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TESIS DOCTORAL

Título
Estudio químico-sensorial de vinos espumosos elaborados con variedades de uva tradicionales de vinos tranquilos
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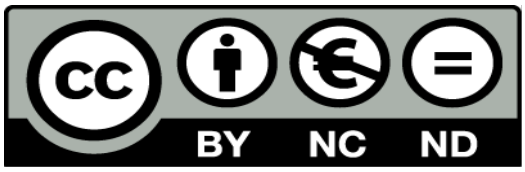

ESTUDIO QUÍMICO-SENSORIAL DE VINOS ESPUMOSOS ELABORADOS CON VARIEDADES DE UVA TRADICIONALES DE VINOS TRANQUILOS

CHEMICO-SENSORY STUDY OF SPARKLING WINES MADE WITH GRAPE VARIETIES TRADITIONALLY EMPLOYED FOR STILL WINES

TESIS DOCTORAL CON MENCIÓN INTERNACIONAL
LETICIA MARTÍNEZ LAPUENTE

UNIVERSIDAD DE LA RIOJA

DEPARTAMENTO DE AGRICULTURA Y ALIMENTACIÓN

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Memoria presentada por

Leticia MARTÍNEZ LAPUENTE

para optar al grado de Doctor con Mención de Doctor Internacional

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UNIVERSIDAD DE LA RIOJA

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CERTIFICAN:

Que la presente memoria, titulada "**Estudio químico-sensorial de vinos espumosos elaborados con variedades de uva tradicionales de vinos tranquilos**", presentada por Leticia MARTÍNEZ LAPUENTE, ha sido realizada en el Departamento de Agricultura y Alimentación de la Universidad de La Rioja, bajo nuestra dirección, y reúne las condiciones exigidas para optar al grado de Doctor con la mención de "Doctor Internacional",

Logroño, abril 2015

Fdo.: Belén Ayestarán Iturbe

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"-¿Qué te parece desto, Sancho? - dijo Don Quijote-

Bien podrán los encantadores quitarme la ventura,

pero el esfuerzo y el ánimo será imposible."

Segunda parte del Ingenioso Caballero

Don Quijote de la Mancha

Miguel de Cervantes

A mis padres y hermano

A Eduardo

Índice

Presentación

Esta memoria de Tesis Doctoral se presenta en forma de compendio de publicaciones científicas y cumple los requisitos para la obtención de la mención de Doctor Internacional de acuerdo con la normativa de la Universidad de La Rioja.

El objetivo de esta Tesis es caracterizar enológicamente los vinos espumosos obtenidos con variedades de uva de Castilla y León tradicionalmente empleadas en la elaboración de vinos tranquilos para la obtención de vinos espumosos de calidad y saludables, elaborados por el método tradicional, con el fin por un lado, de potenciar y diversificar la producción de vino de esta región, y por otro estudiar técnicas que permitan la mejora de la calidad de estos vinos, como la utilización de preparados comerciales a base de levaduras.

Para facilitar la lectura de esta memoria, un primer apartado, *Justificación y objetivos*, centra la temática de la tesis y describe los objetivos de la misma. A continuación, la *Introducción* recoge los estudios más relevantes realizados en relación a la temática de la tesis. En el capítulo de *Materiales y métodos* se describe brevemente la metodología y el plan de trabajo desarrollado para llevar a cabo los objetivos planteados en la tesis, cuyos resultados han dado lugar a las publicaciones científicas que se adjuntan en el capítulo de *Resultados y discusión*, donde también se incluye un resumen de cada artículo y de los resultados más relevantes obtenidos. Posteriormente se realiza un *Resumen y discusión global de los resultados* obtenidos, y finalmente se incluye un capítulo con las *Conclusiones* más destacadas que pueden extraerse de esta Tesis.

Las publicaciones científicas que se encuentran recogidas en esta memoria son:

1. Sparkling wines produced from alternative varieties: sensory attributes and evolution of phenolics during winemaking and aging

American Journal of Enology and Viticulture 64 (2013) 39-49.

2. Multivariate analysis for the differentiation of sparkling wines elaborated from autochthonous Spanish grape varieties: volatile compounds, amino acids and biogenic amines

European Food Research and Technology 236 (2013) 827-841.

3. Changes in polysaccharide composition during sparkling wine making and aging

Journal of Agricultural and Food Chemistry 61 (2013) 12362-12373.

4. 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 (2015) 47-55.

5. Role of major wine constituents in the foam properties of white and rosé sparkling wines

Food Chemistry 174 (2015) 330-338.

6. Use of commercial dry yeast products rich in mannoproteins for white and rosé sparkling wine elaboration

Journal of Agricultural and Food Chemistry, jf-2015-013369 (en revisión).

Presentation

This Doctoral Thesis is presented as a compilation of scientific papers and it meets the requirements for the application of the International Doctor mention according to the University of La Rioja legislation.

The main objective of this Thesis was the enological characterization of sparkling wines produced with grape varieties from Castilla y León (Spain) typically employed to elaborate still wines, which could also have good characteristics to obtain quality sparkling wines, and would allow diversifying the wine production of this region. On the other hand, the thesis studies techniques that allow improving the quality of these wines, such as the use of commercial yeast derivatives.

This Thesis Doctoral begins with a chapter on *Justification and objectives* that explains the subject and describes the objectives of the thesis. Then, the *Introduction* includes the most relevant studies in relation to the subject of the thesis. *Materials and methods* section shows briefly the methodology and work plan used. The scientific papers are included in *Results and discussion* chapter, which also contains a summary of each scientific paper. Then, a section *Summary and global discussion of results* is included. Finally, the *Conclusions* of the thesis are presented.

The papers published as a result of the present Thesis are detailed below:

1. Sparkling wines produced from alternative varieties: sensory attributes and evolution of phenolics during winemaking and aging

American Journal of Enology and Viticulture 64 (2013) 39-49.

2. Multivariate analysis for the differentiation of sparkling wines elaborated from autochthonous Spanish grape varieties: volatile compounds, amino acids and biogenic amines

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Journal of Agricultural and Food Chemistry, jf-2015-013369 (under review).

Resumen

Uno de sectores del vino que más está creciendo en nuestro país en los últimos años es el de los vinos espumosos. Aunque el Cava sigue acaparando el mayor porcentaje de ventas de vino espumoso de calidad español, cada vez son más las bodegas españolas que están elaborando vino espumoso siguiendo el método tradicional con el fin de ampliar su oferta y abrir nuevos mercados. En este sentido, la Comunidad Autónoma de Castilla y León cuenta con un gran número de variedades de vid tradicionalmente empleadas para la elaboración de vinos tranquilos y que podrían presentar buenas aptitudes para la elaboración de vinos espumosos de calidad. Entre las variedades de Castilla y León que pueden ser más adecuadas para la elaboración de vinos blancos espumosos destacan las variedades Viura, Verdejo, Malvasía, Albarín y Godello. Para la elaboración de vinos rosados espumosos destacan, debido a su importancia en la vitivinicultura de la región, las variedades Garnacha, Tempranillo y Prieto Picudo.

Así, el primer objetivo de esta tesis fue caracterizar enológicamente los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional, durante la elaboración y la crianza sobre lías de nueve meses, en términos de compuestos fenólicos monoméricos y poliméricos, aminoácidos, aminas biógenas, compuestos volátiles y polisacáridos. Los resultados obtenidos indicaron que la estabilización por frío y la clarificación de los vinos base produjeron una disminución en el contenido de antocianos y de proantocianidinas. Durante los primeros seis meses de envejecimiento sobre lías se produjeron pérdidas en todos los tipos de compuestos fenólicos, aunque parte de los compuestos fenólicos adsorbidos al inicio de la fase de tiraje fueron liberados durante los últimos tres meses de envejecimiento. Los vinos espumosos de Prieto Picudo mostraron mayor intensidad de color, contenido en antocianos y estabilidad del color que los vinos elaborados con Garnacha, los cuales se caracterizaron por una mayor concentración de ácidos hidroxicinámicos. Entre los vinos espumosos blancos, los elaborados con la variedad

Albarín mostraron el mayor contenido en catequina y en proantocianidinas y junto con los elaborados con Viura, en hidroxicinamatos. Los vinos rosados espumosos de Garnacha y los blancos de Albarín presentaron los mayores valores de polifenoles totales. Por otro lado, durante la crianza sobre lías de los vinos espumosos la concentración aminoácidos permaneció estable o disminuyó y se observó un aumento de los ésteres etílicos de ácidos grasos ramificados, de lactato de etilo y de γ–butirolactona y un descenso de los terpenos. Los vinos espumosos de Albarín, Verdejo, Godello y Prieto Picudo fueron los más ricos en aminoácidos y en la mayoría de los compuestos volátiles, especialmente de ésteres etílicos y alcohol acetatos. El contenido de aminas biógenas permaneció constante durante los primeros tres meses de envejecimiento sobre lías, observándose un incremento de concentración en los siguientes tres meses de envejecimiento. Los vinos espumosos de Albarín y de Prieto Picudo mostraron el mayor contenido de aminas biógenas, aunque fueron inferiores a los límites considerados como un riesgo para la salud. Con respecto al contenido de polisacáridos, entre los vinos blancos espumosos destacaron los elaborados con la variedad Verdejo por su mayor contenido en polisacáridos totales y entre los vinos rosados espumosos, los de Prieto Picudo. Aunque la familia de polisacáridos ramnogalacturonanos tipo II únicamente fue detectada en los vinos elaborados con la variedad Prieto Picudo, todos los vinos estuvieron compuestos por polisacáridos ricos en arabinosa y en galactosa, manoproteínas, glucanos y homogalacturonanos, con porcentajes de 33 ± 5%, 25 ± 9%,36 ± 9% y 6 ± 3%, respectivamente.

El segundo objetivo fue analizar sensorialmente los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, con nueve meses de crianza sobre lías y elaborados por el método tradicional. Los resultados mostraron que aunque todos los vinos espumosos obtuvieron buenas puntuaciones en el análisis sensorial, los vinos obtenidos con Prieto Picudo mostraron una mayor intensidad de color visual, de tonos rojos, de frescor en boca, de intensidad aromática y de calidad de la espuma que los elaborados con la variedad Garnacha. Los vinos espumosos de Albarín y de Godello presentaron mayor intensidad aromática que los elaborados con Viura y Malvasía. Los vinos espumosos de Verdejo mostraron la mejor calidad de la espuma entre los vinos blancos.

El tercer objetivo se centró en analizar los cambios en familias de polisacáridos y en la distribución de sus pesos moleculares durante la elaboración y la crianza sobre lías de treinta meses de vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía,

Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional. Los resultados obtenidos indicaron que el mayor contenido de manoproteínas y de polisacáridos ricos en arabinosa y en galactosa fue obtenido a los seis meses de envejecimiento sobre lías. En este sentido, estos resultados parecen indicar que no son necesarios mayores tiempos de envejecimiento sobre lías para obtener mayor contenido de polisacáridos en el vino. Por otro lado, a los seis meses de envejecimiento sobre lías se observó una disminución en los pesos moleculares de los polisacáridos del vino. La combinación de estos dos fenómenos podría implicar una mejor estabilidad de la espuma y por tanto, una mejor calidad de los vinos.

El cuarto objetivo fue estudiar la influencia de la variedad de uva y del tiempo de crianza en presencia y en ausencia de lías en la composición volátil y en las propiedades espumantes de los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional. Los resultados obtenidos indicaron que los vinos espumosos de Albarín, Verdejo, Godello y Prieto Picudo fueron los más ricos en la mayoría de los compuestos volátiles analizados, especialmente en aquellos que contribuyen a los aromas frutales de los vinos, y mantuvieron sus características varietales durante el envejecimiento en presencia y en ausencia de lías. Los vinos espumosos de Verdejo y de Prieto Picudo mostraron las mejores características espumantes durante el envejecimiento en presencia y en ausencia de lías, seguidos por los vinos de Albarín y de Godello. Dichas propiedades espumantes se mantuvieron estables o mejoraron durante el envejecimiento en presencia de lías, mientras que el envejecimiento en ausencia de lías no provocó una disminución de la calidad de la espuma.

El quinto objetivo fue estudiar la influencia de los constituyentes como polisacáridos, polifenoles y sustancias nitrogenadas del vino espumoso en sus características espumantes. Los resultados obtenidos indicaron una contribución positiva de los antocianos monómeros y de los aminoácidos y una contribución negativa de las proantocianidinas en los parámetros de espumabilidad de los vinos espumosos. Las manoproteínas y los polisacáridos ricos en arabinosa y en galactosa no tuvieron influencia en los parámetros de espumabilidad, sin embargo fueron buenos estabilizadores de la espuma. Los modelos de regresión lineal múltiple revelaron que entre los antocianos, la malvidina-3-glucósido y la malvidina-3-(6-acetil)-glucósido mostraron la mayor influencia en los parámetros de espumabilidad, seguidos por los aminoácidos, principalmente la β-alanina. El modelo que mejor explicó el parámetro de

estabilidad de la espuma fue únicamente predicho por los polisacáridos ricos en arabinosa y en galactosa.

Finalmente, el sexto objetivo se centró en estudiar el efecto de la adición de autolisados de levaduras comerciales a los vinos base en la composición y las características organolépticas de los vinos rosados espumosos monovarietales de las variedades de uva tintas de Tempranillo y Garnacha y blancos de las variedades de uva de Verdejo y Godello elaborados por el método tradicional durante una crianza sobre lías en botella de nueve meses. Los resultados mostraron que la adición de los derivados de levaduras comerciales a los vinos base no tuvo efecto en el contenido fenólico, de amino ácidos, de aminas biógenas ni propiedades espumantes de los vinos. Por el contrario, se observó que la adición de estos productos puede modificar el perfil volátil de los vinos espumosos, aunque este efecto estuvo relacionado con el contenido de manoproteínas y de pureza de los autolisados de levaduras adicionados. Así, los vinos espumosos tratados con el producto de mayor contenido de manoproteínas y de pureza fueron los que mostraron diferencias en el perfil volátil con respecto a sus controles. Además, los vinos blancos espumosos tratados con este producto presentaron concentraciones más altas de terpenos que sus respectivos controles, principalmente α-terpineol, y una mejora de los aromas frutales.

Abstract

In recent years, the sparkling wine market has begun to grow quite fast in our country. However, Cava wines continue cornering the largest percentage of sales of quality Spanish sparkling wines. More and more Spanish wineries are elaborating sparkling wines following the traditional method in order to extend their range of products and open new markets. In this sense, Castilla y León County has an important number of grape varieties typically employed to elaborate still wines, which could also have good characteristics to obtain quality sparkling wines. Among Castilla y León´s grape varieties which may be more suitable for the production of white sparkling wines, Viura, Verdejo, Malvasía, Albarín and Godello stand out. Due to their importance in Castilla y León viticulture, Garnacha, Tempranillo and Prieto Picudo stand out for the production of rosé sparkling wines.

The first objective of this thesis was the enological characterization of rosé monovarietal sparkling wines from Garnacha and Prieto Picudo red grape varieties, and white monovarietal sparkling wines from Malvasía, Verdejo, Godello, Viura and Albarín white grape varieties. The sparkling wines employed in the analysis were manufactured using the traditional method and were evaluated during the nine months of aging on yeast lees. Monomeric and polymeric phenolic compounds, amino acids, biogenic amines, volatile compounds, and polysaccharides were analyzed. Stabilization and clarification of the base wines significantly decreased the concentrations of anthocyanins and proanthocyanidins. During the first six months of aging on yeast lees, all types of polyphenols decreased, although some were released back into the wine during the last three months of aging. Prieto Picudo rosé sparkling wines had greater color intensity, anthocyanin concentrations, and color stability than Garnacha wines, which had more hydroxycinnamic acids. Among whites, Albarín had the most catechin, proanthocyanidins, and, together with Viura, hydroxycinnamates. Garnacha rosé and Albarín white wines had the highest phenolic potentials. During the aging on lees in the bottle, amino acids concentration remained constant or decreased, branched ethyl

esters, ethyl lactate and γ -butyrolactone increased, and a decrease in terpenes was observed. Sparkling wines from Albarín, Verdejo, Godello and Prieto Picudo were the richest in amino acids and in the most of the volatile compounds analyzed, especially ethyl esters and alcohol acetates. Total biogenic amine concentration remained constant during the first three months of aging and a slight increase in total biogenic amines was observed in all the wines during the second three month period of aging. The Albarín and Prieto Picudo sparkling wines showed the highest content of total biogenic amines, although these concentrations were far below of the limits that can cause toxic effects. With regards to polysaccharide content, among white sparkling wines, Verdejo showed the highest amount of total polysaccharides, whereas Prieto Picudo sparkling wines showed the highest quantity among the rosé wines. Although rhamnogalacturonan type II polysaccharide was only present in Prieto Picudo sparkling wines, all wines were essentially composed of polysaccharides rich in arabinose and galactose, mannoproteins, glucans and homogalacturonans, with average percentages of 33 ± 5%, 25 ± 9%, 36 ± 9% and 6 ± 3%, respectively.

The second objective was to analyze the sensory attributes of rosé monovarietal sparkling wines from Garnacha and Prieto Picudo red grape varieties and white monovarietal sparkling wines from Malvasía, Verdejo, Godello, Viura and Albarín white grape varieties, after nine months of aging on yeast lees having been manufactured using the traditional method. All wines obtained good punctuations in the sensory analysis, however, Prieto Picudo had more visual color intensity, red tones, olfactory intensity, freshness and foam quality than Garnacha wines. Albarín and Godello had more olfactory intensity than Viura and Malvasía wines, and Verdejo had better foam quality.

The third objective aimed to analyze the changes occurring on polysaccharide families and molecular weights of polysaccharides of rosé monovarietal sparkling wines from Garnacha and Prieto Picudo red grape varieties, and white monovarietal sparkling wines from Malvasía, Verdejo, Godello, Viura and Albarín white grape varieties. The sparkling wines employed in the analysis were manufactured using the traditional method and were evaluated during thirty months of aging on yeast lees. After six months of aging the highest content of mannoproteins and polysaccharides rich in arabinose and galactose was obtained. In this sense, these results suggest that longer aging time is not necessary to obtain greater amount of polysaccharides in sparkling wines. On the other hand, a shift to lower molecular weights polysaccharides was observed at six months of aging on yeast lees. The combination of these two

characteristics could imply a better foam stability and thus sensory quality of sparkling wines.

The fourth objective was to study the influence of grape variety and aging time in contact with and without lees, on volatile composition and foam properties. This influence was measured in rosé monovarietal sparkling wines from Garnacha and Prieto Picudo red grape varieties, and white monovarietal sparkling wines from Malvasía, Verdejo, Godello, Viura and Albarín white grape varieties. Sparkling wines were elaborated following the traditional method. The results obtained indicated that sparkling wines from Albarín, Verdejo, Godello and Prieto Picudo grape varieties were the richest in most of the volatile compounds analyzed, especially those that contribute to the fruity aroma of wines, and maintained their varietal characteristics both during the aging on lees and during the aging without lees. Verdejo and Prieto Picudo sparkling wines showed the best foam characteristics during the aging on lees and during the aging without lees, followed by Albarín and Godello wines. Foam characteristics were maintained or increased over the aging time on lees. The foam quality did not reduce during aging without lees.

The fifth objective was to study the influence of wine constituents such as polysaccharides, phenolics and nitrogen compounds in the foam properties of sparkling wines. Results obtained indicated positive contribution of monomeric anthocyanins and amino acids and negative contribution of proanthocyanidins to the foamability parameters of sparkling wines. Mannoproteins and polysaccharides rich in arabinose and galactose were poor foam formers but good foam stabilizers. Multiple linear regression analysis showed that anthocyanins were the compounds with a highest positive influence on the foamability parameter, followed by amino acids. Among anthocyanins, malvidin-3-glucoside and malvidin-3-(6-acetyl)-glucoside were those showing the highest influence on the foamability parameters, followed by amino acid compounds, mainly b-alanine. The best model to explain foam stability was only predicted by polysaccharides rich in arabinose and galactose.

Finally, the sixth objective focused on the effect of commercial dry yeast autolysates addition to base wines, on the chemical composition, and sensory properties of rosé and white monovarietal sparkling wines. Rosé sparkling wines were from Tempranillo and Garnacha red grape varieties, and white monovarietal sparkling wines were from Verdejo and Godello white grape varieties, manufactured using the traditional method during nine months of aging on yeast lees in the bottle. The addition of the commercial dry yeast autolysates to base wines did not have any effect on phenolic compounds,

amino acids or biogenic amines content, neither on foam properties. On the contrary, the addition of these products can modify the volatile profile of sparkling wines. However, this effect was associated to the content in mannoproteins and purity of dry yeast autolysates added. So, sparkling wines treated with the product with the highest mannoprotein content and the highest purity showed volatile changes compared to the control wines. In addition white sparkling wines treated with this product showed higher concentrations of terpenes compared with their respective control wines, mainly of α-terpineol, and an improvement in the fruity aromas.

1 JUSTIFICACIÓN Y OBJETIVOS

El sector del vino en Castilla y León ha experimentado en los últimos años un importante auge, como lo demuestra el hecho de que los vinos de esta comunidad alcanzan el 20,9% del mercado nacional en el año 2013, siendo la única región cuya cuota de mercado de vino crece desde 1995. Sin embargo, para que los vinos castellano leoneses puedan seguir posicionándose con fuerza en el mercado nacional e internacional es necesario diversificar su oferta con nuevos productos. En este sentido, uno de sectores del vino que más ha crecido en los últimos años es el de los vinos espumosos elaborados por el método tradicional.

Aunque el Cava sigue acaparando el mayor porcentaje de ventas de vino espumoso de calidad español, cada vez son más las bodegas que están elaborando vino espumoso siguiendo el método tradicional con el fin de ampliar su oferta y abrir nuevos mercados. En este sentido hay que destacar que Castilla y León es una comunidad autónoma con una gran tradición vitivinícola, que cuenta con zonas muy diferentes entre sí y con un gran número de variedades perfectamente adaptadas a cada zona de cultivo. Muchas de estas variedades que no se emplean para la elaboración de vinos de gama media y alta, podrían tener buenas aptitudes para la elaboración de vinos espumosos de calidad, ya que son variedades con una acidez relativamente elevada, que alcanzan una graduación alcohólica moderada y que producen vinos frescos en boca y con aromas finos y afrutados en nariz. Entre las variedades de Castilla y León que podrían ser más adecuadas para la elaboración de vinos blancos espumosos destacan las variedades Viura, Verdejo, Malvasía, Albarín y Godello. Para la elaboración de vinos rosados espumosos destacan, debido a su importancia en la vitivinicultura de la región, las variedades Garnacha, Tempranillo y Prieto Picudo. Sin embargo, existen pocos trabajos científicos sobre las características químicas y propiedades sensoriales de los vinos espumosos obtenidos con estas variedades. Así mismo es necesario estudiar su evolución durante la fase de crianza sobre lías tras la segunda fermentación, así como durante la crianza en ausencia de lías tras el degüelle.

La calidad de los vinos espumosos viene determinada por la calidad de la espuma que se forma y por las características organolépticas de los mismos. La espuma es una

cualidad que define al vino espumoso, que la distingue de otros vinos y es la primera que observa el consumidor. Por esta razón, la espuma ha merecido una especial atención y ha sido objeto de diferentes estudios científicos encaminados a detectar los compuestos que afectan a las propiedades espumantes, aunque aún existe desconocimiento sobre cuáles son los que influyen en la formación y estabilidad de la espuma. Durante la crianza sobre lías en botella se produce la autolisis de las levaduras, con la cesión de determinadas sustancias al vino elaborado, especialmente manoproteínas que mejoran el desprendimiento de gas carbónico y comunican al vino unos caracteres sensoriales singulares, por ello la adición a los vinos de preparados comerciales a base de levaduras ricos en manoproteínas podría mejorar las características organolépticas del vino final. Aunque el empleo de distintos productos comerciales ricos en manoproteínas para la elaboración de vinos tranquilos está bastante extendido y existen trabajos científicos al respecto, hasta el momento pocos estudios evalúan el efecto de estos preparados como aditivos en la composición química y en la calidad sensorial de vinos espumosos.

El interés económico y social que esta tesis tiene es claro, ya que permitiría impulsar la economía del sector vitivinícola de Castilla y León al ampliar la oferta de las bodegas de la región con un tipo de vino muy demandado por los consumidores. El vino obtenido tendría una identidad propia al estar elaborado con variedades diferentes a las tradicionalmente empleadas para la elaboración de vinos espumosos, que le permitiría posicionarse bien en el mercado. Así mismo permitiría sacar del olvido variedades de uva que no se emplean para la elaboración de vinos de calidad, y que están viendo en peligro su supervivencia al ser sustituidas por variedades más comerciales. Además se pretende profundizar en el conocimiento de la implicación de la composición química del vino espumoso sobre sus características espumantes. Hay que indicar que en esta tesis también se pretende mejorar la calidad de los vinos espumosos mediante el uso de distintos derivados comerciales de levadura ricos en polisacáridos. Su uso podría mejorar las características organolépticas de este tipo de vinos, lo cual sería de gran interés e importancia desde el punto de vista cualitativo para las bodegas elaboradoras de vino espumoso. Desde un punto de vista científico se podrá conocer la evolución de la composición química y de las propiedades espumantes de los vinos espumosos durante su crianza en botella en presencia y en ausencia de lías, así como conocer los mecanismos de actuación de los preparados comerciales derivados de levadura, obteniéndose información sobre su efecto en los parámetros que determinan la calidad final de este tipo de vinos.

Por las razones anteriormente expuestas, se ha llevado a cabo este trabajo, cuyos objetivos son:

1. Caracterizar enológicamente los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional, durante la elaboración y la crianza sobre lías de nueve meses, en términos de compuestos fenólicos monoméricos y poliméricos, aminoácidos, aminas biógenas, compuestos volátiles y polisacáridos.
2. Analizar sensorialmente los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, con nueve meses de crianza sobre lías y elaborados por el método tradicional.
3. Analizar los cambios en familias de polisacáridos y en la distribución de sus pesos moleculares durante la elaboración y la crianza sobre lías de treinta meses de vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional.
4. Estudiar la influencia de la variedad de uva y del tiempo de crianza en presencia y en ausencia de lías en la composición volátil y en las propiedades espumantes de los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional.
5. Estudiar la influencia de los constituyentes, como polisacáridos, polifenoles y sustancias nitrogenadas, del vino espumoso en sus características espumantes.
6. Estudiar el efecto de la adición de autolisados de levaduras comerciales a los vinos base en la composición y las características organolépticas de los vinos rosados espumosos monovarietales de las variedades de uva tintas de Tempranillo y Garnacha y blancos de las variedades de uva de Verdejo y Godello, elaborados por el método tradicional, durante una crianza sobre lías en botella de nueve meses.

2 INTRODUCCIÓN

La *Introducción* se ha dividido en nueve apartados. En un primer apartado se describe la situación de mercado de los vinos espumosos a nivel mundial, en España y más concretamente, en Castilla y León, describiéndose brevemente las variedades de uva empleadas en esta tesis. En el *apartado 2.2*. se detalla el proceso de elaboración de los vinos espumosos producidos por el método tradicional. A continuación, y teniendo en cuenta que el proceso biológico más importante que tiene lugar durante el envejecimiento sobre lías de los vinos espumosos es la autolisis de las levaduras, se realiza una breve descripción de este proceso (*apartado 2.3.*). Teniendo en cuenta que la espuma, el color y el aroma de un vino espumoso son características organolépticas que definen su calidad, se hace referencia a cada una de ellas en el *apartado 2.4.*, describiéndose además los compuestos fenólicos (*apartado 2.5.*), volátiles (*apartado 2.6.*), aminoácidos y aminas biógenas (*apartado 2.7.*), y polisacáridos (*apartado 2.8.*), con objeto de resaltar su implicación en el proceso de vinificación y en las características organolépticas de los vinos blancos y rosados espumosos. Por último, y considerando los efectos positivos que pueden tener las manoproteínas en los vinos espumosos, se realiza una descripción bibliográfica sobre el empleo de productos comerciales a base de manoproteínas en vinos espumosos (*apartado 2.9.*).

2.1. Mercado de los vinos espumosos

Uno de sectores del vino que más ha crecido en los últimos años es el de los vinos espumosos. La producción mundial de vinos espumosos ha alcanzado los 17,6 millones de hectolitros en 2013 y registra un aumento del 11% en relación con 2012 y del 40% en los últimos diez años, según el informe de la Organización Internacional de la Viña y el Vino (OIV) sobre estos vinos presentado en el 37º Congreso de esta institución en el año 2014. La parte correspondiente a los vinos espumosos con respecto a la producción total de vino también ha aumentado significativamente estos últimos años. Mientras que en el año 2000 el espumoso no representaba más que el 4% de la producción mundial de vinos, en 2013 ha sobrepasado el 7% (*1*).

La estacionalidad del consumo de los vinos espumosos es su principal característica. Sin embargo, se nota una tendencia a suavizar estos picos de consumo, manteniéndose estable a lo largo de todo el año. Mientras el consumo mundial de vinos aumentó un 4% en estos diez últimos años, los vinos espumosos (que representan un 6% del consumo total de vinos), registraron un incremento del 30%. Tras una disminución debida a la crisis, el consumo vuelve a crecer en estos últimos tres años, para llegar hasta los 15,4 MHL, un progreso del 4% en relación al año anterior (*1*).

Del mismo modo, con respecto a la situación en España y según datos del Ministerio de Agricultura y Medio Ambiente (MAGRAMA), la producción de vino espumoso con Denominación de Origen ha experimentado un crecimiento positivo, destacando el incremento de producción de vinos tintos y rosados espumosos (**FIGURA 1**).

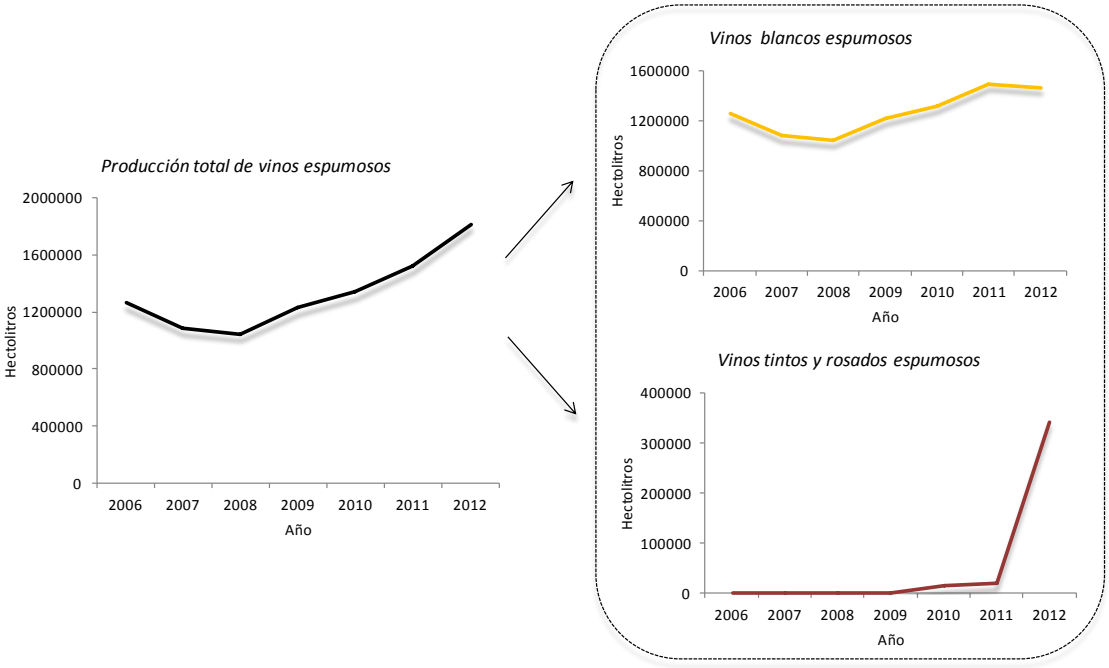


FIGURA 1. Evolución de la producción de vinos espumosos con Denominación de Origen. Elaboración propia con datos del Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA)

La tendencia actual de los últimos años indica un incremento en el consumo de este tipo de vinos en España. Así, en el año 2011 el consumo de vinos espumosos creció un 6,5% en volumen (datos del Observatorio Español del Mercado del Vino, 2011). El informe Market Trends de Nielsen muestra un aumento de las ventas en España del 4,4% en el primer semestre del año 2014, confirmando un aumento continuado año a año de este tipo de vinos, mientras que las ventas de los vinos tranquilos cayeron un 1%.

Las exportaciones de vinos espumosos españoles han crecido en volumen de ventas en los últimos años (desde 2000 hasta 2013) en un 86% y en términos de valor

económico un 122%. Son los vinos espumosos (principalmente el Cava), los que suponen el segundo mayor componente de la facturación exterior, después del vino tranquilo, aunque en un entorno cada vez más competitivo y de rentabilidad más difícil (datos de la Federación Española del Vino).

Un 81,4% de la producción de los vinos espumosos en España corresponden a la D.O. Cava (*2*). Esta D.O. está ampliamente extendida por el territorio nacional, aunque el 98% de los vinos son producidos en la comarca catalana del Penedés (*3*). Las variedades autorizadas por el Consejo Regulador del Cava para elaboración del vino blanco espumoso que recibe esta denominación son Viura, Xarel.lo, Parellada, Malvasía y Chardonnay en blancas y Garnacha, Monastrell y Pinot Noir en tintas. Para la producción de Cava rosado también se permite el uso de Trepat. Sin embargo, las variedades que constituyen la base para la producción de Cava son Macabeo, Xarel.lo y Parellada, ocupando las tres variedades citadas una superficie vitícola que representa más del 90% del total de las variedades permitidas (datos del Consejo Regulador del Cava, 2013). Este hecho provoca la obtención de vinos espumosos homogéneos, y por lo tanto con menos características típicas y diferenciadoras que sólo se pueden conseguir con el empleo de otras variedades. En este sentido, cada vez son más las bodegas que están elaborando vinos espumosos siguiendo el método tradicional, aunque usando otras variedades de uva, con el fin de ampliar su oferta y abrir nuevos mercados. Así, Consejos Reguladores como el de la D.O. de Valdeorras, Vinos de Madrid, Vinos de Alicante, Rueda o La Mancha amparan en su reglamento la elaboración de vinos espumosos, permitiendo el empleo de variedades de uva diferentes a las anteriormente citadas.

2.1.1. Situación de mercado actual de los vinos espumosos en Castilla y León

El sector del vino en Castilla y León ha experimentado en los últimos años un importante auge como lo demuestra el hecho de que los vinos de esta comunidad alcanzan el 20,9% del mercado nacional en el año 2013, siendo la única región cuya cuota de mercado de vino crece desde 1995 (datos de estudios de mercados realizados por la consultora Nielsen).

Esta comunidad autónoma, con sus nueve Denominaciones de Origen (**FIGURA 2**), ofrece una amplia gama de vinos para llegar a todos los consumidores, adaptándose a sus gustos cambiantes. Sin embargo, para que los vinos castellano leoneses puedan seguir posicionándose con fuerza en el mercado nacional e internacional, e incrementar sus ventas, es necesario diversificar su oferta con nuevos productos. De hecho, cada vez

más bodegas están apostando por elaborar vinos espumosos a partir de sus variedades autóctonas.

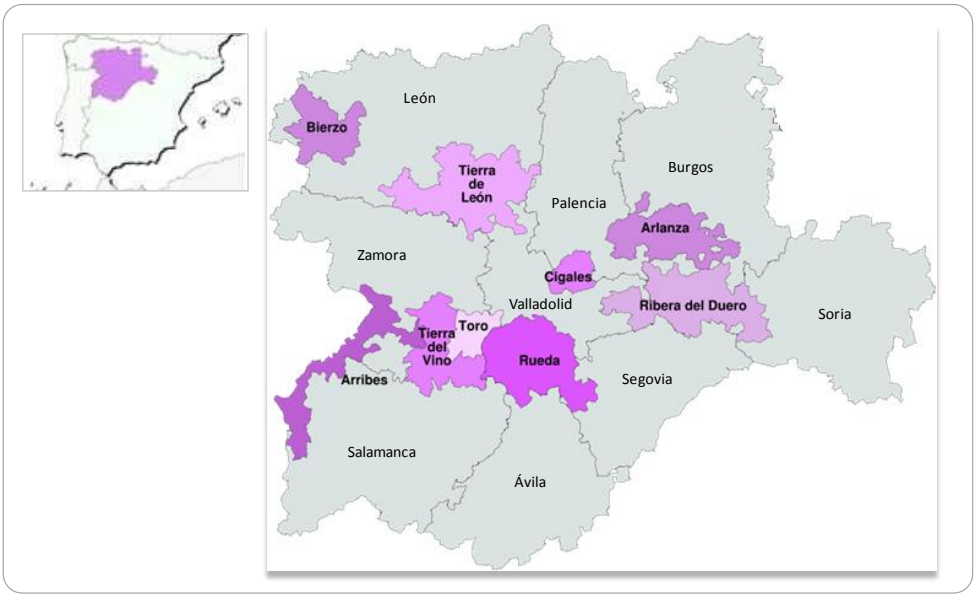


FIGURA 2. Denominaciones de Origen de Castilla y León

Hasta 2011, Rueda era la única D.O. que recogía en su reglamento la elaboración de vinos espumosos en Castilla y León. Recientemente se ha aprobado el Reglamento de la D.O. Cigales y de su Consejo Regulador (orden AYG/1197/2011 del 22 de septiembre), en el que se ha incluido la elaboración de este tipo de vinos. Esto pone de manifiesto el interés creciente de las diferentes zonas vitivinícolas de Castilla y León por elaborar nuevos productos.

La variedad principal con la que se elaboran los vinos espumosos en Castilla y León es la Verdejo. Sin embargo, la gran diversidad vitivinícola de esta región hace que la oferta de vinos espumosos pueda ser muy diversa y amplia, y que se pueda satisfacer la demanda de todo tipo de consumidores. De hecho, muchas de las variedades de Castilla y León tradicionalmente empleadas para la elaboración de vinos tranquilos presentan buenas aptitudes para la elaboración de vinos base para espumosos de calidad, ya que son variedades con una acidez relativamente elevada, que alcanzan una graduación alcohólica moderada y que producen vinos frescos en boca y con aromas finos y afrutados en nariz. Entre las variedades de Castilla y León que podrían resultar adecuadas para la elaboración de vinos blancos espumosos destacan las variedades Viura, Verdejo, Malvasía, Albarín y Godello. Para la elaboración de vinos rosados espumosos destacan, debido a su importancia en la vitivinicultura de la región, las variedades Garnacha, Tempranillo y Prieto Picudo. El empleo de estas variedades de uva permitirá obtener vinos con una personalidad propia, que les permita diferenciarse del

resto de los vinos que existen en el mercado. A continuación se hace una breve descripción de cada una de estas variedades.

Viura

También denominada Macabeo, es la uva blanca más cultivada de España después de la variedad Airén, con una superficie de 38.418 hectáreas (datos del Ministerio de Agricultura y Alimentación, 2009). Se utiliza como vino base para la elaboración de vinos espumosos de la D.O. Cava, junto a las Xarel.lo y Parellada. La planta es de brotación y maduración tardía, bastante productiva, con racimos compactos grandes y bayas esféricas de color amarillo verdoso (**FIGURA 3**). Sus vinos presentan buena acidez, no muy alcohólicos, bastante equilibrados y de delicado aroma, destacando los aromas a manzana verde y herbáceos (*4*).

Verdejo

Es originaria de la comarca vallisoletana de Rueda, encontrándose de forma minoritaria en otras zonas y ocupando una superficie total de 6.034 hectáreas (datos del Ministerio de Agricultura y Alimentación, 2009). Se trata de una de las variedades blancas españolas de mayor calidad y tipicidad, de ahí que suele tratarse sola en vinos monovarietales sin complementar con otra uva. La planta es poco vigorosa, de baja fertilidad, con racimos pequeños y compactos (**FIGURA 3**). Produce unos elegantes vinos pálidos, de color amarillo pajizo verdoso, con aromas bastante intensos a frutas tropicales y vegetales que recuerdan al anís, muy similares aromáticamente a los obtenidos con la variedad Sauvignon Blanc (*5*), manifestando en la boca buen cuerpo y matices almendrados amargos (*4*).

Malvasía

Su cultivo se distribuye por todo el territorio nacional, ocupando una superficie de 4.657 hectáreas (datos del Ministerio de Agricultura y Alimentación, 2009). La variedad es de brotación y maduración temprana, muy poco vigorosa y de baja productividad, con racimos medianos y bayas medianas de color amarillo verdoso (**FIGURA 3**). Se trata de una variedad muy aromática, que contiene un elevado nivel de terpenos, no sólo en el hollejo, sino también en la pulpa (*4*).

Albarín

Es originaria de Asturias y actualmente se encuentra en peligro de extinción. Ocupa una superficie en la D.O. Tierras de León de 30 hectáreas (datos del Consejo Regulador D.O. Tierras de León, 2011), aunque debido a su potencial los kilogramos calificados en la D.O. Tierras de León se han visto incrementados en los últimos tres años en un 87%

(datos del Consejo Regulador de la D.O. Tierra de León, 2014). La variedad es de maduración temprana, los racimos son medianos, poco apretados y las bayas de color amarillo dorado con hollejo delgado (**FIGURA 3**). Produce vinos florales y especiados (*6,7*).

Godello

Es una variedad originaria de Galicia y se cultiva a lo largo de 579 hectáreas en toda España (datos del Ministerio de Agricultura y Alimentación, 2009). Se trata de una variedad de maduración precoz, productiva y de vigor medio, siendo los racimos pequeños con granos de uvas de color verde intenso (*4*) (**FIGURA 3**). Produce unos vinos de gran personalidad, característicos por sus aromas a manzana y fruta tropical (*8*). Debido a sus características sensoriales, la variedad Godello se elabora casi siempre de forma monovarietal, obteniéndose de ella vinos dorados, muy aromáticos y estructurados.

Garnacha

Se trata de una de las variedades tintas más extendidas en España, con 58.757 hectáreas (datos del Ministerio de Agricultura y Alimentación, 2009), siendo la variedad tinta más se cultivada en el mundo. Se utiliza como vino base para la elaboración de vinos rosados espumosos de la D.O. Cava. Tiene racimos de tamaño medio y compactos, las bayas son de tamaño mediano, forma ovalada y color rojo oscuro, morado (**FIGURA 3**). Se trata de una planta de alta productividad, muy rústica y capaz de vivir en condiciones muy adversas en cuanto al clima y al suelo, produciendo vinos no excesivamente ácidos, ni tampoco coloreados y unos excepcionales vinos rosados (*4*), con notas cítricas y frutales (*9*).

Tempranillo

Se trata de la variedad tinta española de mayor calidad y fama, siendo la variedad tinta más cultivada en España, con 201.235 hectáreas. Ocupa el segundo puesto de la totalidad de las variedades, supone casi el 20% del viñedo español y el 37% de la superficie de uva tinta (datos del Ministerio de Agricultura y Alimentación, 2009). Presenta racimo cilíndrico, a menudo con dos alas, muy compacto y de mediano tamaño y bayas esféricas, medianas y de color negro azulado (**FIGURA 3**). El nombre de Tempranillo procede de su maduración temprana, produciendo vinos tintos con un inconfundible aroma a frutos negros y a regaliz (*4*). Debido a su gran importancia en la vitivinicultura española, el presente estudio abre nuevas e interesantes posibilidades para el empleo de esta variedad en el mundo de los vinos espumosos.

Prieto Picudo

La variedad tinta Prieto Picudo (**FIGURA 3**) es originaria de la región leonesa de Valdevimbre-Los Oteros, cultivándose casi exclusivamente en esta provincia, en una superficie de 4.588 hectáreas (datos del Ministerio de Agricultura y Alimentación, 2009). Dado el gran interés que despierta esta variedad, en la actualidad se están plantando viñedos en zonas limítrofes de Castilla y León. Se trata de una de las variedades tintas españolas de mayores expectativas de futuro. Es una variedad de brotación y maduración temprana, poco vigorosa y de fertilidad media, con racimos pequeños compactos (*4*). Tradicionalmente esta variedad se empleaba para la elaboración de vinos rosados de aguja bajo la práctica del madreo, un método que consiste en la adición de racimos enteros durante la fermentación del mosto. Produce vinos de baja graduación alcohólica, acidez alta, frescos y muy aromáticos (*10*), que poseen descriptores volátiles similares a los encontrados en la variedad Sauvignon Blanc (*11*).

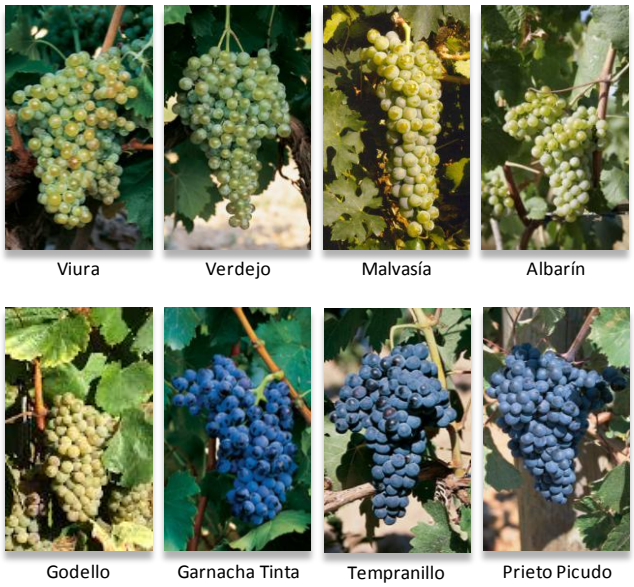


FIGURA 3. Variedades empleadas en este estudio para la elaboración de los vinos espumosos

Teniendo en cuenta que la variedad de uva empleada para la elaboración de los vinos espumosos es uno de los factores que más influyen en su composición (*12-14*) y por tanto en su calidad, resultaría interesante analizar las características químicas y propiedades sensoriales de los vinos espumosos obtenidos con estas variedades. De hecho, aunque son diversos los factores que afectan a la composición del vino espumoso, como las características vitícolas, la tecnología de vinificación aplicada o la duración del periodo de crianza sobre lías, la variedad de uva seleccionada para la elaboración del vino base es considerado uno de los más importantes (*12,14*).

2.2. Elaboración de los vinos espumosos según el método tradicional

Los vinos espumosos naturales, según la Orden del 27 de Julio de 1972 (B.O.E. de 8 de Agosto de 1972) por la que se reglamentan los vinos espumosos naturales y los vinos gasificados, *son aquellos vinos procedentes de uvas de variedades adecuadas, que contienen, como consecuencia de su especial elaboración, gas carbónico de origen endógeno y que al ser descorchada la botella y escanciado el vino forma espuma de sensible persistencia, seguida de un desprendimiento continuo de burbujas. El gas carbónico habrá de proceder de una segunda fermentación, realizada en envase herméticamente cerrado, de azúcares naturales del vino base o azúcares agregados y el producto terminado deberá tener una presión mínima de cuatro atmósferas, medida a 20 °C*.

Dentro de los vinos espumosos naturales se distinguen en España cuatro tipos: *Cava*, *Vino espumoso natural*, *Fermentación en botella* o *Método Transfer*, y *Grandes Envases* o *Granvás* (*15*). Los dos primeros se caracterizan por elaborarse por el método clásico o tradicional. Estos vinos deben efectuar el proceso de elaboración y crianza, desde la segunda fermentación hasta la eliminación de las lías inclusive, en la misma botella en la que se ha realizado el tiraje, siendo el tiempo mínimo de permanencia del vino con las lías de nueve meses. Se diferencian por pertenecer el primero a la Denominación de Origen (D.O.) Cava, mientras que el segundo, se elabora con el mismo sistema, pero no pertenece a dicha denominación.

Por otro lado, en la elaboración de los vinos espumosos *fermentados en botella*, todo el proceso de fermentación y crianza se debe efectuar en la propia botella, durante al menos dos meses, en cavas o locales de condiciones térmicas adecuadas, pudiendo ser trasvasado el vino posteriormente, para efectuar la eliminación de las lías. Los vinos espumosos *Granvás* realizan la segunda fermentación en grandes envases o depósitos de cierre hermético, de los que se transvasa a botellas para su comercialización. El tiempo mínimo de permanencia del vino con las levaduras antes de salir al mercado es de veintiún días.

Los vinos espumosos se denominan, en función de su riqueza en azúcares, como:

- Seco o sec: de 0 a 30 g/L de materias reductoras.
- Semiseco o semidulce: de 30 a 50 g/L de materias reductoras.
- Dulce: superior a 50 g/L de materias reductoras.

Aquellos espumosos naturales con materias reductoras en cantidad inferior a 20 g/L pueden denominarse con la calificación de bruto o *brut*. Según lo dispuesto en el

Reglamento (CE) nº 607/2009, son vinos espumosos *brut nature* aquellos cuyo contenido en azúcar es inferior a 3 g/L y no se le ha añadido azúcar después de la segunda fermentación.

Las principales etapas de la elaboración de los vinos espumosos por el método tradicional son: elaboración del vino base, tiraje, rima, punta, y degüelle. El esquema de elaboración se muestra en la **FIGURA 4**.

FIGURA 4. Esquema del proceso de elaboración de los vinos espumoso mediante el método tradicional. Adaptado de (*10*)

2.2.1. Elaboración del vino base

Para la elaboración del vino base se sigue el proceso tradicional de elaboración de vinos tranquilos. La cosecha se realiza con una madurez de la uva menos avanzada que para la elaboración de vinos tranquilos (*12*), en consecuencia, los procesos de prensado, extracción del mosto y maceración prefermentativa deben ser muy cuidadosos para evitar defectos de amargor, de carácter vegetal y de oxidaciones. Los vinos base deben presentar ciertas características organolépticas, tales como baja tanicidad y aroma afrutado, así como analíticas, como una concentración de oxígeno suficiente para el desarrollo de las levaduras, poco contenido en azúcar residual, contenido en alcohol moderado que permita una adecuada toma de espuma, acidez relativamente elevada que comunique una importante sensación de frescura en boca y una baja acidez volátil. No es habitual que los vinos base realicen la fermentación maloláctica, salvo que su contenido en ácido málico sea excesivo, y convenga entonces reducirlo por motivos sensoriales.

Los vinos base, antes de ser introducidos en la botella con el licor de tiraje deberán estar perfectamente limpios y estabilizados frente a precipitaciones tartáricas y proteicas (*13*), ya que una vez introducidos en la botella junto con el licor de tiraje no se le podrá hacer ningún otro tratamiento. El vino base deberá de tener las siguientes características analíticas:

- Graduación alcohólica: 9,5-11,5% vol.
- Acidez total (en ácido sulfúrico): 3,5-6 g/L
- Extracto seco no reductor: 12,5-20 g/L
- Acidez volátil real (en ácido acético) < 0,7 g/L
- SO_2 libre < 20 mg/L
- SO_2 total < 170 mg/L

2.2.2. Tiraje

Es el llenado de la botella con el vino base y la adición de una disolución que se denomina *licor de tiraje*. Posteriormente las botellas son cerradas con un tapón corona temporal.

El licor de tiraje es una disolución formada por levaduras y sacarosa en proporción adecuada para que la segunda fermentación produzca la presión de dióxido de carbono deseado. La cantidad máxima de sacarosa que puede ser adicionada será de 25 gramos

por litro de vino base. Además, se suele añadir una pequeña cantidad de bentonita con el objetivo de facilitar la floculación de las levaduras para su posterior eliminación (*16*).

Las cepas de levaduras utilizadas en la segunda fermentación deben presentar una serie de características como actividad fermentativa a baja temperatura, resistencia al etanol y a la presión de CO_2, capacidad floculante, etc. (*17*).

2.2.3. Rima

Las botellas se colocan en posición horizontal en locales de crianza especialmente habilitados para ello, llamados cavas. Durante esta etapa se producirá la segunda fermentación, toma de espuma y crianza (envejecimiento sobre lías).

La legislación española establece que un vino espumoso deberá permanecer en presencia de las lías, desde el momento del tiraje hasta el degüelle, como mínimo nueve meses. La D.O. Cava establece las menciones *Reserva* y *Gran Reserva* para aquellos espumosos cuya crianza, contada desde el momento del tiraje hasta el degüelle, no sea inferior a quince y treinta meses, respectivamente.

2.2.4. Punta

En esta etapa las botellas se someten a un proceso de removido para conseguir que todo el sedimento se dirija al cuello de la misma. Este proceso, que se hacía tradicionalmente colocando las botellas sobre pupitres girándolas manualmente un octavo de vuelta cada día durante quince días hasta que quedaban prácticamente perpendiculares al suelo, ha sido sustituido en la actualidad por sistemas más o menos automatizados que permiten manipular un conjunto de botellas de una sola vez.

2.2.5. Degüelle

Consiste en la eliminación de las lías depositadas en el cuello de la botella. En la actualidad este depósito se elimina congelando el cuello de la botella al introducirla en un baño criogénico. A continuación se invierte y se le quita el tapón y por la propia presión interna, el depósito congelado se expulsa. Si se emplean levaduras incluidas o inmovilizadas en bolitas de alginato cálcico o dentro de un cartucho que se coloca en el cuello de la botella, se evita la etapa de punta y se facilita el degüelle, pues no es necesario la congelación del cuello de la botella con las lías (*18,19*). Durante la etapa de degüelle el vino debe quedar perfectamente brillante, sin muestra de sedimento alguno

después de realizar esta operación. Se pueden producir perdidas de vino, las cuales se compensan con la adición, hasta restablecer el volumen inicial, del *licor de expedición*, que puede ser el propio vino espumoso o bien una disolución de sacarosa, mosto de uva, mosto de uva parcialmente fermentado, o mosto de uva concentrado rectificado o no, vino base o una mezcla de dichos productos, con adición en su caso de destilados de vino. De esta manera se consigue dar al vino espumoso el grado de dulzor deseado. Por último se cierra la botella con el tapón definitivo, el cual se sujeta al cuello de ésta con el bozal o morrión.

2.2.6. Envejecimiento en botella en ausencia de lías

Tras el degüelle y el taponado definitivo, los vinos espumosos pueden ser envejecidos en botella durante meses o incluso durante años antes de su consumo. De este modo los sabores pueden evolucionar y las notas más frescas se pueden tornar en florales, siguiendo madurando con matices de frutos secos, tostados o incluso dar lugar a notas oxidativas. Sin embargo, tras el degüelle, pueden producirse defectos en el vino debidos fundamentalmente a fenómenos de oxidación y/o reducción. El degüelle causa un choque oxidativo ya que el potencial redox puede incrementarse bruscamente en 400 mV durante esta operación (*20*). Por otro lado, durante el envejecimiento en ausencia de lías pueden existir fenómenos de pardeamiento, afectando negativamente al color de los vinos espumosos (*21*), además de una pérdida de CO_2 a través del corcho (*22*), ocasionando una depreciación de la calidad de la espuma (*23*).

Los vinos espumosos, terminada su elaboración, deberán tener las siguientes características analíticas:

- Graduación alcohólica: 10,8-12,8% vol.
- Acidez total (en ácido sulfúrico): 3,5-6 g/L
- Extracto seco no reductor: 12-20 g/L
- Acidez volátil real (en ácido acético) < 0,8 g/L
- SO_2 libre < 20 mg/L
- SO_2 total < 170 mg/L
- Presión de CO_2 (a 20 °C) > 4 atm

2.3. Proceso autolítico de las levaduras

Durante la elaboración de los vinos espumosos por el método tradicional tiene lugar un proceso de crianza sobre lías que está estrechamente relacionado con la calidad sensorial del vino espumoso final. De hecho, es durante esta crianza cuando tiene lugar la autolisis de las levaduras, proceso que consiste en la autodegradación enzimática de los constituyentes celulares de las levaduras muertas, en el cual se liberan compuestos al vino que pueden modificar significativamente su composición final (*24-26*).

Normalmente el proceso autolítico se inicia al final de la fase estacionaria de crecimiento de las levaduras y está asociado a la muerte celular (*27*). Está estimado que la autolisis de las levaduras comience entre los dos y cuatro meses después de terminar la segunda fermentación en botella (*24,28*). La autolisis se desarrolla tan sólo en unas horas cuando las condiciones son óptimas (*29*), aunque en las condiciones del vino base (pH ~ 3-3,5; temperatura ~ 10-15 °C; etanol ~ 10% y elevada presión de CO_2) se dilata en el tiempo hasta varios meses o años (*30-34*).

Se distinguen cuatro etapas diferenciadas a lo largo del proceso autolítico de las levaduras. Inicialmente las actividades de las enzimas endo y exo-β-(1,3) glucanasas liberan una mezcla de polisacáridos y de cadenas cortas de oligosacáridos, constituyentes de la pared celular de la levadura no viable. Una fracción de estos polisacáridos corresponde a las manoproteínas unidas covalentemente al glucano de la pared intacta. Posteriormente la hidrólisis parcial del glucano provoca una desestabilización de la estructura de la pared, que supone una liberación de manoproteínas de elevado peso molecular con bajos contenidos de glucosa y que proviene mayoritariamente de la zona periplasmática. En una etapa más tardía continúa la degradación de los glucanos de la pared por las β-(1,3)-glucanasas en los restos de pared y en el medio extracelular. Finalmente, las exo-β-(1,3)-glucanasas solubilizadas en el medio degradan el glucano unido a las manoproteínas y éstas a su vez pueden ser hidrolizadas por α-manosidasas y por otras proteasas que liberan peptidomananos de menor tamaño (*35*).

Como consecuencia de esta ruptura y fragmentación se produce la liberación al vino de compuestos procedentes tanto del interior celular (aminoácidos, péptidos, ácidos grasos y nucleótidos), como de la pared celular de la levadura (glucanos y manoproteínas) (**FIGURA 5**), que resultan fundamentales para las cualidades organolépticas finales del vino espumoso.

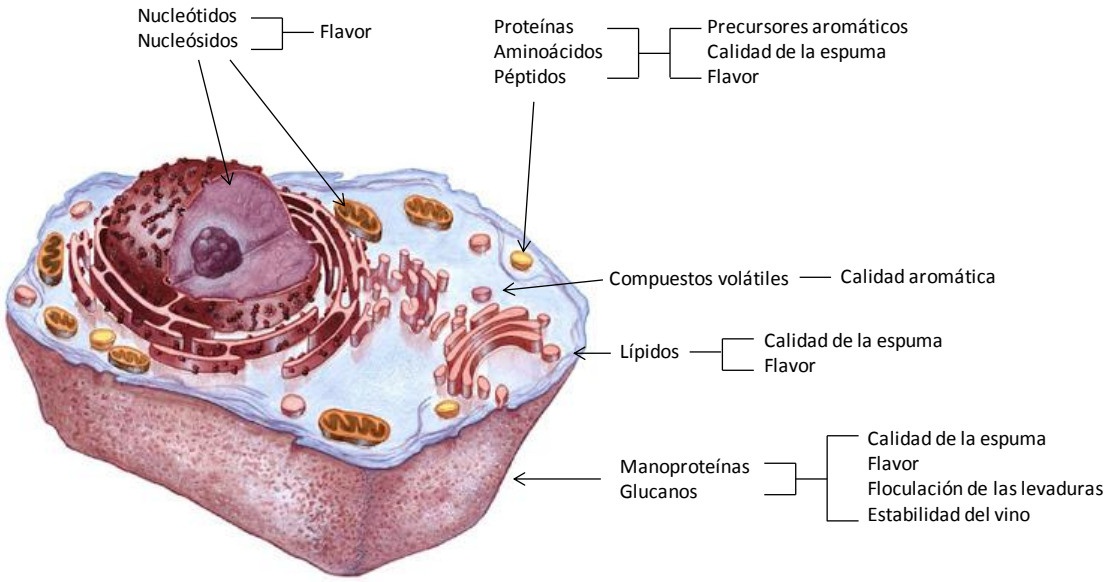


FIGURA 5. Compuestos liberados durante la autolisis y respectivos papeles enológicos. Adaptado de (*26,36,37*)

2.4. Características de los vinos blancos y rosados espumosos

La **espuma**, el **color** y el **aroma** de un vino espumoso son características que definen su calidad y se relacionan con la composición química del vino.

2.4.1. Espuma

Una de las características más apreciadas de un vino espumoso es la calidad de su espuma, definida por los conceptos de espumabilidad, o cantidad de espuma producida, y de persistencia de la espuma, o tiempo de estabilidad. Una espuma de calidad se define como aquella que causa una liberación lenta de CO_2 desde el fondo del líquido, con la formación de rosarios, con pequeñas burbujas que contribuyen a la formación de una corona en la superficie del vino, cubriéndola completamente con burbujas de dos o tres hileras de profundidad (*38*). Además, el CO_2 disuelto en los vinos espumosos no sólo influye en los atributos visuales ligados a la belleza de este tipo de vinos, sino que también influye de manera significativa sobre su aroma (*39,40*), flavor (*41*) y color (*42*).

La presencia de una sobrepresión de CO_2 en el interior de la botella y de compuestos tensoactivos en el vino capaces de formar una interfase líquido-gas que dé a la burbuja su individualidad, son requerimientos indispensables para la formación y la estabilidad de la espuma (*43*) (**FIGURA 6**). Para que una burbuja de CO_2 pueda formarse debe separar

a las moléculas del medio que las rodea, siendo por lo tanto necesaria una energía importante producida por un fenómeno conocido como nucleación. La formación de burbujas puede hacerse por nucleación directa a partir del gas disuelto en el vino, aunque por lo general, la formación de burbujas se hace a partir del gas adsorbido en una partícula sólida, proceso llamado nucleación inducida o heterogénea (*44*). La acumulación de una gran cantidad de burbujas conduce a la formación de espuma y a su persistencia en el tiempo.

La estabilidad de la espuma, es decir, la vida de las burbujas, depende de factores que afectan al espesor de la película que rodea al gas. Esos factores son principalmente el drenaje de líquido entre burbujas, que elimina la interfase líquido-gas, la fusión entre burbujas o coalescencia y el tamaño de éstas. También influyen variables como las características físicas del medio líquido, en especial, la viscosidad, que depende de la composición del vino y la tensión superficial, la cual disminuye con la presencia de moléculas tensoactivas (*43*).

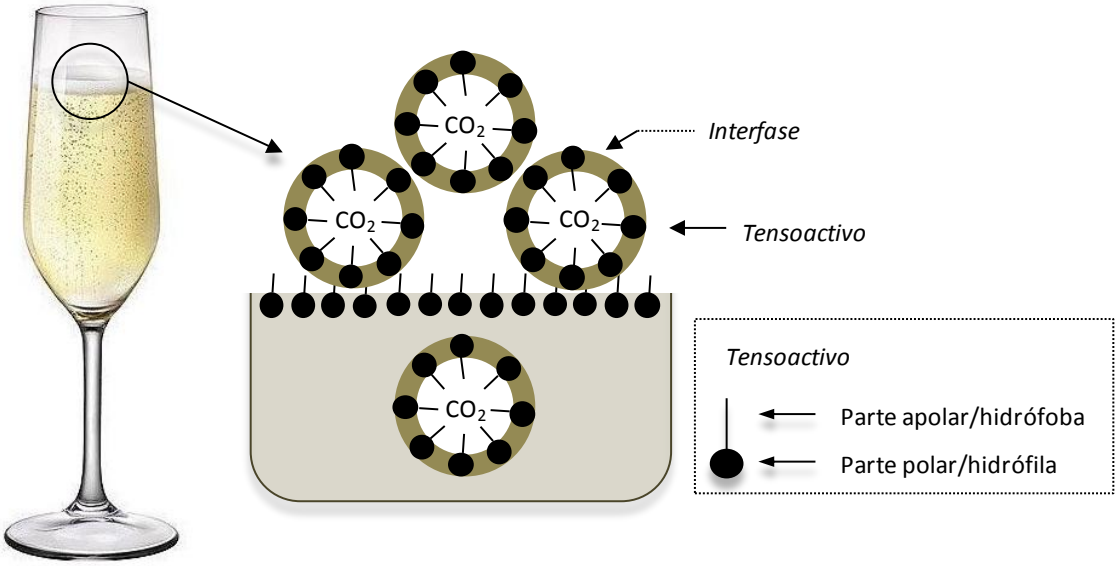


FIGURA 6. Representación esquemática de la interfase líquido-gas. Adaptado de (*43*)

Dada la importancia de la espuma en la calidad de los vinos espumosos, ésta ha merecido una especial atención y ha sido objeto de diferentes estudios científicos encaminados a detectar los principales componentes responsables de su aparición, así como los factores intrínsecos (variedad de uva, estado de maduración y estado sanitario) y extrínsecos (prácticas tecnológicas) que afectan a la composición y a las propiedades espumantes de los vinos. Estos estudios se han llevado a cabo al poder disponer de métodos instrumentales objetivos y normalizados, como el Mosalux diseñado por A. Maujean en 1990 (*45*).

El método Mosalux se basa en inyectar CO_2 para provocar, en el vino previamente desgasificado, la formación de espuma. Está compuesto por una probeta graduada de 40 mm de diámetro y 570 mm de altura, donde en su parte inferior se sitúa un difusor de CO_2. Se introduce en la probeta 100 ml de vino y se hace pasar un caudal de CO_2 de 7 litros/hora a una presión de 1 bar, formándose una espuma cuya altura puede ser medida por un conjunto de emisores y captadores de luz infrarroja colocados a ambos lados de la probeta, y conectados a un ordenador e impresora para recoger los resultados de la prueba en forma gráfica en función del tiempo (**FIGURA 7**).

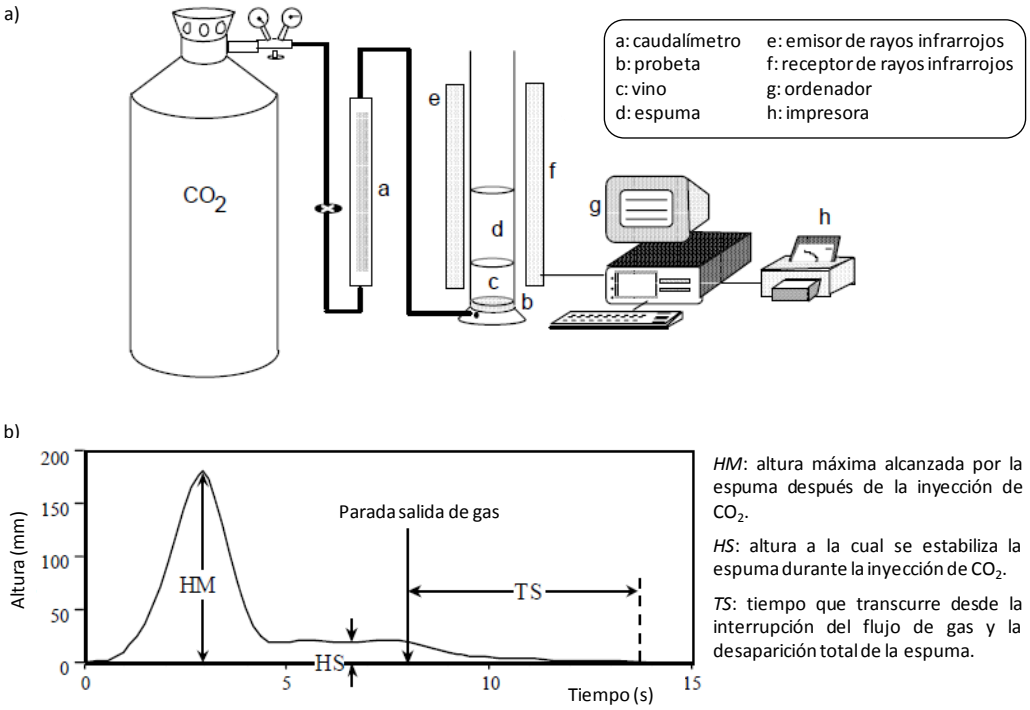


FIGURA 7. Método Mosalux (a) y curva típica de evolución de la espuma (b). Adaptado de (*46*)

El Mosalux determina tres parámetros relacionados con la calidad de la espuma:

- *Altura máxima de la espuma, HM*: se llama también espumabilidad y se corresponde con la altura máxima de la espuma obtenida justo después verter el vino en la copa.
- *Altura estable (o permanencia) de la espuma, HS*: sin variar las condiciones de presión y flujo de CO_2, la altura de espuma disminuye hasta estabilizarse a la altura *HS*. Se corresponde con la permanencia de los cordones de burbujas en la copa y con la presencia de un collar de burbujas en la superficie del líquido. Es un indicador de la capacidad del vino para producir una espuma estable.

- *Tiempo de estabilidad de la espuma, TS*: es el tiempo que tarda en desaparecer completamente la espuma cuando se para la inyección de CO_2. Representa el tiempo de vida de la corona de burbujas antes de desaparecer por drenaje. Con este parámetro se completa la caracterización de la estabilidad de los cordones de burbujas en la copa.

Las medidas físicas obtenidas a través del método Mosalux han sido relacionadas con las características espumantes que muestra el vino espumoso cuando se vierte en la copa y por lo tanto, son representativas de la percepción sensorial de la espuma (*47*). Por otro lado, este sistema de medidas físicas ha permitido relacionar la capacidad espumante de los vinos espumosos con algunas de las moléculas que forman su composición (*43*), aunque no existen estudios científicos que relacionen de forma global la composición química del vino con sus características espumantes.

La variedad de uva seleccionada es una variable decisiva en la calidad de la espuma (*30,48-54*). Así, las distintas características espumantes entre variedades se deben a la diferente composición cualitativa y cuantitativa de sus compuestos tensoactivos, los cuales juegan un papel primordial en la formación y estabilización de la espuma.

De todos los compuestos que presentan características tensoactivas, las proteínas han sido las macromoléculas más estudiadas en cuanto a su relación con las propiedades espumantes de los vinos espumosos (*48,51,53,55-65*). Aunque el efecto de los aminoácidos libres en la calidad de la espuma ha sido menos estudiado que el de las proteínas, se ha observado una influencia positiva en la espumabilidad (*52*), aunque no se conoce su efecto en la estabilidad de la espuma. Por otro lado, las glicoproteínas han sido identificadas como las macromoléculas que más influyen en la espuma de los vinos espumosos (*63,66-69*). Concretamente, las manoproteínas liberadas durante la crianza sobre lías parecen estar implicadas en la estabilidad de la espuma (*64*). Finalmente, debido a la elevada reactividad de los compuestos fenólicos, algunos autores han intentado conocer su influencia en la calidad de la espuma en disoluciones modelo, mostos y vinos base, obteniéndose resultados contradictorios (*30,50,53,57,70,71*).

La paradoja en la elaboración de los vinos espumosos es que cada etapa de vinificación tiende a bajar el poder espumante debido al descenso de macromoléculas. Así, la adición de bentonita para evitar la quiebra proteica en los vinos o facilitar el degüelle en los vinos espumosos producen una disminución de la espumabilidad (*60,72,73*). A pesar de este descenso en la espumabilidad, la crianza sobre lías produce un aumento en la estabilidad de la espuma (*30,49,52*). Por otro lado, diversos autores muestran que cuanto mejor sea la capacidad espumante del vino base, mejor serán las

propiedades espumantes del vino espumoso final (*45,54*). Es por tanto primordial comenzar el proceso de elaboración de los vinos espumosos con una materia prima de gran calidad.

2.4.2. Color

El color es la carta de presentación del vino tranquilo y espumoso, siendo un atributo sensorial indicador de aspectos como su edad, estado de conservación, cuerpo o sabor.

El color de los vinos espumosos depende de la composición fenólica del vino base. Los compuestos fenólicos son más importantes en vinos rosados espumosos por su cantidad y por la presencia de antocianos, pero también están presentes en los vinos blancos espumosos en menor cantidad. Los antocianos y sus pigmentos derivados son los compuestos directamente responsables del color de los vinos rosados espumosos. En el caso de los vinos blancos espumosos, el color depende básicamente de su contenido en flavanoles y flavonoles. Se debe tener en cuenta que los compuestos fenólicos pueden complicar el inicio de la segunda fermentación de los vinos espumosos; por esta razón, los vinos tintos raramente son empleados como vinos base para elaborar espumosos por el método tradicional (*74*). Además, estos compuestos pueden afectar negativamente a las propiedades espumantes de los vinos, y están implicados en un defecto denominado *gushing* (*13,48,53*), fenómeno que consiste en la formación intensa de espuma de forma espontánea durante el degüelle o en el producto terminado.

Diversos estudios muestran que durante la crianza sobre lías existe una disminución en la concentración de los compuestos fenólicos del vino debido, por un lado, a la adsorción de los compuestos fenólicos por la bentonita añadida al licor de tiraje (*72*) y, por otro, a mecanismos de adsorción reversibles entre los compuestos fenólicos y las paredes celulares de las levaduras (*75-79*), así como a una estabilización del color (*29*). La adsorción de los compuestos fenólicos en las paredes celulares de las levaduras y su combinación con otras moléculas puede prevenir la oxidación de los compuestos fenólicos y estabilizar el color de los vinos espumosos (*43,80*). Por otro lado, la elevada concentración de CO_2 y a la presencia de lías en el interior de la botella generan un ambiente reductor que protege al vino espumoso de la oxidación (*81*). Sin embargo, algunos trabajos muestran un incremento del pardeamiento y oxidación de los compuestos fenólicos durante la crianza sobre lías de los vinos espumosos (*21,75,82*). Otros estudios indican que tanto la intensidad de color como la concentración de compuestos fenólicos se mantienen prácticamente constantes durante la crianza sobre lías de los vinos blancos y rosados espumosos (*54,81,83*), por lo que son la variedad de

uva y el sistema de elaboración del vino base los principales factores que influyen en la concentración y composición final de los compuestos fenólicos en los vinos espumosos (*80,81,84,85*).

2.4.3. Aroma

El aroma del vino es uno de los factores más importantes que determinan su carácter y su calidad. La generación del aroma es un proceso muy complejo, en el que intervienen un gran número de reacciones químicas y enzimáticas. En los vinos espumosos elaborados por el método tradicional, debido a sus especiales características de elaboración, existen unas características aromáticas singulares que contribuyen a la calidad y al carácter propio del producto final.

