Crianza sobre lías y uso de preparados comerciales derivados de levadura en la calidad de vinos blancos y tintos

FACULTAD DE FARMACIA

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Rueda, 8 de noviembre de 2011

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JULIÁN C. RIVAS GONZALO, Catedrático de Nutrición y Bromatología de la Universidad de Salamanca, informa que **D. Rubén del Barrio Galán** ha realizado el trabajo titulado "*Crianza sobre lías y uso de preparados comerciales derivados de levadura en la calidad de vinos blancos y tintos*", bajo la dirección de las **Dras. Silvia Pérez-Magariño** y **Miriam Ortega-Heras** y estimo como tutor que el mencionado trabajo reúne los requisitos necesarios para su presentación y defensa como tesis doctoral.

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ÍNDICE

El vino es una cosa maravillosamente apropiada para el hombre si, en tanto en la salud como en la enfermedad, se administra con tino y justa medida.

Hipócrates

I. JUSTIFICACIÓN Y OBJETIVOS

Hoy en día, uno de los principales objetivos que persigue el sector vitivinícola es seguir mejorando la calidad de los vinos, adaptándose por un lado a la demanda de los consumidores y por otro ampliando la oferta de vinos de calidad.

Los consumidores actuales demandan vinos blancos complejos en nariz en los que predominen los aromas afrutados, varietales y frescos y equilibrados en boca, y vinos tintos en los que los tonos violetas perduren a lo largo del tiempo, donde los aromas primarios y secundarios estén perfectamente integrados con los aromas de la madera y estructurados, con volumen y cuerpo en boca.

La crianza sobre lías es una técnica usada hace décadas principalmente en la elaboración de vinos blancos. Su objetivo es mejorar la calidad de los vinos desde el punto de vista sensorial. Esta mejora se debe principalmente a la liberación de compuestos como las manoproteínas durante la autolisis de las levaduras.

Así, desde el punto de vista sensorial, puede reducir la astringencia y amargor; aumentar el cuerpo, la estructura y la redondez en boca; y aumentar la persistencia y complejidad aromática tanto en vinos blancos como tintos. Además, la crianza sobre lías puede favorecer la estabilización tartárica y proteica de los vinos blancos y contribuir a estabilizar el color de los vinos tintos.

Sin embargo, esta técnica también puede plantear una serie de desventajas o inconvenientes, ya que implica un mayor tiempo de envejecimiento, una mayor dedicación de todos los recursos de la bodega, y además tiene un mayor riesgo de aparición de olores a reducción y alteraciones microbiológicas debidas al desarrollo de microorganismos no deseados como *Brettanomyces*.

Con el objetivo de eliminar estos problemas, actualmente se están buscando tanto técnicas que permitan mejorar este proceso como técnicas alternativas que puedan garantizar las mejoras aportadas por la crianza sobre lías.

Por todo ello, desde hace años se están utilizando preparaciones ricas en enzimas β-glucanasas que permitan acelerar el proceso de autolisis de las levaduras durante la crianza sobre lías. De este modo, los compuestos de las paredes celulares de las levaduras se liberarán más rápidamente.

Así mismo, con el fin de reducir la aparición de olores a reducción en la crianza sobre lías, se puede utilizar la técnica de microoxigenación, que permite la adición de pequeñas y controladas cantidades de oxígeno al vino.

Por otro lado, en los últimos años están apareciendo en el mercado diversas preparaciones comerciales obtenidas a partir de levaduras (levaduras inactivas, autolisados de levadura, paredes de levadura y extractos de levadura) que son ricas en polisacáridos (principalmente manoproteínas) y que pueden conseguir las características positivas comentadas anteriormente con la crianza sobre lías. Además, algunas de estas preparaciones comerciales llevan en su composición enzimas β-glucanasas para favorecer la hidrólisis de las paredes celulares de las levaduras.

El uso de otras técnicas diferentes a la crianza sobre lías también puede conseguir sus efectos positivos. Este es el caso de la madera de roble sin tostar, que aporta al vino diferentes compuestos, entre ellos polisacáridos, que pueden mejorar las características sensoriales de los vinos de forma similar a la crianza sobre lías.

Sin embargo, son muchos los interrogantes y las dudas que se les plantean a los enólogos y técnicos sobre la utilización de estas técnicas y su efecto final en los vinos, ya que son muy pocos los trabajos experimentales y científicos que se han realizado hasta el momento sobre estas técnicas y la mayoría están centrados en soluciones modelo.

Por todo ello, para resolver algunos de estos interrogantes, se ha llevado a cabo este trabajo, cuyo objetivo principal ha sido estudiar el efecto de la crianza sobre lías y de otras prácticas alternativas en la composición físico química y en la calidad sensorial de vinos tintos y blancos.

Para la consecución de este objetivo principal se plantearon los siguientes objetivos parciales:

1. Estudiar la interacción de diferentes preparados comerciales derivados de levadura, fragmentos de madera de roble sin tostar y lías finas, con compuestos fenólicos y aromáticos del vino tanto en soluciones hidroalcohólicas (vino sintético) como en un vino tinto.

2. Caracterizar diferentes preparados comerciales de levadura y estudiar su efecto sobre la composición y las características sensoriales de vinos blancos y tintos.

3. Estudiar el efecto de la crianza tradicional sobre lías, la adición de diferentes preparados comerciales derivados de levadura, con o sin la adición de enzimas β-glucanasas, y la adición de fragmentos de madera de roble sin tostar, sobre la composición fenólica y de polisacáridos y las características sensoriales (volumen en boca, astringencia, color, etc.) en vinos blancos y tintos. En vinos tintos se estudiará también la aplicación conjunta de la crianza sobre lías y la microoxigenación.

4. Estudiar la evolución de los vinos tintos tratados con las técnicas indicadas anteriormente durante su crianza en barrica.

II. INTRODUCCIÓN

II.1. LOS COMPUESTOS FENÓLICOS DEL VINO

Los compuestos fenólicos desempeñan un papel importante en las características sensoriales de los vinos, principalmente en el color, aroma, cuerpo, astringencia y amargor (Ribéreau-Gayón et al., 2003), y poseen propiedades antioxidantes, anticancerígenas y antiinflamatorias (Renaud et al., 1992; Chang et al., 2001; Paixao, 2008; Matic et al., 2010; Wang et al., 2010). Estos compuestos son más importantes en el vino tinto por su cantidad y por la presencia de antocianos, pero también están presentes en los vinos blancos en menor cantidad (Ribéreau-Gayón et al., 2003), y por ello han sido menos estudiados.

Los compuestos fenólicos provienen de las diferentes partes del racimo de uva, se extraen durante los procesos de vinificación, fundamentalmente en la maceración, y su contenido va cambiando a lo largo del proceso de envejecimiento debido a su oxidación, precipitación o participación en distintas reacciones de copigmentación, polimerización, etc.

Los compuestos fenólicos se caracterizan por poseer un anillo bencénico aromático con al menos un grupo hidroxilo (-OH) y una cadena lateral funcional (-R). En función de su estructura se clasifican de diferentes maneras debido a su complejidad, pero la mayoría de los autores los dividen en dos grandes grupos: los compuestos fenólicos no flavonoides y los flavonoides **(II.figura 1)** (Cheynier et al., 2003; Ribéreau-Gayón et al., 2003; Ignat et al., 2011).

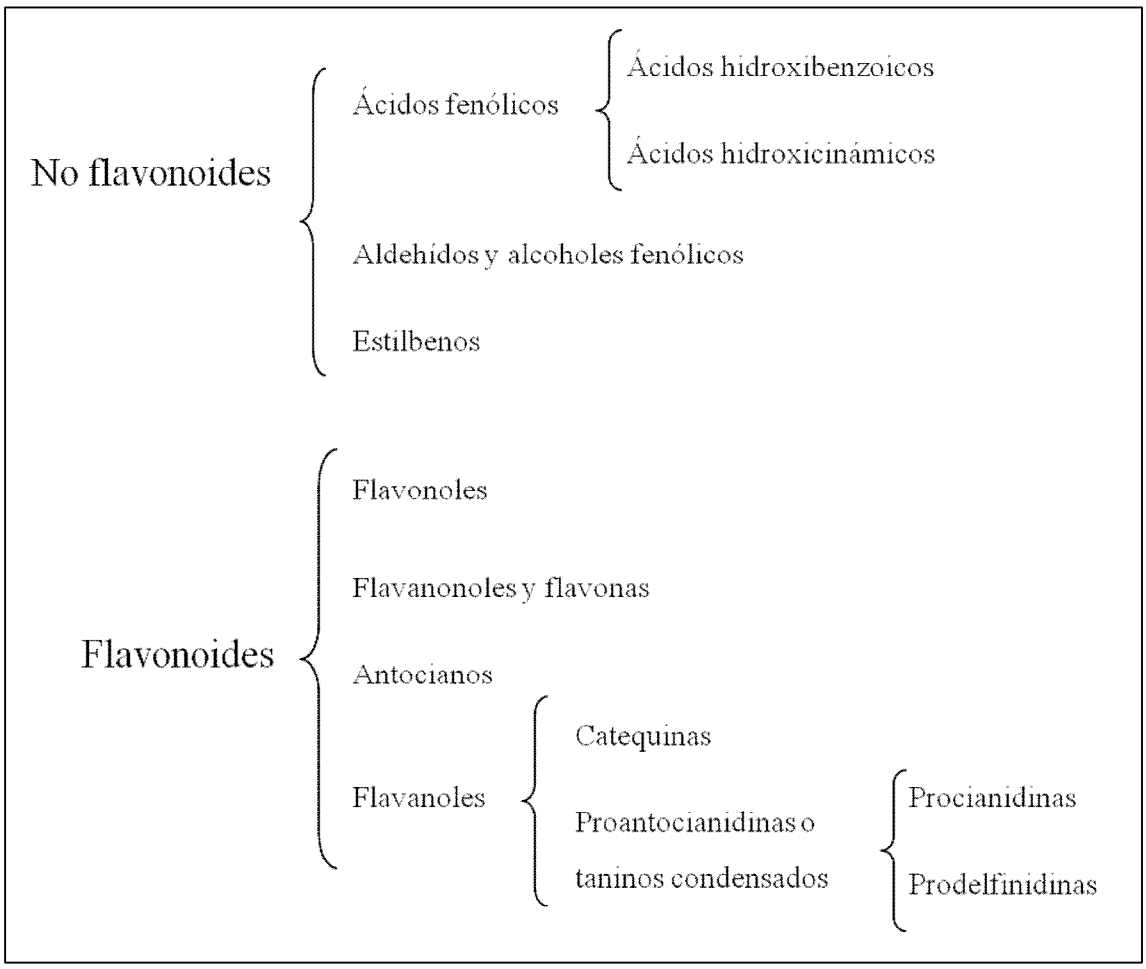


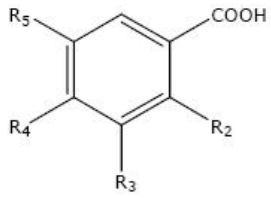
Figura II.1. Clasificación de los compuestos fenólicos.

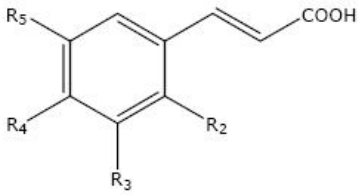
II.1.1. Compuestos fenólicos no flavonoides

II.1.1.1. Ácidos, aldehídos y alcoholes fenólicos

Los ácidos fenólicos se encuentran fundamentalmente en los hollejos, pulpa, semilla y raspón. Son compuestos con un único anillo bencénico y se pueden dividir en: ácidos hidroxibenzoicos, que tienen en común una estructura C6-C1; y ácidos hidroxicinámicos, que tienen en común una estructura C6-C3 **(figura II.2)**. Tanto los ácidos hidroxibenzoicos como los hidroxicinámicos pueden estar en forma libre o esterificada con el ácido tartárico u otros componentes del vino **(figura II.3)**. Los ácidos fenólicos son incoloros, pero la esterificación con el ácido tartárico les hace

particularmente oxidables, y son una de las causas del pardeamiento de los vinos blancos (Singleton et al., 1984). Algunos de estos ácidos fenólicos pueden influir además en la astringencia y amargor de los vinos (Boulton, 2001; Hufnagel y Hoffmann, 2008). Además, estos compuestos pueden tener cierta importancia en el color de los vinos tintos jóvenes, ya que pueden actuar como copigmentos, uniéndose a los antocianos (Brouillard y Dangles, 1994; Gómez-Mínguez et al., 2006).





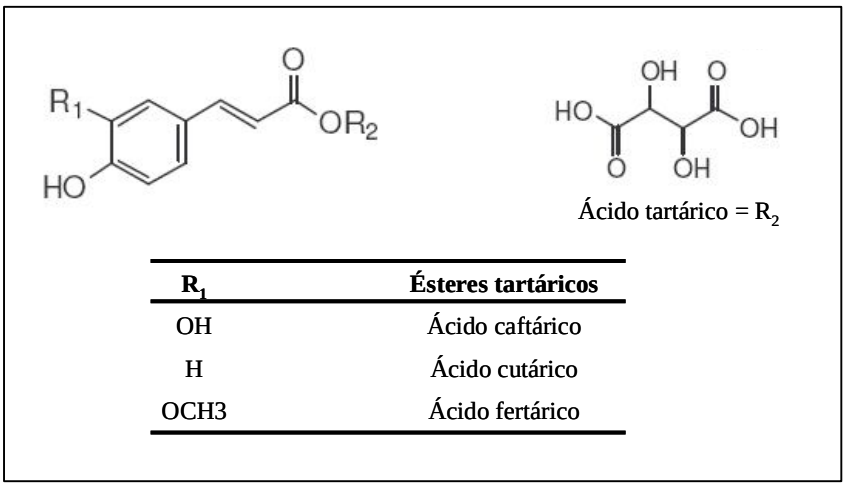
Ácidos hidroxibenzoicos	R2	R3	R4	R5	Ácidos hidroxicinámicos
Ácido p-hidroxibenzoico	H	H	OH	H	Ácido p-cumárico
Ácido protocatéquico	H	OH	OH	H	Ácido cafeico
Ácido vainillínico	H	OCH3	OH	H	Ácido ferúlico
Ácido gálico	H	OH	OH	OH	Ácido sinápico
Ácido siríngico	H	OCH3	OH	OCH3	
Ácido salicílico	OH	H	H	H	
Ácido gentísico	OH	H	H	OH	

Figura II.2. Estructura química de los ácidos fenólicos (Ribéreau-Gayón et al., 2003).

El ácido gálico y el elágico (dímero del ácido gálico) pueden formar polímeros llamados galotaninos y elagitaninos que constituyen los denominados taninos hidrolizables y que proceden de la madera (Cheynier et al., 2003).

Dentro de los aldehídos fenólicos se encuentran la vainillina, siringaldehído, sinapaldehído y coniferaldehído y que proceden principalmente de la madera (Hidalgo, 2003).

Los alcoholes fenólicos están formados por el tirosol, que procede de la desaminación y descarboxilación de la tirosina, y el triptofol, que se forma por una reacción análoga a partir de triptófano durante el proceso de fermentación (Hidalgo, 2003).



R_1	Ésteres tartáricos
OH	Ácido caftárico
H	Ácido cutárico
OCH3	Ácido fertárico

Figura II.3. Estructura química de los ésteres tartáricos (extraído de Guadalupe, 2008).

1.1.2. Estilbenos

Se encuentran mayoritariamente en el hollejo de la uva y están formados por 2 anillos bencénicos unidos por una cadena de etanol **(figura II.4)**. Dentro de este grupo se encuentran el *cis* y *trans*-resveratrol (3,5,4'-trihidroxiestilbeno) y sus derivados glicósidos (piceidos). Además, el resveratrol tiene la capacidad de formar oligómeros, y en los últimos años se han encontrado algunos dímeros (pallidol, ε y δ-viniferin, parthenocissin), un trímero (α-viniferin) (Cantos et al., 2002) y un tetrámero (hopeaphenol) (Guebailia et al., 2006).

El resveratrol es sintetizado por algunas plantas en respuesta a ciertas condiciones adversas como infecciones producidas por ataques patógenos y estrés medioambiental (Bavaresco, 2003). El resveratrol no influye de manera clara en las características sensoriales de los vinos. Sin embargo, en la última década está cobrando

gran interés por los efectos beneficiosos que puede tener sobre la salud, asociados a una gran actividad antioxidante, gracias a la cual tiene un efecto muy positivo en la prevención de enfermedades cardiovasculares, y ciertos tipos de cáncer. Además, posee propiedades antiinflamatorias e inhibe la agregación plaquetaria, etc. (Chang et al., 2001; King et al., 2006; Espin et al., 2007; Pezzuto, 2008).

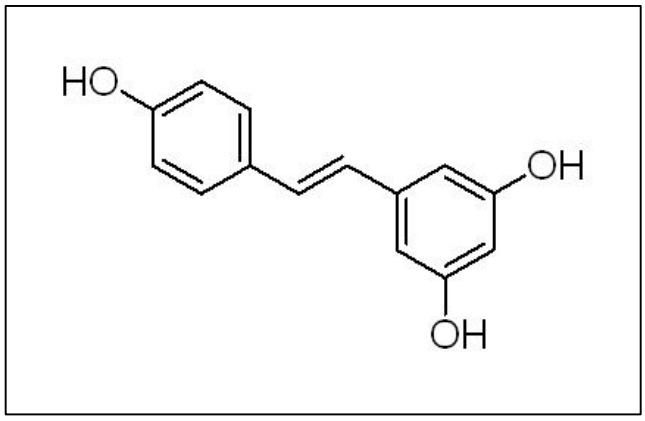


Figure II.4. Estructura molecular del Resveratrol.

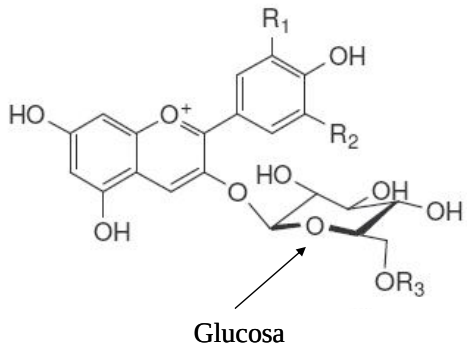
II.1.2. Compuestos fenólicos flavonoides.

Son compuestos con una estructura básica de 15 átomos de carbono dispuestos en una configuración C6-C3-C6. Esencialmente, esta estructura consiste en 2 anillos aromáticos unidos por un puente de 3 átomos de carbono, que normalmente está cerrado formando un anillo heterociclo oxigenado.

II.1.2.1. Antocianos

Son los responsables del color rojo-azulado de la piel de las uvas tintas y por tanto del color del vino tinto (Ribéreau-Gayón, 1964; Glories, 1984). Su localización en la uva se limita a los hollejos, si bien en las variedades tintoreras también están presentes en la pulpa (Souquet et al., 1996; Cheynier et al., 2003).

Los antocianos están formados por una aglicona (antocianidina) que se encuentra unida a un monoglucósido, normalmente glucosa. Así mismo, la glucosa puede estar esterificada con diferentes ácidos, principalmente el ácido acético, *p*-cumárico y *t*-cafeico (González-Sanjosé et al., 1990; Mazza y Miniati, 1993; Cheynier et al., 2003). En el género *Vitis* se pueden encontrar 5 antocianos diferentes en función de los grupos hidroxilos (-OH) y metoxilos (-OCH_3) que haya en el anillo B **(figura II.5)**. El monoglucósido de malvidina es el antociano mayoritario en las uvas de *Vitis vinifera*. Además de estos 5 monoglucósidos, se pueden encontrar sus correspondientes formas esterificadas con los ácidos acético y cumárico, y también las formas esterificadas de los monoglucósidos de peonidina y malvidina con el ácido cafeico (Zamora, 2003).



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

R3= radical acetil, cafeil o cumaril

Figura II.5. Estructura química de los antocianos de la uva.

La concentración de antocianos en la uva puede variar en función de diversos factores como son: la temperatura, la insolación, el terreno (Larice et al., 1989; Esteban et al., 2001; Arozarena et al., 2002), las prácticas culturales o tratamientos del viñedo realizados (sistema de conducción de la poda, fertilización, riego, etc.) (Castia et al., 1992; Keller y Hrazdina, 1998; Sipiora y Gutiérrez-Granda, 1998; De la Hera et al., 2002; De la Hera et al., 2005), así como las diferentes prácticas enológicas (tiempo y

temperatura de maceración y fermentación, prensados, remontados, etc.) empleadas en la elaboración del vino. Aun así, diversos trabajos han mostrado que el perfil de los antocianos es bastante estable para una determinada variedad (Arozarena et al., 2000; Arozarena et al., 2002; Košir et al., 2004; Von Baer et al., 2005; Casavecchia et al., 2007).

II.1.2.2. Flavanoles y proantocianidinas (taninos condensados)

Se encuentran principalmente en el hollejo, en las semillas de la baya y en el raspón. Los principales flavan-3-ol o flavanoles son los monómeros de (+)-catequina, (-)-epicatequina, (+)-galocatequina y (-)-epigalocatequina **(figura II.6)**. Sin embargo, la mayor parte de los flavanoles están en forma de oligómeros (hasta cinco unidades) y polímeros (más de cinco unidades), y se denominan taninos condensados o proantocianidinas. Su nombre se debe a que pueden dar lugar a antocianos por hidrólisis ácida. Así, la hidrólisis de los taninos formados por unidades de catequina y/o epicatequina dan lugar a cianidina (procianidinas) y los formados por unidades de galocatequina y/o epigalocatequina dan delfinidina (prodelfinidinas) (Porter et al., 1986), siendo las primeras las encontradas principalmente en la uva y el vino.

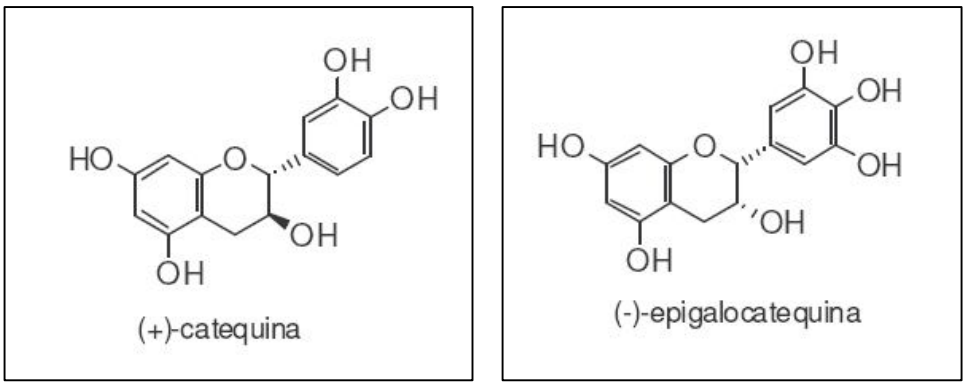


Figura II.6. Estructura química de los flavanoles monómeros de la uva. (extraído de Guadalupe, 2008).

Estos compuestos juegan un papel importante en el color del vino. En los vinos blancos, son responsables, en parte, del pardeamiento oxidativo que aumenta las tonalidades amarillas (Simpson, 1982; Cheynier et al., 1989). En los vinos tintos pueden actuar como copigmentos (Boulton, 2001; Gómez-Mínguez et al., 2006) intensificando el color de los vinos jóvenes. Por otro lado, intervienen en la estabilidad del color de los vinos tintos durante su envejecimiento debido a su interacción con los antocianos mediante las reacciones de polimerización y condensación (Timberlake y Bridle, 1976; Ribéreau-Gayón, 1982; Francia-Aricha et al., 1997; Fischer y Strasser, 1999; Revilla et al., 1999; Atanasova et al., 2002a; Pérez-Magariño y González-San José, 2004).

Los flavanoles son también responsables del cuerpo, astringencia y amargor de los vinos (Vidal et al., 2003b). La sensación de astringencia de un vino se debe a la capacidad que tienen las proantocianidinas de interaccionar con las proteínas de la saliva. Estas interacciones dependen, en gran medida, de la composición y el tamaño de las proantocianidinas (Sarni-Manchado et al., 1999; Vidal et al., 2003b) y de la naturaleza de la proteína. Uno de los factores más importantes es el grado de polimerización de las proantocianidinas. Así, cuanto mayor es su grado de polimerización y el porcentaje de unidades galoiladas de los taninos, mayor es la sensación de astringencia (Herderich y Smith, 2005). Por otro lado, si la polimerización es ordenada y lineal siguen siendo muy astringentes, mientras que si la polimerización es desordenada y cruzada se reduce su astringencia debido a su mayor dificultad para poder interaccionar con las proteínas de la saliva (Sarni-Manchado et al., 1999).

También se ha estudiado el efecto beneficioso para la salud de estos compuestos debido a su capacidad antioxidante (Jordao et al., 2010) y a su efecto protector frente a la úlcera de estómago (Saito et al., 1998).

II.1.2.3. Flavonoles

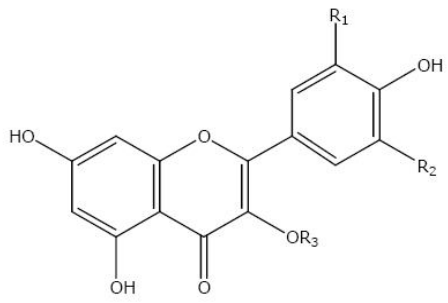
Son pigmentos amarillos que se localizan en los hollejos de las uvas donde solamente existen como forma heterósida (3-*O*-glicósidos), unidos fundamentalmente a glucosa, galactosa y ácido glucurónico. Sin embargo, en el vino pueden encontrarse

tanto los flavonoles glicósidos como sus correspondientes agliconas, debido a la hidrólisis ácida que tiene lugar durante la elaboración y envejecimiento del vino (Castillo-Muñoz et al., 2009).

En la actualidad se conocen seis estructuras flavonoideas diferentes que son: kaempferol, quercetina, miricetina, isorhamnetina, laricitrina y siringetina **(figura II.7)**, además de sus derivados glicósidos. Cheynier et al. (2003) sugerían que los glicósidos de miricetina e isorhamnetina eran flavonoles específicos de uvas tintas, pero estudios recientes han demostrado la presencia de glicósidos de isorhamnetina en variedades de uva blanca (Castillo-Muñoz et al., 2010).

Son fácilmente extraíbles durante los procesos de vinificación aunque son poco solubles en agua y requieren de la presencia del etanol. Tienen carácter amargo y un fuerte poder de copigmentación (Boulton, 2001; Schwarz et al., 2005; Gómez-Mínguez et al., 2006) y también pueden formar parte en las reacciones de polimerización con otros fenoles (Price et al., 1995). Los flavonoles juegan un papel importante en el color del vino blanco, pero en vinos tintos son enmascarados por los pigmentos rojos como los antocianos (Hidalgo, 2003).

Recientemente también se han descrito efectos positivos de la quercetina y miricetina en la prevención de enfermedades cardiovasculares (Angelone et al., 2006) y cómo antioxidantes (Paixao et al., 2008; Hidalgo et al., 2010; Mikstacka et al., 2010).



Flavonol	R_1	R_2	R_3
Miricetina	OH	OH	H
Quercetina	OH	H	H
Kaempferol	H	H	H
Isorhamnetina	OCH_3	H	H
Laricitrina	OCH_3	OH	H
Siringetina	OCH_3	OCH_3	H

Figura II.7. Estructura química de los principales flavonoles del vino.

II.1.2.4. Flavanonoles y flavonas

Los flavanonoles y flavonas presentan una estructura muy similar a la de los flavonoles (Cheynier et al., 2003), solamente se diferencian en que no poseen el doble enlace del heterociclo. Los flavanonoles se han encontrado en la madera en forma de agliconas libres, en uvas de *Vitis vinifera,* en vinos blancos y tintos e incluso en orujos y raspones. Entre los flavanonoles más característicos está la astilbina (dihidroquercetín-3-*O*-rhamnósido) y la engeletina (dihidrokaempferol-3-O-rhamnósido) (Tousdale y Singleton, 1983**).** Sin embargo, las flavonas solamente se han identificado en las hojas de *Vitis vinifera* y no en vinos.

II.2. EL COLOR DEL VINO

II.2.1. El color en los vinos tintos

El color es el primer aspecto que se observa en un vino y por este motivo es uno de los aspectos organolépticos más importantes a tener en cuenta a la hora de evaluar su calidad. Es el primer atributo evaluado en cata y va a ofrecer información sobre la edad, el estado de conservación, la estructura y el cuerpo de los vinos e incluso puede orientar al catador sobre sus posibles cualidades gustativas y aromáticas.

Como ya se ha comentado anteriormente, el color inicial del vino tinto se debe principalmente a los antocianos que son extraídos de la uva durante el proceso de elaboración y al equilibrio entre sus diferentes formas químicas (**figura II.8**). Este equilibrio va a depender en gran medida del pH del vino así como de la temperatura, el contenido en anhídrido sulfuroso (SO_2) y la luz (Zamora, 2003).

El color depende de la proporción de antocianos que existen en la forma de catión flavilio que proporciona el color rojo, y de la base anhidra o quinoidal que es de color azul-violeta, y que está influenciada a su vez por el pH del vino. Así, a bajos pH se produce un aumento de la concentración de la forma flavilio, y por tanto del color rojo de los vinos. A medida que aumenta el pH, la proporción de la forma flavilio (roja) disminuye desplazándose hacia la forma quinoidal (azul/malva). Este aumento de pH puede dar lugar de manera simultánea a la aparición de la forma base carbinol, que es incolora. Al pH del vino (3,5-4,2) existe un equilibrio entre las formas flavilio, quinona y carbinol, pero solamente entre el 20 y el 30% de los antocianos del vino contribuyen al color, es decir, se encuentran en sus formas flavilio y quinona (Glories, 1984).

Por otro lado, un aumento en la temperatura del vino puede hacer que la forma carbinol se transforme en la forma calcona (*cis y trans*) que es de color amarillo y cuya oxidación da lugar a la formación de ácidos fenólicos que son incoloros. A diferencia del resto de reacciones, esta oxidación produce una pérdida irreversible de color (Zamora, 2003).

La presencia de anhídrido sulfuroso (SO_2) en los vinos tintos produce también una decoloración de los antocianos. Al pH del vino, la mayor parte del anhídrido sulfuroso libre se encuentra bajo la forma de anión HSO_3^- que se combina con los antocianos bajo la forma flavilio, produciéndose un complejo incoloro que es estable al pH del vino. Esta reacción es reversible por lo que la pérdida de intensidad de color es temporal (Somers, 1971; Glories, 1984).

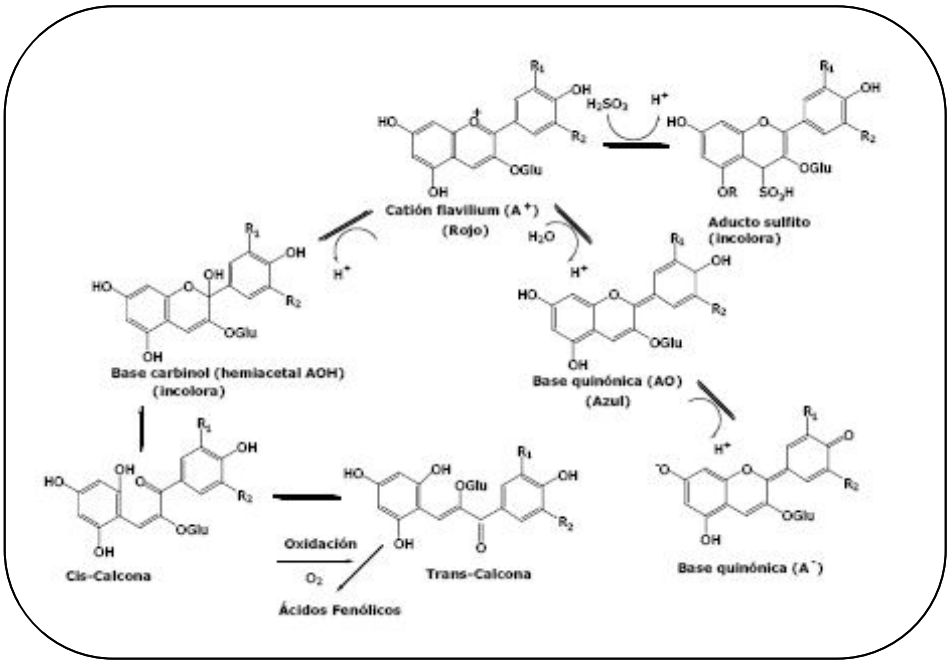


Figura II.8. Cambios en la estructura de los antocianos en función del pH (extraído de Zamora, 2003).

Durante el envejecimiento del vino el color experimenta cambios tanto en la intensidad como en las tonalidades predominantes, de forma que van desapareciendo los tonos azul-violáceos y predominando los amarillo-naranjas.

A continuación, se comentan las principales reacciones que se pueden producir entre los compuestos fenólicos y que afectan al color de los vinos.

II.2.1.1. Reacciones de copigmentación

La copigmentación es un fenómeno característico de los vinos tintos jóvenes que se produce debido a las interacciones hidrofóbicas no covalentes que tienen lugar entre la parte planar y polarizable de los núcleos de las formas coloreadas de los antocianos y otras moléculas, normalmente incoloras, denominadas copigmentos (Boulton, 2001).

Como copigmentos pueden actuar moléculas de naturaleza muy diversa: otros compuestos fenólicos, aminoácidos, ácidos orgánicos, nucleótidos, polisacáridos, alcaloides, iones metálicos, etc. (copigmentación intermolecular) (Brouillard et al., 1991). En general los mejores copigmentos son aquellos que contienen núcleos aromáticos y poseen cierta planariedad, por lo que también pueden intervenir otros antocianos (autoasociación) o incluso una parte de la estructura del propio antociano que copigmenta (copigmentación intramolecular) (González-Manzano et al., 2009).

Algunos estudios realizados en soluciones de vino modelo han demostrado que los flavonoles son los compuestos con mayor poder de copigmentación, los los ácidos hidroxicinámicos presentan un comportamiento intermedio, y los flavanoles son los de poder de copigmentación más bajo (Brouillard et al., 1991; Gómez-Mínguez et al., 2006; González-Manzano et al., 2009; Lambert et al., 2011). Además, se han encontrado diferencias entre los flavanoles monómeros en cuanto a su poder de copigmentación, siendo la epicatequina mejor copigmento que la catequina (Brouillard et al., 1991; Liao et al., 1992; Mirabel et al., 1999).

Las estructuras que se forman adoptan una configuración en “sandwich” que protege al ión flavilio o a la base quinónica del ataque nucleofílico del agua, evitando de este modo la aparición de formas incoloras (calcona y hemiacetal o carbinol) (Liao et al., 1992; Mirabel et al., 1999) **(figura II.9)**. El resultado final es la formación de complejos de apilación vertical que producen dos efectos en el vino que generalmente van asociados:

▪ *Efecto hipercrómico en el color* de los vinos tintos, es decir, produce un aumento de la intensidad colorante, que es mayor de la que cabría esperar de acuerdo con los valores de pH del vino.

▪ *Efecto batocrómico de la tonalidad*, es decir, se produce un viraje de la tonalidad hacia el color púrpura y azul (Vivar-Quintana et al., 2002).

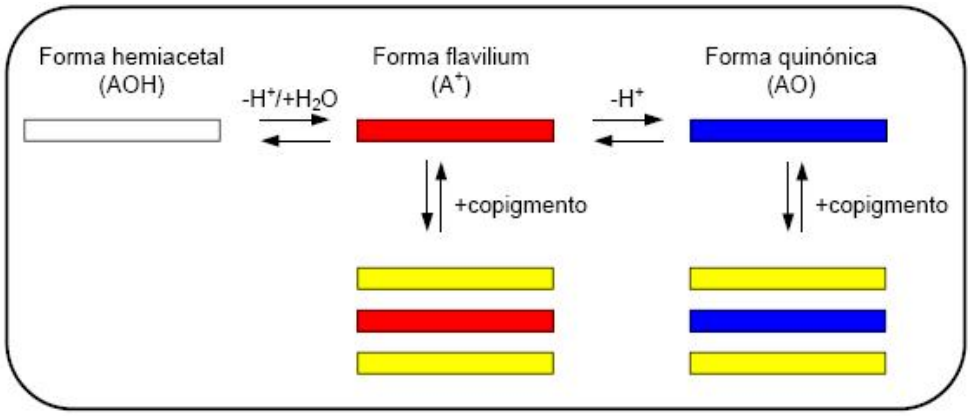


Figura II.9. Esquema del proceso de copigmentación (extraído de Romero-Cascales, 2008).

El fenómeno de la copigmentación ocurre ya en las bayas de las variedades tintas, por lo que este proceso parece tener gran importancia para conocer la relación entre la composición de la uva y el color de los vinos tintos jóvenes (Boulton, 2001). Las reacciones que dan lugar a los complejos de copigmentación se dan con mucha facilidad y son reversibles en el tiempo, pero también se disocian fácilmente por lo que son poco estables (Hermosín-Gutiérrez, 2007).

II.2.1.2. Reacciones de condensación directa entre flavanoles (taninos) y antocianos

Son reacciones basadas en procesos de adición nucleofílica en donde los antocianos y los taninos pueden actuar tanto como agentes electrofílicos como nucleofílicos (Zimman y Waterhouse, 2004), dando lugar a la formación de dos productos diferentes en el vino: antociano-tanino (A-T) y tanino-antociano (T-A)

(figura II.10). Ambos aductos, son más complejos y estables que los antocianos monoméricos (Remy et al., 2000; Salas et al., 2004; González-Sanjosé et al., 2005).

La primera reacción se produce por la adición del tanino sobre el catión flavilio A^+ del antociano, dando lugar al complejo (A-T) que es incoloro. Posteriormente se colorea de rojo en presencia de oxígeno, dando finalmente un aducto antociano-tanino. (Santos-Buelga et al., 1995).

La segunda reacción se produce por la adición de la base hemiacetal o carbinol (AOH) sobre un carbocatión que se forma por la ruptura de las proantocianidinas, produciendo un complejo incoloro que después se colorea de color rojo anaranjado debido a su deshidratación. Esta condensación se ve favorecida por la ausencia de oxígeno, por lo que en ambientes reductores, como el almacenamiento en depósito o el envejecimiento en botella, pueden aumentar su contenido (Ribéreau-Gayón et al., 2003; Zamora, 2003).

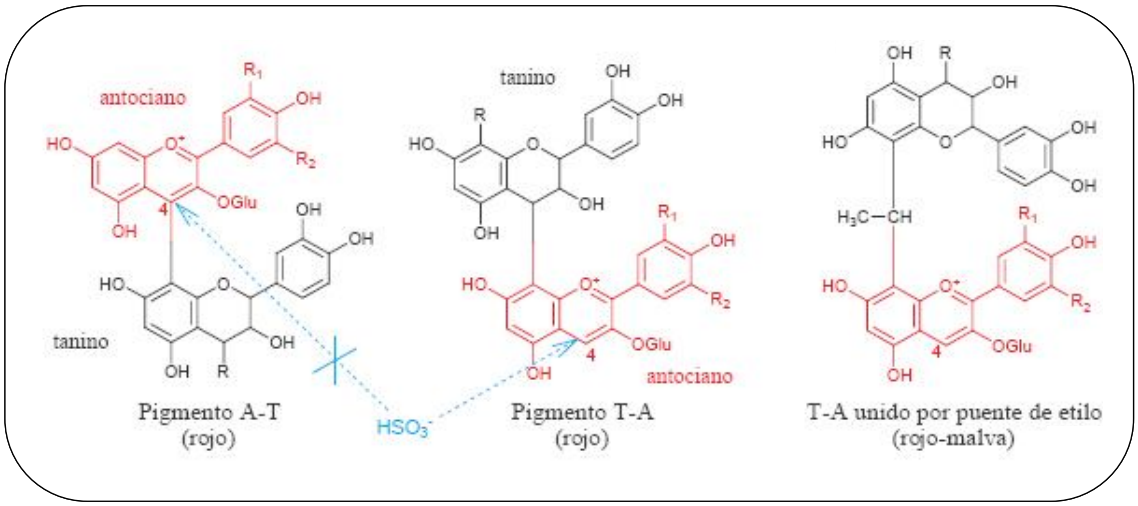


Figura 10. Mecanismos de condensación directa antociano-tanino (A-T) y tanino antociano (T-A) y de condensación mediada por acetaldehído (extraído de Guadalupe, 2008).

Los compuestos resultantes de estos dos tipos de reacciones entre los antocianos y los taninos son de color similar a los antocianos (García-Puente Rivas et al., 2006),

aunque son menos sensibles a los cambios de pH y a la decoloración por SO_2. (Cheynier et al., 2003; Ribéreau-Gayón et al., 2003).

II.2.1.3. Reacciones de condensación entre antocianos y flavanoles (taninos) mediadas por el acetaldehído

En estas reacciones está involucrado el acetaldehído que actúa como puente de unión entre los antocianos y los taninos. El acetaldehído es producido en el vino en pequeñas cantidades por las levaduras durante la fermentación alcohólica; por la acción de las bacterias acéticas; por la oxidación química del etanol; y por la autooxidación de los compuestos fenólicos (Liu y Pilone, 2000).

Estas reacciones fueron descritas en primer lugar por Timberlake y Bridle (1976) y posteriormente demostradas por Fulcrand et al. (1996a). El mecanismo de acción consiste en la condensación del acetaldehído sobre el flavanol, formándose un carbocatión intermedio, el cual puede reaccionar a su vez con otra molécula de flavanol o de antociano. El resultado es la formación de aductos unidos por puentes de etilo del tipo tanino-etil-tanino, tanino-etil-antociano y antociano-etil-antociano **(figura II.10)**. El tamaño molecular de estos compuestos es variable.

- *Aductos tanino-etil-tanino*: Estos compuestos se forman con bastante facilidad en los vinos, y son el resultado de la unión mediante puentes etilo entre las distintas posiciones nucleófilas de los anillos fluoroglucinol (C-6 y C-8) que dan lugar a productos incoloros (Saucier et al., 1997a y b). La formación de este tipo de compuestos parece ser más habitual en las proantocianidinas que en los flavanoles monómeros. Estas uniones tanino-etil-tanino pueden romperse dando lugar a formas etil-flavanol o etil-vinil-flavanol que son reactivas y que pueden reaccionar con otros dímeros de flavanoles, formando compuestos de mayor tamaño, o pigmentos condensados al unirse con antocianos (Guerra, 1997).

- *Aductos tanino-etil-antociano*: Estos compuestos son de color violeta y poseen mayor intensidad colorante que los antocianos monoméricos de los que proceden, ya que producen un desplazamiento batocrómico hasta longitudes de onda de 545

nm (Es-Safi et al., 1999a). Su color es más estable frente a los cambios de pH, a los procesos de hidratación y sólo son parcialmente decolorables por SO_2 (Timberlake y Bridle, 1976). No obstante, algunos autores afirman que estos compuestos son poco estables en disolución por rotura del puente de etilo, y encuentran controvertida su contribución a la estabilidad y evolución del color del vino durante su envejecimiento (Escribano-Bailón et al., 2001; Santos-Buelga, 2001).

- *Aductos antociano-etil-antociano*: La formación de estos compuestos fue indicada por González-Sanjosé y Di Stefano (1990) y finalmente demostrada por Atanasova et al. (2002a) en soluciones modelo. Las reacciones de condensación entre antocianos no se dan con facilidad, especialmente si hay flavanoles en el medio (Bravo-Haro, 1994). Atanasova et al. (2002a) demostraron que las reacciones de condensación entre antocianos mediadas por el acetaldehído, en ausencia de flavanoles, también podían inducir a la polimerización de los antocianos, dando lugar a dímeros, trímeros y tetrámeros. Al igual que los compuestos anteriores, éstos son de color violeta y resistentes a la degradación por hidratación y a la decoloración SO_2.

El pH y la temperatura del vino son dos factores que tienen gran influencia en las reacciones de condensación mediadas por acetaldehído. Así, en el intervalo de pH entre 2 y 5, la velocidad de las reacciones de condensación aumenta a medida que desciende el pH (García-Viguera et al., 1994), debido a que se favorece la formación del catión necesario para que se de la reacción. Por otro lado, la velocidad de las reacciones de formación de estos pigmentos disminuye al bajar la temperatura. Sin embargo, los compuestos que se forman son más estables, debido a que disminuye su tendencia a polimerizarse y precipitar, permitiendo que se acumulen en mayor cantidad (Rivas-Gonzalo et al., 1995).

La aireación excesiva del vino puede producir cantidades elevadas de acetaldehído, provocando por un lado, la oxidación directa de los antocianos, y por otro,

una polimerización excesiva de los compuestos fenólicos que dan complejos poliméricos de elevado tamaño que precipitan (Guadalupe, 2008).

La composición fenólica inicial del vino es uno de los factores que va a condicionar el tipo de reacciones que se producen en el vino. De este modo, si el vino tiene una concentración de antocianos muy superior a la de taninos, predominarán las reacciones de degradación de antocianos por oxidación. En cambio, si la concentración de taninos es mucho mayor que la de antocianos, predominarán las reacciones de polimerización, que producirán un aumento de la componente amarilla del vino. Por último, si la concentración de taninos y de antocianos está equilibrada, todas las reacciones serán igual de probables y por tanto la crianza del vino conducirá a una estabilización de la materia colorante (Guadalupe, 2008).

II.2.1.4. Reacciones de formación de nuevos pigmentos

En los últimos años se han aislado e identificado, primero en soluciones de vino modelo y posteriormente en los vinos de crianza, una serie de compuestos que son derivados de los antocianos monoméricos y que se caracterizan por poseer un anillo de pirano adicional que está unido al esqueleto flavonoideo del catión flavilio del antociano original (Francia-Aricha et al., 1997; Mateus et al., 2002). Estos nuevos pigmentos han sido denominados **piranoantocianos** y se forman mediante una reacción de cicloadición que se produce entre los antocianos y algunos compuestos de bajo peso molecular, principalmente metabolitos procedentes de las levaduras que poseen un doble enlace polarizado como el ácido pirúvico, acetaldehído y vinilfenoles. Además, posteriormente, algunos piranoantocianos pueden reaccionar con flavanoles (Francia-Aricha et al., 1997).

En general, los piranoantocianos son compuestos químicamente más estables a las variaciones de pH, a la decoloración por SO_2, a la degradación oxidativa y a la temperatura que los antocianos monoméricos ya que tienen bloqueada la posición C-4

del anillo de pirano flavonoideo (Sarni-Manchado et al., 1996; Bakker y Timberlake, 1997; Francia-Aricha et al., 1997).

Los piranoantocianos, a diferencia de otros pigmentos poliméricos, son compuestos que suelen tener un tamaño fijo y similar al de los antocianos monoméricos y se mantienen disueltos en el vino. Por tanto tienen poca tendencia a perderse en los precipitados de la materia colorante que se forman en los vinos tintos envejecidos, o a quedarse retenidos en los filtros por los que se pasan los vinos antes de su embotellado (Hermosín-Gutiérrez, 2007).

Existe una gran diversidad de piranoantocianos con una gama de colores desde el rojo-ladrillo al azul intenso y que se describen a continuación.

- *Vitisinas*: El primero de estos pigmentos fue descrito por Bakker y Timberlake (1997) y fue llamado vitisina A, el cual se forma mediante la reacción de cicloadición entre el malvidín-3-monoglucósido y el ácido pirúvico. Posteriormente, Fulcrand et al. (1998) propusieron su estructura **(figura II.11)**, y demostraron que el núcleo antociánico está unido a un nuevo anillo piránico formado por un grupo carboxilo. Por esta razón, las vitisinas del tipo A, son conocidas también como 5-carboxipiranoantocianos. Tienen su máximo de absorbancia desplazado hipsocrómicamente hacia longitudes de onda entre los 505 y 515 nm. Bakker y Timberlake (1997) también detectaron la vitisina B, que se forma por la reacción entre el malvidín-3-monoglucósido y el acetaldehído, y que posee un desplazamiento hipsocrómico a 498 nm.

  Además del ácido pirúvico y el acetaldehído, existen otros compuestos que pueden reaccionar con los antocianos para formar piranoantocianos, como el ácido α-cetoglutárico, acetoína y acetona procedentes de las levaduras (Benabdeljalil et al., 2000), y otras moléculas con función aldehídica como el benzaldehído o formaldehído (Pissarra et al., 2004).

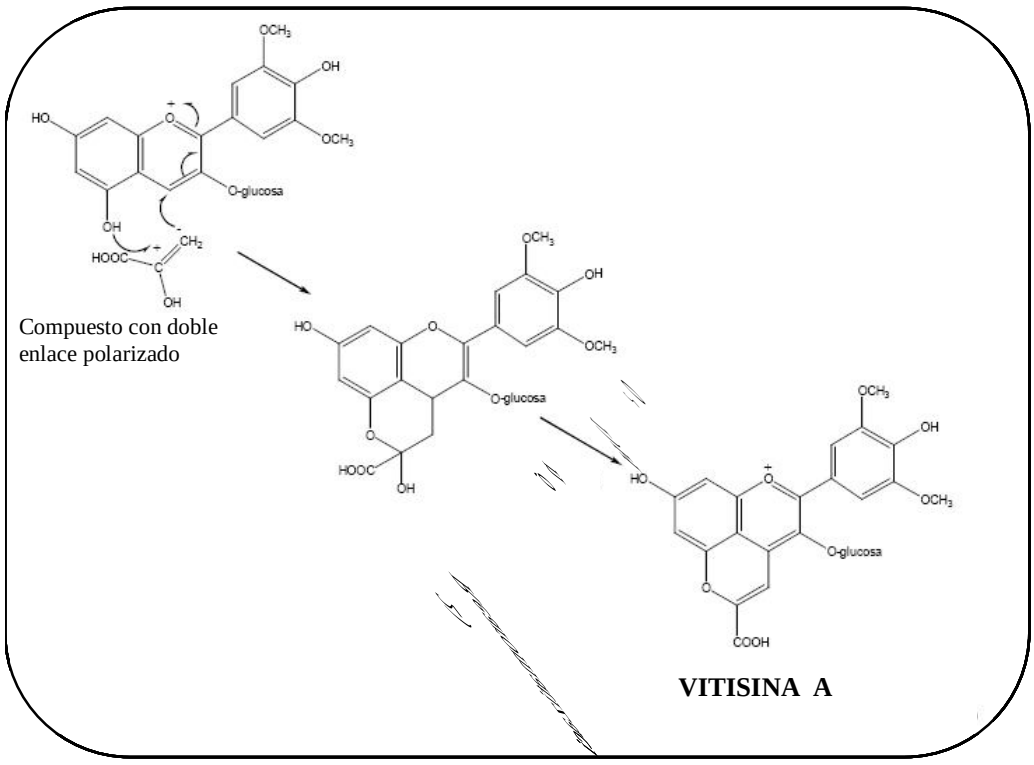
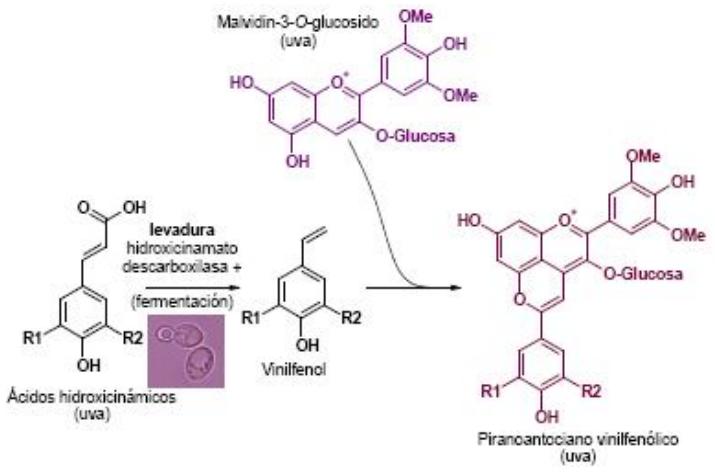


Figura II.11. Mecanismo de reacción de cicloadición entre el malvidín-3-monoglucósido y el ácido pirúvico para formar la vitisina A (Fulcrand et al., 1998).

▪ *Hidroxifenil-piranoantocianos*: Estos compuestos se forman por la reacción entre los antocianos y los ácidos hidroxicinámicos (*p*-cumárico, cafeico, ferúlico y sinápico) o los productos procedentes de su descarboxilación como son los 4-vinilfenoles (4-vinilfenol, 4-vinilcatecol, 4-vinilguaiacol y 4-vinilsiringol) **(figura II.12)**. El primero de estos compuestos fue encontrado por Fulcrand et al. (1996b) y lo llamaron malvidín-3-glucósido-4-vinilfenol. Posteriormente Schwarz et al. (2003a) aislaron la pinotina A, que procede de la unión entre el malvidín-3-glucósido y el 4-vinilcatecol. Sin embargo, en otro trabajo realizado por Schwarz et at. (2003b) se mostró que la pinotina A también puede formarse a partir de una reacción directa entre el ácido cafeico y el malvidín-3-monoglucósido.

Estos compuestos presentan una gran estabilidad del color, pero en general, requieren de elevados tiempos de crianza para su formación (Sarni-Manchado et al., 1996). Presentan un máximo de absorbancia desplazado hipsocrómicamente a 507 nm.

Recientemente se ha demostrado que algunas cepas de *Saccharomyces spp* seleccionadas con alta actividad hidroxicinamato descarboxilasa pueden acelerar la formación de aductos vinilfenólicos durante la fermentación (Morata et al., 2007; Benito et al., 2011). Estas levaduras descarboxilan los ácidos fenólicos formando los vinilfenoles correspondientes de elevada reactividad, y que espontáneamente condensan con los antocianos (Morata et al., 2008) **(figura II.12)**.



Ácido cafeico:	R1=OH; R2=H	malvidín-3-*O*-glucósido-4-vinilcatecol
Ácido *p*-cumárico:	R1=H; R2=H	malvidín-3-*O*-glucósido-4-vinilfenol
Ácido ferúlico:	R1=OCH_3; R2=H	malvidín-3-*O*-glucósido-4-vinilguaiacol
Ácido sinápico:	R1=OCH_3; R2=OCH_3	malvidín-3-*O*-glucósido-4-vinilsiringol

Figura II.12. Formación de piranoantocianos vinilfenólicos o hidróxifenil-piranoantocianos (Morata et al., 2008).

Por otro lado, la formación de piranoantocianos vinilfenólicos permite reducir el contenido de ácidos hidroxicinámicos precursores de los etilfenoles (Suárez et al., 2007). Además, se ha demostrado que las *Brettanomyces* no son capaces de liberar el vinilfenol a partir de un pigmento piranoantociánico. Por lo tanto estos pigmentos son una forma de preservar el color y de reducir el contenido de precursores para la formación de etilfenoles (Morata et al., 2008).

▪ *Vinilflavanol-piranoantocianos*: Estos compuestos son aductos que se forman por la reacción entre el malvidín-3-monoglucósido y los vinilflavanoles (**figura II.13**) (vinilcatequina, vinilepicatequina y vinilproantocianidinas).

Estos compuestos fueron descritos por primera vez por Francia-Aricha et al. (1997) en soluciones modelo, y posteriormente, fueron aislados en vinos de Oporto y en vinos tintos (Mateus et al., 2002; Mateus et al., 2003).

Como todos los anteriores piranoantocianos, presentan una gran estabilidad del color y producen un efecto hipsocrómico, con máximos de absorbancia entre 490 y 511 nm. (Francia-Aricha et al., 1997).

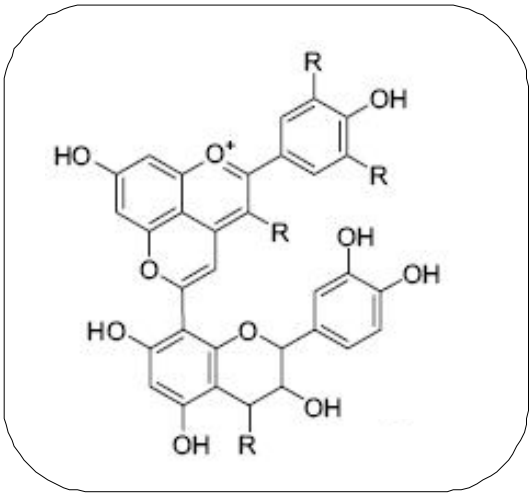


Figura II.13. Estructura de los vinilflavanol-piranoantocianos (extraído de Rentzsch et al., 2007).

▪ *Portisinas*: Estos pigmentos se forman por reacción entre los 5-carboxipiranoantocianos (vitisinas del tipo A) y los vinilflavanoles **(figura II.14 A)**. A diferencia de los anteriores piranoantocianos, estos compuestos tienen un efecto batocrómico con un máximo de absorbancia de 580 nm, y por tanto, muestran un color azul oscuro (Mateus et al., 2003).

Posteriormente, Mateus et al. (2006) encontraron otro tipo de pigmentos que se formaban por la reacción entre un 5-carboxipiranoantociano y un vinilfenol **(figura II.14 B)**, que presentan un máximo de absorbancia de 535 nm, y tienen tonalidades púrpuras.

Ambos tipos de portisinas tienen un color muy estable.

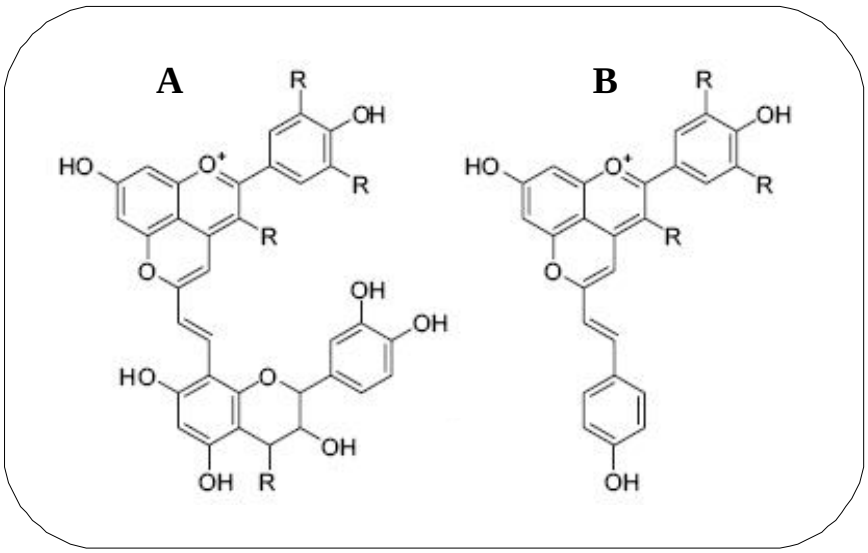


Figura II.14. Estructura de las portisinas del tipo 5-carboxipiranoantociano + vinilflavanol (**A**), y 5-carboxipiranoantociano + vinilfenol (**B**) (extraído de Rentzsch et al., 2007).

▪ *Oaklinas*: Estos pigmentos son aductos de cicloadición, pero no son piranoantocianos propiamente dichos, ya que la reacción de cicloadición ocurre en una unidad de catequina y no de antociano **(figura II.15)**. Estas reacciones se dan entre antocianos y flavanoles o sólo flavanoles y algunos aldehídos furánicos

(furfural e hidroximetilfurfural), cinámicos (coniferaldehído y sinapaldehído) y benzóicos (hidroxibenzaldehído y vainillina) que son aportados por la madera de roble durante la crianza del vino en barrica (Sousa et al., 2005; Sousa et al., 2007).

Estos compuestos también son muy resistentes a los cambios de pH, a la hidratación y a la decoloración por SO_2. Estos pigmentos son generalmente de color rojo ladrillo y presentan unos máximos de absorbancia entre 480-520 nm.

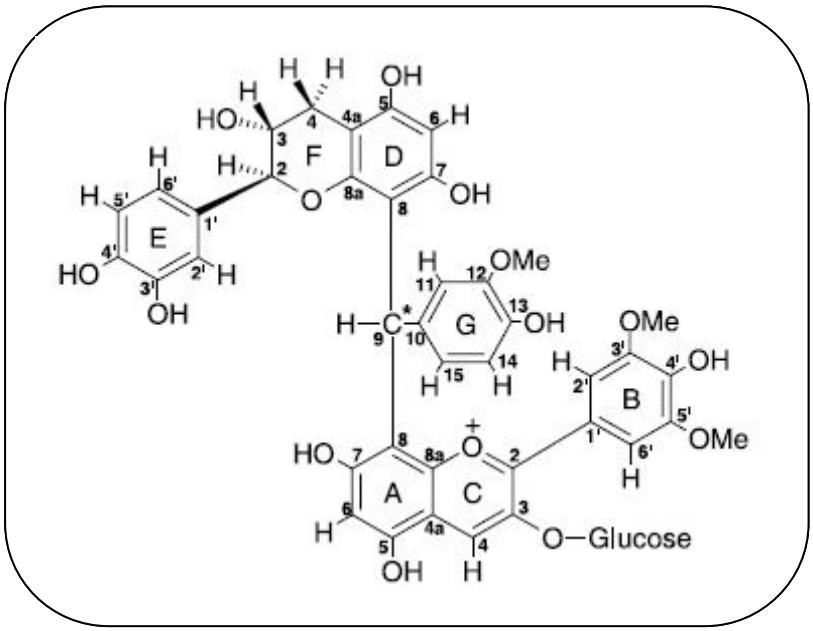


Figura II.15. Estructura del malvidín-3-glucósido-vainillil-catequina (oaklina) propuesta por Sousa et al. (2007).

II.2.1.5. Reacciones de oxidación química y enzimática

Las reacciones de oxidación química y enzimática que ocurren en el vino se caracterizan por tener como sustrato al oxígeno molecular. La oxidación enzimática de los compuestos fenólicos, también conocida como pardeamiento enzimático, es llevada a cabo por la enzima polifenoloxidasa (PPO). Esta enzima actúa en las primeras etapas de la elaboración de los vinos y los ácidos caftárico y cutárico son sus principales substratos (Singleton et al., 1985). La oxidación de estos compuestos da lugar a la

formación de quinonas que al polimerizarse forman pigmentos amarillo-pardos. Estas reacciones son más importantes en vinos blancos, ya que devalúan su calidad. En el caso de los vinos tintos, aunque los antocianos y proantocianidinas son pobres substratos para la PPO, pueden producirse decoloraciones (Cheynier et al., 1994).

Por otro lado, a medida que va transcurriendo el proceso de elaboración del vino, las reacciones de oxidación enzimática cesan debido a la falta de oxígeno o a la inactivación de la PPO, y es entonces cuando comienzan a tener lugar las reacciones de oxidación química (Monagas et al., 2005). Estas reacciones se producen en el vino de manera más lenta que las que ocurren por vía enzimática, y pueden ser catalizadas por el hierro, cobre y los radicales peróxido que pueden activar el oxígeno molecular. Las reacciones de oxidación química sobre los compuestos fenólicos (o-difenoles) dan lugar a pigmentos de la misma naturaleza que los formados por la oxidación enzimática. Sin embargo, además se pueden oxidar otros compuestos no fenólicos como el etanol y el ácido tartárico, e intervenir en reacciones de polimerización antociano-tanino y/o tanino-antociano.

II.2.1.6. Reacciones de condensación mediadas por aldehídos

Otros aldehídos como el ácido glioxílico, el furfural y el hidroximetilfurfural, pueden reaccionar con los flavonoides mediante reacciones de condensación similares a la del acetaldehído (Es-Safi et al., 1999b; Es-Safi et al., 2000a). Los primeros compuestos se encontraron por condensación entre la catequina y el ácido glioxílico (obtenido por la oxidación del ácido tartárico que se encuentra en el vino) (Es-Safi et al., 1999b).

Inicialmente, el compuesto que se forma es incoloro, pero los posteriores procesos de deshidratación y oxidación que se pueden producir en el vino durante el envejecimiento, pueden dar lugar a pigmentos con tonalidad amarillo-anaranjada tipo sales de xantilio (Fulcrand et al., 2006). Estos compuestos tienen un máximo de absorción comprendido entre 440-460 nm, son inestables e intervienen en las reacciones

de pardeamiento debido a la oxidación no enzimática (Es-Safi et al., 2000a). Por tanto, juegan un papel importante en el color de los vinos blancos, aunque también han sido detectados en vinos tintos (Es-Safi et al., 2000b).

II.2.2. El color en los vinos blancos

En los vinos blancos, al igual que ocurre en los tintos, el color es una fuente de información que da una idea al catador sobre el tipo de vino que está examinando. El color de un vino blanco se debe principalmente a su contenido en flavanoles y flavonoles, y puede ir desde el blanco papel (casi incoloro), pasando por diferentes tonalidades hasta el color ámbar fuerte o pardo en función del envejecimiento que haya sufrido.

Las reacciones de oxidación enzimática y no enzimática de los compuestos fenólicos comentadas en el apartado II.2.1.5. que dan lugar a compuestos de color amarillo-pardo son las principales responsables del color de los vinos blancos.

Por otro lado, las reacciones de oxidación de otros compuestos que están presentes en el vino como el etanol y el ácido tartárico, dan lugar a la formación de acetaldehído y ácido glioxílico respectivamente, que pueden reaccionar con los flavanoles del vino, tal y como se ha comentado con anterioridad en los apartados II.2.1.3. y II.2.1.6. Estos compuestos son inicialmente incoloros y posteriormente adquieren tonos amarillos-pardos, y contribuyen de esta manera a aumentar estas tonalidades en los vinos blancos (Fulcrand et al., 1996a; Es-Safi et al., 2000c; López-Toledano et al., 2004).

El pardeamiento del vino blanco durante su almacenamiento es considerado una alteración de la calidad del vino que suele ir acompañada de otras modificaciones en sus propiedades sensoriales, dando como resultado un acortamiento de la vida comercial del vino (López-Toledano et al., 2006).

II.3. POLISACÁRIDOS DE LA UVA Y DEL VINO

Los polisacáridos son componentes de las paredes celulares que recubren y protegen la membrana plasmática, tanto de las células vegetales de la uva, como de los microorganismos que participan en el proceso de vinificación. Por lo tanto, los polisacáridos presentes en el vino pueden provenir de la uva, de las levaduras, de las bacterias (acéticas y lácticas) y de la contaminación por hongos como *Botrytis cinerea* (Guadalupe, 2008). Sin embargo, desde el punto de vista enológico y cuantitativo, los polisacáridos procedentes de la uva y de las levaduras son los más importantes.

