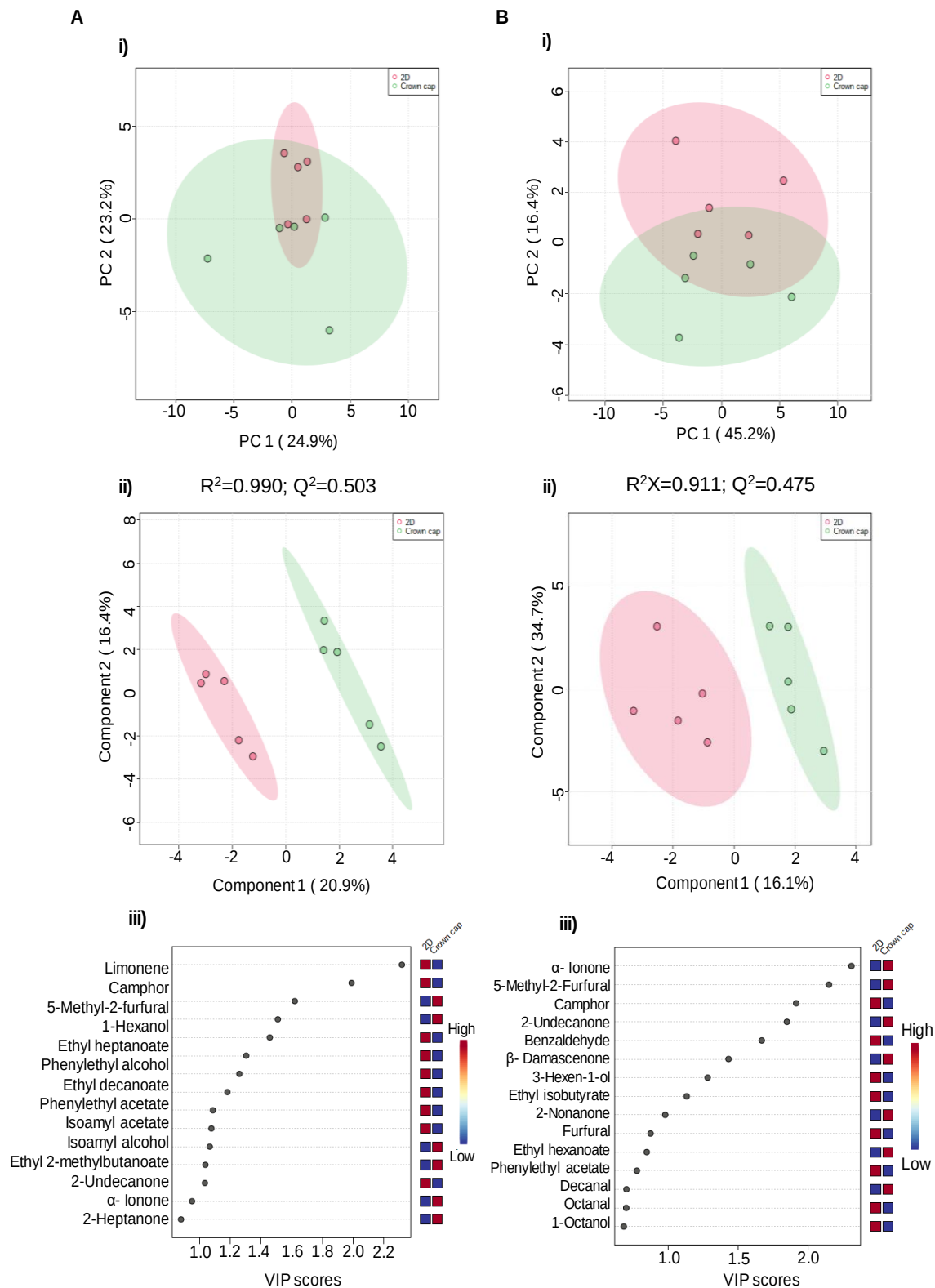


Figure 12. A- Spanish sparkling wine at 24 months sealed with 2D and crown cap stoppers: i) PCA scores scatter plot; ii) PLS-DA scores scatter plot; iii)- Important features; B- Spanish sparkling wine at 80 months sealed with 2D and crown cap stoppers: i) PCA scores scatter plot; ii) PLS-DA scores scatter plot; iii) - Important features.

Table 9. Compounds found altered in the Spanish sparkling wine sealed with 2D stoppers compared with crown cap.

| Compound                                                                                                                                             | % Variation ( <i>p</i> -value) |                          | Descriptor             | Olfactory perception threshold |
|------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|--------------------------|------------------------|--------------------------------|
|                                                                                                                                                      | 2D vs. crown cap at 24 M       | 2D vs. crown cap at 80 M |                        |                                |
| Furans (n=1)                                                                                                                                         |                                |                          |                        |                                |
| 5-Methyl-2-furfural                                                                                                                                  | -                              | -27.8±5.9(**)            | Almond, burnt sugar    | 16 mg/L                        |
| Ketones (n=1)                                                                                                                                        |                                |                          |                        |                                |
| α- Ionone                                                                                                                                            | -                              | -39.9±9.6(**)            | Fruity, violet         | 90 ng/L                        |
| Terpenes (n=2)                                                                                                                                       |                                |                          |                        |                                |
| Camphor                                                                                                                                              | ND in crown cap                | ND in crown cap          | Herbal, camphor        | -                              |
| Limonene                                                                                                                                             | BLOQ in crown cap              | -                        | Flowery, green, citrus | 200µg/L                        |
| BLOQ- Below limit of quantification; ND- Not detected; n-number<br>Statistical significance assessed using the <i>t</i> -test (** - <i>p</i> < 0.01) |                                |                          |                        |                                |

According to these results, it is possible to infer that 2D stoppers revealed a better capacity to preserve the ester content of wine, maintaining the floral, fruity aroma of sparkling wine. In addition, camphor was only found in sparkling wine sealed with 2D stoppers, contributing not only for the discrimination of sparkling wine sealed with 2D stoppers from crown cap but it might also be responsible for a pleasant aroma, favouring positively the aromatic profile.

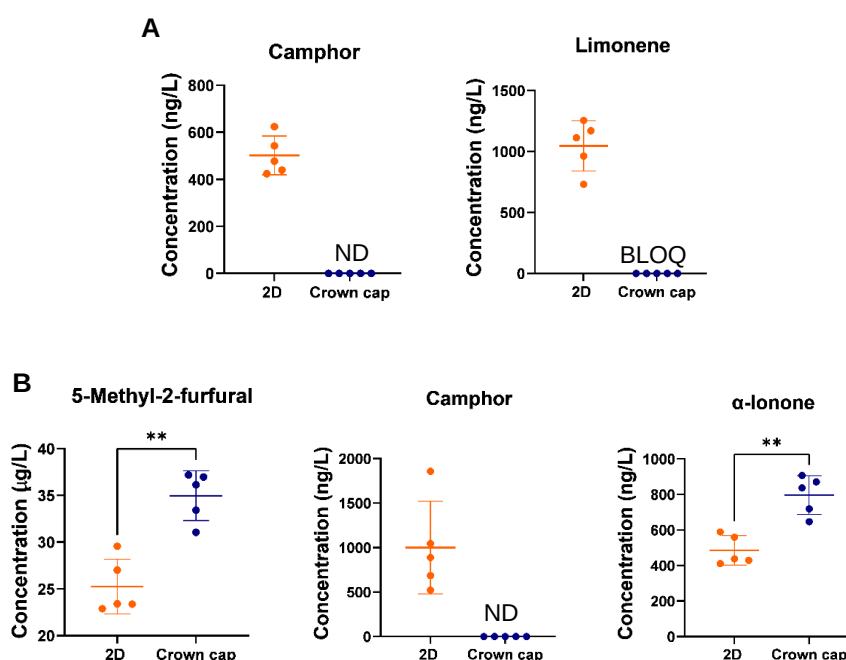


Figure 13. A - Boxplots of compounds found significantly altered in Spanish sparkling wine sealed with 2D and crown cap stoppers at 24 months; B- Boxplots of compounds found significantly altered in Spanish sparkling wine sealed with 2D and crown cap stoppers at 80 months.

In the second study, French sparkling wine was sealed with micro CSP (an agglomerated body of cork), and STO (technical stopper with a body of agglomerated cork and a disc of natural cork in one extremity). The volatile composition of this wine was measured at 18 months post-bottling. In PCA and PLS-DA models (Figure 14.A-i) and ii)), it is possible to see a clear separation between sparkling wine sealed with micro CSP compared with STO. Fourteen compounds were found as potential relevant for the discrimination (VIP score > 1), and the ones that demonstrated statistically significant differences are present in Table 10.

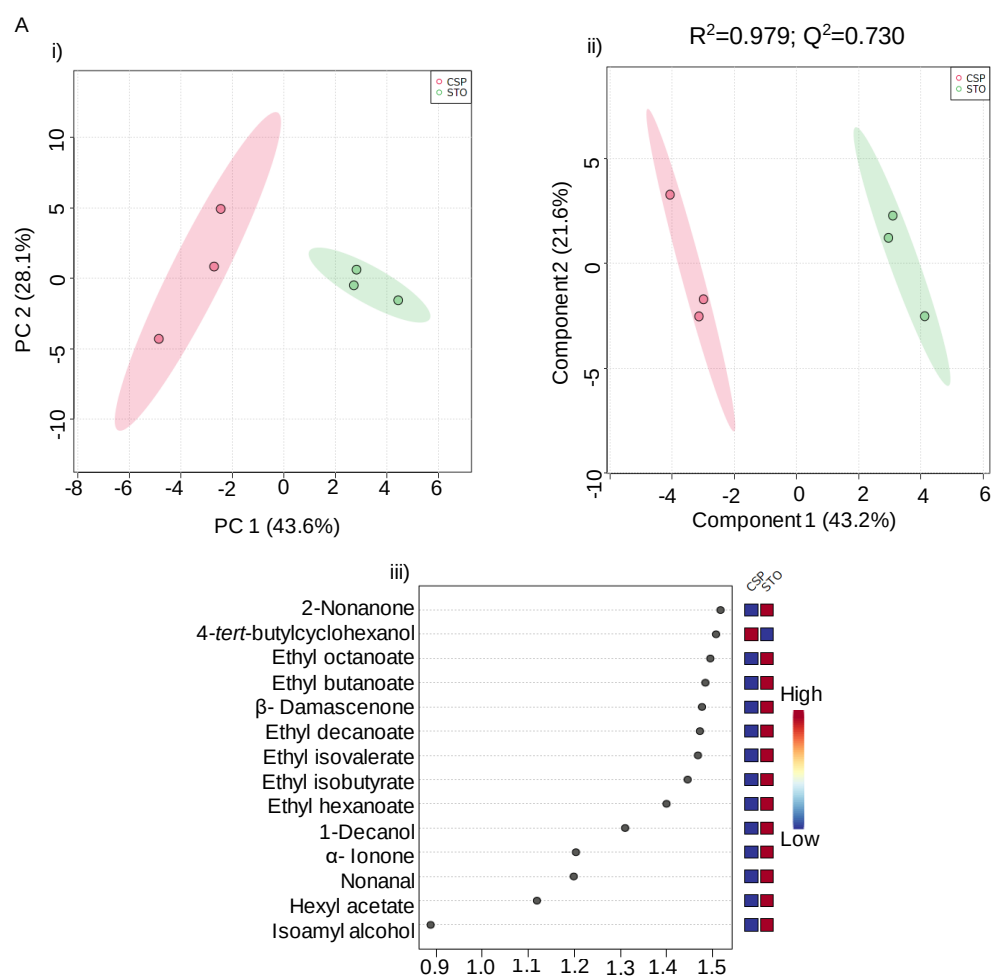


Figure 14.A – French sparkling wine at 18 months post-bottling: i) PCA scores scatter plot; ii) PLS-DA scores scatter plot; iii) Important features.

Table 10. Compounds found altered in the French sparkling wine sealed with micro CSP stoppers compared to STO stoppers.

| Compound                                                                                                                  | % Variation (p-value) | Descriptor           | Olfactory perception threshold |
|---------------------------------------------------------------------------------------------------------------------------|-----------------------|----------------------|--------------------------------|
| <b>Alcohols (n=1)</b>                                                                                                     |                       |                      |                                |
| 1-Decanol                                                                                                                 | -61.6±21.5(*)         | Floral, fruity, waxy | 400 µg/L                       |
| <b>Esters (n=5)</b>                                                                                                       |                       |                      |                                |
| Ethyl butanoate                                                                                                           | -33.8±4.53(**)        | Strawberry, apple    | 20 µg/L                        |
| Ethyl isobutyrate                                                                                                         | -40.0±7.27(**)        | Fruity               | 15 µg/L                        |
| Ethyl hexanoate                                                                                                           | -38.6±8.62(*)         | Apple, banana        | 14 µg/L                        |
| Ethyl octanoate                                                                                                           | -52.7±7.18(**)        | Pineapple, sweet     | 600 µg/L                       |
| Ethyl decanoate                                                                                                           | -66.2±12.1(**)        | Brandy, fruity       | 200 µg/L                       |
| <b>Ketones (n=2)</b>                                                                                                      |                       |                      |                                |
| 2-Nonanone                                                                                                                | -37.2±3.37(***)       | Sweet, herbal        | -                              |
| β-Damascenone                                                                                                             | -88.7±18.9(**)        | Apple, honey, smoky  | 0.05 µg/L                      |
| Statistical significance assessed using the <i>t</i> -test (*- p value ≤ 0.05; ** - p value ≤0.01; *** - p value ≤ 0.001) |                       |                      |                                |

The volatile profile of sparkling wine sealed with STO stoppers showed higher levels of 1-decanol, ethyl butanoate, ethyl isobutyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-nonanone and β-damascenone (Table 10 and Figure 15). These results suggest that STO cork stoppers conserve more efficiently some esters and other volatile compounds, maintaining the freshness and fruity aromas of sparkling wines.