Dentro de los compuestos responsables del aroma de los vinos se encuentran grupos químicos bastante heterogéneos tales como alcoholes, aldehídos, acetonas, ésteres, ácidos volátiles, terpenos, piracinas, etc. Sus orígenes son diversos, pudiéndose distinguir tres tipos de aromas según procedan de la uva (aroma primario), se generen durante la etapa fermentativa (aroma secundario), o bien se originen durante la etapa de crianza sobre lías (aroma terciario o *bouquet*). Sin embargo, está universalmente aceptado que los compuestos que provienen de la uva, y su perfil, juegan el papel más decisivo en la expresión de las características aromáticas de la variedad. Esto es debido a que el aroma varietal está formado por estos compuestos en su estado libre, por precursores específicos y por compuestos como los aminoácidos o los ácidos grasos, cuyo perfil es característico de la variedad. De esta forma, aunque la crianza sobre lías y posterior autolisis marca definitivamente la calidad aromática del vino espumoso (*86-89*), la variedad de la uva empleada para la elaboración del vino base es un factor determinante que influye en su perfil aromático (*90-93*). Así, las notas de nueces, pan tostado, migas de pan y frutos secos son aromas característicos de los vinos espumosos elaborados por el método tradicional, aunque la manzana verde, la pera, los cítricos e incluso los aromas de frutas tropicales son también descriptores citados (*87,93*).

Como se ha visto, los **compuestos fenólicos**, **volátiles**, **aminoacídicos** y **polisacarídicos** influyen en la calidad del vino espumoso final. Se realiza a continuación una breve descripción de cada una de estas familias de compuestos con objeto de resaltar su implicación en el proceso de vinificación y en las características organolépticas de los vinos espumosos.

2.5. Compuestos fenólicos

Desde el punto de vista químico los compuestos fenólicos se caracterizan por tener un núcleo bencénico con uno o varios grupos hidroxilo. Según su estructura química se distinguen dos grupos: fenoles no flavonoides, que son básicamente ácidos fenólicos, y fenoles flavonoides, con un esqueleto del tipo 2-fenil benzopirona (**FIGURA 8**).

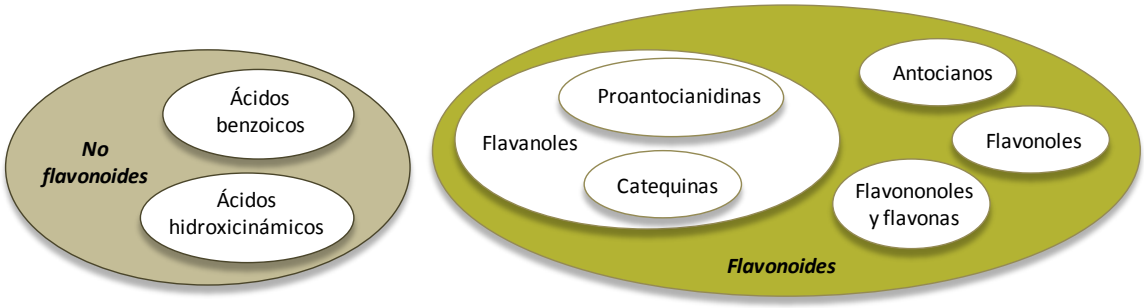


FIGURA 8. Clasificación de los compuestos fenólicos

En general, los compuestos no flavonoides se localizan en todas las partes del racimo, especialmente en la pulpa, mientras que los flavonoides se encuentran en las pepitas, hollejos y raspones (**FIGURA 9**). Debido a las diferencias en su localización y solubilidad, los ácidos fenólicos y, en menor medida, los antocianos, se extraen fácilmente al mosto, mientras que la extracción de los flavanoles se ve favorecida con largas maceraciones en presencia de etanol (*94*).

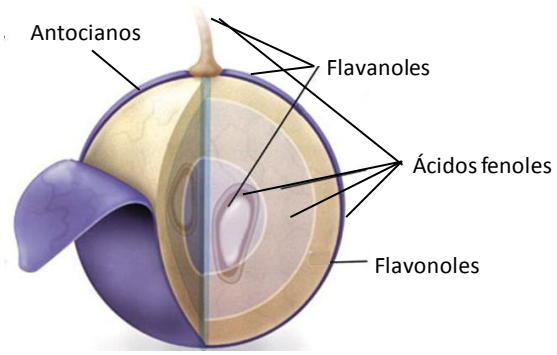


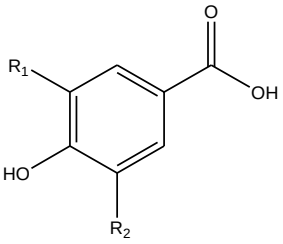
FIGURA 9. Localización de los compuestos fenólicos en la uva

Los compuestos fenólicos han sido ampliamente estudiados en los vinos tintos tranquilos, sin embargo no existe información sobre la presencia y evolución de los compuestos fenólicos durante el proceso tradicional de elaboración de los vinos espumosos. Los estudios descritos en la bibliografía se centran en el perfil fenólico de los vinos base y/o del vino espumoso durante la crianza sobre lías (*75,81,84,85,95-97*) y son escasos los trabajos que describen los compuestos fenólicos en vinos rosados espumosos elaborados por el método tradicional (*80,81,98*).

2.5.1. Compuestos fenólicos no flavonoides

Los fenoles no flavonoides son básicamente ácidos fenólicos de dos tipos, los ácidos benzoicos y los ácidos hidroxicinámicos (**FIGURA 8**).

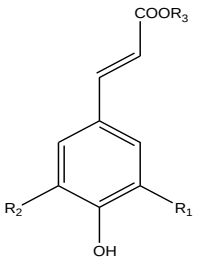
Los **ácidos benzoicos** se encuentran mayoritariamente en el vino en forma de ácido gálico, siríngico y *p*-hidroxibenzoico (**FIGURA 10**). Generalmente el ácido gálico es el mayoritario en los vinos espumosos (*81,83,85*), en concentraciones de 0,3 mg/L y 1,3 mg/L, en vinos blancos y rosados respectivamente (*81*).

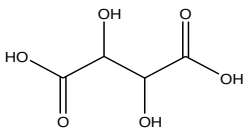


Ácidos benzoicos	R_1	R_2
Ácido gálico	OH	OH
Ácido *p*-hidroxibenzoico	H	H
Ácido siríngico	OCH_3	OCH_3

FIGURA 10. Estructura química de los ácidos benzoicos del vino

Los **ácidos hidroxicinámicos** son los principales fenoles en los vinos blancos y rosados espumosos (*21,75,81,83,85*), representando hasta un 60% del contenido total de los compuestos fenólicos. Los ácidos hidroxicinámicos se encuentran en el vino en forma libre, como el ácido cafeico, cumárico y ferúlico, y en forma esterificada con el ácido tartárico como el ácido caftárico, cutárico y fertárico (**FIGURA 11**). El ácido cafeico y su derivado el ácido caftárico son los ácidos hidroxicinámicos mayoritarios en los vinos blancos y rosados espumosos (*75,81,85*). Durante la segunda fermentación y la crianza sobre lías se produce una hidrólisis de los ácidos esterificados dando lugar a un incremento en la concentración de los ácidos libres (*85*).





ácido tartárico

Ácidos hidroxicinámicos	R_1	R_2	R_3
Ácido cafeico	OH	H	H
Ácido caftárico	OH	H	Ácido tartárico
Ácido *p*-cumárico	H	H	H
Ácido *p*-cutárico	H	H	Ácido tartárico
Ácido ferúlico	OCH_3	H	H
Ácido fertárico	OCH_3	H	Ácido tartárico
Ácido sinápico	OCH_3	OCH_3	H

FIGURA 11. Estructura química de los ácidos hidroxicinámicos

Los ácidos fenólicos son incoloros pero la esterificación con el ácido tartárico les hace particularmente oxidables, y son una de las causas del pardeamiento de los vinos espumosos blancos durante el envejecimiento sobre lías (*75*). Algunos de estos ácidos fenólicos influyen en la astringencia y amargor de los vinos (*99*) y son precursores de los

fenoles volátiles, que pueden provocar defectos en los vinos a nivel olfativo (*100*). Además, estos compuestos pueden tener cierta importancia en el color de los vinos espumosos rosados, ya que pueden actuar como copigmentos, uniéndose a los antocianos (*80*).

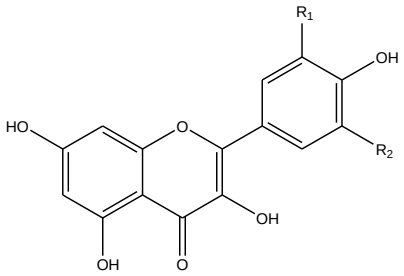
2.5.2. Compuestos fenólicos flavonoides

Los principales fenoles flavonoides del vino incluyen los flavonoles, los flavanoles y los antocianos (**FIGURA 8**).

Los **flavonoles** son pigmentos amarillos que se encuentran en la piel de la uva donde existen como forma heterósida (glucósido, galactósido, ramnósido, rutinósido o glucurónido) de cuatro agliconas principales (quercetina, miricetina, kaempferol e isoramnetina) (**FIGURA 12**). Sin embargo, en el vino pueden encontrarse tanto los flavonoles glicósidos como sus correspondientes agliconas, debido a la hidrólisis ácida que tiene lugar durante la elaboración y envejecimiento del vino (*101*). Son fácilmente extraíbles durante los procesos de vinificación aunque son poco solubles en agua y requieren de la presencia del etanol.

Desde el punto de vista organoléptico, los flavonoles tienen carácter amargo y un fuerte poder de copigmentación (*102,103*). Los flavonoles juegan un papel importante en el color del vino blanco, pero en vinos tintos son enmascarados por los pigmentos rojos como los antocianos (*104*).

Debido a la escasa maceración prefermentativa existente en la elaboración de los vinos base, los únicos flavonoles detectados en vinos blancos y rosados espumosos han sido la quercetina y la quercetina 3-glucurónido, en concentraciones inferiores a 1,20 mg/L (*75,84,85,105*).



Flavonoles	R_1	R_2
Kaempferol	H	H
Quercetina	OH	H
Miricetina	OH	OH
Isorhamnetina	OCH_3	H

FIGURA 12. Estructura química de los flavonoles

Los **flavanoles** se encuentran principalmente en el hollejo, en las semillas de la baya y en el raspón. Los principales flavan-3-ol o flavanoles son los monómeros de (+)-

catequina, (-)-epicatequina, (-)-epigalocatequina y (-)-epicatequina galato (**FIGURA 13**). Sin embargo, la mayor parte de los flavanoles están en forma de oligómeros (hasta cinco unidades) y polímeros (más de cinco unidades), y se denominan taninos condensados o proantocianidinas (**FIGURA 14**). Los únicos flavanoles monómeros detectados en vinos blancos y rosados espumosos han sido la (+)-catequina y la (-)-epicatequina, en concentraciones inferiores a 16,6 y 5,70 mg/L respectivamente (*75,81,84,85,93*). Con respecto al contenido de proantocianidinas, no existen estudios que analicen estos compuestos en vinos rosados espumosos, y tan sólo el estudio de Jordão y col. (*95*) analiza estos compuestos en vinos blancos espumosos portugueses, con concentraciones que varían desde 25,3 a 55,9 mg/L.

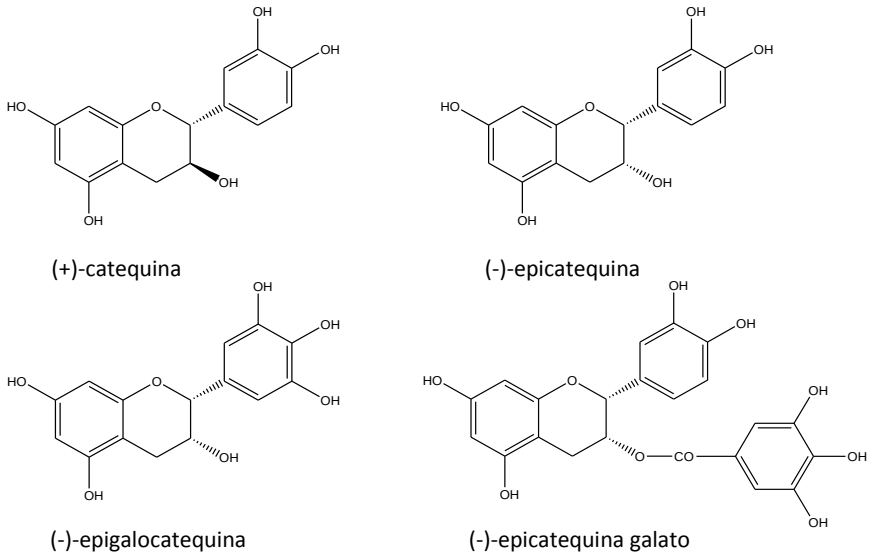


FIGURA 13. Principales flavanoles monómeros de la uva

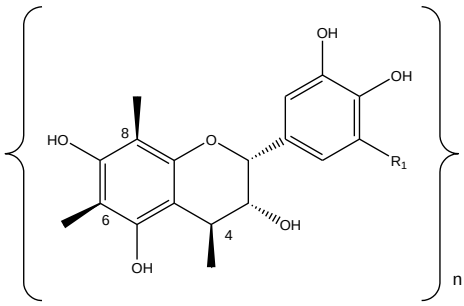


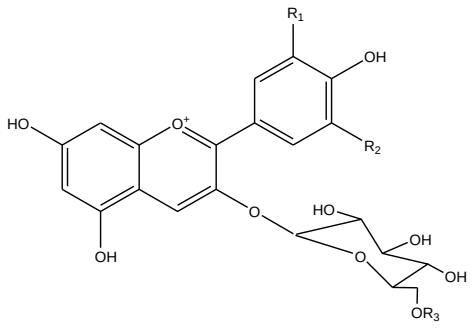
FIGURA 14. Estructura de las proantocianidinas

Los flavanoles juegan un papel importante en el color del vino. En los vinos blancos, son junto con los ácidos hidroxicinámicos responsables del pardeamiento oxidativo que aumenta las tonalidades amarillas (*106*). En los vinos tintos pueden actuar como copigmentos (*102*) intensificando el color de los vinos jóvenes, e intervenir en la

estabilidad del color durante el envejecimiento (*107*). Los flavanoles son también responsables del cuerpo, astringencia y amargor de los vinos (*108*). La sensación de astringencia de un vino se debe a la capacidad que tienen las proantocianidinas de interaccionar con las proteínas de la saliva. El grado de polimerización y el número de unidades galoiladas condiciona notablemente su capacidad astringente y de condensación con las proteínas. Así, cuanto mayor es su grado de polimerización y el porcentaje de unidades galoiladas de los taninos, mayor es la sensación de astringencia (*108,109*).

Los **antocianos** son los responsables del color rojo-azulado de la piel de las uvas tintas y por tanto del color de los vinos rosados espumosos. Su localización en la uva se limita a los hollejos, si bien en las variedades tintoreras también están presentes en la pulpa.

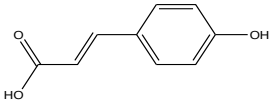
Los antocianos están formados por una aglicona (antocianidina) que se encuentra unida a un monoglucósido, normalmente glucosa. Así mismo, la glucosa puede estar esterificada con diferentes ácidos, principalmente el ácido acético, *p*-cumárico y cafeico (*110*). En las variedades de *Vitis vinífera* se distinguen cinco antocianos diferentes en función de los grupos hidroxilos (-OH) y metoxilos (-OCH_3) que haya en el anillo B (**FIGURA 15**), siendo la malvidina-3-glucósido el antociano mayoritario encontrado en los vinos.



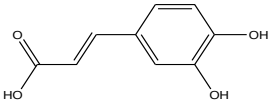
Antocianos	R_1	R_2
Malvidina	OCH_3	OCH_3
Cianidina	OH	H
Delfinidina	OH	OH
Petunidina	OCH_3	OH
Peonidina	OCH_3	H

Esterificación R_3

ácido acético



ácido cumárico



ácido cafeico

FIGURA 15. Estructura química de los antocianos de la uva

La concentración total de antocianos depende de varios factores como la variedad de uva y su grado de madurez, las condiciones climáticas y las técnicas enológicas empleadas (intensidad de prensado, tiempo de maceración, temperatura de fermentación, levadura, clarificación, filtración, etc.). En la elaboración de los vinos base rosados, en la cual existe un periodo de maceración prefermentativa corto, la extracción de antocianos es limitada. La bibliografía describe concentraciones de 0,17 a 60 mg/L en

muestras de vinos rosados tomadas después de la fermentación alcohólica (*21,80,111-113*).

La estructura de los antocianos depende de la composición y de las condiciones del medio donde se encuentran disueltos, y pueden además combinarse entre ellos y con otros compuestos fenólicos dando lugar a modificaciones en su equilibrio estructural y su color (*114*) (**FIGURA 16**). De este modo, las cantidades relativas de cada una de las formas estructurales que coexisten en equilibrio dependen del pH del medio y de su combinación con otras moléculas.

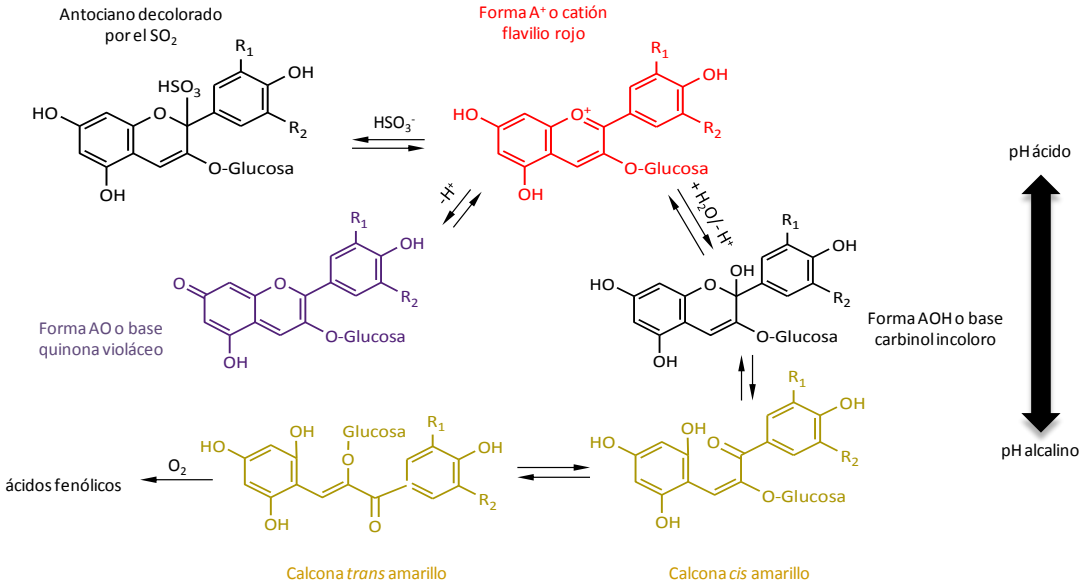


FIGURA 16. Equilibrio y coloración de los antocianos en el vino rosado

Así, a un pH muy ácido, la forma mayoritaria es el catión flavilio que presenta coloración roja. A medida que el pH aumenta, el catión flavilio se transforma en una base quinona de color azulado y en la forma carbinol que es incolora. Ambas reacciones ocurren simultáneamente de acuerdo con sus constantes de equilibrio. Por otra parte, a temperaturas elevadas, la forma carbinol puede transformarse en calcona cuya oxidación da lugar a ácidos fenoles incoloros. A diferencia del resto de reacciones, que son reversibles, las oxidaciones de las *trans*-calconas implican una pérdida irreversible del color del vino. La presencia del anhídrido sulfuroso en los vinos produce también una fuerte decoloración de los antocianos, que mediante una reacción reversible, supone también una pérdida temporal de la intensidad de color.

2.6. Compuestos volátiles

Los compuestos volátiles de los vinos espumosos provienen de la uva, de las levaduras que llevan a cabo la primera y segunda fermentación y del envejecimiento sobre lías. Los compuestos volátiles producidos durante el envejecimiento en presencia de lías se desarrollan mediante reacciones químicas y/o enzimáticas a partir de los compuestos volátiles de las etapas anteriores y son los que proporcionan al vino espumoso unas características aromáticas propias y distinguibles del resto de vinos tranquilos (*115*).

El **aroma primario** o **aroma varietal** del vino es el resultado de los compuestos odorantes presentes en forma libre en la uva o aquellos producidos como consecuencia de los procesos a los que se somete la uva desde su cosecha hasta el comienzo de la fermentación alcohólica (**aroma prefermentativo**). Estos procesos (prensado, despalillado, etc.) provocan la rotura del grano de uva, lo que permite la actuación de algunos sistemas enzimáticos, originando principalmente alcoholes y aldehídos de seis átomos de carbono (1-hexanol, *cis*-3-hexen-1-ol) que están relacionados con aromas vegetativos y herbáceos.

A pesar de tener un aroma muy diferente, en conjunto, la composición volátil de las uvas es bastante similar, y estas diferencias las podemos explicar por la diferente concentración en la que muchos de estos compuestos odorantes aparecen en la uva. Sin embargo, en algunas variedades de uva se han identificado algunos compuestos volátiles, que efectivamente contribuyen de manera decisiva a la tipicidad aromática de una determinada variedad. Algunos ejemplos de moléculas con carácter impacto lo constituyen el 4-mercapto-4-metil-2-pentanona y el 3-metil-2-buteno-1-tiol, con notas a boj y a marihuana respectivamente, en la variedad Prieto Picudo (*11,116*), el acetato de 3-mercaptohexilo, con notas a frutas tropicales en la variedad Verdejo (*5*), o el eugenol, con aroma a regaliz y clavo, característico de las uvas de Tempranillo (*117*), entre otras. Muchos de estos compuestos se encuentran en concentraciones muy bajas tanto en la uva como en el vino (ng/L), pero debido a sus bajos umbrales de detección ejercen un importante impacto aromático.

Aunque los aromas varietales representan en términos de concentración entre el 1 y el 6% de la composición volátil de los vinos espumosos (*91*), los compuestos odoríferos provenientes de la uva y característicos de la variedad juegan un rol determinante en la calidad y en la tipicidad de este tipo de vinos (*91,93*). Sin embargo, la mayoría de los estudios realizados en vinos espumosos se han llevado a cabo con las variedades blancas tradicionalmente empleadas en la elaboración de Cavas, y hasta la fecha muy pocos

trabajos estudian el potencial aromático de variedades tintas para elaborar vinos rosados espumosos por el método tradicional (*90,118,119*).

Además de moléculas odorantes en estado libre, en la uva existen precursores glicosídicos del aroma, que son moléculas no volátiles ni odorantes, susceptibles de liberar aromas por vías químicas o enzimáticas. Así, durante la crianza sobre lías y debido a las enzimas liberadas durante el proceso autolítico de las levaduras, se produce una liberación de los precursores aromáticos y un incremento en muchos aromas varietales, como terpenos, norisoprenoides y alcoholes C6 (*87,90,91*). En este sentido, dos norisoprenoides característicos de los vinos espumosos elaborados por el método tradicional, el vitispirano, de aromas especiados y de té verde, y el 1,2-dihidro-1,1,6-trimetil-1,2-naftaleno (TDN), de aroma a queroseno, se acumulan progresivamente durante la autolisis de las levaduras, por lo que son considerados marcadores del tiempo de crianza de los vinos espumosos sobre sus lías (*90*).

El **aroma secundario** o también llamado **fermentativo** aparece durante la primera y segunda fermentación alcohólica de los vinos espumosos como consecuencia del metabolismo de las levaduras. En esta etapa el vino se enriquece en aromas con respecto al mosto. La mayor parte de los componentes del aroma se forman como productos secundarios del metabolismo de las levaduras durante la fermentación alcohólica (**FIGURA 17**).

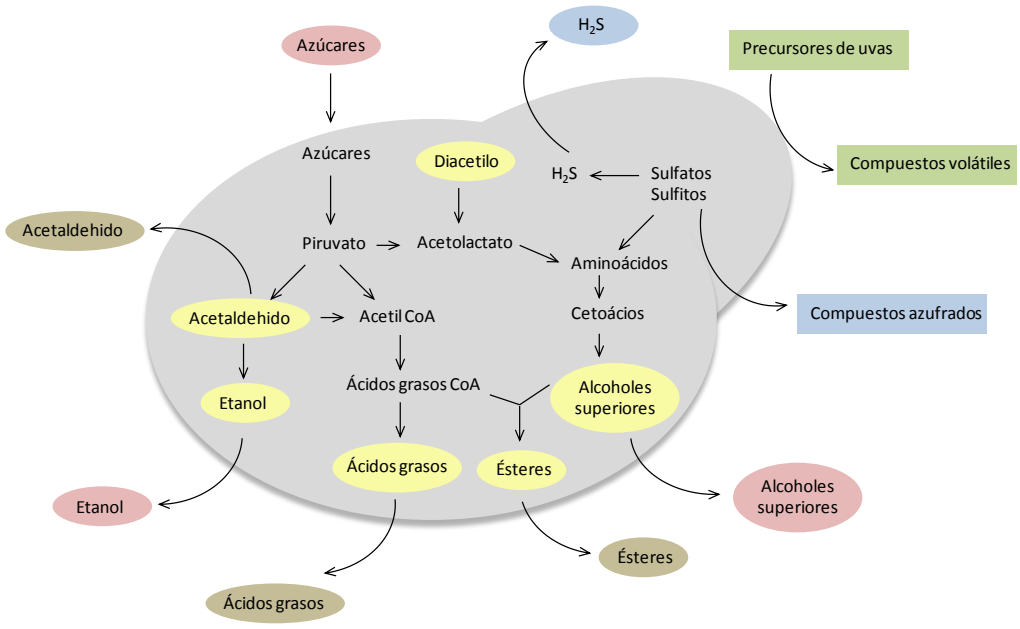


FIGURA 17. Formación de compuestos del aroma por la levadura durante la fermentación alcohólica. Adaptado de (*120*)

El pH, la temperatura y la composición del mosto determinan la biosíntesis de los compuestos volátiles por la levadura durante la primera y segunda fermentación. Sin

embargo, la cepa de levadura de *Saccharomyces cerevisiae* (*121*), la variedad de uva empleada y la composición del mosto (*93,122*) son los factores más influyentes en el aroma secundario de los vinos espumosos elaborados por el método tradicional.

Los compuestos más importantes que se forman en esta etapa son alcoholes, ésteres, ácidos grasos y aldehídos.

Los *alcoholes* son el grupo más importante de compuestos volátiles formados durante la fermentación alcohólica. Se producen principalmente a partir de los aminoácidos presentes en el medio, y aportan aromas acres y a notas herbáceas. Entre los alcoholes superiores de los vinos espumosos destacan el 2- y 3-metil butanol, propanol, 2-metilpropanol, butanol, pentanol y 2-feniletanol. Un contenido total de alcoholes superiores inferior a 300 mg/L añade complejidad al vino, mientras que concentraciones superiores a 400 mg/L disminuyen su calidad (*123*). La mayoría de los *ésteres* del vino son ésteres etílicos de ácidos grasos. Su contribución es positiva en la calidad general de los vinos ya que son los principales responsables del aroma frutal y floral (*124*). Por otro lado, los *ácidos grasos* volátiles lineales de cadena corta (C_2-C_4), media (C_6-C_{10}) y larga (C_{12}-C_{18}), y los ramificados (2-metil propanoico, 2-metil butanoico, etc.) también se producen durante la fermentación, y se ha comprobado que a medida que aumenta la longitud de su cadena, la volatilidad disminuye y el olor cambia de ácido a rancio (*125*). Los principales compuestos carbonílicos que influyen en la percepción aromática de los vinos espumosos elaborados por el método tradicional son el acetaldehído, diacetilo, 3-hidroxipentan-3-ona y aldehído pirúvico. Existen otros compuestos responsables del aroma fermentativo de los vinos espumosos como los compuestos azufrados y fenoles volátiles (*88,126*). Los *aldehídos* proceden de la degradación de los azúcares o se forman durante el envejecimiento, excepto el acetaldehído. Únicamente se pueden encontrar en las etapas iniciales tras la fermentación del mosto ya que rápidamente se reducen a alcoholes. Las cetonas que se encuentran en el vino proceden de la uva.

El tercer grupo de aromas que podemos encontrar en los vinos espumosos elaborados por el método tradicional constituye el **aroma terciario** o ***bouque*t**, y está formado por todos aquellos compuestos que se originan durante la crianza sobre lías de los vinos. Por lo general, la evolución de aroma durante el envejecimiento sobre lías consiste en la pérdida de las notas características varietales y de matices de fermentación, y la aparición de las notas de crianza, como las tostadas y las lácticas (*87,127,128*). Debido a las reacciones autolíticas y enzimáticas que tienen lugar durante este periodo, es este especial envejecimiento el que confiere a los vinos espumosos un

perfil volátil complejo. Por lo tanto, el tiempo de permanencia del vino sobre sus lías determina el tipo y concentración de los compuestos volátiles en el vino espumoso final (*86,90,129*).

La mayoría de los estudios relacionados con los cambios en la composición volátil durante el envejecimiento sobre lías en vinos espumosos son contradictorios. Esto es debido a la simultánea degradación y síntesis de compuestos volátiles que tienen lugar durante esta etapa de elaboración (*14,130*). Algunos autores muestran un incremento en ésteres etílicos y en alcoholes durante la crianza sobre lías (*86,118*). Además, durante esta etapa muchos precursores presentes en el vino pueden ser progresivamente hidrolizados, liberando compuestos aromáticos como los vitispiranos, que están asociados con aromas a frutos secos característicos de los vinos espumosos envejecidos (*14,131*). También durante esta etapa se produce la hidrólisis de algunos ésteres, como los acetatos de alcoholes superiores, con lo que el vino se empobrece en compuestos relacionados con notas aromáticas más frescas y asociadas con aromas florales y frutales (*87,89*). Otros autores observan durante la crianza sobre lías una disminución en ésteres de acetatos y ácidos grasos debido a fenómenos de adsorción con las manoproteínas de las paredes celulares de las levaduras (*87,119,129,132*).

La unión de los compuestos aromáticos formados durante la elaboración de los vinos espumosos, tanto procedentes de la segunda fermentación, como también de la autolisis de las levaduras, junto a los existentes en el vino base procedentes de la variedad de uva o de la fermentación del mosto, y todos ellos arrastrados y potenciados por el desprendimiento del gas carbónico, consiguen el desarrollo de una característica gama de aromas, todos ellos muy sutiles y de gran complejidad, donde destacan además de los perfumes varietales, los desarrollados por la autolisis de las levaduras, que recuerdan a pastelería, tostados, frutos secos, caramelo, etc.

Es importante destacar que sin la **evaluación sensorial**, el mero conocimiento de la composición volátil de un vino es insuficiente para predecir el aroma de todo el sistema tal como lo percibe un catador entrenado. De hecho, las interacciones entre las sustancias olorosas y las interacciones entre el odorante y los diferentes elementos de la matriz no volátil del vino pueden afectar a la volatilidad odorante, a la liberación del aroma y a la intensidad y calidad del *bouquet* percibido (*133*). De ahí la importancia de complementar el análisis instrumental de los compuestos volátiles con el análisis sensorial.

2.7. Aminoácidos y aminas biógenas

Los **aminoácidos** en el vino pueden tener diferentes orígenes. Además de los procedentes de la uva, los aminoácidos también pueden ser secretados al medio por las levaduras al final de la fermentación alcohólica (*134*), o ser liberados durante la autolisis de las levaduras por la hidrólisis de las proteínas de la membrana citoplasmática (*25,26,32,66*). Además, los aminoácidos también pueden proceder de las paredes celulares de las levaduras. Cuando los glucanos son hidrolizados por las glucanasas se liberan manoproteínas, las cuales mediante proteólisis dan lugar a los aminoácidos (*32,135*). Así, los aminoácidos han sido considerados buenos marcadores para seguir el proceso autolítico de las levaduras durante la crianza sobre lías de los vinos espumosos (*32,66,136-138*).

La composición en aminoácidos libres tiene gran importancia en la producción de los vinos espumosos, porque, además de actuar como fuente de nitrógeno para las levaduras durante la primera y segunda fermentación, actúan como precursores de los compuestos aromáticos (*139,140*) e influyen en la calidad de la espuma (*52*), lo que contribuye a las características particulares de este tipo de vinos.

Son muchos los autores que han estudiado la evolución de la composición en aminoácidos libres durante la segunda fermentación y la crianza sobre lías de los vinos espumosos (*32,52,136-138,141*), y aunque existen discrepancias respecto a la variación de la concentración individual, los distintos autores coinciden en el hecho de que durante la segunda fermentación disminuye el contenido de la mayoría de aminoácidos y durante la crianza sobre lías se produce un aumento de éstos debido al proceso autolítico de las levaduras.

La bibliografía describe en vinos espumosos elaborados por el método tradicional y envejecidos sobre lías durante nueve meses concentraciones de aminoácidos totales que varían en un amplio rango, desde 43 mg/L (*137*) hasta 1.500 mg/L (*32*); siendo generalmente los aminoácidos prolina, arginina, α-alanina y ácido γ-amino butírico (GABA) los mayoritarios en los vinos espumosos finales. Sin embargo, excepto para la variedad Viura (*32,72,142*), no existen estudios sobre el contenido y evolución de los aminoácidos en vinos espumosos de las variedades seleccionadas en este estudio. Por lo tanto, y teniendo en cuenta que la variedad de uva empleada en la elaboración del vino base influye en la concentración y composición de los aminoácidos libres del vino espumoso (*32,142*), es necesario analizar la composición aminoacídica de los vinos espumosos obtenidos a partir de las variedades empleadas en esta tesis, así como estudiar su evolución durante la crianza sobre lías.

Por otro lado, hay que tener en cuenta que los aminoácidos pueden ser descarboxilados y dar lugar a la formación de **aminas biógenas** (**FIGURA 18**), compuestos que pueden causar efectos perjudiciales en el organismo como migrañas, hipertensión, aumento de la presión sanguínea, etc. (*143*). Las principales aminas en los vinos son putrescina, cadaverina, agmatina, espermidina y espermina (alifáticas), tiramina, y feniletilamina (aromáticas) e histamina y triptamina (heterocíclicas). La variedad de uva (*144*) y las técnicas de vinificación (*145*) pueden afectar al contenido de aminas biógenas en los vinos. Además de la fermentación maloláctica, principal fuente de formación de aminas, la crianza de los vinos sobre lías es un proceso que puede dar lugar a un aumento en el contenido de estos compuestos, ya que como se ha comentado durante la autolisis de las levaduras se pueden liberar aminoácidos, que posteriormente pueden transformarse en aminas (*25*). Se pueden liberar además enzimas con actividad descarboxilasa, y en las lías se pueden encontrar microorganismos con esta misma actividad enzimática. Varios estudios han puesto de manifiesto que los vinos con crianza sobre lías presentan mayor cantidad de aminas biógenas como tiramina y putrescina (*146,147*); sin embargo, existen muy pocos estudios que evalúen las aminas biógenas en vinos espumosos (*148-150*) y ninguno de ellos estudia su evolución durante la crianza sobre lías en botella.

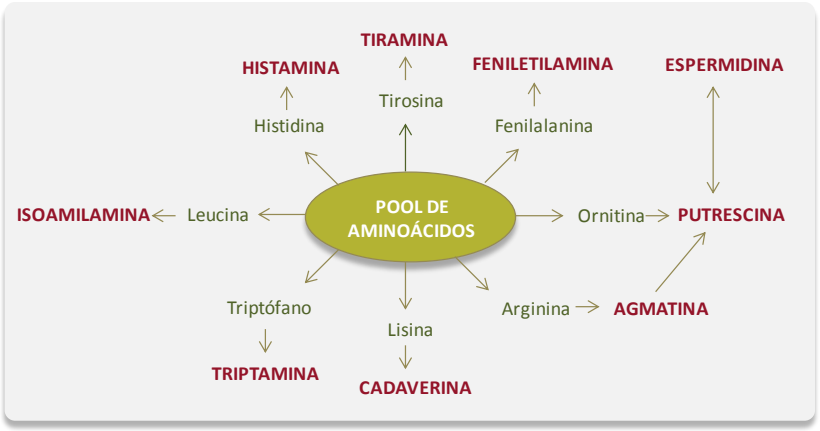


FIGURA 18. Aminas biógenas y sus aminoácidos precursores

La concentración de aminas biógenas también puede ser causa de problemas en la exportación de los vinos, ya que aunque actualmente no existe reglamentación en relación a la concentración de aminas biógenas en vino, la OIV recomienda no sobrepasar los 12 mg/L de histamina, y ya hay países que han establecido límites de importación para la histamina (10 mg/L en Canadá y Suiza, 8 mg/L en Francia o 4 mg/L en Holanda), siendo creciente el número de países que se irán sumando a este tipo de reglamentación. El límite más utilizado es el de 8 mg/L propuesto por Leitao y col. (*151*), por debajo del cual se considera que los vinos no presentan ningún riesgo sanitario. La

cuantificación de las aminas biógenas en los vinos es de suma importancia para la obtención de vinos saludables.

2.8. Polisacáridos

Los polisacáridos son uno de los principales grupos de macromoléculas presentes en los mostos y vinos. Los polisacáridos de los vinos espumosos son liberados durante el prensado y en el curso de la vinificación y de la crianza sobre lías, y proceden tanto de las paredes celulares de la propia uva como de las levaduras y otros microorganismos que actúan durante el proceso de elaboración (**FIGURA 19**). Así, se originan familias de polisacáridos muy diversas tanto en su composición como en su estructura. Desde el punto de vista cuantitativo, los polisacáridos procedentes de la uva y de las levaduras son los más importantes.

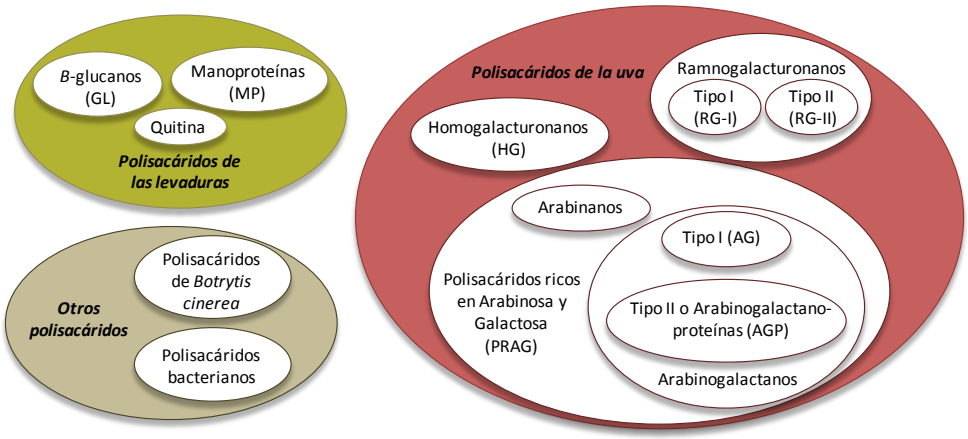


FIGURA 19. Clasificación de los principales polisacáridos del vino según su origen

Considerados coloides protectores, los polisacáridos juegan un papel importante en las propiedades sensoriales (*152-159*) y tecnológicas de los vinos (*160-162*). En el caso de los vinos espumosos, diversos autores han correlacionado las propiedades espumantes de los mostos, vinos base y vinos espumosos con su contenido de polisacáridos (*30,48,51,53,54*). Sin embargo, no todos los polisacáridos tienen el mismo efecto en dichas propiedades ya que su influencia depende no sólo de la cantidad del polisacárido en cuestión, sino también de su peso molecular, estructura y composición (*52,163*). Diversos trabajos han analizado la evolución de las distintas familias de polisacáridos durante la vinificación y envejecimiento de vinos tranquilos (*152,158,164,165*), pero no existen estudios acerca del contenido y evolución de las familias de polisacáridos durante el proceso de elaboración de los vinos espumosos.

La concentración de polisacáridos procedentes de la uva va a depender en gran medida de la variedad de uva, el rendimiento de producción del viñedo, las condiciones edafoclimáticas, el nivel de madurez de la uva, y las técnicas de vinificación empleadas (*166*). En cambio, la concentración de polisacáridos procedentes de las levaduras, principalmente manoproteínas, dependerá de la cepa o cepas de levadura que lleven a cabo la fermentación alcohólica (*166-168*), de la temperatura de fermentación, de la aireación, agitación y del grado de clarificación del mosto (*169*), del estado fisiológico de las levaduras (*170*), del tiempo de contacto de las lías con el vino (*171*) y de las condiciones autolíticas del medio (*26,29,34,36*).

2.8.1. Polisacáridos procedentes de la uva

Los polisacáridos procedentes de la uva son el resultado de la degradación y de la solubilización de las sustancias pécticas contenidas en el hollejo y en la pulpa, debido a la acción de las enzimas pectinolíticas, y se liberan en las primeras etapas del proceso de vinificación (*152,157,172,173*). Estos polisacáridos se clasifican en sustancias pécticas neutras y sustancias pécticas ácidas según contengan o no ácido galacturónico en su molécula. Así, los homogalacturonanos y ramnogalacturonanos pertenecen al grupo de las sustancias pécticas ácidas, mientras que los arabinogalactano-proteínas se engloban dentro de las sustancias pécticas neutras.

Los **homogalacturonanos** están formados por largas cadenas lineales de ácido D-galacturónico unido en α-(1→4), esterificados parcialmente con metanol y/o ácido acético (*4*). Son los polisacáridos más abundantes en las uvas, constituyendo el 80% de los polisacáridos pécticos presentes en las paredes celulares de la pulpa y hollejos (*174*). A pesar de ser muy abundantes en los mostos, en el vino se encuentran en muy bajas cantidades (*152,174,175*), ya que se hidrolizan fácilmente por acción de las enzimas poligalacturonasas de la uva o de la levadura durante la vinificación (*174*).

El **ramnogalacturonano tipo II** (RG-II) posee una estructura muy compleja y una masa molecular pequeña, de aproximadamente 5.400 Dalton. Su estructura química, similar en todos los vegetales, está constituida por una cadena principal bastante corta de unidades de ácido galacturónico unidas por enlaces α-(1→4), unida a su vez a cuatro cadenas laterales de oligosacáridos que contienen arabinosa, ramnosa, fucosa, galactosa, ácidos galacturónico y glucurónico y también diferentes *azúcares raros* como 2-O-metil-xilosa, apiosa, ácido acérico o 3-carboxi-5-deoxi-L-xilosa, Dha o ácido 3-deoxi-D-*liso*-2-heptulosónico, y Kdo o ácido 2-ceto-3-deoxi-D-mano-octulosónico. Dichos

azúcares son exclusivos de la molécula de RG-II y se utilizan para su identificación y cuantificación (*176-178*).

El RG-II, que representa menos del 5% de los polisacáridos de las paredes celulares de las uvas, suele encontrarse en la uva, en los mostos y en los vinos en forma de dímeros unidos por diésteres de ácido bórico (*179-181*), con masas moleculares de 10.000 a 12.000 Dalton (*182,183*).

Los RG-II son más abundantes en las paredes celulares de los hollejos que en las de la pulpa, por lo que su concentración es mayor en los vinos tintos que en los vinos blancos (*177*). En los vinos blancos se obtienen concentraciones inferiores a los 30-50 mg/L y en los vinos tintos de 100-150 mg/L (*175*). El contenido de RG-II en los vinos permanece prácticamente constante desde el final de la fermentación hasta la etapa de envejecimiento en botella (*152*), así como durante la crianza sobre lías en vinos tranquilos (*164*).

Se ha observado que los RG-II son inhibidores de la cristalización de las sales tartáricas en los vinos (*160,184*), fenómeno de gran importancia en los vinos espumosos elaborados por el método tradicional, donde los cristales pueden ser causa del *gushing* (*185*). En relación a su interacción con los compuestos fenólicos, los RG-II favorecen la autoagregación de los taninos en medio sintético (*153*), lo que se traduce en diferentes efectos sobre las propiedades gustativas de los vinos espumosos.

Los **arabinogalatanos tipo II** o **arabinogalactano-proteínas** (AGP) son el grupo de polisacáridos más abundante en mostos y en vinos. Son más abundantes en la pulpa que en los hollejos, por lo que son fácilmente extraídos durante el prensado de la uva (*173*).

Son glicoproteínas, la unidad núcleo del carbohidrato consiste en un esqueleto de β-D-galactano enlazado en (1→3), del cual se ramifican cadenas cortas β-D-galactano en enlace (1→6). Estas cadenas están normalmente fuertemente saturadas con residuos de arabinosa y también otros azúcares como xilosa, ramnosa, fucosa o ácido glucurónico. (**FIGURA 20**). Los AGP típicos aislados de los vinos, con una masa molecular entre 50.000 y 260.000 Dalton contienen menos de un 5% de proteínas, ricas en hidroxiprolina (*186*), y de sus azúcares, sólo entre un 3 y un 10% tienen carácter ácido (*176,177*).

Los AGP están presentes en concentraciones de 100 a 200 mg/L para vinos tintos y de 50-150 mg/L para vinos blancos (*175*). La crianza sobre lías de vinos tintos tranquilos produce descensos en la concentración de AGP, así como modificaciones en su perfil estructural. La actividad enzimática durante la crianza sobre lías produce cambios en las cadenas de β-(1→3)-galactano, y una desarabinosilación parcial de los AGP, que provoca

una disminución en el ratio arabinosa/galactosa (*164*). Así, diversos estudios muestran que la relación molar de arabinosa y galactosa de los AGP en vinos espumosos elaborados por el método tradicional es de ~ 0,2 (*68,186,188*), mientras que la relación en vinos tranquilos es de 0,8 a 1 (*159,166,177,189*).

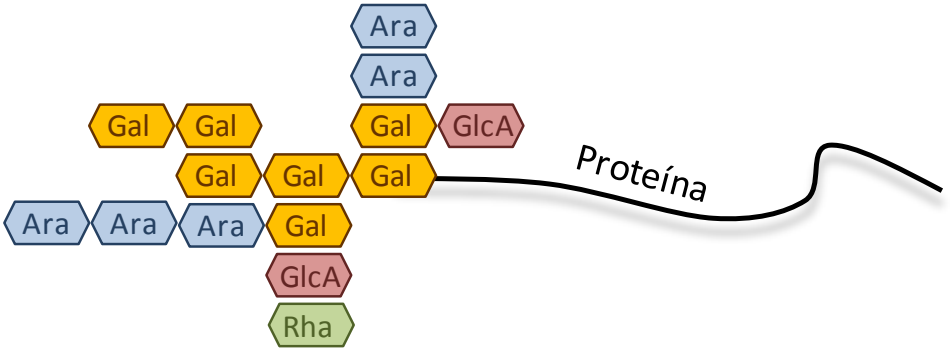


FIGURA 20. Estructura del Arabinogalactano tipo II o Arabinogalactano-proteína (AGP). (Gal: galactosa, Ara: arabinosa, GlcA: ácido glucurónico, Rha: ramnosa). Adaptado de Guadalupe (*187*)

Los AGP tienen influencia en los procesos de filtración de los vinos (*190*) y poseen efectos protectores frente a enturbiamientos proteicos de los vinos blancos (*191,192*). Por otro lado, la goma arábiga, polisacárido natural compuesto por AGP, es empleada por sus propiedades emulsificantes y estabilizadoras de espumas en diversos alimentos (*193-195*). Dichas propiedades están asociadas al carácter anfótero de los AGP, debido al complejo formado por el polisacárido (región hidrofílica) y las proteínas (región hidrofóbica) (*196*). Sin embargo, no se conoce la influencia de los AGP en la calidad de la espuma de los vinos espumosos.

Actualmente, algunos autores engloban a los arabinanos, arabinogalactanos del tipo I y los AGP dentro de un mismo grupo llamado PRAG (Polisacáridos Ricos en Arabinosa y Galactosa) (*159,166,197,198*).

2.8.2. Polisacáridos procedentes de las levaduras

Los polisacáridos procedentes de las levaduras están situados en su pared celular, que representa hasta un 25% de su peso seco (*199*). La composición concreta de la pared parece ser específica de la cepa de levadura y está compuesta por dos capas, una pared externa de carácter elástico formada por manoproteínas y β-1,6 glucanos, y una pared interna de carácter rígido formada por una red tridimensional de β-1,3 glucanos y quitina. (**FIGURA 21**). Así, el 90% de la envoltura celular de las levaduras está constituida por polisacáridos, siendo el resto proteínas y lípidos.

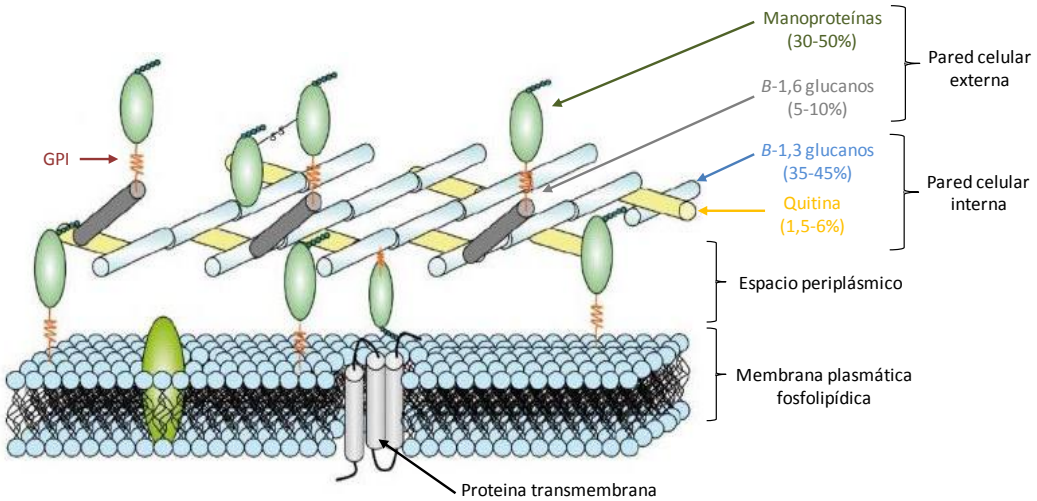


FIGURA 21. Esquema de la envoltura celular de las levaduras. Esquema adaptado de Molina y col. (*200*). Se indica el porcentaje en peso de cada componente según datos obtenidos por Klis y col. (*199*)

Los **glucanos** representan el 50% del peso seco de la pared celular de las levaduras. Son compuestos poliméricos formados principalmente por cadenas de glucosa unidas por enlaces β-(1→3), con ramificaciones laterales de glucosa β-(1→6). En la pared celular de *Saccharomyces cerevisiae* los β-1,3 glucanos constituyen el 85% del total, mientras que el 15% restante son β-1,6 glucanos (*201*).

Los β-(1→3)-glucanos están formados por monómeros de glucosa, llegando a alcanzar hasta las 1.500 unidades, y son los principales responsables de la resistencia mecánica de la pared celular. Poseen una estructura helicoidal y está formada por una o más cadenas de polisacáridos que se unen mediante puentes de hidrógeno, formando así una red a la que se unen otros componentes de la pared celular. En la parte externa se pueden unir moléculas altamente ramificadas de β-(1→6)-glucanos, que a su vez pueden unirse a las manoproteínas. En la parte interna se pueden encontrar cadenas de quitina (*199*).

Los β-(1→6)-glucanos forman un polímero de entre 130 y 350 residuos de glucosa por molécula y poseen una estructura amorfa muy ramificada (*202*). Su principal función es la organización de la pared celular, ya que actúan como unión flexible formando interconexiones con los β-(1→3)-glucanos, con la quitina y con las manoproteínas, enlazando estas últimas con la red β-(1→3)-glucanos (*171,202*).

Las **manoproteínas** representan del 30 al 50% de la pared celular y están unidas covalentemente a la malla de β-1,3 glucanos fundamentalmente de forma indirecta a través de los β-1,6 glucanos (*199*).

Las manoproteínas son glicoproteínas normalmente con un alto grado de glicosilación (80-90%), compuestas por monosacáridos principalmente manosa (> 90%) y glucosa (*157*) y por proteínas (< 10%) (*178*). Estos compuestos son liberados al medio durante la fermentación alcohólica (*152,159,166,177,189,203*) y/o posteriormente durante la crianza sobre lías de los vinos debido al proceso autolítico de las levaduras (*66,67,164,204,205*).

Las manoproteínas poseen una estructura tridimensional basada en un núcleo proteico con dos tipos de cadenas glicánicas: cadenas cortas de manosa unidas a la parte proteica a nivel de residuos de serina o treonina, y cadenas largas polimanosídicas ramificadas con cadenas laterales de manosa que se enlazan a la parte peptídica por intermediación de una N-acetil-glucosamina unida a un residuo de asparragina (*199*) (**FIGURA 22**).

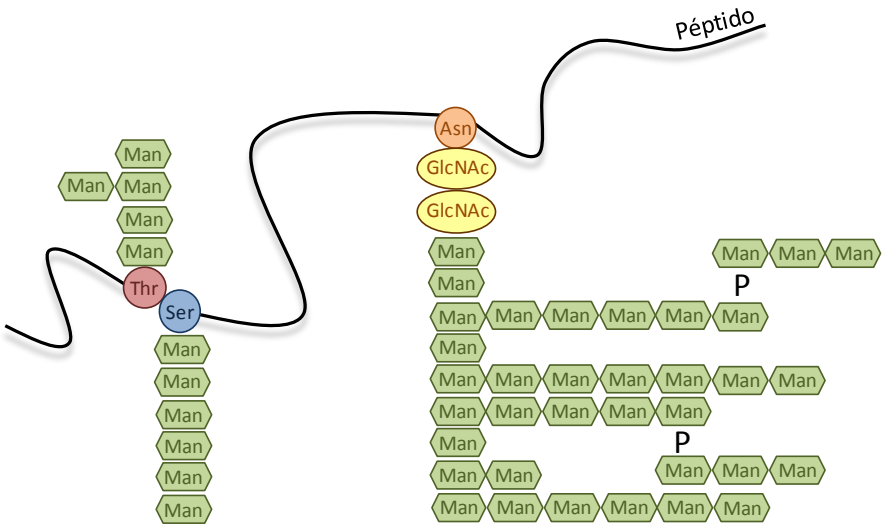


FIGURA 22. Estructura de las manoproteínas exocelulares de levadura. (Man: Manosa, P: fosfato, GlcNAc: N-acetil-glucosamina, Asn: asparragina, Ser: serina, Thr: treonina) (adaptado de Bajard-Sparrow et al. (*206*))

Las manoproteínas presentan tamaños moleculares muy variables, desde 5.000 hasta más de 800.000 Dalton (*164*) y su carga eléctrica varía según el pH del medio. En el rango de pH del vino las manoproteínas están cargadas negativamente, pudiendo establecer interacciones electrostáticas e iónicas con otros componentes del vino (*207*).

La concentración de polisacáridos ricos en glucosa y en manosa incrementa hasta cuatro veces durante el envejecimiento sobre lías de los vinos espumosos (*26*). Así, mientras que en los vinos tranquilos los polisacáridos ricos en arabinosa y en galactosa son los mayoritarios (*152,173,177*), en los vinos espumosos las manoproteínas y los glucanos son los más importantes (*25,208*). Se ha observado que durante el proceso

autolítico de las levaduras se produce un aumento del ratio manosa/glucosa, debido a que la liberación de las manoproteínas ocurre después de la degradación de los glucanos (*26*).

2.9. Efecto de las manoproteínas en la calidad de los vinos espumosos

Las manoproteínas juegan un papel importante en la estabilidad del vino y en las propiedades sensoriales de los vinos. Estudios realizados en vinos tranquilos han puesto de manifiesto que estos compuestos son los principales responsables de los efectos positivos o mejoras obtenidas en los vinos de crianza sobre las lías, como la mejora en la estabilidad tartárica (*209-211*) y proteica (*161,212-216*); favorecen el crecimiento de determinadas cepas de bacterias lácticas (*170,217*); y en cuanto a las características sensoriales, pueden aumentar el cuerpo del vino, y conseguir redondez en boca, suavizando la sensación de astringencia y amargor de los taninos (*153,157,167,218,219*); influir en la intensidad, complejidad y persistencia aromática (*154,220-222*); así como estabilizar el color, aunque se han encontrado resultados contradictorios (*152,156,167,223*).

En los vinos espumosos elaborados por el método tradicional, las manoproteínas liberadas durante el proceso autolítico de las levaduras han sido relacionadas con la mejora en la calidad de la espuma (*63,66-69*). Concretamente, estos compuestos parecen estar implicados en su estabilidad (*64*). Esto es debido a la naturaleza hidrofóbica de las manoproteínas, que favorece su unión a las burbujas de gas. Así, la región hidrofílica de las manoproteínas se localizará en la capa acuosa de la burbuja y la región hidrofóbica correspondiente a la región proteica se situará hacia la cara interior de la burbuja. Esta disposición provoca que cuando la capa acuosa se hace más fina, las manoproteínas aumenten la viscosidad retardando el drenaje (*55*). Se produce así un aumento de la tensión superficial de las burbujas y, con ello, un aumento de la estabilidad de la espuma (*55,64,67,224-226*). Sin embargo, no todas las manoproteínas tienen el mismo comportamiento con respecto a la calidad de la espuma, siendo las más efectivas las que presentan un peso molecular entre 10.000 y 21.500 Dalton y una composición equilibrada en los dominios hidrofóbicos e hidrofílicos de las proteínas (*67*). Por otro lado, las manoproteínas mejoran la floculación de las levaduras, lo que facilita su eliminación de la botella durante la operación de degüelle (*167*). Además, pueden servir como marcadores del proceso autolítico de las levaduras, debido a que son los

principales polisacáridos liberados por las levaduras durante el proceso de crianza sobre lías (*164,204,227*).

Teniendo en cuenta los efectos positivos de las manoproteínas en los vinos espumosos, el conocimiento del efecto de la adición de preparados comerciales a base de levaduras ricos en manoproteínas en la composición química y en las características organolépticas del producto final sería muy deseable para los productores de este tipo de vinos. De este modo, la adición de fórmulas a base de manoproteínas en licor de tiraje de los vinos base podría resultar una alternativa biotecnológica ciertamente interesante.

Hoy por hoy, todas las empresas suministradoras de productos para el sector enológico comercializan uno o varios preparados a base de manoproteínas. Estos productos se obtienen a partir de cepas de levadura *Saccharomyces cerevisiae* tras su inactivación térmica o enzimática, una vez que ha crecido en medios concentrados y ricos en azúcares. Dependiendo del método de obtención, las preparaciones pueden clasificarse en cuatro principales tipos: *a) levaduras inactivas*, se obtienen por inactivación térmica y posterior secado; *b) autolisados de levaduras*, en los que además de la inactivación térmica hay un paso de incubación en el que se permite a los enzimas de la levadura ser liberados de las vacuolas y degradar parte del contenido intracelular; *c) paredes o cortezas de levaduras*, son insolubles y están formados exclusivamente por las paredes de levadura sin contenido citoplasmático, y *d) extractos de levadura*, que es el extracto soluble que se obtiene tras la degradación total del contenido citoplasmático (*228*). Generalmente se encuentran disponibles en el mercado pocas preparaciones comerciales de manoproteínas con alto grado de purificación, principalmente debido a que es un proceso bastante laborioso y costoso. Por ello, la mayor parte de las preparaciones que se comercializan son del tipo autolisados de levadura o paredes de levadura. El uso de las manoproteínas como aditivo enológico durante la vinificación para mejorar la estabilidad tartárica y proteica fue autorizado por la Unión Europea en 2005 (Reglamento CE nº 2165/2005 de 20 de Diciembre de 2005). Además, también está autorizado el uso de preparados comerciales de paredes celulares de levadura hasta un límite de 40 g/HL (Reglamento CE nº 606/2009 de 10 de Julio de 2009).

Diversos autores han estudiado el efecto positivo de estos preparados comerciales ricos en manoproteínas sobre la composición química y las características sensoriales de vinos tranquilos y criados sobre lías (*156-158,222,223,229,230*). Además, se han encontrado trabajos que han estudiado la adición de estos productos y que han permitido obtener vinos tranquilos con unas características tecnológicas y sensoriales

similares a vinos con crianza sobre lías en un menor tiempo (*231,232*). No obstante, la heterogeneidad de estos productos puede dar lugar a efectos muy diferentes en el vino dependiendo del preparado comercial empleado, del tipo de vino y del momento de adición del producto. En este sentido y hasta el momento, pocos estudios evalúan el efecto de estos preparados como aditivos en la composición química y en la calidad sensorial de vinos espumosos, mostrando una mejora en el carácter frutal y floral (233) y en las propiedades espumantes (*67,233*) de los vinos.

2.10. Referencias

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3 MATERIALES Y MÉTODOS

3.1. Vinificación y toma de muestras

La vinificación y toma de muestras de todos los vinos espumosos elaborados por el método tradicional que se han estudiado en esta tesis doctoral, se ha llevado a cabo en la bodega experimental de la Estación Enológica del Instituto Tecnológico Agrario de Castilla y León (ITACyL), situada en el municipio vallisoletano de Rueda.

3.1.1. Cosecha 2009

Se elaboraron diferentes vinos blancos y rosados espumosos monovarietales, siguiendo el método tradicional a partir de 5 variedades de uva blancas y 2 tintas procedentes de Castilla y León. Los vinos blancos espumosos monovarietales se elaboraron con las variedades Verdejo y Viura de la Denominación de Origen (D.O.) Rueda, Malvasía de la D.O. Toro, Albarín de la D.O. Tierra de León y Godello de la D.O. Bierzo. Los vinos rosados espumosos monovarietales se elaboraron con las variedades tintas Prieto Picudo de la D.O. Tierra de León y Garnacha de la D.O. Cigales. Se estudiaron muestras de dos parcelas de Garnacha.

Las uvas se vendimiaron en un estado de maduración adecuado para la elaboración de los vinos base para la obtención de vinos espumosos y en buenas condiciones sanitarias (**TABLA 1**). La vendimia se realizó de forma manual en cajas de 15 Kg de capacidad para evitar aplastamientos y posteriormente fueron transportadas en remolque hasta la bodega. La descarga de las mismas se hizo de forma manual.

TABLA 1. Parámetros enológicos de las uvas en el momento de la entrada en la bodega (mosto) en la cosecha 2009

Variedad	ºBrix	pH	AT[a]	Ácido málico (g/L)	Ácido tartárico (g/L)	Potasio (mg/L)
Verdejo	18,9	3,6	8,0	3,38	6,38	1.420
Viura	19,5	3,3	7,6	3,16	7,20	1.400
Malvasía	20,1	3,1	7,5	3,79	6,40	1.300
Albarín	20,4	3,0	12,0	4,59	9,14	1.210
Godello	20,2	2,9	9,3	3,87	8,45	1.510
Prieto Picudo	20,1	3,1	10,5	4,65	8,18	1.740
Garnacha	21,0	3,1	9,6	4,24	7,71	1.380
Garnacha*	19,7	3,1	9,0	4,26	7,34	1.340

[a]AT: acidez total en gramos de tartárico/L.

La **FIGURA 1** representa el esquema de la vinificación y la toma de muestras en la cosecha 2009 que se detalla a continuación. Los vinos base se elaboraron siguiendo el proceso tradicional de elaboración en blanco y en rosado. Así, las uvas introducidas en la bodega se estrujaron y despalillaron en una despalilladora-estrujadora (modelo ECR-15; grupo CMMC, Madrid, España). La uva estrujada y despalillada fue sulfitada (50 mg/L) y prensada (0,2 a 2 bar; tiempo de prensado de 5 a 6 horas) en una prensa neumática Europress EHS (Scharfenberger, Bad Dürkheim, Alemania) y con un rendimiento del mosto de 50%. En el caso de las uvas tintas se llevó a cabo una maceración prefermentativa de 24 horas a 15 °C antes de obtener el mosto.

El mosto obtenido se llevó a depósitos de acero inoxidable de 150 L a los que se adicionaron enzimas pectinolíticas (1 g/HL Novoclair Speed; Lamothe-Abiet, Burdeos, Francia), para favorecer la precipitación de sustancias coloidales. Tras 24 horas a 12 °C, el mosto se trasegó a depósitos de acero inoxidable de 150 L y por duplicado, que fueron inoculados con 20 g/HL de levadura comercial *Saccharomyces cerevisiae* (Lallemand, Montreal, Canada). La fermentación alcohólica se llevó a cabo a una temperatura controlada de 16 a 18 °C.

Tras la fermentación alcohólica, los vinos fueron estabilizados por frio (-5 °C) y clarificados con PVPP (10 g/HL) y bentonita (80 g/HL) (Laffort, Burdeos, Francia). A continuación se embotellaron los vinos base y se adicionó el licor de tiraje formado por la levadura *S. cerevisiae* var. *bayanus* (30 g/HL) (IOC 18-2007; Lallemand, Montreal, Canada), sacarosa (23 g/L) y bentonita (10 g/HL) (Laffort, Burdeos, Francia). Posteriormente tuvo lugar la segunda fermentación y la crianza sobre lías en botella en una cava subterránea, a una temperatura controlada de 11 a 13 °C y una humedad relativa del 75 a 85% durante 30 meses. A continuación se procedió al removido, al degüelle (DLV 1 TDD Grilliat Machines, Maquinaria Moderna, Barcelona, España) y al

encorchado definitivo. En todos los casos se elaboraron vinos espumosos *brut nature*, con lo cual no fue necesario añadir ningún licor de expedición.

La toma de muestras se realizó una vez acabada la fermentación alcohólica (vino base), en el vino base estabilizado y clarificado, durante la crianza sobre lías en botella (3, 6, 9, 18 y 30 meses de crianza sobre lías) y durante el envejecimiento en botella en ausencia de lías (9 meses de crianza sobre lías + 12 meses en ausencia de lías y 18 meses de crianza sobre lías + 12 meses en ausencia de lías). El análisis sensorial de los vinos espumosos se realizó a los 9 meses de crianza sobre lías.

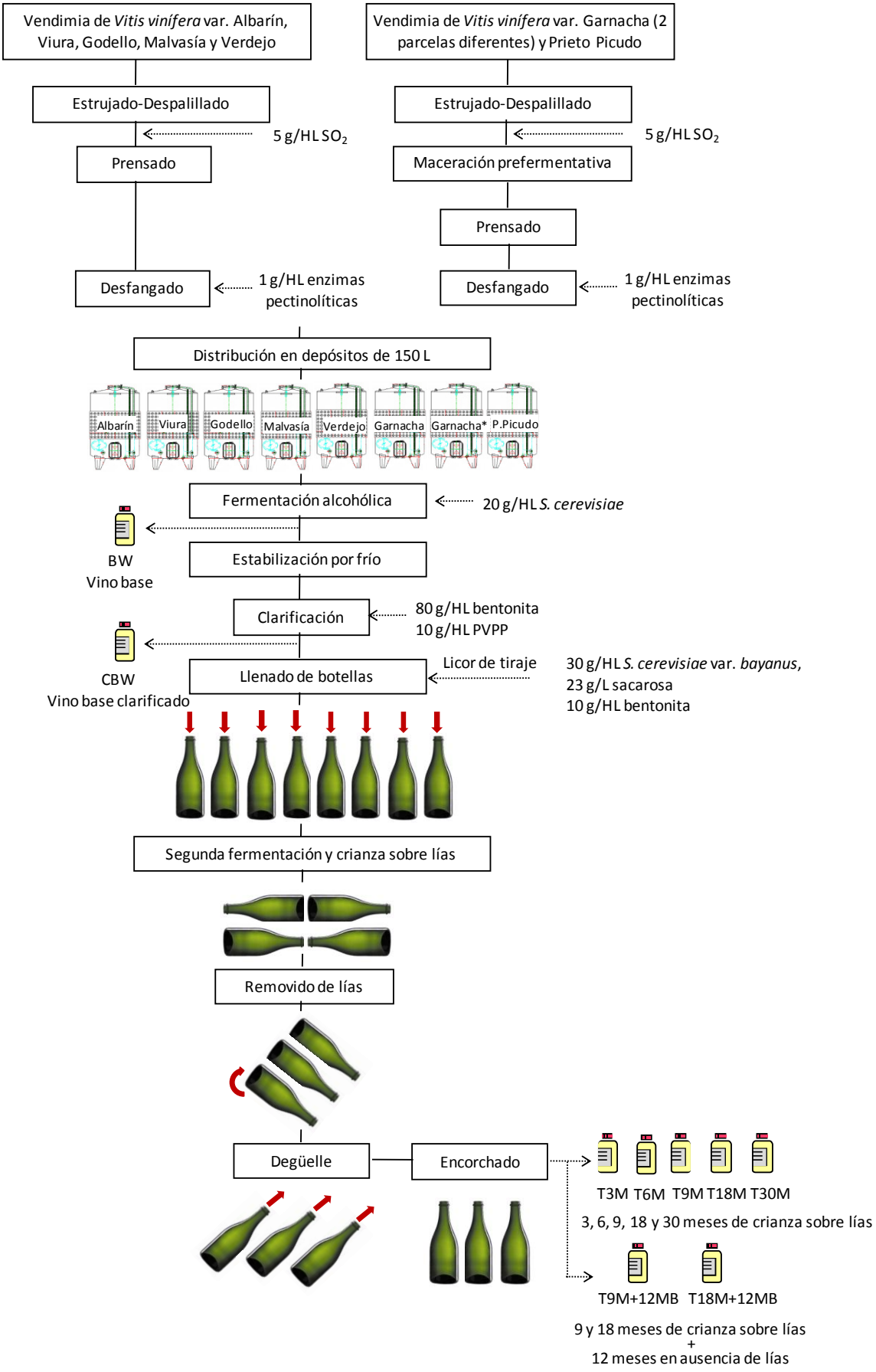

FIGURA 1. Esquema de vinificación y toma de muestras en la cosecha 2009

3.1.2. Cosechas 2010 y 2011

Se analizó el efecto de la adición de la adición de autolisados de levaduras comerciales a los vinos base sobre la composición y las características organolépticas de los vinos espumosos.

En la cosecha 2010 los estudios se realizaron en vinos blancos espumosos elaborados con la variedad Godello de la D.O. Bierzo y con vinos rosados espumosos elaborados con la variedad tinta Garnacha de la D.O. Cigales. En la cosecha 2011, los estudios se realizaron en vinos blancos espumosos elaborados con la variedad Verdejo de la D.O. Rueda y con vinos rosados espumosos elaborados con la variedad tinta Tempranillo de la D.O. Cigales. Las uvas se vendimiaron en un estado de maduración adecuado para la elaboración de los vinos base para la obtención de vinos espumosos y en buenas condiciones sanitarias (**TABLA 2**).

TABLA 2. Parámetros enológicos de las uvas en el momento de la entrada en la bodega (mosto). Cosechas 2010 y 2011

Variedad	Año	ºBrix	pH	AT[a]	Ácido málico (g/L)	Ácido tartárico (g/L)	Potasio (mg/L)
Godello	2010	18,5	3,17	9,3	3,4	10,0	1.750
Garnacha	2010	19,8	3,04	10,2	4,3	8,1	1.410
Verdejo	2011	19,3	3,37	7,7	2,1	8,9	1.730
Tempranillo	2011	19,6	3,14	9,8	4,5	7,2	1.200

[a]AT: acidez total en gramos de tartárico/L

Las **FIGURAS 2** y **3** representan el esquema de la vinificación y la toma de muestras en las cosechas 2010 y 2011, respectivamente. Los vinos base se elaboraron siguiendo el proceso tradicional de elaboración en blanco o en rosado en tanques de acero inoxidable de 2.000 y 2.600 L, respectivamente. El proceso de elaboración fue el mismo que en la cosecha 2009, es decir, se siguió el método tradicional con la única modificación de la adición a los vinos base los preparados comerciales derivados de levaduras a estudiar. Así, tras la fermentación alcohólica y la posterior clarificación y estabilización de los vinos base, cada vino se dividió en cinco depósitos de 150 L y se adicionaron 4 productos comerciales distintos, derivados de levaduras y ricos en polisacáridos (DYA-1, DYA-2, DYA-3 y DYA-4), de diferentes casas comerciales y con diferente composición (**FIGURAS 2** y **3**) dejando uno de los depósitos como testigo o control (C). Los productos comerciales empleados, su fabricante, las características de estos productos y sus efectos en el vino según la información reflejada en sus fichas técnicas, aparecen recogidas en la **TABLA 3**. A continuación se embotellaron los vinos base y se adicionó el licor de tiraje, para posteriormente llevar a cabo la segunda

fermentación y la crianza sobre lías en botella a temperatura y humedad controlada, en las mismas condiciones que en el cosecha 2009. En todos los casos se elaboraron vinos espumosos *brut nature*.

La toma de muestras se realizó una vez estabilizados y clarificados los vinos base y durante la crianza sobre lías en botella (3, 6 y 9 meses de crianza sobre lías). El análisis sensorial de los vinos espumosos se realizó a los 9 meses de crianza sobre lías.

