UNIVERSIDAD DE LA RIOJA

TESIS DOCTORAL

Título
Caracterización enológica de vinos elaborados con variedades minoritarias de La Rioja con diferentes vinificaciones y añadas
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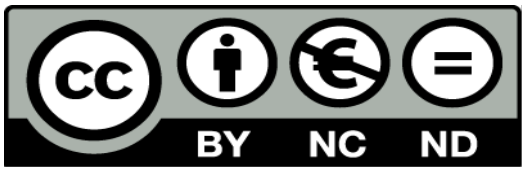
Curso Académico



CARACTERIZACIÓN ENOLÓGICA DE VINOS ELABORADOS CON VARIEDADES MINORITARIAS DE LA RIOJA CON DIFERENTES VINIFICACIONES Y AÑADAS

TESIS DOCTORAL **OLGA MARTÍNEZ PINILLA**

CARACTERIZACIÓN ENOLÓGICA DE VINOS ELABORADOS CON VARIEDADES MINORITARIAS DE LA RIOJA CON DIFERENTES VINIFICACIONES Y AÑADAS

MEMORIA PRESENTADA POR
OLGA MARTÍNEZ PINILLA
PARA OPTAR EL GRADO DE DOCTORA
POR LA **UNIVERSIDAD DE LA RIOJA**
JULIO 2013

BELÉN AYESTARÁN ITURBE, profesora Titular del Área de Tecnología de los Alimentos de la Universidad de La Rioja y ZENAIDA GUADALUPE MÍNGUEZ, profesora Contratada Doctor del Área de Tecnología de los Alimentos de la Universidad de La Rioja,

CERTIFICAN:

Que la memoria titulada *"Caracterización enológica de vinos elaborados con variedades minoritarias de La Rioja con diferentes vinificaciones y añadas"*, que presenta OLGA MARTÍNEZ PINILLA, 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.

Logroño, 18 de julio del 2013

Fdo. Belén Ayestarán Iturbe Zenaida Guadalupe Mínguez

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PRESENTACIÓN

Esta memoria de Tesis Doctoral se presenta en forma de compendio de publicaciones científicas siguiendo la normativa de la Universidad de La Rioja aprobada por Consejo de Gobierno el 2 de febrero del 2012 y modificada el 3 de mayo del 2012.

El objetivo de esta Tesis es caracterizar enológicamente los vinos obtenidos con las variedades minoritarias tintas de La Rioja Monastel, Maturana Tinta y Maturana Tinta de Navarrete, utilizando la variedad Tempranillo como referencia. Para ello, se estudió el perfil sensorial y se analizaron el color y los compuestos fenólicos de los vinos varietales antes y después de la fermentación maloláctica (*Artículo 4.1*), el contenido y perfil de aminoácidos y aminas biógenas de los vinos antes y después de la fermentación maloláctica y en diferentes añadas (*Artículo 4.2)*, y los compuestos volátiles y el perfil olfativo de dichos vinos durante tres añadas consecutivas (*Artículo 4.3).* En el *Artículo 4.4* se evaluó el efecto de la adición de manoproteínas sobre los polisacáridos, polifenoles, composición del color, y perfil sensorial de los vinos varietales de Monastel, Maturana Tinta de Navarrete y Tempranillo, y se estudió la evolución de estos parámetros durante la crianza en barrica y el envejecimiento en botella. Finalmente, en el *Artículo 4.5* se analizó la idoneidad de la cromatografía de gases con detector de masas para la identificación y cuantificación de los monosacáridos constituyentes de los polisacáridos del vino, y evaluó el potencial de la cromatografía de exclusión molecular con detector de índice de refracción como un método rápido y sencillo para estimar el contenido global de polisacáridos en el vino.

Esta memoria se ha dividido en cinco apartados con el fin de facilitar su lectura. La *Introducción* recoge los estudios más relevantes realizados en relación a la temática de la Tesis. A continuación, se describen los *Objetivos* de esta Tesis. En el capítulo de *Materiales y métodos* se describe brevemente la metodología y el plan de trabajo desarrollado para conseguir los objetivos planteados. Se definen así una serie de objetivos parciales cuyos resultados han dado lugar a las publicaciones científicas que forman esta memoria. Dichas publicaciones se adjuntan en el capítulo de *Resultados y discusión*, donde también se incluye un resumen inicial que presenta los resultados y un resumen de cada artículo y de los resultados más relevantes obtenidos. Finalmente, se incluye un capítulo con las *Conclusiones* obtenidas.

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INTRODUCCIÓN

1. 1. SELECCIÓN DE VARIEDADES MINORITARIAS

La calidad de la uva y el potencial enológico de una variedad los define Carbonneau como la respuesta de una variedad a diversos parámetros y factores, tales como el índice de Winkler, la pluviometría, la naturaleza y profundidad del suelo, la amplitud térmica e insolación, los cuidados culturales, y la añada (1). Según Carbonneau y col. (2) e Hidalgo (3) la variedad utilizada, así como el estado de maduración de la uva, van a condicionar la calidad e incluso el tipo de vino obtenido. Por otro lado, Martínez de Toda (4) define la calidad de la uva como la integración de varios aspectos: relación con el vino a elaborar, uva sana, uva madura (tecnológicamente y fenólicamente), y las peculiaridades de la propia variedad. Otros autores definen la calidad dando más importancia al punto de vista vitícola. Así, según Tardáguila y Bertamini (5), en el ecosistema vitícola resulta difícil de analizar de forma separada los efectos de cada factor de producción, y por ello la elección de las distintas técnicas culturales se debe realizar considerando de forma conjunta el sistema planta-ambiente, y sólo una gestión equilibrada de los diferentes factores puede garantizar la máxima calidad y producción de uva. En cambio, otros autores enfocan la calidad priorizando el otro punto de vista, el enológico. Así, Gómez-Gallego y col. (6) evaluaron el potencial enológico de variedades de uva de La Mancha a través del análisis de la composición fenólica, características cromáticas y la actividad antioxidante de los vinos obtenidos de dichas variedades.

La calidad del vino depende fundamentalmente de la calidad de la uva y, por tanto, del funcionamiento del viñedo, por lo que el potencial enológico de una variedad será el resultado de la suma de los factores vitícolas y enológicos. Entre los primeros se encuentran *factores permanentes* (clima, suelo, variedad de uva, portainjerto, densidad y disposición del viñedo, sistema de conducción), *factores variables* (temperatura, humedad, iluminación, edad del viñedo), *factores accidentales* (plagas, enfermedades, accidentes meteorológicos) y *factores modificables* (mantenimiento del suelo, fertilización, riego y gestión en verde del viñedo o canopy management) (7). Estos factores vitícolas tienen influencia sobre el desarrollo vegetativo del viñedo, la producción de uva y las características del mosto, es decir, sobre sus cualidades enológicas: Kg/cepa, racimos/cepa, peso del racimo, peso de la baya, peso de la madera de poda, azúcares, compuestos fenólicos, compuestos aromáticos, ácidos, compuestos nitrogenados, polisacáridos, etc. La interacción adecuada de todos estos factores garantizará la calidad del vino, su estilo y su tipicidad.

Para proteger la biodiversidad en el sector enológico y aumentar la diversificación de la oferta de vinos en el mercado actual, en los últimos años ha surgido un creciente interés por el cultivo de las variedades autóctonas de cada zona en contraposición a la

introducción de variedades foráneas de otras zonas geográficas e internacionalmente extendidas. En este sentido, la elaboración de vinos de calidad procedentes de variedades autóctonas se está extendiendo por varios países, tales como Turquía (8), Grecia (9-12), Italia (13-16) y España (17-22). Concretamente, en España se han realizado estudios recientes sobre el potencial enológico de variedades minoritarias de vid en Canarias (23), La Mancha (6, 22), Castilla-León (24), Galicia (25), Levante, Gerona e Islas Baleares (21) y La Rioja (26, 27).

La Rioja, una comunidad autónoma con una gran tradición vitivinícola, se planteó también la necesidad de preservar y caracterizar sus variedades de vid minoritarias con el fin de aumentar la autenticidad, diversidad y la calidad de sus vinos, y conseguir una mejor adaptación a los recientes cambios climáticos y a la resistencia frente a determinadas plagas o enfermedades. En este sentido, ya hace una década que Martínez de Toda y col. empezaron a evaluar el potencial enológico de un gran número de accesiones de variedades minoritarias con objeto de conocer las posibilidades de su cultivo y elaboración (26, 27). En la Tabla 1 se muestra la relación de las accesiones de las variedades minoritarias tintas conservadas en los bancos de germoplasma de Viña Ijalba y La Grajera.

Tabla 1. Relación de las accesiones preservadas en los bancos de germoplasma de variedades minoritarias tintas de Viña Ijalba y La Grajera con el código y la denominación local, adaptado de Martínez de Toda y col. (27)

Código	Denominación Local	Código	Denominación Local
R-01	Monastel	AR-41	Tempranillo Temprano
R-02	Miguel de Arco	AR-43	Cojón de gato
R-03	Garnacha Tintorera	AR-44	Graciano
R-08	Garnacha tardía	B-47	Tempranillo Royo
R-10	Graciano de Alfaro	B-48	Desconocida
R-11	Morato	B-50	Monastel
R-13	Cojón de gato	B-51	Monastel
R-14	Sabor a menta	B-52	Tintorera
A-15	Tempranillo Temprano	B-53	Graciano
A-16	Tintorera	B-56	Desconocida
A-17	Garnacha Roya	I-57	Graciano
A-20	Garnacha tardía	I-58	Tempranillo Tinto
N-23	Tempranillo del Barón	SO-63	Navarra
SV-28	Garnacha Tinta	SO-64	Teta de Vaca
R-30	Tempranillo Tinto	SO-65	Garnacha Tintorera
R-31	Tempranillo Tinto	G-68	Desconocida
R-32	Tempranillo Tinto	BE-69	Bobal
A-34	Tempranillo Tinto	BE-70	Graciano
AR-37	Desconocida	RA-73	Granegro Tintorera
AR-39	Tempranillo Temprano	RA-74	Desconocida
AR-40	Tempranillo Tinto	NA-102	Maturana Tinta de Navarrete

Para evaluar la aptitud vitícola de las variedades minoritarias de La Rioja, Martínez de Toda y col. (26, 27) estudiaron los estados fenológicos y los parámetros vegetativos y productivos de dichas variedades.

En cuanto a los *estados fenológicos* de las variedades minoritarias tintas estudiadas de La Rioja, la variedad Maturana Tinta de Navarrete NA-102 destacó por ser de brotación más tardía que Tempranillo; mientras que la brotación de Maturana Tinta CI-76 fue más precoz que la de Tempranillo. La variedad Monastel fue similar en brotación a Tempranillo aunque se fue retrasando según avanzaba el ciclo. La variedad Tintorera A-16 resultó ser muy parecida a Tempranillo no sólo en brotación, sino también en el resto de estados fenológicos (26, 27). También se observó que el híbrido Sabor a menta R-14 fue la primera en florecer, y que las variedades Vitis salvaje, Desconocida B-56 y Tempranillo temprano fueron las primeras en madurar (27). En otro estudio reciente que comparaba los estados fenológicos de las variedades Monastel, Maturana Tinta, Maturana Tinta de Navarrete y Tempranillo (28), se obtuvieron resultados similares a los obtenidos por Martínez de Toda y col. (26, 27).

Respecto a los *parámetros vegetativos y productivos*, es necesario conocer cuales son los valores óptimos para la obtención de vinos de calidad. Así, Martínez de Toda (4) considera valores óptimos para la elaboración de vinos de calidad peso de la baya menores de 2 gramos, peso del racimo menores de 250 gramos, entre 8 y 10 racimos por cepa, una producción por cepa de 2,5 Kg, entre 30 y 60 gramos de peso del sarmiento, entre 0,4 y 0,7 Kg de peso de la madera de poda y un índice de Ravaz de entre 4 y 9. Dicho índice ayuda a valorar el equilibrio entre producción y vigor, además de aportar información sobre la relación superficie foliar/peso del fruto, directamente relacionada con el desarrollo vegetativo de la planta.

La evaluación de estos parámetros en las variedades minoritarias tintas estudiadas mostró que el mayor número de racimos correspondió al híbrido Sabor a menta R-14 (16,9); el mayor tamaño de bayas a la variedad Teta de vaca SO-64 (4,99 gramos); y el mayor peso de madera de poda a la variedad Graciano AR-44 (0,5 Kg). Las variedades Monastel R-01 y Desconocida AR-37 presentaron un vigor muy bajo (101 y 95 gramos de madera de poda, respectivamente). En cuanto a la producción, Monastel B-51 resultó ser la variedad más productiva entre las tintas, con los racimos de mayor peso (293 gramos), junto con la otra accesión de Monastel, B-50. Sin embargo, esta última alcanzó una mejor maduración que Monastel B-51. Por el contrario, la variedad Maturana Tinta presentó un elevado número de racimos (13,2) y un tamaño de racimo y de baya pequeño (128 y 1,20 gramos, respectivamente). La variedad Tintorera presentó pocos racimos (7,6), pequeños (160 gramos) y con bayas de tamaño medio (1,72 gramos), por lo que se le consideró poco productiva. La variedad Maturana Tinta de Navarrete presentó un tamaño de baya menor que la de Tempranillo (1,20 gramos versus 2,49

gramos), aunque su índice de Ravaz fue parecido (~10) (26, 27). Otro estudio reciente (28) evaluó también el desarrollo vegetativo y productivo de las variedades Monastel, Maturana Tinta, Maturana Tinta de Navarrete y Tempranillo, mostrando resultados similares en cuanto a los tamaños de baya, pesos de la baya y producción a los obtenidos por Martínez de Toda y col (26, 27). Sin embargo, los valores en el índice de Ravaz, ~ 3,7 en Maturana Tinta y Maturana Tinta de Navarrete y ~ 6,5 en Monastel y Tempranillo (28), difirieron de los encontrados por Martínez de Toda y col. (6,4 en Maturana Tinta, 9 en Maturana Tinta de Navarrete, 12,5 en Monastel, y ~ 10 en Tempranillo) (26, 27).

Martínez de Toda y col. (26, 27) concluyeron que la variedad de mayor aptitud vitícola fue Maturana Tinta de Navarrete por su pequeño tamaño de baya e índice de Ravaz. A distancia le siguen la variedad Maturana Tinta por su pequeño tamaño de racimo y de baya, y por tener un número elevado de racimos; y la variedad Monastel accesión B-50, por su productividad y por madurar mejor que la otra accesión de Monastel. Por ello, en el 2009, fecha de inicio de esta Tesis, se seleccionaron estas tres variedades con el fin de realizar una caracterización enológica en detalle de las mismas y conocer sus posibilidades de elaboración.

Monastel (B-50)

La variedad Monastel (Figura 1) es nombrada ya en 1847 entre las variedades tintas cultivadas en La Rioja (29). También existen referencias de ella en Haro en 1893, y en Logroño, en 1914 (30).

La caracterización ampelográfica y de marcadores moleculares confirma que la variedad Monastel (accesión B-50) es sinonimia de Morate y totalmente diferente de la variedad Monastrel del Levante (31-33), con la que frecuentemente es confundida por su nombre similar (Monastrel, Monastrell, Monastell, Morastel, Morastell, Morratel, Moraster, Moristel, Monastreil). Tampoco guarda ninguna relación con la variedad Moristel de Somontano (26).

No se han encontrado referencias de esta variedad fuera de La Rioja, salvo las erróneas que la confunden con Monastrel (26, 27).

Actualmente no se conoce la superficie total de cultivo de esta variedad, al incluirse junto con otras variedades tintas en el Registro de Viñedo del Consejo Regulador de la D. O. Ca. Rioja (34), pero se cree que su superficie de cultivo es testimonial, existiendo sólo en parcelas experimentales.

Al tratarse de una variedad que no se cultiva en ningún otro lugar de mundo, su cultivo podría ser interesante para aportar biodiversidad. Además, el estudio de su comportamiento en condiciones reales de vinificación podría aclarar cuales son sus

cualidades enológicas peculiares, tanto para la obtención de vinos varietales como para la obtención de vinos de mayor complejidad (coupages), y poder así afianzar su cultivo en la zona.

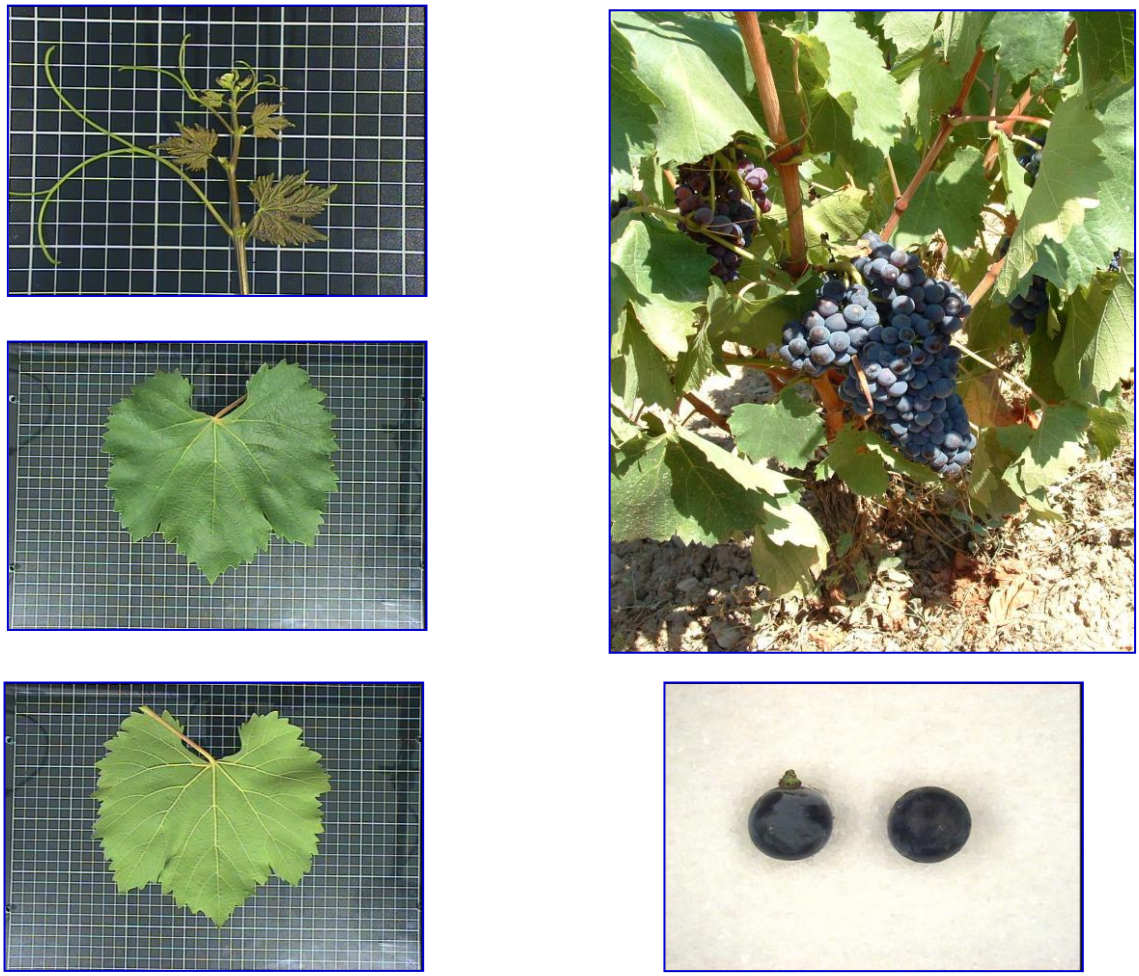


Figura 1. Monastel, extraído de Martínez de Toda y col. (26)

Maturana Tinta

La variedad Maturana Tinta (Figura 2) es nombrada ya en 1669, *Haro recoge 36.266 cántaras de vino blanco, 6.733 de tinto y 309 de "maturano"*, sin especificar si se trata de una variedad blanca o tinta (35). Manso de Zúñiga también comenta la existencia de un tipo de Maturana con la baya negra y más robusta, pero sólo describe la variedad blanca (36).

Los resultados de análisis de marcadores moleculares (31, 33) indicaron que Maturana Tinta no era una mutación de Maturana Blanca (sinonimia de Ribadavia), sino que se trataba de una variedad diferente.

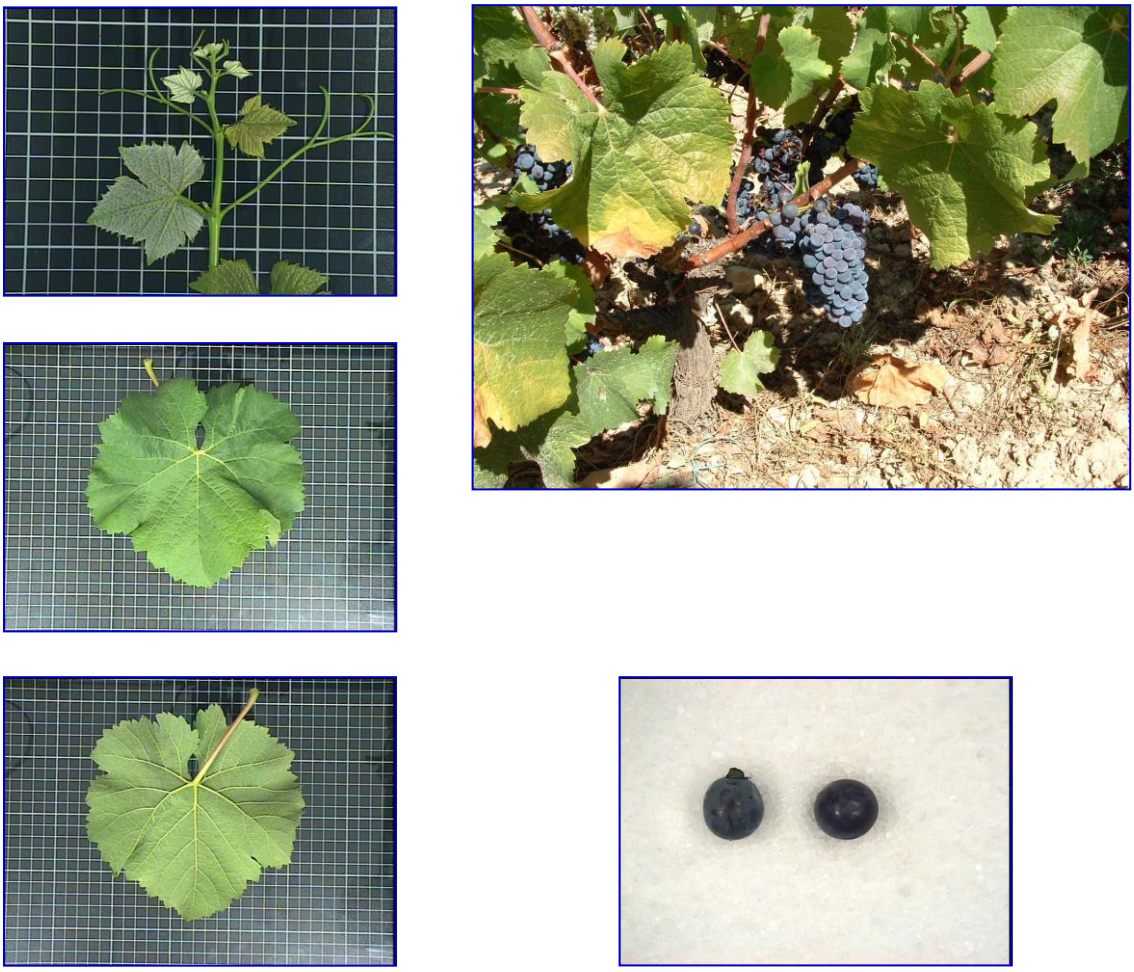


Figura 2. Maturana Tinta, extraído de Martínez de Toda y col. (26)

La accesión de Maturana Tinta CI-76 se ha encontrado en varias zonas del Norte de España. Un estudio reciente sobre la genética y estructura de las vides del Noroeste de España (37) ha demostrado la importancia de los cultivares franceses en el origen de los cultivares gallegos debido al trabajo desarrollado por los monjes de Europa Central, que contribuyeron a las plantaciones en Galicia después de la Reconquista. Este hecho explicaría también la presencia de la variedad Maturana Tinta a lo largo del viejo Camino de Santiago, con distintas sinonimias: Trousseau, Merenzao y Bastardo (Figura 3). La variedad Trousseau se considera originaria de la región de Jura en Francia. En La Rioja se le conoce como Maturana Tinta; en El Bierzo y en el este y sureste de Galicia se le conoce también como Merenzao (D. D. O. O. Valdeorras, Monterrei y Ribera Sacra); y en el suroeste de Galicia y Portugal como Bastardo (38). También se le conoce en Galicia por las sinonimias María Ordoña, María Ardoña (26).

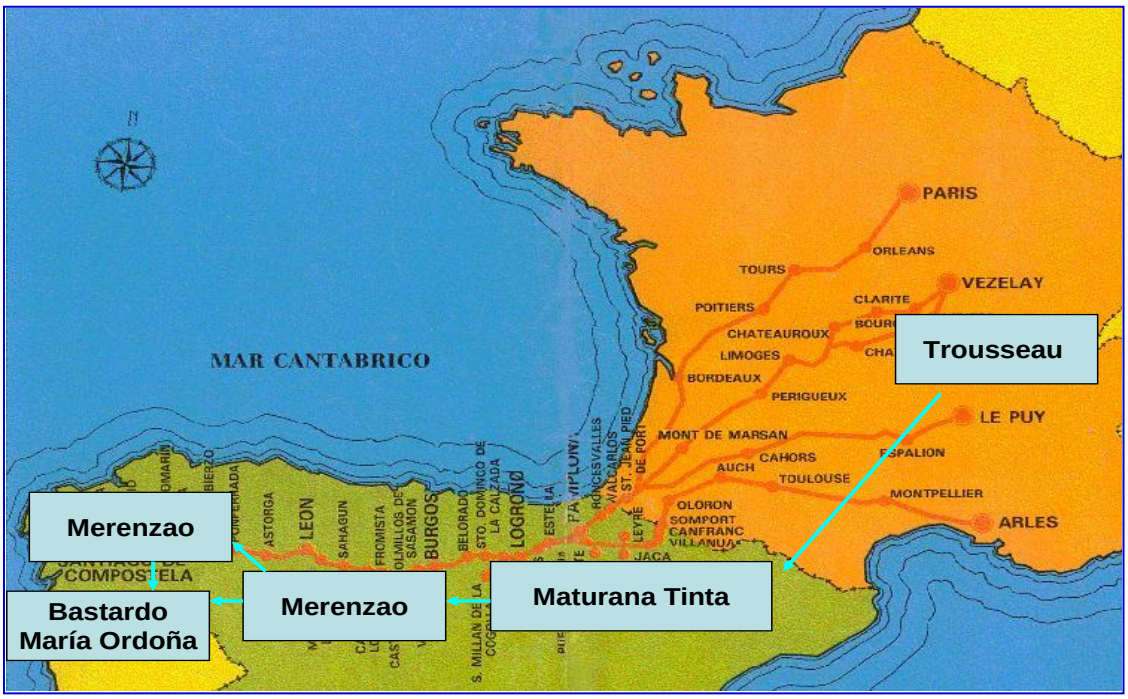


Figura 3. Recorrido de la variedad Trousseau y sus sinonimias a lo largo del camino de Santiago

En cuanto a su superficie de cultivo, el registro de viñedo del Consejo Regulador de la D. O. Ca. Rioja no posee la superficie total de cultivo de esta variedad a fecha enero del 2013 (34), al incluirse junto con otras variedades tintas. Se cree que su cultivo es testimonial, correspondiendo a cepajes aislados en viñedos antiguos, al igual que ocurría con la variedad Monastel de Rioja, y a las parcelas de estudios experimentales. El estudio de su caracterización enológica nos dará idea de su aptitud para obtener vinos de calidad.

Maturana Tinta de Navarrete

En 1991 se encontraron 35 cepas en un viñedo antiguo de la población de Navarrete, (La Rioja), identificándose como Maturana Tinta (39). Posteriormente se comprobó que se trataba de una variedad diferente, por lo que pasó a denominarse Maturana Tinta de Navarrete (Figura 4). Recientemente, en el año 2011, se identificó esta variedad con una variedad francesa prácticamente desaparecida y denominada Castets (40).

Las buenas aptitudes vitícolas observadas en dicha variedad por Martínez de Toda y col. (26, 27) hicieron que el Consejo Regulador de la D. O. Ca Rioja comenzase con los trámites para la inclusión de esta variedad entre las permitidas para su cultivo en La Rioja en el año 2007 (40). La variedad Maturana Tinta de Navarrete se registra en el Registro de Variedades Comerciales de Vid de Madrid con el nombre Maturana Tinta (Servicios Técnicos del Consejo Regulador). A partir de ese momento, se autoriza su

cultivo en la D. O. Ca Rioja con el nombre de Maturana Tinta (Orden ARM/1372/2009 del 27 de mayo), si bien se refiere en todo caso a Maturana Tinta de Navarrete y no a la variedad que presenta sinonimia con Merenzao (Servicios Técnicos del Consejo Regulador). El hecho de que una Denominación de Origen autorice una variedad recuperada en un proyecto de investigación constituye el mejor ejemplo de transferencia de tecnología desde la investigación hasta el sector vitivinícola (40).

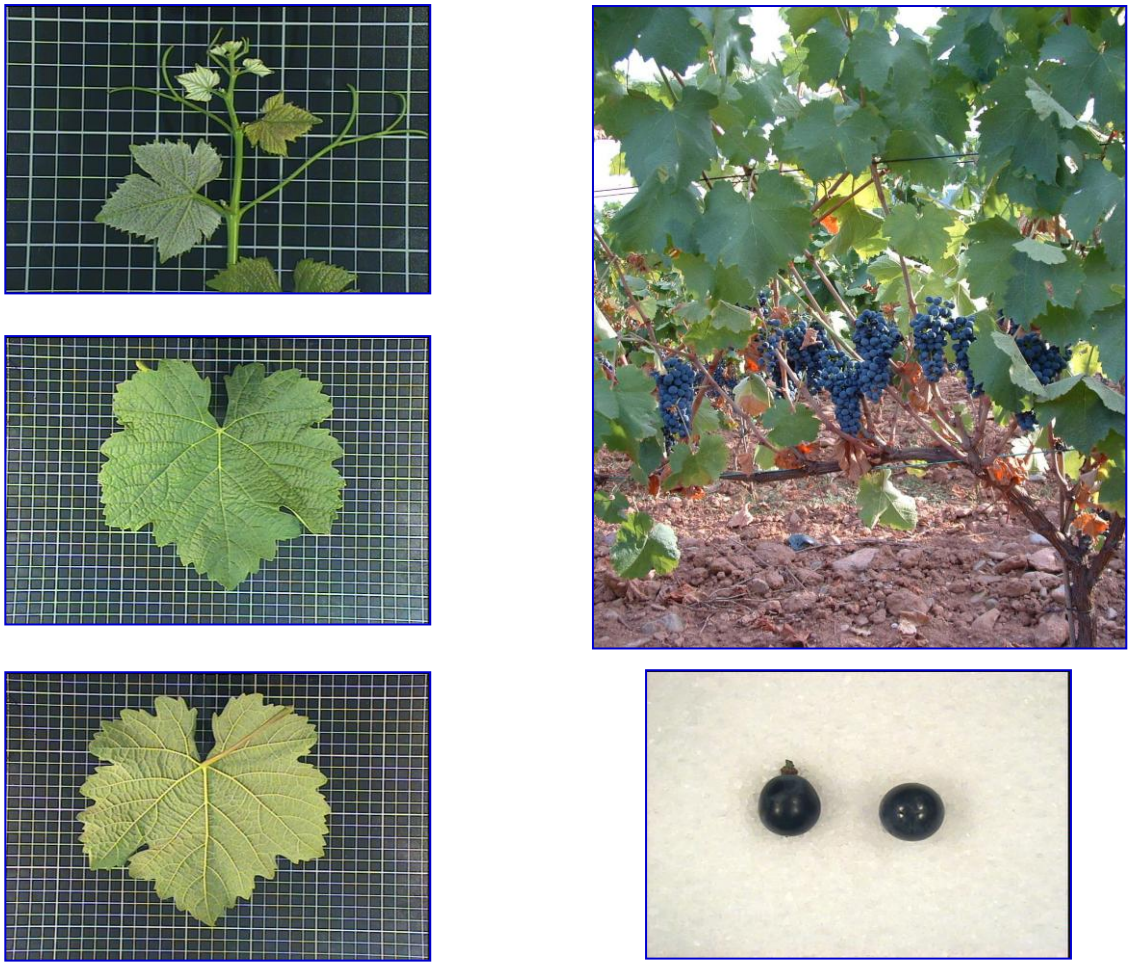


Figura 4. Maturana Tinta de Navarrete, extraído de Martínez de Toda y col. (26)

Debido a la autorización de su cultivo en el año 2009, la superficie de cultivo de esta variedad comienza a incrementarse en La Rioja, alcanzando las 50,29 Has de la actualidad, lo que supone el 0.08% del total de uvas tintas (Tabla 2).

Se produce también un aumento en el número de bodegas que producen vinos de la variedad Maturana Tinta de Navarrete. Así, son seis las bodegas que elaboran y comercializan vinos monovarietales de Maturana Tinta de Navarrete: Viña Ijalba, Juan Carlos Sancha, Pedro Martínez Alesanco, Martínez Bujanda, Vivanco y Barón de Ley. Además, otras bodegas comienzan también a utilizarla como variedad complementaria en la elaboración de vinos plurivarietales (40). Sin embargo, al ser una variedad de reciente autorización en la D. O Ca. Rioja, no existen estudios científicos sobre su

caracterización enológica y su comportamiento en todas y cada una de las etapas de elaboración, incluyendo la crianza y el envejecimiento en botella. El empleo de esta variedad, al no cultivarse prácticamente en ningún otro lugar del mundo, podría ser muy interesante para aumentar la originalidad, diferenciación y diversidad de los vinos de la D. O. Ca. Rioja.

Tabla 2. Situación del viñedo por variedad en La Rioja, adaptado de Registro de Viñedo del Consejo Regulador de la D. O. Ca. Rioja (34)

Variedad	Total (Has)	% Tintas D. O.Ca. Rioja	% Total D. O.Ca. Rioja
Tempranillo	51.252,62	86,49%	80,97%
Garnacha	5.262,42	8,80%	8,31%
Mazuelo	1.446,56	2,44%	2,29%
Graciano	1.049,37	1,77%	1,66%
Maturana Tinta	50,29	0,08%	0,08%
Otras Tinto	200,00	0,34%	0,32%
Total Tinto	59.261,26	100,00%	93,62%

Tempranillo

En 1885 ya se tiene referencia de las variedades denominadas Temprana Blanca, Temprana Negra, Tempranilla, Tempranillo Blanco, Tempranillo de Peralta o de Rioja y Temprano entre otras (41). En 1914 se vincula su origen a la zona del Ebro (30), y parece ser que sólo el Tempranillo de Peralta o de La Rioja es el único material que se puede relacionar con la variedad de Tempranillo conocida hoy (42). Galet considera al Tempranillo como una variedad de maduración temprana que se cultiva desde tiempos muy antiguos en La Rioja, y sugiere que probablemente es propia y peculiar de La Rioja, ó por lo menos es de importación antigua y que al respecto no se tiene ningún dato (43).

La variedad Tempranillo posee un elevado número de sinonimias como Tempranillo de Rioja, Aragonés, Cencibel (La Mancha), Chinchillana, Escobera, Tinta de Madrid (Madrid), Tinta de Toro (Zamora), Tinta del País (D. O. Ribera de Duero), Tinta Fina, Tinta Roriz (Portugal), Tinto Aragonés (Aragón) y Ull de Llebre (42).

Es la variedad tinta más cultivada en la Península Ibérica y en España, existiendo referencias de su cultivo en 45 de las 69 Denominaciones de Origen de vino (42). Ocupa el 18,9% de la superficie vitícola nacional (42) que en La Rioja supone el 80,97% de la superficie total del viñedo, alcanzando el 86,49% de la superficie total de las variedades tintas (Tabla 2).

La variedad Tempranillo se ha elegido como referencia en esta Tesis para compararla con las variedades minoritarias Monastel, Maturana Tinta y Maturana Tinta de

Navarrete debido a que se trata de una variedad profundamente arraigada en La Rioja y cuyo comportamiento en todas las etapas de vinificación es perfectamente conocido.

Figura 5. Tempranillo, extraído de (44)

1. 2. CARACTERIZACIÓN ENOLÓGICA DE LOS VINOS VARIETALES

La calidad del vino depende de la calidad de la uva y, por tanto del desarrollo correcto de la vid, por lo que el potencial enológico de una variedad será el resultado de la suma de factores vitícolas y enológicos. La aptitud enológica de una variedad la determinarán aquellos compuestos que son responsables de la calidad de la propia uva y, por ende, del vino. Estos compuestos son principalmente los compuestos fenólicos, compuestos aromáticos, compuestos nitrogenados, polisacáridos, etc. Los procedimientos tecnológicos influirán en la composición de estos compuestos, que sufrirán modificaciones a lo largo de las etapas de vinificación. No obstante, es importante señalar que cada variedad vinífera presenta una capacidad potencial para sintetizar determinados compuestos que caracterizan a la variedad. Por lo tanto, para conocer las características enológicas adecuadas de una variedad, lo ideal sería conocer el perfil varietal de estos compuestos y su composición idónea para obtener vinos jóvenes de calidad y/o vinos con aptitud para el envejecimiento.

Al contrario de lo que ocurre con la variedad Tempranillo, los estudios previos descritos en la bibliografía sobre las características enológicas de las variedades minoritarias tintas Monastel, Maturana Tinta y Maturana Tinta de Navarrete (26-28) son escasos y se limitan a la evaluación de azúcares, compuestos fenólicos y compuestos aromáticos, únicamente después de la fermentación alcohólica. Sin embargo, existen otras etapas en la elaboración que modifican progresivamente el carácter del vino. Por ello, se hace necesario un estudio detallado de los compuestos que aportan calidad en los vinos elaborados con las variedades seleccionadas en esta Tesis, en diferentes etapas de vinificación, incluida la fermentación maloláctica, la crianza y el envejecimiento en botella.

El color, el aroma, y el sabor de un vino tinto son características que definen su calidad, y se relacionan principalmente con su composición en compuestos fenólicos, compuestos volátiles, compuestos nitrogenados y polisacáridos. Se realiza a continuación una breve descripción de cada uno de ellos con objeto de resaltar tanto su implicación en el proceso de vinificación como el papel que pueden desempeñar como marcadores varietales.

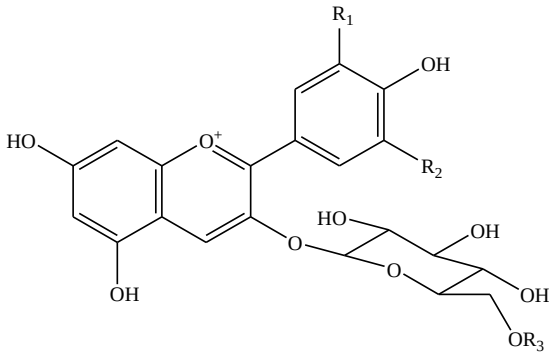
El color es la carta de presentación de un vino tinto, no sólo por ser el primer atributo sensorial que percibe el consumidor, sino también porque es un indicador de otros aspectos como su edad, estado de conservación, cuerpo o sabor.

El color del vino tinto viene determinado por su composición fenólica. De todos los compuestos fenólicos, tanto flavonoides (antocianos, flavanoles y flavonoles) como no

flavonoides (ácidos hidroxibenzoicos, ácidos hidroxicinámicos, estilbenos), son los antocianos y los flavanoles los que mayor influencia tendrán sobre el color y otras características organolépticas definitorias de la calidad del vino tinto, como la astringencia y el aroma.

Los antocianos y sus pigmentos derivados son los responsables directos del color de los vinos. Estos compuestos están sujetos a una serie de transformaciones durante el proceso de vinificación y envejecimiento.

Los compuestos primarios responsables del color del vino son los antocianos monómeros. Estos compuestos están formados por una aglicona (antocianidina) unida a un azúcar, normalmente la glucosa, que puede estar acilada, siendo importantes los derivados acetilados y cumarilados (45). En las variedades de *Vitis vinífera* se distinguen cinco antocianos diferentes (Figura 6), siendo la malvidina-3-glucósido el antociano mayoritario encontrado en los vinos.



R_3= radical acetil, cafeil o cumaril

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

Figura 6. Estructura química de los antocianos de la uva

La concentración total de antocianos depende de varios factores como son la variedad de uva y su grado de madurez, las condiciones climáticas y las técnicas enológicas empleadas (tiempo de maceración, termovinificaciones, levadura, filtración, etc.). Por lo tanto, el rango de valores de la concentración total de antocianos en los vinos es muy amplio. En el caso concreto de los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete, la bibliografía describe concentraciones de 430-520 mg/L, 226-310 mg/L y 742-1.597 mg/L, respectivamente, en muestras tomadas después de la fermentación alcohólica (26, 28).

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 (46) (Figura 7). 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. Así, a un pH muy ácido, la forma mayoritaria es el catión flavilio que presenta coloración roja. Al aumentar el pH, el catión flavilio se transforma en una base quinona de color azul. Estas formas son inestables y dan lugar a bases hidratadas incoloras, llamadas bases carbinol, que ante un aumento de temperatura dan lugar a una estructura abierta llamada chalcona, que puede ser incolora (si es neutra) o amarilla (si está ionizada). La chalcona puede a su vez oxidarse de forma irreversible dando lugar a ácidos fenólicos incoloros, lo que supone una pérdida de color irreversible (47). La presencia del anhídrido sulfuroso en los vinos produce también la formación de un aducto incoloro estable al pH del vino. Esta reacción es reversible dependiendo de la naturaleza del antociano y del pH.

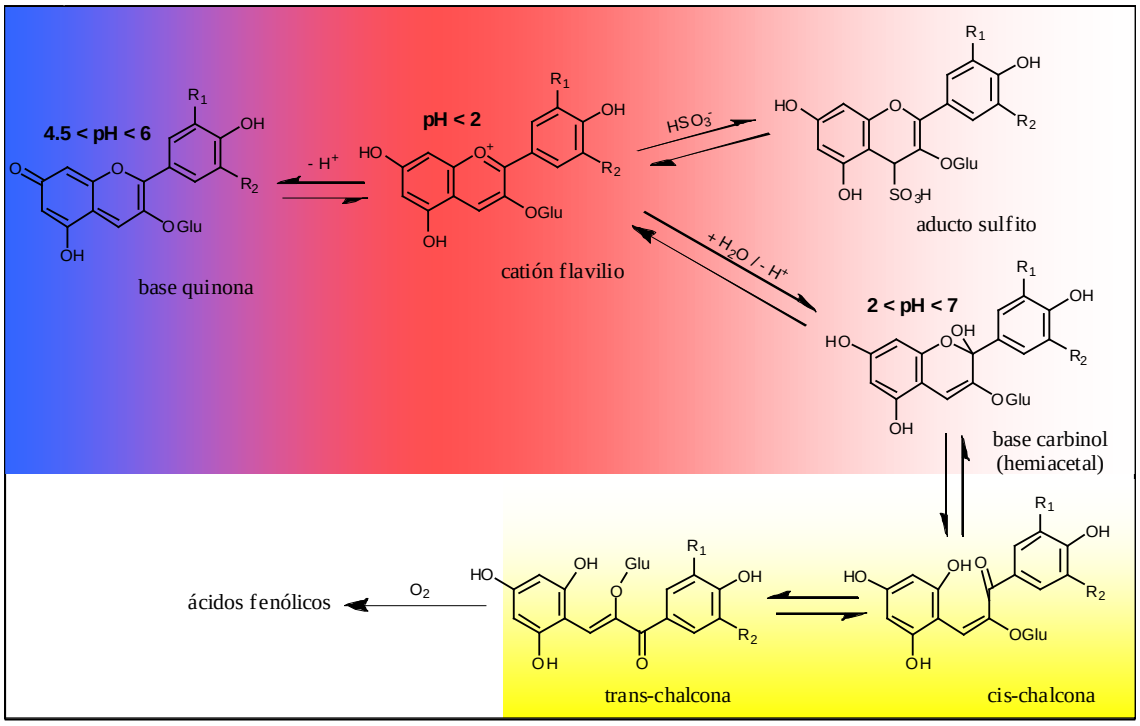


Figura 7. Equilibrio y coloración de los antocianos en el vino, extraído de Guadalupe (48)

Los vinos tintos presentan un color rojo intenso, indicando que existe una estabilización de los pigmentos que permite mantener el color a lo largo de los años (46). Evidentemente, la capacidad de un vino para la crianza vendrá en parte definida por su capacidad para mantener el color a lo largo del tiempo. Los mecanismos principales que intervienen en la estabilización del color son dos: la copigmentación y la formación de pigmentos más estables como los pigmentos poliméricos y los piranoantocianos (49). El primer proceso tiene especial importancia en los vinos

jóvenes, mientras que el segundo participa principalmente en el color de los vinos envejecidos.

La copigmentación supone la formación de asociaciones no covalentes de los antocianos con otros compuestos del vino (Figura 8). La formación del complejo de copigmentación no sólo da lugar a un incremento del color del vino (efecto hipercrómico) sino que modifica su tonalidad hacia tonos más azules (efecto batocrómico) (50).

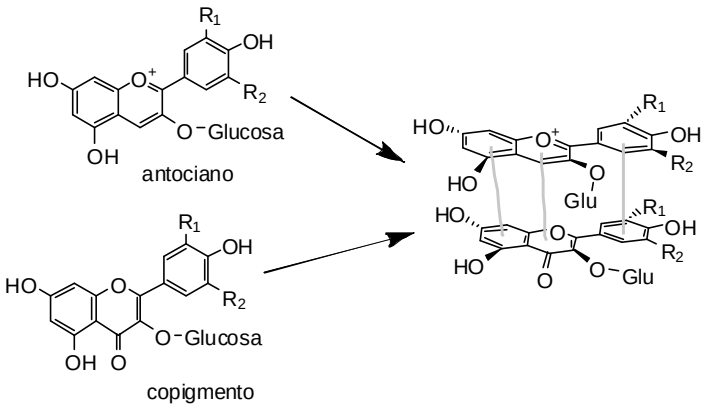


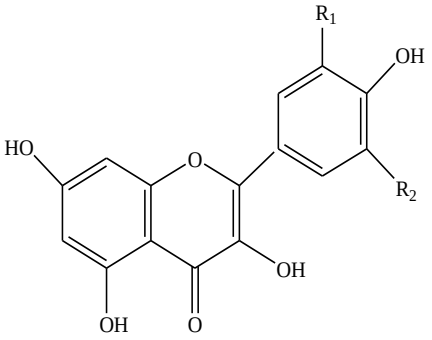
Figura 8. Complejo de copigmentación, extraído de Guadalupe (48)

Diversos autores afirman que el color debido a la copigmentación supone alrededor de la mitad del color observado en los vinos tintos jóvenes y depende del contenido de copigmentos presentes en el vino (51-54). Sin embargo, durante la evolución del vino se produce una disminución de los antocianos y los copigmentos lo que da lugar a un descenso en el color debido a la copigmentación. La bibliografía describe diferencias en la contribución del color debido a la copigmentación de los vinos tintos. Así, en vinos de Cencibel, Cabernet Sauvignon y Syrah la copigmentación supone entre el 32 y el 45% del color de los vinos recién elaborados, para descender al 20-34% después de tres meses y ser prácticamente despreciable (0-5%) transcurridos 9 meses (55). Sin embargo, otros autores describen que el color debido a la copigmentación en vinos de Tempranillo es del 10% después de la crianza y el envejecimiento en botella (24 meses) (56); del 22% y del 19% en vinos de Listan Negro y Negramoll después de uno y dos años de envejecimiento respectivamente (52); y del 18% después de nueve meses de crianza en barrica en co-vinificaciones de Monastrell, Cabernet Sauvignon y Merlot (57).

Como copigmentos pueden actuar sustancias muy distintas, como polisacáridos, ácidos orgánicos, nucleótidos, aminoácidos y sobre todo flavonoides. Los mejores

copigmentos descritos en la bibliografía son los flavonoles (58-60), seguido de los ácidos hidroxicinámicos (61), y por último, los flavanoles, que poseen menor efectividad debido a que no poseen una estructura plana, lo que dificulta su acercamiento a los antocianos.

Los flavonoles, considerados los mejores cofactores de copigmentación, se localizan en los hollejos de las uvas donde sólo aparecen en forma heterósida unidos fundamentalmente a la glucosa, galactosa y ácido galacturónico. En el vino aparecen tanto las formas libres (agliconas) como glicosiladas (Figura 9). Los flavonoles son amargos y pueden formar parte de las reacciones de polimerización de los fenoles (51, 55), lo que provoca una disminución en su contenido a lo largo del proceso de envejecimiento. Su concentración en el vino depende la variedad de uva, del grado de madurez, del proceso de vinificación y del tiempo de envejecimiento (62). La concentración total de flavonoles descrita en la bibliografía varía desde 8 mg/L en vinos de Mencía (63) hasta 175 mg/L en vinos de Syrah (62). En caso de los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete la concentración de flavonoles en microvinificaciones después de la fermentación alcohólica es de 18 mg/L para los dos primeros y de 40 mg/L para los vinos de Maturana Tinta de Navarrete (28).