Moreover, 4-*tert*-butylcyclohexanol was only detected in the sparkling wine sealed with micro CSP stoppers. This compound has been detected in the volatile composition of white wine sealed with one particular microagglomerated stopper (Oliveira et al. 2020). Hence, its presence in sparkling wine might be due to the composition of the binder used in the production of the closure. However, its impact in the evolution of still and sparkling wines is not known so far (Oliveira et al. 2020).

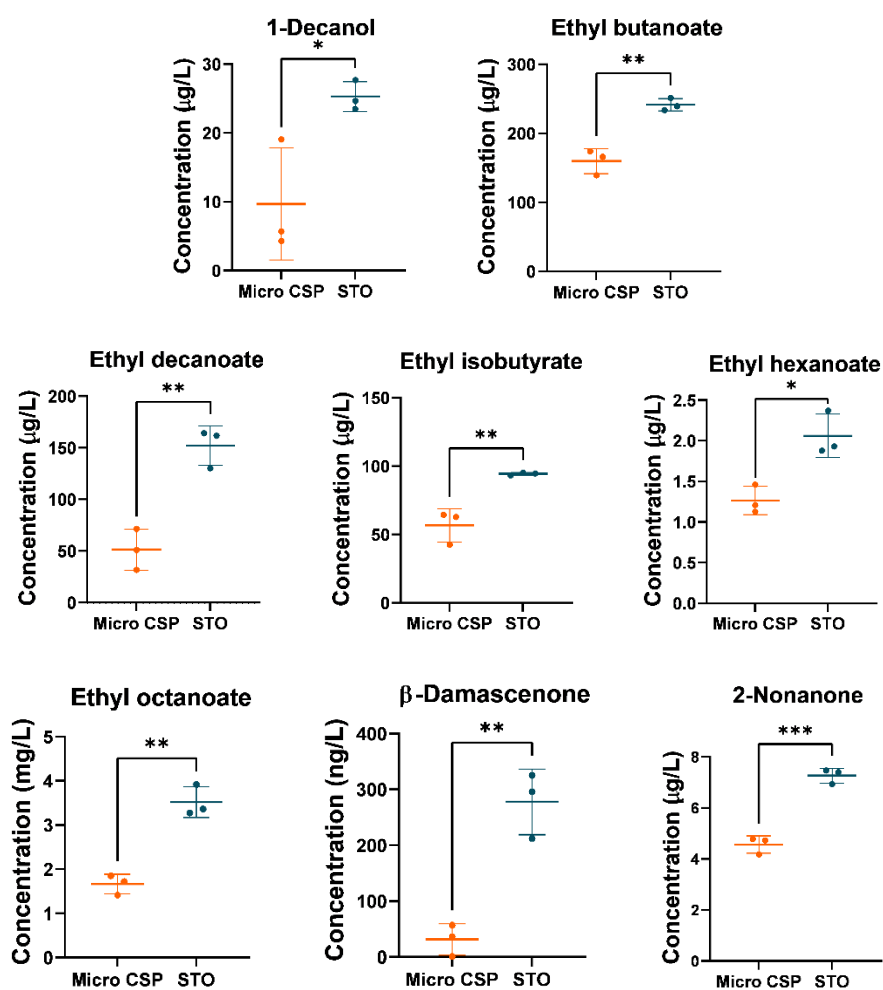


Figure 15. Boxplots of statistically significant compounds found altered between French sparkling wine sealed with Micro CSP and STO stoppers. Statistical significance assessed using the t-test (\*- p value  $\leq 0.05$ ; \*\* - p value  $\leq 0.01$ ; \*\*\* - p value  $\leq 0.001$ )

### 4.3. Impact of different cork stoppers on the volatile composition of white wines

Multivariate analysis was applied to investigate differences in the volatile composition among wines sealed with A (cork stoppers with granules with a density of 150-170 kg/m<sup>3</sup> with wax), B (cork stopper with granules that presented a density of 180-200 kg/m<sup>3</sup>) and C (cork stoppers with granules with a density of 150-170 kg/m<sup>3</sup>) cork stoppers. The values of the respective concentrations of the volatile compounds of each stopper used for multivariate analysis can be found in Annex 5. No separation was observed among groups in the PCA scores scatter plot (Figure 16.A-i)). Contrarily, the PLS-DA models demonstrated a separation between A vs. C cork stoppers (Figure 16.A-i)) and B vs. C cork stoppers (Figure 16.B-i)) with high predictive abilities ( $Q^2$ ). Due to a lack of separation between A and B cork stoppers in PLS-DA model, this group was not

considered for further interpretation.

The volatile compounds with a VIP score higher than 1 were considered relevant to discriminate wines sealed with different closures. A vs. C stoppers presented 20 compounds with a VIP score  $> 1$  (Figure 16.B-ii)), while B vs. C stoppers showed 17 relevant compounds (Figure 16.C-ii)). To confirm the importance of those compounds, a univariate analysis was performed and the ones with statistically significant differences are presented in Table 11. Figure 17 represents boxplots of some of the compounds reported as statistically significant for the discrimination of wine samples.

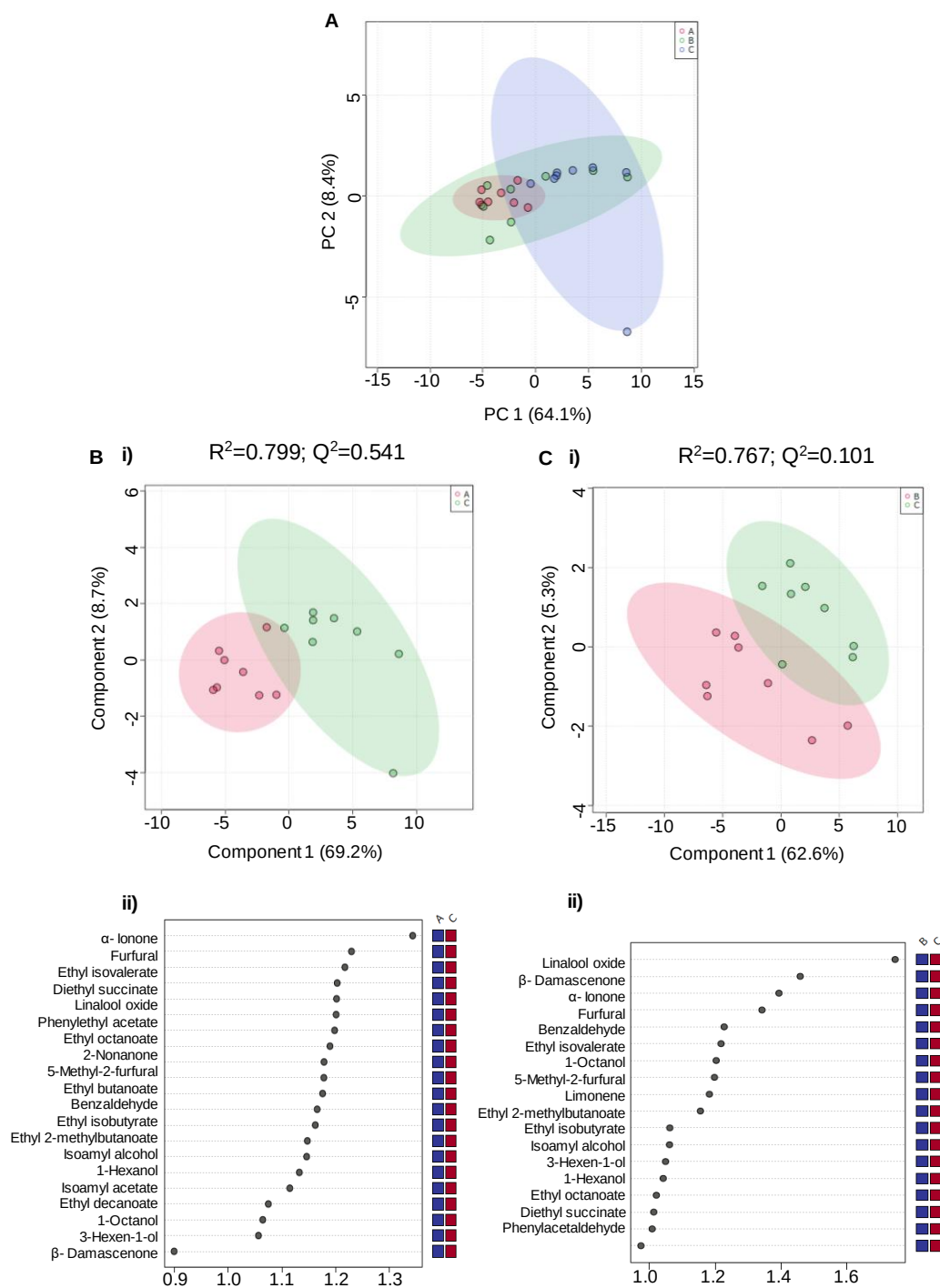


Figure 16. A- PCA of an Italian white wine sealed with A, B and C stoppers; B- Italian white wine sealed with A and C stoppers and their respective i) PLS-DA scores scatter plot, ii) Important features; C- Italian white wine sealed with A and B stoppers and their respective i) PLS-DA scores scatter plot and ii) Important features.

Table 11. Table of compounds found statistically significant between Italian white wine sealed with different stoppers (A, B and C).

| Compound                | % Variation ( <i>p</i> -value) |                 | Descriptor            | Olfactory perception threshold |
|-------------------------|--------------------------------|-----------------|-----------------------|--------------------------------|
|                         | C vs A                         | C vs B          |                       |                                |
| <b>Alcohols (n=4)</b>   |                                |                 |                       |                                |
| Isoamyl Alcohol         | 33.2 ± 7.34 (***)              | -               | Fusel, cheese         | 30 mg/L                        |
| 3-Hexen-1-ol            | 30.6 ± 8.33 (**)               | -               | Green, bitter, fatty  | 400 µg/L                       |
| 1-Hexanol               | 36.6 ± 8.24 (***)              | -               | Grass, resin          | 8 mg/L                         |
| 1-Octanol               | 65.5 ± 15.2 (**)               | -               | Citrus, roses         | 120 µg/L                       |
| <b>Aldehydes (n=1)</b>  |                                |                 |                       |                                |
| Benzaldehyde            | 31.1 ± 6.62 (***)              | 10.3 ± 10.5 (*) | Almond, bitter        | 1-2 mg/L                       |
| <b>Esters (n=9)</b>     |                                |                 |                       |                                |
| Ethyl isobutyrate       | 40.7 ± 8.39 (***)              | -               | Fruity                | 15 µg/L                        |
| Ethyl butanoate         | 42.0 ± 8.82 (***)              | -               | Strawberry, apple     | 20 µg/L                        |
| Ethyl 2-methylbutanoate | 41.0 ± 8.74 (**)               | -               | Apple                 | 18 µg/L                        |
| Ethyl isovalerate       | 39.6 ± 7.09 (***)              | 16.1 ± 12.5 (*) | Sweet, fruity         | 3 µg/L                         |
| Isoamyl acetate         | 40.8 ± 9.40 (***)              | -               | Banana, sweet, fruity | 30 µg/L                        |
| Diethyl succinate       | 29.9 ± 5.81 (***)              | -               | Fruity                | 200 mg/L                       |
| Ethyl octanoate         | 78.6 ± 12.8 (****)             | -               | Pineapple, sweet      | 600 µg/L                       |
| Phenylethyl acetate     | 28.6 ± 5.61 (****)             | -               | Rose, violet          | 250 µg/L                       |
| Ethyl decanoate         | 118 ± 22.4 (***)               | -               | Brandy, fruity        | 200 µg/L                       |
| <b>Ketones (n=3)</b>    |                                |                 |                       |                                |
| 2-Nonanone              | 40.7 ± 8.05 (***)              | -               | Sweet, herbal         | -                              |
| β-Damascenone           | 62.6 ± 20.2 (**)               | 19.5 ± 24.7 (*) | Apple, honey, smoky   | 0.05 µg/L                      |
| α-Ionone                | 43.8 ± 4.72 (****)             | 19.2 ± 11.2 (*) | Fruity, violet        | 90 ng/L                        |
| <b>Terpenes (n=1)</b>   |                                |                 |                       |                                |
| Linalool oxide          | 11.7 ± 2.47 (****)             | 2.14 ± 3.4 (**) | Citrus, green         | 25 µg/L                        |
| <b>Furans (n=2)</b>     |                                |                 |                       |                                |
| Furfural                | 31.3 ± 5.60 (****)             | 10.6 ± 9.24 (*) | Almond, bread         | 14.1 mg/L                      |
| 5-Methyl-2-furfural     | 26.3 ± 5.55 (***)              | -               | Almond, burnt sugar   | 16 mg/L                        |

n-number; Statistical significance assessed using the *t*-test (\* - *p* < 0.05; \*\* - *p* < 0.01; \*\*\* - *p* < 0.001; \*\*\*\* - *p* < 0.0001)

A - Cork stopper density 150-170 kg/m<sup>3</sup> with wax; B - Cork stopper density 180-200 kg/m<sup>3</sup>; C- Cork stopper density 150-170 kg/m<sup>3</sup>