La concentración de polisacáridos procedentes de la uva va a depender en gran medida de la variedad de uva, el rendimiento de producción del viñedo, las condiciones edafoclimáticas, el nivel de madurez de la uva, y las técnicas de vinificación empleadas (prensado, desfangado, clarificación, etc.) (Riu-Aumatell et al., 2002). En cambio, la concentración de polisacáridos procedentes de las levaduras, principalmente manoproteínas, dependerá de la cepa o cepas de levadura que lleven a cabo la fermentación alcohólica, de la temperatura de fermentación, de los carbohidratos disponibles, de la aireación, agitación y del grado de clarificación del mosto (Guilloux-Benatier et al., 1995), y sobretodo del tiempo de contacto de las lías con el vino una vez finalizada la fermentación alcohólica (Caridi, 2006). En general, la concentración de polisacáridos suele oscilar entre 100-400 mg/L en vinos blancos y entre 250-800 mg/L en vinos tintos.

Estos compuestos se pueden dividir en polisacáridos ácidos y neutros, según contienen ácido galacturónico o no.

Dentro de los polisacáridos procedentes de la uva, los homogalacturonanos y ramnogalacturonanos I y II pertenecen al grupo de polisacáridos ácidos y los arabinanos y arabinogalactanos I y II se engloban dentro del grupo de polisacáridos neutros. Los polisacáridos procedentes de las levaduras, mananos y manoproteínas, β-glucanos y quitina, son todos de carácter neutro **(figura II.16)**.

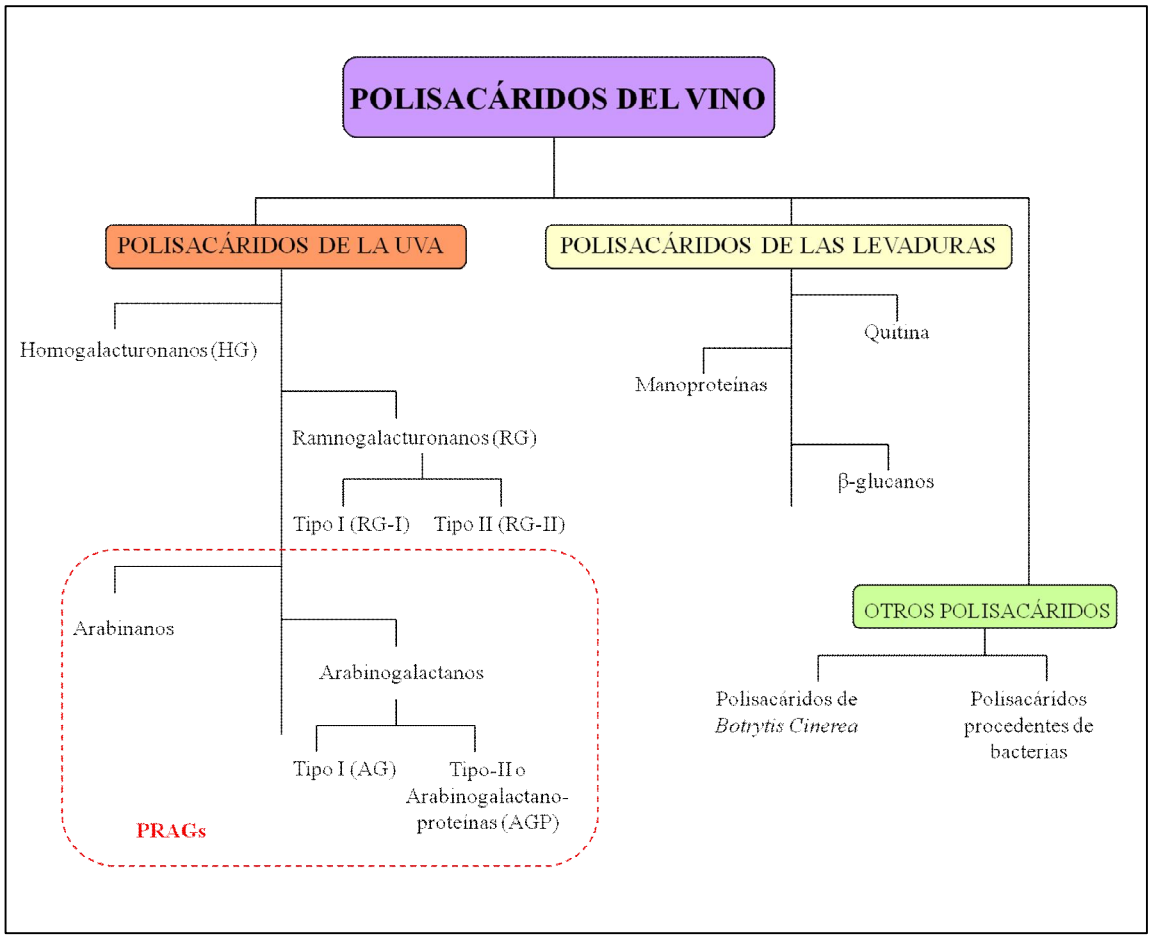


Figura II.16. Clasificación de los polisacáridos del vino.

II.3.1. Polisacáridos de la uva

Los polisacáridos procedentes de la uva son el resultado de la degradación y de la solubilización de las sustancias pécticas contenidas en el hollejo y en la pulpa, debido a la acción de las enzimas pectinolíticas, (Hidalgo, 2006), y se liberan en las primeras etapas del proceso de vinificación (Doco et al., 1999).

II.3.1.1. Homogalacturonanos (HG)

Son los compuestos mayoritarios en la uva (Vidal et al., 2001; Pinelo et al., 2006). Están formados por cadenas lineales de ácido D-galacturónico unidos por enlaces α-(1→4), formando las llamadas zonas "lisas" de las paredes celulares, con un elevado grado de esterificación con metanol y parcialmente con ácido acético **(figura II.17)**. Estos compuestos, a pesar de ser muy abundantes en los mostos (Vidal et al., 2000), en el vino se encuentran en cantidades prácticamente inapreciables (Pellerin y Cabanis, 2003; Vidal et al., 2001; Guadalupe et al., 2007), ya que se hidrolizan fácilmente por acción de las enzimas pectinolíticas (endo y exo-poligalacturonasas, pectín-metilesterasas y endo-pectínliasas). Por ello, se degradan rápidamente liberando otras sustancias pécticas (ramnogalacturonanos) contenidas en las zonas "erizadas" (Vidal et al., 2001; Hidalgo, 2006).

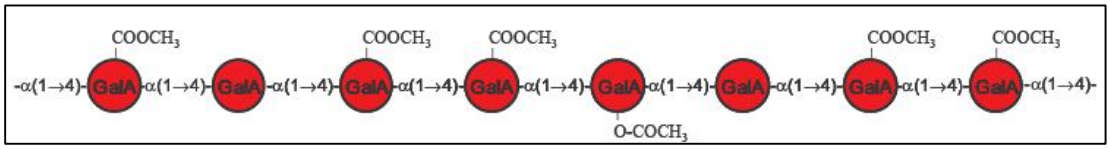


Figura II.17. Estructura del homogalacturonano (extraído de Guadalupe, 2008).

II.3.1.2. Ramnogalacturonanos I (RG-I)

Son los polisacáridos más abundantes en las uvas después de los homogalacturonanos (Vidal et al., 2003a), pero en el vino también se encuentran en cantidades muy pequeñas (Pellerin y Cabanis, 2003) debido a que son poco solubles y muy sensibles a la acción de las enzimas pectinolíticas. Estos compuestos están formados por unidades de α-L-ramnosa y ácido galacturónico unidos linealmente de forma alterna. Además, en esta cadena se insertan lateralmente cadenas de polisacáridos neutros (arabinanos y arabinogalactanos del tipo I y II) en posición α-(1→4) con la ramnosa (Vidal et al., 2003a), que confieren un aspecto ramificado a esta zona de las paredes celulares **(figura II.18).**

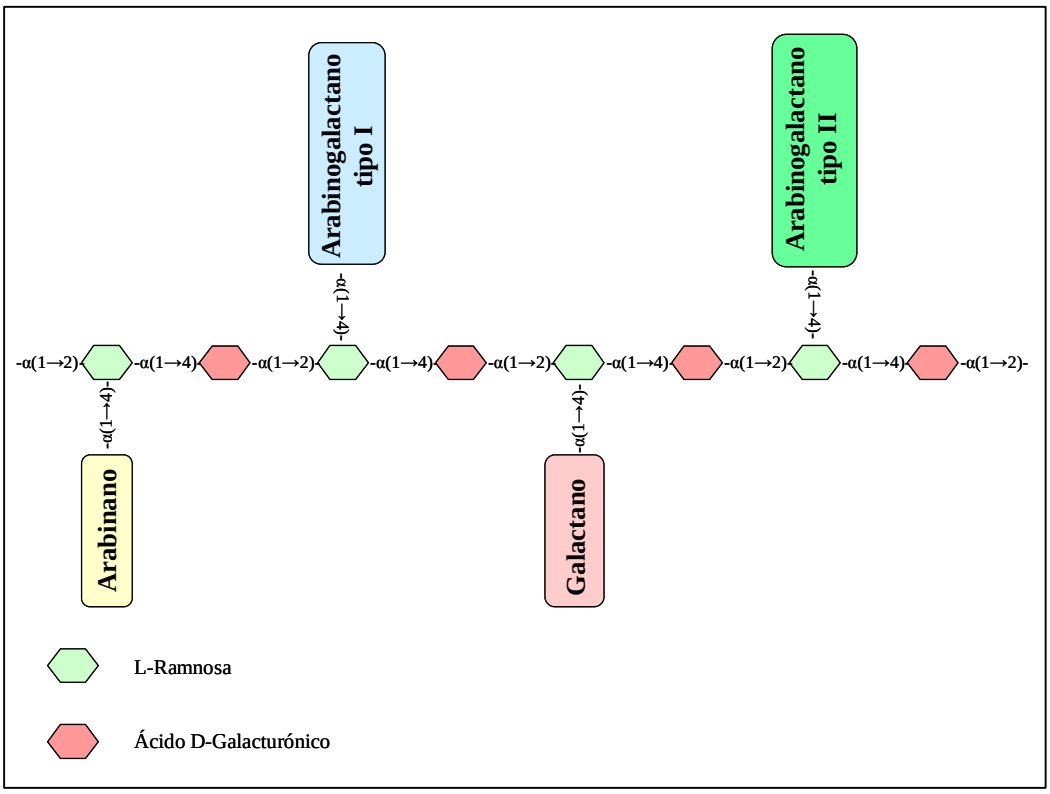


Figura II.18. Estructura de ramnogalacturonano I (adaptado de Guadalupe, 2008)**.**

II.3.1.3. Ramnogalacturonanos II (RG-II)

Son polisacáridos ácidos de estructura muy compleja. Están formados por una cadena de 8 moléculas de ácido galacturónico, unidos por enlaces α-(1→4) a la cual se unen cadenas laterales de oligosacáridos, que contienen arabinosa, ramnosa, fucosa, galactosa, ácido galacturónico, ácido glucurónico y algunos *azúcares raros* (2-*O*-metil-fucosa, 2-*O*-metil-xilosa, apiosa, ácido acérico o 3-carboxi-5-deoxi-L-xilosa, Dha o ácido 3-deoxi-D-liso-2-heptulosónico, y Kdo o ácido 2-ceto-3-deoxi-D-manooctulosónico) **(figura II.19)**.

Estos *azúcares raros* solamente se encuentran en estos compuestos y se utilizan para su identificación en el vino (Pellerin et al., 1996; Pérez et al., 2003; Vidal et al,. 2003a). Se suelen encontrar formando complejos estables con metales pesados como el

boro y el plomo, y son resistentes a la degradación por enzimas pectinolíticas por lo que se encuentran tanto en mostos como en vinos (Doco et al., 1997). Su concentración suele estar entorno al 20% de los polisacáridos solubles totales en el vino tinto (Pellerin et al., 1996; Vidal et al., 2003a).

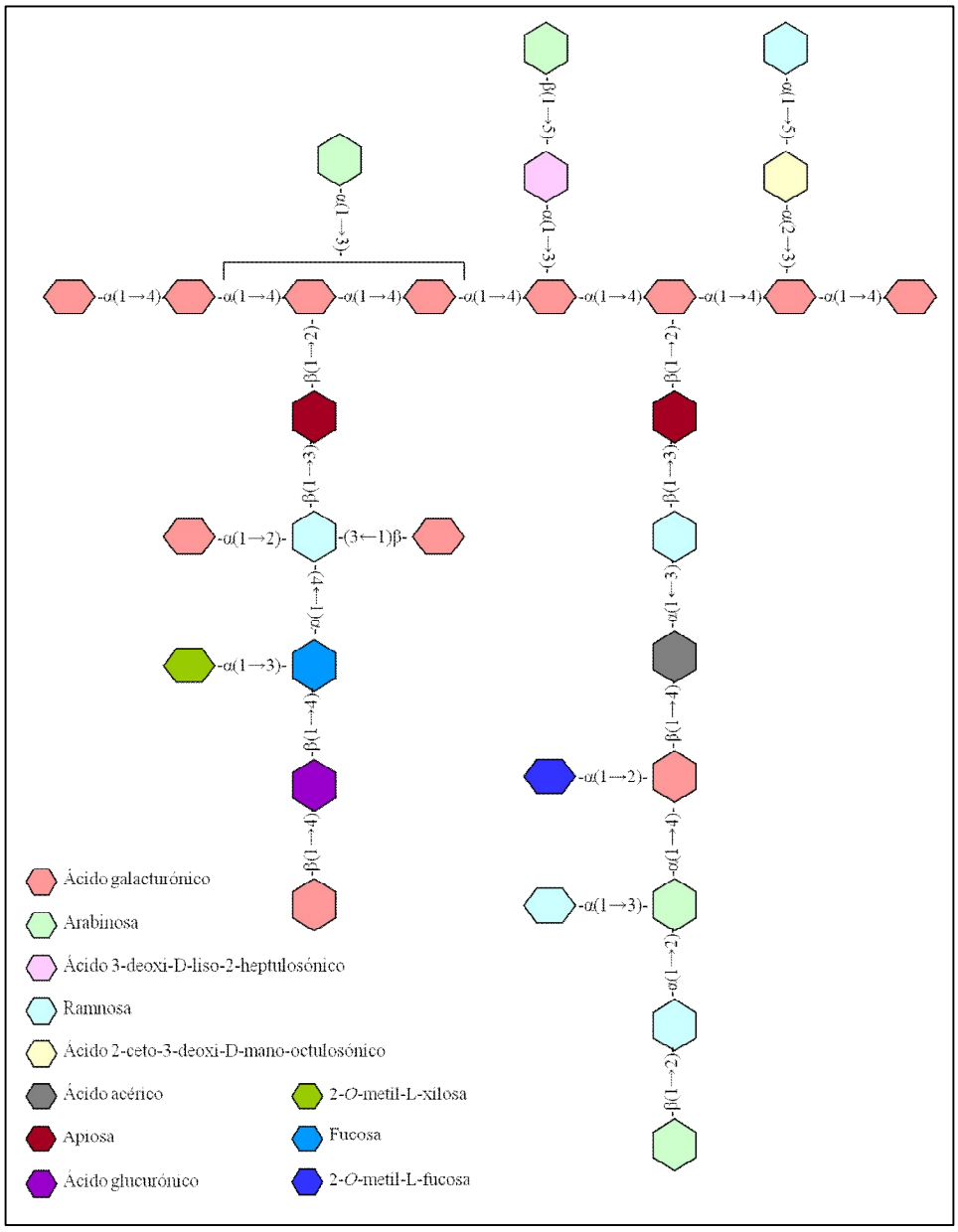


Figura II.19. Representación esquemática de la estructura primaria del ramnogalacturonano II (adaptado de Pérez et al., 2003).

Los ramnogalacturonanos I y II son más abundantes en las paredes celulares de los hollejos que en la pulpa, por lo que su concentración es mayor en los vinos tintos que en los vinos blancos (Vidal et al., 2003a).

II.3.1.4. Arabinanos

Son los componentes principales de las cadenas laterales de los RG-I. Su estructura es bastante simple, ya que es una cadena lineal de L-arabinofuranosa unida por enlaces α-(1→5) con alguna ramificación, consistente en una única unidad de arabinosa en posición 3 (Ribéreau-Gayón et al., 2003). Estos compuestos son poco abundantes en los mostos y en los vinos, ya que son bastante insolubles y son poco hidrolizados por las enzimas correspondientes. Este hecho parece ser debido a la pérdida de los residuos de arabinosa terminales por acción de las enzimas arabinofuranosidasas (Pellerin y Cabanis, 2003).

II.3.1.5. Arabinogalactanos I (AG)

Están formados por cadenas de galactosa (galactanos) o galactopiranosa unidas en posición β-(1→4), estos compuestos se encuentran unidos a los RG-I en posición α-(1→4) con residuos terminales de arabinosa en posición α-(1→3) **(figura II.20)**. Como ya se ha comentado, son abundantes en muchas frutas, pero debido a su baja solubilización, no se encuentran en mostos y en vinos (Hidalgo, 2003).

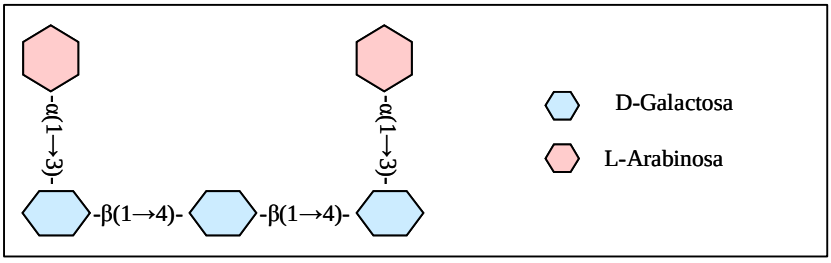


Figura II.20. Estructura del arabinogalactano I (adaptado de Guadalupe, 2008).

II.3.1.6. Arabinogalactanos II o arabinogalactano-proteínas (AGP)

Es el grupo de polisacáridos más abundante en mostos y en vinos. Son más abundantes en la pulpa que en los hollejos por lo que son fácilmente extraídos durante el prensado de la uva (Vidal et al., 2000).

La parte glucídica de estos compuestos está formada por cadenas de galactosa unidas en posición β-(1→3) ramificadas con cadenas cortas de galactosa unidas en β-(1→6), que además se encuentran ramificadas con cadenas de arabinosa en posición α-(1→3) **(figura II.21)**. Estos compuestos se encuentran unidos a un péptido rico en hidroxiprolina (3-4%). Contienen menos de un 5% de ácido glucurónico y no contienen ácido galacturónico (Pellerin et al., 1995; Hidalgo, 2003). Como se ha comentado anteriormente, estos compuestos se encuentran unidos lateralmente a los RG-I.

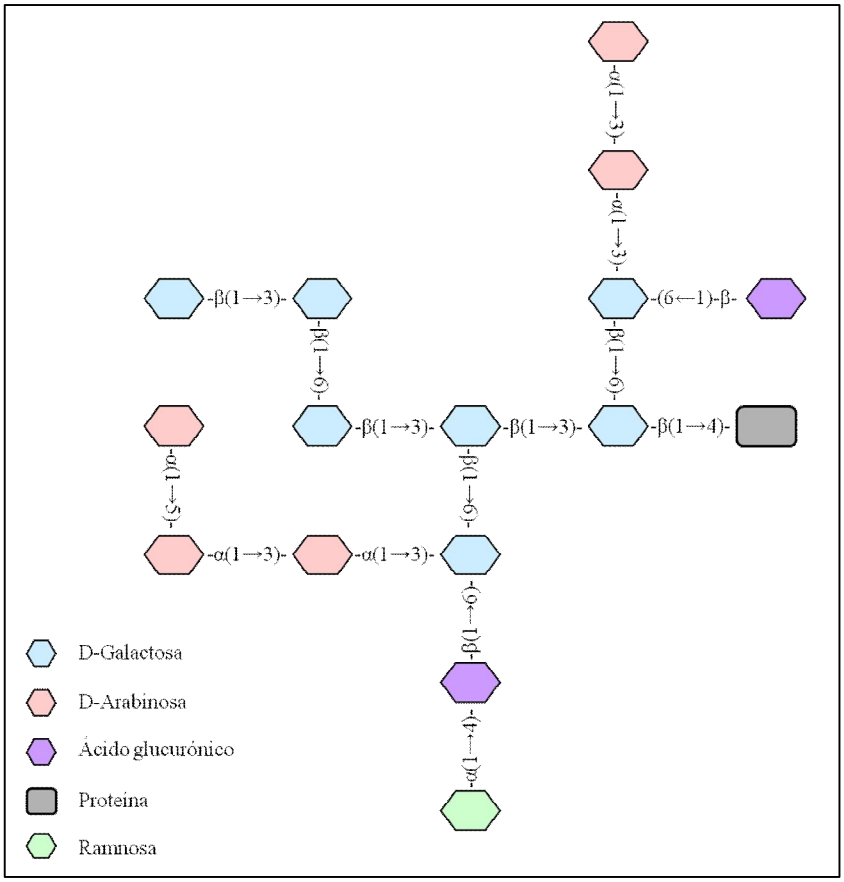


Figura II.21. Estructura de los AGP (adaptado deGuadalupe, 2008).

Actualmente, algunos autores engloban a los arabinanos, arabinogalactanos del tipo I y los AGPs dentro de un mismo grupo llamado PRAGs (polisacáridos ricos en arabinosa y galactosa) (Doco et al., 2007; Ducasse et al., 2010).

II.3.2. Polisacáridos de las levaduras

Los polisacáridos de las levaduras son la segunda fuente de polisacáridos del vino y se encuentran principalmente en su pared celular.

La pared celular representa entre un 10 y un 25% del peso seco de la levadura (Fleet, 1991) dependiendo de la cepa, y está compuesta por dos capas de polisacáridos. La capa interna, transparente y amorfa está, constituida principalmente por β-(1→3) glucanos y quitina, que son los responsables de mantener la forma y rigidez de la célula y favorecer la resistencia a los cambios osmóticos y mecánicos (Cid et al., 1995; Aguilar-Uscanga et al., 2005). La capa externa está formada por β-(1→6) glucanos y manoproteínas **(Figura II.22)**.

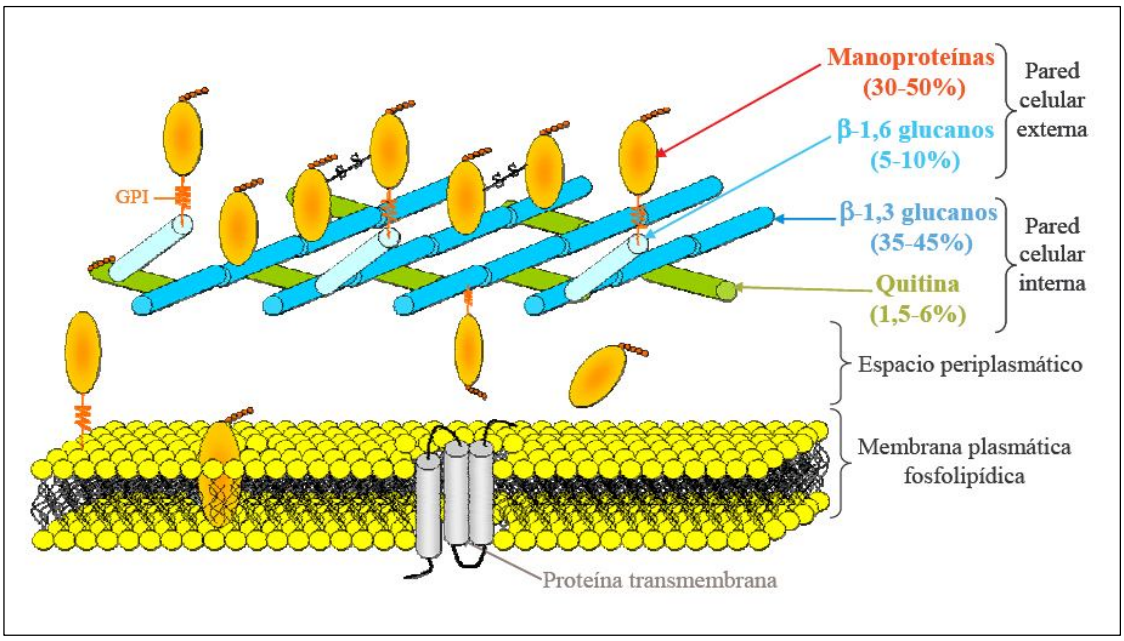


Figura II.22. Estructura de las envolturas celulares de la levadura (Molina et al., 2000). Se indica el porcentage en peso de cada componente (Klis et al., 2006).

La estructura y composición de la pared celular puede variar considerablemente en respuesta al estrés, condiciones de cultivo, edad y modificaciones genéticas (Fleet, 1991; Ha et al., 2002; Klis et al., 2002; Aguilar-Uscanga y Francois, 2003; Aguilar-Uscanga et al., 2005).

II.3.2.1. Quitina

La quitina está formada por cadenas lineales de N-acetilglucosamina unidas en posición β-(1→4). Este polisacárido se encuentra en concentraciones muy bajas en la pared celular y debido a su baja solubilidad en agua, y su cantidad en el vino es inapreciable (Ribéreau-Gayón et al., 2003).

II.3.2.2. β-glucanos

Los β-glucanos son, junto con las manoproteínas, los compuestos más abundantes de la pared celular de las levaduras. Son compuestos poliméricos formados principalmente por cadenas de glucosa unidas por enlaces β-(1→3), con ramificaciones laterales de glucosa β-(1→6). En la pared celular de *Saccharomyces cerevisiae* los β-(1→3) glucanos constituyen el 85% del total, mientras que el 15% restante son β-(1→6) glucanos (Manners et al., 1973).

Los β-(1→3)-glucanos están formados por monómeros de glucosa, llegando a alcanzar hasta las 1500 unidades, y son los principales responsables de la resistencia mecánica de la pared celular. Poseen una estructura helicoidal y está formada por una o más cadenas de polisacáridos que se unen mediante puentes de hidrógeno, formando así una red a la que se unen otros componentes de la pared celular. En la parte externa se pueden unir moléculas altamente ramificadas de β-(l→6)-glucanos, que a su vez pueden unirse a las manoproteínas. En la parte interna se pueden encontrar cadenas de quitina (Klis et al., 2006).

Los β-(1→6)-glucanos forman un polímero de entre 130 y 350 residuos de glucosa por molécula y poseen una estructura amorfa muy ramificada (Lesage y Bussey,

2006). Su principal función es la organización de la pared celular, ya que actúan como unión flexible formando interconexiones con los β-(l→3)-glucanos, con la quitina y con las manoproteínas, enlazando estas últimas con la red β-(l→3)-glucanos (Klis et al., 2002; Lesage y Bussey, 2006).

II.3.2.3. Manoproteínas

Los mananos y manoproteínas constituyen el grupo principal de polisacáridos de las membranas de las levaduras, y se encuentran unidas a los glucanos a través de enlaces covalentes y no covalentes (Valentín et al., 1984). Son responsables de la porosidad de la pared y además juegan un papel de filtro selectivo y de protección contra ataques químicos y enzimáticos de tipo glucanasa (Zlotnik et al., 1984; Aguilar-Uscanga et al., 2005).

Las manoproteínas son glicoproteínas normalmente con un alto grado de glicosilación (80-90%), compuestas por monosacáridos principalmente manosa (>90%) y glucosa (Guadalupe et al., 2010) y por proteínas (<10%) (Vidal et al., 2003a). Estos compuestos son liberados al medio durante la fermentación alcohólica (Doco et al., 1996; Vidal et al., 2003a; Ayestarán et al., 2004) y/o posteriormente durante la crianza de los vinos sobre lías debido a los procesos de autolisis de las levaduras (Doco et al., 2003; González-Ramos et al., 2008).

Las manoproteínas pueden tener un tamaño muy variable (5-800 kDa) (Doco et al., 2003) y pueden constituir entre el 25 y el 50 % del peso seco de la levadura de *Saccharomyces cerevisiae* (Klis et al., 2006; Pozo-Bayón et al., 2009a), aunque su liberación al vino depende de la cepa de levadura utilizada (Rosi et al., 2000), así como de las condiciones nutricionales del mosto (turbidez) (Ribéreau-Gayón et al., 2003).

La estructura de las manoproteínas está formada básicamente por un polipéptido al cual se unen dos tipos de ramificaciones glucídicas **(figura II.23)**. Una ramificación está formada por cadenas cortas de D-manosa (unidas por enlaces α-(1→2) o α-(1→3)) que se unen a la cadena proteica a través de los residuos de serina y/o treonina; y la

segunda ramificación consiste en un polisacárido unido a la parte proteica mediante dos unidades de N-acetilglucosamina que a su vez están unidas entre sí en posición β-(1→4) y unidas covalentemente a un residuo de asparagina (Pérez-Serradilla y Luque de Castro, 2008). El polisacárido unido a las unidades de N-acetilglucosamina consiste en una cadena principal de α-(1→6) manosa con ramificaciones cortas de residuos de manosa unidos en posición α-(1→2) y con manosas terminales en posición α-(1→3), conteniendo algunas de estas ramificaciones enlaces fosfodiéster.

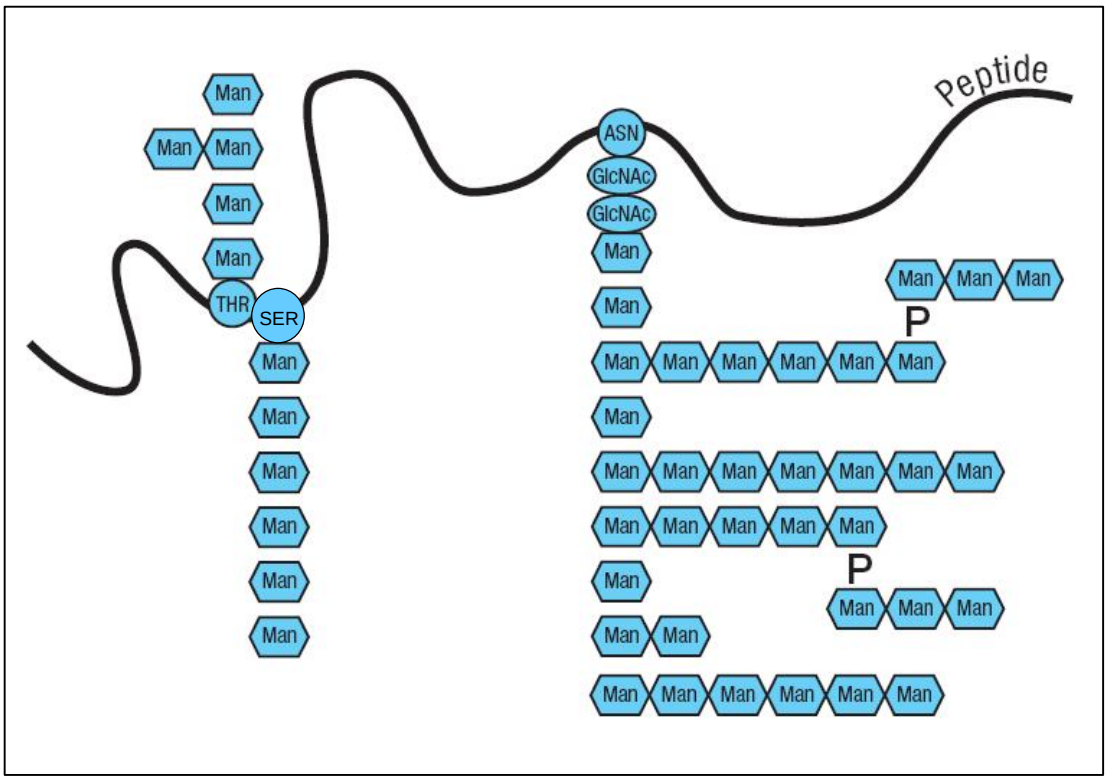


Figura II.23. Estructura de las manoproteínas exocelulares de levadura. (Man: Manosa, P: fosfato, GlcNAc: N-acetil-glucosamina, Asn: asparagina, Ser: serina, Thr: treonina) (extraído de Bajard-Sparrow et al., 2007).

II.3.3. Interés enológico de los polisacáridos

Los polisacáridos del vino en general, y las manoproteínas en particular, juegan un papel importante en las características tecnológicas y sensoriales de los vinos. Sin embargo, no todos ellos tienen el mismo comportamiento, que dependerá del tamaño y origen de estos compuestos. Los efectos positivos que estos compuestos pueden aportar al vino se comentan a continuación:

II.3.3.1. Mejorar la estabilidad proteica

La aparición de turbidez en el vino embotellado se debe a la formación de agregados de proteínas inestables que se forman fundamentalmente por un aumento de la temperatura de conservación del vino. La presencia en los vinos de ciertas manoproteínas puede reducir en gran medida esta turbidez al interaccionar con las proteínas inestables, mejorando por tanto la estabilidad proteica de los vinos blancos (Waters et al., 1994; Moine-Ledoux y Dubourdieu, 1999; Dupin et al., 2000; Waters et al., 2000a y b; González-Ramos et al., 2008; Lomolino y Curioni, 2007; Schmidt et al., 2009).

Un trabajo desarrollado por Moine-Ledoux y Dubourdieu (1999) ha puesto de manifiesto el efecto estabilizante de una manoproteína N-glicosilada de 31,8 kDa, que es un fragmento de la enzima invertasa de *Saccharomyces cerevisiae* que se libera al vino por la acción combinada de las enzimas β-glucanasas y la proteasa A vacuolar durante el proceso de autolisis en la crianza sobre lías. Su efecto es mucho más marcado que el de otros polisacáridos como la goma arábiga u otras manoproteínas con mayor peso molecular. Posteriormente, otros autores han conseguido aislar y purificar otras manoproteínas procedentes de la pared celular de *Saccharomyces cerevisiae*, demostrando su efecto protector frente a la quiebra proteica (Waters et al., 1994; Dupin et al., 2000; Waters et al., 2000a).

Esta mejora en la estabilidad proteica puede suponer una reducción de la dosis de bentonita que se debe añadir a los vinos blancos para conseguir su estabilización.

Esta reducción sería positiva ya que la adición de bentonita trae consigo importantes pérdidas de volumen en lías y de componentes aromáticos presentes en el vino, disminuyendo su calidad (Dupin et al., 2000).

El mecanismo exacto por el que las manoproteínas protegen al vino frente a la quiebra proteica no está del todo claro. Se ha sugerido que las manoproteínas dificultan la formación de los agregados de proteínas inestables al competir con las proteínas por unirse a otros componentes no proteicos del vino, los cuales parecen estar involucrados en la formación de estos agregados proteicos insolubles (Dupin et al., 2000).

Por otro lado, este efecto va a depender de la levadura empleada y de la composición y tamaño de los polisacáridos liberados (Lomolino y Curioni, 2007).

II.3.3.2. Mejorar la estabilidad tartárica

Se han encontrado estudios que han determinado la importancia de las manoproteínas en la estabilidad tartárica de los vinos, especialmente de las manoproteínas altamente glicosiladas con pesos moleculares entre 30 y 50 kDa debido a que producen una disminución de la temperatura de cristalización (Dubourdieu y Moine, 1997; Moine-Ledoux et al., 1997; Moine-Ledoux y Dubourdieu, 2002), y a que inhiben la cristalización de sales de tartrato (Lubbers et al., 1993; Caridi, 2006).

El mecanismo de acción se define como un tipo de inhibición competitiva, limitando la formación de los cristales. Las manoproteínas actúan en la etapa inicial de la formación de cristales de bitartrato potásico, y también pueden actuar durante su crecimiento, inhibiendo su precipitación (Moutounet et al., 1999).

II.3.3.3. Mejorar el perfil aromático de los vinos

Las manoproteínas también pueden influir en el aroma de los vinos, ya que pueden “secuestrar” los compuestos volátiles en su estructura tridimensional o interaccionar con ellos, lo que dará lugar a un aumento de la persistencia aromática en

el tiempo (Chung, 1986; Lubbers et al., 1994b; Dufour y Bayonoue, 1999; Ramírez et al., 2004; Vidal et al., 2004b; Bautista et al., 2007; Chalier et al., 2007). Según Lubbers et al. (1994a) la fracción proteica de las manoproteínas es la principal responsable de la estabilidad aromática, aunque este efecto de retención fue diferente en soluciones modelo que en vinos, y dependió del compuesto volátil y del tratamiento empleado durante el período de vinificación. Sin embargo, Chalier et al. (2007), en un estudio llevado a cabo en soluciones modelo comprobaron, que en la retención de los compuestos volátiles del vino por las manoproteínas, están involucradas tanto la parte glucosídica como la parte peptídica de las mismas. Estos autores también indicaron que la fuerza de estas interacciones parece estar relacionada con la estructura conformacional de las manoproteínas. Además encontraron grandes diferencias en la capacidad de retención de los compuestos aromáticos en función de la cepa de levadura empleada.

Esta interacción entre los compuestos volátiles del vino y las manoproteínas procedentes de las levaduras ha sido observada también en vinos blancos y tintos (Bautista et al., 2007; Del Barrio-Galán et al., 2010, Rodríguez-Bencomo et al., 2010), produciéndose una modificación de la intensidad olfativa de los vinos tras el tratamiento. Este efecto puede implicar una percepción aromática más duradera en el tiempo debido a que estos compuestos volátiles, que son retenidos por las manoproteínas, pueden liberarse a lo largo del tiempo. Del Barrio-Galán et al. (2010) observaron en vinos blancos tratados con diferentes derivados de levadura comerciales, una disminución en la concentración de los ésteres etílicos y ácidos grasos principalmente, disminuyendo su concentración. Por otro lado, Rodríguez-Bencomo et al. (2010) encontraron que, en vinos tintos de la variedad Tempranillo, tanto la crianza sobre lías como la adición de un derivado comercial de levadura modificaban el perfil aromático de los vinos finales. Sin embargo, el efecto de estos tratamientos dependió del compuesto volátil del que se tratase.

II.3.3.4. Mejorar las propiedades sensoriales en boca de los vinos y estabilizar el color de los vinos tintos

Riou et al. (2002) y Poncet-Legrand et al. (2007) observaron que ciertos polisacáridos y manoproteínas de alto peso molecular impedían la autoagregación de los taninos. Los resultados obtenidos en soluciones modelo parecen indicar que estos polisacáridos y manoproteínas pueden unirse a los taninos formando agregados más estables que impiden su polimerización y posterior precipitación **(figura II.24)**. Este fenómeno parece ser dependiente de la concentración y el peso molecular de las manoproteínas y de las condiciones del medio (contenido en etanol y de la fuerza iónica).

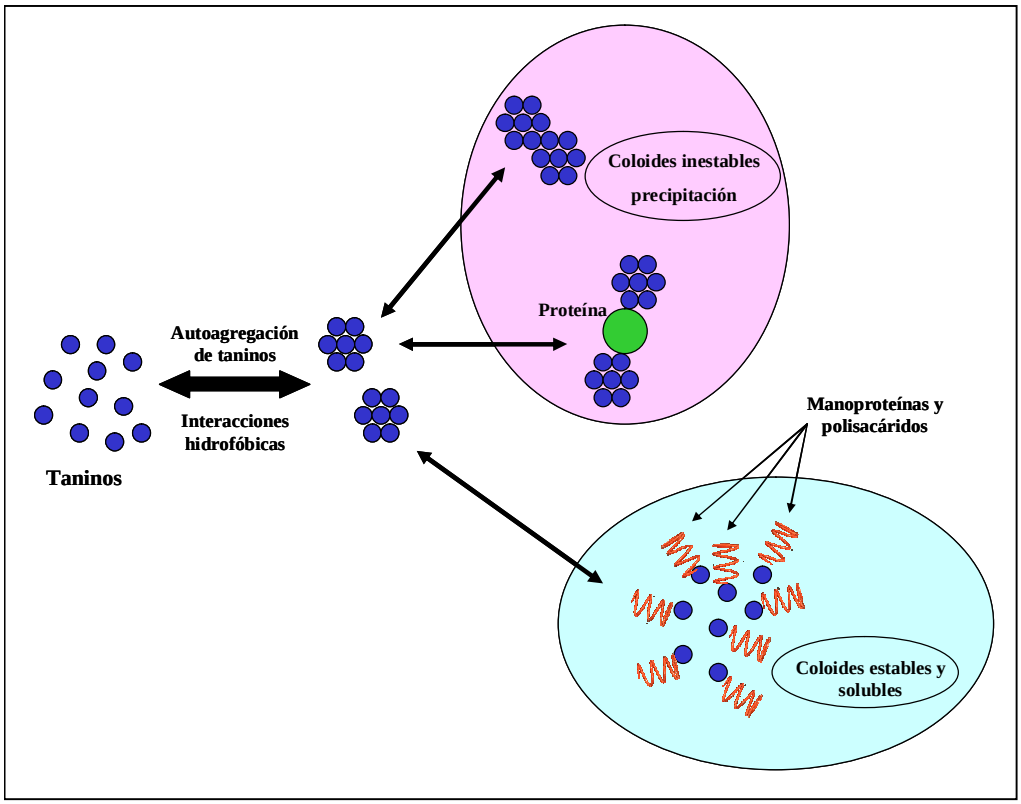


Figura II.24. Esquema de interacción tanino-manoproteína y autoagregación de taninos (adaptado de Guadalupe, 2008).

La presencia de estos complejos polisacárido-tanino puede reducir la astringencia y aumentar la redondez, la estructura y la untuosidad de los vinos en boca. Este hecho ha sido demostrado en soluciones modelo por varios autores usando diferentes fracciones de polisacáridos (Riou et al., 2002; Vidal et al., 2004a y b; Poncet-Legrand et al., 2007). Además, estos mismos estudios han mostrado una reducción del amargor y un aumento del volumen en boca de las soluciones con polisacáridos.

La capacidad de los polisacáridos de interaccionar con los taninos y antocianos puede prevenir su agregación y precipitación (Vidal et al., 2004b) y, de este modo, contribuir a la estabilización del color (Saucier et al., 2000; Escot et al., 2001; Feuillat et al., 2001; Francois et al., 2007) **(figura II.25)**.

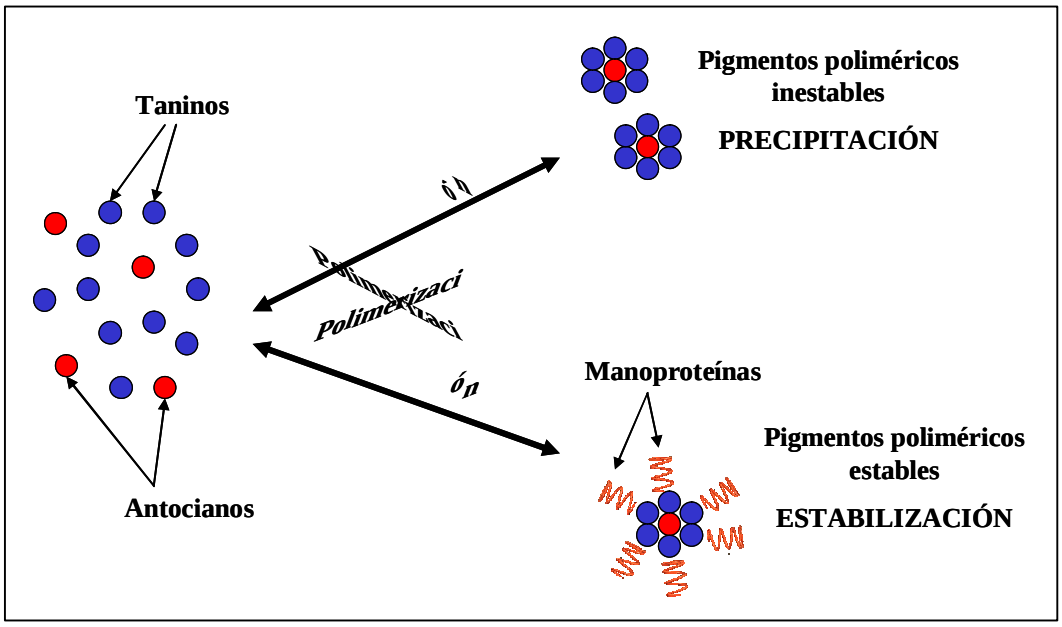


Figura II.25. Modelo de actuación de las manoproteínas sobre la estabilidad de la materia colorante (adaptado de Guadalupe, 2008).

Sin embargo, se han encontrado pocos estudios en vinos. Guadalupe et al. (2007), en un estudio sobre la adición de manoproteínas en vinos tintos, concluyeron que la adición de manoproteínas afecta a la estructura de los vinos, disminuyendo su

astringencia y aumentando la sensación de dulzor y redondez, debido a la disminución del índice de polifenoles totales. Por otro lado, Guadalupe et al. (2010), en un estudio posterior con un vino tinto de la variedad Tempranillo, encontraron evidencias de interacciones entre las manoproteínas secretadas por una cepa de levadura sobreproductora de manoproteínas (BM45) y las proantocianidinas del vino. Sin embargo, no observaron un efecto estabilizante o preventivo de la agregación tánica, sino al contrario, ya que estas manoproteínas parecieron inducir la pérdida de taninos.

No obstante, a día de hoy, no se conocen muchos trabajos científicos que hayan conseguido probar este efecto positivo de los polisacáridos y manoproteínas sobre el color de los vinos. En varios trabajos realizados por un grupo de la Universidad de la Rioja (Guadalupe et al., 2007, Guadalupe y Ayestarán, 2008, Guadalupe et al., 2010) en los que se utilizaron distintas preparaciones ricas en manoproteínas sobre vinos tintos, no se ha observado una mejora de la intensidad y estabilidad del color, incluso alguno de los vinos tratados con estas preparaciones mostró menor intensidad de color que los vinos control (Guadalupe et al., 2007).

II.3.3.5. Favorecer el crecimiento de bacterias lácticas

Diversos trabajos han puesto de manifiesto que las manoproteínas de las levaduras, junto a los aminoácidos y péptidos, favorecen el crecimiento de bacterias lácticas en el vino (Guilloux-Benatier et al., 1995; Rosi et al., 2000; Guilloux-Benatier y Chassagne, 2003; Díez et al., 2010). Esto puede ser debido a que las manoproteínas adsorben algunos ácidos grasos que son sintetizados por las levaduras de *Saccharomyces cerevisiae* y que son inhibidores del crecimiento bacteriano, y/o a que las bacterias lácticas son capaces de hidrolizar las manoproteínas, liberando nutrientes al medio, y favoreciendo de esta forma su crecimiento (Guilloux-Benatier y Chassagne, 2003).

II.3.3.6. Reducir el contenido en ocratoxina A (OTA)

Otro efecto positivo que se ha encontrado en las manoproteínas es que pueden llevar a cabo la adsorción de la ocratoxina A (Baptista et al., 2004; Bejaoui et al., 2004; Ringot et al., 2005; Caridi et al., 2006). Esta micotoxina es neurotóxica y cancerígena y es producida principalmente por algunas especies de hongos de los géneros *Aspergillus* y *Penicillium*. Esta adsorción parece ser más efectiva en vinos blancos que en tintos, debido a la competencia que existe entre los polifenoles y la OTA por los mismos sitios de unión a las paredes de las levaduras (Umarino et al., 2001). Existen diversos factores que pueden afectar de manera importante a la capacidad de adsorción de OTA por parte de las manoproteínas como son: el contenido en manosilfosfato de las manoproteínas de las levaduras, la velocidad de sedimentación celular, dimensión celular, etc. (Caridi, 2007).

II.3.3.7. Otros efectos

II.3.3.7.1. Mejorar las características espumantes

Según diversos estudios, algunos polisacáridos pueden mejorar las características espumantes (altura que alcanza la espuma, estabilidad de la espuma, tamaño de la burbuja, efervescencia del vino, etc.) de los vinos espumosos (Moreno-Arribas et al., 2000; Vanrell et al., 2005; Núñez et al., 2005; Núñez et al., 2006). Dentro de los polisacáridos, las manoproteínas que se liberan durante la autolisis de las levaduras parecen ser, en parte las responsables de los cambios en las características espumantes de estos vinos (Moreno-Arribas et al., 2000).

Por otro lado, Nuñez et al. (2006) observaron que el enriquecimiento de los vinos espumosos con manoproteínas extraídas mediante un tratamiento térmico moderado, contribuyó a mejorar sus propiedades espumantes. Estas manoproteínas presentaban un peso molecular comprendido entre 10 y 21,5 kDa, y su contribución a la calidad de la espuma parece estar relacionada con su hidrofobicidad e hidrofilidad.

II.3.3.7.2. Participar en la formación del velo de flor en la crianza de vinos de Jerez

El envejecimiento de los vinos de Jerez ocurre bajo la formación de una capa de levaduras de *Saccharomyces cerevisiae* que crece espontáneamente en la superficie del vino que está en contacto con el aire. A estas levaduras se les conoce como levaduras de flor, y a la capa que se forma se le denomina velo de flor, y que es fundamental para obtener las características especiales de este tipo de vinos (Gutiérrez et al., 2010).

De acuerdo con los estudios realizados por Alexandre et al. (2000), una manoproteína de 49 kDa y de naturaleza hidrofóbica, aislada de las paredes celulares de las levaduras, parece estar involucrada en la formación de este velo de flor durante la crianza de estos vinos.

II.4. CRIANZA SOBRE LÍAS Y TÉCNICAS QUE PERMITEN MEJORAR ESTE PROCESO

La crianza sobre lías es una técnica enológica que se viene utilizando desde hace décadas en vinos blancos, tanto espumosos como tranquilos, y más recientemente en vinos tintos. El objetivo de esta técnica es mejorar la calidad y las propiedades sensoriales del vino (Dubourdieu, 1992; Feuillat et al., 2001; Fornairon et al., 2002; Feuillat, 2003; Charpentier et al., 2004) debido, principalmente, a la liberación de ciertos compuestos durante la autolisis de las levaduras. Estos compuestos son, péptidos, aminoácidos, ácidos grasos, nucleótidos, nucleósidos y polisacáridos.

De todos los compuestos que las lías aportan al vino durante el proceso de autolisis, las manoproteínas parecen ser las que tienen un mayor interés enológico debido a su mayor influencia sobre las características tecnológicas y sensoriales de los vinos, como se ha comentado anteriormente.

El proceso de autolisis se inicia con la destrucción de las membranas intracelulares (membrana citoplasmática, mesosomas), liberándose al espacio periplásmico las enzimas β-glucanasas. Estas enzimas provocan la rotura de la pared celular, debido a su acción sobre los β-(1→3)-glucanos, y la consecuente liberación de manoproteínas al medio. Sin embargo, este proceso de autolisis es, generalmente, un proceso lento, ya que las condiciones en que suele realizarse (pH≈3,5-4,1 y Tª≈ 15ºC) no son las idóneas (Fornairon et al., 2002). Por ello, la crianza sobre lías se combina con el "batonnage", técnica que consiste en remover el vino con una barra de acero inoxidable para mejorar el contacto de las lías con el vino y permitir una liberación más rápida de las manoproteínas de las levaduras (Doco et al., 2003).

Tras la fermentación alcohólica, las lías consumen cantidades significativas de oxígeno (Fornairon et al., 1999; Salmon et al., 2000), debido a la presencia de lípidos en las membranas celulares (Salmon et al., 2000). Esta interacción entre las lías y el oxígeno no afecta al propio proceso de autolisis (Fornairon et al., 2002), pero sí ayuda a mantener un medio reductor, lo que puede traer consigo tanto efectos positivos como negativos, tal y como se comentará posteriormente.

Debido a este consumo de oxígeno, la técnica de crianza sobre lías suele llevarse a cabo en barrica, ya que de esta forma se favorece la entrada de pequeñas cantidades de oxígeno a través de los poros de la madera, disminuyendo el efecto reductor de las lías.

Ademas, la crianza sobre lías presenta otra serie de ventajas no asociadas únicamente a las manoproteínas liberadas de las levaduras:

- La naturaleza reductora de las lías protege a los compuestos fenólicos de la oxidación (Fornairon y Salmon, 2003; Salmon, 2006), ya que las lías tienen mayor potencial de consumo de oxígeno que los polifenoles (Salmon, 2006). Por ello, la presencia de lías en el medio permite mantener concentraciones de antocianos monoméricos más altas y reducir el pardeamiento de los vinos, debido a que al haber menos oxígeno en el vino estos compuestos fenólicos se oxidan en menor medida (Palomero et al., 2007; Moreno-Arribas et al., 2008). Sin embargo, algunos autores han observado una disminución en la concentración de antocianos monoméricos debido a su adsorción sobre las paredes celulares de las levaduras (Rodríguez et al., 2005; Morata et al., 2005), que va a depender del tipo de cepa de levadura utilizada y del tipo de antociano estudiado. Estos resultados contradictorios pueden ser explicados debido a que las interacciones entre los antocianos y las paredes celulares de las levaduras son débiles y reversibles (Vasserot et al., 1997; Morata et al., 2003). La crianza sobre lías también permite reducir el pardeamiento de los vinos por adsorción de los compuestos fenólicos no antociánicos sobre las paredes celulares de las levaduras. López-Toledano et al. (2006) y Razmkhab et al. (2002) encontraron una reducción de la concentración de los pigmentos poliméricos pardos que se forman por las reacciones de oxidación y condensación de los compuestos fenólicos. Además, Razmkhab et al. (2002) observaron que tanto las levaduras como las paredes celulares de levaduras llevaron a cabo una retención de algunos flavanoles como catequina, epicatequina y algunas proantocianidinas.

- Durante la autolisis de las levaduras también se liberan otros compuestos como aminoácidos y/o lípidos que pueden ser precursores aromáticos, mejorando la fracción aromática de los vinos. Los lípidos liberados de estas levaduras pueden favorecer la formación de ésteres y aldehídos volátiles (Charpentier et al., 1993).
- Las lías pueden adsorber diferentes compuestos volátiles que son causantes de defectos organolépticos en los vinos, como es el caso de los etilfenoles (Chassagne et al., 2005; Pradelles et al., 2008).

Sin embargo, la crianza sobre lías también presenta ciertas desventajas o inconvenientes:

- Es una práctica que implica una mayor dedicación de los recursos de la bodega (mayor cantidad de mano de obra para realizar el "batonnage", depósitos, barricas, inmovilización de los stocks, etc.).
- El consumo de oxígeno por las lías conlleva un mayor riesgo de aparición de olores a reducción (Chatonnet, 2000; Feuillat et al., 2001).
- Pueden producirse desviaciones organolépticas (Chatonnet, 2000; Zamora, 2002) debidas al desarrollo de microorganismos no deseados como *Brettanomyces*, debido principalmente, al mayor aporte de nutrientes que favorece el desarrollo de estos microorganismos.
- Puede favorecerse la formación de aminas biógenas (González-Marco y Ancín-Azpilicueta, 2006; Martín-Álvarez et al., 2006) ya que durante la autolisis se pueden liberar aminoácidos, y las lías pueden contener diferentes microorganismos con actividad descarboxilasa que transforman los aminoácidos en aminas biógenas.

II.4.1. Adición de enzimas β-glucanasas

La adición de enzimas β-glucanasas a los vinos de forma exógena favorece la liberación de los polisacáridos (glucanos) y manoproteínas de las levaduras (Charpentier y Freyssinet, 1989).

Desde hace muchos años, las empresas que suministran productos enológicos vienen desarrollando y comercializando distintas preparaciones enzimáticas con el objetivo de acelerar el proceso de autolisis en los vinos que son envejecidos sobre lías. Estos productos son preparados que principalmente contienen enzimas con actividad β-glucanasa, aunque también pueden llevar enzimas pectinolíticas. Tradicionalmente, el empleo de estas preparaciones comerciales con actividad β-glucanasa ha estado destinado a mejorar la filtración y clarificación de los vinos en vendimias afectadas por *Botrytis cinerea*. Sin embargo, actualmente se están utilizando también para acelerar la liberación de polisacáridos durante la crianza de los vinos sobre lías (Pellerin y Tessarolo, 2001) debido a que se reducen costes en el proceso de elaboración, ya que se logra acelar el proceso de autolisis.

Las enzimas β-glucanasas hidrolizan los β-glucanos que se encuentran unidos a la quitina y a las manoproteínas, y que se encargan de dar forma y estructura a la pared celular de las levaduras. De este modo, además de inducir la liberación de manoproteínas al vino, la acción de estas enzimas produce la liberación de glucosa y de oligosacáridos debido a la ruptura de los enlaces β-glucosídicos que unen las cadenas de β-glucanos (Humbert-Goffard et al., 2004).

Palomero et al. (2009) concluyeron que la cantidad y tipo de polisacáridos liberados por la acción de las enzimas β-glucanasas va a depender tanto de la cepa de levadura como de la enzima comercial utilizada. Además, estos autores pusieron de manifiesto que la fracción de polisacáridos era de menor tamaño en los vinos que fueron adicionados con enzimas β-glucanasas. Estos polisacáridos de menor tamaño no precipitan o lo hacen en menor medida, pudiéndose favorecer, por tanto, la estabilización coloidal de los vinos.

Todas las preparaciones comerciales de enzimas β-glucanasas autorizadas para uso enológico son sintetizadas y aisladas a partir de *Trichoderma ssp.*, cultivada en las condiciones óptimas para su producción y purificación. Sin embargo, si no se lleva a cabo un buen proceso de purificación, estas preparaciones enzimáticas pueden incluir enzimas con cierta actividad β-glucosidasa inespecífica que es perjudicial para la crianza de los vinos tintos sobre lías (Palomero et al., 2007), ya que pueden producir pérdidas de color debido a la ruptura de los enlaces glucosídicos de los antocianos.

II.4.2. Microoxigenación aplicada a la crianza sobre lías

Como se ha comentado anteriormente, las lías consumen oxígeno favoreciendo la aparición de olores a reducción no deseados, y por ello la adición de pequeñas y controladas cantidades de oxígeno podría mejorar el proceso de crianza tradicional sobre lías.

La microoxigenación es una técnica que consiste en aportar al vino pequeñas y controladas cantidades de oxígeno de forma continuada durante un período de tiempo determinado a través de un microdifusor poroso **(figura II.26)** (Moutounet, 2003). Por tanto, el uso combinado de la técnica de microoxigenación y la crianza de vinos sobre lías puede reducir o eliminar la aparición de aromas reductivos, especialmente los azufrados (Moutounet, 2003; Roig y Yerlé, 2003). Es importante eliminar estos compuestos azufrados del vino a tiempo, ya que si no pueden transformarse en otros compuestos volátiles también azufrados que generan olores desagradables en el vino y que son mucho más díficiles de eliminar (Rauhul, 2009).

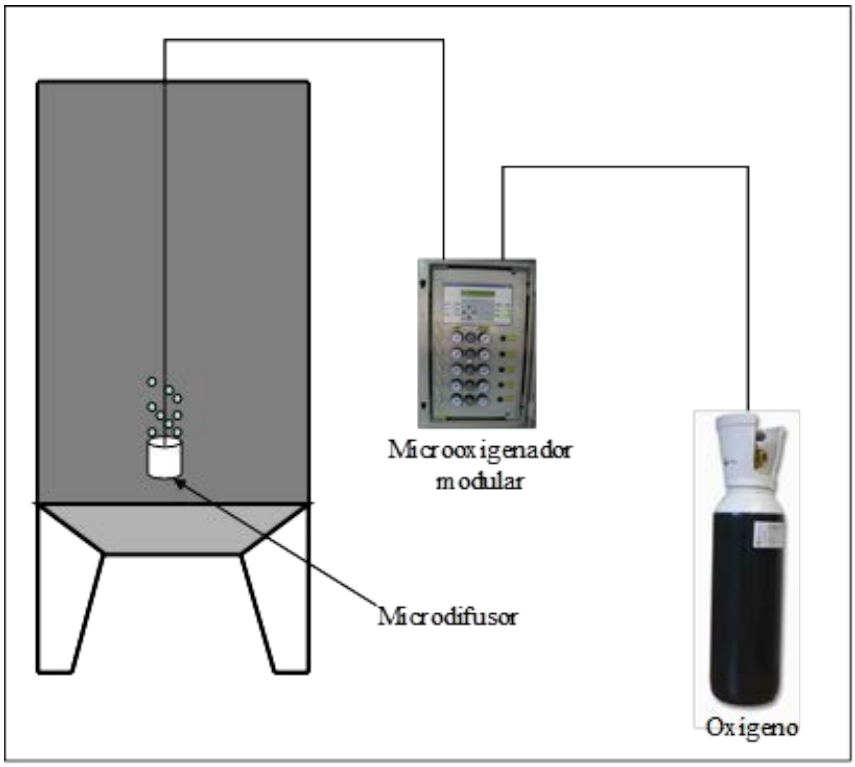


Figura II.26. Representación esquemática del proceso de microoxigenación.

Además, la aplicación de microoxigenación en vinos tintos puede tener otros efectos positivos como son (Roig y Yerlé, 2003):

- **Mejorar y estabilizar la intensidad y color de los vinos**, debido a que favorece las reacciones de condensación entre los antocianos y otros compuestos flavonoideos, bien de forma directa o mediada por el acetaldehído o el ácido glioxílico. Estas reacciones dan como resultado la formación de pigmentos poliméricos rojo-azulados más estables en el tiempo (Atanasova et al., 2002b; Pérez-Magariño et al., 2007; Cano-López et al., 2008; Sánchez-Iglesias et al., 2009: Del Barrio-Galán et al., 2011).

- **Potenciar las notas frutales y favorecer la integración de los aromas, y al mismo tiempo reducir la intensidad de los aromas herbáceos o vegetales del vino** (Moutounet, 2003; Ortega-Heras et al., 2008).
- **Mejorar la palatabilidad del vino**, reduciendo la sensación de aspereza y sequedad producida por los taninos, los cuales reaccionan modificando su estructura y dando lugar a moléculas poliméricas más complejas y voluminosas y de menor capacidad astringente (González-Sanjosé et al., 2008; Parish et al., 2000; Gómez-Plaza y Cano-López, 2011).

Esta técnica fue autorizada por la Comisión Europea en 1996 y desde hace algunos años es utilizada en países como España, Francia, Italia, Australia, Nueva Zelanda, Estados Unidos y Chile.

La microoxigenación se puede aplicar en cualquier etapa del proceso de elaboración, aunque los momentos más adecuados/necesarios son durante la fermentación alcohólica de mostos tanto blancos como tintos, y tras finalizar la fermentación alcohólica y antes de que empiece la fermentación maloláctica en vinos tintos. Las dosis de aplicación son relativamente pequeñas, abarcando un rango de 2-90 mg O_2/litro de vino/mes, dependiendo del tipo de vino y del momento de aplicación (Dykes, 2007).

A la hora de llevar a cabo la microoxigenación con o sin lías, hay que tener en cuenta una serie de parámetros o factores:

- La velocidad de aporte del oxígeno debe ser menor que la velocidad de consumo para evitar principalmente oxidaciones (Moutounet et al., 1995).
- La superficie de contacto gas/líquido va a determinar la cantidad de oxígeno disuelto. Así, cuanto mayor sea la superficie de contacto entre el oxígeno y el vino, mayor será la cantidad de oxígeno disuelto (Moutounet y Vidal, 2006). Esta superficie de contacto va a depender fundamentalmente del tipo de difusor que se utilice, la posición del difusor en el depósito y la altura del depósito

(Ortega-Heras et al., 2007; Sánchez-Iglesias, 2007). La mayor superficie de contacto se consigue con un difusor que permita la formación de burbujas lo más pequeñas posibles, colocado a una altura aproximada de unos 10 o 20 centímetros sobre el fondo del depósito de acero. Además, se tiene que mantener una altura mínima del depósito de 2,5 metros para que las burbujas de oxígeno que salen del difusor tengan el espacio suficiente (altura) para disolverse completamente en el vino, y para que la presión que ejerce la columna de vino impida el aumento de su tamaño (Sánchez-Iglesias, 2007).

- No todos los vinos son aptos para ser microoxigenados, ya que deben ser vinos con un mínimo de 30-40 IPT, y tener una relación adecuada entre la concentración de antocianos y taninos. La dosis a aplicar tanto antes como después de la fermentación maloláctica, será mayor cuanto mayor sea la concentración de compuestos fenólicos del vino, y en especial de taninos verdes (Ortega-Heras et al., 2007).
- La temperatura del vino es otro factor importante ya que influye tanto en la solubilidad del oxígeno como en la velocidad a la que ocurren las reacciones de oxidación. Así, a bajas temperaturas se produce una mayor disolución del oxígeno y además se reduce el consumo de oxígeno ya que las reacciones en las que interviene se producen más lentamente. Por ello, es necesario mantener una temperatura adecuada que permita el equilibrio entre ambos factores y que Roig y Yerlé (2003) establecieron entre 14 y 18 ºC.
- La concentración de SO_2 no afecta directamente a la concentración de oxígeno, pero si se acumula oxígeno, el SO_2 actúa como antioxidante del vino y puede interferir en las reacciones de formación de nuevos pigmentos y compuestos poliméricos.
- Otros parámetros o factores que hay que controlar para llevar a cabo la microoxigenación de forma adecuada son la acidez volátil, la presencia de etanal, y la presencia de otros microorganismos como *Brettanomyces*.

- En el caso de la microoxigenación con lías, además hay que controlar la turbidez del vino con el propósito de evitar un consumo de oxígeno incontrolado, reducir el riesgo de desarrollo de microorganismos indeseables y la obturación de las membranas del difusor (Ortega-Heras et al., 2007). Se recomienda que los valores de turbidez sean inferiores a 100 NTUs.

A día de hoy no se conocen muchos estudios científicos del efecto que el uso combinado de la microoxigenación y la crianza sobre lías tiene sobre las características sensoriales de los vinos. Sartini et al. (2007) y Arfelli et al. (2011) estudiaron el efecto del uso combinado de las técnicas de microoxigenación, lías y chips de madera de roble sobre la composición fenólica, la fracción volátil y el perfil sensorial de vinos tintos. Sartini et al. (2007) observaron ligeras modificaciones en la composición fenólica, y una mayor estabilidad del color debido a la formación de pigmentos poliméricos de color rojo-azulados que son más estables. Sin embargo es difícil establecer si este efecto es debido a la microoxigenación, a las lías, a los chips o a la acción conjunta de estas técnicas. Por otro lado, Arfelli et al. (2011) observaron que el uso combinado de estas técnicas fue más importante en el perfil sensorial del vino que en su fracción volátil, y encontraron cambios significativos solamente en alguno de los compuestos volátiles analizados.

