UNIVERSITAT ROVIRA I VIRGILI

IMPORTANCIA DE LOS PROCESOS PRODUCTIVOS EN LA COMPOSICIÓN QUÍMICA, LA ESTABILIDAD Y LAS CARACTERÍSTICAS ORGANOLÉPTICAS DEL VINO

Ana María Mislata Rodríguez

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Ana María Mislata Rodríguez

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Supervisada por
Dra. Montserrat Mestres Solé y Dr. Raúl Ferrer Gallego

UNIVERSITAT ROVIRA i VIRGILI
Dept. de Química Analítica
i Química Orgànica

Campus Sescelades, Edifici N4
C/ Marcel·lí Domingo, 1
43007 Tarragona
Tel. +34 977 559 769
Fax +34 977 558 446
www.quimica.urv.cat/qaqo

Dra. Montserrat Mestres Solé, Profesora Agregada de Química Analítica de la Universitat Rovira i Virgili, y Dr. Raúl Ferrer Gallego, Coordinador Científico del Centro Tecnológico del Vino (VITEC, Tarragona),

CERTIFICAN

que la Tesis Doctoral titulada "**Importancia de los procesos productivos en la composición química, la estabilidad y las características organolépticas del vino**", presentada por **Ana María Mislata Rodríguez** para obtener el grado de Doctora por la Universitat Rovira i Virgili, se ha realizado bajo nuestra supervisión, en el Departamento de Química Analítica y Química Orgánica de esta Universidad y en el Centro Tecnológico del Vino VITEC, y todos los resultados presentados en esta tesis fueron obtenidos en experimentos realizados por dicha alumna.

Tarragona, 1 de septiembre de 2021

Dra. Montserrat Mestres Solé

Dr. Raúl Ferrer Gallego

Este trabajo forma parte de la investigación desarrollada en el Centro Tecnológico del Vino (VITEC), en colaboración con el Grupo de Investigación Sensometría Instrumental (iSens) de la Universitat Rovira i Virgili.

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“Estoy entre aquellos que piensan que la ciencia tiene una gran belleza”

Marie Curie

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Tabla de contenido

Justificación y Objetivo general

Justificación

El vino es un producto de gran importancia en nuestra cultura ya que la viña es uno de los cultivos que ha ido siempre ligada a la civilización mediterránea. De hecho, el vino nos ha acompañado a lo largo de los siglos como símbolo de trabajo ligado a la tierra, de manera que su profundo conocimiento nos ha llevado a la obtención de productos de gran valor. Hoy en día el mercado del vino es una gran industria que implica la participación de distintos agentes, empezando con el agricultor pasando por su comercialización y llegando al consumidor final.

En el año 2020, la organización internacional de la viña y el vino (OIV) estimó una producción mundial de vino de 260 millones de hectolitros (Mhl), siendo ligeramente superior, en torno a un 1 %, respecto al año 2019. Aunque estos valores están algo por debajo de la media de los últimos 20 años, no necesariamente debe considerarse una mala noticia para el sector vitivinícola, dado el contexto actual, en el que el cambio climático y la pandemia de la Covid-19 generan un alto grado de incertidumbre en el mercado mundial. Respecto a la Unión Europea, cabe destacar que durante el mismo año esta tuvo un fuerte incremento del 8 % lo que implicó una producción de 165 Mhl. Esto se debe sobre todo al aumento de producción de Francia y España, dos de los tres grandes países vinícolas, y gracias a unas condiciones meteorológicas muy favorables. Así, en el caso de España, los 40,7 Mhl de vino producido supusieron un alza del 21 %, siendo la tercera potencia a nivel mundial.

Respecto al comercio internacional de vino, al contrario también de lo que se esperaba en un contexto de pandemia, se contrajo de forma muy moderada en 2020. Concretamente la disminución en volumen sólo fue de un 1,7 %, hasta los 105,8 Mhl, aunque, en valor, el retroceso fue mucho más pronunciado al caer este en un 6,7 %, hasta los 29.600 millones de euros. Entre los principales países exportadores, según la OIV, todos disminuyeron sus ingresos. Sin embargo, ese descenso exportador fue menos pronunciado en España ya que sólo cayó un 3,4 %, hasta 2.626 M€, siendo, además, el primer exportador en volúmenes con algo más de 21 millones de hectolitros (2019), y los terceros mayores exportadores del mundo en valor, con cerca de 2.700 millones de euros exportados (2019).

Aunque como ya se ha dicho, todas estas variaciones en las producciones y valores económicos están muy relacionados con la situación económica mundial, en cada momento hay que tener en cuenta que, como en cualquier otro mercado, el sector agroalimentario también se rige por la ley de la oferta y la demanda. Por lo tanto, resulta evidente que cada vez se dedicarán más esfuerzos para satisfacer los gustos y necesidades de los consumidores quienes demandan productos de mejor calidad con una preocupación creciente por el efecto de su consumo sobre la salud y por las repercusiones del proceso productivo sobre el medio ambiente.

Por lo que al sector enológico se refiere, a lo largo de los años se ha ido investigando sobre los diferentes procesos productivos realizados desde el cultivo de la uva en el viñedo y pasando por las diferentes etapas de vinificación en la bodega (fermentación, embotellado, crianza y almacenamiento). De esta manera, se ha conseguido el desarrollo de nuevas tecnologías y nuevos procesos en el ámbito enológico, con el fin de obtener mejores medios para llegar a conseguir una mayor gama de productos enológicos los cuales presenten mejores cualidades y calidades como producto final. Además, hoy en día, es muy importante aplicar toda esta investigación no solo hasta la obtención del vino, sino que es fundamental aplicar también un plan de gestión de residuos de los subproductos generados durante el proceso.

Por todo ello, a pesar de los años de investigación dentro del sector enológico, en la actualidad, es necesario seguir profundizando y adquiriendo conocimiento para establecer las bases científicas sobre la influencia que tienen ciertas prácticas agronómicas y tecnológicas que se realizan durante el proceso de producción de la uva y de la elaboración del vino para una mejora de las características físico-químicas y sensoriales del producto final.