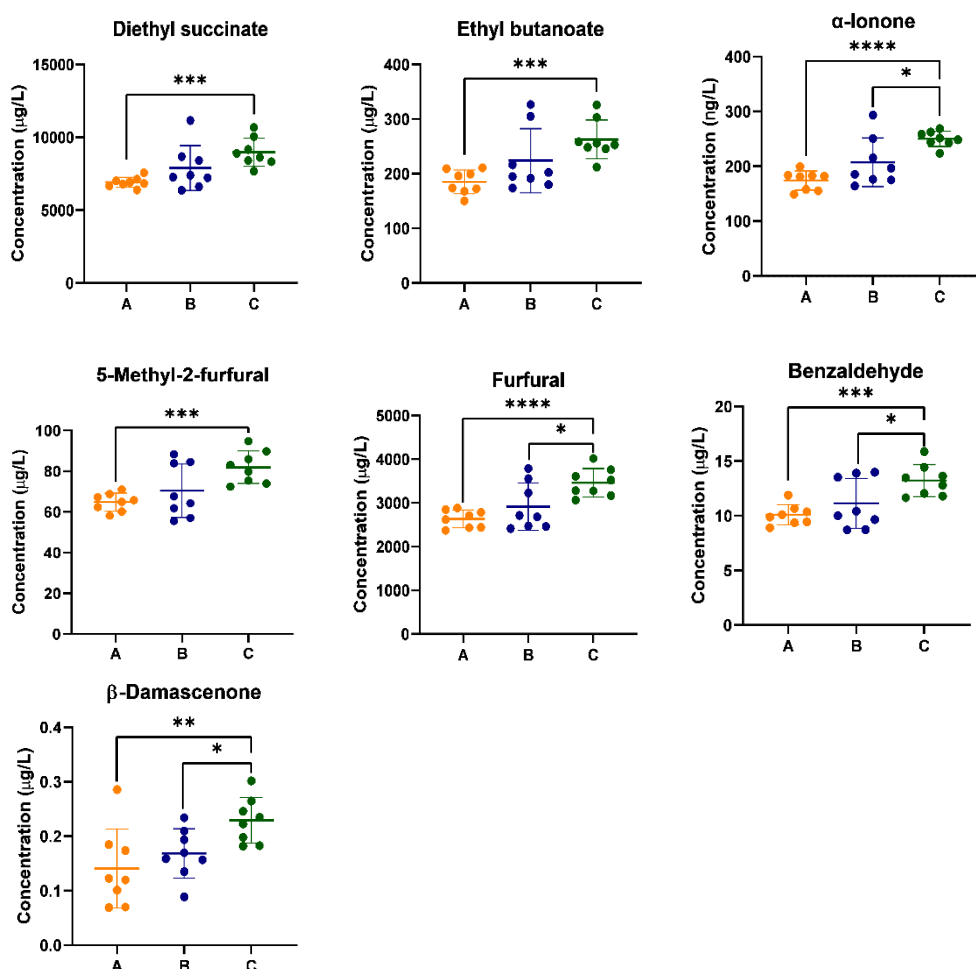


Figure 17. Boxplots of statistically significant compounds found altered between Italian white wine sealed with different cork stoppers (A, B and C). Statistical significance assessed using the t-test (\* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; \*\*\*\* -  $p < 0.0001$ ).

In the heatmaps (Figure 18.A and B), the concentration of the volatile compounds in each wine are displayed, being compounds in red at higher concentration and in blue at lower concentration. Overall, the Italian wine sealed with C stoppers showed higher levels of almost all compounds, mainly esters and alcohols.

At 44 months post-bottling, the greatest differences were obtained for wines sealed with C stoppers in comparison with wines sealed with A stoppers. Of 41 compounds quantified, 20 demonstrated statistically significant differences. Esters were the group that showed more changes, with greater amounts of ethyl isobutyrate, ethyl butanoate, ethyl 2-methylbutanoate, isoamyl acetate, diethyl succinate, ethyl octanoate, phenylethyl acetate, ethyl isovalerate and ethyl decanoate in wines under C stoppers.

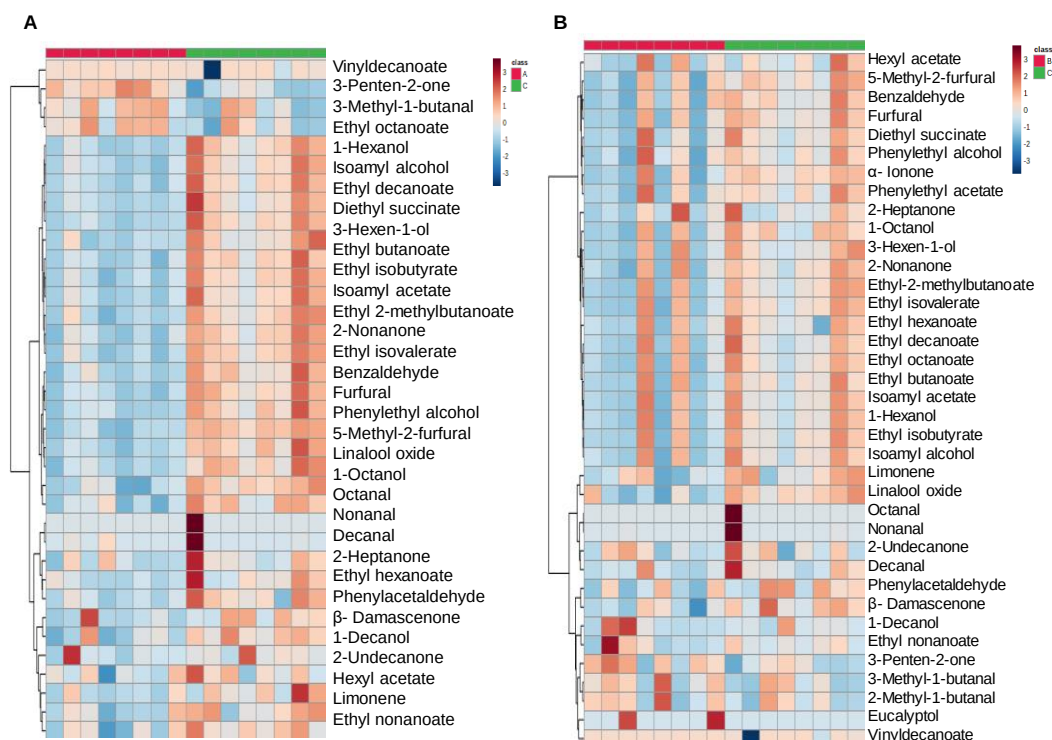


Figure 18.A- Heatmap of the Italian white wine sealed with A and C stoppers; B- Heatmap of the Italian white wine sealed with B and C stoppers. Columns correspond to each sample while rows correspond to the concentration of each discriminant volatile coloured from minimum (−1, dark blue) to maximum (1, dark red).

A reduction in the amount of esters in white wines is attributed to the hydrolysis of the esters (Moreira et al. 2018). However, these differences in the levels of esters in group A might be explained, on one hand by a higher capacity of A stoppers to scalp compounds due to the polarity of the wax, or on the other hand by the permeability of A stoppers to oxygen leading to the loss of ethyl esters. In literature, a reduction in the levels of ethyl octanoate, ethyl decanoate and isoamyl acetate were reported for Chardonnay white wine under semi-oxidative and forced oxidative conditions (Patrianakou and Roussis 2013).

Wines sealed with C stoppers in comparison with B demonstrated statistically significant higher levels of six compounds, namely, benzaldehyde, ethyl isovalerate,  $\beta$ -damascenone, linalool oxide,  $\alpha$ -ionone, and furfural. Camphor has been present in wines sealed with natural cork stoppers or discs wines (Oliveira et al. 2020) and, therefore, it was identified in all wines.

## Chapter 5. Conclusions

The consumption of sparkling wines has increased over the past few years. However, a lack of information regarding the changes that occur in the volatile composition of sparkling wine during ageing in bottle is recognisable, as well as the impact of different closures in the evolution of aroma compounds after bottling.

The study of the volatile composition of two Italian and one Spanish sparkling wines during ageing unveiled significant differences in the level of 7 volatile compounds in common among all three sparkling wines. Overall, diethyl succinate, phenylacetaldehyde, isoamyl alcohol, furfural, ethyl butanoate,  $\alpha$ -ionone and ethyl isovalerate showed an increase in their concentration over time, while only ethyl decanoate unveiled a decrease.

An increase in the concentration of these volatile compounds might be justified by chemical reactions, for example, degradation of carotenoids, hydrolysis between volatile (e.g.  $\beta$ -damascenone) and sugars, alterations in the equilibrium of acid-esters, autolysis of lees or reactions of oxidation. On the other hand, a decrease in the content of ethyl decanoate can be explained by a reaction of hydrolysis to produce decanoic acid and ethanol. These 6 compounds identified, namely diethyl succinate, ethyl isovalerate, furfural, isoamyl alcohol,  $\alpha$ -ionone, and ethyl butanoate might be indicators of long ageing. In addition, ethyl decanoate was identified as possible indicator of young sparkling wines once its concentration decreased over time. Hence, the duration of ageing will determine not only the amount but also the number of volatile compounds present in sparkling wines, since some compounds were not detected after a period of time, namely,  $\beta$ -linalool, 1,4-cineole,  $\beta$ -cyclocitral (in Italian sparkling wine A) and eucalyptol (in Italian sparkling wine B). Overall, the content of esters and terpenes decreased during ageing, which indicates a loss of freshness and fruity notes over time. On the other hand, ketones, furans and alcohols unveiled an increase during ageing, contributing negatively to the aroma of this sparkling wines.

This work also demonstrated that the type of closure has an impact in the evolution of volatile compounds in sparkling and white wines several months post-bottling. Overall, Spanish sparkling wine sealed with 2D cork stoppers compared with crown cap stoppers showed a tendency for higher levels of esters (ethyl heptanoate, ethyl decanoate, phenylethyl acetate and isoamyl acetate at 24 months of storage and ethyl isobutyrate and phenylethyl acetate at 80 months of storage) revealing a better capacity to preserve the fruity aromas. Camphor was the compound that most contribute to differentiate samples, being specifically found in sparkling wine sealed with 2D stoppers. Crown cap stoppers revealed a lower effectiveness in preserving the aroma

composition of sparkling wine after 80 months post-bottling.

The effectiveness of a microagglomerated cork stopper (micro CSP) and a technical cork stopper with a disc of natural cork in one extremity (STO) in preserving the aroma compounds of a French sparkling wine was also evaluated at 18 months post-bottling. The results unveiled statistically significant differences in the concentration of 8 compounds, namely, 1-decanol, ethyl butanoate, ethyl isobutyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-nonanone and  $\beta$ -damascenone. These volatile compounds were found in higher concentrations in sparkling wines sealed with STO stoppers, demonstrating a better capacity of this closures to preserve the volatile composition, maintaining the freshness and fruity aromas.

Finally, the volatile composition of an Italian white wine sealed with three types of cork stoppers differing in density and the presence of wax was evaluated after 44 months of storage in bottle. The results unveiled larger differences in volatile composition of wines sealed with cork stoppers formed with granules of a density 150-170 kg/m<sup>3</sup> with wax (A) compared with cork stoppers constituted of granules with a density of 150-170 kg/m<sup>3</sup> without wax (C). Esters were the class of volatiles most affected, presenting significant lower levels in wines sealed with cork stoppers formed of granules of lower density (150-170 kg/m<sup>3</sup>) and wax (A cork stoppers). One possible explanation may be a higher affinity of A stoppers to scalp these volatiles or higher oxygen transmission rate through the stopper. Besides that, other classes of compounds also demonstrated statistically significant differences in a smaller extent, namely, alcohols (isoamyl alcohol, 3-hexen-1-ol, 1-hexanol and 1-octanol), ketones (2-nonanone,  $\alpha$ -ionone and  $\beta$ -damascenone), furans (furfural and 5-methyl-2-furfural), terpenes (linalool oxide) and aldehydes (benzaldehyde), which were found in higher amounts in white wines under C cork stoppers in comparison to wines under A stoppers. In addition, Italian white wine sealed with cork stoppers with a density of 180-200 kg/m<sup>3</sup> (B) in comparison to cork stoppers with a density of 150-170 kg/m<sup>3</sup> (C) revealed statistically significant differences in the levels of 6 compounds, such as, benzaldehyde, ethyl isovalerate,  $\beta$ -damascenone, linalool oxide,  $\alpha$ -ionone and furfural.

Future studies should entail the analysis of volatile composition in sparkling wines produced using other grape varieties to investigate the behaviour of other volatile compounds specific from grapes. In addition, more studies focused on the impact of different closures are needed to obtain a deeper understanding of their role on the evolution of aroma compounds in sparkling and white wines and select the best sealing for ageing in bottle.

## References

- AMORIM CORK, AMORIM. 'Neutrocork', Accessed 01-07-2021. <https://www.amorimcork.com/pt/portfolio/d/neutrocork/226/>.
- APCOR-1. 'Rolhas de Champanhe', Accessed May. <https://www.apcor.pt/produtos/rolhas/rolhas-de-champanhe/>.
- APCOR-2. 'Technical cork stoppers', Accessed 15 February. <https://www.apcor.pt/en/products/cork-stoppers/technical-cork-stoppers/>.
- APCOR-3. 'Micro-granulated cork stopper', Accessed February. <https://www.apcor.pt/en/products/cork-stoppers/micro-granulated-cork-stopper/>.
- Barros, Elisabete Paula, Nathalie Moreira, Giuliano Elias Pereira, Selma Gomes Ferreira Leite, Claudia Moraes Rezende, and Paula Guedes de Pinho. 2012. 'Development and validation of automatic HS-SPME with a gas chromatography-ion trap/mass spectrometry method for analysis of volatiles in wines', *Talanta*, 101: 177-86.
- Blazquez, Rodrigo. 2004. 'Yeast create other alcohols aside from ethanol; these are fundamental component of brandy aroma', Accessed October. <https://waterhouse.ucdavis.edu/whats-in-wine/higher-alcohols>.
- Bosch-Fusté, Joan, Montserrat Riu-Aumatell, Josep M Guadayol, Josep Caixach, Elvira López-Tamames, and Susana Buxaderas. 2007. 'Volatile profiles of sparkling wines obtained by three extraction methods and gas chromatography–mass spectrometry (GC–MS) analysis', *Food chemistry*, 105: 428-35.
- Boudaoud, Nawal, and Luc Eveleigh. 2003. 'A new approach to the characterization of volatile signatures of cork wine stoppers', *Journal of Agricultural and Food Chemistry*, 51: 1530-33.
- Bullock, John D, Jennifer P Wang, and Gretchen H Bullock. 1998. 'Was dom perignon really blind?', *Survey of ophthalmology*, 42: 481-86.
- Buxaderas, Susana, and Elvira López-Tamames. 2012. 'Sparkling wines: features and trends from tradition', *Advances in food and nutrition research*, 66: 1-45.
- Callejón, RM, C Ubeda, R Ríos-Reina, ML Morales, and AM Troncoso. 2016. 'Recent developments in the analysis of musty odour compounds in water and wine: A review', *Journal of Chromatography A*, 1428: 72-85.
- Campo, Eva, Juan Cacho, and Vicente Ferreira. 2008. 'The chemical characterization of the aroma of dessert and sparkling white wines (Pedro Ximénez, Fino, Sauternes, and cava) by gas chromatography– olfactometry and chemical quantitative analysis', *Journal of Agricultural and Food Chemistry*, 56: 2477-84.
- Capone, D, MA Sefton, IS Pretoius, and PB Høj. 2003. 'Flavour'scalping'by wine bottle closures–the'winemaking'continues post vineyard and winery', *Australian and New Zealand Wine Industry Journal*, 18: 16-20.
- CASCO, JOEY. 2014. 'A bubbly biography-The story of sparkling wine-Part 1: France and Spain', Accessed February.