II.5. TÉCNICAS ALTERNATIVAS A LA CRIANZA SOBRE LÍAS

II.5.1. Preparados comerciales derivados de levadura

En los últimos años se han estado desarrollando diferentes preparaciones comerciales ricas en polisacáridos obtenidas a partir de la levadura *Saccharomyces cerevisiae*, y su aplicación en el vino puede aportar los compuestos responsables de los efectos positivos de una crianza sobre lías.

En la actualidad existen distintos métodos de extracción de estos compuestos de la pared de las levaduras, siendo los más habituales la extracción enzimática y la extracción física por tratamiento térmico. En el método enzimático, primero se lleva a cabo una hidrólisis de las paredes celulares por la acción de enzimas β-glucanasas, seguido de un proceso de aislamiento de las fracciones obtenidas mediante filtración o ultrafiltración, y una última etapa de purificación. El segundo método consiste en tratar las paredes celulares de la levadura a temperaturas muy elevadas (100-120 ºC). Ambos métodos se llevan a cabo tras el crecimiento de las levaduras en unas condiciones de cultivo determinadas (medios concentrados y ricos en azúcares) (Pozo-Bayón et al., 2009a).

La heterogeneidad de estos preparados comerciales en cuanto a su estructura y composición química se debe a las diferencias en los procesos de extracción y purificación empleados y pueden clasificarse en (Pozo-Bayón et al., 2009a):

- Levaduras secas inactivas: El proceso de obtención consiste en la inactivación térmica de la levadura y su posterior secado.
- Autolisados de levadura: Se obtienen por inactivación térmica con un proceso de incubación que permite la liberación de enzimas de la levadura que degradan parte del contenido intracelular. Están formados tanto por materia soluble como insoluble procedente de las paredes celulares.
- Extractos de levadura: Son el extracto soluble que se obtiene tras la degradación total del contenido citoplasmático.

- Paredes o cortezas de levadura: Se obtienen por centrifugación durante el proceso de obtención de los extractos de levadura. Son insolubles y están compuestos únicamente por las paredes de la levadura sin contenido citoplasmático.

Generalmente se encuentran disponibles pocas preparaciones comerciales de manoproteínas con alto grado de purificación, principalmente debido a que es un proceso bastante laborioso y costoso. Por ello, la mayor parte de las preparaciones que se comercializan son del tipo autolisados de levadura o paredes de levadura.

La heterogeneidad de estos compuestos puede dar lugar a efectos muy diferentes en el vino dependiendo del preparado comercial empleado. Además, la falta de información sobre los mecanismos de acción que estos productos tienen sobre los vinos dificulta la elección de un determinado producto. Este hecho ha sido puesto de manifiesto por algunos autores que han estudiado el efecto de diversos preparados comerciales sobre la composición química y las características sensoriales de los vinos (Guadalupe et al., 2007; Guadalupe y Ayestarán, 2008; Pozo-Bayón et al., 2009b; Guadalupe et al., 2010; Del Barrio-Galán et al., 2010). Sin embargo, su verdadero impacto en la calidad de los vinos aún no se conoce en profundidad.

Estas preparaciones comerciales, especialmente las que no han sido sometidas a un elevado grado de hidrólisis, pueden combinarse con la utilización de enzimas β-glucanasas. Francois et al. (2007) observaron que la adición simultánea de preparaciones de levaduras inactivas junto con enzimas β-glucanasas dio lugar a vinos con mayor intensidad de color y más estable a lo largo del tiempo. Además estos vinos fueron ligeramente más ricos en polisácaridos totales.

El uso de las manoproteínas como aditivo enológico durante la vinificación para mejorar la estabilidad tartárica y proteica fue autorizado por la Unión Europea en 2005 (Reglamento CE nº 2165/2005 de 20 de Diciembre de 2005). Además, también está autorizado el uso de preparados comerciales de paredes celulares de levadura hasta un límite de 40 g/HL (Reglamento CE nº 606/2009 de 10 de Julio de 2009).

II.5.2. Utilización de fragmentos, trozos o virutas de madera

La utilización de fragmentos de distinto tamaño de madera de roble, comúnmente llamados chips, es una técnica conocida y aplicada en bodega durante la última década principalmente en países fuera de la Unión Europea como Australia, Estados Unidos, Chile, Argentina o Sudáfrica, donde esta práctica es bastante habitual.

Después de años de discusión, la Unión Europea autorizó el uso de fragmentos de madera de roble en la elaboración de los vinos (CE Reglamento 2165/2005 de 20 de Diciembre de 2005) y reguló la denominación y presentación de los vinos tratados con fragmentos de madera de roble (CE Reglamento 1507/2006 de 11 de Octubre de 2006). Así, la Unión Europea permite su utilización con mayor o menor grado de tostado, siempre que el 95% de los fragmentos supere los 2 mm de tamaño y no liberen sustancias en concentraciones que puedan presentar un riesgo para la salud.

Los productos alternativos de madera de roble se clasifican en función de diversos factores como son el origen botánico y/o geográfico de la madera (principalmente americano y francés), el grado de tostado (sin tostar, tostado ligero, medio y alto) y el tamaño (polvo, virutas o chips, cubos, trozos de madera granulada, dominós, bloques o segmentos, sticks y duelas o travesaños) **(figura II.27)**. Todos estos productos (excepto las duelas) se suelen utilizar en bolsas o sacos microperforados a modo de infusión, que se introducen directamente en los depósitos de acero inoxidable o en barricas ya usadas, pudiendo en este último caso alargar el tiempo de vida útil de dichas barricas (Del Álamo Sanza, 2006).

Además, el uso de estos productos en depósito de acero inoxidable puede combinarse con la aplicación de microoxigenación, con el objetivo de "imitar" la crianza en barrica (Sartini et al., 2007; Arfelli et al., 2011). Así, se conseguirán reducir los costes de producción y tener una mayor flexibilidad y facilidad de manejo.

Figura II.27. Diferentes formatos de productos alternativos de madera de roble existentes en el mercado.

Los efectos que se consiguen con la utilización de los fragmentos de madera son similares a los que se pueden conseguir durante el envejecimiento en barrica:

- **Estabilizar el color**, debido principalmente a la liberación de compuestos fenólicos procedentes de la madera de roble que pueden interaccionar con los antocianos, formando compuestos más estables que evitan la degradación de los antocianos libres. Además, los elagitaninos de la madera pueden actuar como antioxidantes, evitando la oxidación de antocianos y otros compuestos fenólicos.

- **Aumentar el dulzor, la estructura y el volumen en boca** del vino, debido principalmente al aporte de polisacáridos y taninos procedentes de la madera de roble.
- **Aumentar la expresión afrutada del vino**, debido principalmente a las whiskylactonas que aportan notas cítricas y de coco cuando llegan a concentraciones elevadas.
- **Aportar compuestos volátiles** que modifican las características aromáticas del vino, aumentando su complejidad.

A lo largo de los últimos años se han encontrado diferentes trabajos que han estudiado el efecto de la adición de productos alternativos de madera de roble al vino sobre la composición volátil (Pérez-Coello et al., 2000; Arapitsas et al., 2004; Guchu et al., 2006; Ordóñez et al., 2006; Frangipane et al., 2007; Martínez-García et al., 2007; Rodríguez-Bencomo et al., 2008; Rodríguez-Bencomo et al., 2009), la composición fenólica y el color de los vinos tintos (McCord, 2003; Del-Álamo et al., 2004a y b; Del-Álamo y Nevares, 2006; De-Conink et al., 2006; Gómez-Cordovés et al., 2006; Pérez-Magariño et al., 2009; Ortega-Heras et al., 2010) y en las características sensoriales (Ortega-Heras et al., 2010; Pérez-Magariño et al., 2011; Arfelli et al., 2011).

Además, se ha determinado que los parámetros que producen una mayor variabilidad al utilizar alternativos a la madera de roble son el tamaño, la dosis y el grado de tostado, siendo menos importante el origen geográfico del roble utilizado (Del-Álamo et al., 2004a y b; Arapitsas et al., 2004; De-Conink et al., 2006; Guchu et al., 2006; Frangipane et al., 2007; Rodríguez-Bencomo et al., 2008; Pérez-Magariño et al., 2009).

La madera de roble sin tostar puede mejorar las características sensoriales de los vinos como:

- La estabilidad de color debido al aporte de taninos, aunque si la cantidad es elevada puede aumentar la sensación de dureza en boca del vino.
- El aumento del dulzor y volumen en boca, debido a que el contenido en polisacáridos, principalmente celulosa y hemicelulosa es mayor que en la madera tostada, donde muchos de estos compuestos son degradados durante el proceso de tostado. Así, estudios llevados a cabo por Nonier et al. (2005) y Alañón et al. (2010) han indicado que la madera sin tostar es más rica en diversos monosacáridos como la arabinosa, galactosa, xilosa, manosa, glucosa y fructosa. Estos monosacáridos proceden de la hemicelulosa de la madera que se hidroliza fácilmente en las condiciones ácidas del vino (**figura II.28**).
- El aumento de la expresión afrutada, debido a la presencia principalmente de whiskylactonas que se encuentran en mayores concentraciones en la madera sin tostar. Además, aunque en menor medida, la madera sin tostar puede aportar otros compuestos aromáticos que darán al vino una mayor complejidad sensorial (**figura II.28**).

Por todo ello, aunque los compuestos aportados por la madera, en especial los polisacáridos, son diferentes a los procedentes de las paredes celulares de las levaduras (Viriot et al., 1993; Nonier et al., 2005), la adición de chips sin tostar podría conseguir unas características sensoriales (volumen en boca, estabilidad de color, complejidad aromática, etc.) similares a las encontradas en los vinos con crianza sobre lías.

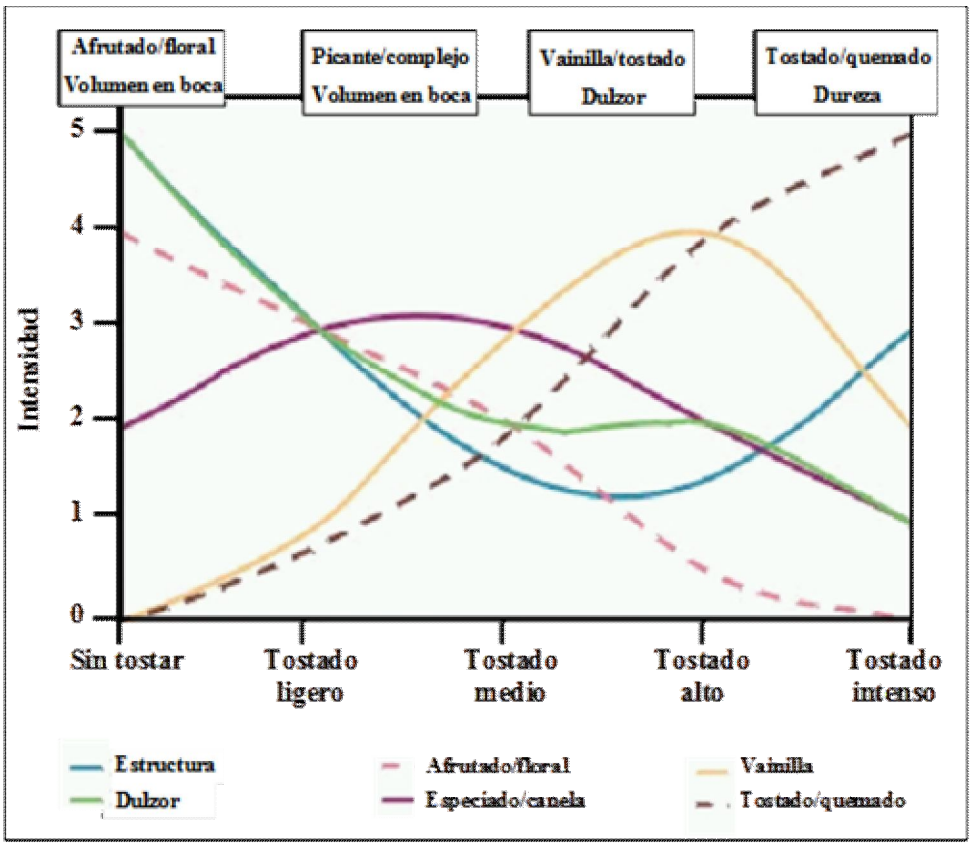


Figura II.28. Características de la madera en función del nivel de tostado (Béteau y Roig-Josa, 2006).

Sin embargo, en la búsqueda bibliográfica realizada no se han encontrado estudios que evalúen el efecto de la madera de roble sin tostar sobre la composición fenólica y de los polisacáridos del vino. Únicamente, Rodríguez-Bencomo et al. (2010) han estudiado el efecto que tiene la adición de chips de madera de roble sin tostar durante la fermentación alcohólica sobre la fracción volátil de un vino tinto. Estos autores, en general observaron un aumento de la mayoría de los compuestos volátiles analizados, excepto en el caso de la γ-butirolactona y la γ-nonalactona y los fenoles volátiles.

II.6. LA CRIANZA EN BARRICA

La crianza o envejecimiento de los vinos en barricas de roble es una práctica tradicional que se ha convertido en uno de los procesos fundamentales que mejoran la calidad de los vinos. Los vinos envejecidos en barrica de roble son muy apreciados por los consumidores, de hecho, un estudio llevado a cabo por Pérez-Magariño et al. (2011) mostró que el 88% de los encuestados prefería los vinos tintos envejecidos en madera a los vinos jóvenes.

La crianza del vino en barricas de roble es un fenómeno complejo en el que tienen lugar diversos procesos. En primer lugar, la madera de roble aporta al vino compuestos aromáticos y fenólicos que mejoran su calidad aromática y gustativa **(figura II.29)**. Por otra parte, la crianza en barricas permite una oxigenación moderada que tiene lugar a través de los poros de la madera, a través de los espacios interduelas y a través del esquive o tampón. Esta microoxigenación natural trae consigo cambios en el color, estructura y aroma del vino, ya que favorece las reacciones de polimerización y condensación de los antocianos y otros compuestos fenólicos del vino (Pontallier et al., 1982; Vivas, 2000; Fulcrand et al., 2006). Así mismo se produce la precipitación de parte de la materia colorante del vino, evitando que precipite posteriormente en la botella (Ribéreau-Gayón et al., 2003).

Desde el punto de vista químico, la madera está compuesta por células muertas que contienen un 50% de celulosa, un 20% de hemicelulosa y un 30% de lignina, aproximadamente (Alañón et al., 2010). Sin embargo, en la madera de roble también hay compuestos fenólicos, fundamentalmente ácidos fenólicos, taninos hidrolizables (galotaninos y elagitaninos), taninos condensados, otros flavanoles y cumarinas (Chatonnet, 1992). Algunos de estos compuestos pueden ser liberados directamente al vino, mientras que otros son originados durante los procesos de secado (Vivas, 1995 a y b) y/o tostado de las duelas (Chatonnet, 1991; Cutzach et al., 1997).

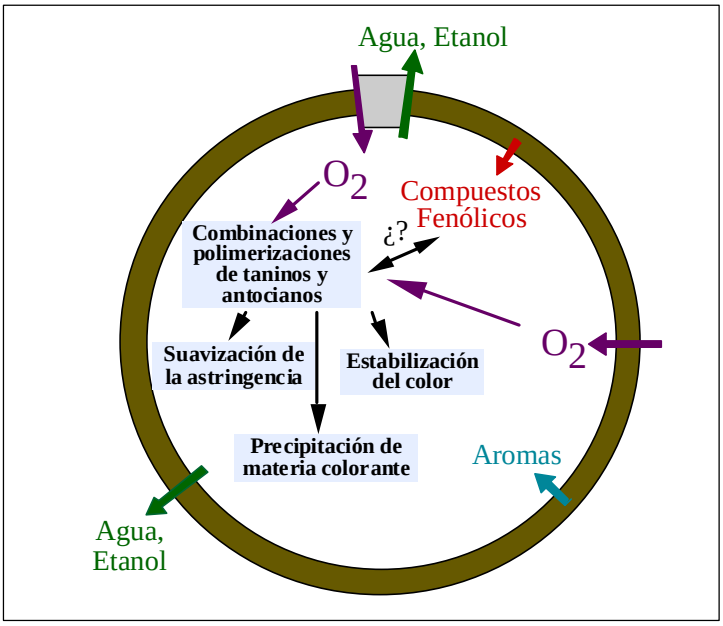


Figura II.29. Influencia de la crianza en barrica sobre la evolución del vino tinto (extraído de Zamora, 2003).

Entre los ácidos fenólicos de la madera de roble destacan fundamentalmente el ácido gálico y su dímero, el ácido elágico. Ambos ácidos pueden actuar como copigmentos, contribuyendo a estabilizar el color de los vinos tintos (Mazza y Brouillard, 1990), y evitar la oxidación de los antocianos (Vivas y Glories, 1996).

Los taninos procedentes de la madera de roble son conocidos como taninos hidrolizables (galotaninos y elagitaninos). Los galotaninos (trigalil-glucosa y pentagalil-glucosa) son poco abundantes en la madera de roble, por lo que no afectan de manera significativa a las propiedades sensoriales de los vinos (Vivas, 1997). En boca, presentan un carácter ácido, ligeramente astringente y muy amargo. La hidrólisis ácida de este tipo de taninos produce la liberación de ácido gálico. Los elagitaninos, como la vescalagina, grandinina, castalagina y roburina D, son más abundantes en la madera de roble, principalmente en la madera sin tostar (Cadahía et al., 2001). Este tipo de taninos se caracterizan por ser muy astringentes. Un aporte moderado de elagitaninos puede

contribuir a reforzar la estructura de los vinos. Sin embargo, un exceso de estos compuestos puede dar lugar a vinos muy maderizados. Por otro lado, la hidrólisis ácida de los elagitaninos da lugar a ácido elágico (Zamora, 2003).

Los taninos condensados y flavanoles monómeros y dímeros no son muy abundantes en la mayoría de especies de roble y están localizados principalmente en la corteza de la madera de roble (Vivas, 1997).

Las cumarinas se forman mediante esterificaciones intramoleculares. En la madera de roble, estos compuestos pueden encontrarse aislados o en forma de heterósido (escopolina y esculina). En la madera verde, se encuentran mayoritariamente en forma de heterósidos, los cuales son muy amargos (Puech y Moutounet, 1988; Ribéreau-Gayón et al., 2003). Sin embargo, durante el secado natural de la madera se produce la hidrólisis de estos heterósidos, debido a la acción de la enzima cumarina esterasa, dando como resultado las correspondientes agliconas (escopoletina y esculetina), las cuales son menos amargas y de carácter más ácido (Vivas, 1995b).

Por otro lado, cuando los vinos son envejecidos en barricas de roble también se produce la liberación de diversas sustancias volátiles de la madera al vino, aportando mayor complejidad a estos vinos y mejorando sus características sensoriales. Estas sustancias suelen agruparse en diversas familias en función de su estructura y origen (Boidron et al., 1988): derivados furánicos, otros heterociclos volátiles y ácido acético, aldehídos fenólicos, lactonas, fenoles volátiles y fenil cetonas.

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III. MATERIALES Y MÉTODOS

III.1. ELABORACIÓN DE LOS VINOS Y EXPERIENCIAS REALIZADAS

La elaboración de los vinos blancos y tintos que se han estudiado en esta Tesis Doctoral se ha llevado a cabo en la bodega experimental de la Estación Enológica del Instituto Tecnológico Agrario de Castilla y León, situada en el municipio vallisoletano de Rueda. Se siguieron los métodos de elaboración tradicional en vinos blancos y tintos. Los vinos tintos se elaboraron con uvas de la variedad Tempranillo procedentes de la D.O. Cigales y los vinos blancos con uvas de la variedad Verdejo de la D.O. Rueda.

Las uvas fueron recolectadas manualmente en su momento óptimo de madurez, basado principalmente en la relación entre el contenido en azúcar (ºBrix) y la acidez total. En todos los casos, las uvas recolectadas presentaron un perfecto estado sanitario. Tras la recolección, las uvas fueron transportadas en cajas de plástico de 15 Kg hasta la bodega experimental de la Estación Enológica. Los racimos de uvas fueron despalillados y estrujados, y a continuación la masa de vendimia obtenida fue ligeramente sulfitada, 0,04 g/L en los vinos tintos y 0,05 g/L en los vinos blancos.

En el caso de los vinos tintos, tras el estrujado, la masa fue llevada a depósitos de acero inoxidable de distinto tamaño dependiendo de la experiencia a realizar, donde se llevó a cabo la fermentación alcohólica mediante la adición de levadura comercial de *Saccharomyces cerevisiae* (20 g/HL de Excellence sp, Lamothe-Abiet) a una temperatura de entre 25-28 ºC.

En el caso de los vinos blancos, tras el despalillado y estrujado, la masa fue prensada, y el mosto obtenido fue llevado a depósitos de acero inoxidable en los que se adicionaron enzimas pectinolíticas (2 g/HL de Vinozym FCE, Novozymes) para favorecer la precipitación de sustancias coloidales. Tras 24 horas a 12 ºC, el mosto se trasegó a diferentes depósitos de acero inoxidable que fueron inoculados con levadura comercial S*accharomyces cerevisiae* (20 g/HL de IOC 18-2007 (Institut Oenologique de Champagne)). La fermentación alcohólica se llevó a cabo a temperatura controlada de 16 ºC ± 2ºC.

Una vez finalizada la fermentación alcohólica, tanto los vinos tintos como los vinos blancos permanecieron 4 días en los depósitos para favorecer la sedimentación de

las lías gruesas. A continuación, estos vinos fueron trasegados a otros depósitos donde permanecieron entre 4 y 5 días para favorecer la sedimentación de las lías finas. Transcurrido este tiempo, los vinos fueron de nuevo trasegados y las lías fueron recogidas para ser usadas en los ensayos de crianza sobre lías.

Tras la fermentación alcohólica, se aplicaron los distintos tratamientos que han sido estudiados en este trabajo y que se indican de forma detallada en los apartados que vienen a continuación. Una vez finalizados los distintos tratamientos, los vinos blancos fueron clarificados con bentonita, filtrados por placas filtrantes de 0,8 µm (KD BECO, Agrovin) y embotellados. Los vinos tintos llevaron a cabo la fermentación maloláctica mediante la inoculación de una preparación comercial de bacterias lácticas de *Oenococcus Oeni* (Viniflora, CHR Hansen). A continuación, los vinos tintos fueron filtrados por placas de 1,5 µm (BM BECO, Agrovin) y embotellados o envejecidos en barrica en función del estudio realizado.

Tanto en los vinos blancos como los vinos tintos, su envejecimiento en botella o en barrica se realizó en condiciones de humedad y temperatura controlada en la bodega subterránea de la Estación Enológica.

A continuación se indican las diferentes experiencias llevadas a cabo:

▪ 1ª. Caracterización de los polisacáridos de preparaciones comerciales de levaduras secas y su efecto sobre la composición de vinos blancos y tintos.

Este estudio se llevó a cabo con un vino blanco de la variedad Verdejo y un vino tinto de la variedad Tempranillo de la vendimia de 2007, siguiendo los procesos tradicionales de vinificación en blanco y en tinto, respectivamente.

Una vez finalizada la fermentación alcohólica, los vinos fueron divididos en diferentes tanques de acero inoxidable de 16 L de capacidad. A cada uno de estos vinos se le adicionaron 6 productos derivados de levadura comerciales distintos, de diferentes casas comerciales y con diferente composición (**figura III.1**). Los productos comerciales utilizados, su fabricante, las características de estos productos y sus efectos

en el vino según la información reflejada en sus fichas técnicas, aparecen recogidas en la **tabla III.1**. Los productos YD 1, YD 2, YD 3, YD 4 y YD 5 fueron adicionados tanto a los vinos blancos como a los vinos tintos. El producto comercial YD 6-R fue aplicado sólo a los vinos tintos y el YD 6-W sólo a los vinos blancos. Ambos productos eran del mismo fabricante, pero según sus indicaciones el primero mejoraba las características organolépticas de los vinos tintos y el segundo las de los vinos blancos. Estos vinos fueron comparados con un vino control (C) al que no se le adicionó ningún producto. La dosis de aplicación de estos productos fue de 40 g/HL (dosis máxima recomendada por los fabricantes).

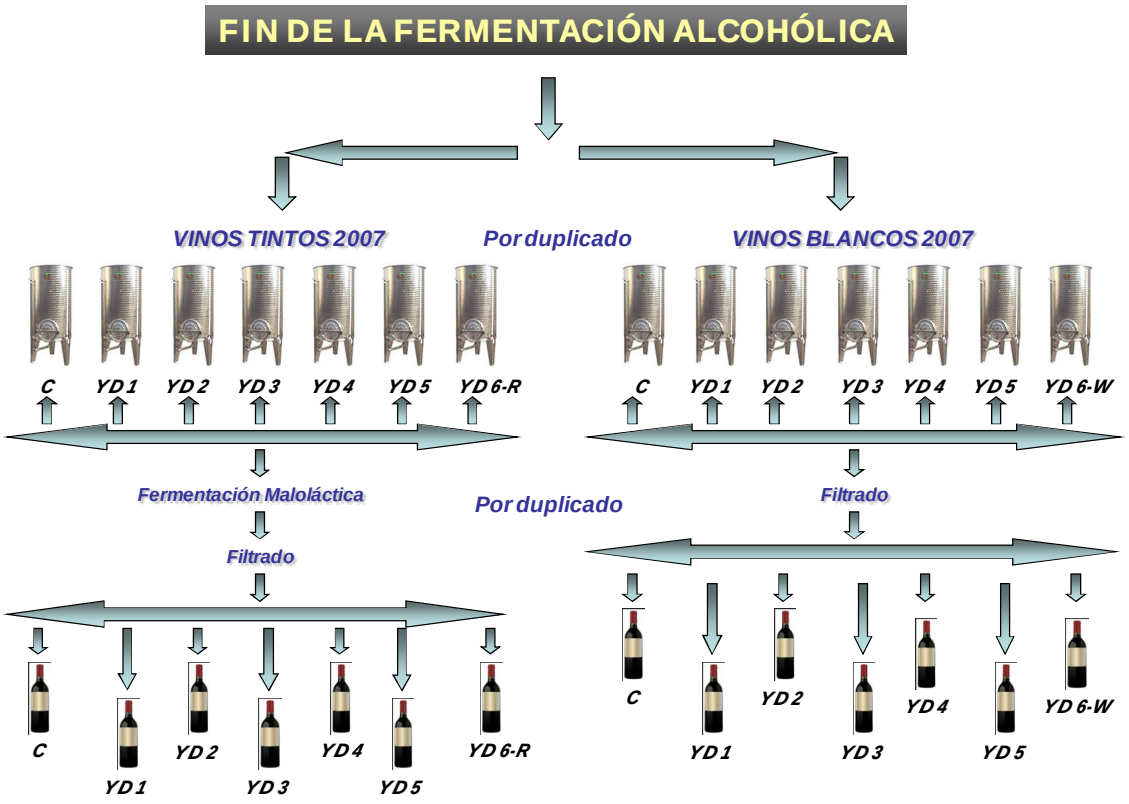

Figura III.1. Esquema de los tratamientos llevados a cabo en vinos blancos y tintos de la 1ª experiencia.

Tabla III.1. Composición y características de los diferentes derivados de levadura comerciales empleados.

Derivado de levadura	Fabricante	Composición y características	Efectos indicados por los fabricantes
YD 1	Agrovin	Producto con levaduras autolisadas ricas en polisacáridos.	Disminuye la astringencia y aumenta las sensaciones de cuerpo y volumen en boca. Aumenta la persistencia y estabilidad de los aromas. Mejora la estabilidad de color. Mejora la estabilidad tartárica y proteica.
YD 2	Agrovin	Producto con levaduras autolisadas ricas en polisacáridos y con actividad β-glucanasa.	Disminuye la astringencia y aumenta las sensaciones de cuerpo y volumen en boca. Aumenta la persistencia y estabilidad de los aromas. Mejora la estabilidad de color. Mejora la estabilidad tartárica y proteica.
YD 3	Sepsa-Enartis	Producto con polisacáridos parietales extraídos enzimáticamente de paredes de levadura seleccionadas.	Aumenta la sensación de volumen y redondez. Disminuye la astringencia y aumenta la persistencia aromática. Mejora la estabilidad tartárica y proteica. Favorece el desarrollo de la fermentación maloláctica.
YD 4	Laffort	Contiene una fracción peptídica encontrada en las levaduras y que posee un gran poder edulcorante.	Aumenta la sensación de dulzor. Favorece la eliminación de determinados polifenoles responsables del amargor y de la astringencia.
YD 5	Bio Springer	Constituido exclusivamente por polisacáridos de la pared celular de las levaduras. Contiene un 25% de manoproteínas libres y altamente solubles.	Aporta redondez y volumen en boca. Disminuye la astringencia. Contribuye a mejorar la estabilización del color, y la estabilización tartárica y proteica. Contribuye a prevenir las desviaciones organolépticas.
YD 6-W (exp. 1) YD 7 (exp. 2)	AEB	Producto a base de paredes celulares de levadura rico en manoproteínas y nucleótidos	Permite redondear y suavizar los vinos, generando un gusto más complejo. Aumenta el volumen en boca.
YD 6-R (exp. 1) YD 6 (exp. 2)	AEB	Producto con paredes celulares de levadura ricas en manoproteínas y nucleótidos. Manoproteínas con un peso molecular medio de 150 KDa.	Permite obtener vinos con más cuerpo, taninos suaves y una mayor persistencia en boca. Previene la formación de tonalidades anaranjadas.

Los tratamientos se llevaron a cabo tras la fermentación alcohólica, en el caso de los vinos blancos, y tras la fermentación alcohólica y antes de la fermentación maloláctica, en el caso de los vinos tintos. En ambos vinos tuvieron una duración de 60 días. Tras finalizar los tratamientos, los vinos blancos fueron filtrados y embotellados. Los vinos tintos, tras finalizar la fermentación maloláctica también fueron filtrados y embotellados. Las muestras para el análisis se tomaron al final del tratamiento y tras la fermentación maloláctica (en el caso de los vinos tintos) y transcurridos 3 meses de envejecimiento en botella.

Con estos vinos se llevó a cabo el trabajo que aparece recogido en el capítulo 1 del apartado de resultados y discusión.

▪ 2ª. Estudio de las interacciones entre los compuestos fenólicos o volátiles y las lías de levadura, derivados comerciales de levadura y chips sin tostar en soluciones modelo y en vinos tintos jóvenes.

Esta experiencia se llevó a cabo sobre dos soluciones hidroalcohólicas de vino modelo. El vino modelo contenía un 13% de etanol (v/v); 4 g/L de ácido tartárico; 3 g/L de ácido DL-málico; 0,1 g/L de ácido acético glacial; 0,1 g/L de sulfato potásico y 0,1 de sulfato de magnesio heptahidratado. El pH fue ajustado a 3,5 con hidróxido sódico 1N. A una de las soluciones de vino modelo se le adicionaron 9 compuestos fenólicos y a la otra se le adicionaron 10 compuestos volátiles, en las concentraciones que aparecen recogidas en la **tabla III.2**. Tras la adición de los compuestos y su homogeneización, las 2 disoluciones fueron dispuestas en botellas a las que se adicionaron los 7 derivados de levadura comerciales ensayados en el estudio anterior (40 g/HL), lías finas (3% v/v) (L) y chips de madera de roble francés sin tostar (Bois Frais, Boise France), (4 g/L) (CH). Las lías fueron obtenidas a partir de un vino blanco de la variedad Verdejo elaborado siguiendo el proceso de vinificación tradicional en blanco. Las botellas de solución de vino modelo sin adición de ninguno de estos productos sirvieron como muestra control (C) (**figura III.2**).

Todas las botellas fueron encorchadas y almacenadas a una temperatura de 15 ºC durante 60 días y fueron homogeneizadas mediante agitación manual 2 veces por semana. Se analizaron 3 botellas por tratamiento a los 15, 30 y 60 días.

Tabla III.2. Compuestos fenólicos y aromáticos (mg/L) adicionados en las soluciones hidroalcohólicas iniciales.

Compuestos fenólicos	**mg/L**	**Compuestos aromáticos**	**mg/L**
Ácido Gálico	22	**Acetato de isoamilo**	1
Ácido Vainillínico	3,88	**Hexanoato de etilo**	0,05
Ácido Elágico	1,92	**Octanoato de etilo**	1
Ácido *trans*-cafeico	9,50	**β-ionona**	0,1
Ácido *trans p*-cumárico	6,00	**4-etilfenol**	0,7
***Trans*-resveratrol**	2,00	**1-hexanol**	1
Triptofol	3,92	**Eugenol**	0,2
Catequina	20	***Cis*-whisky lactona**	0,2
Quercetina	1,50	***Trans*-whisky lactona**	0,3
		Linalol	0,1

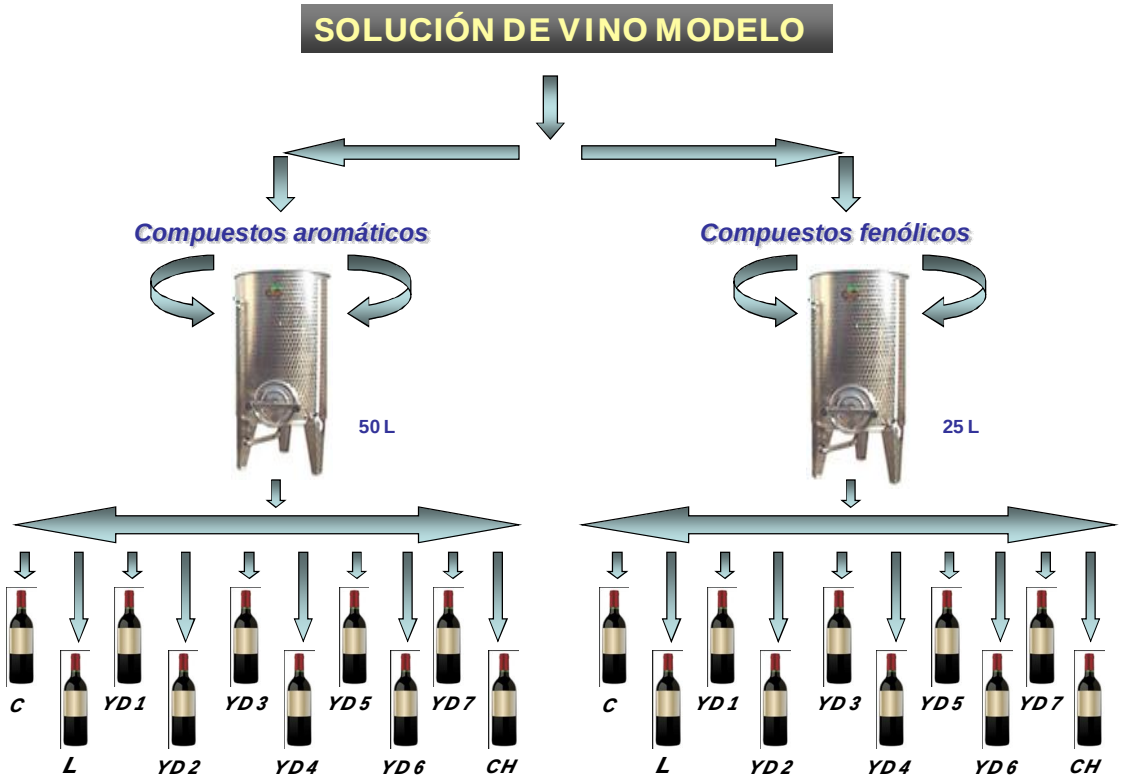

Figura III.2. Esquema de los tratamientos llevados a cabo en las soluciones de vino modelo de la 2ª experiencia.

Los vinos tintos jóvenes estudiados en esta experiencia son los mismos que los de la experiencia 1ª, aunque en este caso se estudiaron también los vinos tintos con lías finas (3% v/v) y los vinos tintos con chips de roble francés sin tostar (Bois Frais, Boise France), (4 g/L).

Con estos vinos se llevó a cabo el trabajo que aparece recogido en el capítulo 2 del apartado de resultados y discusión.

▪ 3ª. Efecto de la crianza sobre lías y de productos derivados de levaduras sobre la composición y las características sensoriales de un vino blanco de la variedad Verdejo.

Para la realización del estudio que aparece recogido en el capítulo 3 del apartado de resultados y discusión, se elaboró un vino blanco con uvas de la variedad Verdejo de la vendimia de 2008. Las experiencias realizadas se llevaron a cabo en depósitos de 150 L y son las siguientes (**figura III.3)**:

- Vinos control (sin adición de ningún producto) (C).
- Vinos con crianza sobre lías finas (3% v/v) (L).
- Vinos adicionados con 3 productos derivados de levadura comerciales diferentes (YD 1, YD 2 y YD 3), todos ellos de la casa comercial Sepsa-Enartis. La **tabla III.3** muestra las características de estos productos y su fabricante.

Tras la fermentación alcohólica se adicionaron las lías (3% v/v) y los derivados de levadura YD 1 y YD 2. La dosis empleada de estos derivados fue de 40 g/HL.

Los tratamientos se llevaron a cabo a una temperatura de 15º C y tuvieron una duración de 60 días, realizándose 2 batonnages semanales tanto a los vinos con lías como a los adicionados con los productos derivados de levaduras comerciales. Una vez finalizados los tratamientos, los vinos fueron clarificados, filtrados y embotellados. A una parte del vino control, antes del embotellado, se le adicionaron 5 g/HL del derivado de levadura YD 3, ya que según las indicaciones proporcionadas por el fabricante, esa era la dosis máxima recomendada y el momento óptimo para su aplicación. Las muestras se tomaron tras el tratamiento y tras 3 y 6 meses de envejecimiento en botella para su análisis.

Tabla III.3. Características de los diferentes derivados de levadura comerciales usados en vinos blancos y tintos de la 3ª y 4ª experiencia.

Derivado de levadura	Fabricante	Características
YD	Agrovin	Producto con levaduras autolisadas ricas en polisacáridos.
YD 1	Sepsa-Enartis	Producto con polisacáridos parietales extraídos enzimáticamente de paredes celulares de levadura selecionadas.
YD 2	Sepsa-Enartis	Producto con polisacáridos parietales de la pared celular de levaduras con alto contenido en manoproteínas libres.
YD 3	Sepsa-Enartis	Producto con polisacáridos de la pared celular de levaduras altamente purificado y completamente soluble en el vino.

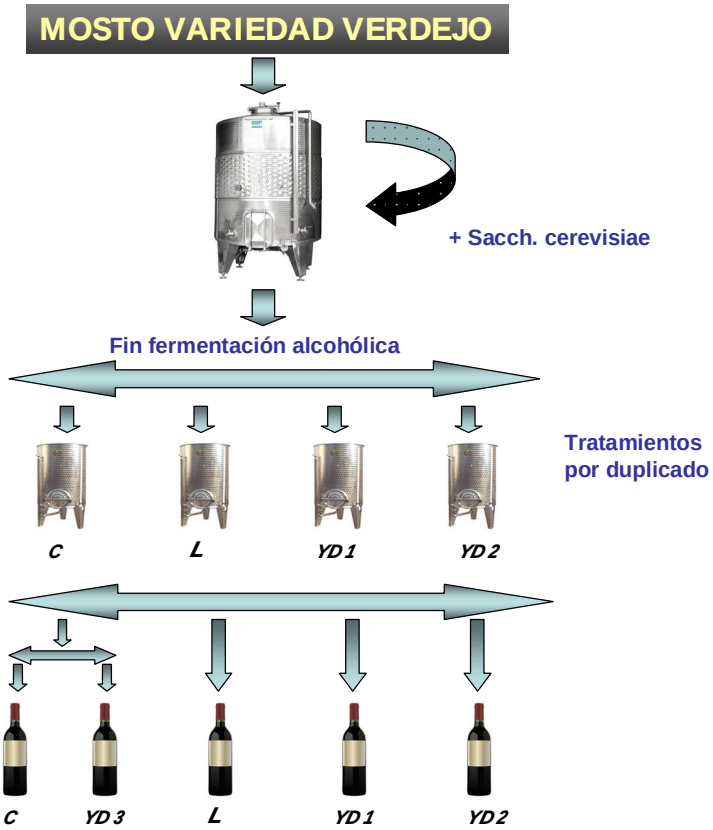

Figura III.3. Esquema de los tratamientos llevados a cabo en los vinos blancos de la 3ª experiencia.

▪ 4ª. Técnicas para mejorar o sustituir la crianza sobre lías de vinos tintos envejecidos en barrica: efectos sobre los polisacáridos y la composición fenólica.

Esta experiencia se llevó a cabo en vinos tintos en las vendimias de 2007 y 2008 **(figura III.4)**, a nivel semi-industrial en depósitos de 500 L.

En la vendimia de 2007 se realizaron las siguientes experiencias:

- Vinos control (C).
- Vinos con crianza sobre lías (L). Se le adicionaron un 3% (v/v) de lías finas.
- Vinos con crianza sobre lías y con la adición de una preparación comercial de enzimas β-glucanasas (L+E) (Enovin Glucan, Agrovin). La dosis aplicada fue de 4 g/HL.
- Vinos con crianza sobre lías y sometidos a un tratamiento de microoxigenación (L+MO). La dosis de oxígeno aplicada fue de 5 mL/L/mes. En este caso, se utilizaron depósitos especiales de acero inoxidable de 300 L de capacidad y de 3 metros de altura, ya que se necesita una altura mínima de 2,5 metros para asegurar una perfecta disolución del oxígeno añadido al vino. El equipo utilizado fue un microoxigenador modular de 5 cabezales VisiO_2 (Oenodev, Francia).
- Vinos adicionados con un derivado de levadura comercial (YD). La dosis aplicada fue de 40 g/HL y fue suministrado por la casa comercial Agrovin.
- Vinos adicionados con el mismo derivado de levadura (40 g/HL) y con la adición de una preparación de enzimas β-glucanasas comerciales (4 g/HL) (YD+E) (Enovin Glucan, Agrovin).
- Vinos a los que se adicionó chips de madera de roble francés sin tostar (CH) (Bois Frais, Boise France). Se añadieron 4 g/L.

Teniendo en cuenta los resultados obtenidos en la vendimia de 2007, en la que no se encontró un efecto significativo de la adición de enzimas β-glucanasas en los parámetros analizados, en la vendimia de 2008 se decidió centrar el trabajo en el estudio

de diferentes derivados de levadura comerciales con diferente composición. Por lo tanto, los tratamientos llevados a cabo en la vendimia de 2008 fueron los siguientes:

- Vinos control (C).
- Vinos con crianza sobre lías (L) (3% v/v).
- Vinos con crianza sobre lías y sometidos a un tratamiento de microoxigenación (L+MO) (5 mL/L/mes).
- Vinos adicionados con 3 productos derivados de levadura comerciales diferentes (YD 1, YD 2 y YD 3) y que fueron suministrados por la casa comercial Sepsa-Enartis. Estos productos y las dosis utilizadas fueron los mismos que los ensayados en los vinos blancos de la experiencia nº 3 **(Tabla III.3)**.
- Vinos adicionados con chips de madera de roble francés sin tostar (Bois Frais, Boise France), (4 g/HL) (CH).

La duración de los distintos tratamientos fue de 60 días con 2 batonnages semanales en el caso de los vinos con crianza sobre lías y con derivados de levadura comerciales en las 2 vendimias, y se llevó a cabo a 15 ºC. Únicamente el tratamiento con lías en combinación con la microoxigenación de los vinos de la vendimia de 2007 duró 5 semanas, ya que comenzó la fermentación maloláctica de forma espontánea.

Tanto en la vendimia de 2007 como en la de 2008, una vez finalizados los tratamientos, los vinos realizaron la fermentación maloláctica y a continuación fueron trasegados a barricas de roble americano nuevas con un grado de tostado medio-alto (Tonelería Victoria) donde permanecieron durante 6 meses. En la vendimia de 2008, una parte del vino control fue adicionada con 5 g/HL del derivado de levadura YD 3 antes de ser envejecido en barrica (**III.figura 4**). Las muestras se tomaron al finalizar la fermentación alcohólica, al finalizar el tratamiento y la fermentación maloláctica, y tras 3 y 6 meses de envejecimiento en barrica para su análisis.

Con estos vinos se llevaron a cabo los estudios que aparecen recogidos en los capítulos 4 y 5 del apartado de resultados y discusión.

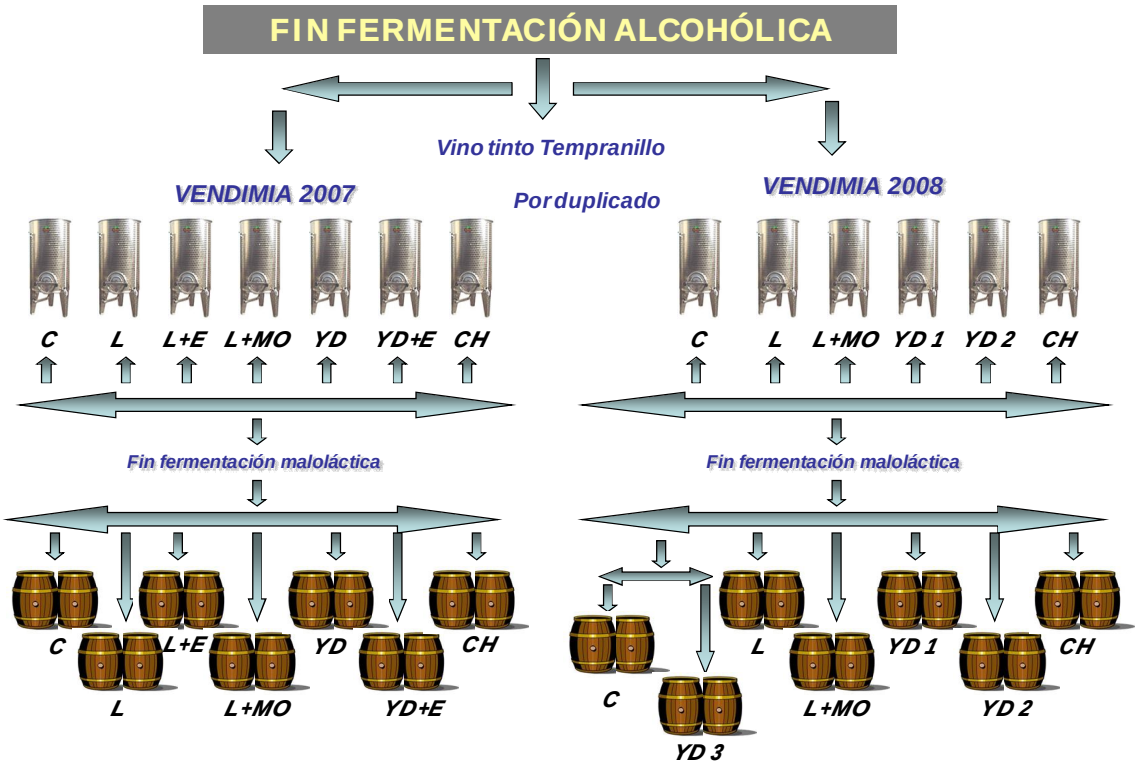

Figura III.4. Esquema de los tratamientos llevados a cabo en los vinos tintos de la 4ª experiencia.

III.2. PARÁMETROS Y MÉTODOS ANALÍTICOS.

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

1- Parámetros enológicos clásicos.

pH (método CEE (Reglamento nº 2676/90)), **acidez total** (método CEE (Reglamento nº 2676/90)), **SO_2 libre y total** (método iodométrico automatizado), **acidez volátil** (método enzimático (Chema-Italia)), **grado alcohólico** (método CEE (Reglamento nº 2676/90)), **ácido málico** (método enzimático (Boehringer-Mannheim)), **ácido tartárico** (método colorimétrico) y **potasio** (método CEE (Reglamento nº 2676/90)). Todos estos métodos están acreditados por la Norma UNE/EN ISO/IEC 17025.

2- Familias fenólicas.

▪ **Polifenoles totales** (Paronetto, 1977). La evaluación de los polifenoles totales se basa en la oxidación de los grupos hidroxilo de los compuestos fenólicos en medio básico, con el reactivo Folin-Ciocalteu, mezcla de ácido fosfomolíbdico y ácido fosfowolfrámico. El complejo azul resultante se mide a 750 nm.

▪ **Antocianos totales** (Paronetto, 1977). La determinación de los antocianos totales se basa en la propiedad que tienen los antocianos de desplazar el equilibrio hacia la forma flavilio y cambiar su color según el pH del medio. La diferencia de absorbancia a 525 nm al desplazar el pH desde 3,5 a un pH inferior a 1 se toma como medida de la concentración de antocianos totales.

▪ **Antocianos monoméricos, poliméricos y copigmentados** (Somers y Evans, 1977; Levengood y Boulton, 2004). La determinación de los antocianos copigmentados se basa en que al diluir la muestra se rompen los complejos antociano-copigmento, reduciéndose la intensidad de color de la muestra y evaluándose únicamente los antocianos libres y poliméricos. La determinación de los antocianos poliméricos se fundamenta en la capacidad

de estos compuestos para resistir la decoloración frente al SO_2. El contenido de antocianos monoméricos se evalúa restando el contenido total de antocianos determinados según el método publicado en Levengood y Boulton (2004), el contenido de antocianos poliméricos y copigmentados. Todas las medidas se realizan a 520 nm.

▪ **Catequinas** (Swain y Hillis, 1959). La determinación de las catequinas se basa en la capacidad de este grupo de fenoles de llevar a cabo reacciones de condensación con los compuestos carbonílicos en medio ácido. Se usa el aldehído vainíllico (vainillina) como compuesto carbonílico, que en medio ácido sólo reacciona con ciclos bencénicos "activados" como es el caso del anillo A de las catequinas y proantocianidinas. La reacción da lugar a un cromóforo rojo que se mide a 500 nm.

▪ **Taninos totales** (Ribéreau-Gayón y Stonestreet, 1966). La determinación de estos compuestos se fundamenta en su capacidad de transformarse en antocianidinas por calentamiento en medio ácido y en presencia de oxígeno. El color rojo formado se debe a los compuestos antociánicos obtenidos por la hidrólisis de las proantocianidinas y se mide a 550 nm.

▪ **Ésteres tartáricos y flavonoles** (Mazza et al., 1999). El contenido de estos dos grupos de compuestos fenólicos se evalúa mediante la medida de la absorbancia en su máximo de absorción, 320 nm para los ésteres tartáricos y 360 nm para los flavonoles.

3- Parámetros de color (Glories, 1984).

El cálculo de los distintos parámetros de color se basa en la medida de las absorbancias a 420, 520 y 620 nm en una cubeta de 1 mm en los vinos tintos y de 1 cm en los vinos blancos. A partir de estas 3 longitudes de onda se determinan los siguientes parámetros de color:

▪ **Intensidad colorante:** En vinos blancos viene dada por la absorbancia a 420 nm y en vinos tintos por la suma de las intensidades a 420, 520 y 620 nm, multiplicado por 10 para corregir el "paso de luz" de la cubeta.

▪ **Tonalidad:** Cociente entre las absorbancias a 420 y 520 nm.

▪ **Porcentaje de amarillo, rojo y azul:** Cociente entre las absorbancias a 420, 520 y 620 nm respectivamente y la intensidad colorante * 100. Estos porcentajes expresan la importancia relativa de cada una de estas tonalidades en el color global del vino.

4- Polisacáridos totales, ácidos y neutros (Segarra et al., 1995).

La determinación de los polisacáridos se basa en su capacidad para precipitar en medio ácido y etanólico, y posteriormente reaccionar con diferentes reactivos: los polisacáridos ácidos con el m-hidroxidifenil (medida a 520 nm) y los polisacáridos totales con el fenol (medida a 490 nm). Los polisacáridos neutros se obtienen por la diferencia de concentración entre los polisacáridos totales y los ácidos.

5- Proteínas (Bradford, 1976).

La determinación de las proteínas se basa en la capacidad del reactivo de Coomassie azul brillante de formar un complejo coloreado con las proteínas. Así, inicialmente, se encuentra en estado libre y es de color verde-azul, mientras que cuando se une a las proteínas pasa a formar un complejo de color azul más intenso. La absorbancia del complejo que se forma se mide a 595 nm.

6- Índice de gelatina (Ribéreau-Gayón et al., 2003).

El cálculo de este índice se basa en las propiedades que tienen los taninos de reaccionar con las proteínas (gelatina). De esta forma se determina el porcentaje de taninos capaces de reaccionar con las proteínas y es una medida de la astringencia del vino.

7- Índice de etanol (Ribéreau-Gayón et al., 2003).

Éste indica el porcentaje de taninos que están combinados con polisacáridos. El cálculo del índice de etanol se basa en la diferencia de las medidas de absorbancias a 280 nm del vino inicial frente al vino previamente precipitado con etanol.

8- Análisis pormenorizado de compuestos fenólicos antociánicos (Pérez-Magariño et al., 2009).

La determinación de los compuestos antociánicos individuales se basa en la inyección directa de la muestra, previa filtración con filtros de PVDF de 0,45 µm, en un cromatógrafo de líquidos de alta resolución. La separación se realiza mediante una columna cromatográfica de fase reversa y su detección se efectúa con un detector de fotodiodos alineados (DAD). El equipo empleado fue un cromatógrafo de Agilent Technologies LC serie 1100 equipado con un inyector automático y una columna cromatográfica NOVA-PACK C18 de 300 mm x 3,9 mm d.i. y 4 µm de tamaño de partícula (Waters). Las condiciones cromatográficas se establecieron según el método publicado en Pérez-Magariño et al. (2009). Los espectros de cada pico se registran en el rango de 240-600 nm, y la identificación de los picos se realiza mediante los tiempos de elución y la relación de absorbancias a 530 nm/313 nm. La cuantificación de los diferentes compuestos antociánicos se realiza a 530 nm usando un patrón externo de malvidin-3-glucósido.

9- Análisis de compuestos fenólicos de bajo peso molecular (Pérez-Magariño et al., 2008).

El análisis de los compuestos fenólicos es complicado debido por un lado al gran número de compuestos que hay en el vino, y por otro a la pequeña concentración en la que se encuentran muchos de ellos. Por ello, en los vinos tintos, para la determinación de los compuestos fenólicos no antociánicos de bajo peso molecular se hace necesario una extracción y concentración previa con el fin de poder cuantificarlos de forma adecuada. En los vinos tintos la

extracción de estos compuestos fenólicos se realizó mediante extracción en fase sólida utilizando cartuchos HLB 6 cc de 500 mg (Waters). La extracción se realizó con un equipo automático de extracción en fase sólida (GX-271 Aspec, Gilson). Los compuestos fenólicos de bajo peso molecular de interés se recogieron en 3 fracciones, con los siguientes eluyentes: acetato de etilo, acetato de etilo:metanol (80:20) y metanol. A continuación, cada una de las fracciones fue concentrada hasta sequedad en un concentrador automático en atmósfera de nitrogéno, redisuelta en una solución de agua miliQ:metanol (80:20) y posteriormente filtrada con filtros de PVDF de 0,45 µm para su inyección en el HPLC.

En el caso de los vinos blancos no se realizó ninguna extracción previa y fueron inyectados directamente en el equipo cromatográfico tras su dilución 1:1 en agua miliQ y su posterior filtración con filtros de 0,45 µm.

El análisis de los compuestos fenólicos de bajo peso molecular se realizó en el mismo equipo en el que se llevó a cabo el análisis de los antocianos individualizados. En este caso se usó una columna cromatográfica Zorbax SB-C18 de 250 mm x 4,6 mm d.i. y 3,5 µm de tamaño de partícula (Agilent).

Las condiciones cromatográficas del método fueron las que aparecen recogidas en Pérez-Magariño et al. (2008). La identificación de los compuestos se realizó comparando los espectros obtenidos con los de los patrones de referencia. La cuantificación se realizó a partir de la recta de calibrado construida para cada compuesto a partir de su patrón comercial a la longitud de onda máxima de cada compuesto.

10- Análisis de la composición en monosacáridos y distribución de los polisacáridos en función de su peso molecular.

El análisis de estos compuestos en los derivados de levadura estudiados en el capítulo 1 del apartado de resultados y discusión fueron realizados por las Doctoras Zenaida Guadalupe y Belén Ayestarán en el Departamento de Agricultura y Alimentación de la Universidad de la Rioja. El análisis de

estos compuestos se realizó mediante cromatografía de exclusión molecular (HPSEC) siguiendo los métodos descritos por Guadalupe et al. (2011, en prensa).

El análisis de las familias de polisacáridos evaluadas en los derivados de levadura y en los vinos blancos estudiados en el capítulo 3 del apartado de resultados y discusión, fue realizado por el doctorando en la unidad de Ciencia para la Enología del INRA (Instituto Nacional de Investigación Agronómica) de Montpellier, bajo la dirección del Doctor Thierry Doco. Su análisis también se realizó por cromatografía HPSEC siguiendo los métodos descritos por Ducasse et al. (2010 a y b).

11- Análisis sensorial.

El panel que llevó a cabo el análisis sensorial estaba formado por 12 catadores, técnicos de distintos Consejos Reguladores de Castilla y León, enólogos de diferentes bodegas de la región y personal de la propia Estación Enológica. Como el panel estaba formado por expertos catadores de vino, las sesiones de entrenamiento se centraron en unificar criterios de terminología y fijar el uso de escalas. Las fichas de cata utilizadas para el análisis descriptivo de los vinos blancos y tintos aparecen recogidas en las **figuras III.5 y III.6**. Se utilizó una escala estructurada de 7 puntos, en la que el valor 1 correspondía a la ausencia del atributo y el valor 7 a la intensidad máxima del mismo.

FECHA DE LA CATA: _____

NOMBRE DEL CATADOR: _____

MUESTRA:

-
- Ausencia +++

VISUAL	**1**	2	**3**	**4**	**5**	**6**	**7**
Intensidad Color							
Tonos Amarillos							
Tonos Verdes							
OLFATIVO	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Inten. olfativa							
Fermentativas							
Varietales							
Frutales							
Frutas exóticas							
Florales							
Verdes (hierba, heno,…)							
Sucio (moho)							
EN BOCA	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Volumen en boca							
Acidez							
Amargor							
Astringencia							
Persistencia							
Equilibrio							
Valoración global							

Figura III.5. Ficha de cata empleada para la evaluación de los vinos blancos.

FECHA DE LA CATA: _____

NOMBRE DEL CATADOR: _____

MUESTRA:

-
-

Ausencia +++

VISUAL	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Intensidad Color							
Azul-violetas							
Rojos (granates)							
Teja							
OLFATIVO	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Inten. olfativa							
Herbáceo (verde)							
Vegetal (col)							
Fruta							
Madera							
Sulfídrico							
Etanal							
Sucio (moho)							
Oxidado							
Reducido							
EN BOCA	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Grasa							
Acidez							
Astringente							
Taninos verdes							
Taninos duros							
Tans. redondos							
Taninos secos							
Equilibrio							
Valoración global							

Figura III.6. Ficha de cata empleada para la evaluación de los vinos tintos.

III.3. REFERENCIAS BIBLIOGRÁFICAS

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IV. RESULTADOS Y DISCUSIÓN

CAPÍTULO 1

Caracterización de los polisacáridos de preparaciones comerciales de levaduras secas y su efecto sobre la composición de vinos blancos y tintos

Actualmente, existen en el mercado una gran variedad de preparados obtenidos a partir de las paredes celulares de las levaduras, ricos en polisacáridos, principalmente manoproteínas, y con diferente grado de purificación (autolisados de levadura, levaduras secas inactivas, extractos de levadura, etc.). Estos compuestos, especialmente las manoproteínas, pueden modificar la composición de los vinos influyendo en las características sensoriales y tecnológicas de los mismos. Además, algunas de estas preparaciones comerciales llevan en su composición enzimas β-glucanasas para favorecer la hidrólisis de las paredes celulares y facilitar la liberación de manoproteínas y glucanos. Sin embargo, estos productos son muy heterogéneos en su composición, y pueden producir efectos muy diferentes en el vino, siendo muy escasos los trabajos publicados sobre el tema.

Por ello, en este capítulo se ha determinado la composición en polisacáridos de 7 preparados comerciales derivados de levadura diferentes (YD 1, YD 2, YD 3, YD 4, YD 5, YD 6-W y YD 6-R). Además se ha estudiado su efecto sobre la composición físico-química y las características sensoriales de un vino blanco de la variedad Verdejo y un vino tinto de la variedad Tempranillo.

Los resultados y conclusiones más destacados en este trabajo son los siguientes:

1- La pureza y composición en polisacáridos de los preparados comerciales derivados de levadura estudiados fue muy heterogénea. Solamente 2 de estas preparaciones (YD 3 y YD 6) mostraron una pureza en polisacáridos superior al 80%.

También se encontraron diferencias importantes en el contenido en manoproteínas y glucanos. Así, el derivado YD 2 presentó concentraciones más altas de glucanos que de manoproteínas (65% vs 34%), mientras que los derivados YD 5 y YD 6 tenían niveles más altos de manoproteínas que de glucanos (72% vs 28% y 44% vs 25%, respectivamente).

2- Los distintos derivados de levadura ensayados no modificaron ninguno de los parámetros enológicos estudiados ni en los vinos blancos ni en los tintos.

3- Los vinos blancos tratados con los derivados de levadura YD 4 y YD 5 presentaron concentraciones más bajas de los distintos compuestos fenólicos evaluados y menor intensidad de color que el vino control y el resto de vinos

estudiados. Esto puede ser debido a la adsorción de algunos de estos compuestos fenólicos sobre las paredes de las levaduras, o a su interacción con las manoproteínas y glucanos liberados por estos preparados. Se observó que esta interacción era mayor cuanto mayor era el contenido en polisacáridos de alto peso molecular de estos preparados.

En el caso de los vinos tintos, la adición de los derivados YD 2, YD 4 y YD 5 favoreció la formación de nuevos pigmentos antociánicos más estables a las variaciones de pH y temperatura, lo que tuvo un efecto positivo en la estabilización del color de estos vinos tintos.

4- Como era de esperar, la adición de derivados de levadura aumentó de manera significativa el contenido en polisacáridos neutros, y por lo tanto en polisacáridos totales. El contenido en estos compuestos siguió creciendo en todos los vinos tratados durante el envejecimiento en botella. Estos resultados indican que la adición de los derivados de levadura no produce una liberación inmediata de polisacáridos, sino que ésta tiene lugar a lo largo del tiempo, debido probablemente a la presencia de enzimas con actividad β-glucanasa. La cantidad de polisacáridos liberados dependió del preparado comercial de levadura adicionado.

5- Desde el punto de vista sensorial, únicamente se encontraron algunos de los efectos positivos descritos por los fabricantes de estos productos. De esta forma, la adición de estos preparados redujo la acidez y mejoró la persistencia aromática de los vinos blancos. En los vinos tintos los catadores únicamente encontraron una reducción del contenido en taninos verdes, lo que mejoró su palatabilidad haciéndolos más suaves y menos astringentes en boca.

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Polysaccharide characterization of commercial dry yeast preparations and their effect on white and red wine composition

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Running title: Commercial dry yeast preparations and their effect on wines

Abstract

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1. Introduction

Nowadays, one of the main targets of the wine sector is to improve wine quality, elaborating wines that satisfy consumer's demand and expanding the offer of quality wines.

Aging of wines on lees is a technique more used in white wines than in red wines. Thank to this technique, wines get rich in some compounds such as polysaccharides, fatty acids, amino acids and peptides. Mannoproteins are the main polysaccharides that are released by yeast during alcoholic fermentation (Doco, Brillouet, & Moutounet, 1996; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003, Ayestarán, Guadalupe, & León, 2004) and also by the autolysis of dead yeasts during the aging of wines on lees (Doco, Vuchot, Cheynier, &, Moutounet, 2003; Gonzalez-Ramos, Cebollero, & González., 2008). These compounds seem to be those that are the most interesting in enology by their positive effects on the quality of the final wine (Doco, et al., 2003, Fournairon, Camarasa, Moutounet, & Salmon, 2002; Feuillat, 2003). Mannoproteins are proteoglycans highly glycosilated mainly composed by mannose (>90%) and glucose (Guadalupe, Martínez, & Ayestarán, 2010) and proteins (<10%) (Vidal et al., 2003). They can have a highly variable size (5-800 kDa) (Doco, et al., 2003) and constitute 25-50% of the dry weight of the *Saccharomyces cerevisiae* walls, but their release into wine depends on the yeast strain (Pozo-Bayón, Andújar-Ortiz, & Moreno-Arribas, 2009).

Different positive effects of these compounds have been described related to sensory characteristics such as stabilization of red wine color (Escot, Feuillat, Dulau, & Charpentier, 2001; Francois, Alexandre, Granes, & Feuillat, 2007), reduction of wine astringency (Escot et al., 2001; Riou, Vernhet, Doco, & Moutounet, 2002; Vidal et al., 2004, Guadalupe, Palacios, & Ayestarán, 2007; Poncet-Legrand, Doco, Williams, &

Vernhet, 2007) and improvement of wine aromatic profile (Lubbers, Charpentier, Feuillat, & Voilley, 1994; Dufour & Bayonoue, 1999; Ramírez, Chassagne, Feuillat, Voilley, & Charpentier, 2004; Bautista, Fernández, & Falqué, 2007; Chalier, Angot, Delteil, Doco, Gunata, 2007). However, most of these works are carried out on model wine solutions.

Other authors have showed that these compounds can also improve tartaric and/or protein stability because they inhibit tartrate salt crystallization (Lubbers, Leger, Charpentier, & Feuillat, 1993; Moine-Ledoux & Dubourdieu, 2002) and/or reduce the protein haze in white wines (Moine-Ledoux & Dubourdieu, 1999, Dupin et al., 2000; Waters, Dupin, & Stockdale, 2000; Lomolino & Curioni, 2007; Schmidt et al., 2009). Furthermore, these wine polysaccharides can have other positive effects such as the reduction of ocratoxin A content (Bejaoui, Mathieu, Taillandier, & Lebrihi, 2004); the improvement of the growth of lactic acid bacteria (Guilloux-Benatier, Guerreau, & Feuillat, 1995; Díez, Guadalupe, Ayestarán, & Ruiz-Larrea, 2010), and foam characteristics of sparkling wines (Moreno-Arribas, Pueyo, Nieto, Martín-Álvarez, & Polo, 2000).

