UNIVERSITAT ROVIRA I VIRGILI

INFLUENCIA DE LA ESPECIE BOTÁNICA, DEL GRADO DE TOSTADO Y DEL USO DE LAS BARRICAS DE ROBLE SOBRE LOS ELAGITANINOS; CONSECUENCIAS ENOLÓGICAS Y SENSORIALES.

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María Navarro Fernández

Influencia de la especie botánica, del grado de tostado y del uso de las barricas de roble sobre los elagitaninos; Consecuencias enológicas y sensoriales

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Tarragona, 15 de Desembre del 2016

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Los análisis de esta Tesis doctoral se realizaron en los laboratorios del departamento de Bioquímica y Biotecnología de la Universidad Rovira i Virgili, en los laboratorios del Instituto Regional de Investigación Científica Aplicada (IRICA) de la Universidad de Castilla-La Mancha, y en los laboratorios del Instituto de la Vid y el Vino de Castilla-La Mancha (IVICAM) bajo la dirección de los profesores Isidro Hermosín Gutiérrez, Joan Miquel Canals Bosch y Fernando Zamora Marín, con la colaboración del Dr. Esteban García Romero.

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- Innovaciones en el estudio de la astringencia del vino relacionadas con los métodos de análisis, la composición y estructura de los taninos, y los polisacáridos (AGL2014-56594-C2-1-R and AGL2014-56594-C2-2-R). De 2015 a 2017.

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Amistades que son ciertas nadie las puede turbar

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1. INTRODUCCIÓN

1.1. Los compuestos fenólicos del vino

Los compuestos fenólicos son una amplia familia de compuestos que se caracterizan por contener en su estructura química al menos un grupo funcional fenol. Presentan una enorme importancia enológica ya que son responsables de numerosos atributos sensoriales tales como el color, la astringencia, el sabor amargo y contribuyen a sensaciones de textura tales como el cuerpo y la estructura del vino (Vidal y col., 2003; Quijada-Morín y col., 2014).

Asimismo, los compuestos fenólicos presentan una gran relevancia tecnológica, ya que son fundamentales para los procesos de crianza y/o están detrás de algunos problemas tecnológicos como las quiebras oxidásicas. También tienen propiedades saludables, que comúnmente se atribuyen a su capacidad antioxidante.

La mayoría de los compuestos fenólicos presentes en el vino proceden de la uva, o del proceso de envejecimiento con madera. La cantidad de estos compuestos en los vinos dependerá de sus niveles en la uva de partida y del proceso de vinificación. Antes de describir las distintas familias, cabe considerar algunas generalidades acerca de los compuestos fenólicos de la uva, también llamados fenoles o polifenoles.

Como ya se ha comentado, la característica química de estos compuestos es que disponen en su molécula de algún anillo bencénico aromático al que se encuentra unido uno o más de un grupo hidroxilo (como su nombre indica, tienen como mínimo un grupo fenol). Se muestra su estructura básica en la figura 1.

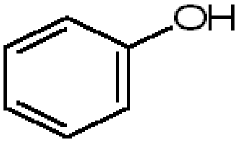


Figura 1. Estructura química del fenol,
constituyente básico de los compuestos fenólicos.

Los grupos hidroxilo (OH^-), al estar unidos a un anillo bencénico, presentan la posibilidad de que los electrones no compartidos del átomo de oxígeno interaccionen con los electrones del anillo bencénico, lo cual les confiere características especiales respecto al resto de los alcoholes. Son más ácidos, presentan la posibilidad de formar puentes de hidrógeno intermoleculares o intramoleculares, formar complejos dimoleculares o trimoleculares con distintos metales y pueden formar ésteres. Cabe señalar que algunos compuestos fenólicos de la familia de los ácidos fenólicos no son polifenoles sino monofenoles.

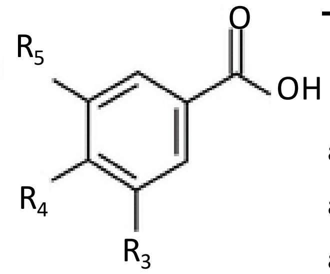
Si realizamos una clasificación de los compuestos fenólicos según su estructura química, se puede hacer una primera distinción entre **no flavonoides** y **flavonoides** (Cheynier y col., 2003; Ribéreau-Gayón y col.2003):

1.1.1. Compuestos No flavonoides

Esta familia comprende los ácidos fenólicos, divididos en *ácidos hidroxibenzoicos* (C_6-C_1) y *ácidos hidroxicinámicos*, portadores de una cadena lateral insaturada (C_6-C_3), y también incluye a otros derivados fenólicos como son los estilbenos con un esqueleto de carbono del tipo (C_6-C_2-C_6).

1.1.1.1. Los ácidos hidroxibenzoicos

Son ácidos carboxílicos aromáticos que tiene un grupo carboxilo unido a un anillo fenólico, como se muestra en la figura 2.



Ácidos Hidroxibenzoicos

	R_3	**R_5**
ácido gálico	OH	OH
ácido vaníllico	OCH_3	H
ácido protocatéquico	OH	H
ácido siríngico	OCH_3	OCH_3

Figura 2. Estructura química de los principales ácidos hidroxibenzoicos.

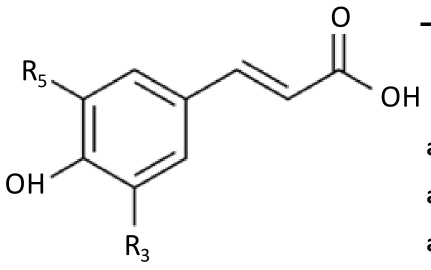
Contribuyen al sabor amargo del vino. El principal componente es el ácido gálico pero en forma de ésteres de flavanol. Es muy abundante en vinos tintos. Tiene propiedades antioxidantes (Kilmartin, Zou y Waterhouse, 2001; Xiang y col., 2014) y por esta razón se ha postulado que ayudan en la conservación del vino. No contribuyen de forma directa al color del vino ya que son incoloros, si bien pueden actuar de forma indirecta como copigmento. Otros ácidos benzoicos son: el vaníllico, protocatéquico, siríngico, elágico. La presencia del ácido elágico (dímero del ácido gálico) en el vino es debida a reacciones de oxidación.

El ácido gálico y el ácido elágico son componentes de los taninos hidrolizables (galotaninos y elagitaninos, respectivamente) que se describirán más adelante.

1.1.1.2. Los ácidos hidroxicinámicos

Son los vinil-análogos de los ácidos hidroxibenzoicos. Están formados por un anillo bencénico aromático, un grupo olefínico (vinilo) y un ácido carboxílico en el extremo. Son denominados hidroxicinámicos por la presencia del grupo -OH (hidroxilo) en el anillo bencénico aromático como se puede observar en la siguiente figura 3.

Se encuentran en forma libre y también en forma de ésteres con el ácido tartárico. También puede encontrarse como restos acilantes de la molécula de glucosa de los antocianos, un grupo de compuestos fenólicos flavonoideos.



Ácidos Hidroxicinámicos

	R_3	R_5
ácido cafeico	OH	H
ácido ferúlico	OCH_3	H
ácido p-cumárico	H	H
ácido sinápico	OCH_3	OCH_3

Figura 3. Estructura química de los principales ácidos hidroxicinámicos.

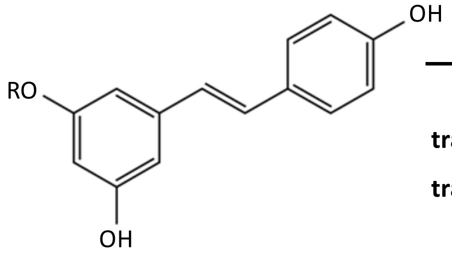
Su oxidación por vía enzimática o química origina el pardeamiento, principalmente en mostos y en vinos blancos. La forma en la que se presentan mayoritariamente en la uva es como ácidos hidroxicinámicos esterificados con ácido tartárico (ácidos caftárico, cutárico y fertárico). Posteriormente, en el vino estos ésteres se hidrolizan y liberan los ácidos hidroxicinámicos libres (ácidos cafeico, *p*-cumárico y ferúlico, respectivamente), que también pueden formar ésteres con el etanol en vinos más envejecidos.

Son los compuestos fenólicos, dentro del grupo de los no flavonoides, mayoritarios de la uva, debido a que se encuentran fundamentalmente en la pulpa y también en la piel. Los más abundantes son el ácido cafeico, ferúlico, *p*-cumárico y sinápico. La exposición al sol es muy importante para el desarrollo de algunos de estos compuestos como por ejemplo el ácido cafeico. Se ha descrito que tienen actividad antioxidante (Xiang y col., 2014) y que son más abundantes en la uvas tintas que en las blancas.

Otros fenoles no flavonoides dignos de destacar serían aquellos que derivan específicamente del contacto del vino con la madera y que por tanto no proceden de la uva. De entre ellos destacaríamos los taninos hidrolizables ya mencionados así como los aldehídos fenólicos y las fenil-cetonas (Zamora, 2003).

1.1.1.3. Los estilbenos

Son hidrocarburos aromáticos, donde los compuestos principales son el *trans*-resveratrol y su derivado 3-glucosilado, conocido como piceido o polydatin, cuyas estructuras se muestran en la figura 4.



Estilbenos	**R**
trans-resveratrol	H
trans-piceido	Gluc

Figura 4. Estructura química básica de los principales compuestos de los estilbenos.

Aparecen en toda la planta pero sobre todo en la piel del fruto y las semillas. Por esta razón es mayor su concentración en vinos tintos que en blancos. Tiene gran importancia como antioxidante y como sistema de resistencia de la planta frente a ataques fúngicos (mayoritariamente de *Botrytis cinerea*) (Chalal y col., 2014). Diversos estudios parecen demostrar que un consumo moderado y habitual de vino, gracias a su contenido en estilbenos, favorece el sistema cardiovascular (Athar y col., 2007; Opie y Lecour, 2007) al disminuir los niveles de colesterol y triglicéridos.

1.1.2. Compuestos Flavonoides

Se caracterizan por tener un esqueleto común que consiste en dos anillos bencénicos aromáticos unidos mediante un heterociclo de tipo pirano, conformando un sistema base de 15 átomos de carbono (C_6-C_3-C_6) de tipo 2-fenilbenzopirona. Esta gran familia se divide en varias subclases, que se distinguen por el grado de oxidación de su núcleo pirano. Podemos ver su estructura general en la Figura 5.

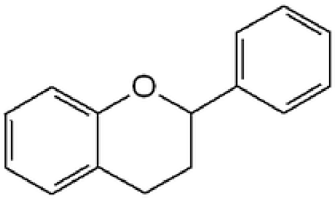


Figura 5. Estructura química común de los flavonoides.

Dentro de los flavonoides se incluyen diversas familias como las flavonas, los flavononoles, los flavonoles, los antocianos y los flavanoles. Dentro de esta última familia se incluyen las proantocianidinas o taninos condensados. Dada la gran importancia de los taninos condensados en el vino, y debido a que los taninos hidrolizables no son flavonoides, se ha optado por incluir un apartado específico para los taninos.

1.1.2.1. Flavanonoles o dihidroflavonoles

Son derivados de la 2-fenilbenzopirona, como se observa en la figura 6. Los compuestos de esta familia han sido identificados en las pieles de uvas blancas. Las más abundantes son la astilbina (3-ramnósido de taxifolin o 3-ramnósido de dihidroquercetina) y la engelatina (3-ramnósido de dihidrokaempferol), cuyas estructuras han sido identificadas por resonancia magnética nuclear de protones.

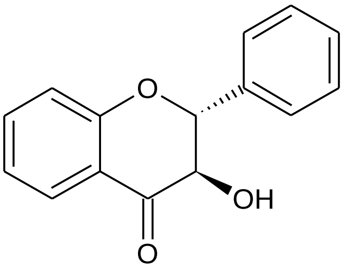
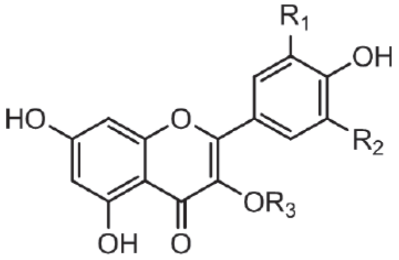


Figura 6. Estructura química básica de un flavanonol.

1.1.2.2. Flavonoles

Dentro de estos compuestos se encuentran los derivados de la quercetina y de la miricetina, que son los mayoritarios, junto con los derivados de kaempferol, isorhamnetina, laricitrina y siringetina (Castillo-Muñoz y col., 2007), estructuras químicas mostradas en la figura 7. Son sustancias de color amarillo que se encuentran en el hollejo. Todos los flavonoles se encuentran mayoritariamente glicosilados en la uva, y en el vino pueden sufrir hidrólisis, por lo que se pueden encontrar también en la forma aglicona libre.



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

Figura 7. Estructura química de los flavonoles.

Parece ser que la síntesis de los flavonoles se ve influenciada por la el grado de iluminación de la baya, del contenido nitrogenado del suelo, de la variedad vinífera y del régimen hidríco (Makris, Kallithraka y Kefalas, 2006).

Los flavonoles sólo existen en la uva como glicósidos (cuando el sustituyente R3 es un carbohidrato), ya sea por unión con monosacáridos (en forma de glucósidos, galactósidos o glucurónidos) o por unión con disacáridos, originando moléculas más complejas como por ejemplo la rutina (quercetina-3-*O*-(6”-ramnosil)-glucósido o quercetina-3-*O*-rutinósido).

Los flavonoles pueden formar complejos con los antocianos propiciando un aumento del color gracias al fenómeno de la copigmentación (Gómez-Mínguez y col., 2006; Lambert y col., 2011). Asimismo, algunos autores postulan que la formación de dichos complejos favorece la extracción de los antocianos durante la maceración de los hollejos (Hermosín-Gutiérrez, 2007).

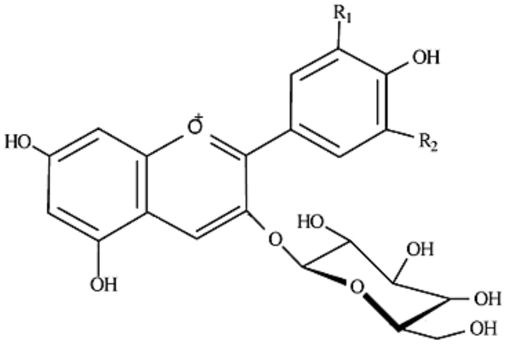
1.1.2.3. Antocianos

Representan una parte importante tanto cualitativa como cuantitativa de los flavonoides en las uvas y en consecuencia de los vinos resultantes, de forma que son los responsables del color rojo-azulado de las uvas tintas y por lo tanto del color de los vinos tintos y rosados (He y col., 2012a; He y col., 2012b). Se localizan en las tres o cuatro primeras capas celulares de la hipodermis, excepto en variedades tintoreras donde también se hallan en la pulpa (Souquet y col., 1996; Cheynier y col., 2003). Su estructura se caracteriza por un esqueleto básico de quince átomos de carbono (C6-C3-C6) dispuestos en 2 anillos aromáticos bencénicos y un heterociclo central, oxigenado, de tipo piránico y también con carácter aromático en la forma de catión flavilio.

Su estructura más común es la de un heterósido, es decir, unido a un grupo azúcar, por lo general glucosa. En *Vitis vinifera* solo se encuentran glicosidados con glucosa en la posición 3 pero en otras especies de *Vitis* o en híbridos existen glicósidos en otras posiciones, siendo muy habituales los 3,5-diglucósidos.

La diferencia entre los antocianos radica en el número de grupos hidroxilos, el grado de metoxilación (grupos –OCH_3), naturaleza de los azúcares y posición que ocupen y de otras moléculas unidas a los azúcares. Cuando se encuentran en forma de aglicona se habla de antocianidinas, mientras que si se hallan glicosiladas se las denomina como antocianos.

Si nos centramos en los antocianos de *Vitis vinifera*, hablaremos de las cinco antocianidinas-3-*O*-glucósidos que se muestran en la figura 8 y que derivan de las antocianidinas denominadas cianidina, delfinidina, peonidina, petunidina y malvidina, siendo esta última la más abundante en la mayoría de variedades. Además, la glucosa puede estar acilada en su posición 6” con los ácidos acético, *p*-cumárico o cafeico. Los antocianos se acumulan en las pieles de las bayas a lo largo del proceso de maduración.



Antocianidina-3-glucósido

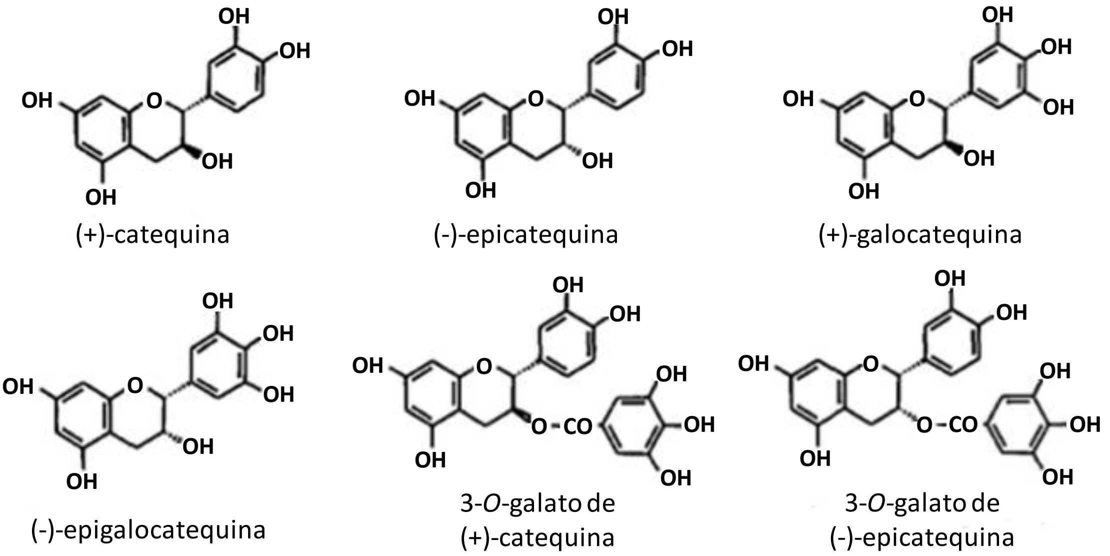
	R_1	R_2
Cianidina	OH	H
Delfinidina	OH	OH
Peonidina	OCH_3	H
Petunidina	OCH_3	OH
Malvidina	OCH_3	OCH_3

Figura 8. Estructura química de antocianidinas-3-*O*-glucósidos, que derivan de las antocianidinas.

1.1.2.4. Flavan-3-oles

Constituyen una compleja familia dentro de los compuestos fenólicos. Pueden estar en forma monomérica o formando oligómeros y polímeros, recibiendo estos últimos la denominación de taninos condensados o proantocianidinas.

Las formas monoméricas más abundantes en uvas y vinos son: (+)-catequina, (-)-epicatequina, (+)-galocatequina, (-)-epigalocatequina, así como los galatos de (+)-catequina y de (-)-epicatequina, cuyas estructuras se exponen en la figura 9.



(+)-catequina (-)-epicatequina (+)-galocatequina

(-)-epigalocatequina 3-*O*-galato de (+)-catequina 3-*O*-galato de (-)-epicatequina

Figura 9. Estructura química de las formas monoméricas de los flavan-3-oles.

Se encuentran en elevadas concentraciones en los raspones, semillas y pieles de las uvas. Tanto los monómeros como los oligómeros y polímeros son extraídos durante la maceración. Los monómeros contribuyen al sabor amargo (Noble, 2009; Ma, 2014) mientras que los polímeros son los principales responsables de la astringencia (de Freitas y Mateus, 2001; Preys y col., 2006; Sáenz-Navajas y col., 2012; Quijada-Morín y col., 2012). La importancia de la composición monomérica de los taninos condensados o proantocianidinas se comentan más adelante.

1.1.3. Taninos

Son metabolitos secundarios de algunos vegetales, que se encuentran en el vino y que proceden de las pieles y pepitas del racimo y/o de la madera de roble de las barricas, siendo más abundantes en los vinos tintos que en los blancos por la diferente forma de elaboración.

El término "tanino" incluye un gran número de moléculas que por definición son capaces de formar enlaces estables con proteínas (Bate-Smith y Swain, 1965; McRae, Falconer y Kennedy, 2010) y, por lo tanto, causar astringencia (Haslam, 1974; Haslam, 1981; McManus y col., 1985; Rinaldi y col., 2015). Los taninos son solubles en agua y proporcionan sabor amargo (Ma y col., 2014). Se clasifican en dos grupos de acuerdo a su estructura química: taninos condensados y taninos hidrolizables (Frazier y col., 2010; Grasel y col., 2016a; Grasel y col., 2016b). Dentro de este último grupo se encuentran los galotaninos y los elagitaninos, cuya estructura general se muestra en la figura 10.

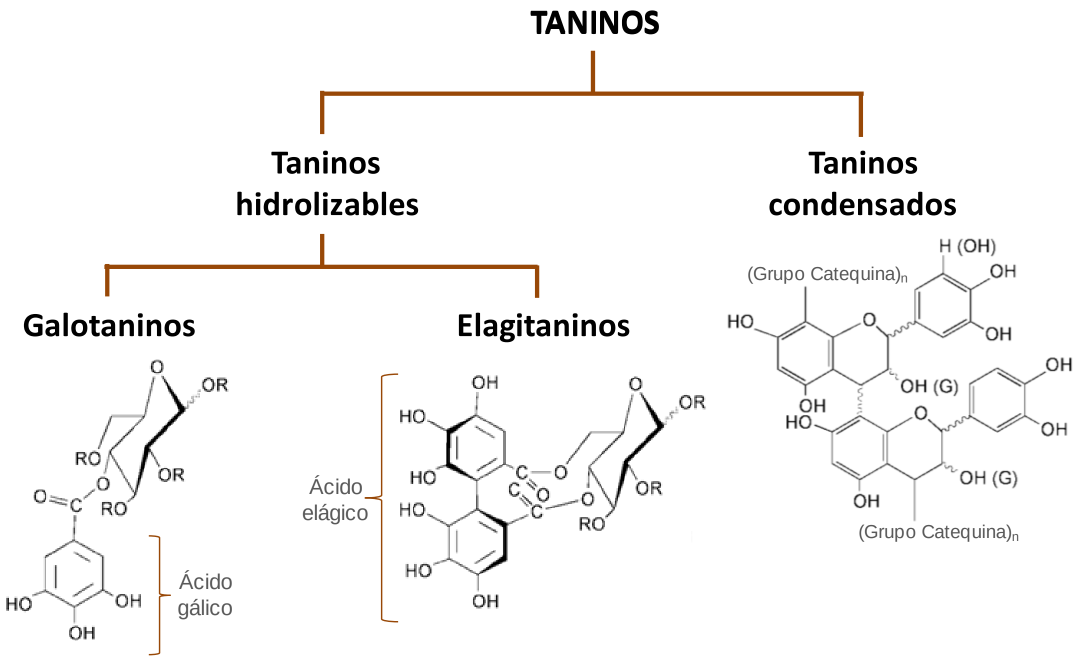


Figura 10. Esquema de los grupos de taninos con estructuras químicas comunes para cada uno de ellos.

1.1.3.1. Taninos condensados o proantocianidinas

Los taninos condensados son polímeros de unidades monoméricas de flavan-3-oles, principalmente de (+)-catequina, (-)-epicatequina, la (-)-epigalocatequina y el galato de (-)-epicatequina, unidas entre sí por enlaces C-C, normalmente de las posiciones 4 y 8 de distintos monómeros, aunque a veces surgen ramificaciones por uniones que implican a las posiciones 4 y 6 de distintos monómeros, estructura general mostrada en la figura 10.

Las formas poliméricas también reciben el nombre de proantocianidinas, ya que en condiciones enérgicas de hidrólisis ácida, a 100 ºC y medio oxidante (reacción de Bate-Smith), se despolimerizan liberando antocianidinas. De hecho la metodología clásica de determinación de taninos en el vino se basa en esta reacción (Ribéreau-Gayon y Stonestreet, 1965). Si las proantocianidinas contienen (-)-epigalocatequina en su composición darán lugar por medio de dicha reacción a delfinidina en lugar de cianidina, por lo que las proantocianidinas se subdividen en procianidinas y prodelfinidinas. Este es un dato importante ya que las proantocianidinas de las semillas contienen prácticamente sólo procianidinas mientras que las de las pieles contienen procianidinas y prodelfinidinas. Por otra parte, los taninos de las semillas contienen una proporción de 3-galato de (-)-epicatequina mucho mayor que los de las pieles y su grado de polimerización es menor (Vidal y col., 2003). Se ha descrito también otros taninos condensados que en hidrólisis ácida liberan otras antocianidinas. Así se han descrito profisetinidinas (quebracho), prorobinetidinas (mimosa) e incluso propelargonidinas (uña de gato) (Celzard y col., 2015).

1.1.3.2. Taninos hidrolizables

Los taninos hidrolizables incluyen a los galotaninos y los elagitaninos. Se denominan hidrolizables porque en medio ácido liberan ácido gálico (galotaninos) o ácido elágico (elagitaninos) (Quideau y col., 1996; Quideau y col., 2010).

1.1.3.2.1. Galotaninos

Los galotaninos son los taninos más simples que existen, están formados por unidades galoil o di-galoil esterificadas a un núcleo de glucosa u otro alcohol polivalente (Figura 10). Los galotaninos, especialmente los aislados de agallas de roble, se emplean como taninos enológicos especialmente como coadyuvantes de la clarificación de los vino blancos (Zamora, 2003b).

1.1.3.2.2. Elagitaninos

Taninos elágicos presentados en la figura 10, están constituidos por la unión de varias moléculas de ácido elágico esterificadas con glucosa en forma lineal. Son muy abundantes en la madera de roble y contribuyen a consolidar la estructura del vino durante su crianza (Moreno y Peinado, 2010).

Se han identificado en la madera de roble ocho elagitaninos: Vescalagina, Castalagina, Grandinina, Roburina A, Roburina B, Roburina C, Roburina D y Roburina E. De los cuales los más abundantes son Castalagina y Vescalagina (Nonier y col., 2005; Ribereau-Gayon y col., 2006) (Figura 11). Estas moléculas son solubles en medio hidroalcohólico. Es por esta razón, que el vino las consigue extraer poco a poco durante su crianza en barricas de roble.

Además, el nivel de los elagitaninos de la madera de roble utilizada para fabricar barricas depende en parte de la especie, edad, origen geográfico, las prácticas de manejo forestal de los árboles, la posición de muestreo en el árbol utilizado y la transformación de la madera en tonelería como el tipo y duración de secado y el tostado (Michel y col., 2011).

De estos compuestos se conoce que se comportan como antioxidantes por su gran capacidad de consumir oxígeno; también que aceleran la condensación de antocianos y taninos con etanal (acetaldehído), por su capacidad de formación de este compuesto; que favorecen la polimerización de los flavanoles del vino, y que pueden unirse a ellos formando complejos flavanoelagitaninos (Quideau y col., 2003 y 2005). Por ello, parece que sólo una pequeña parte de los elagitaninos aportados por la madera se encuentran en el vino en forma libre, contribuyendo a las sensaciones de amargor y astringencia, especialmente Grandinina y Roburina E (Glabasnia y Hofmann, 2006). En el caso de que se llegaran a alcanzar concentraciones muy elevadas, esto daría lugar a vinos con características organolépticas no deseadas, lo que se denomina “sabor a tablón” (Cadahía y col., 2008).

La cantidad de elagitaninos libres presentes en las bebidas envejecidas va a depender también de los tratamientos realizados a la madera, ya que durante el secado natural de la madera al aire libre disminuyen notablemente la cantidad de elagitaninos que aporta la madera, y de igual forma, como veremos más adelante, se ven afectados por el tostado realizado a la barrica durante su fabricación.

En la madera de roble (*Quercus robur, Quercus petraea, Quercus pyrenaica)* y el castaño (*Castenea sativa*), encontramos de forma mayoritaria dos elagitaninos que son isómeros (epímeros): vescalagina y castalagina (Puech y col., 1996; Canas y col., 2000) pueden representar hasta el 60% del contenido de los elagitaninos solubles totales (Fernández de Simón y col., 1999a y 1999b).

Sus estructuras están constituidas por una cadena de glucosa abierta en el que los grupos OH están esterificados por los grupos carboxílicos de las agrupaciones hexahidroxidifenoilo (HHDP) y nonahidroxitrifenoilo (NHTP).

Se han identificado acutisiminas A/B y epiacutisiminas A/B, en las especies de robles anteriormente mencionados, (Ishimaru y col., 1987; Konig y Scholz, 1994), en vinos tintos envejecidos en madera de roble (Saucier y col., 2006, Quideau y col., 2010) y con chips (Jourdes y col., 2011). Su estructura consiste en una unidad de vescalagina unida por enlace C-C a una catequina y epicatequina, respectivamente, en sus posiciones 8 (tipo A) o 6 (tipo B) como se puede observar en la figura 11.

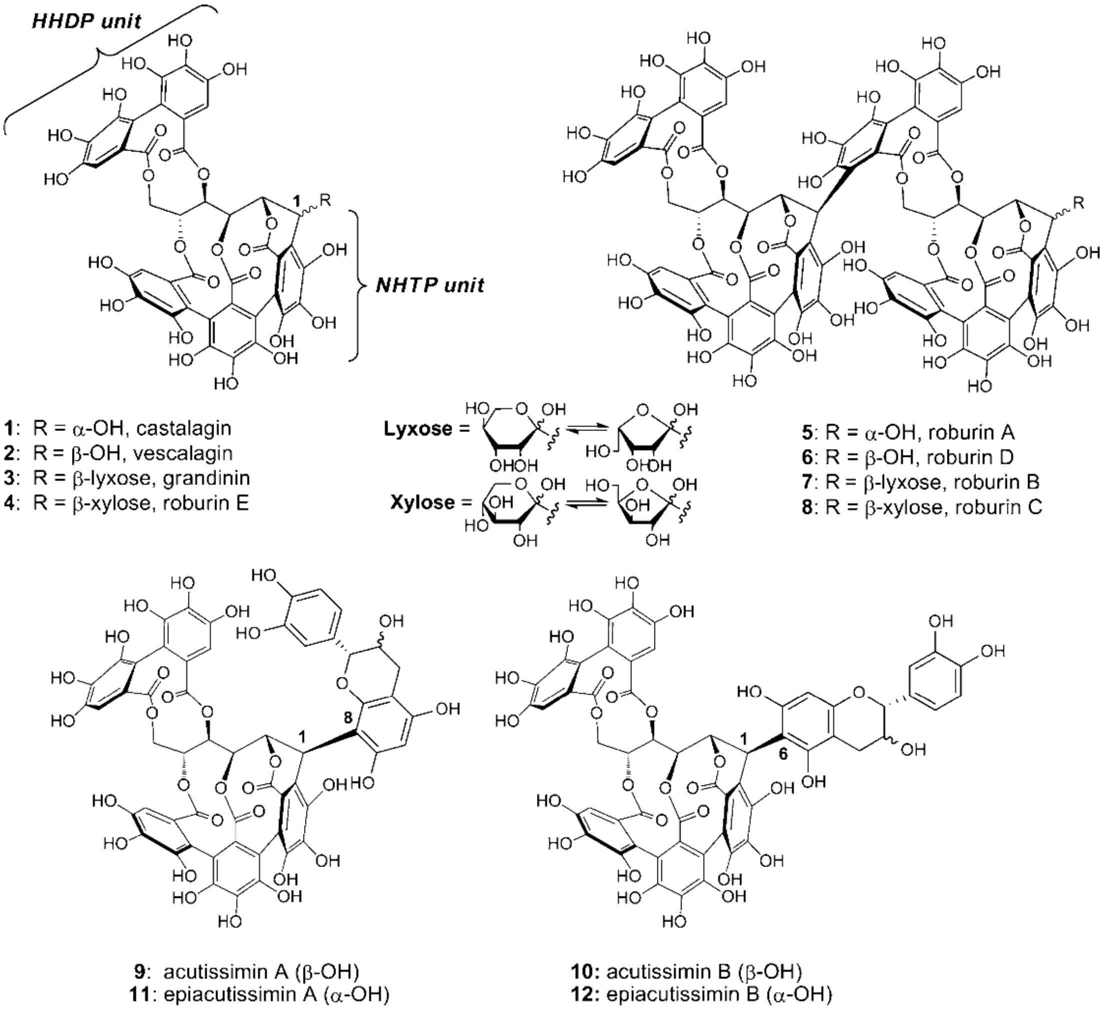
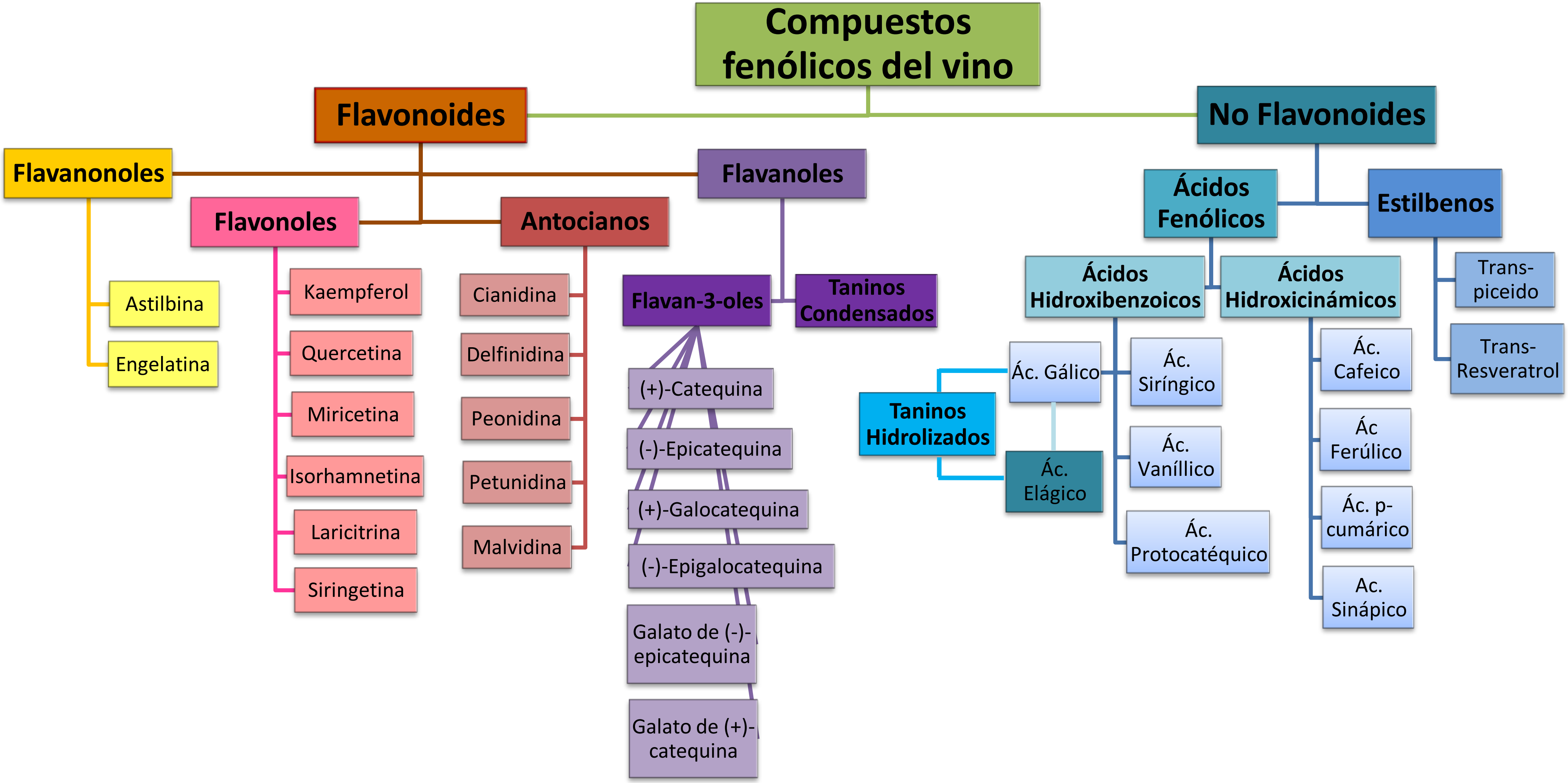


Figura 11. Estructura de elagitaninos presentes en la madera de roble (Jourdes y col.2011).


Compuestos fenólicos del vino
Flavonoides
No Flavonoides
Flavanonoles
Flavanoles
Flavonoles
Antocianos
Astilbina
Engelatina
Kaempferol
Quercetina
Miricetina
Isorhamnetina
Laricitrina
Siringetina
Cianidina
Delfinidina
Peonidina
Petunidina
Malvidina
Flavan-3-oles
Taninos Condensados
(+)-Catequina
(-)-Epicatequina
(+)-Galocatequina
(-)-Epigalocatequina
Galato de (-)-epicatequina
Galato de (+)-catequina
Ácidos Fenólicos
Estilbenos
Ácidos Hidroxibenzoicos
Ácidos Hidroxicinámicos
Trans-piceido
Trans-Resveratrol
Taninos Hidrolizados
Ác. Gálico
Ác. Elágico
Ác. Siríngico
Ác. Vanillico
Ác. Protocatéquico
Ác. Cafeico
Ác Ferúlico
Ác. p-cumárico
Ac. Sinápico

1.2. La madera de roble en enología

La madera de roble tiene propiedades que la hacen idónea para la construcción de barricas. Entre ellas destacan su gran inercia térmica, la resistencia frente a golpes y su composición. También la porosidad y la permeabilidad, que dependerá del sentido de colocación de las fibras, son claves para evitar las fugas y para garantizar una cierta entrada de oxígeno que permita la correcta evolución del vino.

1.2.1. La composición química de la madera de roble

El duramen está formado por 40% de celulosa, 15% de hemicelulosa, 20% de lignina, 10% de elagitaninos, y 5% componentes de naturaleza química diversa (valores referidos al peso seco). El resto de componentes, representada por el 10% restante de la masa de la madera seca, constituyen la llamada fracción extraíble, y pueden hallarse mezclados con los polímeros en la pared celular o como formaciones en los lúmenes celulares. (Fernández de Simón y Cadahía, 2007).

Las concentraciones más altas de taninos de roble se encuentran en los tejidos leñosos, que contienen una alta proporción de células de parénquima, especialmente madera temprana del parénquima, y en los radios de madera. Estos taninos pueden estar presentes de forma libre, o unidos a polisacáridos (Puech y col., 1999). Además contribuyen a propiedades de la madera como: color, sabor y resistencia a la descomposición. Los elagitaninos son los compuestos más abundantes, pero también se encuentran otros componentes de estructuras químicas muy diferentes, como otros polifenoles (lignina), los compuestos furánicos, lactonas, ácidos grasos, entre otros. Algunos de estos compuestos serán el origen de muchas de las sustancias de interés organoléptico que se encuentran en los vinos de crianza (Fernández de Simón y col. 2007).

1.2.2. Sustancias volátiles procedentes del roble y su impacto organoléptico

La madera de roble contiene un gran número de sustancias volátiles, muchas de las cuales se forman durante el tostado de las duelas, que son aportadas al vino aumentando la complejidad aromática de este. El único compuesto de los aportados por la madera que no es positivo respecto a la calidad aromática del vino es el ácido acético. Su origen se encuentra en la estructura de la hemicelulosa de la madera, que libera grupos acetilo por hidrólisis durante el tostado.

La composición volátil que aporta una barrica de roble, varía mucho dependiendo del tipo de madera utilizada para su fabricación (García y col., 2012). Diversos estudios se han realizado para detectar las diferencias aromáticas aportadas por las dos especies de roble utilizadas en Francia (*Quercus petraea y Quercus robur*) y la especie utilizada en EEUU (*Quercus alba*). El potencial aromático del roble americano es mayor que el del roble francés, estando condicionado fundamentalmente por una elevada proporción de whiskey-lactonas (Chatonnet y Dubourdieu, 1998; Prida y col., 2006; Alañon y col., 2012a). Por este motivo, el impacto aromático de las barricas americanas es mayor sobre el vino y enmascara en mayor proporción los aromas frutales y florales que proceden de la uva ó son generados también durante las fermentaciones.

Las sustancias volátiles suelen agruparse en varias familias de moléculas de estructura y origen afines. Los furanos, el ácido acético y otros heterociclos volátiles se originan a partir de los polisacáridos de la madera de roble. Por su parte los aldehídos fenólicos, las fenil-cetonas y los fenoles volátiles tienen su origen en la lignina. Finalmente las lactonas proceden de los lípidos.

1.2.2.1. Compuestos furánicos

Son compuestos que se forman durante el tostado de la madera de roble, por degradación térmica de los polisacáridos de la misma, cediéndose al vino durante el tiempo de contacto madera-vino (Absalon y col., 2000 y 2003; Cacho, 2006; Cadahía y Fernández de Simón, 2004; Garde-Cerdán y col. 2002 y 2006; Pérez-Prieto y col., 2003). Los compuestos que se encuentran en la madera de roble, mostrados en la figura 12, son el furfural, 5-metilfurfural, 5-hidroximetilfurfural y alcohol furfurílico, de los que el furfural es el más abundante.

Presentan aromas a almendra, almendra tostada, caramelo y heno. Algunos autores consideran estos compuestos como un índice de tostado de la madera, ya que sus concentraciones se incrementan con la intensidad de calentamiento (Spillman y col. 2004; Alañon y col., 2012a).

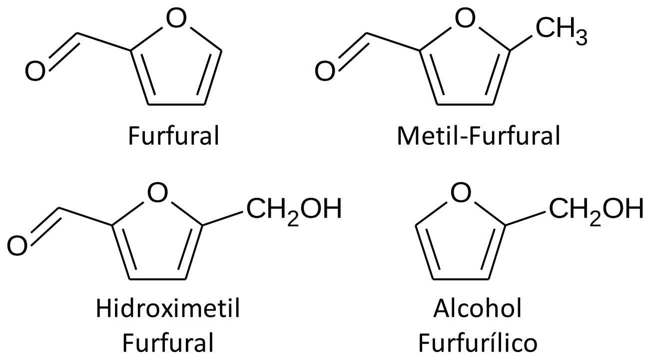
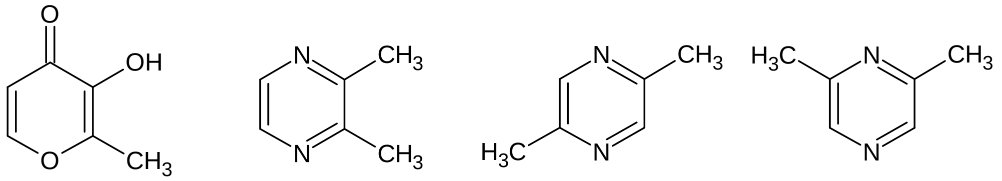


Figura 12. Estructuras químicas de los compuestos furánicos presentes en la madera de roble.

También podemos encontrar en la madera, heterociclos oxigenados como el maltol que otorgan al vino aromas de caramelo y notas de tostado (Aznar, 2002). Heterociclos nitrogenados, las dimetilpirazinas, serán las responsables de la presencia de notas de cacao, avellana, pan tostado y café en los vinos de crianza. Todos ellos mostrados a continuación en la figura 13.



Maltol 2,3-Dimetilpirazina 2,5-Dimetilpirazina 2,6-Dimetilpirazina

Figura 13. Estructuras químicas de los compuestos furánicos con heterociclos oxigenados (maltol) y nitrogenados (dimetilpirazinas) presentes en la madera de roble.

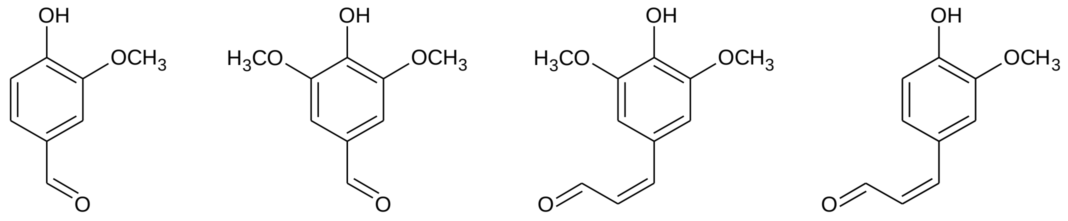
La concentración de estos compuestos parece tener relación con el origen de la madera de roble (Cadahía y Fernández de Simón 2004). Se han observado concentraciones más elevadas de compuestos furánicos en robles de origen americano que en robles europeos a excepción del compuesto furfural, que tiene un comportamiento inverso (Chatonnet y col. 1997; Pérez-Coello y col. 1999; Fernández de Simón y col. 2003; Alañon y col., 2012b).