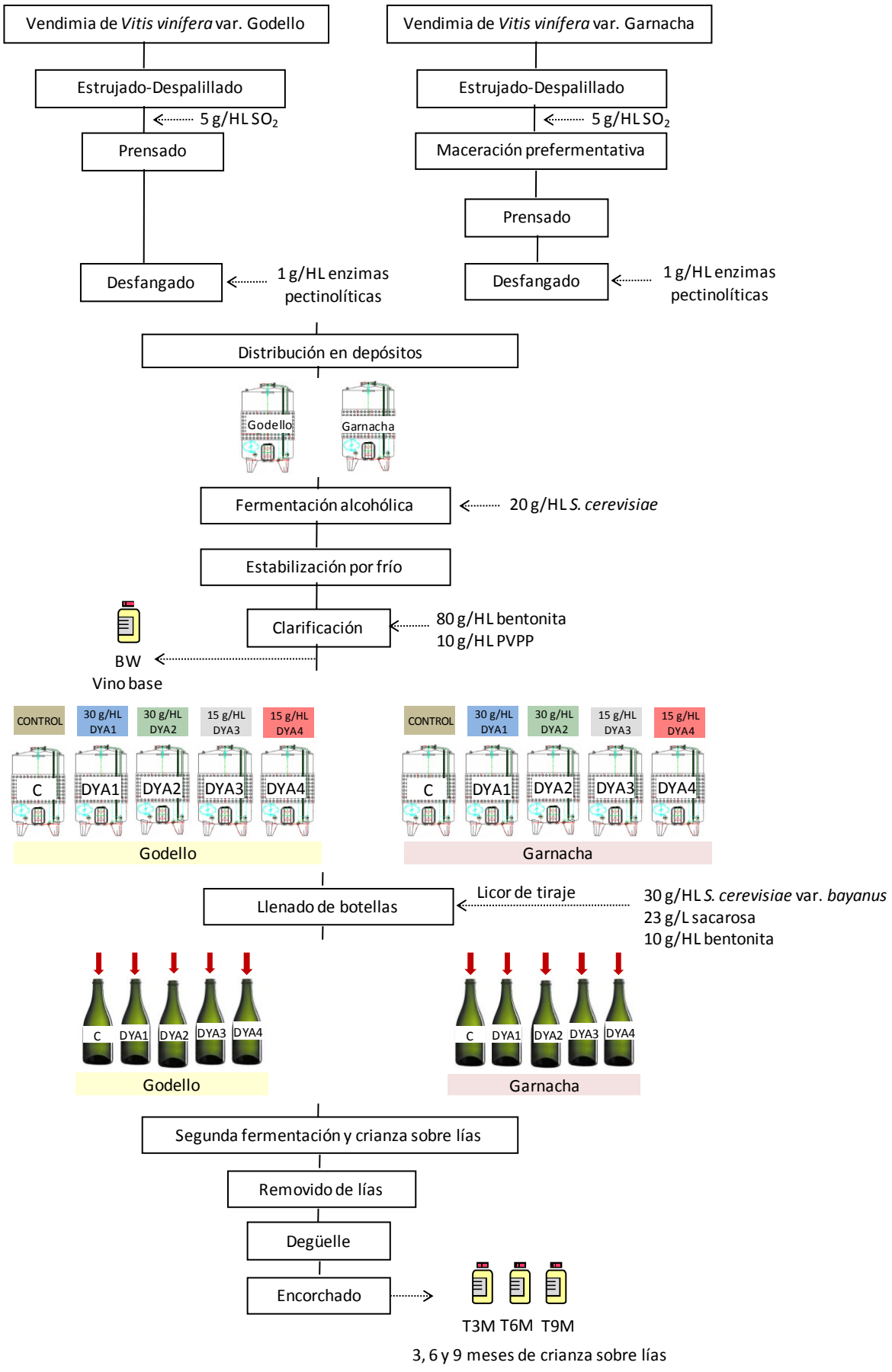

FIGURA 2. Esquema de vinificación y toma de muestras en la cosecha 2010

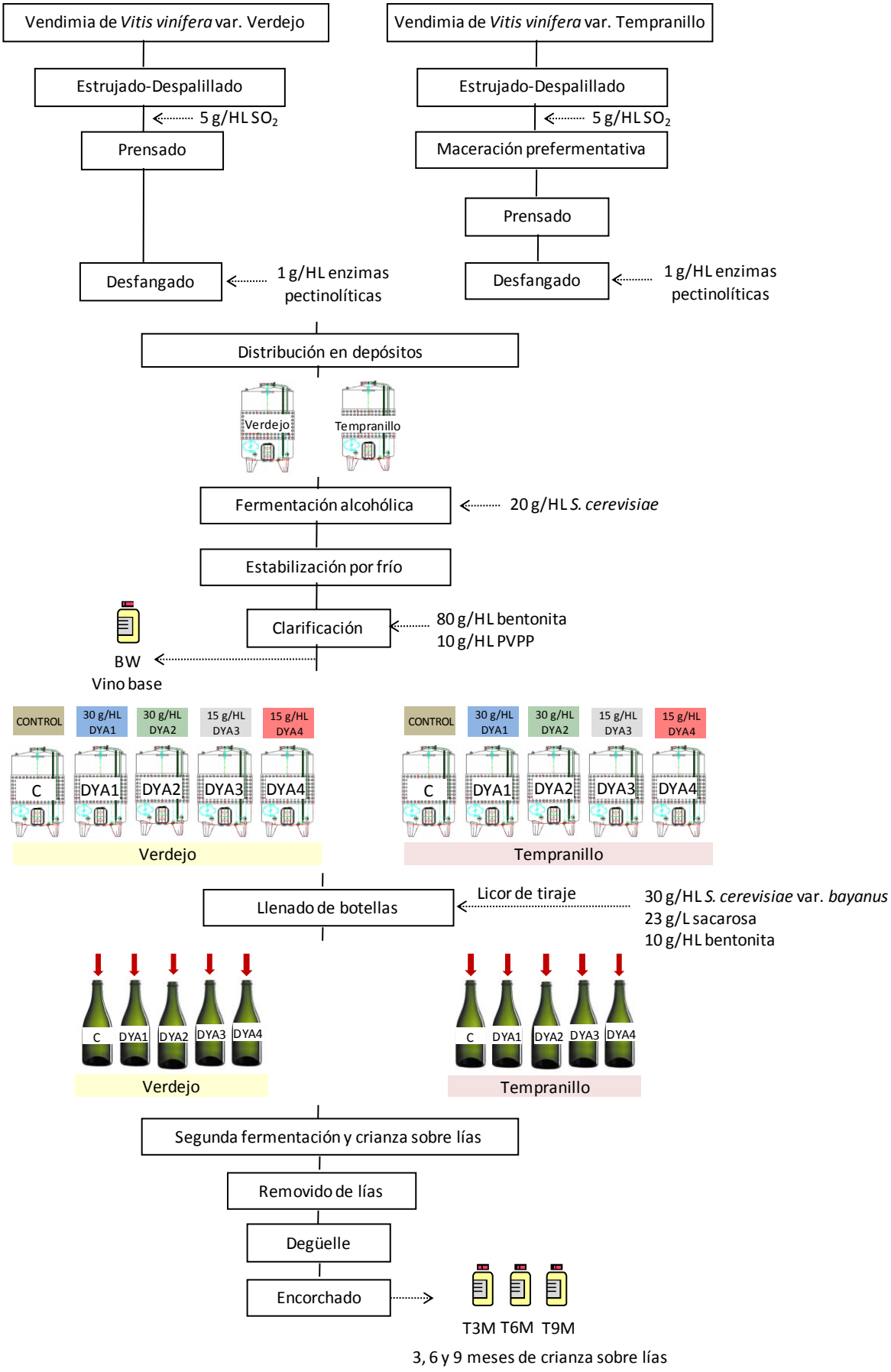

FIGURA 3. Esquema de vinificación y toma de muestras en la cosecha 2011

TABLA 3. Composición y características de los diferentes autolisados de levaduras comerciales empleados

Producto	Fabricante	Composición y características	Efectos esperados
DYA-1	Agrovin	Levaduras autolisadas, inactivadas por calor y ricas en polisacáridos	Aumenta el volumen de las lías, aumentando la sensación de volumen en boca y la persistencia de la espuma
DYA-2	Agrovin	Corteza de levaduras autolisadas inactivadas por calor y ricas en polisacáridos. Alto contenido en manoproteínas solubles (20-22%) de acción rápida. Acelera la crianza sobre lías	Aumenta el volumen de las lías, aumentando la sensación de volumen en boca y la persistencia de la espuma
DYA-3	Sepsa-Enartis	Levadura inactivada seleccionada por su elevado contenido en polisacáridos parietales	Protege los aromas, aumenta el frescor de los vinos y previene el pardeamiento. Elevado contenido en polisacáridos (manoproteínas) que aumentan la estabilidad coloidal del vino. Mejora la estabilidad de la espuma. Aumento de las sensaciones de volumen y suavidad en boca
DYA-4	Sepsa-Enartis	Complejo polisacárido derivado de la pared celular de las levaduras altamente purificadas, con un alto contenido en manoproteína libre y en péptidos sulfurados	Protege los aromas, aumenta el frescor de los vinos y previene el pardeamiento. Elevado contenido en polisacáridos (manoproteínas) que aumentan la estabilidad coloidal del vino. Mejora la estabilidad de la espuma. Aumento de las sensaciones de volumen y suavidad en boca. Protección, estabilidad y persistencia aromática

3.2. Parámetros enológicos y análisis químicos

Los parámetros y compuestos evaluados en esta tesis, así como los métodos de análisis utilizados para su determinación se describen a continuación.

3.2.1. Seguimiento de la maduración de las uvas

Los análisis del seguimiento de la maduración de las uvas fueron realizados en el Instituto Tecnológico Agrario de Castilla y León (Valladolid) por las Doctoras Silvia Pérez Magariño y Miriam Ortega Heras. Se realizó un seguimiento de la maduración de las uvas blancas y tintas desde el envero. Para ello, se tomó una muestra representativa de toda la parcela y sobre cada muestra se realizaron análisis de °Brix, pH, acidez total, ácido málico, ácido tartárico y potasio siguiendo los métodos oficiales de la OIV *(1)*.

3.2.2. Parámetros enológicos generales

Los análisis de parámetros generales fueron realizados en el Instituto Tecnológico Agrario de Castilla y León (Valladolid) por las Doctoras Silvia Pérez Magariño y Miriam Ortega Heras. En todas las muestras de vino se realizaron análisis de grado alcohólico, pH, acidez total, acidez volátil, sulfuroso libre y sulfuroso total, azúcares reductores, ácido málico y ácido tartárico siguiendo los métodos oficiales de la OIV (*1*).

3.2.3. Parámetros de color

El análisis de los parámetros del color se realizó en el Instituto Tecnológico Agrario de Castilla y León (Valladolid) por las Doctoras Silvia Pérez Magariño y Miriam Ortega Heras. Las medidas de intensidad de color y de tonalidad de los vinos rosados espumosos se evaluaron usando los parámetros de Glories (*2*). El color de los vinos blancos espumosos se evaluó mediante la medida de la absorbancia a 420 nm. Estos análisis se llevaron a cabo utilizando el espectrofotómetro Shimadzu serie UV-1700 (Pharmaspec, China). Todas las medidas se realizaron por triplicado y se refirieron a cubetas de cuarzo de 10 mm de espesor.

3.2.4. Compuestos volátiles

El análisis de los compuestos volátiles se realizó en el Instituto Tecnológico Agrario de Castilla y León (Valladolid) por las Doctoras Silvia Pérez Magariño y Miriam Ortega Heras. Los compuestos volátiles se determinaron mediante cromatografía gaseosa con detector de masas (GC-MS) previa extracción líquido-líquido de la fracción volátil siguiendo el método desarrollado por Rodríguez-Bencomo y col. (*3*). Los análisis se realizaron con un cromatógrafo de gases modelo HP-6890N (Agilent Technologies, Waldbronn, Alemania) acoplado a un detector de masas inerte 5973 HP. Se utilizó una columna capilar de

Quadrex 007CWBTR (60 m de longitud, 0,25 mm de diámetro y 0,25 m espesor de la película), y las condiciones cromatográficas establecidas por el método de Rodríguez-Bencomo y col. (*3*). Se identificaron y cuantificaron 44 compuestos volátiles que se agruparon en las siguientes familias de compuestos volátiles: ésteres de etilo, alcoholes, acetatos, terpenos, lactonas, fenoles volátiles, compuestos derivados de la madera, alcoholes de fusel y alcoholes isoamílicos. Todos los análisis se realizaron por triplicado.

3.2.5. Compuestos fenólicos monómeros

La identificación y cuantificación de antocianos, ácidos hidroxicinámicos, ácidos hidroxibenzoicos, flavonoles y flavan-3-oles se realizó por cromatografía líquida de alta presión con detector de fila de diodos (HPLC-DAD). Las muestras de vino se filtraron por filtros de disco de PTFE de 0,45 micras de tamaño de poro y se inyectaron directamente un cromatógrafo líquido modular 1100 Agilent (Agilent Technologies, Waldbronn, Alemania) controlado por el software Agilent Chemstation, equipado con una bomba cuaternaria G1311A, un desgasificador G1379A, un horno de columna G1316A, un inyector automático G1313A y un detector G1315B. La separación se llevó a cabo en una columna ACE (5 C18-HL) (Teknokroma, Barcelona, Spain) de tamaño de partícula de 5 µm (250 mm x 4,6 mm), según la metodología propuesta por Gómez-Alonso y col. (*4*). Este método permitió la identificación y cuantificación del ácido gálico, catequina, 9 ácidos hidroxicinámicos, 12 flavonoles, 15 antocianos y 3 estilbenos. Todos los análisis se realizaron por duplicado.

3.2.6. Proantocianidinas

Las muestras de vino se fraccionaron previamente por cromatografía de permeación en gel a baja presión (GPC) en una columna Toyopearl HP-50F (Tosohaas, Montgomeryville, PA), como describe Guadalupe y col. (*5*). Una primera fracción (F1) se eluyó con una mezcla de etanol/agua/ácido trifluoroacético (55/45/0,05, v/v/v) y la segunda fracción (F2) se obtuvo con una mezcla acetona/agua (60/40, v/v). La fracción rica en taninos (F2) se hidrolizó en presencia de floroglucinol y se analizaron las rupturas (o aductos de floroglucinol) por HPLC-DAD según las condiciones descritas en el método Kennedy y Jones (*6*). El equipo de cromatografía y la columna utilizados para la determinación fue el mismo que el usado para el análisis de los compuestos fenólicos monómeros. Este método permitió identificar y cuantificar los flavan-3-oles catequina, epicatequina, epicatequina-3-O-galato y epigalocatequina y sus respectivos aductos con floroglucinol,

así como calcular el grado medio de polimerización (mDP) de las proantocianidinas. Todos los análisis se realizaron por triplicado.

3.2.7. Aminoácidos y aminas biógenas

El contenido de aminoácidos y aminas biógenas se determinó simultáneamente mediante HPLC-DAD según la metodología descrita por Gómez-Alonso y col. (*7*). Este método consiste en la separación mediante HPLC de las aminoenonas de los aminoácidos y de las aminas formadas por el etoximetilenmalonato de dietilo (DEEMM) como agente derivatizante. El equipo y la columna utilizados para la determinación fueron los mismos que los descritos para la cuantificación de los compuestos fenólicos y de las proantocianidinas. Este método permitió la identificación y cuantificación de 24 aminoácidos y 9 aminas biógenas. Todos los análisis se realizaron por triplicado.

3.2.8. Polisacáridos

Se realizó un análisis cuantitativo de los monosacáridos, de las distintas familias de polisacáridos, así como un estudio del perfil y distribución de sus pesos moleculares en las muestras de vino y en los diferentes autolisados de levaduras empleados según la metodología descrita en Guadalupe y col. (*8*). En los vinos se analizaron tanto los polisacáridos procedentes de las levaduras (manoproteínas y glucanos), como los polisacáridos procedentes de la uva (polisacáridos ricos en arabinosa y galactosa, ramnogalacturonanos tipo II y homogalacturonanos). Los polisacáridos fueron extraídos de las muestras por precipitación con etanol/ácido. La distribución de pesos moleculares de las distintas familias de polisacáridos se analizó mediante cromatografía líquida de exclusión molecular de alta resolución con detector de índice de refracción (HRSEC-RID). Se utilizaron dos columnas en serie OH Shodex, KB-803 y KB-805 (0,8 x 30 cm, Showa Denko, Japón) equilibradas con 1 mL/min de 0,1 M $LiNO_3$. El perfil de la distribución de los pesos moleculares de los polisacáridos se comparó con patrones comerciales (Shodex P-82, Waters, Barcelona, España) de 5,9 KDa (P-5), 11,8 KDa (P-10), 47,3 KDa (P-50), 112 KDa (P-100) y de 212 KDa (P-200). Para la cuantificación de las distintas familias de polisacáridos, los residuos glicosídicos de cada polisacárido se identificaron y cuantificaron por GC-MS de sus trimetilsilil-eter-O-metil glicósidos previa metanolisis ácida y derivatización de la muestra. Se utilizó una columna capilar de sílice fundida (Teknokroma 30 m × 0,25 mm x 0,25 m), con helio a 1 mL/min como gas portador. Las distintas familias de polisacáridos se estimaron a partir de su composición individual de

residuos glicosilados. El equipo de HPLC usado fue el mismo que el descrito previamente acoplado a un detector de índice de refracción modelo G1362. El equipo de GC-MS estaba equipado con un inyector automático 7653B y un cromatógrafo de gases 7890A acoplado a un detector de masas cuadrupolar modelo VL 5975C, de Agilent (Agilent Technologies, Waldbronn, Alemania), controlado por el software ChemStation. Todos los análisis se llevaron a cabo por triplicado.

3.2.9. Medida de la calidad de la espuma

El análisis de la medida de la calidad de la espuma se realizó en el Instituto Tecnológico Agrario de Castilla y León (Valladolid) por las Doctoras Silvia Pérez Magariño y Miriam Ortega Heras. La evaluación de las propiedades espumantes de los vinos se realizó con un equipo Mosalux (Station Oenotechnique de Champagne, Cormontreuil, Francia), según la metodología descrita por Maujean y col. (*9*). Tres parámetros fueron medidos: HM = altura máxima de espuma, expresada en milímetros, es la altura máxima alcanzada por la espuma después de la inyección de CO_2, representa la capacidad de la disolución para producir espuma. HS = altura a la cual se estabiliza la espuma durante la inyección de CO_2, expresado en milímetros, representa la capacidad de la disolución para producir una espuma estable. TS = tiempo de estabilidad de la espuma, expresado en segundos, es el tiempo que transcurre desde la interrupción del flujo de gas y la desaparición total de la espuma. Todos los análisis se llevaron a cabo por triplicado.

3.3. Análisis sensorial

El panel de cata estuvo compuesto por al menos 12 catadores, todos ellos técnicos de distintos Consejos Reguladores y enólogos de diferentes bodegas. Todos los vinos se evaluaron en cuatro fases, en la fase visual (color y calidad de la espuma), en la fase aromática (fracción volátil) y en la fase gustativa (equilibrio en boca).

El análisis sensorial se realizó en dos sesiones. En una primera sesión los catadores definieron los descriptores de los vinos espumosos según la metodología descrita en González-San José y col. (*10*). Los términos descriptivos y sus definiciones se debatieron entre los catadores y, posteriormente se compiló un vocabulario de consenso común. Los catadores seleccionaron seis atributos para evaluar el color, cinco atributos previamente descritos en Gallart y col. (*11*) para la calidad de la espuma, ocho para la

fase aromática, y siete para la gustativa. Todos los términos generados eran términos habituales para definir las características sensoriales de los vinos espumosos.

En la segunda sesión se realizó la fase de evaluación sensorial propiamente dicha. Los catadores fueron entrenados para cuantificar los descriptores elegidos empleando escalas numéricas. El entrenamiento se llevó a cabo de acuerdo con la norma UNE-87-020-93, correspondiente a la norma ISO 4121:1987. Utilizando el vocabulario consenso, se utilizó una escala de respuesta cuantitativa con 7 niveles de intensidad, donde 1 correspondía a la ausencia de percepción de la propiedad considerada, y 7 a la intensidad máxima intensidad del mismo. Los descriptores empleados para determinar la calidad de la espuma se evaluaron en una escala de 1 a 3. Además, los catadores puntuaron la valoración global de los vinos espumosos en una escala de 1 a 7, correspondiendo 1 a la mínima calificación del vino y 7 a la máxima, pudiendo realizar comentarios adicionales sobre las propiedades sensoriales de los vinos. Las fichas de cata que recogen todos los atributos de las catas realizadas de los vinos blancos y rosados espumosos se presentan en las **FIGURAS 4** y **5** respectivamente. En cada sesión se repitió un vino con el fin de determinar la consistencia de cada catador. Cuando los catadores no fueron consistentes, los datos de cata de dichos catadores no se incluyeron en el análisis estadístico.

50-60 mL de vino espumoso se presentaron a 6-8 °C en copas de vidrio estilo flauta de 100 mL sin ningún fallo ni marca. Cada botella de vino espumoso se abrió lentamente y sin agitar la botella. Para evitar la formación de burbujas de aire, el vino se vertió lentamente en la copa.

La evaluación sensorial se llevó a cabo en la sala de catas del Instituto Tecnológico Agrario de Castilla y León, que cumple con la norma ISO 8589:2010.

FECHA DE LA CATA: _____

NOMBRE DEL CATADOR: _____

MUESTRA: Vinos blancos espumosos

	1	2	3	4	5	6	7
VISUAL							
Espuma inicial							
Superficie							
Corona							
Tamaño de burbuja							
Efervescencia							
Intensidad color							
Tonos amarillos							
Tonos verdes							
Tonos pardos							
OLFATIVO							
Intensidad olfativa							
Fermentativas							
Varietales							
Frutales							
Frutas exóticas							
Florales							
Cítricos							
Verdes							
Sucio							
EN BOCA							
Volumen en boca							
Acidez							
Amargor							
Astringencia							
Persistencia							
Frescura							
Equilibrio							

OBSERVACIONES:

Valoración global: Rodee con un círculo su valoración

1=Muy Malo; 2= Malo; 3= Regular; 4 = Correcto; 5= Bueno; 6= Muy Bueno; 7= Excelente

FIGURA 4. Ficha de cata empleada para la evaluación de los vinos blancos espumosos

FECHA DE LA CATA: _____

NOMBRE DEL CATADOR: _____

MUESTRA: Vinos rosados espumosos

	1	2	3	4	5	6	7
VISUAL							
Espuma inicial							
Superficie							
Corona							
Tamaño de burbuja							
Efervescencia							
Intensidad Color							
Tonos rojos							
Tonos teja							
OLFATIVO							
Intensidad olfativa							
Verde							
Fermentativas							
Varietales							
Frutales							
Sucio							
Oxidado							
Reducido							
EN BOCA							
Volumen en boca							
Acidez							
Amargor							
Astringencia							
Persistencia							
Frescura							
Equilibrio							

OBSERVACIONES:

Valoración global: Rodee con un círculo su valoración

1=Muy Malo; 2= Malo; 3= Regular; 4 = Correcto; 5= Bueno; 6= Muy Bueno; 7= Excelente

FIGURA 5. Ficha de cata empleada para la evaluación de los vinos rosados espumosos

3.4. Análisis estadístico

Todo el conjunto de datos obtenidos fueron sometidos, variable por variable, a un análisis de varianza (ANOVA o Kruscal-Wallis). Las diferencias se expresaron con un nivel de confianza mayor del 95%.

Se aplicaron técnicas quimiométricas de Análisis de Componentes Principales (Principal Components Analysis, PCA), de Análisis Discriminantes (Discriminant Analysis, DA), de Análisis Factorial (Factor Analysis, FA) y de Regresión Lineal Múltiple (Multiple Linear Regression analysis, MLR) con el fin de establecer diferencias y similitudes entre los distintos vinos espumosos teniendo en cuenta la variedad de uva empleada, los distintos momentos de elaboración y el tratamiento realizado, así como establecer relaciones entre la composición química de los vinos y sus propiedades espumantes.

Los atributos sensoriales se analizaron mediante un Análisis de Procusters Generalizado (Generalized Procustes Analysis, GPA) que permitió también determinar si los catadores eran precisos (inter-variabilidad) y consistentes (intra-varibilidad). Se realizó además un test de permutación para comprobar que los resultados obtenidos eran significativos.

Los análisis estadísticos se realizaron con distintos paquetes informáticos, SPSS 13.0 (SPSS Inc., Chicago, USA), Statistica 8.0 (Statsoft Inc., Tulsa, USA) y Statgraphics Plus 5.0 (Manugistics Inc., Rockville, USA). Para el análisis estadístico de los datos de cata se utilizó, además de los programas estadísticos comentados, el software específico de análisis sensorial Senstools Versión 3.3.2 (OP&P, Utrecht, The Netherlands).

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4.1. Vinos espumosos elaborados con variedades de uva alternativas: atributos sensoriales y evolución de los compuestos fenólicos durante la vinificación y la crianza sobre lías

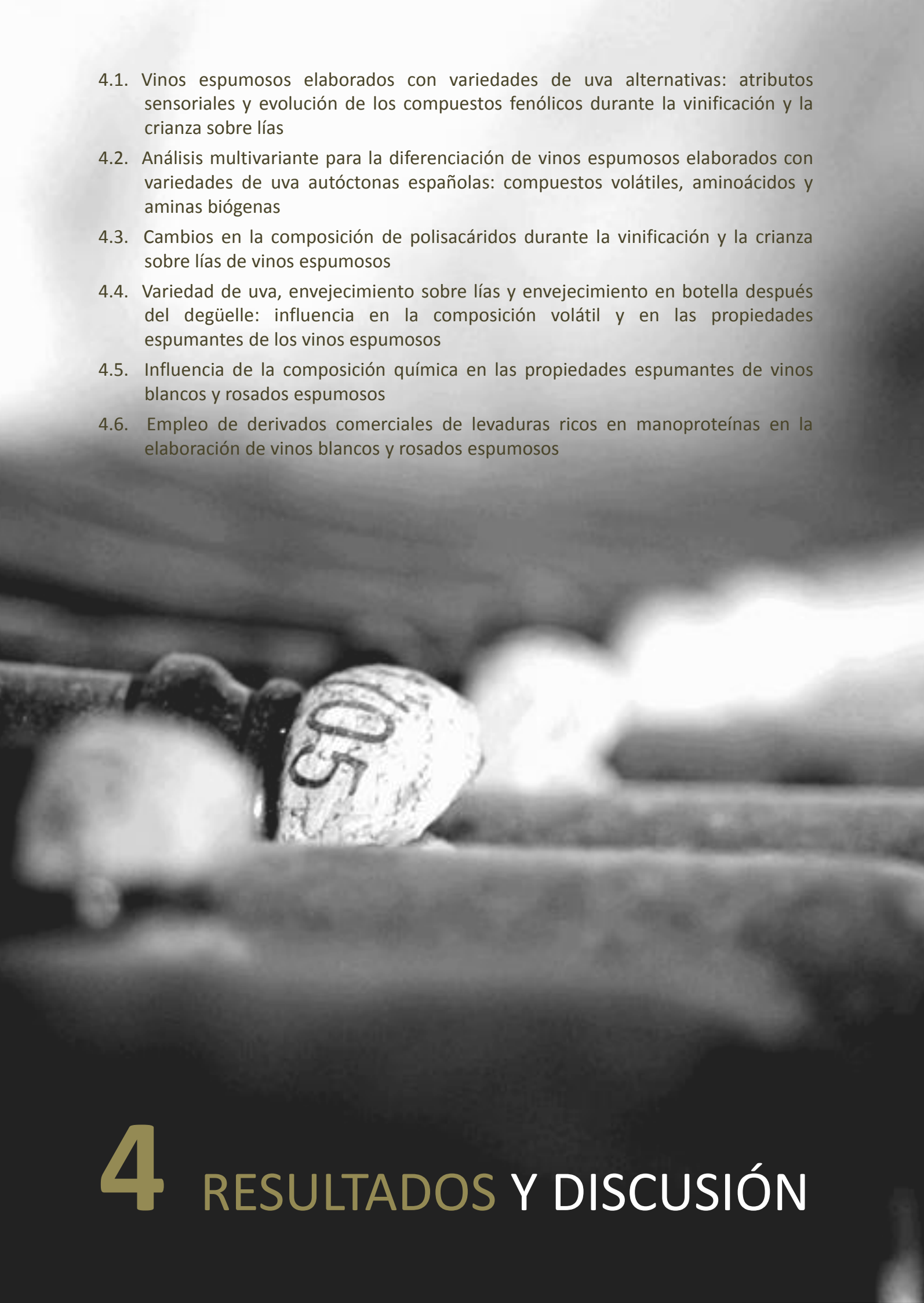
4.2. Análisis multivariante para la diferenciación de vinos espumosos elaborados con variedades de uva autóctonas españolas: compuestos volátiles, aminoácidos y aminas biógenas

4.3. Cambios en la composición de polisacáridos durante la vinificación y la crianza sobre lías de vinos espumosos

4.4. Variedad de uva, envejecimiento sobre lías y envejecimiento en botella después del degüelle: influencia en la composición volátil y en las propiedades espumantes de los vinos espumosos

4.5. Influencia de la composición química en las propiedades espumantes de vinos blancos y rosados espumosos

4.6. Empleo de derivados comerciales de levaduras ricos en manoproteínas en la elaboración de vinos blancos y rosados espumosos



4 RESULTADOS Y DISCUSIÓN

4.1

Vinos espumosos elaborados con variedades de uva alternativas: atributos sensoriales y evolución de los compuestos fenólicos durante la vinificación y la crianza sobre lías

Sparkling wines produced from alternative varieties: sensory attributes and evolution of phenolics during winemaking and aging

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Resumen

Este artículo aborda tres objetivos fundamentales:

1. Caracterizar enológicamente los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional, en términos de composición fenólica durante la vinificación y la crianza sobre lías en botella. En particular, se analizaron antocianos monómeros, ácidos hidroxicinámicos, ácidos hidroxibenzóicos, flavonoles, flavan-3-oles, intensidad de color, tonalidad, absorbancia a 420 nm y proantocianidinas.
2. Evaluar los cambios que se producen en dichos parámetros durante el proceso de elaboración de los vinos espumosos, incluyendo las etapas de vino base, de vino base estabilizado y clarificado y de envejecimiento sobre lías en botella durante nueve meses.
3. Analizar sensorialmente los vinos espumosos obtenidos después de nueve meses de envejecimiento sobre lías en botella, tiempo mínimo necesario para denominarse vinos espumosos.

El estudio se realizó con vinos espumosos de la cosecha 2009.

Todos los vinos base mostraron un adecuado grado alcohólico, elevada acidez y bajo pH, indicando que las variedades estudiadas poseían un buen potencial para la elaboración de vinos espumosos. Durante el proceso de vinificación y envejecimiento sobre lías, en los vinos blancos espumosos se observó una disminución de la absorbancia a 420 nm, indicando ausencia de oxidación y en los rosados una disminución de la intensidad de color.

La composición y el contenido de los ácidos hidroxicinámicos, flavonoles y proantocianidinas en los vinos base fueron independientes del color de las uvas y de la existencia o no de maceración prefermentativa durante la elaboración de los vinos base.

La estabilización por frío y la clarificación de los vinos base antes de la fase de tiraje produjo una disminución en el contenido de antocianos, más acusado en los vinos elaborados con Garnacha que en los obtenidos con Prieto Picudo, y en el contenido de proantocianidinas.

Durante los primeros seis meses de envejecimiento sobre lías se produjeron pérdidas en todos los tipos de compuestos fenólicos analizados debido a su alta reactividad y a los fenómenos de adsorción por las levaduras. Sin embargo, parte de los compuestos fenólicos adsorbidos al inicio de la fase de tiraje fueron liberados durante

los últimos tres meses de envejecimiento debido al proceso autolítico de las levaduras. Los vinos espumosos de Prieto Picudo mostraron mayor intensidad de color, contenido en antocianos y estabilidad del color que los vinos elaborados con Garnacha, los cuales se caracterizaron por una mayor concentración de ácidos hidroxicinámicos. Entre los vinos blancos espumosos, los elaborados con la variedad Albarín mostraron el mayor contenido en catequina y en proantocianidinas y junto con los elaborados con Viura, en hidroxicinamatos. Los vinos rosados espumosos de Garnacha y los blancos de Albarín presentaron los mayores valores de polifenoles totales.

En el análisis sensorial los vinos espumosos de Prieto Picudo mostraron mayor intensidad de color visual, de tonos rojos, de frescor en boca, de intensidad aromática y de calidad de la espuma que los elaborados con la variedad Garnacha. Los vinos espumosos de Albarín y de Verdejo presentaron mayor intensidad de color visual e intensidad aromática que el resto de vinos blancos. Los vinos espumosos de Verdejo mostraron la mejor calidad de la espuma entre los vinos blancos.

Los resultados obtenidos indicaron que las variedades de uva de Prieto Picudo, Albarín y Verdejo fueron las más adecuadas para la elaboración de vinos espumosos, pudiéndose incrementar el potencial enológico de estas variedades tradicionalmente empleadas para la elaboración de vinos tranquilos.

Sparkling Wines Produced from Alternative Varieties: Sensory Attributes and Evolution of Phenolics during Winemaking and Aging

Leticia Martínez-Lapuente,[1] Zenaida Guadalupe,[1]* Belén Ayestarán,[1] Miriam Ortega-Heras,[2] and Silvia Pérez-Magariño[2]

Abstract: Spanish grape varieties that have been traditionally used to produce still wines were examined for their potential to make white and rosé sparkling wines. Sparkling wines manufactured from *Vitis vinifera* cv. Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha, and Prieto Picudo varieties were examined for sensory attributes and for the evolution of monomeric and polymeric phenolics during different stages of winemaking and aging. Stabilization and clarification of the base wines significantly decreased the concentrations of anthocyanins and proanthocyanidins. During the initial months of aging on yeast lees, all types of polyphenols decreased, although some were released back into the wine during the final months. Garnacha rosé and Albarín white wines had high phenolic potential. Garnacha rosé sparkling wines had particularly high hydroxycinnamic acid concentrations, while Prieto Picudo rosé sparkling wines had the highest color intensity and anthocyanin concentrations. Among white sparkling wines, Albarín had the most catechin, proanthocyanidins, and, together with Viura, hydroxycinnamates. In sensory profiling, Prieto Picudo had more visual color intensity, red tones, olfactory intensity, freshness sensations, and foam quality than Garnacha wines. Albarín and Verdejo had more visual color and olfactory intensity than the other white wines, and Verdejo had better foam quality. Prieto Picudo, Albarín, and Verdejo were the most promising varieties for the production of high-quality sparkling wines.

Key words: sparkling winemaking, aging, grape variety, polyphenols, sensory analysis

Sparkling wines produced by traditional methods owe their peculiar characteristics to a double fermentation and to aging with yeast in the bottle. The best-known sparkling wines produced within this premium category are Champagne from France, Talento from Italy, and cava from Spain. Although factors such as winemaking technology, time of aging, and viticultural characteristics can affect sparkling wine composition, grape variety is of key importance (Pozo-Bayón et al. 2009, Cilindre et al. 2010). The majority of sparkling wines manufactured by traditional methods are produced from white grape varieties, but red varieties are used to produce rosé (partially macerated with skins) and *blanc de noir* (without maceration) sparkling wines.

Some studies have examined the Garnacha grape variety for Spanish sparkling wine production (Pozo-Bayón et al. 2003, 2004); however, there has been no evaluation of other red varieties such as Prieto Picudo, an autochthonous variety from northwestern Spain traditionally used for semi-sparkling *aguja* rosé wines and with good characteristics for both young and aged wines (Ortega-Heras et al. 2007). The white varieties Viura and Malvasía have been evaluated for sparkling wine production (Ibern-Gómez et al. 2000, Moreno-Arribas et al. 2000, Andrés-Lacueva et al. 2002, Vanrell et al. 2007, Stefenon et al. 2010) but, to our knowledge, there have not been studies on varieties such as Godello, Albarín, and Verdejo, which are frequently used to make high-quality still white wines (Vilanova 2006, Masa and Vilanova 2008, Sánchez-Palomo et al. 2010) and which have an acidity, aroma, and mouthfeel suitable for sparkling wines.

There is no information on the occurrence and evolution of phenolic compounds during the different phases of the sparkling winemaking: production of base wines, clarification and stabilization, secondary fermentation, and postfermentation aging on yeast lees in bottles. Existing studies focus on the phenolic profile of the base wines or the final product (Ibern-Gómez et al. 2000, Andrés-Lacueva et al. 2002, Chamkha et al. 2003, Pozo-Bayón et al. 2003, Jordão et al. 2010, Stefenon et al. 2010), and several studies describe phenolics in sparkling rosé wines (Pozo-Bayón et al. 2003, 2004). This study examines the changes in monomeric and polymeric phenolic compounds during winemaking and aging and the sensory attributes of sparkling wines produced with different white

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(Verdejo, Viura, Malvasía, Albarín and Godello) and red (Garnacha and Prieto Picudo) grape varieties.

Materials and Methods

Equipment. High-performance liquid chromatography (HPLC) was performed using a modular 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with one G1311A quaternary pump, an online G1379A degasser, a G1316A column oven, a G1313A automatic injector, and a G1315B photodiode-array detector (DAD) controlled by the Agilent software (ChemStation for LC 3D Rev. A. 10.02 [1757]). All spectrophotometric measurements were carried out in quartz cuvettes in a PharmaSpec 1700 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan).

Vinification and sampling. Five monovarietal white sparkling wines and three monovarietal rosé sparkling wines from *Vitis vinifera* varieties were produced in the Instituto Tecnológico Agrario de Castilla y León (vintage 2009). White sparkling wines were made from Verdejo and Viura grapes from the Rueda Denomination of Origin (D.O.), Malvasía grapes from the Toro D.O., Albarín grapes from the Tierra de León D.O., and Godello grapes from the Bierzo D.O. Two rosé sparkling wines were made from Garnacha grapes from different viticultural areas of the Cigales D.O. (designated Garnacha and Garnacha*) and the third was made from Prieto Picudo grapes from the Tierras de León D.O.

Grapes were harvested manually at the optimum ripeness for sparkling wines, under sanitary conditions. They were harvested early in the morning ($T^a < 15°C$) and immediately transported to the enological station cellar in 15-kg plastic bins. Clusters were destemmed and crushed with minimum physical damage in a crusher-destemmer (model ECR-15; CMMC group, Madrid, Spain). The mass obtained was sulfited (50 mg/L) and pressed (0.2 to 2 bars, 5 to 6 hr pressing time) in a Europress EHS pneumatic press (Scharfenberger, Germany) with a juice yield of 50%. Red grapes received 24-hr of prefermentation maceration before pressing. Musts were then transferred to 150-L stainless-steel tanks and a pectinolytic enzyme preparation was added (1 g/100 L Novoclair Speed; Lamothe-Abiet, Bordeaux, France) to favor the precipitation of colloidal substances over 24 hr at 12°C. Fermentation took place in 150-L stainless-steel tanks in duplicate at 16 to 18°C after inoculation with 20 g/100 L *Saccharomyces cerevisiae* yeast (Lallemand, Montreal, Canada). From each of the eight base wines, a batch of sparkling wines was manufactured. Base wines were cold-stabilized (-5°C), clarified with PVPP (10 g/100 L) and bentonite (80 to 90 g/100 L) (Laffort, Bordeaux, France), bottled, and the tirage liquor, consisting of *S. cerevisiae* var. *bayanus* yeast (30 g/100 L) (IOC 18-2007; Lallemand), sucrose (23 g/L), and bentonite (10 g/100 L) (Laffort), was added. Secondary fermentation and aging with yeast for nine months was carried out at 15 to 16°C (cellar temperature). Sparkling wines were then riddled and disgorged. *Liqueur d'expédition* was not added and bottles were filled with the sparkling wine itself to produce Brut nature sparkling wines. Finally, the bottles were closed with the final cork, which was secured to the neck with a wirecap.

Samples for analysis were taken of the base wines (BW), after clarification and stabilization of the base wines (CBW), and then after 3 months (T3M), 6 months (T6M), and 9 months (T9M) of aging on yeast lees, after hand disgorging. For each stage, three bottles were degassed and analyzed. Standard enological parameters in musts and wines were determined using official analysis methods (OIV 1990). Sensory analysis was conducted in the final sparkling wines (T9M), as 9 months is the minimum time established by European Communities regulation (EC Nº 606/2009) for aging.

Analysis of monomeric phenolics. Anthocyanins, hydroxycinnamic acids, flavonols, flavan-3-ols, and gallic acid were analyzed by HPLC-DAD with a direct injection of 25 µL wine previously filtered through 0.45-µm membranes. Separation was achieved with an ACE HPLC (Teknokroma, Barcelona, Spain) (5 C18-HL) particle size 5 µm (250 mm x 4.6 mm) column protected with a guard column of the same material, according to a described method (Gómez-Alonso et al. 2007). The concentration of nonacylated anthocyanins (A) was calculated as the sum of delphinidin, cyanidin, petunidin, peonidin, and malvidin-3-glucosides; the concentration of acetyl-glucoside anthocyanins (A-Ac) as the sum of delphinidin, cyanidin, petunidin, and malvidin-3-(6-acetyl)-glucosides; and the concentration of coumaryl-glucoside anthocyanins (A-Cm) as delphinidin, petunidin, and malvidin-3-(6-*p*-coumaryl)-glucosides. The sum of A, A-Ac, and A-Cm was referred to as total monomeric anthocyanins (T-A). Total hydroxycinnamic acids (T-HA) was calculated as the sum of free caffeic, ferulic, and coumaric acids and hydroxycinnamates (E-Ac): that is, *trans*-caftaric, *cis*-caftaric, *trans*-coutaric, *cis*-coutaric, and *trans*-fertaric acids. Total flavonol concentration (T-Flavo) was calculated as the sum of myricetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-glucuronide, isorhamnetin-3-rutinoside, kaempferol-3-glucoside, isorhamnetin-3-glucoside, myricetin, quercetin, kaempferol, and isorhamnetin. All analyses were performed in duplicate.

Analysis of proanthocyanidins. Wine samples were directly fractionated by gel permeation chromatography on a Toyopearl gel HP-50F column (Tosohaas, Montgomeryville, PA) as described elsewhere (Guadalupe et al. 2006). A first fraction (F1) containing monomeric flavonoids, dimeric anthocyanins, and polymeric pigments was eluted with ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v); a second fraction (F2) containing proanthocyanidins was recovered by elution with acetone/water (60:40, v/v). Fractionation was performed in triplicate and phloroglucinol adducts in F2 fractions were analyzed by reversed-phase HPLC (Kennedy and Jones 2001). The column was an ACE HPLC (Teknokroma) (5 C18-HL) particle size 5 µm (250 mm x 4.6 mm) protected by a guard column containing the same material. Proanthocyanidin cleavage products were estimated using their response factors relative to (+)-catechin, which was used as the quantitative standard. Total proanthocyanidin concentration (PA) was calculated as the sum of extension subunits (phloroglucinol adducts) and terminal subunits (catechin, epicatechin, and epicatechin-gallate). The percentage of terminal subunits was

calculated over the total proanthocyanidins, and the apparent mean degree of polymerization (mDP) as the sum of all subunits divided by the sum of the terminal subunits.

Sensory analysis. After 9 months of aging, panelists rated the sparkling wines for visual, olfactory, gustatory, and foam quality conformance to sparkling wine typology. The sensory analysis was carried out by a tasting panel of eight male and four female judges, all expert tasters from the regulatory councils of various Spanish origin denominations and wineries. Tasters defined the descriptors used in the sensory analysis as described elsewhere (González-San José et al. 2008), and they were then trained to quantify these descriptors using structured numerical scales. The training was carried out in accordance with UNE-87-020-93 Norm, corresponding to ISO 4121:1987 Norm. Tasters selected four attributes for the visual phase, eight for the olfactory, and seven for the gustative, which were agreed upon as best for describing the sensory characteristics of the sparkling wines. A structured numerical scale of seven points was used, with 1 representing no color and weak aroma or taste and 7 representing a very strong color aroma or taste. Particular attention was given to the color and foam characteristics, which many consumers consider the most important characteristics of sparkling wine. To determine foam quality, five previously defined descriptors (Gallart et al. 2004) were evaluated on a scale of 1 to 3. Judges rated two groups of sparkling wines in two different sessions. In the first session, they were given the five white sparkling wines; in the second, they were given the three rosé sparkling wines. To ascertain the consistency of the judges, one sample was replicated in each session. Wine samples of 50 to 60 mL were served at 6 to 8°C in new flute glasses (100 mL) with no faults or marks. Each bottle was opened slowly, with the cork held in the hand and without shaking the bottle. To avoid air bubble formation, the wine was poured slowly into the glass.

Statistical procedures. Significant differences were determined by an analysis of variance (ANOVA) if the data adhered to assumptions of normality. If these assumptions were not adhered to, then a Kruskal-Wallis test was used. Significant differences were always at least $p < 0.05$ (95% confidence level). Sensory data were subjected to ANOVA to determine the within-judges reproducibility in rating two replicated wines. Generalized procrustes analysis (GPA) was then applied on the mean ratings for olfactory, gustatory, and foam quality attributes, and a permutation test determined that the results obtained were significant (85.12%). ANOVA evaluations were performed using Statistica (ver. 8.0 for Microsoft Windows; Statsoft, Tulsa, OK) and the GPA and correlation analyses by using Senstools (ver. 3.3.2.; OP&P, Utrecht, Netherlands).

Results and Discussion

Enological analysis. Standard enological parameters were determined for the musts (Table 1) and the base wines (BW), wines after clarification (CBW), and sparkling wines during aging on yeast lees (T3M, T6M and T9M) (Table 2). For sparkling winemaking, grapes are harvested at low sugar content and thus the base wines had low alcohol concentrations, high acidity, and low pH. The absorbance at 420 nm, customarily used as a measure of browning in white wines, was low in all base wines. In rosé wines, Prieto Picudo base wines had higher color intensity (CI) and hue values than Garnacha. In general, all CI values were lower than typically found in still rosé wines because grapes used for sparkling wine production need to be harvested at low maturity, which is when the phenolic maturity is low, making it difficult to extract phenolic compounds even with long maceration times.

The alcoholic content increased in all the wines during the secondary fermentation in bottle. Total acidity in the final sparkling wines was quite high, although it decreased due to potassium tartrate precipitation during cold stabilization. The low pH and high acidity of the final wines indicated that the grape varieties studied were suitable for sparkling wines because low pH and high acidity have a positive impact on sensory attributes. The final white sparkling wines had almost half of the absorbance at 420 nm of the base wines. These findings contrast with other results (Ibern-Gómez et al. 2000), in which cavas had increased browning (measured as absorbance at 420 nm) during aging due to oxidation of phenolic compounds despite the reducing atmosphere in the bottle. Color intensity decreased in all rosé wines during the winemaking process, but to a greater degree in both Garnacha varieties (76–79%) than in Prieto Picudo (65%). The greatest decrease occurred during two winemaking stages: clarification–stabilization and wine aging in contact with lees. During aging, phenolic compounds responsible for color may adhere to the cell walls of lees or combine with other wine constituents. The CI values of the final sparkling wines were in the range reported in other rosé sparkling wines made from Monastrell and Trepat (Girbau-Sola et al. 2002) and much higher than those reported for one made from Garnacha (Pozo-Bayón et al. 2004).

Monomeric anthocyanins. There were no significant differences in total anthocyanins among base wines (Figure 1). All had less of these compounds than still rosé wines (Salinas et al. 2003) because the grapes were harvested at low phenolic maturity. Nonacylated anthocyanins were the most abundant class in all wines, varying from 85% in Prieto Picudo to 93% in Garnacha. Differences among the varieties were also observed in concentrations of *p*-coumarylated and acetylated anthocyanins, with the acetylated anthocyanins more abundant than the *p*-coumarylated forms in Prieto Picudo base wines (10.4 versus 4.4%) but the reverse was found in both Garnacha wines (2.4 versus 4.4%). Malvidin-3-glucoside was the main anthocyanin in all wines and comprised ~70% of total anthocyanins; hence, its derivatives were also the main acetylated and coumarylated forms. Anthocyanins were drastically reduced during two stages of wine production: the cold stabilization and clarification of the base wines and the first six months of aging in contact with lees (Figure 1). Decreases during clarification–stabilization were attributed to the cold treatment and to the adsorption of phenolic material by the bentonite and PVPP; decreases during aging were attributed to the adsorption of these compounds to the cell walls of lees (Vasserot et al. 1997, Mazauric and Salmon 2006) or to their combination with other wine compounds.

Table 1 Standard enological parameters in musts.

	Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
Brix	20.4	19.5	20.2	20.1	18.9	21.0	19.7	20.1
pH	3.0	3.3	2.9	3.1	3.6	3.1	3.1	3.1
TA[a]	12.0	7.6	9.3	7.5	8.0	9.6	9.0	10.5
Malic acid (g/L)	4.59	3.16	3.87	3.79	3.38	4.24	4.26	4.65
Tartaric acid (g/L)	9.14	7.20	8.45	6.40	6.38	7.71	7.34	8.18
Potassium (mg/L)	1210	1400	1510	1300	1420	1380	1340	1740

[a]Titratable acidity as g tartaric acid equivalents/L.

Table 2 Standard enological parameters in wines during different stages of sparkling wine production: base wines (BW), base wines after clarification and stabilization (CBW), sparkling wines after 3 months (T3M), 6 months (T6M), and 9 months (T9M) of aging on yeast lees.

Parameter[a]	Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
BW								
pH	2.79	2.95	2.84	2.99	2.93	3.03	2.98	3.08
TA	9.5	8.2	7.6	8.0	8.2	7.5	8.7	8.5
SO_2 (mg/L)	56	53	64	65	56	63	58	30
Alcohol	11.1	10.7	11.7	10.6	10.3	11.8	11.1	11.5
VA	0.28	0.16	0.31	0.27	0.21	0.18	0.14	0.17
A420	0.0515	0.0453	0.0503	0.0535	0.0535	-	-	-
CI	-	-	-	-	-	0.59	0.53	0.68
Hue	-	-	-	-	-	0.520	0.517	0.668
CBW								
pH	2.72	2.92	2.85	3.08	2.95	2.89	3.01	3.09
TA	7.1	7.5	7.1	7.2	7.3	6.7	7.2	7.3
SO_2 (mg/L)	53	58	61	40	46	56	57	31
Alcohol	11.2	10.6	11.8	10.3	10.3	11.7	11.0	11.4
VA	0.26	0.18	0.27	0.24	0.25	0.18	0.14	0.17
A420	0.0445	0.0290	0.0285	0.0315	0.0440	-	-	-
CI	-	-	-	-	-	0.23	0.18	0.46
Hue	-	-	-	-	-	0.870	0.890	0.800
T3M								
pH	2.84	2.95	2.87	3.05	2.93	2.90	2.92	3.02
TA	7.0	7.4	7.0	7.2	7.2	6.8	7.3	7.2
SO_2 (mg/L)	48	50	50	38	40	49	52	30
Alcohol	12.0	11.7	12.3	11.5	11.2	12.4	12.1	12.6
VA	0.47	0.27	0.35	0.32	0.36	0.29	0.24	0.34
A420	0.0360	0.0238	0.0248	0.0293	0.0413	-	-	-
CI	-	-	-	-	-	0.18	0.14	0.29
Hue	-	-	-	-	-	0.891	0.854	0.893
T6M								
pH	2.87	2.95	2.85	3.05	2.97	2.91	2.95	3.02
TA	7.1	7.4	7.1	7.0	7.0	6.7	7.3	7.0
SO_2 (mg/L)	27	39	41	29	22	42	38	17
Alcohol	12.0	11.5	12.0	11.5	11.3	12.5	12.0	12.5
VA	0.44	0.22	0.33	0.34	0.35	0.31	0.26	0.34
A420	0.0368	0.0268	0.0263	0.0327	0.0422	-	-	-
CI	-	-	-	-	-	0.17	0.15	0.28
Hue	-	-	-	-	-	0.862	0.853	0.935
T9M								
pH	2.76	2.89	2.82	3.10	2.94	2.85	2.94	3.02
TA	7.2	7.4	7.2	7.4	7.4	6.9	7.4	7.1
SO_2 (mg/L)	35	43	43	31	33	44	41	21
Alcohol	12.2	11.6	12.2	11.6	11.6	12.3	11.9	12.5
VA	0.39	0.24	0.32	0.28	0.30	0.25	0.20	0.29
A420	0.0297	0.0230	0.0207	0.0260	0.0355	-	-	-
CI	-	-	-	-	-	0.14	0.11	0.24
Hue	-	-	-	-	-	0.974	0.880	1.042

[a]TA: titratable acidity as g of tartaric acid equivalents/L. Alcohol: % ethanol by volume at 20°C. VA: volatile acidity as g acetic acid/L. A420: absorbance at 420 nm. CI: color intensity as sum of absorbances at 420, 520, and 620 nm. Hue: A420/A520.

These decreases are consistent with results previously observed for CI values. It is important to point out two phenomena. First, the greatest losses in anthocyanins occurred in the Garnacha wines during clarification. This result and the lower CI in the final Garnacha wines seem to indicate that Prieto Picudo was more stable for color and anthocyanins. Second, total anthocyanins decreased in both Garnacha and Prieto Picudo during the first six months of aging on yeast lees but slightly increased during the last three months of aging in Prieto Picudo. As wine aging progresses, there is a slow autolytic process of yeast (Alexandre and Guilloux-Benatier 2006, Buxaderas and López-Tamames 2010), and phenolics adsorbed in the first stages of aging are released. The final Prieto Picudo sparkling wines had more anthocyanins than the two Garnacha wines, although all three wines had considerably more anthocyanins than those previously reported for a rosé Garnacha sparkling wine (Pozo-Bayón et al. 2004).

Hydroxybenzoic and hydroxycinnamic acids. The concentrations of total and individual hydroxycinnamic acids during different stages of sparkling wine production are shown (Figure 2). Compounds not shown were detected at very low concentrations (<2.4 mg/L gallic acid, <1.2 mg/L caffeic acid, <0.63 mg/L coumaric acid, and <0.5 mg/L ferulic acid). These results agree with those found in other white and rosé sparkling wines (Ibern-Gómez et al. 2000, Chamkha et al. 2003, Pozo-Bayón et al. 2003).

The Garnacha base wines had the most total hydroxycinnamic acids, followed by Albarín and Viura, and lastly by Malvasía, Verdejo, Godello, and Prieto Picudo. Esterified hydroxycinnamic acids were the predominant acids in all wines, representing over 95% of total hydroxycinnamic acids, with esters of caffeic acid the most abundant. Hydroxycinnamic acid composition and concentration were independent of grape color and of whether prefermentation maceration was conducted. Thus, Garnacha rosé base wines contained the most hydroxycinnamic acids of all forms, while Prieto Picudo rosé wines were comparable to the white wines. *trans*-Caftaric acid was the most abundant hydroxycinnamic found in Albarín, Viura, Godello, Malvasía, and both Garnacha wines, but the *cis*-isomer was more abundant in Verdejo and Prieto Picudo. With the exception of Viura, stabilization and clarification of the white base wines reduced total hydroxycinnamic acids, both in the free and esterified forms. In contrast, and unlike monomeric anthocyanins, stabilization and clarification of rosé base wines did not affect the concentration of hydroxycinnamic acids.

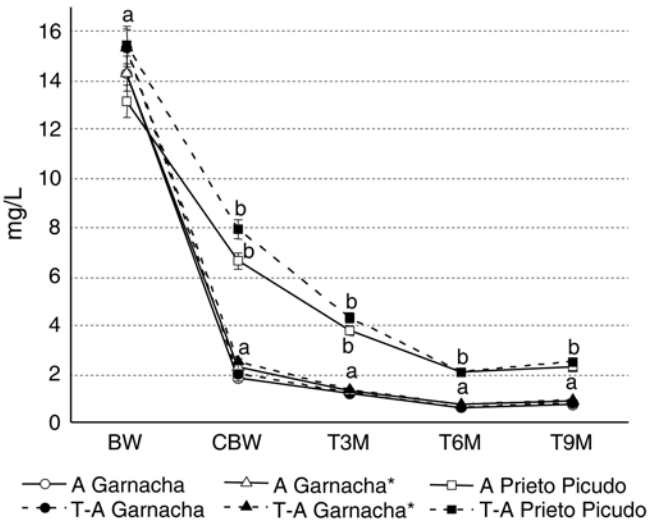

Figure 1 Concentration (mg/L) of total anthocyanins (T-A) and nonacylated anthocyanins (A) during different stages of sparkling wine production: base wines (BW), base wines after clarification and stabilization (CBW), and sparkling wines after 3 months (T3M), 6 months (T6M), and 9 months (T9M) of aging on yeast lees. Values are means ± SD (n = 3). Different letters for the same compound at the same vinification stage represent means significantly different at $p < 0.05$.

Secondary fermentation produced important differences among wines in the evolution of total hydroxycinnamic acids, due to differences in their esterified forms. Total hydroxycinnamic acids decreased during the first three months of aging in all wines, especially in varieties with the highest concentrations of these compounds in the clarified base wine (CBW). Both Garnacha wines, with initial concentrations of 90 mg/L, decreased by ~20%, while Viura, Albarín, and Malvasía (initial concentrations between 15 and 30 mg/L) decreased ~15%. Total hydroxycinnamic acids decreased <10% in Godello and Verdejo, which had the lowest concentrations of these compounds. This trend continued during the next three months of aging: total acids decreased ~45% in Garnacha wines, which had the highest concentrations, but remained stable in the other wines. In the last three months of aging, total hydroxycinnamic acids increased by 70% in both Garnacha wines, but again remained stable in the other wines. Thus, the evolution of the hydroxycinnamic acids seemed to be directly related to their concentration rather than to any other factor.

During the first months of aging on yeast lees, there was loss of hydroxycinnamic acid esters due to their interaction with other wine compounds to give more stable pigments (García-Falcón et al. 2007) and/or because they can be adsorbed by yeast lees (Mazauric and Salmon 2005). The higher the initial concentrations of hydroxycinnamic acids in the base wines, the more adsorption or transformation occurred. Adsorbed hydroxycinnamic acids released as yeast cell walls were degraded during the autolytic process, but only in wines with high concentrations of these compounds. In the final sparkling wines, Garnacha wines had by far the highest concentrations of all hydroxycinnamic acids. With the exception of both Garnachas, all wines had similar hydroxycinnamic acid concentrations as those reported for Chardonnay, Pinot noir, Macabeo, Parellade, and Xarel.lo white sparkling wines and higher concentrations than Trepat or Monastrell white sparkling wines (Ibern-Gómez et al. 2000, Chamkha et al. 2003, Pozo-Bayón et al. 2003). As in the base wines, *trans*-caftaric acid was the most abundant hydroxycinnamic acid in the finished Albarín, Viura, Godello, Malvasía, and Garnacha sparkling wines, while the *cis*- form was the most abundant in Verdejo and Prieto Picudo. These results contrast with other studies in which the *trans*-caftaric form was the most abundant in all wines (Ibern-Gómez et al. 2000, Chamkha et al. 2003, Pozo-Bayón et al. 2003).

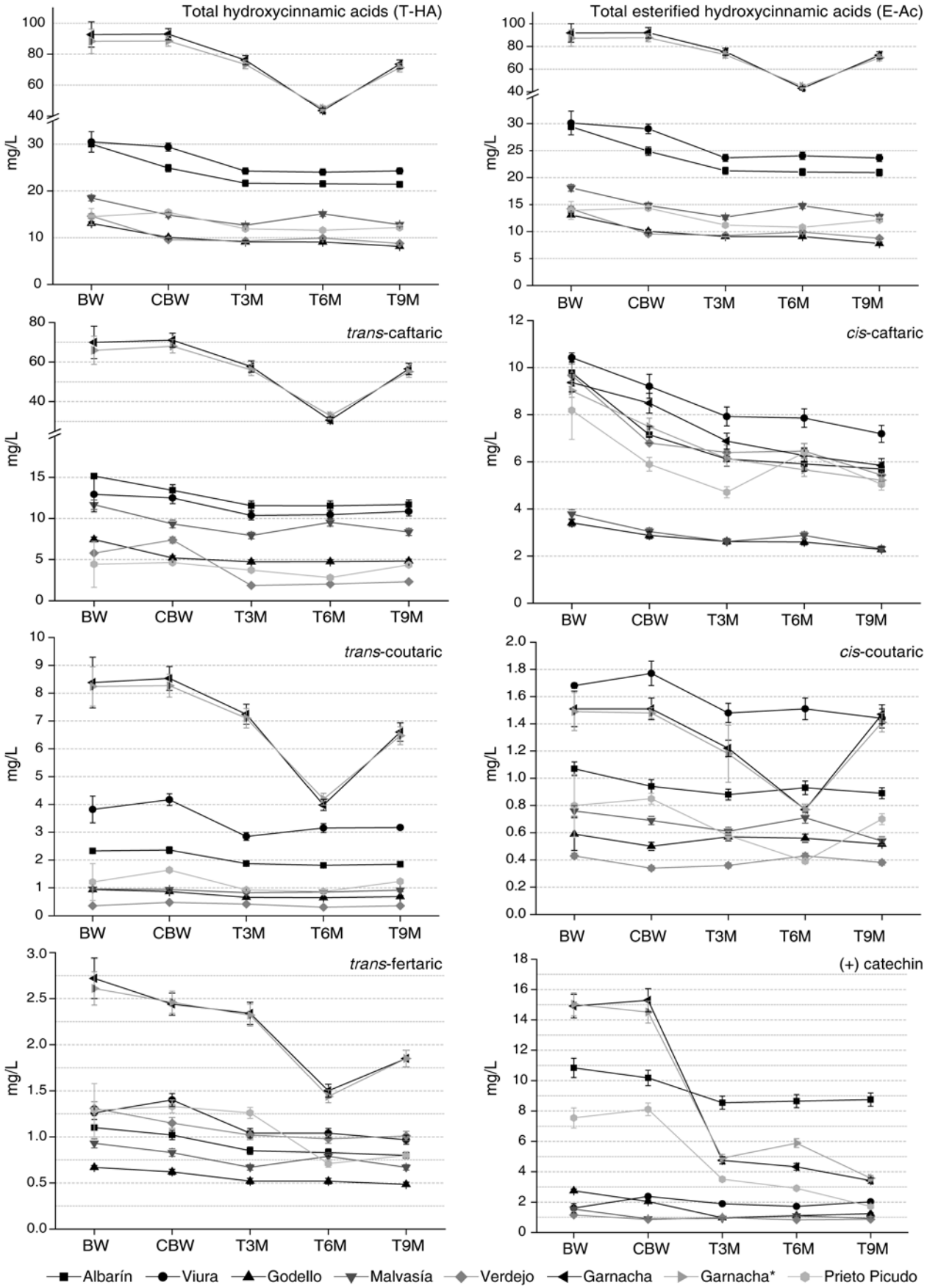

Figure 2 Concentration (mg/L) of total hydroxycinnamic acids, hydroxycinnamates, and catechin in wines during different stages of sparkling wine production: base wines (BW), base wines after clarification and cold stabilization (CBW), and sparkling wines after 3 months (T3M), 6 months (T6M), and 9 months (T9M) of aging on yeast lees. Values are means ± SD (n = 3).

Flavonols and flavan-3-ols. Total flavonols in both white and rosé base wines was <2.5 mg/L. In the final sparkling wines, total flavonols could only be quantified in Garnacha, Garnacha*, and Prieto Picudo wines, with 2.14, 1.73, and 1.08 mg/L, respectively. The only flavan-3-ol detected in the wines within the quantification limits was (+) catechin (Figure 2). Among base wines, the Albarín and both the Garnachas had the most catechin, followed distantly by Prieto Picudo, and then by Viura, Godello, Malvasía, and Verdejo. Contrary to the other phenolics, clarification and stabilization did not affect catechin concentration. Secondary fermentation reduced catechin in the wines that started with the highest concentrations of this compound, likely due the conversion of monomeric flavan-3-ols into more stable polymers by reacting with other flavanols, anthocyanins, and small molecules such as pyruvic acid and vinylphenol. The average concentrations of catechin in the final Viura, Godello, Malvasía, and Verdejo white sparkling wines was within the range reported by other authors in *blanc de blanc* Chardonnay, Macabeo, Parellada, and Xarel.lo, *blanc de noir* Pinot noir, Trepat, and Monastrell, and rosé Garnacha sparkling wines (Chamkha et al. 2003, Pozo-Bayón et al. 2003). However, the concentrations in the Albarín white sparkling wines and in the Prieto Picudo and the two Garnacha rosé sparkling wines was considerably higher.

Proanthocyanidins. Changes occurred in the concentrations of total proanthocyanidins (PA), in the percentages of catechin, epicatechin, and epicatechin-gallate terminal subunits, and in the mean degree of polymerization (mDP) during sparkling winemaking (Table 3). As with catechin, total PA was considerably higher in Albarín and Garnacha base wines than in the other base wines, showing that it is not affected by grape color or prefermentation maceration. The mDP, which helps determine the astringent and bitter character of PA (Vidal et al. 2003), was similar in all base wines. With the exception of Malvasía, the terminal units were primarily comprised of catechin in all base wines, with epicatechin and epicatechin-gallate found in lower quantities. Catechin is the primary terminal subunit in the grape skin, while epicatechin and epicatechin-gallate are found in much lower quantities (Monagas et al. 2003), suggesting that more PA was contributed from grape skins than from grape seeds.

Table 3 Proanthocyanidin concentration (mg/L), % catechin, % epicatechin, % epicatechin-gallate terminal subunits, and mean degree of polymerization (mDP) in wines during different stages of sparkling wine production: base wines (BW), base wines after clarification and stabilization (CBW), and sparkling wines after 3 months (T3M), 6 months (T6M), and 9 months (T9M) of aging on yeast lees.

Parameter[a]	Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
BW								
PA	91.02 ± 10.00 b[b]	61.01 ± 3.70 a	70.86 ± 9.46 ab	48.31 ± 1.96 a	53.01 ± 1.64 a	89.48 ± 10.33 b	90.31 ± 10.33 b	51.92 ± 13.90 a
Cat	8.96 ± 2.51 ab	10.77 ± 2.46 ab	10.13 ± 3.14 ab	5.52 ± 2.57 a	12.45 ± 0.89 b	7.29 ± 0.87 ab	7.01 ± 0.65 ab	9.69 ± 3.97 ab
Epi	3.87 ± 0.95 a	6.06 ± 0.90 a	5.22 ± 1.45 a	7.57 ± 0.79 a	6.68 ± 0.45 a	3.18 ± 1.93 a	3.22 ± 1.45 a	7.53 ± 5.05 a
Epi-gal	3.61 ± 0.82 ab	nd	1.43 ± 1.70 a	nd	7.38 ± 5.68 ab	3.82 ± 1.17 ab	3.93 ± 1.36 ab	9.50 ± 3.99 b
mDP	3.75 ± 2.48 a	9.41 ± 1.84 a	4.91 ± 3.42 a	7.04 ± 1.74 a	4.31 ± 1.32 a	5.51 ± 1.61 a	5.82 ± 1.47 a	5.24 ± 2.36 a
CBW								
PA	65.46 ± 0.75 f	40.47 ± 0.28 c	43.84 ± 0.88 a	46.12 ± 0.11 b	43.07 ± 0.21 a	48.77 ± 0.70 d	57.13 ± 0.91 e	46.35 ± 0.79 b
Cat	3.56 ± 0.08 c	4.23 ± 0.12 a	4.26 ± 0.26 a	3.79 ± 0.07 cd	4.88 ± 0.04 e	2.35 ± 0.14 b	2.37 ± 0.04 b	3.94 ± 0.13 ad
Epi	4.33 ± 0.26 a	7.83 ± 0.15 f	6.75 ± 0.26 e	4.97 ± 0.07 bc	6.48 ± 0.13 de	5.29 ± 0.25 c	4.63 ± 0.18 ab	6.09 ± 0.13 d
Epi-gal	9.77 ± 0.46 bc	11.39 ± 0.50 d	8.53 ± 0.48 a	13.54 ± 0.05 e	8.97 ± 0.27 ab	8.97 ± 0.42 ab	8.47 ± 0.28 a	10.65 ± 0.44 cd
mDP	5.26 ± 0.25 a	5.22 ± 0.15 a	6.50 ± 0.26 bc	5.47 ± 0.03 a	5.21 ± 0.11 a	6.22 ± 0.25 bc	6.69 ± 0.19 c	6.04 ± 0.18 b
T3M								
PA	42.88 ± 1.02 b	38.26 ± 1.01 c	43.04 ± 0.94 b	48.67 ± 0.61 a	48.64 ± 1.21 a	48.17 ± 1.13 a	42.08 ± 1.01 b	46.16 ± 1.16 a
Cat	3.58 ± 0.18 b	5.78 ± 0.75 d	3.65 ± 0.22 b	2.74 ± 0.12 ac	3.33 ± 0.30 ab	2.07 ± 0.15 c	2.66 ± 0.28 ac	2.94 ± 0.15 ab
Epi	1.84 ± 0.27 a	2.63 ± 0.29 b	1.56 ± 0.04 a	2.59 ± 0.26 b	3.47 ± 0.38 c	1.70 ± 0.18 a	1.84 ± 0.05 a	2.64 ± 0.11 b
Epi-gal	12.02 ± 1.08 ac	9.49 ± 0.95 ab	14.56 ± 1.19 c	9.58 ± 0.40 ab	4.67 ± 0.53 d	12.06 ± 1.65 ac	10.93 ± 1.07 a	7.67 ± 0.79 b
mDP	6.70 ± 0.49 ac	6.89 ± 0.52 abc	5.74 ± 0.40 c	8.14 ± 0.29 bd	11.92 ± 0.86 e	7.23 ± 0.83 ab	7.50 ± 0.60 ab	9.44 ± 0.70 d
T6M								
PA	29.19 ± 1.82 a	41.01 ± 4.37 c	30.83 ± 1.78 ab	28.17 ± 1.85 a	39.12 ± 1.53 bc	27.14 ± 0.90 a	28.92 ± 1.12 a	27.54 ± 2.23 a
Cat	3.94 ± 0.54 a	14.34 ± 6.33 b	3.71 ± 1.06 a	7.41 ± 1.18 a	4.98 ± 1.53 a	4.33 ± 0.40 a	4.33 ± 1.10 a	4.05 ± 0.8 a
Epi	6.55 ± 4.51 a	9.10 ± 3.79 ab	12.52 ± 0.78 bc	7.06 ± 0.68 a	6.94 ± 0.66 a	11.46 ± 0.55 abc	16.45 ± 1.20 c	6.55 ± 1.02 a
Epi-gal	18.98 ± 3.38 b	25.85 ± 6.34 c	4.10 ± 0.88 a	19.99 ± 4.75 b	19.14 ± 1.14 b	6.01 ± 0.52 a	6.55 ± 0.28 a	6.60 ± 0.68 a
mDP	4.02 ± 0.71 a	2.32 ± 0.48 c	7.07 ± 0.68 b	3.44 ± 0.60 ac	3.88 ± 0.25 a	6.18 ± 0.34 bd	4.89 ± 0.32 ad	7.67 ± 0.80 b
T9M								
PA	53.13 ± 3.44 c	43.04 ± 1.38 ad	35.21 ± 1.52 b	35.60 ± 1.30 b	47.40 ± 1.68 ac	37.19 ± 1.50 bd	45.97 ± 2.12 a	46.84 ± 2.27 ac
Cat	4.94 ± 0.86 b	11.95 ± 0.67 c	8.93 ± 2.10 a	8.53 ± 1.01 a	4.78 ± 0.27 b	8.11 ± 0.39 a	7.08 ± 1.53 ab	6.91 ± 0.42 ab
Epi	5.23 ± 0.47 a	6.03 ± 0.34 a	13.33 ± 0.81 c	5.21 ± 0.21 a	4.42 ± 0.58 a	10.38 ± 1.73 b	3.82 ± 0.60 a	4.15 ± 0.63 a
Epi-gal	13.17 ± 1.21 ab	17.84 ± 0.46 e	10.59 ± 0.57 c	14.90 ± 2.20 bd	12.58 ± 1.60 abc	11.05 ± 0.87 ac	15.71 ± 0.90 de	13.57 ± 0.74 abd
mDP	5.36 ± 0.49 cd	3.43 ± 0.12 e	3.97 ± 0.29 ae	4.25 ± 0.44 a	5.73 ± 0.53 d	4.41 ± 0.32 ab	4.60 ± 0.33 abc	5.05 ± 0.31 bcd

[a]PA: total proanthocyanidins content (mg/L); Cat: % catechin terminal subunits; Epi: % epicatechin terminal subunits; Epi-gal: % epicatechin-gallate terminal subunits.

[b]Values are means ± SD (n = 3). Different letters in the same row indicate that means significantly differ at $p < 0.05$. nd: below detection limit.

Clarification and stabilization significantly reduced PA in all base wines, particularly in Garnacha. The decreases were attributed to cold stabilization because tannins were associated with the tartrate precipitates, as reported in previous studies (Vernhet et al. 1999). The mDP was maintained during clarification–stabilization, but this stage clearly modified the percentage of terminal subunits; hence, epicatechin-gallate was the major terminal subunit in all wines after clarification–stabilization. As with other phenolics, two different phases were distinguished during secondary fermentation. The concentration of PA decreased in all wines during the first six months of aging, from 9% in Verdejo to 55% in Albarín, then increased during the last three months, from 5% in Viura to 82% in Albarín. Proanthocyanidins are highly reactive compounds involved in condensation and polymerization reactions as well as precipitations (Revilla and González-SanJosé 2003). Tannins are also adsorbed on lees in preference to monomeric phenols, even with low quantities of lees (Mazauric and Salmon 2005). The PA increase during the final months of aging was attributed to the release of adsorbed polyphenol during autolysis of the yeast cell walls. The percentages of catechin, epicatechin, and epicatechin-gallate terminal subunits showed different trends during aging on lees, but with the exception of Godello, epicatechin-gallate remained the predominant subunit in all wines. Although there are no studies on sparkling wines, these results contrasted with research findings on still wines, where concentrations of gallates as terminal units are usually low or even undetectable (Monagas et al. 2003, Fernández et al. 2007). In general, unlike with monomeric polyphenols, the secondary fermentation reduced the differences in PA concentrations among the initial base wines. Consequently, all final sparkling wines contained similar concentrations of PA and similar to those reported in white Portuguese sparkling wines (Jordão et al. 2010).

Total phenolics. Total phenolics were calculated as the sum of total anthocyanins, hydroxycinnamic acids, flavonols, catechin, and proanthocyanidins (Table 4). Garnacha base wines had more total phenols than Prieto Picudo. Albarín

Table 4 Concentration (mg/L) of total phenolics and percentage of total anthocyanins (T-A), total hydroxycinnamic acids (T-HA), total flavonols (T-Flavo), catechin, and proanthocyanidins (PA) during the different stages of sparkling wine production: base wines (BW), base wines after clarification and cold stabilization (CBW), and sparkling wines after 3 months (T3M), 6 months (T6M), and 9 months (T9M) of aging on yeast lees.

	Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
BW								
Total phenolics (mg/L)	133 ± 10 c[a]	94 ± 4 a	86 ± 9 a	70 ± 2 a	71 ± 2 a	214 ± 13 b	212 ± 13 b	90 ± 14 a
T-A (%)	-	-	-	-	-	7.1	7.3	17.1
T-HA (%)	22.5	32.3	15.1	26.3	20.9	43.2	41.8	16.1
T-Flavo (%)	1.1	1.4	nq	2.6	1.8	1.1	1.2	0.9
Catechin (%)	8.1	1.7	3.2	2.2	1.6	6.9	7.1	8.4
PA (%)	68.3	64.6	81.7	68.9	75.7	41.7	42.7	57.5
CBW								
Total phenolics (mg/L)	102 ± 1 e	73.5 ± 0.9 b	55.9 ± 0.9 a	63.1 ± 0.5 d	53.5 ± 0.4 a	161 ± 4 c	166 ± 4 c	78 ± 1 b
T-A (%)	-	-	-	-	-	1.2	1.5	10.2
T-HA (%)	24.4	40.0	18.0	23.5	17.9	57.7	53.6	19.8
T-Flavo (%)	1.3	1.7	nq	2.0	nq	1.3	1.5	nq
Catechin (%)	10.0	3.2	3.7	1.4	1.6	9.5	8.8	10.4
PA (%)	64.3	55.1	78.4	73.1	80.5	30.3	34.6	59.6
T3M								
Total phenolics (mg/L)	73 ± 1 d	64 ± 1 a	53 ± 1 c	62.3 ± 0.7 ab	59 ± 1 b	131 ± 3 f	122 ± 3 e	66 ± 1 a
T-A (%)	-	-	-	-	-	0.9	1.1	6.5
T-HA (%)	29.6	37.7	17.1	20.3	15.8	57.6	59.5	18.0
T-Flavo (%)	nq	nq	nq	nq	nq	1.4	1.4	nq
Catechin (%)	11.7	2.9	1.8	1.5	1.7	3.6	4.0	5.3
PA (%)	58.7	59.4	81.1	78.2	82.6	36.4	34.1	70.1
T6M								
Total phenolics (mg/L)	59 ± 2 d	67 ± 4 e	41 ± 2 a	44 ± 2 ab	50 ± 2 b	75 ± 2 c	80 ± 2 c	44 ± 2 ab
T-A (%)	-	-	-	-	-	0.8	0.9	4.7
T-HA (%)	36.3	36.0	22.1	34.0	19.9	57.6	55.8	26.2
T-Flavo (%)	nq	nq	nq	nq	nq	nq	nq	nq
Catechin (%)	14.6	2.6	2.7	2.5	1.7	5.7	7.3	6.6
PA (%)	49.2	61.4	75.2	63.5	78.5	35.9	35.9	62.5
T9M								
Total phenolics (mg/L)	83 ± 3 e	69 ± 1 b	45 ± 1 a	49 ± 1 a	57 ± 2 d	117 ± 3 c	122 ± 3 c	64 ± 2 b
T-A (%)	-	-	-	-	-	0.7	0.7	3.9
T-HA (%)	25.7	35.0	18.2	25.9	15.4	62.8	57.7	18.9
T-Flavo (%)	nq	nq	nq	nq	nq	1.8	1.4	1.7
Catechin (%)	10.5	2.9	2.8	1.9	1.5	2.9	2.9	2.7
PA (%)	63.8	62.1	79.0	72.2	83.1	31.8	37.2	72.9

[a]Values are means ± SD (n = 3). Different letters in the same row indicate that means significantly differ at $p < 0.05$. nq: compound below the quantification limit.

base wines had the most phenols among the white wines. Final sparkling wines after 9 months of aging had compositions and proportions of phenols that were similar to the base wines. Finished Garnacha rosé wines again had the most total polyphenols, followed by Albarín. With the exception of the Garnacha wines, PA represented >62% of total phenols in all the wines, followed by hydroxycinnamic acids (15 to 35%), catechin (2 to 10%), and other phenols (<2%). In Garnacha wines, hydroxycinnamic acid concentration was considerably higher than PA concentration.

Sensory analysis. After 9 months of aging, sparkling wines were presented to a panel of expert tasters for sensory profiling. Within-judge reproducibility was evaluated using two replicated sparkling wines in the tasting sessions; replications were not a source of variation. White sparkling wines had no significant differences in color intensity or brown tones, but Albarín and Verdejo had significantly more intense green and yellow tones than the other white sparkling wines (Table 5). These results are consistent with the enological measurements, as Albarín and Verdejo sparkling wines had higher absorbance at 420 nm than the other white wines. Among rosé sparkling wines, Prieto Picudo had more intense overall color and red tones than Garnacha. These results correlated with the higher color intensity and anthocyanin concentration in Prieto Picudo sparkling wines. Rosé sparkling wines did not show significant differences in orange tones.

Generalized Procrustes analysis (GPA) provides a consensus configuration of the gustatory, olfactory, and foam quality relationships among the wines (Figure 3). GPA was applied to sensory data to ascertain consistency among the 12 judges and provide information on relationships among sparkling wines and attributes. The gustatory space did not clearly differentiate between white and rosé sparkling wines (Figure 3A). Albarín and Godello were characterized by an overall good equilibrium, full body, and persistence. Prieto Picudo and Verdejo were clearly associated with bitterness and freshness, while neither Garnacha nor Malvasía emphasized any particular sensory descriptor. Among white sparkling wines, Albarín scored the highest in astringency (2.0), bitterness (3.1), and full body (4.3). Although generally it is not easy to relate sensory results with chemical data, these sensory values are consistent with the high concentration of PA in Albarín sparkling wines. In the rosé sparkling wines, the Garnachas and Prieto Picudo

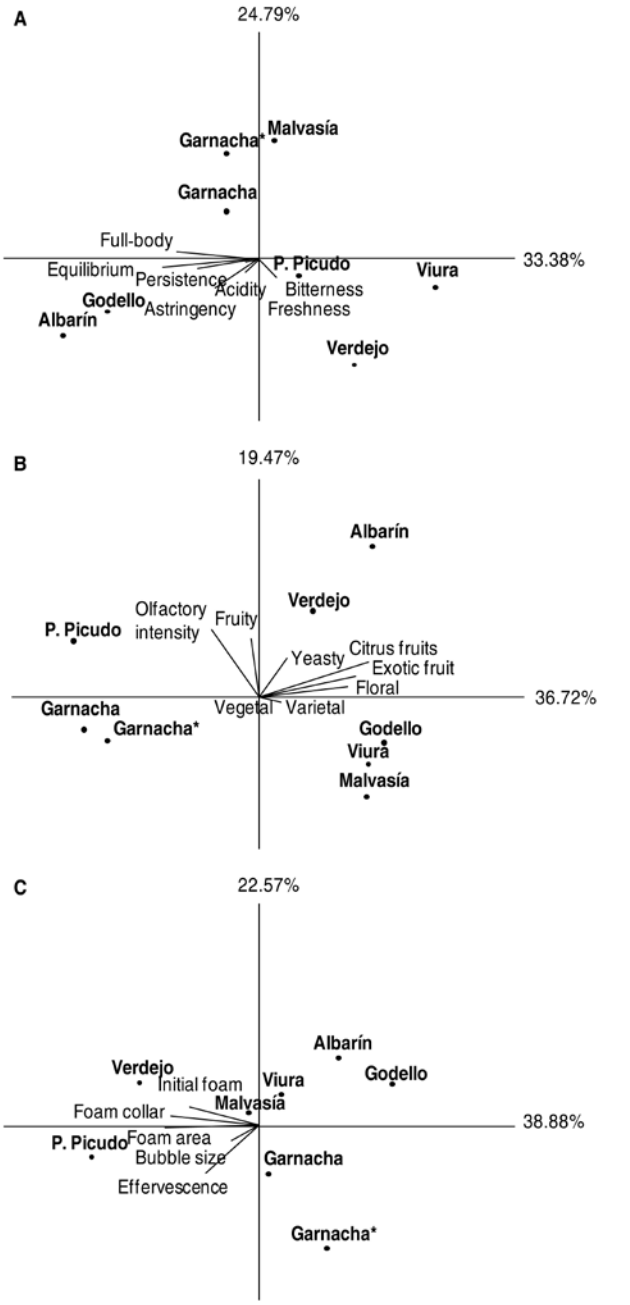

Figure 3 Generalized procrustes analysis of the mean ratings for (**A**) gustatory, (**B**) olfactory, and (**C**) foam quality attributes in the final sparkling wines at 9 months (T9M).

Table 5 Sensory evaluation of color intensity and hue (tone) in the final sparkling wines at 9 months (T9M).

	Visual color intensity	Yellow	Green	Brown	Red	Orange
Albarín	4.3 ± 0.8 a[a]	4.2 ± 0.5 a	2.4 ± 0.5 a	1.2 ± 0.4 a	-	-
Viura	4.0 ± 0.8 a	3.3 ± 0.4 b	1.8 ± 0.6 b	1.0 ± 0.0 a	-	-
Godello	3.9 ± 0.6 a	3.4 ± 0.5 b	1.9 ± 0.9 abc	1.0 ± 0.0 a	-	-
Malvasía	3.8 ± 0.8 a	3.3 ± 0.5 b	1.5 ± 0.5 b	1.2 ± 0.4 a	-	-
Verdejo	4.3 ± 0.6 a	4.3 ± 0.4 a	2.4 ± 0.8 ac	1.2 ± 0.4 a	-	-
Garnacha	3.7 ± 0.5 b	-	-	-	2.6 ± 0.5 b	1.2 ± 0.4 a
Garnacha*	3.6 ± 0.5 b	-			2.7 ± 0.9 b	1.3 ± 0.5 a
Prieto Picudo	4.5 ± 0.8 a	-	-	-	4.0 ± 0.7 a	1.4 ± 0.5 a

[a]Values are means ± SD (12 tasters). Different letters in the same column indicate that means significantly differ at $p < 0.05$. Statistical analysis was performed separately for white and rosé sparkling wines.

had no significant differences in astringency (~2.1), bitterness (~2.5), or full body sensations (~4.1), consistent with their PA concentrations and mDP, which were similar. For olfactory descriptors (Figure 3B), the consensus plot showed a clearly different distribution of rosé and white sparkling wines. Both Garnacha wines were perceived by the tasters as very similar, as were Godello, Viura, and Malvasía. Albarín and Verdejo were located quite close to each other in the GPA space. Prieto Picudo was characterized by a higher olfactory intensity, dominated by fruity aromas, than both Garnacha wines. All white sparkling wines were characterized by citrus, exotic fruit, and floral aromas. Albarín and Verdejo had higher olfactory intensity and a higher correlation with yeasty and fruity aromas. Finally, a clear differentiation among samples was observed in foam quality (Figure 3C). Among white sparkling wines, Verdejo had superior foam quality, followed by Viura and Malvasía. Among rosé sparkling wines, Prieto Picudo had better foam quality than either of the Garnacha wines.

In order to judge more precisely the olfactory attributes of these wines, the volatile composition should be analyzed. Moreover, it would be helpful to conduct a study on consumer preferences.

Conclusions

This paper evaluates the chemical and sensory profile of sparkling wines made with *Vitis vinifera* cv. Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha and Prieto Picudo varieties, with emphasis on changes in phenolic compounds during winemaking and aging. The composition and concentrations of hydroxycinnamic acids, flavonols, and proanthocyanidins in base wines was independent of whether the grapes were red or white and of whether prefermentation maceration was used. The stabilization–clarification of base wines and the first six months of aging in contact with lees drastically reduced the concentrations of all polyphenols in all wines, but these losses were partially reversed during the last months of aging as some of the phenolics were released back into the wine. The chemical composition of the base wines indicated that all the grape varieties studied were suitable for sparkling wine production. Prieto Picudo rosé sparkling wines had greater color intensity, anthocyanin concentrations, and color stability than Garnacha wines, which had more hydroxycinnamic acids. Among whites, Albarín had the most catechin, proanthocyanidins, and, together with Viura, hydroxycinnamates. Garnacha rosé and Albarín white wines had the highest phenolic potentials, but Prieto Picudo, Albarín, and Verdejo were judged superior in sensory profiling. Prieto Picudo, Albarín, and Verdejo were the most promising varieties tested for production of high-quality sparkling wines, increasing the enological potential of these varieties that have been traditionally used for still wines.

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4.2

Análisis multivariante para la diferenciación de vinos espumosos elaborados con variedades de uva autóctonas españolas: compuestos volátiles, aminoácidos y aminas biógenas

Multivariate analysis for the differentiation of sparkling wines elaborated from autochthonous Spanish grape varieties: volatile compounds, amino acids and biogenic amines

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Resumen

Este artículo aborda tres objetivos fundamentales:

1. Caracterizar enológicamente los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional, en términos de compuestos volátiles, aminoácidos y aminas biógenas durante la vinificación y el envejecimiento sobre lías en botella.
2. Evaluar los cambios que se producen en dichos parámetros durante el proceso de elaboración de los vinos espumosos, incluyendo las etapas de vino base y de envejecimiento sobre lías en botella durante nueve meses.
3. Analizar sensorialmente la fase aromática de los vinos espumosos obtenidos después de nueve meses de envejecimiento sobre lías en botella, tiempo mínimo necesario para denominarse vinos espumosos.

El estudio se realizó con vinos espumosos de la cosecha 2009. Se aplicaron técnicas de análisis multivariante con objeto de establecer diferencias entre los vinos espumosos en función de la variedad de uva empleada en su elaboración y el tiempo de envejecimiento sobre lías en botella.

El análisis factorial permitió diferenciar los vinos varietalmente y por el tiempo de envejecimiento sobre lías en función de su composición volátil y aminoacídica. Los vinos base y los vinos espumosos de las variedades de uva Albarín, Verdejo, Godello y Prieto Picudo fueron los más ricos en aminoácidos y en la mayoría de los compuestos volátiles analizados, especialmente de ésteres etílicos y alcohol acetatos, compuestos que contribuyen a los aromas frutales de los vinos. Durante la crianza sobre lías se observó un aumento de los ésteres etílicos de ácidos grasos ramificados, de lactato de etilo y de γ–butirolactona y un descenso de los terpenos, principalmente citronelol y geraniol.

El análisis discriminante por pasos permitió determinar aquellos compuestos más útiles para diferenciar los vinos espumosos en función de la variedad de uva y del tiempo de envejecimiento sobre lías. Al realizar el análisis discriminante por pasos considerando el factor variedad, la glicina, el *trans*-3-hexenol, la acetovanillona y la β-alanina fueron los compuestos con mayor poder discriminante. Por otro lado, al realizar el análisis discriminante por pasos en función del tiempo de crianza, la γ-butirolactona, el decanoato de etilo, el ácido decanoico, la histidina y la treonina fueron los principales responsables de dicha discriminación.

En el análisis sensorial a nivel olfativo, los vinos espumosos de Albarín y de Godello presentaron una mayor intensidad aromática que los vinos espumosos de Viura y de Malvasía, los cuales se caracterizaron por aromas florales y varietales. Los vinos espumosos de Godello se caracterizaron por notas cítricas, de frutas exóticas y vegetales y los de Verdejo por aromas a levadura. Entre los vinos rosados espumosos, los elaborados con la variedad Prieto Picudo presentaron mayor intensidad aromática, con notas frutales y varietales, que los obtenidos con la variedad Garnacha.

Los vinos base y vinos espumosos de Albarín y de Prieto Picudo mostraron el mayor contenido de aminas biógenas, siendo la putrescina la amina biógena mayoritaria. El contenido de aminas biógenas permaneció constante durante los primeros tres meses de envejecimiento sobre lías, observándose un incremento de concentración en los siguientes tres meses de envejecimiento. Los niveles de aminas biógenas en los vinos fueron inferiores a los límites considerados como un riesgo para la salud.

Los resultados obtenidos indicaron que las variedades Albarín, Verdejo, Godello y Prieto Picudo fueron las más apropiadas para la elaboración de vinos espumosos de calidad, pudiéndose incrementar el potencial enológico de estas variedades tradicionalmente empleadas para la elaboración de vinos tranquilos.

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ORIGINAL PAPER

Multivariate analysis for the differentiation of sparkling wines elaborated from autochthonous Spanish grape varieties: volatile compounds, amino acids and biogenic amines

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Abstract The aim of this work was to focus on the study of the volatile composition of white and rosé base wines elaborated from different autochthonous grape varieties in Spain (*Verdejo, Viura*, *Malvasía*, *Albarín, Godello, Prieto Picudo* and *Garnacha*) and the evolution of the sparkling wines elaborated from them following the "champenoise" method. The amino acids and biogenic amines were also studied. Multivariate analyses (factorial and stepwise discriminant) were carried out to study the influence of these compounds all together. Base and sparkling wines from *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* were the richest in most of the volatile compounds analyzed, especially of ethyl esters and alcohol acetates, compounds that contribute to the fruity aroma of wines. During the aging on lees in the bottle of sparkling wines, an increase in branched ethyl esters, ethyl lactate and γ-butyrolactone, and a decrease in terpenes (mainly citronellol and geraniol) were observed. In general, the levels of biogenic amines in all the sparkling wines studied were very low, which does not represent a negative effect on their quality and safety.

Keywords Sparkling wines · Grape variety · Volatile compounds · Amino acids · Biogenic amines

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Introduction

Natural sparkling wines elaborated following the "champenoise" method undergo a second fermentation in closed bottles of base wines, followed by aging of wines with lees for at least 9 months before the disgorging, the minimum aging time legally established (EC Regulation No 606/2009).

During sparkling wine aging, different compounds such as lipids, amino acids, peptides and volatiles can be released due to yeast autolysis. These compounds cause important changes in wine composition, affecting both the foam characteristics and the quality of sparkling wines [1].

Out of foam characteristics, aroma can be considered one of the most important attributes in the final quality of sparkling wines. Wine volatile compounds have different origins, from grape, from yeast metabolism during the first and second fermentation and from aging in contact with lees during the second fermentation in bottle [2]. In sparkling wines obtained by the traditional method, the second fermentation and the aging on lees can lead to important changes in volatile composition [3–5]. Different works have pointed out that ethyl esters, some alcohols and some varietal compounds increase during the second fermentation in bottle [3, 6, 7], while others like acetate esters and fatty acids decrease probably due to their adsorption to the yeast cell walls [5, 7, 8]. In addition, Ganss et al. [9] found differences in the evolution of ethyl esters depending on the length of the chain, showing an increase in short-chain aliphatic acids and their ethyl esters and a decrease in medium-chain compounds. However, the changes occurred during the second fermentation are modified during the aging on lees since the sorption mechanism of the yeast cell walls can change through the time [5, 7]. Therefore, the final aging time will determine the type and amount of the volatile compounds present in the sparkling wine [3, 8, 10].

On the other hand, different authors have found positive correlations between some volatile compounds and their amino acid precursors in musts [11, 12]. Guitart et al. [13], González-Marco et al. [14] and Torrea et al. [15] have not found clear relationships, but they found that musts with higher amino acid concentration and nitrogen compounds gave rise to wines with higher concentrations of ethyl esters and fatty acids. Therefore, taking into account these studies, the amino acids released from yeast lees during the sparkling wine aging [1] could contribute to the volatile profile of the final natural sparkling wine. Besides, the increase in amino acids could also favor the formation of biogenic amines, although there are very few studies on this subject in sparkling wines [16–18].

Different factors can influence sparkling wine composition, being the grape variety one of the most important variables that modify the composition of base and sparkling wines [19], both in volatile [8, 20] and in amino acid composition [21–23].

In Spain, "Cavas" are the most known sparkling wines, which are elaborated mainly from *Macabeo* (*Viura*), *Xarel.lo* and *Parellada* white grape varieties, being also authorized the *Malvasía* and *Chardonnay* between the white varieties and *Garnacha tinta*, *Monastrell*, *Pinot Noir* and *Trepat* between the red ones. Cavas can only be elaborated in specific geographical areas. However, other grape varieties could also present good characteristics to obtain quality natural sparkling wines.

Autonomous Community of "Castilla y León" (North of Spain) has a wide wine-growing area with a great diversity of autochthonous grape varieties. Some of these varieties can allow obtaining different sparkling wines with their own personality and different from those which are in the market. However, no scientific works have been carried out to study the potential of these grape varieties for sparkling wine elaboration.

Considering all the previous comments and studies, the aim of this work was to focus on the study of the volatile composition of white and rosé base wines elaborated from different autochthonous grape varieties of "Castilla y León" and the evolution of the sparkling wines during their aging on lees in bottle. The amino acids and biogenic amines were also studied due to the fact that amino acids, released during the aging on lees of the natural sparkling wines, are precursors of biogenic amines and of some of the volatile compounds present in wines.

Materials and methods

Winemaking process

Different natural sparkling wines were elaborated following the traditional or "champenoise" method, five from white and two from red grape varieties harvested in 2009.

The grape varieties used in this study are cited in the Autonomous Community of Castilla y León, and they were *Verdejo* and *Viura* from the Rueda Designation of Origin (D.O.), *Malvasía* from the Toro D.O., *Albarín* and *Prieto Picudo* from the Tierra de León D.O., *Godello* from the Bierzo D.O. and *Garnacha* from the Cigales D.O. Two different vineyards of *Garnacha* were used.

The base wines were elaborated in the experimental winery of the Enological Station, following the traditional white or *rosé* winemaking process in stainless steel tanks of 150 L. The clusters of white grapes were de-stemmed, crushed, slightly sulfited (0.05 g/L) and pressed. The must obtained was transferred to stainless steel tanks, and a pectinolytic enzyme preparation was added (1 g/hL of Novoclair Speed, Lamothe Abiet) to favor the precipitation of colloidal substances over 24 h at 12 °C. After this period of time, the must was racked off into different stainless steel tanks and inoculated with commercial *Saccharomyces cerevisiae* yeasts (20 g/hL of IOC 18–2007 from Institut Oenologique de Champagne, Lallemand, Spain) to undergo alcoholic fermentation at a controlled temperature (16 ± 2 °C). Once alcoholic fermentation was completed, the wines were kept in the tanks for several days to allow sedimentation of lees, and after that the wines were racked off. The rosé base wines were obtained in the same way, with the exception that the red grapes were macerated 24 h before obtaining the must.

The base wines were cold-stabilized (−5 °C) and clarified with PVPP (0.10 g/L) (Laffort, France) and bentonite (0.80–0.90 g/L) (Laffort, France). Then the wines were bottled, and the tirage liquor, formed by yeast *S. cerevisiae* var. *bayanus* (0.30 g/L, IOC 18–2007 Lallemand, Spain), sucrose (23 g/L) and bentonite (0.10 g/L) (Laffort, France), was added. After that, the bottles were kept in a cellar at a temperature (11–13 °C) and relative humidity (75–85 %) controlled for 9 months. The pressure and residual sugars were measured periodically to control the second fermentation.

Samples for analyses were taken from the base wines, and then after 3, 6 and 9 months of aging on yeast lees. Before the analyses, the wines were riddled and disgorged. The yeast lees were removed from the bottles using a disgorging machine (DLV 1 TDD Grilliat Machines, Maquinaria Moderna, Barcelona, Spain). Since the second fermentation takes place in individual bottles, three bottles of each varietal wine at each sampling time were analyzed.

Chemical reagents

The volatile compound standards were purchased from Fluka (Buchs, Switzerland) (ethyl butyrate, ethyl isovalerate, ethyl hexanoate, ethyl octanoate, β-phenylethyl acetate, isobutanol, benzyl alcohol, 2-methyl-1-butanol,

3-methyl-1-butanol, β-phenylethanol, 1-hexanol, *cis*-3-hexenol, hexanoic acid, octanoic acid, decanoic acid, guaiacol, γ-butyrolactone and citronellol); Sigma-Aldrich (Steinheim, Germany) (ethyl 2-methylbutyrate, ethyl decanoate, isoamyl acetate, *trans*-3-hexenol, 2,6-dimethoxyphenol, γ-nonalactone, acetovanillone, linalool, β-ionone, ethyl cinnamate and methyl octanoate); and Lancaster (Strasbourg, France) (methyl vanillate, ethyl vanillate, 4-ethylphenol, 4-vinylphenol, 4-vinylguaiacol and 3,4-dimethylphenol).

L-cysteine, L-leucine, L-phenylalanine, L-lysine, ammonium chloride, L-histidine, agmatine sulfate, cadaverine, L-arginine, histamine, L-alanine, spermidine, glycine, β-alanine, L-aspartic acid, L-glutamic acid, L-tyrosine, L-valine, L-serine and L-phenylethylamine were purchased from Fluka (Buchs, Switzerland). Isoamylamine, L-proline, diethyl ethoxymethylenemalonate (DEEMM), putrescine, tyramine, tryptamine, *trans*-4-hydroxy-L-proline, L-2-aminoadipic acid, L-ornithine monohydrochloride, L-tryptophan, L-asparagine, L-threonine, γ-aminobutyric acid (GABA), L-isoleucine, L-glutamine, L-methionine and sodium azide were purchased from Sigma-Aldrich (Steinheim, Germany).

HPLC-grade reagents, ethanol, methanol and acetonitrile were provided by Scharlau (Barcelona, Spain), and dichloromethane (HPLC-grade) by Merck (Darmstadt, Germany). The remaining reagents were of analytical quality and were supplied by Panreac (Madrid, Spain). Water Milli-Q was obtained via a Millipore system (Bedford, MA).

Analytical methods

Analysis of the volatile compounds

The volatile compounds were extracted by liquid–liquid extraction following the method developed by Rodríguez-Bencomo et al. [24]. Two hundred and fifty milliliters of wine, 5 mL of dichloromethane and 75 μL of a mixture of two internal IS standards (550 mg/L of methyl octanoate and 450 mg/L of 3,4-dimethylphenol) were added to a flask. The extraction was carried out for 3 h with continuous stirring (150 rpm) in an orbital shaker. The organic phase was then separated and concentrated until 400 μL. After that, the volatile compounds were analyzed by gas chromatography–mass detector (GC–MS). The chromatographic analyses were performed with a HP-6890N GC coupled to a HP-5973 inert MS detector equipped with a Quadrex 007CWBTR capillary column (60 m length, 0.25 mm i.d. and 0.25 μm film thickness), following the chromatographic conditions established by Rodríguez-Bencomo et al. [24].

Quantification ions, retention times and IS chosen for each compound studied are shown in Table 1.

Table 1 Retention times, quantification ions and internal standard (IS) of each compound analyzed

Compound	Retention time (min)	Quantification ion (m/z)	IS
Ethyl butyrate	13.4	88	1
Ethyl 2-methylbutyrate	14.3	57	1
Ethyl isovalerate	15.2	88	1
Isoamyl acetate	18.7	TIC	1
Ethyl hexanoate	26.6	TIC	1
Hexyl acetate	29.3	56	1
Ethyl lactate	34.0	75	2
1-Hexanol	34.7	56	2
trans-3-Hexen-1-ol	35.3	41	2
cis-3-Hexen-1-ol	36.7	67	2
Methyl octanoate (IS = 1)	37.1	74	
Ethyl octanoate	40.4	TIC	1
Linalool	47.3	71	1
g-Butyrolactone	51.9	86	2
Ethyl decanoate	52.8	101	1
Isovaleric acid	55.4	60	2
a-Terpineol	55.7	59	1
Citronellol	59.8	41	1
2-Phenylethyl acetate	62.2	91	1
Geraniol	64.1	69	1
Hexanoic acid	64.0	60	2
Benzyl alcohol	65.5	79	2
2-Phenylethanol	67.4	TIC	2
g-Nonalactone	73.0	85	1
Octanoic acid	75.0	TIC	1
4-Vinylguaiacol	80.9	135	1
3,4-Dimethylphenol (IS = 2)	82.1	107	
Decanoic acid	84.5	TIC	1
4-Vinylphenol	89.6	120	2
Methyl vanillate	98.0	151	1
Ethyl vanillate	99.1	151	1
Acetovanillone	99.5	166	2

Quantification was carried out following the internal standard quantification method. Quantitative data of the relative areas (absolute areas/internal standard area) were subsequently interpolated in the calibration graphs built from results of pure reference compounds.

Analysis of amino acids and biogenic amines

The amino acids and biogenic amines were analyzed by high-performance liquid chromatography with diode array detector (HPLC–DAD) after derivatization with DEEMM, following the method described by Gómez-Alonso et al. [25].

Chromatographic separation was performed in an ACE HPLC column (5 C18-HL) of particle size 5 μm (250 × 4.6 mm) thermostatized at 16 °C through the binary gradient (solvent A: 25 mM acetate buffer pH = 5.8 with 0.02 % sodium azide; solvent B: mixture of acetonitrile and methanol 80:20) as described by Gómez-Alonso et al. [25]. Thirty-four compounds were identified, and they were quantified using the internal standard method. Calibration curves were obtained using the commercial standards, and L-2-aminoadipic acid was used as the internal standard.

Sensory analysis

The sensory analysis was performed in a designed test room in accordance with ISO 8589 Standard (2007) and was carried out by twelve expert tasters from the Regulatory Councils of different Spanish D.O. and wineries (8 male and 4 female between 30 and 55 years old). These tasters defined the descriptors used in this sensory analysis, according to the methodology described in González-Sanjosé et al. [26], and were trained to quantify them using structured numerical scales. This training was carried out in accordance with UNE-87-020-93 Standard (ISO 4121:1987).

This work has focused on the olfactory attributes (olfactory intensity, fruity (exotic and citrus fruits), varietal, floral, vegetal, yeasty, mold, reduced and oxidized notes), and a structured numerical scale of seven points was used (1 representing no intensity and 7 the highest intensity). The sparkling wines were tasted after 9 months of aging on lees in the bottle, since it is the minimum aging time established by EC Regulation N° 606/2009.

Statistical analyses

The data were treated applying the variance analysis (ANOVA), and the least significant difference test, at significant level of $p < 0.05$. Factor analysis is an unsupervised method applied to reduce the dimensionality of the original space and to give an interpretation to the new space. This analysis studies the association of variables and determines similarities or differences between wines by grape variety or by aging time. Varimax rotation criterion was performed, and only factors with eigenvalues greater than unity were selected. Stepwise discriminant analysis is a supervised method that consists to find a linear combination of the variables, which characterizes or separates two or more classes of objects. The forward method was used to select the variables most useful for differentiating the wines according to grape variety or aging time. The F-statistical function was used as the criterion for variable selection. All the statistical analyses were carried out using the Statgraphics Plus 5.0 statistical package.

Generalized Procrustes analysis (GPA) was applied on the mean ratings for olfactory attributes, and a permutation test was also made to explain that the results obtained were significant (85.12 %). These analyses were performed using the Senstools Version 3.3.2. program (Utrecht, the Netherlands).

Results and discussion

Thirty-three volatile compounds were identified and quantified in the sparkling wines that were classified into seven groups: ethyl esters, alcohols, alcohol acetates, acids, terpenes, lactones and volatile phenols. Twenty-four amino acids, the ammonium ion and nine biogenic amines were identified.

Tables 2 and 3 show the data of volatile compounds and amino acids of base and sparkling wines (9 months after tirage). However, due to the high number of variables (compounds) and wine samples, it is more interesting to carry out multivariate analyses in order to study the influence of these compounds all together on the sparkling wines elaborated.

First, a factorial analysis with all data was performed, in order to see whether the information given by these compounds would allow differentiating the wines studied according to the grape variety and/or the aging time.

The factorial analysis selected eight factors with an eigenvalue greater than 1, which explained the 90.8 % of the total variance. However, the variables associated with five factors were enough to explain more than 80 % of total variability. Table 4 shows the loadings for each variable on the selected factor, as well as the eigenvalue and the cumulative variance. The variables with higher loading values contribute most significantly to the explanatory meaning of the factors (marked in bold). The first factor explained the 38.8 % of the data variability and was strongly correlated with most of the amino acids and also with the volatile compounds, alcohol acetates, ethyl vanillate, isobutanol, 1-propanol and isoamyl alcohols. This fact indicates that these volatile compounds are highly correlated with most of the amino acids present in wines. No studies have been found that evaluate the influence of amino acids of wines on the volatile composition of sparkling wines after their aging on lees in the bottle. However, different studies have been found in the literature that showed some relationship between the total amino acid concentration of musts and some volatile compounds of their wines. Higher concentrations of γ-butyrolactone, isobutanol and isobutyric acid [27], and of total esters, isoamyl acetate and 2-phenylethyl acetate [12] were found in white wines elaborated from musts richer in amino acids.

Table 2 Volatile compounds of base wines and sparkling wines 9 months after tirage

Compounds	Albarín	Verdejo	Godello	Viura	Malvasía	Garnacha	Garnacha*	Prieto Picudo
Base wines								
Ethyl esters								
Ethyl butyrate	0.161 de	0.152 d	0.171 e	0.098 bc	0.059 a	0.113 c	0.095 b	0.163 de
Ethyl 2-methylbutyrate	0.009 b	0.007 a	0.014 c	0.010 b	0.010 b	0.009 b	0.008 a	0.007 a
Ethyl isovalerate	0.016 b	0.011 a	0.019 c	0.014 b	0.011 a	0.012 a	0.010 a	0.011 a
Ethyl hexanoate	0.493 f	0.431 e	0.444 e	0.317 bc	0.191 a	0.339 c	0.296 b	0.406 d
Ethyl octanoate	0.694 g	0.687 g	0.579 e	0.400 c	0.167 a	0.432 d	0.367 b	0.654 f
Ethyl decanoate	0.262 e	0.273 e	0.266 e	0.194 bc	0.067 a	0.208 c	0.176 b	0.236 d
Ethyl lactate	12.1 g	7.27 c	7.94 cd	8.89 e	4.09 a	8.20 de	6.22 b	10.1 f
Alcohols								
2-Phenylethanol	34.1 c	24.5 a	29.9 b	27.2 ab	40.4 d	26.9 ab	29.4 b	28.8 b
1-Propanol	41.4 d	50.4 e	30.2 b	37.9 c	14.8 a	35.8 c	36.3 c	37.2 c
Isobutanol	19.8 b	23.5 f	23.0 ef	22.4 de	15.4 a	21.3 c	21.8 cd	29.3 g
Isoamyl alcohols	186 b	194 c	214 e	189 bc	134 a	184 b	183 b	203 d
Benzyl alcohol	0.123 e	0.122 e	0.093 b	0.085 a	0.088 ab	0.113 d	0.103 c	0.086 ab
1-Hexanol	0.993 d	0.965 d	0.847 c	0.709 b	0.735 b	0.934 d	0.837 c	0.565 a
trans-3-Hexen-1-ol	0.135 e	0.087 d	0.130 e	0.028 a	0.044 b	0.048 b	0.040 b	0.057 c
cis-3-Hexen-1-ol	0.133 d	0.114 c	0.076 a	0.193 e	0.077 a	0.116 c	0.094 b	0.084 a
Alcohol acetates								
Isoamyl acetate	0.645 c	1.014 d	0.649 c	0.363 b	0.288 a	0.387 b	0.284 a	1.458 e
Hexyl acetate	0.034 c	0.079 d	0.033 c	0.008 a	0.009 a	0.011 b	0.009 a	0.033 c
2-Phenylethyl acetate	0.191 e	0.179 e	0.157 d	0.136 c	0.080 a	0.116 b	0.101 b	0.374 f
Acids								
Isovaleric acid	0.833 c	0.881 c	1.070 d	1.049 d	1.335 e	0.740 b	0.625 a	0.881 c
Hexanoic acid	5.02 f	4.73 e	4.15 d	3.51 c	1.94 a	3.55 c	3.06 b	3.72 c
Octanoic acid	5.28 f	4.57 e	5.23 f	3.72 d	1.60 a	3.22 c	2.68 b	4.55 e
Decanoic acid	0.887 ef	0.968 g	0.906 f	0.724 d	0.320 a	0.646 c	0.577 b	0.853 e
Terpenes*								
Linalool	8.48 d	4.69 a	5.09 ab	5.39 b	7.19 c	4.91 ab	4.90 ab	6.62 c
α-Terpineol	5.91 g	1.39 a	1.76 b	1.83 bc	3.24 f	2.07 cd	2.18 d	2.73 e
Citronellol	2.99 a	3.16 a	3.42 bc	3.61 cd	3.22 ab	3.68 d	3.49 cd	4.06 e
Geraniol	3.16 b	3.60 c	3.12 b	3.17 b	3.07 b	2.67 a	2.54 a	4.72 d
Lactones								
γ-Butyrolactone	5.94 de	3.78 a	7.28 f	5.60 de	4.44 ab	6.21 e	5.47 cd	4.91 bc
γ-Nonalactone*	2.62 e	1.92 c	1.36 a	1.63 b	1.64 b	2.12 d	2.12 d	1.83 c
Volatile phenols								
Methyl vanillate*	14.59 c	9.51 b	7.47 a	5.76 a	10.51 b	20.11 d	18.16 d	10.94 b
Ethyl vanillate*	nd	nd	nd	nd	nd	2.87 a	2.54 a	13.09 b
Acetovanillone*	17.0 c	13.7 b	5.3 a	17.5 c	18.9 cd	43.5 f	38.7 e	19.4 d
4-Vinylguaiacol	0.547 d	1.058 g	0.723 e	0.797 f	0.412 c	0.155 b	0.160 b	0.024 a
4-Vinylphenol	0.327 c	0.557 f	0.792 g	0.508 e	0.436 d	0.225 b	0.210 b	0.071 a
Sparkling wines (9 months)								
Ethyl esters								
Ethyl butyrate	0.187 e	0.175 d	0.143 b	0.160 c	0.117 a	0.165 c	0.142 b	0.184 e
Ethyl 2-methylbutyrate	0.029 b	0.024 a	0.046 d	0.031 bc	0.033 c	0.032 c	0.023 a	0.026 a
Ethyl isovalerate	0.051 e	0.041 bc	0.066 f	0.048 de	0.040 b	0.046 cd	0.034 a	0.039 ab
Ethyl hexanoate	0.579 e	0.518 d	0.536 de	0.358 bc	0.247 a	0.404 c	0.344 b	0.399 c
Ethyl octanoate	0.592 d	0.558 d	0.566 d	0.312 b	0.199 a	0.386 c	0.326 b	0.390 c

Table 2 continued

Compounds	Albarín	Verdejo	Godello	Viura	Malvasía	Garnacha	Garnacha*	Prieto Picudo
Ethyl decanoate	0.068 d	0.059 c	0.080 e	0.049 b	0.033 a	0.067 d	0.054 bc	0.054 bc
Ethyl lactate	19.7 e	17.4 cd	16.9 c	18.4 de	11.2 a	17.3 cd	13.4 b	23.6 f
Alcohols								
2-Phenylethanol	37.7 c	32.3 a	36.4 bc	30.8 a	42.4 d	36.1 bc	33.7 ab	42.5 d
1-Propanol	28.3 e	34.4 g	20.5 b	24.3 d	11.9 a	22.5 c	20.7 b	33.5 f
Isobutanol	22.3 e	24.0 f	21.6 de	20.9 d	15.1 a	19.6 c	18.3 b	29.2 g
Isoamyl alcohols	179 e	188 f	203 g	170 d	130 a	165 c	155 b	204 g
Benzyl alcohol	0.106 d	0.119 e	0.079 b	0.069 a	0.082 b	0.095 c	0.094 c	0.099 cd
1-Hexanol	0.849 e	0.852 e	0.778 d	0.518 a	0.601 b	0.738 d	0.673 c	0.475 a
trans-3-Hexen-1-ol	0.115 e	0.080 d	0.113 e	0.028 a	0.038 b	0.036 b	0.034 b	0.055 c
cis-3-Hexen-1-ol	0.163 e	0.130 d	0.089 a	0.187 f	0.100 ab	0.127 d	0.115 c	0.109 bc
Alcohol acetates								
Isoamyl acetate	0.273 b	0.434 c	0.258 b	0.183 a	0.198 a	0.192 a	0.170 a	0.596 d
Hexyl acetate	nd	0.015 b	nd	nd	nd	nd	nd	0.007 a
2-Phenylethyl acetate	0.062 b	0.063 b	0.048 a	0.043 a	0.043 a	0.041 a	0.039 a	0.136 c
Acids								
Isovaleric acid	1.247 c	1.141 b	1.315 d	1.251 c	1.186 b	0.863 a	0.905 a	1.283 cd
Hexanoic acid	4.05 f	4.12 g	3.71 e	2.72 c	1.71 a	2.92 c	2.46 b	3.25 d
Octanoic acid	6.28 c	6.76 d	6.29 c	4.49 b	2.40 a	4.08 b	4.28 b	4.45 b
Decanoic acid	0.547 c	0.571 c	0.657 d	0.478 b	0.326 a	0.469 b	0.458 b	0.449 b
Terpenes*								
Linalool	5.42 d	2.75 a	3.33 b	4.92 c	6.89 e	4.83 c	4.95 c	6.68 e
α-Terpineol	9.83 g	3.96 a	4.50 b	4.72 bc	7.58 f	5.24 de	5.05 cd	5.62 e
Citronellol	1.45 a	1.58 b	1.37 a	1.77 c	2.09 d	1.84 c	2.03 d	2.51 e
Geraniol	1.08 d	0.96 c	0.85 ab	0.92 bc	1.40 e	0.77 a	0.99 c	1.67 f
Lactones								
γ-Butyrolactone	10.3 a	14.3 bcd	15.6 d	13.1 b	13.7 bc	11.6 a	11.1 a	15.0 cd
γ-Nonalactone*	2.71 f	2.02 c	1.66 a	1.85 b	1.90 bc	2.42 e	2.29 d	1.99 c
Volatile phenols								
Methyl vanillate*	14.5 c	11.1 b	5.61 a	5.80 a	11.0 b	19.4 d	23.1 e	14.0 c
Ethyl vanillate*	nd	nd	nd	nd	nd	3.44 a	3.24 a	16.5 b
Acetovanillone*	15.5 cd	11.7 b	5.12 a	14.4 c	17.0 d	35.8 e	35.3 e	16.7 d
4-Vinylguaiacol	0.155 c	0.433 f	0.190 d	0.287 e	0.202 d	0.078 b	0.089 b	0.036 a
4-Vinylphenol	0.211 c	0.199 c	0.284 f	0.259 e	0.228 d	0.159 b	0.156 b	0.128 a

Data in mg/L except those marked with an asterisk that are expressed in μg/L. Values with different letters in each compound indicate statistically significant differences at $p < 0.05$

nd not detected

The second factor was mainly correlated positively with branched ethyl esters, ethyl lactate, γ-butyrolactone and 2-phenylethanol and negatively with ethyl decanoate, citronellol and geraniol, and the third one was correlated positively with acids, some alcohols and the aliphatic ethyl esters that were not correlated with factor 2.

Figure 1a shows the distribution of the different sparkling wines studied in the plane defined by the first two factors, which explained the 56.4 % of the total variance. As can be seen in this figure, the variables associated with factor 1 permit to differentiate the sparkling wines by grape variety. The *Prieto Picudo* wines appear on the right side of the plane, showing higher and positive values of factor 1, that is, mainly higher values of amino acid compounds (Table 3). The *Malvasía* wines had the poorest concentration in amino acids. On the other hand, factor 2 allows differentiating the wines by the aging time. The base wines appear on the bottom of the plane, showing higher and negative values for factor 2. During the aging, the wines were taking place in the positive zone of factor 2, which were due to the increase in branched ethyl esters, ethyl lactate and γ-butyrolactone, and the decrease in terpenes

Table 3 Amino acid compounds (mg/L) of base wines and sparkling wines 9 months after tirage

Compounds	Albarín	Verdejo	Godello	Viura	Malvasía	Garnacha	Garnacha*	Prieto Picudo
Base wines								
L-serine	0.551 f	0.806 g	0.152 b	0.209 c	0.103 a	0.276 e	0.240 d	1.196 h
Hydroxyproline	1.557 e	0.916 d	2.052 f	0.714 c	0.306 b	0.198 a	0.193 a	3.420 g
Glycine	0.980 d	2.010 e	0.806 c	0.724 b	0.522 a	0.811 c	0.726 b	3.279 f
L-threonine	1.019 f	1.037 g	0.362 c	0.414 d	0.233 a	0.456 e	0.294 b	1.301 h
α-Alanine	5.844 d	7.335 e	1.492 b	1.494 b	1.074 a	1.995 c	1.584 b	10.17 f
β-Alanine	0.325 e	0.391 f	0.072 b	0.072 b	0.047 a	0.080 c	0.066 b	0.279 d
γ-Aminobutyric acid	7.692 e	7.404 d	1.275 a	1.343 a	1.766 b	2.388 c	1.866 b	12.67 f
L-proline	109 c	133 d	78.3 b	1.685 a	1.002 a	1.492 a	1.058 a	362 e
L-tyrosine	0.704 d	1.382 e	0.278 ab	0.395 c	0.263 a	0.399 c	0.279 b	1.952 f
L-valine	0.955 b	1.504 f	0.887 a	0.995 c	1.004 c	1.083 d	0.984 bc	1.301 e
L-methionine	0.924 f	1.351 g	0.448 b	0.632 d	0.284 a	0.658 e	0.557 c	2.018 h
L-cysteine	0.801 f	0.294 c	0.349 de	0.173 b	0.113 a	0.367 e	0.282 c	0.339 d
L-isoleucine	0.582 e	1.296 f	0.184 a	0.291 d	0.226 b	0.315 d	0.256 c	1.344 g
L-leucine	2.779 f	5.616 g	0.653 b	1.212 d	0.481 a	1.320 e	0.983 c	5.923 h
L-phenylalanine	1.553 d	3.265 f	0.376 a	0.676 b	0.419 a	0.791 c	0.632 b	3.036 e
L-aspartic acid	1.375 e	2.016 f	0.670 b	0.807 c	0.438 a	0.937 d	nd	2.230 g
L-glutamic acid	2.933 e	4.068 f	1.865 d	1.775 c	1.109 a	1.427 b	1.440 b	6.392 g
Ammonium chloride	1.501 e	1.692 f	1.294 c	1.433 d	1.087 a	1.509 e	1.172 b	2.439 g
L-asparagine	4.165 f	4.860 g	0.877 b	1.235 c	0.429 a	2.377 e	1.690 d	11.27 h
L-glutamine	2.569 d	1.339 b	1.951 c	0.875 a	4.532 e	nd	nd	14.94 f
L-histidine	0.490 d	0.331 c	1.323 e	0.136 b	0.038 a	0.148 b	0.129 b	1.945 f
L-arginine	2.059 d	3.919 e	1.009 bc	0.982 bc	0.177 a	1.082 c	0.930 b	5.527 f
L-tryptophan	0.190 d	0.220 e	0.105 b	1.818 g	0.045 a	0.140 c	0.136 c	0.305 f
L-ornithine hydrochloride	1.951 f	1.020 e	0.343 c	0.391 d	0.197 a	0.333 c	0.245 b	2.030 g
L-lysine	2.309 f	4.036 g	0.874 b	1.693 e	0.214 a	1.345 d	1.010 c	7.379 h
Sparkling wines (9 months)								
L-serine	0.456 e	0.410 d	0.370 c	0.403 d	0.185 a	0.371 c	0.286 b	1.120 f
Hydroxyproline	1.597 d	1.011 c	1.944 e	0.405 b	0.361 b	0.319 b	0.201 a	2.190 f
Glycine	1.672 f	1.419 e	1.033 d	0.790 b	0.471 a	0.915 c	0.755 b	3.428 g
L-threonine	2.879 g	0.381 a	1.283 c	1.477 e	0.745 b	1.570 f	1.352 d	2.898 g
α-Alanine	3.119 g	2.327 f	1.945 d	1.753 c	0.827 a	2.060 e	1.422 b	5.772 h
β-Alanine	0.334 e	0.351 f	0.056 c	0.034 b	0.016 a	0.043 b	0.054 c	0.265 d
γ-Aminobutyric acid	0.566 b	1.115 d	0.520 b	0.547 b	0.445 a	0.746 c	0.540 b	1.969 e
L-proline	62.2 b	59.2 b	82.6 c	0.374 a	0.281 a	0.460 a	0.448 a	294 d
L-tyrosine	0.314 e	0.248 d	0.236 c	0.327 f	0.127 a	0.170 b	0.176 b	1.716 g
L-valine	1.568 c	1.385 b	1.252 a	1.211 a	1.676 c	1.258 a	1.251 a	2.083 d
L-methionine	0.462 c	0.422 c	0.332 b	0.435 c	0.248 a	0.287 ab	0.431 c	1.761 d
L-cysteine	0.549 h	0.217 d	0.378 g	0.104 b	0.086 a	0.340 e	0.190 c	0.360 f
L-isoleucine	0.205 cd	0.222 d	0.180 b	0.210 cd	0.195 bc	0.134 a	0.118 a	0.833 e
L-leucine	1.107 c	1.169 d	0.832 b	1.227 d	0.603 a	0.644 a	0.595 a	4.554 e
L-phenylalanine	0.814 f	0.787 f	0.509 d	0.647 e	0.414 b	0.468 c	0.355 a	2.088 g
L-aspartic acid	0.616 d	0.525 c	0.497 c	0.613 d	0.241 a	0.333 b	0.327 b	1.917 e
L-glutamic acid	0.839 f	0.649 d	0.603 d	0.728 e	0.154 a	0.470 c	0.350 b	3.265 g
Ammonium chloride	1.669 d	1.332 ab	1.400 b	1.283 a	1.402 bc	1.849 e	1.517 c	1.641 d
L-asparagine	2.569 f	2.673 g	1.404 d	1.149 c	0.413 a	1.651 e	1.016 b	8.922 h
L-glutamine	0.704 a	0.555 a	0.982 a	0.849 a	0.675 a	1.052 a	0.821 a	7.433 b
L-histidine	2.408 bc	1.799 a	1.953 a	1.853 a	2.304 b	2.612 c	2.494 bc	3.793 d

Table 3 continued

Compounds	Albarín	Verdejo	Godello	Viura	Malvasía	Garnacha	Garnacha*	Prieto Picudo
L-arginine	1.045 e	1.559 f	0.920 cd	0.970 d	0.485 a	0.888 c	0.677 b	3.943 g
L-tryptophan	0.122 d	0.081 c	0.061 a	0.073 bc	0.071 b	0.080 bc	0.056 a	0.209 e
L-ornithine hydrochloride	3.316 e	2.193 d	1.165 c	1.210 c	0.784 a	1.165 c	0.950 b	3.799 f
L-lysine	0.830 d	0.732 c	0.593 b	0.874 e	0.405 a	0.727 c	0.608 b	4.736 f

Values with different letters in each compound indicate statistically significant differences at $p < 0.05$

nd not detected

Table 4 Factor loadings after varimax rotation of the wines studied

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
sEigenvalue	22.1	10.1	7.9	4.3	2.3	2.0	1.8	1.3
Cumulative variance (%)	38.8	56.4	70.4	77.9	81.9	85.4	88.5	90.8
Ethyl butyrate	0.398	0.502	**0.619**					
Ethyl 2-methylbutyrate		**0.905**						
Ethyl isovalerate		**0.940**						
Ethyl hexanoate		0.403	**0.830**					
Ethyl lactate		**0.828**					0.312	
Ethyl octanoate	0.361		**0.883**					
Ethyl decanoate	0.368	**−0.629**	0.480				−0.389	
Isoamyl acetate	**0.877**	−0.290	0.250					
Hexyl acetate	**0.501**	−0.449	0.467			−0.448		
2-Phenylethyl acetate	**0.833**	−0.430						
Isovaleric acid		0.555		**0.669**				
Hexanoic acid	0.264		**0.896**					
Octanoic acid		0.474	**0.771**					
Decanoic acid	0.299	−0.363	**0.707**		−0.328			
1-Hexanol	−0.308	−0.273	**0.756**			−0.342		
trans-3-Hexen-1-ol			**0.881**	0.258	0.258			
cis-3-Hexen-1-ol								**0.908**
Benzyl alcohol			**0.606**	−0.411		−0.445		
Linalool	0.319	−0.274	−0.264		**0.809**			
α-Terpineol		0.524			**0.712**		0.279	
Citronellol	0.453	**−0.780**						
Geraniol	0.496	**−0.800**						
γ-Butyrolactone		**0.899**						
γ-Nonalactone			0.259	**−0.654**	0.334		0.505	
Methyl vanillate				**−0.888**				
Ethyl vanillate	**0.771**					0.383		
Acetovanillone			−0.271	**−0.902**				
2-Phenylethanol		**0.536**			0.493			
1-Propanol	**0.497**	−0.365	**0.495**		−0.336		0.350	0.306
Isobutanol	**0.747**				−0.362	0.361	0.250	
Isoamyl alcohols	**0.457**		**0.561**		−0.412	0.446		
4-Vinylguaiacol		−0.451	0.326	**0.580**		−0.405		0.266
4-Vinylphenol	−0.320	−0.418	0.266	**0.637**				
L-serine	**0.953**							
Hydroxyproline	**0.668**		0.310	0.333		0.471		

Table 4 continued

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
Glycine	**0.905**						0.284	
L-threonine	0.530	**0.576**			0.267			0.254
β-Alanine	0.488		0.542				**0.612**	
α-Alanine	**0.938**		0.266					
γ-Aminobutyric acid	**0.756**	−0.388						
L-proline	**0.908**							
L-tyrosine	**0.962**							
L-valine	0.316	0.416	−0.316					−0.388
L-methionine	**0.964**							
L-cysteine	0.271		**0.792**		0.348			
L-isoleucine	**0.948**							
L-leucine	**0.972**							
L-phenylalanine	**0.951**							
L-aspartic acid	**0.948**							
L-glutamic acid	**0.936**							
L-asparagine	**0.945**							
L-glutamine	**0.747**					0.399		
L-histidine	0.263	**0.632**				0.372	0.301	−0.264
L-arginine	**0.977**							
L-tryptophan								**0.688**
L-ornithine hydrochloride	0.551	0.379				0.297	**0.601**	
L-lysine	**0.982**							

Loadings lower than absolute values of 0.250 are not shown

The bold numbers indicate the higher weight of each compound in each factor

(mainly citronellol and geraniol) (Table 1), compounds associated with the factor 2. The highest differences were found during the first 3 months. Hidalgo et al. [6] also found an increase in some ethyl esters and ethyl lactate during the aging on lees in bottle of *Garnacha* rosé sparkling wines (9 months). In addition, Francioli et al. [3] and Riu-Aumatell et al. [8] showed that long-aged sparkling wines (>20 months) had lower alcohol acetates and higher TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), vitispirane and diethylsuccinate than young ones.

Factors 3 and 4 also permit wine differentiation by grape variety (Fig. 1b). The *Malvasía* wines were the poorest in the volatile compounds associated with factor 3, fatty acids, some alcohols and the aliphatic ethyl esters (Table 2), followed by *Viura* and *Garnacha* wines. *Albarín, Verdejo, Godello* and *Prieto Picudo* wines were the richest in the aforementioned volatile compounds.

Factor 4 differentiates the *Garnacha* wines, sited in the bottom of the plane mainly related to a high concentration of methyl vanillate and acetovanillone and a low concentration of vinylphenols (Table 2). On the contrary, the *Godello* wines are sited in the upper part of the plane.

Therefore, both base and sparkling wines from *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* have in general more volatile compounds than the rest of the wines studied, especially of ethyl esters and alcohol acetates (Table 2), compounds that contribute to the fruity aroma of wines [28].

Stepwise discriminant analysis is a supervised classification technique that was applied to determine the volatile and amino acid compounds most useful for differentiating the wines according to grape variety or aging time. The final model by grape variety selected 16 volatile compounds and 17 amino acids. The variables with the greatest discriminating power were the following: glycine, *trans*-3-hexenol, acetovanillone and β-alanine (with F values between 455 and 191), and linalool, γ-nonalactone, *cis*-3-hexenol, isoamyl alcohols and 1-propanol (with F values between 66 and 21). *Trans*-3-hexenol and *cis*-3-hexenol are C6 alcohols, pre-fermentative compounds that are present in the must and that are clearly associated with the grape variety used. These results agree with those found by Ortega-Heras [29]. Cáceres [30] and Pozo-Bayón et al. [31] also reported *cis*-3-hexenol as a discriminating compound between cultivars. 1-Propanol was also selected to discriminate among different cultivars in a work carried out by Pozo-Bayón et al. [31] and isoamyl alcohols and acetovanillone in a work carried out by Tredoux et al. [32].

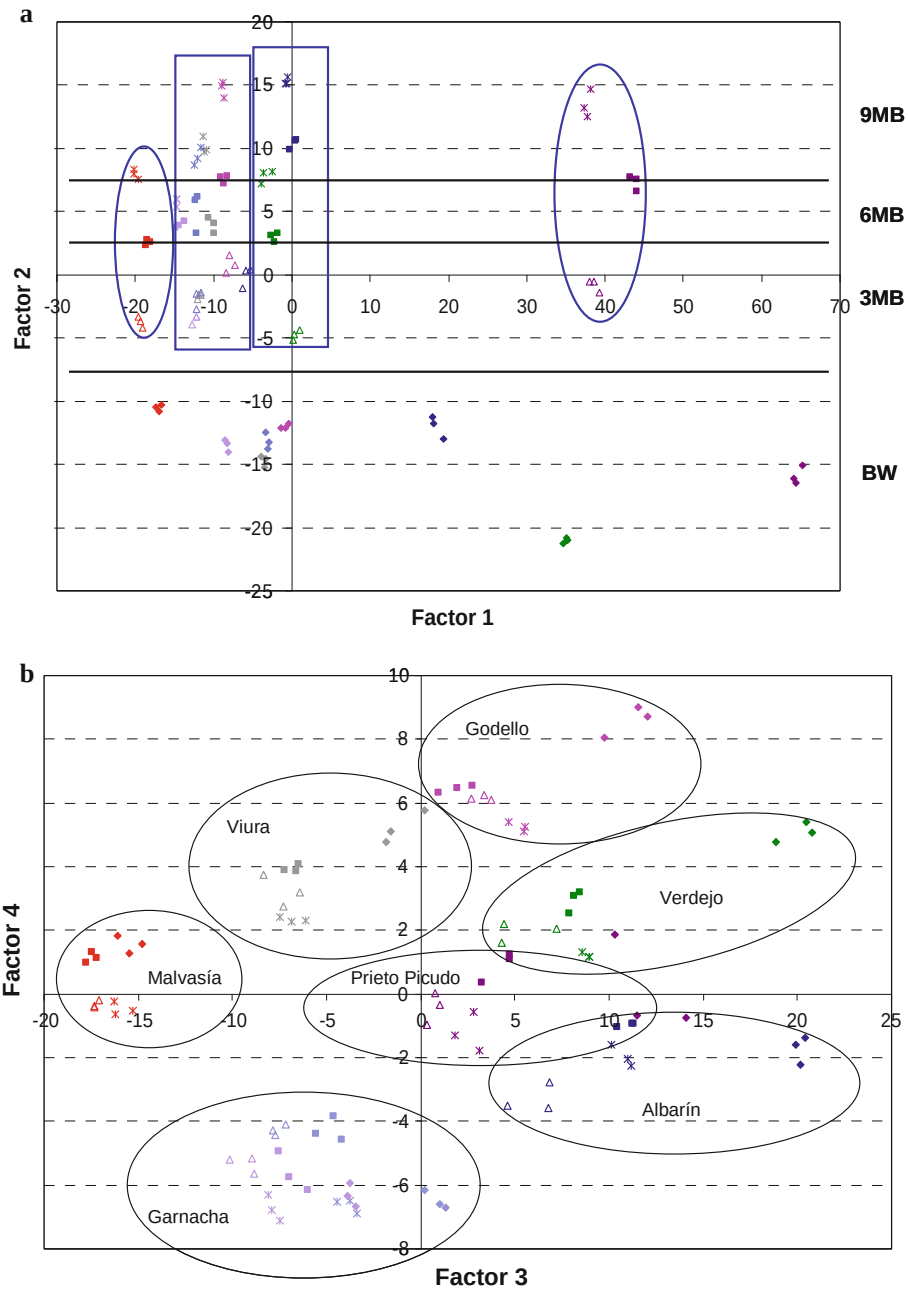

Fig. 1 Distribution of the wines in the plane defined by **a** factor 1 and factor 2 and **b** factor 3 and 4. *Filled diamonds* base wines, *open triangles* 3 months after tirage, *filled squares* 6 months after tirage, *six pointed black stars* 9 months after tirage. *Dark blue diamonds* Albarín, *green diamonds* Verdejo, *pink diamonds* Godello, *gray diamonds* Viura, *red diamonds* Malvasía, *light blue diamonds* Garnacha, *violet diamonds* Garnacha*, *magenta diamonds* Prieto Picudo

The wines on the plane defined by the first two discriminant functions are shown in Fig. 2a. Taking into account that the distance between centroids is proportional to the similarity between groups, *Prieto Picudo* wines are the most different, since they are situated on the left part of the plane, whereas the rest of the wines are placed on the right. According to the weight of variables in the first function, glycine and some other amino acids such as tyrosine, asparagine and lysine are the main variables responsible for the observed distribution of wines. The second function also shows a clear separation between the sparkling varietal wines. The *Albarín* and *Verdejo* are in the upper part of the plane, and the others, with the exception of *Prieto Picudo* wines, are in the bottom part. According to the weight of variables in the second function, β-alanine, 1-propanol and ornithine are the principal variables responsible for this differentiation.

When stepwise forward discriminant analysis was applied to discriminate wines considering the aging factor, the final model selected 22 variables, 11 volatile compounds and 11 amino acids. The variables with the greatest discriminating power were as follows: γ-butyrolactone, ethyl decanoate, decanoic acid, histidine and threonine (with *F* values between 151 and 26). As can be seen in

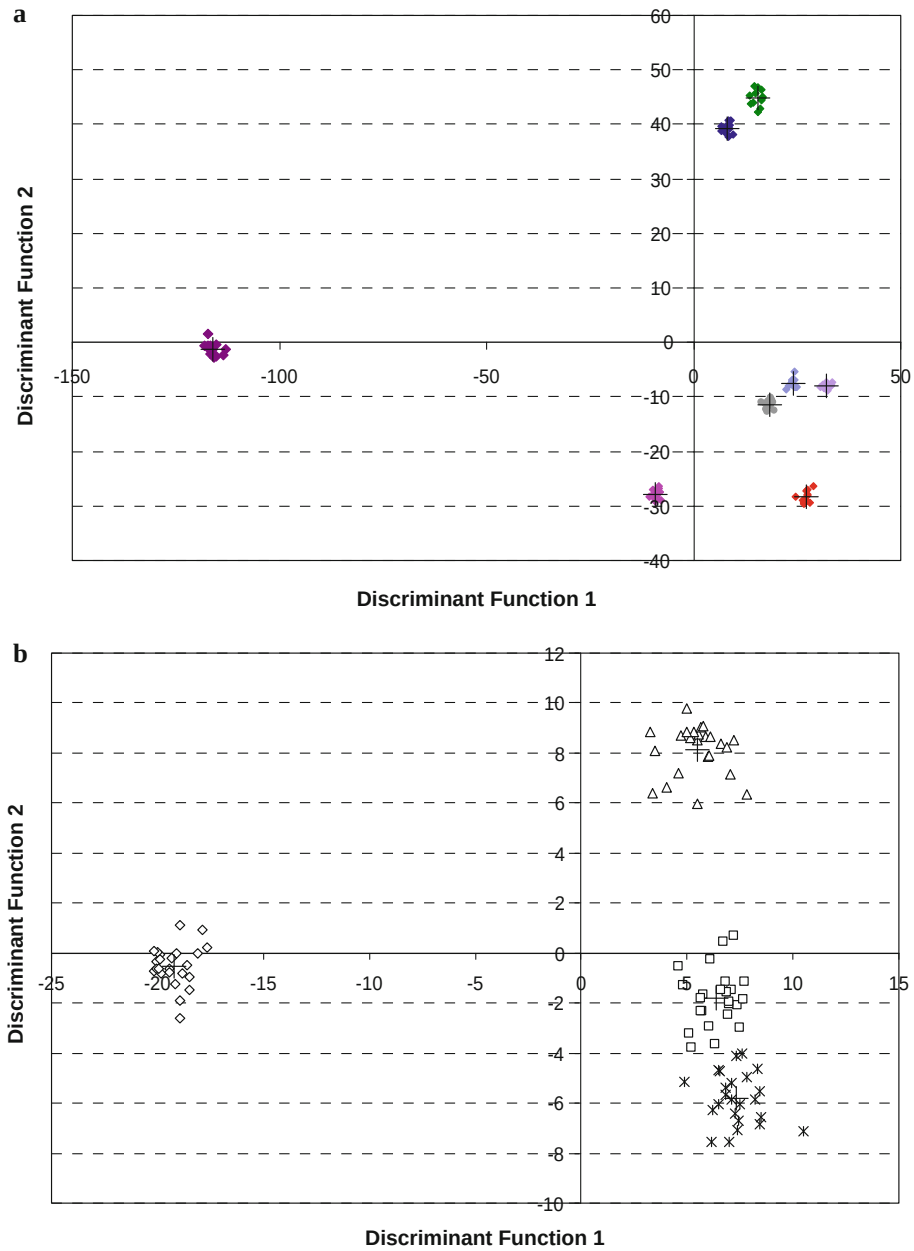

Fig. 2 Distribution of the wines in the plane defined by the first two discriminant functions by **a** grape variety and by **b** aging time. *Plus* centroids, *diamonds* base wines, *triangles* 3 months after tirage, *open triangles* 6 months after tirage, *six pointed black stars* 9 months after triage. *Dark blue diamonds* Albarín, *green diamonds* Verdejo, *pink diamonds* Godello, *gray diamonds* Viura, *red diamonds* Malvasía, *light blue diamonds* Garnacha, *violet diamonds* Garnacha*, *magenta diamonds* Prieto Picudo

Fig. 2b, base wines were clearly separated from all sparkling wines, being situated on the left part of the plane. The second discriminant function was able to separate the sparkling wines by the aging time, being the increase in γ-butyrolactone and ethyl decanoate the main responsible.

Both models were satisfactory with a global classification of 100 % of the wines.

The sensory analysis of the sparkling wines after 9 months of aging on lees in the bottle was carried out. GPA was applied to the olfactory attributes in order to obtain information about the relationship between sparkling wines and these attributes. The white and rosé sparkling wines were represented in the plane defined by the first two factors (Fig. 3 a, b), which accounted for 65.0 and 100 % of the total variance, respectively. The olfactory space of white sparkling wines showed that *Albarín* and *Godello* wines presented higher olfactory intensity than *Malvasía* and *Viura* wines, associated with the fruity

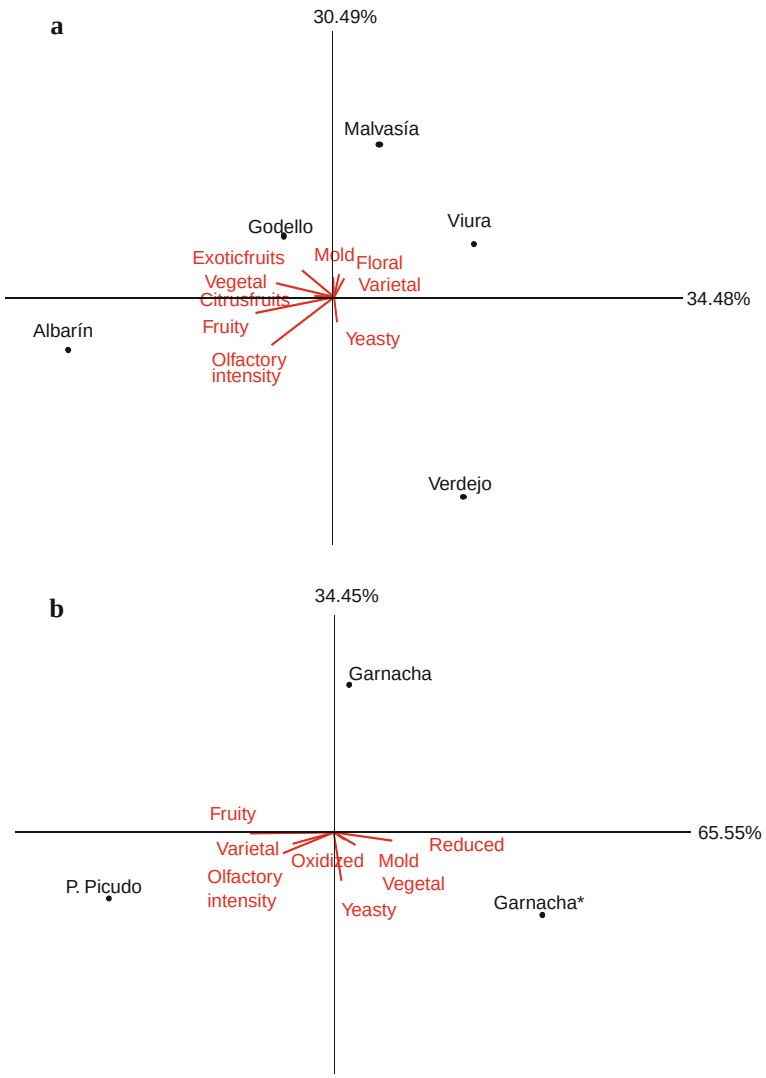

Fig. 3 GPA on the mean ratings for olfactory attributes in the final **a** white and **b** rosé sparkling wines

aromas (Fig. 3a). *Godello* wine was mainly characterized by citrus and exotic fruity aromas and vegetal notes. *Prieto Picudo* sparkling wine was characterized by a higher olfactory intensity, mainly due to the fruity and varietal aromas than *Garnacha* wines (Fig. 3b). All these results are in agreement with the concentrations of volatile compounds found in these wines (Table 1), before mentioned.

As it was said before, the aging on lees can lead an increase in amino acids and then in biogenic amines. Table 5 shows the content of biogenic amines in base and sparkling wines over the aging on lees. Putrescine was the most abundant amine in all base and sparkling wines analyzed (37–80 % of the total content). These results are in agreement with those found by Romero et al. [33], Torrea-Goñi and Ancín-Azpilicueta [34] and González-Marco et al. [35].

Statistically significant differences in the biogenic amine concentrations were found between the varietal base wines, and in general, these differences were maintained over the aging time. Other authors have also found differences in the concentration of these compounds associated with the grape variety [36, 37], although the biogenic amine content of most of the varieties used in this work has not been previously studied.

The *Albarín* and *Prieto Picudo* wines showed the highest content of total biogenic amines, which coincides with the highest amino acid concentration found in these wines. This relationship has also been found by other authors in other types of wines [23, 37].

During the aging on lees, total biogenic amine concentration remained constant during the first 3 months, when the second fermentation in the bottle occurred. These results suggest that the yeast used was unable to produce biogenic amines. However, a slight increase in total biogenic amines was observed in all the wines during the following 3 months of aging. This fact can be

Table 5 Concentration of biogenic amines (mg/L) of the different sparkling wines studied over the aging time

	Albarín	Verdejo	Godello	Viura	Malvasía	Garnacha	Garnacha*	Prieto Picudo
Base wines								
Histamine	nq[a]	nq	nq	nq	nq	0.181 a	0.278 b	nq
Putrescine	3.090 g	1.787 e	0.591 b	0.563 b	0.373 a	1.164 d	1.039 c	2.206 f
Cadaverine	0.091 a	0.119 f	0.114 de	0.111 d	nq	0.095 b	0.108 c	0.115 e
Tyramine	nq	nq	nq	nq	nq	nq	nq	nq
Agmatine	0.274 b	0.251 b	0.248 b	0.352 d	0.203 a	0.885 f	0.784 e	0.317 c
Spermidine	nq	nq	nq	nq	nq	nq	nq	nq
Tryptamine	0.180 a	0.192 a	0.220 b	0.226 b	0.243 c	0.214 b	0.225 b	0.220 b
Phenylethylamine	nq	nq	nq	nq	nq	nq	nq	0.108
Isoamylamine	nq	nq	nq	nq	nq	nq	nq	nq
Total amines	3.635 h	2.348 d	1.173 b	1.252 c	0.818 a	2.539 f	2.434 e	2.966 g
3 months after tirage								
Histamine	0.250 c	0.182 a	nq	nq	nq	nq	nq	0.209 b
Putrescine	2.598 h	1.886 f	0.568 c	0.482 b	0.317 a	1.011 e	0.946 d	2.089 g
Cadaverine	nq	nq	nq	nq	nq	nq	nq	nq
Tyramine	0.081 a	0.123 b	nq	nq	0.126 b	0.169 c	nq	nq
Agmatine	0.182 a	0.207 bc	0.219 c	0.221 cd	0.190 ab	0.244 d	0.200 abc	0.288 e
Spermidine	0.136 c	0.125 b	nq	nq	nq	nq	nq	0.103 a
Tryptamine	nq	nq	nq	nq	nq	nq	nq	0.088
Phenylethylamine	nq	nq	nq	nq	nq	nq	nq	0.092
Isoamylamine	nq	nq	nq	nq	nq	nq	nq	nq
Total amines	3.248 h	2.523 f	0.787 c	0.703 b	0.634 a	1.424 e	1.146 d	2.869 g
6 months after tirage								
Histamine	0.115 b	0.195 d	0.164 c	nq	0.226 e	nq	0.087 a	0.177 cd
Putrescine	3.408 h	1.641 f	0.681 c	0.621 b	0.470 a	1.113 e	0.957 d	2.556 g
Cadaverine	0.102 b	0.138 c	0.098 b	nq	0.098 b	0.150 d	nq	0.087 a
Tyramine	0.240 e	0.103 b	0.098 b	0.164 d	0.130 c	0.173 d	0.080 a	nq
Agmatine	0.317 d	0.211 c	0.151 b	0.382 e	0.203 c	0.703 f	0.704 f	0.097 a
Spermidine	0.097 a	0.300 c	0.104 a	nq	nq	0.247 b	nq	nq
Tryptamine	0.116 b	0.228 c	0.226 c	0.116 b	0.227 c	0.286 d	0.094 a	0.099 a
Phenylethylamine	0.137 c	0.142 cd	0.091 ab	0.091 ab	0.112 b	0.160 d	0.080 a	0.158 cd
Isoamylamine	0.090 b	0.114 d	0.104 c	nq	0.081 a	0.133 e	nq	nq
Total amines	4.621 h	3.073 f	1.718 c	1.374 a	1.546 b	2.966 e	2.001 d	3.173 g
9 months after tirage								
Histamine	0.295 e	0.137 a	nq	nq	nq	0.195 c	0.159 b	0.261 d
Putrescine	3.278 h	1.735 f	0.599 c	0.526 b	0.426 a	1.048 e	0.871 d	2.043 g
Cadaverine	0.156 d	0.152 cd	0.092 ab	nq	0.142 c	0.083 a	nq	0.102 b
Tyramine	0.156 b	0.149 b	nq	0.196 c	0.120 a	0.192 c	nq	0.276 d
Agmatine	0.237 b	0.219 b	0.215 b	0.144 a	0.263 c	0.216 b	0.164 a	0.277 c
Spermidine	0.143 b	0.202 c	nq	nq	0.110 a	0.144 b	0.097 a	0.298 d
Tryptamine	0.090 a	nq	0.082 a	nq	0.084 a	nq	nq	0.139 b
Phenylethylamine	0.133 c	0.113 b	nq	nq	0.092 a	nq	nq	0.129 c
Isoamylamine	nq	nq	nq	nq	nq	nq	nq	nq
Total amines	4.488 g	2.705 e	0.988 b	0.866 a	1.237 c	1.878 d	1.291 c	3.525 f

Values with different letter in the same row indicate statistically significant differences ($p < 0.05$)

* Indicates the second Garnacha grape variety used

[a] Below the quantification limit of the analytical method employed (<0.08 mg/L)

due to the release of amino acids during yeast autolysis [22, 38], some of them being precursors of biogenic amines, as well as the presence in the medium of decarboxylase-positive micro-organisms and/or decarboxylase enzymes [39].