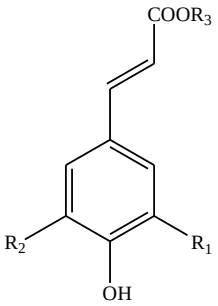
Flavonoles	R_1	R_2
Kaempferol	H	H
Quercetina	OH	H
Miricetina	OH	OH
Isorhamnetina	OCH_3	H

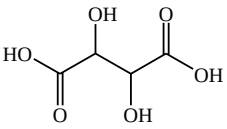
Figura 9. Principales flavonoles del vino

Los ácidos hidroxicinámicos son moléculas constituyentes de los antocianos acilados, actúan como copigmentos (51), participan en la formación de los piranoantocianos (64), y contribuyen a la estabilidad del color (51). A nivel sensorial tienen también importancia por ser precursores de los fenoles volátiles, que pueden provocar defectos en los vinos a nivel olfativo (65).

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 10). El ácido cafeico y su derivado el ácido caftárico son los ácidos hidroxicinámicos mayoritarios en los vinos (66-68). La

concentración total de ácidos hidroxicinámicos en el vino depende de muchos factores como la variedad de uva, el grado de maduración de la uva y las técnicas de vinificación, por lo que es habitual encontrar grandes diferencias en su concentración entre los distintos vinos. La concentración de ácidos hidroxicinámicos totales descrita en la bibliografía varía desde 13,6 mg/L en vinos de Malbec (69) hasta 224 mg/L en vinos de Listán Negro (70). En los vinos de Monastel y Maturana Tinta se encuentran concentraciones de 55 mg/L y de 30 mg/L para la Maturana Tinta de Navarrete (28).





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 10. Estructura química de los ácidos hidroxicinámicos

Los ácidos esterificados son los predominantes en los vinos jóvenes (66, 71) pero su hidrólisis durante la fermentación maloláctica (71-73), la conservación y el envejecimiento de los vinos (55, 74) reduce considerablemente su contenido.

Los flavanoles o flavan-3-oles están presentes en el vino en forma de monómeros (Figura 11), oligómeros y polímeros. Los flavan-3-oles monómeros más abundantes en la uva son la (+)-catequina y su isómero (-)-epicatequina, aunque las formas poliméricas, denominadas taninos condensados o proantocianidinas, son las mayoritarias en los vinos. El término tanino engloba una gran cantidad de compuestos fenólicos que pertenecen fundamentalmente a dos familias: la de las proantocianidinas, que proceden directamente de la uva, y la de los taninos hidrolizables, que provienen de la manera del roble y se encuentran únicamente en los vinos envejecidos en barrica.

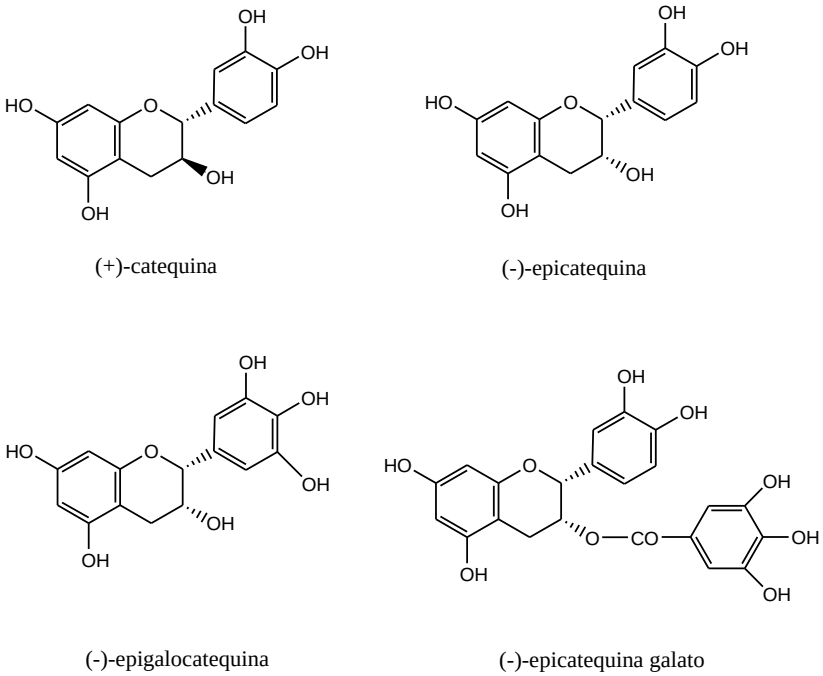


Figura 11. Principales flavanoles monómeros de la uva

Las proantocianidinas son los flavonoides cuantitativamente más importantes en el vino (Figura 12). Se distinguen las procianidinas, derivadas de la catequina y epicatequina, y las prodelfinidinas, derivadas de la galocatequina y epigalocatequina, aunque comúnmente a ambas se les conocen como procianidinas o proantocianidinas.

El contenido en proantocianidinas del vino tinto depende del tipo de variedad de uva empleada, del tiempo de maceración-fermentación y del envejecimiento (75-78). La concentración de proantocianidinas descrita en la bibliografía varía desde 116 mg/L en vinos de Cabernet Sauvignon (78) hasta 2.814 mg/L en vinos de Merlot (75). El contenido de taninos en los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete se describe bibliográficamente (26, 28) pero existen pocos estudios sobre las proantocianidas en dichos vinos (28).

La mayoría de estudios sobre las proantocianidinas dan más importancia a la composición de su perfil y a su grado medio de polimerización (mDP) que a su contenido total (75). Así, los taninos de semilla poseen sólo procianidinas, mientras que los del hollejo están formados por procianidinas y prodelfinidinas. Estas moléculas pueden presentar un número elevado y variable de unidades, siendo el grado medio de polimerización de los hollejos muy superior al de las semillas. Por el contrario, los taninos de los hollejos poseen un menor porcentaje de unidades galoiladas, lo que le confiere al vino una menor astringencia (75). De este modo, la bibliografía describe valores de mDP desde 4,3 en vinos de Cabernet Sauvignon (75) hasta 13 en vinos de

Tempranillo (77), pero no existen estudios en los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete.

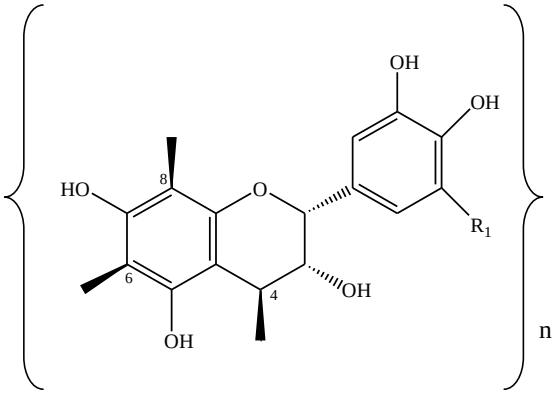


Figura 12. Estructura de las proantocianidinas

Las proantocianidinas son las responsables del sabor amargo y de la astringencia, de la sensación de estructura o cuerpo del vino (47) y de la capacidad del vino para envejecer. De hecho, la astringencia es uno de los atributos sensoriales más importantes de los vinos tintos, y se define como la sensación de sequedad y de aspereza en boca. En los vinos de alta calidad debe estar equilibrada. Una astringencia baja dará lugar a vinos planos y poco interesantes, mientras que un exceso de astringencia puede enmascarar otras características del vino, además de hacerlos ásperos y secos. El nivel de astringencia evoluciona con el tiempo, de manera que la astringencia de un vino joven es muy superior al de un vino envejecido. La bibliografía describe a los vinos jóvenes de Maturana Tinta por poseer una elevada astringencia y amargor, a los vinos jóvenes de Monastel por tener una astringencia media y a los vinos jóvenes de Maturana Tinta de Navarrete por ser ligeramente astringentes (26).

La sensación de sequedad en boca se debe a que los flavanoles son capaces de formar complejos con las proteínas salivales. Esta sensación queda amortiguada con la presencia en el vino de péptidos y de polisacáridos que se agrupan con los taninos formando macroestructuras estables que impiden la reacción con las proteínas salivales. Esto hace que las interacciones de los polisacáridos con las proantocianidinas sean las responsables de que la tanicidad de un vino sea agradable o no (47).

Las proantocianidinas juegan también un papel muy importante en el color del vino debido a que pueden reaccionar con otros compuestos fenólicos como los antocianos para formar pigmentos más estables, responsables del color en los vinos envejecidos (47).

Durante el proceso de envejecimiento de un vino tinto se produce una pérdida de intensidad de color y un desplazamiento de la tonalidad rojo púrpura de los vinos

jóvenes hacia tonalidades más anaranjadas, típicas de los vinos envejecidos. Este cambio se explica por el progresivo desplazamiento de los antocianos de uva hacia la formación de pigmentos más estables (47), que son menos sensibles al pH y a la acción del sulfuroso que los antocianos libres. Por estos motivos, se dice que el color del vino tinto se estabiliza durante el envejecimiento. Los grupos más importantes de estos pigmentos son los pigmentos poliméricos y los piranoantocianos.

Los pigmentos poliméricos se forman o bien por la reacción directa de los antocianos y los taninos, o bien por la reacción mediada por acetaldehído. La combinación de los antocianos con los taninos supondrá, además de la estabilización del color, una importante mejora del gusto del vino, aumentado las sensaciones de redondez y volumen en boca, y disminuyendo la astringencia y el amargor de los vinos recién elaborados.

Los pigmentos poliméricos *derivados de la condensación directa de los antocianos con los flavanoles* dan lugar a dos tipos de pigmentos: pigmento antociano-tanino (A-T), de color rojo (Figura 13a) (79); y, pigmento tanino-antociano (T-A), de color rojo-anaranjado (Figura 13b) que tiene la posición 4 del anillo pirano del antociano libre, lo que hace que puedan reaccionar con el anión bisulfito o el anión hidroxilo del agua. Por ello, estos pigmentos resultan menos estables que los primeros y se pueden decolorar.

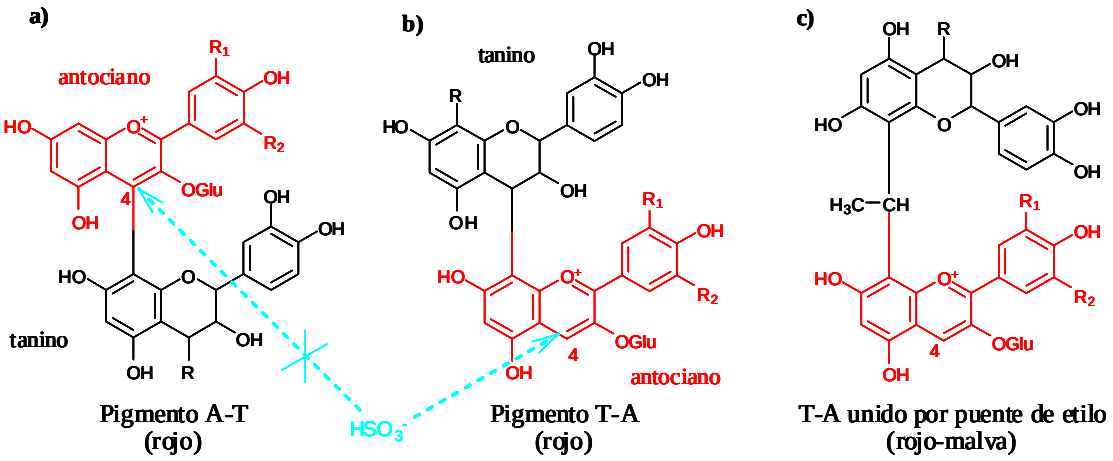


Figura 13. Estructura de los pigmentos poliméricos, extraído de Guadalupe (48)

La formación de pigmentos poliméricos *derivados de las reacciones mediadas por acetaldehído* compite en cierta forma con la polimerización natural de los taninos mediada por etanal y con la formación de otras estructuras poliméricas también mediadas por este compuesto. El etanal, que se genera en el vino durante la fermentación y la crianza, sirve de puente de unión entre muchos polifenoles, bien entre

compuestos de la misma familia o entre compuestos de familias distintas. La condensación entre unidades de antocianos (aducto antociano-etil-antociano) no tiene lugar con la misma facilidad que con los flavanoles pero origina compuestos de color púrpura mucho más resistentes a la decoloración por hidratación y a la adición de SO_2 que los antocianos libres (80). La condensación entre unidades de flavanoles (flavanol-etil-flavanol) tiene lugar fácilmente, pero los polímeros formados son incoloros y se despolimerizan con facilidad, dando lugar a unidades reactivas de flavanol-etilo (vinilflavanol) que pueden volver a condensarse con los taninos o reaccionar con los antocianos (81). El resultado de la condensación de los vinilflavanoles con los antocianos da lugar a pigmentos flavanol-etil-antociano (Figura 13c) de color rojo-violeta más estables frente a los cambios de pH, a la decoloración por hidratación y a la adición de SO_2 que los antocianos libres. Sin embargo, la poca estabilidad de estos pigmentos por la rotura del puente de etilo y su facilidad de precipitación los hace poco importantes en el color de los vinos envejecidos (82), aunque puntualmente podrían intervenir en los tonos azulados de algunos vinos jóvenes.

El que en un vino predominen un tipo u otro de las reacciones descritas depende de su composición original. Si el vino de partida posee una concentración de antocianos muy superior a la de taninos, predominará la degradación de antocianos por oxidación. Por otro lado, si la concentración de antocianos es mucho menor que la de taninos, predominará la polimerización, lo que se traducirá en un aumento de la coloración amarilla a lo largo de la crianza. Por último, si el vino contiene una concentración de antocianos y taninos equilibrada, la situación será ideal para la obtención de vinos de calidad. De hecho, un vino tinto para poder ser apto para la crianza en barrica habrá de tener unas concentraciones mínimas de antocianos (400 mg/L) y taninos (2 g/L) que garanticen la estabilización del color, siendo los valores óptimos para la obtención de vinos de calidad aquellos mayores de 800 mg/L de antocianos y 3 g/L de taninos (47). La relación antociano/tanino de equilibrio óptima es de 1/4 (0,25), es decir, de 0,500 a 0,800 g/L de antocianos y entre 2 y 3,2 g/L de taninos (45). La bibliografía describe ratios antociano/tanino después de la fermentación alcohólica de 0,195-0,247 en vinos de Monastel; 0,087-0,168 en Maturana Tinta y 0,464-0,481 en Maturana Tinta de Navarrete (26, 28). Los vinos de Maturana Tinta parecen poseer un desequilibrio polifenólico, con una mayor tendencia a la polimerización y, por tanto a un incremento de la coloración amarilla durante la crianza (26). Sin embargo, estos estudios se han realizado únicamente en microvinificaciones y en muestras tomadas después de la fermentación alcohólica.

Los piranoantocianos, se originan por reacciones de cicloadición de los antocianos con compuestos de bajo peso molecular como ácido pirúvico, acetaldehído, vinilfenoles diversos, ácidos hidroxicinámicos o vinilflavanoles (83). Todas estas moléculas tienen

un doble enlace polarizado, necesario para la cicloadición (Figura 14). Los piranoantocianos se caracterizan por presentar longitudes de onda desplazadas hipsocrómicamente con respecto a las de sus antocianos precursores, lo que les confiere un color más anaranjado. Se trata de compuestos químicamente más estables, de estructura fija, que se mantienen disueltos en el vino, por lo que no tendrán tendencia ni a precipitar ni a quedar retenidos en las superficies filtrantes antes de su embotellado. Estos compuestos poseen además un color más resistente a los cambios de pH, a la decoloración por SO_2, a la degradación oxidativa, e incluso a la temperatura al tener la posición C4 del antociano bloqueada. Por ello, participan en los cambios de color que se producen en el vino tinto durante su envejecimiento. Estos compuestos no están presentes en la uva, sino que se forman durante la fermentación alcohólica y durante las etapas posteriores de vinificación.

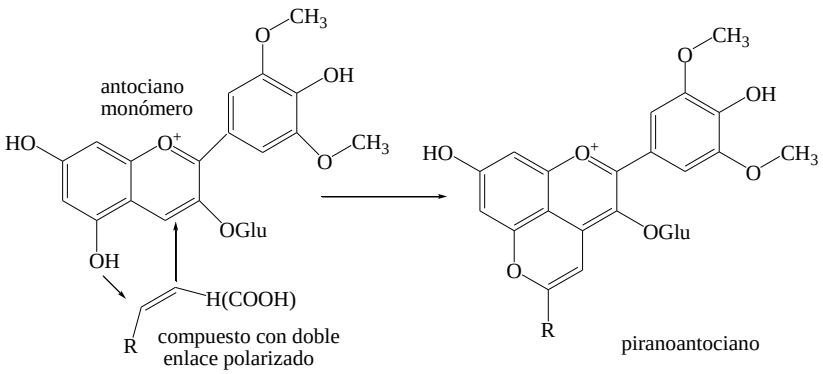


Figura 14. Formación de piranoantocianos

Los polisacáridos son uno de los principales grupos de macromoléculas presentes en los mostos y vinos. Conocidos desde hace tiempo por su efecto sobre la estabilidad coloidal de los vinos, juegan un papel fundamental tanto en las distintas etapas del proceso de vinificación como en las características organolépticas del producto final.

Los polisacáridos del vino son liberados durante el transcurso de la vinificación y durante el prensado, 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. Desde un punto de vista enológico, los polisacáridos procedentes de la uva y de las levaduras son los más importantes.

Los *polisacáridos procedentes de la uva* son el resultado de la degradación y de la solubilización de una parte de las sustancias pécticas contenidas en la pared de las células del hollejo y de la pulpa de la baya de la uva. La concentración de polisacáridos procedentes de la uva en el vino dependerá del grado de madurez de la uva y del uso de determinadas técnicas enológicas como las maceraciones prolongadas y la adición de

enzimas. Estos polisacáridos se clasifican en sustancias pécticas ácidas si tienen al ácido galacturónico en su molécula, y sustancias pécticas neutras, si no poseen ácido galacturónico. A las sustancias pécticas neutras también se les conoce con el nombre de polisacáridos ricos en arabinosa y galactosa (PRAGs) (Figura 15).

Los *homogalacturonanos* (HG) son los polisacáridos más abundantes en la uva, constituyendo el 80% de los polisacáridos pécticos (84, 85). Sin embargo, su concentración es muy baja en los vinos ya que son degradados bajo la acción de enzimas naturales de la propia uva (45). Los *ramnogalacturonanos tipo I* (RG-I) constituyen alrededor del 15% de los polisacáridos pécticos de la uva (85) pero también se encuentran en muy baja concentración en los vinos. Del mismo modo, los *arabinanos* y *arabinogalactanos tipo I* (AG I) sólo aparecen como restos de sus hidrolizados en los turbios del vino (45).

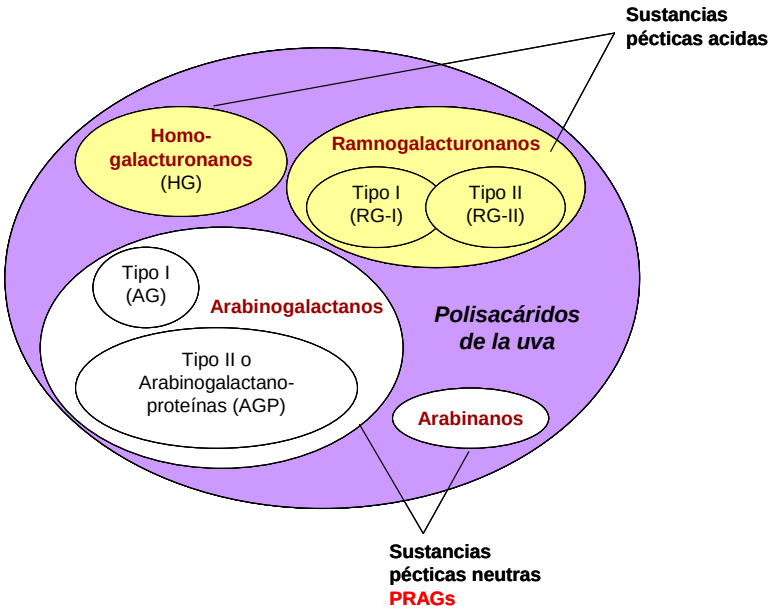


Figura 15. Clasificación de los polisacáridos de la uva, adaptado de Guadalupe (48)

Los *arabinogalactanos tipo II* (AGP) *o arabinogalactanos-proteínas* son glicoproteínas con numerosas ramificaciones que dan lugar a una estructura en forma de "matorral", constituyendo la zona erizada de las sustancias pécticas (45). Su masa molecular es por ello elevada (130-260 KDa) (45). Se encuentran en las paredes vegetales en forma soluble, por lo que se extraen fácilmente durante el prensado (45). En el vino joven su contenido es de 100-250 mg/L (86) lo que supone el 40% (86, 87) de los polisacáridos totales del vino, constituyendo la primera familia de polisacáridos en importancia en el vino recién elaborado. El contenido de AGP disminuye a lo largo de las distintas etapas de elaboración. En este sentido la bibliografía describe concentraciones de AGP en vinos jóvenes de Tempranillo de 300 mg/L, representando

el 50% de los polisacáridos totales, y que van descendiendo a lo largo de la crianza y el envejecimiento en botella hasta alcanzar 160 mg/L, lo que supone el 37% de los polisacáridos totales (86). Los AGP también mostraron descensos en su concentración en vinos de Cariñena envejecidos durante un largo periodo (88), y en vinos de Madiran sometidos a crianza sobre lías (89). En este último caso, además del descenso en su concentración, se observó un cambio en su perfil estructural ya que el ratio arabinosa/galactosa disminuyó con la crianza sobre lías (89).

El *ramnogalacturonano tipo II* (RG-II) posee una estructura muy compleja y una masa molecular pequeña (5 KDa). Su estructura química, similar en todos los vegetales, la forman azúcares raros (2-O-metil-fucosa, apiosa, 2-O-metil-xilosa, KDO o ácido 2-ceto-3-deoxi-D-mano-octulosónico, DHA o ácido 3-deoxi-D-*lixo*-2-heptulosónico, ácido acérico o ácido 3-carboxi-5-deoxi-L-xilosa) que son exclusivos de la molécula de RG-II, por lo que se usan para su identificación y cuantificación (87, 90, 91). La concentración de RG-II en el vino tinto alcanza valores de 80-120 mg/L (45), lo que supone entorno al 20% de los polisacáridos totales del vino (90). Hay que destacar que el contenido de RG-II en los vinos permanece prácticamente constante desde el final de la fermentación hasta la etapa de envejecimiento en botella (86).

Existen estudios que cuantifican los oligómeros de los homo- y ramnogalaturonanos, que suponen solamente un 2% de los polisacáridos totales solubles del vino (86).

En resumen, los compuestos fenólicos y los polisacáridos son responsables del color, del sabor y del aroma del vino tinto, por su participación en distintas reacciones de copigmentación, polimerización, formación de complejos polifenol-polisacárido, etc. Sin embargo, ésta no es la única característica de los compuestos fenólicos. En este sentido, hay que señalar que la composición de compuestos fenólicos en la uva, y por tanto, en el vino, depende de la variedad de vinífera. Así, cada variedad vinífera presenta una capacidad potencial para sintetizar compuestos fenólicos diferentes (47). Por tanto, los compuestos fenólicos pueden utilizarse como herramientas taxonómicas para diferenciar distintos tipos de vinos, ya sean vinos tranquilos (70, 92-96), o espumosos (97).

De hecho, el perfil de antocianos del vino no varía a pesar de las transformaciones que ocurren en los mismos durante la vinificación. Por ello, dicho perfil se ha utilizado como criterio taxonómico para establecer diferencias entre variedades (70, 92, 94, 98). Incluso, existen estudios que han demostrado que el ratio de los antocianos cumarilados/antocianos acetilados permite diferenciar vinos varietales (99, 100). Así, una de las características que diferencian a los vinos de Cencibel de otros vinos varietales, como Cabernet Sauvignon o Merlot, es que la proporción de antocianos cumarilados es superior que la de acetilados (56, 99). También los ácidos

hidroxicinámicos permiten clasificar a los vinos en función de la variedad de uva (97). De hecho, la relación *trans*-cutárico/*trans*-caftárico se ha usado como una herramienta para diferenciar variedades de uva y vino (92, 101). Del mismo modo, el perfil de los flavonoles se ha utilizado como herramienta para diferenciar variedades de uva (62, 92, 102, 103). Sin embargo, no está muy claro si este poder clasificatorio sirve en los vinos (104).

Sin embargo, los polifenoles no son los únicos compuestos presentes en el vino capaces de conseguir clasificar a los vinos en función de la variedad. También existen estudios que indican que los aminoácidos y las aminas biógenas son útiles como herramientas taxonómicas para diferenciar vinos, no sólo en función de la variedad, sino también en función de la zona geográfica, de la etapa de vinificación y de la añada (105-111).

Los aminoácidos presentes en el vino pueden tener diferentes orígenes. Además de los procedentes de la uva, también pueden ser secretados al medio por las levaduras, o ser liberados durante la autolisis de las mismas. Los aminoácidos son fuente de nitrógeno para las levaduras, precursores de aromas (112, 113) y sustrato para el crecimiento de bacterias (114), lo que da lugar a que su concentración se modifique a lo largo de la elaboración del vino. Sin embargo, no existe consenso en la bibliografía sobre si la fermentación maloláctica produce un aumento (115) o un descenso en el contenido total de aminoácidos (105).

La bibliografía describe concentraciones de aminoácidos totales en vinos tintos que varían en un amplio rango, desde 69 mg/L en vinos de Moreto (109) hasta 1.922 mg/L en vinos de Cencibel (116). Sin embargo, no existen estudios sobre el contenido de aminoácidos en los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete.

Las aminas biógenas son bases orgánicas nitrogenadas de bajo peso molecular que se producen principalmente como consecuencia de la descarboxilación de sus aminoácidos precursores por acción de las bacteria lácticas (Figura 16). Están presentes en distintos alimentos como el queso, los productos de la pesca, la cerveza y el vino. Las aminas biógenas pueden encontrarse en el vino a niveles variables dependiendo de la variedad de uva, la añada, el estado sanitario, la cantidad de aminoácidos precursores, el contenido en nitrógeno asimilable y las técnicas de vinificación como el llevar a cabo la fermentación maloláctica o no (108-111, 114). En este sentido, la bibliografía describe concentraciones totales de aminas biógenas en vinos tintos que varían en un amplio rango, desde de 4,5 mg/L en vinos de Moreto (109) hasta 53,61 mg/L en vinos de Carmenere (117). No existen estudios en cuanto a la concentración de aminas biógenas en los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete.

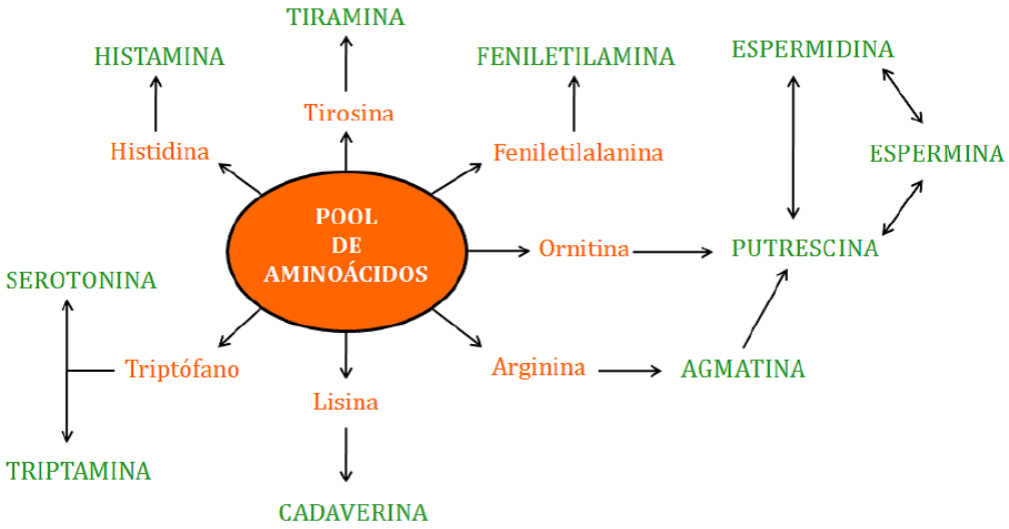


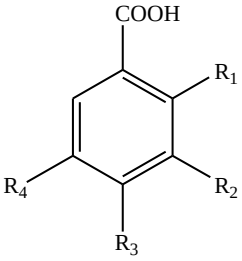
Figura 16. Aminas biógenas y sus aminoácidos precursores

Las principales aminas encontradas en los vinos son: putrescina, cadaverina, agmatina, espermidina y espermina (alifáticas), tiramina, y feniletilamina (aromáticas) e histamina y triptamina (heterocíclicas). La putrescina se relaciona con suelos deficientes en nitrógeno, y junto con la cadaverina, se asocian a deficientes condiciones sanitarias durante la elaboración. Las aminas aromáticas y heterocíclicas, sobre todo la histamina, tienen efectos fisiológicos indeseables en personas sensibles, tales como dolores de cabeza, náuseas, hipo e hipertensión, palpitaciones cardiacas y shock anafiláctico. Por ello, algunos países han establecido formalmente límites legales para el contenido en histamina en los vinos, como por ejemplo: Suiza (10 mg/L), Alemania (2 mg/L), Bélgica (5-6 mg/L) y Francia (8 mg/L) (118). El límite más utilizado es el de 8 mg/L propuesto por Leitao y col. (118), 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 (calidad toxicológica).

Respecto a la obtención de vinos saludables, es ampliamente conocido que los polifenoles tienen propiedades beneficiosas para la salud. En este sentido, son varios los estudios científicos que han evaluado la capacidad antioxidante (68, 78, 119, 120) y vasodilatadora (121) de los flavanoles. Sin embargo, los compuestos de mayor importancia en cuanto a sus características antioxidantes (71, 119, 122-124), anticancerígenas (123, 124) y antiinflamatorias (123) son los ácidos hidroxibenzoicos y los estilbenos (compuestos no flavonoides).

Entre los ácidos hidroxibenzoicos (Figura 17), el ácido gálico, uno de los más potentes antioxidantes del vino (71), es el de mayor concentración en el vino. El ácido gálico no solo proviene de la uva, sino que también puede liberarse a partir de la

hidrólisis de taninos condensados hidrolizables cedidos por la madera al vino, lo que hace aumentar su concentración durante la crianza (65, 71). Por ello, su concentración varía en función de la variedad de uva y del proceso de vinificación (71). En vinos tintos, la bibliografía describe valores de ácido gálico desde 2,4 mg/L en vinos de Syrah (71) hasta 55 mg/L en vinos de Cabernet Sauvignon (71). En los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete sólo se tienen datos del contenido total de ácidos hidroxibenzoicos después de la fermentación alcohólica con concentraciones que oscilan entre 30-40 mg/L (28).



Ácidos hidroxibenzoicos	R_1	R_2	R_3	R_4
Ácido gálico	H	OH	OH	OH
Ácido gentísico	OH	H	H	OH
Ácido p-hidroxibenzoico	H	H	OH	H
Ácido protocatéquico	H	OH	OH	H
Ácido salicílico	OH	H	H	H
Ácido siríngico	H	OCH_3	OH	OCH_3
Ácido vainillínico	H	OCH_3	OH	H

Figura 17. Estructura química de los ácidos hidroxibenzoicos

Los estilbenos, considerados también potentes antioxidantes, se encuentran en las uvas y el vino, siendo éstas las fuentes más importantes de estos compuestos en la dieta (125). Dentro de los estilbenos, se encuentran el *cis* y *trans*-resveratrol (Figura 18) y sus derivados glicósidos (piceidos).

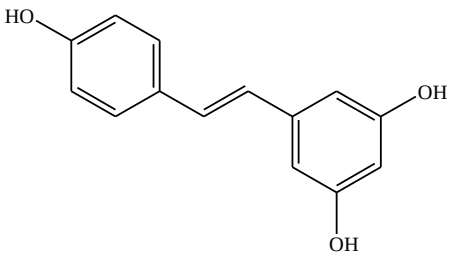


Figura 18. Estructura molecular del resveratrol

El contenido de resveratrol en el vino depende de la variedad de uva (96, 126, 127); del estado de maduración (96, 127) y sanitario de la uva (128); de la añada; de las técnicas de vinificación empleadas como la maceración (129) y de la adición de enzimas de extracción (130); de la cepa de bacteria que lleva a cabo la fermentación maloláctica (72) y del proceso de envejecimiento (131). La concentración de resveratrol en los vinos tintos varía de 0,2 a 13 mg/L (65). La bibliografía describe valores de

resveratrol después de la fermentación alcohólica de 2 mg/L en vinos de Maturana Tinta y Maturana Tinta de Navarrete, y 3 mg/L en vinos de Monastel (28).

Debido a sus propiedades antioxidantes, antiinflamatorias, anticarcinogénicas y antimutágenas, a los estilbenos se les considera de gran importancia en la dieta (138, 132-134), aunque no tenga efectos claros sobre las características sensoriales de los vinos.

Otro de los aspectos organolépticos más importantes en la calidad del vino es el aroma. Por el aroma de un vino podremos reconocer la variedad de la cual procede, su estado de conservación e incluso algunos defectos.

El aroma se forma a través de rutas metabólicas durante la maduración de la uva (aromas varietales o primarios), durante la fermentación (aromas fermentativos o secundarios) y/o durante el envejecimiento de los vinos (aromas derivados de la crianza o terciarios) (135).

Los aromas varietales o aromas primarios son compuestos complejos que pasan de la uva al vino sin transformación. Se trata de sustancias aromáticas libres, o de precursores de aromas combinados, que no son aromáticos en sí mismos, pero que liberan aromas por hidrólisis ácida o enzimática. Su importancia radica en que son característicos de cada variedad, lo que les permite diferenciar y clasificar distintos vinos varietales. A este grupo pertenecen, principalmente, los terpenos, los norisoprenoides los alcoholes C_6, los compuestos bencénicos, y las pirazinas.

Tradicionalmente el estudio de aromas varietales se ha centrado estrictamente en los *compuestos terpénicos*, linalol, nerol, geraniol, citronelol y α-terpineol, asociados con aromas florales, frutales y cítricos; y en los *norisoprenoides*, ß-damascenona relacionada con notas de manzana, rosa y miel, y ß-ionona relacionada con aromas violeta, flores y frambuesa (136) de variedades blancas consideradas más o menos aromáticas como Muscat, Riesling, Sylvanner y Gewürztraminer (137), de Devin (138), o de Boal, Malvazia, Sercial y Verdelho (139). Sin embargo, hay que tener en cuenta que el 90% de la producción mundial de vinos se obtiene a partir de variedades de uva consideradas como neutras o no aromáticas, o, también llamadas no terpeno-dependientes. Por ello, recientemente se realizan estudios sobre el contenido y el perfil aromático de gran cantidad de variedades neutras, la mayoría tintas, que permitan clasificar y definir su potencial aromático para producir vinos de calidad (140-144). En las variedades neutras, los alcoholes C_6, los compuestos bencénicos y las pirazinas adquieren especial importancia.

Los *alcoholes* C_6 se relacionan con la presencia de partes leñosas y de hojas durante la elaboración, y con el uso de las altas presiones en el estrujado (145, 146). Estos

compuestos aportan notas vegetales y herbáceas a los vinos, produciendo un detrimento en su calidad cuando su concentración está por encima de su umbral de detección, 400 μg/L (146). Aleixandre y col. (142) describieron que los vinos de Tempranillo poseían los valores más altos de *cis*-3-hexen-1-ol (0,23 g/L) cuando se comparaban con vinos de Cabernet Sauvignon, Monastrell y Bobal (0,09, 0,06 y 0,10 g/L, respectivamente). Sin embargo, en un estudio sobre Cencibel apenas se encontró la forma *cis*-3-hexen-1-ol, con una mediana de 0,29 μg/L, mientras que la forma *trans* alcanzó 119 μg/L (144). Dentro de los compuestos C_6, el ratio *trans* y *cis*-3-hexen-1-ol se ha considerado como característico de la variedad de uva (140-142), y por tanto, muy importante para clasificar vinos por variedades. La bibliografía describe ratios desde 0,4 en vinos de Tannat y Bobal (140) y Moravia Dulce (141) hasta de más de 4,5 en vinos de la variedad Tortosí (141).

Los *compuestos bencénicos* son un importante grupo que incluye a los alcoholes aromáticos, los aldehídos, los fenoles volátiles y los derivados del ácido shikímico. Los fenoles volátiles pueden provenir bien de las uvas y/o formarse en el proceso de fermentación por reacciones químicas, como la degradación del ácido fenólico, o en el caso de los vinilfenoles, por la contaminación de *Brettanomyces* (147). Su influencia positiva o negativa en la calidad final del vino dependerá de su concentración. Así, el aroma asociado a estos compuestos puede variar desde animal-establo hasta picante (136). En un estudio reciente (143) se describen concentraciones de fenoles volátiles de 133,91 μg/L en vinos de Merenzao, sinonimia de Maturana Tinta.

El benzaldehído, el benzilalcohol y el eugenol son también compuestos bencénicos encontrados en los vinos. Los dos primeros pueden actuar sinérgicamente en el aroma aportando notas frutales y florales. Por otro lado, al eugenol se le relaciona con aromas especiados de clavo. La bibliografía describe concentraciones de benzilalcohol en vinos tintos desde 238 μg/L en Cabernet Franc (148) hasta 1.550 μg/L en vinos de Merenzao (143). En cuanto al eugenol, se describen valores desde 0,25 μg/L en vinos de Sousón (143) hasta 2,1 μg/L en vinos de Brancellao (143). Los derivados del ácido shikímico se forman a partir de los aminoácidos, ya sea en la planta o por acción de las levaduras. Además, también puede extraerse de la madera (149).

En cuanto a las *pirazinas*, otros compuestos aromáticos varietales, son compuestos nitrogenados derivados del catabolismo de algunos aminoácidos como la leucina, isoleucina y valina. En los vinos se caracterizan por sus importantes notas a verdor, relacionándose con el grado de madurez de la uva. Están ligadas al aroma típico del Cabernet Sauvignon (pimiento verde) y al Sauvignon Blanc (retama) (145), con valores que oscilan de 0,4 a 14,9 ng/L (145).

Los aromas fermentativos o aromas secundarios se forman durante el proceso de maceración-fermentación. Su concentración final en el vino está relacionada con la composición inicial del mosto y las condiciones en las que se lleva a cabo la fermentación (150). Los aromas producidos durante la maceración-fermentación alcohólica representan cuantitativamente la mayor parte de los constituyentes del aroma, formando la base aromática común de los vinos tintos. Por otro lado, la fermentación maloláctica da lugar a modificaciones sutiles del aroma, desapareciendo las notas vegetales excesivas y apareciendo notas lácteas. Dentro del grupo de los aromas fermentativos se engloban, principalmente, a los alcoholes, los ésteres y los ácidos grasos.

Los *alcoholes* son el grupo más importante de compuestos volátiles producidos durante la fermentación alcohólica. Estos compuestos se reconocen por aportar aromas acres y notas herbáceas. Entre los alcoholes superiores se encuentran el 2- y 3- metil butanol, el propanol, el 2-metilpropanol, el butanol, el pentanol, el 2-feniletanol, el tirosol y el triptófol. La concentración total de alcoholes superiores por debajo de 300 mg/L añade complejidad al vino, mientras que concentraciones superiores a 400 mg/L disminuyen su calidad (151). Sin embargo, el 2-feniletanol se considera que aporta siempre características positivas, por su agradable aroma a rosa (149). En un estudio reciente (143), se describen concentraciones de alcoholes (butanol, 2-metilpropanol y 2-feniletanol) en vinos de Merenzao de 431 mg/L (143).

La mayoría de los *ésteres* encontrados en el vino son ésteres etílicos de ácidos grasos. Su contribución es positiva en la calidad general de los vinos, ya que son responsables del aroma a fruta de los vinos jóvenes (150), y de notas de mantequilla y lácticos derivadas de la fermentación maloláctica. Existen estudios (26, 27) del contenido total en ésteres después de la fermentación alcohólica en vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete, con valores medios de 170 mg/L, 130 mg/L y 480 mg/L, respectivamente, mientras que los vinos de Tempranillo mostraron valores muy superiores (850 mg/L).

El vino contiene una mezcla de *ácidos grasos* de cadena corta (C_2-C_4), de cadena media (C_6-C_{10}), y de cadena larga (C_{12}-C_{18}) que aportan aromas frutales, de queso, grasos y notas rancias. La volatilidad de estas percepciones cambia con el tamaño de la cadena (152). La bibliografía describe los vinos de Tempranillo y de Maturana Tinta de Navarrete como los vinos de mayor contenido medio en ácidos grasos (suma de los ácidos hexanoico, octanoico y decanoico) en relación a los de Monastel y Maturana Tinta al final de la fermentación alcohólica (26, 27).

Además, existen los aromas derivados de la crianza o aromas terciarios, formados principalmente por las whisky lactonas y los fenoles volátiles, sobre los cuales no

existen estudios en los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete. Durante la maduración o crianza, el aroma afrutado de los vinos jóvenes desaparece para evolucionar hacia aromas más complejos y especiados.

Los isómeros *cis* y *trans* de la ß-metil-γ-lactona, también llamadas *whisky lactonas*, están presentes en la madera verde, incrementándose su contenido durante el tostado de la barrica (153). Contribuyen al aroma final del vino aportando notas de madera, coco y vainilla (154). Los *fenoles volátiles* vainillina, siringaldehído, guaiacol y eugenol se forman por degradación de la lignina, aunque la vainillina se encuentra también en la madera verde, incrementándose con el tostado (154). Los compuestos derivados del *furfural* se forman por termólisis de la celulosa y hemicelulosa dando aromas de almendra. El 4-etilguaiacol, 4-vinilguaiacol, 4-vinilfenol y 4-etilfenol pueden ser cedidos al medio por la madera, pero normalmente su presencia se debe a la acción de *Brettanomyces*, como se ha explicado previamente (147).

En un estudio sobre el efecto de la crianza en barrica de vinos tintos de Tempranillo, Tinta de Toro, Tinta del País, Mencía, Rufete, Juan García, Malbec, Cabernet Sauvignon y Merlot se concluyó que en la cesión de compuestos de la madera al vino influyeron gran cantidad de factores como la especie de roble, su origen geográfico, las variaciones barrica-barrica, el tostado, la estación del año, la edad y volumen de la barrica, el tiempo de contacto con la madera, la capacidad de extracción que posee cada tipo de vino, las condiciones de temperatura y humedad de la bodega, etc., lo que dio lugar a una gran variabilidad en la concentración de los compuestos extraídos de la madera (153).

El equilibrio óptimo entre los atributos sensoriales derivados de la madera y los derivados de la uva y de la fermentación es de suma importancia para obtener el nivel deseado de complejidad aromática de un vino.

Por otra parte, es importante destacar que el mero conocimiento de la composición volátil de un vino, sin la evaluación sensorial, es insuficiente para predecir el sabor 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 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 (155). De ahí la importancia de complementar el análisis instrumental de los compuestos volátiles con el análisis sensorial, máxime cuando se trata de variedades minoritarias que no son ampliamente conocidas. En este sentido, la bibliografía describe a los vinos de Monastel como de baja intensidad aromática; a los de Maturana Tinta por ser ligeramente afrutados, y con predominio de aromas a uvas pasas, uva madura y especias; y, por último, a los de

Maturana Tinta de Navarrete por sus aromas varietales de pimiento verde, herbáceas y de pimienta (26-28).

Como se ha descrito anteriormente, tanto los compuestos fenólicos como los compuestos aromáticos, los compuestos nitrogenados y los polisacáridos van a ser muy importantes para conocer la aptitud enológica de una variedad de uva, y por tanto del vino. En este sentido, es importante señalar que existen muy pocos estudios al respecto en los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete (26-28), y además están realizados en microvinificaciones y en una etapa concreta de la vinificación. Por lo tanto, para conocer las características enológicas de dichos vinos varietales, lo ideal sería conocer en detalle el perfil varietal de estos compuestos en los vinos jóvenes y evaluar como se modifican a lo largo de las distintas etapas de vinificación

1. 3. EFECTO DE LAS MANOPROTEÍNAS EN LA CALIDAD DE LOS VINOS VARIETALES

Los polisacáridos del vino tienen varios orígenes ya que pueden proceder de la uva (arabinogalactano-proteínas, ramnogalacturonanos y homogalacturonanos), de levaduras (manoproteínas y mananos) y de microorganismos como *Botrytis cinerea* y bacterias. Los polisacáridos procedentes de la uva, que se han descrito en el apartado anterior, y de las levaduras son los más importantes desde un punto de vista enológico. Estos compuestos influyen tanto en los procesos tecnológicos de filtración, estabilización y clarificación como en las propiedades organolépticas de los vinos tintos y en su calidad final (90, 156).

Los polisacáridos procedentes de las levaduras se encuentran en su pared celular y son la quitina, los glucanos y las manoproteínas, que son los polisacáridos más interesantes desde un punto de vista enológico y representan entre el 30% y 50% de la pared celular (157).

Desde un punto de vista químico, las manoproteínas son glicoproteínas, normalmente con alto grado de glicosidación (80-90%), compuestas mayoritariamente por manosa (>90%) y glucosa.

Se pueden distinguir en el vino dos tipos de manoproteínas:

a) manoproteínas secretadas durante la fase de crecimiento exponencial de las levaduras y que se acumulan durante la fermentación (158), y

b) manoproteínas liberadas por la autolisis celular de las levaduras durante la crianza del vino sobre lías (159)

La estructura molecular general de las manoproteínas exocelulares de la levadura es similar a la de las manoproteínas localizadas en la pared. 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 (157) (Figura 19).

Las manoproteínas presentan tamaños moleculares muy variables, desde 5 KDa hasta 800 KDa (45), 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 (160).

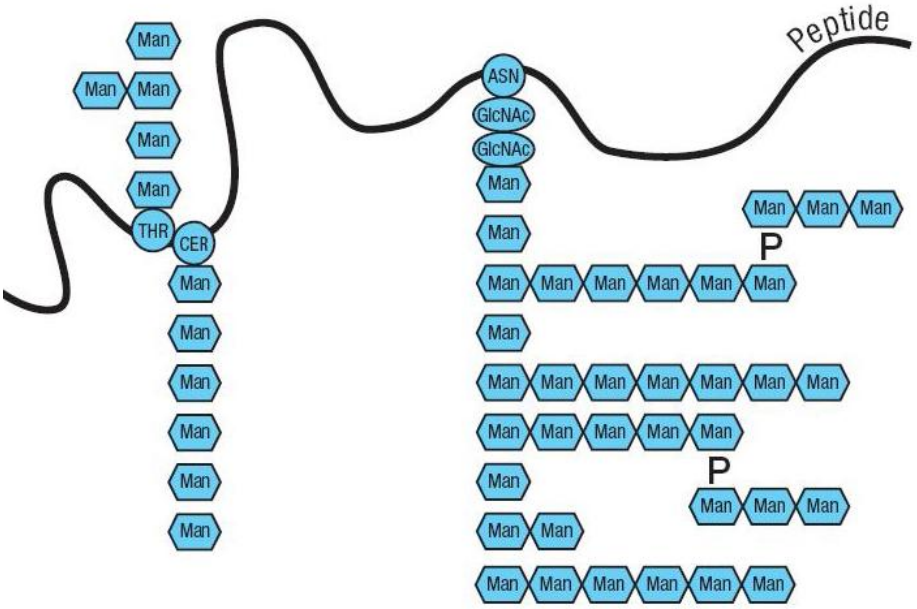


Figura 19. Estructura de las manoproteínas exocelulares de las levaduras, extraído de Bajard-Sparrow y col. (161). (Man: manosa; P: fosfato; GlcNac: N-acetil-glucosamina; Asn: asparagina; Ser: serina; Thr: treonina)

La concentración de manoproteínas en el vino depende de la cepa de levadura y de las condiciones de fermentación y conservación del vino. Así, la liberación de manoproteínas se ve favorecida por temperaturas altas y la realización de battonages frecuentes durante la crianza sobre lías. El contenido de manoproteínas en el vino varía en función de la etapa de vinificación. En el vino joven su contenido es de 100-150 mg/L (45), lo que supone alrededor del 30% de los polisacáridos totales del vino y constituye la segunda familia de polisacáridos en importancia en el vino recién elaborado, después de los arabinogalactano-proteínas. Su contenido se incrementa durante la fermentación maloláctica, la crianza y el envejecimiento, pasando a ser cuantitativamente la primera familia de los polisacáridos del vino y representando el 45% del total de polisacáridos (86, 88).

Las manoproteínas juegan un papel importante en el proceso de elaboración de los vinos y en sus características sensoriales. A nivel tecnológico, además de intervenir en la estabilidad tartárica y proteica, favorecen la fermentación maloláctica, participan en la formación del velo en la crianza de los vinos en flor, mejoran las características espumantes de los vinos espumosos, y adsorben compuestos tóxicos como la ocratoxina A y los metales pesados (162).

Además de las propiedades tecnológicas descritas, las manoproteínas tienen un efecto positivo en las propiedades sensoriales de los vinos. En este sentido, diversos

estudios han descrito su interacción con los compuestos aromáticos, favoreciendo la retención del aroma e incrementando la persistencia aromática de los vinos (163). Sin embargo, Commuzzo y col. (2006) observaron que la adición de manoproteínas no afectaba a todos los vinos por igual; a los vinos con un marcado carácter varietal no les afectaba su adición mientras que los vinos poco aromáticos mejoraban sus características aromáticas (164).

Diversos autores describen también que las manoproteínas reducen la astringencia (90, 165) e incrementan el cuerpo y la redondez del vino en boca (90, 165). Estos cambios organolépticos se han atribuido a la formación de complejos manoproteína-tanino, que disminuyen la reactividad de los taninos e impiden su polimerización y precipitación con las proteínas salivales (166).