Objetivo general

El objetivo general de esta tesis fue profundizar en el conocimiento de las modificaciones sufridas por la composición química de la uva y del vino derivadas de diferentes prácticas realizadas durante el proceso de producción. Concretamente se evaluaron los cambios en la composición aromática y fenólica del vino, así como su impacto en la estabilidad y en las características organolépticas del mismo. Además, con el fin de ampliar el conocimiento a subproductos de la industria enológica, también se evaluó la composición aromática y bioactiva de subproductos derivados del corcho.

Para ello, se realizó un estudio analítico exhaustivo, fundamentalmente de la composición aromática de uvas, de vinos tranquilos (blancos, tintos, rosados) y espumosos, derivada de diferentes prácticas o procesos, en búsqueda de una mejora de la calidad. Además, se realizó un seguimiento de otros compuestos de interés relacionados con la composición química básica, así como de su composición más compleja (composición fenólica, metales, proteínas, aminoácidos, espumabilidad, actividad antioxidante, etc.). En todos los casos, se buscaron correlaciones entre los cambios observados en la composición química y la estabilidad y calidad sensorial de los vinos.

Esta tesis doctoral pretende dar una visión global de la producción del vino de manera que empieza con estudios relacionados con tratamientos en campo, sigue con tratamientos durante la elaboración, embotellado y envejecimiento del vino para acabar con tratamientos de reaprovechamiento de los subproductos del corcho obtenidos en bodega. Todas estas líneas de actuación comprenden estudios de alto interés actual para la industria enológica realizados en VITEC en colaboración con diferentes empresas del sector auxiliar y bodegas de toda España.

Introducción

La obtención de un vino de calidad está directamente relacionada con el control de los procesos productivos (de la uva y del vino) dada la influencia que estos tienen sobre su composición química, su calidad sensorial e incluso sobre su estabilidad a lo largo del tiempo. Por todo ello, hay que tener en cuenta no sólo las características y composición de la uva si no que habrá que saber elegir las tecnologías adecuadas durante la vinificación, la crianza y el correcto almacenamiento en botella, con el fin de conseguir un producto de máxima calidad.

Ello se debe a que la uva posee muchísimos compuestos químicos que pueden ser cedidos al vino, pero los procesos tecnológicos empleados en bodega para la obtención del vino, pueden modificar estos compuestos de manera que interesa buscar aquellas tecnologías que proporcionen la máxima calidad al producto final ya sea manteniendo los compuestos de interés o modificando/eliminando los que no lo sean. Después, durante la crianza de los vinos, ya sea en depósito, barricas o botellas, hará falta controlar la modificación de esas cualidades químicas para conservar o incluso mejorar las propiedades organolépticas. Y finalmente, no hay que olvidar el control del almacenamiento en botella hasta su consumo, donde será muy importante la elección del embalaje (color de botella, tipo de cierre, etc) para conseguir una correcta conservación de manera que factores externos como la temperatura, humedad y la luz, no afecten a la calidad del vino final.

1. Composición y calidad de la uva

1.1. Calidad de la uva

La uva es la materia prima de la cual se parte para la elaboración del vino y se debe de conseguir cosechar en un estado óptimo para poder obtener un vino de calidad.

El estado óptimo de la uva dependerá del tipo de vino que se quiera elaborar, pues las uvas destinadas a vinificar vinos tranquilos (tinto, rosado, blanco) no presentarán las mismas características que las destinadas a elaborar vinos espumosos, dulces o generosos, entre otros. Del mismo modo, el concepto de calidad de la uva no es el mismo para la elaboración de vinos jóvenes que para vinos crianza. Por tanto, una primera valoración de la calidad de la uva está sugestionada según la tipología de vino que se quiera elaborar.

Sin embargo, independientemente del tipo de vino a elaborar, hay un parámetro que indudablemente afectará a la calidad de la uva y este es su estado sanitario. El estado sanitario de los racimos se suele ver afectado por las enfermedades o plagas, así como por las malas condiciones meteorológicas las cuales pueden causar problemas en los racimos o influenciar su crecimiento.

Otro parámetro determinante para obtener un vino de calidad es el estado de madurez de la uva la cual se puede considerar desde el punto de vista de la madurez tecnológica y por otra parte del de la madurez fenólica. La madurez tecnológica se basa en la medida de los azúcares y ácidos de la pulpa de las bayas. La relación óptima entre ambos indica su momento óptimo de cosecha. Por otra parte, también hay que tener en cuenta la madurez fenólica, la cual es mucho más difícil de alcanzar y, sobre todo, imposible de evaluar mediante técnicas sencillas y rápidas. La madurez fenólica se alcanza cuando los compuestos fenólicos como los taninos o antocianos, llegan a su estado óptimo para aportar las mejores propiedades sensoriales de color y sabor al vino final.

Así pues, dado que ambos tipos de madurez son igualmente importantes, lo idóneo sería que la concentración óptima de antocianos y taninos en las uvas (madurez fenólica) coincidiera con el valor óptimo de la relación entre azúcares y acidez total (madurez tecnológica) aunque esta situación no siempre es posible ya que, a veces, una madurez se da de manera anterior o posterior a la otra.

Por último, hay que tener en cuenta también que cada variedad de uva posee unas características que la diferencian del resto por lo que, dependiendo del vino que queramos elaborar, habrá que escoger la variedad más adecuada en cada caso. Dichas características, a su vez, dependen del denominado terruño (en francés, *terroir*) el cual hace referencia a las características climáticas, del suelo, de altitud, de disposición de los viñedos e incluso a las técnicas locales de cultivo de la vid y elaboración del vino.