1.2.2.2. Ácido acético

La madera de roble de las barricas, especialmente en el caso de barricas nuevas, puede aportar cantidades apreciables de ácido acético al vino (Chatonnet, 1994). Aparentemente su origen hay que buscarlo en la propia estructura de la hemicelulosa. Algunas unidades xilosa de las hemicelulosas presentan grupos acetilo en posición 2 y/o 3, los cuales pueden dar lugar a la formación de ácido acético libre, mediante hidrólisis, durante el tostado de las duelas. Este fenómeno implica un aumento de la acidez volátil del vino de crianza. Sin embargo, la principal fuente del ácido acético en el vino nunca es la madera, sino que viene atribuido a las levaduras, bacterias acéticas y eventualmente a las bacterias lácticas.

1.2.2.3. Los aldehídos fenólicos y las fenil-cetonas

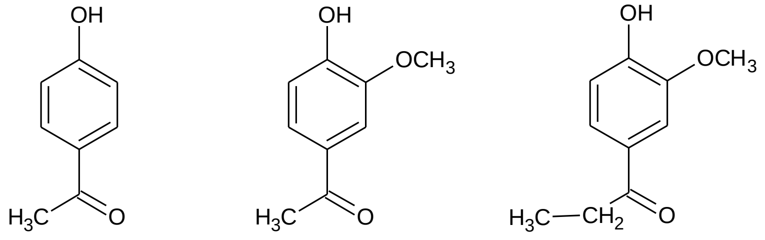
Son otras dos familias que presentan un gran interés como sustancias volátiles procedentes del roble, dentro del conjunto de los aldehídos fenólicos, destaca por su importancia la vainillina, que es la principal responsable del olor a vainilla que caracteriza a muchos vinos de crianza. También se incluyen en esta familia como se puede observar en la figura 14, el siringaldehído, el sinapaldehído y el coniferaldehído, si bien no presentan un gran impacto sensorial en las concentraciones habituales en que se encuentran en los vinos (Chira y col., 2013), debido a que sus umbrales de percepción son superiores.



Vainillina Siringaldehído Sirinpaldehído Coniferaldehído

Figura 14. Estructuras químicas de aldehídos fenólicos, responsables del olor a vainilla.

Las fenil-cetonas (acetofenona, acetovainillona, propiovainillona) cuya estructura se muestra en la figura 15, provienen de la madera pero también de precursores existentes en las uvas. Los niveles cedidos por las barricas son siempre superiores a los generados en depósito. Las cantidades cedidas o generadas suelen estar por debajo de sus umbrales de detección, por lo que su contribución organoléptica en los vinos no será individualmente significativa (Cacho, 2006b), si bien se considera que en su conjunto refuerzan el aroma de vainilla del vino.



Acetofenona Acetovainillona Propiovainillona

Figura 15. Estructuras químicas de las fenil-cetonas.

1.2.2.4. Fenoles volátiles

Son una amplia familia, como se puede ver en la figura 16, de compuestos que aportan al vino aromas muy variados. Así el guayacol aporta olor a tostado ; el 4-metil-guayacol y el 4-etil-guayacol presentan olor a madera quemada; el 4-vinil-guayacol tiene un olor que recuerda al del clavel; el fenol presenta olor de tinta; el eugenol, de gran importancia organoléptica, confiere aroma de clavo de especia; el 4-vinil-fenol aporta olor fenólico y farmacéutico; y el 4-etil-fenol, presenta un desagradable olor animal, descrito como cuero e incluso como sudor de caballo. La presencia de este último compuesto se considera, siempre y cuando sobrepase su umbral de percepción, como un grave defecto del vino (Chatonnet y col., 1997b; Rayne y col., 2007).

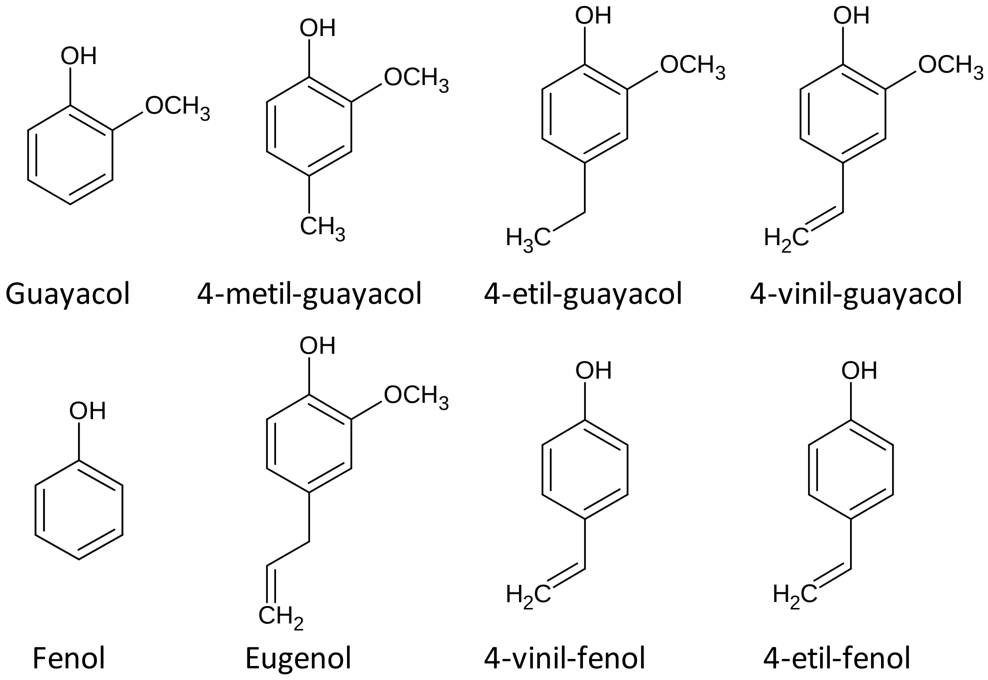


Figura 16. Estructuras químicas de la familia de los fenoles volátiles.

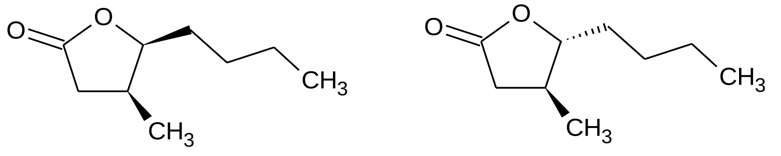
El origen de los fenoles volátiles es variado, por una parte proceden de la pirólisis de las ligninas originadas durante el tostado de las duelas. Pero también pueden originarse mediante la descarboxilación de los ácidos fenólicos presentes en la uva y el vino.

En ese sentido, el desarrollo de ciertos microorganismos como las levaduras *Brettanomyces/Dekkera* puede incrementar enormemente su concentración en el vino otorgándole desagradables notas fenólicas y/o animales ya que son capaces de descarboxilar a los ácidos hidrocinámicos hasta 4-vinil-fenoles y posteriormente reducirlos hasta 4-etil-fenoles (Garde y Ancin, 2005; Palacios y Otaño, 2009).

1.2.2.5. Lactonas

Son sustancias de gran importancia para el aroma de los vinos de crianza, su origen parece estar relacionado con la degradación de los lípidos presentes en la madera durante el tostado de las duelas, las encontradas en madera de roble son la β-nonalactona, β-decalactona y la γ-octolactona, aunque la más importante es la β-metil-γ-octolactona ó más comúnmente conocida como whiskey-lactona. Su isómero *cis* es diez veces más odorante que el *trans*, debido a que presenta un umbral de percepción menor. Las estructuras de ambos isómeros se pueden ver en la figura 17.

Existe una gran variabilidad entre especies, bosques y árboles en cuanto a contenido en whiskey-lactona en madera seca (Masson y col., 1995). Los robles americanos son los que tienen la concentración más elevada de esta lactona, así como una proporción mayor (alrededor del 90 %) del isómero *cis* (Cadahía y col., 2003: Jarauta y col., 2005; Alañón et al., 2012a; Schumacher et al.,2013). Los robles franceses presentan una proporción variable pero siempre menor del isómero *cis* (Masson y col., 1995; Cadahía y col., 2003). Aportan aromas de nuez de coco, coco o melocotón.

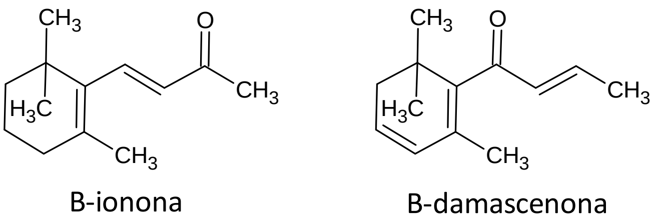


(cis) β -Metil-γ-octolactona (trans) β -Metil-γ-octolactona

Figura 17. Estructuras químicas de β-metil-γ-octolactona, más conocida como whiskey-lactona.

1.2.2.6. Norisoprenoides

La β-ionona (aroma a violetas) y β-damascenona (aroma manzana asada), mostrados es la figura 18, no son considerados normalmente productos cedidos por la madera. Sin embargo Cacho y col. (2006b) demuestran que en la misma existen precursores, posiblemente derivados de los carotenoides, que pueden ser fuente activa de estos odorantes. El tipo de madera no parece que sea importante en esta cesión. Participan en el aroma del vino debido a que poseen un umbral de percepción bajo (Silva-Ferreira y col., 2008).



B-ionona B-damascenona

Figura 18. Estructuras químicas de los norisoprenoides.

1.2.3. Sustancias no volátiles procedentes del roble y su impacto organoléptico

Las concentraciones de los compuestos no volátiles de bajo peso molecular presentes en maderas verdes procedentes de roble son muy variadas (Cadahía y col., 2008). Mediante los análisis químicos de las maderas verdes de las especies de roble, en este caso de origen español, Cadahía y col. (2001) han identificado ácidos, aldehídos fenólicos, cumarinas y elagitaninos que ya habían sido descritos para otras especies y orígenes de roble. La composición en polifenoles de bajo peso molecular y elagitaninos de la madera de roble de origen español en verde, es cualitativamente idéntica a la de origen francés y americano, presentando sólo diferencias cuantitativas (Fernández de Simón y col., 2009). Cabe destacar que la madera procedente de roble francés, cede al menos siete veces más fenoles extraíbles que la madera de roble americano, por lo que los vinos así envejecidos resultarán mucho más "tánicos" (García, 1997).

Estas diferencias permiten distinguir la madera según su origen geográfico, a pesar de la fuerte variabilidad individual, que puede ser mayor que la variabilidad entre especies debido a la gran influencia de las condiciones de suelo, manejo forestal, climatología y edad de los árboles, influyendo sobre los caracteres estructurales de la madera (Fernández de Simón y col., 2009).

Puech y col. (1999) observaron un incremento de la variación en el nivel de elagitaninos entre los diferentes árboles, poblaciones y especies de roble. Sin embargo, pocos estudios han logrado controlar adecuadamente otros factores que pueden influir en el nivel de taninos, como la edad (Vivas y col., 1998) y el tratamiento de la madera (Zhang y col., 2015), garantizando al mismo tiempo la identificación cuidadosa de origen biológico y geográfico de las muestras y el adecuado proceso de muestras replicadas. Se ha encontrado que la diferencia más evidente es el nivel de extractos fenólicos entre las especies de roble europeas y americanas utilizadas para la tonelería.

Tanto los estudios más recientes como los anteriores, indican los niveles más bajos de compuestos fenólicos y elagitaninos extraídos de la madera en el caso de maderas de roble americano en comparación con las maderas de roble europeo empleadas en tonelería (Masson y col., 1995; Chatonnet y Dubourdieu, 1998; Doussot y col., 2000 y 2002; Prida y col., 2006).

1.3. Propiedades sensoriales de los Elagitaninos

Las características sensoriales más reconocidas de los taninos son la astringencia, aspereza y amargor, principalmente presentes en vinos blancos y tintos almacenados en barricas o tratadas con virutas de roble (García, 1997).

Hay numerosos estudios sobre esto, Hervé du Penhoat y col. (1991) realizaron una evaluación sensorial de los taninos de la madera de roble y encontraron sólo una débil sensación de astringencia en los vinos. También Puech y col. (1999) informaron sobre un impacto débil de astringencia por parte de los elagitaninos sobre el vino, pero con umbrales altos de percepción sobre el amargor en vinos madurados en barrica de roble. Debido a la alta reactividad que poseen los elagitaninos, sus niveles en el vino son mucho más bajos de lo que se podría esperar (Michel y col., 2011). Sin embargo su concentración en el vino puede en algunos casos ser suficiente para contribuir de manera notable a la astringencia del vino.

Glabasnia y col. (2006) estudiaron el impacto sensorial de los elagitaninos a nivel individual y encontraron que el umbral más bajo de percepción sobre la astringencia lo tenían la grandinina y roburina E, provocando una sensación astringente a bajas concentraciones. Llegando a ser 5 veces más pequeñas que en el caso de la vescalagina y castalagina, donde el umbral de percepción fue mayor.

Es interesante tener en cuenta que la estereoquímica de los elagitaninos no tiene ninguna influencia sobre su actividad sensorial, los pares de diasteroisómeros mostraron concentraciones de umbral idénticas.

1.4. Aspectos que condicionan el impacto del roble sobre el vino

Los tratamientos a los cuales es sometida la madera en la tonelería pueden condicionar posteriormente la calidad enológica de la barrica. En primer lugar es necesario seleccionar los árboles de los que se va a obtener la madera, y cortarlos de forma adecuada para poder obtener las duelas que van a conformar la barrica.

Una vez obtenidas las duelas es necesario un secado natural apropiado de la madera para proceder a la siguiente fase, la de montaje de la barrica, curvado y tostado de las duelas. (Fernández de Simón y col. 2007).

1.4.1. Origen botánico del roble

La madera de roble presenta unas características físicas y químicas adecuadas para la fabricación de barricas. El género *Quercus*, está formado por más de 600 especies. Las principales especies que se utilizan en la elaboración de barricas son las europeas: *Quercus petraea* y *Quercus robur*; y la americana: *Quercus alba.*

El *Q. petraea* o roble *sessilis* es una madera muy aromática debido a su riqueza en whiskey-lactona y eugenol, y presenta una riqueza en polifenoles menor que el *Q. robur*. En contraste, el *Quercus robur* o roble *pendunculata* es un tipo de madera pobre en compuestos aromáticos, y debido a su grano grueso la cantidad de elagitaninos extraíble es muy alta, alrededor de 7 a 105 mg/g (Kamio, 2012).

Ambas especies se encuentran repartidas por todo el continente europeo, siendo en Francia donde hay una mayor producción (Fernández de Simón y Cadahía, 2007). Por esta razón se acostumbran a denominarlas roble francés.

La especie americana *Quercus alba,* también llamado roble blanco verdadero, se encuentra presente en la costa este de los Estados Unidos (Fernández de Simón y Cadahía, 2007).

Se caracteriza por su escaso aporte en compuestos fenólicos respecto al resto de las especies de origen Europeo y por proporcionar una gran cantidad de whiskey-lactona, especialmente de su isómero *cis*, que es el que mayor impacto odorífero presenta. También posee una cantidad apreciable de compuestos norisoprenoides y de ácido gálico.

En España, además de las especies *Quercus petraea* y *Quercus robur*, existe la especie *Quercus pyrenaica Wild*., que es autóctona de la Península Ibérica. Algunos autores, (Fernández de Simon y Cadahía, 2007; Cadahía y col., 2008) mostraron que la madera de roble español *Quercus pyrenaica* tiene una gran calidad enológica de características intermedias entre el roble francés (*Q. petraea*) y el americano (*Q. alba*). En los últimos años los robles de los países del este europeo (Rusia, Hungría, Rumanía, Polonia…) están empezando a utilizarse, debido a que tienen un menor coste económico y pertenecen a la misma especie que los robles franceses.

La elección de la madera de roble, en tonelería, se realiza no sólo por el origen geográfico de la madera, sino también por el tipo de grano. Se entiende por grano al tamaño y regularidad de los círculos de crecimiento anual del árbol.

Así el roble de grano fino es aquel que presenta un crecimiento de los círculos anuales pequeño, y el roble de grano grueso aquel que los presenta grandes. El tamaño y regularidad del grano dependen de tipo de roble, la edad, las condiciones climatológicas y el terreno donde este plantado (Vivas, 1995).

Los círculos de crecimiento anual se componen de madera de primavera, porosa y rica en grandes vasos, que contiene unas estructuras denominadas tílides. Las tílides son abolladuras de la membrana celular que penetran más o menos profundamente dentro del vaso leñoso y pueden llegar a obstruirlo.

Los vasos de primavera presentan una cierta permeabilidad a los líquidos y en el caso de que no estén lo suficientemente obturados por las tílides como para impedir el paso del líquido, pueden originar que las barricas tengan fugas (Zamora, 2003).

Los robles europeos presentan unas tílides poco abundantes y espesas (Chatonnet y Dobourdieu, 1998) por lo que son más permeables a los líquidos y el riesgo de fugas es alto. Por tanto, los tablones o duelas para confeccionar las barricas se obtienen mediante <u>hendido</u> (figura 19). De este modo, las duelas presentaran una superficie de contacto con el vino paralela a los radios medulares lo que disminuye la posibilidad de fugas.

Por el contrario, el roble americano presenta unas tílides muy abundantes y espesas Chatonnet y Dobourdieu, 1998). Los vasos de primavera son poco permeables al líquido y el riesgo de fugas es mínimo. Por lo que el roble americano se procesa mediante <u>aserrado</u>, proceso que se muestra en la figura 19 presentada a continuación, lo que condicionará que las duelas presenten los radios medulares mayoritariamente oblicuos respecto de la superficie de contacto con el vino.

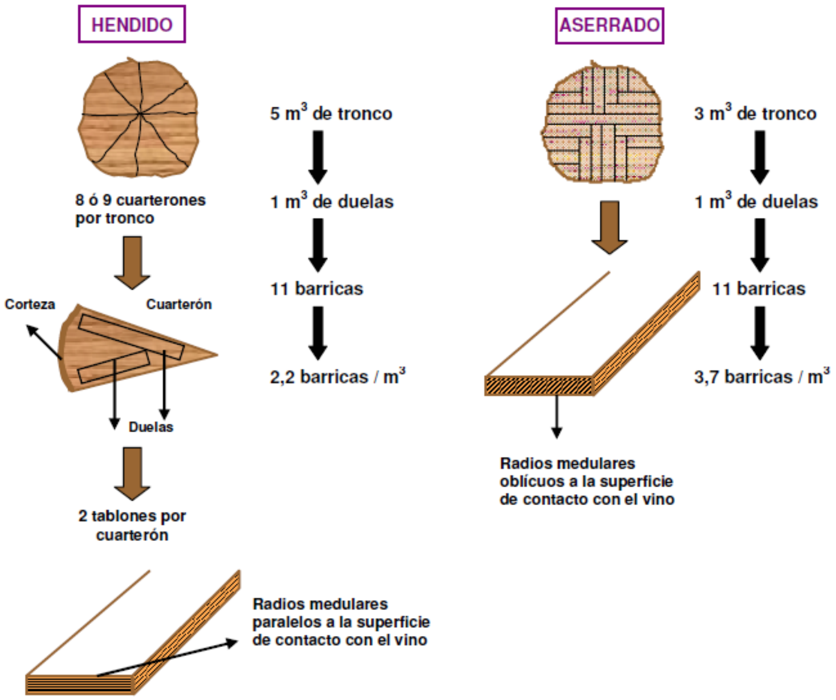


Figura 19. Obtención de duelas para confeccionar las barricas (Zamora, 2003).

Se considera que cuanto más fino es el grano mayor es la porosidad. Los robles de grano fino poseen un mayor número de círculos de crecimiento y por tanto presentan una mayor proporción de vasos leñosos de primavera por unidad de superficie. Por esta razón son más porosos que los robles de grano grueso.

El tipo de grano influirá relativamente poco sobre el aporte de oxigeno ya que la proporción que entra atravesando la propia estructura de la madera no es el principal aporte. Se considera que está en una proporción de alrededor del 16% respecto del total del oxígeno. El resto entraría cruzando las juntas interduelas (63%) y por el esquive (21%) (Vivas y col., 1997). Además, los trasiegos también contribuyen de forma considerable en la cantidad total del aporte de oxígeno durante la crianza.

Por el contrario el tamaño de grano, independientemente de la especie de roble del que se trate, es muy importante en lo que respecta a la aportación de aromas y de taninos elágicos. Al aumentar el tamaño del grano se incrementa el aporte de extracto seco y taninos elágicos, mientras disminuye la concentración de eugenol y whiskey-lactona que aportan al vino los aromas de clavo y de nuez y coco, respectivamente (Vivas 1995).

La tabla 1 (Jordao y col., 2007) muestra los resultados de la evaluación del contenido en los diferentes elagitaninos, de maderas de distintos orígenes y con crecimiento lento (grano fino) y crecimiento más rápido (grano medio). Se observa que el grano medio contiene mayor cantidad de elagitaninos en el caso del roble francés. Esto se debe a que el crecimiento rápido, y por lo tanto grano medio, se centra en la madera de verano que es rica en elagitaninos. Sin embargo, en el caso de la madera americana, presenta menor cantidad de elagitaninos, el anterior resultado sólo se da en el caso de vescalagina.

Tabla 1. Contenido en elagitaninos individuales y totales en virutas de madera de roble de dos diferentes especies botánicas (*Q. petraea* y *Q. alba*) con diferente grosor del grano (Jordao, 2007)

	Q. petraea		***Q. alba***	
Compuesto	Grano Fino	Grano Medio	Grano Fino	Grano Medio
Vescalagina	15.03 ± 0.71	21.80 ± 4.50	1.89 ± 0.42	6.44 ± 1.41
Castalagina	17.87 ± 3.20	24.81 ± 3.20	4.45 ± 1.03	2.49 ± 0.72
Roburina D	1.95 ± 0.02	3.81 ± 0.71	n.d	n.d
Roburina E	5.83 ± 1.20	9.60 ± 1.81	0.59 ± 0.04	n.d
Grandinina	12.20 ± 0.41	13.50 ± 2.34	n.d	n.d
Elag. Totales	52.88 B	73.52 A	6.93 C	8.93 C

Resultados son promedio ± SD calculada para dos muestras de 100 mg de virutas de roble de madera seca. Para elagitaninos totales la comparación de medias se realizó por el método de LSD. Los promedios seguidos por la misma letra no son significativamente diferentes ($p < 0{,}05$). n.d., no se detecta.

En resumen, el grano puede afectar los tres procesos fundamentales que tienen lugar durante la crianza del vino: la microoxigenación natural que favorece la estabilización de la materia colorante y suaviza la astringencia, el aporte de aromas propios de la madera que enriquece la intensidad y la complejidad aromática, y finalmente, el aporte de taninos elágicos que contribuyen a la sensación de estructura.

1.4.2. Influencia del secado del roble

Las duelas de Madera de roble deben ser sometidas a un proceso de secado antes de ser utilizadas para fabricar barricas. La madera reduce su humedad durante el secado desde un 35-60% a un 12-18%. El secado más idóneo de la madera es el que se realiza de modo natural, que consiste en mantener las duelas apiladas a la intemperie durante un período variable de dos o tres años, dependiendo del grosor de la pieza.

Durante este proceso la madera de roble soporta los procesos de deshidratación, rehidratación y humedad constante, que hace que se produzca una cierta contracción de las fibras y una disminución de la aparición de fisuras en las duelas (Fernández de Simón y col., 2009). El secado natural, además de una etapa de deshidratación de la madera, se puede considerar como una etapa de curado, lenta y compleja, comparable a la maduración de la uva. Se pasa de una madera verde y agresiva, a una madera seca y aromática, en la que los elementos solubles son más suaves y más agradables, produciéndose una disminución del amargor y la astringencia. Así, se produce una disminución del contenido en elagitaninos, y de determinados componentes volátiles que se relacionan con la aparición de un carácter vegetal en los vinos. (Fernández de Simón y col., 2007). Asimismo se constata que el secado natural da lugar a mayores niveles de moléculas que participan en el aroma del vino, como serían la vainillina y el eugenol. Y se produce una disminución global de las whiskey-lactonas, aunque en realidad al aumentar el isómero más aromático el *cis*, la contribución final al aroma del vino será también más importante en el caso de secado natural (Sefton y col., 1993; Zamora, 2003).

El comportamiento de cada compuesto es variable durante todo el proceso, debido a una serie de reacciones bioquímicas que se ven influidas por mecanismos físicos asociados a la pluviosidad, las radiaciones ultravioleta y las variaciones de temperatura (amplitudes térmicas), como son los procesos de lixiviación y de degradación hidrolítica oxidativa, y se puede apreciar al final del mismo una tendencia de la mayoría de ellos a reducir su concentración, que se traduce en una disminución del contenido total (Cadahía y col., 2008).

Al mismo tiempo, en la superficie, en los primeros milímetros de las duelas se desarrolla una importante actividad fúngica, que produce una liberación de azúcares por la destrucción de determinadas estructuras fenólicas, como las cumarinas o los taninos hidrolizables, con la consiguiente modificación de las propiedades organolépticas, como es la disminución de la astringencia y del sabor amargo (Fernández de Simón y col., 2007).

La disminución de elagitaninos durante el secado está relacionada con la especie botánica y el origen geográfico de la madera, ya que sus caracteres físico-mecánicos condicionan la efectividad de los mecanismos físico-químicos de degradación. Así, en la madera de roble *Quercus pyrenaica* de origen español y *Quercus petraea*, se produce una disminución de entre el 30-40% de los elagitaninos a lo largo de los 2-3 años que dura el secado natural, en *Quercus robur* (origen francés) la disminución es del 75%, y en la madera de Quercus alba (origen americano) la disminución es menor, en torno al 15% (Fernández de Simón y col., 2007).

Como ya se ha expuesto, el secado natural es el mejor método de secado en cuanto a las modificaciones positivas que produce en la madera, pero este método conlleva unos altos costes de mantenimiento de los stocks de madera inmovilizada durante años.

Por esta razón existen métodos de secado alternativos, como el secado mixto, el cual tiene una etapa de secado natural durante unos meses (de 12 a 15) y una fase final de secado en un horno a temperatura y humedad adecuadas, o bien el secado artificial de la madera, el cual se realiza en un horno durante un tiempo reducido, la madera recibe corrientes de aire caliente entre 30-60 ºC durante 3 o 4 semanas y apenas se mejoran las características sensoriales de la madera.

Estos métodos no son tan buenos como el secado natural, pero reducen el inmovilizado de madera en las tonelerías, abaratando los costes (Hidalgo, 2003; Cadahia y col., 2008). Martinez y col. (2008) confirmaron que el secado natural es más efectivo en cuanto a la reducción del exceso de elagitaninos, especialmente en la madera de roble francés de la especie *Q. petraea*.

García (1997) ha comparado las modificaciones que se producen en la madera en el curso de un secado lento tradicional o bien acelerado por estufa, así como su incidencia en la calidad de los vinos y los espirituosos. En general resulta, aunque no sistemáticamente, que el secado natural permite obtener resultados cualitativamente superiores al secado artificial. A pesar de esto, no siempre es evidente la relación de este resultado con la composición química de la madera desde el punto de vista analítico en el secado natural, ya que éste entraña un empobrecimiento de la madera en relación con el secado artificial.

Un aspecto importante a señalar, es que el nivel de humedad de las duelas alcanzado durante el secado debe ser el adecuado. Si el nivel de humedad es muy bajo, las duelas podrían partirse en el proceso de formación. Por el contrario, si la humedad interna de la madera es muy alta, se corre el riesgo de que aparezcan burbujas de vapor que podrían fragmentar la superficie de las duelas, pudiendo facilitar posteriores fugas o favorecer la acumulación de depósitos que dificultarían su limpieza.

1.4.3. Influencia del tostado de las duelas de roble

Se denomina tostado al proceso en el que se calientan las duelas al fuego para doblarlas ("domarlas") y adaptarlas a la forma característica de la barrica. El calor que llegan a alcanzar las duelas permite su flexibilización, haciendo posible doblarlas mediante la acción mecánica de un cabestrante.

El contacto de la madera con el fuego provoca toda una serie de transformaciones químicas, que dependerán del tiempo de contacto de la madera con el fuego. Según su duración es catalogado normalmente como tostado ligero (tiene una duración de 20 minutos), tostado medio (30 minutos de duración) y tostado fuerte (con un tiempo de 40 minutos). Algunas tonelerías ofrecen un tostado medio plus, extrafuerte e incluso tostado integral, en el que se realiza un tostado de los fondos. No obstante hay que tener en cuenta que cada tonelería tiene su protocolo de tostado y que por tanto sus criterios pueden ser diferentes.

El grado de tostado influye en las características físicas de la barrica pero, también aparecerán una serie de compuestos químicos derivados del calentamiento, que afectaran en mayor o menor medida a las propiedades organolépticas de los vinos contenidos en las mismas (Matricardi y Waterhouse, 1999; Doussot y col., 2002), con variaciones dependiendo del tiempo de contacto del vino con la madera tostada (Chira y col., 2013).

El efecto del tostado se manifiesta también en la variación que experimenta la composición tánica de la madera. Las dos intensidades de tostado más utilizadas en tonelería (media y media plus), suponen reducciones de las concentraciones de elagitaninos más acusadas según se incrementa la intensidad del tostado (Cadahía y col., 2008).

Según Puech y col., (1999), con el nivel más bajo de tostado se reduce, en la capa superficial de la madera, el nivel de vescalagina en un 73% y de castalagina un 46%. El tostado más fuerte eliminará casi por completo los elagitaninos de las capas superficiales de la madera, aunque incluso después del tostado más fuerte el descenso en el nivel de elagitaninos sólo afecta hasta una profundidad de unos pocos milímetros (García-Estévez y col., 2015). Este facto explica en parte los bajos niveles de elagitaninos que se encuentran en los vinos.

Fernández de Simón y col., (2009) encontraron en las maderas de roble español sometidas a un proceso de tostado de intensidad media (durante 35 minutos, a 160-170ºC) que las concentraciones de elagitaninos se redujeron, particularmente en el caso de los monómeros y sus derivados pentosilados. Estos importantes cambios en el perfil polifenólico provocan una disminución de la variabilidad natural entre especies y orígenes de las maderas, resultando al final del proceso de tostado que la composición tánica de las maderas españolas son muy semejantes a las francesas de *Quercus robur y Quercus petraea*.

La especie *Quercus alba* muestra, después del tostado, mayores diferencias respecto a todas las demás. Por lo tanto, el tostado de la madera, además de provocar la formación de nuevos compuestos debido a la degradación térmica de la madera, es capaz de producir la degradación de los elagitaninos, acentuándose este efecto al aumentar el tiempo y la temperatura de tratamiento, hasta llegar incluso a la eliminación total de los elagitaninos en la capa superficial de madera tostada de las duelas (Fernández de Simón y col., 2007).

El tostado influye especialmente sobre los aromas, especialmente los generados a partir de la celulosa y la hemicelulosa, este proceso se comenta más adelante.

El tostado aporta notas de almendra tostada producidas por los furanos, o de caramelo, café y avellana tostado, producidos por el maltol y la dimetilpirazina respectivamente. Algunas unidades xilosa de la hemicelulosa presentan grupos acetilos que se hidrolizan durante el tostado a ácido acético, lo que aumenta la acidez volátil en barricas nuevas, por lo que se precisa de una correcta preparación de estas barricas antes de comenzar la crianza (Vivas y col., 1995). Otros aromas podrían producirse por la degradación térmica de los lípidos presentes en la madera originando las β-metil-γ-octalactona (whiskey-lactonas) con aromas de nuez de coco y coco.

Por último, a partir de la termólisis parcial de la lignina se producen diferentes reacciones que dan lugar aldehídos fenólicos y fenil-cetonas con aromas de vainilla, o a los aromas de tostado, clavo de especias o el desagradable olor animal producido por los fenoles volátiles. Estos últimos también pueden generarse por descarboxilación de ácidos hidroxicinámicos en la uva o el vino, fundamentalmente en presencia de *Brettanomyces.*

A medida que aumenta el grado de tostado, hay un desplazamiento aromático hacia los aromas tostados, especiados y ahumados, disminuyendo los aromas de nuez de coco y coco que son aportados por β-metil-γ-octalactona. El aroma de vainilla se incrementa con el tostado pero si éste es muy fuerte se observa su disminución (Matejicek y col., 2005).

En su conjunto el mayor impacto aromático corresponde al tostado ligero, si bien estará mayoritariamente marcado por los aromas de las β-metil-γ-octalactonas (Cadahía y col., 2003). El tostado medio por su parte, tendrá una cierta disminución del impacto aromático global en relación con el tostado ligero, pero ganará en equilibrio y complejidad, disminuyéndose las notas de nuez de coco de las β-metil-γ-octalactona e incrementándose el resto de las sustancias volátiles, especialmente la vainillina (Chira y col. 2013).

Con respecto al tostado fuerte se produce una ligera pérdida de la intensidad olfativa, pero sobre todo se altera el equilibrio entre las familias de aromas. Básicamente disminuyen las β-metil-γ-octalactona y aumentan las fenil-cetonas (cadahía y col., 2003) los fenoles volátiles y los furanos (Collins y col., 2015), pudiendo marcar un exceso de notas ahumadas, tostadas, que podrían otorgar al vino un carácter excesivamente torrefacto.

1.4.4. Influencia del uso de las barricas

La cantidad de compuestos fenólicos y las sustancias volátiles que aporta el roble de las barricas al vino disminuye con la edad de la barrica, al tiempo que los poros de la misma se obturan, disminuyendo así el aporte de oxígeno al vino y con ello las reacciones de polimerización, combinación y óxido-reducción que se desean con la crianza (Vivas, Zamora y Glories 1993; Vivas, 1999).

En ese sentido, Vivas y Saint-Cricq de Gaulejac (1998) describieron una disminución de la liberación de elagitaninos de alrededor del 40% después del primer año y de alrededor del 80% después de cinco años de uso. Estos datos implican un rápido agotamiento de las barricas y justifican por tanto la necesidad de su renovación.

También resulta obvio que el vino envejecido en barricas nuevas presenta mayor intensidad de aroma, con más sabor a coco, vainilla y carácter especiado, que un mismo vino envejecido en barricas usadas (Garde-Cerdán y col., 2002; Cano-López y col., 2008), incluso aunque el tiempo de contacto con la barrica sea mayor (Gómez-Plaza y col., 2004).

Compuestos como el furfural, 5-metilfurfural, *cis*-whiskey-lactona y vainillina disminuyen considerablemente en barricas usadas, mientras que algunos fenoles volátiles, como 4-etilfenol y 4-etilguaiacol que proporcionan aromas desagradables, se ven aumentados (Bautista-Ortín y col., 2008).

La causa del incremento de los fenoles volátiles se atribuye a la creciente presencia de *Brettanomyces* en el interior de la madera de las duelas. Se ha descrito que pueden estar hasta 8 mm de profundidad y la causa es la gran dificultad de una limpieza minuciosa para eliminarlas de los barriles usados (Malfeito-Ferreira, 2005).

Por estas razones se suele considerar que la vida útil de una barrica es de unos cinco años, transcurridos los cuales se convierte en un mero contenedor que ya no aporta ni aromas, elagitaninos ni apenas microoxigenación. Por el contrario, puede ejercer efectos negativos de índole microbiológica, tal y como se ha comentado, si la limpieza e higiene no hubieran sido las adecuadas (Suárez y col., 2007).

El rejuvenecimiento de una barrica eliminando las capas internas exhaustas (azuelado) ha dado efectos aceptables, pero no reproduce la calidad de una barrica nueva (Puech y cols., 1990). En estas barricas asoladas, el vino entra en contacto con zonas vírgenes de la madera aún no explotadas lo que incrementa la liberación de elagitaninos y algunos aromas. Sin embargo, esas zonas no fueron apenas afectadas por las altas temperatura del proceso de tostado de los barriles y por tanto su contenido en aromas es mucho menor. Algunos toneleros proponen volver a tostar las barricas asoladas para regenerar dichas sustancias volátiles. No obstante, este reacondicionamiento de las barricas usadas, si bien puede ser positivo en algunos aspectos aromáticos y gustativos, presenta el inconveniente de incrementar notablemente las notas quemadas y torrefactas, y sobre todo por otorgar al vino notas animales muy marcadas (Vivas, 1999).

1.4.5. Clasificación de la madera de roble en función de su riqueza en elagitaninos mediante técnicas no invasivas.

Los taninos parecen tener un gran impacto sobre las cualidades organolépticas de los vinos contenidos en las barricas. Hasta ahora, para conocer la composición de la madera, los elagitaninos sólo podían medirse mediante procedimientos químicos de laboratorio. Estas mediciones sólo se hacían mediante toma de muestras de diferentes lotes de madera y el proceso de realización de los ensayos era muy largo.

Tonnellerie Radoux ha desarrollado con los investigadores del INRA y del CEMAGREF un procedimiento único de medida instantánea del contenido en polifenoles de la madera, llamado Oakscan, gracias al cual es posible determinar el potencial tánico de cada barrica según la demanda de los clientes, las exigencias de una variedad o las características de una añada.

Radoux OakScan realiza un análisis rápido, duela por duela, basado en la espectrometría del infrarrojo cercano. Analiza cada duela que entra en producción, asignándole un código y una letra representativa de su índice de polifenoles, es decir, del rango de potencial tánico en el que se encuadra, permitiendo asegurar la homogeneidad de las barricas, como complemento de los procesos tradicionales de selección (origen y grano). Este índice de polifenoles se calcula, según una fórmula confidencial, a partir de las mediciones OakScan y se le asigna un valor entre 0 y 100. Este valor presenta una perfecta correlación con los análisis químicos de referencia (DO280, Folin Ciocalteu y elagitaninos totales).

Esta técnica permite elaborar barricas con diferente potencial en elagitaninos lo que permite a los enólogos elegir su parque de barricas con un nuevo criterio además de los utilizados clásicamente (origen botánico, tamaño de grano, nivel de tostado,…).

1.5. Evolución del color y de los compuestos fenólicos durante la crianza del vino en barricas.

Durante el tiempo que el vino permanece en la barrica se producen numerosas reacciones directas o mediadas por la oxigenación a través de los poros de la madera, la junta de las duelas y el esquive (Glories, 1990). El oxígeno en presencia de cationes de hierro y/o cobre y de *orto*-difenoles genera la oxidación del etanol a etanal (Singleton, 1987), el cual a su vez puede intervenir en la polimerización de los flavanoles o en su combinación con los antocianos mediante la formación de puentes etilideno. El etanal activado provoca la polimerización de las proantocianidinas mediante puentes etilideno, pero también permite las combinaciones entre los antocianos y flavanoles (Jones y col., 2004).

Las reacciones mediadas por etanal se producen de manera simultánea y compiten entre ellas, por lo que la proporción molar entre antocianos y flavanoles definirá el predominio de unas u otras reacciones. En un vino con una proporción mucho menor de antocianos que de flavanoles, el efecto de la oxigenación moderada traerá consigo la polimerización desproporcionada de los flavanoles lo que podría provocar su precipitación. Por el contrario, un vino con proporción mucho menor de flavanoles que de antocianos conllevaría la oxidación de los antocianos con la correspondiente perdida irreversible del color (Llaudy, 2006).

Algunos autores plantean que las uniones antociano-flavanol mediante puentes etilideno es uno de los mecanismos que ayudan a la evolución del color durante la crianza (Ribéreau-Gayon y col., 2003b). Otros, en cambio, plantean que los puentes etilideno son inestables lo que condiciona que se produzca la reestructuración de las proantocianinas y los antocianos después de la crianza en barrica y/o microoxigenación (Escribano-Bailón y col., 2001).

Además, se ha de considerar la formación de piranoantocianos y otros nuevos pigmentos por mecanismo de cicloadición con metabolitos de la levadura (por ejemplo, etanal o ácido pirúvico) y otros compuestos fenólicos, con y sin intermediación del etanal (ácidos hidroxicinámicos, 4-vinilfenoles, flavanoles y etanal, etc.) (Salas y col., 2005; Blanco-Vega et al., 2011). Hay que señalar que la escisión de los puentes etilideno puede originar 8-vinil-flavanoles que pueden a su vez reaccionar con los antocianos generando otro tipo de piranoantocianos denominados aductos vinil-flavanol-antociano (Francia-Aricha y col., 1997; Blanco-Vega et al., 2014).

Dependiendo de la composición del vino, la barrica y las condiciones de la crianza, se modificará el color de los vinos tintos desde los tonos rojos con matices violáceos hasta tonalidades teja-anaranjadas. Cambiarán los aromas, desde los matices varietales a matices más complejos y especiados extraídos de la madera, y tendrá lugar una mejora del gusto aumentando las sensaciones de redondez y volumen, al tiempo que disminuyen las de astringencia y amargor (Pérez-Magariño y González San José, 2004).

1.6. Efecto de los elagitaninos durante el envejecimiento del vino

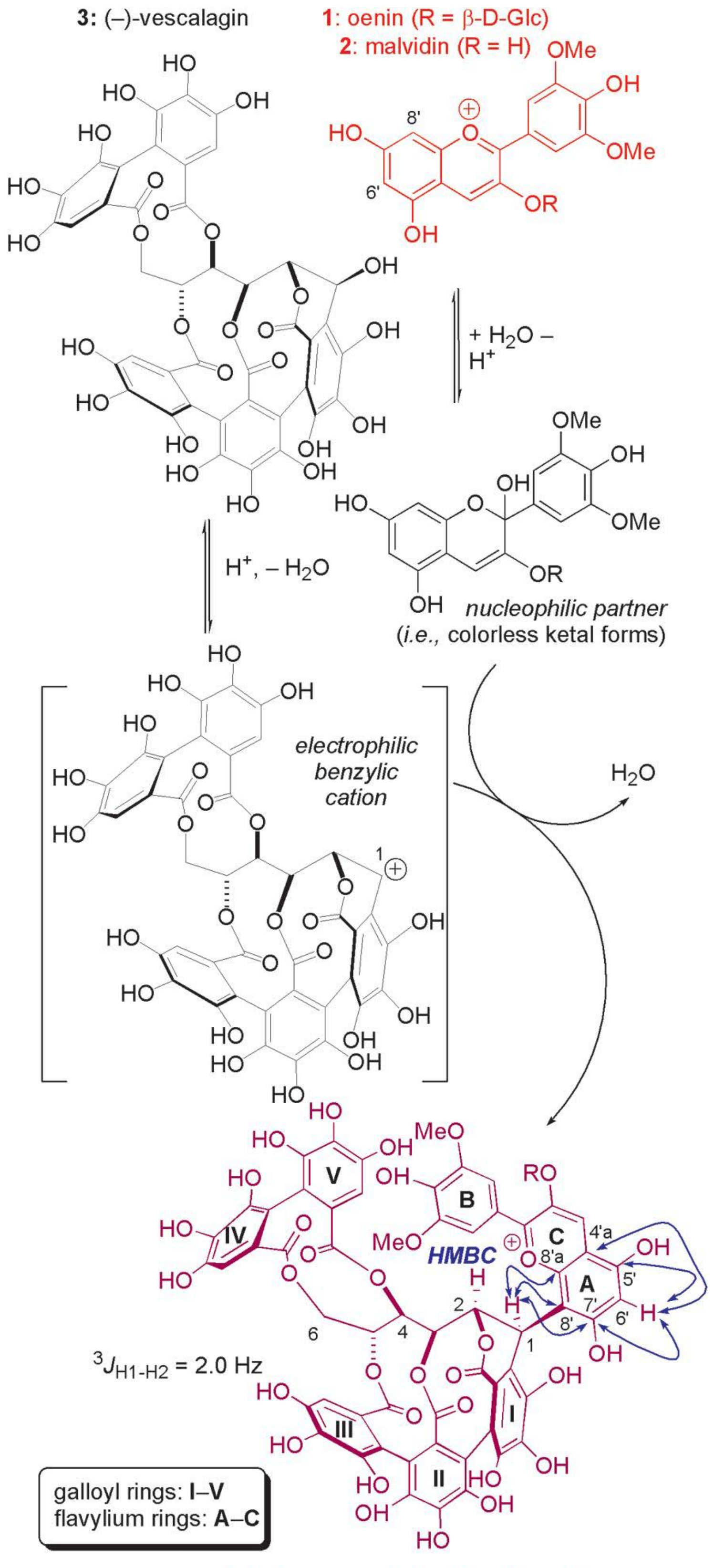
Se pueden distinguir tres cuestiones centrales sobre el efecto de los taninos de roble en el vino. Teniendo en cuenta que los niveles de elagitaninos que se encuentran en los vinos de crianza en roble son bajos, los efectos organolépticos directos e indirectos sobre el sabor del vino dependen del tipo de vino y de madera, así como de la influencia de otras prácticas enológicas realizadas durante el proceso de envejecimiento de vinos. Entre las transformaciones esperadas, las más importantes son la oxidación y la interacción entre elagitaninos con polisacáridos, proteínas de levadura y/o manoproteínas (Puech y col., 1999).