Por último, a las manoproteínas se le atribuyen efectos beneficiosos en la estabilización del color de los vinos tintos (167). La estabilización del color se produciría por medio de interacciones manoproteína-antociano polimérico, que disminuiría la reactividad de los pigmentos poliméricos impidiendo que aumente su polimerización y que precipiten, y evitando así la pérdida de materia colorante inestable. Sin embargo, los estudios científicos que analizan el efecto de las manoproteínas sobre el color del vino muestran resultados contradictorios (156, 165, 168-175). Nuestro grupo de investigación realizó un estudio detallado con objeto de conocer los efectos de las manoproteínas sobre el color del vino tinto. Se realizaron distintos ensayos como la adición de preparados comerciales a base de manoproteínas antes de la fermentación alcohólica (169); el uso de cepas de levaduras superproductoras de manoproteínas (170); el envejecimiento del vino sobre lías lisadas (171) y combinaciones de todos estos tratamientos (165, 171). Todos estos ensayos se realizaron en vinos de Tempranillo. Contrariamente a los resultados obtenidos en medio sintético por otros autores (156, 172), nuestros resultados mostraron que la adición de manoproteínas no mantenía a los polifenoles en dispersión coloidal ni tampoco aseguraban la estabilidad del color (165, 169). Los mismos resultados en cuanto al efecto desestabilizador de las manoproteínas sobre los taninos se obtuvieron cuando se usaron las cepas de levaduras superproductoras, aunque en este caso y en el envejecimiento con lías lisadas no se observó desestabilización del color (170, 171).

Esta controversia, unida a la gran variabilidad de preparados comerciales de manoproteínas que existen en el mercado (168), el amplio rango de dosis usadas y la falta de información sobre su composición química real (168), hace difícil la obtención de conclusiones. Es importante señalar que la mayoría de estudios se han llevado a cabo en medio sintético (156, 172) o en microvinificaciones (169, 171, 175) sin tener en cuenta las condiciones reales de una elaboración en bodega industrial. Además, la

mayoría de los estudios se han realizado añadiendo los productos comerciales antes o después de la fermentación alcohólica (165, 169, 171, 174).

Por ello, resultaría interesante realizar otros ensayos adicionando las manoproteínas en otras etapas de vinificación como después de la fermentación maloláctica y utilizando otros vinos diferentes al Tempranillo.

En vista de la importancia del estudio de las manoproteínas y de los polisacáridos del vino, y con objeto de mejorar los métodos existentes de cuantificación de los mismos (cromatografia de intercambio aniónico de alta resolución con detector amperométrico, espectroscopia infrarroja con transformada de Fourier) en esta Tesis se evaluó la idoneidad del uso de la cromatografía de gases con detector de masas (GC-MS) para la identificación y cuantificación de forma clara e inequívoca de los monosacáridos constituyentes de los polisacáridos del vino. Los resultados obtenidos se compararon con la metodología previamente desarrollada por nuestro grupo de cromatografía de gases con detector de ionización de llama (GC-FID), y se evaluó también el empleo de la cromatografía de exclusión molecular con detector de índice de refracción (HRSEC-RID) como una posible técnica rápida y sencilla para estimar el contenido global de polisacáridos en el vino.

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2

OBJETIVOS

Los principales objetivos de la presente Tesis son:

1.- Caracterizar enológicamente los vinos obtenidos con las variedades minoritarias tintas de La Rioja Monastel, Maturana Tinta y Maturana Tinta de Navarrete, utilizando la variedad Tempranillo como referencia. La caracterización se realizará en términos de:

a) Color

b) Polifenoles monómeros y poliméricos

c) Polisacáridos

d) Aminoácidos y aminas

e) Compuestos volátiles

2.- Estudiar la evolución de dichos compuestos antes y después de la fermentación maloláctica en diferentes añadas.

3.- Analizar sensorialmente los vinos obtenidos.

4.- Evaluar el efecto de la adición de manoproteínas comerciales después de la fermentación maloláctica en los polisacáridos, el color, la composición fenólica y el perfil sensorial de los vinos varietales, y estudiar la evolución de estos parámetros durante la crianza en barrica y envejecimiento en botella.

5.- Analizar la idoneidad del uso de la cromatografía de gases con detector de masas (GC-MS) para la identificación y cuantificación de los monosacáridos constituyentes de los polisacáridos del vino, y evaluar el potencial de la cromatografía de exclusión molecular con detector de índice de refracción (HRSEC-RID) como un método rápido y sencillo para estimar el contenido global de polisacáridos en el vino.

3

MATERIALES Y MÉTODOS

3. 1. VINIFICACIÓN Y TOMA DE MUESTRAS

En esta Tesis se estudiaron los vinos obtenidos de las variedades minoritarias tintas de La Rioja Monastel, Maturana Tinta y Maturana Tinta de Navarrete, usando vinos de la variedad Tempranillo como referencia. La autorización de la variedad Maturana Tinta de Navarrete por el Consejo Regulador de la D. O. Ca. Rioja coincide con la cosecha de inicio del estudio (2009), mientras que las variedades Monastel y Maturana Tinta se encuentran todavía en estudio en parcelas experimentales. La elaboración se realizó durante las añadas 2009 y 2010 en la bodega ecológica de la D. O. Ca. Rioja de Juan Carlos Sancha, S. L.

La bodega de Juan Carlos Sancha, S. L. (latitud: 42º 20´ N; longitud: 2º 45´ O, altitud 580 metros) se encuentra situada en el término de Baños del Río Tobia, La Rioja, en la subzona de Rioja Alta. Las variedades de uva se cultivaron en parcelas propiedad de la bodega situadas alrededor de la misma (polígono 3 parcelas 332, 334, 339-341, 343, 519, 573, 576-579). De estas parcelas, 0,5 hectáreas corresponden a la variedad Tempranillo, 0,5 hectáreas a la variedad Maturana Tinta, 2,5 hectáreas a la variedad Maturana Tinta de Navarrete y 0,5 hectáreas a la variedad Monastel. Todas las parcelas, situadas en terrazas colgadas sobre el Río Najerilla, son de suelo arcilloso-calcáreo, con sistema de conducción en espaldera, y marco de plantación de 2 m x 1 m, lo que supone una densidad de plantación de 5.000 cepas por hectárea. La edad media del viñedo es de 12 años.

Las prácticas de viticultura llevadas a cabo fueron iguales en todas las parcelas y en ambas añadas. La poda de invierno se realizó a seis pulgares, con dos yemas y la ciega; el espergurado y desnietado se realizó en mayo; el deshojado manual hasta el segundo racimo se hizo en junio; y, por último, se realizaron dos despuntados en julio antes del envero. La técnica de aclareo de racimos no se realizó. A nivel fitosanitario se aplicaron 5 tratamientos distribuidos desde mayo hasta 21 días antes de la vendimia. Estos tratamientos sólo incluyeron los productos azufre en polvo, azufre mojable y sulfato de cobre, únicos permitidos en explotaciones ecológicas. La pluviometría fue un 25% superior en el año 2010 que en el 2009 (100 mm del 2009 frente a 125 mm del 2010) (1), considerándose en ambos años escasa.

En ambas añadas las uvas se vendimiaron en óptimo estado de maduración (Tabla 1) y en buenas condiciones sanitarias durante la primera quincena de octubre. La vendimia se realizó de forma manual en cajas de 20 Kg de capacidad que sólo se llenaron hasta 18 Kg para evitar aplastamientos a la hora de apilarlas. 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 vendimia

Variedad	Año	º Brix	pH	AT[1]	AM[2]
Tempranillo	2009	24,7	3,55	5,50	2,8
Monastel	2009	25,3	3,40	6,25	2,6
Maturana Tinta	2009	24,2	3,35	6,70	2,5
Maturana Tinta de Navarrete	2009	23,6	3,35	5,50	2,5
Tempranillo	2010	23,7	3,48	5,80	4,5
Monastel	2010	24,7	3,43	6,35	4,3
Maturana Tinta de Navarrete	2010	23,2	3,35	6,85	4,1

[1] AT: acidez total en gramos de tartárico/L; [2] AM: ácido málico en g/L

Las Figuras 1 y 2 representan los esquemas de la vinificación y la toma de muestras para las añadas 2009 y 2010, respectivamente, que se detallan a continuación. Tras las vendimias, las uvas introducidas en la bodega se estrujaron y despalillaron (2.800 Kg/hora, maquinaria Modelo 30I, Toscana Enológica Mori, Mori Giorgio & C. S. R. L., Tavarnelle Val di Pesa, Italia). La uva estrujada y despalillada se encubó en barricas de roble francés de 500 L de capacidad (*Quercus petraea*, grano fino, tostado medio, espesor de la duela de 27 mm, edad media de la barrica cinco años) y se adicionaron 3g/HL de SO_2. Posteriormente se llevó a cabo la fermentación alcohólica mediante la adición de 25 g/HL de la levadura comercial *Saccharomyces cerevisiae* Uvaferm VRB (Lallemand-Inc., Montreal, Canadá). El proceso de fermentación-maceración duró aproximadamente 10 días a una temperatura de 25 ± 5 ºC. Durante la fermentación alcohólica los remontados se hicieron mediante giros de las barricas (8 veces por la mañana y otras 8 por la tarde). La fermentación alcohólica se controló realizando medidas de densidad y temperatura y se consideró terminada cuando los vinos tenían un contenido en azúcares reductores menor de 2g/L. Después de la fermentación alcohólica, los vinos se descubaron al mismo tipo de barricas de roble francés de 500 L, mezclándose con los vinos de prensa procedentes del prensado de sus hollejos fermentados. El prensado se realizó en una prensa vertical manual Modelo 70 (González Tratamientos, S. L., Logroño, La Rioja), con una presión máxima de trabajo de 400 atmósferas, durante dos horas. El rendimiento de transformación de la uva al vino fue del 65-70%.

La fermentación maloláctica, que duró entre 30 y 60 días, se realizó espontáneamente en las barricas de roble francés a una temperatura de 18 ± 3 ºC. Durante el tiempo que duró la fermentación maloláctica las barricas se giraron una vez al día. Después de la fermentación maloláctica, que se dio por finalizada cuando los vinos tenían un contenido en ácido málico menor de 0,1 g/L, los vinos se trasegaron otra vez a las barricas de roble francés y se corrigieron hasta un contenido de SO_2 libre de 30 mg/L.

En la añada 2009 (Figura 1), los vinos se sometieron a una crianza que duró entre 45 y 65 días a una temperatura aproximada de 15 ± 5 ºC y una humedad relativa del 80 ± 10 %. Durante las dos primeras semanas de la crianza las barricas se giraron una vez por semana; luego se pasó a un giro cada dos semanas y finalmente un giro al mes. Posteriormente se acondicionaron los vinos para su embotellado. Los vinos separados por variedades se trasegaron a un depósito de acero inoxidable siemprelleno de 5.300 L de capacidad (Herpanor, S. A., Laguardia, Álava), donde se realizó una clarificación con claras de huevo (2 claras/HL) durante 15-20 días. Seguidamente se realizó una filtración con un filtro de placas modelo Colombo (González Tratamientos, S. L., Logroño, La Rioja) con placas filtrantes de 20 x 20 cm (Enartis, Navarrete, La Rioja). El vino filtrado se corrigió hasta 30-35 mg/L de SO_2 libre y se embotelló (Maquinaria Toscana Enológica modelo 6LE, Mori Giorgio & C. S. R. L., Tavarnelle Val di Pesa, Italia). Los vinos embotellados se conservaron en la bodega a una temperatura de 15 ± 5 ºC y a una humedad relativa de 80 ± 10 %.

En la añada 2010, después de la fermentación maloláctica los vinos se sometieron a un tratamiento con manoproteínas. La mitad de las barricas de cada variedad se mantuvieron como testigos y a la otra mitad se les adicionó 30 g/HL de un producto a base de manoproteínas (Noblesse, Lallemand Bio S.L., España). El tiempo en que el vino estuvo en contacto con las manoproteínas fue el recomendado por el distribuidor (60 días), así como la frecuencia de los battonages aplicados: dos veces por semana la primera semana de tratamiento y posteriormente una vez por semana. Los vinos testigos se sometieron a la misma frecuencia de battonages que los tratados. Transcurrido el tiempo de tratamiento con manoproteínas, los vinos se acondicionaron y embotellaron del mismo modo que se ha descrito en la añada 2009.

En ambas añadas se tomaron muestras después de la fermentación alcohólica, después de la fermentación maloláctica, después del embotellado y después de seis meses de conservación de los vinos en botella (Figuras 1 y 2). Las muestras se cogieron en botellas tipo borgoña de 0,75 L. Todos los análisis químicos se realizaron en el mismo momento de la toma de muestras. El análisis sensorial de los vinos se realizó después de la fermentación maloláctica y después del embotellado.

Para completar el estudio aromático, se tomaron también muestras de las variedades Monastel, Maturana Tinta de Navarrete y Tempranillo después del embotellado en la añada 2011. La variedad Maturana Tinta sólo se estudió en la añada 2009, porque no pudieron obtenerse muestras el resto de añadas por un problema de disponibilidad de la bodega.

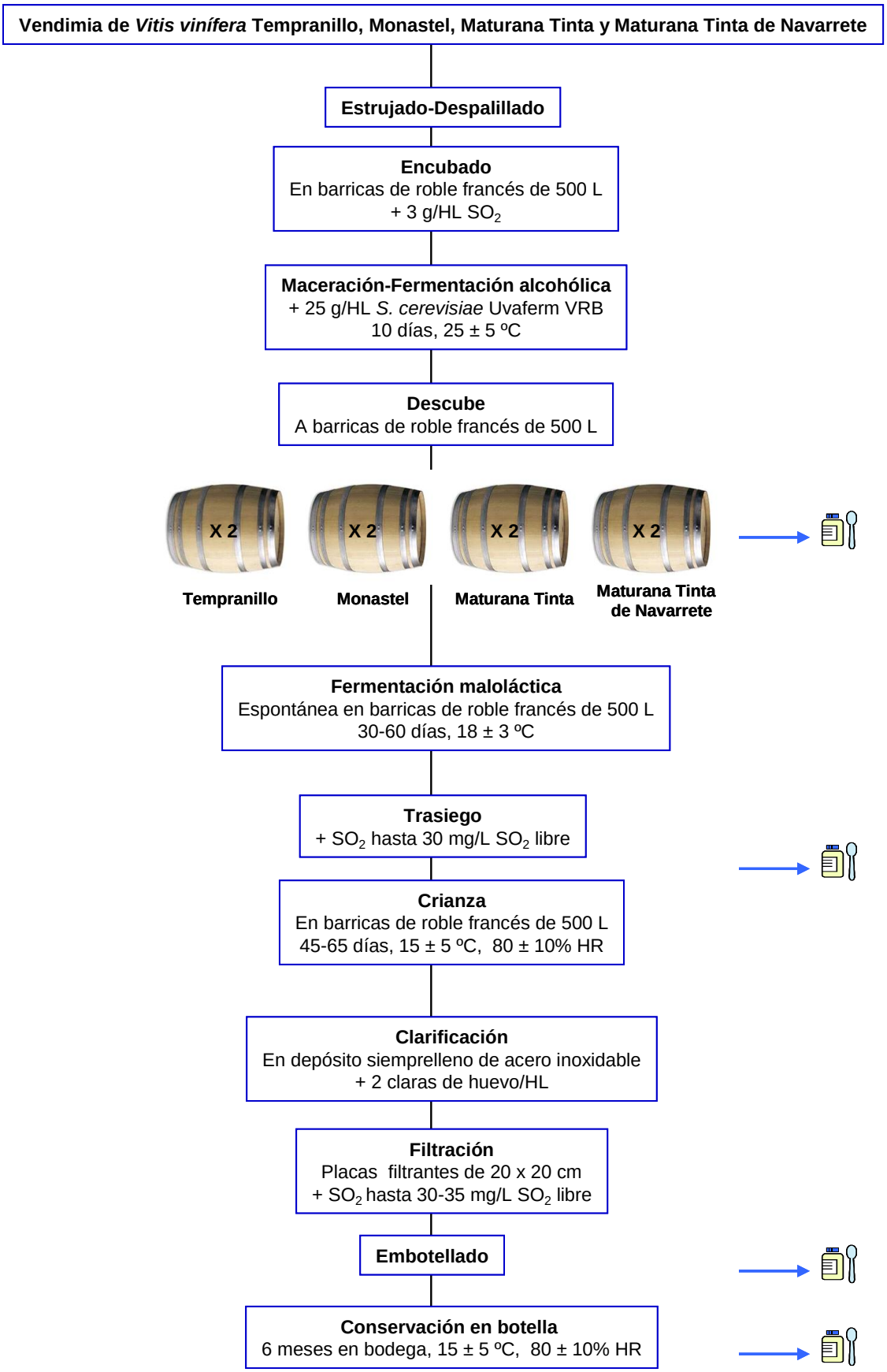

Figura 1. Esquema de vinificación y toma de muestras en la añada 2009

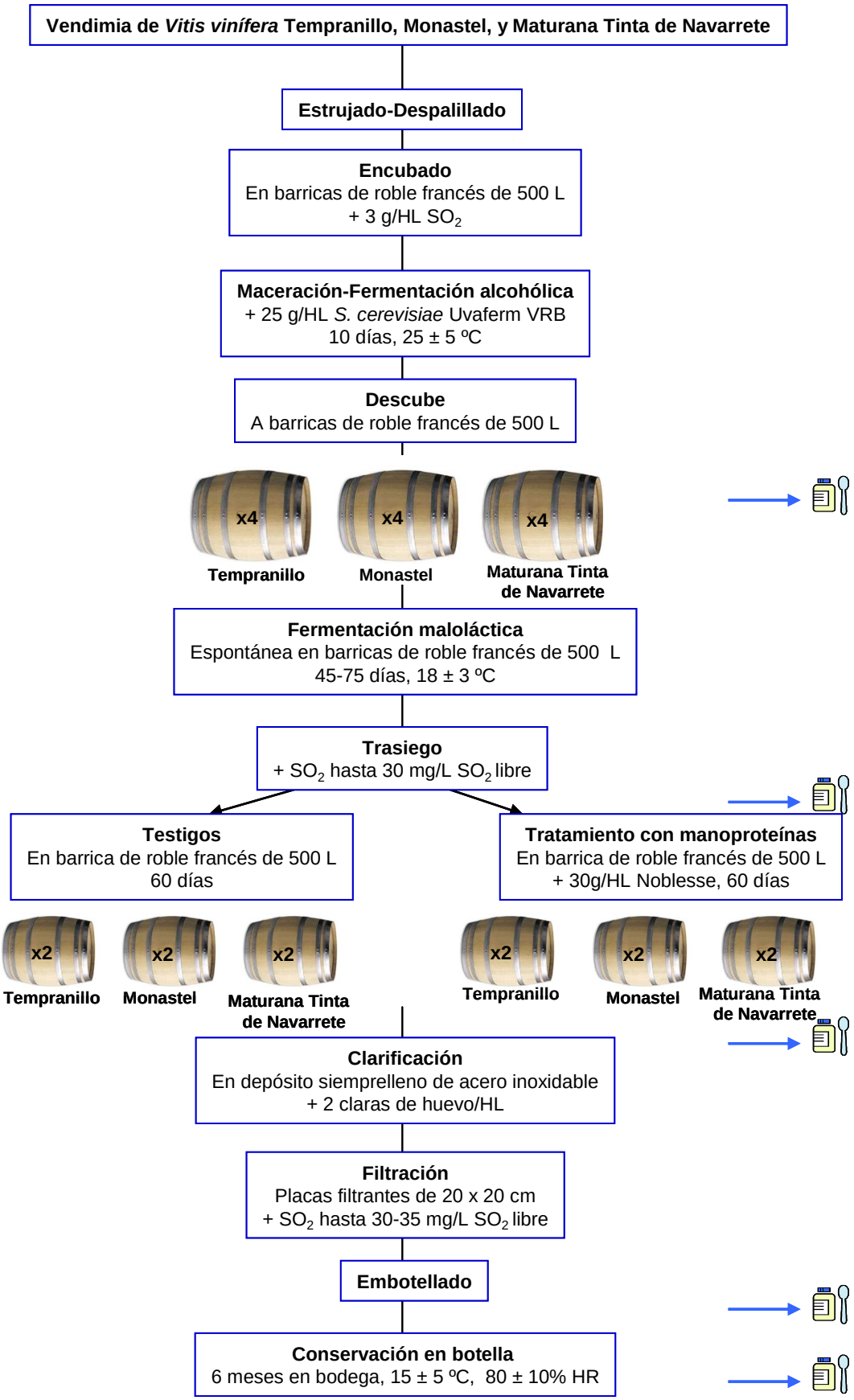

Figura 2. Esquema de vinificación y toma de muestras en la añada 2010

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.

Parámetros enológicos generales

En todas las muestras de vino se realizaron análisis de grado alcohólico, pH, acidez total, acidez volátil, sulfuroso libre y sulfuroso total, siguiendo los métodos oficiales de la OIV (2). Después de terminada la fermentación alcohólica se determinó el contenido de azúcares reductores por métodos enzimáticos usando el autoanalizador LISA 200. Los controles del contenido de ácido málico y ácido láctico hasta la finalización de la fermentación maloláctica también se realizaron por métodos enzimáticos con el mismo autoanalizador.

Análisis químicos

1.- Parámetros de color

Las medidas de intensidad colorante, tonalidad e índice de polifenoles totales se midieron mediante las técnicas recomendadas por la OIV (2). Los parámetros CIELAB se calcularon según el método propuesto por Ayala y col. (3). El color del vino, el color debido a los antocianos monoméricos, el color debido a la copigmentación y el color estable frente a la adición del bisulfito se determinaron por el método propuesto por Levengood y Boulton (4).

Estos métodos usan medidas espectrofotométricas que se llevaron a cabo utilizando el espectrofotómetro CARY 300 Scan UV-VIS (Varian, Madrid, España). Todas las medidas se realizaron por triplicado y se refirieron a cubetas de 10 mm de espesor.

2.- Compuestos volátiles

El análisis de los compuestos volátiles se realizó en la Estación Enológica de Rueda (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. (5). 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. (5). 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 duplicado.

3.- 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 resolución 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) de tamaño de partícula de 5 µm (250 mm x 4,6 mm) según la metodología propuesta por Gómez-Alonso y col. (6). Este método permitió la separación y cuantificación del ácido gálico, catequina, 9 ácidos hidroxicinámicos, 12 flavonoles, 15 antocianos y 3 estilbenos. Todos los análisis se realizaron por duplicado.

4.- Proantocianidinas

Las muestras de vino se fraccionaron previamente por cromatografía de permeación en gel a baja presión (GPC) en una columna Toyopearl HP-50F como describe Guadalupe y col (8). 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 usando una columna ACE (5 C18-HL) de tamaño de partícula de 5µm (250 mm x 4,6 mm) y el resto de condiciones descritas en el método Kennedy y Jones (9). El equipo de cromatografía utilizado 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 duplicado.

5.- 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. (10). 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.

6.- Polisacáridos

Se realizó un análisis cuantitativo de las distintas familias de polisacáridos del vino y un estudio del perfil y distribución de sus pesos moleculares según la metodología del artículo presentado en esta Tesis (11). Se analizaron tanto los polisacáridos procedentes de las levaduras, mananos y manoproteínas, como los polisacáridos procedentes de la uva, arabinogalactano-proteínas, ramnogalacturonanos tipo II y homogalacturonanos. Los polisacáridos se extrajeron del vino por precipitación con etanol/ácido previa concentración de la muestra. 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 1mL/min de 0.1 M $LiNO_3$. El perfil de la distribución de los pesos moleculares de los polisacáridos se comparó con patrones comerciales 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 cromatografía de gases con detector de masas (GC-MS) de sus trimetilsilil-ester-*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 1mL/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. 3. ANÁLISIS SENSORIAL

Se analizaron sensorialmente las muestras de vino tomadas después de la fermentación maloláctica, después del tratamiento con manoproteínas y después del embotellado.

El panel de cata estuvo compuesto por al menos 20 catadores, todos ellos enólogos profesionales de la D. O. Ca. Rioja. Los catadores habían participado previamente en paneles descriptivos de atributos sensoriales y puntuando también la calidad de vinos varietales. Todos los vinos se evaluaron en tres fases, en la fase visual (color), en la fase aromática (fracción volátil) y en la fase gustativa (equilibrio en boca).

Los análisis sensoriales se realizaron en dos sesiones. En una primera sesión los catadores describieron los atributos del vino con sus propias palabras. Los términos descriptivos y sus definiciones se debatieron entre los catadores y, posteriormente se compiló un vocabulario de consenso común. Todos los términos generados eran términos habituales para definir vinos tintos. En la segunda sesión se realizó la fase de evaluación sensorial propiamente dicha. Los catadores utilizaron el vocabulario consenso, marcando la intensidad de cada atributo en una escala de respuesta cuantitativa con 5 niveles de intensidad, donde 0 correspondía a la ausencia de percepción de la propiedad considerada, y 5 a una intensidad muy elevada del mismo. Además, los catadores puntuaron la valoración global de los vinos en una escala de 1 a 5, correspondiendo 1 a la mínima calificación del vino y 5 a la máxima, pudiendo realizar comentarios adicionales sobre las propiedades sensoriales de los vinos. La ficha de cata que recoge todos los atributos de las catas realizadas y la valoración global de los vinos se presenta en la Figura 3. 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.

Los vinos se presentaron a 18 ºC en catavinos codificados de acuerdo a la norma ISO 3591 (1977). La evaluación sensorial se llevó a cabo en la sala de catas de la Universidad de La Rioja, que cumple con la norma ISO 8589 (1998).

FECHA:
NOMBRE:

		VALORACIÓN					
DESCRIPTOR	DEFINICIÓN DE CONSENSO	0	1	2	3	4	5
Aspecto o Fase visual							
Intensidad de color	Pigmentación						
Aromas o Fase olfativa							
Intensidad aromática	Grado de intensidad aromática a copa parada						
Fruta	Fruta negra, mermelada, fresa, ciruela, pasas						
Herbáceos	Vegetal, espárrago, musgo						
Especias	Clavo, pimienta negra, cedro						
Lácteos	Yogurth, queso fresco, leche						
Regaliz							
Pimiento							
Tostado							
Café							
Mineral	Piedra, ceniza						
Vainilla							
Canela							
Crema	Pastelería, natilla, bollería						
Gusto-Textura-Retronasal							
Amargo	Taninos amargos, gusto amargo						
Ácido	Acidez en exceso						
Dulce	Ataque dulce en boca						
Graso	Glicérico, suave, sedosidad, redondo						
Astringente	Sensación táctil de los taninos, fricción labios y dientes						
Persistente	Duración en el tiempo de la percepción retronasal						
Cálido	Alcohólico, percepción de calor						
Largura en boca	Duración en el tiempo de la percepción en boca						
Tanicidad	Intensidad tánica positiva						
Suavidad	Sedosidad, redondez						
Secante	Sequedad en boca, facilidad para ensalivar						

0 equivale a ausencia de percepción; 5 a máxima intensidad

VALORACIÓN GLOBAL	1	2	3	4	5

1 equivale a mínima valoración del vino; 5 a máxima valoración

Figura 3. Ficha de cata con los atributos de todas las sesiones de cata realizadas y valoración global del vino.

3. 4. ANÁLISIS ESTADÍSTICO

Todo el conjunto de datos obtenidos se trataron adecuadamente mediante las técnicas matemáticas más convenientes para poder establecer correlaciones y diferencias entre ellos. Además de la estadística descriptiva, cuando los datos asumían criterios de normalidad, se aplicaron análisis de la varianza. Cuando los criterios de normalidad no se cumplían, se aplicaron análisis de contrastes no paramétricos. Las diferencias significativas encontradas se expresaron con un nivel de confianza superior al 95%. Para medir el grado de relación entre dos variables cuantitativas se realizó un estudio de correlaciones.

Se aplicaron también técnicas quimiométricas de Análisis de Componentes Principales (Principal Components Analyses, PCA) y Análisis Discriminantes (Discriminant Analysis, DA) con el fin de establecer diferencias y similitudes entre los distintos vinos varietales y las distintas añadas. 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 16.0 (SPSS Inc., Chicago, USA), Statistica 8.0 (Statsoft Inc., Tulsa, Oklahoma) 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 Version 3.3.2 (Utrecht, The Netherlands).

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4

RESULTADOS Y DISCUSIÓN

La introducción de variedades de uva ampliamente extendidas internacionalmente ha provocado una pérdida masiva de las variedades tradicionales de cada zona. En La Rioja surge la necesidad de preservar y caracterizar sus variedades minoritarias con el fin de salvaguardar la biodiversidad de su potencial enológico. En este sentido, un estudio sobre la aptitud vitícola de 76 accesiones de variedades minoritarias de La Rioja destacó a las variedades Maturana Tinta de Navarrete, Monastel y Maturana Tinta como las de mayor interés vitícola de entre las variedades tintas estudiadas. Así, los vinos obtenidos de las variedades minoritarias Monastel, Maturana Tinta de Navarrete y Maturana Tinta podrían resultar una buena alternativa para aportar diferenciación y tipicidad a los vinos de La Rioja, ampliando la oferta de vinos al consumidor. Por ello, en la presente Tesis nos planteamos como objetivo principal la caracterización enológica de estos vinos varietales.

Los tres primeros artículos (*Artículos 4.1*, *4.2* y *4.3*) abordan la caracterización enológica de los vinos varietales de Monastel, Maturana Tinta de Navarrete y Maturana Tinta en términos de color, polifenoles monómeros y poliméricos, aminoácidos, aminas biógenas y compuestos volátiles, usando a la variedad Tempranillo como referencia. Asimismo, se estudia la evolución de dichos compuestos antes y después de la fermentación maloláctica en diferentes añadas, y se analizan sensorialmente los vinos obtenidos.

Una vez conocido el potencial enológico de estas variedades minoritarias, se estudia el efecto de determinadas técnicas enológicas, como es la adición de manoproteínas, en la calidad final de estos vinos. De este modo, en el *Artículo 4.4* se evalúan los efectos de la adición de manoproteínas comerciales después de la fermentación maloláctica en los polisacáridos, el color, la composición fenólica y el perfil sensorial de los vinos de Tempranillo, Monastel y Maturana Tinta de Navarrete y se estudia la evolución de estos parámetros durante la crianza y el envejecimiento en botella.

Por último, y ante las limitaciones encontradas en los métodos existentes de identificación y cuantificación de los polisacáridos de mostos y vinos, el quinto artículo (*Artículo 4.5*) tiene como objeto evaluar la idoneidad del uso de la cromatografía de gases con detector de masas (GC-MS) para la identificación y cuantificación de los monosacáridos constituyentes de los polisacáridos de mostos y vinos. En este artículo se evalúa también el empleo de la cromatografía de exclusión molecular con detector de índice de refracción (HRSEC-RID) como una posible técnica rápida y sencilla para estimar el contenido global de polisacáridos en el vino y que podría utilizarse con fines comparativos.

4.1

PERFIL SENSORIAL Y CAMBIOS EN EL COLOR Y EN LA COMPOSICIÓN FENÓLICA PRODUCIDOS POR LA FERMENTACIÓN MALOLÁCTICA EN VARIEDADES MINORITARIAS TINTAS

Sensory profiling and changes in colour and phenolics composition produced by malolactic fermentation in red minority varieties

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Food Research International 2012, Vol. 46, pp. 286-293

RESUMEN

Este artículo aborda tres objetivos fundamentales:

1.- Caracterizar enológicamente los vinos obtenidos de las variedades minoritarias tintas de La Rioja Monastel, Maturana Tinta y Maturana Tinta de Navarrete en términos de color y de composición fenólica antes y después de la fermentación maloláctica.

2.- Evaluar los cambios que se producen en dichos parámetros durante la fermentación maloláctica.

3.- Analizar sensorialmente los vinos obtenidos después de la fermentación maloláctica.

Para la consecución de estos objetivos, se analizaron los antocianos monómeros, ácidos hidroxicinámicos, ácidos hidroxibenzóicos, resveratrol, flavonoles, flavan-3-oles, proantocianidinas, intensidad de color (IC), tonalidad, índice de polifenoles totales (IPT), color del vino (WC), color debido a la copigmentación (CC), color estable a la adición de bisulfito (BSC) y los parámetros CIELAB a*, b* y L*, además del perfil sensorial de los vinos. El estudio se realizó en la añada 2009 usando a la variedad Tempranillo como referencia.

Los resultados obtenidos después de la fermentación alcohólica mostraron que todos los vinos poseían una intensidad de color, IPT y contenido en resveratrol alto, indicando un buen potencial para su envejecimiento y unas buenas características antioxidantes. En esta etapa se observaron diferencias varietales. Los vinos de Monastel se caracterizaron por tener los valores más altos en intensidad de color, IPT, color del vino, antocianos, ácido gálico, resveratrol total y proantocianidinas; los vinos de Maturana Tinta presentaron los valores más altos de flavonoles y los vinos de Maturana Tinta de Navarrete tuvieron los valores más altos de catequina. Los vinos de Tempranillo presentaron los mayores valores de ácidos hidroxicinámicos y grado medio de polimerización de las proantocianidinas (mDP). El análisis de componentes principales reveló que los vinos de Tempranillo y Maturana Tinta de Navarrete eran similares en su color y composición fenólica. Es importante señalar que todos los vinos varietales presentaron también diferencias significativas en el contenido de antocianos totales, así como en el porcentaje de antocianos no acilados, acetilados y cumarilados y en el ratio de los ácidos hidroxicinámicos *trans*-cutárico/*trans*-caftárico.

La fermentación maloláctica afectó a todos los vinos por igual, de manera que las diferencias varietales después de la fermentación alcohólica se mantuvieron después de la fermentación maloláctica. La fermentación maloláctica produjo descensos de alrededor del 10% en la intensidad de color, 20% en el color del vino, 23% en el resveratrol y los flavonoles, 30% en los antocianos, 38% en las proantocianidinas y 40%

su grado medio de polimerización. Por el contrario, el parámetro b* de las coordenadas CIELAB, la tonalidad, el BSC y el ácido gálico se incrementaron significativamente en todos los vinos. Hay que destacar que, a pesar de los cambios que produjo la fermentación maloláctica en las distintas familias de polifenoles, el perfil de antocianos y el ratio *trans*-cutárico/*trans*-caftárico de los diferentes vinos varietales no se modificó, permitiendo diferenciarlos también después de la fermentación maloláctica. Esto indicó que el perfil de antocianos y ácidos hidroxicinámicos dependieron de la variedad de uva y no del proceso de vinificación. De hecho, el perfil de antocianos y el ratio *trans*-cutárico/*trans*-caftárico descrito en este artículo podrían usarse como herramienta taxonómica para diferenciar los vinos obtenidos de estas variedades de estudio.

En el análisis sensorial, todos los vinos presentaron un gran potencial para producir vinos de alta calidad. Los vinos de Monastel mostraron los valores más altos en la fase visual (color). Los vinos se diferenciaron varietalmente tanto en la fase olfativa como en la gustativa. Así, a nivel olfativo, los vinos de Tempranillo se caracterizaron por aromas herbáceos y de regaliz; los vinos de Monastel por fruta, café y tostado; los vinos de Maturana Tinta por lácteos; y, por último, los vinos de Maturana Tinta de Navarrete por pimienta. En la fase gustativa, los vinos de Tempranillo resultaron más amargos y largos en boca; los vinos de Monastel más astringentes; y los vinos de Maturana Tinta y de Maturana Tinta de Navarrete más ácidos, dulces y grasos. Los vinos de Monastel y Maturana Tinta de Navarrete fueron los mejor valorados en la percepción global, debido a su elevada intensidad y complejidad aromática. Los vinos de Monastel destacaron por su mayor persistencia, estructura y largura, y los vinos de Maturana Tinta de Navarrete por resultar frescos y agradables. Aunque los vinos de Tempranillo y de Maturana Tinta se evaluaron como los de menor complejidad aromática, se describieron como muy agradables y equilibrados en boca.

Este artículo presenta por primera vez la caracterización en términos de color y composición fenólica de los vinos de Monastel, Maturana Tinta y Maturana Tinta de Navarrete antes y después de la fermentación maloláctica y proporciona datos que pueden ser usados como criterio químico-taxonómico para identificarlos.

Food Research International 46 (2012) 286–293

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Sensory profiling and changes in colour and phenolic composition produced by malolactic fermentation in red minority varieties

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ARTICLE INFO

Article history:
Received 18 October 2011
Accepted 23 December 2011
Available online 16 January 2012

Keywords:
Red minority varieties
Polyphenols
Colour
Sensory analysis
Alcoholic fermentation
Malolactic fermentation

ABSTRACT

This research studies the sensory properties and chemical composition of varietal wines made with minority varieties from La Rioja (Spain) and it analyses the transformations in phenolic compounds and colour parameters occurring during the malolactic fermentation. In this sense, all the analysed parameters underwent changes of the same magnitude during this stage and both anthocyanin and hydroxycinnamic acid distribution was found to be dependent on the grape variety and not on the winemaking process. Wines made with these varieties showed high values of resveratrol that could lead to healthier wines, and the variety Maturana Tinta de Navarrete was found to share quite similarities in the chemical parameters with Tempranillo, studied as a reference variety. In the sensory analysis, and although all the wines obtained good punctuations, Monastel and Maturana Tinta de Navarrete were the best valued. Monastel had the highest colour intensity and both varieties showed high aromatic intensity and great complexity. In mouth, Monastel showed the greater persistence, mount length and structure and Maturana Tinta de Navarrete was described as fresh and pleasant. In conclusion, this research shows the first characterization of these wines and provides data to be used as a chemotaxonomic tool to fingerprint them. Moreover, it opens the door to the use of minority varieties providing a viable alternative to traditional grape varieties cultivated in La Rioja and favouring consumer offer and wine differentiation.

1. Introduction

Over the last few decades the introduction and spread of world recognized varieties have caused a massive loss of indigenous grapevine varieties traditionally grown in various grape-growing regions. Initiatives have been ongoing in recent years to safeguard biodiversity in the oenological sector via a process of enhancement of ancient varieties, under a pressure of a market strongly oriented towards production deriving from native vines of specific geographical zones. In that sense, La Rioja (Spain), an autonomous community with a large vitiviniculture tradition, has raised the need to preserve and characterize its minority vine varieties in order to maintain the authenticity and quality of its wines. This community has different vine-growing zones with an important number of minority grape varieties, which are perfectly adapted to these zones.

Previous studies of local and minority varieties from La Rioja were carried out in experimental plots in order to know their possibilities of production and winemaking (Martínez de Toda, Martínez, Sancha, Blanco, & Martínez, 2004a,b). The results of these works highlighted for their oenological interest red Maturana Tinta, Monastel and red Maturana Tinta de Navarrete. These minority varieties could provide differentiation of red wines from La Rioja and be a good complement to the widespread and most representative variety of the area, Tempranillo, which implies 85% of the surface of red grapes cultivated in La Rioja.

Although there are a few studies on the growing potential of these minority varieties (Martínez de Toda et al., 2004a), studies on the oenological potential are limited to measures of general oenological parameters and sensory analysis in experimental microvinifications after alcoholic fermentation (Martínez de Toda et al., 2004b). No scientific researches have been carried out to study the composition, behaviour and evolution of these wines during winemaking. Therefore, we aimed to evaluate the oenological characteristics of these minority varieties during winemaking by analysing their behaviour both after the alcoholic fermentation and malolactic fermentation, necessary to elaborate high quality wines that could be aged.

The influence of monomeric and polymeric phenolic compounds in the colour parameters and sensory quality of the wines is obvious. Grape and wine phenolics belong to two main groups: flavonoid and nonflavonoid compounds. Flavonoids, located in grape skins, seeds and stems, include anthocyanins, flavan-3-ol monomers, oligomeric and polymeric proanthocyanidins, flavonols, flavanonols and flavones. Nonflavonoids, which derive primarily from the pulp and skins of grape berries, include hydroxycinnamic and hydroxybenzoic acids and resveratrol and its derivatives. All are important constituents of both grapes and wine due to their direct and indirect

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0963-9969/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.12.030

contribution to wine sensorial properties such as colour, flavour, astringency, bitterness and structure of the wines (Garrido & Borges, 2011). The content and profile of these polyphenols have not yet been studied in these minority varieties, hence the importance of its knowing, could provide information about their oenological potential. Moreover, researches on the effect of malolactic fermentation on phenolics or colour parameters are limited and they are frequently aimed at the study of aromas, biogenic amines or microbiological researches (Bartowsky & Borneman, 2011; Hernádez-Orte et al., 2009; López et al., 2011; Mendoza, Manca de Nadra, & Farías, 2010; Miller, Franz, Cho, & du Toit, 2011; Pan, Jussier, Terrade, Yada, & Mira de Orduña, 2011; Pramateftaki, Metafa, Karapetrou, & Marmaras, 2012; Terrade, Noël, Couillaud, & Mira de Orduña, 2009).

Therefore, the aim of this work was to evaluate the oenological characteristics of the selected varieties by analysing the sensory profiling of the wines obtained as well as the changes occurring in the colour parameters and polyphenolic composition during the malolactic fermentation. Wines were elaborated in an industrial wine cellar. Tempranillo was also studied as a reference variety.

2. Experimental

2.1. Vinifications and samples

Vinifications were carried out in the wine cellar of Juan Carlos Sancha S.L. (Baños de Río Tobia, La Rioja, Spain) using the red grapes *Vitis vinífera cv*. Tempranillo (TE), Maturana Tinta (MA), Monastel (MO) and Maturana Tinta de Navarrete (MNAV). They were harvested on the vintage 2009 at commercial maturity: 24.7°Brix for TE, 24.2°Brix for MA, 25.3°Brix for MO and 23.6°Brix for MNAV.

For the winemaking, grapes were destemmed and distributed into 500 L French oak barrels, sulphited with 3 g/HL SO_2 and inoculated with 25 g/HL *S. cerevisiae* yeast strain. The prefermentation process went on for 72 h at 12 ± 1 °C, the fermentation–maceration process was carried out at a maximum temperature of 28 ± 2 °C and lasted 10 days. Wines were then run off and introduced in eight 500 L French oak barrels, two for each variety. Barrels were maintained at controlled wine cellar temperature and after malolactic fermentation, wines were racked. Malolactic fermentation lasted around 2 months in all wines. Samples were taken at the end of alcoholic fermentation (OH) and at the end of malolactic fermentation (ML). All vinifications were carried out in duplicate and average values of the two barrels are presented.

2.2. Determination of usual oenological parameters

All wines were analysed for pH, ethanol concentration, titratable and volatile acidity according to the OIV official practices (1990). Malic acid was analysed by the autoanalyzer LISA 200 (Biocode Hyad, Le Rhem, France).

2.3. Analysis of colour parameters and total polyphenol index

Wine red colour (WC), monomeric anthocyanin colour (MAC), copigmentation colour (CC), and bisulphite-stable colour (BSC) were determined by the method proposed by Levengood and Boulton (2004) in a Cary 300 Scan UV–vis spectrophotometer (Varian Inc., Madrid, Spain). Wine stable colour (SC) was calculated as the sum of CC and BSC. Colour intensity (CI) was calculated as the sum of absorbances at 420, 520, and 620 nm, and Hue as A_{420}/A_{520}, at wine pH. The CIELAB rectangular parameters (L*, a* and b*, illuminant D65 and 10° observer conditions) were determined according to Ayala, Echávarri, and Negueruela (1997). Total polyphenol index (TPI) was determined by absorbance at 280 nm of diluted wine with synthetic wine. All measurements were performed in triplicate and referred to 10 mm path length quartz cells.

2.4. Analysis of monomeric phenolics

Anthocyanins, hydroxycinnamic and hydroxybenzoic acids, flavonols, flavan-3-ols and resveratrol derivatives were analysed by high performance liquid chromatography in 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. Separation was achieved in an ACE HPLC (5 C18-HL) particle size 5 μm (250 mm × 4.6 mm) column according to the methodology described in Gómez-Alonso, García-Romero, and Hermosín-Gutiérrez (2007). Quantification of non-commercial compounds was made using the calibration curves belonging to the most similar compound: malvidin-3-glucoside for the anthocyanins; quercetin-3-glucoside for myricetin-3-glucoside and quercetin-3-glucuronide; caffeic acid for *cis*- and *trans*-caftaric acids (*cis*- and *trans*-caffeoyl-tartaric acid); *p*-coumaric acid for *cis*- and *trans*-coutaric acids (*cis*- and *trans*-*p*-coumaryl-tartaric acid); ferulic acid for *cis*- and *trans*-fertaric acids (*cis*- and *trans*- ferulic–tartaric acid); and *trans*-resveratrol for its glucoside. The content of non-acylated anthocyanins (A) was calculated as the sum of delphinidin, cyanidin, petunidin, peonidin and malvidin-3-glucosides; the content of acetyl-glucoside anthocyanins (A-Ac) as the sum of delphinidin, cyanidin, petunidin and malvidin-3-(6-acetyl)-glucosides; the content of coumaryl-glucoside anthocyanins (A-Cm) included 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) were calculated as the sum free acids, i. e., caffeic, ferulic and coumaric acid, and hydroxycinnamates, i. e., *trans*-caftaric, *cis*-caftaric, *trans*-coutaric, *cis*-coutaric and *trans*-fertaric. Total flavonol content (T-Flavo) was calculated as the sum of myricetin-3-glucoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-glucuronide, myricetin, quercetin, kaempferol and isorhamnetin. Total Resveratrol (T-resveratrol) was calculated as the sum of *cis*-resveratrol, *trans*-resveratrol and *trans*-resveratrol glucoside. All analyses were performed in duplicate.

2.5. Analysis of proanthocyanidins

Wine samples were directly fractionated by gel permeation chromatography (GPC) on a Toyopearl gel HP-50 F column as described by Fernández, Martínez, Hernández, Guadalupe, and Ayestarán (2011) and Guadalupe, Soldevilla, Sáenz-Navajas, and Ayestarán (2006). Fractionation was performed in triplicate and phloroglucinol adducts were analysed in F2 fractions by reversed-phase HPLC as described by Kennedy and Jones (2001). Proanthocyanidins cleavage products were estimated using their response factors relative to (+)-catechin which was used as the quantitative standard. Total proanthocyanidin content (PA) was calculated as the sum of all the subunits: extension subunits (phloroglucinol adducts) and terminal subunits (catechin, epicatechin and epicatechin-gallate). To calculate the apparent mean degree of polymerization (mDP), the sum of all subunits was divided by the sum of the terminal subunits. All analyses were performed in duplicate.

2.6. Sensory analysis

Wines after malolactic fermentation were analysed for sensory profiling and they were judged for visual (colour), olfactory (volatile fraction), and gustatory (taste and mouth-feel sensations) quality conformance to wine typology.

A panel of twelve tasters, wine professionals from the D. O. Ca. Rioja, was convened. All wine tasters had participated on previous aroma and mouth-feel sensory descriptive panels and had regularly participated in quality scoring varietal wine sensory panels. Tasters

rated 9 attributes for the olfactory phase and 6 for the gustative, scoring the intensity of each attribute on an interval scale with 5 levels of intensity (0 = no aroma or no taste; 1 = weak aroma or weak taste; 5 = strong aroma or strong taste; intermediate values did not bear description). The colour was also judged and blue-red colour was rated according to its intensity on an anchored scale with five levels of intensity (0 = no blue-red colour; 5 = extremely strong blue-red colour). After tasting, panellist were also asked to score the wines according to their global perception on a structured scale from 1 to 5, with 5 being the highest and 1 the lowest; and they were allowed to make any additional comment about the sensory properties of the wines. Wines were presented at 18 °C in coded standard wine-tasting glasses according to standard 3591 (ISO 3591, 1997). Assessment took place in a standard sensory analysis chamber (ISO 8589, 1998) equipped with separate booths. One wine was replicated in order to ascertain judges' consistency.

2.7. Statistical procedures

Significant differences between analytical determinations were analysed by an analysis of variance (ANOVA) if the data adhered to assumptions of normality. If these assumptions were not adhered to, a Kruskal—Wallis test was used. Separate principal component analysis (PCA) was carried out on full data for colour parameters and phenolic compounds, and it was conducted using the correlation matrix with no rotation. Sensory data were subjected to ANOVA analysis to determine the within judges reproducibility in rating two replicated wines. Generalized Procrusters Analysis (GPA) was applied on the mean ratings for olfactory and gustatory attributes, and a permutation test was also made to explain that the results obtained were significant (85.12%). ANOVA evaluations were performed using the Statistica 8.0 programme for Microsoft Windows (Statsoft Inc., Tulsa, Oklahoma) and PCA and GPA analyses by using the Senstools Version 3.3.2. Program (Utrecht, the Netherlands).

3. Results and discussion

3.1. Oenological parameters

Alcohol content, pH, titratable acidity, volatile acidity and malic acid content in wines after alcoholic and malolactic fermentation are shown in Table 1. The values obtained after alcoholic fermentation for volatile acidity confirmed a suitable winemaking with absence of microbial alterations. In general, all the wines reached high levels in the ethanol content after the alcoholic fermentation and Monastel showed the highest value. The highest pH corresponded to Tempranillo, which was attributed to a varietal factor due to the high potassium content usually observed in this variety (Aleixandre, Lizama, Álvarez, & García, 2002). The values of titratable acidity were in agreement with the normal content found in Spanish wines after alcoholic fermentation (Escudero-Gilete, González-Miret, & Heredia, 2010). The content of malic acid differed significantly among varieties, with Tempranillo showing the highest value.