<https://www.thewinestalker.net/2014/10/bubblybio1.html>.

Champagne-1, Comité. 'Appellation d'Origine Controlée (AOC)', Accessed 29-06-2021. <https://www.champagne.fr/en/terroir-appellation/appellation/appellation-origine-controlee-aoc>.

Champagne-2. 'Recognition of the Champagne Appellation', Accessed 29-06-2021. <https://www.champagne.fr/en/terroir-appellation/appellation/recognition-of-the-champagne-appellation>.

Chauhan, Ashish, Manish Kumar Goyal, and Priyanka Chauhan. 2014. 'GC-MS technique and its analytical applications in science and technology', *J. Anal. Bioanal. Tech*, 5: 222.

Chromacademy. 'GC introduction', Accessed November. <https://www.chromacademy.com/channels/gc/principles/gc-introduction/>.

Chromacademy. 'GC sampling techniques-headspace', Accessed 31/05/2021. <https://www.chromacademy.com/channels/gc-training-courses/technique/gc-sampling-techniques-headspace/>.

Coelho, Elisabete, Manuel A Coimbra, JMF Nogueira, and Sílvia M Rocha. 2009. 'Quantification approach for assessment of sparkling wine volatiles from different soils, ripening stages, and varieties by stir bar sorptive extraction with liquid desorption', *Analytica Chimica Acta*, 635: 214-21.

Coelho, Elisabete, Ana Reis, M Rosário M Domingues, Sílvia M Rocha, and Manuel A Coimbra. 2011. 'Synergistic effect of high and low molecular weight molecules in the foamability and foam stability of sparkling wines', *Journal of Agricultural and Food Chemistry*, 59: 3168-79.

Coelho, Elisabete Verde Martins. 2010. 'Relação do aroma e da espuma dos vinhos espumantes com o potencial enológico das uvas e dos vinhos', Aveiro.

The Good Scents Company, The Good Scents. Accessed 1-07-2021. <http://www.thegoodscentscompany.com/>.

Culleré, L, J Cacho, and V Ferreira. 2009. 'Comparative study of the aromatic profile of different kinds of wine cork stoppers', *Food chemistry*, 112: 381-87.

Culleré, Laura, Juan Cacho, and Vicente Ferreira. 2007. 'An assessment of the role played by some oxidation-related aldehydes in wine aroma', *Journal of Agricultural and Food Chemistry*, 55: 876-81.

Di Gianvito, Paola, Giorgia Perpetuini, Fabrizia Tittarelli, Maria Schirone, Giuseppe Arfelli, Andrea Piva, Francesca Patrignani, Rosalba Lanciotti, Lino Olivastri, and Giovanna Suzzi. 2018. 'Impact of *Saccharomyces cerevisiae* strains on traditional sparkling wines production', *Food Research International*, 109: 552-60.

Díaz-Maroto, M Consuelo, Rémi Schneider, and Raymond Baumes. 2005. 'Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging', *Journal of Agricultural and Food Chemistry*, 53: 3503-09.

Esteruelas, Mireia, Elena González-Royo, Nikolaos Kontoudakis, Antonio Orte, Antoni Cantos, Joan Miquel Canals, and Fernando Zamora. 2015. 'Influence of grape

maturity on the foaming properties of base wines and sparkling wines (Cava)', *Journal of the Science of Food and Agriculture*, 95: 2071-80.

Ferreira, Antonio César Silva, Judith Monteiro, Carla Oliveira, and Paula Guedes de Pinho. 2008. 'Study of major aromatic compounds in port wines from carotenoid degradation', *Food chemistry*, 110: 83-87.

Flavornet. Accessed 1-07-2021. <https://www.flavornet.org/flavornet.html>.

Forleo, Tiziana, Alessandro Zappi, Fernando Gottardi, and Dora Melucci. 2020. 'Rapid discrimination of Italian Prosecco wines by head-space gas-chromatography basing on the volatile profile as a chemometric fingerprint', *European Food Research and Technology*, 246: 1805-16.

Gallardo-Chacon, Joan J, Stefania Vichi, Elvira Lopez-Tamames, and Susana Buxaderas. 2010. 'Changes in the sorption of diverse volatiles by *Saccharomyces cerevisiae* lees during sparkling wine aging', *Journal of Agricultural and Food Chemistry*, 58: 12426-30.

Garofalo, Carmela, Carmen Berbegal, Francesco Grieco, Maria Tufariello, Giuseppe Spano, and Vittorio Capozzi. 2018. 'Selection of indigenous yeast strains for the production of sparkling wines from native Apulian grape varieties', *International journal of food microbiology*, 285: 7-17.

Henschen, Charles. 2015. 'A diverse group of aromatic substances: Norisoprenoids', Accessed October. <https://waterhouse.ucdavis.edu/whats-in-wine/norisoprenoids>.

Hidalgo, P, E Pueyo, MA Pozo-Bayón, AJ Martínez-Rodríguez, P Martin-Alvarez, and MC Polo. 2004. 'Sensory and analytical study of rosé sparkling wines manufactured by second fermentation in the bottle', *Journal of Agricultural and Food Chemistry*, 52: 6640-45.

Jagatić Korenika, Ana-Marija, Darko Preiner, Ivana Tomaz, and Ana Jeromel. 2020. 'Volatile Profile Characterization of Croatian Commercial Sparkling Wines', *Molecules*, 25: 4349.

Kemp, Belinda, Hervé Alexandre, Bertrand Robillard, and Richard Marchal. 2015. 'Effect of production phase on bottle-fermented sparkling wine quality', *Journal of Agricultural and Food Chemistry*, 63: 19-38.

Lengyel, Ecaterina, and László Sikolya. 2015. 'The Influence of Aroma Compounds on Sensorial Traits of Wines From the Apold Depression', *Management of Sustainable Development*, 7: 23.

Ling, Meng-Qi, Han Xie, Yu-Bo Hua, Jian Cai, Si-Yu Li, Yi-Bin Lan, Ruo-Nan Li, Chang-Qing Duan, and Ying Shi. 2019. 'Flavor profile evolution of bottle aged rosé and white wines sealed with different closures', *Molecules*, 24: 836.

Liu, N, Y-Y Song, G-F Dang, D-Q Ye, X Gong, and Y-L Liu. 2015. 'Effect of wine closures on the aroma properties of Chardonnay wines after four years of storage', *South African Journal of Enology and Viticulture*, 36: 296-303.

Lopes, Paulo, Maria A Silva, Alexandre Pons, Takatoshi Tominaga, Valérie Lavigne, Cedric Saucier, Philippe Darriet, Pierre-Louis Teissedre, and Denis Dubourdieu. 2009. 'Impact of oxygen dissolved at bottling and transmitted through closures on the

composition and sensory properties of a Sauvignon blanc wine during bottle storage', *Journal of Agricultural and Food Chemistry*, 57: 10261-70.

Marín-San Román, Sandra, Pilar Rubio-Bretón, Eva P Pérez-Álvarez, and Teresa Garde-Cerdán. 2020. 'Advancement in analytical techniques for the extraction of grape and wine volatile compounds', *Food Research International*: 109712.

Martínez-García, Rafael, Juan Carlos Mauricio, Teresa García-Martínez, Rafael A Peinado, and Juan Moreno. 2021. 'Towards a better understanding of the evolution of odour-active compounds and the aroma perception of sparkling wines during ageing', *Food chemistry*, 357: 129784.

Martínez-García, Rafael, Juan Moreno, Andrea Bellincontro, Luna Centioni, Anna Puig-Pujol, Rafael A Peinado, Juan Carlos Mauricio, and Teresa García-Martínez. 2021. 'Using an electronic nose and volatilome analysis to differentiate sparkling wines obtained under different conditions of temperature, ageing time and yeast formats', *Food chemistry*, 334: 127574.

Martínez-Lapuente, Leticia, Zenaida Guadalupe, Belén Ayestarán, Miriam Ortega-Heras, and Silvia Pérez-Magariño. 2013. 'Sparkling wines produced from alternative varieties: sensory attributes and evolution of phenolics during winemaking and aging', *American Journal of Enology and Viticulture*, 64: 39-49.

Mayr, Christine M, Dimitra L Capone, Kevin H Pardon, Cory A Black, Damian Pomeroy, and I Leigh Francis. 2015. 'Quantitative analysis by GC-MS/MS of 18 aroma compounds related to oxidative off-flavor in wines', *Journal of Agricultural and Food Chemistry*, 63: 3394-401.

MetaboAnalyst. 'MetaboAnalyst5.0 - user-friendly, streamlined metabolomics data analysis', Accessed 10-05-2021. <https://dev.metaboanalyst.ca/MetaboAnalyst/upload/StatUploadView.xhtml>.

Moreira, Nathalie, Ana Margarida Araújo, Frank Rogerson, Isabel Vasconcelos, Victor De Freitas, and Paula Guedes de Pinho. 2019. 'Development and optimization of a HS-SPME-GC-MS methodology to quantify volatile carbonyl compounds in Port wines', *Food chemistry*, 270: 518-26.

Moreira, Nathalie, P Lopes, H Ferreira, M Cabral, and P Guedes de Pinho. 2018. 'Sensory attributes and volatile composition of a dry white wine under different packing configurations', *Journal of food science and technology*, 55: 424-30.

OIV-1. 'Special wines-Sparkling wines', Accessed February <https://www.oiv.int/public/medias/3927/e-code-i-44.pdf>.

OIV-2. 'Base wine', Accessed 29-06-2021. <https://www.oiv.int/public/medias/3646/e-code-ii-41-411.pdf>.

OIV 2020. 'OIV Focus-The global sparkling market', Accessed 15/02/2021. <https://www.oiv.int/public/medias/7291/oiv-sparkling-focus-2020.pdf>.

Oliveira, Ana Sofia, Isabel Furtado, Maria de Lourdes Bastos, Paula Guedes de Pinho, and Joana Pinto. 2020. 'The influence of different closures on volatile composition of a white wine', *Food Packaging and Shelf Life*, 23: 100465.

Panighel, Annarita, and Riccardo Flamini. 2014. 'Applications of solid-phase

microextraction and gas chromatography/mass spectrometry (SPME-GC/MS) in the study of grape and wine volatile compounds', *Molecules*, 19: 21291-309.

Patrianakou, M, and IG Roussis. 2013. 'Decrease of wine volatile aroma esters by oxidation', *South African Journal of Enology and Viticulture*, 34: 241-45.

Pawliszyn, Janusz. 1997. *Solid phase microextraction: theory and practice* (John Wiley & Sons).

Pérez-Coello, MS, MA González-Viñas, E Garcia-Romero, MC Diaz-Maroto, and MD Cabezudo. 2003. 'Influence of storage temperature on the volatile compounds of young white wines', *Food control*, 14: 301-06.

Pérez-Magariño, Silvia, Miriam Ortega-Heras, Marta Bueno-Herrera, Leticia Martínez-Lapuente, Zenaida Guadalupe, and Belén Ayestarán. 2015. 'Grape variety, aging on lees and aging in bottle after disgorging influence on volatile composition and foamability of sparkling wines', *LWT-Food Science and Technology*, 61: 47-55.

Pinto, Joana, Ana Sofia Oliveira, Joana Azevedo, Victor De Freitas, Paulo Lopes, Isabel Roseira, Miguel Cabral, and Paula Guedes de Pinho. 2018. 'Assessment of oxidation compounds in oaked Chardonnay wines: A GC–MS and 1H NMR metabolomics approach', *Food chemistry*, 257: 120-27.

Rapp, A. 1998. 'Volatile flavour of wine: correlation between instrumental analysis and sensory perception', *Food/Nahrung*, 42: 351-63.

Riu-Aumatell, M, J Bosch-Fusté, E López-Tamames, and S Buxaderas. 2006. 'Development of volatile compounds of cava (Spanish sparkling wine) during long ageing time in contact with lees', *Food chemistry*, 95: 237-42.

Robinson, Anthony L, Paul K Boss, Peter S Solomon, Robert D Trengove, Hildegard Heymann, and Susan E Ebeler. 2014. 'Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts', *American Journal of Enology and Viticulture*, 65: 1-24.

Ruiz-Moreno, María José, José Manuel Muñoz-Redondo, Francisco Julián Cuevas, Almudena Marrufo-Curtido, Juan Manuel León, Pilar Ramírez, and José Manuel Moreno-Rojas. 2017. 'The influence of pre-fermentative maceration and ageing factors on ester profile and marker determination of Pedro Ximenez sparkling wines', *Food chemistry*, 230: 697-704.

Sánchez-Palomo, Eva, M Consuelo Diaz-Maroto, and M Soledad Perez-Coello. 2005. 'Rapid determination of volatile compounds in grapes by HS-SPME coupled with GC–MS', *Talanta*, 66: 1152-57.

Shellie, R. A. 2013. 'Gas Chromatography.' in Jay A. Siegel, Pekka J. Saukko and Max M. Houck (eds.), *Encyclopedia of Forensic Sciences (Second Edition)* (Academic Press: Waltham).