1.2. Estructura y composición de la baya

El racimo de uva está constituido por la parte leñosa, el raspón y los peciolos, y por los granos de uva o bayas que suelen presentar un aspecto carnoso y redondeado u ovalado. Las bayas están formadas por tres partes bien diferenciadas: el hollejo o la piel que forma una película exterior; la pulpa, la cual rellena el interior de la baya y de la que principalmente se extrae el mosto; y las semillas o pepitas que se encuentran en la parte más interna de la baya (figura 1). Cada una de las partes que conforman la baya contribuye de manera diferente a la composición del mosto y, por lo tanto, del vino, debido a su diferente naturaleza y composición química.

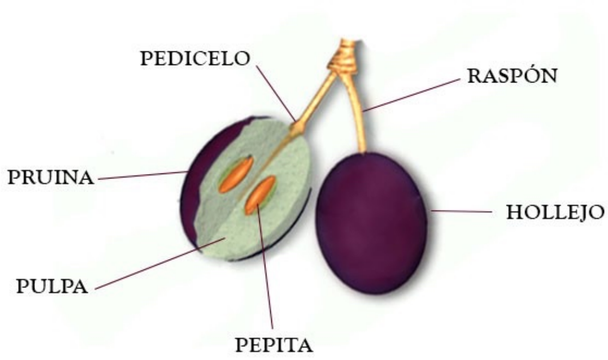


Figura 1. *Partes estructurales de un racimo de uva*.

Las semillas son el elemento de la vid encargado de perpetuar la especie. Las semillas representan alrededor de un 6 % del peso del grano de uva. Los principales componentes de las semillas de uva son: agua, carbohidratos, aceites, compuestos fenólicos, compuestos nitrogenados, minerales y ácidos grasos, siendo los aceites y los compuestos fenólicos los de mayor interés debido a sus propiedades beneficiosas para la salud (1).

La pulpa es la parte más voluminosa del grano de uva y es en este tejido donde se acumulan las sustancias sintetizadas por la vid. Su principal componente es el agua la cual actúa como disolvente del resto de compuestos de la uva. Además de aportar el agua al mosto y al vino, que constituye entre un 80-90 % del volumen total, la pulpa también aporta el resto de componentes mayoritarios del metabolismo primario como son los azúcares y los ácidos orgánicos. Los azúcares son los compuestos más abundantes del mosto (100-350 g/L), siendo los mayoritarios la glucosa y la fructosa. Respecto a los ácidos, los más importantes son los ácidos málico y tartárico que representan el 94 % aproximadamente del total de los ácidos orgánicos de la planta, aunque existen otros en cantidades mucho menores como el succínico, láctico, acético y cítrico. Cabe destacar que la cantidad de los distintos ácidos orgánicos, la relación málico/tartárico y la cantidad de potasio, determinan los valores de acidez y pH, (2). Además, el potasio es un elemento mineral muy importante no sólo por su efecto en el pH, sino también porque es fundamental en el desarrollo y crecimiento de las bayas. Por otro lado, hay que tener en cuenta también que los polifenoles que contiene la pulpa son fundamentalmente de tipo no flavonoides, siendo los ácidos cinámicos y benzoicos los más abundantes.

Por último, la piel u hollejo de la baya que tiene como función principal proteger los tejidos vegetales del fruto y las semillas de las agresiones externas, representa aproximadamente del 8 al 20 % del grano de uva (3). Está formada por tres capas que, desde el exterior al interior, son: cutícula, epidermis e hipodermis. La cutícula está recubierta de una capa cerosa llamada pruina, que le otorga a la uva un aspecto mate, pulverulento. Su función es proteger los granos de uva de las inclemencias meteorológicas, regular la evaporación y ejercer de barrera frente a los microorganismos. Por su parte, las zonas de la epidermis e hipodermis están formadas por unas 10 capas de células, las cuales van disminuyendo gradualmente su tamaño de manera que sus capas más internas se confunden con la pulpa.

Con respecto a la composición química, en el hollejo de las bayas se encuentra las proteínas que sirven como fuente de nitrógeno para los procesos de fermentación y que, posteriormente, pueden persistir en el vino aportándole cuerpo y estabilidad. El nitrógeno también puede encontrarse en la uva en forma de aminoácidos los cuales son fundamentales para el funcionamiento de la levadura y para la producción de ciertos aromas. De entre los diferentes tipos de aminoácidos cabe destacar la importancia de los azufrados, como la cisteína o la metionina, por su influencia en la generación de aromas de reducción que pueden constituir defectos aromáticos muy notorios en los vinos. Por otra parte, en las dos capas más internas del hollejo se ubican la mayor parte de los compuestos fenólicos y sustancias aromáticas que contiene la uva (4). Entre todos ellos, hay que destacar por un lado a los compuestos fenólicos solubles, los cuales contribuyen al color y al sabor del vino, y por otro lado a los precursores de los compuestos aromáticos que contribuyen al sabor y al aroma (2, 5). Estas dos familias de compuestos son especialmente interesantes a tener en cuenta cuando se evalúa la calidad del vino ya que tienen una incidencia directa en sus características sensoriales.

Entre los compuestos fenólicos solubles se distinguen los flavonoides y los no flavonoides (6). En general, los compuestos flavonoides se encuentran en las pepitas, hollejos y raspones mientras que los no flavonoides se localizan en todas las partes del racimo, especialmente en la pulpa. Los antocianos, pertenecen a la familia de los flavonoides y se localizan principalmente en el hollejo. Estos compuestos son los responsables de proporcionar el color de la uva y del vino tinto y rosado (7). Por ello, toda elaboración de vinos tintos requiere la maceración de los mostos junto con los hollejos de las uvas tintas para extraer estos pigmentos. Otros flavonoides importantes son los flavanoles o catequinas, localizados en las partes verdes de la uva, los cuales aportan el sabor amargo y astringencia al vino y por lo tanto contribuyen a las sensaciones organolépticas en boca (6, 8). Cabe señalar que cuando los flavanoles o catequinas polimerizan dan lugar a los denominados taninos condensados o proantocianidinas con efectos organolépticos importantes en los vinos (9).