However, the release of mannoproteins during aging on lees is too slow and some alternatives are being studied to obtain the positive effects above mentioned. Hence, in the last years, a large variety of commercial products which are obtained from the yeast cell walls are being developed to provide similar characteristics that wines aged on lees. These products are obtained by thermal or enzymatic inactivation of *Saccharomyces cerevisiae* yeasts after their growth in aerobic conditions in a highly concentrated sugar medium (Pozo-Bayón et al., 2009). They can be classified as inactive yeasts, yeast autolysates, yeast walls and yeast extracts (mannoproteins with different degree of purification) (Pozo-Bayón et al., 2009). Some of these commercial products also

contain β-glucanase enzymes, which can favor the hydrolysis of the cell walls and the release of mannoproteins.

All these products can be used at different stages of the winemaking process depending on the type of wine that the winemaker wants to make. However, there are different kind of products in the market, with different composition, purity and solubility. Therefore they can cause very different effects on wines depending on the product used.

For all these reasons, the aim of this work was to characterize several commercial dry yeast derivative preparations and to study their effect on the composition of different quality parameters of a white and a red wine.

2. Material and methods

2.1. Winemaking process and treatments

The study was carried out using the *Tempranillo* grape variety from Cigales Designation of Origin (D.O.) for red wines, and the *Verdejo* grape variety from Rueda D.O. for white wines from 2007 vintage. Both D.O.s are sited in the Autonomous Community of Castilla y León in the North of Spain.

The grapes were harvested manually on the optimum harvest date and vinifications were carried out in the experimental winery of the Enological Station, following the traditional white and red winemaking processes.

Once the alcoholic fermentation finished, white and red wines were kept in the tanks for 4 days to allow for the sedimentation of the gross lees. After this time, the wines were racked off and maintained in the tanks for 4-5 days to allow for the sedimentation of the fine lees. The base wine was then again racked off and split into different 16 L tanks in which the different commercial products were added.

The experiences carried out were the control wines, without the addition of any product (C) and wines added with six different commercial yeast derivative products (YDs). All of them were carried out by duplicate.

Table 1 shows the characteristics of the different commercial products studied: commercial supplier, composition, and effects on wine according to the information given by the commercial supplier. The doses applied were the maximum authorized by the European Community: 40 g/hL (EC Regulation Nº 606/2009).

During treatments, two batonnages were performed weekly, and the temperature was maintained at 15 ºC ± 1 ºC. All treatments lasted 8 weeks. After that, the white wines were filtrated and bottled and the red wines were inoculated with a commercial preparation of *O. Oeni* (Viniflora, CHR Hansen, Denmark) to induce the malolactic fermentation. Finally, the red wines were also filtrated and bottled.

Samples were taken and analyzed just after the end of the treatments and at the end of the malolactic fermentation (red wines) and after three months of aging in bottle.

2.2 Chemical reagents

Gallic acid, D-(+)-catechin, Coomassie reactive, *trans*-caffeic acid, D-galacturonic acid, D-glucuronic acid and myo-inositol, lithium nitrate of HPLC, 3-hidroxy-biphenyl, phenol, L-fucose, L-rhamnose, 2-*O*-methyl D-xylose, L-arabinose, D-xylose, D-galactose, D-glucose, D-mannose and Kdo (3-deoxy octulosonic acid) were provided by Sigma-Aldrich (Steinheim, Germany); quercetin, malvidin-3-glucoside and cyanidin chloride by Extrasynthèse (Lyon, France); bovine serum albumine, di-sodium tetraborate decahydrated, dried methanol, pyridine, hexamethyldisilazane and trimethylclorosilane by Merck (Darmstadt, Germany). Acetonitrile and methanol of HPLC grade were provided by Lab Scan (Madrid, Spain). The remaining of reagents

115 was supplied by Panreac (Madrid, Spain) or Scharlab (Barcelona, Spain). Milli-Q water
116 was obtained by a Millipore system (Bedford, MA).

117 2.3. Analytical methods

118 2.3.1. Analysis of monosaccharide and polysaccharide composition

119 In order to characterize the different dry yeast preparations, the monosaccharide
120 composition and their polysaccharide molecular weight distribution and content were
121 analyzed.

122 The monosaccharide composition of the commercial preparations was determined by
123 GC-MS of their trimethylsilyl-ester O-methyl glycosyl residues obtained after acidic
124 methanolysis and derivatization (Guadalupe, Garrido, Carrillo, & Ayestarán, accepted).

125 A high-resolution size-exclusion chromatography (HRSEC) system (1100 Agilent
126 Technologies, Germany) with a refractive index detector was used to obtain the
127 molecular weight distributions of the polysaccharides. Two serial Shodex OHpack KB-
128 803 and KB-805 columns (0.8 x 30 cm, Showa Denko, Japan) equilibrated at 1 mL min^{-1}
129 in 0.1 M $LiNO_3$ were used. Calibration was performed with narrow pullulan molecular
130 weight standards (Shodex P-82, Waters, Barcelona, Spain): P-5, Mw = 5.9 kDa; P-10,
131 Mw = 11.8 kDa; P-20, Mw = 22.8 kDa; P-50, Mw = 47.3 kD; P-100, Mw = 112 kDa; P-
132 200, Mw = 212 kDa; P-400, Mw = 404 kDa. The apparent molecular weights were
133 deduced from the calibration equation $\log M_w = 11.188 - 0.403\ t_R$ (t_R = column retention
134 time at peak maximum, and $r^2 = 0.999$).

135 Polysaccharide contents were estimated using calibration curves constructed from the
136 pullulan standards P-10, P-50, P-100 and P-200, which were chosen because their peaks
137 properly matched with those obtained for the commercial samples.

138 2.3.2. Analyses in wines

139 Oenological parameters were evaluated following the OIV official analysis methods
140 (OIV, 1990).
141 The content of phenolic compounds was evaluated by quantification of several phenolic
142 families: total polyphenols, total anthocyanins, catechins, total tannins, tartaric esters of
143 phenolic acids, flavonols, and monomeric, polymeric and copigmented anthocyanins
144 (Del Barrio-Galán, Pérez-Magariño & Ortega-Heras, 2011).
145 The content of individual anthocyanins and their derivatives were determined by direct
146 injection of the wines previously filtrated through PVDF filters of 0.45 μm (Millipore,
147 Bedford, MA) in a chromatograph Agilent-Tecnologies LC-DAD 1100, following the
148 method described by Pérez-Magariño, Ortega-Heras, Cano-Mozo, & González-Sanjosé
149 (2009). The compounds identified in this study were grouped as it is indicated in
150 Sánchez-Iglesias, González-Sanjose, Pérez-Magariño, Ortega-Heras, & González-
151 Huerta (2009).
152 The color of wines was evaluated using the Glories parameters (Glories, 1984).
153 Acid and total polysaccharides were quantified by the colorimetric method described by
154 Segarra, Lao, López-Tamames, & De La Torre-Boronat (1995). Neutral polysaccharides
155 were calculated as the difference between total and acid polysaccharides.
156 Proteins were determined using the method described by Bradford (1976).
157 All spectrophotometric measurements were carried out in a UV-vis spectrophotometer
158 (Shimadzu series UV-1700 pharmaspec, China).
159 2.4. Sensory analysis
160 The sensory analysis was carried out by a tasting panel made up of twelve persons, all
161 of them expert tasters from the Regulatory Councils of different Spanish D.O. and
162 wineries. These tasters defined the descriptors used in this sensory analysis, according
163 to the methodology described in González-Sanjosé, Ortega-Heras, & Pérez-Magariño

(2008), and were trained to quantify them using structured numerical scales. This training was carried out in accordance with UNE-87-020-93 Norm (ISO 4121:1987). A structured numerical scale of seven points was used, with 1 representing absence of sensation and 7 a very high intense perception. All wines were tasted after the treatment.

2.5. Statistical analyses

All the data were treated applying the variance analysis (ANOVA), and the Least Significant Difference test. Confidence intervals of 95% or significant level of $\alpha = 0.05$ were used. All the statistical analyses were carried out using the Statgraphics Plus 5.0 statistical package.

3. Results and discussion

3.1. Monosaccharide and polysaccharide contents in the commercial yeast products

Table 2 shows the monosaccharide composition of the commercial products evaluated. The YD-1, YD-3, and YD-4 showed very similar monosaccharide compositions. The proportion of mannoproteins in these yeast preparations, estimated directly from their proportion of mannose, was 41%-43%. The percentage of glucose, used to estimate the glucan content, was about 60%, which indicates that during the process to obtain these products more glucans are extracted than mannoproteins. In the case of YD-2, the glucan/mannoprotein relationship was higher (65% vs. 34%). On the other hand, the mannoprotein content in YD-5 and YD-6R was much higher than that of glucan (72% vs. 28% and 44% vs. 25%, respectively). Finally, it is important to note that the YD-6W and YD-6R products showed a high percentage of other monosaccharides, mainly galactose, which are not constituents of parietal polysaccharides from yeasts. This could indicate the presence of some polysaccharide or other glycoside compounds that do not

188 come from yeast. It should be pointed out that both products were provided by the same
189 supplier.
190 **Table 2 also** shows the polysaccharide purity of the commercial products evaluated.
191 This purity was expressed as the total amount of monosaccharides in relation to the
192 weight of the product analyzed. It is interesting to point out that only two products (YD-
193 3 and YD-6R) showed a purity above 80%.
194 **Table 3** shows the percentage of different molecular weights of polysaccharide
195 fractions estimated using HRSEC-RID. With the exception of YD-2 and YD-3, all the
196 products showed a content of high molecular weight polysaccharides significantly
197 higher than that of low molecular weight polysaccharides. In contrast, in both YD-2 and
198 YD-3 the percentage of low molecular weight polysaccharides was similar to or even
199 higher than that of larger polysaccharides. This is in good agreement with the
200 commercial description as both products were extracted enzymatically from the selected
201 yeast walls.
202 3.2. White wines
203 3.2.1. Enological parameters
204 Enological parameters were analyzed in white wines to study the effect of the different
205 techniques assayed on these compounds. The data ranges of these parameters were: pH
206 between 3.2-3.3, total acidity between 6.1-6.2 g/L of tartaric acid, alcoholic degree
207 between 11.8-12.3, volatile acidity average of 0.18 mg/L of acetic acid and potassium
208 between 590-630 mg/L. No statistically significant differences were found between the
209 treated wines and the control wines, which indicate that the commercial yeast
210 preparations used did not have an effect on the enological characteristics of wines.
211 3.2.2. Analysis of phenolic compounds

212 **Figure 1** shows the content of some phenolic families analyzed in white wines.
213 Statistically significant differences were only found in some cases. Only YD-4 and YD-
214 5 wines showed a lower concentration of total polyphenols, tartaric esters of phenolic
215 acids, and flavonols than control wines and the other treated wines at the end of
216 treatment (0 MB). However, the analysis of the tannins did not show any statistically
217 significant differences between treated wines and control wines at the end of treatment.
218 After three months in bottle, the wines treated with YD-4 and YD-5 showed a lower
219 concentration of total polyphenols than the rest of the treated wines. However, these six
220 wines presented higher concentrations of total polyphenols than control wines. On the
221 other hand, wines treated with YD-4 and YD-5 showed a significantly lower
222 concentration of tannins, tartaric esters of phenolic acids, and flavonols than control
223 wines. These results are probably due to the adsorption of some polyphenols on the
224 yeast cell walls (Razmkhab et al., 2002; Márquez, Millán, Souquet, & Salmon, 2009) or
225 to the interaction of some polyphenols with the compounds released to the wine, such as
226 mannoproteins and glucans from yeast derivative products (Riou et al., 2002; Poncet-
227 Legrand et al., 2007). This interaction depends on the type of phenols. In addition, the
228 decrease in these compounds also seems to depend on the type of yeast preparations, the
229 high molecular weight polysaccharides being responsible for this interaction **(Table 3)**.
230 The effect of yeast derivative products was also observed in the color of white wines
231 **(Table 4)**. The YD-4 and YD-5 preparations with 100% of high molecular weight
232 polysaccharides produced a greater decrease in wine color after 3 months in bottle.
233 These results agree with those obtained by Razmkhab et al. (2002), who proposed using
234 yeast cell walls as fining agents for the correction of browning in white wines.
235 3.2.3. Analysis of proteins and polysaccharides

As expected, at the end of the treatment, the wines treated with commercial yeast derivative products presented higher protein concentrations than control wines **(Table 4)**, except for the wines treated with YD-2, which showed a similar content to the control wines. The wines treated with YD-4, YD-5, and YD-6 products showed the highest content. These differences were maintained during the bottle aging. These results suggest that the commercial yeast derivatives obtained from autolyzed yeasts or polysaccharides extracted from the yeast cell wall (YD-1, YD-2, and YD-3) release to wine a lower amount of protein compounds than the other commercial yeast derivatives (YD-4, YD-5, and YD-6) that are theoretically products with higher cell wall degradation.

Polysaccharide concentrations in the wines were also evaluated **(Figure 2)**. A significant increase in total and neutral polysaccharides in all white wines treated with the commercial yeast derivatives was found at the end of treatment and after three months in bottle. This increase depended on the commercial yeast product used; statistically significant differences were observed among the different treatments. The wines treated with YD-1 and YD-4 showed the lowest concentrations of neutral and total polysaccharides. However, it was also observed that total and neutral polysaccharides increased during the bottle aging in all the white wines studied, even in the control wines. This increase was more important in wines treated with YD-2 and YD-3 than in the other treated wines showing the highest content after three months in bottle. In addition, the wines treated with the yeast preparation with the highest mannose content (YD-5) showed the highest concentration of neutral polysaccharides after treatment, while only an 8% increase was observed during bottle aging. These results suggest that the addition of commercial yeast products does not produce an immediate release of these compounds and that this release continues during wine

aging. This is probably due to the presence of endogenous β-glucanase enzymes in the wines, either released from the yeast added to carry out the alcoholic fermentation or present in the commercial products. These enzymes are active and continue working over time, allowing for the release of neutral polysaccharides from more complex soluble compounds or from the autolyzed yeast and/or cell wall extracts added. Consequently, the purer the yeast preparations and the higher their mannose content, the higher the amount of neutral polysaccharides released to wine.

As expected, the concentration of acid polysaccharides was more or less stable in all wines, although slight differences were found among the treatments.

3.2.4. Sensory analysis

Some differences were found in the color parameters between the treated wines and control wines at the end of treatment, although they were not statistically significant. All treated wines showed higher values of color intensity and yellow tones and lower green tones than control wines **(Figure 3A)**.

In the olfactory phase **(Figure 3A)**, all treated wines showed less olfactory intensity than control wines, but no statistically significant differences were found. However, the tasters found less varietal and fruity aromas in all the wines treated with commercial yeast derivatives than in control wines. This was probably due to the interaction of the aromatic compounds with some compounds released from commercial yeast derivatives, such as glucans and mannoproteins, which can produce a decrease in the volatility of these aromatic compounds but that improve the aromatic perception over time. These interactions have been observed by other authors in model wine solutions (Voilley, Beghin, Charpentier, & Peyron, 1991; Chalier et al., 2007) and in red wines (Rodríguez-Bencomo, Ortega-Heras, & Pérez-Magariño, 2010). On the other hand, the

tasters found more exotic fruity notes in treated wines than in control wines, especially in YD-1 and YD-2 wines.

In the gustative phase **(Figure 3B)**, all treated wines showed less acidity than control wines. However, the tasters found no statistically significant differences in balance and overall scores between wines.

3.3. Red wines

3.3.1. Enological parameters

The data ranges of the enological parameters were: pH between 3.5-3.6, total acidity between 4.8-5.1 g/L of tartaric acid, alcoholic degree between 12.4-12.7, volatile acidity average of 0.40 mg/L of acetic acid and potassium between 1100-1200 mg/L. As in white wines, no statistically significant differences between the treated and control wines were found in the enological parameters. Other studies published on the use of different commercial products rich in mannoproteins showed that applying them did not affect these parameters either (Guadalupe et al., 2007; Guadalupe et al., 2010).

3.3.2. Analyses of phenolic compounds

Total polyphenol content, tannins, tartaric esters of phenolic acids, and flavonols showed similar or higher concentrations in treated wines than in control wines **(Figures 4** and **5**). In general, the wines treated with YD-2, YD-3, YD-5, and YD-6 were richer in phenolic composition. Commercial yeast preparations do not release this type of compounds, so these products will avoid their precipitation in these wines. On the other hand, some of the yeast preparations (such as YD-1, YD-4, and YD-5 after treatment and YD-2, YD-4, and YD-6 after three months in bottle) reduced anthocyanin content **(Figure 4)**. These results agree with those described by some authors, who found adsorption of this type of compounds in the yeast (Guadalupe et al., 2007; Mazauric & Salmon, 2005; Mazauric & Salmon, 2006; Lizama, Rodríguez, Álvarez, García, &

Aleixandre, 2006). However, this could be also due to the fact that the compounds released from the yeast preparations studied favor condensation and polymerization reactions between anthocyanins and other phenolic compounds (principally tannins) or with other wine metabolites, forming new polymeric compounds that can contribute to maintaining and stabilizing the color in red wines (De Freitas, Carvalho, & Mateus, 2003). The polymeric anthocyanin results **(Figure 6)** confirm this hypothesis, since the wines treated with YD-2, YD-4, and YD-5 showed higher percentages of these compounds than control wines, and they showed lower content of total anthocyanins. Only wines treated with YD-1 presented lower total anthocyanin and lower polymeric anthocyanin levels than control wines, which can indicate that this yeast preparation really produced a reduction of monomeric anthocyanins by adsorption.

Just after treatment, the detailed analysis of the monomeric anthocyanins **(Figure 7)** only showed statistically significant differences between the different treatments for the cinnamic anthocyanins. However, higher differences were found between treatments after bottle aging. In general, the wines treated with YD-1, YD-2, YD-4, and YD-5 showed lower concentrations of monomeric anthocyanins than the control wines and the other treated wines. These results agree with those found for the total anthocyanins. In addition, the wines treated with YD-1, YD-2, YD-4, and YD-5 presented higher values of new anthocyanin pigment content than the control wines **(Figure 8)**; these compounds are more stable and are partially responsible for wine color stability. The wines treated with these yeast preparations also showed the highest color intensity values both after treatment and after bottle aging **(Figure 6)**. These results are well correlated with the higher percentage of polymeric anthocyanins obtained in these wines. They suggest that these yeast preparations favored the formation of new polymeric pigments, which are more stable and resistant to pH changes and oxidation

reactions (Asenstorfer, Hayasaka, & Jones, 2001) and, thus, contribute to color stability. It can consequently be said that only some of the commercial yeast derivative products used seem to have a positive effect on color stability, probably due to their different composition. The positive effects of mannoproteins and other polysaccharides on color stability have been reported by some authors (Escot et al., 2001; Francois et al., 2007). However, some recent studies did not find an improvement of wine color intensity and color stability using mannoproteins, in some cases, they even found a loss of color in the wines analyzed (Guadalupe & Ayestarán, 2008; Guadalupe et al., 2010).

3.3.3. Analysis of proteins and polysaccharides

The wines treated with YD-2, YD-5, and YD-6 had higher protein content at the end of treatment and especially after bottle aging than the control wines and the remaining treated wines **(Figure 9)**.

At the end of treatment, all treated wines showed higher concentrations of neutral polysaccharides than the control wines. The wines treated with YD-5 presented the highest concentration of these compounds, while those treated with YD-1 showed the lowest **(Figure 9)**. After bottle aging, all treated wines also showed higher neutral polysaccharide content than control wines. The wines treated with YD-4, YD-5, and YD-6 showed the highest concentration and those treated with YD-2, the lowest. It can therefore be said that all yeast derivatives release neutral polysaccharides, but in different amounts and probably with different types of polysaccharides. This could produce different effects on the sensorial characteristics and the quality of wines. These results agree with those obtained by other authors (Guadalupe et al., 2007; Guadalupe & Ayestarán, 2008), who pointed out that adding commercial mannoprotein products to red wines before alcoholic fermentation made the concentration of neutral

(mannoproteins) and total polysaccharides increase or remain constant during the barrel and bottle aging.

3.3.4. Sensory analysis

In red wines, the sensory analysis showed smaller differences than in white wines. No statistically significant differences were found in color between the treated wines and the control wines just after treatment **(Figure 10A)**.

In the olfactory phase **(Figure 10A)**, all wines treated with the commercial yeast derivatives presented lower olfactory intensity values than the control wines. However, no statistically significant differences were found.

In the gustative phase **(Figure 10B)**, statistically significant differences were only found in green tannin values, which were lower in all treated wines than in control wines. This type of tannins produces negative sensations including intense astringent and acid sensations with strong green or herbaceous notes. Consequently, these results can indicate that adding yeast derivatives can reduce aggressive green tannins of red wines, probably due to the interactions between these products and the tannins, increasing roundness and softness on the palate (Escot et al., 2001; Riou et al., 2002; Guadalupe et al., 2007; Poncet-Legrand et al., 2007). The wines treated with YD-4, YD-5, and YD-6 presented the lowest green tannin values, which coincides with their greater overall rating values.

4. Conclusion

In summary, the purity and composition of commercial yeast preparations were very heterogeneous, as were the effects that they can produce in wines.

All the yeast derivative preparations studied increased the content of neutral polysaccharides, although those with greater mannose content reduced the browning in white wines and managed to stabilize the color in red wines.

From a sensory point of view in white wines, some dry yeast preparations reduced acidity and olfactory intensity but improved aromatic persistence. In red wines, yeast derivatives reduced green tannins and increased softness on the palate, especially those that release higher neutral polysaccharides.

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555

FIGURE CAPTIONS

Figure 1. Total polyphenols (TP in mg/L of gallic acid), tannins (mg/L of cyanidin chloride), tartaric esters of phenolic acids (mg/L of caffeic acid) and flavonols (mg/L of quercetin) in white wines. 0 MB: end of treatment, 3 MB: three months in bottle. Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

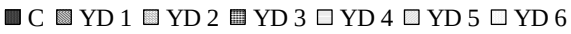

Figure 2. Acid (APS in mg/L of galacturonic acid), neutral (NPS in mg/L) and total (TPS in mg/L of glucose) polysaccharides in white wines. 0 MB: end of treatment, 3 MB: three months in bottle. Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

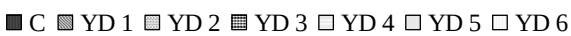

Figure 3. Sensory diagrams of color and olfactory phase (A) and gustative phase (B) in white wines at the end of treatment. The asterisk indicates statistically significant differences for $\alpha < 0.05$. 

Figure 4. Total polyphenols (TP in mg/L of gallic acid), tannins (mg/L of cyanidin chloride), total anthocyanins (mg/L of malvidin-3-glucoside) in red wines. 0 MB: end of treatment and malolactic fermentation, 3 MB: three months in bottle. Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

Figure 5. Catechins (mg/L of D-(+)-catechin), tartaric esters of phenolic acids (mg/L of caffeic acid) and flavonols (mg/L of quercetin) in red wines. 0 MB: end of treatment and malolactic fermentation, 3 MB: three months in bottle. Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

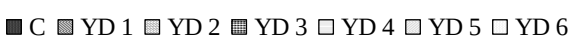

Figure 6. Color intensity values (CI, Absorbance), percentage of blue and red and polymeric anthocyanins in red wines. 0 MB: end of treatment and malolactic fermentation, 3 MB: three months in bottle. Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

Figure 7. Glucoside, acetic and cinnamic anthocyanin concentration (mg/L of malvidin-3-glucoside) in red wines. 0 MB: end of treatment and malolactic fermentation, 3 MB: three months in bottle. Values with different letter indicate statistically significant differences at $\alpha < 0.05$. The absence of letters means that there are not statistically significant differences.

Figure 8. Percentage of new pigments in red wines. 0 MB: end of treatment and malolactic fermentation, 3 MB: three months in bottle. Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

Figure 9. Acid (APS in mg/L of galacturonic acid), neutral (NPS in mg/L) and total (TPS in mg/L of glucose) polysaccharides, protein concentration (mg/L of bovine serum albumine) in red wines. 0 MB: end of treatment and malolactic fermentation, 3 MB: three months in bottle. Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

Figure 10. Sensory diagrams of color and olfactory phase (A) and gustative phase (B) in red wines at the end of treatment and malolactic fermentation. The asterisk indicates statistically significant differences for $\alpha < 0.05$.

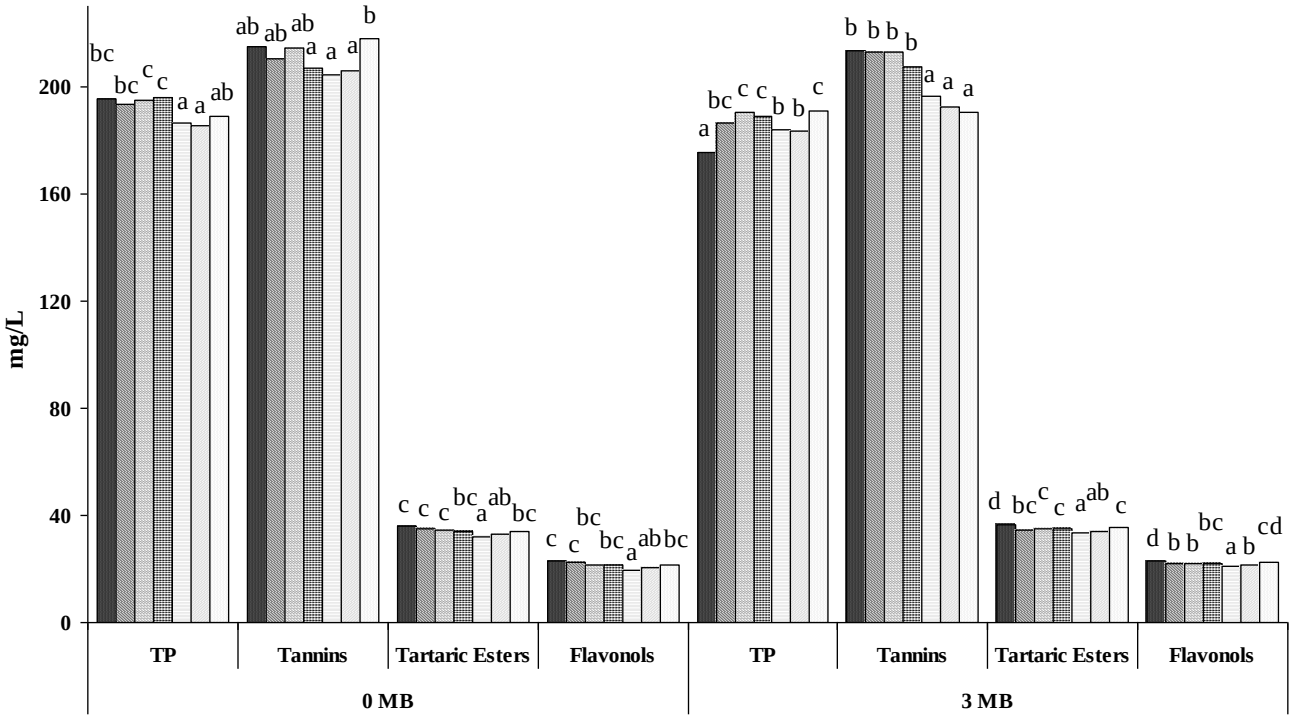
mg/L
0
40
80
120
160
200
TP
Tannins
Tartaric Esters
Flavonols
0 MB
3 MB

Figure 1

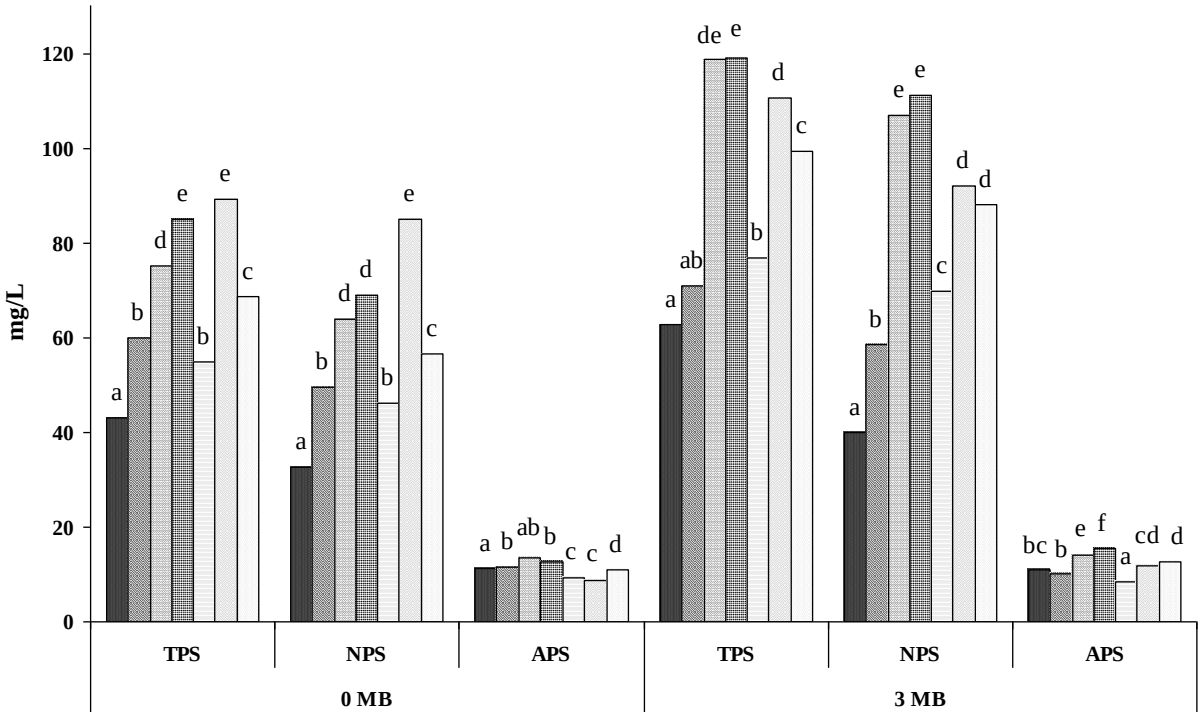

Figure 2

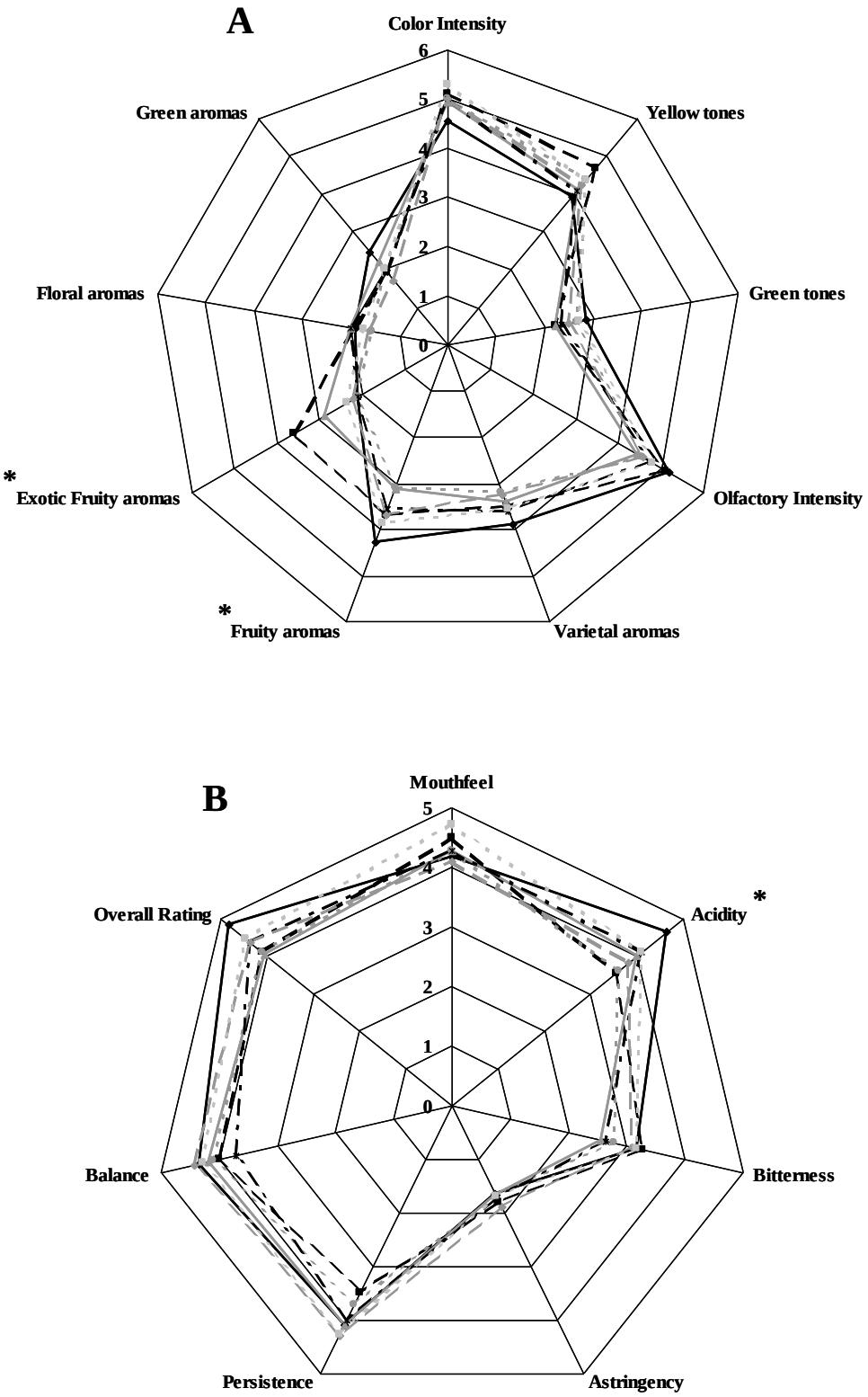
A
Color Intensity
6
5
4
3
2
1
0
Yellow tones
Green tones
Olfactory Intensity
Varietal aromas
Fruity aromas
*
Exotic Fruity aromas
*
Floral aromas
Green aromas
B
Mouthfeel
5
4
3
2
1
0
Acidity
*
Bitterness
Astringency
Persistence
Balance
Overall Rating

Figure 3

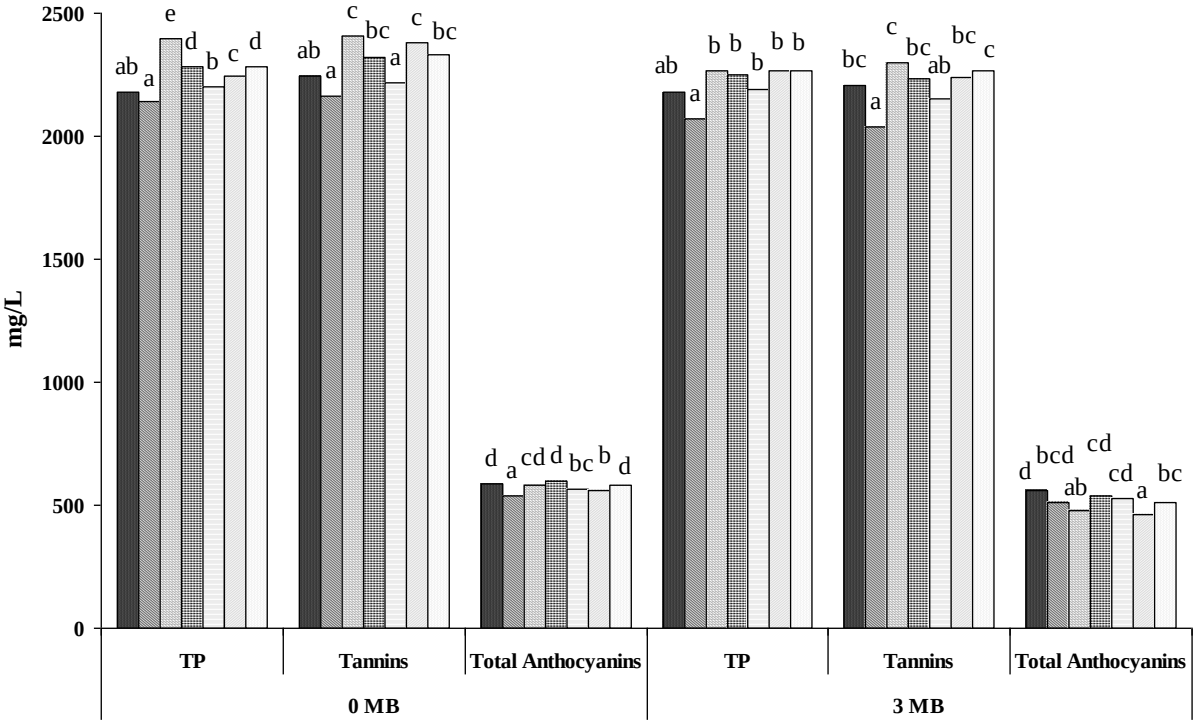
2500
2000
1500
1000
500
0
mg/L
ab a e d b c d
ab a c bc a c bc
d a cd d bc b d
ab a b b b b b
bc a c bc ab bc c
d bcd ab cd cd a bc
TP
Tannins
Total Anthocyanins
TP
Tannins
Total Anthocyanins
0 MB
3 MB

Figure 4

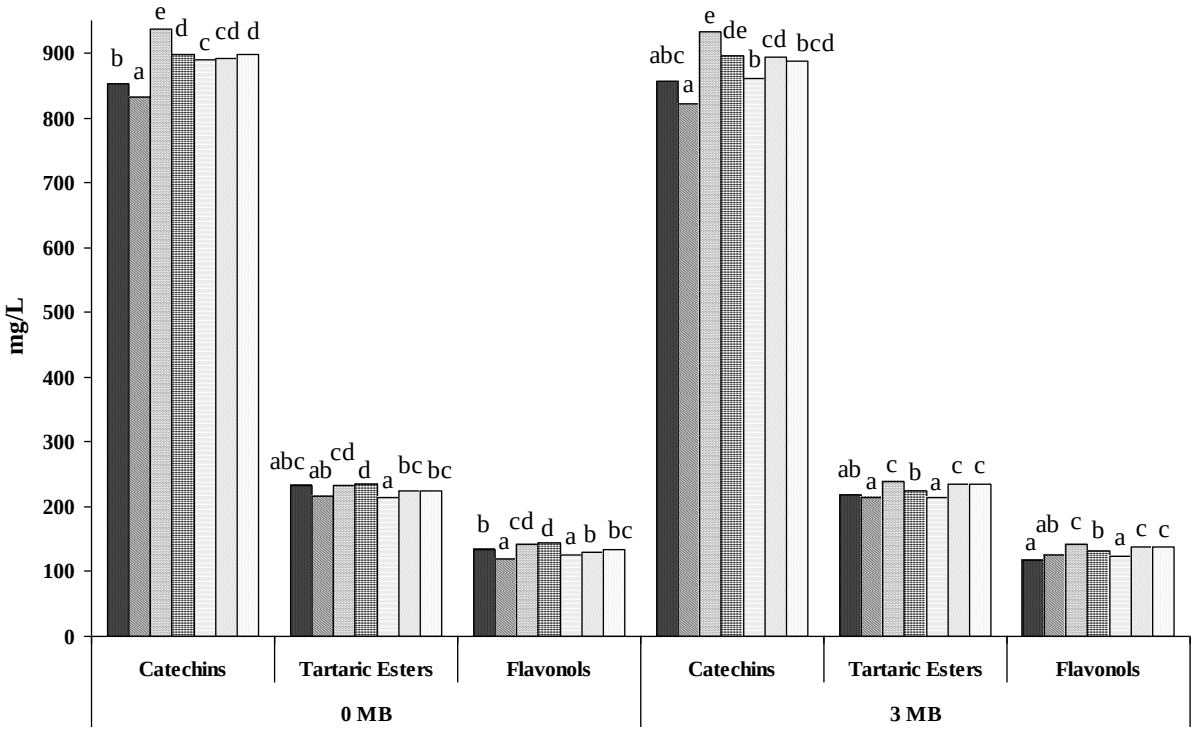
mg/L
0
100
200
300
400
500
600
700
800
900
b a e d c cd d
abc ab cd d a bc bc
b a cd d a b bc
abc a e de b cd bcd
ab a c b a c c
a ab c b a c c
Catechins
Tartaric Esters
Flavonols
Catechins
Tartaric Esters
Flavonols
0 MB
3 MB

Figure 5

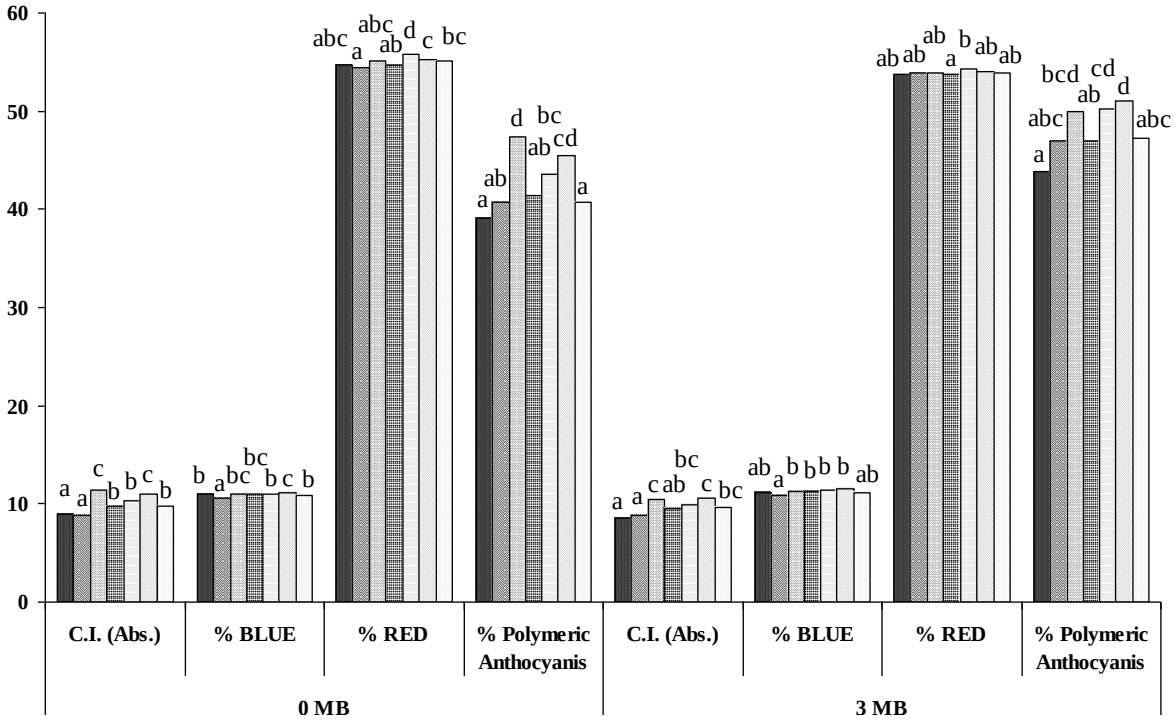
60
50
40
30
20
10
0
C.I. (Abs.)
% BLUE
% RED
% Polymeric Anthocyanis
C.I. (Abs.)
% BLUE
% RED
% Polymeric Anthocyanis
0 MB
3 MB

Figure 6

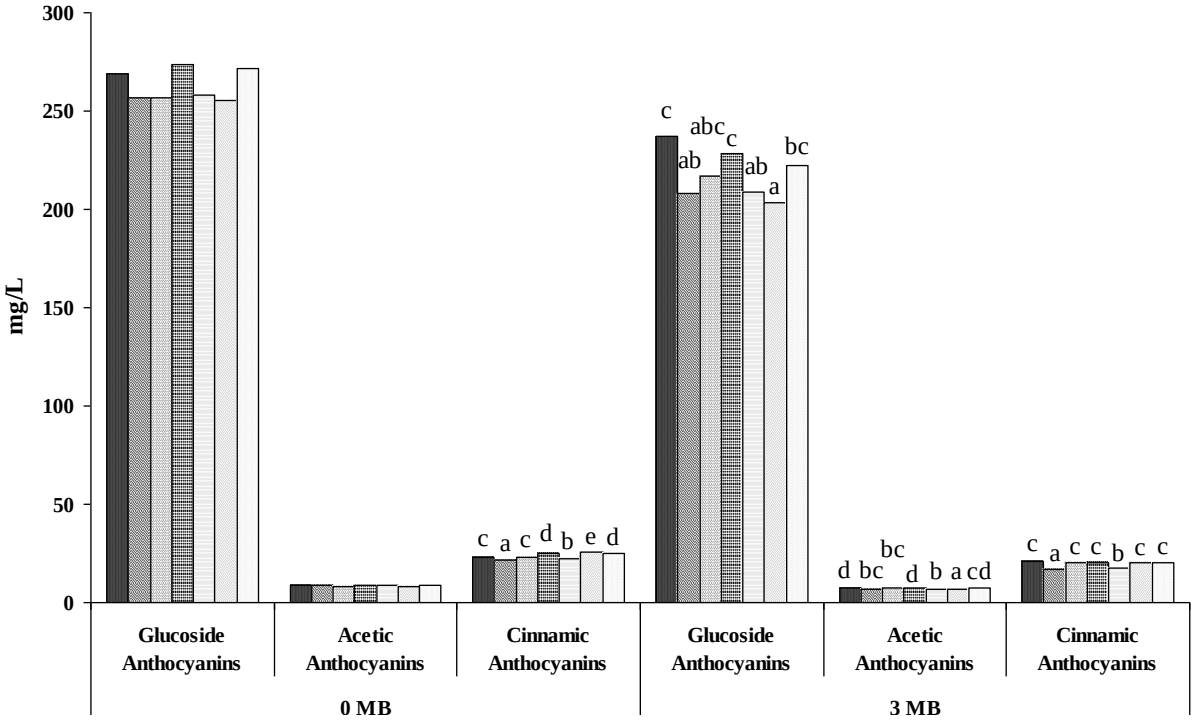
300
250
200
150
100
50
0
mg/L
c a c d b e d
c ab abc c ab a bc
d bc bc d b a cd
c a c c b c c
Glucoside Anthocyanins
Acetic Anthocyanins
Cinnamic Anthocyanins
Glucoside Anthocyanins
Acetic Anthocyanins
Cinnamic Anthocyanins
0 MB
3 MB

Figure 7

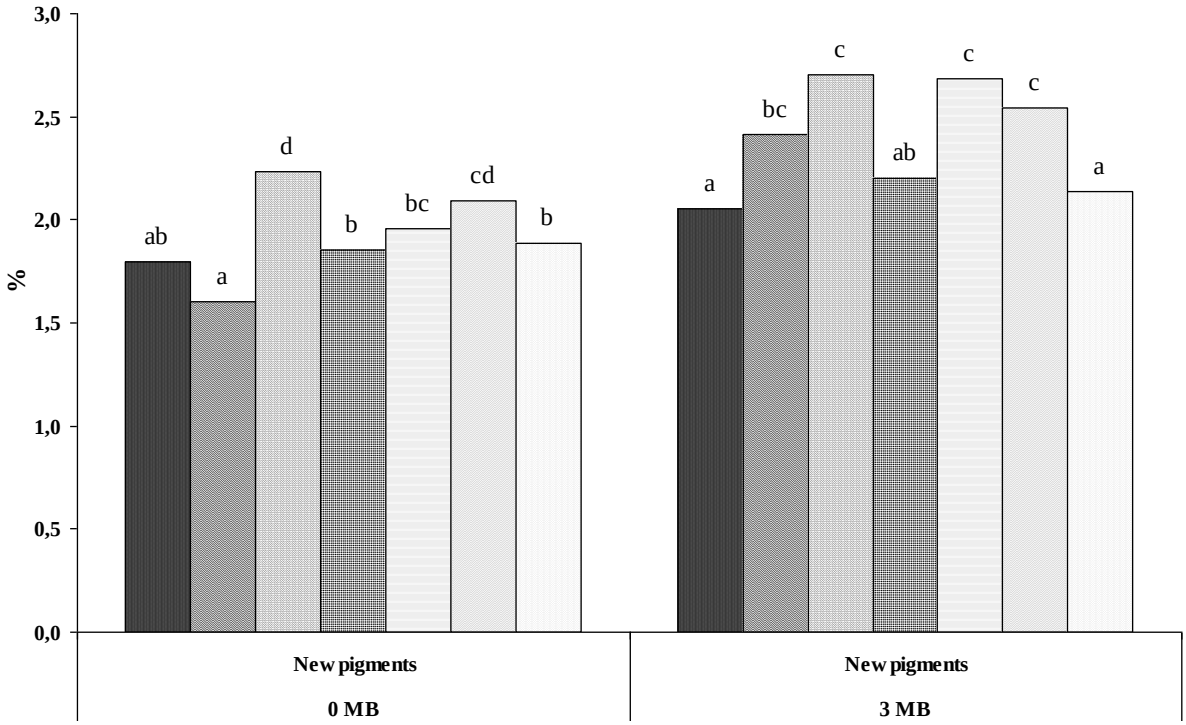
3,0
2,5
2,0
1,5
1,0
0,5
0,0
%
ab
a
d
b
bc
cd
b
a
bc
c
ab
c
c
a
New pigments
0 MB
New pigments
3 MB

Figure 8

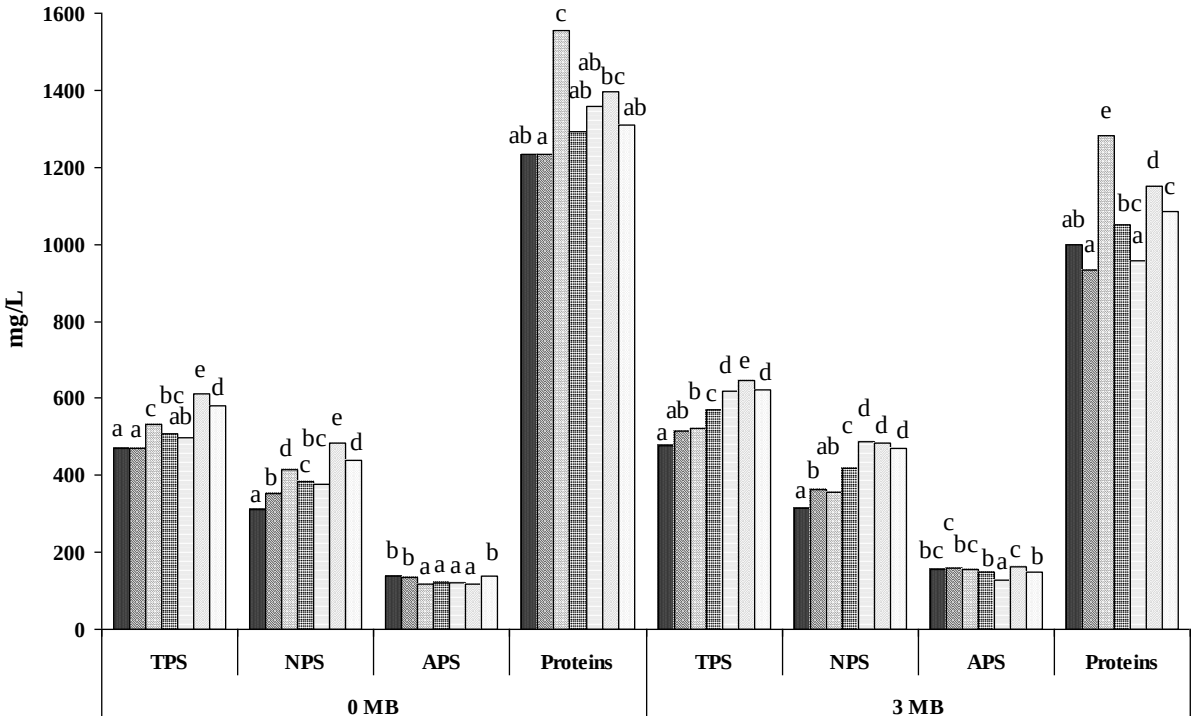
mg/L
1600
1400
1200
1000
800
600
400
200
0
TPS
NPS
APS
Proteins
TPS
NPS
APS
Proteins
0 MB
3 MB

Figure 9

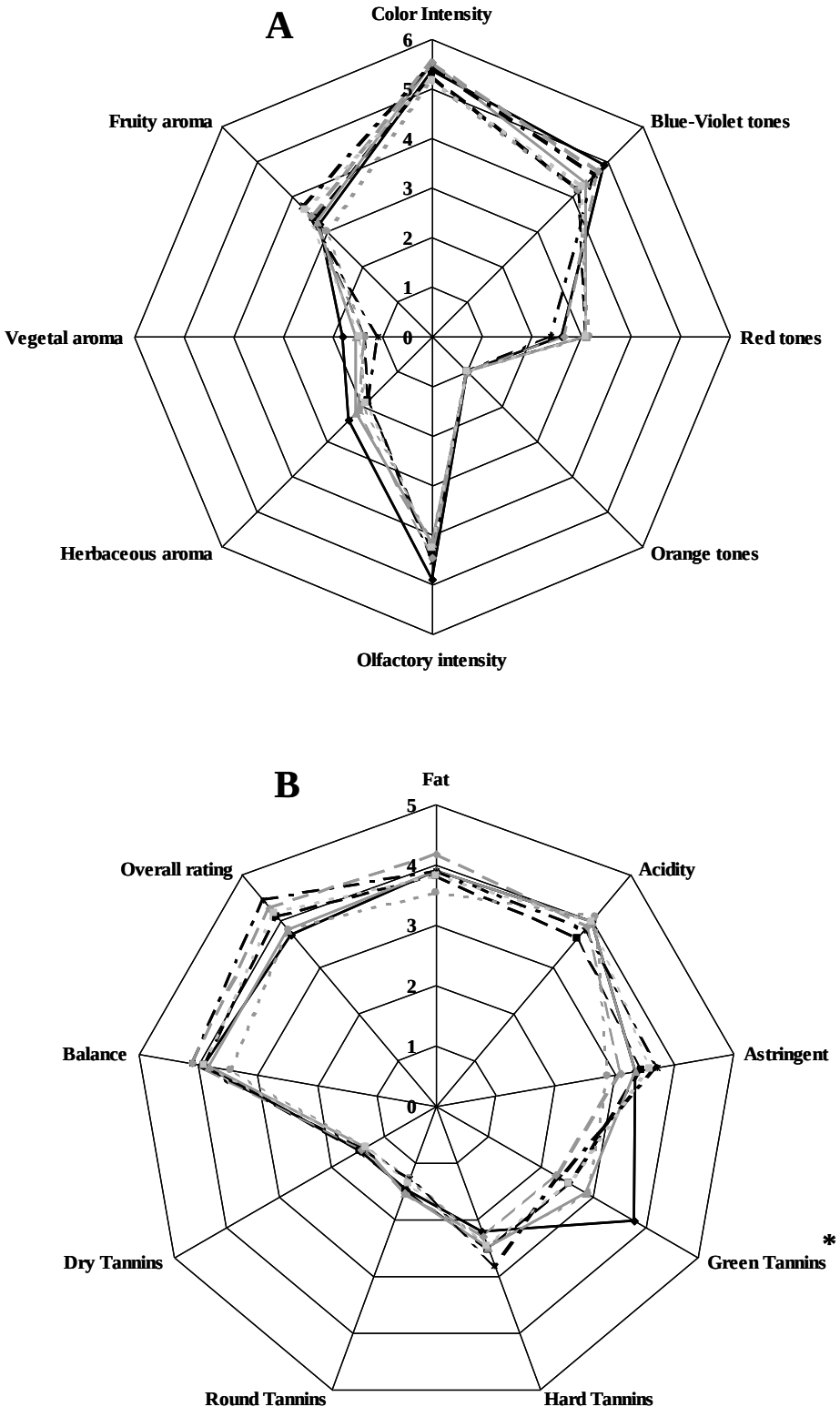
A
Color Intensity
6
5
4
3
2
1
0
Fruity aroma
Blue-Violet tones
Vegetal aroma
Red tones
Herbaceous aroma
Orange tones
Olfactory intensity
B
Fat
5
4
3
2
1
0
Overall rating
Acidity
Balance
Astringent
Dry Tannins
Green Tannins
*
Round Tannins
Hard Tannins

Figure 10

Table 1. Commercial yeast derivative composition and characteristics.

Yeast derivative	Comercial supplier	Composition and characteristics	Expected effect
YD-1	Agrovin	Product with autolysed yeast enriched in polysaccharides.	Decrease astringency and increase mouth-feel. Increases the persistence and aroma stability. Improve color stability, and tartaric and protein stability.
YD-2	Agrovin	Product with autolysed yeast enriched in polysaccharides and with β-glucanase activity.	Decrease astringency and increase mouth-feel. Increase persistence and aroma stability. Improve color stability, and tartaric and protein stability.
YD-3	Sepsa	Product with polysaccharides extracted enzymatically of selected yeast walls.	Increase mouth-feel and roundness sensations. Decrease astringency and increase the aromatic persistence. Improve tartaric and protein stability. Favor the development of malolactic fermentation.
YD-4	Laffort	Contain a peptide fraction found in the yeast which has sweeter power.	Increase sweetness sensation. Favor the elimination of some polyphenols which are responsible for the bitterness and astringency.
YD-5	Bio Springer	Constituted exclusively for polysaccharides from the yeast cell wall. It contains 25 % of free highly soluble mannoproteins.	Provide roundness and mouth-feel. Decrease astringency. Improve the color stability, and tartaric and protein stability. Prevent organoleptic deviations.
[a]YD-6W	AEB	Product with yeast cellular walls rich in mannoproteins and nucleotides.	Allow to obtain round and smooth red wines, generating a more complex taste. Increase mouth-feel.
[b]YD-6R	AEB	Product with yeast cellular walls rich in mannoproteins and nucleotides. Mannoproteins with a medium molecular weight.	Allow to obtain wines with more body, smooth tannins and more persistence in mouth. Prevent the formation of orange tonalities.

[a] Yeast derivative product used in white wines, [b] yeast derivative product used in red wines.

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

Monosaccharides	Comercial products						
	YD1	**YD2**	**YD3**	**YD4**	**YD5**	**YD6R**	**YD6W**
Apiose	nd	nd	nd	nd	nd	nd	1.08±0.13
Arabinose	0.34±0.05a	0.28±0.25a	nd	nd	nd	3.7±0.60b	0.88±0.80a
Rhamnose	nd	nd	nd	nd	nd	0.72±0.34a	2.9±0.50b
Xilose	0.15±0.05a	0.33±0.29a	nd	nd	nd	0.25±0.22a	0.29±0.25a
Manose	41.5±3.6a	34.4±9.6a	42.9±3.9a	40.4±5.4a	72.4±12.5b	33.7±3.6a	43.8±7.7a
Dha [b]	nd	nd	nd	nd	nd	nd	nd
Galactose	0.20±0.34a	0.28±0.30a	nd	nd	nd	11.5±4.8b	11.5±2.9b
Gal. Acid [b]	nd	nd	nd	nd	nd	1.4±0.86a	3.0±0.10b
Glucose	57.8±6.1ab	64.7±7.6b	57.1±6.0ab	59.6±6.9ab	27.6±5.6c	47.9±2.5a	25.5±3.2c
Gluc. Acid [b]	nd	nd	nd	nd	nd	nd	10.9±0.22
	YD1	**YD2**	**YD3**	**YD4**	**YD5**	**YD6R**	**YD6W**
Percentage of polysaccharide purity	58.3±5.7ab	75.9±9.1bc	82.7±10.9c	56.6±7.9ab	42.7±6.3a	98.3±5.7c	54.4±12.2ab

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

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

[c] nd: no detected (≤0.05%).

Table 3. Percentage of different molecular weights of polysaccharide fractions respect to the total soluble polysaccharide contents estimated using HRSEC-RID [a].

Commercial products	∑ (P400-P50)[b] %	P10[b] %
YD1	77.30±0.71d	22.70±1.02a
YD2	35.92±2.92a	64.08±3.30d
YD3	55.62±0.38b	44.38±3.62c
YD4	100.00±4.30e	
YD5	100.00±0.07e	
YD6R	65.00±2.68c	35.00±1.33b
YD6W	100.00±2.88e	

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

[b] Σ(P400-P50): polysaccharides with an average molecular weight between 47.3 kDa and 404 kDa, P10: polysaccharides with an average molecular weight of 11.8 kDa.

Table 4. Color intensity (absorbance at 420 nm.) and protein concentration (mg/L of BSA[a]) in white wines[b].

Color intensity	**C**	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**
0 MB	0.570a	0.570a	0.605ab	0.585a	0.590a	0.590a	0.630b
3 MB	0.606bc	0.620c	0.625c	0.595bc	0.560ab	0.538a	0.615c
Proteins	**C**	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**
0 MB	52.7a	61.2b	57.0ab	59.3b	72.3c	75.5c	80.6d
3 MB	67.2ab	68.3abc	65.0a	65.4a	72.4bc	73.1c	78.2b

[a] BSA: Bovine Serum Albumine.

[b] Values with different letter indicate statistically significant differences at $\alpha < 0.05$.

0 MB (end of treatment, 3 MB (three months in bottle).

CAPÍTULO 2

Estudio de las interacciones entre los compuestos fenólicos o volátiles y las lías de levadura, derivados comerciales de levadura y chips sin tostar en soluciones modelo y en vinos tintos jóvenes

Los polisacáridos que proceden de las levaduras juegan un papel importante en las propiedades sensoriales de los vinos, debido a que pueden interaccionar con los compuestos fenólicos y volátiles presentes en el vino. La interacción de los polisacáridos con los compuestos fenólicos puede dar lugar a una reducción de la astringencia y la estabilización del color de los vinos, mientras que su interacción con los compuestos volátiles puede afectar a su volatilidad y por lo tanto a la percepción aromática de los vinos. Además, hay que tener en cuenta que algunos compuestos volátiles presentes en los preparados de derivados de levadura comerciales pueden ser liberados a los vinos, lo que puede implicar también cambios en la composición aromática del vino.

Por otro lado, la madera de roble sin tostar puede aportar ciertos polisacáridos que, aunque son diferentes a aquellos que proceden de las paredes celulares de las levaduras, también pueden modificar la composición fenólica y volátil de los vinos.

Distintos trabajos han estudiado la interacción entre los polisacáridos procedentes de levaduras y algunos compuestos volátiles y fenólicos presentes en el vino, aunque la mayoría se han realizado en soluciones modelo.

Por este motivo, en este trabajo se ha evaluado la interacción de algunos de los compuestos fenólicos y volátiles más representativos de los vinos con lías, con chips de madera de roble sin tostar y con diferentes preparados comerciales derivados de levadura en soluciones de vino modelo. Además se ha estudiado el efecto de estos productos sobre la composición fenólica y volátil de un vino tinto de la variedad Tempranillo.

Los principales resultados y conclusiones obtenidos en este trabajo son los siguientes:

1- La concentración de la mayoría de los compuestos fenólicos estudiados (ácidos cafeico y cumárico, catequina, resveratrol y triptofol) disminuyó en los vinos modelo tratados con chips de madera de roble sin tostar y en los tratados con lías finas, probablemente debido a la adsorción de estos compuestos sobre los chips y las lías.

Sin embargo, los preparados comerciales derivados de levadura estudiados no modificaron la concentración de los compuestos fenólicos estudiados.

2- El efecto de los distintos tratamientos ensayados en las soluciones de vino modelo sobre los compuestos volátiles dependió del tratamiento empleado y del compuesto.

De esta forma, los derivados de levadura comerciales estudiados únicamente liberaron pequeñas concentraciones de algunos de los compuestos volátiles estudiados. Como era de esperar, el eugenol y los isómeros *cis* y *trans* de las whisky lactonas sólo fueron encontrados en los vinos modelo tratados con chips, ya que estos compuestos son extraídos de la madera de roble.

Así mismo hay que señalar que la mayoría de los compuestos volátiles estudiados fueron adsorbidos por los chips. Sin embargo, los vinos modelo tratados con lías y con los derivados de levadura YD 1, YD 2 y YD 3 presentaron concentraciones más altas de la mayoría de los compuestos volátiles adicionados. El resto de derivados de levadura estudiados no modificaron de forma significativa el contenido de estos compuestos.

De acuerdo con la información dada por los fabricantes, estos resultados parecen indicar que cuanto mayor es el contenido en manoproteínas puras del preparado comercial menor es su efecto sobre la composición volátil del vino de partida.

3- El tiempo que las lías, chips y derivados de levadura comerciales permanecen en las soluciones de vino modelo es uno de los factores que va a influir de manera significativa en la volatilidad de los compuestos aromáticos y en las interacciones con los compuestos fenólicos. De este modo, las interacciones de los compuestos fenólicos con las lías y la madera de roble sin tostar ocurrieron principalmente durante los primeros 15 días de tratamiento y posteriormente se mantuvieron constantes hasta el final del tratamiento (60 días). Sin embargo, en el caso de los compuestos volátiles, éstos fueron retenidos inicialmente (15 días) y posteriormente fueron liberados al medio (30-60 días dependiendo del compuesto analizado y del tratamiento realizado). Estas interacciones reversibles

entre los compuestos volátiles y los compuestos liberados por las lías ya habían sido descritas con anterioridad, pero no se habían estudiado en un período de tratamiento tan largo.

4- Los resultados obtenidos en los vinos modelo no siempre coinciden con los encontrados en los vinos tintos estudiados tanto en los compuestos fenólicos como volátiles.

Así, los vinos tintos tratados con chips y lías presentaron concentraciones más altas de ácidos hidroxicinámicos que los vinos control, mientras que en los vinos modelo se observó una adsorción de estos compuestos por los chips y las lías. Así mismo se observó que el empleo de derivados de levadura comerciales, lías y chips podría retrasar las reacciones de copigmentación de los flavonoles con los antocianos.

En el caso de los compuestos volátiles, se observaron interacciones entre las lías y los ésteres etílicos y acetatos de alcoholes de fusel de los vinos tintos no encontradas en las soluciones de vino modelo.