Sumby, Krista M, Paul R Grbin, and Vladimir Jiranek. 2010. 'Microbial modulation of aromatic esters in wine: Current knowledge and future prospects', *Food chemistry*, 121: 1-16.

Torrens, Jordi, Montserrat Riu-Aumatell, Stefania Vichi, Elvira Lopez-Tamames, and Susana Buxaderas. 2010. 'Assessment of volatile and sensory profiles between base

and sparkling wines', *Journal of Agricultural and Food Chemistry*, 58: 2455-61.

Torrens, Jordi, Pilar Urpí, Montserrat Riu-Aumatell, Stefania Vichi, Elvira López-Tamames, and Susana Buxaderas. 2008. 'Different commercial yeast strains affecting the volatile and sensory profile of cava base wine', *International journal of food microbiology*, 124: 48-57.

Torresi, Sara, Maria Teresa Frangipane, and Gabriele Anelli. 2011. 'Biotechnologies in sparkling wine production. Interesting approaches for quality improvement: A review', *Food chemistry*, 129: 1232-41.

Ubeda, C, RM Callejón, AM Troncoso, A Peña-Neira, and ML Morales. 2016. 'Volatile profile characterisation of Chilean sparkling wines produced by traditional and Charmat methods via sequential stir bar sorptive extraction', *Food chemistry*, 207: 261-71.

Ubeda, Cristina, Ingeborg Kania-Zelada, Rubén del Barrio-Galán, Marcela Medel-Marabolí, Mariona Gil, and Álvaro Peña-Neira. 2019. 'Study of the changes in volatile compounds, aroma and sensory attributes during the production process of sparkling wine by traditional method', *Food Research International*, 119: 554-63.

Ward, Matthew. 2015. 'Aromatic substances from multiple sources', Accessed October. <https://waterhouse.ucdavis.edu/whats-in-wine/volatile-phenols>.

Waterhouse, Andrew L, Gavin L Sacks, and David W Jeffery. 2016. *Understanding wine chemistry* (John Wiley & Sons).

Welke, Juliane Elisa, Mauro Zanus, Marcelo Lazzarotto, Fernando Hepp Pulgati, and Cláudia Alcaraz Zini. 2014. 'Main differences between volatiles of sparkling and base wines accessed through comprehensive two dimensional gas chromatography with time-of-flight mass spectrometric detection and chemometric tools', *Food chemistry*, 164: 427-37.

Zhang, Zhouyao, and Janusz Pawliszyn. 1993. 'Headspace solid-phase microextraction', *Analytical chemistry*, 65: 1843-52.

## **Appendix**

A 1. Quantification of volatile and semi volatile compounds in Italian sparkling wine A at different timepoints (12, 24 and 36 months).

| Compound                | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |             |             | Units | Descriptor            | Olfactory<br>perception<br>threshold |
|-------------------------|-------|-------------------|----------------------------|-------------|-------------|-------|-----------------------|--------------------------------------|
|                         |       |                   | 12 Months                  | 24 Months   | 36 Months   |       |                       |                                      |
| <b>Alcohols</b>         |       |                   |                            |             |             |       |                       |                                      |
| Isoamyl alcohol         | 3.84  | 55                | 172±41.1                   | 248±8.13    | 670±253     | mg/L  | Fusel, cheese         | 30 mg/L                              |
| 3-Hexen-1-ol            | 6.37  | 67                | 33.0±5.96                  | 26.0±5.15   | 23.0±19.9   | µg/L  | Green, bitter, fatty  | 400 µg/L                             |
| 1-Hexanol               | 6.73  | 56                | 1.00±0.105                 | 22.0±0.803  | 10.0±4.98   | mg/L  | Grass, resin          | 8 mg/L                               |
| 1-Octanol               | 12.65 | 56                | 5.00±0.275                 | 5.00±0.301  | 36.0±5.91   | µg/L  | Citrus, roses         | 120 µg/L                             |
| Phenylethyl alcohol     | 13.91 | 91                | 56.0±9.81                  | 19.0±1.65   | 34.0±11.0   | mg/L  | Rose, honey           | 14 mg/L                              |
| 1-Decanol               | 18.52 | 55                | 27.0±12.3                  | 33.0±8.89   | 20.0±11.0   | µg/L  | Floral, fruity, waxy  | 400 µg/L                             |
| <b>Aldehydes</b>        |       |                   |                            |             |             |       |                       |                                      |
| Benzaldehyde            | 9.32  | 77                | 19.0±1.95                  | 24.0±1.89   | 21.0±8.18   | µg/L  | Almond, bitter        | 1-2 mg/L                             |
| Octanal                 | 10.56 | 56                | 0.118±0.174                | BLOQ        | 1.00±1.26   | mg/L  | Citrus                | 2.5 µg/L                             |
| Phenylacetaldehyde      | 11.75 | 91                | 14.0±0.413                 | 34.0±2.89   | 77.0±22.2   | µg/L  | Bitter, green, honey  | 1 µg/L                               |
| Nonanal                 | 13.65 | 57                | 3.00±1.64                  | 8.00±4.59   | 33.0±29.6   | µg/L  | Green, pungent        | 1 µg/L                               |
| Decanal                 | 16.57 | 57                | 5.00±4.97                  | 3.00±1.86   | 2.00±0.668  | µg/L  | Grassy, orange skin   | 1 mg/L                               |
| β-Cyclocitral           | 16.99 | 109               | 9.00±0.0236                | 0.132±0.247 | ND          | µg/L  | Floral, sweet         | -                                    |
| <b>Esters</b>           |       |                   |                            |             |             |       |                       |                                      |
| Ethyl isobutyrate       | 4.21  | 71                | 72.0±44.2                  | 124±64.9    | 146±79.7    | µg/L  | Fruity                | 15 µg/L                              |
| Ethyl butanoate         | 5.01  | 71                | 0.209±0.104                | 0.301±0.144 | 2.03±0.961  | mg/L  | Strawberry, apple     | 20 µg/L                              |
| Ethyl 2-methylbutanoate | 6.22  | 57                | 8.00±4.84                  | 18.0±10.1   | 20.0±12.9   | µg/L  | Apple                 | 18 µg/L                              |
| Ethyl isovalerate       | 6.28  | 88                | 11.8±7.0                   | 5.5±2.9     | 4.2±2.2     | µg/L  | Sweet, fruity         | 3 µg/L                               |
| Isoamyl acetate         | 6.87  | 70                | 326±181                    | 107±52.3    | 454±239     | µg/L  | Banana, sweet, fruity | 30 µg/L                              |
| Ethyl hexanoate         | 10.41 | 88                | 1.00±0.181                 | 1.00±0.306  | 3.00±1.71   | mg/L  | Apple, banana         | 14 µg/L                              |
| Hexyl acetate           | 10.80 | 56                | 2.00±0.240                 | BLOQ        | 0.155±0.170 | µg/L  | Cherry, pear          | 1.5 mg/L                             |
| Ethyl heptanoate        | 13.39 | 88                | 3.00±0.0910                | 0.353±0.240 | 2.00±0.463  | µg/L  | Fruity, melon         | -                                    |

| Compound            | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |             |            | Units | Descriptor                     | Olfactory<br>perception<br>threshold |
|---------------------|-------|-------------------|----------------------------|-------------|------------|-------|--------------------------------|--------------------------------------|
|                     |       |                   | 12 Months                  | 24 Months   | 36 Months  |       |                                |                                      |
| Diethyl succinate   | 15.73 | 101               | 14.0±1.57                  | 28.0±1.63   | 87.0±10.9  | mg/L  | Fruity                         | 200 mg/L                             |
| Ethyl octanoate     | 16.34 | 88                | 2.00±0.541                 | 1.00±0.217  | 5.00±2.57  | mg/L  | Pineapple. sweet               | 600 µg/L                             |
| Phenylethyl acetate | 17.91 | 104               | 25.0±1.12                  | 6.00±0.334  | 4.00±0.548 | µg/L  | Rose. violet                   | 250 µg/L                             |
| Ethyl nonanoate     | 19.07 | 88                | 10.0±0.683                 | 3.00±0.802  | 32.0±8.03  | µg/L  | Rose, fruity                   | -                                    |
| Vinyl decanoate     | 20.94 | 155               | ND                         | ND          | ND         |       |                                |                                      |
| Ethyl decanoate     | 21.69 | 88                | 215±19.8                   | 19.0±8.87   | 30.0±17.6  | µg/L  | Bra0y, fruity                  | 200 µg/L                             |
| <b>Furans</b>       |       |                   |                            |             |            |       |                                |                                      |
| Furfural            | 5.71  | 95                | 1.00±0.0847                | 2.00±0.169  | 3.00±1.50  | mg/L  | Almond, bread                  | 14.1 mg/L                            |
| 5-Methyl-2-furfural | 9.24  | 110               | 39.0±5.66                  | 27.0±2.37   | 42.0±7.90  | µg/L  | Almond, burnt sugar            | 16 mg/L                              |
| <b>Ketones</b>      |       |                   |                            |             |            |       |                                |                                      |
| 2-Heptanone         | 7.20  | 58                | 0.219±0.0854               | 2.00±0.929  | 4.00±2.32  | µg/L  | Sweet, fruity, woody           | -                                    |
| 2-Nonanone          | 13.17 | 58                | 2.00±1.02                  | 4.00±1.10   | 3.00±1.53  | µg/L  | Sweet, herbal                  | -                                    |
| 2-Undecanone        | 18.96 | 58                | 0.0742±0.0774              | 0.453±0.403 | 3.00±1.47  | µg/L  | Green, floral                  | -                                    |
| β-Damascenone       | 21.38 | 69                | 3.00±0.190                 | 4.00±0.345  | 7.00±1.31  | µg/L  | Honey, fruity, stewed<br>apple | 50 ng/L                              |
| α-Ionone            | 22.40 | 121               | ND                         | 1.00±0.121  | 2.00±0.525 | µg/L  | Fruity, violet                 | 90 ng/L                              |
| <b>Terpenes</b>     |       |                   |                            |             |            |       |                                |                                      |
| 1,4-Cineole         | 10.90 | 111               | 52.0±27.6                  | BLOQ        | ND         | ng/L  | Minty, pine                    | -                                    |
| Limonene            | 11.34 | 68                | 513±265                    | ND          | ND         | µg/L  | Flowery, green, citrus         | 200 µg/L                             |
| Eucalyptol          | 11.42 | 81                | 495±853                    | BLOQ        | 286±701    | ng/L  | Fresh, mint,<br>eucalyptus     | 1.1 µg/L                             |
| Linalool oxide      | 12.58 | 59                | 152±189                    | 45.0±14.2   | 158±96.2   | µg/L  | Citrus, green                  | 25 µg/L                              |
| β-Linalool          | 13.47 | 93                | 20.0±3.53                  | ND          | ND         | µg/L  | Flower, muscat,<br>lavender    | 25 µg/L                              |
| Camphor             | 14.86 | 95                | 0.468±0.536                | 0.215±0.239 | 1.00±0.585 | ng/L  | Herbal, camphor                | -                                    |

<sup>a</sup>-Average concentration and standard deviation. BLOQ- below limit of quantification. ND-Not detected.

A 2. Quantification of volatile and semi volatile compounds in Italian sparkling wine B at different timepoints (12, 24 and 36 months).

| Compound                | RT    | Quantifier Ion | Concentration <sup>a</sup> |             |             | Units | Descriptor            | Olfactory perception threshold |
|-------------------------|-------|----------------|----------------------------|-------------|-------------|-------|-----------------------|--------------------------------|
|                         |       |                | 12 Months                  | 24 Months   | 36 Months   |       |                       |                                |
| <b>Alcohols</b>         |       |                |                            |             |             |       |                       |                                |
| Isoamyl alcohol         | 3.84  | 55             | 177±25.1                   | 251±10.1    | 516±215     | mg/L  | Fusel, cheese         | 30 mg/L                        |
| 3-Hexen-1-ol            | 6.37  | 67             | ND                         | 23.0±4.44   | 14.1±16.1   | µg/L  | Green, bitter, fatty  | 400 µg/L                       |
| 1-Hexanol               | 6.73  | 56             | 0.263±0.0138               | 5.64±0.292  | 1.67±0.652  | mg/L  | Grass, resin          | 8 mg/L                         |
| 1-Octanol               | 12.65 | 56             | 6.32±0.564                 | 6.55±0.952  | 34.2±13.1   | µg/L  | Citrus, roses         | 120 µg/L                       |
| Phenylethyl alcohol     | 13.91 | 91             | 66.8±10.9                  | 18.8±3.04   | 32.8±14.7   | mg/L  | Rose, honey           | 14 mg/L                        |
| 1-Decanol               | 18.52 | 55             | 34.2±3.85                  | 43.7±13.6   | 7.22±8.97   | µg/L  | Floral, fruity, waxy  | 400 µg/L                       |
| <b>Aldehydes</b>        |       |                |                            |             |             |       |                       |                                |
| Benzaldehyde            | 9.32  | 77             | 31,2±2,41                  | 40.3±6.65   | 25.2±11.2   | µg/L  | Almond, bitter        | 1-2 mg/L                       |
| Octanal                 | 10.56 | 56             | 0.092±1.61                 | BLOQ        | 0.551±0.927 | mg/L  | Citrus                | 2.5 µg/L                       |
| Phenylacetaldehyde      | 11.75 | 91             | 34,2±1,61                  | 35.5±7.54   | 82.6±36.7   | µg/L  | Bitter, green, honey  | 1 µg/L                         |
| Nonanal                 | 13.65 | 57             | 4.29±3.42                  | 11.0±8.83   | 36.5±59.6   | µg/L  | Green, pungent        | 1 µg/L                         |
| Decanal                 | 16.57 | 57             | 1.70±0.841                 | 2.82±1.51   | 1.98±0.722  | µg/L  | Grassy, orange skin   | 1 mg/L                         |
| β-Cyclocitral           | 16.99 | 109            | 8.95±0.0146                | 0.257±0.357 | ND          | µg/L  | Floral, sweet         | -                              |
| <b>Esters</b>           |       |                |                            |             |             |       |                       |                                |
| Ethyl isobutyrate       | 4.21  | 71             | 108±22.7                   | 177±52.8    | 110±49.5    | µg/L  | Fruity                | 15 µg/L                        |
| Ethyl butanoate         | 5.01  | 71             | 0.460±0.0829               | 0.630±0.152 | 2.41±0.975  | mg/L  | Strawberry, apple     | 20 µg/L                        |
| Ethyl 2-methylbutanoate | 6.22  | 57             | 25.7±5.35                  | 50.4±10.9   | 28.1±13.2   | µg/L  | Apple                 | 18 µg/L                        |
| Ethyl isovalerate       | 6.28  | 88             | 42.7±81.3                  | 159±46.1    | 636±265     | µg/L  | Sweet, fruity         | 3 µg/L                         |
| Isoamyl acetate         | 6.87  | 70             | 101±18.2                   | 130±33.2    | 378±181     | µg/L  | Banana, sweet, fruity | 30 µg/L                        |
| Ethyl hexanoate         | 10.41 | 88             | 1.03±0.364                 | 1.31±0.363  | 3.54±0.826  | mg/L  | Apple, banana         | 14 µg/L                        |