It is difficult to establish the toxic limits of biogenic amines in wines, because they depend on several factors [40]. Therefore, up to date, there are no regulation limits in biogenic amines. However, different countries have established maximum limits only for the histamine levels, being the lower in Germany (2 mg/L). The OIV recommends not exceed of 12 mg/L.

In general, very low levels of biogenic amines were found in the wines studied, and these concentrations are far below of the limits that can cause toxic effects [41, 42]. Therefore, the concentration of biogenic amines found does not represent a negative effect on the quality of sparkling wines.

Conclusions

In summary, base and sparkling wines from *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* were the richest in most of the volatile compounds analyzed, especially of ethyl esters and alcohol acetates, compounds that contribute to the fruity aroma of wines. During the aging on lees in the bottle of sparkling wines, an increase in branched ethyl esters, ethyl lactate and γ-butyrolactone and a decrease in terpenes (mainly citronellol and geraniol) were observed.

The *Albarín* and *Prieto Picudo* wines showed the highest content of total biogenic amines, which coincides with the highest amino acid concentration found in these wines. However, the levels of biogenic amines in all the wines studied were very low, which does not represent a negative effect on the quality and safety of sparkling wines.

Considering these results and those found for other compounds and foam characteristics, the *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* were the most interesting varieties to elaborate natural sparkling wines, which will allow diversifying the wine production in the "Castilla y León" Community.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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4.3

Cambios en la composición de polisacáridos durante la vinificación y la crianza sobre lías de vinos espumosos

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Changes in polysaccharide composition during sparkling wine making and aging

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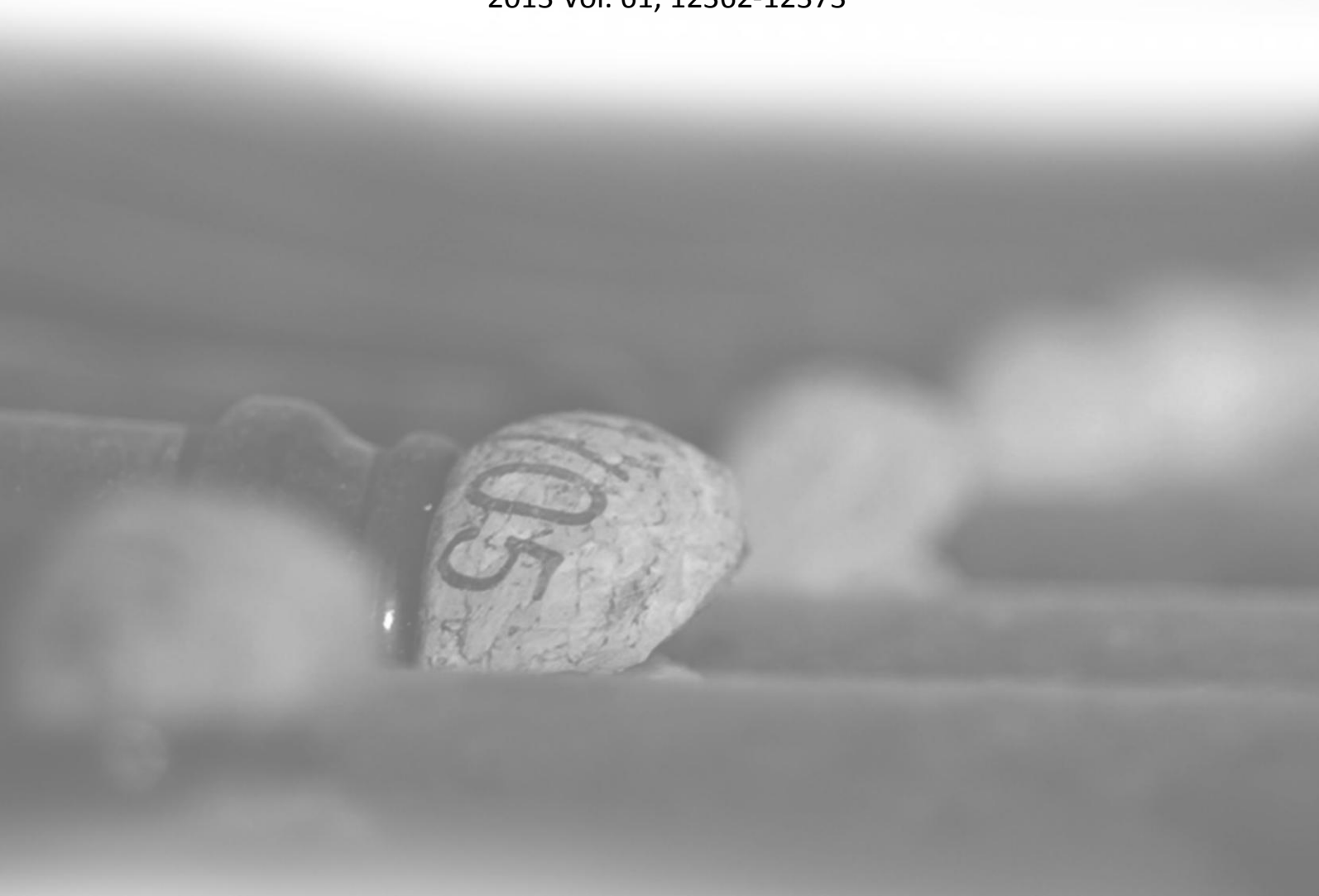

Resumen

Este artículo aborda dos objetivos fundamentales:

1. Caracterizar enológicamente los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional, en términos de familias de polisacáridos, de polisacáridos solubles totales, y de la distribución de sus pesos moleculares durante la vinificación y el envejecimiento sobre lías en botella.
2. Evaluar los cambios que se producen en dichos parámetros durante el proceso de elaboración de los vinos espumosos, incluyendo las etapas de vino base y de envejecimiento sobre lías en botella durante treinta meses.

El estudio se realizó con vinos espumosos de la cosecha 2009.

Se realizó un análisis de componentes principales (PCA) con el objetivo de encontrar diferenciación de los vinos espumosos según la variedad de uva empleada en su elaboración. La separación de los vinos en el espacio PCA no se debió a la variedad de uva empleada, si no que fue debida a la etapa de vinificación. Estos resultados indican que es el envejecimiento sobre lías y no la variedad de uva el factor que condiciona el perfil de monosacáridos y de familias de polisacáridos de los vinos espumosos.

Entre los polisacáridos de las levaduras, los glucanos (GL) fueron los polisacáridos mayoritarios en los vinos base. En general, el contenido de GL en los vinos incrementó desde los tres a los seis meses de envejecimiento en presencia de lías, mientras que el contenido en manoproteínas (MP) mostró aumentos durante los primeros seis meses de envejecimiento. Estos resultados indican que las MP fueron liberadas más fácilmente que los GL durante el proceso autolítico de las levaduras. Por otro lado, en periodos de envejecimiento sobre lías superiores a seis meses se observó que el contenido de MP y de GL permaneció constante o disminuyó gradualmente. La distribución de los pesos moleculares de los polisacáridos pareció indicar que la reducción en MP y en GL ocurrió principalmente en los compuestos de menor peso molecular. El ratio manosa/glucosa permaneció constante hasta los dieciocho meses de envejecimiento e incrementó de los dieciocho a los treinta meses, debiéndose este incremento a una mayor disminución en el contenido de glucosa que de manosa.

Con excepción de los vinos base de Prieto Picudo, el cual mostraba la familia de polisacáridos ramnogalacturonanos tipo II (RG-II), todos los vinos base presentaron polisacáridos ricos en arabinosa y galactosa (PRAG) y homogalacturonanos (HG). Los HG

y los RG-II disminuyeron durante los primeros seis meses de envejecimiento sobre lías y los PRAG se mantuvieron constantes. Periodos de envejecimiento sobre lías superiores a seis meses produjeron una reducción de todas las familias de polisacáridos procedentes de las uvas, siendo los descensos más acusados en los HG. El contenido de polisacáridos procedentes de las uvas estuvo relacionado positivamente con el contenido total de MP ($r=0,792$; $p<0,01$) durante el proceso de elaboración de los vinos espumosos.

El contenido de polisacáridos en los vinos fue independiente del color de las uvas y del tipo de vinificación aplicada (con o sin maceración prefermentativa). Así, entre los vinos base rosados, los elaborados con la variedad Prieto Picudo mostraron el mayor contenido de polisacáridos totales (446,36 ± 18,21 mg/L) y entre los vinos base blancos, destacaron los elaborados con la variedad Albarín (494,29 ± 37,72 mg/L). Sin embargo, los vinos base con el mayor contenido de polisacáridos totales mostraron un mayor descenso de estos compuestos durante el envejecimiento sobre lías. Entre los vinos espumosos finales, destacaron los elaborados con la variedad Garnacha por su mayor contenido de polisacáridos totales (223,11 ± 4,76 mg/L). Los vinos espumosos finales estuvieron formados principalmente por PRAG, MP, GL y HL con porcentajes del 35, 35, 25 y 4% respectivamente.

Los resultados obtenidos en este estudio muestran que el mayor contenido de MP y de PRAG fue obtenido a los seis meses de envejecimiento sobre lías. En este sentido, estos resultados parecen indicar que no son necesarios mayores tiempos de envejecimiento sobre lías para obtener mayores contenidos de polisacáridos en el vino. Por otro lado, a los seis meses de envejecimiento sobre lías se observó una disminución en los pesos moleculares de los polisacáridos del vino. La combinación de estos dos fenómenos podría implicar una mejor estabilidad de la espuma y por tanto, una mejor calidad de los vinos.

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Article

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Changes in Polysaccharide Composition during Sparkling Wine Making and Aging

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ABSTRACT: The evolution in polysaccharide composition and molecular weights during sparkling wine making and aging was studied for the first time in this work. Different autochthonous grape varieties from Spain (Verdejo, Viura, Malvasía, Albarín, Godello, Garnacha and Prieto Picudo) were used to elaborate sparkling wines following the champenoise method. Principal component analysis showed differentiation of wines according to polysaccharide families. This differentiation was due to the process of aging on yeast lees, but not to the variety employed. The content of mannoproteins during aging was positively correlated (r = 0.792) with total polysaccharides from grapes. After six months of aging the highest content of mannoproteins and polysaccharides rich in arabinose and galactose was obtained. Also a shift to lower molecular weights was observed. The combination of these two characteristics could imply a better foam stability and thus sensory quality of sparkling wines.

KEYWORDS: *sparkling wine, grape variety, polysaccharides rich in arabinose and galactose, homogalacturonans, rhamnogalacturonan II, mannoproteins, glucans*

INTRODUCTION

Polysaccharides are one of the main groups of macromolecules in wines. They come from grape berries, yeast, bacteria and fungal grape contamination such as *Botrytis cinerea*. From the enological and quantitative point of view, polysaccharides from grapes and yeast are the most important. Polysaccharides rich in arabinose and galactose (PRAGs) such as type II arabinogalactan-proteins (AGPs) and arabinans, rhamnogalacturonans type I (RG-I) and type II (RG-II), and homogalacturonans (HLs) come from grape berries, while glucans (GLs), mannans and mannoproteins (MPs) are released by yeast either during fermentation or by enzymatic action during aging on yeast lees by autolysis. Exogenous polysaccharides such as arabic gum and carboxymethyl cellulose could also be present in several commercial wines as they are authorized as additives.

Polysaccharides have an important influence on several stages of the winemaking process, including fermentation, filtration and stabilization.[1−3] They are in part responsible for the organoleptic properties of wines.[4−9] However, it has been shown that not all polysaccharides have the same behavior with respect to wines. Their influence on wine processing and sensory properties will depend not only on their quantity but also on the type of polysaccharide. It has been shown that AGPs have greater influence on the filtration procedures than MPs,[10] which are more efficient at reducing protein haze in white wines.[11] RG-II is a stronger accelerator of hydrogen tartrate crystallization than RG-I. RG-II has a concentration-dependent effect on hydrogen tartrate crystallization, accelerating crystallization at low concentrations and inhibition of it at high concentrations.[12] AGPs, on the other hand, have no effect on this phenomenon.[10] Besides, it has been recently shown that RG-II, MPs and AGPs have different influences on aggregation of proanthocyanidins[5] and, therefore, have varied influences on wine characteristics.[6] In the case of sparkling wines, some authors have correlated the foam properties of grape juices, base wines and sparkling wines with the polysaccharide content.[13−17] A connection between the molecular weight and composition of polysaccharides and foaming characteristics has been shown.[18,19] Some authors have even identified the importance of the type of polysaccharide on wine foam properties. Among wine polysaccharides, yeast mannoproteins released during autolysis have been associated with the improvement of foaming properties.[20−23] However it has been shown that not all mannoproteins have the same behavior.[21,22] The positive effect of mannoproteins on foam has been attributed to the presence of a balanced composition of hydrophobic and hydrophilic protein domains. This balance contributes to the creation of points of adsorption to the gas−liquid interface of the bubbles. In this way stability is increased.[21] Moreover, mannoproteins play other roles in sparkling wines since they contribute to the flocculation of yeast strains[24] and improve their elimination from the bottle during disgorging. Finally, these compounds could also serve as markers to follow the autolysis process because they are the major polysaccharides released by yeast.

Given the importance of polysaccharides in the sparkling wine making and sensory properties, an understanding of their content and kinetic release is essential. Different analytical methodologies have been developed to determine grape, must

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and wine polysaccharides. On the one hand, colorimetric methods[25] are frequently used to analyze the global content of neutral and acid polysaccharides. On the other hand, more complex and time-consuming methods based on gas chromatography are used to identify and quantify specific monosaccharides.[26−28] Previous studies have analyzed the evolution of polysaccharide families during the winemaking and aging of still wines.[4,8,29,30] Some research has been carried out on the evolution of neutral or total polysaccharides throughout the sparkling wine making process.[14,18,20] However, none of these studies analyzed the evolution of concrete polysaccharide families.

Therefore, this paper aims to analyze the changes occurring on monosaccharides, polysaccharide families and molecular weights of polysaccharides during the different stages of the sparkling wine processing by the traditional champenoise method. For this purpose different white (Verdejo, Viura, Malvasía, Albarín and Godello) and rosé (Garnacha and Prieto Picudo) sparkling wines were industrially manufactured with maintenance on yeast lees during 30 months. Chemometric techniques were applied to achieve a possible differentiation of the wines according to grape variety along with vinification stage and their monosaccharide and polysaccharide family composition.

MATERIALS AND METHODS

Chemicals. All reagents were analytical grade unless otherwise stated. Standards of different monosaccharides were used to perform the calibration curves. D-(+)-Fucose, L-rhamnose, 2-*O*-methyl-D-xylose, L-(+)-arabinose, D-(+)-galactose, D-(+)-glucose, D-(+)-mannose, Kdo (2-keto-3-deoxyoctonate ammonium salt) and D-apiose solution were supplied by Sigma-Aldrich (Beerse, Belgium), and D-(+)-galacturonic acid, D-glucuronic acid and myo-inositol (internal standard) were obtained from Fluka (Buch, Switzerland). Ethanol 96% (v/v) and acetyl chloride were supplied by Scharlab (Barcelona, Spain), hydrochloric acid 37% was purchased from Carlo Erba (Rodano, Milan, Italy) and hexane, dried methanol, pyridine, hexamethyldisilazane and trimethylclorosilane were obtained from Sigma-Aldrich (Beerse, Belgium). Lithium nitrate of HPLC grade supplied by Sigma (Beerse, Belgium) and Milli-Q deionized water (Millipore, Molsheim, France) were used. A pullulan calibration kit (Shodex P-82) was obtained from Waters (Barcelona, Spain).

Winemaking. All the sparkling wines in this study were manufactured using the traditional method champenoise from grapes from the 2009 harvest in the enological station of Castilla y León (Valladolid, Spain). Five white monovarietal and three rosé monovarietal base wines were prepared using the traditional winemaking process. White base wines were elaborated with *Vitis vinifera* cv. Verdejo and Viura grapes from the Rueda Denomination of Origin (D.O.), *Vitis vinifera* cv. Malvasía grapes from the Toro D.O., *Vitis vinifera* cv. Albarín grapes from the Tierras de León D.O. and *Vitis vinifera* cv. Godello grapes from the Bierzo D.O. Rosé base wines were obtained with *Vitis vinifera* cv. Prieto Picudo grapes from the Tierras de León D.O., and *Vitis vinifera* cv. grapes of Garnacha from the Cigales D.O. Two different viticultural areas of Garnacha were used in this work, and thus two different Garnacha wines were obtained, called Garnacha and Garnacha*, respectively. White grapes were destemmed-crushed and directly pressed to obtain juice. Red grapes were destemmed-crushed and left to prefermentative maceration for 2 days before getting the must. Base wines were made in stainless steel tanks of 150 L by duplicate at 16 to 18 °C after the addition of selected winery yeast strain. The wines were cold-stabilized and clarified, and finally they were bottled and the tirage liquor was added. The bottles were finally kept in the cellar at a temperature (11−13 °C) and relative humidity (75−78%) controlled for 30 months. Stirring was conducted at 29 months of aging in order to remove the lees. Samples for analyses were taken from the base wines (BW) and then after 3 months (T3M), 6 months (T6M), 9 months (T9M), 18 months (T18M) and 30 months (T30M) of aging on yeast lees. These sampling points were selected according to representative aging periods of sparkling wine categories: sparkling wine (≥9 months), Reserve (≥15 months) and Great Reserve (≥30 months). Wines were riddled and disgorged before analysis, and liqueur d'expédition was not added. Three bottles were analyzed at each disgorging time, and all the analyses were conducted in triplicate on wines after centrifugation.

Precipitation of Total Soluble Wine Polysaccharides. Wine polysaccharides were recovered by precipitation after ethanolic dehydration as previously described.[27] Samples were homogenized and centrifuged using a RC-6 Plus Sorvall refrigerated centrifuge (Du Pont, BH, Germany), and 2 mL of the supernatants were taken and introduced into 15 mL falcon-tubes to be concentrated to dryness in a Joan RC10-10 centrifugal evaporator (Fisher Scientific, Madrid, Spain). Polysaccharides were then precipitated by adding 2 mL of cold ethanol/acid (ethanol 96% containing 0.3 M HCl) and kept for 24 h at 4 °C. Thereafter, samples were centrifuged, the supernatants discarded and the pellets washed several times with 96% ethanol to remove the interference materials. The pellet, which corresponded to total soluble polysaccharides (TSP), was finally freeze-dried using a Virtis freeze-drying apparatus (New York, USA). This polysaccharide extraction was performed in triplicate in each sample.

Identification and Quantification of Monosaccharides by GC−MS. The monosaccharide composition of the TSP precipitates was determined by GC−MS of their trimethylsilyl-ester *O*-methyl glycolsyl-residues obtained after acidic methanolisis and derivatization as previously described.[27] GC was controlled by ChemStation software and equipped with a 7653B automatic injector consisting of an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector (MS). Samples were injected in duplicate. The content of each polysaccharide family in the wine samples was estimated from their concentration of individual glycosyl residues which are characteristic of structurally identified wine polysaccharides.[28,31] PRAGs, representing mainly arabinogalactan-proteins and arabinans in wines, were estimated from the sum of galactosyl, arabinosyl, rhamnosyl and glucuronosyl residues. All the mannose content was attributed to yeast mannoproteins (MPs), and all the glucose content was attributed to yeast glucans (GLs). The RG-II content was calculated from the sum of its diagnostic sugars (apiose, 2-*O*-methyl-l-fucose, 2-*O*-methyl-D-xylose, aceric acid (3-c-carboxy-5-deoxy-l-xylose), Kdo (3-deoxy octulosonic acid), and Dha (3-deoxy-D-lyxo heptusolaric acid)), which represent approximately 25% of the RG-II molecule. For one residue of 2-*O*-methyl fucose, RG-II contains 3.5 rhamnosyl, 2 arabinosyl, 2 galactosyl, 1 glucuronosyl and 9 galacturonosyl residues. Taking into account these molar ratios, it was possible to estimate their respective amounts in the RG-II. The remaining part was attributed to the presence of PRAGs in the case of rhamnose, arabinose and galactose; and the remaining galacturonosyl residues was used to estimate the content of oligomers of homogalacturonans (HLs). The content of total polysaccharides was estimated from the sum of PRAGs, MPs, GLs, RG-II and HLs.

Analysis of Polysaccharides by HRSEC-RID. A high-resolution size-exclusion chromatography (HRSEC) system with a refractive index detector was used to obtain the molecular weight distributions of the wine polysaccharides as previously described.[27] Two serial Shodex OHpack SB-803 and SB-805 columns (0.8 × 30 cm, Showa Denko, Japan) equilibrated at 1 mL/min in 0.1 M $LiNO_3$ were used. Chromatographic separation was carried out on an Agilent modular 1100 liquid chromatograph (Agilent Technologies, Waldbronn, Germany) connected to G1362 refractive index detector. Calibration was performed with narrow pullulan molecular weight standards (Shodex P-82, Waters, Barcelona, Spain): P-5, M_w = 5.9 kDa; P-10, M_w = 11.8 kDa; P-20, M_w = 22.8 kDa; P-50, M_w = 47.3 kDa; P-100, M_w = 112 kDa; P-200, M_w = 212 kDa, P-400, M_w = 404 kDa. The apparent molecular weights were deduced from the calibration equation log M_w = 11.027−0.410 tR (tR = column retention time at peak maximum, and r^2 = 0.999).

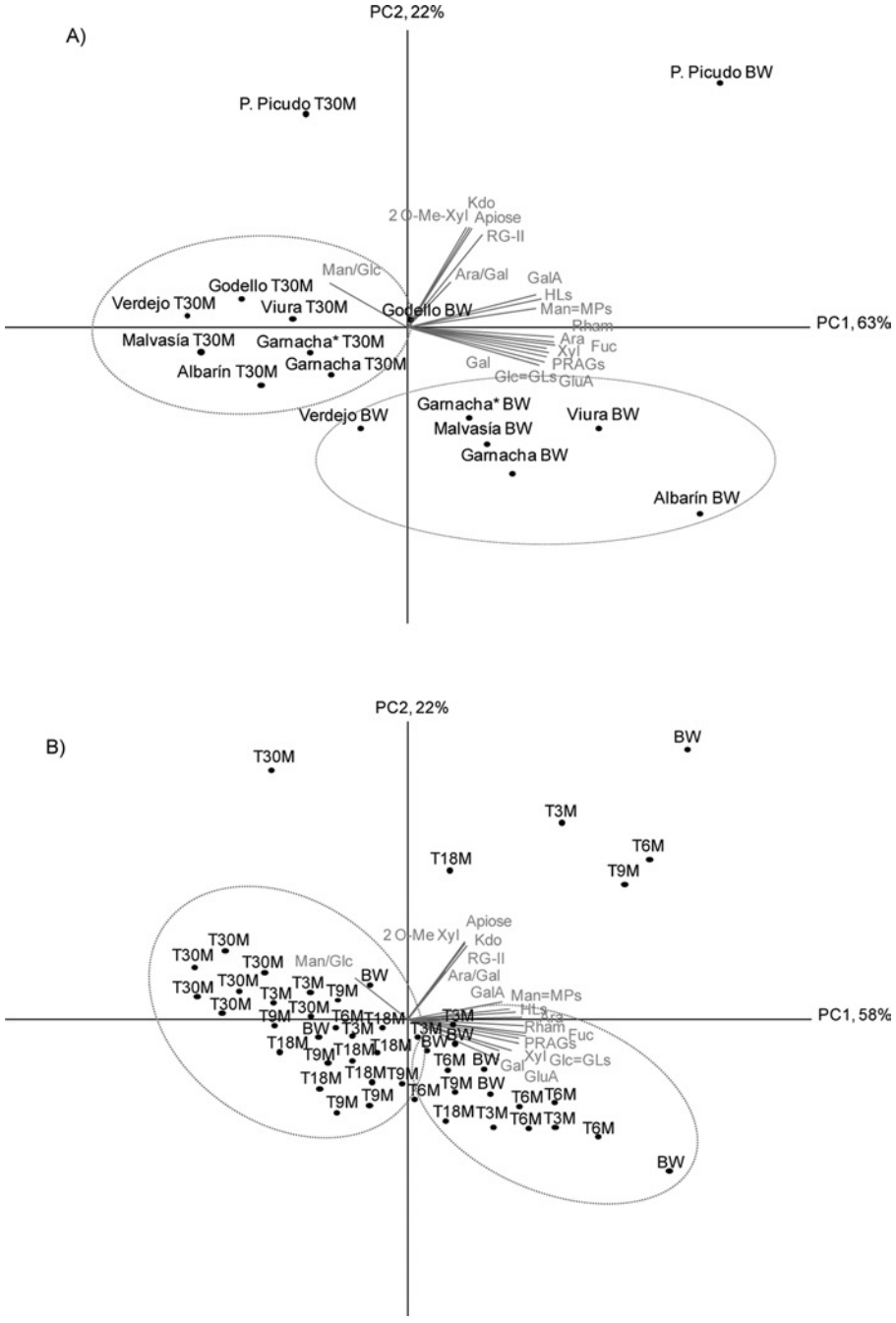

Figure 1. PCA of wines according to the winemaking stage: (A) base wines (BW) and sparkling wines after 30 months of aging on yeast lees (T30M); (B) base wines (BW), and sparkling wines after 3 months (T3M), 6 months (T6M), 9 months (T9M), 18 months (T18M) and 30 months (T30M) of aging on yeast lees. Ara, arabinose; Fuc, fucose; Man, mannose; Gal, galactose; GalA, galacturonic acid; Glc, glucose; Rham, rhamnose; GluA, glucuronic acid; Kdo, 2-keto-3-deoxyoctonate ammonium salt; 2 O-Me-Xyl, 2-*O*-methyl-D-xylose; MP, mannoproteins; PRAG, polysaccharides rich in arabinose and galactose; GL, glucans; HL, homogalacturonans; RG-II, rhamnogalacturonan type II; Ara/Gal ratio; Man/Glc ratio.

Statistical Analysis. Significant differences among samples were analyzed by an analysis of variance (ANOVA) if the data adhered to assumptions of normality. If these assumptions were not adhered to, nonparametric methods were used. Separate principal component analysis (PCA) was carried out on the values of monosaccharide composition, polysaccharide families, arabinose/galactose (Ara/Gal) and mannose/glucose (Man/Glc) ratio grouped according to grape variety and winemaking stage. ANOVA evaluations were performed using the Statistica 8.0 program for Microsoft Windows (Statsoft Inc., Tulsa, Oklahoma) and PCA analysis by using the Senstools Version 3.3.2. Program (Utrecht, The Netherlands).

RESULTS AND DISCUSSION

Differentiation of Sparkling Wines According to Monosaccharide Composition and Polysaccharide Families. Principal component analysis (PCA) was applied to achieve a possible differentiation of the wines according to the variety employed. Figure 1A shows the distribution of base wines and sparkling wines after 30 months of aging on yeast lees, and the monosaccharide composition and polysaccharide families' loads. The two first principal components explained 85% of the accumulative variance. Prieto Picudo wines were

Table 1. Evolution of Yeast Monosaccharides (mg/L) and Mannose/Glucose Ratio during Different Stages of the Sparkling Wine Production: Base Wines (BW), and Sparkling Wines after 3 Months (T3M), 6 Months (T6M), 9 Months (T9M), 18 Months (T18M), and 30 Months (T30M) of Aging in Bottle on Yeast Lees[a]

		Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
BW									
	glucose	146.20 ± 21.45 cd BC	90.61 ± 17.50 ab C	61.23 ± 18.74 a C	87.11 ± 4.97 ab C	49.30 ± 9.27 a B	187.45 ± 21.00 d C	120.64 ± 17.15 bc B	111.69 ± 8.50 bc BC
	mannose	93.62 ± 12.76 d BC	70.69 ± 7.03 c A	37.40 ± 1.25 ab A	55.18 ± 3.63 bc A	28.88 ± 2.63 a A	58.27 ± 6.93 c A	57.13 ± 0.97 c B	106.23 ± 7.68 d B
	mannose/glucose	0.77 ± 0.24 abc ABC	0.94 ± 0.17 bc A	0.73 ± 0.19 ab A	0.76 ± 0.06 abc A	0.70 ± 0.12 ab A	0.37 ± 0.08 a A	0.57 ± 0.07 ab AB	1.14 ± 0.10 c A
T3M									
	glucose	141.76 ± 15.00 cd BC	97.98 ± 12.76 b CD	31.80 ± 9.11 a AB	56.00 ± 12.00 a B	44.89 ± 5.61 a AB	169.89 ± 5.07 d BC	117.17 ± 10.73 bc B	109.95 ± 16.41 bc BC
	mannose	104.18 ± 8.05 cd BC	87.00 ± 0.09 bc B	39.67 ± 0.95 a A	68.00 ± 5.70 b A	44.30 ± 5.18 a B	67.28 ± 14.37 b A	73.55 ± 4.49 b C	120.00 ± 8.89 d BC
	mannose/glucose	0.88 ± 0.15 abc BC	1.07 ± 0.20 bcd A	1.50 ± 0.36 d B	1.46 ± 0.28 cd B	1.18 ± 0.17 bcd A	0.48 ± 0.09 a A	0.75 ± 0.07 ab AB	1.31 ± 0.18 bcd A
T6M									
	glucose	120.00 ± 22.68 ab B	118.00 ± 1.38 ab D	57.10 ± 1.28 a BC	76.09 ± 7.00 ab BC	75.14 ± 18.70 ab BC	210.00 ± 39.90 d C	191.47 ± 42.00 cd C	139.79 ± 17.00 bc C
	mannose	113.05 ± 15.22 de C	98.23 ± 5.61 d BC	42.92 ± 4.60 a A	69.79 ± 10.91 b A	68.00 ± 5.00 b C	94.92 ± 7.00 cd B	73.03 ± 4.24 bc C	134.84 ± 0.03 e C
	mannose/glucose	1.13 ± 0.22 c C	1.00 ± 0.05 c A	0.90 ± 0.08 bc A	1.10 ± 0.17 c AB	1.09 ± 0.23 c A	0.54 ± 0.09 ab AB	0.46 ± 0.09 a A	1.16 ± 0.12 c A
T9M									
	glucose	109.92 ± 14.19 bc AB	52.44 ± 3.47 a B	66.09 ± 7.18 a C	80.54 ± 7.65 ab C	84.76 ± 18.17 ab C	159.11 ± 14.02 d BC	112.66 ± 15.13 bc B	131.32 ± 23.48 cd C
	mannose	40.84 ± 3.02 a A	102.00 ± 2.87 d D	49.00 ± 1.39 ab B	61.00 ± 8.50 bc A	67.80 ± 3.98 c C	65.76 ± 6.23 c A	54.42 ± 1.69 abc B	104.02 ± 8.76 d B
	mannose/glucose	0.45 ± 0.06 a A	2.33 ± 0.14 d C	0.89 ± 0.08 bc A	0.91 ± 0.13 bc AB	0.96 ± 0.18 c A	0.50 ± 0.05 a A	0.58 ± 0.07 ab AB	0.95 ± 0.16 c A
T18M									
	glucose	164.78 ± 6.41 d C	52.69 ± 9.63 a B	57.20 ± 6.22 a BC	55.07 ± 7.00 a B	58.86 ± 10.12 a BC	114.14 ± 20.08 c AB	102.33 ± 15.33 bc B	75.60 ± 5.69 ab B
	mannose	77.87 ± 12.79 c B	68.79 ± 2.54 bc A	41.00 ± 0.56 a A	59.00 ± 4.52 b A	55.30 ± 4.11 ab B	69.39 ± 4.38 bc A	58.25 ± 2.77 b B	83.58 ± 4.28 c B
	mannose/glucose	0.57 ± 0.08 a AB	1.57 ± 0.24 d B	0.86 ± 0.08 ab A	1.29 ± 0.16 cd AB	1.13 ± 0.18 bc A	0.73 ± 0.11 ab B	0.68 ± 0.09 a AB	1.33 ± 0.10 cd A
T30M									
	glucose	70.00 ± 12.00 b A	24.28 ± 0.81 a A	20.64 ± 0.91 a A	26.30 ± 8.99 a A	13.56 ± 3.95 a A	65.84 ± 1.01 b A	24.48 ± 1.56 a A	17.01 ± 1.77 a A
	mannose	23.44 ± 1.30 a A	60.75 ± 3.56 e A	39.00 ± 1.71 cd A	29.27 ± 3.66 ab B	46.19 ± 2.37 d B	68.75 ± 0.86 e A	33.33 ± 2.26 bc A	42.75 ± 6.23 d A
	mannose/glucose	0.40 ± 0.06 a A	3.00 ± 0.17 cd D	2.27 ± 0.12 bc C	1.34 ± 0.41 ab AB	4.09 ± 1.01 c B	1.25 ± 0.02 ab C	1.63 ± 0.13 b C	3.02 ± 0.45 cd B

[a]Values are means ± SD ($n = 3$). Different lowercase letters in the same row indicate that means significantly differ at $p < 0.05$. Different capital letters in the same column indicate that means significantly differ at $p < 0.05$.

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widely separated from the rest of base and sparkling wines because they were highly related to the RG-II polysaccharide and their constituent monosaccharides. However, the rest of the varietal wines could not be separated in the PCA space according to the polysaccharide composition. On the contrary, the process of aging on lees affected the monosaccharide profile differentiation between varieties. Base wines were clearly separated from sparkling wines with 30 months of aging. Except for Man/Glc ratio, base wines were highly related to all studied loads, and the process of aging on yeast lees increased this ratio.

In order to check which stages of aging most influenced the polysaccharide composition of sparkling wines, a new PCA including all the stages was conducted (Figure 1B). Wines were properly located in the vectorial dimension defined by the first two factors, which accounted for 80% of the total variance in the PCA space. Wines were clearly differentiated according to their winemaking stage. There were no differences in the composition of the base wines and the wines obtained after 3 and 6 months of aging on yeast lees. These wines were highly related to all monosaccharide and polysaccharide families. On the contrary, wines after 9, 18, and 30 months of aging showed a weak relation with these compounds only being correlated with the Man/Glc ratio. Therefore, the final months of aging on yeast lees produced a movement of the wines in the PCA space, clearly marked by a decrease in all polysaccharide families but an increase in the Man/Glc ratio.

Evolution of Yeast Monosaccharides and Polysaccharide Families during Sparkling Wine Making and Aging. Table 1 shows the mannose and glucose content (mg/L) and the mannose/glucose ratio in base wines and sparkling wines over aging time. Between both sugars present in the wine glucose was usually found at a higher concentration. It represented more than 60% of the total content of mannose and glucose. Glucose is the prevalent sugar in grape berries[32] being that it is the main component of cellulose and hemicellulosic xyloglucans. However these structural polysaccharides are minor compounds in musts.[33] On the other hand, the presence of glucose in wines may also be related to microbial polysaccharides (*Botrytis cinerea*, *Oenococcus oeni*) or condensed anthocyanins. In this research, grapes were harvested in good sanitary conditions, malolactic fermentation was not conducted, and all wines showed very low anthocyanin content.[34] Therefore, it is reasonable to presume that all the glucose content in the wines was due to yeast glucans released during the fermentation. Thus, we used the content of glucose to estimate the quantity of glucans (GLs) in the same way that the quantity of mannose is used to estimate the quantity of mannoproteins (MPs).[28]

Release of mannoproteins and glucans during aging on yeast lees was attributed to the autolytic process from the yeast. Mannose content increased from 0 to 6 months of aging while glucose content increased only during the 3 to 6 month period of aging. This difference in the release time could be due to the fact that MPs in the cell wall of *Saccharomyces cerevisiae* are trapped or covalently linked to the GLs.[35] Thus MPs are released first by endo- and exo- β-(1,3)-glucanases, after which GLs are released. Therefore, the amount of MPs or GLs released could be regulated to the time in which a sparkling wine is in the bottle.

The content of MPs and GLs remained constant or decreased gradually over periods longer than 6 months. Thus, mannose and glucose concentration was lower in all final sparkling wines than in their corresponding base wines. In fact, the concentration of mannose and glucose were approximately 3 times higher in wines at 6 months of aging than in wines at 30 months of aging. These results contrasted with those obtained by other authors,[14,18] who observed an increase in neutral monosaccharides during 12 months of aging with yeast. This lack of increase of MPs and GLs may be attributed to different aspects. First, the autolytic conditions employed (low pH and low aging temperature, presence of ethanol, and high pressure of CO_2) and the lack of stirring of lees in sparkling wines during the aging time could have caused a reduction of the hydrolytic enzymes activities involved in the autolytic process and a lower release of yeast polysaccharides. Second, the precipitation rate of the released polysaccharides during this period was probably higher than their solubilization into the wine. Thus, decreases in the content of MPs and GLs were attributed to precipitation phenomena as a result of their interaction with other wine components to form unstable colloids. Although these interactions have not been studied regarding wine aging on lees, some authors have described the establishment of unstable colloids between MPs and other wine constituents in still wines at the end of maceration-fermentation.[9] The distribution of the molecular weights of polysaccharides (Figure 3) indicated decreases mainly affected compounds of low molecular weight. These results suggested that small MPs and GLs were more reactive with other wine components.

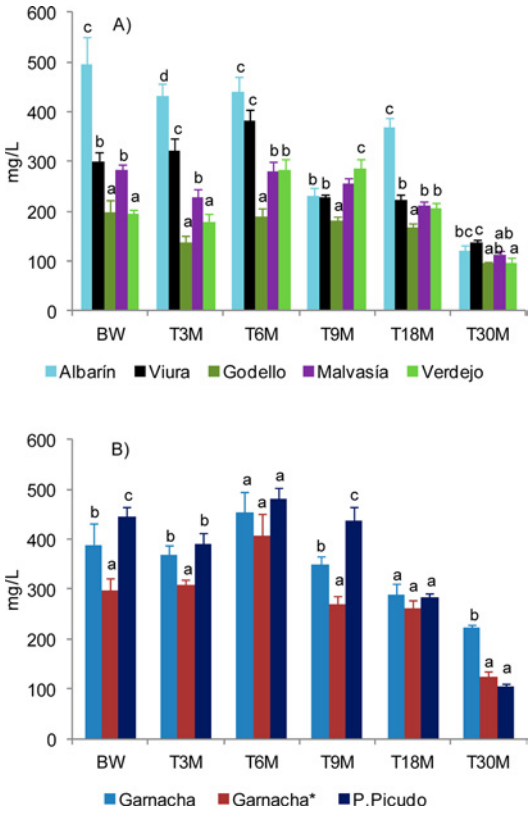

Figure 2. Evolution of total polysaccharide families in (A) white and (B) rosé sparkling wines over the aging time. Base wines (BW), and sparkling wines after 3 months (T3M), 6 months (T6M), 9 months (T9M), 18 months (T18M) and 30 months (T30M) of aging on yeast lees. Values are means ± SD (n = 3). Different letters in the same vinification stage represent means significantly different at $p < 0.05$.

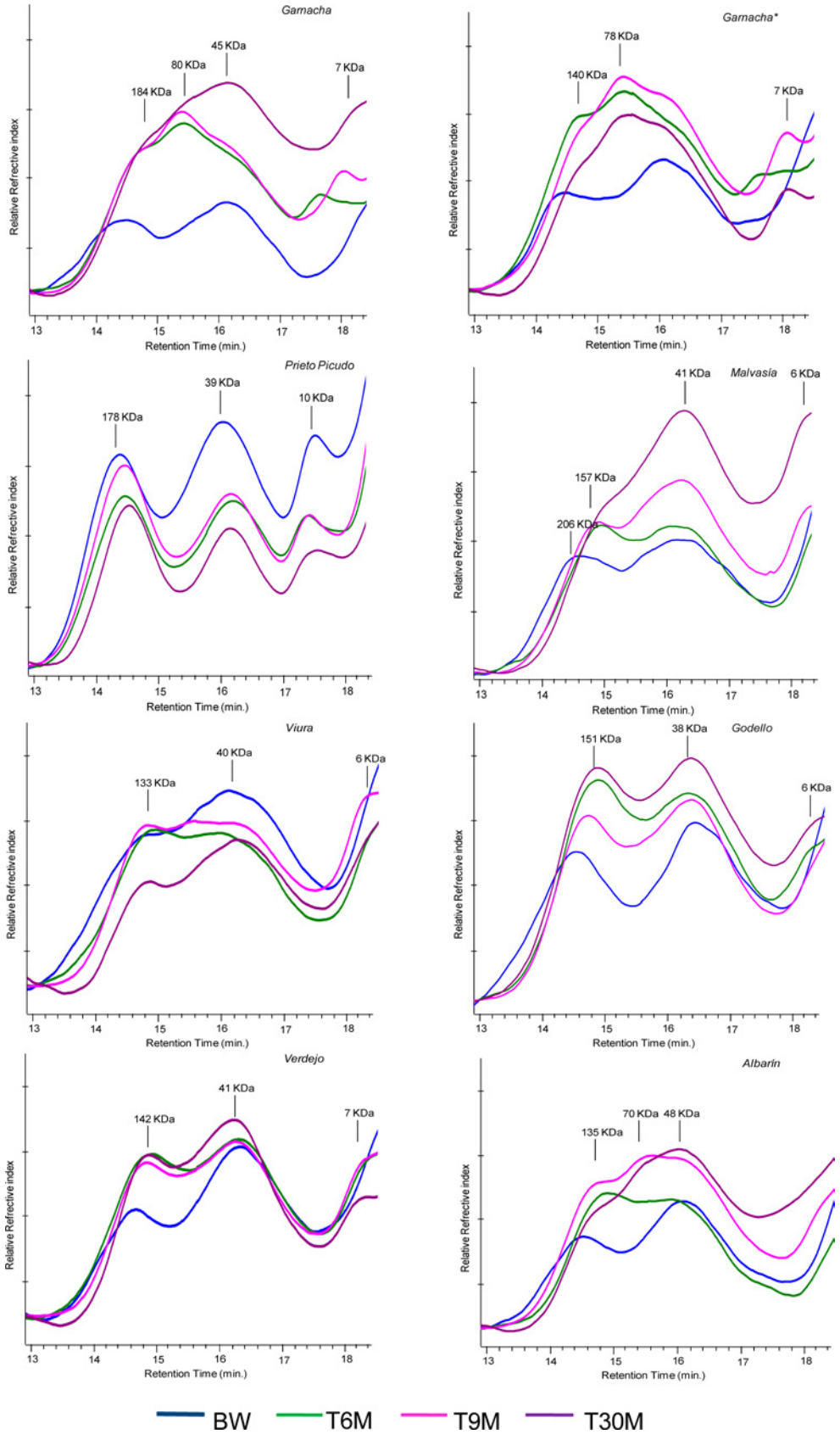

Figure 3. HRSEC-RID chromatograms of total soluble polysaccharides during the sparkling wine winemaking. Base wines (BW), and sparkling wines after 6 months (T6M), 9 months (T9M) and 30 months (T30M) of aging on yeast lees. Chromatograms obtained using two serial Shodex OHpack KB-803 and KB-805 columns.

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The mannose/glucose ratio (Man/Glc) remained constant until 18 months of aging, yet significantly increased from 18 to 30 months of aging (Table 1). Therefore, sparkling wines with 30 months of aging showed a Man/Glc ratio approximately 2.6 times higher than in the rest of the wines. Man/Glc increase from 18 to 30 months of aging was due to a significant reduction in the glucose content, indicating that GLs would form more unstable compounds susceptible to precipitation than MPs.

Evolution of Grape Monosaccharides and Polysaccharide Families during Sparkling Wine Making and Aging. The content of monosaccharides forming the grape polysaccharides and the arabinose/galactose ratio and polysaccharide families from grapes are shown in Table 2. These monosaccharides resulted from the breakdown and solubilization of native grape polysaccharides which were released by enzymatic degradation during the early steps of their processing to base wine.

Among grape monosaccharides, galactose and arabinose were the two most prevalently detected in all base wines samples (41 ± 19% and 26 ± 9%, respectively), indicating a high content of polysaccharides rich in arabinose and galactose (PRAGs). Galacturonic acid, which represented from 10 ± 1% to 37 ± 11%, was used as an indicator of homogalacturonans (HLs). Rhamnose and glucuronic acid were also detected in smaller amounts in wine samples as they also form PRAGs and rhamnogalacturonan type II (RG-II) polysaccharides. Rare sugars such as 2-*O*-methyl-xylose, apiose and Kdo were only detected in Prieto Picudo wines, indicating that the RG-II polysaccharide was only present in this wine. The absence of the RG-II molecule in all white wines was attributed to the winemaking process. RG-II is a molecule tightly bound to the cell wall matrix of grape cell walls, and it is resistant to pectinolytic enzymes. Therefore RG-II needs a longer maceration time to solubilize.[4,33] White base wines were elaborated without prefermentative maceration, and alcoholic fermentation was conducted in total absence of skin contact, which would prevent the extraction of RG-II into the wine. On the contrary, Prieto Picudo and both Garnacha base wines were given two days of prefermentative maceration before obtaining the musts. These rosé wines were elaborated with equal conditions of prefermentative maceration, alcoholic fermentation and grape maturity at time of harvest.[34] The differences observed with respect to RG-II molecule may be due to differences in the weakness of the grape skins that could modulate the extraction of wine components, which suggest a certain varietal characteristic.

Grape monosaccharides decreased similarly in all sparkling wines during the whole period of aging. Therefore, final sparkling wines had lower concentrations of all glycosyl residues than their corresponding base wines. All base wines were composed of grape PRAGs and HLs, which represented 75 ± 26% and 23 ± 18% of total polysaccharide families from grapes, respectively, except for Prieto Picudo base wines, which also contained the RG-II polysaccharide family. PRAGs were the most prevalent polysaccharide family, indicating that they were easily released into the wine by the action of endogenous enzymes as they are localized in soluble form within grape cell walls.[32] The proportion of HLs was higher than that observed by our group in still wines.[4,9] This fact was attributed to the concentration to dryness used to precipitate polysaccharides, which could have resulted in a higher concentration of oligosaccharides and HLs of low molecular weight.[27]

Similar concentrations of PRAGs and HLs were found in rosé base wines and in white base wines, thus indicating a lack of solubilization of these compounds during the prefermentative maceration in rosé base wines. As previously explained, RG-II extraction only occurred in Prieto Picudo base wines, in which it represented 5.5 ± 0.5% of total polysaccharides from grapes.

The evolution of various types of polysaccharide families was different during the stages of the sparkling wine processing. HLs and RG-II decreased during the first 6 months of aging, and PRAGs remained constant. Aging periods of more than 6 months prompted a considerable reduction in all polysaccharide families. As observed with MPs and GLs, grape polysaccharides also reacted with other wine compounds to form unstable colloids during long periods of aging on yeast lees. During this period of more than 6 months of aging, reductions in HLs were higher than in PRAGs and RG-II (86% vs 41%) in all sparkling wines, therefore, indicating a higher reactivity of HLs toward other wine constituents.

The arabinose/galactose ratio (Ara/Gal) is characteristic of the wine arabinogalactan-protein composition. Other authors have described aging on yeast lees produces a decrease in the Ara/Gal ratio because the terminal arabinose residues were removed. This reduction of arabinose residues indicates a dearabinosylation of arabinogalactan-proteins.[29] Although we also observed a significant decrease in this ratio for Viura and Verdejo sparkling wines, the ratio remained constant in the rest of the wines. Therefore, decisive conclusions could not be obtained.

Evolution of Total Polysaccharide Families during Sparkling Wine Making and Aging. Total monosaccharides were calculated as the sum of arabinose, fucose, mannose, galactose, galacturonic acid, glucose, rhamnose, glucuronic acid, 2-keto-3-deoxyoctonate ammonium salt and 2-*O*-methyl-D-xylose. Prieto Picudo had the highest value of total monosaccharides among rosé base wines (439.71 ± 18.21 mg/L) while Albarín base wines showed the highest value among white wines (488.24 ± 34.28 mg/L). Monosaccharide composition was similar in all base wines: it was composed of glucose, followed by galactose, mannose and arabinose. In the same way, monosaccharide composition was similar in all final wines, which were composed of mannose (35 ± 11%), followed by glucose (25 ± 15%), galactose (21 ± 13%) and arabinose (11 ± 5%). These percentages are in agreement with the composition of other sparkling wines obtained by different authors.[20,36]

Total polysaccharide families were calculated as the sum of MPs, GLs, PRAGs, HLs and RG-II (Figure 2). Among rosé base wines, Prieto Picudo showed the highest amount of total polysaccharides (446.36 ± 18.21 mg/L), whereas Albarín base wine showed the highest quantity among the white wines (494.29 ± 37.72 mg/L). However, base wines with the highest concentrations of polysaccharides had a greater drop in their polysaccharide content during aging, compared to base wines with low concentrations. Thus, total polysaccharides decreased 78 ± 6% in Prieto Picudo and 73 ± 9% in Albarín from 6 months of aging on, reaching similar final values as the rest of the sparkling wines. This fact suggests an important quantity of the extra polysaccharides precipitated during aging. Therefore, techniques employed to increase the extraction and release of polysaccharides during winemaking would not be as interesting as expected because the higher initial content of polysaccharides could be related to a higher precipitation. With regard to

Table 2. Evolution of Grape Monosaccharides and Polysaccharide Families (mg/L) and Arabinose/Galactose Ratio during Different Stages of the Sparkling Wine Production: Base Wines (BW), and Sparkling Wines after 3 Months (T3M), 6 Months (T6M), 9 Months (T9M), 18 Months (T18M), and 30 Months (T30M) of Aging in Bottle on Yeast Lees[a]

		Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
BW									
	arabinose	61.41 ± 9.60 e D	42.51 ± 1.95 bc C	24.71 ± 4.10 a B	31.83 ± 1.45 abc C	33.69 ± 1.65 abc B	43.32 ± 0.67 cd C	30.50 ± 0.04 ab B	54.73 ± 5.34 de C
	galactose	125.03 ± 20.2 c C	55.01 ± 0.71 b C	26.98 ± 9.12 a AB	59.84 ± 3.24 b B	61.93 ± 1.36 b CD	66.09 ± 3.36 b BC	50.21 ± 11.89 ab A	73.30 ± 6.83 b C
	rhamnose	18.07 ± 1.95 d D	11.95 ± 0.50 bc BC	8.96 ± 1.00 ab B	9.64 ± 0.76 ab C	4.09 ± 0.41 a AB	12.37 ± 4.21 bc BC	7.57 ± 2.19 ab AB	16.28 ± 1.89 cd C
	fucose	3.95 ± 0.48 e C	1.91 ± 0.05 bc CD	1.58 ± 0.24 b B	2.32 ± 0.11 cd C	0.79 ± 0.11 a A	1.66 ± 0.06 bc A	1.71 ± 0.21 bc AB	2.69 ± 0.26 d D
	galacturonic acid	30.51 ± 4.13 abc C	24.06 ± 0.70 abc BC	37.36 ± 9.35 c C	34.69 ± 8.09 bc B	11.96 ± 1.64 a AB	15.80 ± 2.56 ab B	27.28 ± 11.50 abc B	66.02 ± 11.00 d C
	glucuronic acid	9.46 ± 0.92 c C	5.85 ± 1.48 b BC	2.30 ± 1.46 a AB	5.17 ± 0.31 b B	3.84 ± 0.04 ab AB	5.30 ± 1.08 b A	4.07 ± 1.17 ab B	5.64 ± 0.64 b B
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.21 ± 0.06 B
	apiose	nd	nd	nd	nd	nd	nd	nd	0.75 ± 0.15 C
	Kdo	nd	nd	nd	nd	nd	nd	nd	2.16 ± 0.08 C
	arabinose/ galactose	0.59 ± 0.11 a A	0.93 ± 0.04 ab CD	1.10 ± 0.37 c B	0.64 ± 0.04 a A	0.65 ± 0.03 a B	0.79 ± 0.03 ab C	0.73 ± 0.14 ab A	0.90 ± 0.10 ab A
	PRAGs	204.22 ± 24.94 d D	109.13 ± 7.03 bc CD	57.35 ± 10.74 a B	101.17 ± 3.63 b B	104.03 ± 2.63 b CD	120.58 ± 3.59 bc D	88.91 ± 11.94 ab B	141.09 ± 8.72 c C
	HLs	40.25 ± 13.34 b C	30.25 ± 1.78 ab AB	42.97 ± 9.86 b B	40.02 ± 8.16 b B	11.48 ± 1.65 a BC	22.30 ± 2.63 ab B	30.71 ± 11.50 ab B	74.88 ± 12.74 c C
	RG-II	nd	nd	nd	nd	nd	nd	nd	12.46 ± 0.71 B
T3M									
	arabinose	43.11 ± 2.44 de C	43.25 ± 3.44 de C	15.63 ± 2.54 a A	28.00 ± 4.69 bc BC	23.97 ± 6.72 ab B	36.52 ± 5.00 cd BC	33.72 ± 2.16 bcd B	50.70 ± 5.41 e C
	galactose	97.01 ± 6.13 d C	50.06 ± 4.50 b C	28.33 ± 9.50 a AB	53.00 ± 9.87 bc B	35.71 ± 3.79 ab AB	69.26 ± 4.74 c C	50.25 ± 1.73 b A	69.00 ± 4.85 c BC
	rhamnose	12.28 ± 0.89 c C	9.94 ± 0.66 b AB	4.27 ± 0.05 a A	5.58 ± 0.80 a B	5.57 ± 0.16 a BC	10.07 ± 0.01 b ABC	8.53 ± 0.26 b AB	13.09 ± 1.24 c BC
	fucose	2.50 ± 0.47 b B	1.74 ± 0.30 ab BC	0.85 ± 0.12 a A	1.23 ± 0.80 a AB	1.12 ± 0.09 a AB	1.62 ± 0.39 ab A	1.81 ± 0.05 ab AB	1.88 ± 0.01 ab C
	galacturonic acid	24.42 ± 1.38 ab BC	27.00 ± 10.85 b BC	13.58 ± 5.38 a AB	11.55 ± 1.19 a A	21.05 ± 4.69 ab C	11.26 ± 0.35 a AB	20.25 ± 1.60 ab AB	15.57 ± 2.39 ab A
	glucuronic acid	7.89 ± 0.70 d C	5.20 ± 1.06 bc BC	3.08 ± 0.78 a ABC	4.74 ± 0.28 bc B	2.40 ± 0.21 a A	5.43 ± 0.03 c A	3.70 ± 0.30 ab B	4.98 ± 0.41 bc B
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.13 ± 0.00 AB
	apiose	nd	nd	nd	nd	nd	nd	nd	0.64 ± 0.02 BC
	Kdo	nd	nd	nd	nd	nd	nd	nd	1.42 ± 0.17 B
	arabinose/ galactose	0.53 ± 0.04 a A	1.04 ± 0.10 b D	0.66 ± 0.21 a AB	0.63 ± 0.13 a A	0.81 ± 0.20 ab B	0.63 ± 0.08 a ABC	0.81 ± 0.05 ab A	0.88 ± 0.09 ab A
	PRAGs	153.86 ± 6.64 f C	104.38 ± 5.78 cd C	49.17 ± 9.87 a AB	89.54 ± 10.95 bc B	65.33 ± 15.00 ab B	116.16 ± 6.92 de CD	92.24 ± 2.80 cd B	131.55 ± 7.31 ef C
	HLs	30.86 ± 2.54 b C	31.08 ± 11.02 b AB	15.73 ± 5.43 a A	13.34 ± 1.65 a A	23.37 ± 5.14 ab D	16.38 ± 3.49 ab AB	24.20 ± 1.97 ab AB	21.78 ± 5.15 ab A
	RG-II	nd	nd	nd	nd	nd	nd	nd	7.92 ± 0.68 AB
T6M									
	arabinose	49.55 ± 2.92 de CD	43.79 ± 9.89 cde C	16.95 ± 3.59 a AB	33.39 ± 0.22 cd C	33.00 ± 8.00 ab B	27.93 ± 2.37 ab A	35.80 ± 5.39 cd B	59.23 ± 6.88 e C
	galactose	107.22 ± 7.73 c C	69.08 ± 6.13 b D	32.94 ± 8.77 a AB	61.45 ± 12.00 b B	72.00 ± 10.00 b D	69.00 ± 4.00 b C	58.10 ± 12.00 b A	69.41 ± 2.25 b BC
	rhamnose	14.54 ± 1.15 bcd C	15.42 ± 4.00 cd C	7.96 ± 1.00 ab B	8.71 ± 1.99 abc C	6.53 ± 2.40 a BC	14.89 ± 3.20 bcd C	10.56 ± 1.92 abcd B	17.22 ± 2.51 d C
	fucose	3.16 ± 0.42 b BC	2.41 ± 0.30 ab E	1.49 ± 0.21 a B	2.06 ± 0.35 ab BC	1.51 ± 0.54 a BC	1.47 ± 0.69 a A	2.06 ± 0.48 ab B	2.58 ± 0.01 ab D
	galacturonic acid	26.89 ± 4.46 a BC	31.67 ± 10.41 ab C	27.52 ± 9.45 ab BC	25.09 ± 3.05 a B	20.55 ± 5.00 a C	26.97 ± 6.00 a C	32.05 ± 5.00 ab B	47.12 ± 9.62 b B
	glucuronic acid	8.99 ± 0.64 b C	6.63 ± 0.77 ab C	5.00 ± 0.80 a C	5.10 ± 1.15 a B	6.86 ± 1.80 ab C	8.87 ± 1.57 b B	4.92 ± 1.20 a B	3.91 ± 1.20 a AB
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.14 ± 0.05 AB
	apiose	nd	nd	nd	nd	nd	nd	nd	0.43 ± 0.05 A
	Kdo	nd	nd	nd	nd	nd	nd	nd	0.82 ± 0.02 A
	arabinose/ galactose	0.55 ± 0.04 a A	0.76 ± 0.15 ab ABC	0.62 ± 0.18 a AB	0.65 ± 0.11 a A	0.55 ± 0.13 a AB	0.49 ± 0.04 a A	0.74 ± 0.16 ab A	1.02 ± 0.10 b A

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Table 2. continued

		Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
	PRAGs	172.48 ± 8.30 d CD	125.44 ± 11.74 bc D	57.19 ± 9.52 a B	104.47 ± 16.00 b B	116.34 ± 12.98 bc D	109.59 ± 4.92 bc CD	103.67 ± 13.23 b B	140.58 ± 7.39 c C
	HLs	34.71 ± 5.43 ab C	41.15 ± 16.44 ab B	33.18 ± 9.84 ab B	29.27 ± 3.05 a B	22.61 ± 5.48 a D	38.07 ± 6.98 ab C	37.76 ± 6.51 ab B	56.31 ± 12.88 b BC
	RG-II	nd	nd	nd	nd	nd	nd	nd	8.29 ± 0.04 AB
T9M									
	arabinose	22.52 ± 2.55 ab B	17.58 ± 1.51 a AB	17.51 ± 2.12 a AB	32.92 ± 1.93 c C	34.89 ± 1.53 c B	35.37 ± 0.39 c B	29.68 ± 3.21 bc B	57.75 ± 5.52 d C
	galactose	43.61 ± 4.41 ab B	33.34 ± 1.79 a AB	35.43 ± 2.57 a AB	56.81 ± 0.35 bcd B	67.52 ± 7.11 de CD	58.89 ± 2.76 cde ABC	50.89 ± 3.95 bc A	71.26 ± 9.18 e BC
	rhamnose	5.10 ± 0.57 a B	6.59 ± 0.32 ab A	5.10 ± 0.32 a A	7.82 ± 0.09 ab BC	8.65 ± 1.58 b C	9.39 ± 0.61 b AB	7.57 ± 0.89 ab AB	17.31 ± 2.43 c C
	fucose	1.10 ± 0.10 bc A	0.94 ± 0.04 ab A	0.72 ± 0.02 a A	1.66 ± 0.00 de BC	1.79 ± 0.07 e C	1.71 ± 0.19 e A	1.36 ± 0.15 cd A	2.52 ± 0.13 f D
	galacturonic acid	5.28 ± 0.28 a A	12.81 ± 1.30 bc AB	5.80 ± 0.30 a A	9.96 ± 1.96 ab A	16.18 ± 2.15 c BC	15.57 ± 2.68 bc B	9.57 ± 1.51 ab A	42.11 ± 4.06 d B
	glucuronic acid	4.03 ± 0.54 a B	3.73 ± 0.65 a AB	3.19 ± 0.44 a ABC	5.50 ± 1.07 a B	5.54 ± 1.34 a BC	5.78 ± 0.59 a A	5.24 ± 0.32 a B	5.73 ± 2.00 a B
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.13 ± 0.03 AB
	apiose	nd	nd	nd	nd	nd	nd	nd	0.47 ± 0.03 AB
	Kdo	nd	nd	nd	nd	nd	nd	nd	0.78 ± 0.02 A
	arabinose/ galactose	0.62 ± 0.08 a A	0.63 ± 0.05 a AB	0.59 ± 0.07 a AB	0.70 ± 0.03 a A	0.62 ± 0.06 a AB	0.72 ± 0.03 a BC	0.70 ± 0.08 a A	0.97 ± 0.13 b A
	PRAGs	73.22 ± 5.13 b B	57.04 ± 2.44 a A	58.50 ± 3.37 ab B	99.70 ± 2.25 cd B	112.69 ± 7.40 d D	104.85 ± 2.85 cd BC	89.83 ± 5.12 c B	142.57 ± 10.92 e C
	HLs	7.33 ± 0.76 a AB	17.02 ± 1.56 bc A	8.53 ± 0.84 ab A	13.32 ± 2.15 abc A	20.10 ± 2.30 c CD	20.17 ± 2.69 c B	13.11 ± 2.16 abc A	51.59 ± 8.17 d B
	RG-II	nd	nd	nd	nd	nd	nd	nd	6.96 ± 1.17 AB
T18M									
	arabinose	25.84 ± 3.00 b B	27.37 ± 1.93 bc B	16.27 ± 2.30 a A	23.94 ± 1.85 b B	24.29 ± 2.66 b B	27.71 ± 1.79 bc A	28.36 ± 1.86 bc B	33.76 ± 2.47 c B
	galactose	67.53 ± 9.54 c B	40.04 ± 2.19 a B	40.09 ± 3.30 a B	53.98 ± 2.77 b B	50.96 ± 1.75 ab BC	55.97 ± 6.12 bc AB	51.29 ± 3.23 ab A	56.75 ± 2.36 bc B
	rhamnose	8.15 ± 1.09 cd B	7.93 ± 0.54 bcd AB	4.64 ± 0.32 a A	5.48 ± 0.54 ab B	5.78 ± 0.89 abc BC	7.41 ± 1.06 bcde AB	7.11 ± 0.77 bcd AB	9.52 ± 0.63 e B
	fucose	2.32 ± 0.30 b B	1.46 ± 0.20 a ABC	0.94 ± 0.09 a A	1.39 ± 0.23 a ABC	0.96 ± 0.04 a AB	1.28 ± 0.22 a A	1.23 ± 0.22 a A	1.48 ± 0.10 a B
	galacturonic acid	18.78 ± 7.95 b B	18.28 ± 5.00 b ABC	5.04 ± 0.64 a A	7.73 ± 0.41 a A	7.68 ± 0.59 a A	9.39 ± 1.51 ab AB	8.60 ± 1.40 a A	12.65 ± 0.65 ab A
	glucuronic acid	4.94 ± 0.38 ab B	6.85 ± 0.35 c C	4.07 ± 0.47 ab BC	5.43 ± 0.80 b B	3.69 ± 0.55 a AB	4.39 ± 0.45 ab A	4.80 ± 0.09 ab B	4.40 ± 0.58 ab AB
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.10 ± 0.01 A
	apiose	nd	nd	nd	nd	nd	nd	nd	0.42 ± 0.04 A
	Kdo	nd	nd	nd	nd	nd	nd	nd	0.82 ± 0.02 A
	arabinose/ galactose	0.46 ± 0.07 a A	0.82 ± 0.06 e BCD	0.49 ± 0.07 a A	0.53 ± 0.04 ab A	0.57 ± 0.05 abc AB	0.59 ± 0.06 abc AB	0.66 ± 0.05 bcd A	0.71 ± 0.05 cd A
	PRAGs	101.80 ± 10.02 d B	77.98 ± 2.95 b B	62.64 ± 4.06 a B	86.60 ± 3.43 bc B	82.24 ± 3.25 b BC	91.83 ± 6.39 bcd AB	88.30 ± 3.73 bcd B	99.49 ± 3.48 cd B
	HLs	23.43 ± 8.17 c BC	22.51 ± 5.12 bc AB	7.47 ± 1.00 a A	9.96 ± 0.69 a A	10.16 ± 1.07 a B	13.05 ± 1.75 ab AB	11.86 ± 1.62 a A	17.59 ± 1.78 abc A
	RG-II	nd	nd	nd	nd	nd	nd	nd	7.81 ± 0.05 AB
T30M									
	arabinose	7.03 ± 0.30 a A	12.86 ± 2.70 ab A	13.24 ± 1.83 ab A	15.80 ± 2.87 b A	7.16 ± 0.76 a A	25.79 ± 2.79 c A	15.44 ± 3.88 b A	11.99 ± 1.61 ab A
	galactose	14.12 ± 0.93 a A	27.50 ± 1.06 abc A	18.58 ± 0.82 ab A	31.22 ± 0.89 bc A	26.25 ± 8.89 abc A	47.70 ± 3.51 d A	36.65 ± 10.29 cd A	14.89 ± 3.10 a A
	rhamnose	1.67 ± 0.11 a A	5.11 ± 1.28 cd A	3.81 ± 0.57 bc A	2.06 ± 0.41 ab A	1.84 ± 0.21 a A	4.79 ± 0.66 cd A	5.64 ± 0.56 d A	2.36 ± 0.33 ab A
	fucose	0.52 ± 0.03 a A	1.23 ± 0.24 b AB	0.65 ± 0.20 a A	0.62 ± 0.10 a A	0.54 ± 0.04 a A	0.87 ± 0.16 ab A	1.23 ± 0.20 b A	0.50 ± 0.09 a A
	galacturonic acid	2.66 ± 0.21 a A	4.84 ± 0.87 bc A	nd	4.24 ± 1.04 ab A	nd	6.07 ± 0.60 bc A	6.38 ± 0.64 c A	6.57 ± 1.00 c A
	glucuronic acid	1.01 ± 0.15 a A	2.41 ± 0.12 b A	1.41 ± 0.33 a A	2.41 ± 0.65 b A	1.34 ± 0.36 a A	4.17 ± 0.18 c A	1.52 ± 0.07 a A	1.79 ± 0.19 ab A
	2-*O*-methyl xylose	nd	nd	nd	nd	nd	nd	nd	0.12 ± 0.02 A
	apiose	nd	nd	nd	nd	nd	nd	nd	0.39 ± 0.05 A
	Kdo	nd	nd	nd	nd	nd	nd	nd	0.62 ± 0.01 A

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Table 2. continued

	Albarín	Viura	Godello	Malvasía	Verdejo	Garnacha	Garnacha*	Prieto Picudo
arabinose/galactose	0.60 ± 0.04 ab A	0.56 ± 0.10 ab A	0.85 ± 0.10 bc AB	0.61 ± 0.09 ab A	0.33 ± 0.10 a A	0.65 ± 0.07 abc BC	0.51 ± 0.16 a A	0.97 ± 0.20 c A
PRAGs	23.12 ± 0.99 a A	44.51 ± 2.92 bcd A	35.02 ± 2.05 ab A	51.57 ± 3.10 cd A	35.72 ± 8.93 abc A	81.16 ± 4.50 e A	55.71 ± 11.01 d A	30.29 ± 3.50 ab A
HLs	3.37 ± 0.21 ab A	8.20 ± 1.51 c A	2.01 ± 0.50 ab A	4.16 ± 1.04 b A	0.87 ± 0.09 a A	7.37 ± 0.77 c A	9.92 ± 1.97 c A	7.30 ± 1.01 c A
RG-II	nd	nd	nd	nd	nd	nd	nd	6.32 ± 0.02 A

[a]Values are means ± SD (n = 3). Different lowercase letters in the same row indicate that means significantly differ at $p < 0.05$. Different capital letters in the same column indicate that means significantly differ at $p < 0.05$. nd: below detection limit.

final sparkling wines, Garnacha reached the highest content of total polysaccharides (223.11 ± 4.76 mg/L), followed distantly by Viura (137.74 ± 4.71 mg/L) and last by the rest of sparkling wines (<130 mg/L). These results indicated that the content of polysaccharides was independent of the color of the grapes and the type of winemaking (with or without prefermentative maceration). The values found were in the range described in other studies for sparkling wines.[14,17,18,20] Final sparkling wines were essentially composed of PRAGs, MPs, GLs and HLs, with average percentages of 35 ± 16%, 35 ± 11%, 25 ± 15% and 4 ± 2%, respectively. The sum of MPs and GLs (47−78% of total polysaccharide families) was higher than those found in still wines, obviously due to the lysis process during the aging period. To the best of our knowledge, there is no literature on this aspect relating sparkling wines, and this is the first time concrete polysaccharide families in these types of wines are described.

Despite the foam properties of sparkling wines being controlled by a large number of molecules that act in a synergistic way,[37] MPs released by yeast during autolysis are particularly important because their hydrophobic nature causes them to preferentially adsorb to the gas/liquid interface of foam bubbles.[38] On the other hand, PRAGs could also play an important role in the foam quality and stability due to its protein fraction. The results of our investigation indicated how the highest content of mannoproteins was obtained at 6 months of aging. We also observed how the content of polysaccharides coming from grapes was positively correlated with the content of MPs (r = 0.792; $p < 0.01$) during the entire winemaking and aging process. Therefore, the content of PRAGs and HLs also reached its highest concentrations after 6 months of aging. In this sense, these results suggest that longer aging time is not necessary to obtain greater amount of polysaccharides.

Distribution of the Molecular Weights of Polysaccharides during Sparkling Wine Making and Aging. HRSEC-RID on Shodex column allowed us to follow the qualitative changes in the molecular weight distribution of polysaccharides during sparkling wine making (Figure 3). Chromatograms of base wines were analyzed in order to establish differences due to variety. In this sense, Prieto Picudo base wines showed a different profile than the rest of the base wines. Prieto Picudo base wines were characterized by three populations that eluted at 14.2, 16.0, and 17.2 min and corresponded to fractions of 178, 39, and 10 kDa, respectively. According to the literature,[9,27,28,31,39] the first two populations corresponded to complex mixture of high and medium molecular weight PRAGs from grape berries and high and medium molecular weight MPs and GLs released by the yeast. The third population corresponded mainly to grape RG-II dimers, and also to low molecular weight PRAGs and MPs. The rest of base wines showed two major peaks eluting at 14.2 and 16.1 min. However, they did not show the presence of a third population. These results were in agreement with those obtained by GC−MS, illustrating how Prieto Picudo base wines had the RG-II polysaccharide family. Except for Prieto Picudo, all base wines showed a similar molecular weight distribution as that previously described in white musts.[33]

All samples showed a slight shift from higher to lower molecular weight polysaccharides from base wine to 6 months of aging on yeast lees. This could be attributed to the release of MPs and GLs of lower molecular weights due to the random breaking of the cell wall into a succession of different size

fragments. However, this could also be contributed to the hydrolysis of the macromolecules by exo-β-(1,3)-glucanases, α-manosidases and proteases[40] released into the wine. These results were in agreement with those of other researchers, who also observed a change to lower molecular weights in the polysaccharide size distribution during aging.[30,31,41−43] Moreover, the occurrence of peak tailing at ∼16 kDa was observed, thus, suggesting a partial degradation of the polysaccharides during aging over lees, and modification of their properties and solubilization.

Several authors have observed that small MPs inhibit tannin aggregation[5] and their efficiency as particle stabilizers decreases as their molecular weight increases.[44] Moreover, small MPs have also been shown to be responsible for tartaric stability.[45] The fraction responsible for the foaming properties in sparkling wines is constituted by MPs with a relative molecular weight between 10 and 30 kDa.[21]Therefore, the shift to lower molecular weight polysaccharides could result in an improvement of the wine colloidal stability and foam properties. As the tirage phase went on, no more shifts were observed.