Las diferencias encontradas entre los vinos tintos y los vinos modelo pueden ser debidas a que el vino es una matriz muy compleja que está formada por compuestos de distinta naturaleza que pueden interaccionar con los compuestos fenólicos o volátiles del vino, entre los que pueden tener lugar distintas reacciones (condensación, hidrólisis, etc.).

5- Los derivados de levadura comerciales y los chips adsorbieron cantidades significativas de 4-etilfenol (tanto en las soluciones de vino modelo como en los vinos tintos), compuesto que en cantidades superiores a su umbral de percepción puede aportar al vino olores no deseables a cuadra, establo o sudor de caballo, afectando de forma negativa a la calidad del vino. Por lo tanto, los derivados de levadura comerciales y los chips de roble podrían eliminar una parte del 4-etilfenol presente en los vinos, solucionando uno de los problemas que más preocupa actualmente a bodegueros y enólogos.

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Study of interactions between phenolic or volatile compounds and yeast lees, commercial yeast derivatives and non-toasted chips in model solutions and in young red wines

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Study of interactions between phenolic or volatile compounds and yeast lees, commercial yeast derivatives and non-toasted chips in model solutions and in young red wines

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Abstract

The aim of this work was to evaluate the interaction of some representative phenolic and volatile compounds of wines with lees, non-toasted oak wood chips and different commercial yeast derivative preparations in model wine solutions and their effect on the phenolic and volatile composition of a red wine.

The results found in this study have shown that most of the phenolic and the volatile compounds are adsorbed by wood and lees in model wine solutions. The commercial yeast derivative products did not interact with the phenolic compounds, but they did it with the volatile compounds, and depended on the volatile compound and the product used. The time of treatment influences aroma volatility and phenolic interactions in a different way. The adsorption effect in model wine solution was not always the same than in the red wine studied. The adsorption effect on the phenolic and volatile compounds in the model wine solution was not always the same as in the red wine studied, which highlights the important presence of other wine compounds in these interactions.

Key words: Lees, commercial yeast derivatives, chips, phenolic and volatile compounds, model wine solution, red wine.

INTRODUCTION

It is well known that wine is a complex matrix made up of several compounds which can interact among themselves throughout the wine aging process, thereby modifying their sensorial characteristics. Besides, different processes or techniques can also induce changes in the reactions that can take place between different wine compounds.

One of these techniques is the aging of wines on lees, which permits the release of different compounds such as mannoproteins and polysaccharides into wines during yeast autolysis. In addition, the compounds released during aging on lees can interact with phenolic compounds [1-4] and/or aromatic compounds, [5-7] also modifying wine sensory perception.

The interactions of polysaccharides with phenolic compounds can reduce wine astringency [3, 4, 8]. Some authors have found that aging on lees can also improve the colour stability of red wines [9-12] However, other authors have not found this improvement in wine colour [3, 4, 8] either by using aging on lees or adding products based on yeast [13].

The study of the effect of the compounds released during yeast autolysis on wine aroma has focussed mainly on their capacity to interact with certain volatile compounds modifying their volatility [5, 7, 14, 15, 16]. However, it should also be taken into account that during these processes flavour agents and precursors of many volatile compounds can be released into the medium [17, 18].

Nevertheless, yeast autolysis is a very slow process, and large periods of time are necessary for the release of mannoproteins and polysaccharides. Moreover, the longer the process, the higher the risk of the appearance of certain microbiological and organoleptic alterations. For all these reasons, for the last few years many suppliers of enological products have offered the wineries several preparations rich in

mannoproteins and polysaccharides obtained from *Saccharomyces cerevisiae* cell walls, using physical and/or enzymatic treatment under different names (inactive yeasts, yeast autolysates, yeast walls and yeast extracts (mannoproteins with diverse degrees of purification)) [19]. However, few commercial preparations based on purified mannoproteins are available on the market, and it is more usual to find yeast derivative products of heterogeneous composition [4, 8]. Companies supply these products as an alternative to wine aging on lees, promising the benefits mentioned above regarding the final quality of wines but in a shorter time. However, their real impact on wine quality is still not clear.

Furthermore, the addition of non-toasted oak wood chips can also release some polysaccharides into the wines, although they are not of the same type as those released from the yeast cell walls during the autolysis process [20, 21]. These compounds can also interact with wine compounds, affecting the sensorial characteristics. In addition, oak wood chips can transfer to the wines some phenolic and volatile compounds. The volatile characterization of different commercial inactive dry yeast products has been shown by certain authors, [18, 19, 22] and some have studied the influence of their use on real white and red wines [23, 24]. However, no studies have been found relating to the effect of these products on low molecular phenolic compounds. Therefore, the aim of this research was to evaluate the interaction of several representative phenolic and volatile compounds of wines with lees, non-toasted oak wood chips and different commercial yeast derivative preparations in model wine solutions, and to study their effect on the phenolic and volatile composition of a red wine.

EXPERIMENTAL

Chemical reagents

Gallic acid, *trans*-*p*-coumaric acid, *trans*-resveratrol, (+)-catechin and syringic acid

were provided by Sigma-Aldrich (Steinheim, Germany), protocatechuic acid, vanillic acid, ellagic acid, *trans*-caffeic acid, tyrosol, tryptophol, myricetin and kaempferol by Fluka (Buchs, Switzerland), (-)-epicatechin, ethyl gallate, syringetin-3-glucoside and quercetin by Extrasynthèse (Lyon, France).

Non-commercial available compounds were quantified using the calibration curves corresponding to the most similar compounds: *trans*-p-coumaric for *cis*-p-coumaric acid, *cis*- and *trans* coutaric acid; *trans*-caffeic acid for *trans*-caftaric acid and *trans*-fertaric acid; *trans*-resveratrol for *trans*- and *cis*-resveratrol-3-glucoside; and flavonol aglycones for the respective flavonol glycoside derivatives.

The volatile compound standards were purchased from Fluka (Buchs, Switzerland) (ethyl butyrate, ethyl isovalerate, ethyl hexanoate, ethyl octanoate, β-phenylethyl acetate, isobutanol, benzyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, β-phenylethanol, 1-hexanol, *cis*-3-hexenol, hexanoic acid, octanoic acid, decanoic acid, guaiacol, γ-butyrolactone, citronellol); Sigma-Aldrich (Steinheim, Germany) (ethyl 2-methylbutyrate, ethyl decanoate, isoamyl acetate, *trans*-3-hexenol, eugenol, 2,6-dimethoxyphenol, γ-nonalactone, whiskey-lactone (mixture of *cis* and *trans* isomers), acetovanillone, linalool, β-ionone, ethyl cinnamate, 2-octanol, methyl octanoate); and Lancaster (Strasbourg, France) (methyl vanillate, ethyl vanillate, 4-ethylphenol, 4-vinylphenol, 4-vinylguaiacol, 3,4-dimethylphenol).

Ethanol (HPLC-grade) was provided by Scharlau (Barcelona, Spain), and dichloromethane (HPLC-grade) by Merck (Darmstadt, Germany). Acetonitrile and methanol were provided by Lab Scan (Madrid, Spain) and the remaining reagents by Panreac (Madrid, Spain). Water Milli-Q was obtained via a Millipore system (Bedford, MA).

Hydro-alcoholic solution for model wine

Two model wine solutions were prepared by mixing 13% ethanol ADITIO (v/v), 4 g/L tartaric acid, 3 g/L DL-malic acid, 0.1 g/L acetic acid glacial, 0.1 g/L potassium sulphate and 0.1 g/L magnesium sulphate 7-hydrate. The pH was adjusted to 3.5 with NaOH. Nine phenolic compounds were added to a model wine solution and ten volatile compounds were added to a second model wine solution. The phenolic and volatile compounds were not added together to the same model wine solution in order to avoid interactions between them. The final concentration of the phenolic and volatile compounds in model wines was similar to those found in real wines. One or two compounds of each chemical group were selected (Table 1). Once the phenolic and aromatic solutions were homogenized, each model wine solution was racked in different bottles. Following this, seven different commercial yeast derivative preparations, lees and non-toasted French oak chips were added to the bottles. Samples without any of these products were the control model wine solutions. Table 2 shows the characteristics of the commercial yeast derivatives, lees and chips and the doses that were used. All the bottles were closed and stored at 15ºC for 60 days and were homogenized by manual stirring twice per week. The analyses were carried out at 15, 30 and 60 days of treatment in three different bottles for each sampling date.

In order to check whether some of the products assayed could release some of the volatile compounds studied into the model wine and thus modify the volatile composition, the same experiment mentioned above was carried out, but this time without the addition of the phenolic and volatile compounds. In this case, the model wines were analysed after 60 days of treatment.

Red winemaking

Tempranillo grapes from the 2007 vintage from Cigales Designation of Origin (D.O.) were used to make the red wine. The grapes were harvested manually at the optimum

harvest date and transported to the Oenological Station in 15-Kg-plastic boxes. The clusters were de-stemmed and crushed with minimum physical damage. The mass obtained was slightly sulphited (0.04 g/L) and then transferred to stainless steel tanks to undergo alcoholic fermentation at a controlled temperature. Alcoholic fermentation was carried out through inoculation with commercial yeast S*accharomyces cerevisiae* (Excellence sp, Lamothe-Abiet) and following this fermentation the mass was pressed to obtain the finished red wine.

The wines were kept in tanks for 4 days to allow for sedimentation of the gross lees. After this time, the wines were racked off and maintained in the tanks for 4-5 days to allow for sedimentation of the fine lees. The base wine was again racked off and distributed into different 16 L tanks in which the different treatments were carried out.

The experiments were carried out in duplicate: control wines (without the addition of any product), wines with the addition of lees, ones with non-toasted oak wood chips and wines with six different commercial yeast derivative preparations. The doses used were the same as those used in the model wines. Two batonnages were performed weekly. The temperature was maintained at 15 ºC ± 1 ºC. All treatments lasted 60 days.

After the treatments the red wines were inoculated with a commercial preparation of *Oenococcus Oeni* (Viniflora, CHR Hansen, Denmark) to induce malolactic fermentation. Once this second fermentation had finished, the wines were filtrated and bottled. The samples were analyzed immediately after bottling.

Analytical Methods

Extraction and HPLC-DAD analysis of the phenolic compounds

In the red wines, the phenolic compounds were previously isolated and concentrated by a solid-phase extraction procedure (SPE) using the Oasis HLB cartridges (Waters, Mildforf, Massachusetts, USA) and following the method described by Pérez-Magariño

et al. (2008) [25]. A manifold system (Waters, Barcelona, Spain) was used for SPE. The extracts obtained were filtrated through PVDF filters of 0.45 μm (Symta, Madrid, Spain) and were analyzed with an Agilent-Technologies LC-DAD series 1100.

The chromatographic conditions and quantification of phenolic compounds were established by Pérez-Magariño et al. (2008) [25].

The phenolic compounds in model wine samples were determined by direct injection after filtration through the PVDF filters.

Analysis of the volatile compounds

The volatile compounds were extracted by liquid-liquid extraction following the method developed by Ortega-Heras et al. (2002) [26]. Two hundred and fifty milliliters of wine, 5mL of dichloromethane, and 75 μL of a mixture of two internal IS standards (methyl octanoate, and 3,4-dimethylphenol) were added to a flask. The extraction was carried out for 3 h with continuous stirring (150 rpm). Each extraction was performed in duplicate and the volatile compounds were analysed by gas chromatography-mass detector (GC-MS). The chromatographic analyses were performed with a HP-6890N GC coupled to a HP-5973 inert MS detector equipped with a Quadrex 007CWBTR capillary column (60 m length, 0.25 mm i.d., and 0.25 μm film thickness). The carrier gas was helium at 0.8 mL/min. The oven column program was set to 40 ºC (held for 10 min), raised to 240 ºC by 2 ºC min^{-1}, and kept at this temperature for 45 min. Detection was in EI scan mode (70 eV), and identification was carried out using spectra obtained with commercial standard compounds and from the NIST library. 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.

Statistical analysis

All the data were treated by means of the variance analysis (ANOVA), and the Least Significant Difference test (LSD), which determines statistically significant differences between the means. Confidence intervals of 95% or a significant level of $\alpha = 0.05$ were used.

All the statistical analyses were carried out with the Statgraphics Plus 5.0 statistical package.

RESULTS AND DISCUSSION

Low molecular weight phenolic compounds in model wine solutions

Table 3 shows the results obtained for the different phenolic compounds in the different assays and time studied. In general, none of the products studied modified vanillic acid concentration throughout the two-month period. The highest differences were found in the model wines treated with chips at 60 days, but these differences were lower than 3% with respect to the control model wine.

As for gallic acid, the model wines treated with chips showed the highest concentration of this compound throughout the period of treatment, whereas the model wines treated with lees displayed the lowest content (Table 3). These differences were more noticeable after 30 days of treatment. No differences were found between model wines treated with the different commercial yeast derivative products, which showed a similar concentration to those of the control model wines.

Ellagic acid is an unstable compound that can be hydrolyzed over time. Although it was added to the model wine solution, it was only detected in the model wines treated with chips. Its highest concentration was reached after 15 days of treatment (1.76 mg/L) and from then on it decreased during the treatment (1.44 mg/L at 30 days and 1.15 mg/L at 60 days).

The higher content of gallic and ellagic acids in model wines treated with chips agreed

with the results obtained by other authors, and can be explained by the fact that these compounds can be transferred to the wines by the hydroalcoholysis of oak wood [27-29].

The analysis of hydroxycinnamic acids (*trans*-caffeic and *trans*-*p*-coumaric acids) showed statistically significant differences between the treatments throught the two months (Table 3). The concentration of both compounds in the model wines treated with chips and lees decreased with respect to the control model wines, especially in the ones treated with lees. This decrease was found after 15 days of treatment and was kept up until the end of treatment (60 days). The reduction in the concentration of both acids was approximately 10% in model wines treated with chips and 15% in model wines treated with lees. These hydroxycinnamic acids can react with tartaric acid and give rise to their esterified forms, caftaric and coutaric acids. However, these compounds were not detected in any of the samples. So the decrease in hydroxycinnamic acids in these treatments is due to the adsorption of these compounds into the lees and oak chips. Salameh et al. (2008) [30] also found an adsorption of *p*-coumaric acid into *Brettanomyces* cell walls. All the model wines supplemented with yeast derivative products showed concentrations of these compounds similar to those of the control model wines along all the treatment.

The model wines with lees and chips displayed a lower concentration of catechin during the period of treatment than the control model wine, and the model wines treated with chips had the lowest concentration (Table 3). This decrease was greater after 15 days of treatment. At the end of the process, the model wines treated with lees showed an 18% reduction in catechin and those treated with chips, 22%. The addition of commercial yeast derivative products did not modify catechin content with respect to the control model wines.

As in the case of catechin, the model wines treated with chips and lees showed lower concentrations of tryptophol than the control model wines (Table 3). This reduction was more noticeable in the wines treated with chips (around 12%).

Similar results were found in the analysis of the most important stilbene *trans*-resveratrol (Figure 5C). In this case, the decrease in the concentration of this compound in the model wines treated with lees and chips was greater, with a reduction of 62% and 53%, respectively, with respect to the control model wines. Again, the presence of commercial yeast derivative products did not modify the concentration of this compound.

Quercetin was also evaluated (data not shown), and was detected in all the samples. However, quantification was not possible. This fact could be due to the high instability of this type of compounds (free flavonols) [25].

The results obtained in this study seem to indicate that phenolic compounds can be adsorbed by wood, thus decreasing their concentration in the model wines. This fact has been demonstrated for volatile compounds by different authors [31-32] but, as far as the authors know, this is the first study in which this has been observed for phenolic compounds. A decrease in the concentration of the phenolic compounds in wines aged on lees was also observed. Since this phenomenon was not observed in the wines treated with the different commercial yeast derivative products assayed, more research should be carried out in this area in order to find out and understand the mechanisms that control this interaction.

Aromatic compounds in model wine solution

The compounds released by the different products studied into the model wine are shown in Table 4. The compounds identified were in very low concentrations, findings that agree with those reported previously by Comuzzo et al. (2006) [22] and Pozo-

Bayón et al. (2009) [18]. As was expected, eugenol and *cis* and *trans*-whiskey lactones were only found in chips, since these compounds are released from wood. Of the 10 compounds studied, only hexanol has been previously encountered in commercial yeast derivatives [22].

The addition of the different YDs studied did not modify the concentration of isoamyl acetate and ethyl hexanoate during the first 30 days of treatment. However, after 60 days all the model wines supplemented with YD showed concentrations of these compounds which were higher than those of the control wine (Tabale 5). The presence of chips in the medium did not affect the concentration of isoamyl acetate and ethyl hexanoate. However, the model wine with lees displayed the highest concentrations of these compounds. This fact was due to the presence of these compounds in the wine lees, as can be seen in Table 4. Chalier et al., (2007) [7] also found higher concentrations of isoamyl acetate in a model wine with whole mannoproteins extracts or their fractions; however, they observed a considerable retention of ethyl hexanoate. Lubbers et al. (1994) [5] and Pozo-Bayón et al. (2009) [19] also found a large retention of this ester in model wines supplemented with macromolecules released during fermentation or with different commercial yeast derivatives.

The behaviour of ethyl octanoate was slightly different from that of the other ethyl ester studied. In the case of this compound, after 30 days of treatment all the model wines treated with the different commercial yeast derivatives displayed lower concentrations than the control, with the only exception of YD6 and YD7. However, after 60 days, only the model wine supplemented with YD3 showed concentrations of ethyl octanoate which were lower than the control wine. The other wines with YD displayed concentrations of this compound similar to the control wine (Table 5). Therefore, these results seem to indicate that during the first days of treatment the compounds released

by the different YD bind this ester, thereby reducing their volatility. Nevertheless, after 60 days of treatment this effect seems to be reversible, since the mannoproteins were able to release this compound again into the wine. The amount of volatile compound released would depend on the strength of the interactions, and this strength would depend on several factors such as mannoprotein conformation structure and composition, and volatile compound nature and concentration [7, 14]. The model wines treated with chips and lees showed a similar behaviour to the one described previously for the isoamyl acetate and ethyl hexanoate. However, in the case of the model wine with lees, after 60 days of treatment no statistically significant differences were found between this model wine and the control model wine, although this compound was also detected in the lees (Table 4).

A binding effect of the different compounds released by the yeast derivative products was also found for hexanol after 30 days of treatment (Table 5). Only the model wines with YD5 and YD7 showed concentrations of this compound similar to those of the control wines after one month of contact. This could be due to the fact that hexanol was also detected in trace amounts in these two YDs (Table 4). Again, after 60 days of contact, the differences between the model wines treated with the YD and the control wine disappeared. Neither was any effect observed as a result of the addition of chips to the model wine throughout the 60 days of treatment. The model wine treated with lees only showed higher concentrations of 1-hexanol at the end of the treatment, despite this compound also being present in the lees (Table 5). This might signify that during the first 30 days, the 1-hexanol present in the model wine was adsorbed by lees, but that one month latter it was released again into the medium.

Regarding β-ionone, after 15 days of treatment statistically significant differences were found between the control wine and the model wines with lees and three of the YDs

studied: YD1, YD2 and YD3 (Table 5). The wine with lees showed lower concentrations of this compound than the control wine, while the wines with YD displayed higher contents than the control wine. However, this compound was not detected in any of them (Table 4). After 30 days of treatment, a binding effect of this compound was also found in all the wines treated with the YDs studied. After 60 days, only YD6 and YD7 continued to show lower concentrations of this compound than the control wine, while YD1 and YD2, both from the same supplier, revealed higher concentrations. In the case of all the other wines, no statistically significant differences were found with regard to the control model wine (Table 5).

Several authors have found an important retention of hexanol, and mainly of □-ionone, by lees or commercial yeast derivatives [5, 7, 19] although in these studies shorter contact times were studied.

After 15 days of treatment, all the model wines revealed higher concentrations of linalool than the control wines, with the only exception of the model wines treated with chips and the YD6 (Table 5). It should be also pointed out that this compound was only detected in the lees and the commercial yeast derivative YD5. After 30 days only the model wines treated with lees, YD1, YD3 and YD4, showed higher levels of linalool than the control wine. However, at the end of the treatment, again all the model wines displayed higher concentrations of these compounds than the control wine, with the sole exception of the model wine macerated with chips (Table 5).

Eugenol and *trans* and *cis* oak lactone are genuine wood compounds, and for this reason it should be expected that the model wines macerated with chips showed higher concentrations of these compounds than the control wine. However, this only occurred for the two isomers of whiskey lactones (Table 5). The authors found similar results in a previous study in a red wine macerated with non-toasted chips during alcoholic

fermentation [24]. However, the wine with lees showed higher concentrations of these three oak-wood volatile compounds during the 60 days of treatment. These results contrast with those of Jiménez-Moreno and Ancín-Azpilicueta (2007) [33], who found a binding effect of eugenol by wine lees. The model wines with YD1, YD2 and YD3 also showed higher levels of these compounds than the control wine after 15 and 60 days of treatment.

After 30 days of treatment all the wines supplemented with commercial yeast derivative products showed concentrations of 4-ethyl-phenol that were lower than the control wine (Table 5). This compound was detected in trace amounts in all the YD products. After 60 days of treatment, the binding effect disappeared except for the YD7. Pradelles et al. (2008) [34] and Pradelles et al. (2009) [35] also reported in their studies a decrease in 4-ethyl-phenol concentration in wines containing yeast lees or yeast cell walls as compared with the same model wine without lees. Pradelles et al. (2009) [35] established that sorption of 4-ethylphenol via the yeast surface is a balance between hydrophobic, electron acceptor and electrostatic interactions.

As was the case with eugenol, adsorption of this compound by the chips was observed after 30 and 60 days of treatment (Table 5).

The results obtained in this study indicate that the influence on aroma volatility depends on the type of treatment and on the type of aroma compound, findings that are in agreement with those of Pozo-Bayón et al. (2009) [19]. According to the information given by the suppliers (Table 2), these results seem to indicate that the higher the number of pure mannoproteins and polysaccharides, the lower the effect of the product on the volatile composition of wine and, therefore, on aroma perception. These differences found between the commercial yeast derivative products studied could be related to possible differences in the yeast strain employed for the manufacture of these

products [36] and to the procedure used for the recovery and purification of mannoproteins [37].

Another factor influencing the volatility of aroma compounds seems to be the time these products remained in the wine. Thus, whereas short periods of time (15 days) caused a retention effect, longer exposure times (30-60 days) brought about a release of the previously bound compounds. Again, this should be investigated in future studies.

Low molecular weight phenolic compounds in red wines

The content of low molecular weight phenolic compounds and the statistical analyses are summarized in Table 6.

Hydroxybenzoic acids and derivatives

The statistical analysis showed an effect of the different treatments studied on all the hydroxybenzoic acids evaluated with the exception of syringic acid. The wines treated with YD2, YD5 and lees revealed higher contents of gallic and protocatechuic acids than the rest of the treated wines and the control wine. Besides, the wines treated with chips also showed a high concentration of gallic acid, but a low concentration of protocatechuic acid.

Moreover, the concentration of vanillic acid and ethylgallate was slightly affected by the treatments, and only the wines treated with YD6 showed lower values of these compounds than the control wine.

All the treated wines displayed higher concentrations of ellagic acid than the control wines, with the only exceptions of wines supplemented with YD1 and YD4. The wines treated with chips showed the highest concentration, as was to be expected since this compound can be extracted from oak wood.

Hydroxycinnamic acids and their tartaric esters

In general, the highest concentration of *trans*-caffeic, *trans-p*-coumaric and *cis-p*-

coumaric acids was found in the wines treated with chips, followed by the wines with lees. In addition, the wines treated with chips and lees had a lower concentration of tartaric esters in their hydroxycinnamic acids (*trans*-caftaric, *trans*-coutaric and *cis*-coutaric acids). The increase in the concentration of hydroxycinnamic acid free forms in the wines treated with lees may be due to enzymatic hydrolysis of the respective tartaric esters, as has been postulated by some authors [38, 39]. Furthermore, these authors also reported that hydrolysis of cinnamoyl-glucoside anthocyanins could be another source of hydroxycinnamic acids. In wines treated with oak chips, the increase in these compounds could also result from the hydroalcoholysis of oak wood [29]. The findings are in agreement with those of other authors for wines treated with chips [40] or wines treated with lees [41]. However, these results are in contrast with those obtained in model wine solutions where the treatments with chips or lees reduced the content of hydroxycinnamic acids. This could be due to the fact that wine is a complex matrix, and certain other reactions may occur, such as the enzymatic hydrolysis of tartaric esters of hydroxycinnamic acids.

The effect on hydroxycinnamic acids and their tartaric esters by the addition of the yeast derivatives depended on the product used. The wines treated with YD1 and YD2 displayed a lower concentration of hydroxycinnamic acids and a higher concentration of tartaric esters of hydroxycinnamic acids than the control wines. On the other hand, the yeast derivatives YD4, YD5 and YD6 reduced the content of the tartaric ester derivatives, which could be due to the adsorption of these compounds in the yeast products, since the concentration of free hydroxycinnamic acids did not increase. The latter effect was also observed by Guadalupe and Ayestarán (2008) [4], who showed that red wines treated with commercial mannoproteins had higher concentrations of hydroxycinnamic acid free forms and lower concentrations of their esterified forms than

the control wines.

Flavanols

Flavan-3-ol monomers ((+)-catechin and (-)-epicatechin) and dimmers (procyanidins B1 and B2) were also quantified and different results were obtained depending on the treatment.

Total monomers increased in wines treated with lees and with certain yeast derivatives (YD1, YD3 and YD4), which could be explained by the hydrolysis of tannin polymers, since this was not observed in the model wine study. In addition, the wines treated with chips showed the lowest contents of these compounds, as also occurred in the model wine solutions, which seem to corroborate that these compounds can be adsorbed in the oak chip surface.

A clear effect of the treatment carried out on the flavanol dimmer content was not observed. Thus the wines treated with YD1 displayed the highest dimmer contents, whereas the wines treated with lees and YD6 gave the lowest values. Some authors have explained the decrease in the concentration of these compounds by the capacity of yeast to retain or adsorb this type of phenolic compounds [1, 2] Guadalupe and Ayestarán (2008) [4] and Guadalupe et al. (2010) [8] found a statistically significant lower content of total proanthocyanidins in red wines treated with mannoproteins, but not in the concentration of monomeric flavanols.

However, in this study a decrease in dimmer content was only observed in the wines treated with lees and YD6 and not in the remaining wines treated with yeast derivatives, which in general showed similar contents to the control wines.

Flavonols

Following treatment, kaempferol and isorhamnetin were not detected or values below their detection limit were obtained; for this reason they have not been included in Table

6. The wines treated with chips and lees showed the highest content of myricetin and quercetin. These results agree with those obtained by Hernández et al. (2006) [41], who observed that red wines aged on lees revealed a higher concentration of myricetin and quercetin than the control wines. The use of commercial yeast derivatives also maintained a higher concentration of myricetin than for the control wines with the exception of YD-1. The same effects were observed for the glycoside derivatives of flavonols. Therefore, in general, the total content of flavonols was higher in the treated wines. The higher content of free flavonols found in the treated wines did not result from the hydrolysis of flavonol glycosides because these concentrations were also high in the treated wines. Consequently, as these compounds can interact with anthocyanins acting as co-pigments [42-44], these results seem to indicate that the use of yeast derivatives (except YD-1), lees and chips could delay co-pigmentation reactions.

Stilbenes and alcohols

In general, *trans*-resveratrol*, cis* and *trans*-resveratrol-3-glucoside concentrations were lower in wines treated with chips and lees than in the control wines, as was found in the study of *trans*-resveratrol in the model wine solutions. The same results were encountered in tryptophol content. This fact may be due to the adsorption of these compounds in the lees or the oak chips, as was mentioned before. No statistically significant differences were found in the concentration of tyrosol between the different treatments. In general, the addition of the different commercial yeast derivative products did not affect the content of stilbenes and alcohols.

Volatile compounds in red wines

The results obtained for the volatile compounds studied are listed in Table 7.

In the red wines, a binding effect of lees and chips was observed for all the ethyl esters and fusel alcohol acetates studied, with the only exception of ethyl octanoate and ethyl

decanoate, and ethyl hexanoate for the wine with lees. The results obtained for the chips agree with those observed in the model solutions. However, these results contrast with those found by authors in a previous study [24] and with those reported by Pérez-Coello et al. (2000) [45] in a white wine fermented with chips. However, for wines aged on lees, these results are the complete opposite of those found in the model wines. This binding effect of wine lees has also been observed in previous studies [24, 46]. As has been explained in the case of the model wines, this could be due to the fact that yeast macromolecules and other colloids released in wine during autolysis can interact with aroma compounds, thereby affecting their volatility [5, 6, 7, 14] or the synthesis-hydrolysis activity of esterases, which, as observed by Mauricio et al. (1993) [47] are released by lees yeast within the days following alcoholic fermentation. As for the effect of the different commercial yeast derivatives, generally speaking the control wine showed significantly lower concentrations of all the ethyl esters and fusel alcohol acetates studied than the control wine, with the only exceptions of ethyl hexanoate, ethyl cinnamate and 2-phenylethyl acetate. For these three compounds the control wine displayed concentrations lower than or similar to those found in the wines treated with commercial yeast derivative products. These results seem to agree with those encountered in the model wines. Comuzzo et al. (2006) [22] also found higher concentrations of ethyl acetates in the head space of a wine treated with a commercial yeast derivative product than in the control wine; this was in spite of the fact that these compounds were not detected in the head space of yeast derivative powders.

Regarding fusel alcohols, an effect of the different treatments applied was only found for isobutanol, which showed results similar to those found for ethyl esters. That is, the wines macerated with chips and lees had lower concentrations of these compounds than the control wine, whereas the six wines treated with commercial yeast derivates were

richer in this compound than the control wine.

These results contrast with those found by Pérez-Coello et al. (2000) [45] and Rodríguez-Bencomo et al. (2010) [24], who found higher concentrations of fusel alcohols in the wines fermented with chips. Rodríguez-Bencomo et al. (2010) [24] and Bueno et al. (2006) [46] also found higher concentrations of fusel alcohols in wines aged with YD and lees. Masino et al. (2008) [17] did not find an effect of the presence of lees on the concentration of propanol, isobutanol and isoamylalcohol, but aging on lees enhanced the presence of 2-phenyletanol.

As for the rest of the alcohols studied, the wines treated with chips also showed lower concentrations of the three C6 alcohols studied (hexanol, trans-3-hexen-ol and cis-3-hexen-ol), results that confirm those found in a previous study [24], but they did not affect the concentration of benzyl alcohol. Furthermore, the presence of lees did not affect the C6 alcohols, whereas it did have an effect on benzyl alcohol, which showed higher concentrations than the control wine. Masino et al. (2008) [17] also found higher concentration of benzyl alcohol in wines with lees. Only YD3 and YD4 increased the concentration of hexanol and cis-3-hexen-ol with respect to the control wine.

The results regarding fatty acids indicate that the presence of chips in the medium also decreased the concentration of octanoic and decanoic acid, while a binding effect by the lees was found for hexanoic and decanoic acid. These results contrast with those found in the literature [23, 24, 46]. YD2, YD3, YD4 and YD6 increased the levels of hexanoic acid. However, YD4, YD5 and YD6 showed lower concentrations of decanoic acid than the control wine.

None of the YDs studied modified the concentration of linalool since the differences found between them were not significant. Only the wines with chips and lees showed lower concentrations than the control wine. However, an effect of the treatment was

found for citronellol. In this case, all the wines showed lower concentrations of this compound than the control wine. Bueno et al. (2006) [46] found an increase in the concentration of monoterpenes after contact with lees in Airen wines, but a decrease in Macabeo wines.

As for the lactones studied, no statistically significant differences were found for γ-butyrolactone. For γ-nonalactone, only wines treated with YD1, YD3 and YD4 showed lower concentrations of this compound than the control wine. No effect of aging with lees and fermentation with chips was observed.

As regards the volatile phenols, a binding effect of 4-ethylphenol, 4-vinylphenol and 2,6-dimethoxyphenol was found by all the yeast commercial products. Guilloux-Benatier et al. (2001) [48] Chassagne et al. (2005) [16] and Jiménez-Moreno and Ancín-Azpilicueta (2007) [33] also found a binding of volatile phenolic compounds by lees in wines. Wines treated with chips also showed lower concentrations of these three volatile phenols, probably due to their adsorption by wood [31, 32, 49]. The presence in wines of ethylphenol in concentrations higher than their odour threshold contribute to a wine aroma with unpleasant notes, described as “horse sweat”, “spicy”, “leather” and “stable” [50]. Therefore, both yeast derivative products and chips could help to remove these undesirable compounds from wine. Chassagne et al. (2005) [16] concluded that the effect of wine lees on volatile phenol sorption was sensitive to the yeast autolysis level and physicochemical parameters such as ethanol content, temperature and pH. As for vinylguaiacol, this binding effect was only found in the wines with lees and YD1, YD5 and YD6, whereas the wines treated with the other three yeast derivative products showed higher concentrations of this compound. Also quite surprising was the large amount of ethyl vanillate found in all the wines treated with YD and lees. The higher concentration of these two compounds in the wines treated with YD could be connected

with the addition of spices and vegetable extracts in the manufacture of yeast derivative. They could derive from lignin and malt phenolic precursors (ferulic acid) and, for this reason, might also be related to the use of brewers´ yeasts as starting material [22]. The different treatments studied did not affect the concentration of guaiacol, methyl vanillate or acetovanillone. Since the amount of lees and YD added was not very large, it is likely, according to Jimenez-Moreno and Ancín-Azpilicueta (2007) [33], that there is competition between the different volatile compounds for the binding site of the lees or YD. Thus, the compounds with greatest affinity would saturate those binding sites, preventing the binding of other compounds with less affinity.

CONCLUSIONS

The results found in this study have shown that most of phenolic and volatile compounds studied are adsorbed by wood and bound by lees in model wine solutions. This fact had already been demonstrated for volatile compounds, but, as far as the authors know, this is the first study in which this has been observed for phenolic compounds.

The commercial yeast derivative products studied did not interact with the phenolic compounds but did interact with the volatile compounds. This interaction depended on the volatile compound and the commercial yeast derivative product, which could be related with the different composition of these preparations.

In addition, it has been observed that the time that these products remained in the wine is an important factor to be considered, since it influences aroma volatility and phenolic interactions in a different way. Thus, the adsorption of the phenolic compounds occurred in the first 15 days of treatment, remaining constant for two months; in the case, however, of volatile compounds, these initially displayed a retention effect, but after 30-60 days the release of the previously bound compounds was instigated. This is

the first time that these reversible interactions between the volatile compounds and the compounds released by lees derivative products has been described, since so long a contact time had not been studied previously.

The adsorption effect on the phenolic and volatile compounds in the model wine solution was not always the same as in the red wine studied, which highlights the important presence of other wine compounds in these interactions.

More research should be carried out in this area in order to recognise and understand the mechanisms that control these interactions.

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Table 1. Phenolic and aromatic compounds (mg/L) in the initial hydroalcoholic model wine solutions.

Phenolic compounds	mg/L	Aromatic compounds	mg/L
Gallic acid	22	**Isoamyl acetate**	1
Vanillic acid	3.88	**Ethyl hexanoate**	0.05
Ellagic acid	1.92	**Ethyl octanoate**	1
***Trans*-caffeic acid**	9.5	**β-ionone**	0.1
***Trans* p-coumaric acid**	6	**4-ethylphenol**	0.7
***Trans*-resveratrol**	2	**1-hexanol**	1
Tryptophol	3.92	**Eugenol**	0.2
Catechin	20	***cis*-whiskey lactone**	0.2
Quercetin	1.5	***trans*-whiskey lactone**	0.3
		Linalool	0.1

Table 2. Characteristics of the different commercial yeast derivatives (YD), lees and non-toasted chips used and the doses applied.

Experiences	Doses (g/L)	Supplier	Characteristics
Lees	2% v/v	Obtained in the Oenological Station by settling white wine.	From commercial S*accharomyces cerevisiae* yeast.
Chips	4	(Bois Frais, Boise France)	Non-toasted French oak chips.
YD 1	0.4	Agrovin (Spain)	Product with autolysed yeast enriched in polysaccharides.
YD 2	0.4	Agrovin (Spain)	Product with autolysed yeast enriched in polysaccharides and with β-glucanase activity.
YD 3	0.4	Sepsa (Spain)	Product with parietal polysaccharides extracted enzymatically of the selected yeasts walls.
YD 4	0.4	Laffort (France)	Contain a peptide fraction found in the yeast which has sweeter power.
YD 5	0.4	Bio Springer (France)	Constituted exclusively for polysaccharides from the yeast cell wall. It contains 25% of free highly soluble mannoproteíns.
YD 6	0.4	AEB Pascal-Biotech (Spain)	Product with yeast cellular walls rich in mannoproteins and nucleotides. Mannoproteins with a molecular weight medium (150 Kda.).
YD 7	0.4	AEB Pascal-Biotech (Spain)	Product with yeast cellular walls rich in mannoproteins and nucleotides.

Table 3. Concentration (mg/L) of low molecular weight phenolic compounds in model wine solutions. Values with different letter indicate statistically significant differences at $\alpha = 0.05$. The absence of letters means that there are not statistically significant differences.

Compound	**15 days of treatment**									
	C	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**	**YD7**	**Chips**	**Lees**
Gallic acid	22.0b	21.9b	21.9b	21.9b	22.0b	22.1b	22.0b	22.0b	23.1c	18.5a
Ellagic acid	nd	nd	nd	nd	nd	nd	nd	nd	1.76	nd
Vanillic acid	3.83a	3.86a	3.84a	3.83a	3.84a	3.84a	3.83a	3.84a	3.82a	3.90b
Trans-caffeic acid	9.02bc	8.63b	9.26c	9.16c	9.18c	9.19c	9.21c	9.15c	8.70b	6.92a
Trans-p-coumaric acid	5.78c	5.84c	5.83c	5.82c	5.81c	5.80c	5.83c	5.81c	5.41b	4.87a
Catechin	18.1d	17.8c	18.2d	18.1d	18.3d	18.2d	18.2d	18.1d	16.8b	15.4a
Trans-resveratrol	1.71c	1.76c	1.79c	1.75c	1.77c	1.78c	1.77c	1.79c	1.20b	0.50a
Triptophol	3.65bc	3.80c	3.65bc	3.62b	3.65bc	3.64b	3.66bc	3.61b	3.43a	3.39a
Compound	**30 days of treatment**									
	C	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**	**YD7**	**Chips**	**Lees**
Gallic acid	22.0b	21.8b	22.1b	21.9b	22.0b	21.9b	21.9b	22.0b	23.3c	17.5a
Ellagic acid	nd	nd	nd	nd	nd	nd	nd	nd	1.44	nd
Vanillic acid	3.84	3.83	3.86	3.87	3.83	3.82	3.84	3.84	3.81	3.84
Trans-caffeic acid	9.16c	8.59b	9.12c	9.10c	9.07c	9.08c	9.10c	9.09c	8.52b	6.60a
Trans-p-coumaric acid	5.83c	5.82c	5.84c	5.81c	5.74c	5.73c	5.78c	5.81c	5.31b	4.74a
Catechin	18.2c	17.7c	17.9c	17.9c	17.9c	18.1c	18.1c	17.9c	15.8b	14.6a
Trans-resveratrol	1.81d	1.71cd	1.76d	1.75cd	1.61c	1.74cd	1.73cd	1.79d	1.02b	0.49a
Triptophol	3.63b	3.61b	3.61b	3.61b	3.65b	3.62b	3.63b	3.62b	3.21a	3.27a
Compound	**60 days of treatment**									
	C	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**	**YD7**	**Chips**	**Lees**
Gallic acid	21.9b	21.9b	22.0b	21.8b	21.9b	22.0b	21.9b	22.1b	23.1c	19.2a
Ellagic acid	nd	nd	nd	nd	nd	nd	nd	nd	1.15	nd
Vanillic acid	3.81b	3.86c	3.85bc	3.85bc	3.86c	3.84bc	3.82bc	3.84bc	3.75a	3.82b
Trans-caffeic acid	9.07c	9.01c	9.10c	9.06c	9.10c	9.09c	9.07c	9.11c	8.33b	7.23a
Trans-p-coumaric acid	5.77c	5.79c	5.75c	5.78c	5.80c	5.80c	5.74c	5.79c	5.17b	5.00a
Catechin	18.1d	17.7c	17.7cd	17.7cd	17.9cd	17.9cd	17.9cd	17.8cd	14.1a	14.9b
Trans-resveratrol	1.65b	1.70b	1.75b	1.74b	1.74b	1.74b	1.64b	1.74b	0.771a	0.63a
Triptophol	3.61c	3.60c	3.57c	3.59c	3.62c	3.62c	3.61c	3.60c	3.16a	3.28b

. nd: No detected

675 **Table 4.** Compounds released by the different products studied to the model wine.

Volatile compound	Chips	Lees	YD1	YD2	YD3	YD4	YD5	YD6	YD7
Isoamyl acetate	nd	++	nd	nd	nd	nd	nd	nd	nd
Ethyl hexanoate	nd	++	+	+	+	nd	nd	+	+
Ethyl octanoate	nd	++	+	+	+	nd	nd	+	+
1-hexanol	nd	++	nd	nd	nd	nd	++	++	++
β-ionone	nd	nd	nd	nd	nd	nd	nd	nd	nd
Linalool	nd	++	nd	nd	nd	nd	++	nd	nd
Eugenol	++	nd	nd	nd	nd	nd	nd	nd	nd
cis-whiskey lactone	++	nd	nd	nd	nd	nd	nd	nd	nd
trans-whiskey lactone	++	nd	nd	nd	nd	nd	nd	nd	nd
4-ethylphenol	nd	+	+	+	+	+	+	+	+

676 nd: no detected
677 +: detected below their quantification level
678 ++: detected at concentrations higher than the quantification level.

Table. 5. Concentration (mg/L) of volatile compounds in the model wine solutions. Values with different letter indicate statistically significant differences at $\alpha = 0.05$. The absence of letters means that there are not statistically significant differences.

Compound	15 days of treatment									
	C	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**	**YD7**	**Chips**	**Lees**
Isoamyl acetate	0.248bc	0.246bc	0.287d	0.270c	0.250bc	0.243b	0.250bc	0.249bc	0.202a	0.515e
Ethyl hexanoate	0.222bc	0.223bc	0.267d	0.239c	0.221bc	0.214b	0.221bc	0.220b	0.183a	0.363e
Ethyl octanoate	0.513d	0.493d	0.557e	0.396b	0.460c	0.379ab	0.464c	0.456c	0.356a	0.676f
1-hexanol	1.13b	0.97a	0.97a	0.960a	1.01a	1.12b	1.14b	1.13b	1.03a	1.14b
β-ionone	0.065bc	0.075d	0.093e	0.075d	0.070cd	0.064bc	0.061b	0.063b	0.062a	0.053a
Linalool	0.065a	0.075de	0.094g	0.086ef	0.075cde	0.072bcd	0.068ab	0.072bcd	0.069abc	0.080ef
Eugenol	0.137a	0.167bc	0.218e	0.201d	0.165bc	0.149ab	0.138a	0.151ab	0.144a	0.171c
Cis-whiskey lactone	0.088a	0.103bc	0.136e	0.122d	0.100bc	0.094ab	0.086a	0.092ab	0.106c	0.108cd
Ttrans-whiskey lactone	0.125ab	0.141cd	0.185f	0.165e	0.138bcd	0.129abc	0.120a	0.128abc	0.148de	0.149de
4-ethylphenol	0.703cd	0.599a	0.663b	0.689cd	0.693cd	0.699cd	0.714d	0.693cd	0.684bc	0.664b
Compound	**30 days of treatment**									
	C	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**	**YD7**	**Chips**	**Lees**
Isoamyl acetate	0.241a	0.217a	0.195a	0.232a	0.244a	0.228a	0.239a	0.218a	0.204a	0.477b
Ethyl hexanoate	0.230b	0.203ab	0.198a	0.203ab	0.237b	0.210ab	0.237ab	0.200ab	0.195a	0.364c
Ethyl octanoate	0.533g	0.480e	0.430d	0.292a	0.489ef	0.394c	0.518fg	0.538g	0.329b	0.665h
1-hexanol	1.10cd	0.980b	0.980b	1.00ab	0.860a	1.01bc	1.00b	1.03bc	1.06bc	1.18d
β-ionone	0.071c	0.069bc	0.067bc	0.057ab	0.063ab	0.058a	0.060a	0.060a	0.067bc	0.058a
Linalool	0.071ab	0.080cd	0.074bcd	0.078cd	0.097e	0.068a	0.077bcd	0.072abc	0.076abcd	0.082d
Eugenol	0.168bc	0.187cd	0.169bc	0.176cd	0.161ab	0.146a	0.163ab	0.162ab	0.169bc	0.198d
Cis-whiskey lactone	0.104abc	0.112cd	0.103abc	0.106bcd	0.142f	0.099abc	0.099ab	0.096a	0.131ef	0.123de
Ttrans-whiskey lactone	0.145ab	0.152bcd	0.142ab	0.145bc	0.188e	0.137ab	0.137ab	0.132a	0.166cd	0.168d
4-ethylphenol	0.694f	0.496b	0.607de	0.542bc	0.155a	0.587cd	0.607de	0.648def	0.618de	0.681fe
Compound	**60 days of treatment**									
	C	**YD1**	**YD2**	**YD3**	**YD4**	**YD5**	**YD6**	**YD7**	**Chips**	**Lees**
Isoamyl acetate	0.240ab	0.319e	0.286d	0.308e	0.260bc	0.285cd	0.255bc	0.263c	0.222a	0.565f
Ethyl hexanoate	0.214ab	0.280e	0.261d	0.266de	0.232bc	0.248c	0.232bc	0.234c	0.199a	0.378f
Ethyl octanoate	0.532c	0.467c	0.546c	0.232a	0.502c	0.425bc	0.518c	0.527c	0.318ab	0.529c
1-hexanol	0.969abc	0.965ab	1.01bc	0.949a	0.977abc	1.01abc	1.00abc	1.02cd	0.961a	1.03d
β-ionone	0.066bc	0.088de	0.089e	0.073cd	0.066b	0.066b	0.060ab	0.052a	0.066b	0.068bc
Linalool	0.070a	0.105ef	0.101def	0.104f	0.084bc	0.094d	0.084bc	0.086c	0.078ab	0.100de
Eugenol	0.165a	0.231f	0.231f	0.242g	0.195cd	0.207de	0.187bc	0.180abc	0.178ab	0.225ef
Cis-whiskey lactone	0.098a	0.134c	0.138c	0.136c	0.109ab	0.115b	0.102a	0.105ab	0.130c	0.133c

Table 6. Concentration (mg/L) of low molecular weight phenolic compounds in red wines after bottling.

Compound	C	YD1	YD2	YD3	YD4	YD5	YD6	Chips	Lees
Hydroxybenzoic acids and derivatives									
Gallic acid	21.5ab	21.7abc	23.4e	22.1bc	21.6ab	22.3cd	21.2a	23.4e	23.1de
Protocatechuic acid	5.42bc	6.30de	6.46de	5.56c	5.23bc	6.14d	5.19b	4.48a	6.76e
Vanillic acid	4.08bc	3.72ab	4.38c	4.01bc	4.03bc	4.22bc	3.24a	3.97bc	4.46c
Syringic acid ns	2.97	2.21	3.44	2.99	2.71	2.66	2.04	2.46	2.62
Ethyl gallate	3.26bc	3.25bc	3.39bc	3.28bc	3.19b	3.22b	2.85a	3.56c	3.29bc
Ellagic acid	2.88a	2.79a	4.69cd	3.95bc	3.42ab	4.09cd	4.00bcd	21.87e	4.75d
Total	40.1ab	40.0ab	45.7d	41.9bc	40.2ab	42.7ab	38.6a	59.7e	45.0cd
Hydroxycinnamic acids (HA) and derivatives									
Trans-caffeic acid	5.61de	3.91a	4.40ab	4.70bc	6.15e	5.18cd	4.68bc	11.79g	7.89f
Trans-p-coumaric acid	2.11bc	1.43a	1.78ab	1.85ab	2.80d	2.26c	1.82ab	7.11f	3.66e
Cis-p-coumaric acid	0.560a	0.571a	0.638ab	0.683b	0.830c	0.684b	0.562a	1.86d	0.797c
Total HA	8.28b	5.91a	6.82ab	7.24ab	9.75c	7.87b	7.07ab	20.8e	12.3d
Trans-caftaric acid	30.01d	31.96e	31.93e	30.97de	26.95bc	25.89b	27.64c	16.55a	25.62b
Cis-coutaric acid	3.54cde	3.48cd	3.75e	3.63de	3.38c	3.46c	3.44c	2.65a	3.12b
Trans-coutaric acid	19.1d	20.1ef	20.7f	19.8de	18.1c	19.4de	19.4de	13.4a	16.0b
Trans-fertaric acid	1.14d	1.19d	1.16d	1.16d	1.05c	1.01c	1.02c	0.730a	0.879b
Total HA tartaric esters	53.8e	56.7fg	57.5g	55.6ef	49.5c	49.7c	51.5d	32.7a	45.7b
Flavanols									
Monomers	48.7b	54.0c	52.8bc	53.6c	60.3d	52.3bc	51.3bc	38.3a	54.4c
Dimmers	19.3bc	23.5d	18.6b	18.3b	22.1c	17.8b	15.2a	22.2c	15.0a
Stilbenes									
Trans-resveratrol-3-glucoside	2.03b	1.95b	2.15cd	2.25e	2.16c	2.23de	2.26e	1.79a	1.83a
Cis-resveratrol-3-glucoside	1.39bc	1.35b	1.41bcd	1.42bcd	1.46cd	1.47d	1.45cd	0.16a	0.143a
Trans-resveratrol	2.31bcd	1.96ab	3.20e	3.39e	2.45cd	3.20e	2.64d	1.74a	2.09abc
Total	5.73cd	5.25bc	6.75cd	7.05d	6.08cd	6.10cd	6.35cd	3.69a	4.07ab
Flavonols									
Myricetin	5.35a	6.47ab	11.4c	10.9c	7.6b	10.4c	10.6c	14.2d	14.6d
Quercetin	0.024ab	nd	0.040b	0.027ab	0.032b	0.035b	0.033b	0.299d	0.064c
∑ Myricetin-3-glycosides	3.66ab	3.64ab	4.69e	4.24d	3.50a	3.84bc	3.71ab	4.10cd	3.75abc
∑ Quercetin-3-glycosides	0.261a	0.279ab	0.325d	0.295c	0.286bc	0.319d	0.298c	0.351e	0.298c
Syringetin-3-glucoside	1.17ab	1.10a	1.30c	1.28c	1.21b	1.24b	1.17ab	1.24bc	1.23bc
Total	10.8a	11.5a	17.9bc	17.1b	12.7a	15.9b	15.6b	20.5c	20.0c
Alcohols									
Tyrosol ns	20.4	21.5	20.9	21.2	21.5	20.9	20.6	21.6	19.9
Tryptophol	5.44b	6.04b	4.56b	5.70b	6.46b	6.29b	4.53b	1.63a	1.22a
Total	25.8cd	27.6d	25.5bcd	26.9d	28.0d	27.1d	22.9ab	23.2abc	21.2a

Values with different letter in the same row indicate statistically significant differences (α= 0.05).
ns: no statistically significant differences.
nd: no detected.

Table 7. Concentration (μg/L) of volatile compounds in red wines after bottling.

Compound		C	YD1	YD2	YD3	YD4	YD5	YD6	Chips	Lees
Ethyl esters										
Ethyl butyrate		893cd	868c	933d	1027ef	985e	1029ef	1030f	590b	523a
Ethyl 2-methylbutyrate		2.37b	2.83bc	3.28cd	2.52d	3.33d	3.91e	3.45d	1.90a	1.83a
Ethyl isovalerate		3.91bc	4.44cd	5.49def	5.71ef	5.23de	6.19f	5.58ef	2.79a	3.00ab
Ethyl hexanoate		276bc	280bcd	329cd	334cd	294bcd	360d	301bcd	189a	233ab
Ethyl octanoate		487ab	497abc	517bcd	535cd	537d	495ab	546d	497a	456b
Ethyl decanoate		103ab	131c	167d	126c	120bc	89.1a	126c	120bc	104abc
Ethyl cinnamate		1.89d	1.77b	1.83c	1.72a	1.73ab	1.74ab	1.72a	1.84c	1.87cd
Fusel alcohol acetates										
2-phenylethyl acetate		113c	95.0a	95.9a	99.2ab	96.9a	105b	100ab	95.1a	95.6a
Isoamyl acetate		1456b	1374b	1506b	1755cd	1574bc	1802d	1595bc	984a	782a
Fusel alcohols[a]										
Isobutanol		69.6bc	71.2cd	79.6d	79.6d	79.5d	79.1d	78.8d	56.0a	60.4ab
Isoamyl alcohols	ns	48.8	49.9	50.7	48.0	43.7	49.3	50.9	40.4	46.2
Phenylethanol	ns	90.7	87.1	88.2	91.6	87.0	90.4	86.8	87.8	84.9
Alcohols										
Hexanol		993b	1021bc	1026bc	1058c	1051c	1053c	1034bc	836a	994b
trans-3-hexen-1-ol		43.3bc	51.7bc	50.4bc	53.6c	49.0bc	44.4b	52.0c	32.8a	34.9ab
cis-3-hexen-1-ol		176b	185bc	183bc	191c	191c	185bc	183bc	153a	189bc
Benzyl alcohol		272bc	240a	269abc	249ab	276c	253abc	246ab	252abc	314d
Fatty acids										
Hexanoic acid		1884bc	1991cde	2035de	2090e	2062e	1945cd	2032de	1837b	1683a
Octanoic acid		1990c	1951c	1957c	2005c	1948c	1835b	1961c	1756a	1706ab
Decanoic acid		545de	506bcd	623e	515cd	470abc	449ab	459abc	415a	470abcd
Terpenes										
Linalool		2.95cd	2.83cde	2.98cd	2.70bc	2.74bcd	3.05d	2.85cde	2.44a	2.53ab
Citronellol		7.68d	4.68ab	4.63ab	4.90ab	4.84ab	5.97c	5.37bc	4.08a	5.15abc
Volatile phenols										
Guaiacol	ns	9.32	9.19	9.23	9.38	9.35	9.35	9.28	9.60	9.46
4-vinylguaiacol		25.9c	7.48a	143d	144d	145d	9.35a	10.1a	18.7b	21.7b
4-ethylphenol		4.45b	4.14a	4.24a	4.15a	4.12a	4.14a	4.22a	4.17a	4.49b
4-vinylphenol		25.0bc	12.4a	13.7a	18.0ab	24.3bc	13.5a	17.3ab	50.7c	30.3d
2,6-dimethoxyphenol		153c	7.19ab	7.22ab	7.33ab	7.77ab	8.32b	7.26ab	6.35a	6.98ab
Ethyl vanillate		87,7a	165ef	180f	142d	130bc	138d	116b	97.2a	146de
Methyl vanillate	ns	5.93	6.32	6.71	5.62	6.26	5.35	5.87	5.66	6.20
Acetovanillone	ns	46.6	43.4	45.0	44.3	46.7	42.7	45.1	45.1	48.6
Total										
Lactones										
γ-butyrolactone[a]	ns	10.8	13.8	12.4	12.8	12.2	12.6	12.5	12.5	11.5
γ-nonalactone		20.0de	18.0a	19.3cde	18.1a	18.5ab	19.5cd	18.7abc	19.0abcd	21.0e
Total										

[a] Concentration in mg/L

Values with different letter in the same row indicate statistically significant differences (α= 0.05).

ns: no statistically significant differences.

CAPÍTULO 3

Efecto de la crianza sobre lías y de productos derivados de levaduras secas sobre la composición y las características sensoriales de un vino blanco Verdejo

La crianza de vinos blancos sobre lías se viene utilizando desde hace varias décadas debido a las mejoras que puede producir en sus características sensoriales, asociadas fundamentalmente a los polisacáridos y especialmente a las manoproteínas que se liberan durante el proceso de autolisis de las levaduras. Sin embargo, este proceso es lento, y por este motivo en los últimos años los productos obtenidos a partir de las levaduras, ricos en manoproteínas, están siendo utilizados como una alternativa a la crianza tradicional sobre lías.

Sin embargo, son escasos los trabajos de investigación llevados a cabo sobre el efecto que tienen estos productos sobre la calidad de los vinos blancos. Por todo ello, en este capítulo se ha estudiado el efecto de la crianza sobre lías y de diferentes derivados de levadura comerciales sobre la composición fenólica, el color, las proteínas, los polisacáridos y las características sensoriales de un vino blanco de la variedad Verdejo. Se han estudiado 3 productos comerciales con diferente composición y diferente grado de purificación. También se ha estudiado el efecto de estos tratamientos durante 6 meses de envejecimiento en botella de los vinos.

Los resultados y conclusiones más destacados de este trabajo son los siguientes:

1- Los diferentes tratamientos estudiados no modificaron ninguno de los parámetros enológicos estudiados.

2- Se ha observado que tanto las lías como los diferentes productos comerciales derivados de levadura estudiados, principalmente los derivados YD 1 y YD 2, pueden interaccionar o adsorber algunos de los compuestos fenólicos presentes en los vinos, como polifenoles, flavonoles y taninos, reduciendo su concentración. Este efecto aumentó con el tiempo de permanencia en botella.

3- Los ácidos hidroxicinámicos y el tirosol fueron los principales compuestos fenólicos de bajo peso molecular presentes en los vinos estudiados. En general, los vinos envejecidos con lías y con los derivados de levadura YD 1 y YD 2 mostraron concentraciones más altas de ácidos hidroxicinámicos libres y esterificados que los vinos control. Sin embargo, no se encontró un efecto "tratamiento" claro en los flavanoles.

4- La adsorción de los compuestos mencionados anteriormente por las lías y los derivados de levadura dieron lugar a una reducción de la intensidad colorante de los vinos, tras el tratamiento. Sin embargo, tras el período de envejecimiento en botella, solamente los vinos tratados con el derivado de levadura YD 1 mostraron menor intensidad de color que los vinos control.

Este hecho indica que tanto las lías como los derivados de levadura pueden ayudar a evitar o reducir el pardeamiento de los vinos blancos, que se produce principalmente por los procesos de oxidación química y enzimática de los compuestos fenólicos.

5- El contenido en monosacáridos y polisacáridos analizados mediante HPSEC-GC dependió del producto comercial estudiado. De este modo, se observó que el derivado YD 1 era un producto rico en glucanos (69,5%). Sin embargo, el derivado YD 2 y especialmente el YD 3 fueron productos ricos en manoproteínas (59,8% y 77,1%, respectivamente), lo que puede indicar un mayor grado de purificación. Se encontraron otros monosacáridos en estos productos como son la galactosa y la arabinosa, los cuales son constituyentes de los arabinogalactano-proteínas (PRAGs), polisacáridos que proceden de las uvas. Estos polisacáridos se encontraron en una concentración muy baja (0,6-3%) y su presencia en estos derivados de levadura comerciales podría estar relacionada con su proceso de elaboración.

6- Los derivados YD 1 y YD 2 aportaron polisacáridos neutros a los vinos, mientras que los vinos envejecidos sobre lías presentaron contenidos de polisacáridos neutros similares a los vinos control. Esto puede ser debido a que el tiempo de tratamiento de la crianza tradicional sobre lías fue muy corto para que se llevara a cabo la autolisis de las levaduras. Además, hay que tener en cuenta que el derivado YD 3 tampoco mostró una mayor concentración de polisacáridos neutros que los vinos control. Este hecho pudo ser debido a que, siguiendo las recomendaciones del proveedor, la dosis máxima adicionada fue 8 veces menor a la de los otros 2 derivados de levadura, y a que se adicionó justo antes del embotellado.

Los resultados obtenidos en el análisis de polisacáridos totales, neutros y ácidos en los vinos mediante los métodos HPSEC-GC y espectrofotométrico fueron similares. Por lo tanto, el método espectrofotométrico puede ser propuesto como un método de análisis rápido para determinar la concentración de estos compuestos en el vino.

7- En el análisis sensorial de los vinos, tras el tratamiento únicamente se encontraron diferencias estadísticamente significativas en los aromas frutales. En general, los catadores dieron valores más bajos de estos aromas a los vinos tratados que a los vinos control. Sin embargo, tras 6 meses en botella, los vinos tratados presentaron una mayor intensidad olfativa, con más aromas frutales y varietales que los vinos control. Por otro lado, los catadores observaron que tanto los vinos tratados con lías finas como aquellos tratados con los diferentes derivados de levadura mostraron valores significativamente más bajos de amargor y más altos de volumen en boca, persistencia, equilibrio y valoración global que los vinos control. Por tanto, desde el punto de vista sensorial, tanto los vinos envejecidos sobre lías como los tratados con los derivados de levadura comerciales evolucionaron mejor que los vinos control, mejorando su calidad.

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Effect of the aging on lees and of three different dry yeast derivative products on *Verdejo* white wine composition and sensorial characteristics

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Abstract

A study was made of the effect of aging on lees, and of three different commercial yeast derivative products of different composition and degree of purification on the phenolic compounds, color, proteins, polysaccharides and sensorial characteristics of white wines. The results obtained showed that the lees and yeast derivative products can interact or adsorb some of the phenolic compounds present in wines reducing their concentration. This reduction depends on the treatment applied, the phenolic compound analyzed and on the stage of the vinification or aging process. The use of lees and yeast derivative products can reduce the color intensity and the browning of the wines immediately following treatment. The monosaccharide and polysaccharide content of yeast derivative products depends on the manufacturing process and degree of purification of the product, both of which have an influence on wine treatments.

After six months in the bottle, both the aging on lees and the treatment with commercial yeast derivative products gave rise to wines with better sensorial characteristics than in the case of the control wines.

Keywords: White wine, aging on lees, yeast derivative products, phenolic compounds, polysaccharides.

INTRODUCTION

The aging of white wines on lees has been a well-known vinification technique for several years. During this process yeast autolysis occurs and, as a result, different compounds are released into wines, improving their sensory quality (*1, 2*). Mannoproteins have been described as the most important polysaccharides released during this autolysis process due to their positive effects on the final quality of wines (*2-4*). They are liberated during alcoholic fermentation (*5-7*) and during the aging of wines on lees (*3, 8*).

Mannoproteins are glycoproteins located in the yeast cell walls, and they play an important role in the whole of the vinification process (*9*). They can have, then, an influence on technological characteristics such as the inhibition of tartrate salt crystallization (*10, 11*) and the reduction of protein haze (*12-14*) of white wines, improving their tartaric and protein stability. Furthermore, these compounds can improve the sensorial characteristics of wines, since they affect aroma volatility (*15-17*), reduce astringency and bitterness, and enhance the body, structure and roundness of red wines (*9, 18, 19*) and of model wine solutions (*20-22*). Some authors have also reported the influence of yeast in the browning delay of white wines since yeast can adsorb certain phenolic compounds, preventing oxidation and, therefore, the formation of browning compounds (*23, 24*).

Mannoproteins can have other positive effects on wines, such as the adsorption of some mycotoxins (ochratoxin A) (*25*), or the improvement of foaming characteristics in sparkling wines (*26*). Finally, they are involved in velum formation in the sherry wines (*27*).

However, yeast autolysis is usually a very slow process due to the conditions of pH and temperature at which this process occurs in wines (*28*). For this reason, "batonnage" is a very common technique for allowing a faster release of the yeast compounds.

On the other hand, in recent years, commercial yeast derivative preparations are being used as an alternative technique to aging wines on lees, because they permit a quicker release into the wine of yeast compounds (mainly mannoproteins and glucans). The first preparations that appeared on the market were products composed mainly of inactive yeasts, yeast autolysates and yeast cell walls (*17*). These products have a very heterogeneous composition, and in most cases have a low solubility in wines. Currently, more hydrolyzed and purified products (such as purified mannoproteins) are being offered by commercial suppliers as completely soluble products and with immediate effect on wines.

The addition of yeast mannoproteins for tartaric and protein stability was authorized by the European Community in 2005 (EC Regulation Nº 2165/2005), and the use of yeast cell wall preparations (EC Regulation Nº 606/2009) is also authorized to a limit of 40 g/HL in the different winemaking stages to give wines the positive characteristics mentioned above.

No studies have been found relating to the effect of different commercial yeast derivative products on the quality of white wines. For this reason, the aim of this study was to examine the effect of aging on lees and of different commercial yeast derivative products on the phenolic compounds, color, proteins, polysaccharides and sensorial characteristics of *Verdejo* white wines. Three commercial yeast derivatives of different compositions and degrees of purification were used. The effect of these treatments on white wines during aging in the bottle for six months was also studied.

MATERIAL AND METHODS

Winemaking process and treatments

The study was carried out using the *Verdejo* grape variety from Rueda Designation of Origin, sited in the Autonomous Community of "Castilla y León" in the North of Spain, from the 2008 vintage. The white wines were elaborated in the research winery of the Enological Station of "Castilla y León", following the traditional white winemaking process.

The grapes were harvested manually in accordance with ºBrix and total acidity values (23 ºBrix and 6.7 g/L of tartaric acid), and transported to the Enological Station in 15-Kg plastic boxes.

The clusters of white grapes were de-stemmed, crushed, slightly sulphited (0.05 g/L) and pressed. The must obtained was transferred to stainless steel tanks and a pectinolytic enzyme preparation was added (2 g/HL of Vinozym FCE, Novozymes) to favor the precipitation of colloidal substances over 24 hours at 12 ºC. After this period of time, the must was racked off into different stainless steel tanks and inoculated with commercial *Saccharomyces cerevisiae* yeasts (20 g/HL of IOC 18-2007 from Institut Oenologique de Champagne) to undergo alcoholic fermentation at a controlled temperature (16 ºC ± 2ºC).

Once alcoholic fermentation was completed, the wines were kept in the tanks for 4 days to allow sedimentation of the gross lees. Following this, the wines were racked off and kept in the tanks for 4-5 days to allow sedimentation of the fine lees. The base wine was again racked off, homogenized and distributed into different 150 L tanks in which the different treatments were carried out in duplicate. The wet fine lees decanted in the bottom of the tanks were used in the experiments with lees (L) (3% v/v of fine lees).