| Compound            | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |             |              | Units | Descriptor                            | Olfactory<br>perception<br>threshold |
|---------------------|-------|-------------------|----------------------------|-------------|--------------|-------|---------------------------------------|--------------------------------------|
|                     |       |                   | 12 Months                  | 24 Months   | 36 Months    |       |                                       |                                      |
| Hexyl acetate       | 10.8  | 56                | 1.35±0.0866                | BLOQ        | 0.0662±0.162 | µg/L  | Cherry, pear                          | 1,5 mg/L                             |
| Ethyl heptanoate    | 13.39 | 88                | 3.55±0.0988                | 0.994±0.269 | 2.81±0.446   | µg/L  | Fruity, melon                         | -                                    |
| Diethyl succinate   | 15.73 | 101               | 24.7±8.00                  | 34.6±3.54   | 114±18.5     | mg/L  | Fruity                                | 200 mg/L                             |
| Ethyl octanoate     | 16.34 | 88                | 1.47±0.491                 | 0.835±0.250 | 3.48±1.30    | mg/L  | Pineapple, sweet                      | 600 µg/L                             |
| Phenylethyl acetate | 17.91 | 104               | 26.4±1.63                  | 8.01±0.564  | 5.46±1.09    | µg/L  | Rose, violet                          | 250 µg/L                             |
| Ethyl nonanoate     | 19.07 | 88                | 6.03±0.401                 | 1.74±0.607  | 21.7±9.52    | µg/L  | Rose, fruity                          | -                                    |
| Vinyldecanoate      | 20.94 | 155               | ND                         | ND          | ND           |       |                                       |                                      |
| Ethyl decanoate     | 21.69 | 88                | 237±48.9                   | 33.5±13.9   | 15.6±12.1    | µg/L  | Brandy, fruity                        | 200 µg/L                             |
| <b>Furans</b>       |       |                   |                            |             |              |       |                                       |                                      |
| Furfural            | 5.71  | 95                | 1.77±0.227                 | 4.19±0.294  | 4.39±1.89    | mg/L  | Almond, bread                         | 14.1 mg/L                            |
| 5-Methyl-2-furfural | 9.24  | 110               | 103±18.2                   | 51.5±3.44   | 51.7±16.8    | µg/L  | Almond, burnt sugar                   | 16 mg/L                              |
| <b>Ketones</b>      |       |                   |                            |             |              |       |                                       |                                      |
| 2-Heptanone         | 7.20  | 58                | 0.389±0.0999               | 1.93±0.401  | 3.06±1.74    | µg/L  | Sweet, fruity, woody                  | -                                    |
| 2-Nonanone          | 13.17 | 58                | 2.44±0.810                 | 3.77±1.11   | 1.20±0.516   | µg/L  | Sweet, herbal                         | -                                    |
| 2-Undecanone        | 18.96 | 58                | 0.0769±0.0431              | BLOQ        | 2.56±1.95    | µg/L  | Green, floral                         | -                                    |
| β-damascenone       | 21.38 | 69                | 2.31±0.0447                | 1.55±0.140  | 3.00±0.464   | µg/L  | Honey, sweet, fruity,<br>stewed apple | 50 ng/L                              |
| α-Ionone            | 22.40 | 121               | ND                         | 1.07±0.110  | 1.36±0.431   | µg/L  | Fruity, violet                        | 90 ng/L                              |
| <b>Terpenes</b>     |       |                   |                            |             |              |       |                                       |                                      |
| 1,4-Cineole         | 10.90 | 111               | ND                         | BLOQ        | ND           | ng/L  | Minty, pine                           | -                                    |
| Limonene            | 11.34 | 68                | 581±162                    | ND          | ND           | µg/L  | Flowery, green, citrus                | 200 µg/L                             |
| Eucalyptol          | 11.42 | 81                | 58.0±33.0                  | BLOQ        | ND           | ng/L  | Fresh, mint, eucalyptus               | 1,1 µg/L                             |
| Linalool oxide      | 12.58 | 59                | 57.1±18.7                  | 38.7±17.4   | 155±73.9     | µg/L  | Citrus, green                         | 25 µg/L                              |
| β-Linalool          | 13.47 | 93                | ND                         | ND          | ND           | µg/L  | Flower, muscat, lavender              | 25 µg/L                              |
| Camphor             | 14.86 | 95                | 0.274±0.427                | 0.341±0.413 | 0.335±0.369  | ng/L  | Herbal, camphor                       | -                                    |

| Compound | RT | Quantifier<br>Ion | Concentration <sup>a</sup> |           |           | Units | Descriptor | Olfactory<br>perception<br>threshold |
|----------|----|-------------------|----------------------------|-----------|-----------|-------|------------|--------------------------------------|
|          |    |                   | 12 Months                  | 24 Months | 36 Months |       |            |                                      |

<sup>a</sup>-Average concentration and standard deviation. BLOQ- below limit of quantification. ND-Not detected.

A 3. Quantification of volatile and semi volatile compounds in Spanish sparkling wine at different timepoints (24 and 80 months).

| Compound                | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |             | Units | Descriptor            | Olfactory<br>perception<br>threshold |
|-------------------------|-------|-------------------|----------------------------|-------------|-------|-----------------------|--------------------------------------|
|                         |       |                   | 24 Months                  | 80 Months   |       |                       |                                      |
| <b>Alcohols</b>         |       |                   |                            |             |       |                       |                                      |
| Isoamyl alcohol         | 3.84  | 55                | 130±9.00                   | 478±65.3    | mg/L  | Fusel, cheese         | 30 mg/L                              |
| 3-Hexen-1-ol            | 6.37  | 67                | 315±79.6                   | 389±69.2    | µg/L  | Green, bitter, fatty  | 400 µg/L                             |
| 1-Hexanol               | 6.73  | 56                | 597±73.0                   | 6218±919    | µg/L  | Grass, resin          | 8 mg/L                               |
| 1-Octanol               | 12.65 | 56                | 2.88±0.416                 | 14.2±3.02   | µg/L  | Citrus, roses         | 120 µg/L                             |
| Phenylethyl alcohol     | 13.91 | 91                | 25.1±3.46                  | 45.5±8.08   | mg/L  | Rose, honey           | 14 mg/L                              |
| 1-Decanol               | 18.52 | 55                | 68.6±43.4                  | BLOQ        | µg/L  | Floral, fruity, waxy  | 400 µg/L                             |
| <b>Aldehydes</b>        |       |                   |                            |             |       |                       |                                      |
| Benzaldehyde            | 9.32  | 77                | 4.96±0.621                 | 5.12±0.867  | µg/L  | Almond, bitter        | 1-2 mg/L                             |
| Octanal                 | 10.56 | 56                | 19046±6408                 | 787±1179    | ng/L  | Citrus                | 2.5 µg/L                             |
| Phenylacetaldehyde      | 11.75 | 91                | 18.9±1.63                  | 53.2±6.80   | µg/L  | Bitter, green, honey  | 1 µg/L                               |
| Nonanal                 | 13.65 | 57                | 1.57±3.27                  | 26.5±26.2   | µg/L  | Green, pungent        | 1 µg/L                               |
| Decanal                 | 16.57 | 57                | 3.19±3.11                  | 0.575±0.327 | µg/L  | Grassy, orange skin   | 1 mg/L                               |
| β-Cyclocitral           | 16.99 | 109               | 0.0125±0.0362              | ND          | µg/L  | Floral, sweet         | -                                    |
| <b>Esters</b>           |       |                   |                            |             |       |                       |                                      |
| Ethyl isobutyrate       | 4.21  | 71                | 47.2±68.3                  | 113±20.2    | µg/L  | Fruity                | 15 µg/L                              |
| Ethyl butanoate         | 5.01  | 71                | 184±60.5                   | 2098±332    | µg/L  | Strawberry, apple     | 20 µg/L                              |
| Ethyl 2-methylbutanoate | 6.22  | 57                | 44.1±22.0                  | 25.6±5.12   | µg/L  | Apple                 | 18 µg/L                              |
| Ethyl isovalerate       | 6.28  | 88                | 70.8±41.8                  | 756±120     | µg/L  | Sweet, fruity         | 3 µg/L                               |
| Isoamyl acetate         | 6.87  | 70                | 157±30.4                   | 288±43.5    | µg/L  | Banana, sweet, fruity | 30 µg/L                              |
| Ethyl hexanoate         | 10.41 | 88                | 437±131                    | 27878±478   | µg/L  | Apple, banana         | 14 µg/L                              |
| Hexyl acetate           | 10.80 | 56                | BLOQ                       | BLOQ        | µg/L  | Cherry, pear          | 1.5 mg/L                             |

| Compound            | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |             | Units | Descriptor               | Olfactory<br>perception<br>threshold |
|---------------------|-------|-------------------|----------------------------|-------------|-------|--------------------------|--------------------------------------|
|                     |       |                   | 24 Months                  | 80 Months   |       |                          |                                      |
| Ethyl heptanoate    | 13.39 | 88                | 0.249±0.105                | 2.29±0.247  | µg/L  | Fruity, melon            | -                                    |
| Diethyl succinate   | 15.73 | 101               | 10.7±0.782                 | 43.6±6.80   | mg/L  | Fruity                   | 200 mg/L                             |
| Ethyl octanoate     | 16.34 | 88                | 1848±597                   | 3285±882    | µg/L  | Pineapple, sweet         | 600 µg/L                             |
| Phenylethyl acetate | 17.91 | 104               | 27.8±0.863                 | 4.69±0.786  | µg/L  | Rose, violet             | 250 µg/L                             |
| Ethyl nonanoate     | 19.07 | 88                | 3.14±1.67                  | 5.17±0.821  | µg/L  | Rose, fruity             | -                                    |
| Ethyl decanoate     | 21.69 | 88                | 98.1±25.8                  | 22.4±7.38   | µg/L  | Brandy, fruity           | 200 µg/L                             |
| <b>Ketones</b>      |       |                   |                            |             |       |                          |                                      |
| 2-Heptanone         | 7.20  | 58                | 150±317                    | 2977±2295   | ng/L  | Sweet, fruity, woody     | -                                    |
| 2-Nonanone          | 13.17 | 58                | 1.44±0.417                 | 0.645±0.190 | µg/L  | Sweet, herbal            | -                                    |
| 2-Undecanone        | 18.96 | 58                | 132±35.9                   | 1119±653    | ng/L  | Green, floral            | -                                    |
| β-Damascenone       | 21.38 | 69                | 1.47±0.0987                | 3.78±0.370  | µg/L  | Apple, honey, smoky      | 0.05 µg/L                            |
| α-Ionone            | 22.40 | 121               | 74.3±15.3                  | 641±188     | ng/L  | Fruity, violet           | 90 ng/L                              |
| <b>Terpenes</b>     |       |                   |                            |             |       |                          |                                      |
| 1,4-Cineole         | 10.90 | 111               | BLOQ                       | ND          | µg/L  | Minty, pine              | -                                    |
| Limonene            | 11.34 | 68                | 523±568                    | ND          | ng/L  | Flowery, green, citrus   | 200 µg/L                             |
| Eucalyptol          | 11.49 | 81                | BLOQ                       | ND          | ng/L  | Fresh, mint, eucalyptus  | 1.1 µg/L                             |
| Linalool oxide      | 12.58 | 59                | 1.36±2.30                  | 72.2±33.9   | µg/L  | Citrus, green            | 25 µg/L                              |
| β-Linalool          | 13.47 | 93                | BLOQ                       | ND          | µg/L  | Flower, muscat, lavender | 25 µg/L                              |
| Camphor             | 14.86 | 95                | 251±270                    | 500±631     | ng/L  | Herbal, camphor          | -                                    |
| <b>Furans</b>       |       |                   |                            |             |       |                          |                                      |
| Furfural            | 5.71  | 95                | 483±41.8                   | 987±263     | µg/L  | Almond, bread            | 14.1 mg/L                            |
| 5-Methyl-2-furfural | 9.24  | 110               | 30.8±0.912                 | 30.1±5.75   | µg/L  | Almond, burnt sugar      | 16 mg/L                              |

<sup>a</sup>-Average concentration and standard deviation. BLOQ- below limit of quantification. ND-Not detected.