La lenta y constante penetración del oxígeno a través de las duelas de la barrica y la disolución gradual de los elagitaninos de la madera de roble, forma un tampón efectivo contra la oxidación durante el proceso de envejecimiento, ya que los elagitaninos son fácilmente oxidados. La gran capacidad de estas sustancias para consumir oxígeno es debida a la presencia de varias funciones hidroxilo en posición *orto* en su configuración (García, 1997). Vivas y col., (1996) demostraron que los elagitaninos tienen un mayor poder oxidativo que otros polifenoles del vino, participando en reacciones que dan lugar a quinonas, radicales libres fenólicos, hidroperóxidos orgánicos y agua.

En consecuencia, los elagitaninos se revelan como reguladores de la oxidación, consumiendo rápidamente el oxígeno disuelto y facilitando la formación de etanal. Esta reacción estimula la condensación tanino-antociano vía etanal, favoreciendo la estabilización y aumento del color morado y previniendo el desarrollo del color amarillo (García, 1997)

Además, la estructura de los taninos del vino se modifica, reduciendo su astringencia y limitando la precipitación de materia colorante (García, 1997). En la figura 20 se expone un esquema de la condensación entre antocianos del vino y la vescalagina de la madera propuesto por Chassaing y col. en 2010.

En trabajos sobre los efectos organolépticos del vino como el realizado por Chira y col., (2013) se ha observado que la intensidad del amargor y la astringencia del vino disminuyeron durante el tiempo de contacto con la madera. Esta reducción en la sensación de astringencia seguramente podría ser causada por la formación de un complejo químico entre taninos del vino, polisacáridos y péptidos, o incluso debido a las reacciones entre los elagitaninos de la madera y las moléculas de vino.



4: 1-deoxyvescalagin-(1β→8)-oenin
(3% yield, R = β-D-Glc, MW = 1410 Da)
5: 1-deoxyvescalagin-(1β→8)-malvidin
(25% yield, R = H, MW = 1248 Da)

Figura 20. Esquema de la condensación entre antocianos del vino y la vescalagina de la madera propuesto por Chassaing y col 2010.

1.7. Alternativas a la crianza en barrica

La madera enriquece el vino en sabores y aromas al tiempo que, al ser una materia porosa, permite una oxigenación continua y moderada del vino (Jarauta y col., 2005). El aporte de oxígeno al vino da lugar a procesos de combinación que estabilizan la materia colorante y suavizan la astringencia (Glories, 1999; Pomar y col., 2001). Es evidente que la crianza del vino en barricas de roble es un proceso que implica un elevado coste para las bodegas. En consecuencia, los enólogos siempre han buscado posibles alternativas que fuesen más sencillas y sobre todo más baratas.

Básicamente, las alternativas a la crianza del vino en barricas de roble se centran en la necesidad de cubrir dos aspectos. En primer lugar, es preciso lograr la estabilización de la materia colorante y la suavización de la astringencia, y en segundo lugar, es necesario aportar al vino los aromas y los taninos elágicos que complementen sensorialmente al vino y le aporten suficiente complejidad. Por esta razón las técnicas alternativas a la crianza más manejadas son la microoxigenación y la utilización de fragmentos de madera de roble, siendo ambas técnicas complementarias.

1.7.1. La microoxigenación

El envejecimiento de los vinos en barricas produce una mejora en la estructura y el color de los vinos considerándose como el factor principal de estos cambios la disponibilidad de pequeñas cantidades de oxígeno aportado por su difusión a través de la madera de la barrica, por sus poros o entre duelas (Perez-Prieto y col., 2003; Del Alamo-Sanza y col., 2014). Entre las reacciones que se ven favorecidas están la generación de piranoantocianos y también la formación de acetaldehído, que es responsable de uno de los mecanismos de la unión de proantocianidinas y antocianos (Escribano-Bailón y col., 2001).

Intentando imitar ese fenómeno observado en barrica, nace en la década de los noventa la técnica de la microoxigenación (Ducournau y Laplace, 1993), que consiste en el aporte de pequeñas cantidades de oxígeno al vino a través de un microdifusor poroso, de forma controlada lenta y continua durante su elaboración y/o conservación, tal que la velocidad de este aporte sea inferior a la velocidad de consumo por parte del vino, evitando su acumulación (Moutounet y col., 2000; Bautista-Ortín y col., 2008). Esta técnica permite obtener vinos más aromáticos y con una mejor estabilidad de su estructura y color y más resistentes a la oxidación. El efecto de la microoxigenación sobre el color de los vinos podría ser, por tanto, comparable al obtenido con la crianza de vinos en madera pero es más económico y permite trabajar a mayor escala, permitiendo, al igual que ocurre con los vinos envejecidos en madera, estabilizar el color y la estructura tánica de los vinos tintos (Cano-Lopez y col., 2006; Pérez-Magariño y col., 2009).

Debe señalarse que gran parte de la información es proporcionada por las empresas que comercializan equipos de microoxigenación cuyo interés principal es la venta, comercialización y difusión de su producto que muchas veces se basa más en su experiencia empírica que en evidencias científicas. Por consiguiente hay que tener máxima cautela con algunas de las afirmaciones publicadas. De hecho, suelen afirmar que la microoxigenación incrementa la polimerización de los flavanoles sin aportar resultados experimentales que así lo confirmen. La información científica publicada al respecto es poco concluyente (Atanasova y col., 2002; Llaudy y col., 2006; Kontoudakis y col., 2011), probablemente porque la metodología utilizada para medir el grado de polimerización, la floroglucinolisis, no es capaz de romper todas las estructuras de los flavanoles presentes en el vino (Zeng, 2015).

A pesar de que verdaderamente es complicado elucidar las modificaciones que entraña la microoxigenación en el color y los compuestos fenólicos del vino tinto, sí que parece que se produce una disminución de los antocianos totales debido a que se transforman en nuevos pigmentos (piranoantocianos, aductos flavanol-etil-antocianos, aductos vinilflavanol-antonciano, portisinas,…). En su conjunto estas transformaciones parecen comportar un incremento de la estabilidad del color y una disminución de la astringencia (Singleton, 2000; Cheynier y col., 2002; Zamora, 2003; Santos-Buelga y de Freitas, 2003; Oliveira y col., 2007; He y col., 2012b).

Por otra parte, la microoxigenación, además del color, también puede afectar a otros aspectos sensoriales que condicionan la calidad del vino. Reduce los aromas herbáceos de vinos elaborados con uvas poco maduras (Boulet y Moutounet, 2000; Yerle y Kamio, 2001) y elimina los olores de reducción (Godden y col. 2001).

Se han determinado dos periodos básicos para la aplicación de la microoxigenación: cuando la fermentación alcohólica ha terminado y todavía no ha comenzado la fermentación maloláctica, momento en que la adición de oxígeno induce la polimerización y la suavización de los taninos, en esta etapa la aplicación de oxígeno es bastante segura ya que si hay una producción excesiva de etanal, éste puede ser metabolizado posteriormente por las bacterias lácticas durante la FML (Osborne y col. 2000). Y durante el envejecimiento (después de fermentación maloláctica), etapa en la que el vino necesita oxígeno, normalmente aportado durante las operaciones de trasiego y relleno. Finalizada la FML, la microoxigenación continúa asegurando una suficiente cantidad de etanal para el desarrollo de productos de condensación entre antocianos y taninos (Cottrell y col. 2004), pero hay que ser más cuidadoso, ya que el contenido de antocianos libres es menor, y se podría ver favorecida la polimerización de taninos produciendo vinos más secos o incluso provocar la sobre-oxidación del vino, también puede surgir el riesgo de deterioro microbiano.

Por lo general se aplican cantidades de oxigeno entre 1-3 mL/L/mes, con dosis más elevadas para vinos con estructuras fenólicas fuertes o excesivas, de 2 mL/L/mes para aquellos vinos con notas vegetales pronunciadas y dosis menores de 0.75 mL/L/mes para vinos con bajo contenido fenólico. Estas dosis son orientativas y varían según la evolución del vino (Boulet y Moutounet, 2000).

1.7.2. Utilización de fragmentos de madera de roble

El uso de virutas de roble para aromatizar vinos en una técnica que ha sido aprobada por la O.I.V en el presente siglo XXI. Las posibilidades existentes en el mercado son muy amplias, así podemos encontrarnos con múltiples tamaños y formatos. Así se habla de polvo, granulado, virutas o chips, dominós, bloques, miniduelas o duelas en función de su tamaño y morfología. Estos alternativos se introducen en cubas de acero inoxidable o en el interior de barricas usadas para alargar su vida.

El uso de alternativas de roble junto con la micro-oxigenación puede reproducir los procesos que tienen lugar en los barriles de forma más económica y rápida (Llaudy y col., 2006). Cejudo-Bastante y col. (2011) realizaron varios estudios sobre la aplicación de microoxigenación y virutas de roble en vino tinto, considerando de forma conjunta varios puntos de vista como son el efecto sobre el color, la composición fenólica, la composición volátil y una evaluación sensorial. Los resultados más remarcables fueron los siguientes:

a) una mayor estabilidad del color debido a las concentraciones más altas de piranoantocianos y aductos formados por reacción entre antocianos y flavan-3-oles mediados por etanal.

b) Una mejora en la calidad del aroma del vino tinto (la concentración de eugenol y 4-vinil-guayacol aumentaron, mientras que la del etanal y la de los alcoholes relacionados con mal sabor fueron significativamente inferiores).

c) se produjeron cambios en el perfil sensorial de los vinos (aumentaron las notas de ciruela / grosella y los atributos picantes, así como se detectó la aparición de aromas tabaco y notas de nuez).

d) el sabor amargo y la astringencia se redujeron con el uso de micro-oxigenación, aunque el aroma típico de madera también se redujo significativamente.

La adición de alternativos de roble proporcionan compuestos que, de forma similar a las barricas, modifican significativamente el aroma de los vinos (Chatonnet et al., 1989), entre ellos están los compuestos furanos, formados por la degradación de hemicelulosa durante el tostado de la madera, que además de su papel en el aroma de los vinos también podrían participar en la estabilización del color del vino. También se enriquece el vino con sustancias tales como vainillina y whiskey-lactonas, que dan lugar a un incremento de los aromas de vainilla y de nuez de coco, ambos característicos de la crianza en barrica de roble (Gallego y col., 2012; Hernández-Orte y col., 2014; Pérez-Juan y col., 2015; Baiano y col., 2016).

La elección de la dosis, del tostado de los alternativos, así como de su origen botánico influirá en el resultado final (Pérez-Coello y col., 2000; Fernández de Simón y col., 2010; García y col., 2012). Las virutas tostadas aportan mayor cantidad de vainilla que las no tostadas (García y col., 2012).

Ortega-Heras y col. (2010) mostraron que el uso de virutas de roble podría ser una buena alternativa para la elaboración de vinos jóvenes con unas ligeras notas olfativas y gustativas de madera muy similares a los vinos envejecidos durante cortos períodos de tiempo en barrica nueva. Estos tipos de vinos tienen una buena aceptación de los consumidores debido a su buena relación calidad-precio.

En vinos almacenados en depósitos y microoxigenados, una forma de simular más cercanamente la barrica, sería la adición exógena de un tanino elágico o la adición de virutas de roble. Su uso supone el aporte de elagitaninos que mejoran la estabilidad del color, estructura y perfil organoléptico de los vinos (Bautista Ortín y col., 2008). Los elagitaninos, como son compuestos que se oxidan con mucha facilidad, pueden generar peróxidos y por tanto, grandes cantidades de etanal.

La presencia de elagitaninos en el vino dependerá del tiempo de contacto (Jordao y col., 2005), así como del tamaño y del grado de tostado de los chips que se utilizan. La contribución es mayor para tostados ligeros y tamaño de chips pequeño, debido a su mayor relación superficie volumen (Le Grottaglie y col., 2015; García-Estévez y col., 2015).

Las tasas de extracción de los elagitaninos parecen ser más rápida durante el envejecimiento del vino tinto en depósitos de acero inoxidable con virutas de roble. Sin embargo, sus concentraciones totales en los vinos, se encontraron más altas en el caso del vino envejecido en barricas de roble (Jourdes y col., 2011).

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2. HIPÓTESIS Y OBJETIVOS

The aim of this work was to study the influence of how the main factors considered in the selection of the barrels affect the ellagitannin release into the wine considering the following hypotheses:

"The botanical origin, the toasting level and the use of the barrels have a major influence on the ellagitannin concentration of the wines aged in oak barrels. Subsequently, the different ellagitannin concentrations have a considerable impact on the wine composition and evolution"

With this aim the following objectives were set out.

Objectives:

1. To develop an optimized method for the determination of ellagitannins in wines.
2. To determine the influence of the botanical origin of the oak on the release of ellagitannins into the wines.
3. To determine the influence of toasting level of the staves on the release of ellagitannins into the wines.
4. To compare the ellagitannins levels released by new barrels with those of barrels previously used for one year to establish the depletion rate.
5. To determine the oxygen consumption rate by ellagitannins released by oak chips.

Additional objective:

6. To determine the relationship between the volatile substances released by new and used oak wood barrels of different botanical origin and different toasting level with the sensory appreciation by a trained panel.

3. RESULTADOS

Capítulo I. Método para el análisis cromatográfico de los elagitaninos presentes en el vino envejecido en barricas de roble o alternativos

Los elagitaninos de la madera que se encuentran en los vinos que han envejecido en barricas o con productos alternativos, son un grupo de compuestos que afectan directamente a las propiedades finales del vino debido a su gran facilidad para interaccionar con sus componentes. Aunque existe un buen número de investigaciones que han abordado el análisis de elagitaninos mediante HPLC, optimizando las condiciones de separación y detección, así como el desarrollo de procedimientos de preparación de muestra para asegurar el aislamiento cuantitativo de la fracción de elagitaninos previo a su análisis cromatográfico, la situación dista de estar completamente optimizada para el caso de todos los elagitaninos que pueden encontrarse en el vino.

Siguiendo en esta línea, el objetivo principal que se plantea en este capítulo es mejorar los métodos de análisis utilizados hasta el momento para evaluar la concentración de elagitaninos en el vino envejecido en contacto con madera de roble. Por una parte se han estudiado los diferentes métodos de preparación de las muestras para su posterior análisis, disminuyendo el volumen de muestra necesaria, la cantidad de disolvente utilizado y el número de pasos necesarios para extraer y aislar de forma cuantitativa la fracción de elagitaninos del vino, y también el método de análisis cromatográfico (HPLC) para determinar la cantidad de elagitaninos. Por otro lado también se da una visión de la complejidad de las estructuras de los elagitaninos.

Los ensayos de extracción y de análisis cromatográfico para el desarrollo de este nuevo método fueron realizados en el laboratorio de Análisis Instrumental del Grupo de Investigación en Enología y Productos Naturales, Instituto Regional de Investigación Científica Aplicada (IRICA), Universidad de Castilla-La Mancha (UCLM, Ciudad Real). Los resultados de este capítulo han servido para la redacción del artículo titulado **“Improved Method for the Extraction and Chromatographic Analysis on a Fused-Core Column of Ellagitannins found in Oak-Aged Wine”** que actualmente se encuentra en revisión (“Minor revisions for acceptance”) por la revista *Food Chemistry*.

Improved Method for the Extraction and Chromatographic Analysis on a Fused-Core Column of Ellagitannins found in Oak-Aged Wine

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ABSTRACT: A new method for the analysis of ellagitannins found in oak-aged wine is proposed, showing interesting advantages with regards to previously reported analytical methods. The necessary extraction of ellagitannins from wine was simplified to a single step of solid phase extraction (SPE) on size exclusion chromatography with Sephadex LH-20 without the need for any previous SPE of phenolic compounds using reversed-phase materials. The quantitative recovery of wine ellagitannins requires the combined elution with methanol and ethyl acetate, especially for increasing the recovery of the less polar acutissimins. The chromatographic method was conducted using a fused-core C18 column, thus avoiding the coelution of main ellagitannins like vescalagin and roburin E. However, the very polar ellagitannins, namely the roburins A, B and C still partially coeluted, and their quantification was assisted by an MS detector. This methodology also allowed the analysis of free gallic and ellagic acids in the same chromatographic run.

KEYWORDS: wine aging, ellagitannins, acutissimin, castalagin, grandinin, roburin, vescalagina, fused-core.

CHEMICAL COMPOUNDS STUDIED IN THIS ARTICLE: castalagin (CAS No. 24312-00-3; PubChem CID: 12302513); vescalagin (CAS No. 36001-47-5; PubChem CID: 5458626); grandinin (CAS No. 115166-32-0; PubChem CID: 492392); roburin E (PubChem CID: 101670389); roburin A (CAS No. 132864-75-6; PubChem CID: 101670390); roburin B (PubChem CID: 101670611); roburin C (PubChem CID: 101670612); roburin D (CAS No. 136199-93-4; PubChem CID: 101998420); acutissimin A (CAS No. 108906-66-7; PubChem ID: 101676951); acutissimin B (CAS No. 108906-65-6; PubChem ID: 101676953)

1. INTRODUCTION

Wine aging in oak barrels is a common practice used for improving wine quality and stability, especially for red wines. In addition to the microoxygenation that takes place through the wood pores, the oak barrels also release some substances which are not initially present in young wines. More specifically, toasted oak wood release volatile compounds which enrich wine aroma (Pérez-Coello & Díaz-Maroto, 2009). In addition, both crude and toasted oak wood release ellagitannins which contribute to some texture sensations of wine, such as body and astringency (Stark, Wollmann, Wenker, Lösch, Glabasnia, & Hofmann, 2010; Glabasnia & Hofmann, 2006; Michel, Jourdes, Silva, Giordanengo, Mourey & Teissedre, 2011).

Oak ellagitannins is also found in wines macerated with oak wood chips and other similar alternatives to the use of oak barrels, for instance the immersion of oak wood staves in wine vessels. The ellagitannins found in wine that have been released from oak wood comprise the well-known castalagin and vescalagin, the C-lyxoside and C-xyloside conjugates of vescalagin (grandinin and roburin E respectively), together with C-glycosidic derivatives as the quasi-dimers of all the latter compounds (roburins A, D, B and C respectively). In addition, new ellagitannins can be formed in wine following the reaction of the oak ellagitannins with wine components, like adducts formed by vescalagin with (+)-catechin (acutissimins A and B), ethanol, and even malvidin-3-glucoside (Jourdes, Lefeuvre & Quideau, 2009).

Any study going into detail about the impact of oak wood ellagitannins in the chemical and organoleptic properties of oak-aged red wines need to be based on the knowing of the detailed composition of these compounds. Wine ellagitannins can be separated by high performance liquid chromatography (HPLC). However, the analysis of wine ellagitannins still remains a challenge. Firstly, the level of total ellagitannins in oak-aged red wines can be very variable. Usually, only four wine ellagitannins are quantified, namely castalagin, vescalagin grandinin and roburin E.

The total content of ellagitannins ranges between negligible amounts and a few tenths of mg/L that rarely exceed 25 mg/L (Glabasnia & Hofmann, 2006; Stark et al., 2010; Jourdes, Michel, Saucier, Quideau, & Teissedre, 2011; Michel et al., 2011; García-Estévez, Escribano-Bailón, Rivas-Gonzalo & Alcalde-Eon, 2012; Navarro, Kontoudakis, Gómez-Alonso, García-Romero, Canals, Hermosín-Gutíerrez, & Zamora, 2016). Secondly, a fractionation step of wine ellagitannins is required prior to their analytical determination. Thus, previously proposed methods have used large amounts of sample (100 mL) and time-consuming fractionation on a size exclusion gel column (TSK HW 40F or 50F), which may or may not be preceded by the clean-up of non-phenolic compounds using Amberlite XAD7 HP, although such methods have allowed analyzing up to eight C-glucosidic ellagitannins and five vescalagin derivatives (Saucier, Jourdes, Glories & Quideau, 2006; Michel et al., 2011).

Another method only used 3 mL of wine sample and, after a two-step fractionation method consisting of a solid phase extraction (SPE) in C-18 cartridges followed by size exclusion chromatography in hand-packed Sephadex LH-20 minicolumns, it allowed the quantification of only four of the main C-glucosidic ellagitannins (García-Estevez, Escribano-Bailón, Rivas-Gonzalo & Alcalde-Eon, 2010). Thirdly, ellagitannins are rather polar compounds and reported chromatographic methods based on reversed-phase HPLC show coeluting peaks of the main compounds that make the use of MS detectors necessary. In this case, the use of chlorogenic acid as an internal standard had been suggested (Saucier et al., 2006) but further work demonstrated that chlorogenic acid induced degradation of vescalagin and so the addition of gallocatechin as an internal standard after isolation of the fraction containing ellagitannins was proposed (García-Estévez et al., 2012).

Wine ellagitannins and related compounds, namely gallic and ellagic acids, comprise a complex analytical mixture with regards to their polarity and, subsequently, it is intrinsically difficult to manage their suitable chromatographic separation. Some ellagitannins like the roburins A, B, C and D are very polar compounds, even more than gallic acid, and tend to coelute quickly in C18 reverse phase columns. In contrast, acutissimins are medium polar compounds and ellagic acid is a low polar compound. In order to overcome this expected difficulty, the use of fused-core particle packed C18 columns can be a suitable solution, since they offer initial advantages like diminution of diffusion effects and better resolution (Nuñez, Gallart-Ayala, Martins & Lucci, 2012; Borges, Rostagno & Meireles, 2014). The use of fused-core columns has been reported to improve chromatographic analysis of phenolic compounds in several food products (Rostagno, Manchón, D'Arrigo, Guillamón, Villares, García-Lafuente, Ramos & Martínez 2011; Bayram, Ozcelik, Scultheiss, Frank & Rimbach, 2013; Pellati, Prencipe, Bertelli & Benvenuti, 2013; Gómez-Caravaca, Verardo, Toselli, Segura-Carretero, Fernández-Gutiérrez & Caboni, 2013; Martí, Varcárcel, Herrero-Martínez, Cebolla-Cornejo & Roselló, 2015).

For all the aforementioned reasons, the aim of this work was to improve the previously reported methods for the extraction of wine ellagitannins based on a small scale sample size (3 mL), as well as their chromatographic analysis by HPLC-DAD-ESI-MS/MS mainly based on DAD detection and quantification (García-Estévez, Escribano-Bailón, Rivas-Gonzalo, & Alcalde-Eon, 2010; Jourdes, Michel, Saucier, Quideau & Teissedre, 2011). The improvements achieved in both the extraction and the chromatographic methods were monitored using a mixture of ten ellagitannin standards (castalagin; vescalagin; grandinin; roburins A, B, C, D and E; and acutissimins A and B) and real red wine spiked with these ellagitannins.

2. MATERIALS AND METHODS

2.1. Chemicals, Reagents and Wine Samples

All solvents were of LC-MS quality and all chemicals of analytical grade (>99%). Water was of Milli-Q quality. The following samples of wine ellagitannins were provided by ADERA (Pessac, France): castalagin, vescalagin, grandinin, roburin A, roburin B, roburin C, roburin D, roburin E, and acutissimins A and B (Figure 1). A mother solution containing all the aforementioned ellagitannin standards was conveniently diluted for obtaining the calibration curves necessary for quantification, covering concentration ranges of up to 30 mg/L of each ellagitannin. Sephadex LH-20 was from Sigma-Aldrich (San Luis, MO, USA). The following solid phase extraction (SPE) cartridges were assayed in the extraction trials: PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Palo Alto, CA, USA) and C18 cartridges (Sep-pak Plus C18, Waters Corp., Mildford, MA; cartridges filled with 820 mg of adsorbent). Commercial samples of Tempranillo young wine and Cabernet Sauvignon red wine aged in oak barrel were supplied by Bodegas Naranjo (Carrión de Calatrava, Spain). The commercial sample of Tempranillo young red wine that was elaborated without contact with oak barrels or chips. This young red wine was spiked with the standard solution of ellagitannins (final concentration of 30 mg/L of each ellagitannin) and used for the extraction trials of wine's ellagitannins.

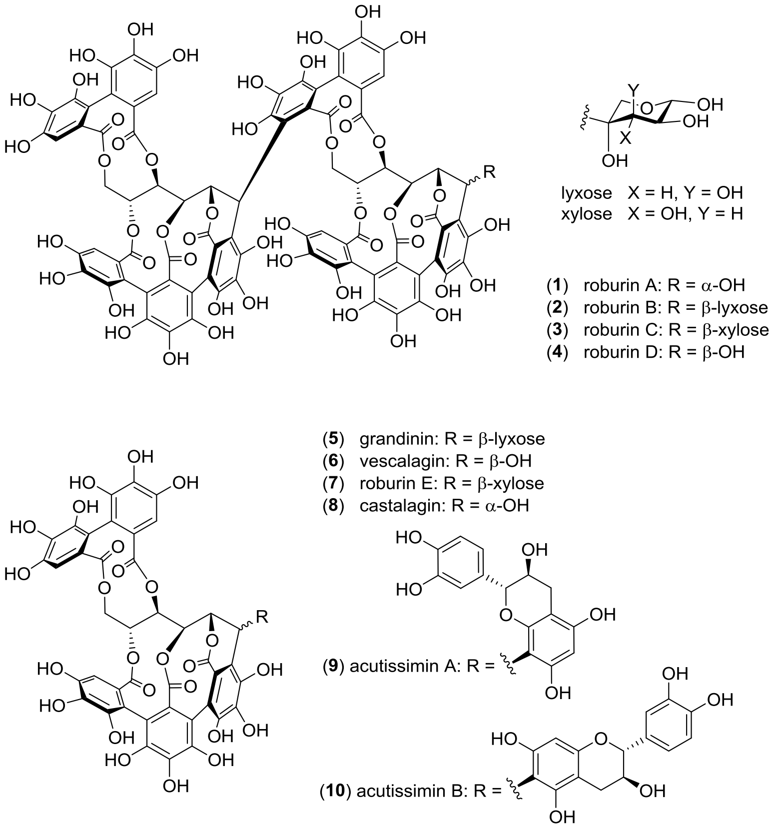


Fig. 1. Structures of the main wine's ellagitannins, originated by transfer from oak wood to wine during aging of wine and also by subsequent reactions in wine.

2.2. Obtaining Wine Ellagitannin Fraction for Chromatographic Analysis

The quantitative recovery of wine ellagitannins was tested after modifications of the two-step SPE fractionation method described by García-Estévez et al. (2010). The best results were obtained by direct extraction of wine ellagitannins on a cartridge filled with Sephadex LH-20 and the extraction method consisted of the following steps: a) 3 mL of red wine sample was dried under reduced pressure at 35 ºC in a rotary evaporator and re-dissolved in 2.5% acetic acid in water to a final volume of 2 mL; b) the prepared wine sample was inserted into a hand-packed 6 mL SPE cartridge (70 × 12 mm) filled with 3 mL of Sephadex LH-20 that was previously activated with methanol (5 mL) and conditioned with water (5 mL); c) then the Sephadex LH-20 bed was successively washed with water (2 mL), 96% ethanol (2 mL) and methanol (1 mL) and their combined eluates were discarded; d) the retained wine ellagitannins were recovered by elution with methanol (10 mL) followed by ethyl acetate (10 mL; an expected decrease of the volume occupied by the adsorbent bed occurred); e) the ellagitannins fraction was dried at 35 ºC in a rotary evaporator under reduced pressure and re-dissolved in 1.5 mL of water/formic acid (996:4, v/v) and placed in a chromatographic vial for HPLC analysis. The Sephadex LH-20 SPE column was re-conditioned for further use with methanol (5 mL; the initial volume of the adsorbent bed was restituted) and water (5 mL).

2.3. Chromatographic Analysis of Wine Ellagitannins by HPLC-DAD-ESI-MS/MS

HPLC separation, identification and quantification of ellagitannins were performed on an Agilent 1100 Series system (Agilent, Waldbronn, Germany) equipped with DAD (G1315B) and a LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MS^n) system, and coupled to an Agilent ChemStation (ChemStation for LC 3D systems, Rev. B.01.03 [204], Agilent Technologies 2001-2005) data-processing station. The mass spectra data was processed with the Agilent LC/MS Trap software (LC/MSD Trap Software 5.3, Build 22.14, Bruker Daltonik GmbH 1993-2005).

The chromatographic separation was performed on a fused-core, small particle size column (Ascentis Express C18, 150 × 4.6 mm, 2.7 µm particle size) thermostated at 40ºC. The solvent system consisted of solvent A [water/formic acid (996:4, v/v)] and solvent B [methanol/formic acid (996:4, v/v)]. The flow was changed during the elution gradient: 0 min , 0% B, flow of 0.55 mL/min; 7.5 min, 0% B, flow of 0.55 mL/min; 8.0 min, 0% B, flow of 0.7 mL/min; 25 min, 20% B, flow of 0.70 mL/min; 35 min, 50% B, flow of 0.70 mL/min; 37 min, 100% B, flow of 0.70 mL/min; 45 min, 100% B, flow of 0.70 mL/min; 50 min, 0% B, flow of 0.55 mL/min; post-run time, 10 min at 0% B and flow of 0.55 mL/min.

The identification of wine ellagitannins was based on the matching of their chromatographic retention times and UV/Vis and MS spectral data with those obtained for the set of standards. The MS ionization and MS/MS fragmentation conditions were optimized by direct infusion of a solution of 30 mg/L of castalagin in solvent A: scan, 100-2100 m/z; negative ionization mode; capillary, 4500 V; nebulizer, 60.0 psi; dry gas, 11.0 L/min; dry temperature, 350 ºC; skim 1, -42.3 V; skim 2, -6.0 V; capillary exit offset, -76.7 V; octopole, -3.04; octopole radiofrequency, 250 Vpp; octopole Δ, -2.40 V; lens 1, 5.0 V; lens 2, 60.0 V; auto MS/MS with fragmentation amplitude of 1.50 V. Calibration curves of all the analyzed compounds were prepared from pure standards. Quantification was performed by the external standard method using the DAD-chromatograms obtained at 250 nm and was assisted by using extracted ion chromatograms at selected *m/z* values in the case of some partially co-eluting ellagitannins.

The calculation of the limits of detection and quantification (LOD and LOQ, respectively) was done following the average height methodology established by the International Organization of Vine and Wine in its Resolution Oeno 7/2000 (International Organisation of Vine and Wine, 2011). The chromatographic resolution (R) between two consecutive peaks of the mixture of ellagitannin standards was calculated from their retention times (t_R) and peak widths at half-height ($w_{1/2}$) (Meyer, 2004).

3. RESULTS AND DISCUSSION

3.1. Chromatographic Separation Method

After various trials, the best chromatographic conditions for the separation of the standard mixture of ten wine ellagitannins combined the typical solvent gradient and a changing flow. The initial flow at 0.55 mL/min using 100% of the very polar solvent A was maintained for 7.5 min to be further quickly increased to 0.70 mL/min (this flow value was reached in only 0.5 min). The method continued with the application of a solvent gradient maintaining the high flow of 0.70 mL/min. Under these conditions, all the studied ellagitannins were separated although partial coelution of the most polar roburins was unavoidable, especially in the case of roburins B and C. It is interesting to remark that the achieved chromatographic separation allowed the quantification of the most relevant wine ellagitannins (Table 1), namely roburin D (peak 4), grandinin (peak 5), vescalagin (peak 6), roburin E (peak 7) and castalagin (peak 8) with the use of only a DAD-detector.

In particular, this chromatographic method separated grandinin, vescalagin and roburin E that partially or totally coeluted in a previously validated method applied to only four wine ellagitannins (García-Estévez et al., 2010) which made the use of a validated mass spectrometry method necessary for quantification (García-Estévez et al., 2012).

Table 1. Chromatographic (t_R, retention time; R, chromatographic resolution referred to the previously eluting peak) and mass spectral data corresponding to a standard mixture of wine ellagitannins. The *m/z* values of the ions marked in bold appearing in the MS spectra were isolated and fragmented for obtaining the MS/MS spectra.

Ellagitannin	Peak	t_R	*R*	MS	MS/MS
Roburin A	**1**	6.39	---	1849.2 (5%; $[M-H]^-$); 933.2 (55%); **924.6** (100%; $[M-2H]^{2-}$); 915.3 (60%)	915.1 (100%); 871.2 (3%); 630.8 (2%); 569.0 (3%)
Roburin B	**2**	6.75	1.0	1981.2 (2%; $[M-H]^-$); 1065.1 (60%); **990.5** (100%; $[M-2H]^{2-}$); 915.3 (95%)	1065.2 (95%); 915.1 (100%); 612.3 (1%); 568.8 (2%)
Roburin C	**3**	6.95	0.5	1981.2 (2%; $[M-H]^-$); 1065.1 (55%); 990.5 (90%; $[M-2H]^{2-}$); **915.3** (100%)	897.0 (20%); 871.0 (100%); 825.0 (17%); 612.9 (35%); 568.9 (38%); 550.8 (15%)
Roburin D	**4**	8.83	5.3	1849.5 (7%; $[M-H]^-$); 933.2 (65%); **924.5** (100%; $[M-2H]^{2-}$); 915.3 (40%); 631.0 (5%)	915.1 (100%); 870.9 (10%); 630.9 (15%); 612.3 (10%)
Grandinin	**5**	10.33	1.5	**1065.5** (100%; $[M-H]^-$); 975.2 (5%)	1047.1 (55%); 1029.0 (90%); 1021.1 (20%); 1005.1 (20%); 987.1 (85%); 975.1 (100%); 945.1 (30%); 931.2 (35%); 915.1 (15%)
Vescalagin	**6**	11.78	1.4	**933.5** (100%; $[M-H]^-$); 915.6 (10%)	915.0 (100%); 871.1 (20%); 612.9 (5%); 568.9 (5%)
Roburin E	**7**	12.74	2.1	**1065.5** (100%; $[M-H]^-$); 975.4 (10%)	1047.1 (55%); 1029.0 (90%); 1021.1 (20%); 1005.1 (10%); 987.1 (50%); 975.1 (100%); 945.0 (18%); 931.1 (20%); 915.1 (5%)
Castalagin	**8**	15.01	6.2	**933.4** (100%; $[M-H]^-$); 613.1 (5%)	915 (80%); 897.0 (45%); 889.1 (22%); 870.9 (20%); 630.9 (100%); 612.9 (12%)
Acutissimin A	**9**	21.53	28.5	**1025.6** (100%; $[M-H]^-$); 915.3 (65%)	915.1 (100%); 871.1 (12%)
Acutissimin B	**10**	25.28	19.0	**1025.7** (100%; $[M-H]^-$); 915.4 (55%)	915.0 (100%); 871.1 (10%); 612.9 (5%)

Because of the lack of characteristic chromophores in ellagitannins, which are characterized by absorbance maxima around 230-235 nm and shoulders around 280 and 320 nm, we used the DAD-chromatograms at 250 nm (Figure 2A) for quantification as previously reported (García Estévez et al., 2012). We also checked the availability of an ion trap MS detector to help identification and quantification of ellagitannins under the aforementioned chromatographic conditions. Under the selected ionization parameters optimized for castalagin, most ellagitannins preferentially formed deprotonated molecules ($[M-H]^-$) in the ESI source of the ion trap MS detector. However, the relatively high molecular weight of some wine ellagitannins, namely roburins A, B, C and D, allowed the formation of bi-deprotonated molecules ($[M-2H]^{2-}$) in the ESI source and their corresponding signals were the predominant ones in their MS spectra, which also showed signals corresponding to some fragment ions (Table 1). All the MS and MS/MS spectra were in agreement with previously reported mass spectral data for the considered ellagitannins (Mämmelä, Savolainen, Lindroos, Kangas & Vartiainen, 2000; Glabasnia & Hofmann, 2006; Saucier et al., 2006; Jourdes et al., 2011).

The extracted ion chromatograms (EIC) at the main *m/z* values ($[M-2H]^{2-}$ in the cases of roburins A, B, C and D; $[M-H]^{-}$ in the rest of considered ellagitannins) were used for identification (Figures 2B to 2F). The quantification was made using the DAD chromatograms, however the observed percent distribution of areas in the EIC chromatograms was used for corroborating the results in the cases of coeluting peaks.

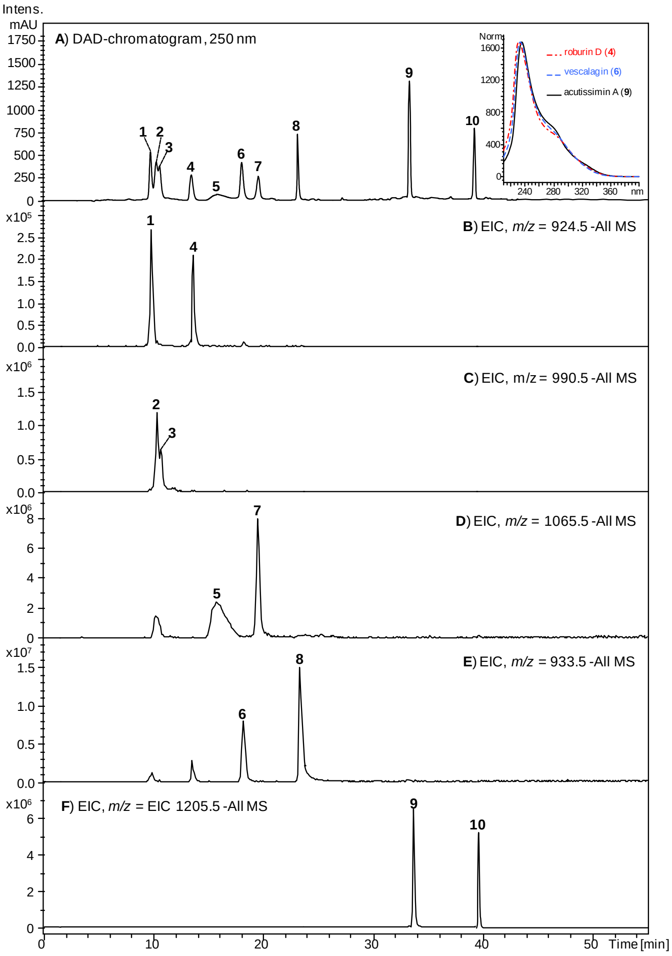


Fig. 2. Chromatographic separation of a standard mixture of ten ellagitannins usually found in wine aged with oak wood:

A) DAD-chromatogram obtained at 250 nm

B) Extracted Ion Chromatogram (EIC) showing the peaks corresponding to roburin A (**1**) and roburin D (**4**)

C) EIC showing the peaks corresponding to roburins B (**2**) and C (**3**)

D) EIC showing the peaks corresponding to grandinin (**5**) and roburin E (**7**)

E) EIC showing the peaks corresponding to vescalagin (**6**) and castalagin (**8**);

F) EIC showing the peaks corresponding to acutissimins A (**9**) and B (**10**).

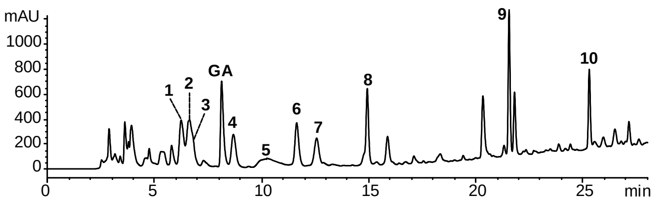
3.2. Extraction Method of Wine Ellagitannins Fraction

Firstly, we checked the application of a previously reported extraction method to a wine containing the ten wine ellagitannins assayed. This method involves two separation steps using different SPE cartridges (García-Estévez et al., 2010). The first step uses a C18 SPE cartridge and the recovery of the fraction containing the wine ellagitannins is carried out by elution with 2.5% acetic acid in water ("fraction a", containing the ellagitannins together with other substances), followed by two more fractions obtained after elution with ethyl acetate ("fraction b", containing ellagic acid, flavanols and flavonols) and methanol ("fraction c", containing anthocyanins).

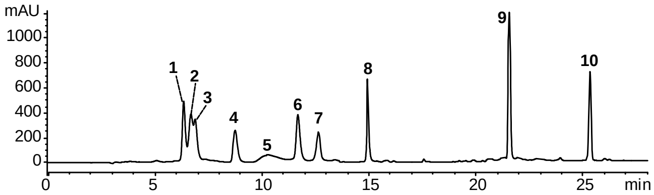
The removing of non-ellagitannin compounds from “fraction a” is achieved in the second extraction step, using a SPE cartridge filled with Sephadex LH-20. Four fractions (“fractions 1 to 4”) are obtained, being the so-called “fraction 4” (recovered by elution with methanol after washing with water, 96% ethanol and a little volume of methanol respectively) that contains wine ellagitannins which were analyzed by HPLC-DAD-MS. The above described extraction method was developed (García-Estévez et al., 2010) and validated (García-Estévez et al., 2012) for only four ellagitannins, namely grandinin (5), vescalagin (6), roburin E (7) and castalagin (8).

We checked the suitability of this extraction method to a more complex mixture of wine ellagitannins, using an ellagitannin-free young red wine that was spiked with the mixture of ten wine ellagitannin standards (Figure 3A). We observed that most polar ellagitannins, namely the roburins A-C (1 to 3) were not present in “fraction b” as expected, however this fraction still contained part of the other ellagitannins (Figure 3C), especially castalagin (8) and, more remarkably, acutissimins A (9) and B (10). In fact, the further SPE step of the “fraction a” to isolate the wine ellagitannins (the so-called “fraction 4”) showed that castalagin (8) was present, but in lower concentration than at first, and that acutissimins A (9) and B (10) were practically non-existent (Figure 3E).

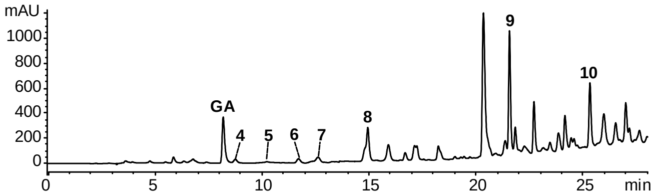
A) Wine spiked with a mixture of ellagitannin standards



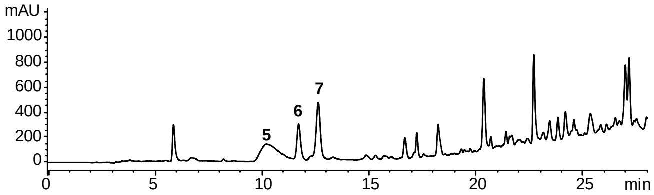
B) Mixture of ellagitannins standards



C) “Fraction b” using C18 cartridges



D) “Fraction b” using PCX cartridges



E) Wine ellagitannins in “fraction 4” using Sephadex LH-20 cartridge

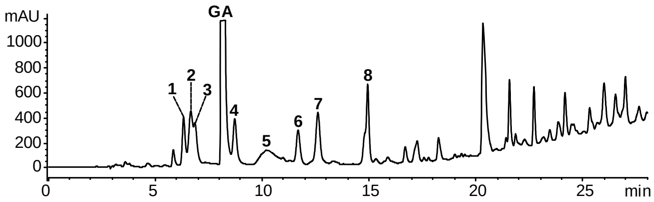


Fig.3. Chromatographic profiles corresponding to the different fractions obtained from the assay of the extraction method proposed by García-Estévez et al. (2010) applied to a red wine spiked with a mixture of standard ellagitannins. “Fraction b” was obtained in the first step of SPE using C18 (C) or PCX (D) cartridges, after the recovery of ellagitannins (“Fraction a”). “Fraction 4” was obtained in the second step of SPE, from “fraction a”, using Sephadex LH-20 cartridge. Peak numbering as in Table 1.

We tested another type of reversed-phase SPE cartridge for the first SPE fractionation step, consisting of a combination of reversed-phase and cationic exchange materials (PCX cartridges). However the equivalent “fraction b” still contained remarkable amounts of grandinin (5), vescalagin (6) and roburin E (7) (Figure 3D). These results suggested that the use of reversed-phase cartridges was not suitable as a first SPE step for the extraction of the expected complex mixture of ellagitannins from wine, necessary for their chromatographic analysis.

Inspired by an extraction method for the analysis of oak ellagitannins released to wine, in which the wine sample was directly subjected to only one sample preparation step by TSK HW 50F gel chromatography (Michel et al., 2011), we checked the suitability of using the SPE cartridge filled with Sephadex LH-20 for developing a similar one-step extraction of wine ellagitannins.

The same sample of spiked young red wine was used in this experiment (Figure 4A). The assay was made using the same fractionation pattern described by García-Estévez et al. (2010) for the second SPE step on Sephadex LH-20. The results demonstrated that the washing fractions obtained by successive passing of water (2 mL), ethanol 96% vol. (2 mL) and methanol (1 mL) did not remove any ellagitannins retained in the Sephadex LH-20 cartridge (Figure 4B).