Three different commercial yeast derivative products (YD), provided by the same commercial manufacturer (Sepsa-Enartis, Spain), were used for this study. They were

selected because, according to the information provided by the commercial manufacturer, these products are rich in glucans and mannoproteins but of a different composition and/or obtained by a different extraction process (Table 1).

Wines without any additional product were used as the control wines (C).

Two "batonnages" per week were performed on each wine. The temperature was maintained at 15 ºC ± 1ºC. All treatments lasted 60 days except those that used YD 3 preparation which was added in the bottling process as it was recommended by its manufacturer as a result of its high degree of purity and solubility.

After the different treatments, the white wines were clarified with bentonite (100 g/HL), filtered through 0.8 μm membrane plates and bottled.

The samples were analyzed immediately following fermentation, at the end of the treatment, and, finally, after three and six months of aging in the bottle.

Chemical reagents

Gallic acid, D-(+)-catechin, glucose, Coomassie reactive, syringic acid, D-(+)-galacturonic acid, 3-hydroxy-biphenyl, phenol and β-D-allose were provided by Sigma-Aldrich (Steinheim, Germany); *trans*-caffeic acid, kaempferol, tyrosol, tryptophol and acetic anhydride by Fluka (Buchs, Switzerland); bovine serum albumine, di-sodium tetraborate decahydrated, trifluoroacetic acid, sodium borohydrure, ethyl acetate, perchloric acid, ammonia, acetone, acetic acid, chloroform and 1-methyl-imidazol by Merck (Darmstadt, Germany); and ethylgallate, quercetin, (-)-epicatechin and cyanidin chloride by Extrasynthèse (Lyon, France). Acetonitrile and methanol were provided by Lab Scan (Madrid, Spain). The remaining reagents were provided by Panreac (Madrid, Spain). Water Milli-Q was obtained through a Millipore system (Bedford, MA).

Analytical methods

Enological parameters were evaluated according to official analysis methods (OIV, 1990).

The content of phenolic compounds was evaluated by the quantification of several phenolic families: total polyphenols, expressed in mg/L of gallic acid (*29*); total tannins, expressed in mg/L of cyanidin chloride (*30*); hydroxycinnamic acid derivatives and flavonols, expressed in mg/L of caffeic acid and quercetin, respectively (*31*).

Low molecular weight phenolic compounds were also analyzed by direct injection of the samples in an Agilent Technologies LC-DAD series 1100 chromatograph, following the chromatographic conditions described by Pérez-Magariño et al. (*32*). The samples were previously diluted in water (1:1) and filtrated through PVDF filters of 0.45 μm (Millipore, Bedford, MA).

Color intensity was evaluated by absorbance measurement at 420 nm (*33*).

Proteins were determined by means of the method described by Bradford (*34*) and the results were expressed in mg/L of bovine serum albumine (BSA).

Global polysaccharide content was evaluated by spectrophotometry following the method described by Segarra et al. (*35*), and was expressed in mg/L of galacturonic acid and glucose for acid and total polysaccharides, respectively. Neutral polysaccharides were calculated as the difference between total and acid polysaccharides.

All spectrophotometric measurements were carried out by means of a UV-vis spectrophotometer (Shimadzu serie UV-1700 pharmaspec, China).

Polysaccharide families were also analyzed in white wines by high-performance size-exclusion chromatography (HPSEC). First, 5 mL of each wine was concentrated in a rotatory vacuum evaporator and re-dissolved in 2 mL of water. HPSEC was carried out by loading the previous 2 mL concentrated fraction on a Superdex 30-HR column (60 x 1.6 cm, Pharmacia, Sweden) with a precolumn (0.6 x 4 cm), equilibrated at 0.6 mL/min

159 in 30 mM ammonium formiate pH 5.6. Chromatographic separation was performed
160 with a refractive index detector (Erma-ERC 7512, Erma, Japan) coupled to a Waters
161 Baseline 810-software following the conditions described by Ducasse et al. (*36*). Two
162 different fractions, containing three different polysaccharide families, were collected
163 according to their elution times. The first fraction contained mannoproteins and
164 polysaccharides rich in arabinose and galactose (PRAGs) (42-53 min.), and the second
165 fraction contained mainly the rhamnogalacturonans II (RG II) (54-61 min.) but also
166 mannoproteins and PRAGs of low molecular weight. These fractions were freeze-dried
167 and re-dissolved in water. This process was repeated four times for complete removal of
168 the ammonium salts. The quantification of polysaccharide families was carried out by
169 quantifying neutral monosaccharide composition by means of gas chromatography (GC-
170 FID) following their release of wine polysaccharides by hydrolysis and conversion in
171 alditol acetates after reduction and acetylation, in accordance with the process described
172 by Ducasse et al. (*37*). Allose was used as the internal standard. The content of each
173 polysaccharide family was estimated from the concentration of individual glycosyl
174 residues characteristic of well-defined wine polysaccharides (*38*).
175 Estimation of polysaccharide families of commercial yeast derivative products was
176 directly made by quantifying their neutral monosaccharides as alditol acetates by gas
177 chromatography, in accordance with the quantity (mg) of each product used.
178 **Sensory analysis**
179 Sensory analysis was carried out by a tasting panel comprising twelve persons, all
180 expert tasters from the Regulatory Councils of various Spanish Designation of Origin
181 and wineries. These tasters defined the descriptors used in this sensory analysis,
182 according to the methodology described by González-Sanjosé et al. (*39*), and were

183 trained to quantify them using structured numerical scales. This training was carried out
184 in accordance with UNE-87-020-93 Norm (ISO 4121:1987).
185 A structured numerical scale of seven points was used, with 1 representing an absence
186 of sensation and 7 a very intense perception.
187 The wines were tasted after the treatments and after six months in the bottle.

188 **Statistical analyses**

189 All the data were examined by the application of variance analysis (ANOVA) and the
190 Least Significant Difference (LSD) test, which determines statistically significant
191 differences between the means. A 95% confidence interval or significant level of p =
192 0.05 was used.
193 All the statistical analyses were carried out using the Statgraphics Plus 5.0 statistical
194 package (Statpoint Technologies, INC., Warrenton, Virginia).

195 **RESULTS AND DISCUSSION**

196 **Enological parameters**

197 Classic enological parameters were analyzed in white wines to study the effect of the
198 different techniques assayed on these compounds. The data ranges of these parameters
199 were: pH between 3.1-3.2, total acidity between 5.8-6.2 g/L of tartaric acid, alcoholic
200 degree between 12.8-13.4, volatile acidity average of 0.2 mg/L of acetic acid and
201 potassium between 590-660 mg/L. No statistically significant differences were found
202 between treated and control wines, which indicates that these treatments did not modify
203 the enological characteristics of the white wines.

204 **Analyses of different phenolic groups and color**

205 Table 2 shows total polyphenol, hydroxycinnamic acid derivatives, flavonol and tannin
206 concentration in white wines. Statistically significant differences were found only in
207 some cases. No statistically significant differences were found between the treated

208 wines and the control wines in total polyphenol content following treatment. However,
209 after six months in the bottle, the wines treated with the yeast derivative products
210 showed a lower content than the control wines. The wines treated with YD 3 displayed
211 the lowest values, followed by YD 2 and YD 1, while the content in the wines treated
212 with lees was similar to that of the control wines.
213 Immediately subsequent to treatment, no statistically significant differences were found
214 in the content of hydroxycinnamic acid derivatives, and, regarding flavonols, the wines
215 treated with YD 1 and YD 2 displayed a lower concentration than in the control wines.
216 After six months of aging, no statistically significant differences were seen in the
217 content of hydroxycinnamic acid derivatives. However, all the treated wines presented
218 lower flavonol content than the control wines, with the exception of those treated with
219 YD 3, which maintained a similar content to that of the control wines. The wines treated
220 with YD 1 showed the lowest values.
221 After treatment, no statistically significant differences were found between the treated
222 wines in total tannin concentration. However, at the end of bottle aging, all the treated
223 wines showed a lower concentration than that of the control wines.
224 These results seem to indicate that lees and certain yeast derivative products can reduce
225 the content of some phenolic compounds, such as total polyphenols, flavonols and
226 tannins. This fact has been pointed out by several studies carried out on model wine
227 solutions (*40-42*), in white wines (*23, 24*) and in red wines (*18*), and it may be due to
228 the capacity of yeast or yeast compounds, such as mannoproteins and/or glucans, to
229 adsorb or interact with different wine phenolic compounds (*20, 22*). The results
230 encountered also suggest that this interaction does not occur immediately after the
231 treatment but over time (Table 2).

232 The different treatments produced some changes in the color of the white wines (Table
233 2). The differences found between the wines just after alcoholic fermentation (EAF) and
234 0MB were due to the fact that following treatment the wines were clarified with
235 bentonite and filtered, which can reduce the color by adsorption of colored compounds.
236 Subsequent to treatment, all the treated wines displayed a lower color intensity than the
237 control wines, with the wines treated with lees and YD 1 presenting the lowest color
238 intensity values. These results are in agreement with those found by some authors (*23,*
239 *24*), who proposed the use of yeast cell walls as fining agents for the correction of
240 browning in white wines. However, after six months of aging, this effect was only
241 observed in wines treated with YD 1.

242 **Analysis of low molecular weight phenolic compounds**

243 Table 3 shows the low molecular weight phenolic compounds identified and quantified
244 in white wines. Hydroxycinnamic acids represented 28.2% of total low molecular
245 weight phenolic compounds after treatment and 38.5% after six months in the bottle,
246 whereas tyrosol represented 43.1% following treatment and 47.7% after six months in
247 the bottle (average values). These compounds were the main phenolic groups in *Verdejo*
248 wines, as was also observed in other varietal white wines (*43, 44*).

249 In general, gallic and protocatechuic acid concentration increased after the treatments.
250 The wines treated with YD 1 and YD 2 presented a higher concentration of both acids
251 than the control wines after treatment. During bottle aging, the concentration of both
252 acids decreased in all the wines, and after six months all the treated wines showed
253 higher concentrations than those of the control wines, the wines treated with YD 1 being
254 the ones with the highest values.

255 No statistically significant differences were found in the concentration of siringic acid
256 after treatment. The concentration of this compound decreased during bottle aging, with

257 a greater loss in wines treated with YD 1 after six months in the bottle. Similar results
258 were obtained for ethylgallate, where the wines treated with YD 1 presented lower
259 concentration than the control wines following treatment and after three and six months
260 of aging.
261 The hydroxycinnamic acids evaluated, *trans*-caffeic and *trans-p*-coumaric acids,
262 increased in all the wines after treatment. However, during bottle aging, *trans-p*-
263 coumaric acid continued to increase whereas *trans*-caffeic acid remained relatively
264 constant. After six months in the bottle, *trans*-caffeic acid concentration was higher in
265 all the treated wines than in the control wines, with the exception of the wines treated
266 with YD 3. The wines treated with YD 1 showed the highest concentration (78% higher
267 than the control wines). As for *trans-p*-coumaric acid, the wines treated with lees and
268 YD 1 displayed a higher concentration than the control wines, whereas the wines treated
269 with YD 3 presented lower contents than the control wines.
270 *Trans*-caftaric acid was the most abundant tartaric ester quantified, contributing to 80%
271 of the total tartaric esters evaluated. This concentration increased slightly in wines after
272 treatment, except in the wines treated with YD 3, which showed the same concentration
273 as that of the controls. *Trans*-caftaric acid concentration remained stable in all the wines
274 during bottle aging, and the differences between treatments stayed the same. The wines
275 treated with lees, YD 1 and YD 2 showed a higher concentration of *trans*-caftaric acid
276 than the control wines, with the wines treated with lees presenting the highest
277 concentrations.
278 *Cis* and *trans*-coutaric acid concentration decreased in all the wines after treatment. At
279 this particular stage, only the wines treated with YD 1 and YD 3 showed a lower
280 concentration of *cis*-coutaric acid than the control wines, and those treated with YD 1
281 also displayed a lower concentration of *trans*-coutaric acid than the control wines.

282 During bottle aging, these compounds showed different trends. Whereas *cis*-coutaric
283 acid continued decreasing, *trans*-coutaric acid increased in all wines up to three months,
284 remaining constant during the last three months. The wines treated with YD 1 also had
285 the lowest concentrations of both acids after six months in the bottle.
286 *Trans*-fertaric acid increased in all wines after treatment, and all the treated wines
287 showed a lower concentration than the control wines. This concentration continued to
288 augment in all the wines during bottle aging, although only the wines treated with YD 1
289 had a statistically significant lower concentration than the control wines after six
290 months in the bottle.
291 The flavanol monomers, (+)-catechin and (-)-epicatechin, and proanthocyanidins B1
292 and B2, were detected and quantified after alcoholic fermentation (Table 3). However,
293 proanthocyanidin B2 was not detected either after treatment or during aging in the
294 bottle. Both flavanol monomers increased in the wines after treatment, and some
295 statistically significant differences were found. Thus, only the wines treated with YD 2
296 showed a lower (+)-catechin concentration than the control wines, while the
297 concentration of (-)-epicatechin was statistically significant, lower in all the treated
298 wines than in the control wines, with those treated with YD 3 showing the lowest
299 concentration. The concentration of proanthocyanidin B1 also increased after treatment
300 in all the wines, with the exception of those treated with YD 2, which showed similar
301 concentrations to the ones found at the end of alcoholic fermentation. These wines also
302 showed a statistically significant lower concentration than the other wines.
303 During bottle aging, (+)-catechin concentration decreased in all the wines. After six
304 months of bottle aging, the wines treated with YD 1 were the only ones that had lower
305 concentrations of this compound than the control wines (a reduction of 12.5%). During
306 bottle aging, the concentration of (-)-epicatechin followed different trends, and after six

307 months all the treated wines displayed similar concentrations to those of the control
308 wines, with the exception of the wines treated with YD 2, which showed the highest
309 concentration.
310 Proanthocyanidin B1 was not detected during bottle aging.
311 No studies have been found relating to the effect of commercial yeast products on the
312 concentration of low molecular weight phenols in white wines. Only Razmkhab et al.
313 (*23*) and López-Toledano et al. (*24*) have examined the use of inactive yeast or yeast
314 cell walls in white wines. Both studies found a reduction of brown polymers. However,
315 contradictory results were obtained regarding the concentration of hydroxycinnamic
316 acids and flavanols. Razmkhab et al. (*23*) observed that the addition of yeast reduced
317 the concentration of *trans*-caftaric acid, catechin, epicatechin and procyanidins, while
318 López-Toledano et al. (*24*) reported higher caftaric acid and catechin concentration in
319 wines with added yeast than in wines without yeast. Moreover, these authors found no
320 effect from the addition of yeast on procyanidin content. General speaking, in this study
321 the wines treated with lees showed a higher content of hydroxycinnamic acids, both in
322 free and esterified forms, than in the case of the control wines. This effect was also
323 observed in the wines treated with YD 1 and YD 2. However, no clear effect was
324 detected for flavanol compounds. These differing results could be due to several causes.
325 On the one hand, each yeast or commercial product may give rise to different
326 compounds or fragments of variable size, with different active sites for retaining
327 phenols (*23*). On the other hand, the concentration of certain phenolic compounds
328 depends on the balance between the oxidation and polymerization reactions that will
329 produce a decrease in the concentration of these compounds, as well as on the
330 hydrolysis of higher oligomers that will increase the presence of these flavanols in
331 wines (*45*).

Kaempferol was the most important flavonol detected after alcoholic fermentation (0.421 mg/L). Other flavonols such as quercetin (0.031 mg/L) and quercetin-3-*O*–glycosides (0.020 mg/L), were also detected albeit at a low concentration (Table 3). However, after treatment and during aging in the bottle, these flavonol compounds were detected below the quantification limit of the method used.

Neither myricetin nor its 3-*O*–glycoside derivatives were found in the white wines. As was reported by other authors, this type of flavonol is considered to be exclusively of red grape varieties (*46*). Castillo-Muñoz et al. (*47*) determined the different flavonol types present in several *Vitis vinifera* white grape varieties, myricetin and its 3-*O*–glycoside derivatives being undetected in any of them.

Tyrosol and tryptophol are alcohols which are formed from deamination and decarboxylation reactions of tyrosine and tryptophan amino acids, respectively, during yeast fermentation (48). Tyrosol was the most abundant and represented about 97% of total alcohols. In general, the content of this compound increased slightly in all the wines after treatment, and several statistically significant differences were found. For instance, the wines treated with YD 1 and YD 2 manifested a higher concentration of tyrosol than in the case of the control wines, with the wines treated with YD 3 representing the poorest in this regard. However, during bottle aging, this compound decreased in all the wines, and no statistically significant differences were encountered between the treated and the control wines.

Tryptophol concentration decreased after treatment and throughout aging in the bottle. In general, statistically significant differences were undetected, and only after treatment did the wines treated with the commercial yeast derivative products reveal a lower concentration of tryptophol than in the case of the control wines and those treated with lees.

Analyses of polysaccharides and proteins

Monosaccharide and polysaccharide content in the commercial yeast derivative products

Table 4 shows the monosaccharide percentage of each commercial yeast derivative preparation. Mannose and glucose were the main monosaccharides quantified in these products, as was to be expected due to their being the main components of microbial polysaccharides (*49*). However, differences in the relationship between glucans and mannoproteins were found. The percentage of glucose, used to estimate glucan content, was highest (69.5%) in YD 1, which indicates that during the process to obtain this product more glucans are extracted than mannoproteins. On the other hand, YD 2, and especially YD 3, showed higher mannose content, 59.8% and 77.1%, respectively; this may indicate a greater purification process. These results agree with the information provided by the manufacturer (Table 1), who points out that YD 2 and YD 3 have a high content of free and highly purified mannoproteins.

The concentration of the different polysaccharide families was estimated from the monosaccharide concentration (Table 4). Thus, mannoprotein concentration was calculated directly from the concentration of mannose and it was observed that YD 3 preparations showed the highest concentration, approximately 2 and 4 times higher than the concentration in YD 2 and YD 1, respectively. RG-II was calculated from the concentration of apiose, 2-*O*-methyl-fucose and 2-*O*-methyl-xylose, which were not detected in the commercial products; this was to be expected, since this type of polysaccharides results from the enzymatical degradation of grape pectins (*49*).

Finally, it is important to point out the presence in these products of other monosaccharides such as galactose and arabinose, which are constituents of arabinogalactan-proteins (PRAGs), a type of polysaccharides that originate from the

pecto-cellulosic cell walls of grape berries (*6*). Consequently, these results seem to indicate the presence of some polysaccharides that do not come from yeast, in spite of the fact that the concentrations found were low, representing between 0.6-3.0% of total polysaccharide concentration. The presence of these compounds could be related to the manufacturing process of the yeast derivative commercial products.

Monosaccharide and polysaccharide content in white wines

Table 5 shows the monosaccharide concentration of white wines and the estimated polysaccharide concentration at the end of bottle aging. Only statistically significant differences in the rhamnose, mannose and galactose monosaccharide concentration were found. Thus, mannoprotein concentration estimated from mannose was higher in the wines treated with YD 1 and YD 2 than in the control and the other treated wines. This indicates that these commercial yeast derivative products release more polysaccharides into the wines than the lees or the YD 3 product. These results demonstrated that lees did not release neutral polysaccharides or mannoproteins from yeast cell walls during autolysis, probably due the short period of time involved in this treatment.

Although the YD 3 product was the richest in mannoprotein content (Table 4), the wines treated with YD 3 showed similar mannoprotein concentration to that of the control wines. This could be due to the fact that, in line with the manufacturers' instructions, the maximum recommended doses of YD 3 were added (5 g/HL). This amount was eight times lower than the added doses of YD 1 and YD 2 (40 g/HL), which was also the maximum dose recommended by the manufacturer. Therefore, although the latter indicates that YD 3 contains highly purified mannoproteins which are completely soluble in wines, the maximum doses recommended are not enough to observe certain effects in the polysaccharide contents of wines.

The wines treated with YD 1 showed a statistically significant lower concentration of PRAGs than the control wines and the other treated wines. This was mainly due to the lower content in galactose encountered in the wines treated with YD 1.

No statistically significant differences were found in the concentration of RG-II.

Polysaccharides by UV-vis spectrophotometry

Figure 1 shows the evolution of neutral (1A) and acid (1B) polysaccharides in elaborated white wines, showing certain statistically significant differences. Total polysaccharides revealed a similar trend to that of neutral polysaccharides. Total (TPS) and neutral polysaccharides increased in all the wines, including the control wines (Figure 1A), from the end of alcoholic fermentation until the end of the treatment. This fact could indicate that these compounds remain in wine in a colloidal state linked to other compounds, or that they originate from the autolysis of the remaining dead yeasts present in the wine. However, this increase was statistically significantly higher in the wines treated with the different commercial yeast derivative products than in the control wines and the wines treated with lees. The wines treated with YD 2 showed the highest content of neutral polysaccharides, followed by the wines treated with YD 1 and YD 3. During bottle aging, all the wines showed a decrease of TPS and NPS, more noticeable in the wines treated with the yeast derivative products, especially in the wines treated with YD 1 and YD 2. However, after six months in the bottle, the wines treated with YD 1 and YD 2 continued to show the highest values for neutral polysaccharides compared with the control wines and then the wines treated with lees. This decrease could be due to the formation of unstable complexes between the polysaccharides and other phenolic compounds, which, as has been pointed out by other authors concerning red wines (*18, 50*), might precipitate.

As expected, the APS concentration remained relatively constant throughout the whole vinification and aging process in all the wines, and only slight differences were found between the different treatments (Figure 1B).

The results of neutral polysaccharides found by spectrophotometry are in agreement with those found by HPSEC and GC, with the wines treated with YD 1 and YD 2 showing the highest concentration. Therefore, the spectrophotometric method, which can be carried out more quickly and easily, might be used by winemakers to estimate the neutral polysaccharide concentration that a certain commercial product could release into a wine.

Proteins

Table 2 shows the protein concentration of the different wines, which decreased strongly in all wines after treatment; this was due to their clarification with bentonite immediately following treatment and prior to being bottled. At this moment, all the treated wines showed a higher concentration of proteins than the control wines. This concentration continued decreasing in all wines during bottle aging, and after six months all the wines showed a protein concentration lower than 5 mg/L, with no statistically significant differences being detected between them.

Sensory analysis

No statistically significant differences were found in the color parameters between the treated and the control wines after the treatment (Figure 2A) or after the aging period (Figure 3A).

In the olfactory phase, some statistically significant differences were seen after treatment (Figure 2A) and the bottle aging period (Figure 3A). Following treatment, all the treated wines presented lower fruity aromas than the control wines, except the wines treated with YD 1. This could be due to the interaction between volatile compounds and

456 other metabolites such as mannoproteins and/or other polysaccharides released by lees
457 and yeast derivatives, which can reduce the volatility of some aromatic wine
458 compounds. Similar interactions have been observed by other authors in model wine
459 solutions (*15, 16, 51*) and in previous studies in white and red young wines using other
460 commercial products (*52, 53*). In general, after six months in the bottle (Figure 3A), all
461 the wines treated displayed stronger varietal, fruity and floral aromas, and higher
462 olfactory intensity than the control wines. This might indicate that these initially
463 retained aromatic compounds are released over time, increasing aroma intensities.
464 In the gustative phase, all the treated wines showed, generally speaking, higher values
465 of mouth-feel and overall rating, and lower values of acidity and astringency than the
466 control wines after treatment (Figure 2B); this was especially the case for wines treated
467 with the commercial yeast derivative products, although no statistically significant
468 differences were detected. However, after six months in the bottle (Figure 3B),
469 statistically significant differences were found. All the wines treated with commercial
470 yeast derivative products and those wines treated with lees showed less bitterness and
471 stronger mouth-feel, persistence, balance and overall rating values than the control
472 wines. This indicates that the treated white wines evolved better than the control wines
473 throughout the aging period.
474 To summarize, the results found in this study have indicated that lees and yeast
475 derivative products can interact or adsorb some of the phenolic compounds present in
476 wines reducing their concentration. This reduction depends on the treatment applied, the
477 phenolic compound analyzed and on the stage of the vinification or aging process.
478 The use of lees and yeast derivative products can give rise to a reduction in the color
479 intensity of wines immediately after treatment, so they can be used as agents for
480 reducing browning in white wines.

The monosaccharide and polysaccharide content of the commercial yeast derivative products depends on the manufacturing process and the product's degree of purification. The results obtained for total, neutral and acid polysaccharides in white wines by means of HPSEC-GC agreed with those obtained by spectrophotometric analysis. Therefore, the spectrophotrometric method could be used as a fast and easy enological method to determine the concentration of total, neutral and acid polysaccharides of a wine. However, a larger number of samples should be analyzed and correlation studies between the results obtained with the two methods should be carried out to corroborate this.

The effects on the chemical composition and sensory characteristics of the wines depended on the YD product used, although in general it can be said that YD-3 does not improve the quality of the wine. The other two YD products and aging on lees gave rise to wines with better sensorial characteristics than the control wines, especially after six months in the bottle, which means it is difficult to establish which one produces the best quality wine.

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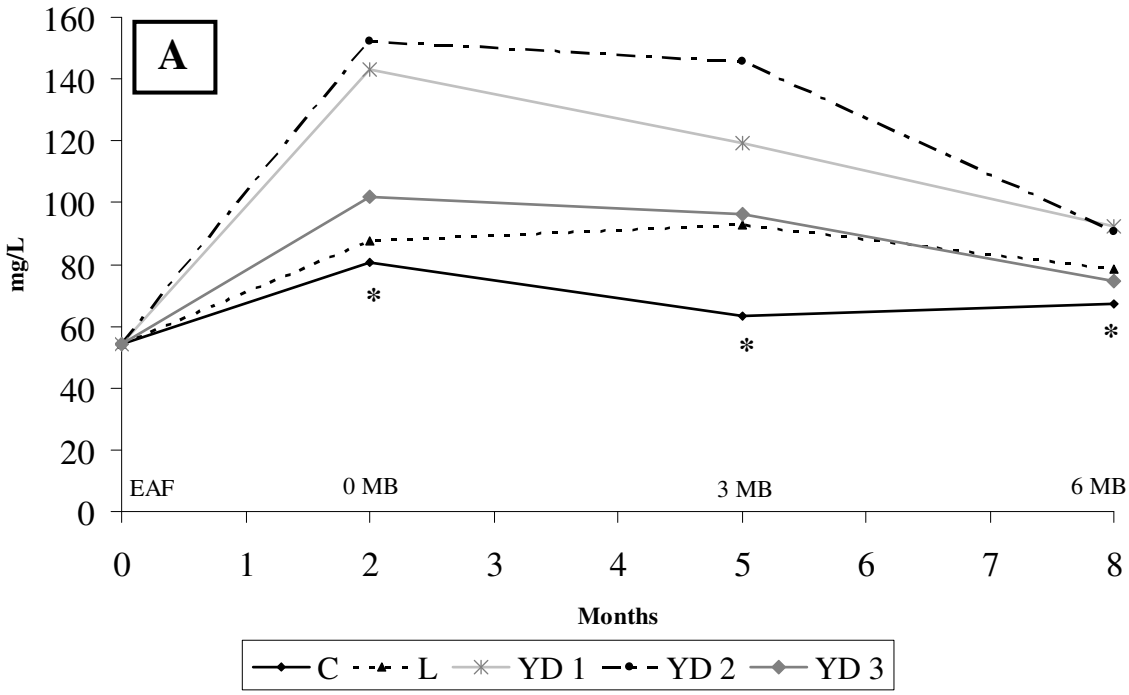
FIGURE CAPTIONS

Figure 1. Neutral (1A) and acid (1B) polysaccharide concentration in white wines. EAF: end of alcoholic fermentation; 0 MB: end of treatment; 3 MB: three months in bottle; 6 MB: six months in bottle. The asterisk indicates statistically significant differences for $p = 0.05$.

Figure 2. Sensory diagrams of the color and the olfactory phase (A) and the gustative phase (B) in white wines at the end of treatment.

Figure 3. Sensory diagrams of the color and the olfactory phase (A) and the gustative phase (B) in white wines after six months in bottle. The asterisk indicates statistically significant differences for $p = 0.05$.


A
160
140
120
100
80
60
40
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mg/L
EAF
0 MB
3 MB
6 MB
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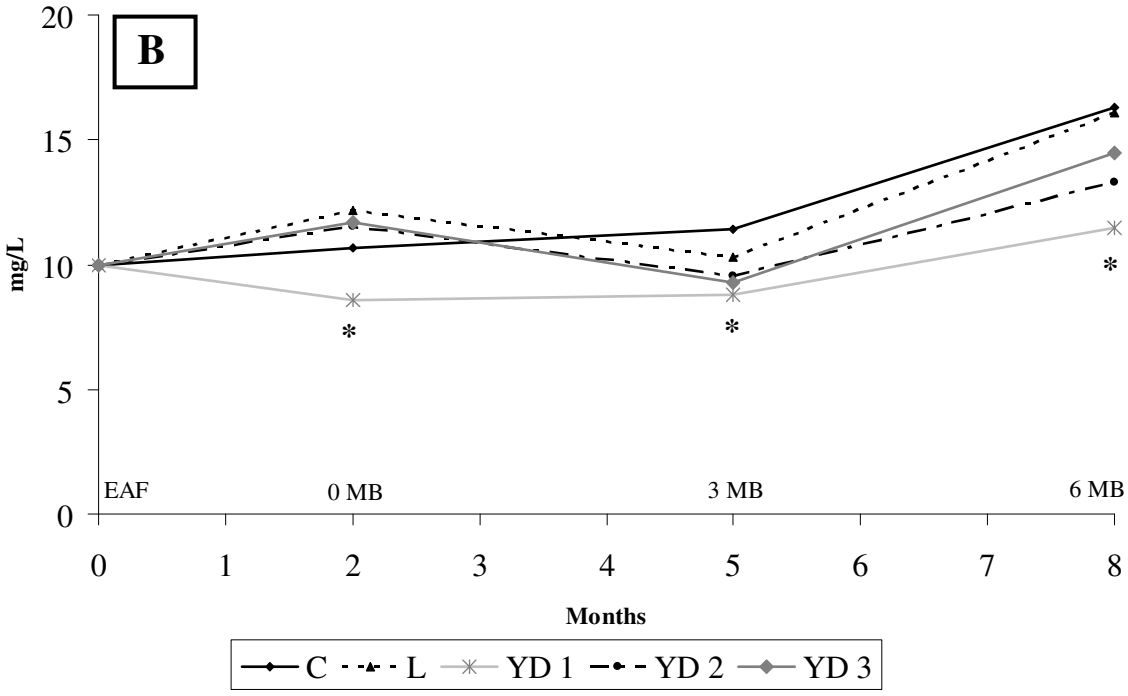
Months
C
L
YD 1
YD 2
YD 3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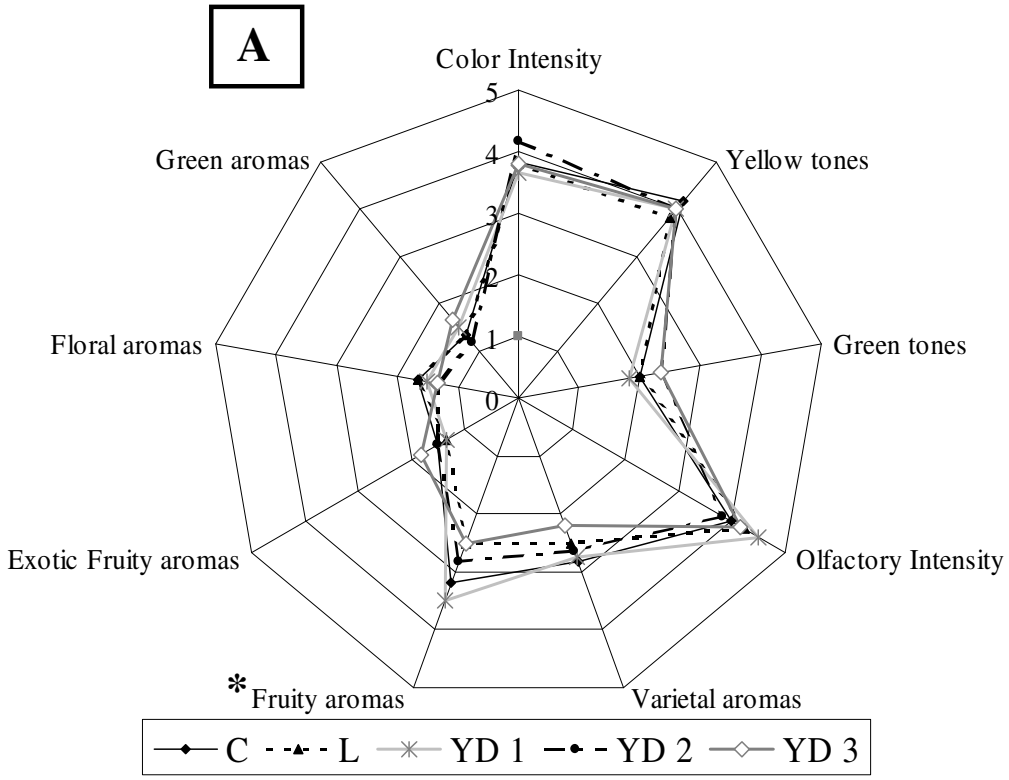
YD 2
YD 3

Figure 1


A
Color Intensity
Yellow tones
Green tones
Olfactory Intensity
Varietal aromas
Fruity aromas
Exotic Fruity aromas
Floral aromas
Green aromas
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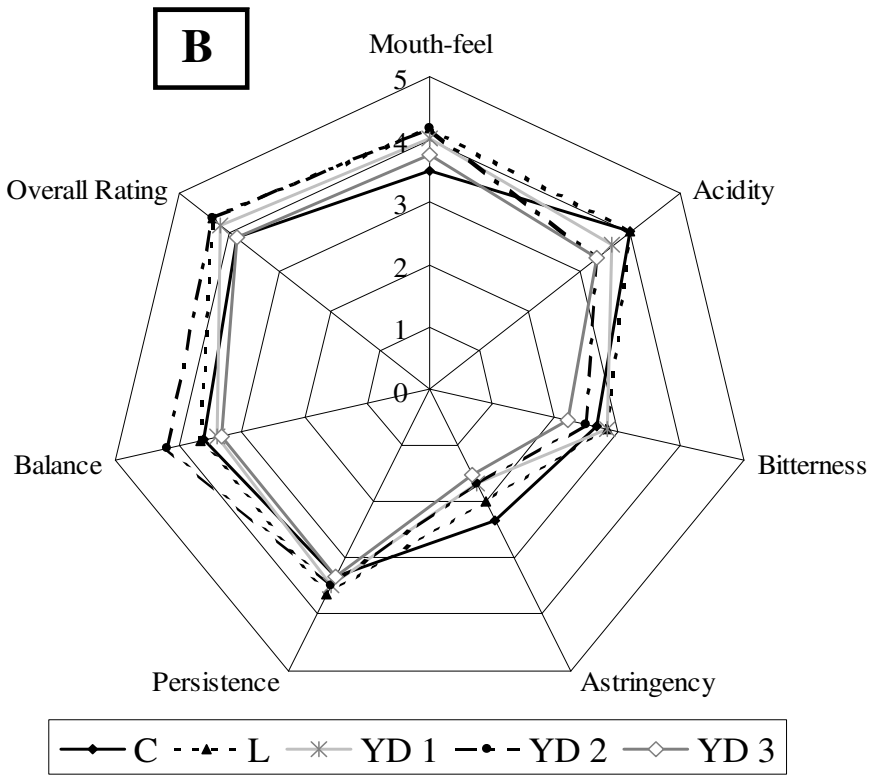
3
4
5
C
L
YD 1
YD 2
YD 3


B
Mouth-feel
Acidity
Bitterness
Astringency
Persistence
Balance
Overall Rating
0
1
2
3
4
5
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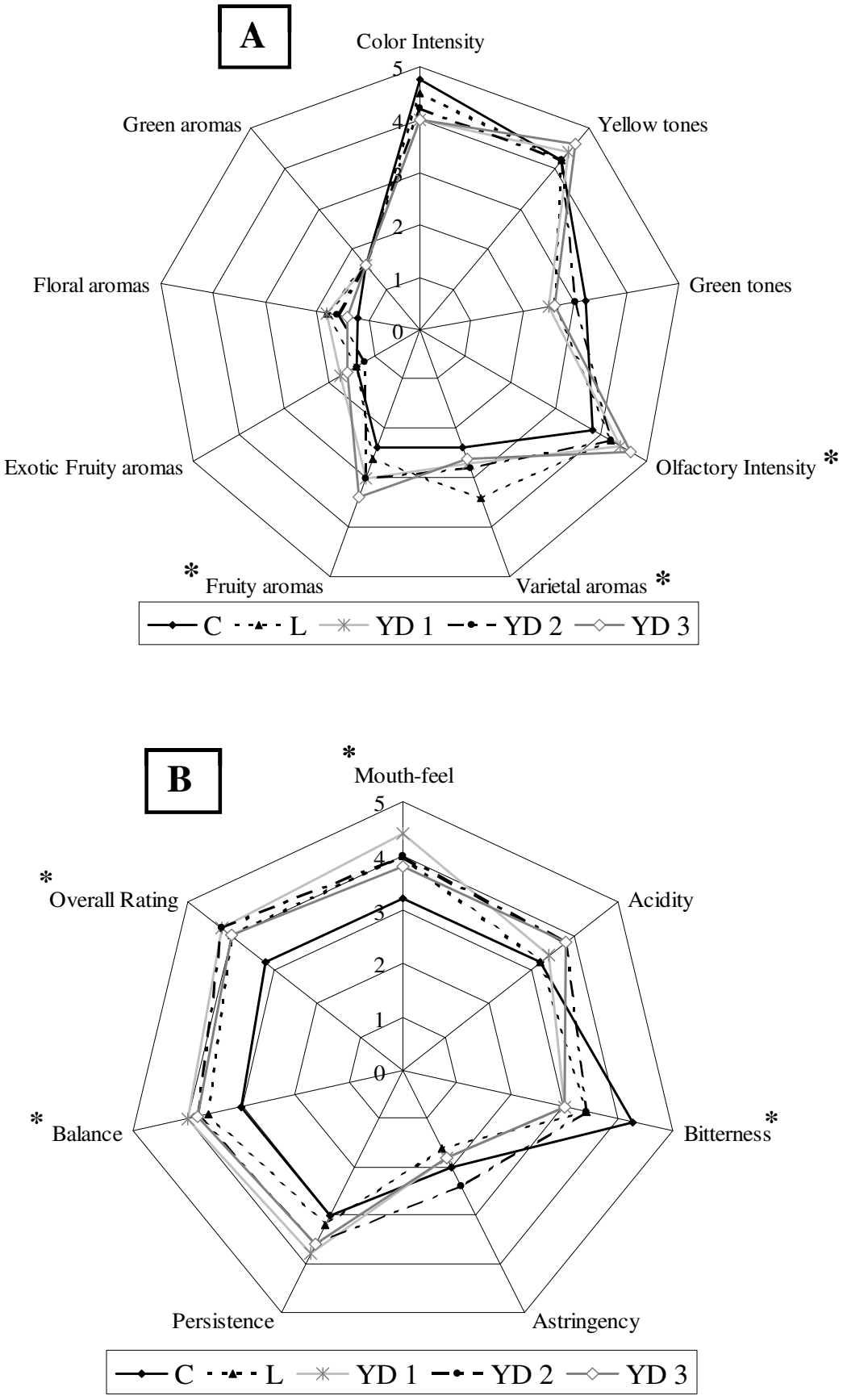
L
YD 1
YD 2
YD 3

Figure 2.


A
Color Intensity
Yellow tones
Green tones
Olfactory Intensity *
Varietal aromas *
Fruity aromas *
Exotic Fruity aromas
Floral aromas
Green aromas
0
1
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YD 1
YD 2
YD 3
B
Mouth-feel *
Acidity
Bitterness *
Astringency
Persistence
Balance *
Overall Rating *
0
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YD 1
YD 2
YD 3

Figure 3

Table 1. Characteristics of the different commercial yeast products used in white wines and the doses applied.

Commercial products	Doses	Expected effect (information provided by the manufacturer)	Characteristics
YD 1	40 g/HL	Increase mouth-feel and roundness sensations. Decrease astringency and increase the aromatic persistence. Improve tartaric and protein stability. Favor the malolactic fermentation	Product with polysaccharides extracted enzymatically from selected yeast walls.
YD 2	40 g/HL	Increase aromatic complexity and persistence, improve mouth-feel and gustative balance, reduce astringency and reduction notes. Increase fruity notes. Improve tartaric and protein stability. Prevent wine oxidation.	Product with parietal polysaccharides from yeast cell walls with high content in free mannoproteins.
YD 3	5 g/HL	Improve mouth-feel and softness and persistence in mouth. Improve tartaric and protein stability. Increase aromatic complexity.	Product with polysaccharides from yeast cellular walls, highly purified and completely soluble in wine.

Table 2. Total polyphenol, hydroxycinnamic acid derivatives, flavonol and total tannin concentration (mg/L), color intensity values, and protein concentration (mg/L of BSA[a]) of white wines elaborated.

Total polyphenols	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
EAF[b]	179	179	179	179	179
0 MB	182	188	190	191	187
3 MB	194a	208c	202b	196ab	196a
6 MB	188d	187cd	180bc	177ab	172a
Hydroxycinnamic acid derivatives	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
EAF	42.8	42.8	42.8	42.8	42.8
0 MB	35.2	35.1	34.5	35.4	36.4
3 MB	35.3b	34.4a	34.4a	35.3b	34.3a
6 MB	35.8	36.0	35.2	35.6	36.2
Flavonols	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
EAF	27.0	27.0	27.0	27.0	27.0
0 MB	20.3c	19.6bc	18.5a	19.4b	19.6bc
3 MB	20.5d	18.7b	18.0a	19.8c	19.1b
6 MB	20.8c	20.1b	18.7a	20.1b	20.8c
Total tannins	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
EAF	358	358	358	358	358
0 MB	314	301	303	307	310
3 MB	282d	268a	279c	278c	272b
6 MB	323b	303a	313a	313a	306a
Color Intensity	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
EAF	0.099	0.099	0.099	0.099	0.099
0 MB	0.042c	0.036a	0.037a	0.040b	0.040b
3 MB	0.043d	0.035a	0.037b	0.041c	0.038b
6 MB	0.040b	0.040b	0.037a	0.040b	0.039b
Proteins	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
EAF	66.0	66.0	66.0	66.0	66.0
0 MB	11.4a	14.7b	14.0b	13.9b	15.2b
3 MB	10.0	10.2	11.2	11.4	14.0
6 MB	<5	<5	<5	<5	<5

Values with different letter in the same row indicate statistically significant differences ($p< 0.05$), and values without letter indicate no statistically significant differences.

[a] BSA: Bovine Serum Albumine

[b] EAF: end of alcoholic fermentation; 0 MB: end of treatment; 3 MB: three months in bottle and 6 MB: six months in bottle.

Table 3. Concentration (mg/L) of low molecular weight phenolic compounds in white wines.

	EAF[a]	0 MB (end of treatment)					3 MB (three months in bottle)					6 MB (six months in bottle)				
Compound	**C**	**C**	**L**	**YD 1**	**YD 2**	**YD 3**	**C**	**L**	**YD 1**	**YD 2**	**YD 3**	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
HBA[b]																
Gallic acid	3.03	3.54a	3.60a	3.80b	3.88b	3.47a	0.51b	0.59c	0.58c	0.56c	0.42a	0.33a	0.46cd	0.48d	0.44c	0.39b
Protocatechuic acid	0.39	0.51a	0.47a	0.61bc	0.62c	0.52a	0.43	0.39	0.40	0.40	0.36	0.40a	0.44bc	0.52d	0.47c	0.42b
Syringic acid	0.40	0.39	0.41	0.46	0.53	0.41	0.34d	0.24b	0.16a	0.29c	0.24b	0.14b	0.17b	0.07a	0.18b	0.18b
Ethyl gallate	1.04	0.99b	1.00b	0.83a	1.04b	0.98b	0.93c	0.88b	0.72a	0.87b	0.90bc	0.84b	0.85b	0.65a	0.86b	0.86b
Total	4.86	5.45a	5.48a	5.69a	6.06b	5.38a	2.21c	2.10b	1.87a	2.11bc	1.93a	1.71a	1.93c	1.72a	1.95c	1.85b
HCA[c]																
Trans-caffeic acid	0.60	0.83b	0.87c	1.48d	0.87c	0.74a	0.79b	0.80b	1.35d	0.83c	0.74a	0.77a	0.85b	1.37c	0.84b	0.79a
Trans-p-coumaric acid	0.08	0.29a	0.29a	0.56c	0.29a	0.36b	0.52a	0.54a	0.88b	0.52a	0.53a	0.60b	0.62c	0.99d	0.59b	0.57a
Total	0.68	1.12ab	1.16b	2.04c	1.16b	1.10a	1.31ab	1.34b	2.24c	1.35b	1.27a	1.37a	1.47c	2.36d	1.43b	1.37a
HCA esters																
Trans-caftaric acid	7.55	7.50a	9.59d	8.91b	9.17c	7.31a	7.36b	9.26e	8.64c	8.77d	7.29a	7.47a	9.39d	8.76b	8.87c	7.47a
Cis-coutaric acid	0.95	0.87c	0.83bc	0.78b	0.84bc	0.64a	0.49c	0.31a	0.31a	0.49c	0.35b	0.41c	0.38b	0.35a	0.43d	0.43d
Trans-coutaric acid	0.78	0.65b	0.67bc	0.42a	0.68c	0.73d	0.78a	1.02d	0.83b	0.79a	0.99c	0.84b	0.90d	0.70a	0.83b	0.86c
Trans-fertaric acid	1.57	1.76c	1.71b	1.70b	1.70b	1.63a	1.93c	1.77b	1.73a	1.89c	1.75ab	1.92b	1.91b	1.84a	1.90b	1.91b
Total	10.8	10.8b	12.8e	11.8c	12.4d	10.3a	10.6b	12.4e	11.5c	11.9d	10.4a	10.6a	12.6d	11.6b	12.0c	10.7a
Flavanols monomers																
(+)-catechin	3.10	3.72bc	3.99cd	4.24d	3.22a	3.63b	2.93b	2.35a	2.39a	2.29a	2.20a	1.91b	1.92b	1.67a	1.89b	1.90b
(-)-epicatechin	0.64	0.97c	0.75ab	0.73ab	0.78b	0.61a	0.81d	0.72c	0.43a	0.63b	0.62b	0.69ab	0.81bc	0.63a	0.88c	0.82bc
Total	3.74	4.69bc	4.74bc	4.97c	4.00a	4.24ab	3.74b	3.07a	2.81a	2.92a	2.82a	2.61b	2.74b	2.30a	2.77b	2.72b
Flavanols dimers																
Proanthocyanidin B1	2.11	2.61b	2.52b	2.59b	2.10a	2.77b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Proanthocyanidin B2	0.679	nd[d]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Alcohols																
Tyrosol	18.64	19.21b	19.76bc	20.62d	20.27cd	18.52a	16.77	16.33	16.06	16.34	16.77	16.05	16.19	15.95	16.11	16.76
Tryptophol	0.62	0.54d	0.51cd	0.46c	0.31a	0.39b	0.38b	0.24a	0.29a	0.26a	0.25a	0.20	0.20	0.21	0.20	0.19
Total	19.26	19.75b	20.27bc	21.08d	20.58cd	18.91a	17.15	16.57	16.35	16.60	17.12	16.25	16.39	16.16	16.31	16.95

Values with different letter in the same row indicate statistically significant differences ($p< 0.05$), and values without letter indicate no statistically significant differences.
[a] EAF: end of alcoholic fermentation; [b] HBA: Hydroxybenzoic acids; [c] HCA: Hydroxycinnamic acids; [d] nd: No detected.

Table 4. Monosaccharide percentage and polysaccharide concentration (mg/g)in the different commercial yeast derivative products.

Monosaccharides	**YD 1**	**YD 2**	**YD 3**
2-*O*-Methyl- fucose	nd[a]	nd	nd
Rhamnose	0.057	0.092	0.073
Fucose	nd	nd	nd
2-*O*-Methyl-xylose	nd	nd	nd
Arabinose	0.475a	1.72b	0.336a
Apiose	nd	nd	nd
Xylose	0.145	0.102	0.099
Mannose	29.3a	59.8b	77.1c
Galactose	0.477	0.154	0.220
Glucose	69.5c	38.1b	22.2a
Polysaccharides	**YD 1**	**YD 2**	**YD 3**
MPs[b]	99.9a	186.9b	407.5c
RG-II	nd[b]	nd	nd
PRAGs	3.02	4.82	2.66
Total	103.0a	191.7b	410.1c

Values with different letter in the same column indicate statistically significant differences ($p < 0.05$), and values without letter indicate no statistically significant differences.

[a] nd: No detected.

[b] MPs: mannoproteins; RG-II: rhamnogalacturonans II; PRAGs: polysaccharides rich in arabinose and galactose.

Table 5. Monosaccharide and estimated polysaccharide concentration (mg/L) in the white wines after six months.

Monosaccharides	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
2-*O*-Methyl- fucose	0.25	0.27	0.25	0.27	0.25
Rhamnose	3.35b	1.40a	1.41a	1.41a	1.50a
Fucose	0.23	0.31	0.15	0.15	0.11
2-*O*-Methyl-xylose	0.18	0.18	0.20	0.20	0.27
Arabinose	3.47	2.85	2.33	3.11	2.57
Apiose	0.31	0.36	0.39	0.40	0.54
Xylose	0.61	0.30	0.16	0.16	0.10
Mannose	58.7a	48.0a	82.7b	90.2b	59.0a
Galactose	17.9c	15.6bc	7.7a	18.0c	14.0b
Glucose	3.50	2.23	2.22	2.98	2.30
Polysaccharides	**C**	**L**	**YD 1**	**YD 2**	**YD 3**
MPs	73.3a	59.9a	103.3b	112.7b	73.8a
RG II	27.7	29.0	28.8	30.4	33.4
PRAGs	25.9b	22.1b	11.1a	25.5b	19.4b
Total	126.9ab	111.0a	143.2b	168.6c	126.6ab

Values with different letter in the same row indicate statistically significant differences ($p < 0.05$), and values without letter indicate no statistically significant differences.

CAPÍTULO 4

Técnicas para mejorar o sustituir la crianza sobre lías de vinos tintos envejecidos en barrica: efectos sobre los polisacáridos y la composición fenólica

Tal y como se ha comentado anteriormente, la crianza sobre lías es una técnica que se viene empleando desde hace varias décadas en la elaboración de vinos blancos. Sin embargo, en los vinos tintos su aplicación es mucho más reciente por lo que también es más escasa la información de que se dispone. Por este motivo, el objetivo del trabajo que se expone en este capítulo ha sido estudiar el efecto de la crianza sobre lías y otras técnicas alternativas (chips, derivados de levadura comerciales, enzimas β-glucanasas y crianza sobre lías en combinación con la microoxigenación) sobre la composición fenólica, color, proteínas, polisacáridos y las características sensoriales de un vino tinto de la variedad Tempranillo. Además, se ha estudiado la evolución de estos vinos durante su crianza en barrica durante 6 meses. Este estudio se llevó a cabo durante 2 vendimias consecutivas, 2007 y 2008.

Una vez concluidos todos los análisis, los resultados y conclusiones más destacados de este trabajo son los siguientes:

1- Ninguno de los tratamientos estudiados modificaron los parámetros enológicos clásicos.

2- Los diferentes tratamientos empleados modificaron la concentración de algunos de los compuestos fenólicos analizados en los vinos tintos, aunque el efecto observado dependió del tipo de compuesto, del tratamiento empleado y de la vendimia. De este modo, en ambas vendimias, tras los 6 meses de envejecimiento en barrica, los vinos tratados con chips mostraron las concentraciones más altas de polifenoles totales, mientras que los vinos tratados con lías finas y con los derivados de levadura presentaron concentraciones más bajas que los vinos control. Esto puede ser debido, como ya se ha comentado anteriormente, a la adsorción de estos compuestos sobre las lías, los derivados de levadura, o los compuestos liberados por ellos.

Tras los 6 meses de envejecimiento en barrica los vinos tratados con lías finas y con los derivados de levadura presentaron mayor concentración de antocianos que los vinos control.

3- Los resultados obtenidos en el análisis individualizado de los antocianos reveló que en la vendimia de 2007, tras los 6 meses de envejecimiento en barrica los vinos tratados con chips, lías y enzimas, y lías con microoxigenación presentaron concentraciones más bajas de antocianos libres (antocianos acéticos, glucósidos y cumáricos) que los vinos control. Sin embargo, en la vendimia de 2008, las diferencias encontradas entre los distintos tratamientos en estos compuestos fueron menores.

La crianza sobre lías y/o el uso de derivados de levadura comerciales no favoreció la formación de nuevos pigmentos antociánicos estables, compuestos que juegan un papel importante en la estabilización del color de los vinos.

El tratamiento de crianza sobre lías con microoxigenación fue el único que mostró un claro efecto sobre la estabilización del color de los vinos tintos, ya que favoreció la formación de nuevos pigmentos. La formación de estos pigmentos permitió aumentar la intensidad de color y los tonos azulados del vino. Estas diferencias respecto al vino control se mantuvieron durante todo el período de crianza en barrica. Sin embargo, es difícil establecer si esta estabilización del color es debida únicamente a la microoxigenación o a la acción conjunta de ambas técnicas.

4- Todos los tratamientos estudiados produjeron un aumento significativo del contenido de polisacáridos totales y neutros en los vinos, y aumentó ligeramente o permaneció constante durante el envejecimiento en barrica.

Por otro lado, el uso de enzimas β-glucanasas no produjo un efecto claro sobre la liberación de polisacáridos al vino.

5- En el análisis sensorial de los vinos, tras el tratamiento, los catadores no encontraron diferencias entre ninguno de los vinos elaborados en ninguna de las 2 vendimias en los parámetros de color. Únicamente tras el envejecimiento en barrica los vinos tratados con chips de la vendimia de 2008 y con los derivados de levadura comerciales mostraron más tonos azulados que el vino control.

Tras el tratamiento, algunos de los vinos tratados tenían menor intensidad olfativa y menores aromas a fruta que los vinos control, probablemente debido a la interacción de los compuestos aromáticos con las manoproteínas y otros polisacáridos liberados por las lías y los derivados de levadura. Tras los 6 meses de envejecimiento en barrica, los vinos tratados con el derivado de levadura en 2007 y todos los vinos tratados en 2008 presentaron valores más altos de aromas a fruta que los vinos control. Esto indica que los compuestos aromáticos retenidos tras el tratamiento son liberados a lo largo del envejecimiento del vino.

Por otro lado, en la fase gustativa, el panel de catadores indicó que los vinos tratados en ambas vendimias presentaban valores más bajos de astringencia y de taninos verdes, y más altos de grasa, equilibrio y valoración global que los vinos control, especialmente aquellos tratados con los derivados de levadura. Tras el período de envejecimiento en barrica, todos los vinos tratados en 2008 mostraron mayor equilibrio y valoración global que los vinos control.

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Techniques for improving or replacing ageing on lees of oak aged red wines: The effects on polysaccharides and the phenolic composition

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ABSTRACT

Alternative techniques to the ageing on lees are being looked for in order to guarantee the improvements provided by this technique but eliminating its disadvantages. The aim of this work was to study the effect of ageing on lees and other alternative techniques (addition of β-glucanase enzymes to the lees; use of different yeast commercial preparations with or without β-glucanase enzymes; use of non-toasted oak chips; and ageing on lees together with micro-oxygenation) on the phenolic compounds, colour, proteins, polysaccharides and sensorial characteristics of red wines during vinification and ageing in oak barrels for 6 months on two consecutive vintages.

Only the use of lees together with micro-oxygenation seemed to have a positive effect on the colour stability, due to the formation of new pigments that allows the intensity and blue notes of wines to be maintained during the barrel ageing process.

All the techniques studied released total and neutral polysaccharides, although the type and content of these compounds depended on the technique used, and the yeast derivative added. No clear effect was observed with the use of β-glucanase enzymes.

The sensory analysis showed that some of the wines treated were better valued than the control wines. The results obtained indicated that is difficult to select the technique that allows us to obtain the best quality wine.

1. Introduction

The ageing of wines on lees is a technique that enhances the sensorial characteristics of wines, due mainly to the compounds that are released during the yeast autolysis as fatty acids, nucleotides and nucleosides, amino acids and peptides, and mannoproteins and polysaccharides. This technique is more widely-used in white wines than in red wines and contributes to improving their organoleptic quality (Charpentier, Santos, & Feuillat, 2004; Feuillat, 2003; Fournairon, Camarasa, Moutounet, & Salmon, 2002; Salmon, Fornairon-Bonnefond, & Mazauric, 2002). The aim of this technique is to reduce astringency and bitterness, as well as enhancing the body, structure and roundness of wines (Feuillat, Escot, Charpentier, & Dulau, 2001; Fuster & Escot, 2002; Riou, Vernhet, Doco, & Moutounet, 2002) and obtaining more persistent (Vidal et al., 2004b) and more aromatic complex wines (Ramírez, Chassagne, Feuillat, Voilley, & Charpentier, 2004). In red wines, ageing on lees can also contribute to colour stability (Escot, Feuillat, Dulau, & Charpentier, 2001; Francois, Alexandre, Granes, & Feuillat, 2007; Fuster & Escot, 2002). This is mainly due to the polysaccharides and mannoproteins which can act as protective colloids (Doco, Patrick, Cheynier, & Moutounet, 2003; Morata, Calderón, González, Colomo, & Suárez, 2005; Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2003). Moreover, the dead yeast consumes oxygen and therefore, prolonged contact with wine lees protects it from oxidation (Fournairon, Mazauric, Salmon, & Moutounet, 1999; Fournairon et al., 2002).

Despite the positive effects referred to above, this technique also has a number of disadvantages, including greater demands on winery resources, namely more staff to carry out the 'batonnage', longer wine storage times, etc., which raise the price of the final product, as well as the appearance of reduction notes (Chattonnet, 2000; Feuillat et al., 2001) and microbiological alterations due to the development of spoilage micro organisms such as Brettanomyces (Chattonnet, 2000; Zamora, 2002).

Nowadays, alternative techniques are being sought in order to guarantee the improvements provided by the ageing on lees but with none of the disadvantages.

One of these techniques is micro-oxygenation, which consists in the addition of small and controlled doses of oxygen to wines (Pérez-Magariño, Sánchez-Iglesias, Ortega-Heras, González-Huerta, & González-Sanjosé, 2007). Therefore, the combined used of micro-oxygenation and ageing on lees could reduce or eliminate the appearance of reductive aroma compounds caused by the lees'

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oxygen consumption (Moutounet, 2003; Roig & Yerlé, 2003). In addition, the micro-oxygenation could provide additional positive effects such as lower astringency and improved colour stability, because the oxygen added favours the condensation and polymerisation reactions between anthocyanins and flavanols (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Cano-López, López-Roca, Pardo-Minguez, & Gómez-Plaza, 2010; Cano-López, Pardo, López-Roca, & Gómez-Plaza, 2006; Cano-López et al., 2008; Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & Freitas, 2003; Pérez-Magariño et al., 2007), as well as the formation of more stable new anthocyanins.

Yeast autolysis is generally a slow process, although it could be accelerated by the addition of exogenous β-glucanase enzymes that act on the yeast cell walls, favouring the release of polysaccharides and mannoproteins (Masino, Montevecchi, Arfelli, & Antonelli, 2008). On the other hand, in the last few years, various commercial preparations based on yeast derivatives with different composition and effects (inactive yeast, yeast autolysates, yeast extracts, etc.) have appeared on the market which could replace traditional ageing on lees. These commercial preparations could confer wines with the positive characteristics mentioned above. A number of authors have studied the use of various commercial products on wine quality (Guadalupe & Ayestarán, 2008; Guadalupe, Martínez, & Ayestarán, 2010; Guadalupe, Palacios, & Ayestarán, 2007; Pozo-Bayón, Andujar-Ortiz, Alcaide-Hidalgo, Martín-Álvarez, & Moreno-Arribas, 2009), although their true impact is not yet well-known.

The use of non-toasted oak wood is a technique that can also add polysaccharides to wine, although they are not the same as those released from the yeast cell walls (Nonier et al., 2005; Viriot, Scalbert, Lapierre, & Moutounet, 1993). However, these compounds extracted from non-toasted wood could also increase the mouth-feel, body and sweet characteristics and reduce the astringent and bitter sensations of wines (Vidal et al., 2004b).

Consequently, the aim of this project is to study the effect of ageing on lees and other alternative techniques on the phenolic compounds, colour, proteins, polysaccharides and sensorial characteristics of red wines during vinification and ageing in oak barrels for 6 months. This study was carried out on two consecutive vintages. The alternative techniques used were: addition of β-glucanase enzymes to the lees; use of different yeast commercial preparations with or without β-glucanase enzymes; use of non-toasted oak chips; and ageing on lees together with micro-oxygenation. This is the first study in which all these vinification techniques have been studied and compared together.

2. Materials and methods

2.1. Winemaking process and treatments

The study was carried out using the Tempranillo red grape variety from Cigales Origin Designation, sited in the Autonomous Community of Castilla y León in the North of Spain, from the 2007 to

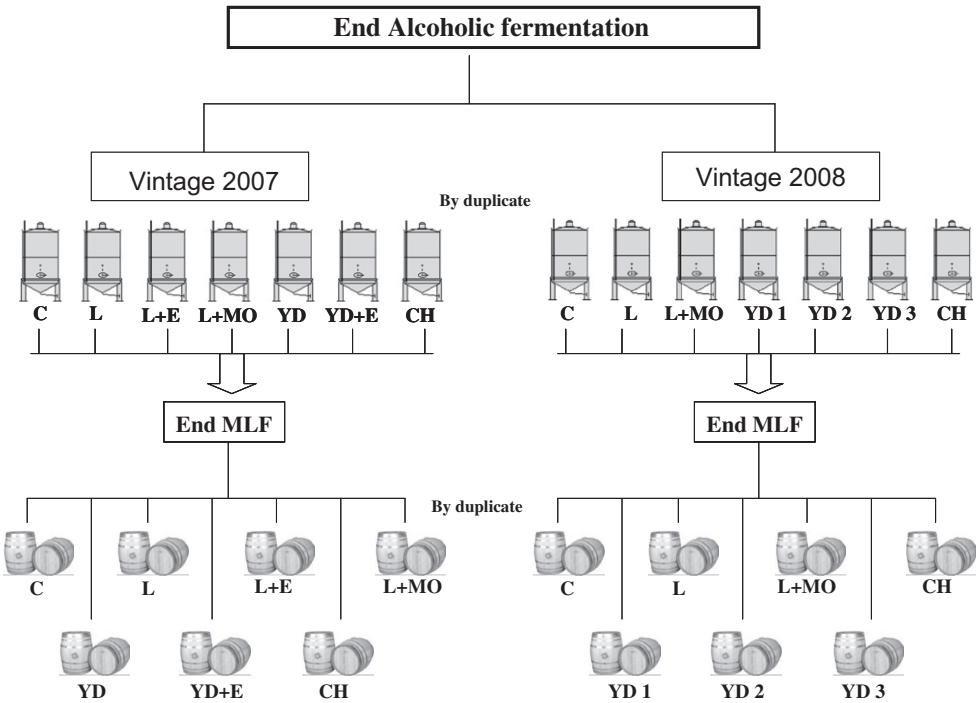

Fig. 1. Scheme of the experiences carried out in each vintage. C (control wine); L (wine aged on lees); L + E (wine aged on lees + enzymes); L + MO (wine aged on lees + micro-oxygenation); YD (wine treated with yeast derivative); YD + E (wine treated with yeast derivative + enzymes); YD 1 (wine treated with yeast derivative 1); YD 2 (wine treated with yeast derivative 2); YD 3 (wine treated with yeast derivative 3) and CH (wine treated with chips).

Table 1
Characteristics of the different commercial yeast derivatives used in this study, and the doses applied considering the recommendations of the suppliers.

Yeast derivative	Vintage	g/Hl	Doses (g/Hl)	Characteristics
YD	2007	20–50	40	Product with autolysated yeast enriched in polysaccharides
YD 1	2008	30–50	40	Product with parietal polysaccharides extracted enzymatically from selected yeasts walls
YD 2	2008	10–30	40	Product with parietal polysaccharides from yeast cell walls with high content in free mannoproteins
YD 3	2008	0.5–5	5	Product with polysaccharides from the yeast cellular walls, highly purified and completely soluble in wine

2008 vintages. The red wines were produced at the Oenological Station of Castilla y León following the traditional red winemaking process.

The grapes (about 12,500 kg.) were harvested manually on the optimum harvest date, based mainly on the relation sugar content (Brix)/total acidity and grape sanitary conditions, and rapidly transported to the Oenological Station in 15-kg-plastic boxes. The clusters were de-stemmed and crushed with minimum physical damage. The mass obtained, was slightly sulphited (0.04 g/l) and then transferred to five 2600 l stainless steel tanks to undergo alcoholic fermentation at a controlled temperature (25–28 °C). Alcoholic fermentation was carried out through inoculation with commercial yeast *Saccharomyces cerevisiae* (Excellence sp., Lamothe-Abiet) in all cases. Once the alcoholic fermentation was complete, the wines were kept in the tanks for 4 days to allow for the sedimentation of the gross lees. After this time, the wines were racked off and maintained in the tanks for 4–5 days to allow the sedimentation of the fine lees. The base wine was then again racked off, homogenised and distributed into a number of 500 l tanks. The wet fine lees decanted in the bottom of the tanks were used in the experiments with lees.

The experiments carried out on the 2007 and 2008 vintage are shown in Fig. 1. In the case of the 2007 vintage, the following experiments were carried out in duplicate: control wines (without the addition of any product) (C); wines aged on lees (3% v/v of fine lees) (L); wines aged on lees and addition of commercial β-glucanase enzymes (L + E) (Enovin Glucan, Agrovin, Spain); wines aged on lees with micro-oxygenation (L + MO) (5 ml/L/month of O_2); wines with a commercial yeast derivative added (YD) (Super Bouquet, Agrovin, Spain); wines with the same commercial yeast derivative and the commercial β-glucanase enzymes added (YD + E); and wines with 4 g/l of non-toasted French oak chips added (CH) (Bois Frais, Boise France, France).