As expected, malolactic fermentation produced an increase of wine pH, a decrease of titratable acidity and an increase in volatile acidity. No important differences were found among varieties after the malolactic fermentation except for the highest alcohol content in Monastel and the lowest titratable acidity in the MNAV wine.

Table 1
Oenological parameters in wines after alcoholic and malolactic fermentation.

Wine	Alcohol content[1]	pH	TA[2]	VA[3]	H_2M[4]
TEOH	14.58 ± 0.2^{ab}	3.64 ± 0.02^{d}	6.01 ± 0.1^{c}	0.28 ± 0.05^{a}	2.64 ± 0.02^{e}
MOOH	15.02 ± 0.3^{a}	3.46 ± 0.01^{ab}	6.75 ± 0.05^{d}	0.29 ± 0.03^{a}	1.64 ± 0.03^{c}
MAOH	14.28 ± 0.2^{bc}	3.41 ± 0.03^{a}	6.96 ± 0.2^{d}	0.38 ± 0.07^{a}	2.22 ± 0.05^{d}
MNAVOH	13.95 ± 0.2^{c}	3.51 ± 0.02^{bc}	5.63 ± 0.05^{b}	0.30 ± 0.03^{a}	1.25 ± 0.02^{b}
TEML	14.55 ± 0.2^{abc}	3.81 ± 0.02^{f}	5.40 ± 0.03^{b}	0.38 ± 0.05^{a}	0 ± 0.01^{a}
MOML	15.00 ± 0.2^{a}	3.65 ± 0.02^{de}	5.52 ± 0.1^{b}	0.40 ± 0.07^{a}	0 ± 0.00^{a}
MAML	14.30 ± 0.2^{bc}	3.54 ± 0.03^{c}	5.54 ± 0.03^{b}	0.39 ± 0.03^{a}	0 ± 0.00^{a}
MNAVML	14.00 ± 0.2^{bc}	3.71 ± 0.01^{e}	4.99 ± 0.1^{a}	0.32 ± 0.05^{a}	0 ± 0.01^{a}

[1]Alcohol content: mL ethanol for 100 mL of wine at 20 °C; [2]TA: titratable acidity as g of tartaric acid per litre; [3]VA: volatile acidity as g of acetic acid per litre; [4]H_2M: malic acid as g of malic acid per litre. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p<0.05$.

3.2. Colour parameters and total polyphenol index

Colour characteristics and total polyphenol index (TPI) in wines after alcoholic and malolactic fermentation are shown in Table 2. After alcoholic fermentation, all wines showed high values of colour intensity (CI) and TPI, indicating a good potential for ageing, but it was Monastel the wine that showed the highest values in both parameters. No noteworthy differences were observed in the CIELAB parameters a^* (redness) and L^* (lightness) among wines although Maturana Tinta wine showed the lowest value in the yellow-blue component b^*, indicating bluish tonalities. Regarding the colour components, and in good agreement with the CI values, Monastel showed the highest wine colour (WC) and it was due to the monomeric anthocyanin colour (MAC) and bisulfite-stable colour (BSC). On the other hand, Maturana Tinta de Navarrete showed the lowest value of WC and MAC and Tempranillo showed the lowest copigmentation colour (CC). In all the wines the contribution of copigmentation colour to WC was around 38% while bisulfite stable colour, attributed in bibliography to polymeric pigments, accounted between 13 and 19% of WC, and monomeric anthocyanin colour represented between 44 and 47%. All these data were found to be in agreement with others studies (Boulton, 2001; Guadalupe & Ayestarán, 2008) except for anthocyanin colour which was found to be slightly lower than data described in literature for young wines (Boulton, 2001; Han et al., 2008).

Malolactic fermentation caused significant decreases in colour intensity in all the wines, ranging between 5–8% in Tempranillo and Monastel to 10–14% in Maturana Tinta and Maturana Tinta de Navarrete, due to changes in absorbance at 520 nm (data not shown). The CIELAB parameters a^* and L^* were maintained while the b^* value decreased by more than 50%, indicating a shift to bluish tonalities. Hue showed a slight increase in all the wines, as well as the TPI, probably due to the contribution of the wood of the oak barrel. With regard to the colour components, malolactic fermentation resulted in a significant decrease in WC (~ 20% in all the wines) due to a decrease of 58 to 65% in the copigmentation colour. On the contrary, MAC was maintained and BSC increased from 17% in Tempranillo to 40% in Maturana Tinta. All these changes were attributed to two different phenomena: firstly, to the dissociation of the copigmentation complexes, probably due to the ionic shift occurring during the malolactic fermentation, and secondly, to the formation of new and stable pigments resistant not only to the bisulfite addition but also to pH changes and oxidation (Asenstorfer, Hayasaka, & Jones, 2001). In this point it is important to highlight that malolactic fermentation produced similar changes in the colour parameters and total polyphenol index in all the wines and they were independent on the variety used. Therefore, the differences observed among varieties after alcoholic fermentation were maintained after malolactic fermentation.

3.3. Monomeric anthocyanins

All wines showed significant differences in the content of anthocyanins after the alcoholic fermentation (Fig. 1). Monastel reached the highest value of total-anthocyanins (T-A), in good agreement with its highest values in colour intensity and red wine colour. Non-acylated anthocyanins (A) were the major anthocyanins in all the wines, varying from 67% in Maturana Tinta de Navarrete to 88% in Tempranillo. Acetylated anthocyanins (A-Ac) were found to be higher

Table 2
Colour parameters and total polyphenol index (absorbance units) in wines after alcoholic and malolactic fermentation.

Wine	CI[1]	Hue[2]	a^*[3]	b^*[3]	L^*[3]	WC[4]	MAC[5]	BSC[6]	CC[7]	TPI[8]
TEOH	19.41 ± 1.1^{b}	0.55 ± 0.01^{d}	45.93 ± 1.8^{ab}	4.02 ± 0.09^{d}	55.30 ± 3.3^{ab}	11.02 ± 0.04^{e}	5.23 ± 0.08^{bcd}	1.99 ± 0.01^{c}	3.79 ± 0.04^{b}	75.3 ± 2.2^{bc}
MOOH	21.48 ± 0.82^{c}	0.52 ± 0.01^{c}	48.04 ± 0.2^{bc}	5.83 ± 0.76^{e}	53.94 ± 2.06^{a}	11.96 ± 0.11^{g}	5.37 ± 0.08^{cd}	2.24 ± 0.01^{d}	4.36 ± 0.11^{d}	80.0 ± 2^{de}
MAOH	19.83 ± 1.30^{b}	0.47 ± 0.01^{a}	49.62 ± 3.58^{c}	-1.51 ± 0.32^{b}	54.72 ± 3.56^{ab}	11.53 ± 0.05^{f}	5.11 ± 0.10^{b}	1.49 ± 0.01^{a}	4.94 ± 0.06^{e}	71.8 ± 4.2^{ab}
MNAVOH	19.81 ± 0.75^{b}	0.5 ± 0.01^{b}	44.75 ± 1.79^{ab}	3.27 ± 1.5^{d}	57.55 ± 2.3^{ab}	10.46 ± 0.09^{d}	4.62 ± 0.06^{a}	1.81 ± 0.01^{b}	4.03 ± 0.09^{c}	71.0 ± 1^{a}
TEML	18.34 ± 0.84^{ab}	0.61 ± 0.01^{f}	45.19 ± 1.4^{ab}	1.73 ± 0.05^{c}	55.78 ± 1.54^{ab}	9.09 ± 0.43^{b}	5.19 ± 0.24^{bc}	2.32 ± 0.11^{d}	1.58 ± 0.08^{a}	78.0 ± 2.6^{cd}
MOML	19.64 ± 0.24^{b}	0.59 ± 0.00^{e}	47.83 ± 0.4^{bc}	3.33 ± 0.14^{d}	54.08 ± 0.19^{a}	10.04 ± 0.2^{c}	5.47 ± 0.07^{d}	2.88 ± 0.01^{e}	1.70 ± 0.08^{a}	84.0 ± 1^{e}
MAML	17.67 ± 0.64^{a}	0.52 ± 0.02^{c}	47.84 ± 1.8^{bc}	-3.46 ± 0.18^{a}	55.94 ± 1.88^{ab}	8.95 ± 0.41^{ab}	5.21 ± 0.25^{bc}	2.09 ± 0.01^{c}	1.65 ± 0.05^{a}	75.5 ± 1.8^{bc}
MNAVML	17.01 ± 0.2^{a}	0.6 ± 0.01^{f}	43.77 ± 1.05^{a}	0.66 ± 0.03^{c}	58.19 ± 0.42^{b}	8.56 ± 0.04^{a}	4.63 ± 0.06^{a}	2.32 ± 0.09^{d}	1.62 ± 0.05^{a}	75.2 ± 1.9^{abc}

[1]CI: colour intensity as sum of absorbances at 420, 520 and 620 nm; [2]Hue: A_{420}/A_{520}; [3]a^*: from green to red; b^*: from blue to yellow; L^*: lightness; [4]WC: red wine colour; [5]MAC: monomeric anthocyanin colour; [6]BSC: bisulfite stable colour; [7]CC: copigmentation colour; [8]TPI: total polyphenol index. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p<0.05$.

than coumarylated anthocyanins (A-Cm) in Monastel (19% vs. 8%), Maturana Tinta (19 vs. 5%) and Maturana Tinta de Navarrete (28% vs. 5%) while Tempranillo showed more coumaryl than acetylated derivatives (7% vs. 5%). These differences were attributed to varietal differences and, although there is no bibliography in this respect for the minority varieties, studies on Tempranillo variety (Gómez-Alonso et al., 2007; Monagas, Gómez-Cordovés, Bartolomé, Laureano, & Ricardo da Silva, 2003) show that the content of coumaryl derivatives is always higher than the concentration of acetylated anthocyanins while other varieties such as Cabernet Sauvignon or Graciano show the opposite (Monagas et al., 2003). The profile of major non-acylated anthocyanins was also studied in detail (Fig. 2) and, as expected, malvidin-3-glucoside was the major anthocyanin in all the varietal wines, representing from 60% to 76%, and its derivatives were also the main anthocyanins in the acetylated and coumarylated forms (data not shown). Some authors consider that anthocyanin profile of varietal wines can be used as an analytical tool to certify their authenticity (Castillo-Muñoz et al., 2009; Ferrandino & Guidoni, 2010; Pérez-Trujillo, Hernández, López-Bellido, & Hermosín-Gutiérrez, 2011). In this sense, it must be noticed that the percentages of each non-acylated anthocyanin varied between wines. Therefore, Tempranillo and Monastel showed quite similar proportions in the major anthocyanins while Maturana Tinta and Maturana Tinta de Navarrete showed more similarities between them (Fig. 2). Moreover, this anthocyanin profile together with the anthocyanin profile of the rest coumaryl and acetylated forms (data not shown) was exactly maintained in the wines after the malolactic fermentation, indicating that the anthocyanin distribution in the wines was dependent on the variety and not on the winemaking process. It should be pointed out that the acylated forms of the non-malvidin pigments seem to be involved in strong copigmentation (Boulton, 2001), which would explain that wines with predominantly malvidin in the anthocyanin profile, i. e., Maturana Tinta and Maturana Tinta de Navarrete, showed the lowest value of red wine colour (Table 2).

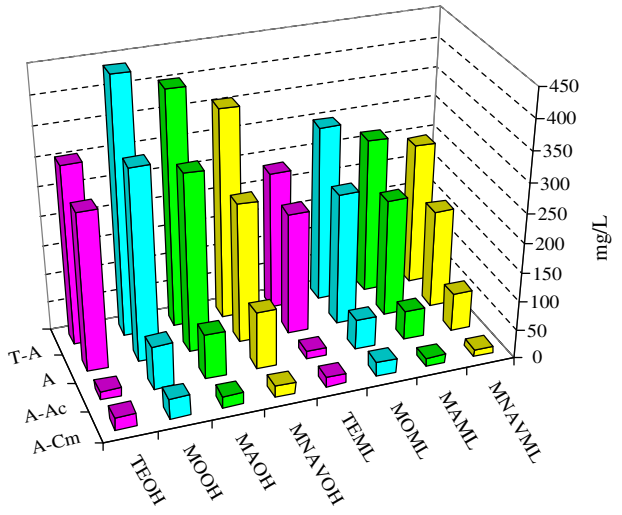

Fig. 1. Concentration (mg/L) of total anthocyanins (T-A), non-acetylated anthocyanins (A), acetyl-glucoside anthocyanins (A-Ac) and coumaryl-glucoside anthocyanins (A-Cm) in wines after alcoholic and malolactic fermentation.

Malolactic fermentation produced a decrease of around 30% in the content of total monomeric anthocyanins (T-A) in all the wines, which coincided with the significant loss observed in CI and WC. This reduction, which affected similarly to A, A-Ac and A-Cm, was attributed to their conversion into polymeric anthocyanins, also in agreement with the BSC increase (Table 2), and to the degradation, oxidation, complexation and precipitation reactions of the monomeric anthocyanins during the malolactic fermentation. It is important to remark again that the changes occurring in the anthocyanin compounds during this winemaking stage were the same, both in quantity and profile, in all the studied varieties. All final wines showed anthocyanin concentrations in the range usually described in bibliography for red wines (Ginjom, D'Arcy, Caffin, & Gidley, 2011; Guadalupe & Ayestarán, 2008).

3.4. Hydroxycinnamic acid derivatives, gallic acid and total resveratrol

All wines after alcoholic fermentation showed similar values of total hydroxycinnamic acids (T-HA) except for Monastel, which showed significantly lower quantity (Table 3). The only cinnamic acids present in all wines were the sterified forms while the free forms were below the quantification limits. In all the analysed wines, the *trans*-form of the acids presented higher concentrations than its *cis* isomer and, as reported in other red wine varieties (Ginjom et al., 2011), the *trans*-caftaric acid was by far the major acid (>50%) followed by the *trans*-coutaric acid (20–30%). Taking into account the ratio *trans*-coutaric/*trans*-caftaric, considered by some authors as a varietal factor and proposed as a possible chemotaxonomic tool (Ferrandino & Guidoni, 2010), a clear difference was observed between Tempranillo, with a ratio of 0.65, and the rest of the wines, with a ratio between 0.34 and 0.39. Curiously, the rest *cis*-caftaric, *cis*-coutaric and *trans*-fertaric acids showed the same proportions in all the wines, i.e., 8–9%, 5–6% and 2–3%, respectively. Regarding the effect of the malolactic fermentation on the content and profile of the hydroxycinnamic acids, different aspects should be highlighted. Firstly, the malolactic fermentation did not produce important effects on the content of total hydroxycinnamic acids. Secondly, and as in the case of anthocyanins, malolactic fermentation did not affect the distribution of hydroxycinnamic acids and without exception the wines showed the same profile as observed after the alcoholic fermentation. This fact confirmed the varietal origin of the acids and indicated that the *trans*-coutaric acid/*trans*-caftaric acid ratio may characterize the wines according to their grape origin.

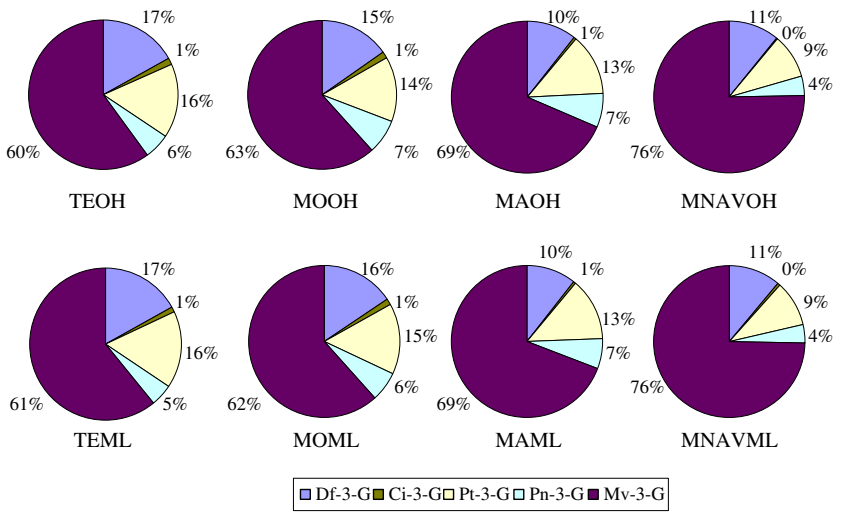

Fig. 2. Relative proportions of non-acylated anthocyanins (A) in wines after alcoholic and malolactic fermentation. Df-3-G: delphinidin-3-glucoside; Ci-3-G: cyaniding-3-glucoside; Pt-3-G: petunidin-3-glucoside; Pn-3-G: peonidin-3-glucoside; Mv-3-G: malvidin-3-glucoside.

Finally, and opposed to other authors that observe that tartaric esters are hydrolysed to their corresponding free forms during malolactic fermentation (Cabrita et al., 2008; Hernández et al., 2007), in the present study the concentration of free acids after malolactic fermentation was below the limit of quantification while the tartaric esters were in the range described for other red varieties (Ginjom et al., 2011).

With regards to benzoic acids, gallic acid, considered one of the most potent antioxidants in wines (Ginjom et al., 2011), was the most abundant both before and after alcoholic fermentation and it was Monastel the wine that showed the highest content (Table 3). Malolactic fermentation prompted an increase in gallic acid in all the wines and it was attributed to the fact that malolactic fermentation was carried out in oak barrels and thus it was released from their tannin galloylated precursors (Ginjom et al., 2011). Therefore, and taking into account its antioxidant activity, conducting the malolactic fermentation in oak barrels would increase the antioxidant capacity of the wines.

Finally, and relative to the content of resveratrol (Table 3), Monastel, Maturana Tinta and Maturana Tinta de Navarrete showed values of total resveratrol above the mean of Spanish wines (Abril, Negueruela, Pérez, Juan, & Estopañán, 2005). In this sense they could be considered as *healthier wines* as the positive biological effect of this compound in human health is widely known. Although malolactic fermentation produced a decrease in the content of total resveratrol in Tempranillo and Monastel, the latter still showed higher values than those described in bibliography for Spanish wines (Abril et al., 2005).

3.5. Flavonols

Table 4 shows the flavonol content in wines after alcoholic and malolactic fermentation. Although all wines showed values in the range of other wines (Hermosín Gutiérrez, Sánchez-Palomo Lorenzo, & Vicario Espinosa, 2005), Maturana Tinta showed the highest content in total flavonols (T-Flavo) at the end of both fermentations followed by far by Tempranillo and finally Monastel and Maturana Tinta de Navarrete. Regarding the flavonol profile, myricetin-3-glucoside was the main flavonol found in Tempranillo both before and after the malolactic fermentation, being in good agreement with other studies (Gómez-Alonso et al., 2007; Hermosín Gutiérrez et al., 2005). Myricetin-3-glucoside was also the main flavonol in Maturana Tinta and Maturana Tinta de Navarrete after the alcoholic fermentation whereas the main flavonols in Monastel corresponded to the aglycones of quercetin and myricetin. Flavonols are present in the grape exclusively in the form of glycosides and the fact the free flavonols were detected in wines could be due to the hydrolysis of their glycosides during the alcoholic fermentation. With the exception of quercetin-3-galactoside and some flavonols present in very low concentrations, all these compounds, considered as the best kind of copigmentation cofactors (Boulton, 2001; Hermosín Gutiérrez et al., 2005), decreased significantly during malolactic fermentation in agreement with a decrease in the copigmentation colour (Table 2). On the other hand, and contrary to what was observed with anthocyanins or hydroxycinnamic acids, the distribution of individual flavonols changed in all the wines during the malolactic fermentation and it was not possible to establish a concrete

Table 3
Concentration of hydroxycinnamic acid derivatives, gallic acid and total resveratrol (mg/L) in wines after alcoholic and malolactic fermentation.

Wine	*cis*-caftaric	*trans*-caftaric	*cis*-coutaric	*trans*-coutaric	*trans*-fertaric	T-HA[1]	t-cout/t-caft[2]	Gallic acid	T-resveratrol[3]
TEOH	5.42 ± 0.05^{bc}	34.3 ± 0.2^{a}	3.82 ± 0.01^{f}	22.23 ± 0.9^{e}	1.86 ± 0.01^{c}	67.6 ± 0.5^{d}	0.65	45.6 ± 0.5^{b}	4.57 ± 0.05^{b}
MOOH	4.72 ± 0.03^{a}	33.93 ± 0.2^{a}	2.57 ± 0.02^{b}	12.72 ± 0.6^{a}	1.82 ± 0.02^{c}	55.76 ± 0.9^{a}	0.37	56.4 ± 0.54^{d}	10.2 ± 0.05^{g}
MAOH	5.60 ± 0.06^{c}	38.97 ± 0.15^{c}	2.96 ± 0.05^{c}	15.95 ± 0.4^{c}	1.14 ± 0.00^{a}	64.62 ± 0.25^{c}	0.39	39.5 ± 0.36^{a}	8.87 ± 0.05^{f}
MNAVOH	5.64 ± 0.14^{c}	40.36 ± 0.32^{c}	3.10 ± 0.09^{d}	13.93 ± 0.45^{b}	1.57 ± 0.01^{b}	64.6 ± 0.07^{c}	0.34	46.2 ± 0.45^{b}	6.03 ± 0.02^{d}
TEML	5.71 ± 0.24^{d}	34.54 ± 1.23^{a}	3.53 ± 0.03^{e}	21.06 ± 0.5^{d}	3.61 ± 0.13^{g}	68.46 ± 1.69^{de}	0.61	50.20 ± 0.96^{c}	3.16 ± 0.13^{a}
MOML	4.84 ± 0.21^{a}	36.39 ± 0.79^{b}	2.86 ± 0.14^{c}	12.79 ± 0.71^{a}	3.40 ± 0.10^{f}	60.28 ± 1.10^{b}	0.35	59.13 ± 2.08^{e}	4.43 ± 0.14^{b}
MAML	5.28 ± 0.21^{b}	42.76 ± 1.75^{d}	3.76 ± 0.05^{f}	15.48 ± 0.43^{c}	2.84 ± 0.08^{d}	70.11 ± 1.95^{e}	0.36	46.04 ± 0.47^{b}	8.39 ± 0.38^{e}
MNAVML	5.69 ± 0.13^{c}	42.96 ± 0.11^{d}	2.03 ± 0.03^{a}	13.87 ± 0.33^{b}	3.12 ± 0.08^{e}	67.64 ± 0.41^{d}	0.33	47.07 ± 1.50^{b}	5.55 ± 0.08^{c}

[1]T-HA: total hydroxycinnamic acids; [2]*t*-cout/*t*-caft: ratio *trans*-coutaric/*trans*-caftaric; [3]T-resveratrol: total resveratrol. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p<0.05$.

Table 4
Concentration of flavonols (mg/L) in wines after alcoholic and malolactic fermentation.

Wine	Myricetin-3-G[1]	Quercetin-3-Gal[2]	Quercetin-3-G[3]	Quercetin-3-Glc[4]	Myricetin	Quercetin	Kaempferol	Isorhamnetin	T-Flavo[5]
TEOH	21.5 ± 0.2^{h}	2.42 ± 0.03^{b}	3 ± 0.03^{e}	6.86 ± 0.07^{b}	6.91 ± 0.07^{f}	1.83 ± 0.02^{a}	0.05 ± 0.01^{a}	0.15 ± 0.01^{a}	42.72 ± 0.4^{e}
MOOH	6.20 ± 0.06^{c}	2.55 ± 0.03^{b}	1.06 ± 0.01^{a}	7.83 ± 0.08^{d}	10.1 ± 0.1^{g}	10.3 ± 0.1^{g}	0.12 ± 0.005^{b}	0.79 ± 0.01^{f}	38.95 ± 0.4^{d}
MAOH	18.9 ± 0.2^{g}	3.61 ± 0.04^{c}	10.73 ± 0.1^{g}	15.06 ± 0.2^{f}	5.33 ± 0.05^{d}	13.06 ± 0.1^{h}	0.57 ± 0.01^{g}	2.05 ± 0.01^{h}	69.31 ± 0.7^{g}
MNAVOH	10.07 ± 0.1^{d}	1.6 ± 0.02^{a}	1.46 ± 0.01^{b}	8.01 ± 0.08^{d}	6.62 ± 0.07^{e}	5.02 ± 0.05^{d}	0.18 ± 0.005^{c}	0.63 ± 0.01^{e}	33.59 ± 0.3^{c}
TEML	12.25 ± 0.08^{e}	4.50 ± 0.09^{d}	2.69 ± 0.09^{d}	6.29 ± 0.20^{a}	3.42 ± 0.06^{a}	2.28 ± 0.07^{b}	0.21 ± 0.01^{d}	0.19 ± 0.00^{b}	31.82 ± 0.2^{b}
MOML	2.01 ± 0.07^{a}	4.95 ± 0.25^{e}	1.66 ± 0.01^{b}	6.55 ± 0.29^{ab}	4.84 ± 0.07^{c}	6.08 ± 0.24^{e}	0.27 ± 0.01^{e}	0.50 ± 0.01^{c}	26.84 ± 0.7^{a}
MAML	14.09 ± 0.65^{f}	5.40 ± 0.25^{f}	8.99 ± 0.41^{f}	13.65 ± 0.2^{e}	3.33 ± 0.14^{a}	7.1 ± 0.13^{f}	0.35 ± 0.02^{f}	1.27 ± 0.04^{g}	54.16 ± 1.3^{f}
MNAVML	5.31 ± 0.13^{b}	3.4 ± 0.12^{c}	2.13 ± 0.08^{c}	7.43 ± 0.24^{c}	4.00 ± 0.15^{b}	4.54 ± 0.2^{c}	0.19 ± 0.01^{c}	0.58 ± 0.02^{d}	27.57 ± 0.07^{a}

[1]Myricetin-3-G: myricetin-3-glucoside; [2]Quercetin-3-Gal: quercetin-3-galactoside; [3]Quercetin-3-G: quercetin-3-glucoside; [4]Quercetin-3-Glc: quercetin-3-glucuronide; [5]T-Flavo: total flavonols. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p<0.05$.

pattern of changes. Decreases in flavonol-glycosides could not be explained by the hydrolysis of glycoside linkages because increases in their correspondent free aglycones were not observed and thus other kind of reactions such as condensation, oxidation and copigmentation with anthocyanins (Hermosín Gutiérrez et al., 2005) may have occurred. Querecetin-3-glucuronide became the major flavonol in Monastel and Maturana Tinta de Navarrete after the malolactic fermentation and, although myricetin-3-glucoside was again the major flavonol in Tempranillo and Maturana Tinta, the proportion of the rest flavonols was not maintained. To sum up, the results of the present study indicated that the flavonol profile was not a good indicator to differentiate grape varieties.

3.6. Catechin and proanthocyanidins

The concentration of (+)-catechin and total proanthocyanidins (PA) as well as the proanthocyanidin mean Degree of Polymerization (mDP) in wines after alcoholic and malolactic fermentation is shown in Table 5. The only flavan-3-ol detected in the wines within the quantification limits was (+)-catechin while epicatechin, epicatechin-gallate and catechin gallate were below the limit of quantification (0.90 mg/L). After alcoholic fermentation, Maturana Tinta de Navarrete reached by far the highest value in catechin, which is involved in the formation of stable colour through copigmentation and formation of polymeric pigments (González-Manzano, Dueñas, Rivas-Gonzalo, Escribano-Bailón, & Santos-Buelga, 2009) and in condensation reactions with other flavanols affecting the astringency and bitterness of the final wines (Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009; Fortes Gris et al., 2011). Although the content of PA was significantly higher in Monastel than in the rest of wines, the mDP, which can influence the flavan-3-ol bioavailability and bioactivity and it is related with the astringent and bitter properties of proanthocyanidins (Vidal et al., 2004), was quite high in all the wines.

Malolactic fermentation prompted a significant decrease in the concentration of catechin, PA and mDP in all the wines and, as it was observed with other polyphenols, both PA and mDP experienced changes in the same magnitude in all the wines. Hence, PA content decreased from 37 to 42% in all wines, while mDP decreased from 35 to 45%. Maturana Tinta de Navarrete continued showing the highest value in catechin and Monastel in proanthocyanidins. Looses in catechin were attributed to their conversion into more stable polymers by reacting with other flavanols, anthocyanins, and small molecules such as pyruvic acid and vinylphenol; decreases in PA were due to tannin–anthocyanin combination and precipitation of unstable colloids tannin–tannin, tannin–polysaccharides and tannin–proteins.

PA values after malolactic fermentation were in the range described in bibliography for other red wines (Cosme, Ricardo-Da-Silva, & Laureano, 2009; Fortes Gris et al., 2011). The results of mDP, also in agreement with other authors (Cosme et al., 2009; Fortes Gris et al., 2011; Monagas et al., 2003), indicated that the proanthocyanidins present in wines after malolactic fermentation were mainly oligomers or short-chain polymers (Fortes Gris et al., 2011). This fact, which could be attributed to a higher precipitation of larger PA and/or to the hydrolysis of higher PA forming lower ones may be related with a change in the astringent sensation (Chira et al., 2009; Vidal et al., 2004).

Table 5
Concentration of catechin and proanthocyanidins (mg/L) and mean degree of polymerization in wines after alcoholic and malolactic fermentation.

Wine	(+)-catechin	PA[1]	mDP[2]
TEOH	78.5 ± 0.75^{d}	828 ± 46^{e}	13.57 ± 0.01^{g}
MOOH	75.9 ± 0.72^{d}	1013 ± 19^{g}	10.53 ± 0.00^{d}
MAOH	66.4 ± 0.63^{c}	903 ± 27^{f}	13.12 ± 0.00^{f}
MNAVOH	90.8 ± 0.68^{f}	715 ± 36^{d}	11.57 ± 0.00^{e}
TEML	55.99 ± 2.91^{b}	483 ± 24^{a}	7.34 ± 0.32^{c}
MOML	69.95 ± 2.7^{c}	584 ± 16^{c}	6.81 ± 0.33^{b}
MAML	44.15 ± 2^{a}	535 ± 16^{b}	7.19 ± 0.36^{bc}
MNAVML	86.15 ± 4.66^{e}	448 ± 8^{a}	6.36 ± 0.22^{a}

[1]PA: total proanthocyanidins; [2]mDP: mean degree of polymerization. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p<0.05$.

3.7. Differentiation of wines in the PCA space

Principal component analysis (PCA) was applied to the chemical data to clarify the interpretation of the data and highlight those variables that best explain the differences between wines. In Fig. 3, a PCA of the varietal wines on colour parameters and phenolic compounds explained the 91% of the accumulative variance. PC1 (69% of variance) was mainly associated with copigmentation colour (CC), stable colour (SC), wine colour (WC), total proanthocyanidin content (PA) and total anthocyanins (T-A) whereas colour intensity (CI), monomeric anthocyanin colour (MAC), bisulphite-stable colour (BSC), total hydroxycinnamic acids (T-HA) and total flavonols (T-Flavo) were associated with both PC1 and PC2 (22% of the variance). It was observed a highly positive correlation between CC, T-A, PA, SC and WC and a

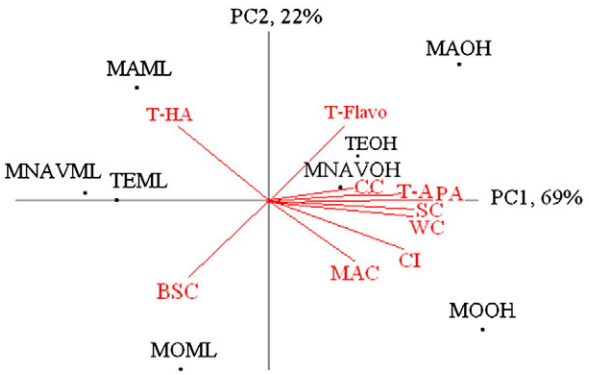

Fig. 3. PCA on the mean ratings for colour parameters and phenolic compounds in wines after alcoholic and malolactic fermentation.

highly negative correlation between CI and T-HA ($r^2 = -0.70$), and between BSC and T-Flavo ($r^2 = -0.74$). The distribution of the wines in the PCA space showed quite interesting aspects to be highlighted. On the one hand, wines were clearly differentiated according to their winemaking stage. Thus, wines after the alcoholic fermentation were located in the right of the PCA space and they were characterised by higher values of CC, T-A, PA, SC, WC, CI and MAC than wines after the malolactic fermentation, which were located in the left of the PCA space. This shift indicated that malolactic fermentation produced a decrease of the values of these parameters and an increase in T-HA and BSC. On the other hand, and taking into account the position of each wine, a similar distribution of the individual wines was observed both before and after the malolactic fermentation. Therefore, Tempranillo and Maturana Tinta de Navarrete were quite close in the PCA space both before and after the malolactic fermentation, indicating that they shared quite similarities in the analytical parameters. However, Monastel and Maturana Tinta were located separately from the rest and just in opposite positions both before and after the alcoholic fermentation, showing that Maturana Tinta was clearly characterised by higher values of T-HA and T-Flavo, whereas Monastel showed higher correlation with BSC, MAC, CI and WC. The results observed in the PCA space showed again that the malolactic fermentation produced the same changes in all the analysed parameters in all the wines, and that the differences observed among wines after the alcoholic fermentation were maintained after the malolactic fermentation.

3.8. Sensory analysis

Sensory evaluations of wines after malolactic fermentation were performed to verify the differences observed between wines on the organoleptic perception. On the visual phase, and in good agreement with what was observed in the analysis of colour, Monastel and Tempranillo wines showed the highest scores in colour intensity although all the wines obtained high punctuations, 4.56 for Monastel, 4.38 for Tempranillo, 3.73 for Maturana Tinta and 3.62 for Maturana Tinta de Navarrete. With regards to judge's comments on colour it is noteworthy that Monastel was described as presenting the highest red tonalities while Maturana Tinta de Navarrete colour intensity was coupled to orange tonalities. Fig. 4 provides a GPA consensus configuration of the relationship of the wines as determined for their olfactory and gustatory perceptions. Generalised Procrustes Analysis (GPA) was applied to sensory data to ascertain consistency among the 12 tasters and provide information on relationship between wines and attributes. Before that, the within judges reproducibility was evaluated by mean of two replicated wines in the tasting session and replications were demonstrated not to be a source of variation.

In the olfactory GPA space (Fig. 4a), wines were properly located in the vectorial dimension defined by the two factors, which accounted for 82% of the total variance. The consensus plot showed the wines quite spread, thus indicating a marked difference among wines. Tempranillo showed a higher correlation with herbaceous and liquorice aromas, the last being a characteristic varietal descriptor of the Tempranillo wines. Monastel was more correlated with fruity, coffee and toasted aromas while Maturana Tinta was described to be related with dairy and also liquorice aromas and Maturana Tinta de Navarrete with pepper odours. Relative to aromatic intensity, Monastel wine was the best valued as it obtained an average score of 3.91 while Tempranillo obtained the lowest score (2.51) and the other wines had values around 3.0. Fig. 4b shows the wine and attribute average space obtained from the gustatory space where PC1 explained 45% of the total variance and PC2 accounted for 33.5%. The first aspect to be highlighted is that Maturana and Maturana Tinta de Navarrete were perceived by the tasters as being very similar in their gustative descriptors as they were located very close in the consensus space. Tempranillo wine showed a higher correlation

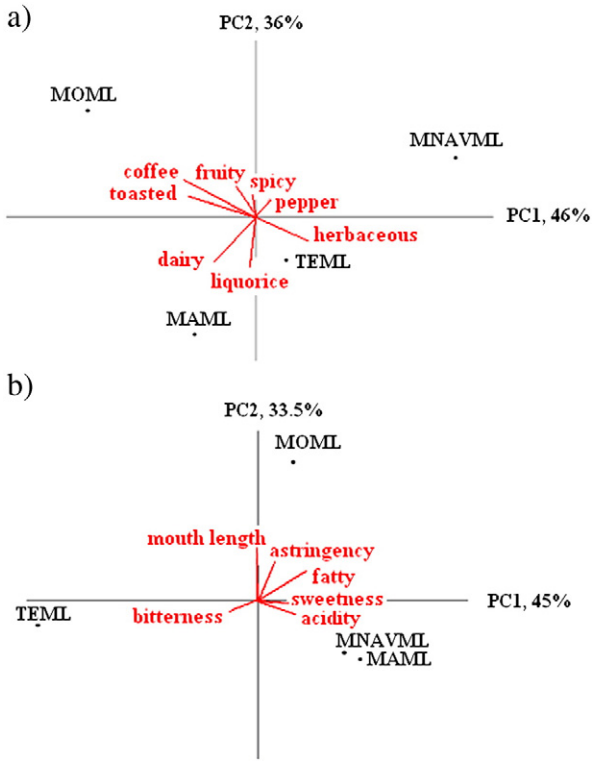

Fig. 4. GPA on the mean ratings for a) olfactory and b) gustatory attributes in wines after malolactic fermentation.

with bitterness and mouth length and obtained low punctuations in relation with fatty, sweet and acid sensations. On the contrary, Monastel was more correlated with mouth length and astringency while Maturana Tinta and Maturana Tinta de Navarrete were related with acidity, sweetness and fatty sensations.

Finally, and although all the wines obtained good punctuations in the global perception, Monastel and Maturana Tinta de Navarrete were best valued as they obtained the highest punctuations. Monastel, with a global score of 4.45, was described by tasters as a wine of high intensity and great aromatic complexity with very pleasant dairy and coffee aromas. In mouth, it resulted the most valuable due to its great persistence, mount length and structure. Maturana Tinta de Navarrete (4.36 in global perception) was also described as highly aromatic and it was fresh and pleasant in mouth. Lastly, Tempranillo and Maturana Tinta obtained lower punctuations in the global perception (3.55 and 3.75 respectively) because they showed lower aromatic complexity although both were described as very pleasant and balanced in the mouth.

4. Conclusions

This work evaluates the sensory profiling of wines Maturana Tinta, Monastel and Maturana Tinta de Navarrete and monitors the chemical changes occurring in phenolic compounds and colour parameters during the malolactic fermentation process. In this sense, it was observed that malolactic fermentation produced changes of the same magnitude in all the analysed compounds in all the wines and both anthocyanin and hydroxycinnamic acid distribution was found to be dependent on the variety and not on the winemaking process. Therefore, this study permitted to characterize for the first time wines manufactured with these minority varieties and provided data that could be used as a chemotaxonomic tool to fingerprint them. Data also revealed that all the varieties produced wines with high values of resveratrol which could lead to healthier wines. Moreover, in sensory analysis all wines were found to present a

great potential to produce high quality wines, which would provide a viable alternative to grape varieties cultivated in La Rioja and would favour the differentiation of the Rioja wines on the national and international markets. However, and in order to complement these findings, further studies would be needed on the biogenic ammine, amino acid and volatile composition of these wines and on consumer preferences.

Acknowledgments

The authors would like to thank to Juan Carlos Sancha for providing the wine samples and to Zenaida Hernández for her help with the statistics.

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4.2

AMINOÁCIDOS Y AMINAS BIÓGENAS EN VINOS TINTOS VARIETALES: EL PAPEL DE LA VARIEDAD DE UVA, LA FERMENTACIÓN MALOLÁCTICA Y LA AÑADA

Amino acids and biogenic amines in red varietal wines: the role of grape variety, malolactic fermentation and vintage

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European Food Research and Technology 2013**,**

DOI 10.1007/s00217-013-2059-x

RESUMEN

Los objetivos de este trabajo son:

1.- Caracterizar los vinos de Monastel, Maturana Tinta de Navarrete y Tempranillo en función de su contenido y perfil de aminoácidos y aminas biógenas.

2.- Estudiar el efecto de la fermentación maloláctica en la composición y perfil de aminoácidos y aminas biógenas de estos vinos.

3.- Estudiar el efecto de la añada en la composición y perfil de aminoácidos y aminas biógenas de dichos vinos.

Este estudio se realizó durante las añadas 2009 y 2010. Los datos obtenidos se analizaron estadísticamente mediante técnicas de análisis de varianza multifactoriales y se aplicaron técnicas quimiométricas con objeto de conseguir una posible diferenciación de los vinos de acuerdo a la variedad, la etapa de vinificación y la añada.

Los vinos de Monastel, Maturana Tinta y Tempranillo pudieron diferenciarse varietalmente antes y después de la fermentación maloláctica en función de su composición de aminoácidos. Así, en las dos añadas los vinos de Maturana Tinta de Navarrete y Monastel presentaron un contenido en aminoácidos totales superior al de los vinos de Tempranillo. Los vinos de Maturana Tinta de Navarrete se caracterizaron por tener los valores más altos de OH-prolina; los vinos de Monastel por los valores más altos en ß-alanina; y los de Tempranillo por los valores más altos en todos los aminoácidos excepto prolina, ß-alanina y OH-prolina. El contenido total de aminas biógenas fue similar en todos los vinos varietales, mostrando niveles de histamina por debajo de los límites considerados como un riesgo para la salud. Al contrario de lo observado con el perfil de aminoácidos, las técnicas de análisis discriminante no permitieron una clasificación de los vinos en función de su composición en aminas biógenas.

Los análisis discriminantes mostraron que tanto el perfil de aminoácidos como el de aminas biógenas podían ser usados para diferenciar a los vinos según su etapa de vinificación. La fermentación maloláctica produjo un aumento en la concentración de todos los aminoácidos con excepción de la arginina y la glutamina, y un incremento de las aminas biógenas entre 2 y 3,7 veces; la putrescina fue la amina biógena mayoritaria en todos los vinos. El análisis de las correlaciones entre la concentración de aminas biógenas después de la fermentación maloláctica y sus precursores antes de la misma dio lugar a resultados contradictorios. Se encontraron correlaciones entre arginina/putrescina, agmatina/putrescina, fenilalanina/feniletilamina y tirosina/tiramina, mientras que no se encontraron entre el resto de aminas biógenas y sus precursores. Tampoco se encontró correlación entre el contenido total de aminoácidos antes de la

fermentación maloláctica y el contenido total de aminas biógenas después de la misma, sugiriendo que una concentración inicial más alta de aminoácidos en el medio no afectaba a la concentración final de aminas biógenas después de la fermentación maloláctica.

Los análisis de la varianza y discriminantes permitieron clasificar a los vinos en función de su contenido en aminoácidos y en aminas biógenas según la añada. El aminoácido γ-aminobutírico (GABA) fue el que experimentó las mayores diferencias entre añadas. Hay que destacar que en la añada 2010 los valores de histamina fueron muy bajos y la triptamina no se detectó.

El análisis estadístico multifactorial permitió estudiar la interacción de los factores variedad, estado de vinificación y añada. La interacción variedad y estado de vinificación no fue significativa para la mayoría de los aminoácidos, indicando que estos compuestos muestran el mismo comportamiento para todos los vinos varietales y en las distintas etapas de vinificación, mientras que sí que se observaron interacciones significativas en el contenido de aminas biógenas. Por el contrario, todos los aminoácidos cuantificados variaron significativamente con respecto a la interacción variedad y añada. El factor variedad tuvo una mayor influencia en los vinos de Tempranillo ya que mantuvieron su perfil de aminoácidos en ambas añadas, mientras que el factor añada tuvo más influencia en los vinos de Monastel y Maturana Tinta de Navarrete ya que su perfil de aminoácidos se modificó en función de la añada. Estos resultados sugieren que los vinos de Monastel y Maturana Tinta de Navarrete tienen un menor carácter varietal que los de Tempranillo, estando más influenciados por las condiciones climáticas. Por último, cuando se estudió la interacción de los tres factores variedad, estado de vinificación y añada, sólo el 29% de los aminoácidos y el 33% de las aminas biógenas presentaron interacciones significativas, correspondiendo a OH-prolina, glutamina, ß-alanina, arginina, prolina, cisteína y triptófano; y a histamina, putrescina y cadaverina, respectivamente.

Este artículo muestra por primera vez la composición de aminoácidos y aminas biógenas de los vinos obtenidos de las variedades minoritarias de La Rioja Monastel y Maturana Tinta de Navarrete, y aporta datos que pueden servir como criterio taxonómico para identificarlos.

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29-Jun-2013

Dear Dr. Ayestaran:

It is a pleasure to accept your manuscript entitled "AMINO ACIDS AND BIOGENIC AMINES IN RED VARIETAL WINES: THE ROLE OF GRAPE VARIETY, MALOLACTIC FERMENTATION AND VINTAGE" in its current form for publication in the European Food Research and Technology.

Thank you for your fine contribution. On behalf of the Editors of the European Food Research and Technology, we look forward to your continued contributions to the Journal.

Sincerely,
Prof. Thomas Henle
Editor-in-Chief, European Food Research and Technology
Thomas.Henle@chemie.tu-dresden.de

Draft Manuscript for Review

AMINO ACIDS AND BIOGENIC AMINES IN RED VARIETAL WINES: THE ROLE OF GRAPE VARIETY, MALOLACTIC FERMENTATION AND VINTAGE

Journal:	*European Food Research and Technology*
Manuscript ID:	EFRT-13-0616.R1
Manuscript Type:	Original paper
Date Submitted by the Author:	24-Jun-2013
Complete List of Authors:	Martínez-Pinilla, Olga; Universidad de La Rioja, Guadalupe, Zenaida; Universidad de La Rioja, Hernández, Zenaida; Universidad de La Rioja, Ayestaran, B.; University La Rioja, Agricultura y Alimentación
Keywords:	red minority varietal wines, amino acid and biogenic amine composition, winemaking stage, vintage

AMINO ACIDS AND BIOGENIC AMINES IN RED VARIETAL WINES: THE ROLE OF GRAPE VARIETY, MALOLACTIC FERMENTATION AND VINTAGE

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Running title: Amino acids and biogenic amines in red minority varietal wines

ABSTRACT

This work studies for the first time the amino acid and biogenic amine composition of Rioja red wines made with the red minority varieties *Vitis vinifera* cv. Monastel and Maturana Tinta de Navarrete, using Tempranillo as a reference variety. The role of malolactic fermentation and vintage on these compounds was also analyzed, and discriminate analyses were applied to achieve a possible differentiation of the wines. Amino acid composition allowed a differentiation of wines according to grape variety. Monastel was characterised by the highest value in ß-alanine and Maturana Tinta de Navarrete by its highest value in OH-proline. However, biogenic amines were no able to classify varietal wines. The malolactic fermentation had significant changes on the amino acid and biogenic amine content, and allowed distinguishing wines that underwent this process from wines without malolactic fermentation. No correlation was found between total amino acids and total biogenic amines after malolactic fermentation, suggesting that a higher initial concentration of amino acids in the medium did no affect the concentration of biogenic amines after malolactic fermentation. Vintage influenced the amino acid and biogenic amine pattern, obtaining a clear separation of wines by vintages. Monastel and Maturana Tinta de Navarrete wines showed a minor varietal character and were more influenced by the climatic conditions of each vintage than Tempranillo wines. All the wines showed histamine levels below the human physiological threshold and implemented regulations.

Keywords: red minority varietal wines, amino acid and biogenic amine composition, winemaking stage, vintage.

Introduction

Amino acids in wine have different origins. Besides those that are present in grapes and that can be partially or totally metabolized by yeasts and lactic acid bacteria during the course of fermentations, some are secreted by yeasts and bacteria at the end of fermentations, some are released by proteolysis during the autolysis of dead yeasts, and other are produced by enzymatic degradation of the grape proteins. Moreover, grape variety, climate, viticulture practices and winemaking techniques, such as maceration time or malolactic fermentation, can affect the amino acid content in wines [1, 2]. Biogenic amines are mainly produced as a consequence of the decarboxylation of amino acids, although they can also be present in grapes. In wine, biogenic amines can be found at variable levels depending on grape variety, vintage, levels of amine-precursor amino acids, assimilable nitrogen content and processing techniques as the occurrence of malolactic fermentation [3-6]. While high concentrations of biogenic amines can cause undesirable physiological effects in sensitive humans, amino acids are precursors for aroma compounds and directly contribute to wine's aroma, taste and appearance [7-9].

Despite the wide range of factors affecting the amino acid and biogenic amine composition in wine, some researchers have successfully employed the amino acid and biogenic amine pattern to differentiate wines according to variety, vintage and winemaking techniques [1, 3, 10, 11]. Hence the importance of using free amino acid and biogenic amine profile to characterize wines made with minority varieties in different regions, which would allow to characterise wines with their own personality and different from the rest that exist in the international market.

In this sense, La Rioja (Spain), an autonomous community with a large vitiviniculture tradition, has raised the need to preserve and characterize its minority

vine varieties in order to maintain the authenticity and quality of its wines. This community has different vine-growing zones with an important number of minority grape varieties, which are perfectly adapted to these zones. Previous studies of local and minority varieties from these areas [12, 13] highlighted for their oenological interest Monastel and Maturana Tinta de Navarrete that could be a good complement to the widespread and most representative variety of the area, Tempranillo comprises 85% of the surface of red grapes cultivated in La Rioja. Although there are some studies on the growing potential of these varieties [12, 13] and on the sensory profiling of the wines produced made from these varietals [14], information on the amino acid and biogenic amine content of these wines is lacking. On the one hand, this study would allow to identify the amino acids and biogenic amines of wines produced with these minority varieties, and on the other hand, to achieve a possible characterization of these wines by means of their amino acid and biogenic amine pattern.

Therefore, the aim of this work was to study the profile and content of amino acids and biogenic amines in red varietal wines made with Monastel and Maturana Tinta de Navarrete, before and after malolactic fermentation. For this purpose, wines were produced in an industrial wine cellar during two different vintages, and Tempranillo was also studied as a reference variety. Stepwise Discriminate Analysis (SDA) were applied in order to achieve a possible differentiation of the wines regarding variety, winemaking stage and vintage.