Por su parte, los compuestos aromáticos de la uva contribuyen al denominado aroma varietal o aroma primario de los vinos. Estos compuestos pueden encontrarse en forma libre o ligada. Esta diferencia estructural es especialmente remarcable cuando hablamos de compuestos aromáticos ya que si aparecen en la forma libre son percibidos por el olfato directamente pero cuando lo hacen en la forma ligada o combinada, están en forma de precursores aromáticos y no son percibidos aromáticamente (no son odorantes). Sin embargo, estos últimos son capaces de transformarse en aromas libres durante la elaboración de los vinos por simple ruptura de un enlace químico lo cual implica la aparición de sus aromas característicos en el vino (3, 10).

Los aromas varietales pueden agruparse atendiendo a distintas propiedades, pero desde el punto de vista estructural y composición química, éstos se pueden dividir en seis familias: alcoholes, aldehídos, compuestos de 6 átomos de carbono comúnmente denominados compuestos C6, los derivados fenólicos o fenoles, los terpenoles, y los C_{13}-norisoprenoides.

Los alcoholes, aldehídos y compuestos C6 se forman a partir de los ácidos grasos linoleico y linolenico cuando las uvas entran en contacto con el aire, y su formación es catalizada por las enzimas lipoxigenasa, peroxidasa y alcohol deshidrogenasa de manera que, cuando se realiza el prensado de la uva, estos compuestos son liberados en los mostos y aumentan su concentración considerablemente. Los compuestos C6 y algunos aldehídos son, en gran parte, los responsables de los aromas herbáceos de mostos y vinos aunque, durante la fermentación, pueden ser transformados en los alcoholes correspondientes que son menos aromáticos (11). Por lo que a los fenoles volátiles respecta, estos se originan en la transformación de determinados ácidos fenólicos presentes en la uva, como el eugenol o el isoeugenol. Estos compuestos se asocian a descriptores aromáticos florales.

Sin embargo, dentro de las seis familias citadas, los terpenoles y C_{13}-norisoprenoides son los que más contribuyen al aroma varietal. Estas familias están formadas a su vez por diferentes subfamilias dependiente del número de moléculas de carbono y oxigeno que contengan. Así, los monoterpenos son los terpenoles más abundantes y se pueden encontrar en la uva en forma libre o en forma glicosilada (12). Hasta la fecha, han sido identificados alrededor de 50 compuestos monoterpenos, siendo de los más importantes aquellos encontrados en la variedad Muscat debido a su gran poder odorante, como son el linalol, geraniol, nerol, citronelol, α-terpineol y terpinen-4-ol. En general los terpenos son los compuestos responsables de los aromas florales y frutales característicos de las distintas variedades de uva

(13, 14), pues se encuentran presente en todas ya sea en mayor o menor medida (15). En cuanto a los C_{13}-norisoprenoides presentes en la uva, estos son tetraterpenos formados por 13 moléculas de carbono. Estos compuestos se caracterizan por ser muy aromáticos, como, por ejemplo, la β-damascenona, y β-ionona que presentan olores característicos de frutas tropicales o violetas respectivamente. La concentración de compuestos norisoprenoides, aumenta durante la fase de crecimiento de la baya y disminuye hasta la madurez (16). Sin embargo en el vino, estos aromas se manifiestan fundamentalmente durante su envejecimiento, siendo los responsables de que variedades no aromáticas den lugar a vinos de alta calidad (17).

Si bien la mayor parte de los aromas primarios son comunes en casi todas las variedades de uva y sólo varían sus proporciones, en ocasiones puede existir una gran variabilidad debida a la influencia de determinados factores como el tipo de suelo, las condiciones climáticas durante el periodo de maduración, e incluso los tratamientos realizados en el viñedo. Si a ello le añadimos el hecho de que cada compuesto aromático varietal evolucionará de forma diferente a lo largo de los diferentes procesos de vinificación y crianza, nos encontramos ante un amplio abanico de posibilidades con respecto a la contribución real de cada uno estos compuestos en el perfil aromático del vino final.

2. Procesos de vinificación

2.1. Elaboración de vinos tranquilos

La elaboración del vino o vinificación implica un conjunto de procesos para transformar el mosto obtenido de las uvas. El proceso principal en esta transformación es la fermentación alcohólica, la cual implica una reacción química en la que los azúcares son transformados fundamentalmente en alcohol y dióxido de carbono. Sin embargo, al mismo tiempo ocurren muchísimas reacciones secundarias que, inevitablemente, también influirán en la composición y calidad del vino final. Todos estos procesos se verán afectados por la tecnología empleada durante la vinificación y el momento de su aplicación. Por ello, actualmente se están llevando a cabo nuevos estudios para profundizar en el conocimiento y mejorar el control de las operaciones tecnológicas de vinificación de manera que se pueda obtener una gran variedad de vinos que se adecuen a las distintas tendencias y preferencias del consumidor, consiguiendo de esta forma una amplia gama de productos que satisfagan la calidad esperada en cada caso.

A continuación, se detallan las diferentes etapas de los procesos de vinificación de vinos tranquilos (figuras 2, 3 y 4):

Vinificación Vino Tinto

1. Despalillado
2. Estrujado
3. Encubado: maceración y Fermentación alcohólica
4. Prensado
5. Descube y F. malolactica
6. Clarificación
7. Estabilización