In the light of the results obtained from the 2007 vintage to other earlier studies (Cano-Mozo et al., 2007; Rodríguez-Bencomo, Ortega-Heras, & Pérez-Magariño, 2010; Rodríguez-Bencomo, Pérez-Magariño, González-Huerta, & Ortega-Heras, 2007) relating to the use of β-glucanase enzymes, in the 2008 vintage, the study of the effect of several commercial yeast derivatives of varying compositions and action modes was considered to be of greater interest than the effect of the addition of β-glucanase enzymes. Therefore, in this second vintage, the following experiments were carried out in duplicate: control wines (C) (with no added products); wines aged on lees (L) (3% v/v of fine lees); wines aged on lees with micro-oxygenation (L + MO) (5 ml/L/month of O_2); wines with three different commercial yeast derivatives added (Surli One (YD-1), Surli Elevage (YD-2) and Velvet (YD-3), Sepsa, Spain); and wines with 4 g/l of non-toasted French oak chips added (CH) (Bois Frais, Boise France, France).

The commercial enzyme preparation was chosen for its high β-glucanase activity (430 BGXU/g, data given by the supplier), and doses of 4 g/HL were used in keeping with manufacturer recommendations. The commercial yeast derivatives used were chosen because of their high concentration in proteins and carbohydrates or different composition. Table 1 shows the data given by the commercial suppliers regarding the different commercial yeast derivatives used in this study, and the doses applied, in accordance with suppliers' recommendations.

Micro-oxygenation was carried out by a modular five-head VisiO2 micro-oxygenator (Oenodev, France).

The "batonnage" is the process of stirring the fine lees or the commercial yeast derivative products added into wine with a stick in order to bring the lees or the products into suspension. Two batonnages per week were performed on each wine. The temperature was maintained at 15 ± 1 °C. In the case of both vintages, all treatments lasted 8 weeks.

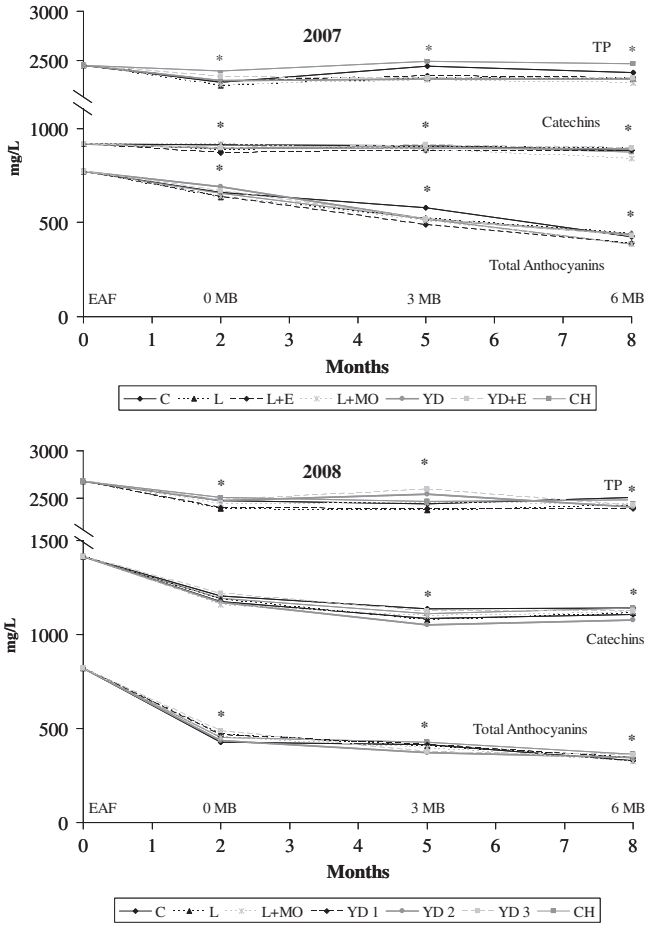

Fig. 2. Evolution of total polyphenol (TP), catechin and total anthocyanin concentration of wines elaborated in each vintage. EAF: end of the alcoholic fermentation; 0 MB: end of the treatment and the malolactic fermentation; 3 MB: 3 months in barrel; 6 MB: 6 months in barrel. The asterisk indicates statistically significant differences for $\alpha = 0.05$.

Following the treatments, all wines were racked off and inoculated with a commercial preparation of *Oenococcus oeni* (Viniflora, CHR Hansen, Denmark) to induce malolactic fermentation. After malolactic fermentation, the wines were racked off into new American oak barrels with a medium–high degree of toasting (two barrels for each treatment and replicate, see Fig. 1).

The samples were analysed immediately after completion of the treatment and the malolactic fermentation, after three and 6 months of ageing in barrels.

2.2. Chemical reagents

Gallic acid, D-(+)-catechin, bovine gelatine, glucose, Coomassie reactive, *trans*-caffeic acid, D-(+)-galacturonic acid, 3-hidroxy-biphenyl and phenol were provided by Sigma–Aldrich (Steinheim, Germany); quercetin, malvidin-3-glucoside and cyanidin chloride by Extrasynthèse (Lyon, France); bovine serum albumine and di-sodium tetraborate decahydrated by Merck (Darmstadt, Germany). Acetonitrile and methanol were provided by Lab Scan (Madrid, Spain) and the remaining reagents by Panreac (Madrid, Spain). Water Milli-Q was obtained through a Millipore system (Bedford, MA).

2.3. Analytical methods

Oenological parameters were evaluated following official analysis methods (OIV, 1990).

The content of phenolic compounds was evaluated by the quantification of several phenolic families: total polyphenols, expressed in mg/l of gallic acid (Paronetto, 1977); total anthocyanins, expressed in mg/l of malvidin-3-glucoside (Paronetto, 1977); catechins, expressed in mg/l of D-(+)-catechin (Swain & Hillis, 1959); total tannins expressed in mg/l of cyanidin chloride (Ribéreau-Gayón & Stonestreet, 1966); tartaric esters and flavonols expressed in mg/l of caffeic acid and quercetin, respectively (Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999) and monomeric, polymeric and copigmented anthocyanins expressed in percentages using the methods proposed by Somers and Evans (1977) and Levengood and Boulton (2004).

The content of individual anthocyanins and their derivatives in red wines were determined by direct injection of the wines, previously filtrated through PVDF filters of 0.45 μm (Millipore, Bedford, MA), in chromatograph Agilent-Technologies LC-DAD 1100 following the method described by Pérez-Magariño, Ortega-Heras, Cano-Mozo, and González-Sanjosé (2009). The compounds identified in this study were grouped as glucoside anthocyanins: delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside; as acetic anthocyanins: delphinidin-3-(6-acetyl)-glucoside, cyanidin-3-(6-acetyl)-glucoside, petunidin-3-(6-acetyl)-glucoside, peonidin-3-(6-acetyl)-glucoside, and malvidin-3-(6-acetyl)-glucoside; as cinnamic anthocyanins: delphinidin-3-(6-p-cumaril)-glucoside, cyanidin-3-(6-p-cumaril)-glucoside, petunidin-3-(6-p-cumaril)-glucoside, malvidin-3-(6-p-cumaril)-glucoside, and malvidin-3-(6-caffeil)-glucoside; as pyruvic anthocyanins: delphinidin-3-glucoside pyruvate, petunidin-3-glucoside pyruvate, peonidin-3-glucoside pyruvate, malvidin-3-glucoside pyruvate (vitisin A) and vitisin B, anthocyanins derived of direct condensation (peonidin-3-glucoside-(epi)catechin and malvidin-3-glucoside-(epi)catechin); malvidin-ethyl-(epi)catechin; malvidin-3-glucoside-vinylphenol and malvidin-3-glucoside-vinylcatechol.

Colour was evaluated using the Glories parameters, namely colour intensity, tonality, percentages of yellow, red and blue (Glories, 1984).

Acid and total polysaccharides were quantified by the colorimetric method described by Segarra, Lao, López-Tamames, and De La Torre-Boronat (1995) and were expressed in mg/l of galacturonic acid and glucose, respectively. Neutral polysaccharides

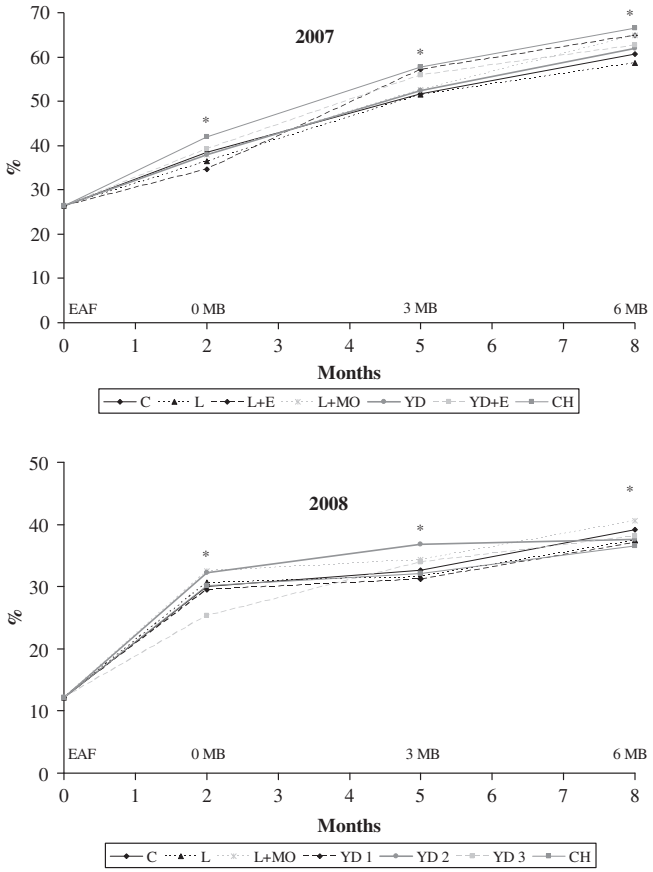

Fig. 3. Evolution of the percentage of polymeric anthocyanins of wines elaborated in each vintage. EAF: end of the alcoholic fermentation; 0 MB: end of the treatment and the malolactic fermentation; 3 MB: 3 months in barrel; 6 MB: 6 months in barrel. The asterisk indicates statistically significant differences for $\alpha = 0.05$.

Table 2
Tartaric esters and flavonols (mg/l) of wines elaborated in each vintage.

	C	L	L + E	L + MO	YD	YD + E	CH
2007							
Tartaric esters							
EAF	238	238	238	238	238	238	238
0 MB	236 b	213 a	214 a	238 b	229 ab	233 b	246 b
3 MB	242 b	232 a	232 a	242 b	237 ab	237 ab	236 ab
6 MB	243 b	232 ab	229a	243 b	235 ab	239 ab	241 ab
Flavonols							
EAF	167	167	167	167	167	167	167
0 MB	150 c	123 a	121 a	154 c	140 b	147 bc	149 bc
3 MB	150 a	150 a	152 a	149 a	152 a	156 a	152 a
6 MB	149 a	142 a	143 a	152 a	143 a	150 a	152 a
	C	L	L + MO	YD1	YD2	YD3	CH
2008							
Tartaric esters							
EAF	306	306	306	306	306	306	306
0 MB	280 d	272 a	278 bcd	274 abc	279 cd	273 ab	278 bcd
3 MB	284 b	275 a	283 ab	275 a	280 ab	279 ab	276 ab
6 MB	285 b	283 b	294 c	285 b	283 b	274 a	283 b
Flavonols							
EAF	215	215	215	215	215	215	215
0 MB	191 b	182 a	187 ab	184 a	191 b	187 ab	191 b
3 MB	189 b	185 ab	185 ab	187 ab	180 a	182 ab	187 ab
6 MB	194 bc	192 b	199 c	196 bc	194 bc	182 a	192 b

Values with different letter indicate statistically significant differences ($p < 0.05$).
EAF (wine at the end of the alcoholic fermentation); 0 MB (end of the treatment and malolactic fermentation; 3 MB (3 months in barrel) and 6 MB (6 months in barrel).

were calculated as the difference between total and acid polysaccharides.

Proteins were determined using the method described by Bradford (1976) and the results were expressed in mg/l of bovine serum albumine (BSA).

The gelatine index, which evaluates the percentage of tannins that are able to react with proteins (astringent tannins) and ethanol index, thereby determining the percentage of tannins that are combined with polysaccharides were also established (Ribéreau-Gayon et al., 2003).

All spectrophotometric measurements were carried out in quartz cuvettes in a UV–vis spectrophotometer (Shimadzu serie UV-1700 pharmaspec, China).

2.4. Sensory analysis

The sensory analysis was carried out by a tasting panel made up of 12 persons, all expert tasters from the Regulatory Councils of various Spanish Origin Designations and wineries. These tasters defined the descriptors used in this sensory analysis, according to the methodology described in González-Sanjosé, Ortega-Heras, and Pérez-Magariño (2008) and were then trained to quantify them using structured numerical scales. This training was carried out in accordance with UNE-87-020-93 Norm (ISO 4121:1987).

A structured numerical scale of seven points was used, with one representing absence of sensation and seven a very high intense perception.

The wines were tasted after the treatments and malolactic fermentation and after 6 months in barrels.

2.5. Statistical analysis

All the data were treated applying the variance analysis (ANOVA). The LSD (Least Significant Difference) test determines statistically significant differences between the means. Confidence intervals of 95% or significant level of $\alpha = 0.05$ were used.

All the statistical analyses were carried out using the Statgraphics Plus 5.0 statistical package.

3. Results and discussion

3.1. Effect on the classic oenological parameters

Classic oenological parameters were analysed to study the effect of the different techniques assayed on these parameters. The data ranges of these parameters are: pH between 3.5 and 3.6, total acidity between 5.1 and 5.5 g/l of tartaric acid, alcoholic degree between 12.5 and 13.0, volatile acidity between 0.5 and 0.7 mg/l of acetic acid and potassium average of 1250 mg/l in wines from 2007 vintage; and pH between 3.6 and 3.7, total acidity between 4.9 and 5.4 g/l of tartaric acid, alcoholic degree between 13.7 and 14.0, volatile acidity between 0.4 and 0.5 mg/l of acetic acid and potassium average of 1250 mg/l in wines from 2008 vintage. No statistically significant differences were detected between the treatments in none of the vintages. Several studies published on the use of different vinification methods (micro-oxygenation, maceration with chips, etc.) (Rodríguez-Bencomo et al., 2010; Sánchez-Iglesias, González-Sanjose, Pérez-Magariño, Ortega-Heras,

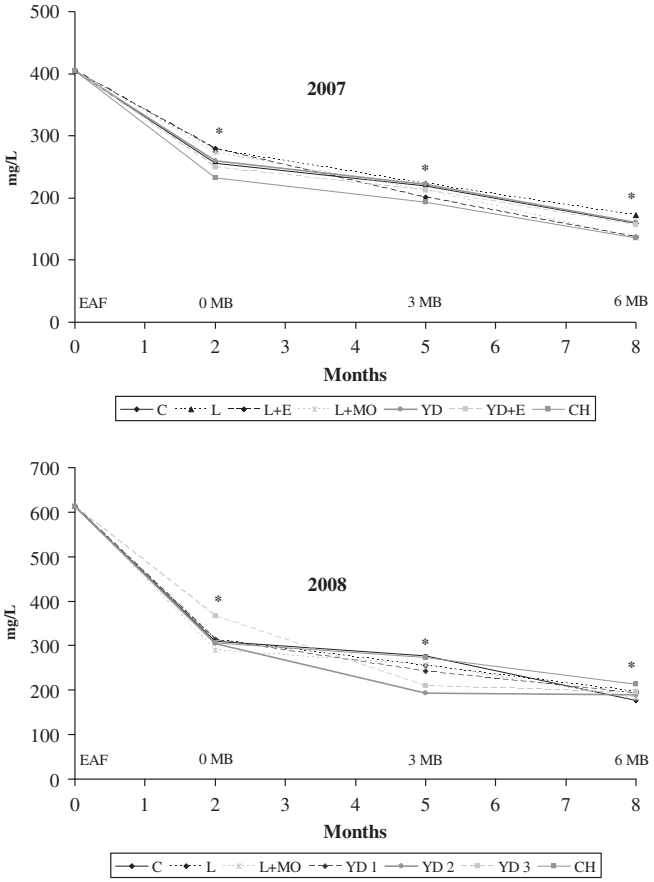

Fig. 4. Evolution of glucoside anthocyanin concentration of wines elaborated in each vintage. EAF: end of the alcoholic fermentation; 0 MB: end of the treatment and the malolactic fermentation; 3 MB: 3 months in barrel; 6 MB: 6 months in barrel. The asterisk indicates statistically significant differences for $\alpha = 0.05$.

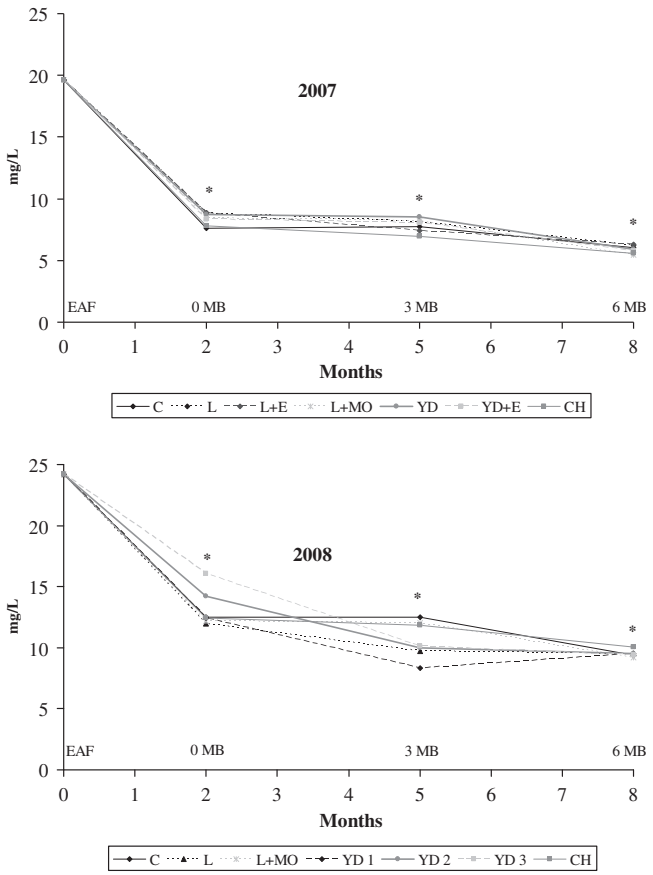

Fig. 5. Evolution of acetic anthocyanin concentration of wines elaborated in each vintage. EAF: end of the alcoholic fermentation; 0 MB: end of the treatment and the malolactic fermentation; 3 MB: 3 months in barrel; 6 MB: 6 months in barrel. The asterisk indicates statistically significant differences for $\alpha = 0.05$.

& González-Huerta, 2009) did not find an effect of their application on the pH value, total acidity or alcoholic degree either. Furthermore, it should be pointed out that the evolution of the oenological parameters quantified was similar in all the treatments studied.

3.2. Effect on the phenolic families studied

Fig. 2 shows the evolution of the various phenolic families analysed in both vintages, with statistically significant differences being detected in almost all cases. In 2007, the total polyphenol concentration decreased slightly in all wines during the treatment period but no statistically significant differences were found between them. During the ageing in barrels, the polyphenol concentration increased slightly due to the extraction of these compounds from the wood. In 2008, the same effects were observed, although in this case, statistically significant differences were found throughout the period of study. In both vintages, after 6 months in barrel, the CH and control wines presented the highest concentrations, whilst wines treated with lees and YD showed a lower polyphenol concentration than the control wines. This is probably attributable to the adsorption of some of these polyphenols on the dead yeasts or yeast derivative products or the interaction of several phenols with the compounds released into the wine such as mannoproteins and polysaccharides from lees to YD. These results agree with those obtained by other authors, which revealed the capacity of the yeast to retain or adsorb different wine phenolic compounds (Guadalupe et al., 2007; Lizama, Rodríguez, Álvarez, García, & Aleixandre, 2006; Mazauric & Salmon, 2005, 2006) and to form mannoprotein–polyphenol colloidal complexes, which produce less astringency, and a greater, roundness and softness in mouth (Feuillat et al., 2001; Fuster & Escot, 2002; Guadalupe & Ayestarán, 2008; Guadalupe et al., 2007, 2010; Poncet-Legrand, Doco, Williams, & Vernhet, 2007; Riou et al., 2002; Saucier, Glories, & Roux, 2000; Vidal et al., 2004b; Wolz, 2005). The changes observed in catechins were similar to those of the total polyphenols, although the differences detected were lower. As with the total polyphenols, the decrease in catechins during treatment was stronger in 2008 than in the 2007 vintage.

As was expected, the anthocyanin concentration also decreased during treatment and ageing time (Fernández de Simón, Hernández, Cadahía, Dueñas, & Estrella, 2003; Pérez-Magariño & González-Sanjosé, 2004; Revilla & González-Sanjosé, 2001; Wang, Edgard, & Shrikhande, 2003). Statistically significant differences were found between the various treatments in both vintages and throughout the time period. In the case of the 2007 vintage this decrease was linear during treatment and ageing, although in the second vintage the decrease was more important during treatment, and lesser during ageing. This decrease was lower in the wines treated with lees and YD, and the anthocyanin concentration of these wines at 6 months of barrel ageing was higher than that of the control and the other treated wines. This was observed in both vintages.

As detected in other earlier researches (Cano-López et al., 2006, 2008; Cano-López et al., 2010; Pérez-Magariño et al., 2007; Sánchez-Iglesias et al., 2009), micro-oxygenation reduced the anthocyanin concentration.

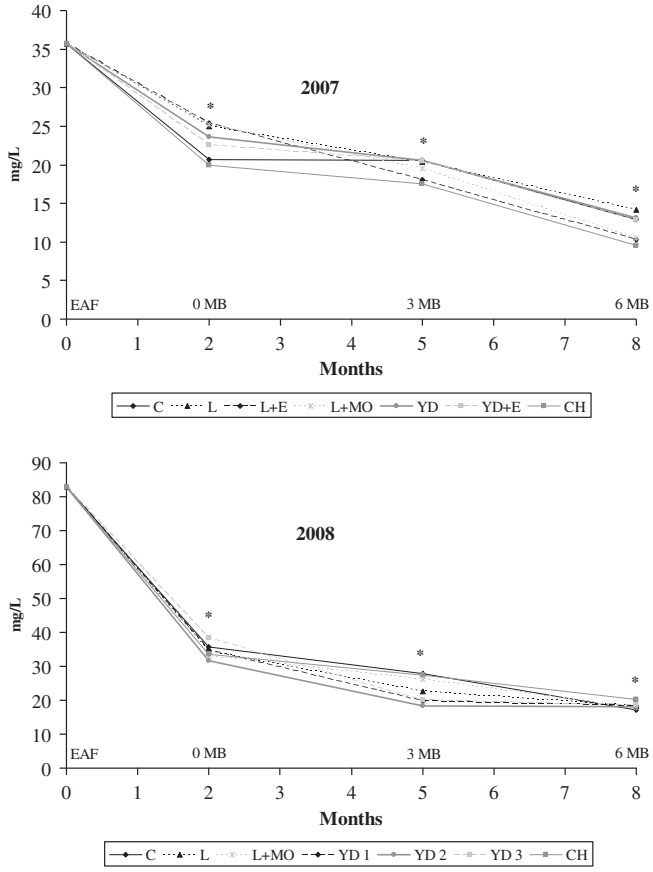

Fig. 6. Evolution of cinamic anthocyanin concentration of wines elaborated in each vintage. EAF: end of the alcoholic fermentation; 0 MB: end of the treatment and the malolactic fermentation; 3 MB: 3 months in barrel; 6 MB: 6 months in barrel. The asterisk indicates statistically significant differences for $\alpha = 0.05$.

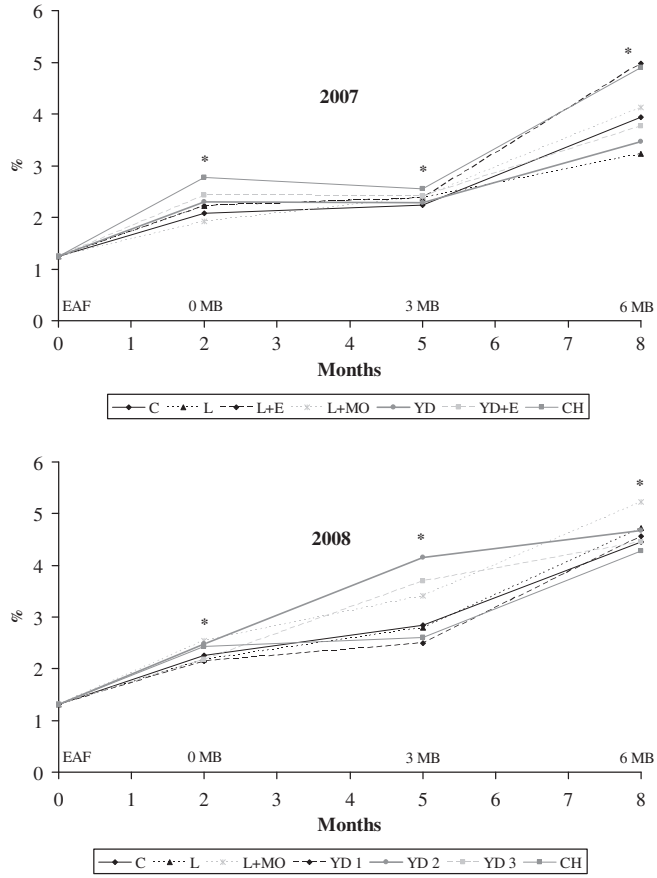

Fig. 7. Evolution of percentage of new anthocyanin pigments of wines elaborated in each vintage. EAF: end of the alcoholic fermentation; 0 MB: end of the treatment and the malolactic fermentation; 3 MB: 3 months in barrel; 6 MB: 6 months in barrel. The asterisk indicates statistically significant differences for $\alpha = 0.05$.

Table 3
Percentage of blue and red notes and values of colour intensity (CI) of wines elaborated in each vintage.

	C	L	L + E	L + MO	YD	YD + E	CH
2007							
% Blues							
EAF	9.1	9.1	9.1	9.1	9.1	9.1	9.1
0 MB	10.5 a	11.1 c	11.3 d	10.6 a	11.2 c	11.0 b	10.4 a
3 MB	11.0 a	11.2 b	11.8 d	11.0 a	11.5 c	11.5 c	11.8 d
6 MB	12.0 c	11.8 a	12.5 f	12.0 e	11.9 b	12.0 d	12.6 g
% Reds							
EAF	60.4	60.4	60.4	60.4	60.4	60.4	60.4
0 MB	57.2 e	52.7 b	52.0 a	57.1 e	53.0 c	54.1 d	57.2e
3 MB	55.2 e	54.7 bc	54.8 d	54.8 cd	54.5 ab	54.6 ab	54.5 a
6 MB	53.8 e	53.8 e	53.3 b	53.4 c	53.5 d	53.3 c	53.1 a
CI							
EAF	15.2	15.2	15.2	15.2	15.2	15.2	15.2
0 MB	11.6 e	10.2 b	10.0 a	12.0 g	10.9 c	11.6 d	11.8 f
3 MB	10.9 b	10.4 a	11.7 f	11.3 d	11.1 c	11.4 e	11.9 g
6 MB	11.8 b	11.5 a	12.5 f	12.4 e	11.9 c	12.1 d	13.0 g
	C	L	L + MO	YD1	YD2	YD3	CH
2008							
% Blues							
EAF	7.9	7.9	7.9	7.9	7.9	7.9	7.9
0 MB	11.6 c	11.5 b	12.0 e	11.5 b	12.3 f	11.3 a	11.8 d
3 MB	11.8 bc	11.7 ab	11.9 cd	11.6 a	12.0 d	11.9 bcd	11.7 abc
6 MB	12.4 b	12.0 a	12.3 b	12.0 a	12.3 b	12.0 a	12.0 a
% Reds							
EAF	66.9	66.9	66.9	66.9	66.9	66.9	66.9
0 MB	58.0 e	58.1 e	56.7 b	57.9 e	54.7 a	57.4 c	57.7 d
3 MB	56.3 b	56.4 bc	55.9 a	56.6 c	55.8 a	55.9 a	56.3 b
6 MB	55.1 a	55.7 c	55.2 a	55.8 c	55.6 b	55.5 b	55.7 c
CI							
EAF	20.1	20.1	20.1	20.1	20.1	20.1	20.1
0 MB	15.4 c	15.2 b	16.2 e	15.4 c	15.8 d	14.9 a	15.9 d
3 MB	14.4 a	14.7 a	15.3 b	14.7 a	15.7 c	15.2 b	14.6 a
6 MB	15.5 d	14.9 a	15.7 d	15.1 b	15.2 bc	15.3 c	14.9 a

Values with different letter indicate statistically significant differences ($p < 0.05$).
EAF (wine at the end of the alcoholic fermentation); 0 MB (end of the treatment and malolactic fermentation; 3 MB (3 months in barrel) and 6 MB (6 months in barrel).

At the end of the ageing process, the wines macerated with chips showed lower (2007 vintage) or similar (2008 vintage) concentrations of anthocyanins in comparison with the control wines.

No clear effect of β-glucanase enzymes was found for any of the phenolic families discussed above.

The tartaric esters and flavonols also decreased during treatment, especially in the case of the 2008 vintage, although they remained stable during ageing in both vintages (Table 2). The different treatments slightly modified the concentration of these compounds at the end of the treatment. Thus, in the 2007 vintage, statistically significant differences were only found at the end of the treatment. As for the 2008 vintage, after the ageing period, the wine treated with YD3 showed the lowest concentration of tartaric esters and flavonols and the L + MO the highest ones. Consequently, the lees, YDs, glucanase enzymes, chips and micro-oxygenation treatment did not affect the composition of this type of phenols. For the rest of the wines studied, no statistical significant differences were detected.

As expected, in both vintages the percentage of polymeric anthocyanins (Fig. 3) increased during the treatment and ageing time, and statistically significant differences were found between treatments. These compounds are formed by the condensation of anthocyanins with other wine compounds and the new polymeric compounds formed stabilized the wine colour (De Freitas, Carvalho, & Mateus, 2003). In the case of the 2007 vintage, after treatments, all the experiments carried out seem to favour the polymerisation process of anthocyanins, which was maintained after 6 months in barrels. The only exceptions to this were the wines with lees, which showed lower concentrations of these compounds than the control wine. It should be pointed out that the wines macerated with chips showed the highest concentration of polymeric anthocyanins, followed by the micro-oxygenated wines and those aged on lees with β-glucanase enzymes.

In 2008, the differences found were lower, and only a clear positive effect on the formation of polymeric anthocyanins was detected in the wines treated with lees and micro-oxygenation.

Therefore, it can be claimed that ageing on lees with micro-oxygenation favours the polymerisation reactions between anthocyanins and other phenols and later the colour stability, as has also been observed by Sartini, Arfelli, Fabiani, and Piva (2007) and other authors in experiments without lees (Cano-López et al., 2008; Pérez-Magariño et al., 2007; Sánchez-Iglesias et al., 2009). However, the treatment with lees only showed lower concentrations of these compounds than control wines. On the other hand, the yeast derivatives used in the 2008 vintage showed different effects, probably due to the varying composition of these products, and no conclusions could be drawn.

3.3. Effect on the anthocyanin compounds quantified by HPLC

The individual analysis of the anthocyanins by HPLC revealed that the concentration of monomeric or free anthocyanins, glucoside, acetic and cinnamic, showed the same trend in all wines (Figs. 4–6). These compounds decreased during the treatment and ageing period due to the oxidation and polymerisation reactions that may occur. In the case of the 2007 vintage, after treatment, all the wines treated showed higher levels of glucoside concentration, acetic and cinnamic anthocyanins than the control wines, with the exception

Table 4
Protein concentration (mg/l of BSA[a]) and values (%) of the gelatine and ethanol indexes of wines elaborated in each vintage.

	C	L	L + E	L + MO	YD	YD + E	CH
2007							
Proteins							
EAF	1149	1149	1149	1149	1149	1149	1149
0 MB	1391 bc	1439 c	1410 bc	1278 a	1354 ab	1369 b	1398 bc
3 MB	1143 b	1096 ab	1108 ab	1050 a	1099 ab	1069 ab	1250 c
6 MB	1129 bcd	1106 bc	1065 ab	1175 cd	1016 a	1055 ab	1179 d
Gelatin index							
EAF	43.4	43.4	43.4	43.4	43.4	43.4	43.4
0 MB	29.7 a	35.3 b	38.5 cd	36.4 bc	29.2 a	30.0 a	40.1 d
3 MB	33.3 bc	30.8 a	33.3 bc	34.8 c	32.1 ab	34.9 c	30.9 a
6 MB	45.1 bc	40.5 a	42.2 ab	41.2 a	48.2 d	47.7 cd	42.4 ab
Ethanol index							
EAF	18.1	18.1	18.1	18.1	18.1	18.1	18.1
0 MB	21.4e	13.2 c	12.4 bc	15.2 d	7.6 a	11.5 b	8.9 a
3 MB	7.6 d	4.9 b	3.9 a	5.6 c	3.7 a	5.1 bc	7.3 d
6 MB	10.0 d	5.8 a	6.5 b	9.9 d	5.9 a	7.0 b	8.3 c
	C	L	L + MO	YD1	YD2	YD3	CH
2008							
Proteins							
EAF	1329	1329	1329	1329	1329	1329	1329
0 MB	1166 ab	1194 bc	1097 a	1207 bc	1241 c	1216 bc	1228 bc
3 MB	1268 a	1290 a	1275 a	1375 b	1275 a	1284 a	1292 a
6 MB	1144 a	1152 a	1119 a	1212 bc	1214 c	1232 c	1155 ab
Gelatin index							
EAF	42.9	42.9	42.9	42.9	42.9	42.9	42.9
0 MB	50.1 d	44.9 bc	47.5 c	46.5 c	42.4 ab	41.8 a	51.6 d
3 MB	41.5 bc	36.6 a	42.1 cd	44.7 de	38.6 ab	47.0 e	41.6 bc
6 MB	60.1 a	69.2 cd	65.6 b	67.3 bc	66.5 bc	72.2 de	72.4 e
Ethanol index							
EAF	8.8	8.8	8.8	8.8	8.8	8.8	8.8
0 MB	14.7 e	12.7 d	9.6 b	9.2 b	10.9 c	8.0 a	12.5 d
3 MB	9.0 bc	8.4 b	11.6 d	13.0 e	9.6 c	10.8 d	5.2 a
6 MB	8.3 bc	8.3 c	10.6 d	6.4 a	7.1 ab	8.8 c	8.0 bc

Values with different letter indicate statistically significant differences ($p < 0.05$).
EAF (wine at the end of the alcoholic fermentation); 0 MB (end of the treatment and malolactic fermentation; 3 MB (3 months in barrel) and 6 MB (6 months in barrel).
[a] BSA: Bovine Serum Albumine.

of the wines treated with chips. However, after the ageing time, the CH, L + E and L + MO wines showed a significantly lower concentration of monomeric anthocyanins than the rest of wines and only the wines treated with lees presented a higher content of these compounds than the control wines. These results agree with the lower values of total anthocyanins found in these wines previously discussed, and with their higher percentage of polymeric anthocyanins than in the rest of wines. Fewer differences were observed in the 2008 vintage. After treatment and ageing time, all the wines treated showed higher monomeric anthocyanins than the control wines. Therefore, in both vintages, no adsorption of anthocyanins on compounds released by lees or yeast derivatives (mannoproteins, polysaccharides, etc.) in the wines treated was observed. These results contrast with those of other authors who found an adsorption of these compounds by lees (Guadalupe et al., 2007; Lizama et al., 2006; Mazauric & Salmon, 2005, 2006). The new anthocyanin pigments formed by direct condensation, anthocyanin-flavanol and anthocyanin-flavanol mediated by ethyl bridges, the pyruvate anthocyanins, the malvidin-3-glucoside vinylphenol and the malvidin-3-glucoside vinylcatechol increased or remained constant until the end of barrel ageing. In order to summarise the results obtained, all these compounds were added and the percentage of these new pigments was shown (Fig. 7). The differences found between wines after treatment increased after the ageing time. In the 2007 vintage, at the end of ageing, the wines treated with CH, L + E and L + MO showed a higher percentage of new pigments than the control wines, results that are in keeping with the lower concentrations of free and total anthocyanins found in these wines. In the 2008 vintage, only the treatment with L + MO increased these compounds. Treatment with lees or yeast derivatives does not favour the formation of these new anthocyanin pigments, which are more stable. It would therefore appear that the use of commercial yeast derivatives does not favour the formation of new pigments that could improve the colour stability of red wines.

3.4. Effect on the colour wines

The detailed results found for the polymeric anthocyanins are well-correlated with the values of colour parameters (Table 3). Thus, in the 2007 vintage, all wines showed higher colour intensity and blue notes during ageing in barrel than the control wine with the sole exception of the wines aged on lees. The wine macerated with chips showed the highest values of these parameters. In the 2008 vintage, only CH, L + MO and YD-2 wines presented higher colour intensity and blues notes than the control wines at the end of the treatment. After the ageing time, no significant differences were found. These results agree with the data obtained by other authors (Guadalupe & Ayestarán, 2008; Guadalupe et al., 2007; Palomero et al., 2009a), who also failed to observe an improvement in colour intensity and stability of wines using mannoproteins or β-glucanase enzymes with lees. Some authors suggest that polysaccharides and mannoproteins can interact with anthocyanins and tannins, thereby preventing their precipitation and improving colour stability (Escot et al., 2001; Feuillat et al., 2001; Francois et al., 2007; Fuster & Escot, 2002; Saucier et al., 2000), whilst other authors (Doco, Williams, & Cheynier, 2007;

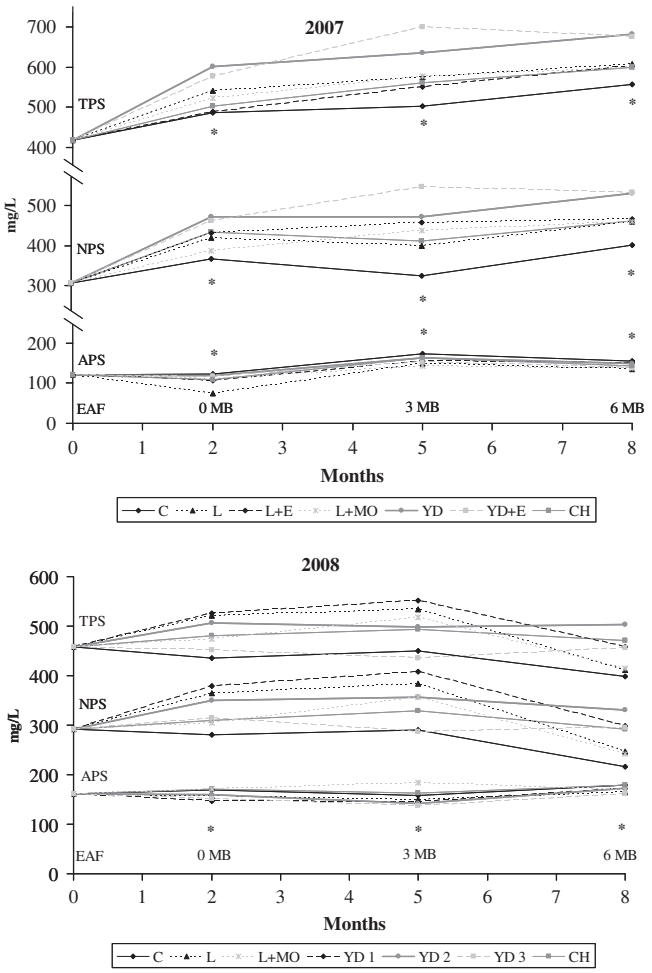

Fig. 8. Evolution of acid (APS), neutral (NPS) and total (TPS) polysaccharide concentration of wines elaborated in each vintage. EAF: end of the alcoholic fermentation; 0 MB: end of the treatment and the malolactic fermentation; 3 MB: 3 months in barrel; 6 MB: 6 months in barrel. The asterisk indicates statistically significant differences for $\alpha = 0.05$.

Poncet-Legrand et al., 2007) indicate that polysaccharides can act as colour stabilizers or enhance the precipitation of the phenolic polymers, causing a loss of colour, depending on their molecular weight, adsorbent character, charge and structure. In the model solution they also found that low molecular weight polysaccharides stabilized polyphenol aggregates. In our study however, the use of YD-3, which is a product with low molecular weight defined by the supplier as "free mannoproteins" did not produce colour stabilization in wines.

3.5. Effect on the protein concentration and the gelatine index

The analysis of proteins (Table 4) showed some statistically significant differences between the various treatments in both vintages. In the 2007 vintage, the proteins decreased slightly during ageing. The treatments did not modify their content to any considerable degree and only the use of yeast derivatives affected the protein content, although this depended on the product used. The yeast derivative used in the 2007 vintage therefore reduced the protein values, in contrast to the ones used in the 2008 vintage, which slightly increased their content at the end of the ageing period. However, in red wines, protein stability is not as important as in white wines, because there are other compounds that can stabilize the proteins. Therefore, the little differences found between treatments are not significant in the protein stability process in red wines.

As Table 4 shows, no positive effects of the various treatments studied on the gelatine index that indicates the percentage of astringent tannins were observed. Only in the case of the 2008 vintage, and immediately following treatment all the wines treated presented a lower gelatine index than the control wines, with the exception of wine treated with chips. During ageing, this index increased in all wines due to the extraction of phenolic compounds from the new barrels to the wines. Therefore, the polysaccharides generated by the treatments did not influence the protein–tannin aggregation, as no decrease in astringency was observed (related to the interactions between tannins and proteins).

These results do not agree with those obtained by other authors in various studies into red wines (Escot et al., 2001; Fuster & Escot, 2002). Escot et al. (2001) studied the effect of the polysaccharides released from three different yeast strains of *S. cerevisiae* in colour stability and wine astringency of red wines of the Pinot Noir variety, concluding that the wines treated with the three different yeast strains were less astringent because these wines showed a lower gelatine index and higher ethanol index than the control wines. Other studies carried out in model wine solutions have revealed the positive effect of some types of polysaccharides and mannoproteins on wine astringency, due to the action of these compounds as protective colloids which limit the autoaggregation of tannins, decreasing their reactivity with salivary proteins (Vidal et al., 2004a, 2004b). However, these authors point out that rhamnogalacturonans II are the main polysaccharides capable of preventing protein–tannin aggregation. However, another study carried out by Riou et al. (2002) indicated that only mannoproteins are able to prevent the tannin aggregation. It is therefore still unclear as to which type of polysaccharides is responsible for tannin aggregation.

3.6. Effect on the polysaccharide concentration and the ethanol index

As discussed above, the natural lees and yeast derivative products are mainly made up of polysaccharides (carbohydrates) and mannoproteins (glycoproteins). It was therefore necessary to evaluate the concentrations of polysaccharides. Fig. 8 shows the evolution of total (TPS), acid (APS) and neutral (NPS) polysaccharides in wines for each vintage. In 2007, the total and neutral polysaccharides showed a significant and progressive increase from the initial time (EAF) until the end of the oak barrel ageing in all treatments, including the control wine. This could indicate that these compounds remain in the wine in a colloidal state linked to other compounds or to the autolysis of the remaining dead yeast present in the wine. This increase was more significant between the stages of EAF and 0 MB, i.e. during the treatment time. After 6 months in barrel, the wines treated with YD and YD + E presented the highest concentrations of these compounds. In the case of the other wines, no statistically significant differences were found. In addition, the results obtained suggest that the addition of β-glucanase enzymes to the natural lees or with the commercial yeast derivatives does not produce a significant effect on the polysaccharide concentration, since no statistically significant differences were found between the treatments. In 2008, the increase of total and neutral polysaccharides was not as significant as in the previous vintage and it occurred mainly during treatment (Fig. 8). This fact could be due to the formation of unstable complexes between the polysaccharides and other phenolic compounds (Guadalupe & Ayestarán, 2007; Guadalupe et al., 2007). The YD-1 and YD-2 contribute to the wine with polysaccharides, while YD3, which commercial suppliers claim has an immediate effect, failed to show statistically significant differences with the control wine in terms of the total and neutral polysaccharide concentration at the end

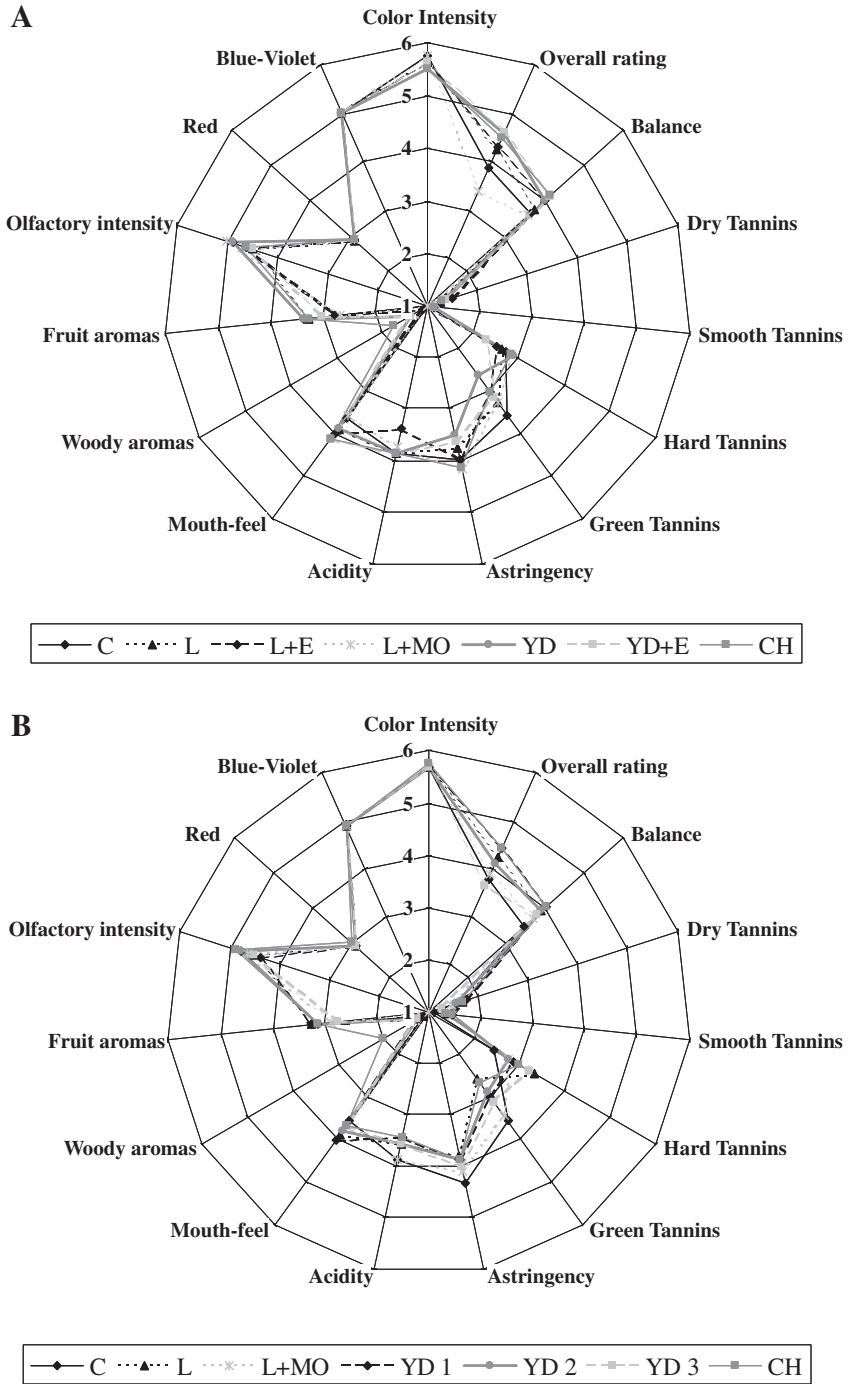

Fig. 9. Sensory analysis diagrams of the different wines elaborated at the end of the treatment and malolactic fermentation (0 MB) in 2007 (A) and 2008 (B) vintage.

of the treatment. However, YD3 showed higher concentrations than the control wine at the end of the ageing process. At this moment, the wine treated with chips also showed a higher concentration of TPS and NPS than the control wine. However, the wine aged on natural lees, with or without micro-oxygenation, showed concentrations of TPS similar to those of the control wine.

A number of authors have studied the evolution of the polysaccharides of wines aged on lees. Palomero, Morata, Benito, Calderón, and Suárez-Lepe (2009b) studied the ageing on lees of different strains of yeast in a model medium and observed a progressive increase in the concentration of polysaccharides over 142 days.

Other authors have also pointed out that the addition of mannoproteins to red wines during or after the alcoholic fermentation increases or maintains the concentration of neutral (mannoproteins) and total polysaccharides during barrel and bottle ageing (Guadalupe & Ayestarán, 2007, 2008; Guadalupe et al., 2007), although these effects can depend on the doses and product used.

As expected, the concentration of acid polysaccharides was more or less stable in both vintages and in all cases studied. In general, the control wine and wines treated with chips showed the highest concentrations of these compounds, mainly at the end of the treatment.

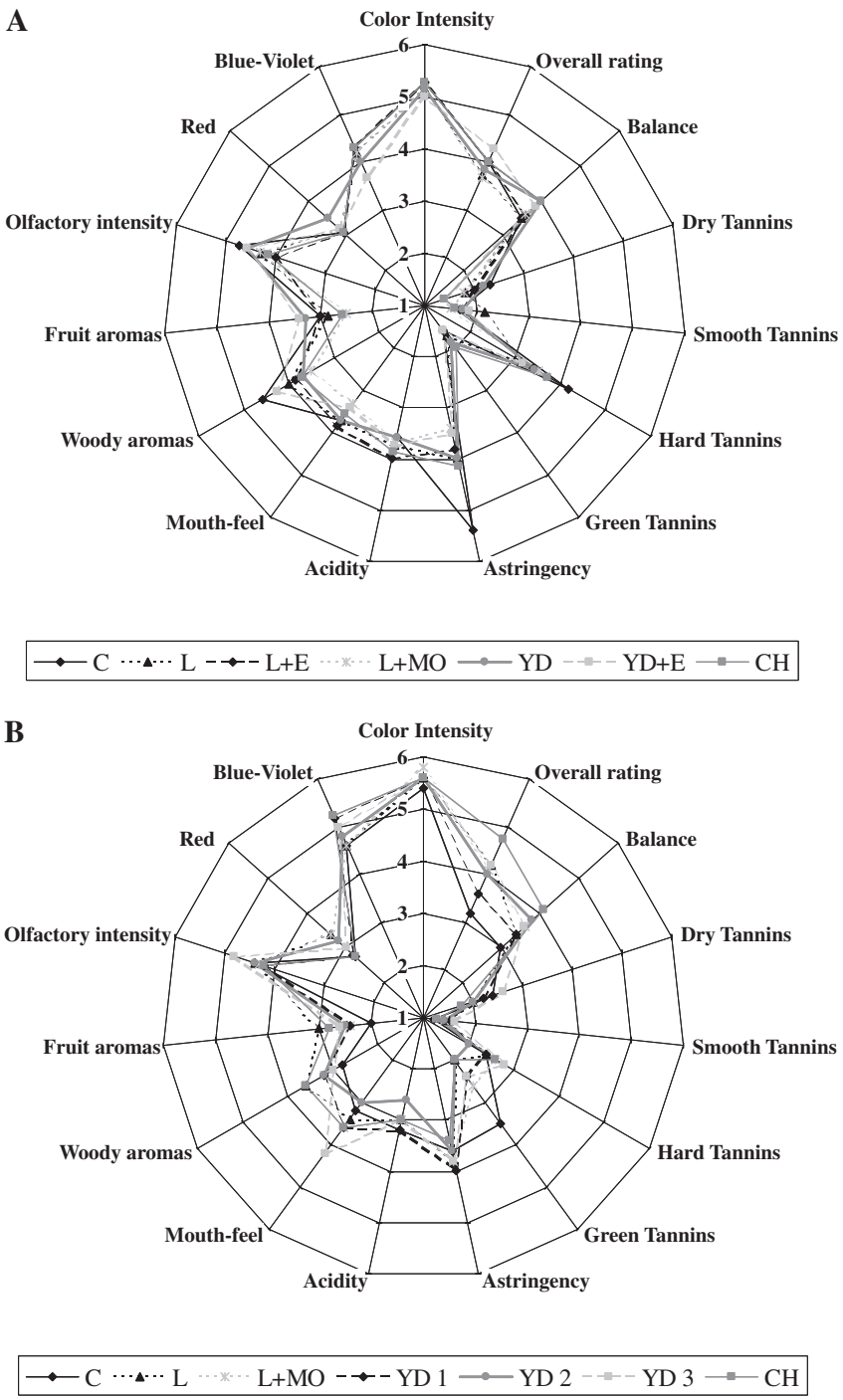

Fig. 10. Sensory analysis diagrams of the different wines elaborated at the end of the 6 months in barrel (6 MB) in 2007 (A) and 2008 (B) vintage.

The ethanol index (Table 4) determines the percentage of tannins combined with polysaccharides. In the case of both vintages, this index remained constant or slightly decreased over time. In the 2007 vintage, the highest values of this index once the ageing process was complete were found in the control wines, followed by the wines macerated with chips and the micro-oxygenated wine. However, in the 2008 vintage, at the end of the ageing, the highest concentration was found in the L + MO wine followed by the wine aged with lees and the one treated with YD-3. It is also important to highlight the fact that in this case, the wines treated with YD-1 and YD-2 showed the lowest percentage of this parameter. These results, together with those obtained for the total, acid and neutral polysaccharides, indicate that each commercial yeast derivative generates different polysaccharides of varying molecular size. Furthermore, they each displayed a varying tannin-binding capacity (De Freitas et al., 2003; Riou et al., 2002; Vidal et al., 2004a).

3.7. Effect on the sensorial characteristics

The sensory analysis did not show significant differences in the colour between the treated wines and the control wine in both vintages at the end of the treatment (Fig. 9). However, after ageing time, a number of slight differences were observed, especially in the case of the 2008 vintage: the wines treated with yeast

derivatives and chips showed higher blue tonalities than the control wines, although no statistically significant differences were found in colour intensity (Fig. 10).

The taster panel indicates that some of the wines had lower olfactory intensity and fruity aromas after treatment than the control wines (Fig. 9). This may be due to the interaction between volatile compounds and mannoproteins or polysaccharides released by the lees or yeast derivatives. Similar interactions have been observed by other authors in model wine solutions (Chalier, Angot, Delteil, Doco, & Gunata, 2007; Lubbers, Charpentier, Feuillat, & Voilley, 1994a; Lubbers, Voilley, Feuillat, & Charpentier, 1994b; Voilley, Beghin, Charpentier, & Peyron, 1991) and by this group in white and red young wines (Del Barrio-Galán, Sánchez-Iglesias, Ortega-Heras, González-Huerta, & Pérez-Magariño, 2010; Rodríguez-Bencomo et al., 2010). After 6 months of ageing, the wines treated with yeast derivatives in 2007, and all wines treated in 2008 showed greater fruity aromas than the control wines, which may indicate that the aromatic compounds retained are released over time (Fig. 10).

In the gustative phase, the taster panel indicates that wines from both vintages showed generally lower values of astringency and green tannins, and higher values of grassy, balance and overall punctuation than the control wines, especially those treated with yeast derivatives. After the ageing period, a significant increase in astringency and dry tannins in control wines was observed. In addition, in the case of the 2008 wines, it was shown that the control wines obtained the overall lowest scores and balance values. Amongst the different wines treated, no statistically significant differences were found in the gustative phase, apart from the fact that the 2008 vintage wine treated with chips obtained the highest values.

4. Conclusion

In summary, the results obtained in this study indicate that neither the traditional ageing on lees nor the use of commercial yeast derivatives seem to favour the formation of new pigments that stabilize wine colour. Only the application of lees and micro-oxygenation enhances the formation of new pigments that allows the intensity and blue notes of wines to be maintained during the barrel ageing process, although it is difficult to establish if this stability is due only to micro-oxygenation, or to the joint use of both techniques.

All the techniques studied released total and neutral polysaccharides, although the type and content of these compounds depended on the technique used, and the yeast derivative added, since each product has a different composition. No clear effect was observed with the use of β-glucanase enzymes.

However, from the sensorial point of view, some of the wines were better valued than the control wines. Further research should therefore be carried out in order to determine both the chemicals and reactions responsible for these sensorial changes.

The results obtained show that it is difficult to select the technique that allows us to obtain the best quality wine. The selection process should take into account the type of wine required as well as economic and winery management factors.

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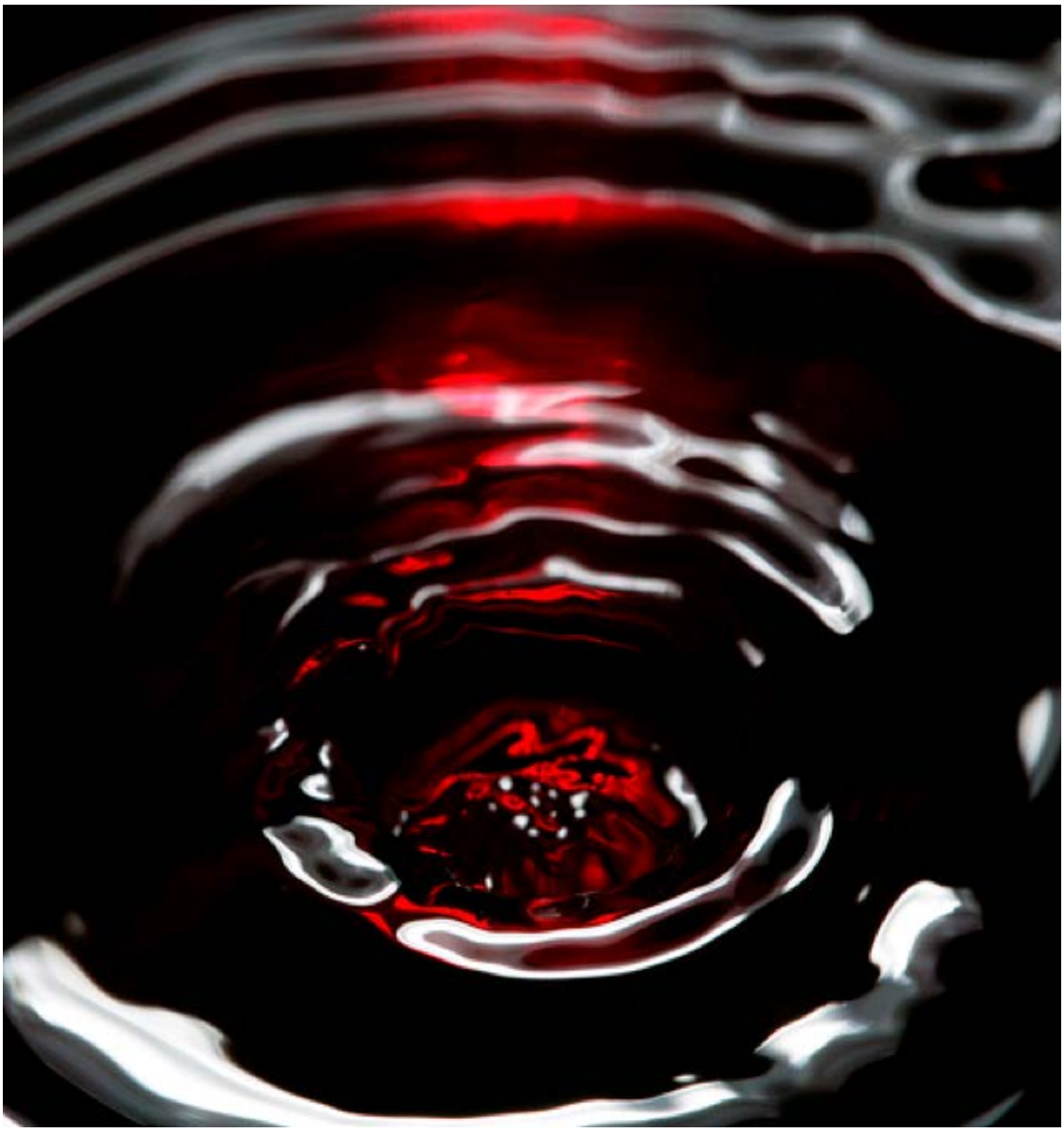

CAPÍTULO 5

Efecto de la crianza sobre lías y otras técnicas alternativas sobre los compuestos fenólicos de bajo peso molecular de un vino tinto envejecido en barrica

El trabajo desarrollado en este capítulo es una continuación del llevado a cabo en el capítulo anterior. En este caso se ha estudiado el efecto de las diferentes técnicas indicadas en el capítulo 4 sobre los compuestos fenólicos de bajo peso molecular.

El interés de este estudio está en que los compuestos que son liberados durante la crianza sobre lías, fundamentalmente las manoproteínas, pueden interactuar con los compuestos fenólicos del vino, disminuyendo su astringencia y aumentando su redondez, estructura y palatabilidad. Además, estas interacciones pueden favorecer la estabilización del color de los vinos tintos, previniendo la agregación de taninos y antocianos.

Además hay que tener en cuenta que son muy escasos, prácticamente inexistentes, los trabajos publicados sobre el efecto de las técnicas mencionadas anteriormente sobre la composición fenólica no antociánica de los vinos tintos. Por tanto, los resultados encontrados en este trabajo pueden ser de gran interés tanto para la comunidad científica como para los profesionales del sector.

Los resultados y conclusiones más destacados de este trabajo son los siguientes:

1- Los ácidos hidroxicinámicos fueron los compuestos fenólicos que más se vieron afectados por los tratamientos realizados. Los vinos tratados con chips de madera de roble sin tostar y con los preparados comerciales derivados de levadura mostraron mayores concentraciones de ácidos hidroxicinámicos libres que los vinos control.

Como era de esperar, los vinos macerados con chips de roble sin tostar presentaron las concentraciones más altas de ácido elágico, ya que este compuesto es extraído de la madera.

2- Durante el envejecimiento en barrica, todos los vinos evolucionaron de forma similar, independientemente del tratamiento aplicado.

3- Se encontró un fuerte efecto vendimia, siendo las diferencias encontradas entre los diferentes tratamientos realizados más importantes en la vendimia

de 2007 que en la de 2008. Esto puede ser debido a las diferencias en el contenido fenólico del vino de partida, que fue superior en la vendimia de 2008 que en la de 2007.

Sin embargo, en la vendimia de 2008, el efecto del tratamiento de microoxigenación sobre los compuestos fenólicos de bajo peso molecular fue más importante que en la vendimia de 2007, probablemente debido a que este tratamiento tuvo una duración más larga en la segunda vendimia.

4- En la vendimia de 2007, los resultados obtenidos en el análisis factorial pusieron de manifiesto que los tratamientos con derivados de levadura y con chips fueron los que produjeron mayores modificaciones en la concentración de los compuestos fenólicos de bajo peso molecular, especialmente en los ácidos hidroxicinámicos. Sin embargo, la crianza sobre lías apenas afectó a la concentración de estos compuestos.

Por otro lado, en la vendimia de 2008, el análisis factorial no mostró grandes diferencias entre los diferentes tratamientos ensayados especialmente durante la crianza en barrica. Solamente los vinos tratados con microoxigenación en combinación con la crianza sobre lías se diferenciaron claramente del resto de vinos tras el tratamiento y tras los 3 y 6 meses de envejecimiento. Este hecho parece indicar que estas diferencias fueron debidas principalmente al efecto de la microoxigenación sobre los compuestos fenólicos, y no a la combinación de la microoxigenación con la crianza sobre lías.

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Effect of the aging on lees and other alternative techniques on the low molecular weight phenols of *Tempranillo* red wine aged in oak barrels

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Abstract

The effect of different alternative techniques to the traditional aging on lees on the low molecular weight phenolic compounds of red wines was study as well as their evolution during the aging in oak wood barrels for six months. The study was carried out with *Tempranillo* red grapes from two consecutive vintages. The techniques assayed were the traditional aging on lees with or without the addition of exogenous β-glucanase enzymes, the use of yeast derivative preparations also with or without the addition of exogenous β-glucanase enzymes, the micro-oxygenation applied together with the aging on lees, and the use of non-toasted oak wood chips.

Hydroxycinnamic acids were the compounds most affected by these treatments, mainly in the wines treated with chips and commercial yeast derivative products, which showed higher concentrations of the free acids, compounds that play an important role in wine stabilization color since they can act as anthocyanin copigments.

The differences found between the assayed treatments were more important in the 2007 vintage than in the 2008. However, a more significant effect of micro-oxygenation in the 2008 vintage was observed, which could be related to the fact that in this vintage the treatment was longer. In the 2008 vintage, the differences between treatments decreased along the aging in barrel. This vintage effect could be associated to the differences in the phenolic concentration of the initial wines. In this sense more research should be done to corroborate this fact.

Key words: aging on lees, yeast derivative, oak chips, glucanase enzymes, red wine, phenolic compounds

1. Introduction

During aging on lees, some interesting metabolites as such as mannoproteins and glucans can be released into wines due to yeast autolysis. The compounds released can interact with wine phenolic compounds, decreasing their astringency [1] and/or acting as protective colloids, enhancing the color stability of red wines [1-3]. Furthermore, Riou et al. [4] and Poncet-Legrand et al. [5] observed in model wine solutions that some high molecular weight polysaccharides and mannoproteins prevented tannin aggregation. The hypothesis proposed by these authors to explain this fact is that this type of polysaccharides can bind proanthocyanidins to give rise to more stable aggregates that prevent their polymerization and precipitation, acting as protective colloids. The presence of these polysaccharide-tannin complexes can reduce astringency and increase roundness, structure and mouth-feel of wines [4-7]. Polysaccharides and mannoproteins can also contribute to wine color stabilization due to their capacity to interact with tannins and anthocyanins preventing their aggregation and precipitation [7].