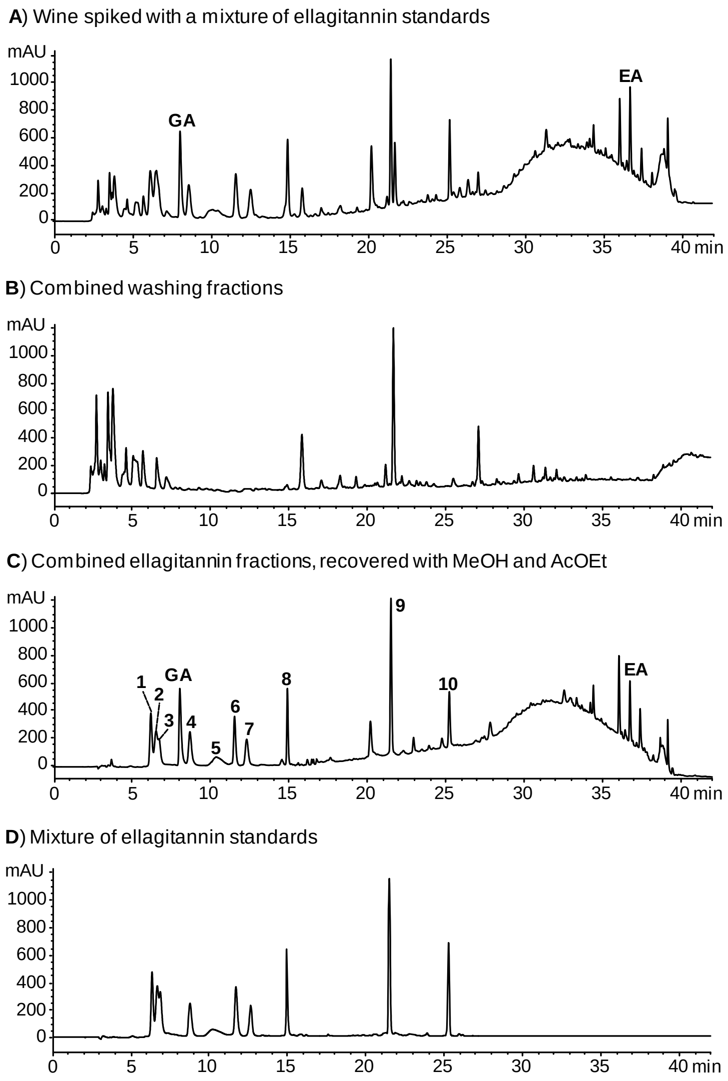


Fig. 4. Chromatographic profiles corresponding to the different fractions obtained from the assay of the extraction method using only one SPE step on Sephadx LH-20 cartridge, applied to a red wine spiked with a mixture of standard ellagitannins. Peak numbering as in Table 1.

However, the recovery of the fraction containing the wine ellagitannins using the prescribed 5 mL of methanol was far from being quantitative (Table 2) and did not even improve when the amount of methanol was increased up to 20 mL. Better results were obtained by combination of consecutive elution with methanol (10 mL) and ethyl acetate (10 mL) (Figure 4C and Table 2). The recoveries of the most relevant wine ellagitannins, namely roburin D (peak 4), grandinin (peak 5), vescalagin (peak 6), roburin E (peak 7) and castalagin (peak 8) was virtually quantitative, ranging between 93% and 100%.

The coeluting roburins A (1), B (2) and C (3) and acutissimin A (9) showed slightly lower recoveries, in the range of 76-85%. In contrast, the recovery of acutissimin B (10) was low at only 44%. The two-step extraction method described by García-Estévez et al. (2012) also allowed quantitative recoveries for castalagin, vescalagin, grandinin and roburin E. However, the recovery experiments were apparently performed directly with a solution of the standards mixture, without the consideration of the expected matrix effects due to the wine.

The other two-step extraction method described by Saucier et al. (2006) has been reported to reach almost quantitative recoveries for the same 10 wine ellagitannins now studied (Jourdes et al., 2011). The lack of quantitative recovery of acutissimin B (10) could be overcome by direct quantification on the DAD-chromatogram of the wine sample before the extraction method (Figure 3A). In fact, most of the wine ellagitannins could be directly quantified in the DAD-chromatogram of the sample wine with the exception of the coeluting roburins A (1), B (2) and C (3) which are overlapped with other interfering compounds, thus making their extraction necessary before chromatographic analysis.

In addition to ellagitannins, the related gallic (GA) and ellagic acids (EA) initially present in the wine sample were almost quantitatively recovered (96.00 ± 0.58% and 94.22 ± 1.17% respectively, calculated from wine samples not spiked with ellagitannins standards and subjected to the same extraction procedure) in the fraction containing the wine ellagitannins as can be surmised by comparing the Figures 4A and 4C. Finally, we also checked the stability of the isolated fractions containing the wine ellagitannins by repeated chromatography of the same sample after 24 and 48 h stored at room temperature, giving rise to integration results that randomly differed between 0.21 and 2.75%.

Table 2. Calibration curves, limits of detection (LOD) and quantification (LOQ), and recovery rates (MeOH, using only 20 mL of methanol; MeOH + AcOEt, using a combination of 10 mL of methanol followed by 10 mL of ethyl acetate) of wine ellagitannins.

Ellagitannin	Quantification factor	R^2	LOD (mg/L)	LOQ (mg/L)	% of recovery (MeOH)	% of recovery (MeOH + AcOEt)
Roburin A	0.0028	0.9999	0.0041	0.0136	51.43	84.57 ± 2.94
Roburin B	0.0025	0.9956	0.0059	0.0196	64.83	79.12 ± 3.18
Roburin C	0.0036	0.9930	0.0052	0.0175	63.04	84.46 ± 2.20
Roburin D	0.0027	0.9999	0.0041	0.0137	75.35	94.59 ± 2.47
Grandinin	0.0041	0.9986	0.2972	0.9905	76.01	99.94 ± 1.08
Vescalagin	0.0042	0.9998	0.0374	0.1248	68.45	98.50 ± 2.45
Roburin E	0.0025	0.9999	0.0795	0.2648	62.30	92.99 ± 3.76
Castalagin	0.0040	0.9999	0.0105	0.0350	65.32	95.49 ± 3.23
Acutissimin A	0.0009	0.9999	0.0025	0.0083	44.56	75.87 ± 0.83
Acutissimin B	0.0014	0.9999	0.0011	0.0037	22.66	44.12 ± 1.26

Regarding the sensitivity of the developed analytical method (Table 2), all the calibration equations for wine ellagitannins fitted well with a linear response, with most of them showing correlation coefficients > 0.998 and only those of roburins B and C (2 and 3) was > 0.993. The limits of quantification (LOQ) were mostly around 0.01-0.12 mg/L; the exception were grandinin (5) and roburin E (7) with LOQ values of 0.99 and 0.26 mg/L respectively, very likely more related to the broad shape of their corresponding chromatographic peak which originated from the standards used for these ellagitannin, especially in the case of grandinin although it appeared to be uniform regarding its DAD and MS spectra. One to two-order lower values of LOQ have been reported for the quantification of ellagitannins using specific mass spectrometer detection with added internal standard (Jourdes et al., 2011; García-Estévez et al., 2012).

The latter results suggest that the use of mass spectrometer detection using internal standard should be preferable to DAD detection. However, the use of chlorogenic acid as internal standard (Jourdes et al., 2011) has been questioned because this compound seems to cause degradation of vescalagin (García-Estévez et al., 2012). Moreover, the alternative use of gallocatechin as internal standard in the analytical method proposed by García-Estévez et al. (2012) should be reconsidered since this internal standard is added after the extraction of wine ellagitannins and we have evidenced that the first step of the extraction procedure with C18 SPE cartridges seems not to be quantitative for less polar ellagitannins, especially the acutissimins.

The developed methods for the extraction and analysis of wine ellagitannins were applied to commercial samples (n=3) of Cabernet Sauvignon red wine aged during 12 months in new barrels of French oak with a light toast. The content of wine ellagitannins was: roburin A, 0.04±0.03; roburin B, 0.14±0.07; roburin C, 0.09±0.03; roburin D, 0.10±0.04; grandinin, 3.99±0.42; vescalagin, 1.32±0.12; roburin E, 2.07±0.34; castalagin, 6.34±0.45; acutissimin A, <0.02; acutissimin B, <0.01.

4. CONCLUSIONS

The newly proposed method for the analysis of wine ellagitannins offers significant advantages over previously reported methods. On the one hand, fused core column allowed optimized separation allowing quantification by means of DAD. The improvement is good enough that most relevant wine ellagitannins (roburin D, grandinin, vescalagin, roburin E and castalagin), and even some vescalagin-derived ellagitannins like acutissimins A and B, could be directly analyzed in wine without any sample preparation.

On the other hand, the sample preparation necessary for the analysis of most polar wine ellagitannins (mainly roburins A, B and C) was simplified to only one single step of solid phase extraction using size exclusion gel (Sephadex LH-20) hand-packed cartridges.

The suggested one-step extraction proved not to cause losses of interesting compounds during loading and washing phases and yielded almost quantitative recovery of most wine ellagitannins, with the exception of the less polar acutissimin B. In addition, the proposed method also allows the analysis of free gallic and ellagic acids in the same chromatographic run.

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Capítulo II. Impacto del origen botánico, tostado y edad del roble en los elagitaninos presentes en el vino envejecido en barricas

El envejecimiento de vinos de calidad en barricas de roble es una práctica tradicional en nuestro país. Durante la crianza en barricas de roble, el vino experimenta cambios en su composición debido a la liberación de moléculas extraídas de la madera, de entre las que destacan por su impacto organoléptico los elagitaninos.

Varios estudios se han llevado a cabo, con el propósito de comprender la importancia de los elagitaninos en el vino, su procedencia e interacciones. La determinación del verdadero impacto de los elagitaninos en el vino sigue siendo un desafío. En primer lugar, el nivel de los elagitaninos totales en los vinos envejecidos en barricas de roble puede ser muy variable ya que se ve influenciado por el origen botánico y geográfico, así como los procesos a los que ve sometida la madera de roble para la fabricación de las barricas, como los procesos de secado y tostado, siendo también muy importantes las veces que es usada la barrica. Así este capítulo se centra en marcar e identificar dichas diferencias para ayudar a enólogos y empresarios del sector en su elección de una barrica adecuada a sus necesidades.

Los análisis de los vinos se realizaron en el laboratorio del grupo de Tecnología Enológica (TECNENOL) del Departamento de Bioquímica y Biotecnología de la Universitat Rovira i Virgili (URV, Tarragona) y en el Instituto Regional de Investigación Científica Aplicada (IRICA), Universidad de Castilla-La Mancha (UCLM, Ciudad Real). Los resultados del estudio fueron publicados en la revista *Food Research International*

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Influence of the botanical origin and toasting level on the ellagitannin content of wines aged in new and used oak barrels

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ABSTRACT: The influence of the botanical origin (French oak: *Quercus petraea* and American oak: *Quercus alba*) and different toasting levels (light, medium and heavy) on the ellagitannin content of wines aged in oak barrels has been studied. This took place in two consecutive vintages in order to study what happens after the barrels have been already used for one year with another wine. This study was carried out with two red wines (Cabernet Sauvignon) and with two discolored white wines (Macabeo) from vintages 2012 and 2013 in order to work with a simpler matrix which facilitates chemical analysis. The results show that the botanical origin, toasting level and the number of times that the barrels have been used exert a major influence on the final ellagitannin concentration. In general, the behavior of all the individual ellagitannins was very similar to that described for the total ellagitannins. Briefly, the levels of total ellagitannins concentration in the discolored white wine aged for 12 months in new French barrels ranged between 31.2 mg/L in the lightly toasted and 4.7 mg/L in the heavy toasted. In contrast, these levels were quite lower in American new barrels ranging between 3.6 mg/L and 0.9 mg/L. Finally, the total ellagitannin concentration decreased an average of 63% in the wines aged in the one year used barrels.

KEYWORDS: Ellagitannins, Wine, Oak, Botanical origin, Toasting level.

1. INTRODUCTION

High-quality red wines are traditionally aged in oak barrels to improve their sensorial characteristics. Oak aging causes some interesting changes in red wines, leading to color stabilization, lower astringency, and the disappearance of excess vegetative notes (Garde-Cerdán & Ancín-Azpilicueta, 2006). These transformations seem to be associated with small quantities of oxygen that penetrate the porosity of the wood, the interstices between staves, and the bunghole (Del Alamo-Sanza & Nevares, 2014).

The contact with oak wood also enriches the wine in many volatile substances such as vanillin, whiskey lactones, furans, volatile phenols, etc that improve the intensity and complexity of the wine's flavor (Rodríguez-Rodríguez & Gómez-Plaza, 2012; Pérez-Juan & Luque de Castro, 2015). Finally, oak wood also releases some non-volatile substances such as ellagitannins into the wine, which contribute to wine texture sensations such as astringency and mouthfeel (Stark et al., 2010; Sáenz-Navajas, Fernández-Zurbano, & Ferreira, 2012; Michel et al., 2013). It has also been reported that ellagitannins can act as natural antioxidant and protect other wine compounds against oxidation thanks to their great capability to react with oxygen (Navarro et al., 2016).

Although there are N150 species of oaks classified in the genus Quercus, only three of them are currently used in cooperage for wine aging. More specifically, the American white oak (*Quercus alba*) and two European oaks, the sessile oak (*Quercus petraea*) and the pedunculate oak (*Quercus robur*), commonly known as the French oaks. Although the great differences between them, Q. *petraea* and Q. *alba* are generally considered to provide greater aromatic richness than Q. *robur*, whereas the latter oak provides more ellagitannins (Masson, Puech, & Moutounet, 1994; Díaz-Maroto, Guchu, Castro-Vázquez, de Torres, & Pérez-Coello, 2008). Q. *petraea* and Q. *alba* have practically monopolized the cooperage market for wines in recent years probably for this reason (Vivas, 2002).

Ellagitannins have a specific structure, consisting of open-chain glucose esterified at position 4 and 6 by a hexahydroxydiphenoyl unit (HHDP) and a nonahydroxyterphenoyl unit (NHTP) esterified at position 2, 3, and 5 with a C-glycosidic bond between the carbon of the glucose and position 2 of trihydroxyphenoyl unit (Quideau et al., 2004; Takuo, Takashi, Tsutomu, & Hideyuki, 2009).

Ellagitannins may reach 10% of the dry weight of oak heartwood, and they contribute to the wood's high durability (Scalbert, Monties, & Favre, 1988; Nonier et al., 2005). Castalagin and vescalagin are the most abundant ellagitannins in oak wood, accounting for between 40% and 60% by weight of the total (Fernández de Simón, Cadahía, Conde, & García-Vallejo, 1999). These two isomers were the first ellagitannins to be isolated and described by Mayer (Mayer, Gabler, Riester, & Korger, 1967). The lyxose/xylose derivatives (grandinin and roburin E) and the dimeric forms (roburins A, B, C and D) were also subsequently described (Herve Du Penhoat, Michon, Ohassan, et al., 1991; Herve Du Penhoat, Michon, Peng, et al., 1991).

The hydroalcoholic nature of wine favors the extraction of these compounds from wood to wine (Moutounet, Rabier, Puech, Verette, & Barillere, 1989; Jordão, Ricardo-da-Silva, & Laureano, 2005) during barrel aging or when wood alternatives are used. However, the high reactivity of ellagitannins means that their levels in wine are much lower than could be expected (Michel et al., 2011).

Nevertheless, according to the described sensory threshold of different ellagitannins (Glabasnia & Hofmann, 2006) their concentration in wine may in some cases be enough to contribute to wine astringency. The level of the ellagitannins in oak wood used to make barrels is also influenced by several factors (Masson, Moutounet, & Puech, 1995; Zhang, Cai, Duan, Reeves, & He, 2015) such as its geographical origin (e.g. American or French oaks) (Canas, Leandro, Spranger, & Belchior, 2000; Prida & Puech, 2006), the oak species (Q. *robur* releases higher concentrations of ellagitannins than Q. *petraea*, and this in turn releases higher concentrations of ellagitannins than Q. alba) (Prida & Puech, 2002), the number of times that the barrels have been used (the levels of ellagitannins extracted are much lower in used barrels) (Vivas & Saint-Cricq de Gaulejac, 1998) and the forest management practices of the tree (Vivas, 2002). Moreover, the manufacture of barrels in cooperage also has a major influence on its composition, with consequent effects on the wine's flavor. First, the logs must be processed to obtain the staves. Due to the different structures of their heartwood, American oak can be sawed, but French oak needs to be split in order to respect the natural grain. Sawing French oak would make it porous and affect its tightness.

Later, the staves must be subjected to a drying process, to ensure the mechanical resistance of the barrels and to eliminate some substances that contribute to bitterness and astringency (Josephe & Marché, 1972; Masson, Baumes, Moutounet, & Puech, 2000). This process usually takes 2 or 3 years. Once the drying time is finished, the staves are heated to form the barrels. The heating time needed to form the barrel is around 15 min. However, cooperages usually extend this time in order to improve the sensory attributes that the barrel gives the wine. Three toasting levels are usually offered: light, medium and heavy, with different sensory styles (Chatonnet, 1992; Rodríguez-Rodríguez & Gómez-Plaza, 2011). Vanillin and smoke flavors (furans, volatile phenols) generally increase with toasting, whereas whiskey lactone decreases (Singleton, 1995; Koussissi et al., 2009; Farrell et al., 2015). The toasting level also exerts amajor influence on the ellagitannins content of oak (Doussot, De Jéso, Quideau, & Pardon, 2002; Chira & Teissedre, 2013a). High temperatures favor the oxidation of ellagitannins and the formation of polymers and copolymers with cell wall components which reduces their solubility (Peng, Scalbert, & Monties, 1991). The ellagitannin potential release therefore decreases when the toasting level is increased.

Finally, the successive use of the barrels leads to a depletion of the substances that the wood can release into the wine. The capacity of the barrels to enrich the wine in aromas and ellagitannins consequently decreases with their use (Garde-Cerdán, Rodríguez-Mozaz, & Ancín-Azpilicueta, 2002).

The interest of wineries and cooperages in how the botanical origin and the toasting level affect wine's composition and quality has led to numerous studies. However, most of them are related with the aromatic aspects (Cadahía, Fernández de Simón, & Jalocha, 2003; García, Soares, Dias, Freitas, & Cabrita, 2012; Fernández De Simón et al., 2014; Collins, Miles, Boulton, & Ebeler, 2015) and only a few have targeted the study of the ellagitannin release (Cadahía, Varea, Muñoz, Fernández de Simón, & García-Vallejo, 2001; Fernández De Simón, Sanz, Cadahía, Poveda, & Broto, 2006), probably because its analysis is quite complicated (Stephen & Kimberly, 2012). Moreover, to our knowledge the recent studies of ellagitannins in wine have mainly been performed with wood chip maceration (Chira & Teissedre, 2013b; Chira et al., 2015; García-Estévez, Alcalde-Eon, Le Grottaglie, Rivas-Gonzalo & Escribano-Bailón, 2015) and only a few with oak barrels (Jourdes, Michel, Saucier, Quideau, & Teissedre, 2011; Chira & Teissedre, 2015). Moreover, these latter studies were performed with new barrels of Q. *petraea*, with no study of Q. *alba* and what happens in used barrels (Saucier, Jourdes, Glories, & Quideau, 2006; Chira & Teissedre, 2015). The aim of this study was to determine the influence of the botanical origin and toasting level on the ellagitannin content of wines aged in new and used oak barrels.

2. MATERIALS AND METHODS

2.1. Chemicals and equipment

Methanol, formic acid and acetic acid of high performance liquid chromatography (HPLC) grade (>99%) and absolute ethanol (96%), ethyl acetate (99%) were purchased from Panreac (Barcelona, Spain). Oenological charcoal and bentonite were purchased from Agrovin (Alcázar de San Juan, Ciudad Real, Spain). Ellagitannins: castalagin (99%), vescalagin (98%), roburin A (98%), roburin B (93%), roburin C (93%), roburin D (98.5%), grandinin (98.5%) and roburin E (97%) were purchased from Adera (Pessac, France).

HPLC separation, identification and quantitation of ellagitannins were performed using an Agilent 1100 Series system (Agilent, Germany), equipped with DAD (G1315B) and an LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MSn) system, and coupled to an Agilent ChemStation (version B.01.03) data processing station. The mass spectra data were processed using Agilent LC/MS Trap software (version 5.3).

2.2. Barrels

A total of 24 new barrels (225 L) were purchased from the Boteria Torner Cooperage (Sant Cugat Sesgarrigues, Barcelona, Spain). The cooperage selected wood staves with the criteria that were as homogeneous as possible within each species. Twelve barrels were made with American white oak (Q. *alba*), and the other 12 with French oak (Q. *petraea*). Three toasting levels (light, medium and heavy) were used for each type of oak, and a similar toasting level was applied to the barrel heads. Each experimental condition was performed in duplicate, using 2 barrels.

2.3. Wines and barrel aging

This study was carried out with two Cabernet Sauvignon red wines and two decolored Macabeo white wines, obtained from the experimental cellar of the Faculty of Enology of the Rovira i Virgili University in Constantí (AOC Tarragona, Spain) from the 2012 and 2013 vintages. The red wines were used without any stabilization treatment, whereas the white wines were drastically decolored with oenological charcoal (2 g/L) and bentonite (1 g/L) in order to minimize the presence of aromas and phenolic compounds. The aim was to obtain a very neutral medium to facilitate chemical analysis.

The analytical characteristics of the wines at the start of the experiment are showed in Table 1.

Table 1. Analytical characteristics of the wines.

Parameter	Discolored white wine		Red wine	
	2012	2013	2012	2013
Ethanol content (%)	13.3	13.5	13.5	13.4
Titratable acidity (g/L) (expressed as g of tartaric acid/L)	6.2	6.5	5.4	5.7
Volatile acidity (g/L) (expressed as g of acetic acid/L)	0.22	0.18	0.42	0.38
pH	3.14	3.09	3.74	3.62
Total phenolic index (TPI)	3.5	2.0	56.0	53.0
Free SO2 (mg/L)	24	27	23	25
Total SO2 (mg/L)	85	78	60	52

In brief, wines of the 2012 vintage were placed in the new barrels in April 2013, and were aged for 12 months. Once the barrels were emptied and cleaned, they were immediately filled with the same wines from the 2013 vintage, which were also aged for 12 months. Samples of all the barrels were taken at 3, 6, 9 and 12 months for ellagitannin analysis.

2.4. General analysis

Analytical methods recommended by OIV (O.I.V., 2001) were used to determine the ethanol content, titratable acidity, volatile acidity, free SO2 and total SO2. The total phenolic index (TPI) was determined by measuring absorbance at 280 nm, and expressed as absorbance units (Ribéreau-Gayon, Glories, Maujean, & Dubordieu, 2006).

2.5. Ellagitannin analysis

All the samples were first fractionated to obtain ellagitannin-rich fractions using a previously described method (García-Estevez, Escribano-Bailon, Rivas-Gonzalo, & Alcalde-Eon, 2010). In brief, the extraction method consisted in: a) 3 mL of red wine sample was dried under reduced pressure (35 °C) and redissolved in 2.5% acetic acid in water to a final volume of 2 mL; b) the prepared wine sample was applied into a hand packed minicolumn (70 × 12 mm) filled with half a volume of Sephadex LH-20 that was previously activated with methanol and conditioned with water (4 mL); c) the minicolumn was successively washed with water (2mL), 96% ethanol (2mL) and methanol (1 mL) and their combined eluates were discarded; d) the retained wine's ellagitannins were recovered by elution with methanol (10 mL) followed by ethyl acetate (10mL) e) the ellagitannins fraction was dried under reduced pressure and redissolved in 1.5 mL of water/formic acid (996:4, v/v) and placed in a chromatographic vial for the HPLC analysis.

A mother solution containing all the individual ellagitannin standards was conveniently diluted for obtaining the calibration curves necessary for quantification, covering concentration ranges up to 40 mg/L of each ellagitannin. The ellagitannins were analyzed by HPLC after the method had been adapted (Michel et al., 2011) to the use of fused core C18 chromatographic columns (Ascentis Express, 150 × 4.6 mm, 2.7 µm particle size), thermostatted at 40 °C (Navarro et al., 2016).

The ellagitannins were identified by matching the retention time and spectral data (DAD-UV–vis and MS/MS) with those of authentic standards. The quantitation was performed using the DAD-chromatograms extracted at 250 nm. The linear regression coefficients (r2) were N0.99 for all individual ellagitannins. The DAD-chromatogram (detection at 250 nm) of a mixture of ellagitannin standards and the MS and MS2 spectra (Ion Trap Mass Detector) of ellagitannin standards used for identification are included as supplementary material (Figs. S1 and S2).

2.6. Calculation of the theoretical sensory impact of ellagitannins present in wines

The estimation of the theoretical sensory impact of the ellagitannins in the different samples was calculated by dividing the individual concentration of each ellagitannin by its corresponding threshold. The thresholds considered for each ellagitannin were those previously reported by Glabasnia and Hofmann (2006) for astringency (grandinin: 0.21 mg/L; vescalagin and castalagin: 1.03 mg/L; roburin D: 5.55 mg/L; roburin E: 0.21 mg/L and finally roburins A, B and C an average of 9.98 mg/L). The theoretical sensory impact of the ensemble of all ellagitannins was obtained by applying the total of all the quotients of the individual ellagitannin concentrations by their corresponding threshold ($\Sigma Ei/Ti$). It must be highlighted that these thresholds were determined in water and not in wine so that should be considered with caution. However, given the unavailability of having enough amounts of each individual ellagitannin for determining the real thresholds in wine, we decided to use these values considering that, at least, are the best available indicators.

2.7. Statistics

All data are expressed as the arithmetic mean± standard deviation of the samples from two barrels. The multifactor analysis of variance (ANOVA) was carried out using XLSTAT software (2015 Edition (mac & win) Cracked FULL).

3. RESULTS AND DISCUSSION

Table 1 shows the analytical characteristics of wines at the start of the experiment. In both vintages the analytical parameters of the discolored white wines and red wines were very similar between them and can be considered as normal values in these types of wines.

Table 2 shows the total ellagitannin content of the discolored white wines and the red wines aged in the different oak barrels over time. This table presents information about how the botanical origin, toasting level and aging time affect the ellagitannin concentration of both the wines, and also considers the comparison between new and 1-year used barrels. This table also presents the significance level of the different factors in order to facilitate discussion of the results, owing to the complexity of the experimental approach.

Table 2. Total ellagitannins concentration of the different samples.

Medium	Toasting Level	Aging time (months)	French Oak		American Oak	
			New Barrel	1 year barrel	New Barrel	1 year barrel
Discolored white wine	LIGHT	3	4.00 ± 1.89	0.99 ± 0.14	0.70 ± 0.16	0.16 ± 0.03
		6	12.10 ± 1.81	0.26 ± 0.09	2.09 ± 0.24	0.04 ± 0.01
		9	17.24 ± 2.75	7.39 ± 2.03	6.19 ± 0.41	0.87 ± 0.07
		12	31.19 ± 11.61	9.12 ± 2.60	3.62 ± 0.57	1.50 ± 0.11
	MEDIUM	3	0.97 ± 0.07	0.38 ± 0.05	0.21 ± 0.02	0.05 ± 0.01
		6	3.69 ± 0.38	0.14 ± 0.01	0.67 ± 0.05	0.02 ± 0.01
		9	9.02 ± 0.72	2.78 ± 0.13	1.18 ± 0.07	0.43 ± 0.23
		12	9.02 ± 1.10	4.62 ± 0.18	1.09 ± 0.14	0.62 ± 0.03
	HEAVY	3	0.60 ± 0.12	0.25 ± 0.01	0.11 ± 0.02	0.03 ± 0.01
		6	3.62 ± 0.50	0.06 ± 0.01	0.54 ± 0.07	0.01 ± 0.01
		9	4.89 ± 0.88	1.98 ± 0.18	0.89 ± 0.14	0.17 ± 0.01
		12	4.67 ± 0.41	2.93 ± 0.19	0.92 ± 0.20	0.33 ± 0.06
Red wine	LIGHT	3	3.20 ± 0.16	0.05 ± 0.01	0.66 ± 0.01	0.06 ± 0.02
		6	7.37 ± 0.82	0.06 ± 0.01	2.06 ± 0.36	0.01 ± 0.01
		9	15.13 ± 0.86	1.79 ± 0.12	3.02 ± 0.69	0.22 ± 0.02
		12	14.10 ± 1.59	4.57 ± 0.18	2.46 ± 0.51	0.79 ± 0.06
	MEDIUM	3	1.07 ± 0.16	0.03 ± 0.01	0.46 ± 0.08	n.d
		6	3.03 ± 0.39	0.02 ± 0.01	0.74 ± 0.08	n.d
		9	5.24 ± 0.43	0.79 ± 0.07	1.38 ± 0.08	0.12 ± 0.01
		12	6.20 ± 0.70	2.27 ± 0.27	1.07 ± 0.17	0.15 ± 0.01
	HEAVY	3	0.74 ± 0.02	0.02 ± 0.01	0.33 ± 0.05	n.d
		6	1.93 ± 0.08	0.02 ± 0.01	0.32 ± 0.02	n.d
		9	4.54 ± 0.75	0.61 ± 0.07	0.77 ± 0.17	0.06 ± 0.01
		12	4.09 ± 0.63	1.89 ± 0.06	0.58 ± 0.13	0.07 ± 0.01

Significance level of the different factors (***p***)

Medium	Aging time	Botanical origen	Toasting Level	New/Used
Decolored white wine	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Red wine	< 0.0001	< 0.0001	< 0.0001	< 0.0001

All data are expressed as the average values (mg/L) of two barrels ± standard deviation.

In general, the ellagitannin levels detected in the discolored white wines were higher than those measured in the red wines in both vintages and in all the experimental conditions. In specific terms, the average ellagitannin concentration of the discolored white wines was 63% higher than the average observed in the red wines. These differences may be due to the heterogeneity of the wood staves used in the different barrels but can also be attributed to the differences between the two wine matrixes, i.e. discolored white wines and red wines.

The main differences between them were the pH, which was lower in the discolored white wines (an average of 3.12) than in the red wines (an average of 3.68), and obviously the presence of phenolic compounds in red wines. Consequently, one possible explanation might be that a lower pH favors ellagitannin extraction, and another possibility is that some of the ellagitannins released may have reacted with naturally occurring polyphenols to form new compounds cannot be detected by the method applied. In that respect, it has been reported that vescalagin can form adducts with (+)-catechin (acutissimins A and B), and even with malvidin-3-glucoside (Jourdes, Lefeuvre, & Quideau, 2009).

The influence of aging time on total ellagitannin concentration was very clear, with a significant increase over time in all the experimental conditions. However, this increase was not always observed between consecutive sampling times, probably because ellagitannins can be hydrolyzed (Peng et al., 1991; Michel et al., 2011; Jourdes et al., 2011) or transformed into other compounds, as mentioned above (Jourdes et al., 2009). The final ellagitannin concentration is therefore a balance between their release from oak wood and their disappearance.

French oak released significantly higher amounts of total ellagitannins than American oak under all experimental conditions. This data is consistent with previously reported studies, which reported that Q. *alba* is relatively poorer than Q. *petraea* in ellagitannins (Fernández de Simón et al., 1999; Cadahía et al., 2001; Prida & Puech, 2002). However these differences were greater than expected. In fact, our results show that the wines aged in new barrels of French oak have an average of 5.4 folds more ellagitannins than wines aged in new barrels of American oak. This ratio even reaches 9 folds in the case of the discolored white wines, after 9 months of aging in light toasted new barrels.

The toasting level also exerts a significant influence on total ellagitannin concentration. In brief, the higher the toasting level the lower the total ellagitannin concentration under all experimental conditions. In specific terms, after 12 months of aging in French oak new barrels, the total ellagitannin concentration was 3 times higher in the wines from the light toasting level than for the same wines from the medium toasting level, and this ratio increased to 5 times in comparison with the wines from the heavy toasting levels. These differences were somewhat lower for the wines aged for 12months in new American oak barrels, at 2.5 and 4 times respectively. After one year, the differences between the ellagitannin concentrations of the new wines aged in the same barrels maintained this tendency, although the absolute values were logically lower. This effect, which had been previously reported (Chira & Teissedre, 2015), confirms that ellagitannins from oak heartwood are degraded or transformed by high temperatures, reducing their extractability (Peng et al., 1991)

Table 2 also shows the comparison between the total ellagitannin concentration observed in the wines in the first year, when the barrels were new, and the wines in the second year, when the barrels have already been used for one year. As expected, the ellagitannin concentration of both wines in the first year was significantly higher than in the wines for the second year, which confirms that the barrels' ability to release ellagitannins is depleted with use. However the decrease observed after one year may be considered very drastic. In fact, the total ellagitannin concentration detected in the wines aged for 12 months in the 1-year used French oak barrels was on average around 3 times lower than in the wines aged in new barrels for the same time.

There are some references as to how the aromatic contribution is depleted over successive uses of the barrels (Garde-Cerdán et al., 2002). However, to our knowledge, only one previous study has reported on how the release of ellagitannins decreases over the barrels' time of use. Vivas and Saint-Cricq de Gaulejac (1998) described a decrease of around 40% after the first year, and of around 80% after five years of use. Our results show that the depletion of ellagitannins is even quicker, because after the first year of use, the decrease observed was already an average of 63%.

The individual concentrations of the different ellagitannins, namely castalagin, vescalagin, grandinin, roburin E, roburin D, and the ensemble of roburins A, B and C, is shown in Tables S1–S6 (Supporting information). In general, the behavior of all the individual ellagitannins was very similar to that described for the total ellagitannins. In specific terms, their concentration increased with the time of aging, it was higher in French oak than in American oak, it diminished as the toasting level increased and with the use of the barrels, and all these factors were statistically significant in all cases. For this reason, all the comments in the discussion of the results for total ellagitannins are also valid for individual ellagitannins.

However, the ratio between castalagin and vescalagin concentrations showed an interesting and different behavior depending on the botanical origin. Table 3 shows the comparison of this ratio in the wines aged for 12 months in new barrels depending on the botanical origin and the toasting level. In overall terms, the castalagin/vescalagina ratio was significantly higher in American oak (8.95 ± 2.53) than in French oak (4.64±1.26). This ratio is higher in American oak, although it is poorer in total ellagitannins than French oak because it has a higher proportion of castalagin, and a smaller proportion of vescalagin in particular.

Meanwhile, the toasting level also seems to have an influence on this ratio. In the case of French oak, the ratio tended to increase, with significant differences reached between light and heavy toasting levels.

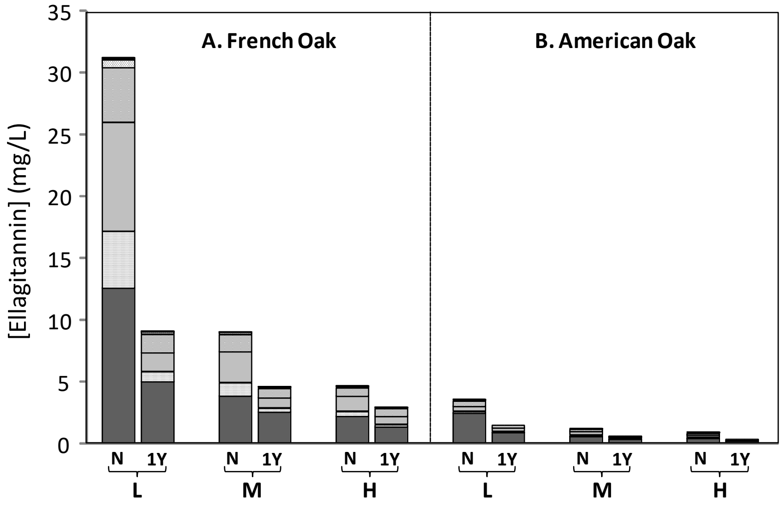
This trend should indicate that vescalagin is degraded to a greater extent than castalagin during the toasting process. However, American oak showed the opposite trend, although the differences were not significant in that case. None of the other ratios between the other individual ellagitannins showed a clear trend.

Table 3. Ratio castalagin/vescalagin.

Toasting level	French oak		American oak	
Light	3.77 ± 1.47	**a** α	11.02 ± 0.79	**a** β
Medium	4.34 ± 1.11	**ab** α	8.31 ± 3.2	**a** α
Heavy	5.81 ± 0.22	**b** α	7.51 ± 2.74	**a** α
Global	4.64 ± 1.26	α	8.95 ± 2.53	β

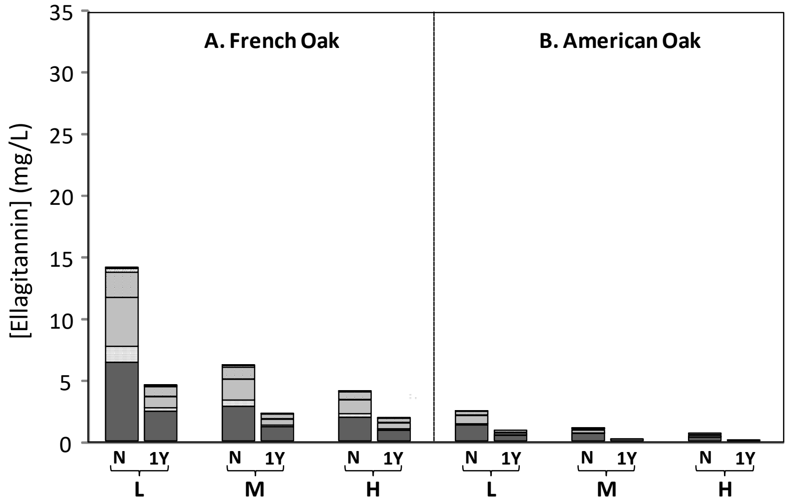
All data are expressed as the average values (mg/L) ± standard deviation. Different letters indicate statistical differences ($p < 0.05$). Small Roman letters are used to compare toasting levels. Greek letters are used to compare botanical origins.

The total and individual ellagitannin concentrations after 12months of barrel aging are shown in Fig. 1 (discolored white wines) and Fig. 2 (red wines). These figures graphically illustrate what really happens after the most common time period for wine aging. These graphics clearly show the importance of the selection of the characteristics of the barrel in terms of their contribution of ellagitannins to wine, as discussed above.



All data is the mean of two barrels. ■: Roburin D; ▩: Roburins A, B and C; ▨: Roburin E; ▨: Grandinin; ▤: Vescalagin; ■: Castalagin; N: New barrels; 1Y: one year used barrels; L: Light toasting level; M: Medium toasting level; H: heavy toasting level.

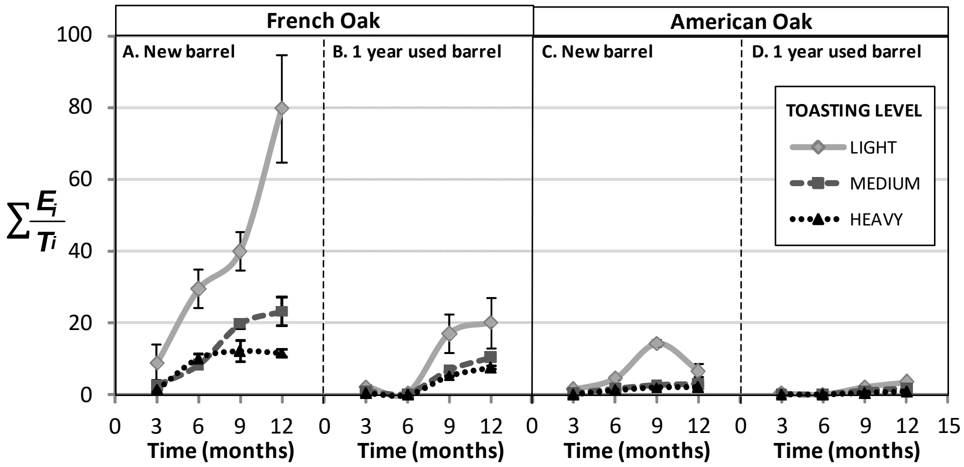
Fig. 1. Ellagitannin content of discolored white wine after 12 months of oak aging.



All data is the mean of two barrels. ■: Roburin D; ☐: Roburins A, B and C; ▨: Roburin E; ▨: Grandinin; ☐: Vescalagin; ■: Castalagin; N: New barrels; 1Y: one year used barrels; L: Light toasting level; M: Medium toasting level; H: heavy toasting level.

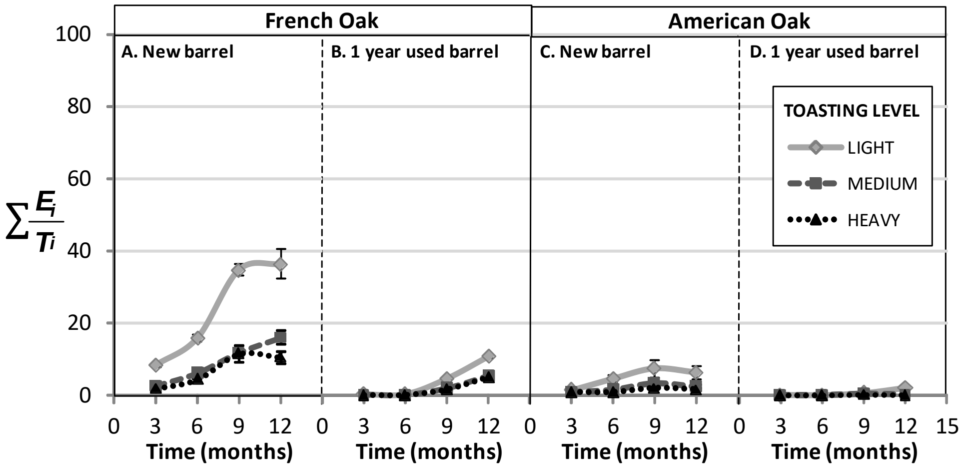
Fig. 2. Ellagitannin content of red wine after 12 months of oak aging.

The theoretical sensory impact of the ellagitannins present in the different wines (ΣEi/Ti) was calculated in order to show the hypothetic importance of the contribution of the different barrels with regard to this family of phenolic compounds. Fig. 3 discolored white wine) and Fig. 4 (red wine) show these results. As expected, the sensory impact of ellagitannins increased during the aging time, reaching an average value of around 6 times higher in the wines aged for 12 months in new barrels than in the wines aged in similar barrels for 3 months. Looking at the graphics, this effect is very clear in the case of French oak, especially in new barrels, but is not so clear in the American oak, due to its much lower ellagitannin impact.



All data are expressed as the average values of two barrels ± standard deviation. The theoretical sensory impact of the ensemble of all ellagitannins ($\sum E_i/T_i$) was obtained by applying the summation of all the quotients of the individual ellagitannin concentrations by their corresponding threshold.

Fig. 3. Theoretical sensory impact of ellagitannins in the discolored white wine.



All data are expressed as the average values of two barrels ± standard deviation. The theoretical sensory impact of the ensemble of all ellagitannins ($\sum E_i/T_i$) was obtained by applying the summation of all the quotients of the individual ellagitannin concentrations by their corresponding threshold.

Fig. 4. Theoretical sensory impact of ellagitannins in the red wine.

All things considered, it can be stated that ellagitannins released by French oak could really provide a sensory impact far higher than that of American oak, at on average six times greater, although this varies depending on the wine, the aging time, the toasting level and the time of use of the barrel. This ratio even reaches a maximum value of 12 times greater in the case of the discolored white wine aged for 12 months in new barrels with a light toasting level.

The toasting level of the barrels also has a noticeable effect on ellagitannin sensory impact, which is on average 3 times higher in light toasting barrels than in medium toasting barrels, and 4 times higher than in heavy toasting barrels. These ratios also vary depending on the wine, the botanical origin, the time of aging and the time of use of the barrel, but in general it can be considered as a reference value.

Finally, the use of barrels entails a decrease of the sensory impact of the ellagitannins due to their progressive depletion. On average, the sensory impact decreases by around 64%, and this value is very similar to that observed in the total ellagitannin concentration.

4. CONCLUSIONS

In short, it can be concluded that the barrel aging time and especially the selection of the barrel characteristics has a very significant influence on the final ellagitannin concentration in wine, and probably on its sensory impact. In specific terms, the botanical origin, the toasting level and the number of times that the barrels have been used have a very major influence on the final ellagitannin concentration. As an example, the total ellagitannin concentration of a wine after 12 months of aging in a new French oak barrel with light toasting can be >100 times higher, and the sensory impact >125 times higher than in the same wine aged in an American oak barrel with heavy toasting that has been previously used for one year. The potential impact of ellagitannin concentration in wine should consequently be a criterion by wine makers when selecting the botanical origin, the toasting level and the number of times that the barrels are used, as with other more common criteria, such as the aromatic profile or the wood's oxygen permeability.