In conclusion, it is important to point out that the highest amount of polysaccharides was obtained at 6 months of aging along with a change to lower molecular weights. These changes could imply a better foam stability and thus better sensory quality.

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Notes

The authors declare no competing financial interest.

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4.4

Variedad de uva, envejecimiento sobre lías y envejecimiento en botella después del degüelle: influencia en la composición volátil y en las propiedades espumantes de los vinos espumosos

Grape variety, aging on lees and aging in bottle after disgorging: influence on volatile composition and foamability of sparkling wines

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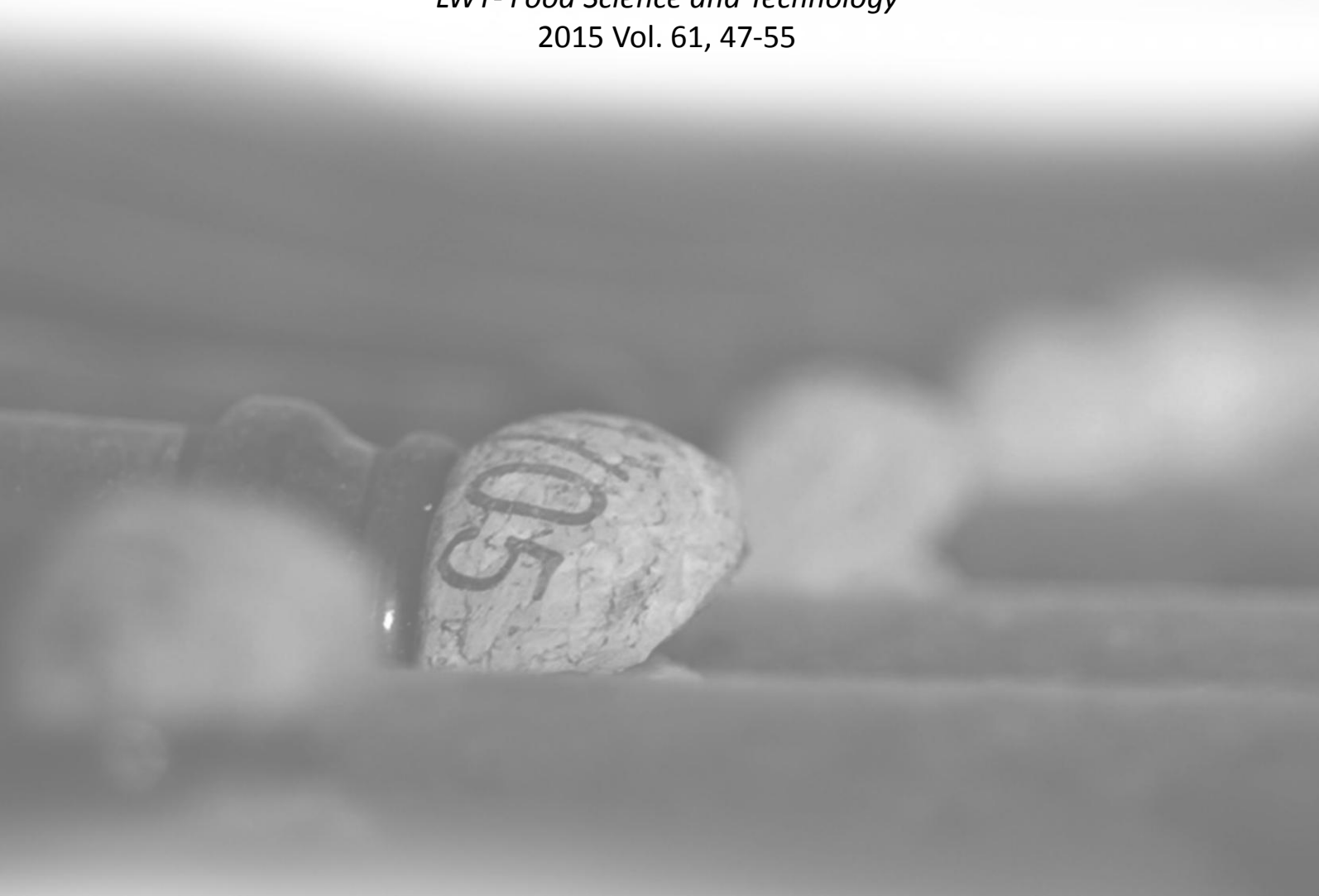

Resumen

Este artículo aborda dos objetivos fundamentales:

1. Estudiar la influencia de la variedad de uva y del tiempo de crianza en presencia y en ausencia de lías en la composición volátil y en las propiedades espumantes de los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional.
2. Evaluar los cambios que se producen en la composición volátil y en las propiedades espumantes de los vinos espumosos durante el envejecimiento sobre lías en botella de treinta meses y durante el envejecimiento en ausencia de lías tras de doce meses después del degüelle.

El estudio se realizó con vinos espumosos de la cosecha 2009. Se aplicaron técnicas de análisis multivariante con objeto de estudiar la influencia de la variedad de uva y del tiempo de envejecimiento en presencia y en ausencia de lías en la composición volátil de los vinos espumosos.

El análisis factorial permitió diferenciar los vinos varietalmente y por el tiempo de envejecimiento en botella en presencia y en ausencia lías en función de su composición volátil. Los vinos espumosos de Albarín, Verdejo, Godello y Prieto Picudo fueron los más ricos en la mayoría de los compuestos volátiles analizados, especialmente en ésteres etílicos y acetatos de alcoholes superiores, compuestos que contribuyen a los aromas frutales de los vinos. Durante el envejecimiento en presencia de lías se observó un aumento de los ésteres etílicos de ácidos grasos ramificados y un descenso de acetatos de alcoholes superiores y de terpenos. En general, los vinos envejecidos sobre lías durante nueve y dieciocho meses mostraron valores más altos de lactato de etilo y de alcoholes isoamílicos que los envejecidos durante treinta meses. Durante el envejecimiento en ausencia de lías se observó un descenso en los ésteres etílicos de ácidos grasos de cadena lineal, acetatos de alcoholes superiores, ácidos grasos, alcoholes C6 y terpenos, y un incremento en ésteres etílicos de ácidos grasos ramificados y vainillina. Aunque se observaron algunas diferencias entre los vinos espumosos en función del tiempo y tipo de envejecimiento, los resultados obtenidos indicaron que los vinos espumosos mantuvieron sus características varietales durante el envejecimiento en presencia y en ausencia de lías.

Las propiedades espumantes de los vinos fueron similares a las obtenidas en vinos espumosos de alta calidad, como *Champagne* y Cava y se mantuvieron estables o

mejoraron durante el envejecimiento en presencia de lías. El envejecimiento en ausencia de lías no provocó una disminución de la calidad de la espuma. Los vinos espumosos de Verdejo y de Prieto Picudo mostraron las mejores características espumantes durante el envejecimiento en presencia y en ausencia de lías, seguidos por los vinos de Albarín y de Godello.

Los resultados obtenidos en este estudio indicaron que las variedades Albarín, Verdejo, Godello y Prieto Picudo fueron las más apropiadas para la elaboración de vinos espumosos de calidad, pudiéndose incrementar el potencial enológico de estas variedades tradicionalmente empleadas para la elaboración de vinos tranquilos.

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Grape variety, aging on lees and aging in bottle after disgorging influence on volatile composition and foamability of sparkling wines

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ABSTRACT

The aim of this work was focused on the study of the influence of grape variety and aging time in contact with lees and without lees, on volatile composition and foamability of white and rosé sparkling wines. Seven different grape varieties were used and the sparkling wines were studied until 30 months of aging on lees and after 12 months of aging in bottle after disgorging.

Sparkling wines from *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* grape varieties were the richest in most of the volatile compounds analyzed, especially those that contribute to the fruity aroma of wines, and maintained their varietal characteristics even after long aging time (30 months). *Verdejo* and *Prieto Picudo* sparkling wines presented the best foam characteristics, followed by *Albarín* and *Godello* wines.

Considering all the results, *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* were the most interesting grape varieties to elaborate sparkling wines, following the traditional or "champenoise" method.

1. Introduction

Sparkling wines are obtained after a second fermentation of a base wine that can be carried out in closed bottles or in hermetically sealed tanks. High quality sparkling wines, such as Champagne wines in France, Cava wines in Spain or Talento in Italy, are fermented in closed bottles following the traditional or "champenoise" method, and they remain in contact with the yeast lees in a bottle for at least 9 months (EC Regulation N° 606/2009). The greatest differences among sparkling wines are mainly due to the grape varieties and the aging time on lees (Andres-Lacueva, Gallart, Lopez-Tamames, & Lamuela-Ravento;s, 1996; Pozo-Bayón, Martínez-Rodríguez, Pueyo, & Moreno-Arribas, 2009; Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006; Torrens, Riu-Aumatell, Vichi, López-Tamames, & Buxaderas, 2010).

During the sparkling wine aging, yeast autolysis leads to significant changes in wine composition (Alexandre and Guilloux-Benatier, 2006), and especially in the volatile compounds that could have a great effect on the final quality of these wines (Francioli, Torrens, Riu-Aumatell, López-Tamames, & Buxaderas, 2003; Pozo-Bayon, Pueyo, Martin-Alvarez, Martinez-Rodriguez, & Polo, 2003; Pozo-Bayón, Martín-Álvarez, Moreno-Arribas, Andujar-Ortiz, & Pueyo, 2010). During this process, different enzymatic and chemical reactions can lead to the formation or degradation of some volatile compounds, and others can be released into the wine (Del Barrio-Galán, Ortega-Heras, Sánchez-Iglesias, & Pérez-Magariño, 2012; Riu-Aumatell et al., 2006; Torrens et al., 2010), modifying the aroma profile of sparkling wines. On the other hand, some volatile compounds can be adsorbed on the yeast lees, reducing their concentration in sparkling aged wines, mainly the most hydrophobic ones (Gallardo-Chacón, Vichi, López-Tamames, & Buxaderas, 2009, 2010). Gallardo-Chacón et al. (2009) determined the volatile compounds retained by lees during the second fermentation of sparkling wines and found that esters, aldehydes and terpenes were retained by the lees surface. Sorption depends not only on the physicochemical characteristics of the volatile compounds but also on the structure of the yeast cell walls, hence the retention of volatile compounds by the lees surface can be reversible and the volatile composition of these wines can change over long aging time (Gallardo-Chacón et al., 2010). Therefore, the final aging time will determine the type and amount of the volatile compounds present in sparkling wines (Francioli et al., 2003;

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Table 1
Volatile compounds of the sparkling wines at the different aging times: T9, T18, T30: nine, eighteen, thirty months of aging on lees; 12 MB: twelve months in bottle after disgorging. Data in mg/L except those marked with an asterisk that are expressed in μg/L.

	Albarín					Verdejo				
	T9	T18	T30	T9+12 MB	T18 + 12 MB	T9	T18	T30	T9+12 MB	T18 + 12 MB
Ethyl butyrate	0.187	0.182	0.154	0.173	0.180	0.175	0.134	0.147	0.179	0.150
Ethyl 2-methylbutyrate	0.029	0.057	0.057	0.050	0.046	0.024	0.049	0.047	0.039	0.043
Ethyl isovalerate	0.051	0.070	0.084	0.097	0.074	0.041	0.059	0.070	0.068	0.066
Ethyl hexanoate	0.579	0.548	0.513	0.558	0.423	0.518	0.555	0.460	0.525	0.413
Ethyl lactate	19.7	23.7	22.8	18.3	20.9	17.4	22.4	20.1	14.7	19.1
Ethyl octanoate	0.592	0.501	0.480	0.557	0.380	0.558	0.566	0.443	0.563	0.402
Ethyl decanoate	0.068	0.052	0.036	0.084	0.027	0.059	0.058	0.040	0.077	0.035
Isoamyl acetate	0.273	0.226	0.150	0.175	0.117	0.434	0.367	0.156	0.180	0.127
2-Phenylethyl acetate	0.062	0.026	0.023	0.042	0.022	0.063	0.040	0.022	0.040	0.021
Isovaleric acid	1.247	1.100	1.078	1.468	0.990	1.141	1.186	1.101	1.177	1.059
Hexanoic acid	4.052	4.335	4.313	3.658	3.766	4.124	4.268	4.428	3.346	4.201
Octanoic acid	6.281	6.793	5.469	5.858	5.229	6.757	7.396	5.072	6.396	5.083
Decanoic acid	0.547	0.616	0.508	0.428	0.471	0.571	0.680	0.588	0.478	0.519
1-Hexanol	0.849	1.455	0.969	0.709	0.843	0.852	1.178	0.956	0.604	0.821
trans-3-hexen-1-ol	0.115	0.202	0.141	0.091	0.124	0.080	0.132	0.085	0.052	0.078
cis-3-hexen-1-ol	0.163	0.153	0.128	0.104	0.115	0.130	0.148	0.106	0.082	0.099
Benzyl alcohol	0.106	0.114	0.104	0.120	0.102	0.119	0.136	0.103	0.102	0.106
Linalool*	5.418	1.739	0.808	1.854	0.693	2.746	1.664	0.614	1.414	0.582
α-Terpineol*	9.828	9.303	8.336	10.835	8.133	3.960	4.678	3.108	4.501	3.396
Citronellol*	1.446	1.103	0.577	0.812	0.493	1.577	1.419	0.647	0.821	0.592
γ-Butyrolactone	10.30	13.58	9.45	9.57	9.72	14.25	12.29	10.27	11.54	11.03
γ-Nonalactone*	2.705	2.111	2.502	3.299	2.626	2.021	1.777	1.907	2.438	2.082
Vanillin*	2.799	nd	nd	9.260	17.24	1.949	nd	3.159	18.77	16.14
Methyl vanillate*	14.45	8.10	11.03	12.65	11.62	11.12	5.61	5.33	11.74	7.40
Ethyl vanillate*	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acetovanillone*	15.5	10.4	12.8	11.0	14.1	11.7	7.3	9.9	14.4	11.7
2-Phenylethanol	37.7	66.6	49.7	43.9	45.3	32.3	58.2	37.3	29.8	35.5
1-propanol	28.3	23.8	21.0	25.2	26.1	34.4	27.6	25.9	34.5	31.1
Isobutanol	22.3	19.7	19.0	19.8	19.5	24.0	24.0	21.7	26.8	23.4
Isoamyl alcohols	179	175	182	166	187	188	186	193	202	196
4-vinylguaiacol	0.155	0.120	0.178	0.213	0.197	0.433	0.166	0.125	0.219	0.121
	Godello					Viura				
	T9	T18	T30	T9+12 MB	T18 + 12 MB	T9	T18	T30	T9+12 MB	T18 + 12 MB
Ethyl butyrate	0.143	0.159	0.180	0.127	0.200	0.160	0.154	0.154	0.178	0.161
Ethyl 2-methylbutyrate	0.046	0.073	0.084	0.063	0.081	0.031	0.048	0.063	0.049	0.076
Ethyl isovalerate	0.066	0.080	0.109	0.098	0.109	0.048	0.056	0.085	0.083	0.104
Ethyl hexanoate	0.536	0.492	0.462	0.487	0.431	0.358	0.389	0.353	0.379	0.374
Ethyl lactate	16.9	21.1	19.2	14.3	17.5	18.4	25.8	23.6	16.6	30.0
Ethyl octanoate	0.566	0.520	0.468	0.511	0.408	0.312	0.353	0.302	0.359	0.310
Ethyl decanoate	0.080	0.066	0.050	0.087	0.040	0.049	0.044	0.034	0.070	0.031
Isoamyl acetate	0.258	0.203	0.136	0.138	0.128	0.183	0.163	0.093	0.095	0.109
2-Phenylethyl acetate	0.048	0.018	0.017	0.027	0.017	0.043	0.027	0.016	0.026	0.016
Isovaleric acid	1.315	1.459	1.271	1.349	1.121	1.251	1.094	1.198	1.211	2.312
Hexanoic acid	3.712	5.694	3.952	3.059	3.378	2.716	3.123	3.470	2.268	3.014
Octanoic acid	6.290	6.111	5.629	4.837	4.676	4.485	4.259	3.958	4.721	4.015
Decanoic acid	0.657	0.466	0.577	0.474	0.530	0.478	0.538	0.524	0.428	0.597
1-Hexanol	0.778	1.126	0.866	0.575	0.730	0.518	0.722	0.673	0.401	0.642
trans-3-hexen-1-ol	0.113	0.187	0.127	0.078	0.114	0.028	0.059	0.033	0.019	0.043
cis-3-hexen-1-ol	0.089	0.102	0.062	0.049	0.058	0.187	0.275	0.174	0.129	0.257
Benzyl alcohol	0.079	0.104	0.077	0.077	0.072	0.069	0.091	0.065	0.075	0.111
Linalool*	3.329	1.040	0.000	0.886	0.327	4.923	1.965	0.617	1.379	0.829
α-Terpineol*	4.497	3.294	3.382	4.802	3.616	4.716	4.572	4.048	5.863	6.048
Citronellol*	1.368	0.696	0.625	0.522	0.402	1.772	1.343	0.678	0.878	1.272
γ-Butyrolactone	15.56	15.62	11.42	11.37	11.92	13.12	15.13	10.82	7.83	18.68
γ-Nonalactone*	1.659	1.336	1.544	1.865	1.672	1.847	1.644	1.798	2.193	1.996
Vanillin*	1.441	nd	3.953	23.41	14.95	1.630	nd	3.055	23.27	31.83
Methyl vanillate*	5.61	1.92	2.53	5.91	3.26	5.80	1.38	2.35	6.42	6.95
Ethyl vanillate*	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acetovanillone*	5.1	3.2	3.9	4.6	4.7	14.4	9.6	12.3	16.9	18.3
2-Phenylethanol	36.4	65.0	44.8	32.0	41.5	30.8	52.1	41.0	27.9	52.1
1-propanol	20.5	18.3	13.9	19.5	17.8	24.3	20.6	19.8	23.3	22.9
Isobutanol	21.6	20.8	18.1	23.3	19.8	20.9	21.7	18.7	23.0	21.2
Isoamyl alcohols	203	205	202	218	211	170	174	183	182	187
4-vinylguaiacol	0.190	0.053	0.075	0.119	0.076	0.287	0.097	0.100	0.166	0.170
	Malvasía					Garnacha-A				
	T9	T18	T30	T9+12 MB	T18 + 12 MB	T9	T18	T30	T9+12 MB	T18 + 12 MB
Ethyl butyrate	0.117	0.137	0.113	0.154	0.142	0.165	0.183	0.151	0.180	0.149
Ethyl 2-methylbutyrate	0.033	0.065	0.064	0.055	0.066	0.032	0.063	0.061	0.049	0.056
Ethyl isovalerate	0.040	0.056	0.067	0.071	0.071	0.046	0.064	0.073	0.073	0.076

Table 1 (*continued*)

	Malvasía					Garnacha-A				
	T9	T18	T30	T9+12 MB	T18 + 12 MB	T9	T18	T30	T9+12 MB	T18 + 12 MB
Ethyl hexanoate	0.247	0.258	0.227	0.258	0.217	0.404	0.546	0.361	0.408	0.334
Ethyl lactate	11.2	16.3	14.6	10.7	22.2	17.3	18.9	19.8	14.2	16.2
Ethyl octanoate	0.199	0.222	0.173	0.205	0.144	0.386	0.495	0.317	0.387	0.308
Ethyl decanoate	0.033	0.027	0.019	0.042	0.017	0.067	0.058	0.032	0.079	0.033
Isoamyl acetate	0.198	0.186	0.098	0.121	0.096	0.192	0.154	0.097	0.107	0.087
2-Phenylethyl acetate	0.043	0.034	0.021	0.036	0.021	0.041	0.021	0.015	0.026	0.015
Isovaleric acid	1.186	1.294	1.142	1.349	1.671	0.863	1.014	0.952	1.198	0.833
Hexanoic acid	1.711	2.942	1.984	1.563	2.637	2.919	4.536	3.146	2.329	2.525
Octanoic acid	2.402	3.454	1.428	1.974	2.743	4.081	3.903	3.508	4.255	2.053
Decanoic acid	0.326	0.316	0.370	0.288	0.469	0.469	0.450	0.455	0.388	0.436
1-Hexanol	0.601	0.872	0.716	0.450	0.713	0.738	0.996	0.826	0.504	0.592
trans-3-hexen-1-ol	0.038	0.054	0.047	0.027	0.062	0.036	0.063	0.040	0.023	0.034
cis-3-hexen-1-ol	0.100	0.123	0.072	0.061	0.109	0.127	0.121	0.088	0.068	0.074
Benzyl alcohol	0.082	0.101	0.081	0.085	0.144	0.095	0.112	0.087	0.094	0.082
Linalool*	6.894	4.182	1.774	3.679	1.830	4.834	1.533	0.516	1.255	0.524
α-Terpineol*	7.584	8.489	6.412	10.466	9.896	5.241	5.606	3.992	6.564	4.483
Citronellol*	2.089	1.516	0.891	1.418	1.596	1.840	1.079	0.535	0.735	0.621
γ-Butyrolactone	13.65	12.39	10.71	12.44	17.72	11.57	12.96	9.81	7.37	9.85
γ-Nonalactone*	1.896	1.450	1.782	2.448	1.953	2.418	2.073	2.227	2.869	2.495
Vanillin*	1.276	nd	3.516	24.43	19.14	0.867	nd	4.983	22.81	20.00
Methyl vanillate*	10.99	5.76	6.09	12.40	13.62	19.39	8.49	11.18	27.63	14.79
Ethyl vanillate*	nd	nd	nd	nd	nd	3.44	1.48	2.79	6.01	3.87
Acetovanillone*	17.0	9.7	12.9	17.5	19.9	35.8	20.0	27.4	40.4	30.3
2-Phenylethanol	42.4	73.0	52.9	38.4	69.6	36.1	55.6	46.2	32.3	39.6
1-propanol	11.9	9.4	7.1	11.5	10.7	22.5	19.1	19.3	20.3	21.0
Isobutanol	15.1	14.5	11.7	17.2	14.5	19.6	18.5	17.8	19.2	18.7
Isoamyl alcohols	130	137	130	146	150	165	171	182	157	175
4-vinylguaiacol	0.202	0.077	0.103	0.195	0.169	0.078	0.051	0.078	0.122	0.090
	Garnacha-B					Prieto Picudo				
	T9	T18	T30	T9+12 MB	T18 + 12 MB	T9	T18	T30	T9+12 MB	T18 + 12 MB
Ethyl butyrate	0.142	0.103	0.134	0.161	0.153	0.184	0.178	0.170	0.189	0.173
Ethyl 2-methylbutyrate	0.023	0.026	0.046	0.038	0.049	0.026	0.051	0.050	0.041	0.052
Ethyl isovalerate	0.034	0.028	0.058	0.059	0.064	0.039	0.054	0.065	0.067	0.072
Ethyl hexanoate	0.344	0.299	0.315	0.375	0.306	0.399	0.603	0.369	0.411	0.363
Ethyl lactate	13.4	16.0	17.7	12.3	15.4	23.6	27.1	27.7	20.5	24.3
Ethyl octanoate	0.326	0.240	0.298	0.370	0.293	0.390	0.579	0.349	0.403	0.344
Ethyl decanoate	0.054	0.034	0.031	0.064	0.031	0.054	0.051	0.032	0.068	0.032
Isoamyl acetate	0.170	0.081	0.081	0.096	0.082	0.596	0.537	0.202	0.268	0.188
2-Phenylethyl acetate	0.039	0.020	0.015	0.026	0.015	0.136	0.083	0.048	0.088	0.046
Isovaleric acid	0.905	1.229	0.873	1.304	0.771	1.283	1.600	1.154	1.581	1.050
Hexanoic acid	2.456	3.643	2.910	2.016	2.517	3.251	4.398	3.381	2.565	2.937
Octanoic acid	4.276	4.461	3.578	3.699	2.345	4.451	4.430	3.694	4.109	2.114
Decanoic acid	0.458	0.684	0.456	0.376	0.433	0.449	0.575	0.457	0.387	0.421
1-Hexanol	0.673	0.846	0.758	0.459	0.599	0.475	0.854	0.519	0.365	0.428
trans-3-hexen-1-ol	0.034	0.048	0.041	0.020	0.035	0.055	0.098	0.057	0.037	0.049
cis-3-hexen-1-ol	0.115	0.112	0.085	0.059	0.076	0.109	0.116	0.078	0.066	0.068
Benzyl alcohol	0.094	0.116	0.092	0.082	0.086	0.099	0.114	0.089	0.095	0.080
Linalool*	4.948	1.596	0.783	1.571	0.746	6.675	3.381	1.797	3.531	1.786
α-Terpineol*	5.051	5.209	4.610	5.912	4.633	5.618	6.248	5.632	7.257	5.912
Citronellol*	2.028	1.217	0.700	0.814	0.756	2.510	2.265	1.300	1.806	1.212
γ-Butyrolactone	11.10	12.63	10.21	9.91	10.73	14.99	16.49	10.67	14.67	10.97
γ-Nonalactone*	2.287	1.876	2.141	2.746	2.361	1.990	1.727	1.839	2.496	2.016
Vanillin*	1.711	nd	3.690	25.71	27.27	2.409	nd	2.366	23.07	20.33
Methyl vanillate*	23.13	13.65	12.82	24.09	14.83	14.03	5.33	7.68	11.00	8.61
Ethyl vanillate*	3.24	1.70	2.91	5.49	3.77	16.53	7.83	11.03	16.15	14.45
Acetovanillone*	35.3	19.7	28.0	38.4	31.5	16.7	9.7	12.5	11.2	14.1
2-Phenylethanol	33.7	67.7	47.5	34.6	42.6	42.5	68.1	50.5	41.1	44.5
1-propanol	20.7	15.7	18.1	19.4	19.1	33.5	33.9	30.2	34.1	34.4
Isobutanol	18.3	15.8	18.4	19.4	18.7	29.2	30.3	26.1	27.8	28.3
Isoamyl alcohols	155	144	179	173	172	204	217	215	188	211
4-vinylguaiacol	0.089	0.068	0.095	0.115	0.101	0.036	0.099	0.120	0.182	0.149

nd: no detected.

Gallardo-Chacón et al., 2010; Riu-Aumatell et al., 2006), and it can last from a minimum of 9 months to years.

Foam is the characteristic that differentiates sparkling wines from still wines, being the first sensory attribute that tasters and consumers perceive and that determines the final quality of sparkling wines (Buxaderas & López-Tamames, 2012). The foaming properties mainly depend on the chemical composition of wines (Gallart, Lopez-Tamames, Suberbiola, & Buxaderas, 2002; López-Barajas, López-Tamames, Buxaderas, Tomás, & De La Torre, 1999; Moreno-Arribas, Pueyo, Nieto, Martín-Álvarez, & Polo, 2000), and different factors involved in wine composition will have an effect on foam quality, among them the grape variety used (Andrés-Lacueva et al., 1996; Cilindre, Liger-Belair, Villaume, Jeandet, & Marchal, 2010; Girbau-Solà, López-Barajas, López-Tamames, &

Table 2
Multifactor analysis of variance carried out considering all the data of all the sparkling wines studied.

	Grape variety		Aging time		Grape variety × aging time	
	F-ratio	*P*-value	F-ratio	*P*-value	F-ratio	*P*-value
Ethyl butyrate	49.8	**0.000**	15.0	**0.000**	11.8	**0.000**
Ethyl 2-methylbutyrate	141	**0.000**	287	**0.000**	9.1	**0.000**
Ethyl isovalerate	144	**0.000**	287	**0.000**	7.8	**0.000**
Ethyl hexanoate	243	**0.000**	71.1	**0.000**	10.6	**0.000**
Ethyl lactate	147	**0.000**	136	**0.000**	10.3	**0.000**
Ethyl octanoate	450	**0.000**	130	**0.000**	16.7	**0.000**
Ethyl decanoate	143	**0.000**	550	**0.000**	7.3	**0.000**
Isoamyl acetate	537	**0.000**	691	**0.000**	53.7	**0.000**
2-Phenylethyl acetate	570	**0.000**	602	**0.000**	28.3	**0.000**
Isovaleric acid	30.8	**0.000**	15.6	**0.000**	14.5	**0.000**
Hexanoic acid	181	**0.000**	186	**0.000**	10.8	**0.000**
Octanoic acid	349	**0.000**	128	**0.000**	10.8	**0.000**
Decanoic acid	7.9	**0.000**	6.6	**0.000**	1.4	0.142
1-Hexanol	164	**0.000**	401	**0.000**	8.2	**0.000**
trans-3-hexen-1-ol	623	**0.000**	315	**0.000**	15.4	**0.000**
cis-3-hexen-1-ol	801	**0.000**	481	**0.000**	35.2	**0.000**
Benzyl alcohol	62.3	**0.000**	65.4	**0.000**	18.0	**0.000**
Linalool	420	**0.000**	2242	**0.000**	21.7	**0.000**
α-Terpineol	391	**0.000**	85.3	**0.000**	8.2	**0.000**
Citronellol	357	**0.000**	870	**0.000**	20.7	**0.000**
γ-Butyrolactone	55.3	**0.000**	112	**0.000**	23.3	**0.000**
γ-Nonalactone	166	**0.000**	208	**0.000**	2.4	**0.001**
Vanillin	29.1	**0.000**	1397	**0.000**	17.0	**0.000**
Methyl vanillate	436	**0.000**	325	**0.000**	22.0	**0.000**
Ethyl vanillate	2215	**0.000**	108	**0.000**	39.8	**0.000**
Acetovanillone	2231	**0.000**	474	**0.000**	49.8	**0.000**
2-Phenylethanol	66.4	**0.000**	463	**0.000**	10.6	**0.000**
1-propanol	881	**0.000**	107	**0.000**	5.4	**0.000**
Isobutanol	547	**0.000**	71.3	**0.000**	8.4	**0.000**
Isoamyl alcohols	490	**0.000**	35.5	**0.000**	13.9	**0.000**
4-vinylguaiacol	440	**0.000**	513	**0.000**	138	**0.000**

Values in bold showed statistically significant differences in each compound and factor considered (*P*-values < 0.05).

Buxaderas, 2002; López-Barajas, López-Tamames, Buxaderas, & De La Torre, 1998). In addition, the foamability of sparkling wines can be influenced by the aging time on lees (Andres-Lacueva, Lamuela-Ravento;s, Buxaderas, & De La Torre-Boronat, 1997; Girbau-Sola et al., 2002; Moreno-Arribas et al., 2000).

However, few studies have been found in relation to the volatile composition of sparkling wines after aging times longer than 9 months (Francioli et al., 2003; Riu-Aumatell et al., 2006; Torrens et al., 2010), as well as the changes in their foamability (Andrés-Lacueva et al., 1996; Girbau-Sola et al., 2002), and no one has been found in relation to the changes that can occur in sparkling wines after disgorging, i.e. without lees.

Therefore, the aim of this work was focused on the study of the influence of grape variety and aging time in contact with lees and without lees, on volatile composition and foamability of white and rosé sparkling wines. Seven different grape varieties were used and the sparkling wines were studied until 30 months of aging on lees. In addition, the sparkling wines were also analyzed after 12 months in bottle after disgorging.

2. Material and methods

2.1. Winemaking process

The grape varieties used in this study were: *Verdejo* and *Viura* from the Designation of Origin (D.O.) Rueda, *Malvasía* from the D.O. Toro, *Albarín* from the D.O. Tierra de León and *Godello* from the D.O. Bierzo, for the elaboration of white sparkling wines, and *Prieto Picudo* from the D.O. Tierra de León and *Garnacha* from the D.O. Cigales for rosé sparkling wines. Two different vineyards of *Garnacha* grape variety were used. Only *Verdejo* and *Godello* grape varieties have been used to elaborate sparkling wines, but all of them produced high quality still wines in these D.O.s.

The base wines were elaborated in the experimental winery of the Enological Station sited in Rueda (Valladolid), following the traditional white or *rosé* winemaking process in stainless steel tanks of 150 L. The same commercial *Saccharomyces cerevisiae* yeasts (IOC 18-2007, Lallemand, Spain) were used.

The sparkling wines were elaborated following the traditional or "champenoise" method, therefore the base wines, after cold-stabilization (at −5 °C) and clarification (with PVPP and bentonite), were bottled and the tirage liquor was added. The tirage liquor were formed by yeast *S. cerevisiae* var. *bayanus* (0.30 g/L, IOC 18-2007 Lallemand, Spain), sucrose (23 g/L) and bentonite (0.10 g/L) (Laffort, France). After that, the bottles were kept in a cellar at a temperature of 11–13 °C and at a relative humidity of 75–85% controlled for 30 months. The pressure and residual sugars were measured periodically to control the second fermentation.

Sparkling wines were analyzed after 9, 18 and 30 months of aging on lees. These sampling points were selected according to representative aging periods of sparkling wine categories: sparkling wine (≥9 months), Reserve (≥15 months) and Great Reserve (≥30 months). In addition, the sparkling wines aged on lees for 9 and 18 months were also analyzed after 12 months in bottle after disgorging. Before the analyses, the wines were riddled and disgorged. Brut Nature sparkling wines were obtained, i.e. no expedition liqueur was added.

Therefore, eight sparkling wines were elaborated and were analyzed in five aging times.

2.2. Chemical reagents

The volatile compound standards were purchased from Fluka (Buchs, Switzerland) (ethyl butyrate, ethyl isovalerate, ethyl hexanoate, ethyl octanoate, ethyl lactate, 2-phenylethyl acetate, isobutanol, benzyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-propanol, 2-phenylethanol, 1-hexanol, *cis*-3-hexenol, hexanoic acid, octanoic acid, decanoic acid, isovaleric acid, γ-butyrolactone, citronellol, α-terpineol, vanillin); Sigma–Aldrich (Steinheim, Germany) (ethyl 2-methylbutyrate, ethyl decanoate, isoamyl acetate, *trans*-3-hexenol, γ-nonalactone, acetovanillone, linalool, methyl octanoate); and Lancaster (Strasbourg, France) (methyl vanillate, ethyl vanillate, 4-vinylguaiacol, 3,4-dimethylphenol).

The remaining reagents were supplied by Panreac (Madrid, Spain). Water Milli-Q was obtained via a Millipore system (Bedford, MA).

2.3. Analysis of the volatile compounds

Volatile compounds were extracted by liquid–liquid extraction following the method developed by Rodríguez-Bencomo, Ortega-Heras, & Pérez-Magariño (2010). 250 mL or two hundred and fifty mL of wine, 5 mL of dichloromethane, and 75 μL of a mixture of two internal IS standards (550 mg/L of methyl octanoate, and 450 mg/L of 3,4-dimethylphenol) were added to a flask. The extraction was carried out for 3 h with continuous stirring (150 rpm) in an orbital shaker. Chromatographic analyses were performed with a HP-6890N GC coupled to a HP-5973 inert MS detector equipped with a Quadrex 007CWBTR capillary column (60 m length, 0.25 mm i.d., and 0.25 μm film thickness), following the chromatographic conditions established by Rodríguez-Bencomo et al. (2010).

Quantification was carried out following the internal standard quantification method. Quantitative data of the relative areas

Table 3
Factor loadings after varimax rotation of the sparking wines aged on lees.

Compounds	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
Ethyl butyrate	0.547		0.563		−0.301			
Ethyl 2-methylbutyrate		**−0.714**		0.447		−0.400		
Ethyl isovalerate	0.274	**−0.793**		0.299	−0.281			
Ethyl hexanoate	**0.887**		0.333					
Ethyl lactate		−0.270	**0.809**					0.320
Ethyl octanoate	**0.907**		0.324					
Ethyl decanoate	**0.841**	0.399						
Isoamyl acetate	0.318	**0.625**	0.565					
2-Phenylethyl acetate		**0.745**	**0.572**					
Isovaleric acid				**0.807**				
Hexanoic acid	**0.742**	−0.291	0.267		0.317			
Octanoic acid	**0.841**					0.290		
Decanoic acid	0.435						−0.415	
1-Hexanol	**0.632**	−0.373			0.547			
trans-3-hexen-1-ol	**0.761**	−0.256		0.302	0.281			
cis-3-hexen-1-ol								**0.954**
Benzyl alcohol	0.434				**0.800**			
Linalool		**0.892**					0.301	
α-Terpineol							**0.894**	
Citronellol		**0.942**						
γ-Butyrolactone	0.263	0.561		**0.585**				
γ-Nonalactone				**−0.866**			0.325	
Vanillin	−0.275	−0.338			−0.556			−0.449
Methyl vanillate		0.453		**−0.829**				
Ethyl vanillate		0.396	**0.751**			−0.312		−0.277
Acetovanillone	−0.279			**−0.857**				
2-Phenylethanol				0.403	**0.733**	−0.419		
1-propanol	0.378	0.296	**0.784**			0.287		
Isobutanol	0.340	0.321	**0.838**					
Isoamyl alcohols	0.487		**0.733**					
4-vinylguaiacol						**0.883**		
Eigenvalue	8.83	6.53	3.34	3.15	2.17	1.44	1.40	1.05
Cumulative variance (%)	28.5	49.6	60.3	70.5	77.5	82.1	86.6	90.0

Loadings lower than absolute values of 0.250 are not shown.
Values in bold indicate the highest weight of each compound in each factor.

(absolute areas/internal standard area) were subsequently interpolated in the calibration graphs built from results of pure reference compounds (Pérez-Magariño, Ortega-Heras, Martínez-Lapuente, Guadalupe, & Ayestarán, 2013).

Three bottles of each varietal sparkling wine at each sampling time were analyzed, and one extraction for each bottle was carried out.

2.4. Measurement of foaming properties by instrumental method

The foam measurement of sparkling wines was carried out using the Mosalux procedure (Maujean, Poinsaut, Dantan, Brissonet, & Cossiez, 1990). This equipment consists in a glass cylinder with a glass frit in the bottom. This cylinder was filled with 100 mL of wine and CO_2 was injected through the glass frit at a rate of 7 L/h under a constant pressure of 1 bar for 15 min. Then the gas injection was stopped.

Three parameters were measured: HM (expressed in mm) was the maximum height reached by the foam after CO_2 injection that represents the foamability; HS (expressed in mm) was the foam stability height during CO_2 injection that represents the persistence of the foam collar; and TS (expressed in sec) was the foam stability time until all bubbles collapse after the CO_2 flow has stopped.

Three bottles of each varietal sparkling wine at each sampling time were analyzed, and three measurements for each bottle were carried out.

2.5. Statistical analyses

Multifactor analyses of variance were performed. Factor analysis was applied in order to study the association of volatile compounds and to determine similarities or differences between wines by grape variety or by aging time. Varimax rotation criterion was performed and only factors with eigenvalues greater than 1 were selected. Foam parameters were treated applying the variance analysis (ANOVA), and the Least Significant Difference test at significant level of $p < 0.05$. These statistical analyses were carried out using the Statgraphics Plus 5.0 statistical package.

3. Results and discussion

3.1. Volatile compounds of sparkling wines during aging on lees and aging in bottle after disgorging

Table 1 shows the data of volatile compounds of sparkling wines at the different aging times studied. Due to the high number of data, initially, multifactor analysis was carried out with all the data. Table 2 shows the effects of grape variety, aging time, and the interaction of grape variety-aging time for each compound. In general, it can be observed that there are strong grape variety and aging time effects on all volatile compounds evaluated.

Therefore, factorial analyses were carried out in order to study the influence of grape variety and aging time on the volatile profile of the sparkling wines elaborated. Two different factorial analyses were carried out, one considering only the sparkling wines aged on lees and other considering the wines aged on lees and those aged in bottle after disgorging (without lees).

The factorial analysis carried out with the sparkling wines only aged on lees selected eight factors with an eigenvalue greater than 1, which explained the 90.0% of the total variance. However, the first six factors were enough to explain more than 80% of total variability. Table 3 shows the loadings of each compound in each

one of the selected factors, as well as the eigenvalue and the cumulative variance of each factor. The compounds with higher loading values contribute most significantly to the explanatory meaning of the factors (marked in bold).

Fig. 1a shows the distribution of the different sparkling wines studied in the plane defined by the first two factors, which explained the 49.6% of the total variance. As it can be seen in this figure, the variables mainly associated with factor 2 allow differentiating the sparkling wines by the aging time on lees. The sparkling wines aged on lees for 9 months were sited in the positive zone of factor 2, and along the aging (18 and 30 months), the values of factor 2 decreased. This fact was due to the increase of ethyl esters of branched-chain fatty acids, and to the decrease of higher alcohol acetates and terpenes (mainly linalool and citronellol), compounds associated to factor 2 (Table 3). Rodríguez-Bencomo et al. (2010) also observed these changes in still red wine aged on lees, and Hidalgo et al. (2004) also found an increase of some ethyl esters of *Garnacha* rosé sparkling wines during the aging on lees for 9 months. In addition, Francioli et al. (2003) and Riu-Aumatell et al. (2006) observed lower concentrations of higher alcohol acetates and higher of TDN (1,1,6-trimethyl-l,2-dihydronaphthalene), vitispirane and diethylsuccinate in Cava wines aged on lees for long period of time (more than 20 months) than in young ones (9 months of aging). Torrens et al. (2010) also found a decrease of higher alcohol acetates along the aging of Cava wines (until 24 months of aging on lees). Riu-Aumatell et al. (2006) also asserted that these compounds could be used as age markers.

In general, wines aged on lees for 9 and 18 months showed slightly higher values of factor 3 than wines aged for 30 months in each grape variety (Fig. 1b), which was mainly due to the increase of ethyl lactate and isoamyl alcohols. These results agree with those obtained by Riu-Aumatell et al. (2006) and Torrens et al. (2010) in white and rosé Cava wines.

Considering the results obtained by Gallardo-Chacón et al. (2009 and 2010), the decrease of terpenes and ethyl esters of long-chain fatty acids could be due to their adsorption on the yeast cell walls, since they concluded that the most hydrophobic compounds were more retained by the lees surface.

On the other hand, the plane defined by the factors 1 and 3, which explained the 39.3% of the data variability, allows differentiating the wines by grape variety (Fig. 1b). *Prieto Picudo* sparkling wines showed the highest values of factor 3, which indicates that these wines had the highest concentrations of volatile compounds associated with factor 3, ethyl lactate, ethyl vanillate, and higher alcohols (Table 3). *Albarín*, *Verdejo and Godello* sparkling wines were the richest in volatile compounds associated with factor 1, ethyl esters of straight-chain fatty acids, fatty acids, 1-hexanol and *trans*-3-hexen-1-ol (Table 3). On the contrary, *Malvasía* sparkling wines were the poorest in volatile compounds associated with factors 1 and 3, followed by *Viura* and *Garnacha* sparkling wines. Higher alcohols, mainly 1-propanol and isoamyl alcohols, were also selected as volatile compounds to differentiate among varietal wines in different studies carried out in still wines (Pozo-Bayón, Pueyo, Martín-Álvarez, & Polo, 2001; Ortega-Heras, González-Huerta, Herrera, & González-Sanjosé, 2004; Tredoux et al., 2008).

Besides, there are other compounds, such as higher alcohol acetates and terpenes that were influenced by aging time, as it has been previously commented, but also they are influenced by grape variety (data not shown). In this way, Ferreira, López, & Cacho, 2000 concluded that the volatile compounds derived from yeast amino

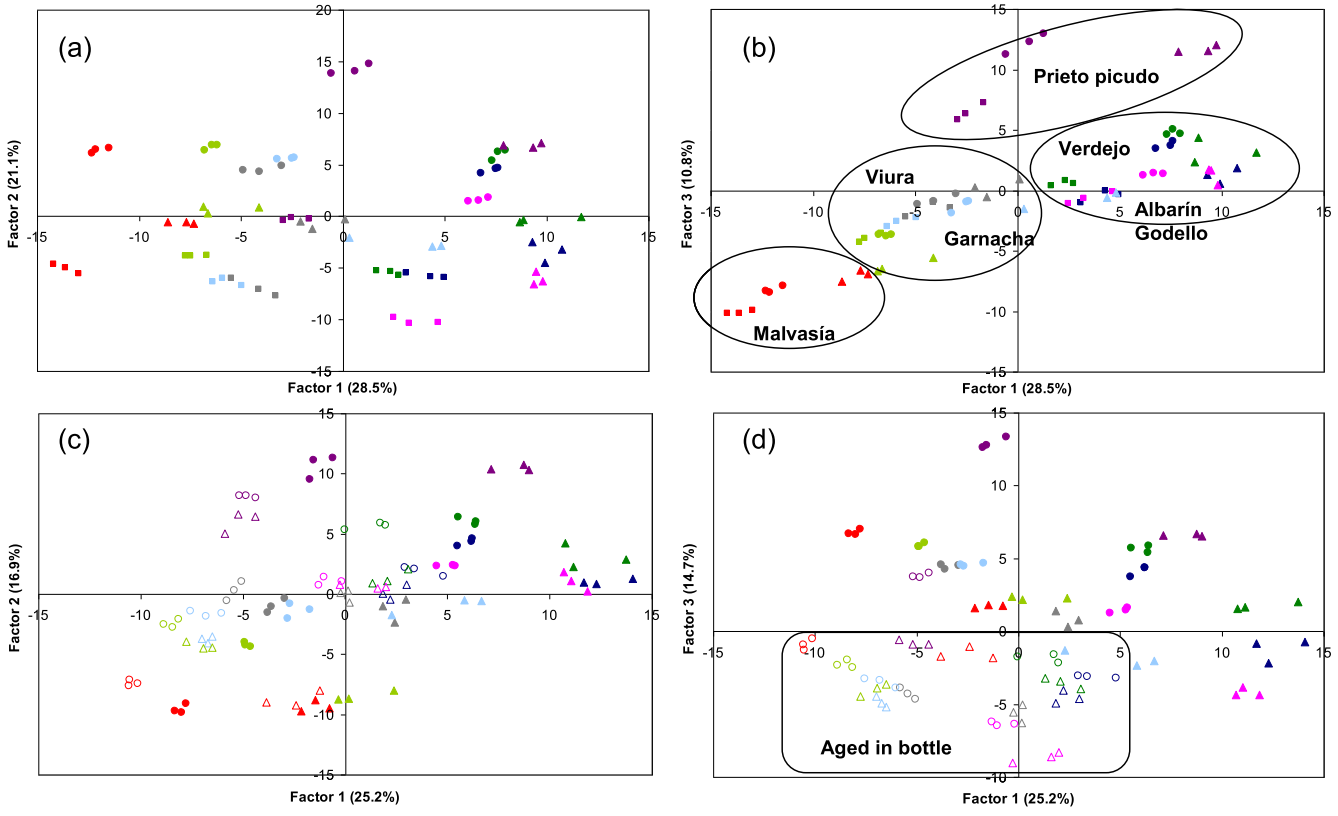

Fig. 1. Distribution of the sparkling wines aged on lees in the plane defined by (a) factor 1 and 2, and (b) factor 1 and 3; and of the sparkling wines aged on lees and in bottle after disgorging in the plane defined by (c) factor 1 and 2, and (d) factor 1 and 3. ● nine months of aging on lees, ▲ eighteen months of aging on lees, ■ thirty months of aging on lees, ○ nine months of aging on lees + twelve months of aging in bottle after disgorging, △ eighteen months of aging on lees + twelve months of aging in bottle after disgorging. ● Albarín ● Verdejo ● Godello ● Viura ● Malvasía ● Garnacha-A ● Garnacha-B ● Prieto Picudo.

Table 4
Factor loadings after varimax rotation of the sparking wines aged on lees and in bottle after disgorging.

Compounds	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
Ethyl butyrate		**0.704**					0.355	
Ethyl 2-methylbutyrate			**−0.695**	−0.416	0.308			−0.324
Ethyl isovalerate			**−0.842**					
Ethyl hexanoate	**0.668**	0.487				0.459		
Ethyl lactate	0.357	0.514			0.488	−0.489		
Ethyl octanoate	**0.610**	0.498				0.541		
Ethyl decanoate						**0.871**		
Isoamyl acetate	0.275	0.534	**0.668**					
2-Phenylethyl acetate		**0.574**	**0.728**					
Isovaleric acid					**0.903**			
Hexanoic acid	**0.855**	0.250		−0.257				
Octanoic acid	**0.736**					0.382		0.348
Decanoic acid	0.508				0.281		−0.509	
1-Hexanol	**0.927**							
trans-3-hexen-1-ol	**0.813**			−0.385				
cis-3-hexen-1-ol	0.287				0.328	−0.476		0.452
Benzyl alcohol	**0.650**			0.266	0.463			
Linalool			**0.899**					
α-Terpineol							**0.876**	
Citronellol			**0.895**		0.319			
γ-Butyrolactone			0.266	−0.390	**0.712**			
γ-Nonalactone				**0.739**			0.422	
Vanillin	**−0.592**		**−0.657**					
Methyl vanillate				**0.887**				
Ethyl vanillate	−0.362	**0.638**	0.323					−0.462
Acetovanillone	−0.328			**0.832**				
2-Phenylethanol	0.485				0.392	−0.449		−0.442
1-propanol		**0.895**						
Isobutanol		**0.904**						
Isoamyl alcohols		**0.749**	−0.253	−0.364			−0.297	
4-vinylguaiacol								**0.846**
Eigenvalue	7.83	5.24	4.56	3.09	2.20	1.77	1.51	1.00
Cumulative variance (%)	25.2	42.1	56.8	66.8	73.9	79.6	84.5	87.7

Loadings lower than absolute values of 0.250 are not shown.
Values in bold indicate the highest weight of each compound in each factor.

acid metabolism (higher alcohols, ethyl esters of isoacids and higher alcohol acetates) are the most important to differentiate wines by grape variety. *Prieto Picudo* sparkling wines presented the highest concentrations of higher alcohol acetates, which agrees with the higher concentrations in amino acids of this varietal wine (Pérez-Magariño et al., 2013), and terpenes, with the exception of α-terpineol. This fact corroborate the results of the study carried out by Álvarez-Pérez et al. (2012), who found that rosé wines from *Prieto Picudo* have a complex aromatic profile with a relatively high concentrations of ethyl esters and terpenes.

Therefore, the sparkling wines from *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* have in general higher concentrations of most of the volatile compounds quantified than the rest of the wines studied, especially of ethyl esters and higher alcohol acetates, compounds that contribute to the fruity aroma of wines (Ferreira, Fernández, & Cacho, 1996).

The results of factorial analysis carried out with the wines aged only on lees (for 9 and 18 months) and those aged on lees and bottle (for 9 and 18 months aged on lees and 12 months aged in bottle after disgorging) are shown in Table 4.

The distribution of the different sparkling wines studied in the plane defined by the first two factors, which explained the 42.1% of the total variance, permits to observe that the varietal characteristics of each wine were maintained both during the aging on lees and during the latter aging in bottle (without lees), as it is shown in Fig. 1c. The compounds associated with these factors are the responsible for the varietal differences of the wines, which were in general the same that those previously commented in the factorial analysis carried out with the sparkling wines aged only on lees.

On the other hand, the plane defined by the factors 1 and 3 that explained the 39.9% of the total variance, allows differentiating the sparkling wines aged on lees from those with additional aging in bottle (without lees), being the latter sited on the bottom of the plane (Fig. 1d). This means that the compounds associated positively with the factor 1 and 3, ethyl esters of straight-chain fatty acids, higher alcohol acetates, fatty acids, C6 alcohols and terpenes (mainly linalool and citronellol), decreased during the aging in bottle, while those associated negatively, ethyl esters of branched-chain fatty acids and vanillin increased (Table 4). Some of the changes observed during the aging in bottle of the sparkling wines agree with those found during the bottle aging of still wines (Díaz-Maroto, Schneider, & Baumes, 2005; Pérez-Coello, González-Viñas, García-Romero, Díaz-Maroto, & Cabezudo, 2003).

3.2. Foaming properties of sparkling wines during aging on lees and aging in bottle after disgorging

The mean values of the foam instrumental parameters of the sparkling wines during the aging on lees are shown in Table 5. After 9 months of aging on lees, *Verdejo* and *Prieto Picudo* sparkling wines presented the highest values of foam maximum height (HM), foam stability height (HS), and foam stability time (TS), followed by *Albarín* wines.

No studies have been reported in the literature focus on the foamability of sparkling wines elaborated from these grape varieties. Only some works have been found that study the foam properties of Cava or Champagne wines evaluated by Mosalux method (Andres-Lacueva et al., 1996, 1997; Girbau-Solà et al., 2002; Vanrell et al., 2007). Andres-Lacueva et al. (1996) and Vanrell et al. (2007) found that *Chardonnay* Cava wines showed higher HM values and lower TS values than *Macabeo*, *Xarel.lo* and *Parellada* Cava wines. The white sparkling wines aged on lees for 9 months of

Table 5
Foam parameters determined by the Mosalux method and ANOVA results of sparkling wines aged on lees.

	Grape variety	T9[a]	T18[a]	T30[a]
HM[b] (mm)	Verdejo	97 a D	121 b E	129 b D
	Godello	77 a BC	113 b DE	117 b C
	Malvasía	64 a A	85 b A	104 c A
	Albarín	82 a C	112 b DE	108 b AB
	Viura	74 a B	109 b CD	103 b A
	Prieto Picudo	96 a D	120 b DE	119 b C
	Garnacha-A	72 a B	96 b A	107 b AB
	Garnacha-B	70 a AB	100 b B	113 b BC
HS[b] (mm)	Verdejo	39.3 b D	27.1 a D	47.1 c D
	Godello	13.0 a A	12.7 a B	17.8 b A
	Malvasía	13.0 b A	9.6 a A	15.3 c A
	Albarín	19.8 ab B	16.7 a C	24.1 b B
	Viura	13.7 a A	12.1 a B	16.4 b A
	Prieto Picudo	30.0 a C	30.8 a E	36.7 b C
	Garnacha-A	14.0 b A	11.6 a B	15.3 b A
	Garnacha-B	13.6 b A	11.2 a B	15.2 c A
TS[b] (sec)	Verdejo	132 a C	212 b D	205 b C
	Godello	5.8 a A	6.9 b A	7.8 c A
	Malvasía	5.5 b A	4.7 a A	7.0 c A
	Albarín	8.0 a A	32.8 b B	11.3 a A
	Viura	5.6 a A	6.7 b A	7.6 c A
	Prieto Picudo	101 a B	162 b C	156 b B
	Garnacha-A	6.3 a A	7.8 b A	7.3 b A
	Garnacha-B	5.9 a A	6.6 ab A	7.2 b A

Values with different small letters in each grape variety and each parameter or with different capital letters in each aging time and each parameter indicate statistically significant differences at $p < 0.05$.
[a] T9, T18, T30: nine, eighteen, thirty months of aging on lees.
[b] HM: foam maximum height; HS: foam stability height; TS: foam stability time.

Table 6
Foam parameters determined by the Mosalux method and ANOVA results of sparkling wines aged on lees and in bottle after disgorging.

	Grape variety	T9[a]	T18[a]	T9 +12 MB[a]	T18 + 12 MB[a]
HM[b] (mm)	Verdejo	97 a D	121 b E	117 b B	119 b B
	Godello	77 a BC	113 d DE	90 b A	103 c A
	Malvasía	64 a A	85 b A	85 b A	100 c A
	Albarín	82 a C	112 bc DE	127 d C	119 cd B
	Viura	74 a B	109 b CD	111 b B	105 b A
	Prieto Picudo	96 a D	120 bc DE	128 c C	115 b B
	Garnacha-A	72 a B	96 b AB	124 d BC	106 c A
	Garnacha-B	70 a AB	100 b BC	127 c C	100 b A
HS[b] (mm)	Verdejo	39.3 b D	27.1 a D	25.4 a E	37.0 b B
	Godello	13.0 a A	12.7 a B	11.9 a AB	15.8 b A
	Malvasía	13.0 b A	9.6 a A	10.9 a A	17.3 c A
	Albarín	19.8 a B	16.7 a C	18.7 a D	34.0 b B
	Viura	13.7 a A	12.1 a B	12.6 a BC	16.5 b A
	Prieto Picudo	30.0 a C	30.8 ab E	32.9 b F	42.4 c C
	Garnacha-A	14.0 b A	11.6 a B	13.0 ab C	16.0 c A
	Garnacha-B	13.6 b A	11.2 a B	13.2 b C	14.7 c A
TS[b] (sec)	Verdejo	132 a C	212 b D	137 a B	200 b C
	Godello	5.8 a A	6.9 b A	6.1 a A	7.0 b A
	Malvasía	5.5 b A	4.7 a A	6.1 b A	7.3 c A
	Albarín	8.0 a A	32.8 b B	14.6 a A	33.7 b B
	Viura	5.6 a A	6.7 b A	6.6 b A	7.7 c A
	Prieto Picudo	101 a B	162 b C	176 b C	227 c D
	Garnacha-A	6.3 a A	7.8 b A	7.7 b A	7.7 b A
	Garnacha-B	5.9 a A	6.6 ab A	7.3 b A	6.8 b A

Values with different small letters in each grape variety and each parameter or with different capital letters in each aging time and each parameter indicate statistically significant differences at $p < 0.05$.
[a] T9, T18: nine, eighteen months of aging on lees; 12 MB: twelve months in bottle after disgorging.
[b] HM: foam maximum height; HS: foam stability height; TS: foam stability time.

this study showed mean values of HM similar to those of *Chardonnay* Cava wines, with the exception of *Malvasía* sparkling wines; and *Verdejo* sparkling wines showed TS values similar than those obtained in *Chardonnay* Cava wines (Andres-Lacueva et al., 1996; Vanrell et al., 2007).

Andres-Lacueva et al. (1996) did not showed differences in HS values by grape variety, while Vanrell et al. (2007) found that sparkling wines from *Chardonnay* showed the highest HS values. Taking into account the data obtained in this study, the HS values of *Verdejo* sparkling wines were also more similar to those of *Chardonnay* sparkling wines (Vanrell et al., 2007).

For red grape varieties, *Prieto Picudo* sparkling wines showed similar foam characteristics (HM and HS) than *Pinot Noir* (Vanrell et al., 2007) and *Trepat* (Girbau-Solà et al., 2002), red grape varieties traditionally used in sparkling wine elaboration.

Considering the aging time, the HM values of the sparkling wines increased until the 18 months, keeping constant until the 30 months of aging, with the exception of sparkling wines from *Malvasía* that continue increasing until 30 months (Table 5). On the other hand, HS values maintained constant or slightly decreased until the 18 months of aging on lees and increased after 30 months of aging. TS values also increased with the aging time, and some differences among wines were found. Sparkling wines from *Verdejo*, *Albarín*, *Prieto Picudo* and *Garnacha-A* presented their maximum TS values at 18 months after the aging on lees, while the other wines obtained their maximum values at 30 months. Although some differences were detected in the evolution of the foam parameters of the sparkling wines depending on the grape variety, it can be pointed out that in general, the quality and stability of the foam of all the sparkling wines increased over the aging on lees or were maintained stable for 30 months of aging on lees. However, Andres-Lacueva et al. (1996 and 1997) and Moreno-Arribas et al. (2000) found that the foaming properties of white Cavas depended on the aging time. They observed an increase in foamability and stability of foam at 18 months of aging on lees, but after this time the sparkling wines showed a decrease in foamability and an increase in foam persistence, and concluded that the optimum time of aging for the best and most stable foam appears to be 18 months. On the other hand, these authors suggested that the increase in foamability could be due to autolysis of the yeast, in agreement with the results obtained by Maujean et al. (1990). Yeast autolysis is a slow natural process that is characterized by the hydrolysis of intracellular polymers by yeast enzymes activated after cell death, and the release of several compounds from cytoplasm and from cell wall that gradually occurs over the aging time (Alexandre and Guilloux-Benatier, 2006). Some of the compounds that stabilize the foam were released from the yeasts, such as polysaccharides, although their influence on foamability will depend on the type and molecular weight of each polysaccharide (Martínez-Lapuente, Guadalupe, Ayestarán, Ortega-Heras, & Pérez-Magariño, 2013; Moreno-Arribas et al., 2000; Núñez, Carrascosa, González, Polo, & Martínez-Rodríguez, 2006; Vanrell et al., 2002).

Table 6 shows the foam parameters of the sparkling wines aged on lees and in bottle after disgorging, and some differences were observed depending on the aging time on lees. In the sparkling wines aged on lees for 9 months, the HM parameter increased during the aging in bottle (without lees), although the HS values remained relatively constant. On the contrary, the sparkling wines aged on lees for 18 months maintained the HM values during the aging in bottle but increased their foam stability height (HS). The aging time in bottle after the aging on lees of the studied sparkling wines did not have a clear influence on the foam stability time (Table 6), being the TS values maintained constant or slightly increased, with the exception of the TS values of *Prieto Picudo* sparkling wines that increased between 40 and 70%, in both type of wines. Therefore, the aging time on lees and in bottle after disgorging did not reduce the foam characteristics of the sparkling wines in the period of time studied.

The varietal differences in the foam characteristics were maintained over the aging time on lees and in bottle, being *Verdejo* and *Prieto Picudo* sparkling wines those with the highest values of foam parameters.

4. Conclusions

In summary, sparkling wines from *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* grape varieties were the richest in most of the volatile compounds analyzed, especially those that contribute to the fruity aroma of wines. Although some differences were observed between the sparkling wines depending on the aging time on lees, the results obtained indicate that the sparkling wines maintain their varietal characteristics even after long aging time (at least until 30 months).

Verdejo and *Prieto Picudo* sparkling wines presented the best foam characteristics, followed by *Albarín* and *Godello* wines. These differences are maintained over the aging time on lees and in bottle after disgorging.

The aging time on lees improved the foam instrumental parameters of sparkling wines at least until 18 or 30 months.

Considering all the results obtained, *Albarín*, *Verdejo*, *Godello* and *Prieto Picudo* were the most interesting grape varieties to elaborate sparkling wines, following the traditional or "champenoise" method. In addition, taking into account the foam data found in the bibliography, these wines have similar foam properties than high quality sparkling wines as Champagne and Cava wines.

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4.5

Influencia de la composición química en las propiedades espumantes de vinos blancos y rosados espumosos

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Role of major wine constituents in the foam properties of white and rosé sparkling wines

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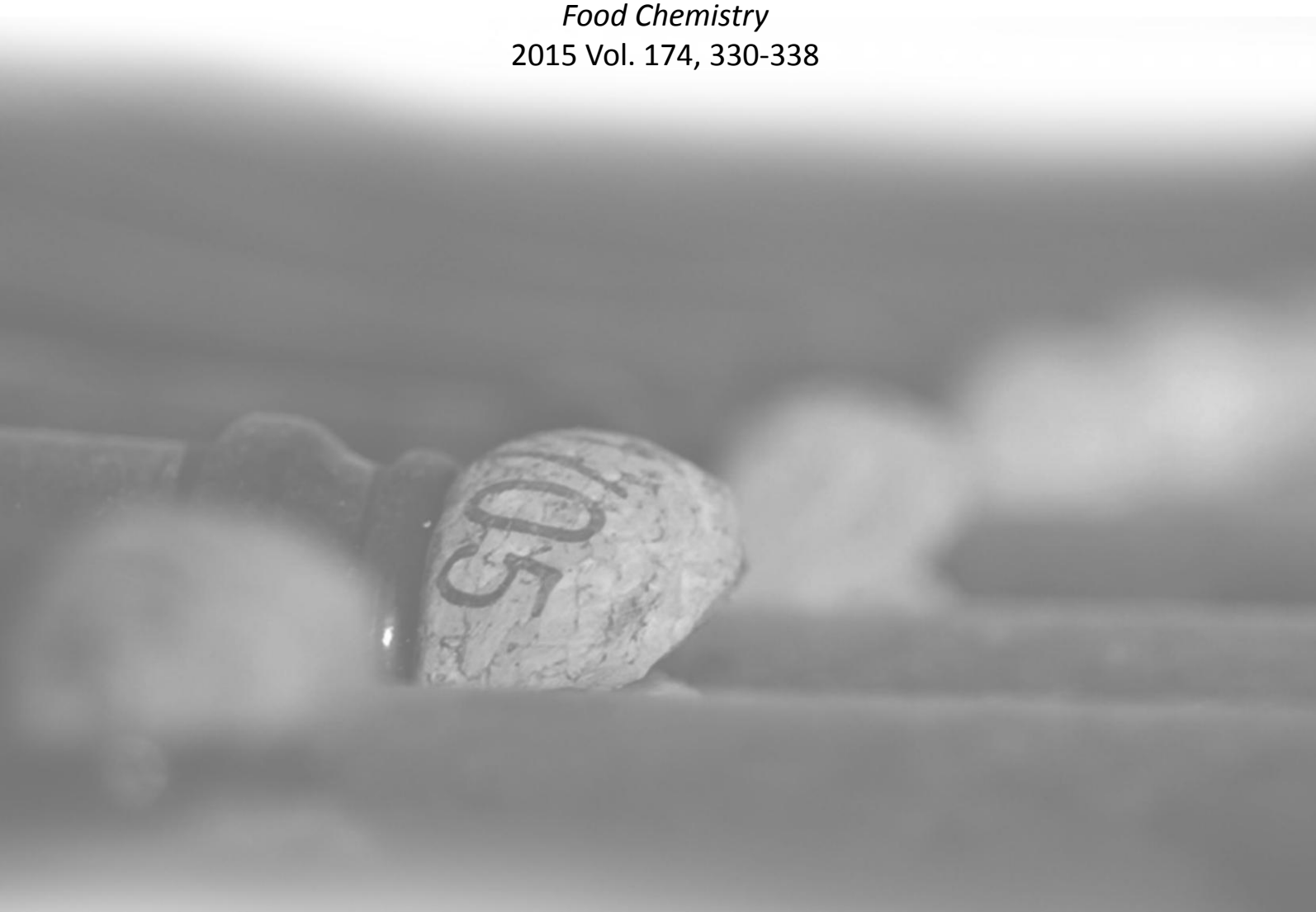

Resumen

Este artículo analiza la influencia de los constituyentes del vino espumoso como son los polisacáridos, los polifenoles y las sustancias nitrogenadas, en sus características espumantes. La calidad de la espuma de los vinos, definida por la espumabilidad (ligada a los parámetros HM, altura máxima de la espuma después de la inyección de CO_2; y HS, altura a la cual estabiliza la espuma durante la inyección de CO_2) y la estabilidad de la espuma (ligada al parámetro TS, tiempo necesario para la desaparición completa de la espuma cuando se para la inyección de CO_2) fue determinada por el método Mosalux. Se evaluaron los coeficientes de correlación individuales entre los compuestos químicos y dichas propiedades espumantes. Posteriormente se aplicaron técnicas de análisis multivariante para estudiar el efecto combinado de todos los compuestos evaluados y establecer qué moléculas fueron las más influyentes en la espumabilidad y en la estabilidad de la espuma de los vinos espumosos.

El estudio se realizó con vinos espumosos de las cosechas 2009, 2010 y 2011, obtenidos después de nueve meses de envejecimiento sobre lías en botella

Los resultados obtenidos indicaron una contribución positiva de los antocianos monómeros y de los aminoácidos y una contribución negativa de las proantocianidinas en los parámetros de espumabilidad de los vinos blancos y rosados espumosos. La influencia positiva de los antocianos en la espumabilidad podría atribuirse a la interacción de las antocianinas con las proteínas del vino a través de interacciones hidrofóbicas y puentes de hidrógeno. Debido a la naturaleza anfifílica del producto formado, éste podría ser retenido en la interfase líquido/aire, disminuyendo la tensión interfacial e incrementándose la formación de espuma. En general, los aminoácidos con cadenas laterales no polares mostraron valores más altos de correlación que los aminoácidos con cadenas laterales polares. Al pH del vino, los aminoácidos están protonados y actuarían como agentes tensoactivos catiónicos según la hidrofobicidad de sus cadenas laterales. El carácter anfifílico de los aminoácidos les permitiría concentrarse en la interfase líquido/aire y mejorar la espumabilidad de los vinos. Finalmente, y debido a la influencia positiva de las proteínas en la espumabilidad de los vinos, las correlaciones negativas entre las proantocianidinas y el parámetro HM se atribuyeron a la capacidad de los taninos de unirse con proteínas para formar precipitados insolubles.

Las manoproteínas (MP) y los polisacáridos ricos en arabinosa y en galactosa (PRAG) no tuvieron influencia en los parámetros de espumabilidad, sin embargo fueron buenos estabilizadores de la espuma. Este comportamiento se debió a la naturaleza hidrofóbica

de las MP y PRAG, que favoreció la unión de estos a las burbujas de gas; así, los monosacáridos hidrofílicos se localizarían en la capa acuosa entre las burbujas y la región hidrofóbica correspondiente a la región proteica se situaría hacia la cara interior de la burbuja. Esta disposición provoca que cuando la capa acuosa se hace más fina, las glicoproteínas aumenten la viscosidad retardando el drenaje. Se produce así un aumento de la tensión superficial de las burbujas y, con ello, un aumento de la estabilidad de la espuma. El hecho de que los PRAG mostraran coeficientes de correlación más altos que las MP se debió a diferencias en sus estructuras y conformaciones, así como en sus cargas.

Los modelos de regresión lineal múltiple revelaron que los antocianos fueron los compuestos con mayor influencia positiva en la espumabilidad, seguidos por los aminoácidos. Entre los antocianos, la malvidina-3-glucósido y la malvidina-3-(6-acetil)-glucósido mostraron la mayor influencia en los parámetros HM y HS, mientras que la β-alanina fue el aminoácido que más contribuyó a explicar el parámetro HM. El modelo que mejor explicó el parámetro de estabilidad de la espuma fue únicamente predicho por los PRAG.

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Role of major wine constituents in the foam properties of white and rosé sparkling wines

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ABSTRACT

The chemical composition of sparkling wines is directly related to their foam quality, but the compounds responsible are not yet completely established. This work aims at identifying the contribution of the different wine compounds to the foaming properties of white and rosé sparkling wines. Our results demonstrated the positive contribution of anthocyanins and amino acids to the foamability parameters HM (maximum height reached by foam after CO_2 injection) and HS (foam stability height during CO_2 injection), and the negative contribution of proanthocyanidins. Mannoproteins and polysaccharides rich in arabinose and galactose (PRAG) were poor foam formers but good foam stabilizers. The different forms of malvidin showed the highest influence on the HM and HS parameters, followed by amino acid compounds, mainly β-alanine. The model to explain foam stability was only predicted by polysaccharides from grapes, concretely PRAG. To our knowledge, this is the first time these correlations in sparkling wines have been described.

1. Introduction

Natural sparkling wine produced with the *champenoise* method is the final product after two fermentations followed by an ageing period of at least 9 months with yeast inside the bottle (EC Regulation N° 606/2009). Foam characteristic is the first attribute observed by the consumer, so the foam of a sparkling wine is a key parameter of its quality. Quality foam can be defined as one that causes a slow release of CO_2, in ring shapes from the depths of the liquid, with small bubbles that contribute to the formation of a crown over the surface of the wine, covering it completely, and with bubbles two or three rows deep (Martínez-Rodríguez & Pueyo, 2009). Foam duration is directly related to bubble stability, and stability is itself dependent on the composition of the film that supports it. It was established that foaming properties depend on compounds that decrease surface tension and increase the viscosity of the film between the bubbles. This factor contributes to foam stabilization and renders the bubbles more resistant to coalescence (López-Barajas, Viu-Marco, López-Tamames, Buxaderas, & de la Torre-Boronat, 1997).

The foam properties have been correlated with the sparkling wine chemical composition, although the compounds that are directly involved in foam formation and stabilization are not yet completely established.

Proteins were the first compounds correlated with foam characteristics due to their surfactant properties. Although most studies indicate a positive correlation between protein concentration and foamability, correlations with foam stability have shown contradictory results (Blasco, Viñas, & Villa, 2011; Coelho, Reis, Domingues, Rocha, & Coimbra, 2011a; Coelho, Rocha, & Coimbra, 2011b; García, Aleixandre, Álvarez, & Lizama, 2009; Girbau-Sola, López-Tamames, Buján, & Buxaderas, 2002; Pueyo, Martín-Alvarez, & Polo, 1995; Vanrell et al., 2007). Therefore, some proteins have been described as good foam formers but poor stabilizers while others are poor foam formers but good stabilizers (Andrés-Lacueva, López-Tamames, Lamuela-Raventós, Buxaderas, & de la Torre-Boronat, 1996; Lao et al., 1999; López-Barajas, López-Tamames, Buxaderas, & de la Torre-Boronat, 1998). Among wine glycoproteins, the yeast mannoproteins released during fermentation and autolysis have been described as the major foam promoters due to their structure, which favours adsorption to the foam bubbles gas/liquid interface (Blasco et al., 2011; Nuñez, Carrascosa, González, Polo, & Martínez-Rodríguez, 2005; Núñez, Carrascosa, González, Polo, & Martínez-Rodríguez, 2006; Vincenzi, Crapisi, & Curioni, 2014). However, according to our knowledge, the influence of yeast mannoproteins on foam stability of sparkling wines has only been analysed in model solutions

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(Coelho et al., 2011b), and there are no studies regarding other wine glycoproteins or polysaccharide families.

Regarding amino acids, some authors agree that these molecules confer hydrophobicity to the peptides and improve the quality of the foam (Bartolomé, Moreno-Arribas, Pueyo, & Polo, 1997; Moreno-Arribas, Pueyo, & Polo, 1996). Contrarily, Moreno-Arribas et al. (2000) observed low correlations between free amino acids and foamability parameters and they did not find any relationship with the concentration of wine peptides. The influence of free amino acids on foam stability has not been described, and no precedents have been found of research into the influence of biogenic amines on the foam properties.

In addition to proteins, peptides and polysaccharides, polyphenols are highly reactive compounds; some authors have tried to establish a correlation between them and the quality of foam in sparkling wines. Most of these studies have shown contradictory results (Andrés-Lacueva, Lamuela-Raventós, Buxaderas, & de la Torre-Boronat, 1997; Girbau-Sola et al., 2002; Lao et al., 1999; López-Barajas et al., 1997; Sarker, Wilde, & Clark, 1995; Sausse, Aguié-Béghin, & Douillard, 2003). Moreover, they are carried out in model systems, juices or base wines, and refer to global measurements of polyphenols. No scientific studies have been conducted to ascertain which phenolic compounds are those that influence foam quality, which could be critical in the production of rosé and red sparkling wines.