However, one of the disadvantages of aging on lees is that consumes oxygen. Oxygen plays an important role in the condensation reactions between flavonoids mediated by acetaldehyde and/or glioxylic acid [8-9], and in the cicloaddition reactions [10]. As result of these polymerization and condensation reactions, new polymeric structures are formed that enhance wine sensorial characteristics such as color stability and astringency. Therefore, the consumption of oxygen can reduce the condensation and polymerization reactions between phenolic compounds, and also favors the formation of reduction aromas. The application of the micro-oxygenation technique together with the traditional aging on lees could avoid these problems since this technique consists in the addition of small and controlled amounts of oxygen [11]. In this way, micro-

oxygenation combined with aging on lees could reduce the presence of reductive aromas [12-13] and could favor the formation or more stable colored pigments improving the color stability of red wines over the time [14-15]. The positive effects of this technique on wine color and the formation of new polymeric pigments have been corroborated by several authors [10, 14-17].

On the other hand, aging on lees can be improved by the addition of exogenous β-glucanase enzymes to wines, accelerating the yeast autolysis process, and favoring the release of polysaccharides (glucans) and mannoproteins [18].

Nowadays, the use of commercial yeast derivative preparations from *Saccharomyces cerevisiae* has increased for the last three years. The goal of these preparations is to release the mannoproteins and glucans from the yeast cell walls more quickly into wines, reducing in this way the time needed to obtain wines with physico-chemical and sensory characteristics similar to those aged on lees. Some authors have studied the effect of these products on the volatile compounds [19-21], the color and anthocyanin pigments of wines [22-24]. However, few published papers have focused on studied the effect of commercial yeast preparations on the non anthocyanin phenolic compounds of red wines. Only Guadalupe and Ayestarán [22] and Guadalupe et al. [23] studied the effect of commercial mannoproteins on some non anthocyanin phenolic compounds of red wines such as hydroxycinnamic acids and flavanols.

Other vinification technique that gives rise to red wines with sensory characteristics similar to the wines aged on lees is the use of non-toasted wood chips after alcoholic fermentation and before the beginning of the malolactic fermentation. This fact is due to the polysaccharides [25-26], and the phenolic compounds that are released into wines from wood, and which can also interact with wine phenolic compounds.

Non anthocyanin phenolic compounds of wines play also an important role in color and taste characteristics of wines. They can be classified as phenolic acids (hydroxybenzoic and hydroxycinnamic acids and their derivatives), flavanols, flavonols, stilbenes and phenolic alcohols. The hydroxycinnamic acids, flavanols and flavonols can act as copigments in copigmentation reactions with anthocyanins, improving in this way the color of mainly young red wines [27-29].

Besides, in the last years, the non-anthocyanin phenolic compounds, especially flavonols and stilbenes, have been recognized by their importance in the health human due to their high antioxidant activity, anticarcinogenic potential, anti-inflammatory properties and because they can prevent cardiovascular diseases [30-34].

For all these reasons, the aim of this work was to study the effect of different alternative techniques to the traditional aging on lees on the low molecular weight (non anthocyanin) phenolic compounds of red wines and to study their evolution during the aging in oak wood barrels for six months. The study was carried out in two consecutive vintages. The techniques assayed were the traditional aging on lees with or without the addition of exogenous β-glucanase enzymes, the use of yeast derivative preparations also with or without the addition of exogenous β-glucanase enzymes, the micro-oxygenation applied together with the aging on lees, and the use of non-toasted oak wood chips.

2. Material and methods

2.1. Winemaking process and treatments

The study was carried out using *Tempranillo* red grapes from Cigales Designation of Origin sited in the Autonomous Community of Castilla y León in the North of Spain, from two consecutive vintages (2007 and 2008). The red wines were elaborated in the

research winery of the Enological Station of Castilla y León, following the traditional red winemaking process.

The grapes (about 12,500 kg) were harvested manually on the optimum harvest date, based mainly on the relation sugar content (ºBrix) and total acidity, and transported to the Enological Station in 15-Kg-plastic boxes. The clusters were de-stemmed and crushed with minimum physical damage. The mass obtained, was slightly sulphited (0.04 g/L) and then transferred to five 2,600 L stainless steel tanks to undergo alcoholic fermentation at controlled temperature (25-28 ºC). Alcoholic fermentation was carried out through the inoculation with commercial yeast S*accharomyces cerevisiae* (Excellence sp, Lamothe-Abiet). Once the alcoholic fermentation was completed, the mass was pressed and the wines were kept in the tanks for 4 days to allow for the sedimentation of the gross lees. After this time, the wines were racked off and maintained in the tanks for 4-5 days to allow for the sedimentation of the fine lees. The base wine was then again racked off, homogenized and distributed into several 500 L tanks, except for the micro-oxygenation treatments in which special tanks were used. These tanks are 3 meters high, since at least this height is necessary to get a good dissolution of the oxygen applied with the micro-oxygenation. The wet fine lees decanted in the bottom of the tanks were colleted and used in the experiments with lees.

The experiments carried out in the 2007 and 2008 vintage are shown in Figure 1. In the case of the 2007 vintage, the following experiments were carried out in duplicate: control wines (without the addition of any product) (C); wines aged on lees (L); wines aged on lees and with the addition of commercial β-glucanase enzymes (L+E); wines aged on lees with micro-oxygenation (L+MO); wines with a commercial yeast derivative added (YD); wines with the same commercial yeast derivative and the

commercial β-glucanase enzymes added (YD+E); and wines with non-toasted French oak chips added (CH).

Considering the results obtained in the 2007 vintage, and those obtained in a previous study relating to the use of β-glucanase enzymes [21, 35, 36], and taking into account the high number and variety of commercial yeast preparations that are appearing in the market, different studies were carried out in the 2008 vintage. In this vintage, three commercial yeast derivative products with different composition, purity and effect on wines were studied **(Figure 1)**. The aging on lees combined with the micro-oxygenation and the use of non-toasted oak chips were also studied.

Table 1 shows the information provided by the commercial manufactures regarding the commercial products used in this study, and the doses applied, in accordance with the manufactures' recommendations.

Micro-oxygenation was carried out by means of a modular five-head VisiO2 micro-oxygenator (Oenodev, France). In the two vintages studied, the same doses were applied: 5 mL/L/month of O_2. However, in the first vintage the length of the treatment was 35 days and in the second vintage was 60 days.

Two batonnages per week were performed on each wine. The temperature was maintained at 15 ºC ± 1 ºC. All treatments lasted 60 days, with the exception of the YD3 product that was added just before the barrel aging, because according to its manufacture it is a soluble product with direct action.

After the treatments, all the wines were racked off and inoculated with a commercial preparation of *O. Oeni* (Viniflora, CHR Hansen, Denmark) to induce malolactic fermentation. After that, the wines were racked off into new American oak barrels with a medium–high degree of toasting (two barrels for each treatment and replicate, see Figure 1).

147 The samples were analyzed immediately after the malolactic fermentation (end of
148 treatment), and after three and six months of aging in barrels.

149 **2.2. Chemical reagents**

150 Gallic, syringic and *trans*-*p*-coumaric acids, *trans*-resveratrol and catechin were
151 provided by Sigma-Aldrich (Steinheim, Germany); protocatechuic, vanillic, ellagic and
152 *trans*-caffeic acids, tyrosol, tryptophol, myricetin and kaempferol by Fluka (Buchs,
153 Switzerland); epicatechin, ethyl gallate, syringetin-3-glucoside, and quercetin by
154 Extrasynthèse (Lyon, France).

155 The ethanol HPLC-grade was provided by Scharlau (Barcelona, Spain). Acetonitrile and
156 methanol HPLC-grade were from Lab Scan (Madrid, Spain) and the remaining reagents
157 from Panreac (Madrid, Spain). Water Milli-Q was obtained through a Millipore system
158 (Bedford, MA).

159 **2.3. Analytical methods**

160 Low molecular weight phenolic compounds were isolated and concentrated by a solid-
161 phase-extraction process (SPE) using the Oasis HLB cartridges (Waters, Mildforf,
162 Massachusetts, USA) as it was previously described by Pérez-Magariño et al. [37]. An
163 automatic SPE equipment was used (GX-271 Aspec, Gilson, USA).

164 The extracts obtained were filtrated through PVDF filters of 0.45 μm (Symta, Madrid,
165 Spain) and were analyzed with an Agilent-Technologies LC-DAD series 1100
166 (Germany). The chromatographic conditions and the quantification of phenolic
167 compounds were also established by Pérez-Magariño et al. [37].

168 **2.4. Statistical analyses**

169 All the data were treated applying the variance analysis (ANOVA) and the Least
170 Significant Difference test, which determines statistically significant differences
171 between the means. Confidence interval of 95% or significant level of $p = 0.05$ were

used. Factor analysis was applied in order to study the association of variables. Varimax rotation criterion was performed and only factors with eigenvalues greater than unity were selected.

All the statistical analyses were carried out using the Statgraphics Plus 5.0 statistical package.

3. Results and discussion

Tables 2 and 3 show the hydroxybenzoic and hydroxycinnamic acids and their derivatives quantified in the studied wines from 2007 and 2008 vintages, respectively.

Some differences were found between treatments that depended on the compound and the vintage. Thus in 2007, after the treatments, the wines treated with L and L+E presented in general, the highest content of hydroxybenzoic acids, except of ellagic and gallic acids. The wines treated with CH also showed higher concentrations of vanillic acid, ethyl gallate and ellagic acid than the control wines. The remaining assayed treatments did not modify the content of these compounds. However, in 2008, the wines treated with L+MO showed the lowest values of vanillic and syringic acids. The presence of CH also modified the wine composition in hydroxybenzoic acids, mainly increasing the content of ellagic acid. Neither the aging on lees nor the addition of YDs affected the content of these compounds in this vintage.

The increase in the ellagic acid concentration found in the wines treated with chips was expected since this acid can be transferred into wines by the hydroalcoholysis of oak wood [38-42].

The aging of wines in barrels produced an increase in the concentration of all the hydroxybenzoic acids in all the wines and in both vintages, and in general, the differences found between treatments just after the treatment were maintained during the aging.

197 *Trans*-caffeic and *trans*-*p*-coumaric acids and their respective tartaric esters (*trans*-
198 caftaric and *trans*-coutaric) were the most abundant hydroxycinnamic acids quantified
199 in wines from both vintages. A vintage factor was found in the effect of the different
200 treatments studied on these compounds. Thus, in 2007 after treatment, *trans*-caffeic,
201 *trans*-*p*-coumaric and *cis*-*p*-coumaric acid concentration was higher in all the treated
202 wines than in the control wines, except in the L+MO wines that showed similar values
203 than the controls. The wines treated with CH, YD and YD+E showed the highest values.
204 During barrel aging, *trans*-caffeic and *cis*-*p*-coumaric acid concentration increased in all
205 the wines, whereas *trans*-*p*-coumaric acid was maintained stable. In general, the
206 differences found after treatment in the free forms of hydroxycinnamic acids between
207 the treated wines were maintained. Regarding the hydroxycinnamic acid tartaric esters,
208 after treatment all the treated wines, with the only exception of the L+MO wines
209 showed lower concentration of these compounds than the control wines. The lowest
210 concentrations were found in the wines treated with YD, YD+E and CH.
211 During barrel aging, in general terms, the concentration of *trans*-caftaric, *trans*-coutaric,
212 *trans*-fertaric acids increased in all wines, being this increase more important for *trans*-
213 caftaric acid. The concentration of *cis*-coutaric acid kept relatively constant. After six
214 months in barrel, in general, all the treated wines presented also lower concentration of
215 these compounds than the control wines, with the exception of *trans*-fertaric acid. The
216 wines treated with YD, YD+E and CH continued showing the lowest values. The
217 increase of the content of *trans*-fertaric acid during barrel aging can be due to the
218 extraction of ferulic acid from wood. However, no explication has been found for the
219 increase of the hydroxycinnamic acids commented before. These results disagree with
220 those found by other authors in red wines who observed that the concentration of these
221 compounds decreased during the aging in oak barrels [22, 39].

In general, an opposite trend was observed between hydroxycinnamic acids and their esterified forms. Thus, the treated wines that showed higher hydroxycinnamic acids in their free form presented lower concentration of hydroxycinnamic acids in their esterified form. The higher content of free hydroxycinnamic acids can be due to the hydrolysis of their esterified derivatives or to the hydrolysis of cinnamoyl glucoside anthocyanins [43-45]. This effect was more important in the wines treated with YD, YD+E and CH. Only a scientific work was found that studied the addition of commercial mannoproteins during alcoholic fermentation [22] on the hydroxycinnamic acid concentration. These authors also showed an increase of free hydroxycinnamic acids and a decrease of their sterified form (*trans* isomers).

In 2008, after treatment, *trans*-caffeic, *trans*-*p*-coumaric, and *cis*-*p*-coumaric acid concentration was higher in the wines treated with L+MO, YD-1 and CH than in the control wines, showing the wines treated with L+MO the highest values. On the other side, the wines treated with L and YD-3 showed the lowest content of *trans*-caffeic and *trans*-*p*-coumaric acids, and those treated with L and YD-2 the lowest of *cis*-*p*-coumaric acid.

During barrel aging, *trans*-caffeic and *trans*-*p*-coumaric acid concentration decreased in all the wines, and after six months only the wines treated with L+MO showed higher concentration than the control wines. The *cis*-*p*-coumaric acid concentration increased in all the wines during the aging in barrel, especially after three months. However, only the wines treated with L+MO and YD-1 showed higher content than the control wines after six months in barrel, whereas the remaining treated wines maintained similar content to the control ones.

As occurred in the 2007 vintage, the wines that showed higher content of hydroxycinnamic acid free forms had lower content of their tartaric esters. Thus, after

247 treatment, wines treated with L+MO, YD-1, and CH presented lower concentration of
248 their hydroxycinnamic acid tartaric esters than the control wines. The remaining treated
249 wines showed similar or higher content to the controls. As happened in the 2007 vintage
250 during aging in barrel, all hydroxycinnamic acid tartaric esters increased in all wines.
251 After six months in barrel, no statistically significant differences were found for the
252 *trans*-caftaric, *cis*-coutaric and *trans*-coutaric acids between the control wines and the
253 treated wines, except for the L+MO wines that showed lower concentration than the
254 control wines. As for the *trans*-fertaric acid, the L, YD-1, YD-2 and CH wines were
255 richer in this compound than the control wines.
256 The comparison of the results found in 2007 and in 2008 for these acids highlighted the
257 existence of a vintage effect, which can be due to the fact that the 2008 wines were
258 richer in hydroxycinnamic acids than the 2007 wines.
259 In general, a lower effect of the different assayed treatments was found on the 2008
260 wines, since only L+MO wines showed statistically significant differences in
261 hydroxycinnamic acids. However, these changes can be attributed mainly to the oxygen
262 addition. In 2007, the effect of micro-oxygenation on these compounds was not
263 noticeable since it had to be stopped before the end of the remaining treatments due to
264 the beginning of malolactic fermentation. Then, the micro-oxygenation only lasted five
265 weeks, instead of the eight weeks that lasted in the 2008 vintage.
266 Flavanol monomers, (+)-catechin and (-)-epicatechin, were summed up since they
267 showed the same evolution and behavior. Procyanidins (dimmers B1 and B2) were also
268 summed up for the same reason (**Tables 4 and 5**).
269 In the 2007 vintage, after treatment, all the treated wines showed similar concentration
270 of total flavanol monomers than the control wines, except the wines treated with YD
271 and CH that showed the lowest concentration. During barrel aging flavanol monomers

concentration followed different trend depended on the treatment. In general, their content increased, except for the wines treated with L+E and L+MO which maintained the initial values. Therefore, these wines and those treated with YD showed statistically lower flavonol monomers than controls in contrast with what happened after treatment. In the 2008 vintage, the total flavanol monomer concentration was lower in all the treated wines than in the control wines, with the exception of those treated with YD-3, which maintained similar content respect to the control, since this product was added just before barrel aging. The wines treated with CH showed the lowest values. However, during barrel aging, all wines decreased the flavanol monomers, except wines treated with CH which showed a highlighted increase. The aging in barrel modified the differences found between treatments, since after six months in barrel all the treated wines presented higher concentrations of these compounds than the control wines, especially those treated with CH. Both the changes of flavanols between treatments and during barrel aging can be due to the reactivity of these compounds. Thus the concentration of these flavanols can decrease due to their oxidation and polymerization reactions, and can increase due to the hydrolysis of higher oligomers [46].

Different evolution during barrel aging was found in procyanidin concentration between 2007 and 2008 wines. Then, an important increase was found in all the wines from the 2007 vintage, which initially has lower concentration than 2008 wines, whereas the latter showed a slight decrease during aging. This fact can be related to the equilibrium reactions between flavanols occurred over the time [46].

Only Guadalupe and Ayestarán [22] and Guadalupe et al. [23] have studied the effect of mannoprotein overproducing yeast strains and commercial mannoproteins during alcoholic fermentation of red wines on these compounds. They showed that the use of these products did not modify flavanol monomers but reduced procyanidin content after

297 treatment and after aging in barrel. Fernández et al. [47] also corroborated a reduction of
298 the tannin content in red wines treated with lysated lees with or without the presence of
299 pectinases and β-glucanase enzymes. Hernández et al. [48] did not find significant
300 differences in the concentration of flavanol monomers and procyanidins of red wines
301 after six months in barrel with or without lees either.
302 The different flavonols identified and quantified are shown in **tables 4 and 5**.
303 Myricetin, quercetin, kaempferol and isorhamnetin aglycones were summed up and
304 called flavonol aglycones in order to facilitate the understanding of the results obtained.
305 In the 2007 vintage after treatment, the flavonol aglycones concentration was
306 statistically significant lower in all the treated wines than in the control wines, except in
307 those treated with L+MO. In general the flavonol glycosides concentration (∑
308 myricetin-3-glycosides, quercetin-3-glycosides and syringetin-3-glucoside) was higher
309 in wines treated with YD, YD+E and CH than in control wines, being those treated with
310 YD+E that showed the highest values. The remaining treated wines maintained similar
311 content than the control. None of the treatments studied modified the concentration of
312 syringetin-3-glucoside. During barrel aging, in general, the concentration of flavonol
313 aglycones decreased in all the wines being more important in the wines with higher
314 concentration (C and L+MO). Then, after six months, only the YD+E wines showed
315 higher values of these compounds than the control wines, and no statistically significant
316 differences were found between the rest of the wines. On the contrary, flavonol
317 glycosides slightly increased, with the exception of quercetin derivatives. After six
318 months only the wines treated with YD+E showed statistically significant higher
319 concentration than the control wines, while those treated with L showed lower flavonol
320 glycoside content than the control wines.

Lower differences were found in the 2008 vintage for aglycone and glycoside flavonols. After treatment, only the wines treated with CH showed a statistically significant reduction of the flavonol aglycones respect to the control wines. As for the flavonol glycosides, only statistically significant differences were found in myricetin derivatives, being the wines treated with CH those that showed the lowest values. During aging, the concentration of both type of flavonols (aglycones and glycosides) decreased in the all wines especially of aglycones. The control wines showed an important decrease of flavonol aglycones, and after six months all the treated wines showed statistically significant higher concentration of these compounds than the control wines. In general, no statistically significant differences were found between the treated and the control wines in the flavonol glycosides.

Fernández de Simón et al. [40] also found a decrease of some flavonols after 12 months in different types of oak wood, especially in the American one. This can be due to the fact that flavonols can act as copigments and can take part in the copigmentation reactions with anthocyanins [27-29].

Hernández et al. [48] studied the evolution of several flavonol compounds in red wines aged with or without lees during aging in barrel, and in general, a clear effect of aging on lees during aging in barrel for six months was not found either.

The different treatments assayed also caused some statistically significant differences in the content of stilbenes in both vintages (**tables 4 and 5)**. In 2007, in general, all the treated wines showed similar or higher concentration of *trans*-resveratrol and *trans*-resveratrol-3-glucoside than the control wines, with the only exception of those treated with L+MO, which showed a highlighted reduction of *trans*-resveratrol-3-glucoside concentration respect to the control and the remaining treated wines. On the other hand, the concentration of *cis*-resveratrol-3-glucoside was lower in all the treated wines than

346 in the control ones, except those treated with YD+E. During barrel aging, *trans*-
347 resveratrol and the two isomers of resveratrol-3-glucosides increased in all the wines,
348 and in general, the differences found between treatments after the treatments were
349 maintained after six months, except for the *trans*-resveratrol-3-glucoside. In contrast to
350 after treatment, the wines treated with L, L+E and CH showed lower concentration of
351 *trans*-resveratrol-3-glucoside than the control and the remaining treated wines.
352 In the 2008 vintage after treatment, the wines treated with CH, L, L+MO and YD-1
353 showed a lower concentration of *trans*-resveratrol-3-glucoside than the control wines
354 and those treated with YD-2 and YD-3. The concentration of *trans*-resveratrol was
355 similar in all the treated wines, with the exception of those treated with L, which
356 showed higher values than the control wines, and those treated with CH, which showed
357 the lowest values. Barrera-García et al. [49] also observed a reduction of this compound
358 in model solutions added with non toasted oak chips during the first days of contact.
359 This fact can be due to the adsorption of *trans*-resveratrol on the oak surface. However,
360 this effect was only found in the 2008 vintage. No statistically significant differences
361 were found in the concentration of *cis*-resveratrol-3-glucoside between the treated and
362 the control wines. The evolution of total tilbenes showed a slight decrease during barrel
363 aging, opposite to what happened in 2007. The decrease of stilbenes during oak aging
364 was also observed by Fernández de Simón et al. [40] and Hernández et al. [48]. After
365 six months, a significant effect of the different treatments studied was not found, with
366 the exception of *trans*-resveratrol. For this compound, the wines treated with L and YD-
367 1 showed higher content than in the control wines. In spite of the fact that the 2008
368 wines were initially richer in stilbenes, after six months of oak aging, they showed
369 similar or even a lower content than the 2007 wines.

No great differences were found in the content of phenolic alcohols, tyrosol and tryptophol **(tables 4 and 5)** as it was expected because these compounds are mainly obtained during alcoholic fermentation [50].

In order to see if the information given by all the variables would allow to differentiate the wines studied according to the treatment applied, a factorial analysis was carried out. Due to the important vintage effect found, it was considered necessary to study the data from the two vintages separately.

The factorial analysis for the 2007 wines selected five factors with an eigenvalue greater than 1, which explained the 84.9% of the total variance. Table 6 shows the loadings for each variable on the selected factor. The variables with higher loading values contribute most significantly to the explanatory meaning of the factors. Figure 2 shows the distribution of the different wines studied in the first vintage in the plane defined by factors 1 and 2 which explained the 64.3% of the total variance. As can be seen in this figure, the variables associated with factor 1 permit separating YD, YD+E and CH wines from the control ones and the wines treated with lees (L, L+E and L+MO). That means that the aging on lees hardly modify the content of low molecular weight phenolic compounds. However, the treatment with chips and commercial yeast derivative products induced important changes in the concentration of some of these compounds, mainly in the hydroxycinnamic acids (compounds associated with factor 1). Thus, these wines showed higher concentrations of their free forms and lower of their sterified forms. The free hydroxycinnamic acids play an important role in color stabilization since they can act as anthocyanin copigments [27, 51].

On the other hand, factor 2 permits differentiating wines by the time that they have been aged in barrel (Figure 2). That means that most of the phenolic compounds positively associated with factor 2 increased during wood aging, and those negatively correlated

(quercetin glycosides and flavonol aglycones) decreased over the time, as it was commented before in the discussion of the individual phenolic compounds.

In the factorial analysis with the data of the 2008 vintage, no so important differences were found between the wines. In this case the factorial analysis selected four factors that explained the 85.4% of the total variance (Table 7). Figure 3 shows the distribution of the wines in the plane defined by the two first factors. As can be seen in this figure, factor 1 permits differentiating the wines by the treatment applied just before the wines were aged in oak casks. Thus, wines treated with lees, YD-2 and YD-3 showed a phenolic composition relatively close to that of the control wines as indicated by the proximity on the plane defined by factor 1 and 2 (Figure 3). The L+MO wines were placed quite far from the rest of the wines. However factor 1 was not able to separate by treatment after three and six months of aging barrel and again, only L+MO wines separated from the other wines. These results seem to indicate that the differences found in this vintage between the L+MO wines and the rest of the wines are due mainly to the effect of microoxygenation on the phenolic compounds and not to the combination of aging on lees and microoxygenation. It should be also taken into account that microoxygenation diminished the concentration of all the phenolic compounds positively associated with factor 1, and increased the *trans*-caffeic acid and *trans*-p-coumaric acid (Table 7). This decrease can be due to the combination of these compounds with anthocyanins through copigmentation reactions [15-16]. The authors ina previous work in which the same treatments were studied, also found that the use of lees together with micro-oxygenation seemed to have a positive effect on the color stability [24]. In this vintage most of the variables associated with factor 1 were the same than those associated with factor 1 of the factorial analysis of 2007 vintage, although with opposite signs. Factor 2 permits separating the wines after the treatment

from the wines aged in barrel, although in this vintage the factorial analysis did not differentiate the wines aged three months in oak barrels from those aged six months. However, it should be pointed out that the variables associated with this factor in this vintage were not exactly the same than those associated with factor 2 in the factorial analysis of the first vintage.

4. Conclusions

The effect of the different treatments studied on wine phenolic composition depended on the vintage, the phenolic compound and the treatment applied. Hydroxycinnamic acids were the compounds most affected by these treatments, mainly in the wines treated with chips and commercial yeast derivative products, which showed higher concentrations of the free acids, compounds that play an important role in wine stabilization color since they can act as anthocyanin copigments.

The assayed treatments did not affect the changes occurred along barrel aging.

The differences found between the assayed treatments were more important in the 2007 vintage than in the 2008. However, a more significant effect of micro-oxygenation in the 2008 vintage was observed, which could be related to the fact that in this vintage the treatment was longer. In the 2008 vintage, the differences between treatments decreased along the aging in barrel. This vintage effect could be associated to the differences in the phenolic concentration of the initial wines. In this sense more research should be done to corroborate this fact.

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FIGURE CAPTIONS

Figure 1. Scheme of the experiences carried out in each vintage. C (Control wine); L (wine aged on lees); L+E (wine aged on lees+Enzymes): L+MO (wine aged on lees+Micro-oxygenation): YD (wine treated with Yeast Derivative); YD+E (wine treated with Yeast Derivative+Enzymes), YD 1 (wine treated with Yeast Derivative 1), YD 2 (wine treated with Yeast Derivative 2), YD 3 (wine treated with Yeast Derivative 3) and CH (wine treated with Chips).

MLF: Malolactic fermentation.

Figure 2: Distribution of all the wines studied in 2007 vintage in the plane defined by factor 1 and factor 2. 3MB: three months in barrel; 6MB: six months in barrel.

Figure 3: Distribution of all the wines studied in 2008 vintage in the plane defined by factor 1 and factor 2. 3MB: three months in barrel; 6MB: six months in barrel.

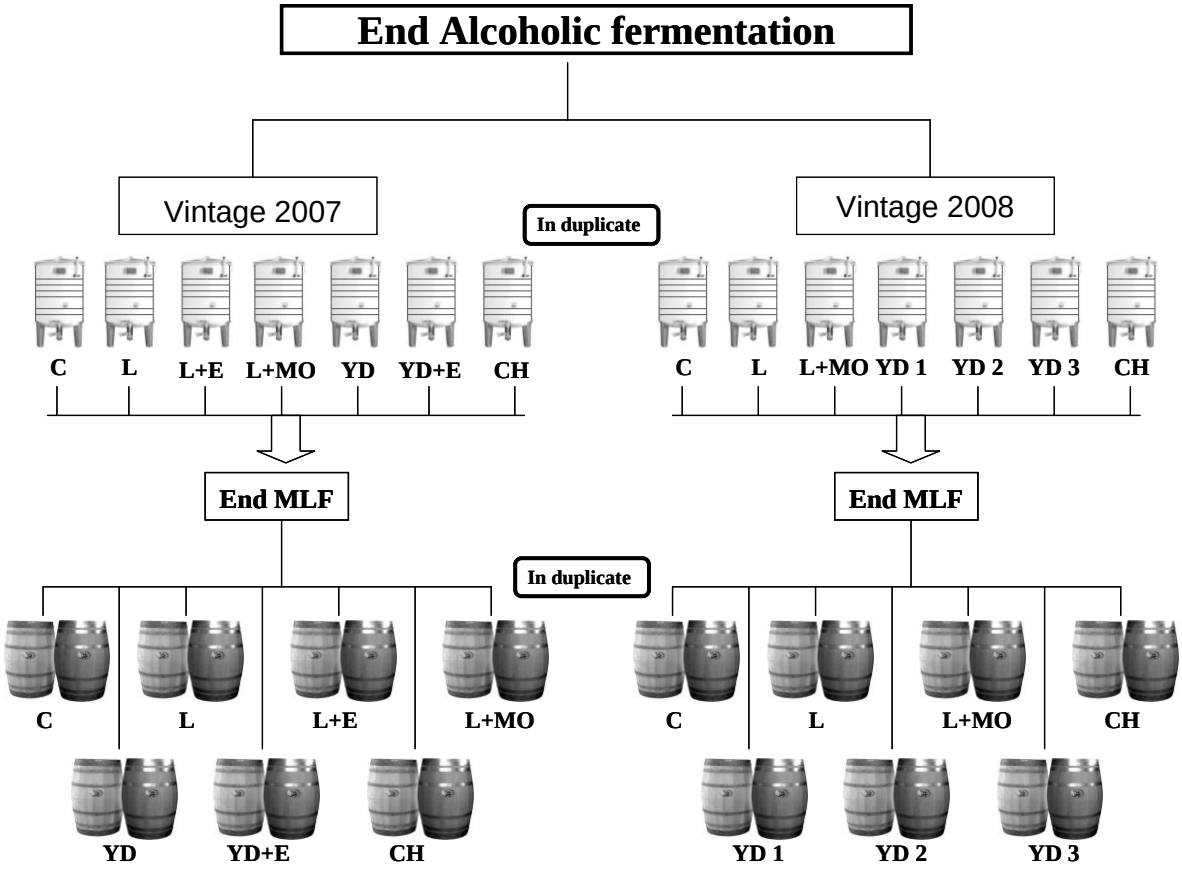
End Alcoholic fermentation
Vintage 2007
In duplicate
Vintage 2008
C
L
L+E
L+MO
YD
YD+E
CH
C
L
L+MO
YD 1
YD 2
YD 3
CH
End MLF
End MLF
In duplicate
C
L
L+E
L+MO
C
L
L+MO
CH
YD
YD+E
CH
YD 1
YD 2
YD 3

Figure 1

Figure 2

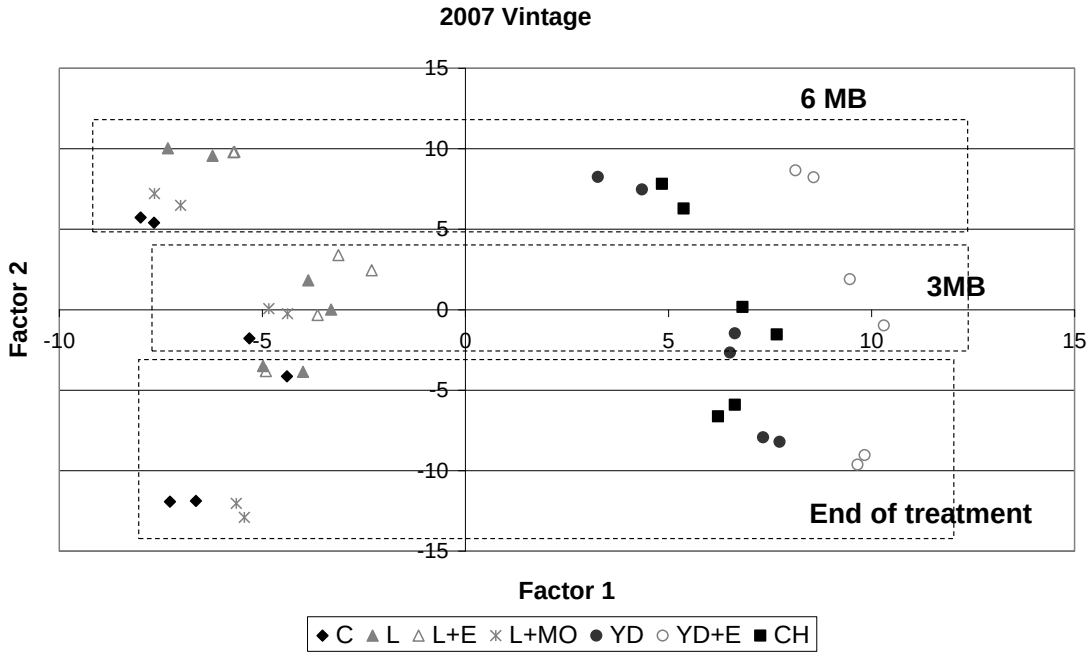
2007 Vintage
6 MB
3MB
End of treatment
Factor 2
Factor 1
15
10
5
0
-5
-10
-15
-10
-5
0
5
10
15
C L L+E L+MO YD YD+E CH

Figure 3

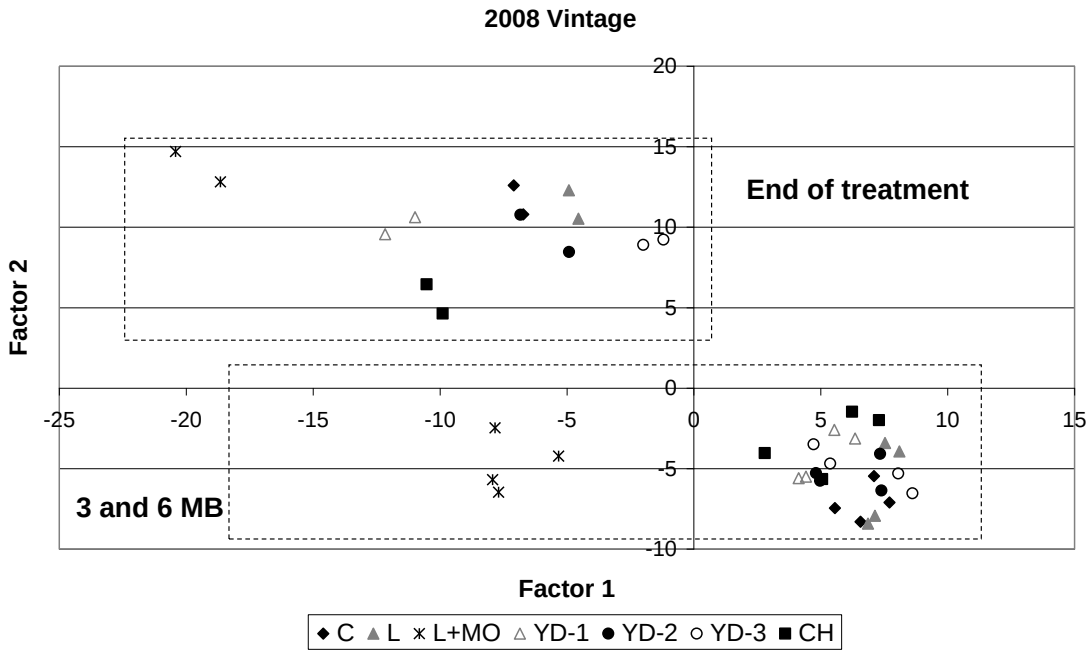
2008 Vintage
End of treatment
3 and 6 MB
Factor 2
Factor 1
20
15
10
5
0
-5
-10
-25
-20
-15
-10
-5
0
5
10
15
C
L
L+MO
YD-1
YD-2
YD-3
CH

Table 1. Characteristics of the natural lees, β-glucanase enzyme, non-toasted oak wood chips and the different commercial yeast derivatives used in this study, and doses applied.

Products added	**Doses** (g/L)	**Manufacture**	**Characteristics**
Lees	3% v/v	Obtained in the Enological Station by settling red wine	From commercial S*accharomyces cerevisiae* yeast.
β-glucanase enzyme preparation	0.04	Enovin Glucan. Agrovin (Spain)	High β-glucanase activity (430 IU[a]/g). Act on the yeast cell walls, allowing the extraction of polysaccharides. Accelerate the aging on lees
Chips	4.0	(Bois Frais, Boise France)	Non-toasted French oak chips
YD	0.4	Agrovin (Spain)	Product with autolysated yeast enriched in polysaccharides
YD 1	0.4	Sepsa Enartis (Spain)	Product with polysaccharides extracted enzymatically from selected yeast cell walls
YD 2	0.4	Sepsa Enartis (Spain)	Product with polysaccharides from yeast cell walls with high content in free mannoproteins
YD 3	0.05	Sepsa Enartis (Spain)	Product with polysaccharides from the yeast cell walls, highly purified and completely soluble in wine

[a] IU: International Units.

Table 2. Phenolic acid concentration (mg/L) in wines from 2007 vintage.

Compound	**End of treatment**						
Hydroxybenzoic acids (HBA)	**C**	**L**	**L+E**	**L+MO**	**YD**	**YD+E**	**CH**
Gallic acid	22.6	23.1	23.4	22.0	20.3	20.5	23.4
Protocatechuic acid	3.78a	6.76c	6.55c	5.35b	4.39ab	3.97a	4.48ab
Vanillic acid	2.91a	4.46c	4.62c	2.95a	3.35ab	3.33ab	3.97bc
Syringic acid	1.90a	2.62c	3.30d	1.99ab	3.31d	2.36abc	2.46bc
Ethyl gallate	3.05ab	3.29bc	3.53c	3.39bc	2.73a	2.84a	3.56c
Ellagic acid	6.23a	4.75a	4.82a	6.23a	4.46a	5.79a	21.9b
Total HBA	40.4ab	45.0bc	46.2c	42.0abc	38.6a	38.8a	59.7d
Hydroxycinnamic acids (HCA) and derivatives							
Trans-caffeic acid	5.43a	7.89b	7.43b	5.88a	10.6c	10.6c	11.8d
Trans-*p*-coumaric acid	2.92a	3.66b	3.78b	3.23ab	7.30c	8.34d	7.11c
Cis-*p*-coumaric acid	0.350a	0.797b	0.798b	0.211a	1.58c	1.85d	1.86d
Total HCA	8.70a	12.3b	12.0b	9.32a	19.4c	20.9d	20.8d
Trans-caftaric acid	27.1d	25.6d	23.0c	25.9d	13.9a	13.0a	16.6b
Cis-coutaric acid	3.33cd	3.12c	3.44d	3.17c	2.50ab	2.33a	2.65b
Trans-coutaric acid	19.0f	16.0d	16.6d	18.1e	12.2b	10.8a	13.4c
Trans-fertaric acid	0.430a	0.879c	0.857bc	0.415a	0.399a	0.430a	0.730b
Total HCA tartaric esters	49.9e	45.7cd	44.1c	47.6de	29.1a	26.6a	33.3b
Compound	**3 months in barrel**						
Hydroxybenzoic acids (HBA)	**C**	**L**	**L+E**	**L+MO**	**YD**	**YD+E**	**CH**
Gallic acid	21.7bc	23.5d	23.0cd	22.0bc	19.9a	20.8ab	22.9cd
Protocatechuic acid	4.46ab	6.68e	5.97d	5.25c	4.04a	4.90bc	4.27ab
Vanillic acid	3.48ab	3.23a	4.55d	3.98c	3.39ab	3.61abc	3.65bc
Syringic acid	2.37a	3.44b	4.43c	3.14b	3.61b	3.65b	3.70b
Ethyl gallate	3.13	3.02	3.26	3.14	2.89	3.07	3.27
Ellagic acid	7.13a	5.22a	4.86a	6.87a	4.97a	5.99a	17.7b
Total HBA	42.2b	45.0c	46.1c	44.4c	38.8a	42.0b	55.4d
Hydroxycinnamic acids (HCA) and derivatives							
Trans-caffeic acid	5.94a	8.29b	8.21b	6.24a	11.8c	17.1e	14.7d
Trans-*p*-coumaric acid	2.82a	3.70b	3.61b	3.02a	6.40c	7.79e	6.87d
Cis-*p*-coumaric acid	0.907ab	0.831a	0.878a	1.02b	1.67c	1.99d	1.77c
Total HCA	9.64a	12.9b	12.7b	10.3a	19.9c	26.9e	23.3d
Trans-caftaric acid	28.8d	26.2c	26.1c	26.1c	15.2a	13.9a	19.3b
Cis-coutaric acid	3.26c	3.18c	3.08c	3.24c	2.11a	2.39b	2.32b
Trans-coutaric acid	19.3b	16.6b	16.3b	18.3b	12.0a	11.0a	12.8a
Trans-fertaric acid	1.06b	0.830ab	1.00ab	1.04b	0.767a	0.995ab	0.962ab
Total HCA tartaric esters	52.5f	46.8de	46.5d	48.6e	30.2b	28.3a	35.5c
Compound	**6 months in barrel**						
Hydroxybenzoic acids (HBA)	**C**	**L**	**L+E**	**L+MO**	**YD**	**YD+E**	**CH**
Gallic acid	24.5	25.5	25.7	24.3	24.5	24.6	25.5
Protocatechuic acid	5.69c	7.57e	6.66d	5.56c	5.23b	4.94a	5.35b
Vanillic acid	3.85a	5.13e	4.94de	4.69cd	4.53bc	4.36b	4.42b
Syringic acid	3.29a	4.15d	3.97cd	3.65b	3.71bc	3.69bc	3.79bc
Ethyl gallate	3.52	3.60	3.63	3.55	3.64	3.68	3.78
Ellagic acid	8.65a	6.07a	5.56a	7.72a	6.17a	6.92a	20.3b
Total HBA	49.5ab	52.0b	50.5ab	49.5ab	47.8a	48.1a	63.1c
Hydroxycinnamic acids (HCA) and derivatives							
Trans-caffeic acid	6.24a	8.86c	8.87c	7.13b	14.7d	17.5e	15.2d
Trans-*p*-coumaric acid	2.86a	3.61c	3.58c	3.21b	6.71d	8.21f	7.32e
Cis-*p*-coumaric acid	0.933ab	0.893a	0.960b	1.08c	1.75d	2.06f	1.94e
Total HCA	10.0a	13.4c	13.4c	11.4b	23.2d	27.8e	24.5d
Trans-caftaric acid	32.1e	28.2c	28.2c	30.9d	21.4b	19.3a	20.8b
Cis-coutaric acid	3.58d	3.42cd	3.39c	3.46cd	2.62b	2.31a	2.41a
Trans-coutaric acid	20.1f	17.2d	17.4d	19.3e	14.0c	12.4a	13.2b
Trans-fertaric acid	1.46b	1.63d	1.60cd	1.46b	1.59cd	1.52bc	1.32a
Total HCA tartaric esters	57.2e	50.5c	50.5c	55.1d	39.6b	35.6a	37.7b

Values with different letter in the same row indicate statistically significant differences (p= 0.05), and values without letter indicate no statistically significant differences.

Table 3. Phenolic acid concentration (mg/L) in wines from 2008 vintage.

Compound	**End of treatment**						
Hydroxybenzoic acids (HBA)	**C**	**L**	**L+MO**	**YD 1**	**YD 2**	**YD 3**	**CH**
Gallic acid	23.8ab	24.5bc	23.0a	24.3bc	24.3bc	23.7ab	24.9c
Protocatechuic acid	3.21b	3.29bc	3.39c	3.05a	3.54d	3.20b	2.96a
Vanillic acid	4.07bc	4.10bc	2.88a	3.96c	3.97c	4.36c	3.55b
Syringic acid	2.21c	2.16bc	1.98a	2.17c	2.17c	2.23c	2.06ab
Ethyl gallate	3.80bc	3.81bc	3.81bc	3.48a	3.59ab	3.79bc	3.98c
Ellagic acid	4.47a	4.20a	4.55a	3.87a	4.22a	4.70a	15.0b
Total HBA	41.6a	42.0a	39.6a	40.8a	41.8a	42.0a	52.5b
Hydroxycinnamic acids (HCA) and derivatives							
Trans-caffeic acid	6.49b	5.29a	16.4e	11.2d	6.50b	6.38b	10.0c
Trans-p-coumaric acid	4.35b	3.31a	10.9d	7.9c	3.93ab	4.25b	7.5c
Cis-p-coumaric acid	0.198b	0.116a	0.277c	0.297c	0.087a	0.202b	0.267c
Total HCA	11.0b	8.7a	27.6d	19.4c	10.5b	10.8b	17.8c
Trans-caftaric acid	30.7d	33.7e	20.4a	27.2b	32.1d	31.1d	28.8c
Cis-coutaric acid	3.36c	3.55c	2.22a	2.83b	3.49c	3.25c	2.95b
Trans-coutaric acid	20.7c	23.2d	15.0a	18.4b	22.6d	20.3c	18.8b
Trans-fertaric acid	1.04d	1.14e	0.270a	0.805b	1.13e	1.00d	0.919c
Total HCA tartaric esters	55.8c	61.7d	37.9a	49.3b	59.3d	55.7c	51.5b
Compound	**3 months in barrel**						
Hydroxybenzoic acids (HBA)	**C**	**L**	**L+MO**	**YD 1**	**YD 2**	**YD 3**	**CH**
Gallic acid	26.3cde	26.7e	25.1a	25.8bc	25.8bcd	25.5ab	26.4de
Protocatechuic acid	4.24c	4.75e	4.75e	4.30cd	4.39d	4.12b	3.89a
Vanillic acid	4.89bc	5.42d	4.26a	4.61b	5.04c	5.01c	4.16a
Syringic acid	3.63bc	3.53bc	3.79c	2.86a	3.42b	2.88a	2.82a
Ethyl gallate	4.13	4.16	3.95	3.89	3.94	4.03	4.13
Ellagic acid	5.46a	5.55a	4.80a	5.64a	5.87a	5.68a	16.6b
Total HBA	48.6bc	50.1c	46.7a	47.1ab	48.5bc	47.2ab	58.0d
Hydroxycinnamic acids (HCA) and derivatives							
Trans-caffeic acid	1.52a	1.59a	14.0b	1.71a	1.55a	1.64a	2.06a
Trans-p-coumaric acid	0.926a	0.950a	8.03c	0.990a	0.921a	0.931a	1.34b
Cis-p-coumaric acid	0.634a	0.580a	1.25b	0.620a	0.648a	0.600a	0.650a
Total HCA	3.08a	3.126a	23.3b	3.32a	3.20a	3.17a	4.05a
Trans-caftaric acid	37.6b	37.8b	24.6a	37.9b	38.4b	38.5b	37.8b
Cis-coutaric acid	3.80b	3.79b	3.06a	3.77b	3.86b	3.76b	3.78b
Trans-coutaric acid	26.3bc	26.3bc	16.6a	25.9b	26.5c	26.3bc	26.4bc
Trans-fertaric acid	1.16b	1.24c	0.902a	1.25c	1.23c	1.12b	1.17b
Total HCA tartaric esters	68.9b	69.1b	45.2a	68.8b	70.0b	69.7b	69.2b
Compound	**6 months in barrel**						
Hydroxybenzoic acids (HBA)	**C**	**L**	**L+MO**	**YD 1**	**YD 2**	**YD 3**	**CH**
Gallic acid	25.7	26.7	24.8	24.8	26.4	26.3	26.5
Protocatechuic acid	4.22b	4.40d	4.60e	4.24bc	4.30bcd	4.35cd	3.76a
Vanillic acid	4.62b	4.83b	3.89a	4.94bc	4.61b	5.27c	4.14a
Syringic acid	2.73de	2.69cd	2.54bc	2.64cd	2.48ab	2.86e	2.39a
Ethyl gallate	4.12	4.07	3.84	3.94	4.00	4.30	4.19
Ellagic acid	6.12a	6.86ab	6.59ab	6.72ab	7.36b	7.31b	21.5c
Total HBA	47.5ab	49.6c	46.3a	47.2a	49.1bc	50.3c	62.4d
Hydroxycinnamic acids (HCA) and derivatives							
Trans-caffeic acid	1.85a	2.07a	14.6b	2.04a	1.98a	1.96a	2.46a
Trans-p-coumaric acid	0.911a	0.924a	7.66b	1.04a	0.900a	0.980a	1.17a
Cis-p-coumaric acid	0.546ab	0.509a	1.17d	0.687c	0.621bc	0.614bc	0.570ab
Total HCA	3.31a	3.50a	23.4b	3.77a	3.50a	3.55a	4.20a
Trans-caftaric acid	38.4b	39.4b	25.7a	39.7b	39.5b	39.5b	39.3b
Cis-coutaric acid	4.11bc	4.20c	3.20a	4.11bc	4.02b	4.13bc	4.11bc
Trans-coutaric acid	26.3b	27.1b	17.0a	26.7b	27.0b	27.1b	26.9b
Trans-fertaric acid	1.71b	1.85c	1.41a	1.77bc	1.79bc	1.71b	1.84c
Total HCA tartaric esters	70.5b	72.6b	47.3a	72.3b	72.3b	72.4b	72.2b

Values with different letter in the same row indicate statistically significant differences (p= 0.05), and values without letter indicate no statistically significant differences.

Table 4. Flavanol, flavonol, stilbene and phenolic alcohol concentration (mg/L) in wines from 2007 vintage.

Compound	**End of treatment**						
Flavanols	**C**	**L**	**L+E**	**L+MO**	**YD**	**YD+E**	**CH**
Total flavanol monomers	42.9b	43.0b	42.1b	42.2b	38.4a	40.5ab	38.2a
Total procyanidins	18.7c	15.0b	16.0b	16.1b	12.6a	14.4ab	22.2d
Flavonols							
Total flavonol aglycones	18.4c	14.7ab	13.1a	17.6bc	14.6ab	14.7ab	14.8ab
∑ Myricetin-3-glycosides	3.77a	3.75a	3.90ab	4.21c	4.02bc	4.51d	4.10bc
∑ Quercetin-3-glycosides	0.297a	0.298a	0.304a	0.296a	0.364b	0.365b	0.351b
Syringetin-3-glucoside	1.32	1.23	1.19	1.22	1.23	1.25	1.24
Total flavonol glycosides	5.39ab	5.28a	5.34ab	5.73c	5.61bc	6.13d	5.69c
Stilbenes							
Trans-resveratrol-3-glucoside	1.51b	1.83c	1.73c	0.85a	1.59b	1.51b	1.79c
Cis-resveratrol-3-glucoside	0.204c	0.143a	0.150ab	0.161b	0.155ab	0.189c	0.160b
Trans-resveratrol	1.68a	2.29c	2.09bc	1.69a	2.13bc	2.04bc	1.74b
Total stilbenes	3.39b	4.27d	3.97cd	2.70a	3.88cd	3.74c	3.70c
Phenolic alcohols							
Tyrosol	18.9a	19.9ab	21.1bc	20.9bc	20.3abc	20.1ab	21.6c
Tryptophol	1.96	1.41	2.39	2.04	2.49	2.17	1.63
Compound	**3 months in barrel**						
Flavanols	**C**	**L**	**L+E**	**L+MO**	**YD**	**YD+E**	**CH**
Total flavanol monomers	34.6a	48.5d	45.4c	45.5c	40.9b	40.8b	39.7b
Total procyanidins	24.3a	36.9d	34.1c	27.4a	30.3b	35.8d	29.8b
Flavonols							
Total flavonol aglycones	13.4	14.4	14.2	13.0	13.2	13.6	14.2
∑ Myricetin-3-glycosides	4.18	3.92	4.03	4.03	3.74	4.13	4.07
∑ Quercetin-3-glycosides	0.286	0.270	0.281	0.265	0.276	0.312	0.308
Syringetin-3-glucoside	1.28	1.29	1.21	1.35	1.15	1.26	1.23
Total flavonol glycosides	5.60	5.48	5.52	5.65	5.17	5.70	5.60
Stilbenes							
Trans-resveratrol-3-glucoside	2.12d	2.00c	1.86a	2.17d	1.91ab	1.96bc	1.93abc
Cis-resveratrol-3-glucoside	0.162	0.205	0.173	0.185	0.166	0.182	0.167
Trans-resveratrol	1.93a	2.71b	2.59b	2.01a	2.20a	2.63b	2.04a
Total stilbenes	4.21a	4.92c	4.62bc	4.37ab	4.28a	4.77c	4.14a
Phenolic alcohols							
Tyrosol	19.9	20.2	20.2	20.0	19.6	20.3	20.5
Tryptophol	4.49b	4.57b	5.48d	3.87a	4.33ab	4.77bc	5.21cd
Compound	**6 months in barrel**						
Flavanols	**C**	**L**	**L+E**	**L+MO**	**YD**	**YD+E**	**CH**
Total flavanol monomers	45.9b	45.1b	41.0a	41.2a	41.8a	50.0c	45.2b
Total procyanidins	39.7a	42.9ab	41.4ab	40.0a	43.7bc	47.2c	41.7ab
Flavonols							
Total flavonol aglycones	12.3ab	12.3ab	10.9a	11.5a	13.9bc	14.5c	11.9ab
∑ Myricetin-3-glycosides	4.34c	3.92a	4.17bc	4.09ab	4.36c	4.66d	4.25bc
∑ Quercetin-3-glycosides	0.233b	0.217ab	0.230b	0.212a	0.255c	0.274d	0.258cd
Syringetin-3-glucoside	1.34a	1.40a	1.73b	1.44a	1.65b	1.72b	1.41a
Total flavonol glycosides	5.92bc	5.54a	6.13c	5.74ab	6.27c	6.65d	5.93bc
Stilbenes							
Trans-resveratrol-3-glucoside	2.59c	2.26a	2.28a	2.54c	2.57c	2.58c	2.39b
Cis-resveratrol-3-glucoside	0.329ab	0.299a	0.338ab	0.338ab	0.332ab	0.401c	0.356bc
Trans-resveratrol	2.12a	2.64cd	2.47bc	2.12a	2.79d	3.21e	2.31ab
Total stilbenes	5.04a	5.20a	5.09a	5.00a	5.69b	6.19c	5.06a
Phenolic alcohols							
Tyrosol	19.9	20.1	20.6	19.6	20.4	20.7	19.9
Tryptophol	4.78bc	4.92cd	4.86bcd	3.41a	4.27b	5.43d	4.27b

Values with different letter in the same row indicate statistically significant differences (p= 0.05), and values without letter indicate no statistically significant differences.

Table 5. Flavanol, flavonol, stilbene and phenolic alcohol concentration (mg/L) in wines from 2008 vintage.

Compound	**End of treatment**						
Flavanols	**C**	**L**	**L+MO**	**YD 1**	**YD 2**	**YD 3**	**CH**
Total flavanol monomers	67.6e	59.9d	58.2cd	55.5c	49.9b	65.2e	45.2a
Total procyanidins	53.2bc	51.3abc	55.4c	50.6ab	50.9ab	53.1bc	48.3a
Flavonols							
Total flavonol aglycones	28.8b	28.6b	29.5b	26.2b	29.5b	28.1b	23.2a
∑ Myricetin-3-glycosides	24.4b	24.5b	23.2b	23.4b	22.9b	24.2b	18.0a
∑ Quercetin-3-glycosides	0.600	0.609	0.602	0.581	0.623	0.601	0.566
Syringetin-3-glucoside	2.40	2.46	2.38	2.43	2.44	2.40	2.29
Total flavonol glycosides	27.4b	27.6b	26.2b	26.4b	26.0b	27.2b	20.9a
Stilbenes							
Trans-resveratrol-3-glucoside	2.06c	1.97b	1.93ab	1.97b	2.06c	2.06c	1.90a
Cis-resveratrol-3-glucoside	0.233	0.231	0.243	0.224	0.226	0.233	0.238
Trans-resveratrol	2.44b	2.72c	2.48b	2.44b	2.61bc	2.53bc	1.43a
Total	4.73bc	4.92c	4.65b	4.63b	4.90bc	4.82bc	3.57a
Phenolic alcohols							
Tyrosol	18.1a	18.9ab	18.5ab	18.9ab	18.6ab	19.1b	25.3c
Tryptophol	19.7	21.4	18.9	18.9	21.1	19.5	19.1
Compound	**3 months in barrel**						
Flavanols	**C**	**L**	**L+MO**	**YD 1**	**YD 2**	**YD 3**	**CH**
Total flavanol monomers	46.1a	49.8bc	47.0a	50.5cd	51.7de	52.3e	48.6b
Total procyanidins	43.7	42.1	45.1	39.9	45.1	45.2	43.7
Flavonols							
Total flavonol aglycones	24.9	24.6	27.0	26.4	25.9	27.1	26.5
∑ Myricetin-3-glycosides	19.7d	19.3abc	19.3ab	19.6bcd	19.6cd	20.4e	19.0a
∑ Quercetin-3-glycosides	0.534abc	0.526a	0.554e	0.529ab	0.540cd	0.549de	0.539bcd
Syringetin-3-glucoside	2.20ab	2.26bc	2.26c	2.16a	2.18a	2.21abc	2.17a
Total flavonol glycosides	22.5b	22.1ab	22.1ab	22.3b	22.4b	23.2c	21.8a
Stilbenes							
Trans-resveratrol-3-glucoside	2.03b	1.90a	2.09b	2.04b	2.03b	2.03b	1.93a
Cis-resveratrol-3-glucoside	0.205	0.202	0.204	0.196	0.208	0.209	0.202
Trans-resveratrol	1.79	1.92	1.94	1.79	1.94	1.87	1.64
Total	4.03b	4.02b	4.23b	4.03b	4.18b	4.11b	3.77a
Phenolic alcohols							
Tyrosol	15.8a	16.7ab	19.4c	18.1bc	18.7bc	14.9a	18.9bc
Tryptophol	13.2	14.9	14.6	14.9	13.6	13.9	13.7
Compound	**6 months in barrel**						
Flavanols	**C**	**L**	**L+MO**	**YD 1**	**YD 2**	**YD 3**	**CH**
Total flavanol monomers	48.7a	52.9b	55.7cd	53.2b	52.4b	53.6bc	56.2d
Total procyanidins	39.7	44.0	41.4	42.3	42.5	43.8	44.6
Flavonols							
Total flavonol aglycones	17.4a	20.2bc	19.5b	21.6c	21.2c	24.1d	20.9bc
∑ Myricetin-3-glycosides	18.5ab	19.1bc	17.9a	19.3c	18.1a	19.0bc	18.9bc
∑ Quercetin-3-glycosides	0.441a	0.467b	0.446a	0.446a	0.434a	0.445a	0.471b
Syringetin-3-glucoside	2.55bc	2.65cd	2.47ab	2.54abc	2.43a	2.43a	2.76d
Total flavonol glycosides	21.5ab	22.2bc	20.9a	22.3c	21.0a	21.9bc	22.2bc
Stilbenes							
Trans-resveratrol-3-glucoside	2.27	2.24	2.30	2.30	2.58	2.25	2.17
Cis-resveratrol-3-glucoside	0.206	0.224	0.218	0.221	0.216	0.219	0.219
Trans-resveratrol	1.92ab	2.18c	2.00abc	2.16c	2.06bc	1.94ab	1.85a
Total	4.40	4.64	4.52	4.68	4.86	4.41	4.24
Phenolic alcohols							
Tyrosol	17.8	17.7	18.2	18.2	17.3	18.0	17.7
Tryptophol	13.2a	13.3a	14.6bc	15.3c	13.3ab	13.1a	13.3ab

Values with different letter in the same row indicate statistically significant differences (p= 0.05), and values without letter indicate no statistically significant differences.

Table 6. Factor loadings after varimax rotation of the wines elaborated in 2007.

Loadings lower than absolute value of 0.250 are not shown.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Eigenvalue	8.98	6.46	1.95	1.71	1.28
Cumulative variance	37.4	64.3	72.5	79.6	84.9
Gallic acid	-0.340	0.373	**0.650**	0.477	
Protocatechuic acid	-0.487	0.414		**0.570**	0.411
Vanillic acid		**0.657**		0.543	
Syringic acid		**0.857**			
Ethyl gallate		0.299	**0.595**	**0.570**	-0.289
Ellagic acid	0.283				**-0.722**
Trans-caffeic acid	**0.877**	0.254	0.265		
Trans-*p*-coumaric acid	**0.975**				
Cis-*p*-coumaric acid	**0.904**	0.273			
Trans-caftaric acid	**-0.928**		0.264		
Cis-coutaric acid	**-0.949**				
Trans-coutaric acid	**-0.972**				
Trans-fertaric acid		**0.766**	0.558		
Total flavanol monomers			0.537		**0.641**
Total procyanidins		**0.723**	0.605		
Total flavonol aglycones		**-0.845**			
∑ Myricetin-3-glycosides	0.386		**0.670**		
∑ Quercetin-3-glycosides	0.553	**-0.642**	-0.409		
Syringetin-3-glucoside			**0.758**		
Trans-resveratrol-3-glucoside		**0.768**	0.512		
Cis-resveratrol-3-glucoside		0.441	**0.841**		
Trans-resveratrol	0.386	**0.728**			0.383
Tyrosol				**0.777**	
Tryptophol		**0.752**	0.271		

Table 7. Factor loadings after varimax rotation of the wines elaborated in 2008.

Loadings lower than absolute value of 0.250 are not shown.

	Factor 1	Factor 2	Factor 3	Factor 4
Eigenvalue	11.5	3.84	3.00	2.17
Cumulative variance	47.9	63.9	76.4	85.4
Gallic acid	**0.588**	**-0.544**		-0.368
Protocatechuic acid		**-0.846**	0.317	
Vanillic acid	**0.754**	-0.487		
Syringic acid		**-0.874**		
Ethyl gallate	**0.467**	**-0.442**		**-0.411**
Ellagic acid				**-0.851**
Trans-caffeic acid	**-0.985**			
Trans-*p*-coumaric acid	**-0.970**			
Cis-*p*-coumaric acid		**-0.809**	0.349	
Trans-caftaric acid	**0.960**			
Cis-coutaric acid	**0.892**		0.356	
Trans-coutaric acid	**0.970**			
Trans-fertaric acid	0.640		**0.717**	
Total flavanol monomers		**0.654**		0.475
Total procyanidins	-0.341	**0.724**	-0.414	
Total flavonol aglycones			**-0.809**	0.312
∑ Myricetin-3-glycosides		**0.686**	-0.425	0.537
∑ Quercetin-3-glycosides	-0.268	0.447	**-0.798**	
Syringetin-3-glucoside		0.488	**0.764**	
Trans-resveratrol-3-glucoside			**0.868**	
Cis-resveratrol-3-glucoside	-0.338	**0.751**		
Trans-resveratrol		0.637		**0.703**
Tyrosol	-0.455	0.304		**-0.589**
Tryptophol	-0.369	**0.745**	-0.372	

V. CONCLUSIONES

Las conclusiones más destacadas de este trabajo son las siguientes:

1- No existe una estandarización respecto a la pureza y la composición de los diferentes preparados obtenidos a partir de las paredes celulares de las levaduras que existen en el mercado. La composición en glucanos y manoproteínas varía de un fabricante a otro debido probablemente al método de extracción usado, al grado de purificación, y a la cepa de levadura empleada. Por ello, estos preparados producen efectos muy diversos sobre los vinos, siendo difícil establecer una relación significativa entre su composición y su efecto en vinos blancos y tintos.

2- Los chips de madera sin tostar, las lías y los preparados comerciales derivados de levadura interaccionan con algunos de los compuestos fenólicos y volátiles presentes en el vino. En el caso de los compuestos volátiles estas interacciones desaparecen a lo largo del tiempo. De forma generalizada, los resultados obtenidos de estas interacciones en soluciones modelo no permiten extrapolarlos a lo que ocurre en un vino real.

3- La crianza sobre lías y la adición de los derivados de levadura comerciales disminuyen la intensidad colorante de los vinos blancos debido a que se limita la oxidación de los compuestos fenólicos y por lo tanto el pardeamiento de los vinos. Este efecto se reduce durante el envejecimiento en botella.

4- La crianza tradicional sobre lías o el uso de preparados comerciales de levadura no favorece la formación de nuevos pigmentos que estabilizan el color de los vinos tintos. Únicamente la aplicación conjunta de la microoxigenación y la crianza sobre lías permite mantener una mayor intensidad de color y tonos azulados, aunque este efecto positivo parece ser debido a la microoxigenación, y no a la aplicación conjunta de ambas técnicas.

5- La crianza tradicional sobre lías y la adición de derivados de levadura comerciales producen la liberación de polisacáridos neutros al vino. La cantidad de polisacáridos neutros liberados por los preparados de derivados de levadura comerciales depende de la composición de cada preparado.

6- Desde el punto de vista sensorial, la crianza tradicional sobre lías o la utilización de derivados de levadura comerciales permite mejorar la palatabilidad de los vinos tanto blancos como tintos, haciéndolos menos astringentes, más grasos y con más volumen en boca. Esta mejora en la calidad sensorial de los vinos parece ser debida a los polisacáridos neutros liberados por las lías y los derivados de levadura comerciales. Estos tratamientos también mejoran la persistencia aromática de los vinos. Estas mejoras sensoriales de los vinos son más importantes al aumentar el tiempo de envejecimiento en barrica o en botella.

7- La utilización de madera sin tostar en forma de chips antes de la fermentación maloláctica favorece la estabilización del color de los vinos tintos, al aumentar el contenido de antocianos poliméricos.

8- En las condiciones ensayadas, el uso de enzimas β-glucanasas en combinación con la crianza sobre lías o con preparados comerciales de levadura no modifica significativamente la composición físico-química ni las características sensoriales de los vinos blancos o tintos.

9- No es posible concluir que la utilización de preparados comerciales de derivados de levaduras permite obtener vinos con una composición similar a la de los vinos con crianza tradicional sobre lías, ya que dependerá del producto utilizado y del tiempo de crianza. Sin embargo los resultados obtenidos en el análisis sensorial de los vinos parecen indicar que la adición de estos preparados comerciales permite obtener vinos con unas características sensoriales similares o incluso mejores a las de los vinos con crianza tradicional sobre lías.

10- El efecto de los distintos tratamientos ensayados en este trabajo en los compuestos fenólicos, especialmente en los de bajo peso molecular, va a depender de su contenido en el vino de partida, siendo menor el efecto cuanto mayor sea la concentración de estos compuestos.

11- Teniendo en cuenta los resultados obtenidos en esta tesis, es difícil determinar cuál de las técnicas estudiadas permitirá mejorar las características sensoriales del vino de partida. La selección de la técnica debe tener en cuenta las características del vino inicial, el tipo de vino que se desea elaborar, así como factores económicos y de gestión de la bodega.