A 4. Quantification of volatile and semi volatile compounds in Spanish sparkling wine sealed with 2D and crown cap.

| Compound                | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |                |               |               | Units | Descriptor            | Olfactory<br>perception<br>threshold |
|-------------------------|-------|-------------------|----------------------------|----------------|---------------|---------------|-------|-----------------------|--------------------------------------|
|                         |       |                   | 24M                        |                | 80 M          |               |       |                       |                                      |
|                         |       |                   | 2D                         | Crown cap      | 2D            | Crown cap     |       |                       |                                      |
| <b>Alcohols</b>         |       |                   |                            |                |               |               |       |                       |                                      |
| Isoamyl alcohol         | 3.84  | 55                | 132 ± 10 .0                | 128 ± 6.00     | 46 9 ± 55.0   | 487 ± 79.8    | mg/L  | Fusel, cheese         | 30 mg/L                              |
| 3-Hexen-1-ol            | 6.37  | 67                | 290 ± 63 .0                | 340 ± 79.0     | 393 ± 44.9    | 386 ± 93.5    | µg/L  | Green, bitter, fatty  | 400 µg/L                             |
| 1-Hexanol               | 6.73  | 56                | 0.553 ± 0 .067             | 0 .640 ± 0 .04 | 6.15 ± 0.752  | 6.28 ± 1.15   | mg/L  | Grass, resin          | 8 mg/L                               |
| 1-Octanol               | 12.65 | 56                | 2.84 ± 0 .270              | 2 .93 ± 0 .480 | 13.9 ± 3.43   | 14.6 ± 2.90   | µg/L  | Citrus, roses         | 120 µg/L                             |
| Phenylethyl alcohol     | 13.91 | 91                | 26.4 ± 3 .00               | 23 .8 ± 3 .00  | 45.7 ± 7.87   | 45.3 ± 9.22   | mg/L  | Rose, honey           | 14 mg/L                              |
| 1-Decanol               | 18.52 | 55                | 48.8 ± 19 .3               | 88.4 ± 47 .3   | BLOQ          | BLOQ          | µg/L  | Floral, fruity, waxy  | 400 µg/L                             |
| <b>Aldehydes</b>        |       |                   |                            |                |               |               |       |                       |                                      |
| Benzaldehyde            | 9.32  | 77                | 5.26 ± 0 .280              | 4 .67 ± 0 .670 | 5.62 ± 0.644  | 4.63±0.813    | µg/L  | Almond, bitter        | 1-2 mg/L                             |
| Octanal                 | 10.56 | 56                | 19.7 ± 2 .20               | 18.3 ± 8 .30   | 1.06 ± 1.45   | 0.514 ± 0.909 | µg/L  | Citrus                | 2.5 µg/L                             |
| Phenylacetaldehyde      | 11.75 | 91                | 19.8 ± 1 .20               | 18.1 ± 1 .40   | 54.2 ± 6.41   | 52.3 ± 7.78   | µg/L  | Bitter, green, honey  | 1 µg/L                               |
| Nonanal                 | 13.65 | 57                | 2.00 ± 3.99                | 1 .15 ± 1 .73  | 29.7 ± 26.3   | 23.2 ± 28.7   | µg/L  | Green, pungent        | 1 µg/L                               |
| Decanal                 | 16.57 | 57                | 2.52 ± 1.24                | 3.85 ± 3 .88   | 0.494 ± 0.356 | 0.655 ± 0.313 | µg/L  | Grassy, orange skin   | 1 mg/L                               |
| β-Cyclocitral           | 16.99 | 109               | BLOQ                       | 25 .0 ± 45 .2  | ND            | ND            | µg/L  | Floral, sweet         | -                                    |
| <b>Esters</b>           |       |                   |                            |                |               |               |       |                       |                                      |
| Ethyl isobutyrate       | 4.21  | 71                | 38.4 ± 48 .0               | 56.1 ± 77.0    | 114 ± 18.0    | 112 ± 24.4    | µg/L  | Fruity                | 15 µg/L                              |
| Ethyl butanoate         | 5.01  | 71                | 0.178 ±0 .031              | 0 .190 ±0 .075 | 2.10 ± 0.286  | 2.10 ± 0.408  | mg/L  | Strawberry, apple     | 20 µg/L                              |
| Ethyl 2-methylbutanoate | 6.22  | 57                | 34.9 ± 17.0                | 53.3 ± 20.3    | 25.8 ± 4.25   | 25.3 ± 6.38   | µg/L  | Apple                 | 18 µg/L                              |
| Ethyl isovalerate       | 6.28  | 88                | 69 .4 ± 19.1               | 72 .2 ± 52 .7  | 767 ± 108     | 745 ± 142     | µg/L  | Sweet, fruity         | 3 µg/L                               |
| Isoamyl acetate         | 6.87  | 70                | 170 ± 32                   | 144 ± 17       | 296 ± 52.4    | 280 ± 37.1    | µg/L  | Banana, sweet, fruity | 30 µg/L                              |
| Ethyl hexanoate         | 10.41 | 88                | 0 .450 ±0.046              | 0 .423 ±0 .168 | 2.65 ± 0.486  | 2.93 ± 0.478  | mg/L  | Apple, banana         | 14 µg/L                              |

| Compound            | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |                |               |               | Units | Descriptor              | Olfactory<br>perception<br>threshold |
|---------------------|-------|-------------------|----------------------------|----------------|---------------|---------------|-------|-------------------------|--------------------------------------|
|                     |       |                   | 24M                        |                | 80 M          |               |       |                         |                                      |
|                     |       |                   | 2D                         | Crown cap      | 2D            | Crown cap     |       |                         |                                      |
| Hexyl acetate       | 10.80 | 56                | BLOQ                       | BLOQ           | 0.132±0.229   | BLOQ          | µg/L  | Cherry, pear            | 1.5 mg/L                             |
| Ethyl heptanoate    | 13.39 | 88                | 0 .308 ±0.041              | 0 .190 ±0.106  | 2.43 ± 0.237  | 2.15 ± 0.185  | µg/L  | Fruity, melon           | -                                    |
| Diethyl succinate   | 15.73 | 101               | 10 .8 ± 0 .6               | 10 .5 ± 0.8    | 44.4 ± 5.82   | 42.9 ± 8.29   | mg/L  | Fruity                  | 200 mg/L                             |
| Ethyl octanoate     | 16.34 | 88                | 1 .91 ± 0.22               | 1 .78 ± 0 .76  | 3.04 ± 0.812  | 3.53 ± 0.973  | mg/L  | Pineapple, sweet        | 600 µg/L                             |
| Phenylethyl acetate | 17.91 | 104               | 28 .2 ± 0 .5               | 27 .4 ± 0.9    | 4.77 ± 0.754  | 4.62 ± 0.898  | µg/L  | Rose, violet            | 250 µg/L                             |
| Ethyl nonanoate     | 19.07 | 88                | 2 .94 ± 1 .77              | 3 .35 ± 1.35   | 5.21 ± 0.651  | 5.13 ± 1.04   | µg/L  | Rose,f ruity            | -                                    |
| Vinyl decanoate     | 20.94 | 155               | ND                         | ND             | ND            | ND            | µg/L  | -                       | -                                    |
| Ethyl decanoate     | 21.69 | 88                | 111 ± 14                   | 85 .3 ± 26 .1  | 22.4 ± 6.66   | 22.4 ± 8.84   | µg/L  | Brandy, fruity          | 200 µg/L                             |
| <b>Furans</b>       |       |                   |                            |                |               |               |       |                         |                                      |
| Furfural            | 5.71  | 95                | 0 .489 ±0 .009             | 0 .481 ±0 .055 | 1.07 ± 0.222  | 0.908 ± 0.301 | mg/L  | Almond, bread           | 14.1 mg/L                            |
| 5-Methyl-2-furfural | 9.24  | 110               | 30 .4 ± 0 .4               | 31 .1 ± 1 .0   | 25.3 ± 2.92   | 35.0 ± 2.65   | µg/L  | Almond, burnt sugar     | 16 mg/L                              |
| <b>Ketones</b>      |       |                   |                            |                |               |               |       |                         |                                      |
| 2-Heptanone         | 7.20  | 58                | BLOQ                       | 0.301 ±0 .369  | 2.31 ± 0.521  | 3.64 ± 3.24   | µg/L  | Sweet, fruity, woody    | -                                    |
| 2-Nonanone          | 13.17 | 58                | 1 .33 ± 0 .12              | 1.56 ± 0 .52   | 0.581 ± 0.188 | 0.709 ± 0.189 | µg/L  | Sweet, herbal           | -                                    |
| 2-Undecanone        | 18.96 | 58                | 0.118 ±0 .032              | 0 .147 ±0 .030 | 0.800 ± 0.449 | 1.44 ± 0.711  | µg/L  | Green, floral           | -                                    |
| β-damascenone       | 21.38 | 69                | 1.47 ± 0.07                | 1.47 ± 0 .11   | 3.75 ± 0.344  | 3.82 ± 0.432  | µg/L  | Apple, honey, smoky     | 0.05 µg/L                            |
| α-Ionone            | 22.40 | 121               | 69 .6 ± 13 .7              | 79 .0 ± 13 .7  | 486 ± 83      | 797 ± 109     | ng/L  | Fruity, violet          | 90 ng/L                              |
| <b>Terpenes</b>     |       |                   |                            |                |               |               |       |                         |                                      |
| 1 ,4-Cineole        | 10.90 | 111               | BLOQ                       | BLOQ           | ND            | ND            | ng/L  | Minty, pine             | -                                    |
| Limonene            | 11.34 | 68                | 1050 ± 180                 | BLOQ           | ND            | ND            | ng/L  | Flowery green, citrus   | 200 µg/L                             |
| Eucalyptol          | 11.42 | 81                | BLOQ                       | BLOQ           | ND            | ND            | ng/L  | Fresh, mint, eucalyptus | 1.1 µg/L                             |
| Linalool oxide      | 12.58 | 59                | 1.79 ± 2 .40               | 0.925 ± 1 .851 | 70.8 ± 29.1   | 73.6 ± 41.7   | µg/L  | Citrus, green           | 25 µg/L                              |

| Compound          | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |                  |            |                   | Units | Descriptor               | Olfactory<br>perception<br>threshold |
|-------------------|-------|-------------------|----------------------------|------------------|------------|-------------------|-------|--------------------------|--------------------------------------|
|                   |       |                   | 2D                         | 24M<br>Crown cap | 2D         | 80 M<br>Crown cap |       |                          |                                      |
| $\beta$ -Linalool | 13.47 | 93                | BLOQ                       | BLOQ             | ND         | ND                | µg/L  | Flower, muscat, lavender | 25 µg/L                              |
| Camphor           | 14.86 | 95                | 502 ± 74                   | ND               | 1000 ± 519 | ND                | ng/L  | Herbal, camphor          | -                                    |

<sup>a</sup>-Average concentration and standard deviation. BLOQ- below limit of quantification. ND-Not detected.

A 5. Quantification of volatile and semi-volatile compounds in Italian white wine sealed with different stoppers (A, B and C).

| Compound            | RT            | Quantifier<br>Ion | Concentration <sup>a</sup> |               |               | Units | Descriptor           | Olfactory<br>perception<br>threshold |
|---------------------|---------------|-------------------|----------------------------|---------------|---------------|-------|----------------------|--------------------------------------|
|                     |               |                   | A                          | B             | C             |       |                      |                                      |
| <b>Alcohols</b>     |               |                   |                            |               |               |       |                      |                                      |
| Isoamyl alcohol     | 3.84          | 55                | 603 ± 48.3                 | 687 ± 152     | 803 ± 105     | mg/L  | Fusel, cheese        | 30 mg/L                              |
| 3-Hexen-1-ol        | 6.37          | 67                | 53.6 ± 5.73                | 59.1 ± 14.4   | 70.0 ± 9.99   | µg/L  | Green, bitter, fatty | 400 µg/L                             |
| 1-Hexanol           | 6.73          | 56                | 0.791 ± 0.820              | 0.915 ± 0.225 | 1.08 ± 0.152  | mg/L  | Grass, resin         | 8 mg/L                               |
| 1-Octanol           | 12.65         | 56                | 542 ± 169                  | 652 ± 273     | 897 ± 177     | ng/L  | Citrus, roses        | 120 µg/L                             |
| Phenylethyl alcohol | 13.91         | 91                | 33.9 ± 2.01                | 38.7 ± 8.30   | 43.1 ± 4.34   | mg/L  | Rose, honey          | 14 mg/L                              |
| 1-Decanol           | 18.52         | 55                | 3.38 ± 2.38                | 4.74 ± 3.58   | 4.06 ± 1.71   | µg/L  | Floral, fruity, waxy | 400 µg/L                             |
| <b>Aldehydes</b>    |               |                   |                            |               |               |       |                      |                                      |
| Benzaldehyde        | 9.32          | 77                | 10.1 ± 0.929               | 11.1 ± 2.30   | 13.2 ± 1.45   | µg/L  | Almond, bitter       | 1-2 mg/L                             |
| Octanal             | 10.56         | 56                | BLOQ                       | BLOQ          | 296 ± 837     | ng/L  | Citrus               | 2.5 µg/L                             |
| Phenylacetaldehyde  | 11.75         | 91                | 3.35 ± 1.25                | 3.47 ± 0.849  | 4.18 ± 0.802  | µg/L  | Bitter, green, honey | 1 µg/L                               |
| Nonanal             | 13.65         | 57                | 0.221 ± 0.451              | 0.027 ± 0.075 | 0.861 ± 2.33  | µg/L  | Green, pungent       | 1 µg/L                               |
| Decanal             | 16.57         | 57                | 2.10 ± 1.60                | 2.66 ± 1.62   | 3.95 ± 2.35   | µg/L  | Grassy, orange skin  | 1 mg/L                               |
| β-Cyclocitral       | 16.99         | 109               | BLOQ                       | BLOQ          | BLOQ          | ng/L  | Floral, sweet        | 5 ng/L                               |
| 2-Methylbutanal     | 17.40 + 17.50 | 57                | 26.8 ± 3.51                | 25.5 ± 5.80   | 22.9 ± 4.40   | µg/L  | Green, grass, fruity | 16 µg/L                              |
| 3-Methylbutanal     | 18.90 + 19.10 | 230               | 1.01 ± 0.174               | 0.945 ± 0.298 | 0.776 ± 0.264 | µg/L  | Fatty, sour, peach   | 4.6 µg/L                             |
| <b>Esters</b>       |               |                   |                            |               |               |       |                      |                                      |