Finally, it is important to point out that foam behaviour results from the synergistic interaction between the different foam active compounds which, due to aggregation or complex formation, may modify their surface-active properties. For this reason, and in order to ascertain which compounds affect foam quality of sparkling wines, it is necessary to evaluate as many compounds together as possible.

Therefore, this paper aims to determine the influence of free amino acids, biogenic amines, proanthocyanidins, anthocyanins, hydroxycinnamic acids, flavan-3-ols, flavonols and polysaccharide families on the foamability and foam stability of sparkling wines. In this work, different white and rosé sparkling wines were produced under the same winemaking conditions during three consecutive vintages, and their foaming properties evaluated using a Mosalux-based device. Firstly, an evaluation of the individual correlations between the chemical compounds and the foam properties was made. To study the combined effect of all the chemical compounds, a multiple linear regression model was created to evaluate which molecules were the major contributors to both foamability and foam stability.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were analytical grade unless otherwise stated. L-cysteine, L-leucine, L-phenylalanine, L-lysine, L-histidine, cadaverine, L-arginine, histamine, L-alanine, spermidine, glycine, β-alanine, L-aspartic acid, L-glutamic acid, L-tyrosine, L-serine and L-phenylethylamine, D-(+)-galacturonic acid, D-glucuronic acid, myo-inositol, trifluoroacetic acid, HPLC grade *o*-phosphoric 85% acid and HPLC grade acetic acid 50% were purchased from Fluka (Buchs, Switzerland). Isoamylamine, L-proline, diethyl ethoxymethylenemalonate (DEEMM), putrescine, tyramine, tryptamine, *trans*-4-hydroxy-L-proline, L-2-aminoadipic acid, L-ornithine monohydrochloride, L-tryptophan, L-asparagine, L-threonine, γ-aminobutyric acid (GABA), L-isoleucine, L-glutamine, L-methionine and sodium azide, D-(+)-fucose, L-rhamnose, 2-O-methyl-D-xylose, L-(+)-arabinose, D-(+)-galactose, D-(+)-glucose, D-(+)-mannose, Kdo (2-keto-3-deoxyoctonate ammonium salt), hexane, dried methanol, pyridine, hexamethyldisilazane, trimethylchlorosilane and D-apiose solution were purchased from Sigma–Aldrich (Steinheim, Germany). Acetyl chloride, gallic acid, ethanol 96% (v/v), acetone, acetic acid glacial and sodium acetate were supplied by Scharlab (Barcelona, Spain). Hydrochloric acid 37% and sodium hydroxide were purchased from Carlo Erba (Rodano, Milan, Italy). Malvidin-3-glucoside, peonidin-3-glucoside, isorhamnetin, isorhamnetin-3-glucoside, isorhamnetin-3-rutinoside, quercetin-3-O-galactoside, *trans*-ferulic acid, syringic acid, *trans*-caffeic acid, *trans*-*p*-coumaric acid, (+)-catechin, (−)-epicatechin, myricetin, myricetin-3-O-rhamnoside, quercetin, quercetin-3-glucopyranoside, quercetin-3-rutinoside, kaempferol, kaempferol-3-glucoside, *trans*-resveratrol and quercetin-3-arabinoglucoside were purchased from Extrasynthèse (Lyon, France). Ascorbic acid and ammonium di-hydrogen phosphate were obtained from Panreac (Barcelona, Spain), and Toyopearl gel HW-50F was obtained from Tosoh Corporation (Tokyo, Japan). HPLC-grade reagents, ethanol, methanol, acetonitrile, ascorbic acid and ammonium di-hydrogen phosphate were provided by Scharlau (Barcelona, Spain). Water Milli-Q was obtained via a Millipore system (Bedford, MA).

2.2. Equipment

High performance liquid chromatography (HPLC) was performed using a modular 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with one G1311A quaternary pump, an on-line G1379A degasser, a G1316A column oven, a G1313A automatic injector, and a G1315B photodiode-array detector (DAD) controlled by the Chemstation Agilent software. The gas chromatography (GC) system controlled by the Chemstation software and equipped with a 7653B automatic injector consisted of an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector (MS). Foaming properties of sparkling wines were evaluated using a Mosalux apparatus (Station Oenotechnique de Champagne, Cormontreuil, France).

2.3. Sparkling wine samples

In this study, all the sparkling wines were produced in the Instituto Tecnológico Agrario de Castilla y León (Spain) following the traditional or *champenoise* method. White monovarietal sparkling wines were produced with *Vitis vinifera* cv. Verdejo and Viura grapes from the Rueda Denomination of Origin (D.O.), *V. vinifera* cv. Malvasía grapes from the Toro D.O., *V. vinifera* cv. Albarín grapes from the Tierras de León D.O. and *V. vinifera* cv. Godello grapes from the Bierzo D.O. Rosé monovarietal sparkling wines were obtained with *V. vinifera* cv. Prieto Picudo grapes from the Tierras de León D.O., *V. vinifera* cv. Tempranillo grapes from Cigales D.O., and *V. vinifera* cv. Garnacha grapes from different viticultural areas of the Cigales D.O. All wines were made under the same winemaking conditions during three consecutive vintages (2009, 2010 and 2011). Samples for analyses were taken after 9 months of ageing on yeast lees. Wines were riddled and disgorged before analysis and liqueur d'expédition was added. Three bottles were analysed at each disgorging time.

2.4. Measurement of foam properties

Foamability and foam stability were measured using the Mosalux procedure (Maujean, Poinsaut, Dantan, Brissonnet, & Cossiez, 1990). The gas flow rate was controlled at 7 L/h (Cole-Parmer Instruments Company, IL, USA) and under a constant pressure of 1 bar for 15 min. Then the gas injection was stopped. Foamability was evaluated as the increase in height of 100 ml of wine placed inside the glass column, after CO_2 injection through

the glass-frit. Two parameters of foamability were measured: (1) maximum height reached by foam after CO_2 injection through the glass frit (HM, expressed in mm), which represents the solution's ability to foam; (2) foam stability height during CO_2 injection (HS, expressed in mm), which represents the wine's ability to produce stable foam persistence as a foam collar. Foam stability time (TS), expressed in seconds, was evaluated as the time until all bubbles collapsed when CO_2 injection was interrupted, and could represent the foam stability time once effervescence decreased. Mosalux parameters were determined three times for each bottle of sparkling wine, which were analysed in triplicate (3 bottles × 3 replicates per bottle).

2.5. Chemical analysis

2.5.1. Analysis of monomeric phenolics

Anthocyanins, hydroxycinnamic acids, flavonols, flavan-3-ols, and gallic acid were analysed with a direct injection of the wine previously filtered through 0.45-μm membranes. Separation was achieved with an ACE HPLC (Teknokroma, Barcelona, Spain) (5 C18-HL) particle size 5 μm (250 mm × 4.6 mm) column protected with a guard column of the same material, according to the method described by Gómez-Alonso, García-Romero, and Hermosín-Gutiérrez (2007). The content of non-acylated anthocyanins was calculated as the sum of delphinidin, cyanidin, petunidin, peonidin and malvidin-3-glucosides; the content of acetyl-glucoside anthocyanins defined as the sum of delphinidin, cyanidin, petunidin and malvidin-3-(6-acetyl)-glucosides; the content of coumaroyl-glucoside anthocyanins included delphinidin, petunidin, and malvidin-3-(6-*p*-coumaryl)-glucosides. The sum of all anthocyanin forms was referred to as total monomeric anthocyanins. Total hydroxycinnamic acids were calculated as the sum of caffeic, ferulic and coumaric acid, and the hydroxycinnamates *cis*-caftaric, *trans*-caftaric, *cis*-coutaric, *trans*-coutaric, and *trans*-fertaric. Total flavonol content was calculated as the sum of myricetin-3-galactoside, myricetin-3-glucuronide, myricetin-3-glucoside, quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-glucuronide, kaempferol-3-glucoside, isorhamnetin-3-glucoside, kaempferol-3-glucuronide, myricetin, quercetin, kaempferol and isorhamnetin. All analyses were performed in duplicate.

2.5.2. Analysis of proanthocyanidins

Wine samples were firstly fractionated by gel permeation chromatography (GPC) on a Toyopearl gel HP-50F column (Tosohaas, Montgomeryville, PA) as described by Guadalupe, Soldevilla, Sáenz-Navajas, and Ayestarán (2006). A first fraction (F1) containing monomeric flavonoids, dimeric anthocyanins, and polymeric pigments was eluted with ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v); a second fraction (F2) containing proanthocyanidins was recovered by elution with acetone/water (60:40, v/v). Fractionation was performed in triplicate and phloroglucinol adducts in F2 fractions were analysed by reversed-phase HPLC (Kennedy & Jones, 2001). The column was an ACE HPLC (Teknokroma, Barcelona, Spain) (5 C18-HL) particle size 5 μm (250 mm × 4.6 mm) protected by a guard column containing the same material.

Proanthocyanidin cleavage products were estimated using their response factors relative to (+)-catechin, which was used as the quantitative standard. Total proanthocyanidin content was calculated as the sum of all the subunits: extension subunits (phloroglucinol adducts) and terminal subunits (catechin, epicatechin and epicatechin-gallate). The mean degree of polymerisation (mDP) was calculated as the sum of all subunits divided by the sum of the terminal subunits. All analyses were performed in triplicate.

2.5.3. Total phenolics

Total phenolics were calculated as the sum of total anthocyanins, hydroxycinnamic acids, flavonols, catechin and proanthocyanidins.

2.5.4. Analysis of amino acids and biogenic amines

Amino acid and biogenic amine content was determined simultaneously using the method described by Gómez-Alonso, Hermosín-Gutiérrez, and García-Romero (2007). Analyses were performed using an ACE HPLC column (Symta, Madrid, Spain) (5 C18-HL) of particle size 5 μm (250 mm × 4.6 mm). Neutral amino acids were calculated as the sum of serine, OH-proline, glycine, threonine, α-alanine, β-alanine, GABA, proline, tyrosine, methionine, cysteine, leucine, isoleucine and phenylalanine. Basic amino acids were calculated as the sum of asparagine, glutamine, histidine, arginine, tryptophan, ornithine and lysine. Acid amino acids were calculated as the sum of aspartic acid and glutamic acid. Total amino acids content was calculated as the sum of neutral, basic and acid amino acids. Total biogenic amines were calculated as the sum of histamine, spermidine, tyramine, putrescine, tryptamine, cadaverine, phenylethylamine and isoamylamine. All analyses were performed in triplicate for each sparkling sample.

2.5.5. Analysis of polysaccharide families

Wine polysaccharides were recovered by precipitation after ethanolic dehydration and their monosaccharide composition was determined by GC–MS of their trimethylsilyl-ether O-methyl glycolsyl-residues obtained after acidic methanolysis and derivatization as previously described (Guadalupe, Martinez-Pinilla, Garrido, Carrillo, & Ayestarán, 2012). The chromatographic column was a Teknokroma fused silica capillary column (30 m × 0.25 mm × 0.25 μm) of phase 5% phenyl – 95% methylpolysiloxane. Polysaccharide extraction was performed in triplicate for each sparkling bottle.

The content of polysaccharides rich in arabinose and galactose (PRAG), representing mainly arabinogalactan-proteins and arabinans in wines, rhamnogalacturonans type II (RG-II), homogalacturonans (HG), and yeast mannoproteins (MP) and glucans (GL), was estimated from the concentration of their constituent sugars (Martínez-Lapuente, Guadalupe, Ayestarán, Ortega-Heras, & Pérez-Magariño, 2013). Polysaccharides from grapes were estimated from the sum of PRAG, HG and RG-II. Polysaccharides from yeasts were estimated from the sum of MP and GL. The content of total polysaccharides was estimated from the sum of PRAG, RG-II, HG, MP and GL.

2.6. Statistical analysis

Pearson correlation was applied to foam parameters and instrumental data to examine the linear relationships between chemical composition and foam characteristics. Multiple linear regression analysis (MLR) was carried out in a stepwise manner in order to develop a model for HM, HS and TS foaming properties, using as independent variables chemical compounds showing significant correlations. The SPSS 13.0 for Windows software package (SPSS, Inc., Chicago, IL) was used for data processing.

3. Results and discussion

3.1. Influence of the global chemical composition on the foam properties of sparkling wines

Table 1 shows the correlations (r) and the level of significance (p) between the foam instrumental properties (HM, HS and TS) and the global chemical composition of sparkling wines.

Table 1
Correlation coefficients (r) and significance levels (p) between parameters that determine foam properties (HM, HS, TS) and the global chemical composition of sparkling wines.

	HM[a]		HS[a]		TS[a]	
	r	p	r	p	r	p
Total phenolics	−0.287	0.090	−0.141	0.413	0.004	0.984
Total monomeric anthocyanins[b]	**0.961**	**0.000**	**0.795**	**0.000**	0.168	0.551
Non-acylated anthocyanins[b]	**0.967**	**0.000**	**0.808**	**0.000**	0.151	0.590
Acetyl-glucoside anthocyanins[b]	**0.937**	**0.000**	**0.754**	**0.001**	0.211	0.450
Coumaryl-glucoside anthocyanins[b]	**0.882**	**0.000**	**0.671**	**0.006**	0.275	0.322
Total hydroxycinnamic acids	−0.125	0.468	−0.236	0.165	−0.171	0.319
Total flavan-3-ols	**0.500**	**0.002**	0.212	0.214	**0.415**	**0.012**
Total flavonols	0.177	0.300	0.058	0.737	0.053	0.760
Total proanthocyanidins	−**0.732**	**0.000**	−0.148	0.390	−0.012	0.946
mDP	−0.211	0.216	0.315	0.061	0.297	0.079
Total amino acids	**0.849**	**0.000**	**0.634**	**0.000**	0.317	0.059
Acid amino acids	**0.817**	**0.000**	**0.582**	**0.000**	0.422	0.010
Neutral amino acids	**0.849**	**0.000**	**0.677**	**0.000**	0.312	0.064
Basic amino acids	**0.748**	**0.000**	**0.624**	**0.000**	0.315	0.062
Total biogenic amines	**0.655**	**0.000**	**0.640**	**0.000**	**0.475**	**0.003**
Total polysaccharides	0.071	0.679	0.189	0.270	**0.641**	**0.000**
Polysaccharides from yeasts	−0.081	0.637	0.054	0.753	**0.533**	**0.001**
Polysaccharides from grapes	0.184	0.283	0.280	0.098	**0.684**	**0.000**

The significance levels ($p < 0.05$) are indicated in bold.
[a] Foamability, HM (maximum height reached by foam after CO_2 injection) and HS (foam stability height during CO_2 injection), and foam stability time (TS, time elapsed before bubble collapse until the liquid appears after the interruption of CO_2).
[b] Correlation coefficient and significance level evaluated in rosé sparkling wines.

Total phenolics did not shown correlation with any foam instrumental properties. However, the different anthocyanin families showed very high correlations with foamability, both with HM and HS parameters. The highest correlations were found between monomeric anthocyanins and the sparkling wine ability to foam (HM), with values ranging from 0.882 to 0.961. These families of compounds also showed high positive correlations with the HS parameter, which is an indicator of the ability of the sparkling wine to produce stable foam persistence as a foam collar. Therefore, all anthocyanin families positively affected foamability but they did not show any effect on the foam stability time as no correlation was found between these compounds and the TS parameter. Among the different families of anthocyanins, non-acetylated anthocyanins showed the highest correlations with both HM and HS, followed by acetyl-glucoside anthocyanins and coumaroyl glucoside anthocyanins. On the other hand, total proanthocyanidins showed high negative correlation ($r = -0.732$) with sparkling wine ability to foam (HM), although no correlation was observed with their mean degree of polymerisation. Total hydroxycinnamic acids and total flavonols did not correlate with any foam instrumental properties.

The correlations observed between anthocyanins and the foamability of sparkling wines could be critical in the elaboration of rosé and red sparkling wines. To the best of our knowledge, no reports regarding the effect of these compounds on foamability have been published. This effect could be attributed to the interaction of anthocyanins with wine proteins through hydrophobic interactions and hydrogen bonds. Attachment of a long aliphatic (lipophilic) chain could confer interesting surfactant behaviour on (hydrophilic) flavylium cations. Due to the amphoteric nature of the product formed, it is retained in the liquid/air interface, resulting in a reduction of the interfacial tension and an increase in the foamability. The foamability of the solution is enhanced as it increases the number of amphiphilic molecules adsorbed on the interface (Carrera Sánchez & Rodríguez Patino, 2005), which could explain the problem of gushing in the winemaking of red sparkling wines. On the other hand, copigmentation complexes formed by anthocyanins during ageing on lees adopt a sandwich configuration that produces a hydrophobic environment (Boulton, 2001; He et al., 2012), and the adsorption of other wine surfactant molecules on the surface of these hydrophobic particles may also improve sparkling wine foamability. These observations could explain the fact that other authors have found that rosé sparkling wines exhibit better foam characteristics than white ones (Girbau-Sola et al., 2002; Hidalgo et al., 2004; Pozo-Bayón, Martín-Álvarez, Moreno-Arribas, Andujar-Ortiz, & Pueyo, 2010). These authors instrumentally measured the foam characteristics of rosé sparkling wines but they did not analyse the content of monomeric anthocyanins. High negative correlations were found between total proanthocyanidins and HM, attributed to the capability of tannins binding with proteins to form insoluble tannin-protein precipitates (Ma et al., 2014), although correlation with HS was not found. Despite the observations that total flavonols did not correlate with any foam instrumental properties, total flavan-3-ols showed positive correlation with both HM and TS. In this sense, Sarker et al. (1995) demonstrated that (+)-catechin together with proteins produced an increase in foamability and foam stability in model systems by increasing the rigidity of the interfacial air–liquid layers.

Total amino acids showed high positive correlation coefficients with both HM ($r = 0.849$) and HS ($r = 0.634$), in agreement with Moreno-Arribas et al. (2000). At wine pH, amino acids carry a net positive charge, so they are surfactant with hydrophilic and hydrophobic groups. This property causes amino acids to be retained in the air/liquid interface, and thus reduces the wine surface tension, improving the sparkling wine ability to foam. All acid, neutral and basic amino acids were positively correlated with the foamability parameter, suggesting that they were not selectively retained at the gas–liquid interface. Regarding HM and HS values, biogenic amines showed the same behaviour as free amino acids, although lower correlation values were observed. As in the case of anthocyanins, none of these compounds influenced the TS parameter.

Total polysaccharides were the only compounds affecting the foam stability time, but they did not show any effect on foamability parameters. All polysaccharide families showed positive correlation with TS, and polysaccharides from grapes showed higher correlation coefficients than polysaccharides from yeast. The effect of polysaccharides from grapes was attributed to polysaccharides rich in arabinose and galactose (PRAG) while the influence of polysaccharides from yeast was probably due to mannoproteins (MP).

The proteinaceous fraction of MP and PRAG is able to bind to the liquid/air interface and interact with others by means of electrostatic or hydrophobic forces, hydrogen bonds, or covalent linkages (Blasco et al., 2011). These interactions could lead to the formation of a strong viscoelastic film that could be highly resistant to tension and able to withstand the film's thickness (Blasco et al., 2011). This fact could prevent coalescence of bubbles, leading to more stable foams.

3.2. Influence of phenolic compounds on the foam properties of sparkling wines

Table 2 shows the correlations (r) and the level of significance (p) between the foam instrumental properties (HM, HS, and TS) and the phenolic composition of sparkling wines. Firstly, it is important to point out that there is no literature relating to the phenolic composition of a sparkling wine with its foam properties.

The formation of hydrogen bonds between the hydroxyl groups of the phenolic compounds and the polar head groups of proteins can be particularly relevant for the interaction with the air/liquid interface of the bubble film (Sarker et al., 1995; Sausse et al., 2003). These formed compounds could adsorb at the interface and form a stabilizing film around bubbles, which could promote foam formation and, therefore, HM. The results found in the present study (Table 2) suggested that each phenolic compound exhibits different behaviour patterns on foam instrumental properties.

All anthocyanin compounds, monoglucosides, acetyl-glucosides and coumaroyl-glucosides, showed high positive correlations with HM and HS, and no relevant differences were found in the influence of each individual compound. As commented above, this effect could be attributed to hydrophobic interactions and hydrogen bonds of anthocyanins with proteins. Moreover, hydrophilic interactions between the glucose components of the anthocyanin molecules and the hydrophobic repulsion that takes place between their aromatic nuclei could promote self-aggregation of anthocyanins (He et al., 2012). Therefore, anthocyanin complexes could form amphiphatic layers on hydrophobic or hydrophilic surfaces. This property could help foam formation and stabilization of gas bubbles, and thus lead to an increase of HS. The reduction of the surface tension could also lead to the formation of bubbles and therefore, to higher HM values.

Although total hydroxycinnamic acids were not correlated with any foam parameter (Table 1), *cis*-caftaric acid showed a negative correlation with HM while coumaric and gallic acid showed a positive correlation, higher than 0.75 in the case of coumaric acid. Catechin and quercetin also showed positive correlations with HM although they were not high, and isorhamnetin showed a correlation with HM higher than 0.84. The positive effect of all these compounds on the HM parameter was attributed to their lower molecular weight and planar structure, which modulate their apolarity. As in the case of anthocyanins, these monomeric phenols could create hydrophobic molecular interactions through vertical

Table 2
Correlation coefficients (r) and significance levels (p) between parameters that determine foam properties (HM, HS, TS) and the phenolic composition of sparkling wines.

Chemical compound	HM[a]		HS[a]		TS[a]	
	r	p	r	p	r	p
Delphinidin-3-glucoside[b]	**0.937**	**0.000**	**0.707**	**0.003**	0.043	0.878
Cyanidin-3-glucoside[b]	**0.843**	**0.000**	**0.603**	**0.017**	0.283	0.307
Petunidin-3-glucoside[b]	**0.947**	**0.000**	**0.728**	**0.002**	0.026	0.926
Peonidin-3-glucoside[b]	**0.870**	**0.000**	**0.652**	**0.008**	0.295	0.286
Malvidin-3-glucoside[b]	**0.978**	**0.000**	**0.852**	**0.000**	0.180	0.521
Delphinidin-3-(6-acetyl)-glucoside[b]	**0.912**	**0.000**	**0.674**	**0.006**	0.145	0.606
Cyanidin-3-(6-acetyl)-glucoside[b]	**0.894**	**0.000**	**0.618**	**0.014**	−0.036	0.900
Petunidin-3-(6-acetyl)-glucoside[b]	**0.919**	**0.000**	**0.691**	**0.004**	0.096	0.734
Peonidin-3-(6-acetyl)-glucoside[b]	**0.886**	**0.000**	**0.648**	**0.009**	0.200	0.474
Malvidin-3-(6-acetyl)-glucoside[b]	**0.886**	**0.000**	**0.924**	**0.000**	0.551	0.033
Delphinidin-3-(6-*p*-coumaryl)-glucoside[b]	**0.757**	**0.001**	**0.523**	**0.045**	0.384	0.158
Cyanidin-3-(6-*p*-coumaryl)-glucoside[b]	**0.917**	**0.000**	**0.684**	**0.005**	0.120	0.671
Petunidin-3-(6-*p*-coumaryl)-glucoside[b]	**0.782**	**0.001**	**0.549**	**0.034**	0.354	0.196
Peonidin-3-(6-*p*-coumaryl)-glucoside[b]	**0.905**	**0.000**	**0.665**	**0.007**	0.163	0.561
Malvidin-3-(6-*p*-coumaryl)-glucoside[b]	**0.935**	**0.000**	**0.762**	**0.001**	0.278	0.316
Cis-caftaric	−**0.654**	**0.000**	−0.159	0.353	−0.122	0.478
Trans-caftaric	−0.125	0.468	−0.259	0.127	−0.145	0.400
Cis-coutaric	0.299	0.077	0.097	0.573	−0.081	0.639
Trans-coutaric	0.210	0.220	0.003	0.988	−0.215	0.207
Caffeic acid	0.323	0.055	−0.167	0.330	−0.273	0.107
Trans-fertaric	**0.345**	**0.039**	0.038	0.825	0.146	0.395
Coumaric acid	**0.772**	**0.000**	**0.374**	**0.024**	−0.144	0.403
Ferulic acid	−**0.389**	**0.019**	−0.170	0.323	−**0.406**	**0.014**
Gallic acid	**0.618**	**0.000**	0.217	0.204	0.112	0.515
Catechin	**0.500**	**0.002**	0.212	0.214	**0.415**	**0.012**
Myricetin-3-glucuronide + myricetin-3-glucoside	0.165	0.335	0.151	0.380	0.112	0.515
Quercetin-3-rutinoside	−**0.429**	**0.009**	0.184	0.284	0.181	0.290
Quercetin-3-galactoside	−0.329	0.050	−0.226	0.186	−0.119	0.490
Quercetin-3-glucoside	−0.105	0.541	0.134	0.437	−0.064	0.710
Quercetin-3-glucoside + quercetin-3-glucuronide	0.005	0.977	−0.116	0.502	0.080	0.641
Isorhamnetin-3-glucoside + kaempferol-3-glucoside + kaempferol-3-glucuronide	0.031	0.856	−0.128	0.457	0.011	0.950
Myricetin	0.321	0.056	**0.357**	**0.032**	0.230	0.178
Quercetin	**0.583**	**0.000**	0.228	0.180	0.264	0.120
Kaempferol	0.289	0.088	0.091	0.600	**0.534**	**0.001**
Isorhamnetin	**0.840**	**0.000**	0.317	0.059	−0.104	0.545
Total proanthocyanidins	−**0.732**	**0.000**	−0.148	0.390	−0.012	0.946

The significance levels ($p < 0.05$) are indicated in bold.

[a] Foamability, HM (maximum height reached by foam after CO_2 injection) and HS (foam stability height during CO_2 injection), and foam stability time (TS, time elapsed before bubble collapse until the liquid appears after the interruption of CO_2).

[b] Correlation coefficient and significance level evaluated in rosé sparkling wines.

stacking. Through this interaction, the nucleophilic attack of water on the molecule could be partially reduced and, therefore, hydrophobicity could be increased. This is consistent with several studies which emphasize the importance of the hydrophobicity of molecules on the foamability of wine (Brissonnet & Maujean, 1993; Coelho et al., 2011a, 2011b; Moreno-Arribas et al., 1996). However, molecules with non-planar structures could impede a close approach to other molecules, and could reduce the potential surface area available for hydrophobic stacking. This would explain the negative correlations observed in the case of *cis*-caftaric acid and proanthocyanidins, which also showed a negative correlation with the HM parameter ($r = -0.732$). *Cis*-caftaric was the only hydroxycinnamic acid showing a negative correlation with HM ($r = -0.654$), although the *trans*-isomer did not show any correlation. This different behaviour could be attributed, on the one hand, to differences in chemical reactivity due to their different polarity, and, on the other hand, to a lower steric effect of *cis*-caftaric than *trans*-caftaric.

It is widely known that proteins play a major role on the foam properties of beverages due to their surface properties. Therefore, the negative correlation of proanthocyanidins with HM was due to the precipitation of wine proteins by tannins.

3.3. Influence of amino acids and biogenic amines on the foam properties of sparkling wines

Table 3 shows the correlations (r) and the level of significance (p) between the foam instrumental properties (HM, HS, and TS) and the amino acids and biogenic amines of sparkling wines.

In general, all amino acid compounds showed positive correlations with both HM and HS parameters although, as in the case of phenolic compounds, correlations with HM were higher. In good agreement with our previous results (Table 1), biogenic amines did not influence foam properties.

Moreno-Arribas et al. (2000) also observed correlations between free amino acids and foamability, but they found lower correlation coefficients.

Among all amino acids, glycine, β-alanine, and methionine showed the highest correlation with HM ($r > 0.878$). In general, amino acids with non polar side chains showed higher values of correlation than amino acids with polar side chains. At wine pH, amino acids are protonated and they act as cationic surfactants according to the hydrophobicity of their side chains. Their amphiphilic character could cause amino acids to become concentrated at the liquid–gas interfaces, improving the sparkling wine foamability.

During the second fermentation of sparkling wines and their ageing on yeast lees, peptides are released into the wine and they give rise to smaller-sized peptides and free amino acids (Moreno-Arribas et al., 1996). Therefore, the increase of free amino acids during ageing on lees could explain the improvement on the foam quality with the ageing time.

3.4. Influence of polysaccharide families on the foam properties of sparkling wines

Table 4 shows the correlations (r) and the level of significance (p) between the foam instrumental properties (HM, HS, and TS) and the polysaccharide composition of sparkling wines.

None of the wine polysaccharides correlated with the HM or HS parameter, indicating that they do not influence the foamability of sparkling wines. On the contrary, positive correlations were found between foam stability time (TS) and all wine polysaccharides, with the exception of rhamnogalacturonans type II (RG-II).

Polysaccharides rich in arabinose and galactose (PRAG) showed the highest correlations ($r = 0.723$).

Table 3
Correlation coefficients (r) and significance levels (p) between parameters that determine foam properties (HM, HS, TS) and the amino acids and the biogenic amines of sparkling wines.

Chemical compound	HM[a]		HS[a]		TS[a]	
	r	p	r	p	r	p
Aspartic acid	**0.862**	**0.000**	**0.625**	**0.000**	0.268	0.114
Glutamic acid	**0.765**	**0.000**	**0.542**	**0.001**	**0.455**	**0.005**
Asparagine	**0.793**	**0.000**	**0.682**	**0.000**	**0.450**	**0.006**
Serine	**0.616**	**0.000**	**0.586**	**0.000**	**0.575**	**0.000**
Hydroxyproline	**0.460**	**0.005**	0.270	0.111	0.297	0.078
Glutamine	−0.037	0.832	0.228	0.181	**0.416**	**0.012**
Histidine	−0.114	0.509	0.110	0.524	0.139	0.419
Glycine	**0.878**	**0.000**	**0.662**	**0.000**	**0.346**	**0.039**
Threonine	**0.557**	**0.000**	**0.417**	**0.011**	−0.023	0.892
β-Alanine	**0.920**	**0.000**	**0.547**	**0.001**	0.018	0.916
ι-Arginine	**0.828**	**0.000**	**0.652**	**0.000**	0.121	0.481
α-Alanine	**0.827**	**0.000**	**0.647**	**0.000**	**0.389**	**0.019**
GABA	**0.768**	**0.000**	**0.522**	**0.001**	−0.077	0.656
Proline	**0.819**	**0.000**	**0.604**	**0.000**	**0.336**	**0.045**
Histamine	**0.385**	**0.020**	**0.420**	**0.011**	**0.431**	**0.009**
Tyrosine	**0.810**	**0.000**	**0.598**	**0.000**	0.196	0.251
Methionine	**0.888**	**0.000**	**0.579**	**0.000**	0.322	0.056
Cysteine	**0.791**	**0.000**	**0.485**	**0.003**	−0.033	0.847
Isoleucine	**0.670**	**0.000**	**0.637**	**0.000**	**0.471**	**0.004**
Tryptophan	**0.845**	**0.000**	**0.587**	**0.000**	0.052	0.765
Leucine	**0.416**	**0.012**	**0.546**	**0.001**	**0.546**	**0.001**
Phenylalanine	**0.840**	**0.000**	**0.621**	**0.000**	**0.361**	**0.031**
Ornithine	**0.793**	**0.000**	**0.639**	**0.000**	0.065	0.706
Lysine	**0.655**	**0.000**	**0.607**	**0.000**	0.328	0.051
Spermidine	**0.717**	**0.000**	**0.409**	**0.013**	0.282	0.095
Tyramine	−0.188	0.272	0.199	0.245	**0.350**	**0.037**
Putrescine	**0.511**	**0.001**	**0.588**	**0.000**	**0.425**	**0.010**
Tryptamine	−0.289	0.088	−0.014	0.936	0.268	0.115
Cadaverine	−**0.349**	**0.037**	0.229	0.178	0.139	0.420
Phenylethylamine	0.242	0.154	**0.603**	**0.000**	0.324	0.054
Isoamylamine	−**0.546**	**0.001**	−0.038	0.827	−0.084	0.628

The significance levels ($p < 0.05$) are indicated in bold.

[a] Foamability, HM (maximum height reached by foam after CO_2 injection) and HS (foam stability height during CO_2 injection), and foam stability time (TS, time elapsed before bubble collapse until the liquid appears after the interruption of CO_2).

Both PRAG and MP present a protein moiety with hydrophobic and hydrophilic domains and sugar moieties, which are hydrophilic. Therefore, they could interact with surface-active materials and be absorbed at the gas/liquid interface. Blasco et al. (2011) explain that the hydrophilic glycans are located at the liquid layer, among the bubbles, corresponding to the oxidic zone of the protein. Hence, when the layer surrounding the bubbles becomes thinner, the viscosity increases and drainage of the liquid is delayed. Thus, an increment of the surface tension of the bubbles occurs, resulting in a stabilized foam. Moreover, the proteinaceous fraction of the polysaccharide could interact with other proteins by means of electrostatic or hydrophobic forces, hydrogen bonds, or covalent linkages. These interactions lead to the formation of a viscoelastic film that is highly resistant to tension and able to withstand the film's thickness (Blasco et al., 2011).

The fact that PRAG showed higher correlation coefficients than MP could be due to the PRAG structure. Since foam stability is related to hydrophobicity of polypeptides (Ferreira, Jorge, Nogueira, Silva, & Trugo, 2005), the proteins with higher hydrophobicity would be the most appropriate for stabilizing the film. For example, other rich plant arabinogalactan-proteins (Redgwell, Curti, Fischer, Nicolas, & Fay· 2002), PRAG isolated from wines, concretely from Champagne wines, show a high content of the hydrophobic amino acid hydroxyproline in their structure (Doco & Williams, 2013). Moreover, the presence of glucuronic acid in the terminal positions of the PRAG could improve their capacity of foam stabilization. At wine pH, glucuronic residues could be protonated and lead to a less stiff molecule, allowing them to be

Table 4
Correlation coefficients (r) and significance levels (p) between parameters that determine foam properties (HM, HS, TS) and the polysaccharide composition of sparkling wines.

Chemical compound	HM[a]		HS[a]		TS[a]	
	r	p	r	p	r	p
Mannoproteins (MP)	0.150	0.383	0.157	0.360	**0.465**	**0.004**
Polysaccharides rich in arabinose and galactose (PRAG)	0.042	0.806	0.285	0.092	**0.723**	**0.000**
Homogalacturonans (HG)	0.290	0.087	0.209	0.221	**0.577**	**0.000**
Glucans (GL)	−0.225	0.187	−0.041	0.811	**0.396**	**0.017**
Rhamnogalacturonans type II[b] (RG-II)	0.602	0.589	0.240	0.846	0.204	0.869
Mannose/glucose ratio	0.317	0.060	0.101	0.556	−0.103	0.550
Arabinose/galactose ratio	−0.287	0.090	−0.003	0.984	0.273	0.108

The significance levels ($p < 0.05$) are indicated in bold.
[a] Foamability, HM (maximum height reached by foam after CO_2 injection) and HS (foam stability height during CO_2 injection), and foam stability time (TS, time elapsed before bubble collapse until the liquid appears after the interruption of CO_2).
[b] Correlation coefficient and significance level evaluated in rosé sparkling wines.

Table 5
Results of the multiple linear regression analysis for the foam properties.

Dependent variable	Parameter	B[a]	β[b]	p-Value	R^2
(a) The global composition of sparkling wines					
Rosé sparkling wines					
HM	Constant	84.882		0.000	0.902
	Total amino acids	0.065	0.330	0.001	
	Non-acylated anthocyanins	5.242	0.456	0.000	
	Total proanthocyanidins	−0.477	−0.322	0.000	
HS	Constant	9.730		0.000	0.974
	Basic amino acids	0.331	0.427	0.000	
	Acetyl-glucoside anthocyanins	2.492	0.694	0.000	
	Total biogenic amines	0.995	0.152	0.001	
	Neutral amino acids	0.013	0.192	0.006	
TS	Constant	−22.277		0.098	0.467
	Polysaccharides from grapes	0.489	0.684	0.000	
White sparkling wines					
HM	Constant	66.997		0.000	0.733
	Total amino acids	0.206	0.856	0.000	
HS	Constant	13.258		0.001	0.192
	Total biogenic amines	2.906	0.438	0.047	
TS	Constant	−7.348		0.658	0.339
	Polysaccharides from grapes	0.359	0.582	0.006	
(b) The phenolic composition of sparkling wines					
Rosé sparkling wines					
HM	Constant	61.982		0.000	0.956
	Malvidin-3-glucoside	15.754	0.978	0.001	
HS	Constant	13.859		0.000	0.854
	Malvidin-3-(6-acetyl)-glucoside	84.225	0.924	0.000	
TS	Constant	105.558		0.000	0.506
	Ferulic acid	−367.981	−0.711	0.003	
White sparkling wines					
HM	Constant	74.163		0.000	0.633
	Coumaric acid	122.577	0.796	0.001	
(c) The amino acids and biogenic amines composition of sparkling wines					
HM	Constant	70.706		0.000	0.904
	β-Alanine	37.090	0.725	0.000	
	Glutamic acid	3.896	0.310	0.000	
HS	Constant	14.947		0.000	0.465
	Asparagine	1.546	0.682	0.000	
TS	Constant	−14.623		0.343	0.331
	Serine	101.255	0.575	0.000	
(d) The polysaccharide families in sparkling wines					
TS	Constant	−49.978		0.004	0.523
	Polysaccharides rich in arabinose and galactose (PRAG)	0.933	0.723	0.000	

[a] Non-standardised regression coefficients.
[b] Standardised regression coefficients.

more closely distributed at the gas/liquid surface (Castellani et al., 2010). The interfacial layer formed by PRAG could provide stability against bubble aggregation through steric and electrostatic repulsion. Therefore, PRAG molecules create more points of adsorption to the bubble interface than MP, which avoids gas diffusion and increments the stability of the film.

In conclusion, the different behaviour of the polysaccharide families regarding foam stability could be explained not only by

differences in their structures and conformations, but also differences in their charges.

3.5. Multiple linear regression analysis

Multiple linear regression analysis (MLR) was carried out in a stepwise manner to select suitable variables in the model, and only the variables showing significant correlations ($p < 0.05$) in the previous univariate analysis were included in the study. Since anthocyanins are not found in white sparkling wines, white and rosé sparkling wines were differentiated in models in which anthocyanins were included (Table 5). In order to determine the compounds that better explain the foam properties in sparkling wines, the variables HM, HS and TS were considered as dependent and the compounds as independent variables. Parameters such as B (non-standardised regression coefficients), β (standardised regression coefficients), p-value (significance level), and R^2 (multiple correlation coefficient) were obtained.

The MLR obtained by only taking into account the global composition of the sparkling wines is shown in Table 5a. In rosé sparkling wines, from the initial independent variables for HM parameter, only three were statistically significant in the final fitted model ($p < 0.05$). The regression coefficient R^2 took a value of 0.902, which indicates that the fitted model explains 90.2% of the variability observed in the data. Non-acylated anthocyanins presented the highest β values, indicating that they were the most influent variables in the model. The positive sign of the β value indicated that there was a positive relationship between the amounts of non-acylated anthocyanins and HM. Regarding the influence of total proanthocyanidins and total amino acids, similar strength but opposite effects were observed. When the HS parameter was considered, basic amino acids, acetyl-glucoside anthocyanins, total biogenic amines and neutral amino acids were statistically significant in the final fitted model, which explained 97.4% of the variability observed. Acetyl-glucoside anthocyanins displayed the highest contribution to the model, followed by basic amino acids. Total biogenic amines and neutral amino acids presented the lowest contributions to the model. Regarding TS descriptor, only polysaccharides from grapes were statistically significant, and the fitted model explained 46.7% of the variability observed in the data. In white sparkling wines, when the HM parameter was considered as dependent variable, only total amino acids were statistically significant in the final fitted model and the regression coefficient R^2 took a value of 0.733 Regarding HS and TS descriptors, the regression coefficients R^2 took very low values.

In order to predict the effect of the phenolic composition on the foam properties, white and rosé sparkling wines were differentiated in MLR analysis (Table 5b). In rosé sparkling wines, only malvidin-3-glucoside was included in the HM model, with R^2 of 0.956. In HS parameter, malvidin-3-(6-acetyl)-glucoside was statistically significant in the final fitted model, which explained 85.4% of the variability observed. In white sparkling wines, only the HM parameter could be predicted but it showed a low regression value.

To identify which amino acid or biogenic amine better explain the foam properties, all sparkling wines were included in the model. When the HM descriptor was considered, β-alanine and glutamic acid were statistically significant in the final fitted model, which explained 90.4% of the variability observed. Both amino acids showed a positive relationship between their amounts in sparkling wines and HM, although β-alanine displayed the highest contribution to the model. Asparagine and serine were the only amino acids which made a positive contribution to the prediction of HS and TS, respectively, although fitted models only explained 46.5% and 33.1% of the variation (Table 5c).

Finally, in order to determine which family of polysaccharide compounds better explain the foam properties, all sparkling wines were included in the model (Table 5d). Only TS parameter could be predicted, and only polysaccharides rich in arabinose and galactose (PRAG) were included in the model.

4. Conclusions

In conclusion, anthocyanins were the compounds with a highest influence on the HM and HS parameter, followed by amino acids compounds, in good agreement with the correlation data. Among anthocyanins, the different forms of malvidin were those showing the highest influence in both HM and HS, which was expected as they exhibit the highest concentrations. β-alanine followed by glutamic acid were the major amino acid compounds contributing to HM. Total proanthocyanidins were only included in the HS model of rosé sparkling wines, showing a negative correlation. None of these compounds were included in the TS model, which was only predicted by polysaccharides from grapes, concretely PRAG, also in good agreement with the correlation data, as these compounds showed the highest correlations with the TS values (Table 4).

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4.6

Empleo de derivados comerciales de levaduras ricos en manoproteínas en la elaboración de vinos blancos y rosados espumosos

Use of commercial dry yeast products rich in mannoproteins for white and rosé sparkling wine elaboration

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Resumen

Las manoproteínas juegan un papel importante en la estabilidad del vino y en las propiedades sensoriales de los vinos. Por ello, actualmente existen en el mercado una gran variedad de preparados comerciales a base de levaduras con diferente grado de purificación. De hecho, la efectividad de estos tratamientos para mejorar las propiedades tecnológicas y sensoriales de los vinos tranquilos ha sido objeto de numerosos estudios. Sin embargo son escasos los trabajos de investigación llevados a cabo sobre el efecto que tienen estos productos sobre la composición química y calidad sensorial de los vinos espumosos.

Por todo ello, en este artículo se ha estudiado el efecto de la adición de autolisados de levaduras comerciales sobre la composición fenólica, aminoácidos, aminas biógenas, manoproteínas, compuestos volátiles, propiedades espumantes y características sensoriales de vinos rosados espumosos monovarietales de las variedades de uva tintas de Tempranillo y Garnacha y blancos de las variedades de uva de Verdejo y Godello elaborados por el método tradicional durante nueve meses de crianza sobre lías. Se han estudiado cuatro productos comerciales con diferente composición y grado de purificación.

El estudio se realizó con vinos espumosos de la cosecha 2010 y 2011.

Todos los preparados comerciales derivados de levadura estuvieron formados por manosa y glucosa, siendo el producto DYA-2 el más rico en manoproteínas (85,9%) y el que mostró la mayor pureza (88,8%).

La adición de los preparados comerciales a base de levaduras no produjo ningún cambio significativo en el contenido fenólico, de amino ácidos, de aminas biógenas ni propiedades espumantes de los vinos. Por el contrario, todos los vinos tratados con el producto DYA-2 mostraron mayor contenido de manoproteínas que sus respectivos controles durante la crianza sobre lías. Por otro lado, se observó que la adición de estos productos puede modificar el perfil volátil de los vinos espumosos. Sin embargo, este efecto estuvo relacionado con el contenido en manoproteínas y de pureza del producto empleado, siendo los vinos tratados con el producto de mayor pureza y contenido de manoproteínas los que mostraron diferencias con respecto a sus controles.

En general, los vinos tratados con autolisados de levaduras mostraron menor contenido de ésteres etílicos de ácidos grasos que los vinos controles. Además, los vinos espumosos tratados con el producto DYA-2 mostraron el menor contenido de decanoato de etilo y de ácido decanoico. Esto pudo ser debido a la elevada hidrofobicidad de

dichos compuestos que pudieron ser retenidos por las paredes celulares de las levaduras y al elevado contenido de manoproteínas del producto DYA-2. Asimismo, los vinos tratados con autolisados de levaduras presentaron concentraciones más altas de terpenos que sus respectivos controles, principalmente α-terpineol en vinos blancos espumosos tratados con el producto DYA-2. En el análisis sensorial de los vinos espumosos se observó que la adición del producto DYA-2 mejoró los aromas frutales en los vinos blancos, aunque este efecto no fue observado en los vinos rosados.

Use of commercial dry yeast products rich in mannoproteins for white and rosé sparkling wine elaboration

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Use of commercial dry yeast products rich in mannoproteins for white and rosé sparkling wine elaboration

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ABSTRACT

In sparkling wines, mannoproteins released during yeast autolysis largely affect their final quality. This process is very slow and may take several months. The aim of this work was to study the effect of several commercial dry yeast autolysates on the chemical composition, foam and sensory properties of white and rosé sparkling wines aged on lees for 9 months during two consecutive vintages. The addition of these products in the tirage phase did not affect either the content of phenolic compounds, amino acids, biogenic amines or the foam properties. The commercial product with the highest mannoprotein content and the highest purity caused significant changes in the volatile composition of the wines and enhanced the fruity aromas in both Verdejo and Godello sparkling wines.

Keywords: sparkling wines; dry yeast products; volatile compounds; foam; sensory analysis

INTRODUCTION

Natural sparkling wines are obtained after a second fermentation in closed bottles, and they remain in contact with the yeast lees for at least 9 months (EC Regulation Nº 606/2009). During sparkling wine aging, mannoproteins can be released into wines due to yeast autolysis. Mannoproteins are highly glycosylated proteoglycans mainly composed of mannose (>90%) and glucose[1] and proteins (<10%)[2]. They can have a highly variable size (5-800 kDa)[3] and constitute 25-50% of the dry weight of the *Saccharomyces cerevisiae* walls. These compounds appear to be those that are the most interesting in oenology due to their positive effects on the quality of the final wines.[3-4] In fact, different positive effects of mannoproteins have been described in still wines related to sensory characteristics such as improvement of wine aroma profile[5-8], reduction of astringency and bitterness, and enhancement of red wine body, structure and roundness[1,9-13], as well as influence on the red wine color.[9,14,15]

In sparkling wines, mannoproteins released during yeast autolysis largely affect the final quality of the wines. They can bind volatile compounds and thus retain wine aroma and they have also shown a positive effect on foam stability.[16-17] During sparkling wine aging, interactions between aroma compounds and yeast cell walls can reduce the concentration of some volatile compounds, mainly the most hydrophobic ones.[18-19] In fact, mannoproteins released from yeast can also affect the solubility of aroma compounds, therefore modifying the volatility of certain compounds that could have effects on the final aroma of sparkling wines.[6] In addition, yeast mannoproteins have been associated with the improvement of the foaming properties of sparkling wines.[20-25] The hydrophobic nature of these compounds causes them to adsorb preferentially to the gas/liquid interface of foam bubbles[26], resulting in foam stabilization.[24] Moreover, as

observed in red still wines, mannoproteins could influence the phenolic composition and color of rosé sparkling wines, although there are not studies evaluating this effect.

Mannoprotein release depends on the autolysis process, which is very slow and may take several months. Moreover, the longer the process takes, the higher the production costs and the risk of the appearance of certain microbiological and organoleptic alterations.[27] Therefore, many suppliers of oenological products offer several preparations rich in mannoproteins and polysaccharides obtained from *Saccharomyces cerevisiae* cell walls by different ways.[28] Besides, in the last years, some products have been developed more specifically to sparkling wines in order to improve the quality characteristics of these wines. The use of commercial yeast autolysates to improve the technological and sensory properties of still wines has been widely studied.[1,13-15,29]. However, relatively few papers evaluate the effect of commercial yeast autolysates on the foam properties and chemical characteristics of sparkling wines.[21,30]

On the basis of these considerations, the aim of this work was to study the effect of several commercial dry yeast autolysates on the phenolic compounds, free amino acids, biogenic amines, mannoproteins, volatile compounds, foam properties and sensory properties of white and rosé sparkling wines aged on lees for 9 months during two consecutive vintages. For this purpose, four commercial dry yeast products with different composition were used.

MATERIAL AND METHODS

Polysaccharide commercial dry yeast products

Table 1 shows the information provided by the commercial manufactures regarding the commercial dry yeast autolysates (DYA) used in this study, and the doses applied. These products were selected since there were more specifically developed for sparkling wine elaboration according to the manufactures' information.

Winemaking process

All sparkling wine production was carried out in the experimental winery of the Enological Station (ITACyL) sited in Rueda (Valladolid, Spain), following the traditional or *champenoise* method. White monovarietal base wines were elaborated with *Vitis vinifera* cv. Verdejo grapes from the Rueda Denomination of Origin (D.O.), and *Vitis vinifera* cv. Godello grapes from the Bierzo D.O. Rosé monovarietal base wines were obtained with *Vitis vinifera* cv. Tempranillo and Garnacha grapes from the Cigales D.O. Godello and Garnacha grape varieties were harvested in 2010 and Verdejo and Tempranillo in 2011.

The base wines were elaborated following the traditional white or *rosé* winemaking process in stainless steel tanks of 2000 and 2600 L, respectively. After cold-stabilization and clarification of base wines, the tirage liquor was added and the wines were bottled. The tirage liquor was formed by yeast *S. cerevisiae* var. *bayanus* (0.30 g/L, IOC 18–2007 Lallemand, Spain), sucrose (23 g/L), bentonite (0.10 g/L) (Laffort, France), and the different commercial dry yeast products in the doses indicated in Table 1. The experiences carried out were the control wines (C), without the addition of any product, and the sparkling wines made with the addition of the four different commercial dry yeast autolysates (DYA-1, DYA-2, DYA-3, and DYA-4). After that, the bottles were kept in a cellar at a temperature (11-13 ºC) and relative humidity (75-85%) controlled for 9 months. The pressure and residual sugars were measured periodically to control the second fermentation.

Wines were analyzed after 3, 6 and 9 months of aging on lees. Wines were riddled and disgorged and no expedition liqueur was added. Since the second fermentation takes place in individual bottles, three bottles of each varietal sparkling wine at each sampling time were analyzed.

Chemical reagents

All reagents were analytical grade unless otherwise stated.

The volatile compound standards were purchased from Fluka (Buchs, Switzerland), Sigma-Aldrich (Steinheim, Germany), and Lancaster (Strasbourg, France).[31]

The monosaccharides, amino acids, biogenic amines and the necessary reactives to analyze these compounds were purchased from Fluka (Buchs, Switzerland), and Sigma-Aldrich (Steinheim, Germany).[31,32]

The phenolic compounds were purchased from Extrasynthèse (Lyon, France), and Toyopearl gel HW-50F was obtained from Tosoh Corporation (Tokyo, Japan).

HPLC-grade reagents, ethanol, methanol, acetonitrile, ascorbic acid and ammonium di-hydrogen phosphate were provided by Scharlau (Barcelona, Spain). Dichloromethane (HPLC-grade) was provided by Merck (Darmstadt, Germany), and the remaining reagents were supplied by Panreac (Madrid, Spain). Water Milli-Q was obtained via a Millipore system (Bedford, MA).

Analysis of polysaccharide composition of commercial dry yeast products and sparkling wine samples

In order to characterize the different dry yeast preparations, the monosaccharide composition and their polysaccharide molecular weight distribution were analyzed. The monosaccharide composition of the commercial preparations was determined by GC-MS of their trimethylsilyl-ester O-methyl glycosyl residues obtained after acidic methanolysis and derivatization.[33] GC was controlled by ChemStation software and equipped with a 7653B automatic injector consisting of an Agilent 7890A gas chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5975C VL quadrupole mass detector (MS). A high-resolution size-exclusion chromatography (HRSEC) system (1100 Agilent Technologies, Waldbronn, Germany) connected to a

refractive index detector (RID) was used to obtain the molecular weight distributions of the polysaccharides. Two serial Shodex OHpack KB-803 and KB-805 columns (0.8 x 30 cm, Showa Denko, Japan) equilibrated at 1 mL/ min in 0.1 M $LiNO_3$ were used, and calibration was performed with narrow pullulan molecular weight standards as previously described.[33] All analyses were performed in triplicate.

Wine polysaccharides were recovered by precipitation after ethanolic dehydration and their monosaccharide composition was determined by GC-MS as commented before. The content of mannoproteins was estimated from the content of mannose as described by Guadalupe et al.[33]

Analysis of phenolic compounds

Anthocyanins, hydroxycinnamic acids, flavonols, and flavan-3-ols were analyzed by HPLC in a 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a photodiode-array detector (DAD). Separation was achieved in an ACE HPLC column (Teknokroma, Barcelona, Spain) (250 mm×4.6 mm, particle size 5 μm), according to the methodology previously described.[34]

For analysing proanthocyanidins, wine samples were fractionated by gel permeation chromatography (GPC) on a Toyopearl gel HP-50 F column (Tosohaas, Montgomeryville, PA) as described by Guadalupe et al.[35]. A first fraction was eluted with ethanol/water/trifluoroacetic acid; a second fraction containing proanthocyanidins was recovered by elution with acetone/water. Phloroglucinol adducts in the second fraction were analyzed by reversed-phase HPLC-DAD.[36] The column was an ACE HPLC (Teknokroma, Barcelona, Spain) (250 mm x 4.6 mm, particle size 5 μm) protected by a guard column containing the same material. Total proanthocyanidin content was calculated as the sum of all the subunits: extension subunits (phloroglucinol adducts) and terminal subunits (catechin, epicatechin and epicatechin-gallate).

Analysis of amino acids and biogenic amines

Amino acid and biogenic amine content was determined simultaneously using the method described by Gómez-Alonso et al.[37] HPLC was performed using a 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a photodiode-array detector (DAD). Chromatographic separation was performed in an ACE HPLC column (Teknokroma, Barcelona, Spain) (250 mm x 4.6 mm, particle size 5 μm), thermostatized at 16 ºC.

Analysis of volatile compounds

The volatile compounds were extracted by liquid-liquid extraction following the method developed by Rodríguez-Bencomo et al.[7], and were quantified by GC–MS. The chromatographic analyses were performed with a HP-6890N GC coupled to a HP-5973 inert MS detector equipped with a Quadrex 007CWBTR capillary column (60 m length, 0.25 mm i.d., and 0.25 μm film thickness), following the chromatographic conditions established by Rodríguez-Bencomo et al.[7]

Quantification was carried out following the internal standard quantification method. Quantitative data of the relative areas (absolute areas/ internal standard area) were subsequently interpolated in the calibration graphs built from results of pure reference compounds.[31]

Measurement of foaming properties by instrumental method

Foam properties of sparkling wines were evaluated using a Mosalux equipment (Station Oenotechnique de Champagne, Cormontreuil, France) according to Mosalux procedure.[38] This equipment consists in a glass cylinder with a glass frit in the bottom. This cylinder was filled with 100 mL of wine and CO_2 was injected through the glass frit at a rate of 7 L/h under a constant pressure of 1 bar for 15 minutes. Then the gas injection was stopped.

Three parameters were measured: (1) Maximum height reached by foam after CO_2 injection through the glass frit (HM, expressed in mm) that represents the foamability; (2) Foam stability height during CO_2 injection (HS, expressed in mm) that represents the wine´s ability to produce stable foam persistence of foam collar; (3) Foam stability time (TS, expressed in seconds) was evaluated as the time until all bubble collapsed when CO_2 injection was interrupted, and could represent the foam stability time once effervescence has decreased. Three bottles of each varietal sparkling wine at each sampling time were analyzed, and three measurements for each bottle were carried out.

Sensory analyses

The sensory analysis was performed in a designed test room in accordance with ISO 8589 Standard (2010), and was carried out by twelve expert tasters from the Regulatory Councils of different Spanish D.O. and wineries (8 male and 4 female between 30 and 55 years old). These tasters defined the descriptors used in this sensory analysis, according to the methodology described in González-Sanjosé et al.[39], and were trained to quantify them using structured numerical scales. This training was carried out in accordance with UNE-87-020-93 Standard (ISO 4121:1987).

Samples were presented in standard glasses in random order, and a structured numerical scale of seven points was used (1 representing no intensity and 7 the highest intensity). This work has focused on olfactory attributes (olfactory intensity, varietal, vegetal, yeasty, fruity, exotic fruit, citrus fruit, floral, oxidized, and reduced notes).

Sensory foam properties were valuated using the descriptors defined by Gallart et al.[40]: initial foam, foam area, foam collar, bubble size and effervescence speed with scores from 1 to 3.

The sparkling wines were tasted after 9 months of aging on lees.

Statistical analyses

The data were treated applying the variance analysis (ANOVA), the Least Significant Difference test at significant level of $p< 0.05$, and multifactor analyses of variance. Factor analysis was applied in order to study the association of variables and to determine similarities or differences between wines by aging time or by treatment. Varimax rotation criterion was performed and only factors with eigenvalues greater than 1 were selected. Stepwise discriminant analysis is a supervised method that consists to find a linear combination of the variables, which characterizes or separates two or more classes of objects. The forward method was used to select the variables most useful for differentiating the wines according to treatment. The F-statistical function was used as the criterion for variable selection. These statistical analyses were carried out using the Statgraphics Plus 5.0 statistical package.

Generalized Procrusters Analysis (GPA) was applied on the mean ratings for olfactory attributes by using the Senstools 3.3.2. program (Utrecht, The Netherlands).

RESULTS AND DISCUSSION

Monosaccharide and polysaccharide contents in the commercial dry yeast products

Table 2 shows the monosaccharide composition, polysaccharide purity and polysaccharide composition of the commercial dry yeast products. Only mannose and glucose could be quantified in the commercial products. The percentage of mannose in these preparations, which is used to estimate the content of mannoproteins, was between 53%-86%. The percentage of glucose, used to estimate the glucan content, was between 14-47%, which indicated that during the process to obtain these products more mannoproteins were extracted than glucans. DYA-2 was the product with the highest percentage of mannoproteins, and it showed the highest mannoprotein/glucan relationship (6.1). The other mannoprotein/glucan values were 1.1 for DYA-1, 1.65 for

215 DYA-4 and 3.3 for DYA-3.

216 Table 2 also shows the polysaccharide purity of the commercial products evaluated,
217 expressed as the total amount of monosaccharides in relation to the weight of the
218 product analyzed. Although all products showed values of purity above 60%, it is
219 important to point out that the DYA-2 product showed significantly the highest value,
220 showing a polysaccharide purity of 88.8%. With the exception of DYA-1, the
221 commercial products were only composed of large polysaccharides with an average
222 molecular weight of 47.3 kDa. In contrast, DYA-1 showed a similar content of low
223 molecular weight polysaccharides (average molecular weight of 11.8 kDa) and high
224 molecular weight polysaccharides.

225 **Effect of the addition of the commercial dry yeast products on the phenolic**
226 **compounds, amino acids and biogenic amines and mannoprotein content of the**
227 **sparkling wines**

228 In any of the varieties analyzed, no significant differences were found between control
229 and sparkling wines treated with DYA in non-acylated, acetyl-glucoside, coumaryl-
230 glucoside and total monomeric anthocyanins, free, esterified and total hydroxycinnamic
231 acids and total flavonols ($p < 0.05$). No significant differences were either observed in
232 the content of amino acids and biogenic amines ($p < 0.05$) or in the amount of total
233 proanthocyanidins ($p < 0.05$) between control and treated sparkling wines. Therefore, it
234 could be concluded that the added products did not have any effect on phenolic
235 compounds, amino acids or biogenic amines.

236 Contrary to that was observed with these chemical compounds, significant differences
237 were observed in the content of mannoproteins (Figure 1). In order to know the
238 effectiveness of the addition of the commercial products, the content of mannoproteins
239 was assessed in control and treated sparkling wines before and after the addition of the

product. Except for Verdejo sparkling wines, the samples treated with the DYA-2 preparation showed significantly the highest concentrations of mannoproteins after 3 months of aging. In the same way, Garnacha, Tempranillo and Verdejo treated with DYA-2 showed the highest content of mannoproteins after six months of aging. At the end of the aging, all the wines treated with DYA-2 showed the highest concentrations of mannoproteins, showing 21 to 34% more mannoproteins than their respective controls. These results were attributed to the fact that the DYA-2 was the commercial product with both the highest polysaccharide purity and mannoprotein content. Therefore, the content of mannoproteins added to the wine was higher with the addition of DYA-2 than with the rest of the products.

Effect of the addition of the commercial dry yeast products on the volatile composition of the sparkling wines

Due to the high number of data, initially, multifactor analysis was carried out with all the volatile compound data. Table 3 shows the effects of grape variety, treatment and aging time for each compound. It can be observed that there are strong grape variety and aging time effects on all volatile compounds evaluated. In spite of these effects, the treatment with DYA also showed effect on most of the compounds. Therefore, the volatile data were treated by grape variety separately due to the great influence of this factor.

Factorial analyses were performed, in order to see whether the information given by these compounds would allow differentiating the sparkling wines by the treatment with DYA.

The four factorial analyses (one for each grape variety) selected five factors with an eigenvalue greater than 1, which explained between the 85-91 % of the total variance.

Figure 2 shows the distribution of the different sparkling wines studied in the plane defined by the first two factors, and the percentage of the total variance explained by each factor. In general, the variables associated with factor 1 mainly permit to differentiate the sparkling wines by the aging time. These changes were due to the increase in ethyl esters of branched-chain fatty acids, ethyl lactate, and 2-phenylethanol, and the decrease in ethyl esters of straight-chain fatty acids, alcohol acetates, and terpenes (mainly citronellol and linalool). These results are in agreement with those obtained in a previous work with several grape varieties,[31] and with those obtained by other authors in Garnacha rosé sparkling wines.[41] Therefore, it seems that the grape variety does not affect the changes in volatile composition that occur during the aging time on lees of the sparkling wines.

The aging time is an important factor in the volatile composition of the sparkling wines and it does not allow observing clear differences by the addition of different DYA Therefore, discriminant analyses were carried out in order to see if volatile compounds would allow differentiating the sparkling wines treated with the different commercial products.

These discriminant analyses were carried out by each grape variety, and therefore four different models were obtained. The forward stepwise discriminant analysis was applied to determine the volatile compounds most useful for differentiating the sparkling wines according to the treatment.

The final model for Godello sparkling wines selected 20 volatile compounds. The variables with the greatest discriminating power were decanoic acid, ethyl hexanoate, ethyl decanoate, citronellol, ethyl butyrate and methyl vanillate. Figure 3A shows the distribution of the Godello sparkling wines in the plane defined by the first two discriminant functions, which explained the 68.2% of the total variance. The wines

289 treated with the different commercial products were well separated, and taking into
290 account that the distance between centroids is proportional to the similarity between
291 groups, the wines treated with DYA-2 were more different than control wines. The final
292 model for Verdejo sparkling wines selected 16 volatile compounds, being the first
293 variables selected with the greatest discriminating power ethyl butyrate, decanoic acid,
294 methyl vanillate, terpineol, acetovanillone and ethyl decanoate. Figure 3B shows the
295 distribution of the Verdejo sparkling wines in the plane defined by the first two
296 discriminant functions, which explained the 93.2% of the total variance. In these wines,
297 the treatment with DYA-2 showed also the major differences compared to the control
298 wines.
299 The final model for Garnacha rosé sparkling wines selected 13 volatile compounds. The
300 variables with the greatest discriminating power were decanoic acid, acetovanillone, 2-
301 phenylethyl acetate, ethyl butyrate, ethyl decanoate and isoamyl acetate. Figure 3C
302 shows the distribution of these sparkling wines in the plane defined by the first two
303 discriminant functions, which explained the 87.0% of the total variance. The final
304 model for Tempranillo rosé sparkling wines selected 13 volatile compounds, being the
305 first variables selected with the greatest discriminating power ethyl decanoate, ethyl
306 lactate, octanoic acid, decanoic acid, isovaleric acid and isobutanol. Figure 3D shows
307 the distribution of these sparkling wines in the plane defined by the first two
308 discriminant functions, which explained the 93.6% of the total variance. In both rosé
309 sparkling wines, the treatment with DYA-2 showed also the major differences
310 compared to the control wines, as it was observed in white sparkling wines.
311 On the other hand, the differences observed between the control wines and those treated
312 with the rest of DYA depended on the varietal wine.
313 All the models were satisfactory with a global classification of 100% of the wines.

As it was indicated, the final models for each grape variety were slightly different, and the selected variables were not exactly the same for each model. However, in general the main variables responsible for the observed distribution of wines were some ethyl esters, terpenes (mainly terpineol), decanoic acid and some alcohols.

Considering the results obtained with the factorial and discriminant analyses, the data of the volatile compounds of sparkling wines aged on lees for 9 months were studied in order to see the effect of the addition of DYAs in the sparkling wines, focusing on the more discriminating compounds. Table 4 shows the mean values by treatment and the ANOVA results. It was observed differences depending on the varietal wine and commercial product used. In general, it was observed that the sparkling wines treated with DYAs had a lower content of the ethyl esters of fatty acids than the control wines, with the exception of Verdejo wines. These differences could be due to the sorption phenomenon onto the yeast lees and/or DYAs is a reversible process, and several factors can influence in these interactions.[6,7,42] In addition, all the sparkling wines treated with DYA-2 showed the lowest content mainly in ethyl decanoate. This compound has a high hydrophobity,[43] and therefore they can be retained by the yeast cell walls and mannoproteins.[18,19]

As regards the fatty acids, the decanoic acid presented lower levels in wines treated with DYA-2 than in control wines that indicated the possible adsorption on the DYA surface. This fact could be due to the high hydrophobity of this compound and to the high content of mannoproteins of the DYA-2.

In general, the addition of DYAs in the tirage phase of sparkling wine elaboration maintained higher concentrations of terpenes in the treated wines compared with their respective control wines, and mainly of α-terpineol in white sparkling wines treated with DYA-2. Therefore these products reduced the losses of these compounds occurred

339 during the aging in bottle,[31] and could favor the presence of higher fruity notes in these
340 wines.

341 **Effect of the addition of the commercial dry yeast products on the foam**
342 **parameters determined by Mosalux equipment of the sparkling wines**

343 Figure 4 shows the data of the different Mosalux parameters of the four grape variety
344 sparkling wines and the ANOVA and LSD results.

345 The DYA addition did not modify the foam maximum height (HM) in the white
346 sparkling wines, and only slight differences were observed in rosé sparkling wines. The
347 average values of HM were higher than the obtained in other vintages,[44] which can be
348 due to their different composition in aminoacids and in phenolic compounds in the case
349 of rosé sparkling wines.[45]

350 Slight differences were found in the foam stability (HS) of the white sparkling wines,
351 being the addition of the DYA positive in the Verdejo sparkling wines. In the rosé
352 sparkling wines, only the Tempranillo wines treated with DYA-4 showed statistically
353 significant higher HS values than the rest of the wines.

354 Nuñez et al.[21] showed a positive effect of one mannoprotein thermal extract to the
355 foaming properties of sparkling wines. These different results could be due to they used
356 this type of product in final sparkling wines, not during the aging time on lees.

357 The measurements of time stability (TS) did not show a good repeatability, and
358 therefore it cannot be obtained any conclusion of the effect of DYAs addition in the
359 sparkling wines.

360 Therefore, although some differences were detected in the foam parameters of the
361 different sparkling wines depending on the DYA treatment, it can be pointed out that in
362 general, these commercial products did not modify the foam properties of sparkling
363 wines, and the few differences detected were not justified.

Effect of the addition of the commercial dry yeast products on the sensory analyses of the sparkling wines

The sensory foam properties evaluated using the descriptors defined by Gallart et al.[40] are shown in Table 5. No differences were found in the foam attributes of the different sparkling wines by treatment with DYA. Only the foam area and foam collar of Verdejo sparkling wines showed statistically significant differences.

Generalized Procrustes Analysis (GPA) was applied to the olfactory data to provide information about the relationships between sparkling wines and olfactory attributes (Figure 5). The effects produced on the aroma by the addition of the commercial products were different in the white or rosé sparkling wines. Therefore, the addition of the DYA-2 product enhanced the fruity aromas in both the Verdejo and Godello sparkling wines. On the contrary, the increment in the fruity aromas was not observed in the sparkling rosé wines of Tempranillo or Garnacha.

In summary, the addition of the DYAs used in this study in the tirage phase for sparkling wine elaboration can modify the volatile profile of these wines. However, this effect appears to be associated to the content in mannoproteins of these products, since the volatile changes were mainly observed in DYA-2 that has the highest mannoprotein content and the highest purity. In addition, the fruity aromas of white sparkling wines were enhanced by the addition of DYA-2. On the other hand, the addition of these products did not have any effect on phenolic compounds, amino acids or biogenic amines, as well as on foam properties.

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FIGURE CAPTIONS

Figure 1. Concentration (mg/L) of mannoproteins during the different stages of the aging on lees of the sparkling wines. C: control wines; DYA: wines treated with the commercial dry yeast autolysates; 3M, 6M, 9M: three, six, nine months of aging on lees.

Figure 2. Distribution of the sparkling wines from the four grape varieties in the plane defined by factor 1 and 2. C: control wines; DYA: wines treated with the commercial dry yeast autolysates; 3M, 6M, 9M: three, six, nine months of aging on lees.

Figure 3. Distribution of the sparkling wines from the four grape varieties in the plane defined by the first two discriminant functions of the stepwise model. C: control wines; DYA: wines treated with the commercial dry yeast autolysates.

Figure 4. Foam parameters determined by the Mosalux method and ANOVA results of sparkling wines aged on lees for nine months after tirage. C: control wines; DYA: commercial dry yeast autolysates; HM: foam maximum height; HS: foam stability height. Values with different letters in each parameter and grape variety indicate statistically significant differences at $p < 0.05$ and parameters marked with an asterisk * indicate no statistically significant differences.

Figure 5. Generalized Procrustes Analysis of the mean ratings for olfactory phase in the different sparkling wines after nine months of aging on lees. C: control wines; DYA: wines treated with the commercial dry yeast autolysates.

Table 1. Characteristics of the commercial dry yeast products used in the elaboration of white and rosé sparkling wines and doses applied.

Products added	**Commercial supplier**	**Doses** (g/L)	**Expected effect** (information provided by the manufacturer)	**Characteristics**
DYA-1[a]	Agrovin	0.30	Increase the volume of the lees, increase mouthfeel and foam persistence	Product with autolysated yeast enriched in polysaccharides
DYA-2	Agrovin	0.30	Increase the volume of the lees, increase mouthfeel and foam persistence	Cell wall autolysated yeast enriched in polysaccharides and with 20-22% of soluble mannoproteins
DYA-3	Sepsa-Enartis	0.15	Aromatic protection, increase freshness and prevent browning. Increase colloidal stability, mouthfeel and roundness sensations, improve foam stability	Inactivate yeast with high content in parietal polysaccharides
DYA-4	Sepsa-Enartis	0.15	Aromatic protection, increase freshness and prevent browning. Increase colloidal stability, mouthfeel and roundness sensations, improve foam stability. Protection stability and aromatic persistence	Product with polysaccharides from the yeast cell walls, highly purified and with high content in free mannoproteins

[a] DYA: commercial dry yeast autolysates

Table 2. Monosaccharide composition (%), polysaccharide purity (%) and polysaccharide composition (%) of the different commercial products[a]

% Monosaccharides	Commercial product			
	DYA-1	DYA-2	DYA-3	DYA-4
Apiose	nd[c]	nd	nd	nd
Arabinose	nd	nd	nd	nd
Rhamnose	nd	nd	nd	nd
Xylose	nd	nd	nd	nd
Mannose	52.9±5.1a	85.9±6.1d	77.1±4.5cd	62.3±6.8ab
Dha[b]	nd	nd	nd	nd
Galactose	nd	nd	nd	nd
Gal. Acid[b]	nd	nd	nd	nd
Glucose	47.1±3.2b	14.1±1.7a	22.9±4.3a	37.7±4.6b
Gluc. Acid[b]	nd	nd	nd	nd
% polysaccharide purity[d]	71.3±6.2a	88.8±10.2b	63.2±2.8a	73.6±4.3ab
%∑ (P400-P50)[e]	52.5±4.0a	100.0±0.0b	100.0±7.2b	100.0±7.1b
% P10[e]	47.5±3.0			

[a] The data shown are the average and standard deviation of three analysis of each product. Values with different letter indicate statistically significant differences at $p < 0.05$.

[b] Dha: 3-deoxy-D-lyxo-heptulosaric acid, Gal. Acid: galacturonic acid, Gluc. Acid: glucuronic acid.

[c] nd: no detected

[d] Polysaccharide purity expressed as the total amount of monosaccharides in relation to the dry weight of the product analyzed.

[e]∑ (P400-P50): polysaccharides with an average molecular weight between 47.3 kDa and 404 kDa; P10: polysaccharides with an average molecular weight of 11.8 kDa. Polysaccharide fractions estimated by HRSEC as described in the text.

Table 3. Multifactor analysis of variance carried out considering all the volatile compounds of all the sparkling wines studied.