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SUPPLEMENTARY DATA

Figs. S1 and S2, the DAD-chromatogram (detection at 250 nm) of a mixture of ellagitannin standards and the MS and MS2 spectra (Ion Trap Mass Detector) of ellagitannin standards used for identification. Table S1–S6, individual ellagitannin concentrations. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.foodres.2016.07.016.

NOTE: Supplementary tables are also included just at the end of this article.

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Figure S1. DAD-chromatogram (detection at 250 nm) of a mixture of ellagitannin standards

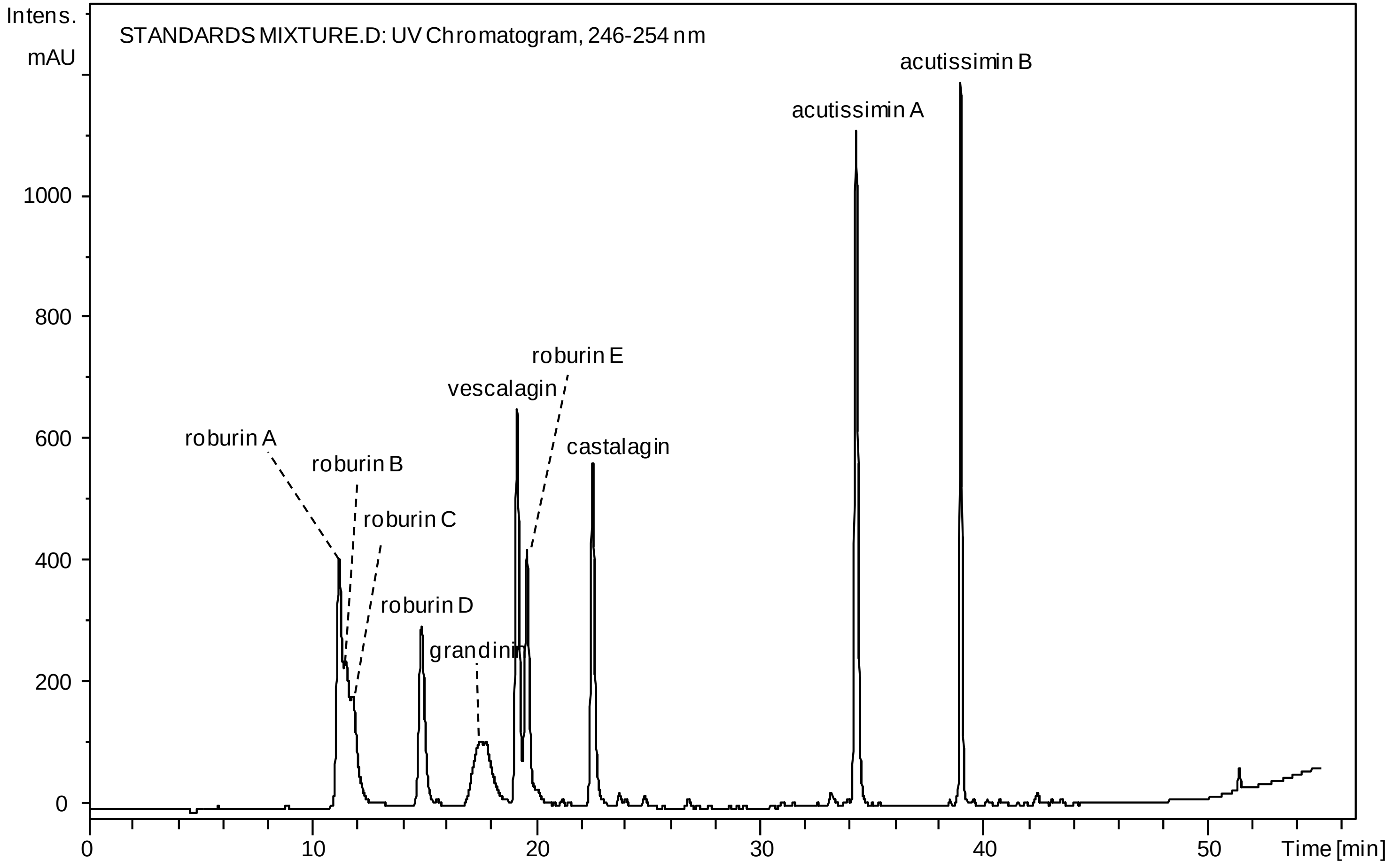

Figure S2. MS and MS2 spectra (Ion Trap Mass Detector) of ellagitannin standards used for identification.

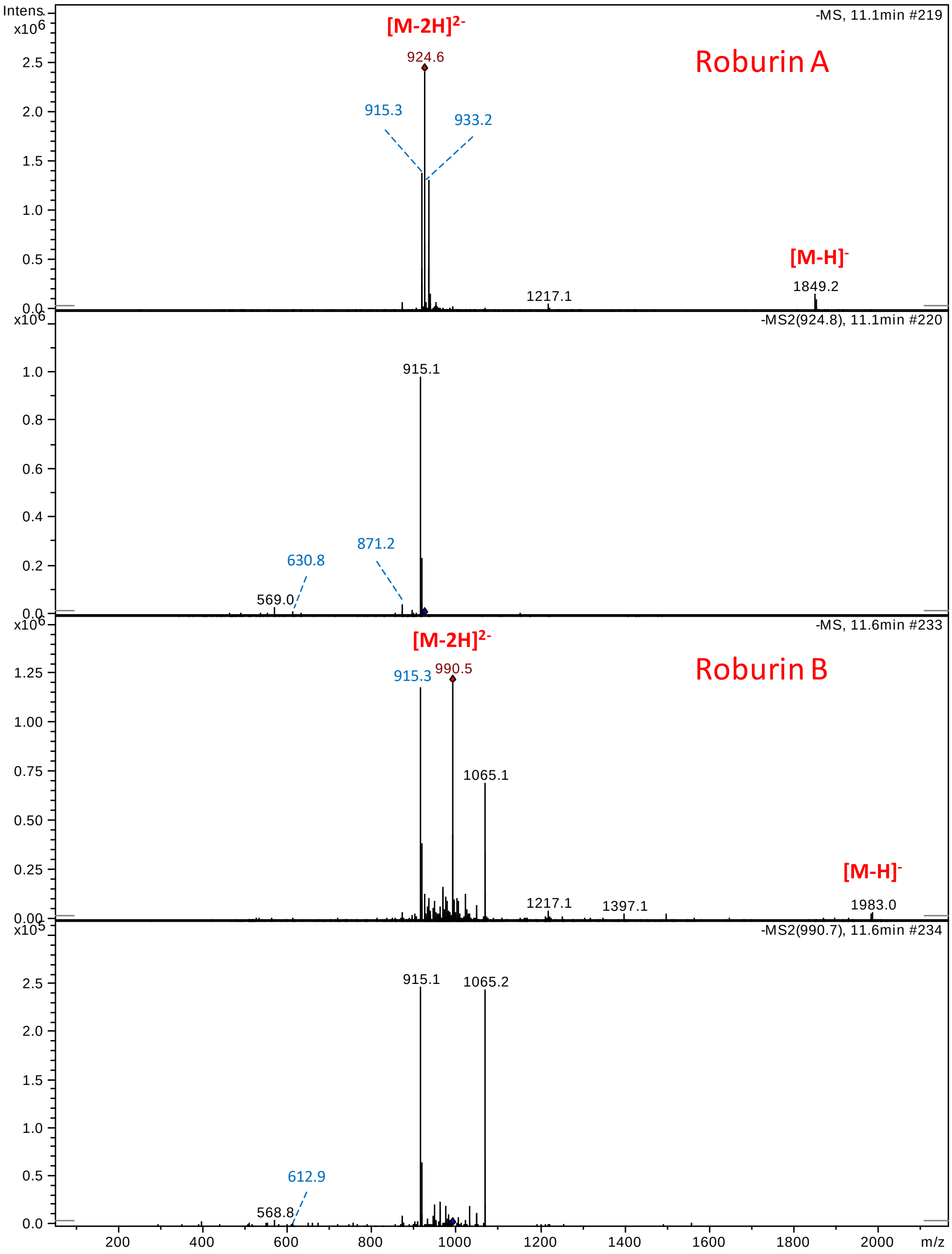

Figure S2 (cont.). MS and MS2 spectra (Ion Trap Mass Detector) of ellagitannin standards used for identification.

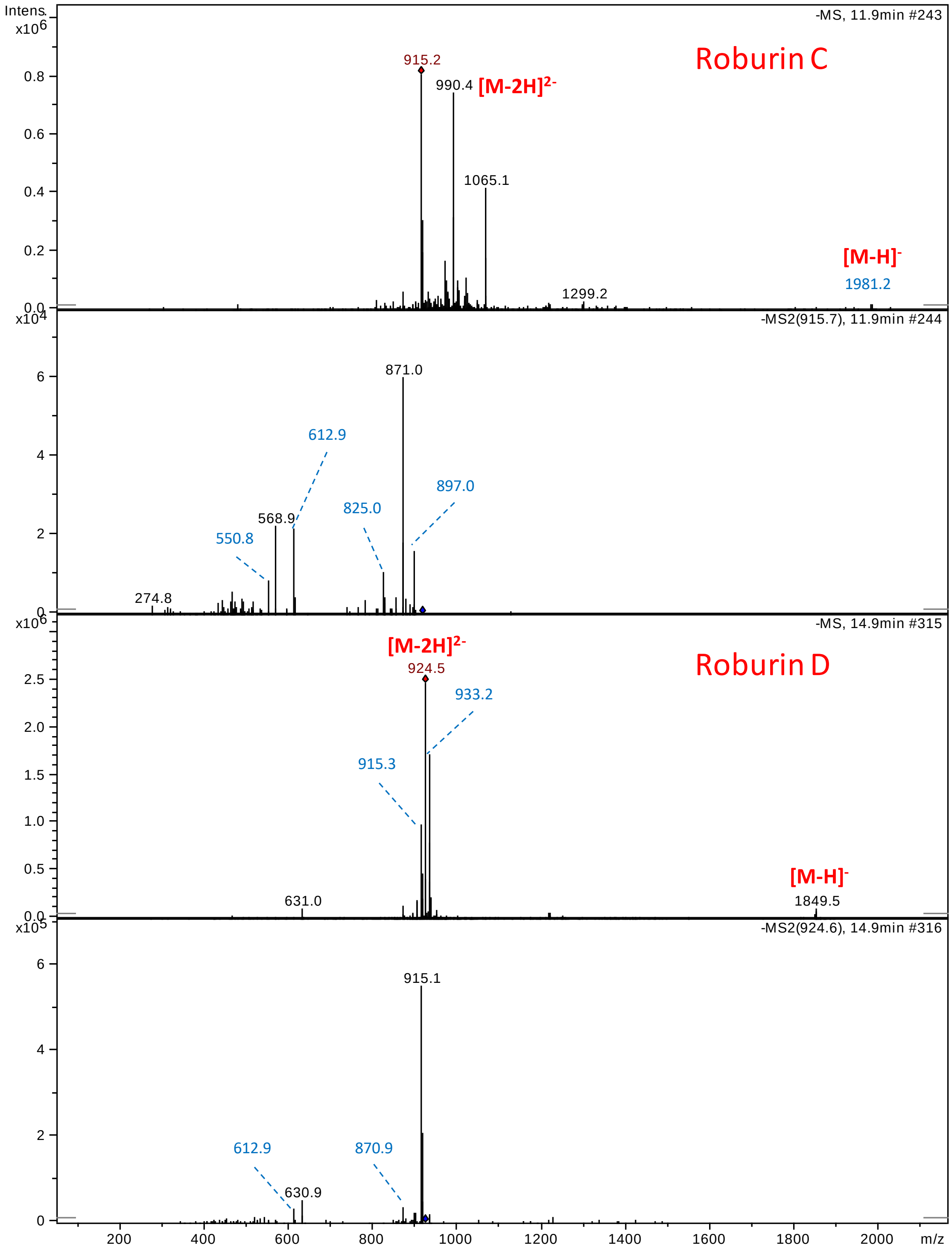

Figure S2 (cont.). MS and MS2 spectra (Ion Trap Mass Detector) of ellagitannin standards used for identification.

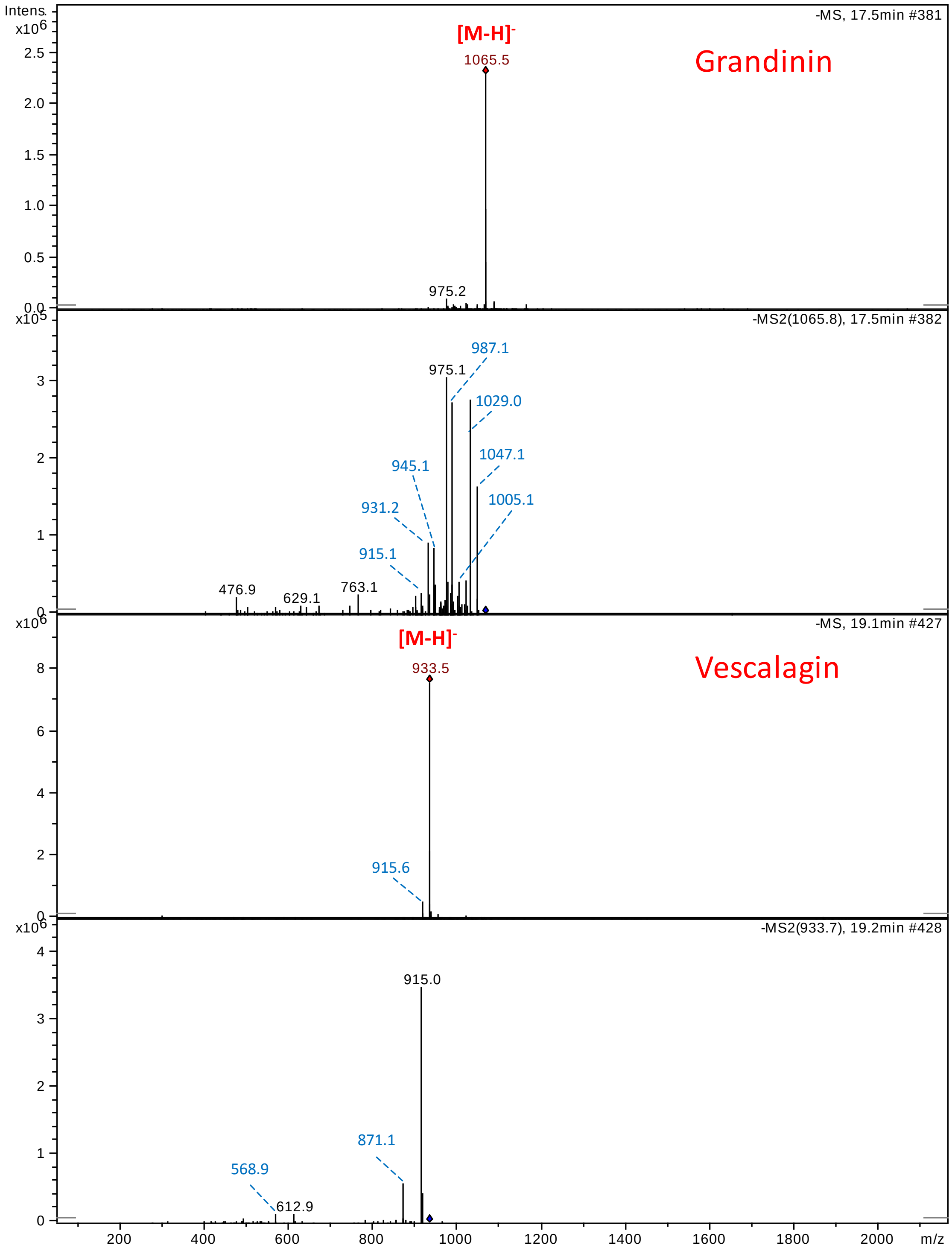

Figure S2 (cont.). MS and MS2 spectra (Ion Trap Mass Detector) of ellagitannin standards used for identification.

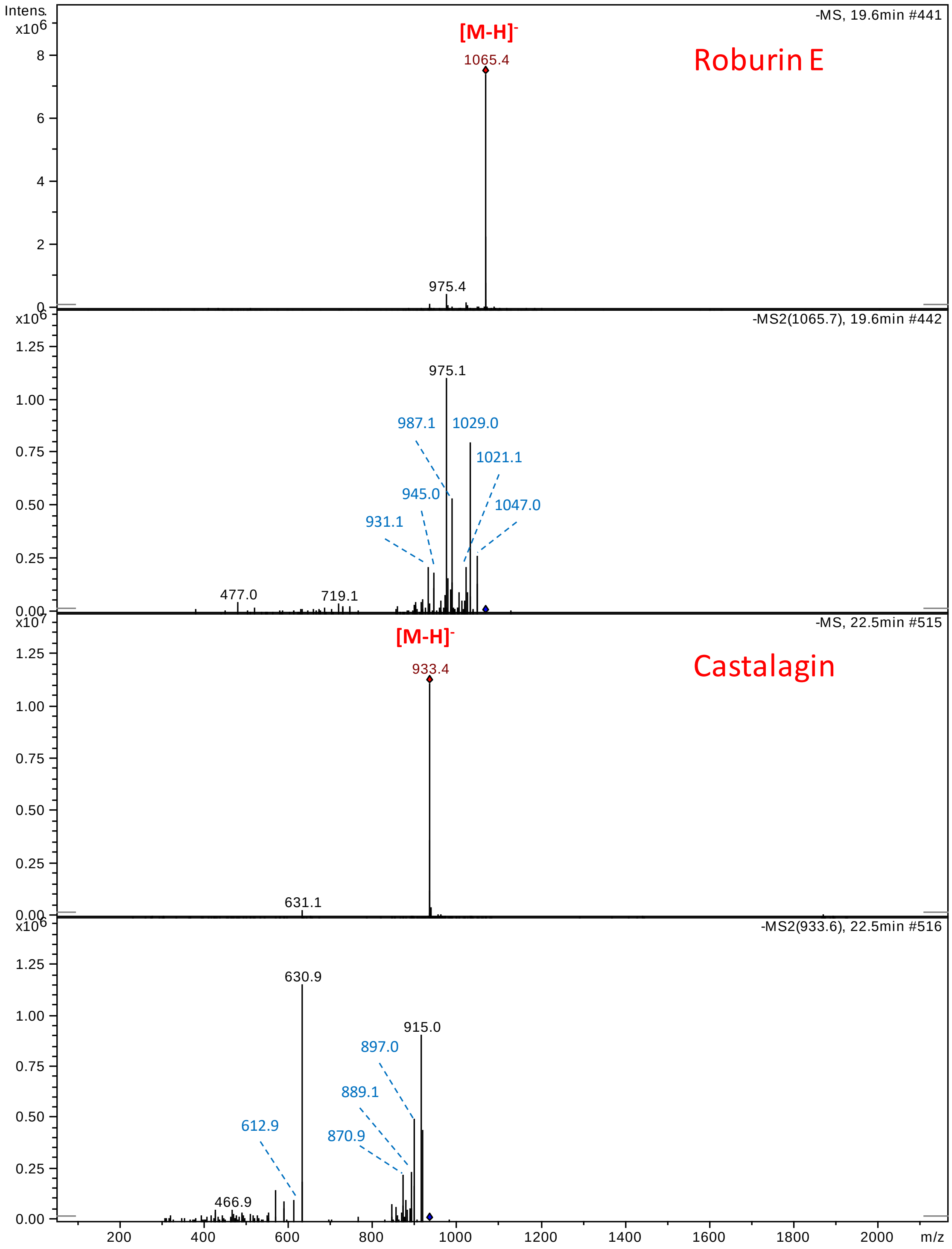

Figure S2 (cont.). MS and MS2 spectra (Ion Trap Mass Detector) of ellagitannin standards used for identification.

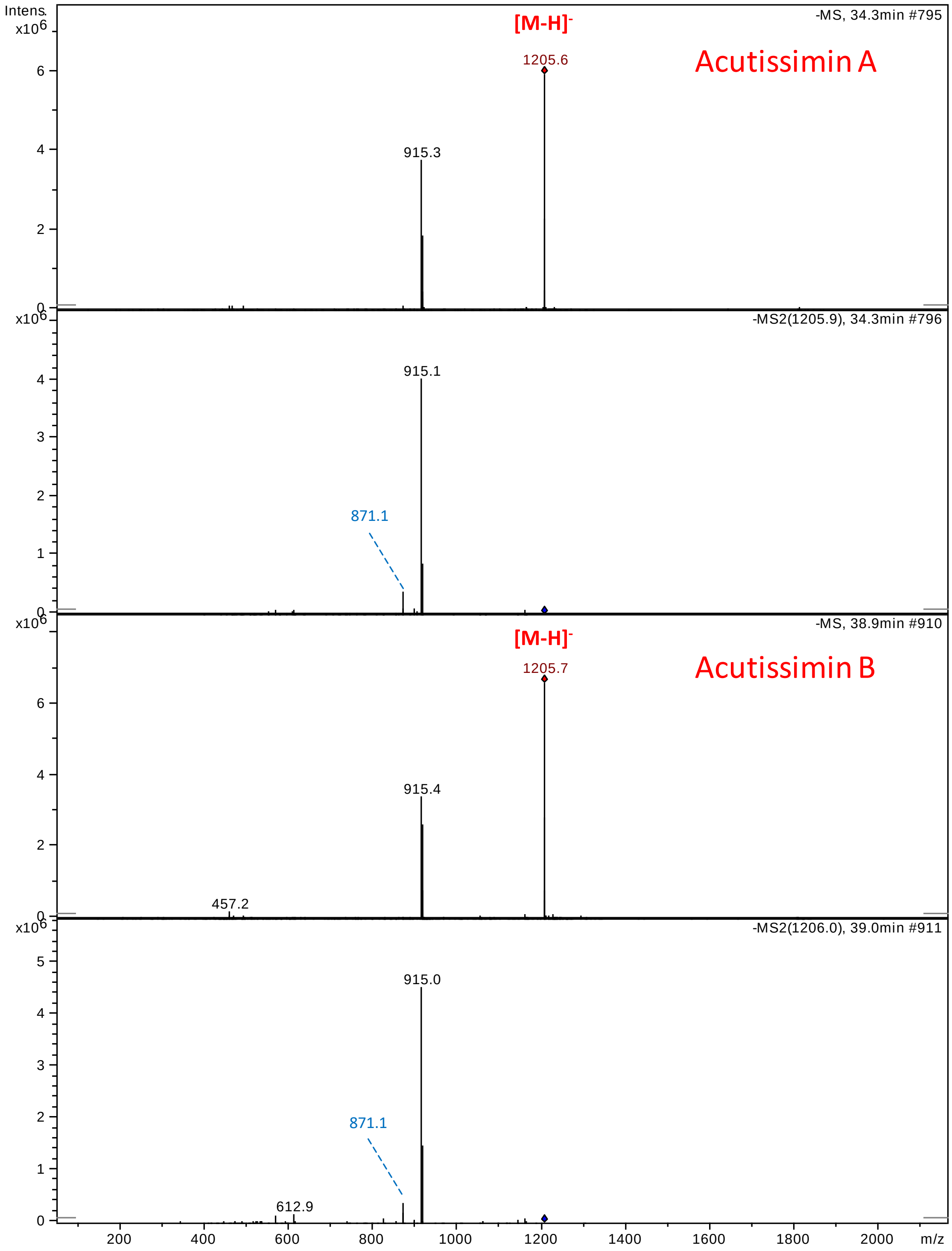

Table S1. Castalagin concentration of the different samples

Medium	Toasting Level	Aging time (months)	French Oak		American Oak	
			New Barrel	1 year barrel	New Barrel	1 year barrel
Decolored white wine	LIGHT	3	2.10 ± 0.75	0.45 ± 0.02	0.36 ± 0.11	0.07 ± 0.02
		6	5.98 ± 0.33	0.12 ± 0.04	1.19 ± 0.15	0.02 ± 0.01
		9	7.03 ± 1.60	3.67 ± 0.77	3.16 ± 0.01	0.44 ± 0.04
		12	12.57 ± 2.75	4.98 ± 1.18	2.43 ± 0.15	0.85 ± 0.07
	MEDIUM	3	0.39 ± 0.02	0.14 ± 0.01	0.10 ± 0.01	0.02 ± 0.01
		6	1.76 ± 0.01	0.06 ± 0.01	0.37 ± 0.01	0.01 ± 0.01
		9	4.18 ± 0.50	1.27 ± 0.08	0.57 ± 0.02	0.22 ± 0.14
		12	3.83 ± 0.21	2.51 ± 0.12	0.54 ± 0.07	0.32 ± 0.01
	HEAVY	3	0.21 ± 0.02	0.09 ± 0.01	0.06 ± 0.01	0.01 ± 0.01
		6	1.38 ± 0.21	0.03 ± 0.01	0.26 ± 0.03	0.01 ± 0.01
		9	1.95 ± 0.18	0.81 ± 0.13	0.34 ± 0.02	0.08 ± 0.01
		12	2.19 ± 0.11	1.31 ± 0.07	0.39 ± 0.01	0.17 ± 0.03
Red wine	LIGHT	3	1.14 ± 0.03	0.02 ± 0.01	0.41 ± 0.01	n.d
		6	3.95 ± 0.71	0.02 ± 0.01	1.18 ± 0.10	n.d
		9	7.07 ± 0.36	0.85 ± 0.05	1.58 ± 0.14	0.12 ± 0.01
		12	6.34 ± 0.45	2.38 ± 0.10	1.26 ± 0.09	0.43 ± 0.05
	MEDIUM	3	0.58 ± 0.13	0.01 ± 0.01	0.27 ± 0.05	n.d
		6	1.70 ± 0.29	0.01 ± 0.01	0.37 ± 0.03	n.d
		9	2.53 ± 0.26	0.39 ± 0.01	0.74 ± 0.01	0.06 ± 0.01
		12	2.77 ± 0.25	1.14 ± 0.19	0.60 ± 0.08	0.15 ± 0.01
	HEAVY	3	0.36 ± 0.01	0.01 ± 0.01	0.17 ± 0.03	n.d
		6	0.89 ± 0.05	0.01 ± 0.01	0.17 ± 0.01	n.d
		9	1.93 ± 0.21	0.28 ± 0.05	0.40 ± 0.09	0.03 ± 0.01
		12	1.89 ± 0.29	0.85 ± 0.04	0.29 ± 0.08	0.07 ± 0.01

Significance level of the different factors (***p***)

Medium	Aging time	Botanical origen	Toasting Level	New/Used
Decolored white wine	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Red wine	< 0.0001	< 0.0001	< 0.0001	< 0.0001

All data are expressed as the average values (mg/L) of two barrels ± standard deviation

Table S2. Vescalagin concentration of the different samples

Medium	Toasting Level	Aging time (months)	French Oak		American Oak	
			New Barrel	1 year barrel	New Barrel	1 year barrel
Decolored white wine	LIGHT	3	0.35 ± 0.14	0.12 ± 0.02	0.04 ± 0.02	n.d
		6	0.80 ± 0.21	0.03 ± 0.01	0.06 ± 0.01	n.d
		9	2.17 ± 0.19	0.81 ± 0.20	0.40 ± 0.06	0.06 ± 0.01
		12	4.61 ± 1.79	0.81 ± 0.12	0.21 ± 0.07	0.08 ± 0.01
	MEDIUM	3	0.06 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	n.d
		6	0.16 ± 0.05	0.02 ± 0.01	0.03 ± 0.01	n.d
		9	0.91 ± 0.07	0.26 ± 0.01	0.07 ± 0.01	0.03 ± 0.02
		12	1.08 ± 0.10	0.32 ± 0.02	0,09 ± 0.02	0.04 ± 0.02
	HEAVY	3	0.04 ± 0.01	0.02 ± 0.01	n.d	n.d
		6	0.16 ± 0.05	0.01 ± 0.01	0.02 ± 0.01	n.d
		9	0.39 ± 0.06	0.18 ± 0.02	0.05 ± 0.01	0.02 ± 0.01
		12	0.39 ± 0.03	0.20 ± 0.02	0.07 ± 0.02	0.02 ± 0.01
Red wine	LIGHT	3	0.40 ± 0.05	n.d	0.05 ± 0.02	n.d
		6	0.53 ± 0.08	0.01 ± 0.01	0.07 ± 0.01	n.d
		9	1.34 ± 0.22	0.14 ± 0.03	0.17 ± 0.04	0.02 ± 0.01
		12	1.32 ± 0.12	0.31 ± 0.01	0.12 ± 0.01	0.05 ± 0.02
	MEDIUM	3	0.12 ± 0.01	n.d	0.04 ± 0.01	n.d
		6	0.19 ± 0.05	n.d	0.05 ± 0.02	n.d
		9	0.40 ± 0.04	0.06 ± 0.02	0.07 ± 0.02	0.01 ± 0.01
		12	0.54 ± 0.03	0.15 ± 0.01	0.06 ± 0.02	n.d
	HEAVY	3	0.08 ± 0.01	n.d	0.04 0.01	n.d
		6	0.10 ± 0.01	n.d	0.02 ± 0.01	n.d
		9	0.40 ± 0.07	0.04 ± 0.01	0.05 ± 0.02	n.d
		12	0.32 ± 0.01	0.13 ± 0.01	0.03 ± 0.01	n.d

Significance level of the different factors (***p***)

Medium	Aging time	Botanical origen	Toasting Level	New/Used
Decolored white wine	0.003	< 0.0001	0.001	0.004
Red wine	0.001	< 0.0001	< 0.0001	< 0.0001

All data are expressed as the average values (mg/L) of two barrels ± standard deviation

Table S3. Grandinin concentration of the different samples

Medium	Toasting Level	Aging time (months)	French Oak		American Oak	
			New Barrel	1 year barrel	New Barrel	1 year barrel
Decolored white wine	LIGHT	3	0.90 ± 0.54	0.21 ± 0.05	0.17 ± 0.02	0.04 ± 0.01
		6	2.73 ± 0.71	0.06 ± 0.02	0.39 ± 0.04	0.01 ± 0.01
		9	3.04 ± 0.59	1.35 ± 0.47	1.13 ± 0.07	0.20 ± 0.01
		12	8.81 ± 4.42	1.54 ± 0.57	0.43 ± 0.18	0.31 ± 0.03
	MEDIUM	3	0.27 ± 0.02	0.10 ± 0.01	0.04 ± 0.01	n.d
		6	0.70 ± 0.12	0.03 ± 0.01	0.15 ± 0.02	n.d
		9	1.48 ± 0.12	0.63 ± 0.02	0.23 ± 0.01	0.10 ± 0.04
		12	2.51 ± 0.32	0.85 ± 0.01	0.33 ± 0.04	0.15 ± 0.01
	HEAVY	3	0.16 ± 0.05	0.07 ± 0.01	0.02 ± 0.01	n.d
		6	1.11 ± 0.18	0.02 ± 0.01	0.12 ± 0.01	n.d
		9	1.07 ± 0.30	0.46 ± 0.02	0.18 ± 0.04	0.05 ± 0.01
		12	1.26 ± 0.12	0.68 ± 0.06	0.22 ± 0.09	0.08 ± 0.01
Red wine	LIGHT	3	0.78 ± 0.02	0.01 ± 0.01	0.11 ± 0.01	n.d
		6	1.21 ± 0.03	0.01 ± 0.01	0.37 ± 0.12	n.d
		9	2.85 ± 0.14	0.40 ± 0.03	0.62 ± 0.23	0.05 ± 0.01
		12	3.99 ± 0.42	0.89 ± 0.01	0.67 ± 0.39	0.16 ± 0.01
	MEDIUM	3	0.19 ± 0.01	0.01 ± 0.01	0.08 ± 0.01	n.d
		6	0.48 ± 0.01	0.01 ± 0.01	0.13 ± 0.02	n.d
		9	0.97 ± 0.03	0.16 ± 0.02	0.25 ± 0.01	0.03 ± 0.01
		12	1.69 ± 0.08	0.46 ± 0.01	0.21 ± 0.03	n.d
	HEAVY	3	0.17 ± 0.01	n.d	0.07 ± 0.01	n.d
		6	0.40 ± 0.01	n.d	0.07 ± 0.01	n.d
		9	1.17 ± 0.29	0.15 ± 0.01	0.17 ± 0.04	0.02 ± 0.01
		12	1.11 ± 0.15	0.48 ± 0.01	0.13 ± 0.03	n.d

Significance level of the different factors (***p***)

Medium	Aging time	Botanical origen	Toasting Level	New/Used
Decolored white wine	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Red wine	< 0.0001	< 0.0001	< 0.0001	< 0.0001

All data are expressed as the average values (mg/L) of two barrels ± standard deviation

Table S4. Roburin E concentration of the different samples

Medium	Toasting Level	Aging time (months)	French Oak		American Oak	
			New Barrel	1 year barrel	New Barrel	1 year barrel
Decolored white wine	LIGHT	3	0.51 ± 0.33	0.18 ± 0.04	0.08 ± 0.01	0.03 ± 0.01
		6	2.09 ± 0.33	0.04 ± 0.01	0.35 ± 0.01	0.01 ± 0.01
		9	3.45 ± 0.13	1.32 ± 0.48	1.15 ± 0.09	0.17 ± 0.01
		12	4.41 ± 2.35	1.49 ± 0.64	0.43 ± 0.15	0.25 ± 0.01
	MEDIUM	3	0.18 ± 0.02	0.08 ± 0.01	0.03 ± 0.01	n.d
		6	0.66 ± 0.14	0.03 ± 0.01	0.12 ± 0.01	n.d
		9	1.65 ± 0.05	0.52 ± 0.01	0.20 ± 0.02	0.08 ± 0.04
		12	1.39 ± 0.43	0.76 ± 0.01	0.17 ± 0.01	0.12 ± 0.01
	HEAVY	3	0.13 ± 0.03	0.06 ± 0.01	0.02 ± 0.01	n.d
		6	0.74 ± 0.04	0.01 ± 0.01	0.09 ± 0.01	n.d
		9	1.03 ± 0.30	0.46 ± 0.01	0.17 ± 0.02	0.03 ± 0.01
		12	0.66 ± 0.08	0.63 ± 0.04	0.13 ± 0.01	0.06 ± 0.01
Red wine	LIGHT	3	0.66 ± 0.05	0.01 ± 0.01	0.09 ± 0.01	n.d
		6	1.20 ± 0.01	0.01 ± 0.01	0.32 ± 0.09	n.d
		9	2.69 ± 0.08	0.35 ± 0.01	0.55 ± 0.20	0.04 ± 0.01
		12	2.07 ± 0.34	0.82 ± 0.05	0.35 ± 0.02	0.16 ± 0.01
	MEDIUM	3	0.17 ± 0.02	n.d	0.07 ± 0.01	n.d
		6	0.40 ± 0.01	n.d	0.11 ± 0.01	n.d
		9	0.90 ± 0.06	0.15 ± 0.01	0.27 ± 0.01	0.02 ± 0.01
		12	0.99 ± 0.24	0.41 ± 0.03	0.16 ± 0.04	n.d
	HEAVY	3	0.14 ± 0.01	n.d	0.04 ± 0.01	n.d
		6	0.34 ± 0.01	n.d	0.05 ± 0.01	n.d
		9	0.78 ± 0.14	0.12 ± 0.01	0.13 ± 0.01	0.01 ± 0.01
		12	0.66 ± 0.14	0.38 ± 0.01	0.12 ± 0.02	n.d

Significance level of the different factors (***p***)				
Medium	Aging time	Botanical origen	Toasting Level	New/Used
Decolored white wine	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Red wine	< 0.0001	< 0.0001	< 0.0001	< 0.0001

All data are expressed as the average values (mg/L) of two barrels ± standard deviation

Table S5. Σ Roburin A, B and C concentration of the different samples

Medium	Toasting Level	Aging time (months)	French Oak		American Oak	
			New Barrel	1 year barrel	New Barrel	1 year barrel
Decolored white wine	LIGHT	3	0.10 ± 0.05	0.02 ± 0.01	0.03 ± 0.01	n.d
		6	0.34 ± 0.18	0.01 ± 0.01	0.07 ± 0.02	n.d
		9	1.18 ± 0.22	0.16 ± 0.08	0.26 ± 0.13	n.d
		12	0.63 ± 0.25	0.19 ± 0.03	0.10 ± 0.01	n.d
	MEDIUM	3	0.05 ± 0.01	0.01 ± 0.01	n.d	n.d
		6	0.27 ± 0.03	0.01 ± 0.01	n.d	n.d
		9	0.59 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	n.d
		12	0.19 ± 0.03	0.10 ± 0.02	0.03 ± 0.01	n.d
	HEAVY	3	0.03 ± 0.01	n.d	n.d	n.d
		6	0.16 ± 0.01	n.d	0.03 ± 0.02	n.d
		9	0.33 ± 0.01	0.04 ± 0.01	0.10 ± 0.02	n.d
		12	0.14 ± 0.04	0.07 ± 0.01	0.06 ± 0.03	n.d
Red wine	LIGHT	3	0.16 ± 0.01	n.d	n.d	n.d
		6	0.35 ± 0.02	n.d	0.08 ± 0.02	n.d
		9	0.91 ± 0.02	0.03 ± 0.01	0.06 ± 0.05	n.d
		12	0.28 ± 0.13	0.12 ± 0.01	0.03 ± 0.01	n.d
	MEDIUM	3	0.00 ± 0.01	n.d	n.d	n.d
		6	0.19 ± 0.02	n.d	0.04 ± 0.01	n.d
		9	0.33 ± 0.03	0.01 ± 0.01	0.02 ± 0.03	n.d
		12	0.14 ± 0.09	0.07 ± 0.01	0.02 ± 0.01	n.d
	HEAVY	3	0.00 ± 0.01	n.d	n.d	n.d
		6	0.15 ± 0.01	n.d	n.d	n.d
		9	0.19 ± 0.01	0.01 ± 0.01	n.d	n.d
		12	0.08 ± 0.02	0.06 ± 0.01	n.d	n.d

Significance level of the different factors (***p***)

Medium	Aging time	Botanical origen	Toasting Level	New/Used
Decolored white wine	< 0.0001	< 0.0001	0.005	< 0.0001
Red wine	0.010	< 0.0001	0.004	< 0.0001

All data are expressed as the average values (mg/L) of two barrels ± standard deviation

Table S6. Roburin D concentration of the different samples

Medium	Toasting Level	Aging time (months)	French Oak		American Oak	
			New Barrel	1 year barrel	New Barrel	1 year barrel
Decolored white wine	LIGHT	3	0.04 ± 0.05	n.d	0.03 ± 0.02	n.d
		6	0.16 ± 0.05	n.d	0.03 ± 0.01	n.d
		9	0.37 ± 0.01	0.08 ± 0.03	0.09 ± 0.04	n.d
		12	0.16 ± 0.06	0.11 ± 0.05	0.02 ± 0.01	n.d
	MEDIUM	3	0.03 ± 0.01	n.d	n.d	n.d
		6	0.14 ± 0.02	n.d	n.d	n.d
		9	0.22 ± 0.03	0.03 ± 0.01	0.03 ± 0.01	n.d
		12	0.04 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	n.d
	HEAVY	3	0.02 ± 0.01	n.d	0.01 ± 0.01	n.d
		6	0.06 ± 0.01	n.d	0.01 ± 0.01	n.d
		9	0.13 ± 0.03	0.02 ± 0.01	0.06 ± 0.04	n.d
		12	0.04 ± 0.02	0.04 ± 0.01	0.05 ± 0.04	n.d
Red wine	LIGHT	3	0.06 ± 0.01	n.d	n.d	n.d
		6	0.13 ± 0.01	n.d	0.04 ± 0.01	n.d
		9	0.28 ± 0.04	0.01 ± 0.01	0.04 ± 0.02	n.d
		12	0.10 ± 0.14	0.06 ± 0.01	0.02 ± 0.01	n.d
	MEDIUM	3	n.d	n.d	n.d	n.d
		6	0.07 ± 0.02	n.d	0.03 ± 0.02	n.d
		9	0.11 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	n.d
		12	0.07 ± 0.01	0.05 ± 0.03	0.02 ± 0.01	n.d
	HEAVY	3	n.d	n.d	n.d	n.d
		6	0.05 ± 0.01	n.d	n.d	n.d
		9	0.07 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	n.d
		12	0.03 ± 0.02	n.d	n.d	n.d

Significance level of the different factors (***p***)

Medium	Aging time	Botanical origen	Toasting Level	New/Used
Decolored white wine	< 0.0001	< 0.0001	0.005	< 0.0001
Red wine	0.005	< 0.0001	< 0.0001	< 0.0001

All data are expressed as the average values (mg/L) of two barrels ± standard deviation

Capítulo III. Impacto de los compuestos volátiles liberados en vino por barricas de roble de diferente origen botánico, tostado y edad en la apreciación sensorial de un panel entrenado

El aroma del vino evoluciona y gana en complejidad durante su periodo de crianza debido a que la madera de roble libera al vino numerosas sustancias volátiles. El aroma es, posiblemente, una de las características más importantes ligadas a la calidad y a las preferencias de los consumidores, ya que el vino constituye un producto que es fundamentalmente consumido por puro placer sensorial.

Los principales compuestos volátiles susceptibles a la migración desde la madera de roble al vino son los isómeros de whiskey-lactona, que aportan aroma a coco, los aldehídos fenólicos tales como la vainillina, principal responsable del aroma a vainilla, y los fenoles volátiles que son los responsables del aroma tostado. En este capítulo se estudian estos compuestos volátiles del vino envejecido en barrica de roble, cómo se ven afectados por el origen botánico del roble, por el nivel de tostado de las duelas y por el uso de la barrica. Así mismo, se completa el estudio con un enfoque sensorial mediante el estudio de correlación entre la concentración de las substancias volátiles, su impacto olfativo teórico y la percepción por parte de un panel entrenado.

La degustación y los análisis de los vinos fueron realizados en el laboratorio del grupo de Tecnología Enológica (TECNENOL); en la sala de cata del Departamento de Bioquímica i Biotecnología de la Universitat Rovira i Virgili (URV, Tarragona) y en el Instituto de la Vid y el Vino de Castilla-La Mancha (IVICAM, Tomelloso). Los resultados del ensayo han sido enviados a la revista *American Journal of Enology and Viticulture* bajo el título

“Influence of the volatile substances released by oak barrels into wine on sensory appreciation by a trained panel”

Influence of the volatile substances released by oak barrels into wine on sensory appreciation by a trained panel

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ABSTRACT

The analytical and sensory analysis of two wines aged in new and 1-year-used barrels of French and American oak with three different levels of toasting was performed in order to determine the relationship between the volatile substances released by oak wood and sensory appreciation by a trained panel. A significant correlation was found between the appreciation of coconut and smoked/toasted notes by the panel and the theoretical sensory impact of β-methyl-γ-octalactone and volatile phenols respectively in two different wines aged in new oak barrels. Vanillin correlated significantly in only one of the wines tasted. The panelists generally preferred wines aged in medium-toasted new barrels for both oak species. In a triangle test, tasters could distinguish between wines aged in new American and French oak barrels when the toasting level was light or medium but not when it was heavy. They were also able to distinguish between wines aged in new and 1-year-used barrels. It can be concluded that the botanical origin of the oak, the toasting level of the staves and the number of times that the barrels have been used previously have a real impact on the volatile composition of the wine and in its sensory impact.

KEYWORDS: Wine; Oak aging; Oak botanical origin; Toasting level; Barrel use; Aroma perception.

INTRODUCTION

Aging in oak barrels is a common practice used to improve the quality of wine because of its beneficial effects on flavor, aromatic complexity, color stabilization and astringency modulation (Fernández de Simón et al. 2014, González-Centeno et al. 2016). Different phenomena take place inside the barrel during aging.

First, oak wood releases several volatile substances into the wine which enrich the aroma, making it more intense and complex (Návojská et al. 2012, Martínez-Pinilla et al. 2013, Pérez-Juan and Luque de Castro 2015). Second, oak wood also releases phenolic compounds, especially ellagitannins, which contribute to texture sensations such as astringency and mouthfeel (Stark et al. 2010, Jourdes et al. 2011, Fernández de Simón et al. 2014). Third, the slow and continuous diffusion of oxygen through the wood pores, the interstices between staves and the bunghole causes different reactions between anthocyanins and proanthocyanidins which stabilize the wine color and smooth wine astringency (Singleton 2000, Pomar and Gonzalez-Mendoza 2001, Del Alamo-Sanza and Nevares 2014). Finally, the repose of the wine inside the barrels allows the natural sedimentation of unstable colloidal matter, leaving the wine limpid and stable (Ribéreau-Gayon 2003, Zamora 2003).

The volatile compounds that oak barrels transfer to the wine may be naturally present in the original oak wood or may derive from other wood compounds during the barrel manufacture (Chira and Teissedre 2013a). Many volatile compounds have been described belonging to very different chemical families (Cadahía et al. 2003), but few of them are significant as regards their impact on the sensory characteristics of wines. From the point of view of their sensory impact, the main volatile substances released by oak wood are furans, phenolic aldehydes and ketones, volatile phenols and β-methyl-γ-octalactones (Garde-Cerdán et al. 2004, Prida and Chatonnet 2010).