Experimental

Wine samples

Thirty six red wine samples were analyzed in this study (Table 1). All samples belonged to three red grape varieties cultivated in the autonomous community of La Rioja (Spain): 12 of them corresponded to Tempranillo, used as a reference variety, and

the others corresponded to the red minority varieties Monastel (12 samples) and Maturana Tinta de Navarrete (12 samples). All the samples were collected in two different vintages (2009 and 2010), and among them, half were collected from different deposits after the alcoholic fermentation while the other half were taken after the malolactic fermentation. Grapes were grown in the same vineyard under similar conditions to eliminate possible variations due to different soils, climatic conditions and viticulture procedures. All grapes were vinified under the same controlled procedures to avoid variations during the winemaking. Grapes were destemmed, sulphited with 3 g/HL SO_2 and inoculated with 25 g/HL *S. cerevisiae* yeast strain (Uvaferm VRB, Lallemand Inc. Spain). The fermentation-maceration process was carried out at a maximum temperature of 28 ± 2 ºC and lasted 10 days. Wines were then run off and they were maintained at controlled wine cellar temperature for undergoing spontaneous malolactic fermentation. Malic acid content was 3 g/L ± 0.2 and 4 g/L ± 0.5 in vintage 2009 and 2010, respectively. After malolactic fermentation, malic acid was below 0.1 g/L and the lactic acid content was 2 g/L ± 0.2 in vintage 2009 and 2.6 g/L ± 0.4 in vintage 2010.

Chemical analysis

L-malic and L-lactic acid were analyzed by a multiparametric enzymatic autoanalyzer LISA 200 (Analytical Methodology LISA 200, Biocode Hyad, Le Rhem, France).

Amino acid and biogenic amine analysis

Amino acid and biogenic amine content were determined simultaneously using the method described by Gómez-Alonso et al. [15]. 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. Twenty four amino acids and nine biogenic amines were identified on the basis of the aminoenone derivative retention times of the corresponding patterns (Sigma-Aldrich Chemie, Steinhein, Germany), and quantified using the internal pattern method. All analyses were performed in triplicate in each wine sample.

Statistical analysis

Significant differences between analytical determinations were analyzed by a factorial analysis of variance (ANOVA) taking in account variety, winemaking stage and vintage. For comparison of wines, a two-tailed Pearson correlation test was carried out. Stepwise discriminate analysis (SDA) following the forward method was used to select the variables most useful for differentiating wines according to grape variety, winemaking stage and vintage. The F-statistical function was used as the criterion for variable selection. ANOVA evaluations were performed using the Statistica 8.0 program for Microsoft Windows (Statsoft Inc., Tulsa, Oklahoma). Discriminate analysis and two-tailed Pearson correlation test were carried out using the IBM SPSS Statistic 16.0 program (New York, USA).

Results and discussion

Amino acids and biogenic amines composition of wines by grape variety, winemaking stage and vintage

Table 2 and Table 3 show the concentration (mg/L) of amino acids and biogenic amines and the significance of the ANOVA analysis by variety, winemaking stage and vintage, respectively. Data in the tables has been arranged by amino acids and biogenic amines compounds and listed according to their order elution. Total amino acids and

total biogenic amines were calculated as the sum of the concentration of the individual compounds.

From the 36 wines analysed, 12 corresponded to each studied variety (Table 1). Regarding amino acids, wines made from Monastel and Maturana Tinta de Navarrete showed the highest concentrations of total amino acids while Tempranillo wines showed the lowest (Table 2). Tempranillo showed similar values to those obtained in other studies for this variety [2, 15]. Regarding differences among varieties in the concentration of individual amino acids (Table 2), several aspects should be highlighted. Firstly, proline was by far the major amino acid in all the wines, and was significantly higher in Maturana Tinta de Navarrete and Monastel than in Tempranillo wines (96%, 90% and 83%, respectively). Some studies have observed that proline is not consumed under anaerobic conditions during alcoholic fermentation [16], and it can even be released during this stage [9]. These facts could explain that the resulting wines after fermentation had a higher amount of proline than the initial musts. Secondly, it is worth highlighting that, with the exception of proline, OH-proline and ß-alanine, the concentration of all the remaining amino acids was higher in Tempranillo than in Monastel and Maturana Tinta de Navarrete. Thus, Monastel wines showed the highest value in ß-alanine. Despite the low content observed in individual amino acids in Maturana Tinta de Navarrete wines, they could be differentiated from the rest of the wines by its highest content in OH-proline. It is also worth mentioning that all the amino acids analyzed varied significantly among samples with respect to the grape variety factor (V) when the significance of the ANOVA analysis was made (Table 3). These results suggested that distinctive amino acids for each grape variety could be found.

Malolactic fermentation (ML) is part of the traditional winemaking techniques for red wines and it is necessary to elaborate high quality wines that could be aged. In the present study, we investigated if this winemaking stage had a marked influence on the amino acid and biogenic amine content.

The malolactic fermentation caused significant changes in the concentrations of individual amino acids (Table 2). Thus, malolactic fermentation produced increases in nearly all the amino acids studied, and mainly in valine, methionine, isoleucine, phenylalanine, asparagine, serine, glycine, threonine, leucine and lysine. The increase in these amino acids was attributed to their release from wine proteins or peptides by the action of proteases from the lactic acid bacteria [17]. On the contrary, glutamine and arginine were the only amino acids which showed reductions (around 60%), leading to changes in the amino acid profile after this winemaking stage. There is no consensus on the bibliography as regards the evolution of the different amino acids during malolactic fermentation, with the exception of arginine, in which case all authors report a considerable decrease [2], probably due to the fact that arginine is one of the nutritional requirements for the lactic acid bacteria [18]. Hence, some authors found a decrease in total amino acids after this winemaking stage [1, 19], while others found an increase [2, 16, 20, 21]. Results of the ANOVA (Table 3) showed that the effect of the winemaking stage (S) was also important, due to significant differences were found in all studied amino acids, except in GABA and ornithine.

From the 36 wine samples analysed, 12 were produced in vintage 2009 and 24 in vintage 2010 (Table 1). Taking in account individual amino acids, the most pronounced change observed was in GABA, which showed double quantity in wines of vintage 2009 (Table 2). Regarding to the vintage factor (VI), significant differences were found in 18 of the 24 amino acids studied (Table 3).

It is to point out that the interaction variety x winemaking stage (V x S) was not significant for most of the amino acids (Table 3). However, when the interaction V x VI was studied, all the amino acids quantified varied significantly (Table 3). Tempranillo wines maintained its amino acid profile in both vintages, whereas different profiles were obtained for Monastel and Maturana Tinta de Navarrete (Table 2). Thus, for Tempranillo wines major amino acids after proline were glutamine, glutamic acid, α-alanine, arginine and GABA. Similar profile was described by López et al. [16] in other Tempranillo wines from La Rioja. Monastel and Maturana Tinta de Navarrete wines showed the same major amino acids GABA, glutamic acid, glutamine, glycine and arginine in vintage 2009. However, in vintage 2010, the major amino acids for Monastel wines were α-alanine, glutamine, glutamic acid, arginine and glycine, whereas glutamic acid, OH-Proline, α-alanine, glycine and glutamine were the major ones for Maturana Tinta de Navarrete wines. Remaining amino acids were present in amounts that did not exceed 20 mg/L in any variety. These results suggested that Monastel and Maturana Tinta de Navarrete amino acid profiles had a minor varietal character and were more influenced by the climatic conditions of each vintage when the interaction of both factors was studied. However, Tempranillo wines amino acid profiles showed similar patterns from the same location in different years, in agreement with the work of Huang and Ough [22].

The interaction S x VI was significant in 17 of the 24 individual amino acids studied (Table 3). An overall increase in their total content was observed after malolactic fermentation in vintage 2009, whereas an overall decrease was observed in vintage 2010.

Finally, when the interaction V x S x VI was studied only the 29% of amino acids varied significantly (Table 3) corresponding to OH-proline, glutamine, ß-alanine, arginine, proline, cysteine and tryptophan.

In relation to the concentration of biogenic amines (Table 2), varietal wines showed similar values, agreeing with those obtained in other studies for Tempranillo wines [2, 4, 16] and in other red wines [4, 23-27]. Although no legal limit has been established for total biogenic amines content in wines, some countries have formerly defined limits for histamine, e.g. Switzerland (10 mg/L), Germany (2 mg/L), Belgium (5-6 mg/L), and France (8 mg/L) [4, 24]. Taking into account the limit of 8 mg/L proposed by Leitao et al. [28], all varietal wines did no represent a health risk. When the significance of the ANOVA analysis by variety was studied, 7 of 9 biogenic amines and total biogenic amines content showed significant differences (Table 3), suggesting that these differences could be due to the different amino acid precursors and their respective amounts in grape varieties, and/or the presence of the natural bacteria microflora present on grapes [29].

Malolactic fermentation produced increases in six of the nine biogenic amines analysed because latic acid bacteria are able to descarboxylate amino acids [30]. Consequently, changes in the biogenic amine pattern were produced, affecting mainly to putrescine, tyramine, spermidine and isoamylamine. Total biogenic amine content was between 2 and 3.7 fold in wines that underwent malolactic fermentation, in agreement with other researches [2, 4, 29, 31-33]. Putrescine was the most abundant amine after the malolactic fermentation, as was found in other studies [2, 4, 19, 34]. Putrescine can be produced by decarboxylation of ornithine or by metabolism of arginine, through being the agmatine intermediary. The Pearson correlation test revealed no correlation between ornithine and putrescine, whereas a negative correlation was found between

arginine and putrescine (r = -0.38, α = 0.01) and a positive one between agmatine and putrescine (r = 0.477, α = 0.01). This result supports the argument that the biosynthetic pathway for putrescine is via arginine-agmatine rather than ornithine. The same result was obtained by Bauza et al. [35] regarding polyamines and their precursors in Grenache noir and Syrah grapes and wine of the Rhône Valley. It should be also highlighted that phenylethylamine showed a negative correlation with its amino acid precursor phenylalanine (r = -0.521, α = 0.01), while tyramine showed a positive correlation with its precursor tyrosine (r = 0.415, α = 0.01). On the contrary, no correlation between the rest of amine/amino acid precursor was found, in good agreement with bibliography, where there is no consensus as regards the correlation between amines and their precursors [2, 6, 19, 36]. It is important highlight that no correlation was found between total amino acids and total biogenic amines, suggesting that a higher initial concentration of amino acids in the medium did no affect the concentration of biogenic amines after malolactic fermentation. However, this result contradicts others studies [1, 2, 6]. Other factors such the type of bacteria performing malolactic fermentation [33] and/or a lack of hygiene during the winemaking process or associated with poor sanitary conditions of grapes could influence the final biogenic amine content in wines [28]. It is worth mentioning that all biogenic amines varied significantly among samples with respect to the winemaking stage factor, except tryptamine and phenylethylamine (Table 3).

The content of biogenic amines also varied between vintages. Thus, wines of vintage 2009 showed more total biogenic amine content than wines of vintage 2010, leading to a change in their amine profile. These results were in good agreement with other studies that reported that different amine content in wines from different years can be due to the diversity of wine microorganism that are naturally differently selected

each year, probably due to climatic conditions [24, 29]. It is to point out that tryptamine was no detected in vintage 2010 and histamine showed very low values. Within all the biogenic amines studied, phenylethylamine was the only which did not show significant differences when the vintage factor was studied (Table 3).

The interaction V x S showed significant differences in all amines, except spermidine, tryptamine and isoamylamine (Table 3). Thus, agmatine showed an increase of more than 190% in Tempranillo and Monastel wines and a decreased of 40% in Maturana Tinta de Navarrete wines after malolactic fermentation. Furthermore, Maturana Tinta de Navarrete wines showed the highest values in histamine after malolactic fermentation. Nevertheless, histamine showed lower values than in other studies [2] and below the limit of human health risk.

Tryptamine and phenylethylamine were no significant when the interactions V x VI was studied. Besides the formers, histamine and agmatine were also no significant in the interaction S x VI (Table 3).

Finally, when the interaction V x S x VI was analyzed, only the 33% of amines varied significantly (Table 3) corresponding to histamine, putrescine and cadaverine.

Discriminant analysis of wines according to their amino acids and biogenic amines content

Stepwise linear discriminate analysis (SDA) was applied as a supervised classification technique in order to determine the amino acids and biogenic amines most useful for differentiating wines according to grape variety, winemaking stage and vintage.

Taking in account grape variety, the final model selected 12 amino acids: HO-proline, ß-alanine, threonine, cysteine, histidine, asparagine, leucine, tyrosine, lysine, α-alanine, proline and arginine. An accurate classification of wines by grape variety was

obtained, with a global classification of 100% of the wines (Fig. 1). This result suggests that wine amino acids may play an important role, as oenological compounds, to differentiate varietal wines. Besides, they contribute to the overall taste of wines. However, when biogenic amines were used to discriminate varietal wines, a clear differentiation could not be achieved (Fig. 2). The final model selected 5 biogenic amines: phenylethylamine, cadaverine, agmatine, isoamylamine and spermidine, with a global classification of only 83.3% of the wines.

Taking in account the winemaking stage, only with the three amino acids methionine, ornithine and cysteine were enough to discriminate wines. Four biogenic amines: isoamylamine, putrescine, spermidine and histamine were also able to differentiate wines that underwent malolactic fermentation. In other studies, putrescine and histamine, together with tyramine increased significantly after malolactic fermentation [2, 19, 31] and they could be used as chemical descriptors to distinguish malolactic fermented-wines. Both models showed a global classification of 97.2%.

When SDA was applied to discriminate wines by vintage, the final model selected 6 amino acids: GABA, ornithine, ß-alanine, glutamine, valine and isoleucine. GABA and alanine were discriminate variables by vintage in other studies [1]. Finally, tryptamine, histamine, cadaverine, spermidine and tyramine were able to group wines by vintage. Both models showed a satisfactory classification of 100%.

Conclusions

This work showed for the first time the amino acid and biogenic amine profile and content of wines made from the red varieties Monastel and Maturana Tinta de Navarrete. Grape variety, winemaking stage and vintage affected the amino acids and biogenic amines content in the two years of study. Therefore, Monastel was characterised by the highest value in ß-alanine, while Maturana Tinta de Navarrete

showed the greatest content in OH-proline. Both wines had a less varietal character and were more influence by climatic conditions than Tempranillo wines in the two years of study. All the wines showed histamine levels below the human physiological threshold, fact of extreme importance from a health point of view. No correlation was found between total amino acids and total biogenic amines after malolactic fermentation, suggesting that a higher initial concentration of amino acids in the medium did no affect the concentration of biogenic amines after malolactic fermentation during the study. When the interactions of the factors variety, winemaking stage and vintage were studied in this paper, only the 29% of amino acids and 33% of biogenic amines varied significantly.

When discriminate analyses were applied, amino acids were able to differentiate wines by variety, winemaking stage and vintage. On the contrary, amines were not able to discriminate varietal wines. However, they could differentiate wines by winemaking stage and vintage in the studied period. Consequently, the amino acid profile could be used as a tool to differentiate wines according to grape variety, winemaking stage and vintage, whereas amines might be used as descriptors of malolactic-fermented wines and to discriminate wines from different vintages.

Acknowledgements

The authors would like to thank to Juan Carlos Sancha for providing the wine samples.

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FIGURE CAPTIONS

Fig. 1 Distribution of the wines in the plane defined by the two first discriminate functions by amino acids and grape variety. T: Tempranillo wine; O: Monastel wine; V: Maturana Tinta de Navarrete wine

Fig. 2 Distribution of the wines in the plane defined by the two first discriminate functions by biogenic amines and grape variety. T: Tempranillo wine; O: Monastel wine; V: Maturana Tinta de Navarrete wine

Table 1. Distribution of the wine samples according to variety, vintage and winemaking stage

Variety	Vintage 2009		Vintage 2010		
	OH[1]	ML[2]	OH[1]	ML[2]	*Total*
Tempranillo	2	2	4	4	12
Monastel	2	2	4	4	12
Maturana Tinta de Navarrete	2	2	4	4	12
Total	6	6	12	12	36

[1] OH: wines samples taken after alcoholic fermentation; [2] ML: wines samples taken after malolactic fermentation.

Table 2. Concentration (mg/L) of amino acids and biogenic amines by variety, winemaking stage and vintage

	Vintage 2009						Vintage 2010					
	Tempranillo		Monastel		Maturana Tinta de Navarrete		Tempranillo		Monastel		Maturana Tinta de Navarrete	
	OH	ML	OH	ML	OH	ML	OH	ML	OH	ML	OH	ML
Amino acids												
Aspartic acid	5.9 ± 1.9	12.7 ± 3.8	2.1 ± 0.9	5.6 ± 1.6	1.4 ± 0.4	4.8 ± 1.0	4.3 ± 0.1	4.5 ± 0.2	4.8 ± 0.1	4.9 ± 0.2	1.8 ± 0.0	1.9 ± 0.2
Glutamic acid	46.5 ± 13.5	59.1 ± 12.7	22.4 ± 9.1	35 ± 10	11.3 ± 1.4	20.0 ± 2.6	22.7 ± 0.6	21.7 ± 0.6	34.5 ± 1.0	28.9 ± 0.6	8.6 ± 0.3	10.4 ± 0.4
Asparagine	13.2 ± 4.4	17.9 ± 4.0	8.0 ± 2.9	15.2 ± 3.5	4.1 ± 0.9	9.2 ± 2.4	8.6 ± 0.5	11.8 ± 0.3	13.9 ± 0.4	18.4 ± 0.3	3.4 ± 0.1	4.2 ± 0.1
Serine	6.0 ± 1.9	8.5 ± 2.2	2.8 ± 1.2	5.6 ± 1.6	1.3 ± 0.4	3.5 ± 0.8	3.3 ± 0.1	3.8 ± 0.1	5.1 ± 0.2	5.5 ± 0.1	1.2 ± 0.1	1.7 ± 0.1
OH-Proline	1.9 ± 0.5	3.0 ± 0.1	2.9 ± 0.3	6.0 ± 1.1	5.6 ± 0.2	13.7 ± 0.5	3.1 ± 0.1	3.7 ± 0.3	4.1 ± 0.2	4.6 ± 0.3	7.9 ± 0.1	7.7 ± 0.2
Glutamine	48.8 ± 13.7	22.0 ± 2.1	18.6 ± 8.2	8.0 ± 2.9	6.7 ± 1.5	4.8 ± 0.7	26.8 ± 0.5	1.1 ± 0.1	36.8 ± 1.2	1.1 ± 0.0	6.2 ± 0.3	0.3 ± 0.0
Histidine	11.0 ± 2.9	16.8 ± 3.3	5.3 ± 1.7	7.7 ± 4.2	4.3 ± 0.7	3.0 ± 0.2	7.5 ± 0.2	8.8 ± 0.3	9.1 ± 0.3	9.9 ± 0.2	4.8 ± 0.1	4.9 ± 0.1
Glycine	17.6 ± 5.3	28.1 ± 4.2	9.3 ± 2.0	22.0 ± 3.6	6.1 ± 1.1	15.5 ± 1.6	11.8 ± 0.4	13.5 ± 0.4	18.0 ± 0.5	19.4 ± 0.2	6.9 ± 0.2	7.6 ± 0.1
Threonine	7.5 ± 2.1	13.2 ± 3.2	2.9 ± 1.2	7.9 ± 1.9	2.1 ± 0.5	6.4 ± 1.9	4.7 ± 0.1	6.3 ± 0.2	6.2 ± 0.2	7.2 ± 0.1	1.8 ± 0.1	3.7 ± 0.2
ß-Alanine	1.9 ± 0.4	2.9 ± 0.4	7.2 ± 0.8	14.1 ± 0.6	5.3 ± 0.7	10.9± 2.1	1.1 ± 0.1	1.2 ± 0.0	8.1 ± 0.2	7.7 ± 0.1	2.9 ± 0.1	3.0 ± 0.3
Arginine	28.1 ± 8.1	6.0 ± 1.0	8.4 ± 4.0	3.3 ± 0.9	6.1 ± 1.5	6.2 ± 1.7	15.5 ± 0.5	2.4 ± 0.2	19.3 ± 0.5	2.1 ± 0.0	6.0 ± 0.2	2.1 ± 0.4
α-Alanine	32.1 ± 7.8	61.4 ± 16.0	5.5 ± 1.3	37.6 ± 11.3	8.4 ± 2.8	21.1 ± 6	24.5 ± 1.2	26.8 ± 0.7	42.8 ± 1.3	42.0 ± 0.6	7.1 ± 0.2	8.3 ± 0.2
GABA	48.2 ± 14.0	44.9 ± 6.0	25.9 ± 5.2	11.1 ± 3.2	10.7 ± 0.7	15.1 ± 4.6	14.8 ± 0.9	16.1 ± 0.3	14.7 ± 0.4	15.1 ± 0.2	5.2 ± 0.2	5.8 ± 0.3
Proline	841 ± 150	1857 ± 358	1549 ± 467	3558 ± 553	1713 ± 173	4849 ± 145	1628 ± 88	1126 ± 358	3076 ± 30	1979 ± 41	2651 ± 89	1548 ± 64
Tyrosine	2.31 ± 0.2	6.3 ± 2.6	0.7 ± 0.3	2.3 ± 0.9	0.4 ± 0.2	1.9 ± 1.5	3.5 ± 0.1	3.1 ± 0.1	5.5 ± 0.3	4.6 ± 0.1	1.8 ± 0.2	1.8 ± 0.1
Valine	4.3 ± 1.4	7.8 ± 2.8	2.1 ± 1.0	3.7 ± 1.1	1.8 ± 0.4	4.0 ± 1.5	3.8 ± 0.3	7.0 ± 0.8	4.7 ± 0.2	9.3 ± 0.2	2.2 ± 0.2	6.1 ± 0.3
Methionine	2.7 ± 0.9	5.1 ± 2.5	1.4 ± 0.5	2.9 ± 0.4	1.3 ± 0.0	2.9 ± 1.0	1.9 ± 0.1	6.2 ± 1.0	2.8 ± 0.0	8.8 ± 0.1	1.3 ± 0.0	5.3 ± 0.1
Cysteine	1.3 ± 0.3	1.3 ± 0.1	1.0 ± 0.3	1.1 ± 0.1	0.7 ± 0.1	1.0 ± 0.6	1.4 ± 0.0	2.1 ± 0.5	1.7 ± 0.1	5.1 ± 0.0	1.2 ± 0.1	3.7 ± 0.1
Isoleucine	2.2 ± 0.7	4.6 ± 2.6	1.3 ± 0.4	2.5 ± 0.6	1.2 ± 0.1	2.4 ± 0.6	1.4 ± 0.1	3.3 ± 0.5	1.9 ± 0.2	4.7 ± 0.0	0.6 ± 0.0	2.8 ± 0.0
Tryptophan	3.0 ± 0.9	3.8 ± 0.8	1.9 ± 0.3	2.8 ± 0.8	1.6 ± 0.2	2.8 ± 0.3	2.0 ± 0.1	5.6 ± 1.0	3.4 ± 0.1	9.1 ± 0.1	1.1 ± 0.1	3.9 ± 0.0
Leucine	5.5 ± 2.7	9.6 ± 5.2	2.6 ± 1.0	4.6 ± 1.9	3.2 ± 0.6	7.2 ± 2.3	3.1 ± 0.2	5.8 ± 0.3	5.4 ± 2.7	8.2 ± 0.1	2.1 ± 0.1	3.4 ± 0.1

(continued)

	Vintage 2009						Vintage 2010					
	Tempranillo		Monastel		Maturana Tinta de Navarrete		Tempranillo		Monastel		Maturana Tinta de Navarrete	
	OH	ML	OH	ML	OH	ML	OH	ML	OH	ML	OH	ML
Phenylalanine	4.0 ± 1.9	6.4 ± 3.3	2.2 ± 0.9	3.8 ± 1.2	1.9 ± 0.4	4.4 ± 1.4	2.5 ± 0.1	6.8 ± 1.0	4.2 ± 0.2	9.5 ± 0.1	1.5 ± 0.0	4.4 ± 0.1
Ornithine	16.9 ± 5.0	12.6 ± 0.5	2.9 ± 1.8	0.95 ± 0.2	0.8 ± 0.7	0.3 ± 0.1	9.0 ± 0.5	16.8 ± 0.1	7.2 ± 0.2	19.9 ± 0.5	1.4 ± 0.2	1.1 ± 0.0
Lysine	7.9 ± 3.2	16.7 ± 6.0	4.2 ± 1.9	10.6 ± 3.3	3.1 ± 0.6	8.8 ± 1.1	5.6 ± 0.3	8.4 ± 0.1	8.3 ± 0.3	11.7 ± 0.2	2.9 ± 0.1	3.6 ± 0.1
Total	1160 ± 74	2227 ± 454	1690 ± 424	3772 ± 502	1803 ± 162	5019 ± 502	1811 ± 93	1313 ± 47	3339 ± 33	2236 ± 43	2731 ± 89	1646 ± 64
Biogenic amines												
Histamine	3.3 ± 1	1.2 ± 0.1	1.9 ± 0.7	2.2 ± 1.9	1.1 ± 0.1	6.2 ± 1.6	n.d.	0.08 ± 0.01	n.d.	0.06 ± 0.01	n.d.	0.3 ± 0.0
Agmatine	5.3 ± 1.0	11.0 ± 0.1	8.7 ± 0.5	11.8 ± 0.4	4.6 ± 1.4	3.3 ± 0.3	1.88 ± 0.13	8.9 ± 0.2	2.4 ± 0.1	7.9 ± 0.2	2.3 ± 0.0	1.3 ± 0.3
Spermidine	1.16 ± 0.63	1.9 ± 1.2	1.11 ± 0.47	1.75 ± 0.16	1.2 ± 0.2	2.3 ± 0.2	0.73 ± 0.07	5.9 ± 1.9	1.6 ± 0.1	9.7 ± 0.4	0.39 ± 0.09	6.1 ± 0.1
Tyramine	0.16 ± 0.06	1.4 ± 0.7	0.10 ± 0.03	0.82 ± 0.1	0.5 ± 0.3	1.0 ± 0.0	0.12 ± 0.01	0.64 ± 0.04	0.17 ± 0.01	0.84 ± 0.02	0.11 ± 0.03	0.3 ± 0.1
Putrescine	2.4 ± 0.6	25.1 ± 0.5	2.8 ± 0.4	12.8 ± 3.0	5.7 ± 0.8	15.7 ± 2.9	3.1 ± 0.0	7.5 ± 0.2	5.2± 0.1	8.5 ± 0.2	5.6 ± 0.1	7.8 ± 0.2
Tryptamine	0.34 ± 0.04	0.10 ± 0.02	0.24 ± 0.04	0.37 ± 0.35	0.3 ± 0.1	0.13 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cadaverine	0.52 ± 0.09	0.6 ± 0.06	0.16 ± 0.10	0.27 ± 0.10	0.6 ± 0.1	1.7 ± 0.4	0.39 ± 0.01	0.43 ± 0.01	0.33 ± 0.01	0.36 ± 0.00	0.37 ± 0.01	0.4 ± 0.0
Phenylethylamine	0.34 ± 0.03	0.42 ± 0.04	0.19 ± 0.03	0.29 ± 0.01	0.11 ± 0.01	0.20 ± 0.02	0.37 ± 0.02	0.31 ± 0.01	0.25 ± 0.02	0.11 ± 0.01	0.54 ± 0.03	0.37 ± 0.01
Isoamylamine	1.07 ± 0.04	1.13 ± 0.09	0.12 ± 0.06	0.20 ± 0.10	0.09 ± 0.02	0.10 ± 0.02	0.29 ± 0.00	1.6 ± 0.3	0.18 ± 0.00	1.47 ± 0.06	0.22 ± 0.02	1.6 ± 0.1
Total	14.6 ± 2.1	42.8 ± 2.1	14.3 ± 2.3	30.5 ± 1.9	13.9 ± 0.4	30.6 ± 5.0	6.9 ± 0.2	25.4 ± 1.8	10.2 ± 0.3	28.9 ± 0.37	9.6 ± 0.2	18.2 ± 0.2

OH: after alcoholic fermentation; ML: after malolactic fermentation; n.d.: no detectable.

Table 3. Significance of ANOVA for the factors variety (V), winemaking stage (S) and vintage (VI)

	Variety (V)	Winemaking stage (S)	Vintage (VI)	V x S	V x VI	S x VI	V x S x VI
Amino acids							
Aspartic acid	***	***	***	ns	***	***	ns
Glutamic acid	***	*	***	ns	***	**	ns
Asparagine	***	***	ns	ns	***	ns	ns
Serine	***	***	**	ns	***	**	ns
OH-Proline	***	***	*	***	***	***	***
Glutamine	***	***	***	***	***	**	**
Histidine	***	*	ns	*	***	ns	ns
Glycine	***	***	***	ns	***	***	ns
Threonine	***	***	***	ns	***	***	ns
ß-Alanine	***	***	***	***	***	***	***
Arginine	***	***	ns	***	***	ns	***
α-Alanine	***	***	ns	ns	***	***	ns
GABA	***	ns	***	ns	***	ns	ns
Proline	***	***	***	**	***	***	***
Tyrosine	***	**	**	ns	***	***	ns
Valine	***	***	***	ns	***	ns	ns
Methionine	**	***	***	ns	***	***	ns
Cysteine	***	***	***	***	***	***	***
Isoleucine	**	***	ns	ns	**	ns	ns
Tryptophan	***	***	***	*	***	***	**
Leucine	*	***	ns	ns	***	ns	ns
Phenylalanine	***	***	*	ns	***	*	ns
Ornithine	***	ns	*	ns	***	**	ns
Lysine	***	***	*	ns	***	**	ns
Total	***	***	***	***	***	***	***
Biogenic amines							
Histamine	*	*	***	***	*	ns	***
Agmatine	***	***	***	***	**	ns	ns
Spermidine	*	***	***	ns	**	***	ns
Tyramine	ns	***	***	*	*	*	ns
Putrescine	***	***	***	***	***	***	***
Tryptamine	ns	ns	***	ns	ns	ns	ns
Cadaverine	***	***	***	***	***	***	***
Phenylethylamine	**	ns	ns	*	ns	ns	ns
Isoamylamine	***	***	***	ns	***	***	ns
Total	**	***	***	***	**	*	*

*, **, *** indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. ns indicates no significant difference at $p < 0.05$.

Figure 1

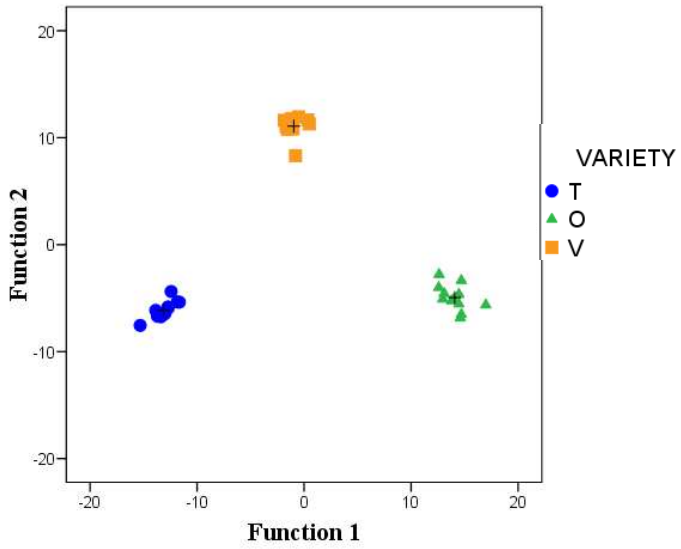
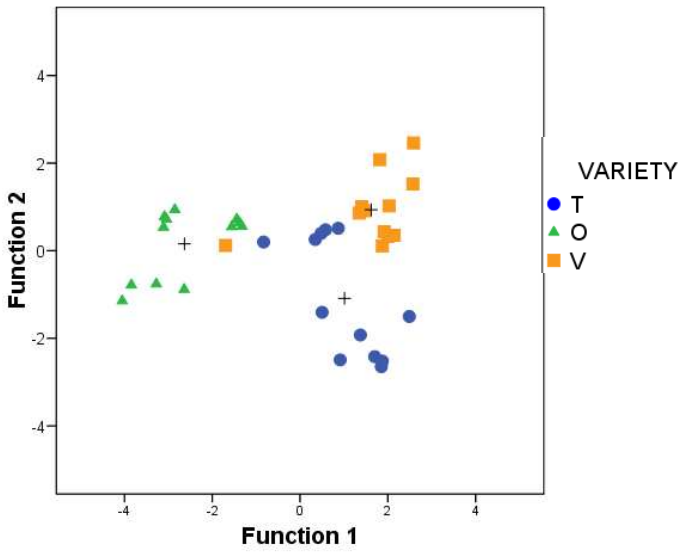


Figure 2



4.3

CARACTERIZACIÓN DE LOS COMPUESTOS VOLÁTILES Y PERFIL OLFATIVO DE VINOS TINTOS DE VARIEDADES MINORITARIAS DE LA RIOJA

Characterization of volatile compounds and olfactory profile of red minority varietal wines from La Rioja

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Journal of the Science of Food and Agriculture 2013,

DOI 10.1002/jsfa.

RESUMEN

Los objetivos de este trabajo son:

1.- Caracterizar los compuestos volátiles y el perfil olfativo de los vinos obtenidos a partir de las variedades minoritarias tintas de La Rioja Monastel y Maturana Tinta de Navarrete, usando a la variedad Tempranillo como referencia.

2.- Estudiar el efecto de la añada en la composición volátil y perfil olfativo de estos vinos.

El estudio se realizó durante las añadas 2009, 2010 y 2011 en muestras tomadas en el momento del embotellado. Se aplicaron técnicas de análisis de la varianza multifactoriales y técnicas quimiométricas con objeto de establecer diferencias entre los vinos en función de la variedad y de la añada.

Los vinos de Monastel, Maturana Tinta de Navarrete y Tempranillo pudieron diferenciarse varietalmente en función de sus compuestos volátiles. Así, en las tres añadas los vinos de Maturana Tinta de Navarrete destacaron por tener la mayor concentración de compuestos volátiles y se caracterizaron fundamentalmente por presentar los valores más altos de ésteres de etilo, 4 etilfenol y alcoholes C_6, considerados como marcadores varietales. Los vinos de Monastel presentaron los valores más altos de γ-nonalactona y ácido decanoico, y los vinos de Tempranillo los valores más bajos de lactonas, 2-etil-metilbutirato y etil isovalerato. Las técnicas quimiométricas de análisis de componentes principales y de análisis discriminantes también diferenciaron a los vinos en función de la variedad, siendo fundamentalmente los aromas propios de la variedad (alcoholes C6, terpenos y compuestos bencénicos) los compuestos volátiles que permitieron discriminar dichos vinos. La caracterización de los vinos varietales mediante el análisis de componentes principales mostró los mismos resultados que los obtenidos por el análisis multifactorial.

Los análisis de la varianza, de componentes principales y discriminantes también permitieron clasificar a los vinos en función de la añada. Los compuestos aromáticos formados durante la fermentación (alcohol isoamílico, 1-propanol, acetato de hexilo) y/o los extraídos de la madera de la barrica durante el proceso de elaboración (furfural, eugenol, siringaldehído, 4-propilguaiacol) fueron los principales responsables de dicha discriminación. Los vinos de la añada 2009 se caracterizaron por los valores más bajos en alcoholes C_6 y más altos en compuestos derivados de la madera. Los vinos del 2010 mostraron las concentraciones más bajas de ésteres etílicos lineales, alcoholes de fusel y lactonas, pero fueron los más ricos en acetatos. Los vinos del 2011 destacaron por elevados niveles de fenoles volátiles.

El análisis estadístico multifactorial realizado para estudiar la interacción de los factores variedad y añada, mostró que la mayoría de los alcoholes de fusel, lactonas, ácido octanoico, 1-hexanol, vainillato de metilo, vainillato de etilo, 2,6-dimetoxifenol y siringaldehído no presentaron interacciones significativas. Esto indica que estos compuestos presentan el mismo comportamiento en todos los vinos varietales durante las tres añadas.

En el análisis olfativo, los vinos de Tempranillo de la añada 2009 se caracterizaron por aromas de regaliz; los vinos de Monastel por aromas especiados, lácteos, de café y tostados; y los vinos de Maturana Tinta de Navarrete por pimienta y notas herbáceas. Los vinos de Tempranillo de la añada 2010 mostraron otra vez notas de regaliz; los de Monastel aromas lácteos y afrutados mientras que los de Maturana Tinta de Navarrete fueron los menos afrutados y los más herbáceos, especiados y con más notas de pimienta. Los vinos de Tempranillo de la añada 2011 se caracterizaron por aromas de regaliz, fruta y vainilla; los de Maturana Tinta de Navarrete por ser los menos afrutados y los más correlacionados con aromas herbáceos, de pimienta y especias; y por último, los de Monastel por ser los menos aromáticos. En resumen, el perfil olfativo de los vinos de Tempranillo y Maturana Tinta de Navarrete parece no variar con las añadas, mientras que sí que lo hace el de los vinos de Monastel.

Este artículo estudia por primera vez la composición volátil y el perfil olfativo de los vinos tintos obtenidos de las variedades de uva Monastel y Maturana Tinta de Navarrete. Los vinos de Maturana Tinta de Navarrete podrían ser una buena alternativa para producir vinos con un perfil aromático característico y diferente al resto de vinos que existen en el mercado.

Research Article

Received: 31 October 2012 Revised: 18 April 2013 Accepted article published: 2 May 2013 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6211

Characterization of volatile compounds and olfactory profile of red minority varietal wines from La Rioja

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Abstract

BACKGROUND: The aim of this work was to study for the first time the volatile compounds and olfactory profile of La Rioja red wines made with the local varieties *Vitis vinifera* cv. Monastel and Maturana Tinta de Navarrete, using Tempranillo as a reference variety. The impact of vintage on these compounds was also evaluated, and chemometric techniques were applied to achieve a possible differentiation of the wines.

RESULTS: A clear classification of wines according to grape variety and vintage was obtained. Volatile compounds that differentiated wines by grape variety were varietal aromas whereas vintage was mainly differentiated by compounds formed during the alcoholic fermentation and extracted from wood during the elaboration process in wooden barrels. Sensory analysis also allowed differentiation of wines by grape variety. Tempranillo wines were characterized by liquorice notes, whereas Maturana Tinta de Navarrete wines were the least fruity and showed herbaceous and pepper notes. The sensory profile of Monastel varied between vintages.

CONCLUSION: These minor grape varieties could provide a good alternative to the most widespread variety in La Rioja: Tempranillo. The use of these varieties produced wines with their own personality and different aromatic profile from other wines on the market.

Keywords: volatile compounds; grape variety; red minority varietal wines; vintage; sensory profile

INTRODUCTION

Wine aroma is one of the most important properties when it comes to consumer preference, and it is mainly determined by the volatile compounds. These volatile compounds are produced through metabolic pathways during ripening and harvesting of grapes, during their fermentation and/or during the storage of wines.[1]

Although the overall composition of most grape varieties is very similar, there are clear and distinct aroma and flavour differences among most cultivars.[2] Varietal aromas are some of these compounds which allow differentiating wines by their variety. However, several studies support the contribution of other volatile compounds such as fusel alcohols, esters and fatty acids to varietal differentiation.[3–5]

The phenomenon of replacement of local grape varieties with widely spread international cultivars is coming to a standstill. In addition, wine consumers' taste and preferences have changed during the last few years, since there are other values and motivations apart from aroma and taste for drinking wines such as marketing attributes and new wine styles. Having in mind these new tendencies, several Denominations of Origin are starting to promote varieties linked to specific locations which produce original and high-quality wines. Minor varieties, perfectly adapted to the local environmental conditions, may represent a good option. In this sense, in La Rioja (Spain) – an autonomous community with a large vitiviniculture tradition – has increased the need to preserve and characterize its minority grape varieties in order to maintain the authenticity and differentiation of its wines. Previous studies of local red varieties from this region[6,7] highlighted the vine-growing interest of Monastel and Maturana Tinta de Navarrete grape varieties, which could be a good complement to the most widespread variety of the area – Tempranillo – which implies 85% of the surface of red grapes cultivated in La Rioja. Therefore, studies on the sensory properties and phenolic composition of varietal wines made with these varieties have recently been carried out.[8] However there is no published information about the aromatic profile of these wines. Moreover, it is important to highlight that the mere knowledge of the volatile composition of a wine, without sensory evaluation, is

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inadequate to predict the flavour of the whole system as perceived by a trained sensory judge. In fact, interactions among odorants and interactions between the odorant and different elements of the wine's non-volatile matrix can affect the odorant volatility, flavour release and overall perceived flavour, intensity and quality.[9]

Considering all the previous comments and studies, the aim of this work was to identify the aroma characteristics of red varietal wines made from the minor varieties Monastel and Maturana Tinta de Navarrete, using Tempranillo as a reference variety. Wines were elaborated in a real winery during three consecutive vintages, and both the sensory olfactory profile and the volatile composition were studied. Multivariate techniques of data analysis were employed in order to establish differentiation criteria among the wines as a function of the grape variety or the vintage.

MATERIALS AND METHODS

Chemicals

Volatile standards were of analytical quality. 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 were purchased from Fluka (Buchs, Switzerland); ethyl 2-methylbutyrate, ethyl decanoate, isoamyl acetate, *trans*-3-hexenol, 2,6-dimethoxyphenol, γ-nonalactone, acetovanillone, linalool, β-ionone, ethyl cinnamate and methyl octanoate were obtained from Sigma-Aldrich (Steiheim, Germany); and finally methyl vanillate, ethyl vanillate, 4-ethylphenol, 4-vinylphenol, 4-vinylguaiacol and 3,4-dimethylphenol were purchased from Lancaster (Strasbourg, France). Dichloromethane (HPLC grade) was supplied by Merck (Darmstadt, Germany).

Vinifications and samples

Vinifications were carried out in the wine cellar *Juan Carlos Sancha SL* (Baños de Río Tobia, La Rioja, Spain) using the grape varieties *Vitis vinífera* L. cv. Tempranillo (T), Monastel (O) and Maturana Tinta de Navarrete (V) harvested in 2009, 2010 and 2011. All grape varieties were harvested in good health conditions at their optimal stage of ripeness, with sugar concentrations ranging between 22.2 and 24.9 °Brix, and total acidity of 4.80–7.15 g L^{-1} tartaric acid. All grapes were vinified under the same controlled winemaking techniques.

Grapes were destemmed and distributed into 500 L French oak barrels/containers, sulfited with 3 g hL^{-1} SO_2 and inoculated with 25 g hL^{-1} *Saccharomyces cerevisiae* yeast strain (Uvaferm VRB, Lallemand Inc., Spain). The fermentation–maceration process was carried out at a maximum temperature of 28 ± 2 °C and lasted around 10 days. Wines were then run off and introduced again into the same 500 L French oak containers, where they were maintained at controlled wine cellar temperature. After spontaneous malolactic fermentation, which lasted from 1 to 2 months, wines were racked, clarified and bottled. In 2009, two batches for each grape variety were studied. In 2010 and 2011 vintages, four batches for each variety were collected. Samples after bottling were analysed for aromatic compounds and tasted. A total amount of 30 samples were taken for this study from the three vintages; 10 corresponded to Tempranillo, 10 to Monastel and 10 to Maturana Tinta de Navarrete wines. The same barrels (*Quercus petraea*, fine grain, medium toasting, thickness of stave 27 mm) were used in the three vintages; vintage 2009 was their fourth use, 2010 their fifth use and 2011 their sixth use. Thus both the year of harvest and the time of use of the barrel are included in the term *vintage*.

Analysis of volatile compounds

Volatile compounds were extracted by liquid–liquid extraction following the method developed by Rodríguez-Bencomo *et al.*[10] Chromatographic analyses were performed with an HP-6890 N GC (Agilent Technologies, Waldbronn, Germany) 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 the method.

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. Forty-four volatile compounds were identified and quantified in the red wines that were classified into ten groups: ethyl esters, alcohols, alcohol acetates, acids, terpenes, lactones, volatile phenols, oak compounds, fusel alcohols and isoamilic alcohols. All analyses were performed in duplicate.

Sensory analysis

A panel of 12 tasters, wine professionals from the DOCa Rioja, was formed. All wine tasters had participated in previous wine-tasting panels. Wines were presented at 18 °C in coded standard wine-tasting glasses according to standard 3591 (ISO 3591, 1997). The tasting sessions took place in a standard sensory analysis chamber (ISO 8589, 1998) equipped with separate booths. In a first session, the panellists were asked to describe the olfactory attributes in their own words. Descriptive terms and their definitions were debated among the assessors, and a common consensus vocabulary was then compiled and discussed further with panellists. Tasters selected eight attributes for vintage 2009 and 2010, and 11 for vintage 2011, which were agreed upon as best for describing the olfactory characteristics of the wines. All the generated terms were usual wine terms for describing red wines. In the following sessions, assessors used the consensus vocabulary, scoring the intensity of each attribute on an interval scale with five levels of intensity (0 = no aroma; 1 = weak aroma; 5 = strong aroma; intermediate values did not bear description). One wine was replicated in order to ascertain judges' consistency.

Statistical analysis

Significant differences between analytical determinations were analysed by a two-way analysis of variance (ANOVA) taking in account variety and vintage. Tukey HSD tests were performed to determinate the statistically significant effect of the parameters with a value of $P < 0.005$. Principal component analyses (PCA) were carried out with the data of the volatile compounds. Only factors with eigenvalues greater than unity were selected. Stepwise discriminant analysis (SDA) following the forward method was used to select the variables most useful for differentiating wines according to grape variety and vintage. The *F*-statistical function was used as the criterion for variable selection. Generalized Procrustes analysis (GPA) was applied on the full data for sensory olfactory attributes, and a permutation test was also made to explain that the results obtained were significant (83.07%).

ANOVA evaluations were performed using the Statistica 8.0 program for Microsoft Windows (Statsoft Inc., Tulsa, OK, USA).

PCA and GPA were carried out using the Senstools Version 3.3.2. Program (Utrecht, Netherlands). Discriminant analysis was carried out using the Statgraphics Plus 5.0 statistical package.

RESULTS AND DISCUSSION

Table 1 presents the concentrations of the 44 volatile compounds quantified by variety and vintage. Data in the table have been arranged into nine chemical families (ethyl esters, acetates, acids, C_6 alcohols, terpenes, lactones, volatile phenols, oak compounds and fusel alcohols). Alcohols were the major group of volatile compounds in all the wines, followed by ester compounds. In the three vintages, Maturana Tinta de Navarrete wines were characterized by the highest concentration of wine volatile compounds because they reached the highest values in ethyl esters and C_6 alcohols (hexanol and *cis*- and *trans*-3-hexen-ol). Also, they reached the highest values in 4-ethyl phenol. The high values found in ethyl esters were due to the high values of ethyl lactate. Ethyl lactate concentrations agreed with those found in other red varietal wines that underwent malolactic fermentation.[11] C_6 alcohol contents allowed differentiating red varietal wines.[4,12,13] Although the ratio *cis*-3-hexen-1-ol/*trans*-3-hexen-1-ol has been also used as a varietal marker,[13] our results indicated that these compounds were also influenced by the vintage. The low values of vinyl and ethyl phenols, which are frequently found in wines aged in barrels, were as expected for young red wines.[14] Monastel wines stood out by a high content in γ-nonalactone and decanoic acid, with similar values to those found in other red varietal wines.[11,14] However, Tempranillo wines showed the lowest contents in lactones, isoamyl alcohols, ethyl-2-methylbutyrate and ethyl isovalerate in the three vintages. It should be pointed out that the aroma of the three varietal wines did not seem to be terpene dependent, as all of them showed terpene values below their odour thresholds.

Wines from the 2009 vintage showed the lowest values in C_6 alcohols and the highest in oak compounds. In spite of the higher relation area/volume of the barrels and taking into account that they had 4 years of use, wines from the 2009 vintage showed more compounds from wood than the rest of the wines. Wines from 2010 showed the lowest concentrations of linear ethyl esters, fusel alcohols and lactones but they were the richest in acetates. Wines from 2011 stood out by the highest levels of volatile phenols. The content of acids and C_6 alcohols were similar in the last two vintages.

Table 2 shows the significance of the ANOVA results for the factor variety and vintage. It is worth mentioning that all the volatile compounds analysed, except eugenol and *trans* whisky lactone, varied significantly among samples with respect to the variety factor. Results of the ANOVA showed that the effect of the vintage on volatiles was also important as significant differences among vintages were found in 39 of the 44 volatiles analysed. It was remarkable that three of the five volatiles with no significance when the factor vintage was analysed corresponded to C_6 compounds (hexanoic acid, 1-hexanol and *trans*-3-hexen-1-ol). Finally, a total of 31 volatile compounds presented a significant interaction between the two factors variety and vintage. Interaction variety $\times$ vintage was not significant for most of the fusel alcohols, lactones, octanoic acid, 1-hexanol, methyl vanillate, ethyl vanillate, 2,6-dimethoxyphenol and siringaldehyde, indicating that these compounds showed the same behaviour for all the varieties in the three vintages of study.