	Grape variety		Treatment		Aging time	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Ethyl butyrate	45.5	**0.000**	2.23	0.069	35.9	**0.000**
Ethyl 2-methylbutyrate	369	**0.000**	1.10	0.357	142	**0.000**
Ethyl isovalerate	538	**0.000**	1.38	0.242	86.6	**0.000**
Ethyl hexanoate	84.2	**0.000**	5.73	**0.000**	181	**0.000**
Ethyl octanoate	124	**0.000**	10.0	**0.000**	229	**0.000**
Ethyl decanoate	36.9	**0.000**	98.1	**0.000**	194	**0.000**
Ethyl lactate	248	**0.000**	11.3	**0.000**	317	**0.000**
Isoamyl acetate	657	**0.000**	0.52	0.721	406	**0.000**
2-Phenylethyl acetate	452	**0.000**	10.1	**0.000**	542	**0.000**
Isovaleric acid	436	**0.000**	4.55	**0.002**	41.3	**0.000**
Hexanoic acid	36.7	**0.000**	4.53	**0.002**	120	**0.000**
Octanoic acid	303	**0.000**	5.35	**0.001**	103	**0.000**
Decanoic acid	398	**0.000**	109	**0.000**	391	0.142
1-Hexanol	2028	**0.000**	4.78	**0.001**	39.3	**0.000**
trans-3-hexen-1-ol	1387	**0.000**	2.04	0.092	14.6	**0.000**
cis-3-hexen-1-ol	1290	**0.000**	2.69	**0.033**	20.3	**0.000**
Benzyl alcohol	536	**0.000**	9.13	**0.000**	53.1	**0.000**
Linalool	1170	**0.000**	11.2	**0.000**	132	**0.000**
α-Terpineol	1791	**0.000**	16.5	**0.000**	306	**0.000**
Citronellol	560	**0.000**	10.9	**0.000**	137	**0.000**
γ-Butyrolactone	299	**0.000**	4.15	**0.003**	37.5	**0.000**
Methyl vanillate	1180	**0.000**	2.67	**0.034**	16.9	**0.000**
Acetovanillone	3602	**0.000**	2.63	**0.037**	46.5	**0.000**
2-Phenylethanol	811	**0.000**	4.93	**0.001**	100	**0.000**
1-propanol	2566	**0.000**	1.93	0.109	48.4	**0.000**
Isobutanol	392	**0.000**	1.14	0.339	58.0	**0.000**
Isoamyl alcohols	330	**0.000**	2.19	0.073	77.2	**0.000**
4-vinylguaiacol	1878	**0.000**	5.46	**0.000**	81.8	**0.000**

Values in bold showed statistically significant differences in each compound and factor considered (P-values < 0.05).

Table 4. Volatile compounds of sparkling wines aged on lees for nine months after tirage[a]

	Godello					Verdejo				
	C[b]	DYA-1	DYA-2	DYA-3	DYA-4	C	DYA-1	DYA-2	DYA-3	DYA-4
Ethyl butyrate	0.293 b	0.329 c	0.372 d	0.181 a	0.373 d	0.096 a	0.116 a	0.234 b	0.251 b	0.243 b
Ethyl 2-methylbutyrate	0.020 b	0.021 b	0.021 b	0.015 a	0.021 b	0.005 a	0.006 b	0.008 c	0.010 d	0.008 c
Ethyl isovalerate	0.031 a	0.038 b	0.038 b	0.028 a	0.037 b	0.012 a	0.014 a	0.018 b	0.018 b	0.018 b
Ethyl hexanoate	1.226 b	1.311 c	1.208 b	0.954 a	1.190 b	0.555 a	0.563 a	0.776 c	0.770 c	0.638 b
Ethyl octanoate	1.237 d	1.222 cd	1.007 b	0.851 a	1.137 c	0.575 a	0.582 a	0.848 c	0.899 c	0.707 b
Ethyl decanoate	0.081 a	0.126 b	0.077 a	0.154 c	0.117 b	0.077 b	0.076 b	0.062 a	0.119 d	0.094 c
Σ ETHYL ESTERS	2.888 c	3.047 d	2.723 b	2.182 a	2.875 bc	1.320 a	1.357 a	1.946 c	2.067 c	1.708 b
Ethyl lactate	18.6 b	15.8 a	14.7 a	14.5 a	14.7 a	11.6	11.1	12.7	10.4	10.7
Isoamyl acetate	0.826 b	0.881 b	0.853 b	0.723 a	0.915 b	0.590	0.599	0.905	0.811	0.681
Hexyl acetate	0.052 a	0.074 d	0.066 bc	0.060 b	0.070 cd	0.049	0.053	0.071	0.087	0.066
2-Phenylethyl acetate	0.111 a	0.178 b	0.182 b	0.177 b	0.179 b	0.119	0.127	0.119	0.132	0.123
Σ ALCOHOL ACETATES	0.989 a	1.134 b	1.100 b	0.960 a	1.164 b	0.758 a	0.780 a	1.095 c	1.029 c	0.870 b
2-Phenylethanol	40.5	41.7	39.8	38.5	39.0	28.4 a	27.0 a	40.7 b	25.7 a	26.9 a
1-propanol	16.5 a	18.7 ab	21.0 b	21.1 b	19.3 b	23.0 ab	22.9 a	24.1 c	23.6 b	22.8 a
Isobutanol	11.6 a	12.5 ab	13.2 b	13.5 b	13.1 b	12.5	12.8	13.0	13.2	13.4
Isoamyl alcohols	158 a	169 ab	176 b	178 b	168 ab	139	140	139	140	142
Benzyl alcohol	0.074	0.082	0.079	0.077	0.082	0.073 a	0.075 a	0.086 b	0.069 a	0.073 a
Σ FUSEL ALCOHOLS	226 a	242 b	250 b	251 b	240 b	203 a	202 a	217 b	203 a	205 a
1-Hexanol	2.294	2.109	2.084	2.086	2.013	1.081 a	1.057 a	1.195 b	1.020 a	0.984 a
trans-3-hexen-1-ol	0.162 c	0.138 b	0.129 ab	0.123 a	0.132 ab	0.117 a	0.112 a	0.132 b	0.111 a	0.111 a
cis-3-hexen-1-ol	0.227	0.235	0.229	0.224	0.231	0.125	0.117	0.134	0.115	0.117
Σ C6 ALCOHOLS	2.68	2.48	2.44	2.43	2.38	1.32 a	1.29 a	1.46 b	1.25 a	1.21 a

Isovaleric acid	1.220 b	1.086 a	1.042 a	1.041 a	1.059 a	0.647	0.641	0.695	0.645	0.634
Hexanoic acid	10.46 b	8.38 a	8.09 a	8.00 a	7.99 a	7.89 a	7.67 a	10.13 b	7.57 a	7.92 a
Octanoic acid	14.29d	10.31 b	10.05 ab	9.47 a	11.90 c	12.53 c	11.87 bc	10.82 b	9.69 a	10.60 ab
Decanoic acid	1.432 b	1.353 b	1.138 a	1.383 b	1.428 b	1.944 c	1.716 b	1.107 a	1.537 b	1.657 b
Σ FATTY ACIDS	27.4 d	21.1 b	20.3 ab	19.9 a	22.4 c	23.0 b	21.9 b	22.8 b	19.4 a	20.8 ab
Linalool *	2.66 a	4.96 b	5.07 b	4.78 b	5.01 b	2.10 a	2.36 b	2.17 ab	2.82 c	2.29 ab
α-Terpineol *	4.04 a	5.36 c	6.03 d	4.49 b	5.78 cd	1.10 a	1.10 a	1.49 c	1.29 b	1.05 a
Citronellol *	1.63 a	2.33 b	2.33 b	2.36 b	2.24 b	1.58 a	1.89 b	1.61 a	2.31 c	1.72 ab
Σ TERPENES	8.32 a	12.66 c	13.43 d	11.63 b	13.03 cd	4.78 a	5.36 b	5.26 b	6.42 c	5.05 ab
γ-Butyrolactone	10.5 b	8.5 a	8.9 a	8.2 a	8.6 a	5.4 b	5.5 b	6.8 c	5.0 a	5.3 ab
γ-Nonalactone *	2.18 a	2.92 b	2.89 b	2.71 b	2.71 b	nq [c]	nq	nq	nq	nq
Methyl vanillate *	2.20 a	4.51 c	6.17 d	3.22 b	4.07 c	8.4 d	8.1 cd	5.0 a	7.5 b	7.8 bc
Acetovanillone *	6.1 a	7.1 bc	6.7 ab	7.3 bc	7.7 c	16.6 b	16.5 b	18.7 c	15.1 a	16.7 b
4-vinylguaiacol	0.167 a	0.434 b	0.433 b	0.413 b	0.448 b	0.184 a	0.209 b	0.235 c	0.237 c	0.195 ab

	Garnacha					Tempranillo				
	C	DYA-1	DYA-2	DYA-3	DYA-4	C	DYA-1	DYA-2	DYA-3	DYA-4
Ethyl butyrate	0.376 c	0.179 b	0.164 b	0.162 b	0.092 a	0.289 a	0.364 c	0.324 b	0.319 b	0.281 a
Ethyl 2-methylbutyrate	0.013 c	0.006 b	0.006 b	0.007 b	0.003 a	0.009 ab	0.010 c	0.009 a	0.010 bc	0.009 bc
Ethyl isovalerate	0.018 d	0.010 b	0.009 b	0.011 c	0.005 a	0.016	0.019	0.019	0.018	0.017
Ethyl hexanoate	1.239 c	0.560 b	0.542 b	0.520 b	0.306 a	0.728 ab	0.777 b	0.684 a	0.731 ab	0.700 a
Ethyl octanoate	1.137 c	0.459 b	0.449 b	0.444 b	0.320 a	0.875 a	0.990 b	0.811 a	0.820 a	0.860 a
Ethyl decanoate	0.078 a	0.161 c	0.092 a	0.152 bc	0.138 b	0.127 b	0.129 b	0.093 a	0.121 b	0.129 b
Σ ETHYL ESTERS	2.861 c	1.375 b	1.262 b	1.296 b	0.865 a	2.043 b	2.289 c	1.939 a	2.019 ab	1.997 ab
Ethyl lactate	18.6 c	15.5 ab	16.5 b	14.8 a	15.6 ab	17.6 c	18.5 d	16.3 a	16.9 b	17.5 c
Isoamyl acetate	1.914 d	0.796 b	0.753 b	0.964 c	0.403 a	1.273 a	1.630 d	1.421 bc	1.561 cd	1.375 ab
Hexyl acetate	0.067 c	0.036 b	0.035 b	0.034 b	0.019 a	0.042	0.044	0.044	0.044	0.044

2-Phenylethyl acetate	0.197 a	0.245 c	0.219 b	0.261 c	0.224 b	0.117 a	0.126 b	0.127 b	0.121 ab	0.116 a
Σ ALCOHOL ACETATES	2.177 d	1.077 b	1.008 b	1.259 c	0.646 a	1.431 a	1.800 d	1.592 bc	1.726 cd	1.535 ab
2-Phenylethanol	45.3 b	40.2 a	43.0 ab	40.9 a	42.2 a	16.4 a	16.9 ab	17.5 c	16.8 ab	17.0 b
1-propanol	19.2	22.2	21.1	21.0	20.6	43.2	45.1	43.3	43.7	44.8
Isobutanol	16.8	18.0	17.2	17.6	17.5	16.6	17.5	17.1	16.4	17.5
Isoamyl alcohols	171 a	188 b	184 b	186 b	179 ab	164 ab	174 d	170 cd	161 a	168 bc
Benzyl alcohol	0.078 a	0.089 b	0.092 bc	0.088 b	0.098 c	0.045 a	0.073 b	0.043 a	0.043 a	0.044 a
Σ FUSEL ALCOHOLS	252 a	268 b	265 b	266 b	259 ab	241 ab	253 c	248 bc	238 a	247 bc
1-Hexanol	2.041 b	1.693 a	1.682 a	1.731 a	1.714 a	0.617 a	0.692 b	0.582 a	0.620 a	0.614 a
trans-3-hexen-1-ol	0.057 b	0.046 a	0.046 a	0.048 a	0.049 a	0.194 a	0.278 b	0.189 a	0.189 a	0.189 a
cis-3-hexen-1-ol	0.439	0.416	0.441	0.413	0.415	0.285 a	0.440 b	0.283 a	0.282 a	0.284 a
Σ C6 ALCOHOLS	2.54 b	2.16 a	2.17 a	2.19 a	2.18 a	1.10 a	1.41 b	1.05 a	1.09 a	1.09 a
Isovaleric acid	1.044 b	0.959 ab	1.036 b	0.940 a	0.934 a	0.634 a	0.660 b	0.642 a	0.643 a	0.646 a
Hexanoic acid	10.49 b	8.43 a	8.75 a	8.55 a	8.48 a	6.87	7.30	7.44	7.26	7.34
Octanoic acid	13.15 c	8.79 ab	7.97 a	9.03 b	8.32 ab	10.55 a	13.37 c	12.13 b	10.88 a	11.36 ab
Decanoic acid	1.267 b	1.407 c	1.017 a	1.258 b	1.186 b	1.984 cd	2.161 d	1.351 a	1.780 b	1.967 c
Σ FATTY ACIDS	25.9 b	19.6 a	18.8 a	19.8 a	18.9 a	20.0 a	23.5 c	21.6 b	20.6 ab	21.3 b
Linalool *	3.59 a	5.04 c	4.79 b	5.13 d	4.78 b	2.29	2.65	2.56	2.42	2.27
α-Terpineol *	3.18 a	4.03 c	3.54 b	3.91 c	3.28 a	1.14 ab	2.33 d	1.75 c	1.26 b	0.97 a
Citronellol *	2.99 a	3.19 ab	2.98 a	3.50 b	3.19 ab	1.25 a	1.86 d	1.48 c	1.39 b	1.25 a
Σ TERPENES	9.77 a	12.26 c	11.31 b	12.54 c	11.25 b	4.69 ab	6.84 d	5.79 c	5.07 b	4.49 a
γ-Butyrolactone	9.1	9.1	10.2	10.0	9.6	6.3	6.5	6.5	6.4	6.4
γ-Nonalactone *	3.3 a	4.4 c	4.1 bc	4.1 bc	4.0 b	nq	nq	nq	nq	nq
Methyl vanillate *	9.0 a	12.6 c	10.6 b	11.9 c	10.3 b	1.5 c	1.0 a	1.2 b	1.3 bc	1.3 bc
Acetovanillone *	22.8 a	24.7 ab	26.9 bc	27.0 bc	30.2c	13.1	12.9	13.2	13.1	13.1
4-vinylguaiacol	0.028 a	0.045 c	0.039 b	0.042bc	0.040 b	0.005 a	0.008 c	0.006 b	0.005 a	0.005 a

[a] data in mg/L except those marked with an asterisk * that are expressed in µg/L. Values with different letters in each compound and grape variety indicate statistically significant differences at $p < 0.05$ and values without letters indicate no statistically significant differences.
[b] C: control wine; DYA: sparkling wines treated with the different dry yeast autolysates.
[c] nq: below the quantification limit (< 1.2 µg/L)

Table 5. Sensory foam attributes of sparkling wines aged on lees for nine months after tirage[a]

	Godello					Verdejo				
	C[b]	DYA-1	DYA-2	DYA-3	DYA-4	C	DYA-1	DYA-2	DYA-3	DYA-4
Initial foam	2.5	2.7	2.4	2.5	2.6	1.7	2.3	2.3	2.2	1.5
Foam area	2.0	1.9	1.8	2.0	1.9	1.2a	1.8b	2.2b	2.2b	2.0b
Foam collar	2.1	2.2	1.7	2.1	2.3	1.7a	2.3b	2.7b	2.7b	2.2b
Bubble size	2.1	2.2	2.3	1.9	2.3	2.3	2.5	2.3	2.2	2.0
Effervescence	2.3	2.2	2.2	2.2	2.1	1.8	2.2	2.0	2.5	1.8
	Garnacha					Tempranillo				
	C[b]	DYA-1	DYA-2	DYA-3	DYA-4	C	DYA-1	DYA-2	DYA-3	DYA-4
Initial foam	2.3	2.5	2.2	2.3	2.4	2.3	2.7	2.5	2.5	2.5
Foam area	2.1	2.2	2.1	2.1	2.1	2.3	2.5	2.7	2.3	2.3
Foam collar	2.2	2.4	2.4	2.4	2.6	2.7	2.7	2.7	2.7	2.7
Bubble size	2.5	2.4	2.3	2.1	2.3	2.8	2.5	2.5	2.5	2.3
Effervescence	1.9	2.3	2.2	2.3	2.1	2.7	2.3	2.2	2.7	2.2

[a] Values with different letters in each attribute and grape variety indicate statistically significant differences at $p < 0.05$ and values without letters indicate no statistically significant differences.
[b] C: control wine; DYA: sparkling wines treated with the different dry yeast autolysates.

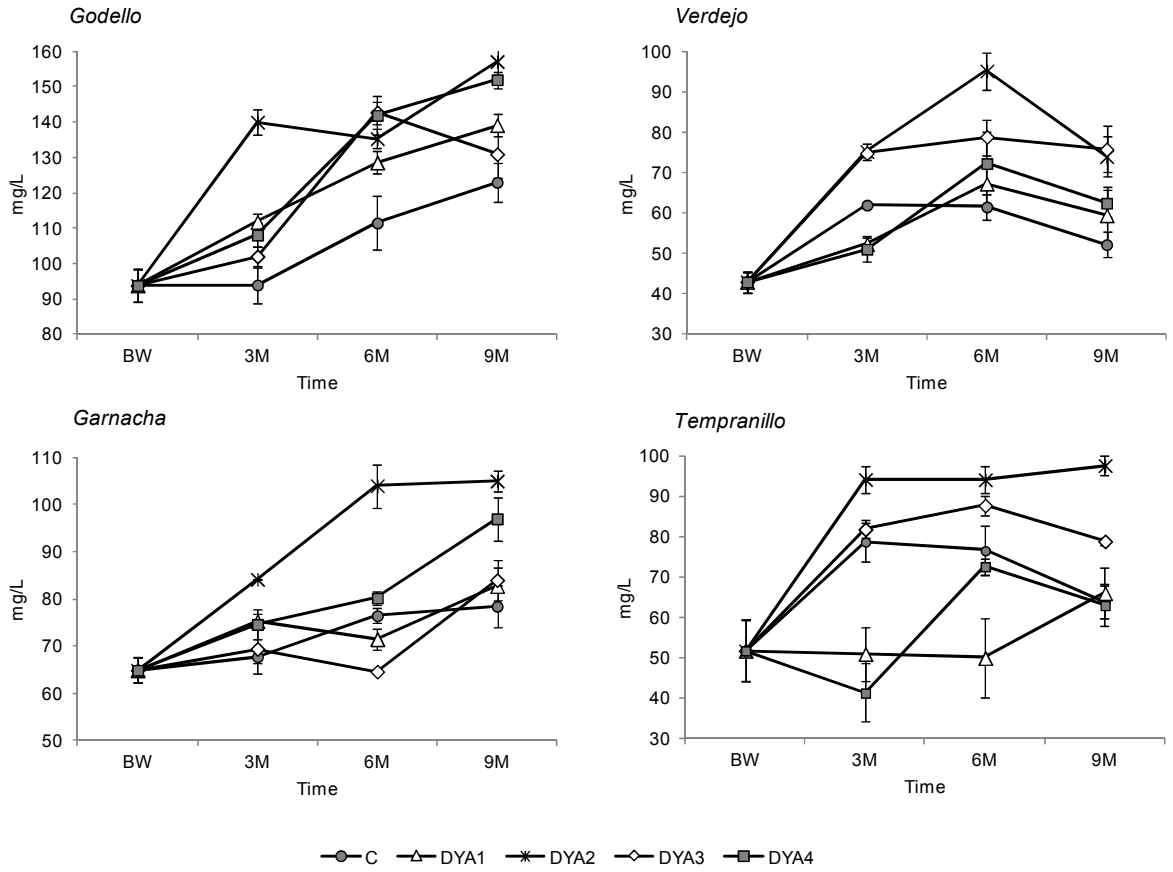

Godello
Verdejo
Garnacha
Tempranillo
mg/L
Time
BW
3M
6M
9M
C
DYA1
DYA2
DYA3
DYA4

Figure 1

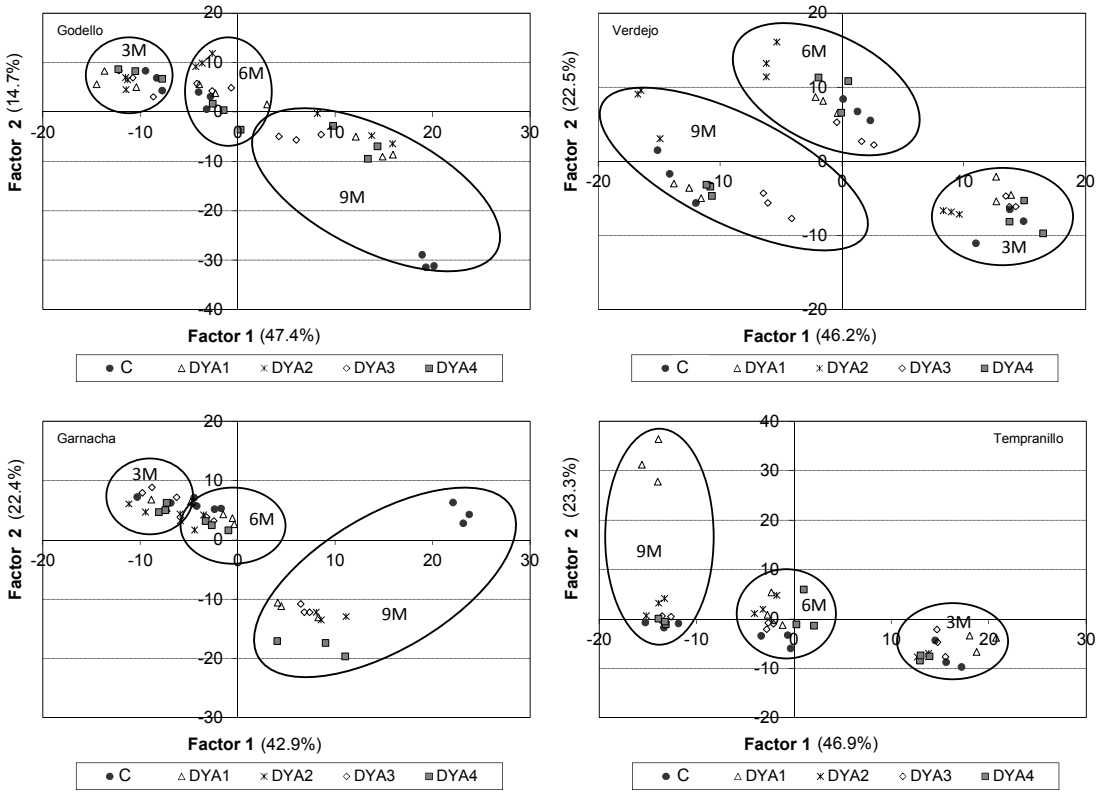

Godello
3M
6M
9M
Factor 2 (14.7%)
Factor 1 (47.4%)
Verdejo
6M
9M
3M
Factor 2 (22.5%)
Factor 1 (46.2%)
Garnacha
3M
6M
9M
Factor 2 (22.4%)
Factor 1 (42.9%)
Tempranillo
9M
6M
3M
Factor 2 (23.3%)
Factor 1 (46.9%)
C
DYA1
DYA2
DYA3
DYA4

Figure 2

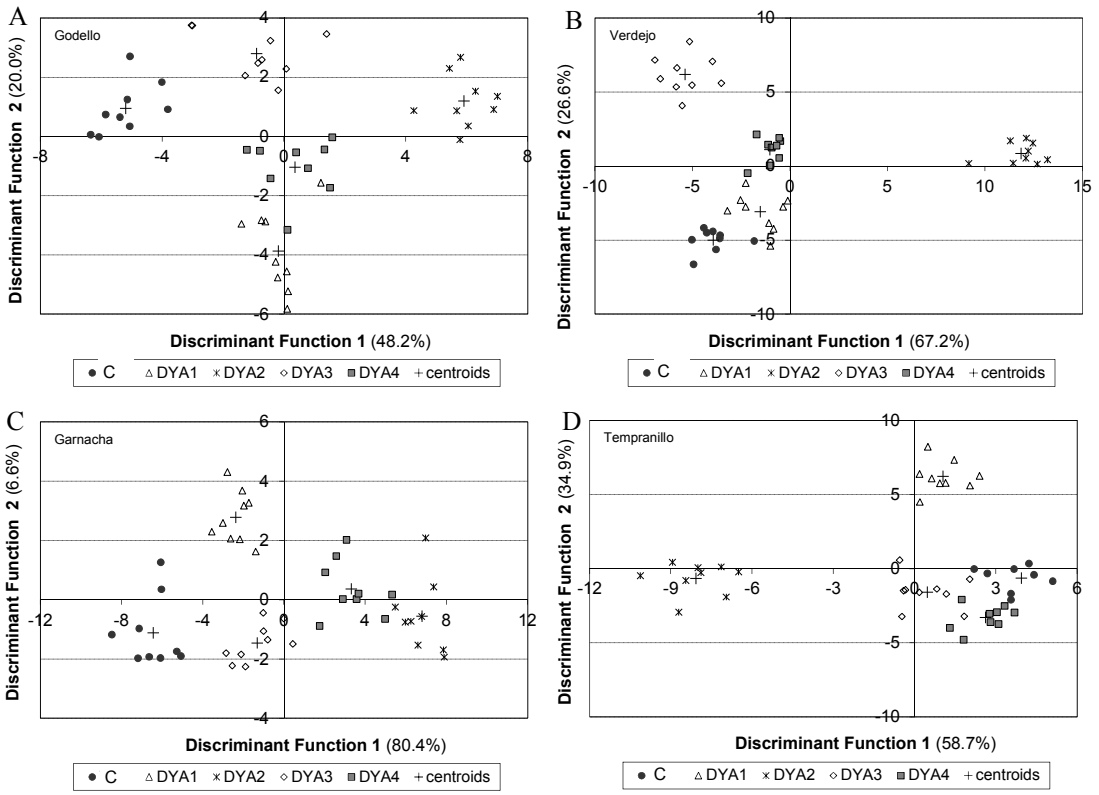

A
Godello
Discriminant Function 2 (20.0%)
Discriminant Function 1 (48.2%)
B
Verdejo
Discriminant Function 2 (26.6%)
Discriminant Function 1 (67.2%)
C
Garnacha
Discriminant Function 2 (6.6%)
Discriminant Function 1 (80.4%)
D
Tempranillo
Discriminant Function 2 (34.9%)
Discriminant Function 1 (58.7%)
C DYA1 DYA2 DYA3 DYA4 centroids

Figure 3

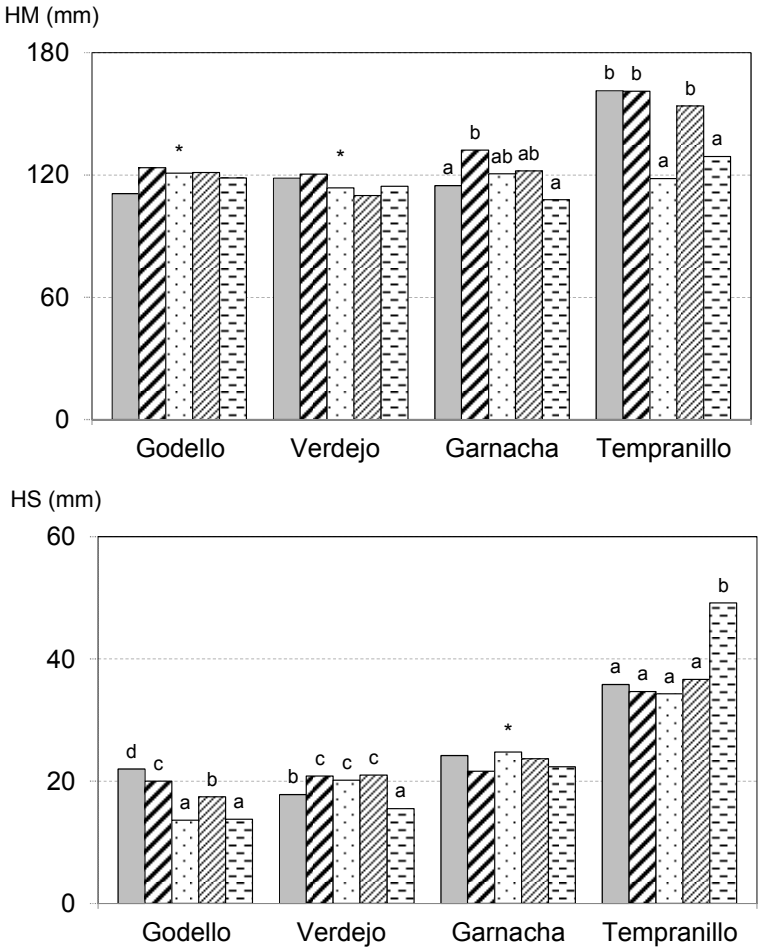

HM (mm)
180
120
60
0
Godello
Verdejo
Garnacha
Tempranillo
HS (mm)
60
40
20
0
Godello
Verdejo
Garnacha
Tempranillo

Figure 4

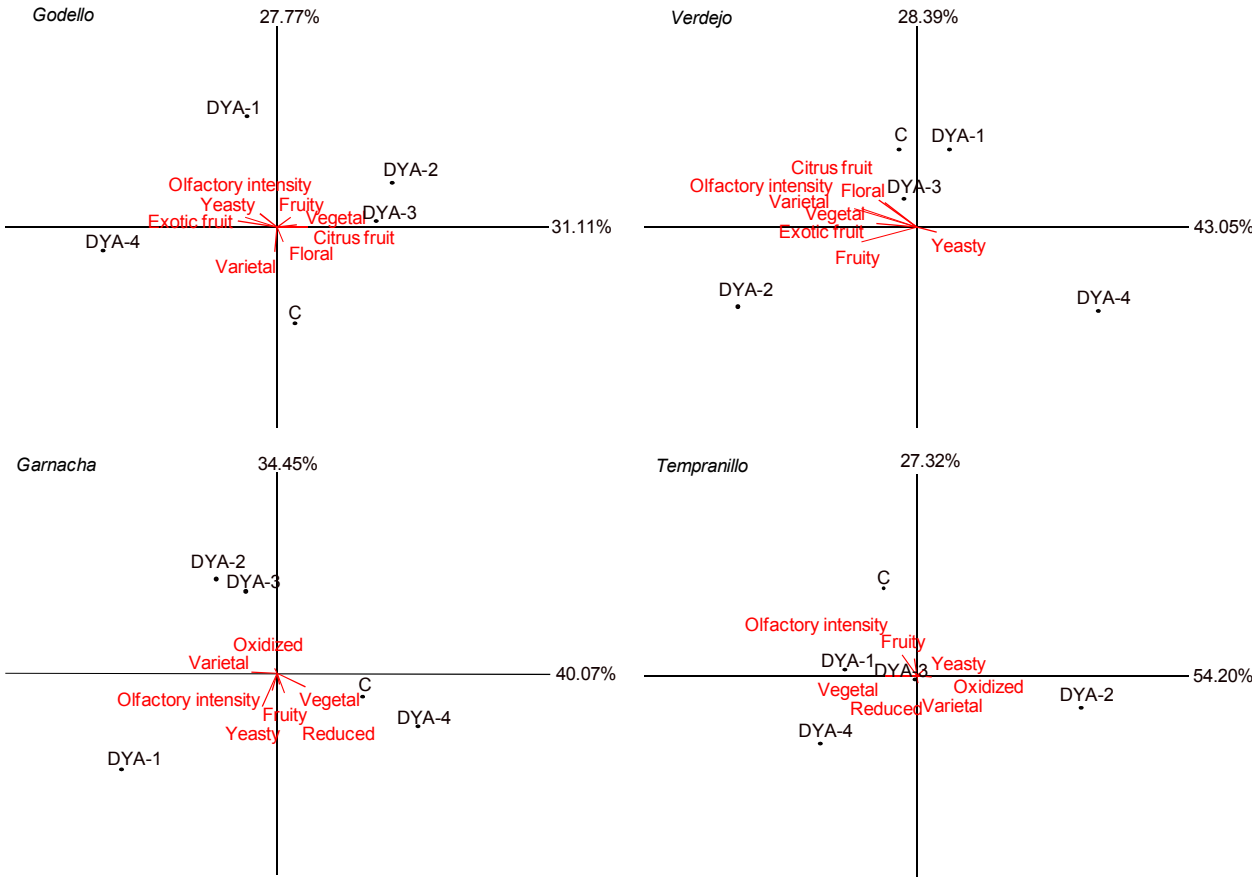
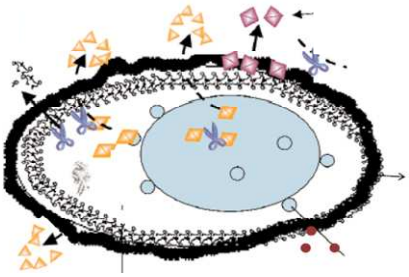

Godello
27.77%
DYA-1
DYA-2
Olfactory intensity
Yeasty
Fruity
Exotic fruit
Vegetal
DYA-3
DYA-4
31.11%
Citrus fruit
Floral
Varietal
C
Verdejo
28.39%
C
DYA-1
Citrus fruit
Olfactory intensity
Floral
DYA-3
Varietal
Vegetal
Exotic fruit
Yeasty
Fruity
DYA-2
DYA-4
43.05%
Garnacha
34.45%
DYA-2
DYA-3
Oxidized
Varietal
40.07%
C
Olfactory intensity
Vegetal
Fruity
DYA-4
Yeasty
Reduced
DYA-1
Tempranillo
27.32%
C
Olfactory intensity
Fruity
DYA-1
DYA-3
Yeasty
54.20%
Vegetal
Oxidized
DYA-2
Reduced
Varietal
DYA-4

Figure 5

TOC grafic

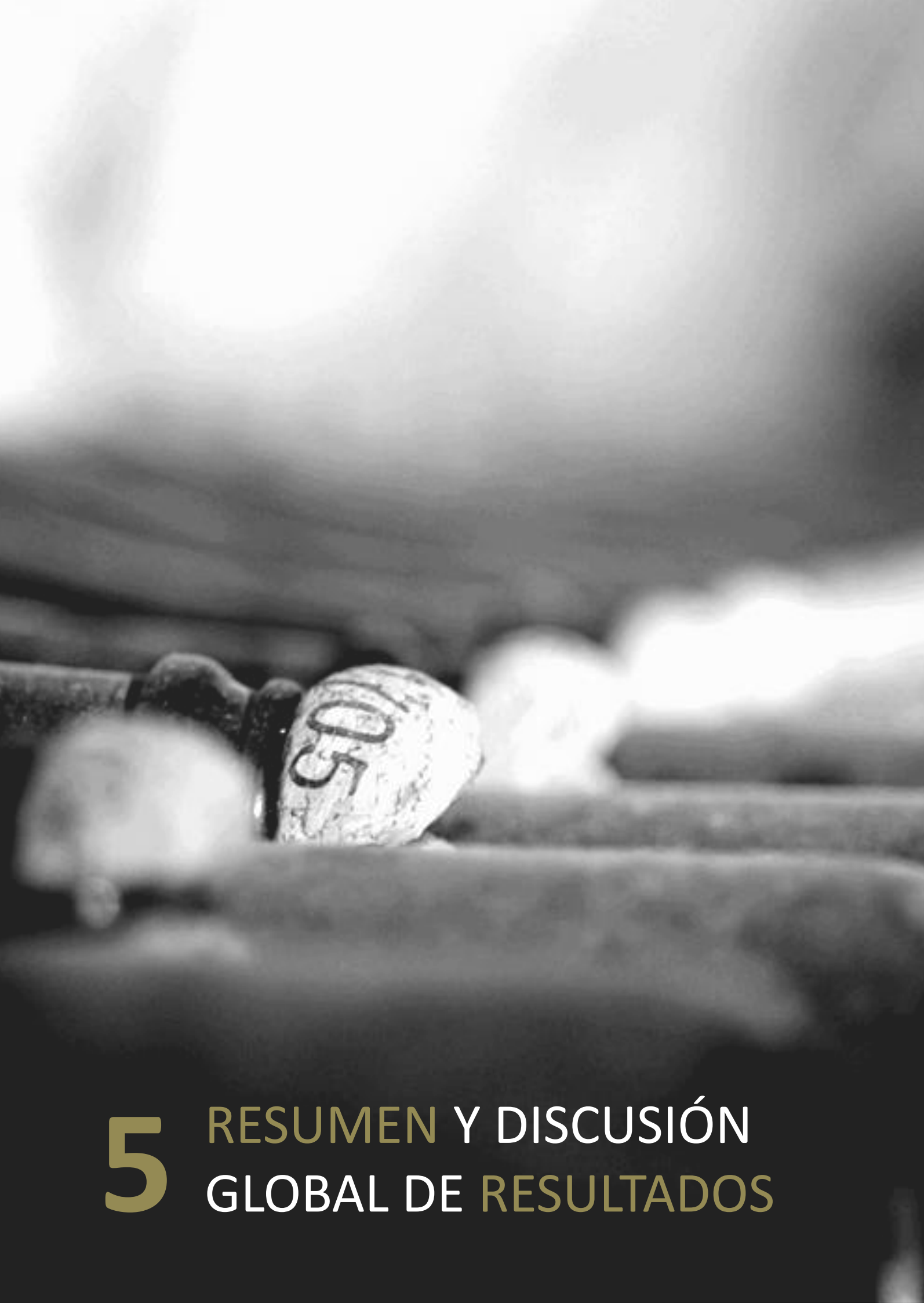
SPARKLING WINE ELABORATION



YEAST AUTOLYSES / COMMERCIAL DRY YEAST PRODUCTS

Has effects on..?

- Volatile compounds
- Mannoproteins
- Foam properties
- Phenolic compounds
- Aminoacids
- Biogenic amines
- Sensory analyses



5 RESUMEN Y DISCUSIÓN GLOBAL DE RESULTADOS

Diversos factores afectan a la composición química del vino espumoso tales como las características vitícolas, la tecnología de vinificación aplicada o la duración del periodo de crianza sobre lías, aunque la variedad de uva seleccionada para la elaboración del vino base es considerado uno de los factores más importantes (*1,2*). Sin embargo, la mayoría de los trabajos científicos dedicados a la elaboración de vinos espumosos elaborados según el método tradicional se han realizado con variedades de uvas autorizadas en la región de *Champagne* y de Cava. Aunque existen estudios científicos sobre la adaptabilidad de las variedades Viura, Malvasía (*3-8*) y Garnacha (*9-11*) para la elaboración de este tipo de vinos, no existen trabajos que realicen una caracterización en términos de compuestos fenólicos, aminoácidos y aminas biógenas, compuestos volátiles, polisacáridos, características espumantes y análisis sensorial de dichas variedades y de otras seleccionadas en esta tesis, tales como Prieto Picudo, Verdejo, Albarín y Godello.

Por lo anteriormente expuesto, y dado que el tiempo mínimo de crianza sobre lías de los vinos espumosos es de nueve meses, se caracterizaron enológicamente los vinos rosados espumosos monovarietales de las variedades de uva tintas de Garnacha y Prieto Picudo, y blancos de las variedades de uva de Malvasía, Verdejo, Godello, Viura y Albarín, elaborados por el método tradicional a los nueve meses de crianza sobre lías, en términos de compuestos fenólicos monoméricos y poliméricos, aminoácidos, aminas biógenas, compuestos volátiles, polisacáridos, características espumantes y análisis sensorial.

A los nueve meses de crianza sobre lías, todos los vinos rosados espumosos mostraron una mayor concentración de antocianos totales a la descrita previamente en la bibliografía en vinos rosados espumosos elaborados con la variedad Garnacha (*11*), siendo los elaborados con la variedad Prieto Picudo los que tuvieron un mayor contenido (2,49 ± 0,10 mg/L). Al igual que lo observado en vinos espumosos elaborados con variedades diferentes a las empleadas en la tesis (*4,9,12*), los ácidos hidroxicinámicos libres, cafeico, cumárico y ferúlico, y el ácido hidroxibenzoico, ácido gálico, fueron detectados en muy bajas concentraciones en todas las muestras de vino

analizadas. A excepción de los vinos elaborados con la variedad Garnacha, que destacaron por un mayor contenido en ácidos hidroxicinámicos totales (73,85 ± 2,86 y 71,64 ± 2,79 mg/L), todos los vinos espumosos mostraron concentraciones de ácidos hidroxicinámicos similares a las obtenidas en vinos blancos espumosos de Chardonnay, Pinot noir, Macabeo, Parellada, y Xarel.lo, y superiores a las obtenidas en vinos blancos espumosos de Trepat y Monastrell (*4,9,12*). El ácido *trans*-caftárico fue el mayoritario en los vinos espumosos de Albarín, Viura, Godello, Malvasía y Garnacha, mientras que la forma *cis*- fue la más abundante en los vinos de Verdejo y de Prieto Picudo. Estos resultados contrastan con otros estudios que indican que la forma *trans*- es la más abundante en los vinos (*4,9,12*). El contenido de flavonoles totales sólo pudo ser cuantificado en los vinos rosados espumosos de Garnacha (2,14 ± 0,08 y 1,73 ± 0,07 mg/L) y de Prieto Picudo (1,08 ± 0,02 mg/L) y el único flavan-3-ol detectado dentro de los límites de cuantificación fue la (+) catequina. Así, la concentración de catequina en los vinos espumosos de Viura, Godello, Malvasía y Verdejo estuvo dentro del rango obtenido en otros vinos espumosos (*9,12*). Sin embargo, el contenido de (+) catequina en los vinos espumosos de Albarín, Garnacha y Prieto Picudo fue considerablemente mayor. Al igual que lo observado en otros compuestos fenólicos, el contenido de proantocianidinas fue independiente del color de la uva empleada y del proceso de vinificación seguido para elaborar vinos espumosos, mostrando todos los vinos concentraciones de proantocianidinas similares a las obtenidas en vinos blancos espumosos (*13*). Los vinos rosados espumosos de Garnacha y los blancos de Albarín presentaron los mayores valores de polifenoles totales. A excepción de los vinos elaborados con la variedad Garnacha, que mostraron mayor concentración de ácidos hidroxicinámicos que de proantocianidinas, en todos los vinos espumosos las proantocianidinas representaron más del 62% de los polifenoles totales, seguidas por los ácidos hidroxicinámicos (15-35%), catequina (2-10%), y otros fenoles (< 2%).

Tras los nueve meses de crianza sobre lías, los vinos blancos espumosos de Godello, Verdejo y Albarín presentaron valores significativamente más altos de aminoácidos totales (103 ± 0,3; 83 ± 4 y 92 ± 1 mg/L, respectivamente) que Viura y Malvasía (20 ± 1 y 14 ± 0,1 mg/L, respectivamente). Entre los vinos rosados espumosos destacaron los elaborados con la variedad Prieto Picudo, que presentaron un valor de aminoácidos totales 20 veces superior a los vinos elaborados con la variedad Garnacha (365 ± 10; 17 ± 0,1 y 21 ± 0,4 mg/L, respectivamente). El aminoácido L-prolina fue el mayoritario en los vinos espumosos de Albarín, Verdejo, Godello y Prieto Picudo, mientras que el aminoácido L-histidina, fue el prevalente en los vinos de Viura, Malvasía y Garnacha. Tal y como han indicado diversos autores (*14-16*) estos resultados ponen de manifiesto que

la variedad de uva tiene gran influencia sobre la composición cualitativa y cuantitativa de los aminoácidos presentes en los vinos. Los niveles de aminas biógenas en los vinos espumosos fueron inferiores a los límites considerados como un riesgo para la salud (*17,18*), siendo la putrescina la amina biógena mayoritaria en todos los vinos analizados (34-73% del contenido total).

Al igual que lo observado en los aminoácidos, los vinos espumosos de Albarín, Godello, Verdejo y Prieto Picudo presentaron las mayores concentraciones de compuestos volátiles, especialmente de ésteres etílicos y alcohol acetatos, compuestos que son responsables de los aromas frutales de los vinos (*19*).

Con respecto a los polisacáridos procedentes de las levaduras, destacaron los vinos espumosos elaborados con la variedad Viura y Prieto Picudo por su mayor contenido de manoproteínas (MP), y los elaborados con las variedades Garnacha y Prieto Picudo, por su mayor concentración en glucanos (GL). A excepción de los vinos espumosos elaborados con la variedad Viura, los GL fueron los polisacáridos mayoritarios procedentes de las levaduras en todos los vinos estudiados.

Entre las familias de polisacáridos procedentes de las uvas, la familia de polisacáridos ramnogalacturonanos tipo II (RG-II) únicamente fue detectada en los vinos rosados espumosos elaborados con la variedad Prieto Picudo. La ausencia de la molécula de RG-II en todos los vinos blancos espumosos se debió al proceso de elaboración de los vinos base blancos. La molécula RG-II se encuentra fuertemente unida a la matriz de la pared celular de las paredes celulares de uva y es resistente a las enzimas pectinolíticas, necesitando largos tiempos de maceración para solubilizar (*20,21*). La ausencia de maceración prefermentativa, así como una fermentación alcohólica en ausencia de hollejos, impidieron la extracción de los RG-II en los vinos base blancos. Sin embargo, los vinos base rosados de Prieto Picudo y de Garnacha fueron elaborados bajo las mismas condiciones de madurez de la uva, maceración prefermentativa y fermentación alcohólica. Por lo tanto, las diferencias observadas con respecto a la molécula de RG-II pueden deberse a diferencias en la debilidad de los hollejos de la uva que podrían modular la extracción de componentes al vino, sugiriendo una cierta característica varietal.

Aunque el contenido de homogalacturonanos (HL) fue superior a lo observado en vinos tranquilos (*20,22*), entre los polisacáridos procedentes de las uvas, destacaron por su mayor concentración los polisacáridos ricos en arabinosa y en galactosa (PRAG). Los vinos espumosos de Prieto Picudo destacaron por su mayor contenido en PRAG y en HL, no obstante, todos los vinos blancos y rosados espumosos mostraron concentraciones

similares de HL y PRAG, indicando la ausencia de solubilización de estos compuestos durante la maceración prefermentativa de los vinos base rosados.

Entre los vinos blancos espumosos destacaron los elaborados con la variedad Verdejo por su mayor contenido en polisacáridos totales (285,34 ± 20,15 mg/L), y entre los vinos rosados espumosos, los de Prieto Picudo (436,45 ± 28,56 mg/L). Todos los vinos estuvieron compuestos por PRAG, MP, GL y HL, con porcentajes de 33 ± 5%, 25 ± 9%,36 ± 9% y 6 ± 3%, respectivamente. Del mismo modo, los vinos espumosos de Verdejo y de Prieto Picudo presentaron los mayores valores en cuanto a los parámetros determinados por el método Mosalux y relacionados con la calidad de la espuma.

El trabajo de caracterización enológica se completó con un análisis sensorial de los vinos espumosos a los nueve meses de crianza sobre lías. Así, se pudo constatar que los vinos espumosos de Prieto Picudo mostraron una mayor intensidad de color visual, de tonos rojos, de frescor en boca, de intensidad aromática, principalmente debida a aromas frutales y varietales, y de calidad de la espuma que los elaborados con la variedad Garnacha. Por otro lado, los vinos espumosos de Albarín y de Verdejo presentaron mayor intensidad de color visual e intensidad aromática que el resto de vinos blancos. Los vinos blancos espumosos de Albarín y de Godello mostraron mayor intensidad aromática que los elaborados con Viura y Malvasía, caracterizándose por los aromas frutales; mientras que en los vinos espumosos de Godello predominaron los aromas cítricos, de frutas exóticas y vegetales. Los vinos espumosos de Verdejo mostraron la mejor calidad de la espuma entre los vinos blancos.

Además de la caracterización enológica de los vinos espumosos a los nueve meses de crianza sobre lías, se evaluaron los cambios que se producen en términos de composición fenólica, aminoácidos, aminas biógenas, compuestos volátiles, polisacáridos y calidad de la espuma durante el proceso de elaboración de los vinos espumosos. Así, se estudió la evolución de los compuestos fenólicos, aminoácidos y aminas biógenas durante nueve meses de crianza sobre lías. Debido a la ausencia de trabajos científicos con respecto a las familias de polisacáridos en vinos espumosos, se estudió la evolución de estos compuestos durante treinta meses de crianza sobre lías. Del mismo modo, y dada la importancia de la calidad de la espuma y de la composición volátil en la evaluación cualitativa de los vinos espumosos, y de que no existe bibliografía con respecto a su evolución durante largos periodos de crianza en presencia y en ausencia de lías, se estudiaron los cambios que se producen en dichos parámetros durante treinta meses en presencia de lías, así como después del degüelle.

Todas las familias de compuestos fenólicos disminuyeron durante el proceso de elaboración de los vinos espumosos. La concentración de todas las formas de antocianos se vio reducida durante dos estados del proceso de vinificación: el proceso de estabilización-clarificación y durante los primeros seis meses de envejecimiento de los vinos sobre sus lías. Los descensos durante la clarificación-estabilización se atribuyeron al tratamiento por frío y a la adsorción del material fenólico por la bentonita y el PVPP, mientras que las disminuciones durante el envejecimiento se debieron a la adsorción de estos compuestos sobre las paredes celulares de las lías de las levaduras (*23,24*) y/o a su combinación con otros componentes del vino. Cabe destacar que la mayor pérdida de compuestos antociánicos ocurrió en los vinos elaborados con la variedad Garnacha y se debió al proceso de clarificación-estabilización. Este hecho parece indicar que los vinos elaborados con Prieto Picudo fueron más estables en términos de color y compuestos antociánicos. Además, el contenido de antocianos totales disminuyó en ambas variedades durante los primeros seis meses de envejecimiento sobre lías. Sin embargo, parte de los compuestos antociánicos adsorbidos al inicio de la fase de tiraje fueron liberados durante los últimos tres meses de envejecimiento debido al proceso autolítico de las levaduras (*25,26*).

Con la excepción de los vinos elaborados con Viura, los procesos de clarificación-estabilización en los vinos blancos produjeron una disminución de ácidos hidroxicinámicos totales. Por el contrario, los procesos de clarificación-estabilización no produjeron ningún efecto en el contenido de ácidos hidroxicinámicos de los vinos rosados. Durante los primeros tres meses de envejecimiento se observó un descenso en el contenido de ácidos hidroxicinámicos totales en todos los vinos. Sin embargo, este descenso fue más acusado en aquellos vinos que mostraban mayor contenido de estos compuestos tras el proceso de clarificación-estabilización. Siguiendo esta tendencia, el contenido de ácidos hidroxicinámicos totales disminuyó aproximadamente un 45% en los siguientes tres meses de envejecimiento sobre lías en aquellos vinos que mostraban los mayores contenidos (los elaborados con Garnacha), mientras que en el resto de vinos la concentración de ácidos se mantuvo constante. Finalmente, en los últimos tres meses de envejecimiento se observó un incremento del 70% en ácidos hidroxicinámicos totales en los vinos elaborados con la variedad Garnacha, mientras que en el resto de vinos la concentración se mantuvo constante. Teniendo en cuenta estas diferencias, parece ser que la evolución de los ácidos hidroxicinámicos estuvo relacionada con su concentración en el vino más que con cualquier otro factor. Los primeros meses de envejecimiento sobre lías se observaron pérdidas en la concentración de ésteres de ácidos hidroxicinámicos debido a su interacción con otros componentes del vino para

dar pigmentos estables (*27*) y/o porque estos compuestos pueden ser adsorbidos por las lías de las levaduras (*28*). En este sentido, se pudo observar que en los vinos base que mostraban mayores valores en ácidos hidroxicinámicos, ocurrían mayores fenómenos de adsorción o transformación. Finalmente, como se observó en el caso de los antocianos monómeros, los ácidos hidroxicinámicos adsorbidos fueron liberados al medio durante el proceso autolítico de las levaduras, aunque este fenómeno sólo fue observado en aquellos vinos que mostraban elevadas cantidades de estos compuestos. Contrariamente a lo observado en otros compuestos fenólicos, los procesos de clarificación-estabilización no afectaron a la concentración de (+) catequina en los vinos. Sin embargo, la fase de tiraje produjo una disminución en su concentración en aquellos vinos que mostraban mayores contenidos iniciales.

Los procesos de clarificación-estabilización redujeron significativamente el contenido de proantocianidinas en todos los vinos base, siendo los elaborados con la variedad Garnacha los que mostraron mayores pérdidas. El grado medio de polimerización de las proantocianidinas (mDP) se mantuvo durante el proceso de clarificación-estabilización, aunque este proceso modificó el porcentaje de unidades terminales en los vinos. Como ya se observó en otros compuestos fenólicos, se distinguieron dos tendencias durante la fase de tiraje: la concentración de proantocianidinas disminuyó en todos los vinos durante los primeros seis meses de envejecimiento, para aumentar durante los últimos tres meses. Esta pérdida de proantocianidinas se puede atribuir a fenómenos de adsorción sobre las lías de las levaduras (*28*) y a reacciones de condensación, polimerización y precipitación de taninos (*29*). El aumento de proantocianidinas observado durante los últimos meses de envejecimiento se puede atribuir a la liberación de estos compuestos durante el proceso autolítico de las levaduras.

Al igual que lo observado por otros autores durante el mismo periodo de crianza sobre lías (*30,31*), la concentración de aminoácidos libres permaneció estable o disminuyó a los nueve meses de envejecimiento. La disminución del contenido de aminoácidos en los vinos espumosos con respecto a los vino base puede deberse a reacciones de desaminación de los aminoácidos o a su participación en la formación de diferentes compuestos (*30,32,33*). El contenido de aminas biógenas permaneció constante durante los primeros tres meses de envejecimiento sobre lías, periodo en el cual tuvo lugar la segunda fermentación en botella. Estos resultados sugieren que las levaduras empleadas en la elaboración no produjeron aminas biógenas. Sin embargo, se observó un ligero incremento de concentración en aminas biógenas totales a los seis meses de crianza sobre lías. Este hecho pudo ser debido a la liberación de aminoácidos precursores de aminas biógenas durante el proceso autolítico de las levaduras (*34,35*),

así como a la presencia en el medio de microorganismos con actividad descarboxilasa y a enzimas descarboxilasas liberadas al medio durante la autolisis de las levaduras (*36*).

En general, el contenido de GL en los vinos incrementó desde los tres a los seis meses de envejecimiento en presencia de lías, mientras que el contenido en MP mostró aumentos durante los primeros seis meses de envejecimiento. Estos resultados indican que las MP fueron liberadas más fácilmente que los GL durante el proceso autolítico de las levaduras. Por otro lado, en periodos de envejecimiento sobre lías superiores a seis meses se observó que el contenido de MP y de GL permaneció constante o disminuyó gradualmente. Así, la concentración de manosa y glucosa fue aproximadamente tres veces superior en los vinos con seis meses de crianza sobre lías que en los de treinta meses. Estos resultados contrastan con los obtenidos por otros autores (*3,37*), quienes observaron un incremento de monosacáridos neutros a los dieciocho meses de crianza sobre lías. La ausencia de incremento de MP y GL puede atribuirse a diferentes aspectos. Por un lado, que las condiciones autolíticas del medio (bajo pH y temperatura, presencia de etanol y elevada presión de CO_2), así como la ausencia de movimiento de las lías durante la crianza, causaran una reducción de la actividad hidrolítica de las enzimas implicadas en el proceso autolítico y una menor liberación de polisacáridos de las levaduras. Por otro lado, que el ratio de precipitación de los polisacáridos durante este periodo fuera mayor que su solubilización en el vino, atribuyéndose éstas disminuciones a fenómenos de precipitación como resultado de su interacción con otros constituyentes del vino para formar coloides inestables. La distribución de los pesos moleculares de los polisacáridos pareció indicar que la reducción en MP y en GL ocurrió principalmente en los compuestos de menor peso molecular.

Los HG y los RG-II disminuyeron durante los primeros seis meses de envejecimiento sobre lías y los PRAG se mantuvieron constantes. Periodos de envejecimiento sobre lías superiores a seis meses produjeron una reducción de todas las familias de polisacáridos procedentes de las uvas, siendo los descensos más acusados en los HG.

Cabe destacar que los vinos base con el mayor contenido de polisacáridos totales mostraron un mayor descenso de estos compuestos durante el envejecimiento sobre lías. Este hecho sugiere que una importante cantidad de polisacáridos “extra” precipita durante la crianza sobre lías. Por lo tanto, las técnicas de vinificación empleadas para incrementar la extracción y liberación de polisacáridos durante la vinificación no serían tan interesantes como se esperaba, dado que un contenido superior de polisacáridos podría estar relacionado con una mayor precipitación. Los resultados obtenidos en este estudio muestran que el mayor contenido de MP y de PRAG fue obtenido a los seis

meses de envejecimiento sobre lías. En este sentido, estos resultados parecen indicar que no son necesarios mayores tiempos de envejecimiento sobre lías para obtener mayores contenidos de polisacáridos en el vino. Por otro lado, a los seis meses de envejecimiento sobre lías se observó una disminución en los pesos moleculares de los polisacáridos del vino. La combinación de estos dos fenómenos podría implicar una mejor estabilidad de la espuma y por tanto, una mejor calidad de los vinos.

Durante la crianza sobre lías se observó un aumento de los ésteres etílicos de ácidos grasos ramificados y un descenso de acetatos de alcoholes superiores y de terpenos, principalmente citronelol y linalol. En general, los vinos envejecidos sobre lías durante nueve y dieciocho meses mostraron valores más altos de lactato de etilo y de alcoholes isoamílicos que los envejecidos durante treinta meses. Durante el envejecimiento en ausencia de lías se observó un descenso en los ésteres etílicos de ácidos grasos de cadena lineal, acetatos de alcoholes superiores, ácidos grasos, alcoholes C6 y terpenos, y un incremento en ésteres etílicos de ácidos grasos ramificados y vainillina.

Algunos de estos cambios observados durante el envejecimiento de los vinos espumosos en ausencia de lías están de acuerdo con los observados por otros autores durante el envejecimiento en botella de vinos tranquilos (*38,39*). Aunque se observaron algunas diferencias entre los vinos espumosos en función del tiempo y tipo de envejecimiento, los resultados obtenidos indicaron que los vinos espumosos mantuvieron sus características varietales durante el envejecimiento en presencia y en ausencia de lías.

Las propiedades espumantes de los vinos fueron similares a las obtenidas en vinos espumosos de alta calidad, como *Champagne* y Cava (*6,40,41*) y se mantuvieron estables o mejoraron durante los treinta meses de crianza en presencia de lías. Por otro lado, el envejecimiento en ausencia de lías no provocó una disminución de la calidad de la espuma. Las diferencias varietales en cuanto a las características espumantes se mantuvieron durante el periodo de crianza en presencia y en ausencia de lías, siendo los vinos espumosos de Verdejo y de Prieto Picudo los que mostraron las mejores características espumantes, seguidos por los vinos de Albarín y de Godello.

Teniendo en cuenta los resultados obtenidos, puede deducirse que las variedades Albarín, Verdejo, Godello y Prieto Picudo son las más apropiadas para la elaboración de vinos espumosos de calidad, pudiéndose incrementar el potencial enológico de estas variedades tradicionalmente empleadas para la elaboración de vinos tranquilos.

La espuma es una cualidad que define el vino espumoso, que le distingue de otros vinos y es la primera que observa el consumidor. Por tanto, resulta evidente que la

calidad de un vino espumoso está fuertemente condicionada por sus características espumantes. Por esta razón, el conocimiento de los factores que tienen un papel sobre la espumabilidad y la estabilidad de la espuma es un tema de gran interés para la enología. Así, la espuma ha merecido una especial atención y ha sido objeto de diferentes estudios científicos encaminados a detectar los componentes principalmente responsables de su aparición (*3,42-51*), así como de los factores intrínsecos (variedad de uva, vendimia) (*40,41,52,53*) y extrínsecos (prácticas tecnológicas) (*6,54,55*) que afectan a la composición y a las propiedades espumantes. De hecho, aunque la bibliografía existente es abundante, los conocimientos actuales no han permitido establecer cuáles son los compuestos implicados en la formación y estabilidad de la espuma. Teniendo en cuenta lo anterior, se determinó la influencia de los polisacáridos, los polifenoles y las sustancias nitrogenadas en las características espumantes de vinos blancos y rosados espumosos a los nueve meses de crianza sobre lías. La calidad de la espuma de los vinos, definida por la espumabilidad (HM, altura máxima de la espuma después de la inyección de CO_2; y HS, altura a la cual estabiliza la espuma durante la inyección de CO_2) y la estabilidad de la espuma (TS, tiempo necesario para la desaparición completa de la espuma cuando se para la inyección de CO_2) fue determinada por el método Mosalux.

Los resultados obtenidos indicaron una contribución positiva de los antocianos monómeros y de los aminoácidos y una contribución negativa de las proantocianidinas en la espumabilidad de los vinos blancos y rosados espumosos.

La influencia positiva de los antocianos en la espumabilidad se atribuyó a la interacción de las antocianinas con las proteínas del vino a través de interacciones hidrofóbicas y puentes de hidrógeno. Debido a la naturaleza anfifílica del producto formado, éste podría ser retenido en la interfase líquido/aire, disminuyendo la tensión interfacial e incrementándose la formación de espuma. Este efecto podría explicar el problema de *gushing* o *rebullit* en la elaboración de vinos tintos espumosos. Aunque no existen estudios científicos que evalúen el efecto de los antocianos en las características espumantes de los vinos espumosos, diversos autores han observado que los vinos rosados espumosos muestran mejores características espumantes que los vinos blancos espumosos (*10,44,56*). En general, los aminoácidos con cadenas laterales no polares mostraron valores más altos de correlación que los aminoácidos con cadenas laterales polares. Al pH del vino, los aminoácidos están protonados y actuarían como agentes tensoactivos catiónicos según la hidrofobicidad de sus cadenas laterales. El carácter anfifílico de los aminoácidos les permitiría concentrarse en la interfase líquido/aire y mejorar la espumabilidad de los vinos. Finalmente, y debido a la influencia positiva de las proteínas en la espumabilidad de los vinos, las correlaciones negativas entre las

proantocianidinas y éste parámetro, se atribuyeron a la capacidad de los taninos de unirse con proteínas para formar precipitados insolubles (*57*).

Las MP y los PRAG no tuvieron influencia en los parámetros de espumabilidad, sin embargo fueron buenos estabilizadores de la espuma. Este comportamiento se debió a la naturaleza hidrofóbica de las MP y PRAG, que favoreció la unión de estos a las burbujas de gas. Así, los monosacáridos hidrofílicos se localizarían en la capa acuosa entre las burbujas y la región hidrofóbica correspondiente a la región proteica se situaría hacia la cara interior de la burbuja. Esta disposición provoca que cuando la capa acuosa se hace más fina, las glicoproteínas aumenten la viscosidad retardando el drenaje (*45*). Se produce así un aumento de la tensión superficial de las burbujas y, con ello, un aumento de la estabilidad de la espuma. Contrariamente a lo esperado, las MP presentaron coeficientes de correlación más bajos que los PRAG. Los resultados indicaron que los PRAG son mejores estabilizadores de la espuma que las MP. Este hecho pudo deberse a diferencias en sus estructuras y conformaciones, así como en sus cargas.

Los modelos de regresión lineal múltiple revelaron que los antocianos fueron los compuestos con mayor influencia positiva en la espumabilidad, seguidos por los aminoácidos. Entre los antocianos, la malvidina-3-glucósido y la malvidina-3-(6-acetil)-glucósido mostraron la mayor influencia en los parámetros HM y HS, mientras que la β-alanina fue el aminoácido que más contribuyó a explicar el parámetro HM. El modelo que mejor explicó el parámetro de estabilidad de la espuma fue únicamente predicho por los PRAG.

Según nuestra información, este es el primer trabajo en el que se analiza la influencia de los polisacáridos, los polifenoles y las sustancias nitrogenadas en las características espumantes de vinos blancos y rosados espumosos. A partir de los resultados obtenidos en este trabajo, y teniendo en cuenta que la calidad de la espuma es de suma importancia en la elaboración de los vinos espumosos, sería interesante realizar más ensayos para dilucidar el efecto de los antocianos sobre la espumabilidad y de los PRAG sobre la estabilidad de la espuma, así como los mecanismos implicados en estas propiedades.

Por último, y teniendo en cuenta por un lado el papel que juegan las manoproteínas en las características sensoriales y tecnológicas de los vinos (*48,58,59*, y por otro, la escasez de trabajos científicos que evalúen el efecto de preparados comerciales a base de levaduras en la composición química y en la calidad sensorial de los vinos espumosos (*46,60*), se estudió el efecto de la adición de autolisados de levaduras comerciales a los

vinos base en la composición y las características organolépticas de los vinos rosados espumosos monovarietales de las variedades de uva tintas de Tempranillo y Garnacha y blancos de las variedades de uva de Verdejo y Godello, durante una crianza sobre lías en botella de nueve meses.

El análisis de los productos comerciales indicó que todos los preparados comerciales derivados de levadura estaban formados por manosa y glucosa, siendo el producto DYA-2 el más rico en manoproteínas y el que mostró la mayor pureza. Los resultados obtenidos indicaron que la adición de autolisados de levaduras a los vinos base puede modificar la composición volátil de los vinos espumosos. Sin embargo este efecto parece estar relacionado con el contenido en manoproteínas y de pureza de los autolisados de levaduras adicionados, siendo los vinos tratados con el producto de mayor pureza y contenido de manoproteínas los que mostraron diferencias con respecto a sus controles.

En general se observó que los vinos tratados con autolisados de levaduras mostraron menor contenido de ésteres etílicos de ácidos grasos que los vinos controles, siendo los vinos espumosos tratados con el producto DYA-2 los que tuvieron el menor contenido de decanoato de etilo y de ácido decanoico. Esto pudo ser debido a la elevada hidrofobicidad de dichos compuestos (*61,62*), los cuales pudieron ser adsorbidos por las paredes celulares de las levaduras y las manoproteínas (*63-65*) y al elevado contenido de manoproteínas que mostraba el producto DYA-2. Asimismo, los vinos tratados con autolisados de levaduras presentaron concentraciones más altas de terpenos que sus respectivos controles, principalmente α-terpineol en vinos blancos espumosos tratados con el producto DYA-2. Por lo tanto, estos productos pueden disminuir las pérdidas de compuestos terpénicos que se producen durante la crianza sobre lías (*64,66,67*), lo que puede tener consecuencias importantes para el aroma frutal de los vinos espumosos. Por otro lado, en el análisis sensorial de los vinos espumosos se observó que la adición del producto DYA-2 mejoró los aromas frutales en los vinos blancos, aunque este efecto no fue observado en los vinos rosados. Por el contrario, la adición de los preparados comerciales a base de levaduras no produjo ningún cambio significativo en el contenido fenólico, de amino ácidos, de aminas biógenas ni propiedades espumantes de los vinos.

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6 CONCLUSIONES
CONCLUSIONS

Las conclusiones de esta Tesis Doctoral pueden resumirse en los siguientes puntos:

1. Entre las variedades blancas estudiadas, Albarín, Verdejo y Godello fueron las más apropiadas para la elaboración de vinos espumosos de calidad.
   - A los nueve meses de crianza sobre lías, los vinos blancos espumosos elaborados con Albarín destacaron por un alto contenido en aminoácidos, compuestos volátiles y polifenoles totales. Los vinos de Verdejo tuvieron un alto contenido de aminoácidos, compuestos volátiles y polisacáridos, y los valores más altos de las propiedades espumantes. Los vinos de Godello destacaron por un alto contenido en aminoácidos y compuestos volátiles. En el análisis sensorial los vinos de Albarín y de Godello mostraron mayor intensidad aromática que los elaborados con Viura y Malvasía, y los vinos espumosos de Verdejo mostraron la mejor calidad de la espuma entre los vinos blancos.
2. Entre las variedades tintas estudiadas, Prieto Picudo resultó la más apropiada para la elaboración de vinos espumosos de calidad.
   - A los nueve meses de crianza sobre lías, los vinos rosados espumosos de Prieto Picudo mostraron mayor contenido en antocianos, aminoácidos, compuestos volátiles, polisacáridos y valores más altos de las propiedades espumantes. En el análisis sensorial mostraron mayor intensidad de color visual, de tonos rojos, de frescor en boca, de intensidad aromática y de calidad de la espuma que los elaborados con la variedad Garnacha.
3. La utilización de las variedades Albarín, Verdejo, Godello y Prieto Picudo, tradicionalmente empleadas para la elaboración de vinos tranquilos, incrementaría su potencial enológico. Sus propiedades espumantes fueron similares a las obtenidas en vinos espumosos de alta calidad como *Champagne* y Cava.
4. La composición química mostró modificaciones durante la crianza sobre lías de los vinos espumosos.
   - Todas las familias de compuestos fenólicos disminuyeron, la concentración de aminoácidos permaneció estable o disminuyó ligeramente, se produjo un

aumento de los ésteres etílicos de ácidos grasos ramificados y un descenso de acetatos de alcoholes superiores y de terpenos.

- El mayor contenido en manoproteínas y polisacáridos ricos en arabinosa y en galactosa y se obtuvo a los seis meses de crianza sobre lías. En este momento se produjo también una disminución en los pesos moleculares de los polisacáridos. La combinación de estos dos fenómenos podría implicar una mejor estabilidad de la espuma y por tanto, una mejor calidad de los vinos.
- Las propiedades espumantes de los vinos determinadas por el método Mosalux se mantuvieron estables o mejoraron al cabo de treinta meses de envejecimiento en presencia de lías.

5. Durante el envejecimiento en ausencia de lías se produjo un descenso en los ésteres etílicos de ácidos grasos de cadena lineal, acetatos de alcoholes superiores, ácidos grasos, alcoholes C6 y terpenos, un incremento en ésteres etílicos de ácidos grasos ramificados y vainillina, y no afectó a la calidad de la espuma.
6. Se encontraron correlaciones entre las características espumantes de los vinos y su composición química.
    - Los antocianos monómeros y aminoácidos se correlacionaron positivamente con los parámetros de espumabilidad mientras que las proantocianidinas mostraron una contribución negativa. Las manoproteínas y los polisacáridos ricos en arabinosa y en galactosa no tuvieron influencia en los parámetros de espumabilidad, aunque fueron buenos estabilizadores de la espuma.
    - La malvidina-3-glucósido y la malvidina-3-(6-acetil)-glucósido mostraron la mayor influencia positiva en los parámetros de espumabilidad, seguidos por los aminoácidos, principalmente la β-alanina. Los polisacáridos ricos en arabinosa y en galactosa fueron los compuestos con mayor influencia positiva en la estabilidad de la espuma.
7. La adición de autolisados de levaduras a los vinos base no afectó ni al contenido de compuestos fenólicos, amino ácidos y aminas ni a las propiedades espumantes de los vinos espumosos.
    - Los cambios observados en la composición volátil de los vinos dependieron de la composición y pureza del producto comercial empleado, siendo los vinos tratados con el producto de mayor pureza y contenido de manoproteínas los que mostraron diferencias con respecto a sus controles. Los vinos blancos espumosos tratados con este producto presentaron concentraciones más altas de terpenos que sus respectivos controles, principalmente α-terpineol, y una mejora de los aromas frutales.

The conclusions of this Doctoral Thesis are summarized in the following points:

1. Among white grape varieties studied, Albarín, Verdejo and Godello were the most appropriate for the production of quality sparkling wines.
    - At nine months of aging, white sparkling wines from Albarín stand out for high amino acids content, volatile compounds, and total polyphenols. Sparkling wines from Verdejo had high amino acids content, volatile compounds and polysaccharides, and the highest values of foaming properties measured. Sparkling wines from Godello stand out for high amino acids content and volatile compounds. In sensory analysis, Albarín and Godello presented higher olfactory intensity than Malvasía and Viura wines. Verdejo sparkling wines had the best foam quality.
2. Among red grape varieties studied, Prieto Picudo was the most appropriate for the production of quality sparkling wines.
    - At nine months of aging, rosé sparkling wines from Prieto Picudo had more anthocyanins, amino acids, volatile compounds, polysaccharides and higher values of foaming properties than Garnacha sparkling wines. In sensory analysis, Prieto Picudo sparkling wines had more visual color intensity, red tones, freshness, olfactory intensity and higher foam quality than Garnacha wines.
3. Use of the varieties, Albarín, Verdejo, Godello and Prieto Picudo which have been traditionally employed for still wines, have increased enological potential when used in sparkling wines. These wines had similar foam properties than high quality sparkling wines such as *Champagne* and Cava.
4. Chemical composition measurements of the sparkling wines showed changes during the period of aging on yeast lees.
    - All families of phenolics compounds decreased. Amino acids concentration remained constant or slightly decreased. An increase of ethyl ester branched-

chain fatty acids was seen. A decrease of higher alcohol acetates and terpenes was observed.

- After six months of aging the highest content of mannoproteins and polysaccharides rich in arabinose and galactose was obtained. Also a shift to lower molecular weights was observed. The combination of these two characteristics could imply a better foam stability and thus sensory quality of sparkling wines.
- Foaming properties of sparkling wines determined with Mosalux were maintained at stable levels or increased over the thirty months of aging on lees.

5. During the aging without lees in the bottle ethyl ester straight-chain fatty acids, higher alcohol acetates, fatty acids, C6 alcohols and terpenes decreased along with an increase in ethyl ester branched chain fatty acids and vanillin. The aging without lees in the bottle did not affect the foam characteristics of the sparkling wines.
6. Correlations between wine foaming properties and their chemical composition were found.
   - Monomeric anthocyanins and amino acids were positively correlated with the foamability parameters, while proanthocyanidins showed a negative contribution. Mannoproteins and polysaccharides rich in arabinose and galactose were poor foam formers but good foam stabilizers.
   - Malvidin-3-glucoside and malvidin-3-(6-acetyl)-glucoside showed the highest positive influence on the foamability parameters, followed by amino acid compounds, mainly b-alanine. Polysaccharides rich in arabinose and galactose showed the highest positive influence on the foam stability.
7. The addition of dry yeast autolysate products to the base wines did not have any effect on either the content of phenolic compounds, amino acids, biogenic amines or the foam properties.
   - Changes observed in volatile composition of wines were associated with the composition and purity of commercial products. So, sparkling wines treated with the product of highest mannoprotein content and purity showed the major volatile changes compared to the control wines. White sparkling wines treated with this product showed higher concentrations of terpenes compared with their respective control wines, mainly of α-terpineol, and an improvement in the fruity aromas.

"Venid, deprisa, estoy bebiendo *estrellas"*