The family of furans, which includes mainly furfural, methyfurfural, hydroxymethyfurfural and furfurilic alcohol, are produced from wood polysaccharides by means of the Maillard reaction during the toasting of the staves needed for barrel assembly (Garde-Cerdán et al. 2002, Absalon et al. 2003, Garde-Cerdán and Ancín-Azpilicueta 2006). Furans contribute to smoked and toasted nut notes (Cutzach et al. 1997, Spillman et al. 2004). The family of phenolic aldehydes and ketones, which mainly includes vanillin, syringaldehyde, acetovanillone and propiovanillone, contributes to the characteristic vanilla aroma of aged wines, with vanillin being the major contributor. Phenolic aldehydes and ketones result from the thermal degradation of lignin during barrel toasting. The family of volatile phenols includes ethylphenol, guaiacol, methylguaiacol, ethylguaiacol, vinylguaiacol, eugenol and trans-isoeugenol. Eugenol contributes with an interestingly spicy note of clove, while all the other volatile phenols contribute with smoked/toasted notes. These volatile phenols are also originated from lignin during barrel toasting. Finally, β-methyl-γ-octalactones (also known as whiskey lactones), which are present in the form of two isomers (*cis* and *trans*), are responsible for the coconut flavor. It should be noted that the *cis* isomer has a much lower perception threshold than the *trans* isomer, making its contribution to coconut perception much more important (Günther and Mosandl 1986, Abbott et al. 1995).

The release of volatile compounds from oak barrels into the wine depends on several factors including the botanical and geographic origin of the wood (Chatonnet and Dubourdieu 1998, Mosedale et al. 1999, Garde et al. 2002, Chira and Teissedre 2015), seasoning (Masson et al. 2000, Cadahía et al. 2003), the degree of oak toasting (Hale et al. 1999, Chatonnet et al. 1999, Fernández de Simón et al. 2010a, Fernández de Simón et al 2010b, Chira and Teissedre 2013a,b) and the number of times the barrel was previously used (Towey and Waterhouse 1996). In addition, the quantity of volatile compounds released is influenced by the aging time of the wine in the barrels (García et al. 2012).

Although the genus *Quercus* is composed of more than 150 species of oak, only three are currently used in cooperage (Fernández de Simón et al. 2006, Fernández de Simón et al. 2008), specifically the American white oak (*Quercus alba*) and two European species, the sessile oak (*Quercus petraea*) and the pedunculated oak (*Quercus robur*), usually known as French oaks. It is generally accepted that *Q. petraea* and *Q. alba* provide greater aromatic richness than *Q. robur*, whereas *Q. robur* releases more ellagitannins (Masson et al. 1994, Díaz-Maroto et al. 2008). Since winemakers today tend to be seeking wines with aromatic complexity rather than high structure, Q. *petraea* and *Q. alba* are the main oaks used for wine aging (Vivas, 2002). Much scientific research has therefore been carried out to study the volatile compounds released by the wood of both oaks and also their organoleptic impact (Pérez-Coello et al. 1999, Garde-Cerdán et al. 2002, Fernández de Simón et al. 2003, Guchu et al. 2006, Prida and Puech 2006, Rodríguez-Rodríguez and Gómez-Plaza 2011, Chira and Teissedre 2015). These studies have shown that *Q. alba* contributes mainly with coconut flavor because it releases high amounts of β-methyl-γ-octalactone, especially from its isomer *cis* (more than 90%) (Garde Cerdán et al. 2004, Jarauta et al. 2005, Prida and Puech 2006, Alañón et al. 2012, Schumacher et al. 2013), whereas *Q. petraea* provides a more complex aroma because it releases lower amounts of β-methyl-γ-octalactone with a lower proportion of the *cis* isomer, which produces a better balance with the other flavors (vanillin, eugenol,…) (Sefton et al. 1993, Masson et al. 1995, Cadahía et al. 2003, Chira and Teissedre 2013b, 2015, González-Centeno et al. 2016).

The toasting process necessary for the construction of the barrels is also reported to have a major influence on the substances that oak wood releases into the wine (Jordão et al. 2006). Toasting the inner surface of the barrels severely modifies the chemical composition of the wood, which in turn will influence the composition of the wine during aging. Consequently winemakers consider the level of toasting to be a key point when they choose the barrels for wine aging. In short, furanic compounds, vanilla and volatile phenols increase with higher toasting levels, although the vanilla decreases when the level of toasting is very high (Matejicek et al. 2005, Alañon et al. 2012, Collins et al, 2015).

In contrast, the opposite happens with β-methyl-γ-octalactones and ellagitannins (Cadahía et al. 2001, Cadahía et al. 2003, Fernández de Simón et al. 2010a, Chira and Teissedre 2013b). For that reason, cooperages usually commercialize at least three levels of toasting – light, medium and heavy – so as to offer different sensory profiles. However, medium-toasted is the level most requested by winemakers because it provides a good balance between the different sensory attributes that oak wood can release into wine.

The successive use of the barrels leads to a depletion of these substances. The capacity of the barrels to enrich the wine with aromas and ellagitannins consequently decreases with use (Garde-Cerdán et al. 2002). The number of times a barrel has previously been used is therefore another key point considered by winemakers.

The interest of wineries and cooperages to discover how a barrel's botanical origin, toasting level and age affects the composition and quality of the wine has led to numerous studies being carried out. However, most of these have focused on chemical composition (Vivas and Saint-Cricq de Gaulejac 1998, Cadahía et al. 2003, Jourdes et al. 2011, García et al. 2012, Fernández De Simón et al. 2014, Collins et al. 2015, Navarro et al. 2016), with only a few looking into the sensory aspects (Chira et al. 2015, González-Centeno et al. 2016). It is important to remember that knowledge of a wine's volatile composition alone, without sensory evaluation, is not enough when it comes to predicting the flavor of the whole system as perceived by a trained sensory panel. Indeed, the interactions between odorants and between the odorant and different elements of the wine's non-volatile matrix can affect odorant volatility, flavor release and the overall perceived flavor, intensity and quality (Saénz-Navajas et al. 2010).

In light of the above considerations, the aim of this study is to determine the influence of botanical origins and toasting levels on the volatile composition and sensory appreciation of wines aged in new and used oak barrels and the relationship between the volatile substances released by oak wood and sensory appreciation by a trained panel.

MATERIALS AND METHODS

Chemicals. Enological charcoal and bentonite were purchased from Agrovin (Alcázar de San Juan, Ciudad Real, Spain). Dichloromethane and methanol (LiChrosolv quality) were purchased from Merck (Darmstadt, Germany), pentane from Fluka (Buchs, Switzerland), and ethyl acetate, absolute ethanol and sodium hydroxide from Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, USA). The chemical standards used were purchased from Aldrich and PolyScience (Niles, USA).

The internal standard solution contained 4-methyl-2-pentanol at a concentration of 300 µg/g of dichloromethane.

Barrels. A total of 24 new barrels (225 L) were purchased from the Boteria Torner Cooperage (Sant Cugat Sesgarrigues, Barcelona, Spain). The cooperage selected wood staves with the criterion that they were as homogeneous as possible within each species. Twelve barrels were made with American white oak (*Quercus alba*) and the other 12 with French oak (*Quercus petraea*). Three toasting levels (light, medium and heavy) were used for each type of oak, and a similar toasting level was applied to the barrel heads. Each experimental condition was performed in duplicate, using 2 barrels.

Wines and barrel aging. The study was carried out with two Cabernet Sauvignon red wines and two discolored Macabeo white wines obtained from the experimental cellar of the Faculty of Enology of the Rovira i Virgili University in Constantí (AOC Tarragona, Spain) from the 2012 and 2013 vintages. The red wines (RW) were used without any stabilization treatment, whereas the white wines were drastically decolored (DWW) with enological charcoal (2 g/L) and bentonite (1 g/L) in order to minimize the presence of aromas and phenolic compounds. The aim was to obtain a very neutral medium to facilitate chemical and sensory analysis.

The analytical characteristics of the wines at the start of the experiment were as follows: DWW, 2012 vintage: ethanol content: 13.3%; titratable acidity: 6.2 g of tartaric acid/L; volatile acidity: 0.22 g of acetic acid/L; pH: 3.14; total phenolic index (TPI): 3.5, free SO2, 24 mg/L; total SO2, 85 mg/L. DWW, 2013 vintage: ethanol content: 13.5%; titratable acidity: 6.5 g of tartaric acid/L; volatile acidity: 0.18 g of acetic acid/L; pH: 3.09; total phenolic index (TPI): 2, free SO2, 27 mg/L; total SO2, 78 mg/L.

Red Wine, 2012 vintage: ethanol content: 13.5%; titratable acidity: 5.4 g of tartaric acid/L; volatile acidity: 0.42 g of acetic acid/L; pH: 3.74; total phenolic index (TPI): 56, free SO2, 23 mg/L; total SO2, 60 mg/L. RW, 2013 vintage: ethanol content: 13.4%; titratable acidity: 5.7 g of tartaric acid/L; volatile acidity: 0.38 g of acetic acid/L; pH: 3.62; total phenolic index (TPI): 53, free SO2, 25 mg/L; total SO2, 52 mg/L.

Experimental design. The wines of the 2012 vintage were placed in the new barrels in April 2013 and aged for 12 months. Once the barrels were emptied and cleaned, they were immediately filled with the same wines from the 2013 vintage, which were also aged for 12 months. Samples from all barrels were taken and bottled at 12 months for volatile compound and sensory analysis.

General analysis. Analytical methods recommended by the International Organisation of Vine and Wine (O.I.V. 2001) were used to determine the ethanol content, titratable acidity, volatile acidity, free SO_2 and total SO_2. The total phenolic index (TPI) was determined by measuring absorbance at 280 nm and expressed as absorbance units (Ribéreau-Gayon et al. 2006).

Volatile compound analysis. Volatile compounds released from the oak wood were analyzed by GC/MS using a gas chromatograph TraceGC Ultra coupled to a mass spectrometer DSQ II with electron impact ionization source and quadrupole analyzer equipped with an autosampler TriPlus, all from ThermoQuest.

The conditions of the detector were as follows: electron multiplier voltage, 1592 V; impact energy, 70 eV; ion source temperature 250ºC; and mass scanning range, 39-400 amu. A BP21 column (SGE) 50m-0.32mm internal diameter and 0.25mm thick of free fatty acid phase (FFAP) (polyethylene glycol treated with nitroterephthalic acid) was used.

Volatile compounds were extracted using the method developed by Ibarz et al. (2006). 25 mL of wine were passed through columns filled with 0.2 g of LiChrolut EN (40-120 µm, Merck) using 4-nonanol as internal standard. The columns were then washed with 25 mL of water to remove sugars, acids and other polar substances. The fraction of volatile compounds was eluted with 15 mL of pentane: dichloromethane (2:1 v/v). Extracts were concentrated by distillation in a Vigreux column and then under nitrogen stream to 100 µL and then kept at -20ºC until analysis.

Separated compounds were identified by their mass spectra and chromatographic retention times, using commercial products as a standard. Quantification was performed by analyzing the characteristic *m/z* fragment for each compound using the internal standard method. Results for non-available compounds were expressed in concentration units (µg/L or mg/L) as internal standard equivalents obtained by normalizing the compound peak to that of the internal standard and multiplying by the concentration of the internal standard. Only volatile compounds released by oak wood (furanic compounds, phenolic aldehydes and ketones, β-methyl-γ-octalactones and volatile phenols) are shown because the aim of this study focuses on the impact of oak aging.

Sensory analysis. All sensory analyses were performed in the tasting room of the Faculty of Enology of Tarragona (University Rovira i Virgili), which was designed in accordance with UNE 87004.197 (AENOR, 2010). Tasting was carried out using ISO official tasting glasses (ISO 3591, 1997).

Each sample consisted of 30 ml of wine presented at room temperature (20°C) covered with clear plastic petri dishes to minimize the escape of volatile components and randomly coded with three-digit numbers.

All the samples were tasted by 16 students on the final year of the Bachelor in Enology at the University Rovira i Virgili (four-year degree). This panel was made up of 10 males and 6 females aged between 21 and 25, who had been training together for three years while studying sensory analysis as part of the enology degree.

Six months after bottling, different descriptive sensory analyses of the wines aged in new barrels were carried out in two sessions to prevent fatigue in the panelists. During the first session two trials were carried out to determine the influence of the toasting level of both French and American oaks on the discolored white wines. Similar tasting was performed with red wines in the second session.

For each sample, tasters were required to evaluate the intensity of three olfactory sensory attributes on a scale of 1 to 5 (1 = ‘slight intensity’, 5 = ‘maximum intensity’): coconut, vanilla and smoked/toasted. The intensity level of each descriptor was then expressed as the mean value of all the judges. A sensory training session was held beforehand so that the panelists could agree on the criteria for each of the different sensory attributes. Samples were served randomly to avoid the influence of the tasting order. Dark glasses were used in the case of the samples corresponding to the DWW to avoid the influence of the color. Normal glasses were used in the case of the RW samples because the color was similar in all of them. Tasters were also asked which of the samples they preferred.

Table 1.Triangle tests to compare wines according to botanical origin

Matrix	Test	New French Oak		New American Oak
Discolored white wine	1	Light toasted	vs	Light toasted
	2	Medium toasted	vs	Medium toasted
	3	Heavy Toasted	vs	Heavy Toasted
Red wine	4	Light toasted	vs	Light toasted
	5	Medium toasted	vs	Medium toasted
	6	Heavy Toasted	vs	Heavy Toasted

Two trials of sensory triangle tests were also conducted in accordance with UNE ISO 4120 (2004). The first consisted of six triangle tests to compare the wines aged in new French oak barrels with those aged in new American oak barrels. Table 1 shows the comparisons carried out in each triangle test in this trial. The second trial also consisted of six triangle tests, but in this case the aim was to compare wines aged in new and 1-year-used barrels. This was only performed with the DWW in order to minimize the effect of the vintage.

Table 2 shows the comparisons made in each of the triangle tests in this trial. This experiment was carried out over two consecutive years because the aim was to determine the effect of the depletion caused by using the barrels. Since the difference between the red wines from the 2012 and 2013 vintages was so obvious, a triangle test was of no use. In contrast, the discoloration of the white wines with charcoal and bentonite minimized the differences in color and aroma between the two vintages in such a way that the differences were mainly due to the aromas released by oak aging.

Table 2. Triangle tests to compare wines aged in new and 1-year-used barrels

Oak	Test	New Barrel		1 year used Barrel
French	7	Light toasted	vs	Light toasted
	8	Medium toasted	vs	Medium toasted
	9	Heavy Toasted	vs	Heavy Toasted
American	10	Light toasted	vs	Light toasted
	11	Medium toasted	vs	Medium toasted
	12	Heavy Toasted	vs	Heavy Toasted

All these triangle tests were performed to determine whether tasters were able to recognize the wine that was different. The second objective was to determine which wine was preferred by the panelists who correctly identified the different wines.

Calculation of the theoretical sensory impact of aromas present in wines. The estimation of the theoretical sensory impact of the main aromas in the different samples was calculated by dividing the individual concentration of each aroma by its corresponding threshold. These odor activity values (Escudero et al. 2004) were obtained using the average of the thresholds previously reported by Spillman et al. (2004) for the different volatile compounds in different matrices. Since we have not determined the specific thresholds of the different volatile compounds in the matrix used (DDW and RW), we decided to use the term 'theoretical sensory impact of aroma' (TSIA) instead of odor value.

Specifically, the thresholds considered were for coconut: *cis* β-methyl-γ-octalactones: 83 µg/L; *trans* β-methyl-γ-octalactones: 390 µg/L; for vanilla: vanillin: 65 µg/L; and for smoked/toasted: guayacol and the other volatile phenols: 20 µg/L. Furanic compounds were not considered because their concentration in the samples was much lower than their sensory thresholds. The TSIA of each sensory attribute was considered as the summatory of the total of all the quotients of the individual compound concentrations divided by their corresponding threshold (∑Ai/Ti). It should be noted that these thresholds were determined in different matrices to those of this study, so they should be considered with caution. We decided to use these values in the belief that they would at least be good indicators.

Statistics. All analytical data are expressed as the arithmetic mean ± standard deviation of the samples from two barrels. The analysis of variance (ANOVA) was carried out using XLSTAT software (2015 Edition (mac & win) Cracked FULL). All sensory data are expressed as the arithmetic mean ± standard deviation of the scores of the 16 tasters.

The performance and homogeneity of criteria between the different panelists with respect to different sensorial attributes were analyzed by Tucker's method (Tucker 1966, Tomic et al. 2010) using PanelCheck software (PanelCheck software 2006, Tomic et al. 2010). The Tucker correlation plot is essentially a principal component analysis (PCA) on an unfolded data matrix, and is very useful for visualizing similarities and dissimilarities between the tasters' criteria for the different sensory attributes.

Pearson's correlation of data and principal component analysis were carried out using IBM SPSS Statistics 19 software (International Business Machines S.A., Madrid, Spain). The level of significance of the sensory triangle tests was determined following Jackson's method (Jackson, 2002).

RESULTS

Volatile compounds released into the wine by the different barrels. Table 3 shows a synthesis of the concentration of the main volatile substances released by oak wood from the different barrels into the different wines. It only shows the global furanic compounds, vanillin, *trans* and *cis* β-methyl-γ-octalactones, the *cis*/*trans* ratio and the global volatile phenols so as to simplify the great amount of information. The full data are shown in supplementary tables S1 (furanic compounds), S2 (phenolic aldehydes and ketone compounds), S3 (β-methyl-γ-octalactones) and S4 (volatile phenols).

Table 3.Main volatile compounds of oak wood from the different samples

Matrix	Compounds	Oak origin	Barrel age	Light Toast		Medium Toast		Heavy Toast	
Discolered white wine	**Total Furanic compounds**	French	New Barrel	1231.7 ± 603.7	a B α	3717.2 ± 767.7	b B α	3232.6 ± 370.6	b B α
			1 year barrel	166.1 ± 57.2	a A α	421.4 ± 131.6	b A α	1095.7 ± 157.4	c A α
		American	New Barrel	716.1 ± 196.6	a B α	4554.1 ± 1298.7	b B α	5688.8 ± 1642.3	b B β
			1 year barrel	121.6 ± 15.0	a A α	435.9 ± 77.2	b A α	1713.9 ± 421.1	c A β
	Vanillin	French	New Barrel	547.1 ± 157.5	a B α	621.2 ± 16.7	a B α	497.0 ± 148.9	a B α
			1 year barrel	185.7 ± 54.5	a A α	239.1 ± 76.4	ab A α	281.1 ± 27.3	b A α
		American	New Barrel	390.1 ± 90.4	a B α	1152.6 ± 100.0	b B β	568.2 ± 128.2	a B α
			1 year barrel	147.1 ± 17.4	a A α	361.6 ± 34.0	c A β	288.2 ± 25.5	b A α
	***trans*-β-methyl-γ-octalactone**	French	New Barrel	183 ± 58	b B α	155 ± 10	b B α	58 ± 20	a B α
			1 year barrel	38 ± 8	b A	38 ± 4	b A α	24 ± 5	a A α
		American	New Barrel	366.3 ± 56.9	b B β	282.9 ± 60.5	b B β	79.0 ± 12.3	a B α
			1 year barrel	74.3 ± 7.6	b A β	60.6 ± 7.6	b A β	29.3 ± 3.7	a A α
	***cis*-β-methyl-γ-octalactone**	French	New Barrel	349 ± 231	ab B α	267 ± 53	b B α	103 ± 25	a B α
			1 year barrel	60 ± 32	ab A α	56 ± 10	b A α	32 ± 8	a A α
		American	New Barrel	2507.5 ± 96.9	b B β	2597.4 ± 754.7	b B β	712.6 ± 107.6	a B β
			1 year barrel	472.0 ± 34.0	b A β	495.4 ± 82.4	b A β	224.2 ± 27.9	a A β
	***cis*/*trans* ratio**	French	New Barrel	1.8 ± 0.7	a A α	1.7 ± 0.2	a A α	2.0 ± 1.1	a A α
			1 year barrel	1.5 ± 0.6	a A α	1.5 ± 0.4	a A α	1.4 ± 0.6	a A α
		American	New Barrel	6.9 ± 1.3	a A β	9.0 ± 1.0	a A β	9.0 ± 1.7	a A β
			1 year barrel	6.4 ± 1.1	a A β	8.1 ± 0.5	b B β	7.7 ± 0.3	ab A β
	Volatile Phenols	French	New Barrel	125.3 ± 34.0	a B α	165.3 ± 39.8	a B α	248.4 ± 23.4	a B α
			1 year barrel	25.7 ± 7.5	a A α	35.8 ± 4.3	a A α	61.6 ± 4.9	a A α
		American	New Barrel	173.8 ± 43.7	a B α	246.5 ± 46.5	b B β	313.3 ± 61.8	a B α
			1 year barrel	81.5 ± 13.7	a A β	99.7 ± 16.7	a A β	117.2 ± 27.7	a A β
Red wine	**Total Furanic compounds**	French	New Barrel	253.0 ± 14.6	a B α	1273.4 ± 150.0	b B β	1480.4 ± 307.5	b B α
			1 year barrel	48.3 ± 7.4	a A α	138.4 ± 16.4	b A β	381.3 ± 98.6	c A α
		American	New Barrel	205.1 ± 36.1	a B α	493.1 ± 159.9	b B α	1724.9 ± 100.7	c B α
			1 year barrel	47.9 ± 5.4	a A α	80.8 ± 13.2	b A α	457.0 ± 49.3	c A α
	Vanillin	French	New Barrel	437.7 ± 27.1	a B β	490.6 ± 46.7	a B α	330.2 ± 120.1	a A α
			1 year barrel	122.0 ± 7.1	a A α	206.4 ± 12.5	b A α	197.9 ± 16.9	b A α
		American	New Barrel	324.5 ± 70.6	a B α	502.6 ± 102.8	b B α	361.4 ± 1.9	a B α
			1 year barrel	120.3 ± 13.6	a A α	208.2 ± 10.5	b A α	227.0 ± 17.7	b A α
	***trans*-β-methyl-γ-octalactone**	French	New Barrel	157 ± 19	c B α	82 ± 51	b A α	27 ± 3	a B α
			1 year barrel	34 ± 3	a A α	25 ± 14	a A α	12 ± 2	a A α
		American	New Barrel	365.1 ± 79.6	c B β	195.7 ± 62.3	b B α	79.2 ± 8.9	a B β
			1 year barrel	75.4 ± 15.2	b A β	61.7 ± 22.5	b A β	31.0 ± 2.6	a A β
	***cis*-β-methyl-γ-octalactone**	French	New Barrel	275 ± 179	ab B α	297 ± 1	b B α	136 ± 42	a B α
			1 year barrel	51 ± 25	ab A α	59 ± 4	b A α	33 ± 7	a A α
		American	New Barrel	2887.3 ± 51.2	c B β	1837.5 ± 160.2	b B β	760.6 ± 42.7	a B β
			1 year barrel	466.0 ± 19.8	b A β	459.3 ± 11.0	b A β	234.9 ± 11.0	a A β
	***cis*/*trans* ratio**	French	New Barrel	2 ± 1.4	a A α	4 ± 2.8	ab A α	5 ± 1.0	b B α
			1 year barrel	2 ± 0.9	a A α	3 ± 1.6	a A α	3 ± 0.3	a A α
		American	New Barrel	8 ± 1.9	a A β	10 ± 4.0	a A α	10 ± 1.6	a A β
			1 year barrel	6 ± 1.5	a A β	8 ± 3.0	a A β	8 ± 0.5	a A β
	Volatile Phenols	French	New Barrel	122.5 ± 10.0	b B β	140.3 ± 12.1	a B α	201.8 ± 67.0	a B β
			1 year barrel	98.3 ± 8.7	b A α	55.7 ± 6.6	a A α	117.4 ± 23.7	b A α
		American	New Barrel	172.4 ± 11.1	b B α	191.1 ± 51.1	b B α	257.0 ± 26.0	a B α
			1 year barrel	147.8 ± 11.7	b A α	240.9 ± 81.9	c A β	130.0 ± 10.3	a A α

All data are expressed as the average of 2 barrels ± standard deviation. Different letters indicate statistical differences ($p < 0.05$). Roman lower case letters are used to compare toasting levels. Roman capital letters are used to compare new and 1-year-used barrels. Greek letters are used to compare the botanical origin of the oak barrels

Generally speaking, both matrices (DWW and RW) showed a similar trend. In brief, American oak released significantly higher amounts of β-methyl-γ-octalactones, especially of the *cis* isomer, than the French oak at all toasting levels and in new and 1-year-used barrels. In contrast, no clear differences were found between the two oak species for furanic compounds, vanillin and volatile phenols. The toasting level clearly affected all volatile substances in both species in a similar way β-methyl-γ-octalactones decreased when the toasting level was higher, whereas furanic compounds and volatile phenols did the opposite. Vanillin also increased between light and medium toasting but decreased in the heavy toasting. Finally, the levels of all volatile substances were significantly lower in wines aged in 1-year-used barrels than in new barrels in all the experimental conditions.

Evaluation of panel performance and of the homogeneity of criteria between the different panelists. Figure 1 shows the results of the multivariate analysis following the Tucker method that was applied to verify panel performance and assess the homogeneity of criteria between the different panelists as regards the different sensorial attributes. This analysis produced a total of six Tucker correlation plots. The first three show how the 16 panelist are aligned according to the sensory attribute: coconut (Tucker-1), vanilla (Tucker-2) and smoked/toasted (Tucker-3) for the DWW. The other three are for the same sensory attributes but for the RW.

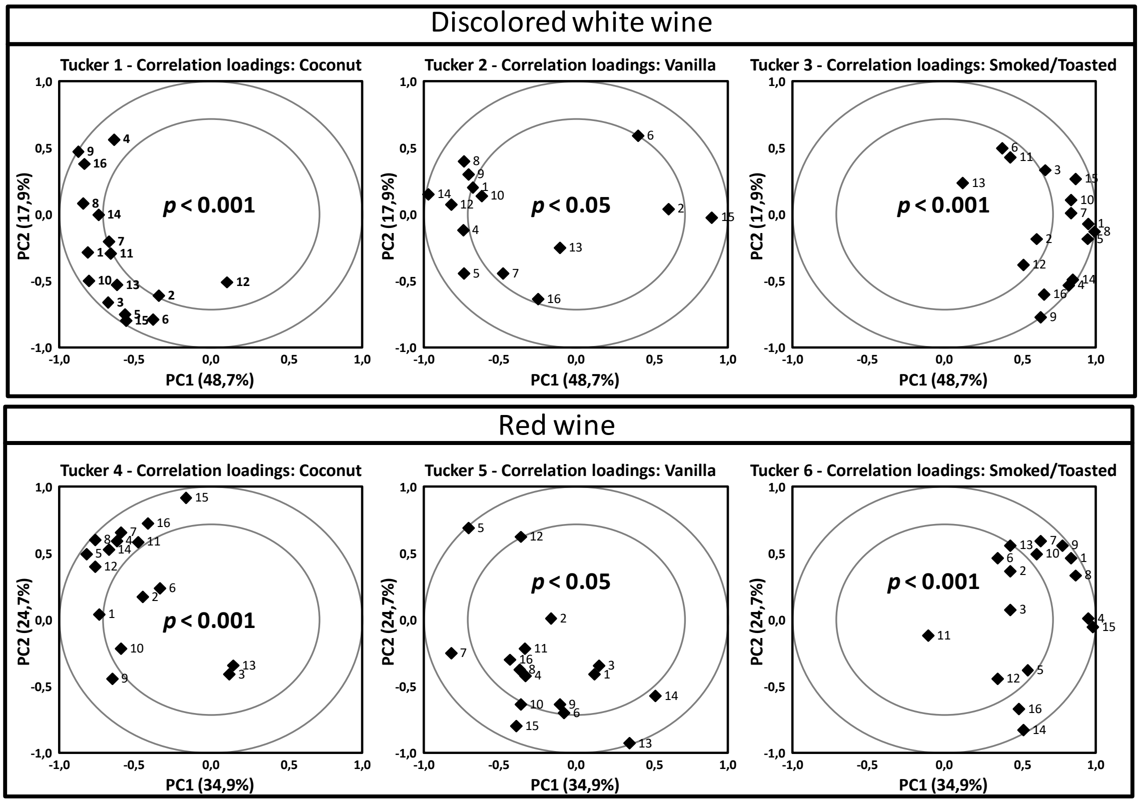
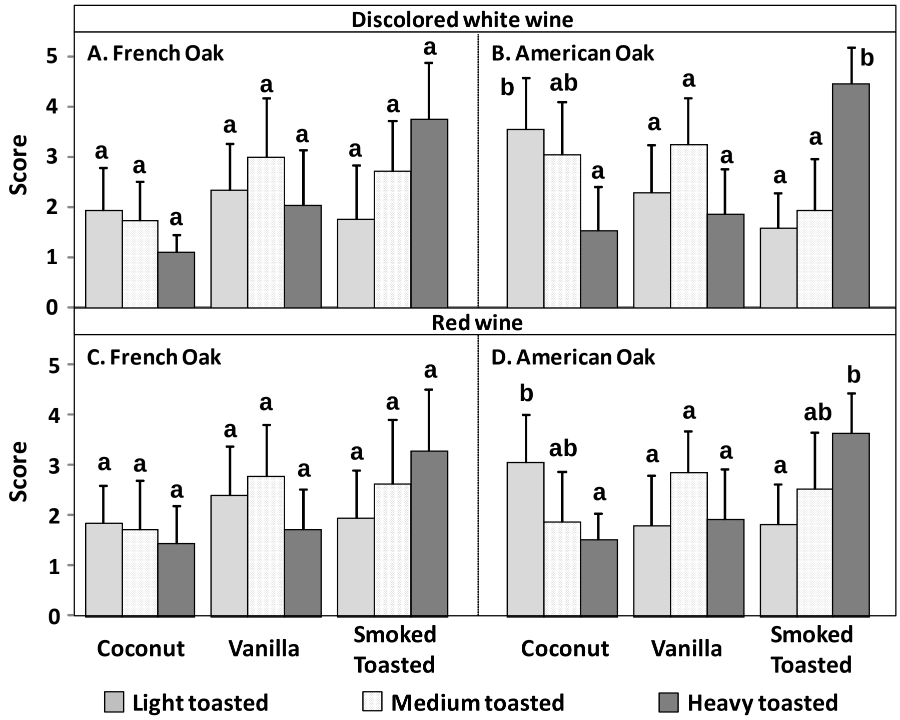


Fig.1. Assessment of the panelists' homogeneity of criteria with respect to the different sensorial attributes

By screening through the plots, it can be seen that the overall performance by the 16 tasters can be considered reasonably good for the three sensory attributes in both matrices since in all cases the results were statistically significant ($p < 0.05$). The different Tucker correlation plots show that the discolored white wine presents a better arrangement with fewer tasters straying from the common alignment than in the case of the red wine. The plots also indicate that tasters are better aligned as regards the coconut and smoked/toasted notes than with the vanilla.

Sensory evaluation of the main oak aromas and preference selection. Figure 2 shows the panelists' sensory evaluation of the main oak aromas of the wines aged in new barrels. No significant differences were found in any of the sensory attributes in the wines aged in French oak. In contrast, significant differences were found in the coconut and smoked/toasted notes of the wines aged in American oak.



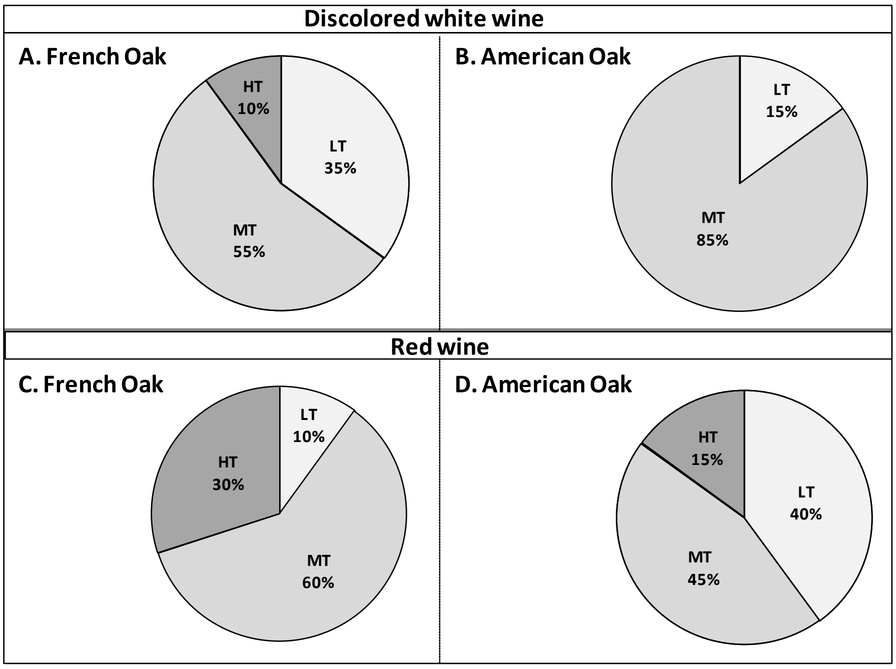
All data are expressed as the average of 16 tasters. Different letters indicate statistical differences ($p < 0.05$).

Fig.2. Sensory evaluation of the main oak aromas

However, despite the lack of significance in some of these data, a number of interesting trends can be pointed out. Briefly, coconut was scored higher in wines aged in American oak than in French oak in both matrices, and the intensity of this sensory attribute decreased in line with the toasting level in a similar way as observed with the levels of β-methyl-γ-octalactones.

Vanilla tended to increase between light and medium-toasted barrels in both oak species and in both matrices, thus showing a similar trend to that determined with the levels of vanilla. Finally, smoked/toasted notes increased with toasting in both oak species and both matrices in a similar way as for volatile phenols.

The panelists were also asked to indicate their preferences during the sensory analysis of the wines aged in the various different new barrels. Figure 3 shows the results obtained. Briefly, in the case of French oak the panelists clearly preferred the wine aged in medium-toasted barrels regardless of the matrix (55 % in DWW and 60 % in RW). However, a different order of preference was obtained for the other two levels of toasting depending on the matrix. In the case of DWW, 35 % of the tasters preferred the wine aged in light-toasted barrels and only 10 % preferred the wine aged in the heavy-toasted. In contrast, in the case of RW 30 % of the tasters preferred the wine aged in heavy-toasted barrels and only 10 % selected the wine aged in light-toasted oak.

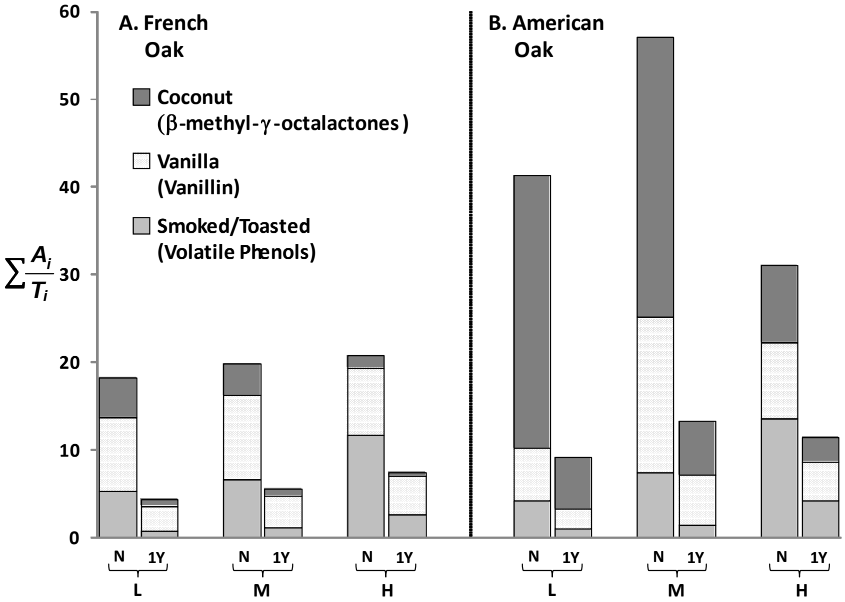


All data are expressed as the average and percentage of 16 tasters. LT: light toasting; MT: medium toasting; HT: heavy toasting

Fig.3. Preferences during the sensory analysis of the wines aged in the different new barrels.

In the case of American oak, important differences were found in the tasters' preferences depending on the matrix. Specifically, in the case of DWW 85 % of the panelists preferred the wine aged in medium-toasted barrels, while the remaining 15 % selected the wine aged in light-toasted oak and none preferred the wine aged in heavy-toasted barrels. In contrast, in the case of RW no clear preferences were obtained between wines aged in medium and light-toasted barrels (45 and 40 % respectively), and 15 % of the tasters preferred the wine aged in heavy-toasted barrels.

Theoretical sensory impact of the main aromas. Figures 4 and 5 show the theoretical sensory impact of the main aromas (TSIA) released by the different barrels in the DWW and RW respectively. The three main olfactory sensory attributes associated with oak aging were selected: coconut, vanilla and smoked/toasted notes.



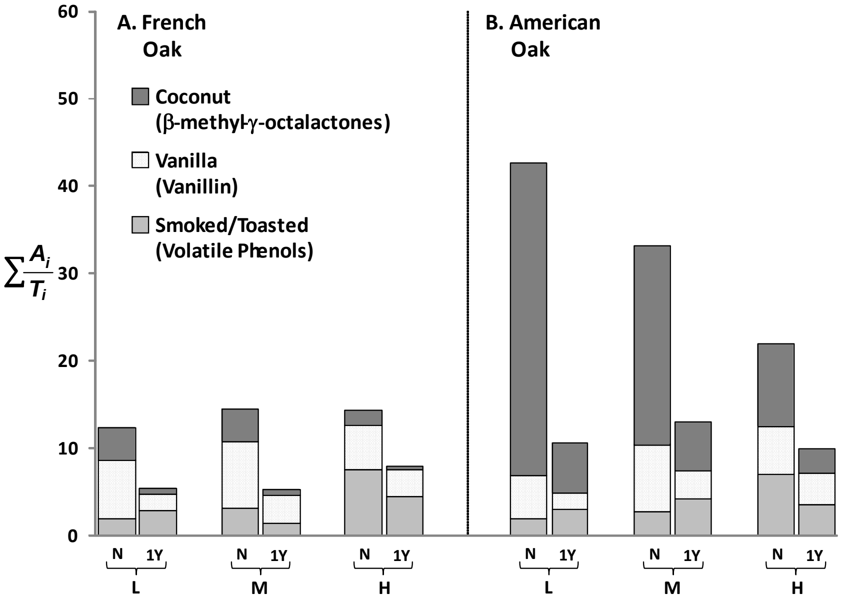
All data are expressed as the average of two barrels. N: new barrels; 1Y: one-year-used barrels; L: light toasting; M: medium toasting; H: heavy toasting. The theoretical sensory impacts of the coconut, vanilla and smoked/toasted aromas (∑Ai/Ti) were obtained by applying the summatory of all the quotients of the corresponding individual volatile compounds concentrations by their corresponding threshold.

Fig.4. Theoretical sensory impact of the main aromas in a discolored white wine

The total TSIA was clearly higher in wines aged in new American oak barrels than in new French oak barrels. This higher total TSIA was mainly due to the coconut attribute resulting from the higher amounts of β-methyl-γ-octalactones released by this specie. This trend was observed in both matrices, DWW and RW. The wines aged in 1-year-used American barrels also showed a higher total TSIA, but the differences were much smaller.

The toasting level also had an important effect on the total TSIA, but the trend was different in the French and American oaks. In French oak the total TSIA increased with the toasting level because the global increase in smoked/toasted and vanilla was higher than the decrease in coconut. In contrast, the decrease in coconut notes in the case of American oak was more drastic than the increase in smoked/toasted and vanilla.

This balance means that the total TSIA tended to decrease when the toasting level was higher in the case of the RW. In the case of the DWW, the total TSIA increased between the light-toasting and medium-toasting levels but clearly decreased in the heavy-toasted level.



All data are expressed as the average of two barrels. N: new barrels; 1Y: one-year-used barrels; L: light toasting; M: medium toasting; H: heavy toasting. The theoretical sensory impacts of the coconut, vanilla and smoked/toasted aromas (∑Ai/Ti) were obtained by applying the summatory of all the quotients of the corresponding individual volatile compounds concentrations by their corresponding threshold.

Fig.5. Theoretical sensory impact of the main aromas in a red wine

Finally, the total TSIA of the new barrels was clearly higher than for the 1-year-used barrels in both wines and at all toasting levels.

Statistical correlation between sensory appreciation of the different descriptors and the corresponding TSIA value. The similarities observed between chemical analysis, TSIA and sensory evaluation by the panel suggested a Pearson's correlation analysis between the sensory evaluation and the TSIA of each attribute. The results obtained (Table 4) confirm the existence of a significant correlation ($p < 0.05$) with high Pearson's correlation coefficients between coconut perception and coconut TSIA in both matrices (0.964 in DWW and 0.903 in RW). The other Pearson's correlation coefficients between coconut perception and the other TSIA attributes were not significant.

Similar results were obtained for smoked/toasted perception, which showed significant correlation, and high Pearson's correlation coefficients with volatile phenol TSIA (0.945 in DWW and 0.959 in RW), but no significant correlations with other TSIA attributes were found. Vanilla sensory perception also showed a significant correlation and high Pearson's correlation coefficient with vanilla TSIA (0.915) in RW, but was not significant in DWW. No other significant correlation was found between vanilla perception and other TSIA attributes.

Table 4. Statistical correlation between sensory appreciation of the different descriptors and the corresponding odor activity value

Matrix	Sensory evaluation	TSIA					
		Coconut		Vanilla		Smoked/Toasted	
		PCC	*p*	PCC	*p*	PCC	*p*
Discolored white wine	Coconut	**0.964**	**0.002**	0.362	0.481	-0.591	0.217
	Vanilla	0.324	0.531	0.686	0.132	-0.294	0.572
	Smoked/Toasted	-0.634	0.177	-0.633	0.177	**0.945**	**0.004**
Red wine	Coconut	**0.903**	**0.014**	-0.097	0.854	-0.415	0.413
	Vanilla	-0.194	0.713	**0.915**	**0.011**	-0.360	0.483
	Smoked/Toasted	-0.627	0.182	-0.699	0.123	**0.959**	**0.003**

All values correspond to Pearson's correlation coefficients. Values in **bold** indicate the existence of a statistically significant correlation ($p < 0.05$). TSIA: theoretical sensory impact of aroma.

Sensory comparisons using triangle tests. Table 5 shows the results of sensory comparisons by triangle test between wines aged in new barrels of French and American oak at the different toasting levels. The panelists were able to significantly distinguish between wines of both matrices, DWW and RW, aged in new French and new American oak barrels in the case of light and medium toasting levels but not in the case of the heavy toasting level.

Specifically, the number of tasters out of a total of sixteen that were capable of distinguishing between DWW aged in new French and American oak barrels was 12 in the case of light-toasted ($p = 0.001$), 10 in the case of medium-toasted ($p = 0.05$) and only 7 in the case of heavy-toasted ($p> 0.05$). Similar results were obtained in the case of RW, since 12 tasters were able to differentiate between wines aged in new French and American oak barrels ($p = 0.001$) in the case of light and medium-toasted barrels, but only 8 ($p > 0.05$) when the barrels were heavy-toasted.

Table 5 also shows the preferences of the tasters who correctly identified the different samples in the different triangle test comparisons. In the case of DWW aged in light-toasted new barrels, the wine aged in French oak was clearly preferred over the wine aged in American oak (8 and 4 respectively). However the preferences tended to become more balanced between both oak species when the toasting level increased.

Specifically, 5 panelists selected the wine aged in French oak and 5 the wine aged in American oak when the barrels were medium-toasted, and, although the panel was not able to significantly distinguish when the barrels were heavy-toasted, 3 of the 7 tasters who correctly identified the different sample preferred the wine aged in French oak and 4 the wine aged in American oak.

Table 5. Sensory comparison by triangle test between wines aged in new barrels of French and American oak at the different toasting levels

Matrix	Triangle test	Positive identifications	*p*	Preferences	
				French	American
Discolored white wine	Light toasted	12/16	0.001	8	4
	Medium toasted	10/16	0.05	5	5
	Heavy toasted	7/16	NS	3	4
Red wine	Light toasted	12/16	0.001	4	8
	Medium toasted	12/16	0.001	8	4
	Heavy toasted	8/16	NS	1	7

In the case of RW, the preferences were not similar. Only 4 of the 12 tasters who correctly identified the different samples preferred wines aged in French oak and 8 preferred wines aged in American oak. However, this preference changed completely when the barrels were medium-toasted, since 8 tasters preferred the wine aged in French oak and only 4 selected the wine aged in American oak. Finally, when the barrels were heavy-toasted, although the panel did not give statistically significant results, the tasters who correctly identified the different sample clearly preferred the wine aged in American oak (7 out of a total of 8).