PCA was used in order to clarify data and highlight those variables that better explained the compositional differences among varietal wines and vintage. The PCA selected seven factors with an eigenvalue greater than 1, which explained 93% of the total variance. However, the variables associated into five factors were enough to explain more than 87% of total variability. Table 3 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 contributed most significantly to the explanatory meaning of the factors (marked in bold). The first factor (PC1) explained 33% of data variability and it was strongly correlated with acetates, acids (except hexanoic and octanoic acid), ethyl esters, oak compounds, fusel alcohols, γ-butyrolactone, acetovanillone and vanillin. Except for oak compounds that are extracted from wood, the rest of the compounds are formed during the winemaking process.[2,15]

PC2 was positively correlated with C6 alcohols (except *cis*-3-hexen-1-ol), hexanoic acid, octanoic acid, α-terpineol and methyl vanillate, and negatively with citronellol. C6 alcohols which are produced from unsaturated linoleic and linolenic acids by grape enzymes during destemming and crushing[16] have been related to vegetal and herbaceous aromas of wines.[17] Terpene and benzene compounds have been also associated with the grape variety,[15] but hexanoic and octanoic acids are related to the fermentation process.[15] PC3 was positively correlated with *cis*-3-hexen-1-ol, the varietal terpene geraniol, and most of the volatile phenols (with the exception of vanillin, methyl vanillate and acetovanillone), and negatively correlated with β-phenylethyl alcohol. PC4 was positively correlated with the varietal volatile compounds linalool and ethyl vanillate, and with γ-nonalactone, which derives from precursors present in grapes[14] and it is associated with the fermentation process.

Figure 1(1a) shows the distribution of the different wines in the plane defined by the first two components, which explained 53% of the total variance. Variables associated with PC2 allowed separation of varietal wines. Thus Maturana Tinta de Navarrete wines were mainly characterized by higher concentrations of C6 alcohols, hexanoic acid, octanoic acid, linalol and methyl vanillate, whereas Tempranillo wines showed the opposite behaviour. Monastel wines were located between Maturana Tinta de Navarrete and Tempranillo wines. These results were in agreement with data of Table 1. Conversely, variables associated with PC1 were less suitable for differentiating varietal wines.

Figure 1(1b) shows the distribution of wines according to variety taking into account PC3 and PC4. A good separation was only achieved for Monastel wines, placed in the second quadrant. Monastel wines were characterized for the lowest contents in *cis*-3-hexen-1-ol, 4-ethyl-2-methoxyphenol, 4-ethyl phenol, 2-metoxy-4-vinylphenol guaiacol and eugenol and 2,6 dimethoxyphenol and the highest contents in γ-nonalactone and ethyl vanillate, in agreement with the data of Table 1.

Regarding the factorial analysis for vintage, Fig. 1(2a, 2b) shows the distribution of wines in the plane defined by PC1 and PC2, and PC3 and PC4, respectively. A good separation by vintage was achieved in both figures. In Fig. 1(2a), factor 1 allowed separation of wines by vintage. Wines from 2009 showed the highest values of this factor and 2010 wines the lowest. Differences among the three vintages could be explained by different reasons. Firstly, it is important to highlight that important families of compounds derived from yeast amino acid metabolism, i.e. isoacids, fusel alcohols, ethyl esters of isoacids and fusel alcohol acetates,[5] were associated with this factor, and it is well known that the

Table 1. Concentration (mg L^{-1}) of wine volatile compounds by variety and vintage

	Vintage 2009			Vintage 2010			Vintage 2011		
	T	O	V	T	O	V	T	O	V
Ethyl esters									
Ethyl butyrate (13.72)	0.131 ± 0.011a	0.136 ± 0.001a	0.262 ± 0.004cde	0.227 ± 0.035bcd	0.319 ± 0.023de	0.222 ± 0.014bcd	0.196 ± 0.013abc	0.266 ± 0.015cd	0.188 ± 0.021ab
Ethyl 2-methylbutyrate (14.54)	0.011 ± 0.001ab	0.025 ± 0.002f	0.022 ± 0.000ef	0.010 ± 0.000a	0.016 ± 0.001cd	0.016 ± 0.000cd	0.014 ± 0.000bc	0.020 ± 0.002e	0.018 ± 0.002de
Ethyl isovalerate (15.52)	0.017 ± 0.002a	0.038 ± 0.002d	0.036 ± 0.001d	0.015 ± 0.000a	0.028 ± 0.000c	0.024 ± 0.000bc	0.023 ± 0.003b	0.034 ± 0.002d	0.028 ± 0.003c
Ethyl hexanoate (26.96)	0.268 ± 0.019a	0.295 ± 0.003ab	0.430 ± 0.001cd	0.486 ± 0.019de	0.536 ± 0.023e	0.542 ± 0.017e	0.400 ± 0.032c	0.476 ± 0.008d	0.373 ± 0.037bc
Ethyl lactate (34.42)	73.49 ± 10.53ab	61.15 ± 1.81ab	108.66 ± 12.74b	49.01 ± 2.356a	49.85 ± 6.22a	70.96 ± 9.35ab	85.40 ± 23.40ab	79.43 ± 12.46ab	104 ± 28b
Ethyl octanoate (40.45)	0.221 ± 0.015a	0.256 ± 0.002ab	0.327 ± 0.002bc	0.466 ± 0.015ef	0.518 ± 0.016f	0.464 ± 0.012ef	0.383 ± 0.030cd	0.450 ± 0.012de	0.295 ± 0.060ab
Ethyl decanoate (53.15)	0.045 ± 0.006a	0.058 ± 0.005a	0.051 ± 0.000a	0.169 ± 0.005c	0.221 ± 0.005d	0.145 ± 0.002bc	0.172 ± 0.018c	0.216 ± 0.016d	0.120 ± 0.024b
Acetates									
Isoamyl acetate (19.27)	0.546 ±0.021abc	0.501 ± 0.002ab	0.637 ± 0.022abc	1.40 ± 0.24f	1.18 ± 0.06ef	1.034 ± 0.113de	0.745 ± 0.048bc	0.868 ± 0.015cd	0.460 ± 0.016a
Hexyl acetate (29.72)	ND1	ND1	0.004 ± 0.000a	0.023 ± 0.006c	0.015 ± 0.001b	0.012 ± 0.005ab	0.006 ± 0.000a	0.009 ± 0.000ab	ND1
β-Phenylethyl acetate (62.76)	0.032 ± 0.004a	0.041 ± 0.001a	0.033 ± 0.000a	0.115 ± 0.006d	0.086 ± 0.002c	0.083 ± 0.009bc	0.073 ± 0.005bc	0.070 ± 0.002b	0.043 ± 0.009a
Acids									
Isovaleric acid (55.41)	1.57 ± 0.12ab	2.18 ± 0.15c	2.49 ± 0.11c	1.13 ± 0.03a	1.49 ± 0.07ab	1.28 ± 0.06ab	1.15 ± 0.11a	1.44 ± 0.08ab	1.58 ± 0.40b
Hexanoic acid (64.78)	1.67 ± 0.05a	1.89 ± 0.01ab	3.63 ± 0.04c	1.92 ± 0.04ab	2.41 ± 0.15ab	2.46 ± 0.10ab	2.13 ± 0.08ab	2.39 ± 0.06ab	2.76 ± 0.61b
Octanoic acid (75.82)	2.19 ± 0.11a	2.54 ± 0.16ab	2.99 ± 0.32abc	3.03 ± 0.09bc	3.40 ± 0.17c	3.31 ± 0.19bc	2.99 ± 0.24abc	3.37 ± 0.40bc	3.12 ± 0.27bc
Decanoic acid (85.35)	0.18 ± 0.02a	0.23 ± 0.00ab	0.21 ± 0.00ab	0.450 ± 0.01de	0.487 ± 0.008de	0.417 ± 0.012cd	0.379 ± 0.052c	0.434 ± 0.016cde	0.283 ± 0.035b
C6 alcohols									
1-Hexanol (35.07)	1.60 ± 0.06ab	1.51 ± 0.04ab	2.33 ± 0.03ab	1.92 ± 0.063ab	1.68 ± 0.10a	2.36 ± 0.10ab	1.88 ± 0.14ab	1.61 ± 0.06a	2.57 ± 0.91b
trans-3-hexen-1-ol (35.64)	0.057 ± 0.002ab	0.055 ± 0.001ab	0.107 ± 0.000cd	0.054 ± 0.002a	0.075 ± 0.004abc	0.085 ± 0.003bcd	0.058 ± 0.001a	0.071 ± 0.008ab	0.101 ± 0.025d
cis-3-Hexen-1-ol (36.96)	0.200 ± 0.005bc	0.032 ± 0.000a	0.085 ± 0.003a	0.274 ± 0.007c	0.133 ± 0.009ab	0.388 ± 0.011d	0.287 ± 0.005c	0.129 ± 0.011ab	0.412 ± 0.077d
Benzyl alcohol (65.89)	0.218 ± 0.004a	0.53 ± 0.01bc	0.65 ± 0.03cd	0.270 ± 0.019a	0.688 ± 0.046de	0.661 ± 0.013cd	0.444 ± 0.016b	0.836 ± 0.048f	0.784 ± 0.090ef
Terpenes									
Linalool (47.61)	0.006 ± 0.001abc	0.007 ± 0.000bcd	0.005 ± 0.000ab	0.006 ± 0.000ab	0.007 ± 0.000cd	0.005 ± 0.000a	0.008 ± 0.001d	0.008 ± 0.000cd	0.008 ± 0.001d
α-Terpineol (56.06)	0.003 ± 0.000ab	0.005 ±0.000abc	0.004 ± 0.000abc	0.003 ± 0.000a	0.004 ± 0.000bc	0.010 ± 0.000e	0.006 ± 0.001c	0.008 ± 0.000d	0.013 ± 0.001f
Citronellol (60.11)	0.010 ± 0.001e	0.010 ± 0.001de	0.007 ± 0.000abc	0.007 ± 0.000bc	0.008 ± 0.000cd	0.005 ± 0.000a	0.008 ± 0.001bc	0.008 ± 0.000bc	0.007 ± 0.001b
Geraniol (64.56)	0.006 ± 0.001a	0.003 ± 0.000a	0.003 ± 0.000a	0.006 ± 0.001a	0.005 ± 0.000a	0.006 ± 0.000a	0.011 ± 0.002b	0.006 ± 0.000a	0.006 ± 0.002a
Lactones									
γ-Butyrolactone (52.16)	17.37 ± 1.62abc	26.48 ±3.03bc	26.59 ± 3.50bc	8.31 ± 0.913a	12.77 ± 1.06ab	14.62 ± 1.62ab	12.11 ± 1.92ab	17.07 ± 2.32ab	29.86 ± 10.57c
γ-Nonalactone (73.21)	0.013 ± 0.002ab	0.016 ± 0.001ab	0.011 ± 0.000a	0.013 ± 0.001a	0.019 ± 0.001b	0.014 ± 0.000a	0.014 ± 0.001a	0.019 ± 0.001b	0.015 ± 0.004ab

Table 1. (*Continued*)

	Vintage 2009			Vintage 2010			Vintage 2011		
	T	O	V	T	O	V	T	O	V
Volatile phenols									
Vanillin (96.78)	0.102 ± 0.003c	0.098 ± 0.000bc	0.024 ± 0.001a	0.044 ± 0.007a	0.038 ± 0.006a	0.045 ± 0.008a	0.071 ± 0.011bc	0.070 ± 0.008b	0.078 ± 0.016bc
Methyl vanillate (98.25)	0.008 ± 0.000a	0.015 ± 0.000ab	0.018 ± 0.000b	0.021 ± 0.002bc	0.026 ± 0.002cd	0.029 ± 0.002d	0.019 ± 0.001b	0.026 ± 0.001cd	0.026 ± 0.003cd
Ethyl vanillate (99.36)	0.164 ± 0.021a	0.188 ± 0.018a	0.137 ± 0.015a	0.138 ± 0.030a	0.157 ± 0.008a	0.145 ± 0.008a	0.146 ± 0.015a	0.173 ± 0.008a	0.159 ± 0.014a
Acetovanillone (99.61)	0.041 ± 0.001abc	0.045 ± 0.000bcd	0.030 ± 0.001a	0.068 ± 0.002f	0.053 ± 0.003cd	0.055 ± 0.003de	0.063 ± 0.007ef	0.056 ± 0.005de	0.043 ± 0.006ab
4-Ethyl-2-methoxyphenol (73.45)	0.006 ± 0.000c	0.004 ± 0.000abc	0.004 ± 0.000abc	0.004 ± 0.001bc	0.001 ± 0.000a	0.014 ± 0.000d	0.005 ± 0.000bc	0.003 ± 0.000ab	0.014 ± 0.002d
4-Ethyl phenol (80.45)	0.024 ± 0.001c	0.007 ± 0.000ab	0.020 ± 0.000c	0.009 ± 0.002b	0.002 ± 0.001a	0.069 ± 0.002d	0.020 ± 0.001c	0.008 ± 0.000b	0.072 ± 0.002d
2-Methoxy-4-vinylphenol (81.26)	0.048 ± 0.002a	0.090 ± 0.001abc	0.042 ± 0.002a	0.161 ± 0.012abc	0.122 ± 0.010ab	0.272 ± 0.020de	0.216 ± 0.023cd	0.190 ± 0.043bcd	0.327 ± 0.081e
Guaiacol (65.02)	0.005 ± 0.000bcd	0.004 ± 0.000bc	0.004 ± 0.000a	0.005 ± 0.000cd	0.003 ± 0.000a	0.005 ± 0.000d	0.007 ± 0.000e	0.004 ± 0.000b	0.007 ± 0.000e
4-Propylguaiacol (77.22)	0.000 ± 0.000abc	0.000 ± 0.000abc	0.000 ± 0.000bcde	0.000 ± 0.000cd	0.000 ± 0.000a	0.001 ± 0.000de	0.001 ± 0.000e	0.000 ± 0.000ab	0.001 ± 0.000de
Eugenol (79.89)	0.011 ± 0.001a	0.014 ± 0.001ab	0.012 ± 0.000a	0.013 ± 0.001a	0.011 ± 0.000a	0.013 ± 0.002a	0.020 ± 0.001b	0.014 ± 0.002a	0.015 ± 0.004a
2,6-Dimethoxyphenol (84.39)	0.017 ± 0.001de	0.014 ± 0.000bcd	0.016 ± 0.000de	0.012 ± 0.000b	0.009 ± 0.000a	0.012 ± 0.001bc	0.020 ± 0.000e	0.015 ± 0.001cd	0.018 ± 0.002e
Oak compounds									
Furfural (42.36)	0.097 ± 0.003b	0.134 ± 0.005c	ND	ND	ND	ND	ND	ND	0.058 ± 0.016a
trans Whisky lactone (66.40)	0.072 ± 0.009bc	0.084 ± 0.001c	0.067 ± 0.001abc	0.041 ± 0.006a	0.036 ± 0.001a	0.048 ± 0.009ab	0.066 ± 0.010bc	0.052 ± 0.004ab	0.079 ± 0.018c
cis Whisky lactone (69.83)	0.068 ± 0.009ab	0.110 ± 0.001c	0.082 ± 0.002bc	0.047 ± 0.007a	0.050 ± 0.001a	0.048 ± 0.006a	0.086 ± 0.010bc	0.077 ± 0.005b	0.079 ± 0.016b
Siringaldehyde (111.99)	0.116 ± 0.015b	0.061 ± 0.004ab	0.111 ± 0.003b	ND	ND	0.004 ± 0.000a	0.032 ± 0.031a	0.004 ± 0.000a	0.029 ± 0.011a
Fusel alcohols									
β-Phenylethyl alcohol (67.82)	36.4 ± 0.6ab	57.4 ± 5.4bc	59.34 ± 0.95c	37.2 ± 1.2abc	41.3 ± 4.9abc	41.5 ± 1.3abc	34.65 ± 3.92a	37.12 ± 3.50abc	42.58 ± 15.40abc
1-Propanol	32.8 ± 1.2b	22.77 ± 0.5a	21.97 ± 0.62a	26.18 ± 0.81ab	26.26 ± 0.45ab	21.11 ± 1.65a	26.29 ± 7.16ab	24.41 ± 1.63ab	23.57 ± 0.16ab
Isobutanol	56.23 ± 2.1a	59.82 ± 2.1a	49.37 ± 2.03a	43.32 ± 4.59a	52.20 ± 3.83a	40.87 ± 6.94a	43.78 ± 12.42a	53.73 ± 2.05a	50.96 ± 0.24a
2-Methyl-1-butanol	46.11 ± 1.0ab	76.00 ± 2.2d	75.15 ± 1.91cd	44.75 ± 4.18a	69.09 ± 7.64cd	58.87 ± 6.23bcd	57.86 ± 4.25bc	60.09 ± 2.75bcd	70.76 ± 6.23d
3-Methyl-1-butanol	201.57 ± 3.71ab	261.8 ± 5.1abc	290.6 ± 7.0c	195.7 ± 16.1a	221.3 ± 12.8abc	218.5 ± 26.9abc	205.1 ± 43.7a	224.4 ± 16.2abc	260.8 ± 1.6bc
Isoamyl alcohols	247.68 ± 5.3a	337.8 ± 6.7bcd	365.7 ± 9.1d	240.4 ± 20.2a	290.4 ± 5.2abcd	277.4 ± 33.2abc	262.9 ± 39.5ab	284.5 ± 18.9abc	331.6 ± 4.6cd

ND, not detectable; values are means ± standard deviations. Different letters in the same row indicate that means significantly differ at $P < 0.05$.

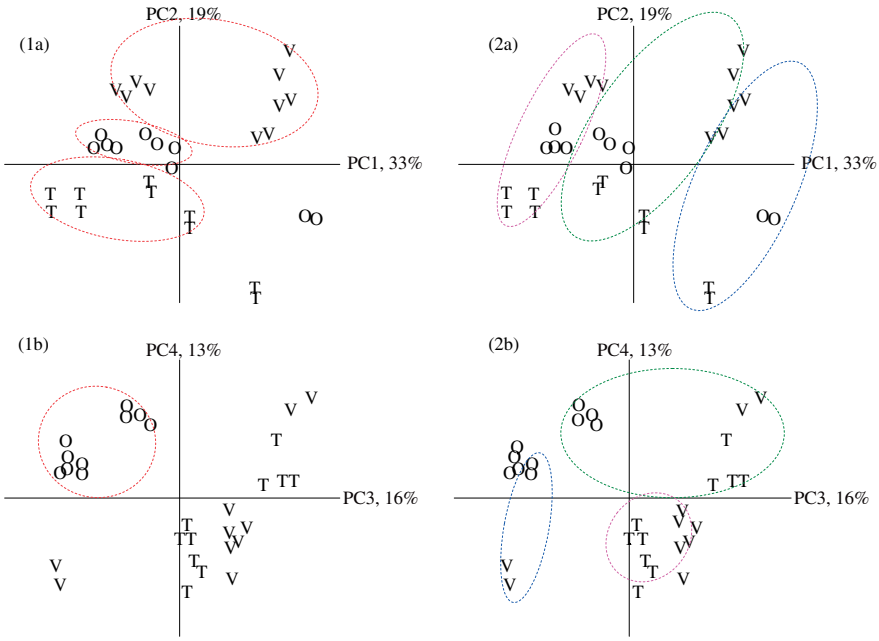

Figure 1. Distribution of the wines in the plane defined by variety: (1a) factors 1 and 2 and (1b) factors 3 and 4; and by vintage: (2a) factors 1 and 2 and (2b) factors 3 and 4. T, Tempranillo wines; V, Maturana Tinta de Navarrete wines; O, Monastel wines. ----, vintage 2009; ----, vintage 2010; ----, vintage 2011.

concentration of amino acids in grape depends on the climatic conditions of each year. Secondly, changes during the alcoholic and malolactic fermentation (temperature, aeration, etc.) could have been occurred among vintages. Finally, oak compounds were also associated with this factor. The number of times the barrels were used could have determined the release of oak wood compounds into wine as their quantity and rate of extraction generally diminish with the utilization of the barrel over successive years. Therefore, this could explain why 2009 wines had higher concentrations of furfural, *cis* and *trans* whisky lactones and siringaldehyde than 2010 wines. However, 2011 wines were richer in these compounds than 2010 wines, which could be due to differences between years in the microbial activity in extractable compounds of the wood. In this way, Hernández-Orte *et al.*[18] found a significant decrease of furfural and 5-methylfurfural during the malolactic fermentation. Figure 1(2b) shows that the variables associated with PC3, mainly related to volatile phenols, also permitted differentiating wines by vintage.

Stepwise linear discriminant analysis was applied as a supervised classification technique in order to determine the volatile compounds most useful for differentiating wines according to grape variety and vintage. The final model by grape variety selected six volatile compounds: linalool, octanoic acid, 4-ethyl phenol, guaiacol, ethyl isovalerate and methyl vanillate (with *F*-values between 41 and 7). Linalool is a varietal terpene characteristic of aromatic varieties.[15] Octanoic acid and ethyl isovalerate are mainly formed during alcoholic fermentation due to yeast metabolism. Many researchers have found the importance of some esters in the differentiation of red varietal wines, resulting in a fruity character of the final wines.[1,15] Guaiacol and 4-ethylpenol can be extracted from wood[1] but they can also be released from non-aromatic precursors present in wine through the fermentation process.[19] Guaiacol and 4-ethyl phenol have also been found in young wines without wood contact, and they may arise from degradation of the lignin of the herbaceous part of the cluster or from the release of their glycosidic precursors.[20] Also, it is important to take into account that 4-ethyl phenol may be formed from ferulic acid,[21] whose levels show important variability among grape varieties. The relationship between grape variety and the latter compound has previously been described in the literature.[22] Methyl vanillate is a varietal compound originating from the precursors present in grapes.[14] It is important to highlight that these results contrasted with those obtained in the PCA, where some C6 alcohols, hexanoic and octanoic acid, and α-terpineol and linalool were able to differentiate wines according to variety, especially Maturana Tinta de Navarrete wines. However, these results agreed with those found by Ortega-Heras *et al.*,[23] who observed that not all wines have the same capacity to extract volatile compounds from the oak wood.

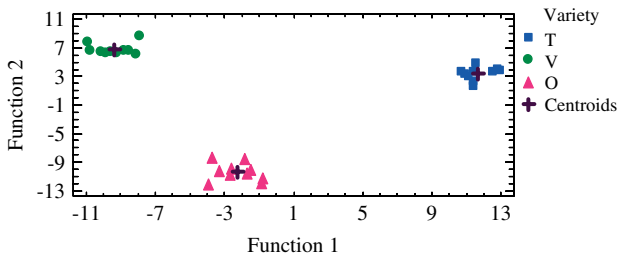

Figure 2. Distribution of wines in the plane defined by the two first discriminate functions by grape variety.

The distribution of wines in the plane defined by the first two discriminant functions is shown in Fig. 2. Applying discriminant analysis, an accurate classification of wines by grape variety was obtained. Taking into account that the distance between centroids is proportional to the similarity between groups, Maturana Tinta de Navarrete wines differed most from the rest of the varieties studied, since they were situated on the left part of the plane.

When stepwise forward discriminant analysis was applied to discriminate wines by vintage, the final model selected nine variables: β-phenylethyl alcohol, furfural, eugenol, ethyl

Table 2. Significance of ANOVA for the factors variety and vintage

	Significance		
	Variety	Vintage	Variety × Vintage
Ethyl esters			
Ethyl butyrate	***	***	***
Ethyl 2-methylbutyrate	***	***	***
Ethyl isovalerate	***	***	***
Ethyl hexanoate	***	***	***
Ethyl lactate	***	***	ns
Ethyl octanoate	***	***	***
Ethyl decanoate	***	***	***
Acetates			
Isoamyl acetate	**	***	***
Hexyl acetate	*	*	*
β-Phenylethyl acetate	***	***	***
Acids			
Isovaleric acid	***	***	*
Hexanoic acid	***	ns	***
Octanoic acid	**	***	ns
Decanoic acid	***	***	**
C6 alcohols			
1-Hexanol	***	ns	ns
trans-3-Hexen-1-ol	***	ns	*
cis-3-Hexen-1-ol	***	***	***
Benzyl alcohol	***	***	*
Terpenes			
Linalool	***	***	***
α-Terpineol	***	***	***
Citronellol	***	***	***
Geraniol	***	***	**
Lactones			
γ-Butyrolactone	***	***	ns
γ-Nonalactone	***	*	ns
Volatile phenols			
Vanillin	***	***	***
Methyl vanillate	***	***	ns
Ethyl vanillate	**	ns	ns
Acetovanillone	***	***	***
4-Ethyl-2-methoxyphenol	***	***	***
4-Ethyl phenol	***	***	***
2-Methoxy-4-vinylphenol	***	***	**
Guaiacol	***	***	***
4-Propylguaiacol	***	***	***
Eugenol	ns	***	*
2,6-Dimethoxyphenol	***	***	ns
Oak compounds			
Furfural	*	*	*
trans Whisky lactone	ns	***	*
cis Whisky lactone	*	***	***
Siringaldehyde	*	***	ns
Fusel alcohols			
β-Phenylethyl alcohol	**	**	ns
1-Propanol	***	ns	ns
Isobutanol	*	*	ns
2-Methyl-1-butanol	***	**	***
3-Methyl-1-butanol	***	**	ns
Isoamyl alcohols	***	***	*

Asterisks indicate significance at
* $P < 0.05$,
** $P < 0.01$,
*** $P < 0.001$; ns indicates no significant difference.

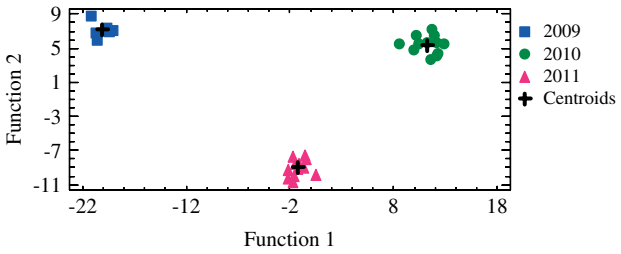

Figure 3. Distribution of wines in the plane defined by the two first discriminate functions by vintage.

2-methylbutyrate, hexyl acetate, siringaldehyde, 4-propylguaiacol, isoamyl alcohol and 1-propanol. (with *F*-values between 43 and 6). Furfural, eugenol, siringaldehyde and 4-propylguaiacol are compounds extracted from the oak wood.[23] Differences between 2009 and 2010 vintages in siringaldehyde and furfural could be explained as the extraction of these compounds decreases due to the depletion of the oak barrel with the years of use. Eugenol showed an irregular behaviour between vintages. Besides the fact that it can be extracted from wood, eugenol is also a varietal aroma belonging to benzene compounds, whose identification in wines is related to a sweet, spicy aroma, especially clove.[15] Isoamyl alcohol and 1-propanol are fusel alcohols and they are correlated with the initial amino acid content in grapes,[1] and thus the ripeness stage and climatic factors can affect the amount of these compounds. Hexyl acetate can change among vintages due to differences in the factors that affect the development of the alcoholic and malolactic fermentation.[24] β-phenylethyl alcohol shows three origins: variety and fermentation (fusel alcohol), and it can also be extracted in small concentrations from oak wood.[23] These results showed that the selected variables to discriminate wines by vintage were strongly dependent on the initial must characteristics, which are strongly dependent on the climatic factors but also on the compounds than can be extracted from the oak wood. Three groups representing each vintage were clearly differentiated in the discriminant analysis by vintage (Fig. 3). It is noteworthy that this distribution matched those obtained in the PCA (Fig. 1(2b)).

Both models, discriminating analysis by grape variety and vintage, were satisfactory, with a global classification of 100% of the wines. However, the mere knowledge of the volatile composition of a wine without a sensory evaluation is inadequate to predict the flavour of the whole system as it is perceived by a trained sensory judge. For that reason, a sensory analysis of the different varietal wines in each vintage was carried out.

Figure 4 provides a GPA consensus configuration of the relationship of the wines in each vintage as determined from their olfactory perceptions. GPA was applied to sensory data to ascertain consistency among the 12 tasters. Before that, the within-judges reproducibility was evaluated by mean of two replicated wines in the tasting session and replications were demonstrated not to be a source of variation.

In the olfactory GPA space of wines from the 2009 vintage (Fig. 4a), wines were properly located in the vectorial dimension defined by the two factors, which accounted for 93.2% of the total variance. The resulting plot showed the wines quite spread, indicating a marked difference among wines. Tempranillo showed a higher correlation with liquorice aromas. Monastel was more correlated with spicy, dairy, coffee and toasted aromas, whereas Maturana Tinta de Navarrete was more correlated with pepper

Table 3. Factor loadings of the wines

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Eigenvalue	14.56	8.57	7.09	5.66	2.25	1.62	1.27
Cumulative variance (%)	33	53	69	82	87	90	93
Ethyl esters							
Ethyl butyrate	**−0.54**	0.45	−0.53		0.29		
Ethyl 2-methylbutyrate	**0.60**	0.34	−0.42	0.45		−0.27	
Ethyl isovalerate	**0.54**	0.35	−0.45	0.51			
Ethyl hexanoate	**−0.79**	0.51					
Ethyl lactate	**0.59**	0.42	0.27		0.35		−0.38
Ethyl octanoate	**−0.89**	0.26		0.25			
Ethyl decanoate	**−0.76**			0.56			
Acetates							
Isoamyl acetate	**−0.92**						
Hexyl acetate	**−0.88**						
β-Phenylethyl acetate	**−0.94**						
Acids							
Isovaleric acid	**0.71**		−0.58				
Hexanoic acid	0.32	**0.79**			0.38		
Octanoic acid	−0.47	**0.65**		0.33			
Decanoic acid	**−0.91**			0.34			
C6 alcohols							
1-Hexanol		**0.68**	0.32	−0.53			
trans-3-Hexen-1-ol	0.37	**0.84**					
cis-3-Hexen-1-ol		0.38	**0.83**	−0.27			
Benzyl alcohol		**0.78**		0.47	−0.34		
Terpenes							
Linalool	0.28			**0.81**			
α-Terpineol	0.32	**0.63**	0.53	0.35	−0.27		
Citronellol	0.29	**−0.79**	−0.32	0.28			
Geraniol		−0.29	**0.65**	0.41	0.43		
Lactones							
γ-Butyrolactone	**0.82**	0.41					
γ-Nonalactone				**0.91**			
Volatile phenols							
Vanillin	**0.55**	−0.52	0.34	0.34	−0.27		
Methyl vanillate	−0.43	**0.74**		0.41			
Ethyl vanillate	0.34			**0.58**	−0.40		
Acetovanillone	**−0.81**		0.30				
4-Ethyl-2-methoxyphenol	0.27	0.44	**0.68**		−0.41		
4-Ethyl phenol	0.29	0.56	**0.65**		−0.32		
2-Methoxy-4-vinylphenol		0.46	**0.76**	0.36			
Guaiacol			**0.92**				
4-Propylguaiacol							
Eugenol			**0.60**	0.45	0.46	−0.28	
2,6-dimethoxyphenol	0.55		**0.60**		0.45		
Oak compounds							
Furfural	**0.72**	−0.40			−0.50		
trans Whisky lactone	**0.83**		0.33				0.26
cis Whisky lactone	**0.74**	−0.26		0.43	0.27		
Siringaldehyde	**0.74**	−0.39		−0.31			
Fusel alcohols							
β-Phenylethyl alcohol	**0.49**	0.36	**−0.49**		−0.25		
1-Propanol		−0.67				**0.71**	
Isobutanol	**0.53**	−0.26	−0.44	0.33		0.49	
2-Methyl-1-butanol	**0.52**	0.45	−0.30	0.41			
3-Methyl-1-butanol	**0.71**	0.36	−0.26				0.42
Isoamyl alcohols	**0.71**	0.41	−0.29	0.26			0.37

Loadings lower than absolute values of 0.250 are not shown. Bold numbers indicate the higher weight of each compound in each factor.

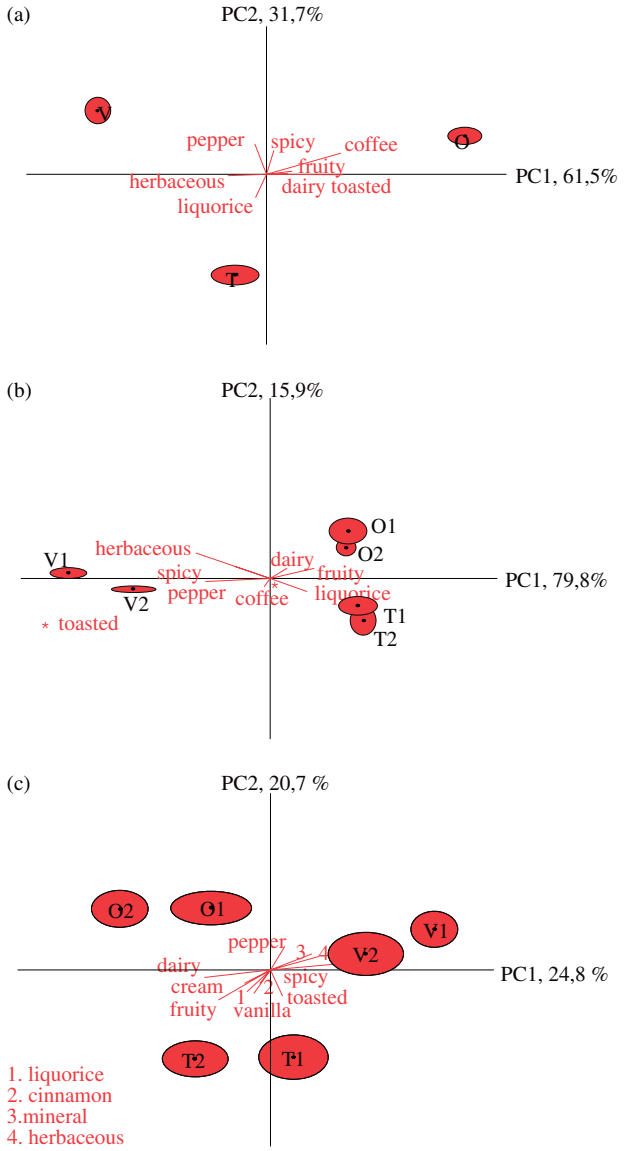

Figure 4. GPA on the mean ratings for olfactory attributes with individual variances of wines in (a) vintage 2009, (b) vintage 2010 and (c) vintage 2011. T, T1, T2: Tempranillo wines; V, V1, V2: Maturana Tinta de Navarrete wines; O, O1, O2: Monastel wines.

and herbaceous odours. In the GPA space of wines from the 2010 vintage (Fig. 4b), 95.7% of the total variance was explained. A good differentiation among varietal wines was also achieved. Maturana Tinta de Navarrete wines were the least fruity, probably due to its low values in acetates, and they were more correlated with herbaceous, pepper and spicy aromas, as well as with C6 alcohols, in good agreement with the results found in Table 1 and Fig. 1(1a). Monastel wines were mainly characterized by fruity and dairy aromas, whereas Tempranillo wines were correlated with liquorice aromas. Furthermore, all tasters gave low scores to coffee and toasted attributes in all wines, which could be related to non-detectable amounts of furfural and other oak volatile compounds in the samples. These results also agreed with those obtained in Table 1 and Fig. 1-2a), where wines of vintage 2010 were poor in volatile compounds extracted from wood. Finally, GPA space of wines from the 2011 vintage only explained 45.5% of the total variance. A higher variability in the wines from the same grape variety was found. As in the 2010 vintage, Maturana Tinta de Navarrete wines were again more related to herbaceous, pepper and spicy attributes and less to fruity notes. Tempranillo wines were characterized by liquorice, fruity and vanilla aromas, whereas Monastel wines were the least aromatic in that year.

CONCLUSIONS

A clear differentiation of the wines according to grape variety and harvesting year was achieved both with PCA and stepwise linear discriminant analysis. The volatile compounds that allowed differentiating wines by grape variety were mainly varietal aromas, whereas vintages were mainly differentiated by volatile compounds formed during the alcoholic fermentation and/or extracted from wood during the elaboration process in wooden barrels. The sensory analysis also allowed differentiating wines by grape variety. Tempranillo wines were characterized by liquorice notes, whereas the Maturana Tinta de Navarrete wines were the least fruity and showed high herbaceous and pepper notes. The sensory profile of Monastel varied between vintages.

ACKNOWLEDGEMENTS

The authors would like to thank to Juan Carlos Sancha for providing the wine samples and Zenaida Hernández for her help with statistics.

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4.4

EFECTO DE LA ADICIÓN DE MANOPROTEÍNAS COMERCIALES EN LOS POLISACÁRIDOS, POLIFENOLES, LA COMPOSICIÓN DEL COLOR Y EL PERFIL SENSORIAL DE VINOS TINTOS VARIETALES

Effect of commercial mannoproteins addition on polysaccharyde, polyphenolic, colour composition and on sensory profile in red varietal wines

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Journal of the Science of Food and Agriculture,

enviado en Julio 2013

RESUMEN

Este artículo aborda tres objetivos fundamentales:

1.- Estudiar el perfil y la composición de los polisacáridos procedentes de la uva en los vinos varietales de Monastel, Maturana Tinta de Navarrete y Tempranillo después de la fermentación maloláctica.

2.- Evaluar el efecto de la adición de manoproteínas comerciales después de la fermentación maloláctica en los polisacáridos, el color, la composición fenólica y el perfil sensorial de los vinos de estudio.

3.- Estudiar la evolución de los polisacáridos, el color y la composición fenólica de estos vinos durante la crianza en barrica y el envejecimiento en botella.

Este estudio se realizó en la añada 2010.

Todos los vinos varietales presentaron un perfil similar de polisacáridos procedentes de la uva: 53-62% de polisacáridos ricos en arabinosa y galactosa (PRAGs), 25-36% de homogalacturonanos (HG) y 6-13% de ramnogalacturonanos tipo II (RG-II). Los vinos de Tempranillo y Monastel mostraron el triple de contenido de RG-II que los de Maturana Tinta de Navarrete. Los PRAGs liberados durante la vinificación presentaron diferencias varietales ya que el ratio arabinosa/galactosa fue de 1,90 para los vinos de Monastel, 1,65 para los vinos de Tempranillo y 1,28 para los vinos de Maturana Tinta de Navarrete.

La adición de manoproteínas no produjo ningún cambio significativo en el contenido de los polisacáridos procedentes de la uva, los antocianos monómeros, los flavonoles o las proantocinidinas. Por el contrario, dicho tratamiento produjo una disminución en el color total del vino, en el color estable y en el contenido de ácidos hidroxicinámicos. Por lo tanto, el empleo de manoproteínas no produjo los efectos esperados y no estabilizó el color ni las proantocianidinas. La disminución en el contenido de ácidos hidroxicinámicos fue atribuida a una adsorción de los mismos por el producto. Así, la adición de manoproteínas podría servir como estrategia para reducir los etilfenoles en los vinos al reducir sus ácidos hidroxicinámicos precursores. En el análisis sensorial, el tratamiento con manoproteínas afectó de distinta forma a los vinos varietales, por lo que no se pudieron obtener conclusiones al respecto.

Los PRAGs y los RG-II descendieron durante la crianza en barrica, siendo los RG-II la familia de polisacáridos más reactiva con disminuciones del 68%. Se observó que cuanto mayor era la concentración inicial de PRAGs y RG-II en los vinos, más descendía su concentración durante el proceso de crianza y envejecimiento. Este hecho indicaba que las técnicas encaminadas a favorecer la extracción de los polisacáridos durante la vinificación no serían tan interesantes como se esperaba. La composición de

los PRAGs se modificó en todos los vinos durante la crianza en barrica, fundamentalmente debido a una pérdida de residuos de arabinosa, lo que hizo descender el ratio arabinosa/galactosa. Con excepción de la tonalidad, el índice de polifenoles totales, y los parámetros de CIELAB b* y L*, todos los parámetros de color y compuestos fenólicos analizados descendieron significativamente en esta etapa.

Los cambios en los polisacáridos de la uva fueron menos intensos durante el envejecimiento en botella que durante la crianza en barrica. El ratio arabinosa/galactosa permaneció constante en esta etapa y se mantuvo también el perfil de polisacáridos de los vinos de Tempranillo y Maturana Tinta de Navarrete. Además, el perfil de polisacáridos de los vinos de Tempranillo después de la conservación en botella fue muy similar a su perfil inicial. El color total del vino disminuyó en esta etapa en todos los vinos debido a una pérdida del 10% en el color debido a los antocianos monómeros. Por el contario, el color estable se incrementó entre un 3-12% debido a un aumento del color estable frente a la adición de bisulfito, que supuso un 35% del color total del vino, y al aumento del color debido a la copigmentación, que supuso un 6% del color total del vino. El resto de compuestos fenólicos disminuyó durante esta etapa.

Finalmente es importante destacar que en todas las etapas en las que se analizaron los vinos, fermentación maloláctica, crianza y envejecimiento en botella, se observaron diferencias varietales en el ratio *trans*-cutárico/*trans*-caftárico, coincidiendo con los resultados obtenidos en el *Artículo 4.1.* de la presente Tesis.

Draft Manuscript for Review

EFFECT OF COMMERCIAL MANNOPROTEIN ADDITION ON POLYSACCHARYDE, POLYPHENOLIC, COLOUR COMPOSITION AND ON SENSORY PROFILE IN RED VARIETAL WINES

Journal:	*European Food Research and Technology*
Manuscript ID:	EFRT-13-0977
Manuscript Type:	Original paper
Date Submitted by the Author:	17-Jul-2013
Complete List of Authors:	Martínez-Pinilla, Olga; Instituto de Ciencias de la Vid y del Vino (Universidad de la Rioja, Gobierno de La Rioja y CSIC), C/ Madre de Dios 51, 26006, Logroño, La Rioja, España., Agricultura y Alimentación Guadalupe, Zenaida; Instituto de Ciencias de la Vid y del Vino (Universidad de la Rioja, Gobierno de La Rioja y CSIC), C/ Madre de Dios 51, 26006, Logroño, La Rioja, España., Agricultura y Alimentación Ayestaran, B.; University La Rioja, Agricultura y Alimentación
Keywords:	mannoproteins, polysaccharides, wine colour, polyphenols, sensory analysis , red varietal wines

EFFECT OF COMMERCIAL MANNOPROTEIN ADDITION ON POLYSACCHARYDE, POLYPHENOLIC, COLOUR COMPOSITION AND ON SENSORY PROFILE IN RED VARIETAL WINES

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ABSTRACT

The aim of this work was to evaluate the effect of commercial mannoproteins on wine polysaccharides, colour stability, polyphenol composition and wine sensory properties. Commercial mannoprotein derivatives were added after malolactic fermentation in three different red varietal wines elaborated under real winemaking conditions. Varietal wines could not be differentiated according to their polysaccharide composition. A higher initial content of polysaccharides was related with a higher precipitation. PRGAs changed their composition during the evolution of wines in barrels. The addition of mannoproteins produced a significant decrease in wine colour, stable colour and in total hydroxycinnamic acids, and it did not produce any change in the rest of the analysed compounds. No significant conclusions could be obtained regarding the sensory analysis. The addition of commercial mannoproteins did not produce either the stabilization of wine colour or the stabilization of proanthocyanidins. However, they could be used after malolactic fermentation to reduce precursors of ethyl phenols in wines.

Keywords: mannoproteins, polysaccharides, wine colour, polyphenols, sensory analysis and red varietal wines.

INTRODUCTION

Wine polysaccharides have a positive influence on several stages of the winemaking process (must racking, fermentation, wine filtration and wine stabilization) and on the organoleptic properties of red wines affecting the final quality of the product [1-3]. In order to increase the final amount of polysaccharides in wines, several winemaking techniques have been used by oenologists: (1) addition of commercial mannoprotein rich preparations in different stages of the winemaking [4]; (2) use of mannoprotein overproducing yeast strains [5]; (3) use of enzymes [6-9]; (4) ageing of wine on yeast lees [10, 11] and (5) a combination of these treatments [10, 12-14].

Nowadays ageing wines on lees is being reconsidered by oenologists because it requires substantial investment in resources (time, vats, barrels, labour, sensorial analyses and batonnâges) and it is not free of problems, disagreeable scents of reduction and risk of microbial deviation [15, 16], even with the addition of enzymes which could favour a faster release of polysaccharides. For this reason, the use of commercial inactive yeast preparations that include in their composition mannoproteins is gaining interest in recent years.

Therefore, the use of mannoproteins has been regulated legislatively for winemaking. Recently, resolutions OENO 416-2011 and OENO 417-2011 from OIV (International Organisation of Vine and Wine) authorised yeast protein extracts as new oenological treatments for the clarification of musts and wines. However, differences in legislation exist between countries.

Although the use of mannoprotein based-products to improve the technological properties of wines has been widely described, their effects on the stabilization of colour and polyphenols, on the reduction of proanthocyanidins and on the improvement of the wine sensory properties have shown contradictory results [1]. Contrary to what it is

described in model solutions by using mannoprotein purified preparations [3, 17], some authors have found that the use of commercial mannoproteins in real vinification situations do not maintain the extracted polyphenols in colloidal dispersion, and neither seem to ensure colour stability [4, 14, 18]. Although the use of mannoproteins is usually associated to the reduction of wine astringency, only a few papers have evaluated their real effect on wine sensory properties [4, 13, 14, 19]. This coupled with the wide variability of commercial preparations of mannoproteins and its derivatives offered by different companies, the dosages used, and the lack about its real composition make difficult the obtainment of conclusions, as recently reviewed Pozo-Bayón et al. [1] Finally, it is important to highlight that almost all the experiments are generally conducted in small cellar [4, 5, 13, 19] without taking into consideration the real winemaking conditions. Furthermore, most of the studies are carried out by adding mannoprotein-based products at the beginning or after the alcoholic fermentation [4, 5, 13, 14].

Therefore, the aim of this work was to evaluate the effects of the addition of commercial yeasts derivatives after malolactic fermentation on polysaccharides, wine colour, polyphenol composition and sensory properties of three different red varietal wines manufactured under real winemaking conditions and dosages, and their evolution over the time. The commercial mannoprotein product was previously characterized.

MATERIALS AND METHODS

Vinifications and samples

Vinifications were carried out in the wine cellar of Juan Carlos Sancha S.L. (Baños de Río Tobia, La Rioja, Spain) using the red grapes Vitis vinífera cv. Tempranillo (TE), Monastel (MON) and Maturana Tinta de Navarrete (MAT). They

were harvested on the vintage 2010 at their optimal stage of ripeness and in good health conditions.

Grapes were destemmed and distributed into 500 L French oak barrels, sulphited with 3 g/HL SO_2 and inoculated with 25 g/HL S. *cerevisiae* yeast strain (Uvaferm VRB, Lallemand Inc. Spain). The fermentation–maceration process was carried out at 25 ± 5 °C and lasted 10 days. Wines were then run off and introduced again into the same 500 L French oak barrels. Barrels were maintained at controlled wine cellar temperature and after malolactic fermentation, wines were racked and corrected to 30 mg/L free SO_2 and introduced again in the 500 L French oak barrels. Half of barrels were maintained as control, labelled as TE, MON and MAT. The other half were treated with 30 g/HL of Noblesse, a commercial yeast derivative from Lallemand Bio S.L. (Spain), and they were labelled as TE-CM, MON-CM and MAT-CM. Dosages, battonage frequency and time of treatment were those recommended by the commercial manufacturer. Both control and treated barrels were battonaged twice a week and the treatment with the commercial product lasted 60 days. After the treatment, wines were racked, clarified, filtered and bottled.

Samples were taken at the beginning of the treatment ($TE_{0\ days}$, $MON_{0\ days}$ and $MAT_{0\ days}$) and at the end of the treatment ($TE_{60\ days}$, $MON_{60\ days}$, $MAT_{60\ days}$, $TE\text{-}CM_{60\ days}$, $MON\text{-}CM_{60\ days}$ and $MAT\text{-}CM_{60\ days}$). To check the treatment effect through the time, samples were taken after wines were aged in bottle during six months ($TE_{240\ days}$, $MON_{240\ days}$, $MAT_{240\ days}$, $TE\text{-}CM_{240\ days}$, $MON\text{-}CM_{240\ days}$ and $MAT\text{-}CM_{240\ days}$). All experiments were made in duplicate.

Determination of usual oenological parameters

All wines were analysed for ethanol concentration, pH, titratable and volatile acidity and free sulphurous acid (Table 1) according to the OIV official practices (1990).

Analysis of the commercial yeast derivative used

Monosaccharide composition of the mannoprotein-based product was determined by gas chromatography-mass spectrometry (GC-MS) [20]. High-resolution size-exclusion chromatography (HRSEC) with a refractive index detector (HRSEC-RID) was used to obtain the molecular weight distributions of the commercial mannoprotein-rich product [20]. All analyses were performed in triplicate.

Analysis of wine polysaccharides

Wine polysaccharides were recovered by precipitation after ethanolic dehydration [20] and the monosaccharide composition of the polysaccharide soluble precipitates was determined by GC-MS [20]. The content of each polysaccharide family was estimated from their concentration of individual glycosyl residues which are characteristic of structurally-identified wine polysaccharides. Total polysaccharides were estimated from the sum of polysaccharides rich in arabinogalactan-proteins and arabinans (PRAGs), mannoproteins (MP), glucans (GU), rhamnogalacturonan-II dimmers (RG-II) and oligomers of homogalacturonans (HG). The molecular weight distributions of the wine polysaccharides was also analysed by HRSEC-RID [20]. All analyses were performed in triplicate.

Analysis of colour parameters and total polyphenol index

Wine red colour (WC), monomeric anthocyanin colour (MAC), copigmentation colour (CC), and bisulphite-stable colour (BSC) were determined by the method proposed by Levengood and Boulton [21]. Wine stable colour (SC) was calculated as the sum of CC and BSC. Colour intensity (CI) was calculated as the sum of absorbances

at 420, 520, and 620 nm, and Hue as A420/A520, at wine pH. The CIELAB rectangular parameters (L*, a* and b*, illuminant D65 and 10° observer conditions) were determined according to Ayala et al. [22]. Total polyphenol index (TPI) was determined by absorbance at 280 nm of diluted wine with synthetic wine. All measurements were performed in triplicate and referred to 10 mm path length quartz cells.