a) 8. Filtración
   9. Embotellado

b) 8. Crianza en barricas
   9. Embotellado
   10. Crianza en botella

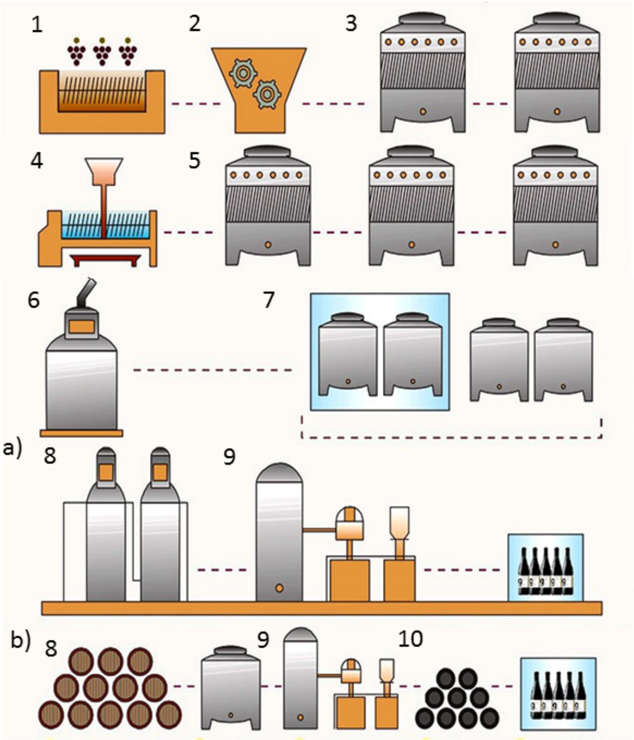


***Figura 2**. Etapas del proceso de elaboración del vino tinto.*

Vinificación Vino Rosado

1. Despalillado
2. Estrujado
3. Encubado: maceración corta
4. Prensado
5. Descube y F. alcohólica
6. Clarificación
7. Filtración
8. Estabilización
9. Embotellado

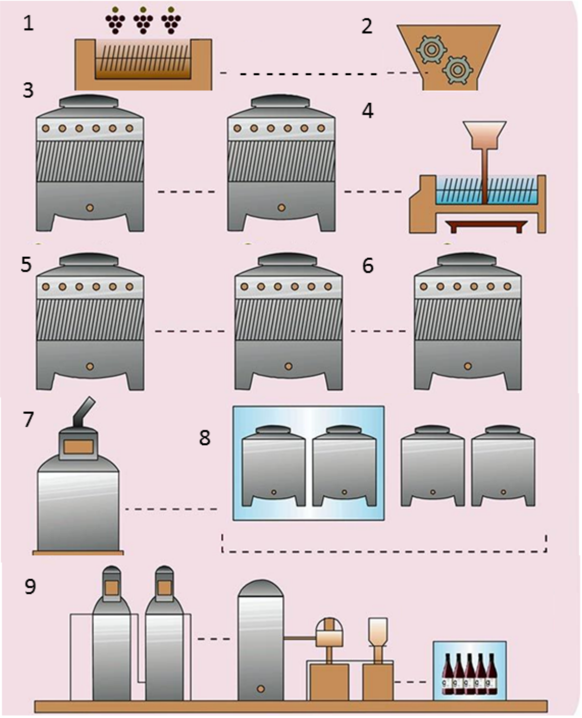


***Figura 3**. Etapas del proceso de elaboración del vino rosado.*

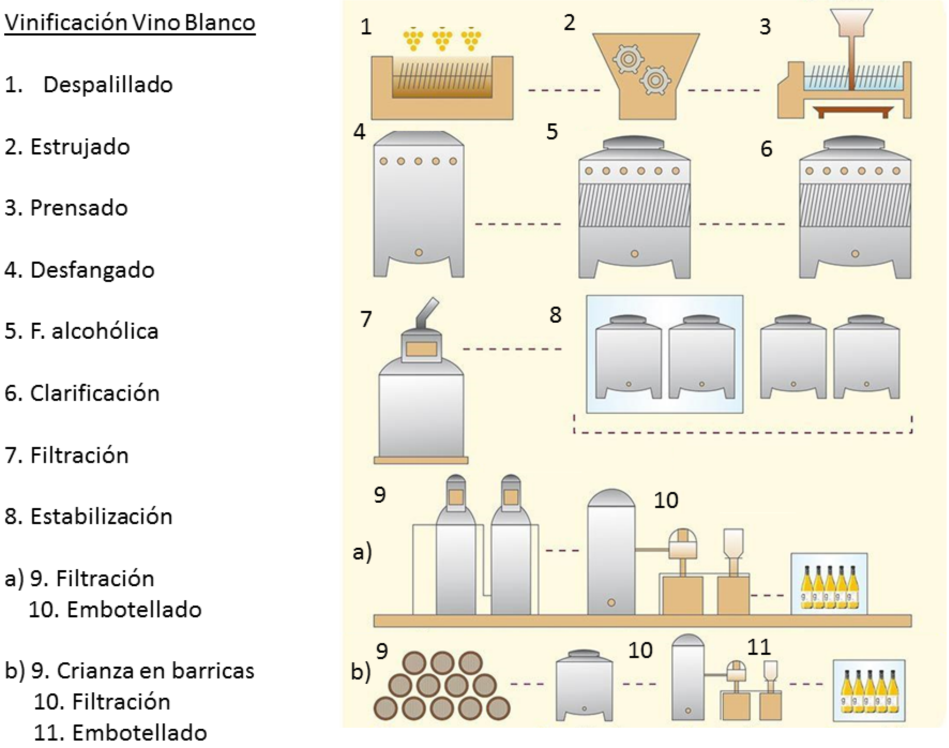


Figura 4. *Etapas del proceso de elaboración del vino blanco.*

2.1.1. Despalillado

La función principal del despalillado consiste en la separación de toda la parte leñosa del racimo de uva, principalmente de los raspones, aunque también de hojas, pedúnculos u otro material que haya podido caer junto a los racimos en la vendimia. El despalillado es una práctica habitual en la elaboración de vinos tranquilos, principalmente porque cuando los raspones son triturados junto a las uvas, estos suelen ceder al mosto compuesto con carácter astringente y sustancias con carácter aromático vegetal. Por tanto, el despalillado supone una mejora organoléptica en los mostos y vinos, evitando sensaciones astringentes, amargos, vegetales y herbáceos al mismo tiempo que reduce la oxidación de los vinos, proporcionándoles finura y redondez (18).