Table 6 shows the results of the sensory comparison by triangle test between DWW aged in new and 1-year-used barrels of French and American oak at the different toasting levels. Panelists were able to distinguish between wines aged in new barrels and wines aged in 1-year-used barrels in all cases, in different oak species and at different toasting levels.

To be specific, 13 ($p = 0.01$) out of a total of sixteen tasters were capable of distinguishing between new and 1-year-used French oak barrels in the case of light-toasted, 14 ($p = 0.001$) in the case of medium-toasted and 16 ($p < 0.001$) in the case of heavy-toasted. Similar results were found in the case of American oak: 16 ($p < 0.001$) for light-toasted, 13 ($p = 0.01$) for medium-toasted and 16 ($p < 0.001$) for heavy-toasted.

Table 6. Sensory comparison by triangle test between decolored white wines aged in new barrels and 1-year-used barrels of French and American oak at the different toasting levels

Oak	Triangle test	Positive identifications	*p*	Preferences	
				New	1 year
French	Light toasted	13/16	0.01	8	5
	Medium toasted	14/16	0.001	12	2
	Heavy toasted	16/16	< 0.001	3	13
American	Light toasted	16/16	< 0.001	13	3
	Medium toasted	13/16	0.01	10	3
	Heavy toasted	16/16	< 0.001	10	6

Table 5 also shows the preferences of the tasters who correctly identified the different samples in the various triangle test comparisons. In the case of French oak, tasters clearly preferred the wines aged in new barrels when the toasting level was light (8 out of 13) and medium (12 out of 14).

However, this changed completely when the barrels were heavy-toasted, since 13 out of 16 preferred the wine aged in the 1-year-used barrel. As for the American oak, tasters always preferred the wine aged in the new barrels: 13 out of 16 in the case of light-toasted, 10 out of 13 in the case of medium-toasted and 10 out of 16 in the case of heavy-toasted.

DISCUSSION

The aim of the present study was to determine the influence of botanical origins and toasting levels on the volatile composition and sensory appreciation of wines aged in new and 1-year-used oak barrels and also to study the relationship between the volatile substances released by oak wood and sensory appreciation by a trained panel.

In general terms, the results obtained for the main volatile aroma levels released by new and 1-year-used French and American oak barrels with different toasting levels into the wines analyzed match previously reported data (Cadahía et al. 2003, Garde-Cerdán and Ancín-Azpilicueta 2006, Díaz-Maroto et al. 2008, Fernández de Simón et al. 2010a, Chira and Teissedre 2015, González-Centeno et al. 2016).

The panel's performance in the sensory analysis of the three main aromas released by oak wood can be considered quite satisfactory in all cases. However, their performance was better as regards the DWW matrix than the RW matrix, probably because the red wine was rather more complex than the decolored white wine. Indeed, the total amount of variance explained by PC1 and PC2 is 66.6% (PC1: 48.7% and PC2: 17.9%) in the case of the DWW, whereas in the case of the RW they explained only 59.6% (PC1: 34.9% and PC2: 24.7%). It was also observed that the panelists were more aware of and had more homogeneous criteria for the sensory attributes of coconut and smoked/toasted notes than for vanilla. In fact the dispersion of panelists in the Tucker plot for vanilla in the RW matrix is very clear. In any case, the variance analysis was statistically significant ($p < 0.05$) in all cases, which confirms that the panel is suitably trained for the sensory evaluation of these attributes.

The trained panel's sensory evaluation of the main aromas released by oak wood in the different wines generally followed a similar behavior to the calculated TSIA. Thus the correlation analysis between both data was performed with very satisfactory results in the case of coconut and smoked/toasted notes, since in all cases the Pearson's correlation coefficients were high and significant. However, in the case of vanillin the results were rather surprising. The Tucker plots for vanilla indicated that the panel performed better with the DWW than the RW matrix.

However, the Pearson's correlation coefficient between the sensory score of the panel and the TSIA was only significant in the case of the red wine. In the case of DWW, the Pearson's correlation coefficient for vanilla was relatively high (0.686) but did not reach a significant level ($p = 0.132$). Another interesting fact arising from these correlation analyses is that vanilla and coconut showed relatively high negative Pearson's correlation coefficients (lower than –0.6 in all cases) with the sensory perception of smoked/toasted notes, which suggests a possible antagonism between "sweetish" and "smoky" aromas.

Regarding preferences, the panelists almost always preferred the medium-toasted barrels independently of the matrix (DWW or RW) or the oak species, probably because this toasting level gives the best balance of the three main aromas. However, the light toasting level was also preferred by a substantial proportion of the tasters in the case of the DWW aged in French oak and the RW aged in American oak. The heavy-toasted level was also relatively preferred in the case of the red wine aged in French oak. These data explain why medium-toasted barrels are the most frequently used for wine aging in wineries.

The triangle test comparisons between wines aged in new French and American oak barrels at different levels of toasting produce some very interesting results. Tasters were able to distinguish significantly between French and American oak when the barrels were light or medium-toasted.

In contrast, when the barrels were heavy-toasted the panel could not significantly identify the differences. This suggests that the excess of toasted notes masks the other aromas, making it impossible to distinguish between oak species.

The panel could also significantly distinguish between DWW wines aged in new and 1-year-used barrels of both oak species at all toasting levels. This was to be expected, since the concentrations and TSIAs of the different aromas were significant higher in the new barrels than in the 1-year-used barrels. However, the panelists' preferences produced more interesting data.

In the case of American oak barrels, the tasters mostly preferred the DDW aged in new barrels for the three toasting levels. However, in the case of French oak, the tasters preferred the DDW aged in new barrels only when the toasting level was light or medium but not when it was heavy. This suggests that the excess of smoked/toasted notes caused by heavy toasting is considered a negative characteristic by tasters, since they prefer wines aged in the used barrel in which these aromas have a noticeably lower impact.

CONCLUSIONS

It has been possible to establish a significant correlation between a trained panel's sensory appreciations of coconut and smoked/toasted notes and the theoretical sensory impact of β-methyl-γ-octalactone and volatile phenols respectively in two different wines aged in new oak barrels. The correlation between the sensory appreciation of vanilla and the theoretical sensory impact of vanillin was significant in the case of the red wine but not in the discolored white wine. In general, the panelists clearly preferred wines aged in medium-toasted new barrels for both oak species. However a substantial number of tasters selected discolored white wine aged in light-toasted French oak barrels and the red wine aged in light-toasted American oak barrels. The wines aged in heavy-toasted new barrels tended to be the least appreciated, although a non-negligible proportion of the panelists selected this toasting level in the case of French oak. In a triangle test they were able to distinguish between wines aged in new American and French oak barrels when the toasting level was light or medium but not when it was heavy. The panelists generally selected wines aged in French oak, but not in all the cases. They were also able to distinguish between wines aged in new and 1-year-used barrels and preferred wines aged in new barrels, the only exception being the discolored white wine aged in heavy-toasted French oak barrels.

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SUPPLEMENTARY DATA

Supplementary data associated with this article will be found, in the online version: tables S1 (furanic compounds), S2 (phenolic aldehydes and ketone compounds), S3 (β-methyl-γ-octalactones) and S4 (volatile phenols).

NOTE: Supplementary tables are also included just at the end of this article.

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Table S1. Furanic compounds concentration of the different samples

Matrix	**Furanic compounds**	**Oak origin**	**Barrel age**	**Light Toast**				**Medium Toast**				**Heavy Toast**			
Discolered white wine	Furfural	French	New Barrel	137.9 ± 8.3	a	B	β	943.3 ± 343.6	b	B	α	1045.7 ± 147.7	b	B	α
			1 year barrel	46.8 ± 11.9	a	A	α	114.3 ± 38.4	b	A	α	418.1 ± 76.8	c	A	α
		American	New Barrel	94.1 ± 1.2	a	B	α	502.9 ± 228.5	b	B	α	2589.5 ± 934.3	c	B	β
			1 year barrel	40.1 ± 3.6	a	A	α	122.9 ± 35.7	b	A	α	635.7 ± 293.9	c	A	α
	5-methylfurfural	French	New Barrel	205.2 ± 165.1	a	A	α	968.3 ± 48.8	c	B	α	736.4 ± 78.6	b	B	α
			1 year barrel	29.5 ± 21.4	a	A	α	174.5 ± 42.1	b	A	α	411.5 ± 45.0	c	A	α
		American	New Barrel	118.2 ± 32.9	a	B	α	977.4 ± 235.5	b	B	α	1386.9 ± 206.0	b	B	β
			1 year barrel	13.2 ± 2.5	a	A	α	149.9 ± 20.7	b	A	α	636.8 ± 82.3	c	A	β
	5-hydroxymethylfurfural	French	New Barrel	554 ± 149	a	B	α	1090 ± 80	b	B	α	898 ± 142	b	B	α
			1 year barrel	50 ± 12	a	A	α	56 ± 38	a	A	α	141 ± 16	b	A	α
		American	New Barrel	331.9 ± 114.0	a	B	α	2149.3 ± 732.5	b	B	β	1137.7 ± 310.8	b	B	α
			1 year barrel	34.8 ± 3.2	a	A	α	86.8 ± 14.2	b	A	α	200.7 ± 21.0	c	A	β
	Furfuryl alcohol	French	New Barrel	334 ± 282	a	B	α	716 ± 295	a	B	α	552 ± 2	a	B	α
			1 year barrel	40 ± 12	a	A	α	77 ± 14	b	A	α	125 ± 20	c	A	α
		American	New Barrel	171.9 ± 48.5	a	B	α	924.5 ± 102.2	c	B	α	574.7 ± 191.1	b	B	α
			1 year barrel	33.6 ± 5.6	a	A	α	76.3 ± 6.5	b	A	α	240.7 ± 24.0	c	A	β
	Total	French	New Barrel	1231.7 ± 603.7	a	B	α	3717.2 ± 767.7	b	B	α	3232.6 ± 370.6	b	B	α
			1 year barrel	166.1 ± 57.2	a	A	α	421.4 ± 131.6	b	A	α	1095.7 ± 157.4	c	A	α
		American	New Barrel	716.1 ± 196.6	a	B	α	4554.1 ± 1298.7	b	B	α	5688.8 ± 1642.3	b	B	β
			1 year barrel	121.6 ± 15.0	a	A	α	435.9 ± 77.2	b	A	α	1713.9 ± 421.1	c	A	β
Red wine	Furfural	French	New Barrel	62.3 ± 2.8	a	B	α	435.4 ± 90.2	b	B	β	605.0 ± 85.0	b	B	α
			1 year barrel	21.3 ± 3.2	a	A	α	44.5 ± 5.1	b	A	β	133.8 ± 43.7	c	A	α
		American	New Barrel	55.7 ± 8.3	a	B	α	88.2 ± 56.7	a	B	α	738.2 ± 35.2	b	B	β
			1 year barrel	25.5 ± 2.7	a	A	α	26.1 ± 1.8	a	A	α	146.4 ± 13.5	b	A	α
	5-methylfurfural	French	New Barrel	57.8 ± 2.7	a	B	β	538.3 ± 33.6	b	B	β	557.1 ± 99.7	b	B	α
			1 year barrel	5.2 ± 1.4	a	A	α	52.3 ± 3.9	b	A	β	169.0 ± 42.5	c	A	α
		American	New Barrel	38.9 ± 15.5	a	B	α	201.0 ± 90.2	b	B	α	715.6 ± 54.2	c	B	β
			1 year barrel	4.8 ± 0.7	a	A	α	17.7 ± 6.9	b	A	α	217.6 ± 24.6	c	A	α
	5-hydroxymethylfurfural	French	New Barrel	80 ± 4	a	B	β	121 ± 14	b	B	β	137 ± 48	b	B	α
			1 year barrel	9 ± 1	a	A	α	10 ± 3	a	A	α	25 ± 3	b	A	α
		American	New Barrel	69.9 ± 4.5	a	B	α	71.8 ± 3.4	a	B	α	110.9 ± 9.2	b	B	α
			1 year barrel	8.2 ± 0.8	a	A	α	12.2 ± 0.6	b	A	α	23.3 ± 2.0	c	A	α
	Furfuryl alcohol	French	New Barrel	53 ± 5	a	B	α	179 ± 12	b	B	β	181 ± 75	b	B	α
			1 year barrel	13 ± 2	a	A	β	32 ± 5	b	A	α	53 ± 9	c	A	α
		American	New Barrel	40.5 ± 7.8	a	B	α	132.1 ± 9.6	b	B	α	160.2 ± 2.1	c	B	α
			1 year barrel	9.5 ± 1.2	a	A	α	24.8 ± 3.9	b	A	α	69.6 ± 9.3	c	A	α
	Total	French	New Barrel	253 ± 14.6	a	B	α	1273 ± 150.0	b	B	β	1480 ± 307.5	b	B	α
			1 year barrel	48 ± 7.4	a	A	α	138 ± 16.4	b	A	β	381 ± 98.6	c	A	α
		American	New Barrel	205 ± 36.1	a	B	α	493 ± 159.9	b	B	α	1725 ± 100.7	c	B	α
			1 year barrel	48 ± 5.4	a	A	α	81 ± 13.2	b	A	α	457 ± 49.3	c	A	α

All data are expressed as the average of 2 barrels ± standard deviation. Different letters indicate statistical differences ($p < 0.05$). Roman lower case letters are used to compare toasting levels. Roman capital letters are used to compare new and 1-year-used barrels. Greek letters are used to compare the botanical origin of the oak barrels.

Table S2. Phenolic aldehydes and ketones compounds concentration of the different samples

Matrix	Phenolic aldehydes and ketones	Oak origin	Barrel age	Light Toast				Medium Toast				Heavy Toast			
Discolered white wine	Vanillin	French	New Barrel	547.1 ± 157.5	a	B	α	621.2 ± 16.7	a	B	α	497.0 ± 148.9	a	B	α
			1 year barrel	185.7 ± 54.5	a	A	α	239.1 ± 76.4	ab	A	α	281.1 ± 27.3	b	A	α
		American	New Barrel	390.1 ± 90.4	a	B	α	1152.6 ± 100.0	b	B	β	568.2 ± 128.2	a	B	α
			1 year barrel	147.1 ± 17.4	a	A	α	361.6 ± 34.0	c	A	β	288.2 ± 25.5	b	A	α
	Syringaldehyde	French	New Barrel	408.2 ± 130.2	a	A	α	739.1 ± 14.9	b	A	α	617.3 ± 237.5	ab	A	α
			1 year barrel	453 ± 167	a	A	α	650 ± 380	ab	A	α	1078 ± 139	b	B	α
		American	New Barrel	351.1 ± 53.6	a	A	α	1280.7 ± 60.4	c	B	β	885.2 ± 180.5	b	A	α
			1 year barrel	404 ± 19	a	A	α	1029 ± 124	b	A	α	1458 ± 185	c	B	β
	Acetovanillone	French	New Barrel	22.2 ± 11.4	a	B	α	53.5 ± 4.6	b	B	α	83.3 ± 18.5	c	B	α
			1 year barrel	4.2 ± 1.4	a	A	α	7.8 ± 2.6	a	A	α	23.0 ± 1.4	b	A	α
		American	New Barrel	21.6 ± 3.9	a	B	α	74.0 ± 2.8	b	B	β	104.8 ± 8.8	c	B	α
			1 year barrel	4.0 ± 0.2	a	A	α	9.5 ± 1.0	b	A	α	28.5 ± 3.9	c	A	β
	Propiovanillone	French	New Barrel	58.4 ± 55.5	a	A	α	294.3 ± 43.8	b	A	α	425.8 ± 127.1	b	B	α
			1 year barrel	3.6 ± 2.6	a	A	α	26.9 ± 8.4	b	A	α	98.6 ± 7.7	c	A	α
		American	New Barrel	28.7 ± 13.8	a	B	α	355.3 ± 41	b	A	α	545.8 ± 60.4	c	B	α
			1 year barrel	1.7 ± 0.5	a	A	α	28.7 ± 3.2	b	A	α	153.3 ± 20.1	c	A	β
	Total	French	New Barrel	1035.9 ± 354.5	a	A	α	1708.0 ± 80.0	b	B	α	1623.5 ± 531.9	ab	A	α
			1 year barrel	646.8 ± 225.9	a	A	α	924.1 ± 467.3	ab	A	α	1481.1 ± 175.4	b	A	α
		American	New Barrel	791.4 ± 161.8	a	B	α	2862.6 ± 204.4	c	B	β	2104.0 ± 377.9	b	A	α
			1 year barrel	556.4 ± 37.2	a	A	α	1429.1 ± 161.8	b	A	α	1928.6 ± 234.6	c	A	β
Red wine	Vanillin	French	New Barrel	438 ± 27	a	B	β	491 ± 47	a	B	α	330 ± 120	a	A	α
			1 year barrel	122 ± 7	a	A	α	206 ± 12	b	A	α	198 ± 17	b	A	α
		American	New Barrel	324 ± 71	a	B	α	503 ± 103	b	B	α	361 ± 2	a	B	α
			1 year barrel	120 ± 14	a	A	α	208 ± 11	b	A	α	227 ± 18	b	A	α
	Syringaldehyde	French	New Barrel	247 ± 12	a	A	α	365 ± 84	b	A	α	318 ± 134	ab	A	α
			1 year barrel	232 ± 11	a	A	α	452 ± 93	b	A	α	646 ± 74	c	B	α
		American	New Barrel	205 ± 41	a	A	α	391 ± 106	b	A	α	407 ± 31	b	A	α
			1 year barrel	249 ± 26	a	A	α	575 ± 28	b	B	β	915 ± 66	c	B	β
	Acetovanillone	French	New Barrel	152.0 ± 8.0	a	B	β	149.5 ± 25.5	a	B	α	155.5 ± 48.4	a	B	α
			1 year barrel	44.6 ± 1.1	a	A	α	41.8 ± 3.0	a	A	α	62.0 ± 4.3	b	A	α
		American	New Barrel	138.0 ± 0.7	a	B	α	120.7 ± 21.9	a	B	α	166.1 ± 4.2	b	B	α
			1 year barrel	42.3 ± 1.6	a	A	α	50.6 ± 1.0	b	A	β	55.5 ± 4.3	b	A	α
	Propiovanillone	French	New Barrel	36.0 ± 4.7	a	B	α	295.0 ± 34.5	b	B	β	353.8 ± 115.0	b	B	α
			1 year barrel	3.0 ± 0.2	a	A	α	31.6 ± 1.7	b	A	β	97.3 ± 12.8	c	A	α
		American	New Barrel	26.4 ± 9.4	a	B	α	156.3 ± 10.0	b	B	α	424.7 ± 23.6	c	B	α
			1 year barrel	2.5 ± 0.3	a	A	α	17.4 ± 4.6	b	A	α	92.9 ± 10.0	c	A	α
	Total	French	New Barrel	872.3 ± 52.1	a	B	β	1300.5 ± 191.0	b	B	α	1157.6 ± 417.2	ab	A	α
			1 year barrel	401.5 ± 19.7	a	A	α	731.9 ± 110.5	b	A	α	1003.3 ± 107.8	c	A	α
		American	New Barrel	693.6 ± 121.7	a	B	α	1170.5 ± 240.9	b	B	α	1359.1 ± 60.5	b	A	α
			1 year barrel	413.6 ± 41.4	a	A	α	851.5 ± 44.0	b	A	α	1290.2 ± 97.7	c	A	β

All data are expressed as the average of 2 barrels ± standard deviation. Different letters indicate statistical differences ($p < 0.05$). Roman lower case letters are used to compare toasting levels. Roman capital letters are used to compare new and 1-year-used barrels. Greek letters are used to compare the botanical origin of the oak barrels.

Table S3. β-methyl-γ-octalactones concentration of the different samples

Matrix	β-methyl-γ-octalactones	Oak origin	Barrel age	Light Toast		Medium Toast		Heavy Toast	
Discolered white wine	*trans*-*β*-methyl-*γ*-octalactone	French	New Barrel	183.5 ± 58.3	b B α	155.4 ± 10.1	b B α	57.6 ± 19.7	a B α
			1 year barrel	37.8 ± 7.5	b A α	37.7 ± 4.3	b A α	24.4 ± 5.4	a A α
		American	New Barrel	366.3 ± 56.9	b B β	282.9 ± 60.5	b B β	79.0 ± 12.3	a B α
			1 year barrel	74.3 ± 7.6	b A β	60.6 ± 7.6	b A β	29.3 ± 3.7	a A α
	cis-*β*-methyl-*γ*-octalactone	French	New Barrel	348.7 ± 230.6	ab B α	266.7 ± 53.2	b B α	103.1 ± 24.6	a B α
			1 year barrel	59.6 ± 32.0	ab A α	56.2 ± 10.1	b A α	32.3 ± 7.9	a A α
		American	New Barrel	2507.5 ± 96.9	b B β	2597.4 ± 754.7	b B β	712.6 ± 107.6	a B β
			1 year barrel	472.0 ± 34.0	b A β	495.4 ± 82.4	b A β	224.2 ± 27.9	a A β
	cis/trans ratio	French	New Barrel	1.8 ± 0.7	a A α	1.7 ± 0.2	a A α	2.0 ± 1.1	a A α
			1 year barrel	1.5 ± 0.6	a A α	1.5 ± 0.4	a A α	1.4 ± 0.6	a A α
		American	New Barrel	6.9 ± 1.3	a A β	9.0 ± 1.0	a A β	9.0 ± 1.7	a A β
			1 year barrel	6.4 ± 1.1	a A β	8.1 ± 0.5	b B β	7.7 ± 0.3	ab A β
	Total	French	New Barrel	532.2 ± 288.9	b B α	422.1 ± 63.3	b B α	160.7 ± 44.4	a B α
			1 year barrel	97.4 ± 39.5	ab A α	93.9 ± 14.4	b A α	56.7 ± 13.3	a A α
		American	New Barrel	2873.8 ± 153.9	b B β	2880.3 ± 815.2	b B β	791.5 ± 119.9	a B β
			1 year barrel	546.3 ± 41.6	b A β	556.0 ± 90.0	b A β	253.5 ± 31.6	a A β
Red wine	*trans*-*β*-methyl-*γ*-octalactone	French	New Barrel	157.4 ± 19.0	c B α	82.3 ± 51.1	b A α	27.3 ± 2.8	a B α
			1 year barrel	34.0 ± 3.4	a A α	25.2 ± 13.9	a A α	12.4 ± 1.8	a A α
		American	New Barrel	365.1 ± 79.6	c B β	195.7 ± 62.3	b B α	79.2 ± 8.9	a B β
			1 year barrel	75.4 ± 15.2	b A β	61.7 ± 22.5	b A β	31.0 ± 2.6	a A β
	cis-*β*-methyl-*γ*-octalactone	French	New Barrel	274.8 ± 178.7	ab B α	296.6 ± 1.1	b B α	135.6 ± 42.0	a B α
			1 year barrel	51.4 ± 24.9	ab A α	58.6 ± 3.8	b A α	33.1 ± 7.1	a A α
		American	New Barrel	2887.3 ± 51.2	c B β	1837.5 ± 160.2	b B β	760.6 ± 42.7	a B β
			1 year barrel	466.0 ± 19.8	b A β	459.3 ± 11.0	b A β	234.9 ± 11.0	a A β
	cis/trans ratio	French	New Barrel	1.8 ± 1.4	a A α	4.5 ± 2.8	ab A α	4.9 ± 1.0	b B α
			1 year barrel	1.6 ± 0.9	a A α	3.0 ± 1.6	a A α	2.7 ± 0.3	a A α
		American	New Barrel	8.1 ± 1.9	a A β	10.0 ± 4.0	a A α	9.7 ± 1.6	a A β
			1 year barrel	6.4 ± 1.5	a A β	8.3 ± 3.0	a A β	7.6 ± 0.5	a A β
	Total	French	New Barrel	432.2 ± 197.7	b B α	378.9 ± 52.2	b B α	163.0 ± 44.7	a B α
			1 year barrel	85.3 ± 28.3	b A α	83.8 ± 17.7	b A α	45.5 ± 8.9	a A α
		American	New Barrel	3252.4 ± 130.8	c B β	2033.2 ± 222.4	b B β	839.8 ± 51.5	a B β
			1 year barrel	541.4 ± 35.0	b A β	521.0 ± 33.5	b A β	265.9 ± 13.5	a A β

All data are expressed as the average of 2 barrels ± standard deviation. Different letters indicate statistical differences ($p < 0.05$). Roman lower case letters are used to compare toasting levels. Roman capital letters are used to compare new and 1-year-used barrels. Greek letters are used to compare the botanical origin of the oak barrels.

Table S4. Volatile phenols concentration of the different samples

Matrix	Volatile phenols	Oak origin	Barrel age	Light Toast	Medium Toast	Heavy Toast
Discolered white wine	4-ethylphenol	French	New Barrel	2.6 ± 0.8 a B α	2.0 ± 0.5 a A α	1.6 ± 0.2 a B α
			1 year barrel	1.0 ± 0.2 a A α	1.0 ± 0.5 a A α	0.9 ± 0.1 a A α
		American	New Barrel	3.0 ± 0.6 b B α	3.1 ± 0.6 b B α	1.3 ± 0.2 a A α
			1 year barrel	2.0 ± 0.2 b A β	1.6 ± 0.5 ab A α	1.0 ± 0.1 a A α
	Guaiacol	French	New Barrel	3.7 ± 2.5 a A α	14.5 ± 1.5 b B α	46.0 ± 2.5 c B α
			1 year barrel	1.6 ± 0.2 a A α	2.9 ± 0.5 b A α	9.2 ± 0.6 c A α
		American	New Barrel	9.7 ± 2.1 a B β	19.2 ± 0.1 b B β	62.8 ± 10.8 c B β
			1 year barrel	3.7 ± 0.3 a A β	5.7 ± 0.4 b A β	18.2 ± 3.5 c A β
	4-methylguaiacol	French	New Barrel	4.4 ± 4.2 a A α	12.6 ± 0.1 b B β	25.8 ± 1.5 c B α
			1 year barrel	0.6 ± 0.4 a A α	2.4 ± 0.3 b A β	6.4 ± 0.5 c A α
		American	New Barrel	1.2 ± 0.2 a B α	6.9 ± 0.4 b B α	22.8 ± 5.3 c B α
			1 year barrel	0.4 ± 0.1 a A α	1.4 ± 0.1 b A α	7.3 ± 2.3 c A α
	4-ethylguaiacol	French	New Barrel	31.4 ± 14.4 a B α	22.8 ± 15.7 a B α	15.9 ± 0.8 a B α
			1 year barrel	3.2 ± 1.4 ab A α	2.8 ± 0.1 a A α	4.2 ± 0.3 b A α
		American	New Barrel	20.7 ± 7.4 ab B α	29.2 ± 8.3 b B α	16.4 ± 0.6 a B α
			1 year barrel	3.6 ± 0.6 a A α	4.1 ± 1.2 ab A α	6.1 ± 1.6 b A α
	4-vinylguaiacol	French	New Barrel	65.8 ± 10.3 a B α	83.2 ± 13.8 a B α	146.0 ± 15.3 b B α
			1 year barrel	9.0 ± 3.5 a A α	13.6 ± 1.6 a A α	33.0 ± 2.3 b A α
		American	New Barrel	52.6 ± 16.0 a B α	92.2 ± 14.6 b B α	170.0 ± 32.8 c B α
			1 year barrel	12.8 ± 1.7 a A α	18.7 ± 2.9 b A β	52.6 ± 12.3 c A β
	Eugenol	French	New Barrel	16.8 ± 1.6 b B α	21.2 ± 0.7 c B α	9.9 ± 1.4 a B α
			1 year barrel	10.1 ± 1.6 b A α	12.0 ± 1.1 b A α	7.3 ± 1.0 a A α
		American	New Barrel	85.8 ± 17.3 b A β	88.1 ± 21.1 b A β	31.2 ± 8.6 a A β
			1 year barrel	58.9 ± 10.7 b A β	66.5 ± 11.0 b A β	29.6 ± 6.9 a A β
	trans -isoeugenol	French	New Barrel	0.6 ± 0.3 a A α	9.0 ± 7.5 b B α	3.3 ± 1.6 b B α
			1 year barrel	0.2 ± 0.1 a A α	1.0 ± 0.2 c A α	0.6 ± 0.1 b A α
		American	New Barrel	0.9 ± 0.1 a B α	7.8 ± 1.4 b B α	8.8 ± 3.5 b B β
			1 year barrel	0.1 ± 0.1 a A α	1.6 ± 0.5 b A α	2.4 ± 1.0 b A β
	Total	French	New Barrel	125.3 ± 34.0 a B α	165.3 ± 39.8 a B α	248.4 ± 23.4 a B α
			1 year barrel	25.7 ± 7.5 a A α	35.8 ± 4.3 a A α	61.6 ± 4.9 a A α
		American	New Barrel	173.8 ± 43.7 a B α	246.5 ± 46.5 b B β	313.3 ± 61.8 a B α
			1 year barrel	81.5 ± 13.7 a A β	99.7 ± 16.7 a A β	117.2 ± 27.7 a A β
Red wine	4-ethylphenol	French	New Barrel	61.2 ± 1.7 b B β	35.1 ± 5.0 a B α	32.4 ± 21.3 a A α
			1 year barrel	22.5 ± 2.7 b A β	7.6 ± 1.0 a A α	11.5 ± 3.5 a A α
		American	New Barrel	32.7 ± 2.0 a B α	31.0 ± 15.5 a A α	61.5 ± 7.9 b B α
			1 year barrel	13.9 ± 1.1 a A α	64.0 ± 52.8 a A β	15.5 ± 2.2 a A α
	Guaiacol	French	New Barrel	14.2 ± 1.1 a A α	21.8 ± 1.0 b B α	62.0 ± 16.3 c A α
			1 year barrel	35.3 ± 1.3 b B α	14.9 ± 2.8 a A α	49.6 ± 8.2 c A α
		American	New Barrel	19.1 ± 1.0 a A β	24.5 ± 0.3 b A β	59.7 ± 5.2 c B α
			1 year barrel	40.0 ± 3.9 a B α	45.5 ± 2.5 a B β	40.9 ± 2.1 a A α
	4-methylguaiacol	French	New Barrel	2.9 ± 0.5 a B α	16.9 ± 1.2 b B β	27.9 ± 6.6 c B α
			1 year barrel	0.9 ± 0.1 a A α	3.5 ± 0.1 b A β	9.7 ± 2.9 c A β
		American	New Barrel	2.5 ± 0.6 a B α	6.5 ± 0.1 b B α	23.4 ± 3.9 c B α
			1 year barrel	1.2 ± 0.4 a A α	2.1 ± 0.4 b A α	6.2 ± 0.5 c A α
	4-ethylguaiacol	French	New Barrel	12.3 ± 1.2 b B β	8.8 ± 1.1 a B α	20.8 ± 5.6 c B α
			1 year barrel	6.6 ± 1.6 b A β	3.1 ± 0.2 a A α	8.3 ± 2.4 b A α
		American	New Barrel	7.3 ± 0.6 a B α	10.1 ± 5.4 a A α	22.3 ± 3.5 b B α
			1 year barrel	4.2 ± 0.3 a A α	14.9 ± 10.3 b A β	6.8 ± 0.6 b A α
	4-vinylguaiacol	French	New Barrel	9.8 ± 0.9 a A α	15.8 ± 1.1 b B α	39.9 ± 9.5 c B α
			1 year barrel	14.3 ± 1.0 b B α	7.2 ± 1.0 a A α	22.5 ± 4.5 c A α
		American	New Barrel	9.6 ± 0.7 a A α	13.7 ± 2.0 b A α	35.1 ± 4.2 c B α
			1 year barrel	15.1 ± 1.5 a B α	20.8 ± 4.4 a B β	18.0 ± 1.1 ab A α
	Eugenol	French	New Barrel	19.7 ± 3.8 b A α	23.9 ± 1.9 b B α	12.7 ± 2.6 a A α
			1 year barrel	18.1 ± 1.8 b A α	16.3 ± 1.1 b A α	13.3 ± 1.6 a A α
		American	New Barrel	98.8 ± 5.7 b B β	83.3 ± 22.8 b A β	34.7 ± 1.2 a A β
			1 year barrel	73.0 ± 4.4 b A β	90.0 ± 9.8 c A β	36.6 ± 3.3 a A β
	trans -isoeugenol	French	New Barrel	2.5 ± 0.7 a B α	18.0 ± 0.8 b B α	6.0 ± 5.0 a A α
			1 year barrel	0.5 ± 0.2 a A α	3.1 ± 0.3 b A α	2.5 ± 0.6 b A α
		American	New Barrel	2.4 ± 0.5 a B α	22.1 ± 4.8 b B α	20.4 ± 0.2 b B β
			1 year barrel	0.4 ± 0.1 a A α	3.6 ± 1.6 b A α	6.0 ± 0.5 c A β
	Total	French	New Barrel	122.5 ± 10.0 b B β	140.3 ± 12.1 a B α	201.8 ± 67.0 a B β
			1 year barrel	98.3 ± 8.7 b A α	55.7 ± 6.6 a A α	117.4 ± 23.7 b A α
		American	New Barrel	172.4 ± 11.1 b B α	191.1 ± 51.1 b B α	257.0 ± 26.0 a B α
			1 year barrel	147.8 ± 11.7 b A α	240.9 ± 81.9 c A β	130.0 ± 10.3 a A α

All data are expressed as the average of 2 barrels ± standard deviation. Different letters indicate statistical differences ($p < 0.05$). Roman lower case letters are used to compare toasting levels. Roman capital letters are used to compare new and 1-year-used barrels. Greek letters are used to compare the botanical origin of the oak barrels.

Capítulo IV. Consumo de oxígeno por chips de roble en un vino modelo e impacto del origen botánico y tostado de los elagitaninos presentes

La madera de roble, además de enriquecer el vino en aromas y elagitaninos, es un material poroso que permite una microoxigenación moderada. El aporte de oxígeno es el origen de una serie de transformaciones en el vino tinto que permiten estabilizar el color y suavizar la astringencia. No obstante en la elaboración de vinos blancos, esta microoxigenación puede causar una cierta oxidación que malogre su calidad sensorial.

Por otra parte, se ha descrito que todos los taninos poseen una capacidad antioxidante que, al menos en teoría, debería proteger al vino frente a la oxidación. Dado que la madera de roble es una fuente de elagitaninos, nos planteamos estudiar el consumo de oxígeno por parte de la madera de roble de diversos orígenes botánicos y niveles de tostado a fin de cuantificar cuál es su verdadero impacto en la posible protección del vino frente a la oxidación.

Este estudio se realizó con virutas de roble proporcionadas por la tonelería Radoux-Pronektar y mediante el uso de un sensor de oxígeno no invasivo.

Los análisis de los vinos fueron realizados en el laboratorio del grupo de Tecnología Enológica (TECNENOL) del Departamento de Bioquímica i Biotecnología de la Universitat Rovira i Virgili (URV, Tarragona) y en el Instituto Regional de Investigación Científica Aplicada (IRICA), Universidad de Castilla-La Mancha (UCLM, Ciudad Real). Los resultados del estudio han sido publicados en la revista *Food Chemistry*

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Oxygen consumption by oak chips in a model wine solution; Influence of the botanical origin, toast level and ellagitannin content

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ABSTRACT: The botanical origin, toast level and ellagitannin content of oak chips in a model wine solution have been studied in terms of their influence on oxygen consumption. French oak chips released significantly higher amounts of ellagitannins than American oak chips at any toast level. The release of ellagitannins by oak chips decreased as the toast level increased in the French oak but this trend was not so clear in American oak. Oxygen consumption rate was clearly related to the level of released ellagitannins. Therefore, oak chips should be chosen for their potential to release ellagitannins release should be considered, not only because they can have a direct impact on the flavor and body of the wine, but also because they can protect against oxidation.

KEYWORDS: Oak chips, Oxygen consumption, Ellagitannins, Botanical origin, Toast level.

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1. INTRODUCTION

Oak chips have been widely used to flavor wine for many years now (Bertrand, Barbe, & Gazeau, 1997; Zamora, 2003a). They enrich the wine with the same substances that are released during oak barrel aging (Zamora, 2003b). It is well known that contact with oak wood, either as barrels or as alternatives (staves, chips, etc.), enriches the wine with substances that improve its aroma such as furfural (toasted almonds), dimethyl pyrazines (cacao), maltol (caramel), whiskey-lactones (cocoa), vanillin (vanilla), eugenol (cloves) and volatile phenols (smoky notes) (Ferreira, 2010; Gallego et al., 2012; Pérez-Juan & Luque de Castro, 2015). Oak also releases other non-volatile compounds that participate in the flavour and texture of wine. Ellagitannins are one of these such non-volatile compound, and they contribute to astringency and mouthfeel (Chira & Teissedre, 2013; De Simón, Sanz, Cadahía, Poveda, & Broto, 2006).

Aging in oak barrels also oxygenates wine because small quantities of oxygen can reach the wine through the pores in the wood, the interstices between the staves, and the bunghole (Del Alamo-Sanza & Nevares, 2014; Nevares, Gonzalez, Crespo, & Del Alamo-Sanza, 2014; Vivas, Glories, & Raymond, 1997). This supply of oxygen is positive for red wine because it stabilizes color, reduces astringency, and removes excess vegetal notes. The dissolved oxygen leads to the formation of ethanal from ethanol. The ethanal can then react with flavanols to form a very reactive carbocation which, in turn, quickly reacts with either another flavanol molecule or an anthocyanin to produce ethyl-bridged flavanol–flavanol and/or flavanol–anthocyanin oligomers (Escribano-Bailón, Alvarez-Garcia, Rivas-Gonzalo, Heredia, & Santos-Buelga, 2001). It has been reported that ethanal also participates in the formation of new pigments such as vitisin B and other pyranoanthocyanins (Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & Freitas, 2002). However, in white wines this oxygen supply may be negative because it can lead to wine oxidation. White wines have no anthocyanins and their flavanol concentration is very low (Cáceres-Mella et al., 2013). For this reason white wines are usually aged in oak barrels in contact with the lees, which consume oxygen and protect the wine against oxidation (Comuzzo et al., 2015; Salmon, Fornairon-Bonnefond, Mazauric, & Moutounet, 2000).

The use of oak alternatives coupled with micro-oxygenation can reproduce the processes taking place in the barrels more economically and quickly (Llaudy et al., 2006; Cejudo-Bastante, Hermosín-Gutiérrez, & Pérez-Coello, 2011). As has been mentioned above, red wines require oxygen to induce a series of reactions between phenolic compounds so that color can be stabilized and astringency decreased (Kontoudakis et al., 2011; Ribéreau-Gayon et al., 1998). When white wines are kept in contact with the lees, reduction odour needs to be prevented (Rodríguez et al., 2005; Zamora, 2002). Particular care should be taken to ensure that the oxygen supply is appropriate since an excess may cause oxidation and affect the quality. In this regard, it has been postulated that the ellagitannins released from oak can consume oxygen and protect the wine from excessive oxidation (Poinsaut, 2000; Vivas, 2001).

Several studies have been made on how oak alternatives can contribute to wine flavor (Chassin, 1999; Pérez-Coello et al., 2000; Wilker & Gallander, 1988), but little is known about how they affect the release of ellagitannins and even less is known about the oxygen they consume (Vivas & Glories, 1996). Therefore, the objective of our work was to study the relationship between the release of phenolic compounds, especially ellagitannins, from oak chips and the oxygen consumption of a model wine solution In particular, the influence of botanical origin, toast level and ellagitannin release was analyzed.

2. MATERIALS AND METHODS

2.1. Chemicals and equipment

Methanol, formic acid and acetic acid of high performance liquid chromatography (HPLC) grade (>99%) and absolute ethanol, ethyl acetate, L(+)-tartaric acid and sodium hydroxide pellets were purchased from Panreac (Barcelona, Spain); gallic acid, copper (II) sulfate pentahydrate and iron (III) chloride hexahydrate from Sigma–Aldrich (Madrid, Spain); ellagic acid from Fluka (Sigma-Aldric, Madrid, Spain). Ellagitannins (Vescalagin, Castalagin, Roburin A, Roburin D, Granidin and Roburin E) were purchased from Adera (Pessac, France).

All spectrophotometric measurements were performed with a Helios Alpha UV–vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

HPLC separation, identification and quantitation of ellagitannins were performed on an Agilent 1100 Series system (Agilent, Germany), equipped with DAD (G1315B) and an LC/MSD Trap VL (G2445C VL) electrospray ionization mass spectrometry (ESI-MSn) system, and coupled to an Agilent ChemStation (version B.01.03) data-processing station. The mass spectra data were processed with the Agilent LC/MS Trap software (version 5.3).

2.2. Oak chips

For this study we used 2, 5 and 10 g/L of French oak (*Quercus petraea*) and American oak (*Quercus alba*) chips and three toast levels (low, medium and high). The chips were quadrangular with a size of 5–8 mm long; 5–8 mm wide and 3.5–4.5 mm deep. They were provided by the company Tonnellerie Radoux-Pronektar (Jonzac, France). In the case of medium toast French oak, chips with three levels of potential ellagitannin release (PER). The PER was determined by the supplier company itself (Tonnellerie Radoux-Pronektar) using its non-invasive measurement method based on infrared spectrometry, (Radoux OakScanTM) (Michel et al., 2011, 2013) high, medium and low PER were also used. Hence, high, medium and low PER staves were employed in this experiment.

2.3. Experimental design

A model wine solution was used to estimate the oxygen consumption. The model wine solution was composed of ethanol: 12% (v/v); tartaric acid: 4 g/L; pH = 3.5; iron: 3 mg/L, in the form of iron (III) chloride hexahydrate and copper: 0.3 mg/L in the form of copper (II) sulfate pentahydrate. Three white wines and three red wines were also used to compare the kinetics of oxygen consumption of the different oak chips with those of these wines.

The ethanol content (ET), Titratable acidity expressed as tartaric acid (TA), free sulfur dioxide (FS) and Total phenolic index (TPI) were measures to characterize these wines. The chemical characteristics of these wines was: white wine 1: ET: 10.7% (v/v); TA: 6.7 g/L; FS: 22 mg/L and TPI: 4.7; white wine 2: ET: 11.2% (v/v); TA: 6.3 g/L; FS: 18 mg/L; TPI: 4.3; white wine 3: ET: 12.7% (v/v); TA: 5.3 g/L; FS: 28 mg/L; TPI: 6.2; red wine 1: ET: 13.2%; TA: 5.9 g/L; FS: 18 mg/L; TPI: 56,1; red wine 2: ET: 12.6%; TA: 5.6 g/L; FS: 23 mg/L; TPI: 48,6; red wine 3: ET: 14.3%; TA: 4.8 g/L; FS: 32 mg/L; TPI: 71.3. None of these wines had been in contact with oak or had been supplemented with ascorbic acid or oenological tannin.

The oak chips were placed in clear glass bottles into which a pill had previously been inserted (PreSens Precision Sensing GmbH, Ordering Code: SP-PSt3-NAU-D5-CAF; Batch number: 1203-01_PSt3-0828-01, Regensburg, Germany) for the non-invasive measurement of dissolved oxygen by luminescence (NomasenseTM O2 Trace Oxigen Analyzer by Nomacorc S.A., Thimister Clermont, Belgium). Three doses of oak chips (2, 5 and 10 g/L) were assayed. The bottleswere completely filledwith the modelwine solution previously saturated with oxygen. In parallel, other bottles were filled with the different wines without supplementation with oak chips.

The wine model solution and the various wines were saturated in oxygen by bubbling with air for 10 min. Once the bottles had been closed with a crown cap and bidule, to minimize the volume of headspace, oxygen (Diéval, Vidal, & Aagaard, 2011) was measured periodically. Oxygen was measured every day during the first 5 days and after every 2–3 days until the end of the experiment. Fig. 1 shows a schematic of the experimental design. All assays were performed in triplicate taking control bottles with the oxygen-saturated model wine solution without added oak chips as control reference. After 140 days of maceration, the bottles with 10 g/L of oak chips were opened for ellagitannins analysis. The various samples were strained and centrifuged (12,000xg 10 min).