Analysis of monomeric phenolics

Anthocyanins, hydroxycinnamic and hydroxybenzoic acids, flavonols and total resveratrol were analysed by HPLC [23]. The sum of non-acylated anthocyanins, acetylglucoside anthocyanins and coumaryl-glucoside anthocyanins was referred as total monomeric anthocyanins (T-AN). Total hydroxycinnamic acids (T-HA) were calculated as the sum of caffeic, ferulic and coumaric acid, and hydroxycinnamates, as the sum of trans-caftaric, cis-caftaric, trans-coutaric, cis-coutaric and trans-fertaric. Total flavonol content (T-Fla) was calculated as the sum of myricetin-3-glucoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-glucuronide, myricetin, quercetin, kaempferol and isorhamnetin. All analyses were performed in duplicate.

Analysis of proanthocyanidins

Wine samples were firstly fractionated by gel permeation chromatography (GPC) [24]. Fractionation was performed in triplicate and phloroglucinol adducts were analysed in F2 fractions by reversed-phase HPLC [25]. Total proanthocyanidin content (T-PAs) 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 polymerization (mDP) was calculated as the sum of all subunits divided by the sum of the terminal subunits. All analyses were performed in duplicate.

Sensory analysis

Wines taken at the end of treatment ($t_{60\ days}$) were analysed for sensory profiling, and they were judged for visual (colour), olfactory (volatile fraction), and gustatory (taste and mouth-feel sensations) quality conformance to wine typology. A panel of 15 tasters, wine professionals from the D. O. Ca. Rioja, was convened. All wine tasters had participated on previous aroma and mouth-feel sensory descriptive panels and had regularly participated in quality scoring varietal wine sensory panels. Tasters rated 11 attributes for the olfactory phase and 9 for the gustative, scoring the intensity of each attribute on an interval scale with 5 levels of intensity (0 = no aroma or no taste; 1 = weak aroma or weak taste; 5 = strong aroma or strong taste; intermediate values did not bear description). The colour was also judged and blue-red colour was rated according to its intensity on an anchored scale with five levels of intensity (0 = no blue-red colour; 5 = extremely strong blue-red colour). Wines were presented at 18 °C in coded standard wine-tasting glasses according to standard 3591 (ISO 3591, 1977). Assessment took place in a standard sensory analysis chamber (ISO 8589, 1998) equipped with separate booths. One wine was replicated in order to ascertain judges' consistency.

Statistical analysis

Significant differences between analytical determinations were analysed by a two-way analysis of variance (ANOVA). Sensory data were subjected to ANOVA analysis to determine the within judges reproducibility in rating two replicated wines and to find significant differences between wine attributes. ANOVA evaluations were performed using the Statistica 8.0 programme for Microsoft Windows (Statsoft Inc., Tulsa, Oklahoma) and sensory data by using the Senstools Version 3.3.2. Program (Utrecht, the Netherlands).

RESULTS AND DISCUSSION

Characterization of the commercial mannoproteins used

The characterization of the commercial product was firstly carried out by its molecular weight distribution, showing two populations of molecular sizes of 152 KDa (81%) and 12 KDa (19%). Secondly, the polysaccharide purity of the commercial product was evaluated and it was found to be 89.7%, expressed as the total amount of monosaccharides in relation to the weight of the product analyzed. Only mannose and glucose could be quantified in the preparation. The proportion of mannose, used to estimate the mannoprotein content, was found to be 70.6%. The percentage of glucose, used to estimate the glucan content, was 29.4%. Those results confirmed the prevalence of parietal mannoproteins and some glucans.

Effect of commercial mannoprotein addition on the molecular weight distribution of polysaccharides

HRSEC-RID on Shodex column allowed us to follow the qualitative changes in the molecular weight distribution of polysaccharides during the winemaking process (Fig. 1). Therefore, chromatograms of wines were analyzed in order to establish differences due to varietal characteristic (Fig. 1a), treatments and times (Fig. 1b, 1c and 1d). Polysaccharides isolated from the three varietal wines were characterized by three peaks before treatment (Fig. 1a). Hence, the first peak corresponded to populations of 120 KDa in TE and MON, and of 99 KDa in MAT. The second and third peak corresponded to populations of 30 and 12 KDa, respectively. Similar areas were obtained for the first two populations, which have been previously related to a complex mixture of high and medium molecular weight PRAGs from grape berries and high-and medium molecular weight MP from yeast [26]. On the contrary, the third peak, which has been related with RG-II and to low-molecular-weight PRAGs and MP [26], showed a higher population in MON wines than in the rest, probably because MON wines showed the highest ripeness degree (24.9 ºBrix), that could favour a higher extraction of

RG-II due to an extended cell wall degradation in the winemaking. The profile of the varietal wine polysaccharides was in agreement with that found by other authors for other red varieties [8, 20].

Regarding the effect of the addition of commercial mannoproteins on the distribution of the molecular weight of polysaccharides, no significant differences were found between control and treated wines, and the same polysaccharide profile was also obtained after ageing in bottle (Fig. 1b, 1c and 1d). Therefore, the treatment with mannoproteins did not affect the molecular weight distribution of wine polysaccharides.

It is important to point out that the molecular weight distribution of polysaccharides varied after 60 days of treatment (Fig. 1). Thus, MON and TE wines showed a shift to lower molecular weight (from 120 to 99 KDa) after 60 days of study in both control and treated wines, indicating that PRAGs and MP have been partially degraded (Fig. 1c and 1d), while MAT wines (Fig. 1b) did not change their molecular weight distribution. Changes to lower molecular weights could result in more stability because mannoproteins of lower size have been associated with the improvement of colloidal stability [3]. No differences were observed in the molecular weight distribution of polysaccharides during the stage of bottle aging (Fig. 1b, 1c and 1d), in good agreement with previous observations of our group [26]. The differences observed in the peak areas during the different stages of study indicated that changes in the polysaccharide contents were occurring.

Effect of commercial mannoprotein addition on yeast polysaccharide families

In order to know the effectiveness of the addition of the commercial product, the content of mannoproteins and glucans was assessed in control and treated wines before and after the addition of the product (Fig. 2). Mannoprotein-treated wines just after addition showed between 117 to 174 mg/L more mannoproteins and 46 to 69 mg/L

more glucans than control wines, indicating that around 56 to 80% of the added product remained in solution after their addition. However, both control and treated wines showed similar contents of mannoproteins and glucans after 60 days of treatment because their content significantly decreased in the treated wines (around 70% in mannose and 57% in glucose). As no differences were found between control and treated wines on the molecular weight distribution of polysaccharides, the reduction in the MP content could not be attributed to enzymatic fragmentation, suggesting instead a molecular precipitation and/or a reaction of these molecules with other wine compounds. The content of MP after 60 days of treatment was between 10.5 to 13.2% of total polysaccharides and the content of GU was between 14.3 to 16.6%, maintaining similar percentages than those obtained before treatment. No noteworthy changes were observed in MP and GU during ageing in bottle.

Effect of commercial mannoprotein addition on grape polysaccharide families

Grape polysaccharide families were firstly studied in wines before the treatment (0 days) in order to search for varietal differences. The values found in total grape polysaccharides before treatment were 435 ± 10 mg/L in Maturana Tinta de Navarrete wines, 607 ± 278 mg/L in Tempranillo and 720 ± 5 mg/L in Monastel wines (data not shown). These values were in the range described in other studies for other red varieties [8, 20, 27]. All wines showed similar polysaccharide compositional patterns. PRAGs, localized in soluble form within grape cell walls, were the most prevalent polysaccharide family in all the varietal wines, indicating that these molecules were easily extracted by endogenous enzymes during the winemaking. However, Tempranillo and Monastel wines contained higher percentages of RG-II (~2 fold more) than Maturana Tinta de Navarrete wines. Hence, Tempranillo wines profile showed 53% of PRAGs, 36% of HG and 11% of RG-II. Monastel wines were composed of 62% of

PRAGs, 25% of HG and 13% of RG-II, whereas Maturana Tinta de Navarrete wines were characterized by 59% of PRAGs, 35% of HG and 6% of RG-II. RG-II is tightly bound to the cell wall matrix of grape cell walls and it is resistant to pectinolytic enzymes, needing long maceration times to solubilize. Although all wines were elaborated under the same conditions, differences in RG-II could be related, on the one hand, to differences in maturity stages between the cultivars (Table 1), and on the other hand, to differences in the weakness of the grape skins that could modulate the extraction of wine components, which suggest a certain varietal characteristic. It is important to point out that the polysaccharide profiles obtained did not agree which those found by others studies [4, 5, 26, 27], probably due to the high content of HG (from 105 to 370 mg/L). The type of concentration to dryness used to obtain the polysaccharide fraction probably resulted in a major content of homo- and rhamnogalacturonans of lower molecular weight [20]. Differences were also observed in the arabinose/galactose ratio among the varietal wines. This ratio is characteristic of the wine arabinogalactan-protein composition and it seems to be different depending on the type of winemaking, being around 1 in red winesc[11, 26, 27], 0.2 to 0.4 in white wines and sparkling wines [28], or red wines aged on lees [11]. Monastel wines showed a ratio arabinose/galactose of 1.90 while it was 1.65 in Tempranillo and 1.28 in Maturana Tinta de Navarrete wines, (Fig. 3d), indicating that the PRGAs released during the winemaking differed between varietal wines.

Regarding the effect of the mannoprotein addition on the grape polysaccharides, no noteworthy differences were found between control and treated wines in PRAGs, HG or RG-II (Fig. 3) neither after 60 days of treatment nor after ageing in bottle (240 days).

Grape polysaccharides evolved differently during the studied period. Thus, a great decrease in both PRAGs and RG-II (Fig. 3a and 3c) was observed from 0 to 60 days of treatment. On the contrary, HG significantly increased. Decreases in PRAGs and RG-II could be attributed to electrostatic and ionic interactions with other wine constituents that could lead to the formation of unstable complexes and precipitation phenomena. The highest decreases were observed for RG-II molecules (68% versus 45% in PRAGs), which could be due to the fact that RG-II have the highest negative charge density among wine polysaccharides, being the most reactive family. Moreover, we observed that the highest the initial content of both PRAGs and RG-II in wines, the highest they decreased during treatment, showing a Pearson correlation of r=0.91 (p<0.01) and r=0.948 (p<0.01), respectively. These results suggested that techniques employed to increase the extraction of polysaccharides during the winemaking would not be as interesting as expected because a higher initial content could be related with a higher precipitation. It is important to point out that the ratio arabinose/galactose significantly decreased after the treatment, reaching values of 1.08 in Maturana Tinta de Navarrete wines, 1.14 in Tempranillo and 1.33 in Monastel wines. Therefore, PRAGs content decreased during this period but also its composition was also strongly modified due to an important loss of arabinose residues, which would suggest an enzymatic activity. Loss of terminal arabinose residues have been also reported during the ageing of wines on lees [11]. Structural modification of PRAGs is likely to have a direct incidence on their physicochemical properties and thus on the quality and the organoleptic properties of the wines. HG increases from 0 to 60 days of treatment were attributed to the hydrolysis of hemicelluloses from the oak barrel, probably due to an enzymatic polygalacturonase activity. A similar result was also found in the winemaking of old cider brandies storage in French oak barrels [29]. Wines after

treatment showed a similar polysaccharide composition, with average percentages of 49% in PRAGs, 46% in HG and 5% in RG-II.

Regarding the stage of aging in bottle (60 to 240 days), decreases of 24% in PRAGs and 46% in HG (Fig. 3a and 3b) were observed. On the contrary, RG-II remained almost constant (Fig. 3c). Decreases in PRAGs and HG were again attributed to precipitation reactions due to the formation of unstable complexes between polysaccharides and other wine compounds and/or a colloidal sedimentation in bottle. It is important to highlight that grape polysaccharide families were less reactive during ageing in bottle than in the previous stage, suggesting that reactions occurred more slowly, probably due to the reductive conditions found in bottle. Doco et al. [30] also found slow hydrolytic phenomena of polysaccharides in bottle-aged wines. The arabinose/galactose ratio remained constant after ageing in bottle, in good agreement with Guadalupe and Ayestarán [26] but showing higher values than those obtained by Doco et *al.* [30]. Tempranillo and Maturana Tinta de Navarrete wines after bottle ageing showed a similar polysaccharide profile, 58% PRAGs, 32% HG and 10% RG-II; whereas Monastel wines were composed of 49 % PRAGs, 45% HG and 6% RG-II. Thus, the polysaccharide prolife of Tempranillo wines after the ageing in bottle was very similar to their initial profile (0 days).

Effect of the commercial mannoprotein addition on colour parameters

Table 2 shows the colour parameters of the control and treated wines over the time. No significant differences were found between control and treated wines in colour intensity (CI), hue, a*, L* or in monomeric anthocyanin colour (MAC), either after the treatment with mannoproteins or after ageing in bottle. On the contrary, significant differences were found in b*, wine red colour (WC) and stable colour (SC). Tempranillo treated wines showed a tendency towards yellow nuances both after the

treatment and ageing in bottle. Values of SC were significantly lower in treated wines than in control ones after 60 days treatment. Monastel and Maturana Tinta de Navarrete also showed lower values of SC in treated wines than in control ones after the period of ageing in bottle. Therefore, the use of mannoprotein did not increase colour stability but the wine stable colour was maintained or even decreased. Although the use of mannoproteins is usually associated with the stabilization of wine colour, scientific researchers do not observe this effect when mannoproteins are tested under real winemaking conditions. Thus, no positive effects were found when different moments of mannoprotein addition were tested, either before the alcoholic fermentation [4, 5, 14] or after the alcoholic fermentation [13, 19] or malolactic fermentation [18]; or when different mannoprotein products were used. However, Rodrigues et al. [18] found a higher content of total anthocyanins in treated wines after 21 months of ageing in bottle, which suggests that reactions would be very slow and long times are needed to ensure colour stability.

Regarding the evolution of colour parameters (Table 2), significant differences were found between 0 to 60 days of treatment. Thus, CI, a*, WC, MAC and SC decreased in all the wines, whereas hue, b* and L* increased, indicating a tendency toward yellow nuances and higher values of lightness. Although CI, hue and CIELAB parameters remained constant during bottle-aging (60 to 240 days of study), WC decreased in all the wines due to a loss of MAC of around 10%. On the contrary, the stable colour increased between 3 to 12% in all the wines as a result of an increase in bisulphite stable colour (BSC) and copigmentation colour (CC) (data not shown). Increases in BSC, which contributed to WC with around 35%; indicated that reactions that lead to the formation of new and stable pigments resistant to the bisulphite addition were occurring. The increase in CC, which contributed around 6% to WC (data not

shown), indicated that copigmentation reactions were occurring during bottle ageing, although this phenomenon has been described as decreasing during bottle ageing in other studies [31].

Effect of the commercial mannoprotein addition on wine polyphenols

The polyphenol content in both control and treated wines through the time is shown in Fig. 4. No noteworthy differences were found between control and treated wines after the treatment with mannoproteins (60 days) and after the ageing in bottle (240 days) in total monomeric anthocyanins (T-AN), total flavonol content (T-Fla), total proanthocyanidin content (T-PAs) and their mean degree of polymerization (mDP), and total polyphenol index (TPI) (Fig. 4a, 4c, 4d, 4e and 4f). On the contrary, the content of total hydroxycinnamic acids (T-HA) was lower in treated wines that in control wines (Fig. 4b). An adsorption of hydroxycinnamic acid tartaric esters in the yeast products was probably occurring in the treated wines because they showed lower values of these compounds than their respective controls. Therefore, the use of mannoproteins could be proposed as a possible strategy to reduce ethyl phenols in wines because they could adsorb the precursors of these types of compounds (coumaric and ferulic acids released from their esters form).

It is also important to highlight that, contrary to expectations, the treatment with mannoproteins did not stabilize the T-PAs content. Mannoproteins had shown stabilizing effects on grape seed tannin aggregation in model solutions depending on its molecular weight, suggesting that the stabilisation mechanism implied steric stabilisation, and explaining why the medium and low molecular weight molecules were more efficient [3]. Furthermore, Rodrigues et al. [18] had observed that low molecular weight mannoproteins were able to delay tannin polymerisation in red wines. However, both studies were not carried out under real winemaking conditions because either they

used synthetic medium [3] or long times of treatment [18]. On the other hand, our results could have been due to the composition of the commercial mannoprotein-rich preparation we used because it had a high proportion of high and medium molecular weight molecules (81% with an average size of 152 KDa and 19% with an average size of 12 KDa). Moreover, the time might not be enough to produce stabilizing reactions. Our results were in agreement with that obtained by Guadalupe and Ayestarán [4] in a real winemaking situations and conditions, where no protective effect toward tannins was found.

With regards to the evolution of polyphenols, noteworthy differences were found in T-AN, T-HA, T-PAs, TPI and mDP from 0 to 60 days of treatment (Fig. 4). With the exception of flavonols and TPI, all polyphenols decreased during treatment. The increase in the TPI was attributed to the extraction of polyphenols from the oak wood. On the contrary, total monomeric anthocyanins suffered the highest reduction, around 27%, indicating that it was the most reactive family during this period. All type of phenols also decreased during the ageing in bottle, as it was found in other studies of bottle-aged wines [31, 32]. The highest reduction corresponded again to total monomeric anthocyanins (around 50%), probably due to their combination with other compounds to give more stable pigments, in agreement with the decrease we observed in WC and the increase in SC. Flavonols, considered as the best kind of copigmentation cofactors [31], were more reactive in this stage than during treatment, in good agreement with the increase we observed in SC. With regards to proanthocyanidins, the evolution of wine in bottle produced a precipitation of T-PAs formed by long chains, as the mDP decreased.

Finally, it is interesting to point out that the wines showed marked varietal differences, as it was found in previous studies of our workgroup [33]. Thus, the ratio

trans-coutaric acid/trans-caftaric acid (Fig. 4g), considered by some authors as a varietal factor, was maintained over the time, reaching the highest value in Tempranillo wines (0.60), followed by Monastel wines (0.51) and Maturana Tinata de Navarrete wines (0.41). Regarding resveratrol (Fig. 4h), of great importance due to its positive biological effect in human health, Monastel wines showed the highest values, as it was also found in our previous study [33].

Effect of the commercial mannoproteins addition on wine sensory analysis

Sensory evaluation of wines was made after mannoprotein treatment in order to find differences in the sensory perceptions between control and treated wines. No differences were found in the visual phase between control and treated wines, in good agreement with the chemical data obtained in CI. Moreover, no significant differences were found among varietal wines.

In the olfactory phase (Fig. 6a), significant differences were found between control and treated wines. Tempranillo treated wines had more fruity and liquorice aromas than their control wines, whereas Maturana Tinta de Navarrete treated wines showed less spicy and mineral and more herbaceous character than their controls. On the other hand, Monastel treated wines had lower olfactory values in fruity, toasted and liquorice aromas than their controls.

In the gustatory phase (Fig. 6b), Tempranillo treated wines had higher values in warm and dryness, and lower in sweetness and astringency than their controls. On the contrary, Monastel treated wines showed higher values in astringency, acidity and retronasal length than their controls, and lower values in warm. Maturana treated wines showed higher values in mouth length, sweetness, and acidity and lower values in dryness. These results indicated that the use of mannoprotein-rich preparation affected wines in a different way and no significant conclusions could be obtained.

CONCLUSIONS

This paper evaluates the effect of the addition commercial mannoproteins after malolactic fermentation on polysaccharides, colour stability, polyphenol composition and sensory analysis in three varietal wines elaborated under real winemaking conditions. The highest the initial content of polysaccharides, the highest they decrease during aging, suggesting that a higher extraction of polysaccharides would not be as interesting as expected. Varietal wines could not be differentiated according to their polysaccharide composition and thus these compounds cannot be used as taxonomic tools to for classification. Contrary to expectations, the addition of commercial mannoproteins did not stabilize wine colour or total proanthocyanidins, and produced a significant decrease in wine colour, stable colour and in total hydroxycinnamic acids. Mannoprotein addition did not produce any change in the rest of the analysed compounds, and no significant conclusions could be obtained regarding the sensory analysis. Taking to account the results of the present study, it would be interesting to analyse the use of mannoproteins to reduce precursors of ethyl phenols in future researches.

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FIGURE CAPTIONS

Fig. 1. Molecular weight distribution of polysaccharides in a) the three varietal wines before treatment (0 days); b) Maturana Tinta de Navarrete wines over the time; c) Monastel wines over the time and d) Tempranillo wines over the time. TE: Control Tempranillo; TE-CM: Tempranillo treated with commercial mannoprotein; MON: Control Monastel; MON-CM: Monastel treated with commercial mannoprotein; MAT: Control Maturana Tinta de Navarrete; MAT-CM: Maturana Tinta de Navarrete treated with commercial mannoprotein.

Fig. 2. Evolution of the content of a) Mannoproteins (MP) and b) Glucans (GU) in control and treated wines before the addition of the product, after the addition of the product (0 days), at the end of treatment (60 days) and after ageing in bottle (240 days). TE: Control Tempranillo; TE-CM: Tempranillo treated with commercial mannoprotein; MON: Control Monastel; MON-CM: Monastel treated with commercial mannoprotein; MAT: Control Maturana Tinta de Navarrete; MAT-CM: Maturana Tinta de Navarrete treated with commercial mannoprotein.

Fig. 3. Evolution of the content of a) polysaccharides rich in arabinose and galactose (PRAGs); b) oligomers of homogalacturonans (HG); c) rhamnogalacturonan-II dimmers (RG-II) and d) arabinose/galactose ratio in control and treated wines at the beginning of treatment (0 days), at the end of treatment (60 days) and after ageing in bottle (240 days). TE: Control Tempranillo; TE-CM: Tempranillo treated with commercial mannoprotein; MON: Control Monastel; MON-CM: Monastel treated with commercial mannoprotein; MAT: Control Maturana Tinta de Navarrete; MAT-CM: Maturana Tinta de Navarrete treated with commercial mannoprotein.

Fig. 4. Evolution of the content of a) total monomeric anthocyanins (T-AN); b) total hydroxycinnamic acids (T-HA); c) total flavonols (T-Fla); d) total proanthocyanidins

(T-PAs); e) total polyphenols index (TPI); f) mean degree of polymerization (mDP); g) ratio trans-coutaric/trans-caftaric (ratio t-cout/t-caft) and h) total Resveratrol in control and treated wines at the beginning of treatment (0 days), at the end of treatment (60 days) and after ageing in bottle (240 days). TE: Control Tempranillo; TE-CM: Tempranillo treated with commercial mannoprotein; MON: Control Monastel; MON-CM: Monastel treated with commercial mannoprotein; MAT: Control Maturana Tinta de Navarrete; MAT-CM: Maturana Tinta de Navarrete treated with commercial mannoprotein.

Fig. 5. Sensory analysis diagrams of wines at the end of treatment (60 days) of a) olfactory phase and b) gustatory phase. The asterisk indicates statistically significant differences for $p < 0.05$. TE: Control Tempranillo; TE-CM: Tempranillo treated with commercial mannoprotein; MON: Control Monastel; MON-CM: Monastel treated with commercial mannoprotein; MAT: Control Maturana Tinta de Navarrete; MAT-CM: Maturana Tinta de Navarrete treated with commercial mannoprotein.

Table 1. Enological parameters in control and treated wines at the beginning of treatment (0 days), at the end of treatment (60 days) and after ageing in bottle (240 days)

Wine	Stage	% v/v[1]	pH	TA[2]	VA[3]	Free SO_2[4]
Tempranillo	$TE_{0\ days}$	13.6 ± 0.2^{a}	3.58 ± 0.01^{a}	5.6 ± 0.2^{b}	0.41 ± 0.03^{a}	35 ± 1^{a}
	$TE_{60\ days}$	13.5 ± 0.2^{a}	3.59 ± 0.01^{a}	5.6 ± 0.2^{ab}	0.45 ± 0.01^{ab}	34 ± 1^{a}
	$TE_{240\ days}$	13.6 ± 0.2^{a}	3.73 ± 0.01^{b}	5.1± 0.2^{ab}	0.47 ± 0.01^{ab}	33 ± 2^{a}
	$TE\text{-}CM_{0\ days}$	13.6 ± 0.1^{a}	3.58 ± 0.01^{a}	5.5 ± 0.2^{ab}	0.42 ± 0.03^{ab}	35 ± 1^{a}
	$TE\text{-}CM_{60\ days}$	13.5 ± 0.2^{a}	3.60 ± 0.02^{a}	5.5 ± 0.2^{ab}	0.44 ± 0.04^{ab}	33 ± 1^{a}
	$TE\text{-}CM_{240\ days}$	13.5 ± 0.3^{a}	3.72 ± 0.01^{b}	5.0 ± 0.2^{a}	0.49 ± 0.03^{b}	33 ± 3^{a}
Monastel	$MON_{0\ days}$	14.8 ± 0.2^{a}	3.48 ± 0.01^{ab}	5.9 ± 0.1^{ab}	0.41 ± 0.01^{a}	35 ± 1^{b}
	$MON_{60\ days}$	14.7 ± 0.2^{a}	3.53 ± 0.01^{b}	5.8 ± 0.2^{ab}	0.49 ± 0.01^{bc}	33 ± 1^{b}
	$MON_{240\ days}$	14.8 ± 0.3^{a}	3.64 ± 0.01^{c}	5.5 ± 0.1^{a}	0.52 ± 0.01^{c}	26 ± 3^{a}
	$MON\text{-}CM_{0\ days}$	14.8 ± 0.1^{a}	3.47 ± 0.01^{a}	5.8 ± 0.2^{ab}	0.41 ± 0.01^{a}	35 ± 1^{b}
	$MON\text{-}CM_{60\ days}$	14.7 ± 0.2^{a}	3.50 ± 0.03^{ab}	6.0 ± 0.2^{b}	0.47 ± 0.02^{b}	34 ± 1^{b}
	$MON\text{-}CM_{240\ days}$	14.7 ± 0.2^{a}	3.60 ± 0.03^{c}	5.6 ± 0.2^{ab}	0.52 ± 0.01^{c}	25± 2^{a}
Maturana Tinta de Navarrete	$MAT_{0\ days}$	13.2 ± 0.2^{a}	3.45 ± 0.01^{a}	5.8 ± 0.1^{ab}	0.51 ± 0.01^{a}	36 ± 1^{b}
	$MAT_{60\ days}$	13.1 ± 0.3^{a}	3.45 ± 0.01^{a}	6.2 ± 0.2^{b}	0.52 ± 0.04^{a}	35 ± 1^{b}
	$MAT_{240\ days}$	13.2 ± 0.2^{a}	3.58 ± 0.01^{b}	5.9± 0.2^{ab}	0.57 ± 0.04^{a}	26 ± 3^{a}
	$MAT\text{-}CM_{0\ days}$	13.1 ± 0.2^{a}	3.46 ± 0.01^{a}	5.7 ± 0.1^{a}	0.52 ± 0.01^{a}	35 ± 1^{b}
	$MAT\text{-}CM_{60\ days}$	13.2 ± 0.3^{a}	3.46 ± 0.01^{a}	5.9 ± 0.1^{ab}	0.55 ± 0.03^{a}	34 ± 1^{b}
	$MAT\text{-}CM_{240\ days}$	13.2 ± 0.2^{a}	3.57 ± 0.01^{b}	5.9 ± 0.2^{ab}	0.56± 0.03^{a}	27 ± 2^{a}

[1] Millilitres of ethanol for 100 mL of wines at 20ºC; [2] Titratable acidity as g of tartaric acid per litre; [3] Volatile acidity as g of acetic acid per litre; [4] Free sulphurous acid expressed as mg per litre. Values are means± standard deviations. Different letters in the same column for each varietal wines indicate that means significantly differ at $p<0.05$.

Table 2. Colour parameters in control and treated wines at the beginning of treatment (0 days), at the end of treatment (60 days) and after ageing in bottle (240 days)

Wine	Stage	CI[1]	Hue[2]	a*[3]	b*[3]	L*[3]	WC[4]	MAC[5]	SC[6]
Tempranillo	$TE_{0\ days}$	17.9 ± 0.2^{b}	0.65 ± 0.01^{a}	42.3 ± 1.1^{a}	1.35 ± 0.03^{a}	56.9 ± 1.1^{a}	9.09 ± 0.02^{e}	5.95 ± 0.04^{c}	3.15 ± 0.00^{c}
	$TE_{60\ days}$	16.7 ± 0.5^{a}	0.72 ± 0.01^{b}	37.5 ± 2.2^{a}	2.01 ± 0.02^{b}	59 ± 1.5^{a}	8.29 ± 0.01^{d}	5.18 ± 0.03^{b}	3.10 ± 0.01^{b}
	$TE_{240\ days}$	16.3 ± 0.2^{a}	0.75 ± 0.01^{b}	37.5 ± 1.5^{a}	2.01 ± 0.01^{b}	59 ± 0.8^{a}	7.92 ± 0.01^{b}	4.65 ± 0.02^{a}	3.27 ± 0.01^{d}
	$TE\text{-}CM_{0\ days}$	17.7 ± 0.1^{b}	0.64 ± 0.01^{a}	42.3 ± 1.1^{a}	1.35 ± 0.03^{a}	57.0 ± 1.1^{a}	9.11 ± 0.01^{e}	5.96 ± 0.03^{c}	3.16 ± 0.01^{c}
	$TE\text{-}CM_{60\ days}$	16.5 ± 0.3^{a}	0.73 ± 0.02^{b}	36.1 ± 1.2^{a}	3.10 ± 0.05^{c}	59.7 ± 1.1^{a}	8.03 ± 0.01^{c}	5.12 ± 0.04^{b}	3.01 ± 0.01^{a}
	$TE\text{-}CM_{240\ days}$	16.1 ± 0.4^{a}	0.74 ± 0.02^{b}	36.1 ± 0.8^{a}	3.10 ± 0.02^{c}	59.7 ± 0.5^{a}	7.79 ± 0.05^{a}	4.54 ± 0.09^{a}	3.26 ± 0.00^{d}
Monastel	$MON_{0\ days}$	18.5 ± 0.5^{b}	0.65 ± 0.02^{a}	43.4 ± 1.2^{b}	2.94 ± 0.02^{a}	56.1 ± 0.8^{a}	9.37 ± 0.03^{d}	6.22 ± 0.03^{d}	3.16 ± 0.01^{b}
	$MON_{60\ days}$	17.0 ± 0.1^{a}	0.74 ± 0.02^{b}	36.8 ± 0.8^{a}	5.11 ± 0.04^{b}	58.9 ± 1.7^{b}	8.33 ± 0.01^{c}	5.18 ± 0.05^{b}	3.15 ± 0.01^{b}
	$MON_{240\ days}$	16.6 ± 0.3^{a}	0.74 ± 0.01^{b}	36.8 ± 0.5^{a}	5.11 ± 0.05^{b}	58.9 ± 0.3^{b}	8.14 ± 0.02^{b}	4.68 ± 0.02^{a}	3.46 ± 0.00^{d}
	$MON\text{-}CM_{0\ days}$	18.3 ± 0.3^{b}	0.65 ± 0.01^{a}	43.2 ± 1.1^{b}	2.94 ± 0.02^{a}	56.1 ± 0.8^{a}	9.33 ± 0.05^{d}	6.19 ± 0.03^{d}	3.14 ± 0.01^{b}
	$MON\text{-}CM_{60\ days}$	17.0 ± 0.2^{a}	0.73 ± 0.03^{b}	36.8 ± 1.1^{a}	5.05 ± 0.02^{b}	59.1 ± 0.6^{b}	8.34 ± 0.01^{c}	5.23 ± 0.04^{ab}	3.10 ± 0.00^{a}
	$MON\text{-}CM_{240\ days}$	16.5 ± 0.2^{a}	0.74 ± 0.02^{b}	36.8 ± 0.2^{a}	5.05 ± 0.03^{b}	59.1 ± 1.2^{b}	8.05 ± 0.02^{a}	4.68 ± 0.05^{a}	3.37 ± 0.01^{c}
Maturana Tinta de Navarrete	$MAT_{0\ days}$	19.5 ± 0.4^{c}	0.62 ± 0.01^{a}	45.8 ± 2.1^{b}	1.51 ± 0.01^{a}	54.2 ± 0.7^{a}	10.31 ± 0.11^{d}	6.38 ± 0.04^{c}	3.93 ± 0.01^{e}
	$MAT_{60\ days}$	18.3 ± 0.6^{ab}	0.71 ± 0.01^{b}	39.4 ± 1.2^{a}	3.51 ± 0.09^{b}	56.4 ± 1.1^{ab}	9.10 ± 0.03^{c}	5.60 ± 0.06^{b}	3.51 ± 0.01^{d}
	$MAT_{240\ days}$	17.2 ± 0.5^{a}	0.74 ± 0.02^{b}	39.4 ± 0.5^{a}	3.51 ± 0.06^{b}	56.4 ± 0.6^{ab}	8.39 ± 0.03^{a}	4.90 ± 0.09^{a}	3.48 ± 0.01^{c}
	$MAT\text{-}CM_{0\ days}$	19.2 ± 0.2^{c}	0.61 ± 0.01^{a}	45.5 ± 2.1^{b}	1.51 ± 0.01^{a}	54.2 ± 0.7^{a}	10.25 ± 0.07^{d}	6.30 ± 0.04^{c}	3.95 ± 0.01^{e}
	$MAT\text{-}CM_{60\ days}$	17.9 ± 0.3^{ab}	0.72 ± 0.02^{b}	38.8 ± 1.1^{a}	3.37 ± 0.04^{b}	57 ± 1.1^{b}	8.88 ± 0.01^{b}	5.56 ± 0.02^{b}	3.33 ± 0.01^{a}
	$MAT\text{-}CM_{240\ days}$	17.2 ± 0.1^{a}	0.73 ± 0.01^{b}	38.8 ± 1.2^{a}	3.37 ± 0.05^{b}	57 ± 1.2^{b}	8.38 ± 0.01^{a}	4.95 ± 0.04^{a}	3.43 ± 0.01^{b}

[1] CI: colour intensity as sum of absorbances at 420, 520 and 620 nm; [2] Hue: A420/A520; [3] a*: from green to red; b*: from blue to yellow; L*: lightness; [4] WC: red wine colour; [5] MAC: monomeric anthocyanin colour; [6] SC: stable colour. Values are means± standard deviations. Different letters in the same column for each varietal wine indicate that means significantly differ at $p<0.05$.

Figure 1

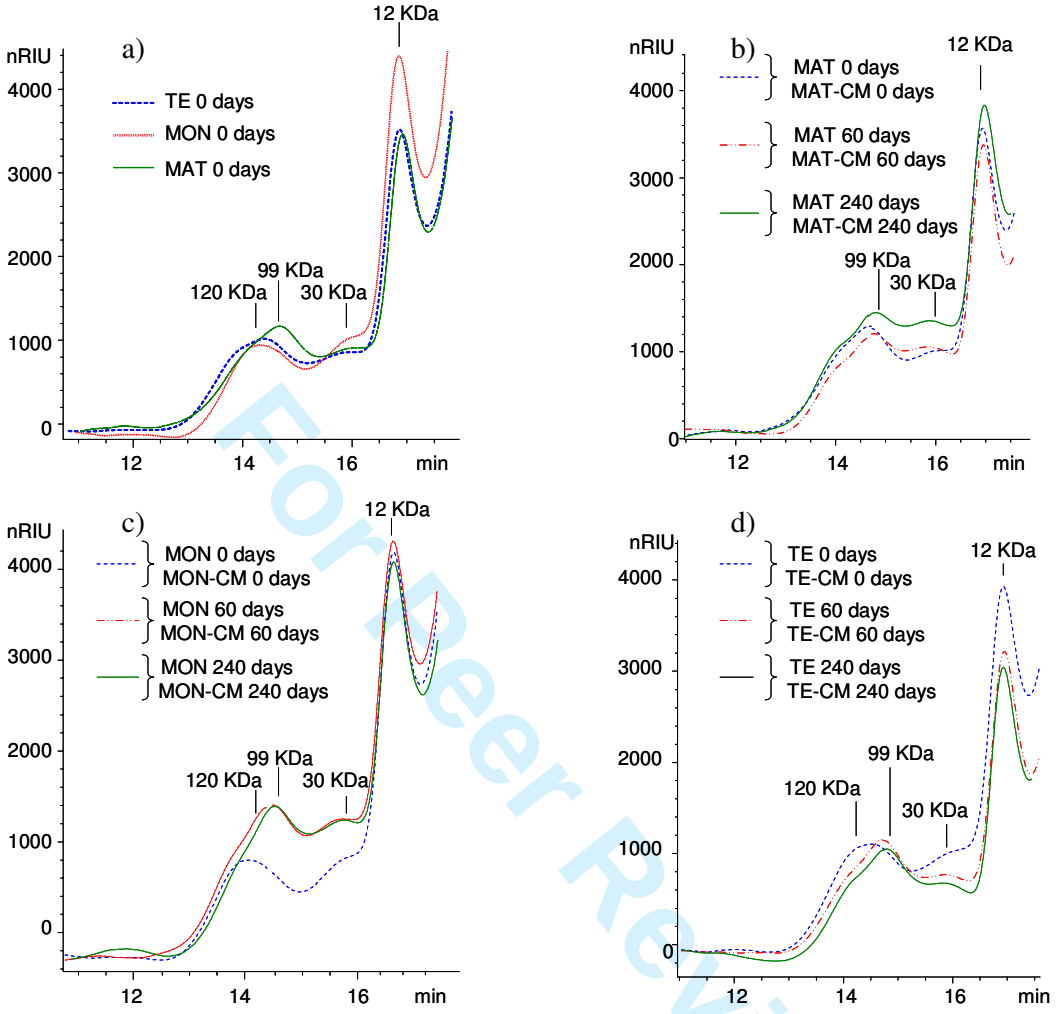


Figure 2

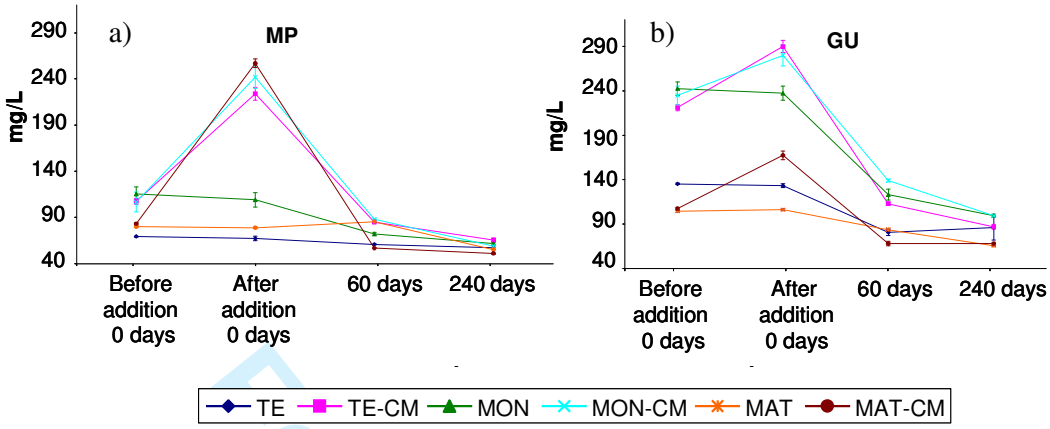


Figure 3

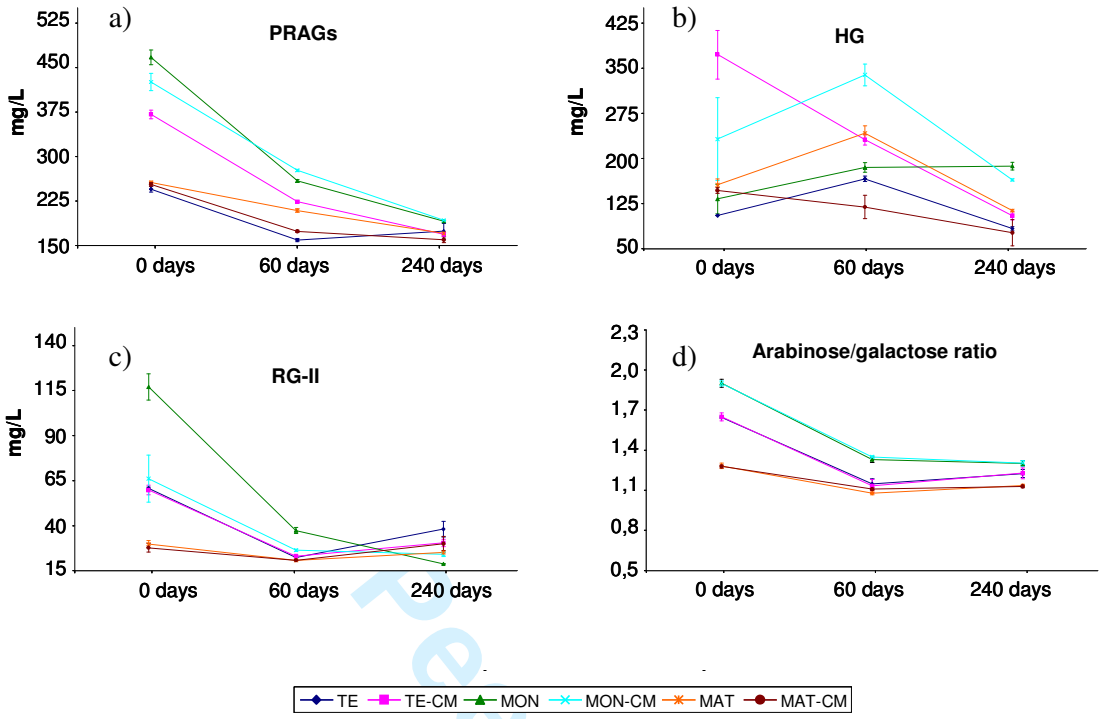


Figure 4

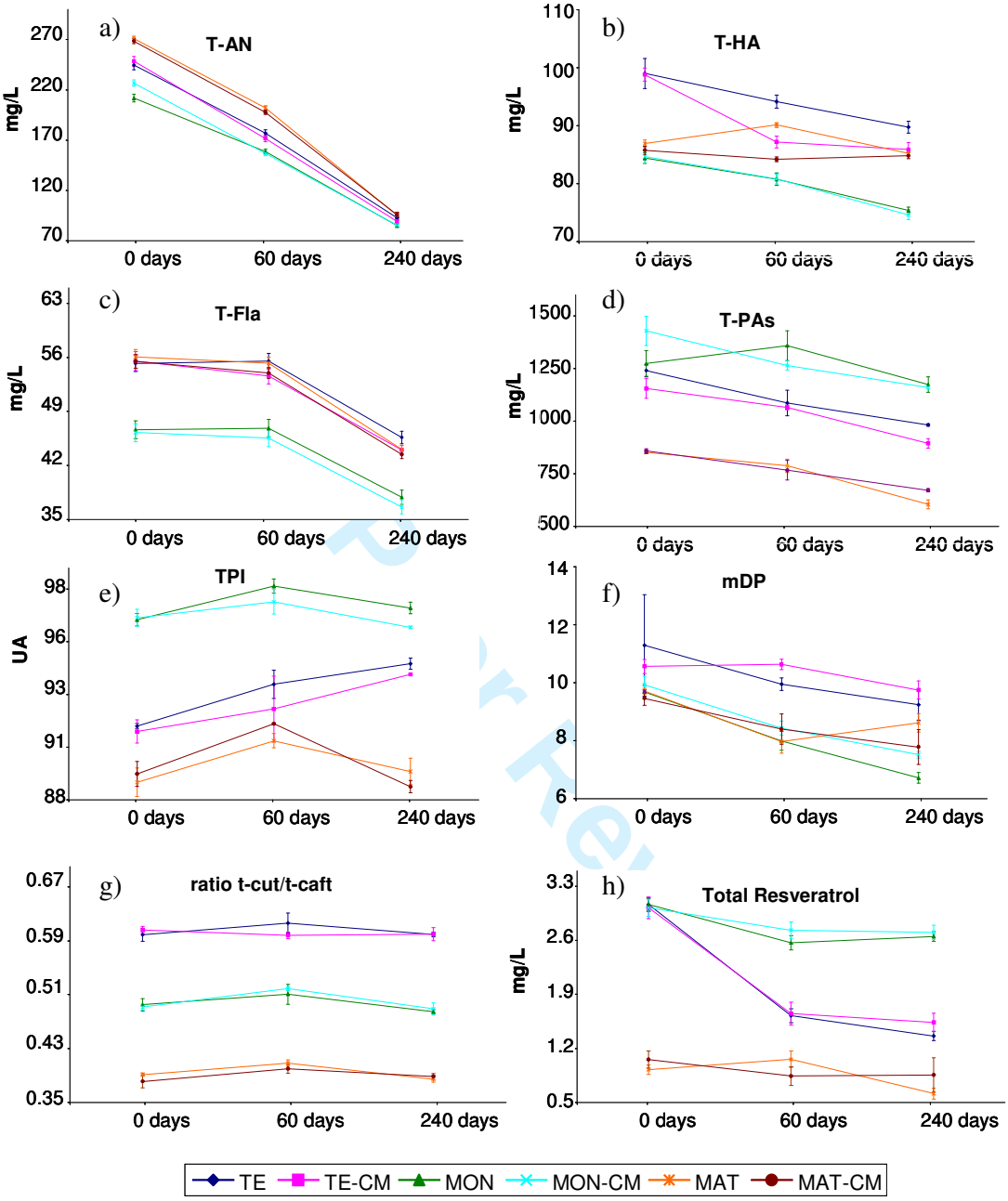
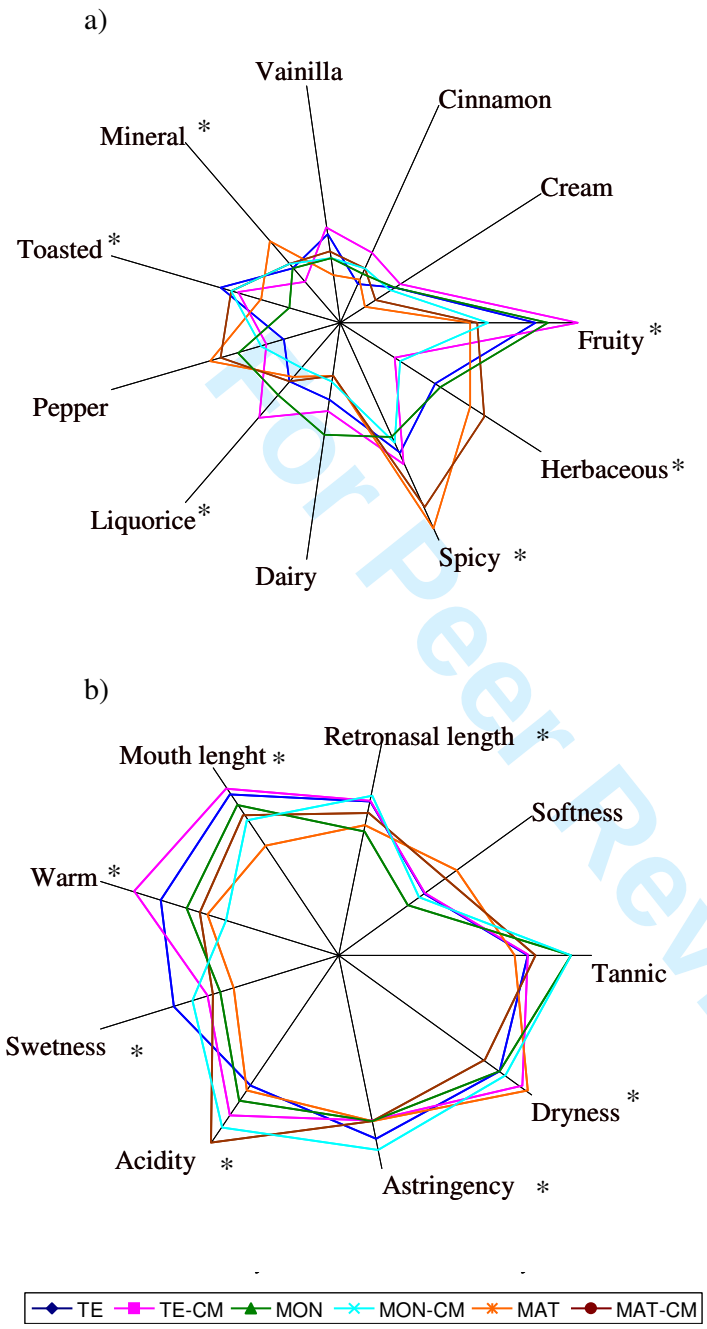

a)
T-AN
mg/L
270
220
170
120
70
0 days
60 days
240 days
b)
T-HA
mg/L
110
100
90
80
70
0 days
60 days
240 days
c)
T-Fla
mg/L
63
56
49
42
35
0 days
60 days
240 days
d)
T-PAs
mg/L
1500
1250
1000
750
500
0 days
60 days
240 days
e)
TPI
UA
98
96
93
91
88
0 days
60 days
240 days
f)
mDP
14
12
10
8
6
0 days
60 days
240 days
g)
ratio t-cut/t-caft
0.67
0.59
0.51
0.43
0.35
0 days
60 days
240 days
h)
Total Resveratrol
mg/L
3.3
2.6
1.9
1.2
0.5
0 days
60 days
240 days
TE
TE-CM
MON
MON-CM
MAT
MAT-CM

Figure 5



4.5

DETERMINACIÓN CUANTITATIVA DE LOS POLISACÁRIDOS DEL VINO POR CROMATOGRAFÍA DE GASES-ESPECTROMETRÍA DE MASAS (GC-MS) Y CROMATOGRAFÍA DE EXCLUSIÓN MOLECULAR (SEC)

Quantitative determination of wine polysaccharides by gas chromatography-mass spectrometry (GC-MS) and size exclusión chromatography (SEC)

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Food Chemistry 2012, Vol. 131, pp. 367-374

RESUMEN

Este artículo aborda dos objetivos fundamentales:

1.- Analizar la idoneidad del uso de la cromatografía de gases con detector de masas (GC-MS) para determinar el contenido en monosacáridos del vino, y por tanto en las familias de polisacáridos, comparándolo con la cromatografía de gases con detector de ionización de llama.