2.1.2. Estrujado

Una vez despalillada la uva, los granos se pasan por una máquina estrujadora o pisadora. Este proceso consiste en romper la piel de la uva para liberar el mosto del interior de la baya. En este proceso se favorece la difusión de los constituyentes odorantes de la piel de la baya a los mostos (19). Sin embargo, el estrujado no debe ser demasiado exhaustivo, con el fin de evitar que se rompan las semillas de las uvas, algo que podría aportar efectos negativos como amargor y astringencia al mosto (18).

2.1.3. Encubado: maceración y fermentación

En la elaboración de vino después del estrujado se realiza el encubado del mosto. Consiste en llevar el mosto a los depósitos para que se lleve a cabo la maceración y la fermentación.

La maceración es un proceso que se inicia en el momento en que el mosto entra en contacto con los hollejos de la uva. Este proceso es de gran importancia debido a que a través de este contacto con los hollejos el mosto va adquiriendo su color, además de otros compuestos como antocianinas, taninos y aromas, que aportaran estructura y cualidades organolépticas al vino. En el caso del vino tinto, el proceso de maceración dura varios días, ya que se encadena con el arranque de la fermentación alcohólica. Sin embargo, en el caso del vino blanco este proceso suele ser inexistente, o durar tan solo unas horas (entre 12 y 16 h) y se produce a una temperatura controlada de unos 16 ºC, para evitar el inicio del proceso de fermentación. En el caso de los rosados, el tiempo de contacto con los hollejos suele ser mayor (unas 24 h) y también se mantiene la maceración a baja temperatura.

La fermentación alcohólica es la etapa principal en la transformación del mosto en vino y de hecho, la Organización Internacional de la Viña y el Vino (OIV) define el vino como el producto obtenido exclusivamente por fermentación alcohólica, total o parcial, de uva fresca, estrujada o no, o de mosto de uva. Durante la fermentación alcohólica los azúcares del mosto, principalmente glucosa y fructosa, son consumidos por levaduras convirtiéndolos en alcohol, principalmente en etanol. Además de esta reacción principal, las distintas rutas metabólicas que se activan dan lugar a una serie de reacciones secundarias que dan como resultado una gran cantidad de metabolitos. Estos metabolitos van a constituir la estructura del vino y van a ser en gran parte responsables de las características sensoriales y de la calidad de los vinos (color, aroma, gusto) (20). Respecto a la composición aromática, hay que destacar que durante el proceso de fermentación se generan los aromas secundarios o fermentativos. Estos compuestos constituyen la base aromática del vino, aportando las principales notas frutales, florales y en algunas ocasiones lácticas y dulces, que definen su calidad sensorial. Los aromas secundarios provienen del proceso de fermentación alcohólica pero también pueden provenir de la segunda fermentación o fermentación maloláctica, y suelen variar en función del género, especie y cepa de las levaduras empleadas, y de la temperatura de fermentación de los vinos. Por tanto, es muy importante realizar un control de la fermentación para que ésta se desarrolle de manera óptima. Para ello, se ha de conseguir un buen arranque, un buen desarrollo y un buen seguimiento de la fermentación, ya que cualquier desviación durante la misma puede producir alteraciones en la calidad de los productos (18).

La fermentación puede llevarse a cabo mediante forma espontánea con levaduras indígenas propias de la uva lo cual suele llevar a resultados bastante aleatorios, o mediante la adición de levadura seca seleccionada (LSA). Esta última proviene de aislar y seleccionar determinadas cepas de levaduras a partir de fermentaciones espontáneas, dando excelentes resultados (19). Desde ya hace años, el uso de levaduras secas activas (LSA) permite al enólogo disponer de una población viable, muy elevada y controlada en todo el proceso de fermentación, mejorando el control de la calidad organoléptica que ofrece la flora microbiana y evitando la aparición de posibles alteraciones.

En enología, las levaduras más importantes son las pertenecientes al género *Saccharomyces*, las cuales son las grandes responsables de transformar el mosto en vino, principalmente las especies *Saccharomyces cerevisiae, Saccharomyces bayanus* o *Saccharomyces uvarum*. Sin embargo, la especie de levadura comúnmente empleada en la elaboración del vino es la *Saccharomyces cerevisiae* por garantizar ésta la mejor calidad y estabilidad de los vinos. Se ha visto que este género sintetiza mayores cantidades de ésteres superiores que juegan un papel positivo en la calidad del aroma secundario (21).

En la actualidad, existen muchos estudios que demuestran que el uso mixto de especies *Saccharomyces* y no-*Saccharomyces*, pueden mejorar el proceso de vinificación, la calidad y diferenciación de los vinos (22, 23). Entre las levaduras no-*Saccharomyces*, los géneros más estudiados son *Candida, Metschnikowia, Delbrueckii, Pichia, Torulaspora, Starmerella* y *Lachancea*, porque ayudan a mejorar las características organolépticas de los vinos como color, aroma y características sensoriales. El empleo de levaduras no-*Saccharomyces* en las primeras etapas de la fermentación mejora la calidad del aroma del vino debido a los productos metabólicos resultantes, como terpenoides, ésteres, glicerol, y ácidos orgánicos, entre otros. Por lo tanto, debido a que las levaduras no-*Saccharomyces* producen vinos con muchas características distintivas, hoy en día existen muchas más LSA comerciales de diferentes especies, como *T. delbrueckii*, *M. pulcherrima* y *P. kluyveri*. Algunos estudios apuestan por la tecnología del cultivo mixto durante la fermentación para obtener buenos perfiles aromáticos en los vinos (24). Todos estos estudios demuestran que la elección de la levadura juega un papel muy importante en la composición organoléptica final del vino.

Por último, no hay que olvidar que durante la evolución de la fermentación alcohólica es indispensable realizar un seguimiento y control de los siguientes parámetros: densidad, temperatura del mosto, acidez volátil en caso de parada o ralentizado, y azúcares residuales al final de la fermentación (18).