| Compound                | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |              |              | Units | Descriptor            | Olfactory<br>perception<br>threshold |
|-------------------------|-------|-------------------|----------------------------|--------------|--------------|-------|-----------------------|--------------------------------------|
|                         |       |                   | A                          | B            | C            |       |                       |                                      |
| Ethyl isobutyrate       | 4.21  | 71                | 209 ± 26.3                 | 249 ± 59.9   | 295 ± 39.2   | µg/L  | Fruity                | 15 µg/L                              |
| Ethyl butanoate         | 5.01  | 71                | 185 ± 21.8                 | 224 ± 58.5   | 263 ± 35.5   | µg/L  | Strawberry, apple     | 20 µg/L                              |
| Ethyl 2-methylbutanoate | 6.22  | 57                | 30.1 ± 4.45                | 35.0 ± 8.84  | 42.4 ± 5.52  | µg/L  | Apple                 | 18 µg/L                              |
| Ethyl isovalerate       | 6.28  | 88                | 24.5 ± 3.10                | 28.5 ± 6.72  | 34.3 ± 3.48  | µg/L  | Sweet, fruity         | 3 µg/L                               |
| Isoamyl acetate         | 6.87  | 70                | 50.2 ± 5.96                | 59.5 ± 16.3  | 70.6 ± 11.2  | µg/L  | Banana, sweet, fruity | 30 µg/L                              |
| Ethyl hexanoate         | 10.41 | 88                | 1.48 ± 0.202               | 1.81 ± 0.529 | 2.09 ± 0.535 | mg/L  | Apple, banana         | 14 µg/L                              |
| Hexyl acetate           | 10.80 | 56                | 2.11 ± 0.439               | 2.52 ± 0.936 | 2.77 ± 0.738 | µg/L  | Cherry, pear          | 1.5 mg/L                             |
| Ethyl heptanoate        | 13.39 | 88                | BLOQ                       | BLOQ         | BLOQ         | µg/L  | Fruity, melon         | -                                    |
| Diethyl succinate       | 15.73 | 101               | 6.91 ± 347                 | 7.89 ± 1.53  | 8.98 ± 0.973 | mg/L  | Fruity                | 200 mg/L                             |
| Ethyl octanoate         | 16.34 | 88                | 2.35 ± 0.405               | 3.25 ± 1.31  | 4.19 ± 0.841 | mg/L  | Pineapple, sweet      | 600 µg/L                             |
| Phenylethyl acetate     | 17.91 | 104               | 8.88 ± 0.550               | 10.1 ± 2.09  | 11.4 ± 1.15  | µg/L  | Rose, violet          | 250 µg/L                             |
| Ethyl nonanoate         | 19.07 | 88                | 230 ± 70.2                 | 320 ± 178    | 313 ± 51.2   | ng/L  | Rose, fruity          | -                                    |
| Vinyl decanoate         | 20.94 | 155               | 34.3 ± 0.183               | 34.2 ± 0.089 | 32.7 ± 3.53  | µg/L  | -                     | -                                    |
| Ethyl decanoate         | 21.69 | 88                | 144 ± 32.3                 | 233 ± 138    | 313 ± 110    | µg/L  | Brandy, fruity        | 200 µg/L                             |
| <b>Furans</b>           |       |                   |                            |              |              |       |                       |                                      |
| Furfural                | 5.71  | 95                | 2.64 ± 0.201               | 2.92 ± 0.537 | 3.46 ± 0.324 | mg/L  | Almond, bread         | 14.1 mg/L                            |
| 5-Methyl-2-furfural     | 9.24  | 110               | 64.8 ± 4.35                | 70.4 ± 13.2  | 81.9 ± 7.99  | µg/L  | Almond, burnt sugar   | 16 mg/L                              |
| <b>Ketones</b>          |       |                   |                            |              |              |       |                       |                                      |

| Compound        | RT            | Quantifier<br>Ion | Concentration <sup>a</sup> |              |              | Units | Descriptor              | Olfactory<br>perception<br>threshold |
|-----------------|---------------|-------------------|----------------------------|--------------|--------------|-------|-------------------------|--------------------------------------|
|                 |               |                   | A                          | B            | C            |       |                         |                                      |
| 2-Heptanone     | 7.20          | 58                | 1.57 ± 0.649               | 2.74 ± 2.37  | 3.58 ± 2.01  | µg/L  | Sweet, fruity, woody    | -                                    |
| 2-Nonanone      | 13.17         | 58                | 1.34 ± 0.190               | 1.58 ± 0.408 | 1.88 ± 0.218 | µg/L  | Sweet, herbal           | -                                    |
| 2-Pentanone     | 18.60 + 18.80 | 253               | BLOQ                       | BLOQ         | BLOQ         | µg/L  | Banana, sweet, fruity   | -                                    |
| 2-Undecanone    | 18.96         | 58                | 428 ± 167                  | 502 ± 134    | 550 ± 189    | ng/L  | Green, floral           | -                                    |
| 3-Penten-2-one  | 20.80 + 20.90 | 56                | 19.5 ± 1.48                | 17.8 ± 2.51  | 15.6 ± 1.84  | µg/L  | Phenolic, fishy         | -                                    |
| β-Damascenone   | 21.38         | 69                | 141 ± 72.3                 | 168 ± 45.2   | 229 ± 41.9   | ng/L  | Apple, honey, smoky     | 0.05 µg/L                            |
| α-Ionone        | 22.40         | 121               | 174 ± 17.6                 | 207 ± 44.4   | 250 ± 13.9   | ng/L  | Fruity, violet          | 90 ng/L                              |
| <b>Terpenes</b> |               |                   |                            |              |              |       |                         |                                      |
| Limonene        | 11.34         | 68                | 4.65 ± 1.52                | 4.83 ± 1.77  | 6.60 ± 1.76  | µg/L  | Flowery, green, citrus  | 200 µg/L                             |
| Eucalyptol      | 11.42         | 81                | ND                         | 25.7 ± 47.8  | ND           | µg/L  | Fresh, mint, eucalyptus | 1.1 µg/L                             |
| Linalool oxide  | 12.58         | 59                | 14.5 ± 0.678               | 14.9 ± 0.888 | 16.3 ± 0.516 | µg/L  | Citrus, green           | 25 µg/L                              |
| Camphor         | 14.86         | 95                | BLOQ                       | BLOQ         | BLOQ         | µg/L  | Herbal, camphor         | -                                    |

A - Cork stopper density 150-170 kg/m<sup>3</sup> with wax; B - Cork stopper density 180-200 kg/m<sup>3</sup>; C- Cork stopper density 150-170 kg/m<sup>3</sup>

<sup>a</sup>-Average concentration and standard deviation. BLOQ- below limit of quantification. ND-Not detected.

A 6.Quantification of volatile and semi-volatile compounds in French sparkling wines sealed with Micro CSP and STO.

| Compound                | RT    | Quantifier<br>ion | Concentration <sup>a</sup> |               | Units | Descriptor            | Olfactory<br>perception<br>threshold |
|-------------------------|-------|-------------------|----------------------------|---------------|-------|-----------------------|--------------------------------------|
|                         |       |                   | CCCS                       | CCST          |       |                       |                                      |
| <b>Alcohols</b>         |       |                   |                            |               |       |                       |                                      |
| Isoamyl alcohol         | 3.84  | 55                | 429 ± 98.3                 | 515 ± 38.4    | mg/L  | Fusel, cheese         | 30 mg/L                              |
| 3-Hexen-1-ol            | 6.37  | 67                | 117 ± 23.5                 | 128 ± 2.44    | µg/L  | Green, bitter, fatty  | 400 µg/L                             |
| 1-Hexanol               | 6.73  | 56                | 601 ± 146                  | 704 ± 53.9    | µg/L  | Grass, resin          | 8 mg/L                               |
| 1-Octanol               | 12.65 | 56                | 1.05 ± 0.298               | 0.807 ± 0.045 | µg/L  | Citrus, roses         | 120 µg/L                             |
| Phenylethyl alcohol     | 13.91 | 91                | 19.0 ± 5.11                | 17.9 ± 2.96   | mg/L  | Rose, honey           | 14 mg/L                              |
| 1-Decanol               | 18.52 | 55                | 9.70 ± 8.14                | 25.3 ± 2.16   | µg/L  | Floral, fruity, waxy  | 400 µg/L                             |
| <b>Aldehydes</b>        |       |                   |                            |               |       |                       |                                      |
| Benzaldehyde            | 9.32  | 77                | 16.1 ± 0.786               | 15.3 ± 1.58   | µg/L  | Almond, bitter        | 1-2 mg/L                             |
| Octanal                 | 10.56 | 56                | 8.48 ± 12.3                | 20.0 ± 16.6   | µg/L  | Citrus                | 2.5 µg/L                             |
| Phenylacetaldehyde      | 11.75 | 91                | 416 ± 32.5                 | 429 ± 399     | ng/L  | Bitter, green, honey  | 1 µg/L                               |
| Nonanal                 | 13.65 | 57                | 11.7 ± 14.7                | 36.5 ± 9.36   | µg/L  | Green, pungent        | 1 µg/L                               |
| Decanal                 | 16.57 | 57                | 15.9 ± 12.8                | 22.2 ± 6.45   | µg/L  | Grassy, orange skin   | 1 mg/L                               |
| <b>Esters</b>           |       |                   |                            |               |       |                       |                                      |
| Ethyl isobutyrate       | 4.21  | 71                | 56.6 ± 12.2                | 94.4 ± 0.945  | µg/L  | Fruity                | 15 µg/L                              |
| Ethyl butanoate         | 5.01  | 71                | 160 ± 18.2                 | 242 ± 8.94    | µg/L  | Strawberry, apple     | 20 µg/L                              |
| Ethyl 2-methylbutanoate | 6.22  | 57                | 9.11 ± 4.67                | 9.20 ± 0.932  | µg/L  | Apple                 | 18 µg/L                              |
| Ethyl isovalerate       | 6.28  | 88                | 0.792 ± 0.707              | 3.33 ± 0.304  | µg/L  | Sweet, fruity         | 3 µg/L                               |
| Isoamyl acetate         | 6.87  | 70                | 153 ± 18.2                 | 174 ± 22.6    | µg/L  | Banana, sweet, fruity | 30 µg/L                              |
| Ethyl hexanoate         | 10.41 | 88                | 1.26 ± 0.173               | 2.06 ± 0.269  | mg/L  | Apple, banana         | 14 µg/L                              |
| Hexyl acetate           | 10.80 | 56                | 52.2 ± 5.38                | 62.1 ± 6.11   | µg/L  | Cherry, pear          | 1.5 mg/L                             |
| Ethyl heptanoate        | 13.39 | 88                | BLOQ                       | BLOQ          | µg/L  | Fruity, melon         | -                                    |
| Diethyl succinate       | 15.73 | 101               | 3.87 ± 0.561               | 4.27 ± 0.159  | mg/L  | Fruity                | 200 mg/L                             |
| Ethyl octanoate         | 16.34 | 88                | 1.66 ± 0.224               | 3.52 ± 0.351  | mg/L  | Pineapple, sweet      | 600 µg/L                             |

| Compound            | RT    | Quantifier<br>Ion | Concentration <sup>a</sup> |              | Units | Descriptor               | Olfactory<br>perception<br>threshold |
|---------------------|-------|-------------------|----------------------------|--------------|-------|--------------------------|--------------------------------------|
|                     |       |                   | CCCS                       | CCST         |       |                          |                                      |
| Phenylethyl acetate | 17.91 | 104               | 46.3 ± 15.9                | 37.1 ± 10.6  | µg/L  | Rose, violet             | 250 µg/L                             |
| Ethyl nonanoate     | 19.07 | 88                | 239 ± 18.1                 | 224 ± 59.5   | ng/L  | Rose, fruity             | -                                    |
| Ethyl decanoate     | 21.69 | 88                | 51.4 ± 19.9                | 152 ± 19.0   | µg/L  | Brandy, fruity           | 200 µg/L                             |
| <b>Furans</b>       |       |                   |                            |              |       |                          |                                      |
| Furfural            | 5.71  | 95                | BLOQ                       | BLOQ         | µg/L  | Almond, bread            | 14.1 mg/L                            |
| <b>Ketones</b>      |       |                   |                            |              |       |                          |                                      |
| 2-Heptanone         | 7.20  | 58                | 2.78 ± 0.806               | 2.72 ± 0.097 | µg/L  | Sweet, fruity, woody     | -                                    |
| 2-Nonanone          | 13.17 | 58                | 4.56 ± 0.337               | 7.27 ± 0.291 | µg/L  | Sweet, herbal            | -                                    |
| 2-Undecanone        | 18.96 | 58                | 75.0 ± 130                 | 163 ± 30.2   | ng/L  | Green, floral            | -                                    |
| β-Damascenone       | 21.38 | 69                | 31.3 ± 28.3                | 278 ± 58.7   | ng/L  | Apple, honey, smoky      | 0.05 µg/L                            |
| α-Ionone            | 22.40 | 121               | 88.6 ± 8.51                | 116 ± 17.2   | ng/L  | Fruity, violet           | 90 ng/L                              |
| <b>Terpenes</b>     |       |                   |                            |              |       |                          |                                      |
| Limonene            | 11.34 | 68                | 106 ± 183                  | 15.7 ± 27.1  | ng/L  | Flowery, green, citrus   | 200 µg/L                             |
| Linalool oxide      | 12.58 | 59                | 14.2 ± 1.53                | 13.6 ± 0.111 | µg/L  | Citrus, green            | 25 µg/L                              |
| β-Linalool          | 13.47 | 93                | 3.03 ± 0.572               | 2.69 ± 0.401 | µg/L  | Flower, muscat, lavender | 25 µg/L                              |
| Camphor             | 14.86 | 95                | BLOQ                       | BLOQ         | µg/L  | Herbal, camphor          | -                                    |

<sup>a</sup>-Average concentration and standard deviation. BLOQ- below limit of quantification. ND-Not detected.