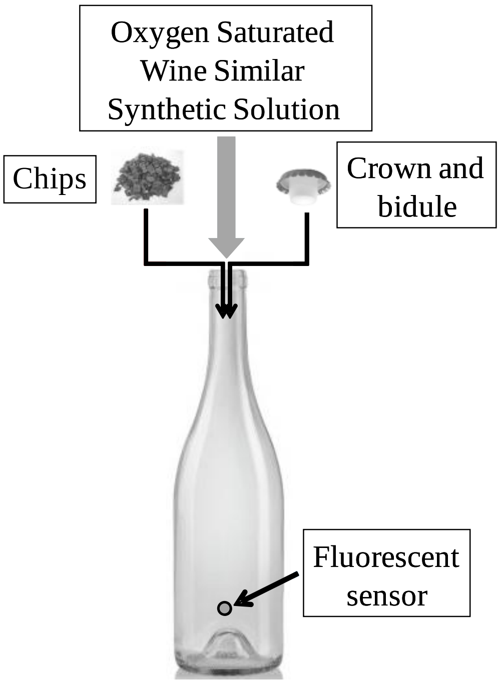

Fig. 1. Experimental design for oxygen consumption measurement.

2.4. Color analysis

The color intensity (CI) was estimated as described by Glories (1984). The CIELab coordinates, lightness (L*), chroma (C*) and hue (h*) were determined according to Ayala, Echávarri, and Negueruela (1997) and data were processed with MSCV software (Ayala, Echávarri, & Negueruela, 2014).

2.5. Total phenolic index

The total phenolic index (TPI) was determined by measuring the absorbance at 280 nm of a diluted (1:10) model wine solution with a spectrophotometer after centrifugation (15,900g for 20 min) using a 10-mm quartz cuvette (Ribéreau-Gayon, Glories, Maujean, & Dubordieu, 2006).

2.6. Ellagitannins analysis

The real or model wine samples were first fractionated to obtain ellagitannin-rich fractions using a previously described method (Garcia-Estevez, Escribano-Bailon, Rivas-Gonzalo, & Alcalde-Eon, 2010). Ellagitannins, together with gallic and ellagic acids, were analyzed by HPLC after the method had been adapted (Michel et al., 2011) to the use of fused core C18 chromatographic columns (Ascentis Express, 150 x 4.6 mm, 2.7 lmparticle size), thermostatted at 40 ºC. Elution solvents A (0.4% formic acid in water) and B (0.4% formic acid in methanol) were used with the following linear gradient of elution: initial conditions, flow of 0.28 mL/min and 0% solvent B maintained for 3 min; 25 min, flow of 0.23 mL/min and 12.5% solvent B; 40 min, flow of 0.23 mL/min and 50% solvent B; 45 min, flow of 0.23 mL/min and 100% solvent B; 50 min, flow of 0.23 mL/min and 100% solvent B; 55 min, return to initial conditions (flow of 0.28 mL/min and 0% solvent B); post-run time of 10 min between injections to equilibrate the column.

The ellagitannins, gallic acid and ellagic acid were identified by matching the retention time and spectral data (DAD-UV–vis and MS/MS) with those of authentic standards. The quantitation was performed using the DAD-chromatograms extracted at 250 nm. The DAD-chromatogram (detection at 250 nm) of a mixture of ellagitannin standards and the MS and MS2 spectra (Ion Trap Mass Detector) of ellagitannin standards used for identification are included as Supplementary material (Figs. S1 and S2).

2.7. Statistics

All data are expressed as the arithmetic average ± standard deviation of three replicates. One-factor analysis of variance (ANOVA) was carried out using SPSS 15.0 software (SPSS Inc., Chicago, IL).

3. RESULTS AND DISCUSSION

Table 1 shows how the botanical origin and toast level of oak chips influence the color and phenolic composition of the model wine solution after 140 days of maceration. These measurements were only performed in samples with 10 g/L in order to facilitate the analysis. In general, color intensity (CI) and chroma (C*) were significantly higher and luminosity (L*) significant lower for French oak than for American oak at all toast levels. The hue (h*) was significantly higher in American oak than in French oak but only at low and medium toast levels.

Table 1. Influence of botanic origin and toast level of oak chips on color and phenolic composition of the model wine solution after 140 days of maceration with 10 g/L of oak chips.

Parameters	Botanic origin	Low Toast			Medium Toast			High Toast		
CI	American Oak	0.25 ± 0.00	a	A	0.17 ± 0.01	b	A	0.07 ± 0.01	c	A
	French Oak	0.82 ± 0.02	a	B	0.57 ± 0.03	b	B	0.09 ± 0.00	c	A
L*	American Oak	95.6 ± 0.0	a	A	97.5 ± 0.2	b	A	99.3 ± 0.1	c	A
	French Oak	84.3 ± 0.3	a	B	89.5 ± 0.5	b	B	98.8 ± 0.1	c	B
C*	American Oak	6.30 ± 0.18	a	A	5.32 ± 0.11	b	A	3.06 ± 0.11	c	A
	French Oak	15.48 ± 0.33	a	B	12.52 ± 0.62	b	B	3.68 ± 0.07	c	B
h*	American Oak	96.8 ± 0.6	a	A	97.9 ± 0.3	ab	A	99.8 ± 1.8	c	A
	French Oak	91.1 ± 0.2	a	B	93.0 ± 0.5	b	B	98.8 ± 0.8	c	A
TPI	American Oak	5.23 ± 0.01	a	A	4.91 ± 0.10	b	A	3.66 ± 0.03	c	A
	French Oak	13.45 ± 0.12	a	B	10.03 ± 0.52	b	B	5.93 ± 0.12	c	B
Vescalagin (mg/L)	American Oak	0.07 ± 0.01	a	A	0.02 ± 0.00	b	A	n.d.		
	French Oak	3.33 ± 0.89	a	B	1.38 ± 0.18	b	B	0.02 ± 0.01	c	
Castalagin (mg/L)	American Oak	1.02 ± 0.24	a	A	0.36 ± 0.07	b	A	0.03 ± 0.00	c	A
	French Oak	13.44 ± 3.50	a	B	9.21 ± 0.62	b	B	0.10 ± 0.02	c	B
Roburin A (mg/L)	American Oak	n.d.			n.d.			n.d.		
	French Oak	0.07 ± 0.03	a		0.07 ± 0.01	a		n.d.		
Roburin D (mg/L)	American Oak	n.d.			n.d.			n.d.		
	French Oak	0.09 ± 0.02	a		0.24 ± 0.03	b		n.d.		
Grandinin (mg/L)	American Oak	0.07 ± 0.02		A	n.d.			n.d.		
	French Oak	0.64 ± 0.07	a	B	0.11 ± 0.02	b		n.d.		
Roburin E (mg/L)	American Oak	0.06 ± 0.01		A	n.d.			n.d.		
	French Oak	0.91 ± 0.13	a	B	0.16 ± 0.03	b		n.d.		
Total Ellagitannins (mg/L)	American Oak	1.21 ± 0.28	a	A	0.43 ± 0.17	b	A	0.03 ± 0.00	c	A
	French Oak	18.48 ± 4.64	a	B	11.18 ± 0.88	b	B	0.12 ± 0.03	c	B
Gallic Acid (mg/L)	American Oak	1.05 ± 0.09	a	A	0.81 ± 0.02	b	A	0.15 ± 0.01	c	A
	French Oak	2.76 ± 0.23	a	B	1.99 ± 0.06	b	B	0.15 ± 0.01	c	A
Ellagic Acid (mg/L)	American Oak	0.26 ± 0.06	a	A	0.24 ± 0.04	a	A	0.10 ± 0.01	b	A
	French Oak	1.30 ± 0.11	a	B	1.20 ± 0.11	a	B	0.18 ± 0.01	b	A

Results are expressed as mean ± standard deviation. Different lowercase letters in a row indicate a statistical difference ($p < 0.05$) between toasting levels. Different uppercase letters indicate a statistical difference ($p < 0.05$) between botanic origin. CI: Color intensity; L*: lightness value (CIELab coordinates); C*: chroma value (CIELab coordinates); h*: hue value (CIELab coordinates); TPI: total phenolic index

This data indicates that at similar toast levels, French oak releases more colored compounds than American oak. On the other hand, CI and C* decreased and L* increased significantly when the toast level was higher in both oak species, thus suggesting that the higher the toast level the lower the release of colored compounds.

This data was confirmed by the total phenolic indexes of the various samples. The TPI was significantly higher in French oak than in American oak at all toast levels, and it also decreased when the toast level was higher in both oak species. Consequently, our results seem to confirm that French oak is richer in phenolic compounds than American oak, and that the toasting process decreases the capacity of the wood to release phenolics. In general these results were in agreement with previous studies (Chatonnet & Dubourdieu, 1998; Vivas, 1995).

Table 1 also shows the ellagitannin content of the various samples. Castalagin was the major ellagitannin in all the experimental conditions, representing more than 70% of total ellagitannins in all cases. Vescalagin was the second ellagitannin in concentration in nearly all the samples although its values were much lower (between 0% and 18%). Other minor ellagitannins, Roburin A, Roburin D, Grandinin and Roburin E, were also detected in some of the samples, usually accounting for proportions below 5%. In general, our results were in agreement with previously reported levels of the oak ellagitannins (Chira & Teissedre, 2013; De Simón et al., 2006; Michel et al., 2011).

In addition, our results confirmed that French oak released many more ellagitannins than American oak at all toast levels (Chatonnet & Dubourdieu, 1998) and that toasting drastically diminished the capacity of the wood to release these substances (Vivas, 1995). Gallic and ellagic acids were also analyzed and, in general, they showed a similar trend to the ellagitannins: i.e. higher levels when French oak was used instead of American oak, and lower levels when the toast level increased.

Fig. 2 shows how the botanical origin and toast level of the oak chips influence the oxygen consumption of a model wine solution. The oxygen levels of the control samples without added chips oscillated slightly, especially at the beginning of the experiment, but remained quite stable over time, confirming that this solution did not consume oxygen. In contrast, the oxygen concentration of all the samples containing oak chips decreased over time demonstrating that the presence of these oak alternatives involved oxygen consumption. Moreover, the oxygen consumption was faster when more oak chips were added.

In the presence of French oak, the oxygen consumption was faster than with American oak at all amounts of added chips and all toast levels. Since French oak released higher concentrations of ellagitannins and other phenolic compounds, the oxygen consumption kinetics seems to be related to the capacity of the wood to release these substances.

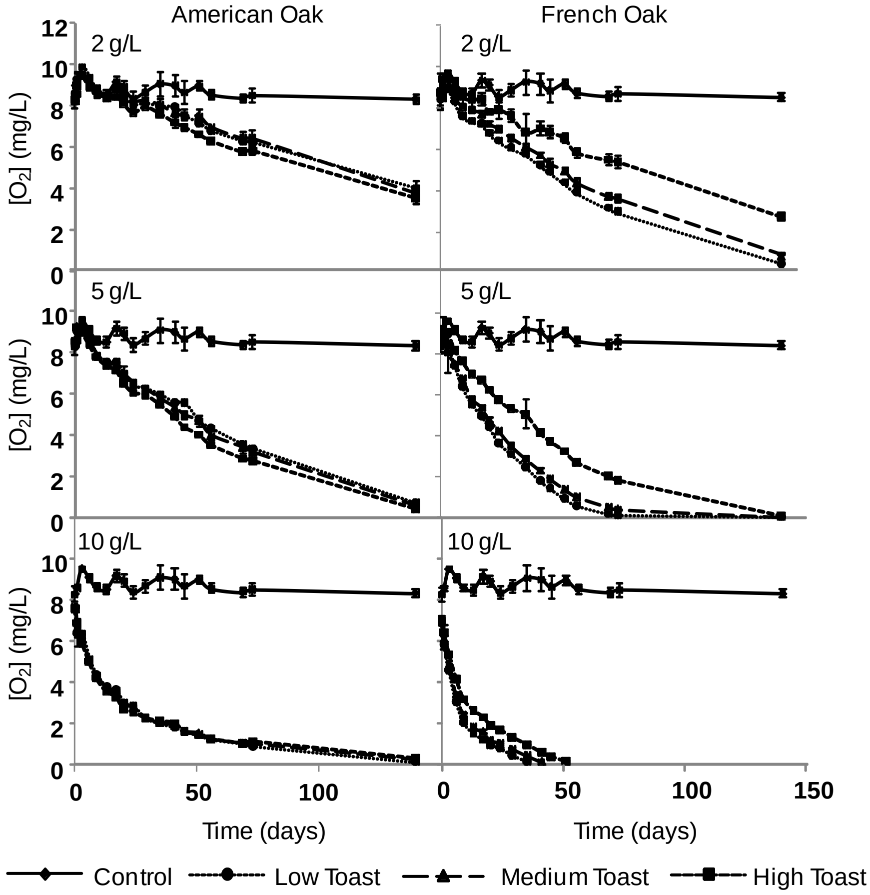

Fig. 2. Influence of botanic origin and toast level of oak chips on oxygen consumption of a model wine solution during maceration with different doses of added chips.

In the case of French oak, the toast level seems to have an additional effect on the oxygen consumption rate. Specifically, the lower the toast level of the chips, the faster the oxygen consumption. This also suggested that the amount of released ellagitannins and other phenolic compounds conditioned the oxygen consumption rate, since toasting decreased the release of these compounds. However, the toast level was not observed to influence the oxygen consumption rate in the case of American oak probably because it released much lower amounts of ellagitannins and other phenolic compounds than French oak.

Table 2 shows how the potential ellagitannin release (PER) of medium toast French oak chips influences the color and phenolic composition of the model wine solution after 140 days of maceration.

Table 2. Influence of the potential of ellagitannin release (PER) of medium toast French oak chips on color and phenolic composition of a model wine solution after 140 days of maceration with 10 g/L of chips.

Parameters	Low IP	Medium IP	High IP
CI	0.25 ± 0.01 a	0.57 ± 0.03 b	0.89 ± 0.01 c
L*	95.8 ± 0.3 a	89.5 ± 0.5 b	82.8 ± 0.1 c
C*	6.69 ± 0.16 a	12.52 ± 0.62 b	16.00 ± 0.28 c
h*	96.7 ± 0.4 a	93.0 ± 0.5 b	89.7 ± 0.2 c
TPI	5.12 ± 0.08 a	10.03 ± 0.52 b	15.07 ± 0.20 c
Vescalagin (mg/l)	0.11 ± 0.01 a	1.38 ± 0.18 b	4.13 ± 0.48 c
Castalagin (mg/l)	1.28 ± 0.22 a	9.21 ± 0.62 b	20.38 ± 2.71 c
Roburin A (mg/l)	n.d.	0.07 ± 0.01 a	0.08 ± 0.01 a
Roburin D (mg/l)	n.d.	0.24 ± 0.03 a	0.18 ± 0.04 a
Grandinin (mg/l)	0.08 ± 0.01 a	0.11 ± 0.02 a	0.32 ± 0.03 b
Roburin E (mg/l)	0.09 ± 0.02 a	0.16 ± 0.03 b	0.36 ± 0.02 c
Total Ellagitannins (mg/l)	1.56 ± 0.25 a	11.18 ± 0.88 b	25.46 ± 3.29 c
Gallic Acid (mg/l)	0.52 ± 0.03 a	1.99 ± 0.06 b	4.87 ± 0.03 c
Ellagic Acid (mg/l)	0.38 ± 0.02 a	1.20 ± 0.11 b	1.14 ± 0.69 b

Results are expressed as mean ± standard deviation. Different letters in a row indicate a statistical difference ($p < 0.05$) . CI: Color intensity; L*: lightness value (CIELab coordinates); C*: chroma value (CIELab coordinates); h*: hue value (CIELab coordinates); TPI: total phenolic index

These measurements were only performed in samples with 10 g/L of oak chips in order to facilitate the analysis. The CI, C* and TPI increased and L* and h* decreased when the PER of the French oak chips increased.Overall, this trend was also observed for gallic acid, ellagic acid and total ellagitannin concentrations. As was also observed in previous experiments, vescalagin and specially castalagin were the major ellagitannins (around 90% of total ellagitannins). These results confirmed that the non-invasive measurement method using infrared spectrometry (Radoux OakScanTM) (Michel et al., 2011) allows oak wood to be correctly classified on the basis of its PER.

Fig. 3 shows how the PER of medium toast French oak chips influence the oxygen consumption of a model wine solution. As expected, the rate of oxygen consumption increased as the amount of French oak chips increased, whatever the PER value. The PER also proved to have a considerable effect on the rate of oxygen consumption. Specifically, the higher the PER was, the faster the oxygen was consumed. Consequently, these results reinforced the suggestion that there is a direct relationship between the oxygen consumption kinetics and the capacity of the wood to release ellagitannins and other phenolic compounds.

Overall, these results suggest that the ellagitannins and other phenolic compounds released by oak wood can consume some of the oxygen entering the wine during barrel aging or other winemaking operations, therefore protecting it against oxidation.

However, is this protection really important for the oxygen consumption by the wine itself or, conversely, is it negligible? In an attempt to respond to this question, the oxygen consumption of three white wines and three red wines without added oak chips was compared to that of a model wine solution with 10 g/L of added French and American oak chips (both medium toast and medium PER) (Fig. 4).

As expected, the red wines consumed oxygen faster than the white wines although a great variability between the wines was observed, very likely because of their different qualitative and quantitative phenolic composition. Specifically, the time it took for the oxygen to be completely consumed in red wines oscillated between 1 and 6 days whereas in white wines it took between 5 and 20 days. Since red wines contain higher concentrations of phenolic compounds than white wines and phenolic compounds are probably the main oxygen consumers in wine (Ginjom, D'Arcy, Caffin, & Gidley, 2010), these results were completely logical and only to be expected.

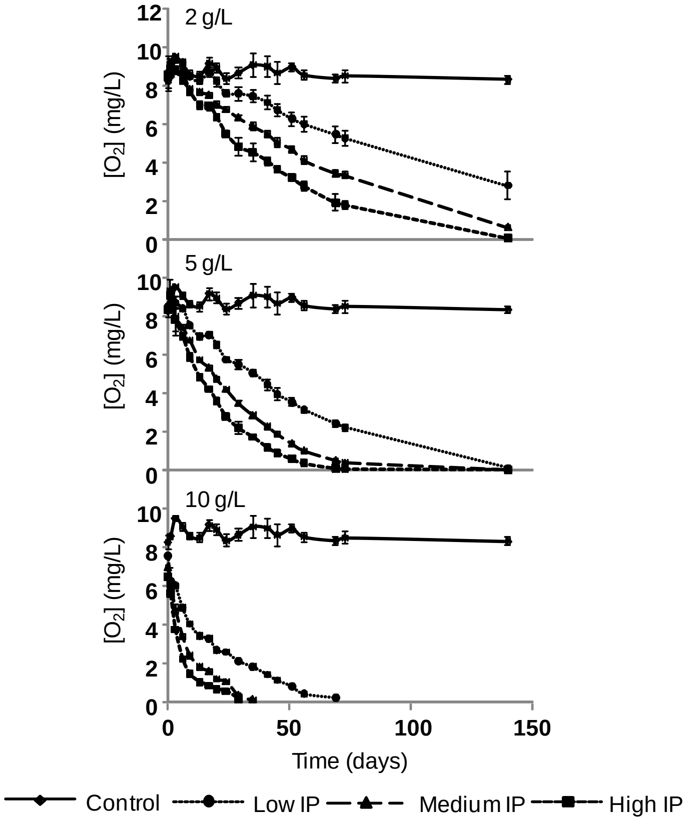

Fig.3. Influence of the potential of ellagitannin release (PER) of medium toast French oak chips on oxygen consumption of a model wine solution during maceration with different doses of added chips.

Oak chips consumed oxygen more slowly than wine. Specifically, the time it took for the oxygen to be completely consumed by model wine solutions with 10 g/L of added oak chips was 28 days in the case of French oak and 52 days in the case of American oak. These times are much longer than those measured in red wines but are not too different from those observed in some of the white wines assayed. Therefore, the oxygen consumed by ellagitannins and the other phenolic compounds released from oak chips may effectively compete with the oxygen consumed by the wine itself.

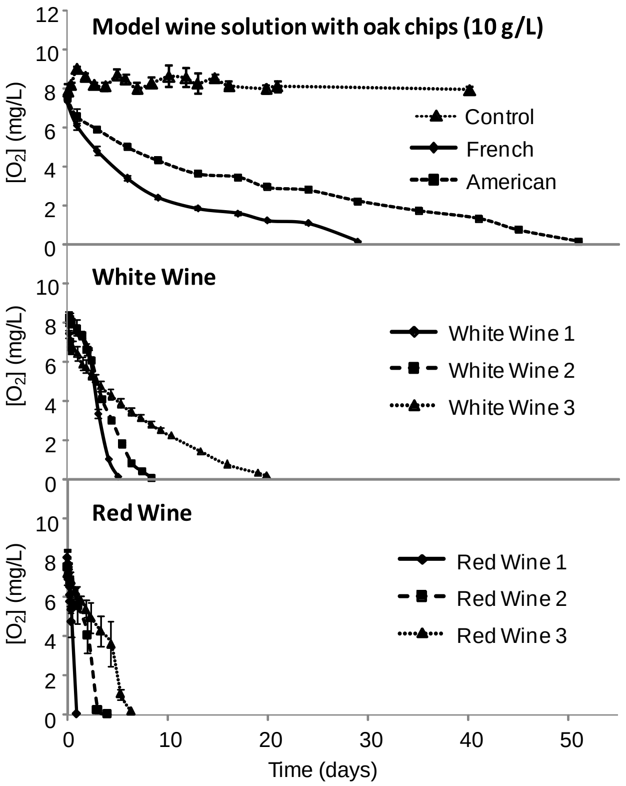

Fig.4. Comparison between the oxygen consumption by a model wine solution with added oak chips and different red and white wines without added chips.

Consequently, the contact of the wine with oak wood, either in the form of barrels or alternatives, can play a major role in protecting the wine against oxidation. This protection can be negligible in the case of red wines but important in the case of some white wines. It may even help to reduce sulfite levels during wine aging. These results also suggest that oak wood should be chosen for its potential ellagitannin release, because it can have a direct impact on the flavor and body of the wine, and also a possible protective effect against oxidation.

4. CONCLUSIONS

The maceration of oak chips in a model wine solution led to dissolved oxygen being consumed at a rate that seems to be closely associated with the release of ellagitannins and other phenolic compounds from the wood. In this regard, botanical origin and toast level clearly influenced the amount of released ellagitannins as well as the rate of oxygen consumption. The rate of oxygen consumption was higher when French oak was used instead of American oak and also decreased as the toast level increased. The same behavior was observed when oak chips were selected for their potential ellagitannin release (PER), the oxygen consumption rate increasing in parallel to the PER. In a model wine solution, the oxygen was consumed by oak wood much more slowly than in assayed real red wines, but at a rate that was not so different from rates observed in some of the assayed real white wines. Consequently, the contact of wine with oak wood, in barrels or other alternatives, may be an effective enological tool for protecting wine against oxidation and can also be considered useful for reducing sulfites during wine aging. Therefore, oak wood should be chosen for its potential ellagitannin release because it can have a direct impact on the flavor and body of the wine, and also a possible protective effect against oxidation.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.12.081.

NOTE: Supplementary figures S1 and S2 are included at the end of Chapter II, because they are the same as used for this article.

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4. DISCUSIÓN GENERAL

Para poder realizar una discusión general de los resultados presentados en esta memoria, es necesario contextualizarlos dentro de la línea de investigación sobre análisis de los vinos de crianza, y en general, de la calidad global del vino tinto, que se desarrolla desde hace años en el grupo de tecnología enológica (TECNENOL) del Departamento de Bioquímica y Biotecnología de la Universidad Rovira i Virgili, y que en los últimos años se ha complementado con la cooperación con el Instituto Regional de Investigación Científica Aplicada (IRICA), perteneciente a la Universidad de Castilla-La Mancha UCLM), y con el Instituto de la Vid y el Vino de Castilla-La Mancha (IVICAM).

El objetivo fundamental de este trabajo era generar información científica sobre los elagitaninos que la madera de roble libera al vino, así como la influencia del origen botánico del roble, del grado de tostado de las duelas y de la influencia del uso previo de las barricas, a fin de conocer mejor cómo todos estos factores afectan a la composición final del vino de crianza y de este modo generar conocimiento que pueda resultar útil tanto para el mundo académico como para los profesionales del sector.

Está generalmente aceptado, gracias a la experiencia acumulada durante años, que el contacto del vino con la madera de roble, ya sea en barricas o mediante el uso de alternativos como los virutas, implica el enriquecimiento del vino en elagitaninos, los cuales parecen contribuir a la astringencia, al sabor amargo y a la sensación de cuerpo del vino. Estos compuestos se liberan de forma relativamente rápida y posteriormente pueden desaparecer por hidrólisis u oxidación en un medio ácido como el vino.

El avance y la generalización de las técnicas instrumentales de análisis, especialmente en el caso de HPLC-MS, ha permitido el desarrollo de métodos analíticos mucho más potentes y eficaces por lo que su empleo para un estudio como el planteado permite revisar y profundizar en la investigación sobre los elagitaninos liberados por el roble. Gracias a las nuevas técnicas analíticas se ha descrito recientemente que los elagitaninos pueden combinarse con los flavan-3-oles monómeros e incluso con los antocianos para originar nuevas moléculas. Dado que algunos de los flavan-3-oles monómeros contribuyen al sabor amargo, su transformación por medio de estas reacciones podría contribuir a la disminución de dicha sensación durante el proceso de crianza.

Por otra parte, la variedad estructural y complejidad de los elagitaninos comporta una gran dificultad para el desarrollo de métodos de aislamiento y análisis suficientemente eficaces y selectivos para el análisis de estos compuestos en los distintos tipos de madera y/o en los vinos. En dicho sentido, investigadores de diferentes campos han dirigido su atención hacia la implementación de mejores metodologías para la extracción, purificación y detección de elagitaninos.

Varios autores han coincidido que el uso de mezclas de como agua/metanol resultan ser eficaces para la extracción de taninos de la madera, siendo un factor muy importante la selección del extractante, para que permita una completa extracción de los taninos presentes en cada muestra.

Para detectar y cuantificar las cantidades de los compuestos se han empleado mayoritariamente el análisis por cromatografía líquida de alta eficacia (HPLC) y la detección por Espectroscopía Ultravioleta y por Espectrometría de Masas, usando como fases móviles mezclas de solventes como agua:metanol ligeramente acidificadas, que han demostrado ser eficaces para separar la gran mayoría de los taninos hidrolizables que son polifenoles hidrosolubles. Sin embargo, se trata de procesos de preparación de muestra y cuantificación laboriosos y que consumen mucho tiempo, por lo que todavía hay una necesidad general de nuevos métodos que sean rápidos, precisos y eficaces para el aislamiento, la identificación y la cuantificación de los elagitaninos.

Así pues, la principal aportación del capítulo I recae sobre el hecho de haber desarrollado una mejora de los métodos ya existentes sobre el aislamiento, la identificación y la cuantificación de los elagitaninos presentes en vinos gracias a la experiencia en HPLC-MS que posee el grupo de la Universidad de Castilla-La Mancha y del IVICAM que nos ha permitido abordar el análisis de elagitaninos, así como de sus posibles aductos con flavanoles o antocianos.

Aunque existe abundante literatura científica relacionada con la presencia de elagitaninos en el vino no existía ningún artículo, al menos en nuestro conocimiento, que contemplara conjuntamente a los principales factores que condicionan la presencia y liberación de elagitaninos al vino: origen botánico del roble, nivel de tostado de las duelas y utilización de barricas nuevas o previamente usadas.

El estudio mostrado en el capítulo II, se llevó a cabo mediante la utilización de dos matrices diferentes, una de vino tinto (Cabernet Sauvignon) que nos proporciona unas condiciones reales y otra de vino blanco (Macabeo) decolorado completamente con carbón enológico y bentonita a fin de obtener una matriz mucho más simple que nos facilitara tanto el análisis químico como el sensorial debido a que se minimiza la presencia de aromas y compuestos fenólicos. Los vinos fueron llevados a crianza durante 12 meses en barricas nuevas o usadas durante 1 año, de roble francés (*Quercus petraea*) y americano (*Quercus alba*), y con tres niveles de tostado. Cada 3 meses se extraía muestra y se procedía a su análisis completo.

Es necesario señalar que se realizaron análisis de color (coordenadas CIELab), así como de antocianos y de proantocianidinas mediante HPLC pero en ningún caso se observaron diferencias significativas en función del tipo de roble, del grado de tostado ni entre barricas nuevas o usadas durante un año. Tan sólo se observaron diferencias en las concentraciones de elagitaninos (capítulo II) y aromas (Capítulo III). Por esta razón no se ha considerado necesario presentar dichos resultados, ya que hubiesen alargado la memoria sin aportar datos de interés. Sin embargo, sí que se ha considerado conveniente incluir los resultados obtenidos en un apéndice (Anexo A) y reflejar estos datos en una de las conclusiones. Tan sólo se presentan los resultados a los 12 meses de crianza para no complicar excesivamente la memoria.

En todos los casos, los niveles de elagitaninos fueron superiores en el vino blanco decolorado que en el vino tinto. Estas diferencias, en función de la matriz, podrían deberse a que el pH más bajo del vino decolorado podría favorecer la extracción o a que los polifenoles presentes en el vino tinto hubieran reaccionado con los elagitaninos para generar nuevos compuestos no detectados.

El vino criado en barricas de roble francés mostro concentraciones significativamente mayores de elagitaninos que en el caso del roble americano, en todas las condiciones experimentales. Estos datos son consistentes con los estudios reportados anteriormente, en los que se encuentra que *Q. petraea* (roble francés) es relativamente más rico que *Q. alba* (roble americano) en elagitaninos.

También en el capítulo II, hemos podido confirmar que el nivel de tostado ejerce una influencia significativa sobre la presencia de elagitaninos en el vino, probablemente a causa de la degradación o transformación que sufren los elagitaninos cuando la madera de roble es sometida altas temperaturas durante el tostado. Así, cuanto mayor es el nivel de tostado de las duelas, menor es su capacidad de liberación y, por lo tanto, menor es la concentración total de elagitaninos.

Los vinos criados en barricas nuevas, presentan unos niveles de elagitaninos y un teórico impacto sensorial muy superiores a los vinos similares criados al año siguiente en las mismas barricas, lo que confirma el agotamiento progresivo de la barrica tal y como ya se había descrito. Consecuentemente, todos estos hechos deben ser un criterio más de los elaboradores de vino a tener en cuenta en el momento de seleccionar el origen botánico, el nivel de tostado y el número de veces que se han utilizado las barricas.

La crianza del vino en barricas de roble permite también su enriquecimiento en substancias volátiles que intensifican su aroma y le otorgan una mayor complejidad sensorial. Como ya se ha comentado, el objetivo principal de este trabajo era el estudio de los elagitaninos liberados durante la crianza del vino en barrica y no el de los aromas que aporta el roble. No obstante, al disponer de un protocolo experimental como el empleado y al contar con un gran volumen de muestras creímos interesante abordar también un objetivo adicional como era el de la influencia de la crianza del vino en barricas de roble sobre la componente aromática del vino. Este es un tema ya muy desarrollado sobre el que hay numerosas referencias en la bibliografía por lo que decidimos realizar un enfoque original tratando de interrelacionar la composición de los vinos con los diversos compuestos volátiles y la percepción sensorial por parte de un panel de cata entrenado.

Por otra parte, al haber planteado la experiencia con dos matrices distintas, un vino tinto y un vino blanco decolorado, se disponía de un modelo experimental en el que había un medio complejo en el que los aromas propios del vino tinto coexisten con los liberados por la madera de roble, y un medio simple en el que mayoritariamente los aromas procedían sólo de la madera de roble.

Una evaluación sensorial de los catadores sobre los principales aromas de roble de los vinos envejecidos en barricas mostró puntuaciones más altas para el atributo de coco en los vinos que habían estado en contacto con roble americano que con el roble francés, resultados que coinciden con los descritos en la bibliografía ya que el roble americano posee mayores niveles del isómero *cis* de las β-metil-γ-octolactonas. Asimismo, la intensidad de este atributo sensorial disminuyó al aumentar el nivel de tostado, resultados que también coinciden con investigaciones anteriores.

La percepción de vainilla aumentó entre las barricas con un tostado ligero y medio en ambas especies de roble y en ambas matrices, produciéndose una disminución cuando tratamos con barricas de tostado fuerte. Esta disminución está relacionada con la disminución en la concentración de vainillina que el tostado fuerte genera, pero también probablemente con que su percepción se ve enmascarada por las notas ahumadas/tostadas, acordes con un aumento de los fenoles volátiles en el vino. Estos resultados se confirmaron a través de una correlación significativa entre la percepción de coco, ahumado y vainilla con los correspondientes impactos sensoriales teóricos de las β-metil-γ-octolactonas, vainillina y fenoles volátiles respectivamente.

Se constató que los catadores eran más conscientes y tenían criterios más homogéneos para los atributos sensoriales de coco y de notas ahumadas/tostadas que para la vainilla, y que su rendimiento fue mejor cuando se trataba con el vino blanco decolorado que con el vino tinto, probablemente debido a que la matriz de tinto era bastante más compleja. Sin embargo, se comprobó que el panel de catadores estaba adecuadamente capacitado para la evaluación sensorial de estos atributos ya que las diferencias encontradas fueron significativas.

El panel distinguió con claridad entre los vinos envejecidos en barricas nuevas, dado que su impacto teórico sensorial fue claramente superior, y aquellos envejecidos en las barricas de 1 año utilizadas, con ambos vinos y en todos los niveles de tostado.

El panel de catadores fue capaz de distinguir significativamente, mediante test triangular, los vinos criados en barricas de roble americano y francés cuando los tostados eran ligero o medio, pero no cuando el tostado era fuerte, lo que indicaría que el exceso de fenoles volátiles llega a enmascarar las características asociadas al origen botánico del roble. Por lo general los vinos criados en roble francés eran preferidos a los criados en roble americano, aunque no en todos los casos.

Las preferencias de los catadores fueron casi siempre dirigidas a los vinos envejecidos en barricas con un nivel de tostado medio, independientemente de la matriz o de las especies de roble, probablemente porque este nivel de tostado otorga al vino el mejor equilibrio de los tres tipos de aromas principales que se encuentran en los vinos tintos de crianza (coco, vainilla y ahumados/tostados). Estos datos explicarían por qué las barricas de tostado medio son las más utilizadas en la crianza del vino en las bodegas.

Aunque hasta el momento sólo se hablado de la presencia de elagitaninos y aromas en el vino, no hay que olvidar que la microoxigenación moderada que tiene lugar en las barricas es uno de los puntos clave de la crianza del vino. Por esta razón, en el capítulo IV se aborda el estudio del consumo de oxígeno disuelto en una solución de vino modelo en maceración con virutas de roble de ambos orígenes botánicos y con diferente grado de tostado, todo ello en relación con la liberación de elagitaninos.

Los resultados confirman de nuevo que el origen botánico y el nivel de tostado de las virutas de roble influyen claramente en la cantidad de elagitaninos liberados de forma similar a lo observado en el capítulo II con los vinos criados en barricas. Se determinó también una clara relación entre la velocidad de consumo de oxígeno y la concentración de elagitaninos liberada por las virutas de roble. Específicamente, cuando mayor era la concentración de elagitaninos el consumo de oxígeno era más rápido.

Estos resultados se confirmaron utilizando virutas de roble francés con diferente capacidad potencial de liberación de elagitaninos (potential ellagitannin release – PER), seleccionadas mediante un método no invasivo basado en la espectrofotometría infrarroja (Radoux OakScan^{TM}).

El resultado anterior es muy interesante y debe tenerse en consideración, ya que una mayor liberación de elagitaninos implicaría una mayor protección del vino frente a la oxidación, especialmente en el caso de los vinos blancos mucho más sensibles a la degradación oxidativa. Por consiguiente, se deduce que la capacidad potencial para aportar elagitaninos al vino debería ser considerada como un criterio de selección de las barricas debido a que contribuyen a la estructura y cuerpo del vino y también a una posible protección contra la oxidación.

De todo lo expuesto se puede afirmar que los resultados presentados en esta memoria confirman satisfactoriamente la hipótesis planteada, ya que se ha podido verificar la gran influencia del origen botánico, nivel de tostado y el uso de la barrica en la liberación de los elagitaninos al vino y en su aroma.

5. GENERAL CONCLUSIONS

1. An optimized method for the ellagitannin analysis has been developed. This method offers some advantages:
    - The use of a fused core column allowed quantification by means of DAD.
    - The sample preparation was simplified to only one single step of solid phase extraction.
    - The method allows the analysis of free gallic and ellagic acids in the same chromatographic run.

2. The botanical origin, the toasting level and the number of times that the barrels have been used have a very major influence on the final ellagitannin concentration, and probably on its sensory impact. Briefly:
    - French oak released higher amounts of ellagitannins than American oak.
    - The higher the toasting level the lower the ellagitannin release.
    - The depletion of the barrels during 1-year of use has a drastic impact on the ellagitannin release.

    The potential impact of ellagitannin concentration in wine should consequently be a criterion by winemakers when selecting the botanical origin, the toasting level and the number of times that the barrels has been used, as with other more common criteria, such as the aromatic profile or the wood's oxygen permeability.

3. No significant differences were found in color, anthocyanin and proanthocyanidin composition between the red wines aged in French and Amercian oak barrels which indicate that botanic origin do not play any relevant role in the evolution of these parameters. There were also no differences between wines aged in light, medium or heavy toasted barrels which indicate that the toasting level does not influence these aspects either. The comparison between red wines aged in new or 1-year-used barrels was not possible because they were wines from different vintage.

4. American oak barrels released significant higher amounts of β-methyl-γ-octalactones, especially its *cis* isomer, than French oak barrels independently of the toasting level. In contrast, no differences were found between both oak species in vanillin and volatile phenols release. The higher the toasting level the lower the β-methyl-γ-octalactones and the higher the volatile phenol release in both oak species. Vanillin increased between light and medium toasting level but decreased in heavy toasted barrels independently of the oak species.

5. It has been possible to establish a significant correlation between the sensory appreciations of coconut and smoked/toasted notes with the theoretical sensory impact of β-methyl-γ-octalactone and volatile phenols respectively. This correlation was not so clear for vanilla and vanillin. In general, the trained tasters clearly preferred wines aged in medium-toasted new barrels for both oak species. Tasters were able to distinguish between wines aged in new American and French oak barrels when the toasting level was light or medium but not when it was heavy. The panelists generally preferred wines aged in French oak, but not in all the cases. Tasters were also able to distinguish between wines aged in new and 1-year-used barrels and generally preferred wines aged in new barrels.

6. It has been demonstrated the existence of a close relationship between the release of ellagitannins by oak chips and the oxygen consumption rate in a model wine solution. In this regard, botanical origin and toast level clearly influenced the amount of released ellagitannins as well as the rate of oxygen consumption.
    - The oxygen consumption rate was higher when French oak was used instead of American oak.
    - The oxygen consumption rate also decreased as the toast level increased.

- The same behavior was observed when oak chips were selected for their potential ellagitannin release.

The contact of wine with oak wood, in barrels or other alternatives, may be an effective enological tool for protecting wine against oxidation and can also be considered useful for reducing sulfites during wine aging.

7. Finally, the ellagitannin potential release of the oak wood should be therefore a major criterion for choosing the barrels because it can have a direct impact on the structure and body of the wine, and also a possible protective effect against oxidation.

6. APÉNDICE

Table Anexo A. Different parameters of the red wine samples aging 12 months

Matrix	Parameter	Oak origin	Barrel age	Light Toast			Medium Toast			Heavy Toast		
Red wine	TPI	French	New Barrel	60.10 ± 0.49	b	β	60.50 ± 1.84	b	β	56.20 ± 0.14	a	α
			1 year barrel	54.30 ± 0.85	a	α	54.40 ± 1.13	a	α	55.20 ± 0.57	a	α
		American	New Barrel	57.90 ± 0.57	a	α	57.70 ± 0.52	a	α	59.40 ± 0.71	b	β
			1 year barrel	54.20 ± 0.85	a	α	54.30 ± 0.71	a	α	54.50 ± 0.28	a	α
	Anthocyanins (mg/l)	French	New Barrel	267 ± 9	a	α	277 ± 8	a	β	269 ± 11	a	α
			1 year barrel	335 ± 5	a	α	336 ± 11	a	α	338 ± 11	a	α
		American	New Barrel	272 ± 9	ab	α	258 ± 6	a	α	279 ± 1	b	α
			1 year barrel	340 ± 2	a	α	328 ± 16	a	α	351 ± 5	b	α
	IC	French	New Barrel	12.10 ± 0.19	a	α	12.00 ± 0.16	a	α	12.20 ± 0.16	a	α
			1 year barrel	12.35 ± 0.01	b	α	12.53 ± 0.01	c	α	12.28 ± 0.06	a	α
		American	New Barrel	12.10 ± 0.05	a	α	12.50 ± 0.01	c	β	12.20 ± 0.01	b	α
			1 year barrel	12.40 ± 0.28	a	α	12.67 ± 0.23	a	α	12.33 ± 0.64	a	α
	L*	French	New Barrel	47.00 ± 0.42	a	α	47.05 ± 0.49	a	β	46.60 ± 0.42	a	α
			1 year barrel	46.20 ± 0.00	b	α	45.60 ± 0.07	a	α	46.10 ± 0.28	b	α
		American	New Barrel	47.10 ± 0.14	c	α	46.20 ± 0.14	a	α	46.85 ± 0.07	b	α
			1 year barrel	46.10 ± 0.78	a	α	45.40 ± 0.71	a	α	46.20 ± 1.77	a	α
	C*	French	New Barrel	49.93 ± 0.25	a	α	49.68 ± 0.06	a	α	49.56 ± 0.18	a	α
			1 year barrel	49.40 ± 0.03	a	β	49.30 ± 0.14	a	α	48.70 ± 0.67	a	α
		American	New Barrel	49.92 ± 0.24	ab	α	50.17 ± 0.18	b	β	49.59 ± 0.14	a	α
			1 year barrel	48.50 ± 0.58	a	α	49.50 ± 0.08	a	α	49.30 ± 0.51	a	α
	h*	French	New Barrel	13.51 ± 0.36	a	α	13.73 ± 0.08	a	α	13.55 ± 0.33	a	α
			1 year barrel	11.60 ± 0.02	b	α	11.20 ± 0.35	a	α	10.80 ± 0.03	c	α
		American	New Barrel	13.87 ± 0.27	a	α	14.36 ± 0.14	b	β	13.74 ± 0.01	a	α
			1 year barrel	11.90 ± 0.41	ab	α	12.20 ± 0.08	b	β	11.20 ± 0.81	a	α
	Proanthocyanidin (mg/l)	French	New Barrel	427.1 ± 57.2	a	α	430.8 ± 73.6	a	α	429.6 ± 67.5	a	α
			1 year barrel	703.3 ± 71.4	a	α	696.7 ± 108.7	a	α	726.9 ± 22.0	a	α
		American	New Barrel	451.6 ± 56.4	a	α	455.8 ± 55.0	a	α	427.3 ± 54.5	a	α
			1 year barrel	691.0 ± 49.5	a	α	712.4 ± 72.2	a	α	738.3 ± 39.2	a	α
	mDP	French	New Barrel	9.49 ± 1.33	a	α	8.14 ± 1.01	a	α	8.77 ± 0.61	a	α
			1 year barrel	6.70 ± 0.18	a	α	7.04 ± 0.16	a	β	6.86 ± 0.16	a	α
		American	New Barrel	8.55 ± 0.55	a	α	9.44 ± 0.94	a	α	8.69 ± 0.38	a	α
			1 year barrel	6.98 ± 0.18	a	α	6.70 ± 0.10	a	α	6.89 ± 0.10	a	α
	%Gal	French	New Barrel	2.05 ± 0.16	a	α	2.28 ± 0.46	a	α	2.01 ± 0.36	a	α
			1 year barrel	3.25 ± 0.01	b	α	3.10 ± 0.03	a	α	3.31 ± 0.01	c	α
		American	New Barrel	2.06 ± 0.11	a	α	1.87 ± 0.17	a	α	2.19 ± 0.35	a	α
			1 year barrel	3.36 ± 0.09	a	β	3.25 ± 0.03	a	β	3.29 ± 0.07	a	α
	%PD	French	New Barrel	13.25 ± 2.86	a	α	11.70 ± 1.71	a	α	12.84 ± 2.03	a	α
			1 year barrel	18.78 ± 0.38	a	α	18.98 ± 0.20	a	α	19.00 ± 0.16	a	α
		American	New Barrel	13.99 ± 1.02	a	α	12.39 ± 1.57	a	α	11.92 ± 1.12	a	α
			1 year barrel	19.19 ± 0.15	b	α	19.25 ± 0.09	b	α	18.82 ± 0.11	a	α

All data are expressed as the average of 2 barrels ± standard deviation. Different letters indicate statistical differences ($p < 0.05$). Roman lower case letters are used to compare toasting levels. Greek letters are used to compare the botanical origin of the oak barrels. CI: Color intensity; L*: lightness value (CiELab coordinates); C*: chroma value (CiELab coordinates); h*: hue value (CiELab coordinates); TPI: total phenolic index; Gal: Galloylation; PD: Prodelphinidines. Total anthocyanins are expressed as mg/L of malvidine-O-3-glucoside.

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