En el caso de la elaboración de vinos tintos, y en ciertos casos más puntuales en la de blancos y rosados, después de la fermentación alcohólica se realiza la fermentación maloláctica. Esta fermentación es necesaria para la crianza y envejecimiento de los vinos tintos. Sin embargo, su desarrollo en vinos blancos se realiza para suavizar la acidez en ocasiones concretas. La fermentación maloláctica consiste en la transformación del ácido málico en ácido láctico mediante la función de las bacterias lácticas (25). La actividad bacteriana disminuye la acidez del vino, realzando su aroma y complejidad. El ácido láctico resulta más suave que el málico, por lo que tiene lugar un aumento de la untuosidad, alcanzando el vino mayor redondez y cuerpo. Sin embargo, también puede disminuir el carácter afrutado, y pueden aparecer ciertas desviaciones organolépticas como olores demasiado lácticos o de mantequilla (26).

2.1.4. Descube, escurrido y prensado

El descube consiste en separar el vino, en la mayoría de ocasiones mosto ya fermentado, del contacto de los hollejos. Para ello se traspasa el vino mediante un trasiego a otro depósito. En el caso de los vinos blancos, en general estos no son macerados con las pieles, sino que ya son fermentados sin los hollejos. Por tanto, en la elaboración de vino blanco se realiza el escurrido, que consiste en la separación del mosto liberado por el estrujado.

El prensado tiene gran importancia sobre el vino final. Es la operación cuyo objetivo es la extracción de mosto por efecto de la presión. Después del descube, la pasta extraída del depósito debe llevarse a la prensa para obtener el vino que aún contienen. El líquido que se saca del sangrado es el de mejor calidad, el mosto 'lágrima' o mosto 'flor', que se destinará al mejor de los vinos. Durante el prensado se obtienen distintas fracciones de mostos que presentaran diferentes calidades con diferencias en su composición química. Por tanto, la calidad del vino dependerá del tipo de prensa y del modo operativo empleado. Cuanto mayor es la presión efectuada, mayor es la extracción mecánica de las sustancias procedentes de las pepitas y del hollejo, por lo que peores cualidades químicas y organolépticas presentará.

2.1.5. Crianza

Otra de las etapas de mayor importancia en la elaboración de un vino es el proceso de envejecimiento o de crianza. Para ello, el vino obtenido durante los pasos anteriores es trasvasado a barricas de roble. La madera de roble es seleccionada teniendo en cuenta su dureza, permeabilidad y porosidad. Las barricas también son seleccionadas dependiendo del tipo de tostado que presente su madera. Tanto el tipo de roble utilizado, siendo el más habitual el francés o americano, como el grado de tostado o la cantidad de usos que hayan tenido las barricas a lo largo del tiempo, modificarán el carácter del vino. Durante este tiempo de crianza, el vino consigue una perfecta maduración, afinando sus características organolépticas. Durante este envejecimiento, tienen lugar diversos procesos mediante los cuales el vino gana complejidad y estabilidad. En la estancia en barrica se producen una serie de procesos físico-químicos en el vino relacionados con la microoxigenación. Esta entrada de oxígeno de manera moderada es muy importante para que se den las reacciones de polimerización y combinación entre antocianos y taninos que conllevan a la estabilización del color y a la corrección de la astringencia. Por otra parte, durante la crianza en barrica de roble el vino adquiere notas aromáticas que durante la cata podremos identificar como tostadas, ahumadas, avainilladas y, cómo no, notas amaderadas. Esto es debido a que el roble aporta gran cantidad de compuestos volátiles, tanto nativos de la madera como formados durante la evolución del vino en la misma. Estos compuestos pertenecen al grupo de los aromas terciarios o de evolución, los cuales pueden mejorar la calidad y complejidad organoléptica del vino que solo se consiguen mediante el envejecimiento.

2.1.6. Clarificación, estabilización y filtración

Después de la fermentación y/o crianza del vino es necesario clarificar, estabilizar y filtrar el vino con el fin de eliminar las posibles alteraciones de la limpidez y microbiológicas, para obtener un vino de calidad.

El proceso de clarificación consiste en arrastrar al fondo del envase o depósito todos los compuestos sólidos flotantes, con el fin de conseguir limpiar el vino. La clarificación se realiza normalmente de forma natural manteniendo al vino a baja temperatura, y dejando que vaya precipitando de forma estática poco a poco a lo largo del tiempo. Sin embargo, para acelerar este proceso se emplea la incorporación de determinados productos capaces de arrastrar las sustancias en suspensión de los vinos. A este proceso también se le denomina encolado, que consiste en disminuir la turbidez e inestabilidad de los vinos mediante la adición de sustancias que favorezcan la floculación o sedimentación de las partículas en suspensión. De esta forma el vino presentará un aspecto menos turbio. Entre todos los productos clarificantes se suelen utilizar de tres tipos: los proteicos (gelatina, cola de pescado, albúmina de huevo y caseína), los minerales (bentonita y sílice coloidal) y los sintéticos (PVPP), estos últimos suelen ser empleados en vino blanco.

Sobre todo, es muy importante realizar una correcta elección del tipo de cola y de dosis a utilizar ya que puede tener efectos negativos sobre las características del producto final.

Para evitar estos posibles problemas, se debe emplear la cola que mejor se adapte al tipo de vino que se quiere clarificar y estabilizar, evaluando mediante análisis sensorial la cola más apropiada a la calidad del producto final, pues el uso excesivo de colas puede eliminar muchos aromas (27).

Mediante la clarificación ya se consigue una estabilización del vino respecto a su turbidez. Sin embargo, existen ciertos compuestos como los cristales de tartratos que pueden precipitar si los vinos no son previamente estabilizados. La estabilización del vino frente a las sales del ácido tartárico puede llevarse a cabo mediante la estabilización por frío, la adición de coloides protectores, y la eliminación de los cationes responsable (K^+ y Ca^{+2}).

La estabilización por frío ha sido y es el procedimiento más utilizado por la mayoría de las bodegas. Esta técnica consiste en la refrigeración del vino a temperaturas próximas a su punto de congelación durante un período aproximado de una semana (28, 29). Esta técnica basada en el enfriamiento del vino es muy efectiva frente al bitartrato potásico, pero no garantiza la estabilización frente al tartrato neutro de calcio. Es por ello que para asegurar la completa estabilización es necesario realizar tests conductimétricos que nos verifiquen la estabilidad. Económicamente este tratamiento de estabilización supone un coste muy elevado y además presenta una duración excesiva de tiempo.