2.- Evaluar el potencial de la cromatografía de exclusión molecular con detector de índice de refracción (HRSEC-RID) como un método rápido y sencillo para estimar el contenido global de polisacáridos en el vino.

El método analítico de cuantificación de polisacáridos por GC-MS, que permite la identificación y cuantificación de los arabinogalactanoproteínas, ramnogalacturonanos tipo II, homogalacturonanos y manoproteínas, se desarrolló en tres etapas:

1.- Extracción de los polisacáridos del vino por precipitación con etanol-ácido previa concentración de la muestra. Se evaluaron diferentes factores que podrían influir en el grado de extracción de los polisacáridos. Se observó que la concentración de la muestra es una etapa fundamental para que no se produzca una subestimación en el contenido en polisacáridos, y se concluyó que la concentración a sequedad es la mejor opción ya que simplifica el método y se obtiene una mayor respuesta de los monosacáridos del vino.

2.- Identificación y cuantificación de los residuos glicosídicos mediante GC-MS de sus trimetil-silil-ester *O*-metil glicósidos (TMS) previa metanolisis ácida y derivatización de la muestra.

3.- Cuantificación de las diferentes familias de polisacáridos de la uva (arabinogalactano-proteínas, ramnogalacturonanos y oligómeros de homo- y ramnogalacturonanos) y de las levaduras (manoproteínas), a partir de su composición individual de residuos glicosilados.

El método propuesto permitió obtener una identificación clara e inequívoca de los monosacáridos del vino, evitando las interferencias y solapamientos que se producían usando el detector de llama. Además, el detector de masas resultó ser más sensible y selectivo que el FID, con menores límites de detección (0,1-0,9 µg MS versus 1-14 µg FID) y de cuantificación (0,4-4,4 µg MS versus 1-21 µg FID), lo que resulta muy útil para cuantificar los azúcares raros de los ramnogalacturonanos tipo II, que están presentes en el vino en concentraciones muy bajas. La precisión de ambos detectores fue muy similar, con valores de reproducibilidad entre 1% y 14% y repetibilidad entre 2% y 12%. El estudio de recuperación de la muestra mostró valores del 81% al 116%, resultados muy buenos teniendo en cuenta que el método se desarrolla en varias etapas.

El método analítico de estudio de polisacáridos por HRSEC-RID se desarrolló en dos etapas:

1.- Extracción de los polisacáridos del vino por precipitación con etanol-ácido previa concentración de la muestra.

2.- Análisis de la fracción de polisacáridos obtenida mediante HRSEC-RID.

Los resultados obtenidos en este estudio indicaron que este método es adecuado para conocer las distribuciones de pesos moleculares de los polisacáridos, y permite además obtener una estimación global del contenido de polisacáridos del vino ya que se obtuvieron buenas correlaciones con los datos obtenidos por GC-MS ($r = 0,746$, $p < 0,05$). A pesar de que la concentración de polisacáridos obtenida por HRSEC-RID fue menor que la obtenida por GC-MS y podría por tanto dar lugar a una subestimación de la cantidad total de polisacáridos, se trata de un método rápido y sencillo que puede usarse con fines comparativos.

Food Chemistry 131 (2012) 367–374

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Quantitative determination of wine polysaccharides by gas chromatography–mass spectrometry (GC–MS) and size exclusion chromatography (SEC)

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ARTICLE INFO

Article history:
Received 7 July 2010
Received in revised form 19 May 2011
Accepted 18 August 2011
Available online 25 August 2011

Keywords:
Wine
Monosaccharides
Polysaccharides
GC–MS
GC–FID
HRSEC-RID

ABSTRACT

Wine polysaccharides play an important role on a number of technological and quality properties of wines and thus several methods have been proposed for their quantification. The present study evaluates the suitability of gas chromatography with mass spectrometry detector (GC–MS) for determining the content of wine monosaccharides and thus polysaccharide families. Factors affecting the yield of polysaccharide precipitation were firstly evaluated and the GC–MS method was characterised and compared with the previously reported GC–FID method. Repeatability and reproducibility values were similar in both methods, with values ranging from 1% to 14%. LODs obtained by MS were below 1.0 μg for all monosaccharides and LOQs were below 1.8 μg. Moreover, a recovery study of the whole method was carried out and it provided absolute recoveries between 81% and 116% for different wine samples, very good values taking into account the multi-step procedure. Both GC–MS and GC–FID were applied to determine the content of wine polysaccharide families in three wine samples and no significant differences were observed. Finally, high-resolution size exclusion chromatography with refractive index detector (HRSEC-RID) was applied to obtain the molecular weight distributions of the wine polysaccharides and to estimate their global content. The correlation observed between the polysaccharide values obtained with the GC method and the HRSEC-RID method ($r = 0.746$, $p < 0.05$) indicated that the latter could serve as a rapid and simple method to give an estimation of total wine polysaccharides although it can not be used to quantify in a precise way.

1. Introduction

Wine and must polysaccharides are a subject of a number of studies since they play an important role on a number of technological and quality properties of wines. Considered as *protective colloids*, they can significantly modify several colloidal phenomena during the winemaking process, such as tartrate salt crystallization (Gerbaud et al., 1997; Gerbaud et al., 1996; Lubbers, Leger, Charpentier, & Feuillat, 1993; Moine-Ledoux & Dubourdieu, 2002), protein haze (Dupin et al., 2000a; Dupin et al., 2000b; González-Ramos & González, 2006; Moine-Ledoux & Dubourdieu, 1999; Waters, Pellerin, & Brillouet, 1994), retention of aromatic compounds (Chalier, Angot, Delteil, Doco, & Gunata, 2005; Dufour & Bayonoue, 1999; Lubbers, Voilley, Feuillat, & Charpentier, 1994; Wolz, 2005), colour stabilization (Escot, Feuillat, Dulau, & Charpentier, 2001; Feuillat, Escot, Charpentier, & Dulau, 2001; Fuster & Escot, 2002; Saucier, Glories, & Roux, 2000), or tannin aggregation and precipitation (Guadalupe, Palacios, & Ayestarán, 2007a; Saucier et al., 2000; Vidal et al., 2004; Wolz, 2005).

Polysaccharides in wine originate mainly from grape primary cell walls and microorganisms acting during the winemaking. The main polysaccharides coming from grape berries cell walls are arabinans and arabinogalactan-proteins (AGP), homogalacturonans (HL) and rhamnogalacturonans (RG-I and RG-II) whereas those released by microorganisms are mainly mannans and mannoproteins (MP) produced by yeasts during alcoholic fermentation or ageing on lees, and glucans produced by *Botrytis cinerea* on infected grapes. Exogenous polysaccharides such as arabic gum and carboxymethyl cellulose could also be present in several commercial wines as they are authorised as additives.

Not all polysaccharides show the same behaviour with respect to wines and their influence on wine will depend not only on their quantity but also on the type of polysaccharide. It has been shown that AGP have greater influence on the filtration procedures than MP (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2002), which are more efficient at reducing protein haze in white wines

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doi:10.1016/j.foodchem.2011.08.049

(Dupin et al., 2000b; Moine-Ledoux & Dubourdieu, 1999; Waters et al., 1994). RG-I and II inhibit hydrogen tartrate crystallization (Gerbaud et al., 1997) whereas AGP do not affect this phenomenon (Ribéreau-Gayon et al., 2002). Among the MP classes present in wine, some have been found to act as protective factors with regards to tartaric acid precipitation (Gerbaud et al., 1997; Moine-Ledoux & Dubourdieu, 1999) and it has also been described that the dimer RG-II/boron can form complexes with di- and trivalent cations which could reduce the level of toxic cations (e.g., Pb^{2+}) in wines (Pérez, Rodríguez-Carvajal, & Doco, 2003; Vidal et al., 2000a). Regarding to wine sensory properties, RG-II dimer seems to favour the self-aggregation of grape seed proanthocyanidins in wine-like solutions, whereas wine MP, acidic AGP and other ionic carbohydrates tend to inhibit tannin aggregation (Carvalho et al., 2006; De Freitas, Carvalho, & Mateus, 2003; Mateus, Carvalho, Luis, & de Freitas, 2004; Riou, Vernhet, Doco, & Moutounet, 2002), and therefore have a different influence on wine astringency and fullness (Vidal et al., 2004).

To determine the content of grape, must or wine polysaccharides, all the methods proposed begin with an extraction step by either direct precipitation with ethanol-acid, concentration-precipitation, dialysis or ultrafiltration. After the extraction step, two alternatives can be chosen to analyse the polysaccharides in the extract: (a) using rapid, simple and global methods for total polysaccharide quantification, (b) using more complex and time-consuming methods to quantify specific monosaccharides present in the wine. Direct quantification of wine polysaccharides are usually based on the precipitation of total wine colloids, followed by colorimetric assays (Segarra, Lao, López-Tamames, & de la Torre-Boronat, 1995) or by the determination of peak areas by size-exclusion chromatography (Dubourdieu, Llauberes, & Ollivier, 1986; López-Barajas, López-Tamames, & Buxaderas, 1998; Palomero, Morata, Benito, Calderón, & Suárez-Lepe, 2009; Palomero, Morata, Benito, González, & Suárez-Lepe, 2007). However, these global methods do not allow the quantification of the different families of polysaccharides present in wines, which can be estimated by assessing its monosaccharide profile. Several methods have been proposed for the identification and quantification of grape and wine monosaccharides: High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Arnous & Meyer, 2009), Fourier transform infrared spectroscopy (FTIR) (Boulet, Williams, & Doco, 2007; Coimbra et al., 2005) and Gas chromatography (GC).

When GC is used two different detectors have been used: flame ionisation detector (FID) and mass spectrometry detector (MS). Although MS is expected to be more sensitive and selective than FID, wine monosaccharide derivatives are usually quantified by GC–FID (Ayestarán, Guadalupe, & León, 2004; Chalier et al., 2005; Doco, Quellec, Moutounet, & Pellerin, 1999; Doco, Vuchot, Cheynier, & Moutounet, 2003; Doco, Williams, & Cheynier, 2007; Dols-Lafargue et al., 2007; Guadalupe & Ayestarán, 2008; Nuñez, Carrascosa, González, Polo, & Martínez-Rodríguez, 2005; Nuñez, Carrascosa, González, Polo, & Martínez-Rodríguez, 2006; Nuñez, Pueyo, Carrascosa, & Martínez-Rodríguez, 2008; Vicens et al., 2009; Vidal, Doco, Pellerin, & Moutounet, 2000b; Vidal, Williams, ÓNeill, & Pellerin, 2003) while GC–MS is generally reported to confirm the identity of each peak (Ayestarán et al., 2004; Doco, ÓNeill, & Pellerin, 2001; Doco et al., 1999). Previous studies of our workgroup have described the quantification of wine polysaccharides by GC–FID and reported the characteristics of the method (Ayestarán et al., 2004), but to our knowledge, the suitability of GC–MS for direct quantification of wine monosaccharide derivatives has not been reported. Therefore, the aim of the present study was to evaluate the suitability of the GC–MS detection for determining the content of wine monosaccharides and polysaccharides. The proposed method was characterised in terms of linearity, detection and quantification limits and repeatability and reproducibility, comparing these results with those previously obtained with the GC–FID method. In order to evaluate the accuracy of the method a recovery study was carried out by using commercial polysaccharides. Moreover, the monosaccharide and polysaccharide composition in three red wines was determined using the GC–MS detection and the FID, and the results were compared. Finally, the potential use of size-exclusion chromatography with refractive index detector (HRSEC-RID) as a simple and rapid method for estimating total polysaccharides in wine samples was evaluated.

2. Materials and methods

2.1. Chemicals

All reagents were analytical grade unless otherwise stated. Standards of different monosaccharides were used to perform the calibration curves. L-fucose, L-rhamnose, 2-*O*-methyl D-xylose, L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, Kdo (3-deoxy octulosonic acid) 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), hexane and acetyl chloride were supplied by Scharlab (Barcelona, Spain), hydrochloric acid 37% was purchased from Carlo Erba (Rodano, Milan, Italy), and dried methanol, pyridine, hexamethyldisilazane and trimethylclorosilane were obtained by Merck (Darmstadt, Germany). Lithium nitrate of HPLC grade supplied by Sigma (Beerse, Belgium) and MilliQ deionized water (Millipore, Molsheim, France) were used. A pullulan calibration kit (Shodex P-82) was obtained from Waters (Barcelona, Spain). All the solutions were filtered through a 0.45 μm filter before use in the HPLC.

2.2. Equipments

High-resolution size-exclusion chromatography (HRSEC) 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 G1362 refractive index detector, a G1313A automatic injector, and 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) and a flame ionisation detector (FID).

2.3. Samples

Commercial polysaccharides were purchased from Lallemand (Lallemand-Inc., Montreal, Canada). Wine samples were elaborated in the wine cellar of Juan Carlos Sancha S.L. (Baños de Río Tobia, La Rioja, Spain) using grapes harvested on the vintage 2008. Three grape varieties from the qualified origin denomination Rioja (D.O.Ca. Rioja) were used: *Vitis Vinífera* L. Cv. red Tempranillo (Wine 1), Monastel (Wine 2) and red Maturana (Wine 3). For the red winemaking, grapes were destemmed and distributed into 500 L French oak barrels, sulphited with 3 g/HL SO_2 and inoculated with 25 g/HL *S. cerevisiae* yeast strain. The prefermentation process went on for 24 h at 12 ± 1 °C; the alcoholic fermentation–maceration process was carried out at a maximum temperature of 28 ± 2 °C and lasted 10 days. During this period, barrels were battonaged sixteen times a day. Wines were then racked and introduced in the same 500 L

French oak barrels and samples were taken and analysed. All vinifications were carried out in triplicate.

2.4. Precipitation of total soluble wine polysaccharides

Wine polysaccharides were recovered by precipitation after ethanolic dehydration. Samples were centrifuged (14,000 rpm for 5 min) using a RC-5B Sorvall refrigerated centrifuge (Du Pont, BH, Germany) and 2.5 mL of the supernatants were taken and introduced into 15 mL falcon-tubes. Three assays were then carried out: (a) polysaccharides were directly precipitated by adding 10 mL of cold acidified ethanol (ethanol of 96% containing HCl 0.3 M) and kept for 18 h at 4 °C (non-concentrated samples); (b) the supernatants were concentrated to dryness in a Jouan RC10-10 centrifugal evaporator (Fisher Scientific, Madrid, Spain), the residues were dissolved in 0.5 mL of water to obtain wine concentrated five times and then 2.5 mL of cold acidified ethanol was added and kept for 18 h at 4 °C (samples concentrated five times); (c) the supernatants were concentrated to dryness in a centrifugal evaporator and the residues directly precipitated with 2.5 mL of cold acidified ethanol and kept for 18 h at 4 °C (samples concentrated to dryness). Thereafter, all the samples were centrifuged (14,000 rpm for 20 min), the supernatants discarded, and the pellets washed several times with 96% ethanol to remove the interference materials. The precipitates were finally dissolved in ultrapure water and freeze-dried using a Virtis freeze drying (New York, USA). The freeze-dried precipitates obtained contained the total soluble polysaccharides (TSP). This polysaccharide extraction was performed in triplicate in each sample.

2.5. Identification and quantification of monosaccharides by GC–MS and GC–FID

The monosaccharide composition of the TSP precipitates was determined by GC–MS and GC–FID of their trimethylsilyl-ester *O*-methyl glycolsyl-derivades (TMS) obtained after acidic methanolyisis and derivatization.

TSP fractions were treated with 1.5 mL of the methanolysis reagent (MeOH containing HCl 0.5 M) in order to hydrolyse neutral and acidic monosaccharides to their corresponding methyl glycosides. The reaction was conducted in nitrogen atmosphere at 80 °C for 16 h and thereafter the excess of reagent was removed using a stream of nitrogen gas. The conversion of the methyl glycosides to their trimethylsilyl (TMS) derivatives was performed by adding 0.5 mL of a mix pyridine: hexamethyldisilazane: trimethylclorosilane (10:2:1 v/v) to the dried material. The reaction was carried out at 80 °C for 30 min and the reagent removed using a stream of nitrogen gas. A solution (25 μL) of derivatized myo-inositol was then added as internal standard and the derivatized residues were extracted with 1 mL of hexane. GC–MS and GC–FID was performed with 1 μL of these solutions and samples were injected in duplicate. Different standard carbohydrates were also converted to their corresponding TMS derivatives and analysed by GC–MS and GC–FID in order to obtain patterns for identification and the standard calibration curves.

The chromatographic column was a Teknokroma fused silica capillary column (30 m × 0.25 mm × 0.25 μm) of phase 5% phenyl – 95% methylpolysiloxane. The oven program started at an initial temperature of 120 °C which was increased at a rate of 1 °C min^{-1} to 145 °C and then to 180 °C at a rate of 0.9 °C min^{-1} and finally to 230 °C at 40 °C min^{-1}. The GC injectors were equipped with a 3.4 mm I.D. and were maintained at 250 °C with a 1:20 split ratio. The carrier gas was helium (99.996%) at a flow rate of 1 mL min^{-1}. Ionisation was performed by electron impact (EI) mode at 70 eV. The temperatures used were 150 °C for the MS Quad, 230 °C for the MS Source, and 250 °C for the transfer line.

2.6. Analysis of polysaccharides by HRSEC-RID

The high-resolution size-exclusion chromatography (HRSEC) system with a refractive index detector was used to obtain the molecular weights and molecular weight distributions of the wine polysaccharides. Two serial Shodex OHpack KB-803 and KB-805 columns (0.8 × 30 cm, Showa Denko, Japan) were used. TSP precipitates were dissolved in 2.5 mL of $LiNO_3$, filtered through a membrane with a 0.45 μm pore size, and 100 μL was injected and eluted with a 0.1 M solution of $LiNO_3$ at a flow rate of 1 mL min^{-1}. Calibration was performed with narrow pullulan molecular weight standards (Shodex P-82, Waters, Barcelona, Spain): P-5, Mw = 5.9 KDa; P-10, Mw = 11.8 KDa; P-20, Mw = 22.8 KDa; P-50, Mw = 47.3 KDa; P-100, Mw = 112 KDa; P-200, Mw = 212 KDa; P-400, Mw = 404 KDa.

2.7. Statistical analysis

Performance of the method and significant differences between samples were evaluated by an analysis of variance (ANOVA). Statistical evaluations were performed using the SPSS 15.0 program for Microsoft Windows (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Extraction of wine polysaccharides

Wine samples were first centrifuged to remove insoluble material and three assays were then carried out: (a) direct precipitation of polysaccharides in non-concentrated samples, (b) precipitation of polysaccharides in samples concentrated five times, and (c) precipitation of polysaccharides in samples concentrated to dryness. Previous studies had proven the concentration step to be critical for the quantitative precipitation of all soluble polysaccharides since some polysaccharide families seemed to be precipitated only partially in non-concentrated wines (Ayestarán et al., 2004; Doco et al., 1999). Concentration by filtration could cause the loss of material on microfilters and heating was avoided to prevent from degradations or losses. Samples were thus concentrated in a centrifugal evaporator with controlled temperature being less than 35 °C. Concentrated samples showed higher concentrations in all monosaccharides than non-concentrated samples indicating that non-concentration could lead to an underestimation. Besides, HRSEC-RID of non-concentrated samples provided very small areas in all the peaks corresponding to high and low molecular weight polysaccharides, to the point that some peaks were even lost (data not shown). When comparing samples concentrated to dryness with samples concentrated five times, the former showed higher concentrations of monosaccharides, which means higher signal/noise ratio in the GC–MS chromatogram and thus higher sensitivity for the lower concentrated monosaccharides. When analysed by HRSEC-RID, both sample preparations showed identical areas in the peaks corresponding to high molecular weight polysaccharides but signals corresponding to low molecular weight compounds were significantly lower in samples concentrated five times, indicating that the precipitation of oligosaccharides and small fragments of wine polysaccharides had been affected by the concentration step. In conclusion, concentration of samples is recommended in order to achieve the quantitative determination of all wine polysaccharides. Concentration to dryness was chosen in order to simplify the method and obtain higher monosaccharide responses, although five-time concentrated samples also ensured the quantitative precipitation of wine polysaccharides. Anyway, and regardless the concentration-step chosen, all the samples must be treated in the same way for comparative purposes.

3.2. Identification and quantification of glycosyl residues by GC–MS detection

The monosaccharide composition of the TPS fractions was determined by GC–MS of their trimethylsilyl (TMS) residues. The identification of the peaks was carried out by comparing retention times and mass spectra with those obtained by injections of pure standards. Typical GC–MS chromatogram for a wine polysaccharide extract is shown in Fig. 1.

In order to be able to quantify the monosaccharides in the GC–MS chromatograms, calibration curves of monosaccharide standards were needed. Standard curves of L-fucose, L-rhamnose, 2-*O*-methyl D-xylose, L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose, Kdo (3-deoxy octulosonic acid), D-galacturonic acid and D-glucuronic acid were used for monosaccharide quantification and myo-inositol was used as internal standard. MS fragmentation patterns reported in Ref. (Doco et al., 2001) were used to identify those monosaccharides for which no commercial standards were available. 2-*O*-methyl fucose, aceric acid and Dha (3-deoxy-D-*lyxo*-heptulosaric acid) and apiose were quantified using the 2-*O*-methyl xylose calibration curve. The different monosaccharides were quantified in selected ion monitoring (SIM) mode, selecting the appropriate number of ions for each compound (m/z) in one segment from 3 to 65 min. D-galacturonic acid, L-rhamnose, L-fucose, D-galactose, D-glucose, D-mannose and D-xylose with 204 ion and D-glucuronic acid, L-arabinose, Kdo, 2-O-methyl-L-fucose, Dha and aceric acid with 217 ion, 2-O-methyl D-xylose with 146 ion, apiose with 191 and myo-inositol (internal standard) with 305 ion. For all the spectra, these ions showed the highest signal/noise ratio and were selected for recording SIM mode chromatograms.

3.3. Performance of the GC–MS detection

The features of the GC–MS detection including equation, the slope with its standard deviation, the correlation coefficients (r^2), the linear range and the limits of detection (LOD) and quantification (LOQ), for the carbohydrate standards are listed in Table 1. The features of the GC–MS method were established after a linearity study using solutions of standard carbohydrates. The analyte to internal standard peak area ratio was used as analytical signal for constructing the calibration graphs. The limit of detection was calculated as the concentration of a signal to noise of three and the limit of quantification from the signal to noise of ten.

The correlation coefficients obtained from the calibration curves were all higher than 0.96 ($p < 0.001$). These curves were, therefore, considered to be linear for the range of concentrations studied (to 1500 μg for majority monosaccharides, i.e., arabinose, rhamnose, mannose galactose, galacturonic acid and glucose, around 500 μg for fucose and glucuronic acid, and 125–270 μg for the rest of monosaccharides). The limits of detection and quantification were good and in all the cases they were below the values obtained for the monosaccharides present in the wine samples.

A validation of the proposed method was carried out by analysing real wine samples. The precision of the GC–MS method was checked in terms of repeatability and reproducibility (Table 1) by means of an analysis of variance (ANOVA). Repeatability was evaluated by the analysis of six aliquots of the same wine under normal operating conditions and it was expressed as relative standard deviation values (inter-wine standard deviation). The reproducibility of the method was expressed as the relative standard deviation values obtained for the three different wines (intra-wine standard deviation). As described in the experimental section, samples were centrifuged and the supernatants concentrated to dryness and precipitated. The residues obtained were freeze-dried, methylated, derivatizated and submitted to GC–MS analysis to calculate the amount of carbohydrates. Both repeatability and reproducibility values were good taking into account that a multi-step procedure was performed with values ranging between 2–12% and 1–14%, respectively.

In order to test the accuracy of the whole method, a recovery study was also carried out (Table 2). Recovery studies were accom-

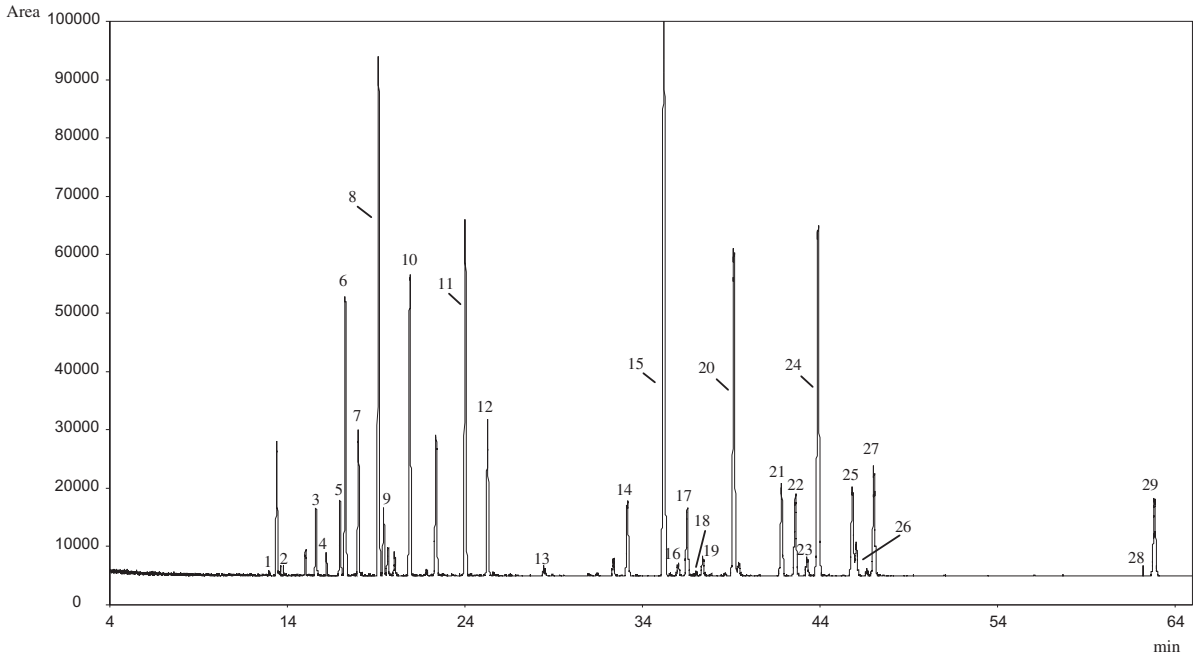

Fig. 1. GC–MS chromatogram obtained for a wine polysaccharide extract. For extraction, chromatographic and detection conditions see Sections 2.4 and 2.5. Peak identification: Aceric acid (peak 1), 2-*O*-methyl-fucose (peak 2), 2-*O*-methyl-xylose (peak 3), apiose (peak 4), arabinose (peaks 5–7, 10, 11), rhamnose (peak 8), fucose (peak 9), xylose (peaks 12–14), mannose (peaks 15, 19), galacturonic acid (peaks 16, 23), Dha (peak 18), galactose (peaks 17, 20, 21, 22), glucose (peaks 24, 26), glucuronic acid (peaks 25, 27), Kdo (peak 28), myo-inositol (peak 29).

Table 1
Calibration curve data[a], calculated detection (LOD) and quantification (LOQ) limits[a] and precision (repeatability and reproducibility) of the GC–MS detection.

Compound	Equation[b]	Sd_{slope}[c]	Correlation coefficient (r^2)	Linear range (μg)	LOD (μg)	LOQ (μg)	Repeatability (%)[d]	Reproducibility (%)[e]
Aceric acid							9	1
2-*O*-methyl fucose							10	1
2-*O*-methyl xylose	A = 0.1278 C	0.0015	0.998	1.3–275	0.4	1.3	12	1
Apiose							3	9
Arabinose	A = 0.5083 C	0.0067	0.998	0.7–1550	0.2	0.7	11	5
Rhamnose	A = 0.6812 C	0.0959	0.960	0.4–1500	0.1	0.4	11	13
Fucose	A = 0.6456 C	0.0109	0.997	0.6–525	0.2	0.6	10	7
Xylose	A = 0.5602 C	0.0083	0.997	0.4–500	0.1	0.4	9	9
Mannose	A = 0.6312 C	0.0861	0.970	2.5–1500	0.1	0.5	8	4
Dha[f]							5	8
Galactose	A = 0.2630 C	0.0031	0.998	1.6–1500	0.5	0.6	6	4
Galacturonic acid	A = 0.1741 C	0.0016	0.999	1.9–1300	0.6	0.9	6	14
Glucose	A = 0.8499 C	0.0165	0.996	1.5–1500	0.5	1.5	8	3
Glucuronic acid	A = 0.2382 C	0.0029	0.998	4.4–475	0.9	4.4	5	2
Kdo[f]	A = 0.1216 C	0.0002	0.999	1.7–125	0.5	1.7	10	13

[a] Values calculated for the monosaccharide standards.
[b] A denote the peak area and C denote concentration in mg.
[c] $n = 3$ replicates.
[d] Calculated as RSD values, $n = 6$ replicates.
[e] Calculated as RSD values, $n = 3$ replicates.
[f] Dha: 3-deoxy-D-*lyxo*-heptulosaric acid; Kdo: 3-deoxy octulosonic acid.

Table 2
Recoveries for the three wine samples.

Compound	Wine 1 % recovery	Wine 2 % recovery	Wine 3 % recovery
Mannose	99.37	80.64	88.73
Galactose	84.13	87.33	89.22
Glucuronic acid	116.45	106.63	95.3
TM[a]	90.25	94.54	103.5

[a] TM, total monosaccharide content.

plished with the three wine samples by using a commercial wine polysaccharide composed of galactose, mannose, glucose and glucuronic acid. Each sample was divided in two fractions, one of them was spiked with the commercial polysaccharide at the 400 mg L^{-1} level, and the other one considered as a blank. Samples were then treated as previously described and recoveries were calculated for monosaccharides which form wine polysaccharides. The obtained recoveries ranged from 81 to 116% (Table 2), again good values for this complex analysis. In contrast to previous studies, this is the first time to study the recovery of the whole method and not just of the chromatographic determination.

3.4. Comparison of the GC–MS detection with the GC–FID detection

Fig. 1 shows the chromatogram obtained for the glycosyl residues of a wine sample using the GC–MS detection. All the chromatograms obtained showed good chromatographic peaks and revealed no evidences of interferences or overlaps between compounds. Although good chromatographic peaks were also obtained for FID (chromatogram not shown), the presence of interferences or overlaps was difficult to detect with this type of detector. Taking into account that the co-elution of compounds is frequent in real wine samples, MS detector was preferred to solve this difficulty.

Features of the GC–MS method were compared with those previously reported for the GC–FID method (Ayestarán et al., 2004). Although the studied linear range differed between MS and FID, the correlation coefficients of the calibration curves were similar for both methods. In principle, both detectors provided similar precisions, with values of repeatability and reproducibility ranging from 1% to 14%; however, detection and quantification limits of the MS method were lower than those from the FID in all cases. LOQ values were even around ten times lower 10 times for the majority of monosaccharides. Thus, the LOQ of some monosaccharides which are present in wines in very low amounts but whose concentrations are essential for RG-II quantification, i.e. 2-*O*-methyl xylose and Kdo, were reduced from 7 to 1.3 μg and from 9 to 1.7 μg, respectively, and the LOQ for glucose, one of the major monosaccharides of wine and must samples, was reduced from 21 to 1.5 μg.

In conclusion, GC–MS quantification was preferred to provide unambiguous identification and quantification of the complex mixtures of monosaccharides present in grape derived beverages. Besides, MS detector showed to be more sensitive and selective than FID, allowing lower detection limits which may be useful when quantifying rare RG-II polysaccharides as they are present in must and wines in very low concentrations.

Table 3
Monosaccharide composition (mg L^{-1}) of three wines determined by GC–MS of their TMS derivatives.

Compound	Wine 1	Wine 2	Wine 3
Aceric acid	1.35 ± 0.14	6.1 ± 0.1	5.3 ± 1.1
2-*O*-methyl fucose	1.8 ± 0.4	7.0 ± 0.2	1.7 ± 0.1
2-*O*-methyl xylose	1.99 ± 0.35	4.8 ± 0.8	4.1 ± 0.2
Apiose	1.54 ± 0.20	10.3 ± 2.4	5.1 ± 0.9
Arabinose	70 ± 8	174 ± 5	186 ± 10
Rhamnose	90.7 ± 7.4	226 ± 47	53 ± 13
Fucose	3.2 ± 0.2	7.0 ± 0.2	4.7 ± 0.9
Xylose	15.0 ± 3.6	11.0 ± 0.1	25.4 ± 1.7
Mannose	179 ± 13	275 ± 35	150 ± 23
Dha[a]	2.53 ± 0.37	14 ± 2	7.7 ± 2.5
Galactose	174 ± 26	228 ± 13	237 ± 19
Galacturonic acid	198 ± 6	72 ± 11	377 ± 29
Glucose	136 ± 9	280 ± 31	315 ± 24
Glucuronic acid	4.3 ± 0.4	14.2 ± 0.1	13.6 ± 1.4
Kdo[a]	4.9 ± 1.6	4.2 ± 0.26	2.18 ± 1.1

Values are means ± standard deviations ($n = 3$).
[a] Dha, 3-deoxy-D-*lyxo*-heptulosaric acid; Kdo, 3-deoxy octulosonic acid.

3.5. Method application: analysis of wine polysaccharides by GC–MS and GC–FID

The described method was applied to analyse polysaccharide families in the three wine samples. Table 3 shows the glycosyl residue composition of the three wines determined by the GC–MS method proposed. Without any exception, all monosaccharides were detected in the wines at the levels above the quantification limits of the method, and all the concentrations were always

Table 4
Polysaccharide concentration (mg L^{-1}) of three wines determined by GC of their TMS derivatives.

Sample	GC detection	Polysaccharide families				
		AGP[a]	MP[a]	RG-II[a]	GL[a]	TPC[b]
Wine 1	GC–MS	324 ± 26[a]	179 ± 13[a]	147 ± 16[a]	182 ± 13[a]	832 ± 36[a]
Wine 2		578 ± 13[b]	275 ± 35[b]	171 ± 13[a]	9 ± 1[b]	1033 ± 44[b]
Wine 3		477 ± 14[c]	150 ± 23[a]	181 ± 13[a]	361 ± 30[c]	1169 ± 41[c]
Wine 1	GC–FID	321 ± 31[a]	160 ± 16[a]	158 ± 18[a]	201 ± 17[a]	840 ± 42[a]
Wine 2		553 ± 36[b]	244 ± 39[b]	197 ± 17[b]	3.82 ± 1[b]	999 ± 56[b]
Wine 3		476 ± 21[c]	132 ± 23[a]	184 ± 17[b]	390 ± 28[c]	1184 ± 46[c]

Values are means ± standard deviations ($n = 3$). For each detection, means in the same column and the same letter are not significantly different ($p < 0.05$).
[a] AGP, arabinans and arabinogalactan-proteins; MP, mannoproteins; RG-II, rhamnogalacturonans-II; GL, oligomers of homo- and rhamnogalacturonans.
[b] TPC, total polysaccharide content estimated as the sum of AGP, MP, RG-II and GL.

within the linear range. Wine samples contained all the monosaccharides that form wine polysaccharides and they were present in similar proportions than those found in other studies (Ayestarán et al., 2004; Doco et al., 1999; Doco et al., 2007; Guadalupe & Ayestarán, 2007b). Aceric acid, 2-O-methyl xylose, 2-O-methyl fucose, apiose, Dha and Kdo, the rare monosaccharides markers of the rhamnogalacturonan II molecule (RG-II), were present in all the samples in lower amounts. On the opposite, rhamnose and galacturonic acid, principal components of rhamnogalacturonans; mannose, the main component of yeast mannoproteins (MP); and arabinose and galactose, the glycosyl residues found in arabinogalactans and arabinogalactan-proteins (AGP), were present in high concentrations. The high quantities of glucose were attributed to the presence of grape and yeast polysaccharides but also to anthocyanins; the presence of xylosyl residues indicated that traces of hemicelluloses (arabinoxylans or xyloglucans) were solubilised from grape cell walls. 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 (Ayestarán et al., 2004; Doco et al., 1999). AGP were therefore estimated from the sum of galactosyl, arabinosyl, rhamnosyl and glucuronosyl residues; all the mannose content was attributed to yeast mannoproteins; the RG-II content was calculated from the sum of its diagnostic monosaccharides, which represent approximately 25% of the RG-II molecule. Taking into account the molar ratios of the RG-II (1 residue of 2-*O*-methyl fucose, 3.5 rhamnose, 2 arabinose, 2 galactose, 1 glucuronic acid and 9 galacturonic acid), the remaining part was attributed to the presence of AGP in the case of rhamnose, arabinose and galactose, and the remaining galacturonosyl residues was used to estimate the content of oligomers of homo- and rhamnogalacturonans (GL). The results of the analysis are shown in Table 4, which shows that except for RG-II, the concentrations of the different polysaccharide families were significantly different depending on the wine sample ($p < 0.05$). The content of total polysaccharides was estimated from the sum of AGP, MP, RG-II and GL.

In order to compare the results of the method using the MS detection and the FID, the monosaccharide (data not shown) and polysaccharide composition of the three wine samples was also analysed by GC–FID. Table 4 shows the results for polysaccharide concentrations analysed by both methods. The comparison of the data obtained with the two methods was carried out by an analysis of variance (ANOVA) as all the data met random and normality assumptions. No significant differences were found in the content of monosaccharide residues and thus in any polysaccharide family ($p < 0.05$), indicating that both methods provided similar results when determining wine polysaccharides.

3.6. Analysis of wine polysaccharides by HRSEC-RID

HRSEC of wine samples on two serial Shodex columns was performed in order to obtain the molecular weights and molecular weight distributions of the wine polysaccharides. Moreover, we tried to evaluate if this method, quite frequent for wine polysaccharide evaluation, could serve as a rapid and simple method to estimate the content of total wine polysaccharides.

The sizes and content of the polysaccharides were compared to those of known pullulan standards. The apparent molecular weights were deduced from the calibration equation $\log M_w = 11.188 - 0.403\ t_R$ (t_R = column retention time at peak maximum, and $r^2 = 0.999$) with a correlation coefficient r^2 of 0.998. Polysaccharide contents were estimated using calibration curves constructed from the pullulan standards; the pullulan P-10 of 11.8 KDa, P-50 of 47.3 KDa, P-100 of 112 KDa, and P-200 of 212 KDa were chosen to obtain the calibration curves because their peaks properly matched with those obtained for the wine samples. The four calibration curves showed a correlation coefficient r^2 higher than 0.998 and they were linear for all the range of concentrations detected (0–1000 mg L^{-1}).

In the wine samples analysed, the HRSEC fractionation allowed the separation of soluble polysaccharides in different peaks (Fig. 2). According to previous work (Guadalupe & Ayestarán, 2007b), higher-molecular-weight polysaccharides, eluting between P50 and P400, corresponded to molecules with an average molecular weight higher than 47.3 kD (average of 212, 112 and 50 kD), and corresponded to a complex mixture of high-molecular-weight AGP from grape berries and high-molecular-weight MP from yeasts. Polysaccharides with an average molecular weight of 12 kD (P10) corresponded to grape RG-II dimers and lower molecular-weight AGP and MP. Signals eluting after P5 corresponded to a molecular weight of less than 6 kD and it was attributed to oligosaccharides and small fragments of AGP, MP and RG-II. Therefore, and in order

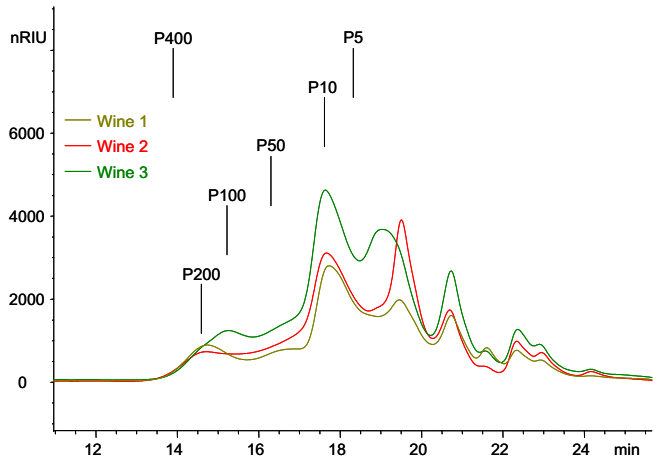

Fig. 2. HRSEC-RID chromatograms of total soluble polysaccharides in three wine samples. Chromatogram obtained using two serial Shodex OHpack KB-803 and KB-805 columns. Elution times for the molecular weight markers (P5 → P400) are shown.

Table 5
Estimated polysaccharide concentration (mg L^{-1} of pullulans) of three wines determined by HRSEC-RID on two serial Shodex molecular exclusion columns.

	Mean molecular mass (kD)				EPC[a]
	212	112	50	12	
Wine 1	89.6 ± 1.8		68.2 ± 1.3	267.6 ± 5.2	425.4 ± 5.7
Wine 2	75.6 ± 1.2		99 ± 0.9	285 ± 4.8	459.9 ± 5.0
Wine 3		126.9 ± 2.7	107.1 ± 3.1	371.7 ± 13.7	605.7 ± 14.5

[a] EPC, estimated polysaccharide concentration as the sum of polysaccharides of different molecular mass. Values are means ± standard deviations ($n = 3$).

to estimate the total polysaccharide content from the HRSEC profile, these last signals were not taking into account and total polysaccharides were estimated from the sum of signals with a higher molecular weight than P5. Table 5 shows the quantities of polysaccharides estimated by HRSEC-RID for the three wines analysed. It was observed again that the estimated polysaccharide content was dependent on the wine sample ($p < 0.05$). The results of total polysaccharide content (TPC) obtained with the GC–MS method and estimated polysaccharide content (EPC) obtained by the HRSEC-RID method were compared by using a correlation matrix. Although the polysaccharide content estimated by HRSEC was considerably lower than that obtained with GC–MS, a good correlation was found between the two methods ($r = 0.746$, $p < 0.05$), indicating that the former could serve as a rapid and simple method for total wine polysaccharide estimation. It is important to notice that HRSEC-RID provided almost half of the value obtained by the chromatographic method and thus it led to an underestimation of real wine polysaccharides; however, it could be valid for comparative purposes.

4. Conclusions

Factors affecting the yield of polysaccharide precipitation were carefully evaluated, concluding that concentration of wine samples was essential to ensure the quantitative precipitation of all wine polysaccharides The suitability of gas chromatography with mass spectrometry detector (GC–MS) for determining the content of wine monosaccharides and thus polysaccharide families was proved. It provided good values of quantification and detection limits and suitable values of repeatability, reproducibility and overall recoveries. The comparison of the results obtained by GC–MS and GC–FID revealed that both methods were suitable for determining the content of monosaccharides in wine samples but MS detector showed to be more sensitive and selective than FID, allowing lower detection limits which may be useful when quantifying rare RG-II monosaccharides which are present in musts and wines in very low concentrations. A further advantage of the MS detection is that it provides unambiguous identification and quantification of the complex mixtures of monosaccharides present in grape derived beverages. Finally, three wine samples were analysed by GC–MS, GC–FID and HRSEC-RID. No significant differences were found for polysaccharide families quantified by MS or FID, indicating that both methods provided similar results. The good correlation observed between the polysaccharide values obtained with the GC method and the HRSEC-RID method ($r = 0.746$, $p < 0.05$) indicated that the latter could serve as a rapid and simple method for estimating the content of total wine polysaccharides although it does not provide information about the concentration of specific polysaccharide families.

Acknowledgements

The authors would like to thank the *Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria* (INIA) for the funding provided for this study through the project RTA2009-00029-C02-01 (with FEDER funds).

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5

CONCLUSIONES

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

1.- En esta Tesis se presenta por primera vez la caracterización enológica de los vinos varietales obtenidos de las variedades minoritarias tintas de La Rioja Monastel, Maturana Tinta y Maturana Tinta de Navarrete en términos de color, composición fenólica, composición de aminoácidos y aminas biógenas, composición volátil, polisacáridos, y análisis sensorial.

Todos los vinos presentaron un alto contenido en polifenoles y resveratrol, indicando un buen potencial para su envejecimiento y buenas características antioxidantes. Los niveles de histamina se encontraron por debajo de los límites considerados como un riesgo para la salud.

Los vinos de Monastel se caracterizaron por tener los valores más altos en intensidad de color, índice de polifenoles totales, color del vino, antocianos, ácido gálico, resveratrol, proantocianidinas, ß-alanina, γ-nonalactona, ácido decanoico y en el ratio arabinosa/galactosa, y por los valores más bajos en flavonoles.

Los vinos de Maturana Tinta se caracterizaron por tener los valores más altos en flavonoles.

Los vinos de Maturana Tinta de Navarrete se caracterizaron por tener los valores más altos en catequina, OH-prolina, ésteres de etilo, 4 etilfenol, alcoholes C_6 y los más bajos en ramnogalacturonanos tipo II.

Los vinos de Tempranillo se caracterizaron por tener los valores más altos de ácidos hidroxicinámicos, grado medio de polimerización de las proantocianidinas y en todos los aminoácidos salvo prolina, OH-prolina y ß-alanina, y por los valores más bajos en lactonas, 2-etilmetilbutirato y etil isovalerato.

Los vinos de Tempranillo y Maturana Tinta de Navarrete fueron similares en los componentes del color y la composición fenólica.

En el análisis sensorial en los vinos de Monastel, Maturana Tinta, Maturana Tinta de Navarrete y Tempranillo durante las distintas sesiones de cata, los vinos no presentaron diferencias significativas en la fase visual (color). En el perfil olfativo, los vinos de Maturana Tinta de Navarrete destacaron por tener un perfil aromático característico, especiado y de pimienta, y diferente al resto de vinos que existen en el mercado. En el perfil gustativo, los vinos de Tempranillo y Maturana Tinta de Navarrete fueron los mejor valorados por su suavidad y equilibrio en boca.

2.- En esta Tesis se presentan datos que pueden servir como criterio taxonómico para diferenciar los vinos varietales Tempranillo, Monastel, Maturana Tinta y Maturana Tinta de Navarrete.

Los vinos de Monastel, Maturana Tinta, Maturana Tinta de Navarrete y Tempranillo pudieron diferenciarse varietalmente en función del perfil de antocianos y el ratio de los ácidos hidroxicinámicos *trans*-cutárico/*trans*-caftárico.

Los vinos de Monastel, Maturana Tinta de Navarrete y Tempranillo pudieron diferenciarse varietalmente en función de su composición en aminoácidos.

Los vinos de Monastel, Maturana Tinta de Navarrete y Tempranillo pudieron diferenciarse varietalmente en función de su composición volátil.

El perfil de estos compuestos se mantuvo en los distintos vinos varietales con independencia del proceso de vinificación o de la añada. Por lo tanto, los datos proporcionados en este estudio pueden servir como criterio químico-taxonómico para identificarlos.

Los vinos estudiados no pudieron diferenciarse varietalmente en función del perfil de aminas biógenas y de polisacáridos.

3.- En esta Tesis se estudia la evolución del color, los polifenoles, los aminoácidos y aminas biógenas y compuestos volátiles antes y después de la fermentación maloláctica en diferentes añadas.

La fermentación maloláctica no modificó las diferencias varietales encontradas en el color y los compuestos fenólicos después de la fermentación alcohólica.

Tanto el perfil de aminoácidos como el de aminas biógenas podrían ser usados para diferenciar los vinos antes y después de la fermentación maloláctica.

El perfil de aminoácidos, aminas biógenas y compuestos volátiles (aromas fermentativos y derivados de la madera) permitió también clasificar a los vinos según la añada.

Los datos proporcionados en este estudio pueden servir como criterio taxonómico para diferenciar a los vinos varietales estudiados por etapa de vinificación (fermentación maloláctica) y añada.

4.- En esta Tesis se evalúa el efecto de la adición de manoproteínas a los vinos varietales de Tempranillo, Monastel y Maturana Tinta de Navarrete después de la fermentación maloláctica.

El empleo de manoproteínas no favoreció la estabilidad del color ni de las proantocianidinas, y produjo por el contrario un descenso en el color total del vino, el color estable y el contenido de ácidos hidroxicinámicos. Se propone por tanto su uso como una posible estrategia para reducir los etilfenoles en los vinos. Los cambios producidos por la adición de las manoproteínas se mantuvieron durante el envejecimiento de los vinos en barrica y en botella.

En el perfil sensorial, la utilización de manoproteínas no tuvo un efecto claro y no se pudieron obtener resultados concluyentes.

La crianza en barrica produjo cambios más acusados en el contenido de polisacáridos, color y compuestos fenólicos que el envejecimiento en botella.

El 35% de los antocianos se encontró en forma de pigmentos estable en los vinos envejecidos en botella, donde aún se detectó un 6% de color debido a la copigmentación.

5.- En esta Tesis se analiza la idoneidad del uso de la cromatografía de gases con detector de masas para determinar el contenido en monosacáridos del vino, y por tanto en las familias de polisacáridos.

La sustitución del detector de ionización de llama por el detector de masas mejoró las características analíticas del método en términos de límites de detección y cuantificación, y permitió obtener una identificación clara e inequívoca de los monosacáridos del vino.

Se evaluó además el empleo de la cromatografía de exclusión molecular con detector de índice de refracción. Se propone esta técnica como un método rápido y sencillo para estimar el contenido global de polisacáridos del vino.