La segunda técnica mencionada para estabilizar el vino es mediante la adición de substancias que inhiban el crecimiento de los cristales. Los productos más utilizados son el ácido metatartárico (MTA) y más recientemente la carboximetilcelulosa (CMC). Ambos aditivos son muy efectivos en la estabilización, sin embargo, hay que destacar que también presentan algunos inconvenientes. Por una parte, el ácido metatartárico se hidroliza progresivamente lo que hace que su efecto protector desaparezca con el tiempo (30). Sin embargo, aunque la CMC sí que mantiene su efecto protector durante largo tiempo, puede presentar ciertos inconvenientes. En primer lugar, no puede ser utilizada en vinos tintos dado que precipita en presencia de taninos. Es por ello que la OIV solo autoriza su uso en vinos blancos. En segundo lugar, el uso de CMC comporta un mayor riesgo de quiebra proteica en los vinos blancos lo que obliga a realizar una clarificación exhaustiva con bentonita si después se le va añadir CMC (31). Y por último, la adición de CMC puede dificultar la filtración del vino si no se emplea de forma adecuada según sus indicaciones de uso. Además, del empleo de MTA y CMC, también se ha propuesto el uso de manoproteínas (MP) para la estabilización tartárica del vino, aunque su efectividad depende en gran medida del tipo de vino a tratar. Más recientemente, ha sido aprobado el uso de poliaspartato de potasio (KPA) para la estabilización del vino frente a las cristalizaciones de las sales del ácido tartárico. Su efecto estabilizador es similar al de MTA o al de la CMC con la diferencia y ventaja que no interacciona con los taninos y por tanto puede aplicarse a vinos tintos y rosados. Además, su eficacia es duradera y no complica el proceso de filtrado de los vinos (32).

Y por último, la tercera técnica de estabilización es la técnica de intercambio iónico, basada en el empleo de resinas de intercambio catiónico de carácter fuerte regeneradas en ciclo ácido (33). En la resina, los iones potasio, principales causantes de la precipitación de sales de bitartrato, son intercambiados por iones hidrogeno, por lo que en el vino desaparece una sal muy inestable (el bitartrato potásico) y aparece el ácido tartárico, que a su vez causa el efecto de una bajada de pH. Este incremento de la acidez permite una primera corrección lo cual es útil sobre todo en vinificaciones con escasa acidez, como puede ser el caso de las obtenidas en climas cálidos. Actualmente está autorizado su uso por la OIV aunque sólo bajo unas condiciones concretas que son: que las resinas se regeneren con un ácido, que el tratamiento se emplee en combinación con un tratamiento por frío, se limita la intensidad del tratamiento a bajar el pH como máximo 0,3 unidades, y que el pH final del vino sea igual o superior a 3,0. Aunque la implementación en bodega de esta técnica pueda conllevar a una primera gran inversión, una de sus ventajas es su reversibilidad, por lo que un intercambiador de este tipo puede regenerarse y actuar indefinidamente (34).

2.2. Elaboración de vinos espumosos mediante el método tradicional o *Champenoise*

Los vinos espumosos naturales y los vinos gasificados son vinos con gas disuelto. Por una parte están los vinos gasificados que son aquellos a los que se les añade artificialmente el gas del mismo modo que se hace con los refrescos gaseosos. Por otra parte, encontramos los denominados vinos espumosos naturales que son aquellos cuyo gas tiene una procedencia endógena como consecuencia de su especial elaboración que implica una segunda fermentación realizada en el envase (depósito o botella). Dado que el envase donde se da este proceso está herméticamente cerrado, el dióxido de carbono que se produce no puede escapar y se disuelve en el líquido. Concretamente esta segunda fermentación se lleva a cabo utilizando un vino al cual se le añade de nuevo levadura y una cantidad concreta de azúcar ya que, para poder considerar un vino espumoso como natural, es necesario que el gas carbónico se genere en esta segunda fermentación, realizada en envase herméticamente cerrado, con azúcares naturales del vino base o azúcares agregados, consiguiendo un producto terminado con una presión mínima de cuatro atmósferas (a 20 ºC).

Entre todos los vinos espumosos naturales, los más reconocidos a nivel internacional están el Champagne (elaborado en la zona noreste francesa de Champagne) y el Cava (elaborado en diferentes zonas geográficas de España: Penedés (Cataluña), Requena (Valencia), Calatayud (Aragón), Almendralejo (Extremadura), La Rioja, Colmenar de Oreja (Madrid) y Villanueva de Alcardeta (Toledo). Tanto el Champagne como el Cava son elaborados mediante el método tradicional denominado *Champenoise*. Ambos espumosos están protegidos bajo una Denominación de Origen Controlada, que es quien determina las variedades aptas para su elaboración, el proceso de elaboración donde se realizará la segunda fermentación en botella, así como los tiempos mínimos de crianza sobre lías en la botella antes de salir al mercado.

En el método *Champenoise* después de una primera fermentación de un vino base, el vino es embotellado junto con la adición de levadura y azúcar (licor de tiraje), para que tenga lugar la segunda fermentación alcohólica en la botella.

Las principales etapas realizadas durante el método tradicional para la elaboración de los vinos espumosos son: elaboración del vino base, tiraje, rima, punta y degüelle. El esquema de elaboración se muestra en la figura 5.

Vinificación vino espumoso
Método champanoise - tradicional

Segunda etapa, se parte del vino base:

1. Adición del licor de tiraje
2. Segunda fermentación y crianza
3. Almacenamiento en Rima
(9 meses mínimo para cavas y 15 para Champagnes)
4. Removido
5. Almacenamiento en Punta
6. Degüelle
7. Adición licor de expedición
8. Taponado
9. Almacenamiento/crianza en botella

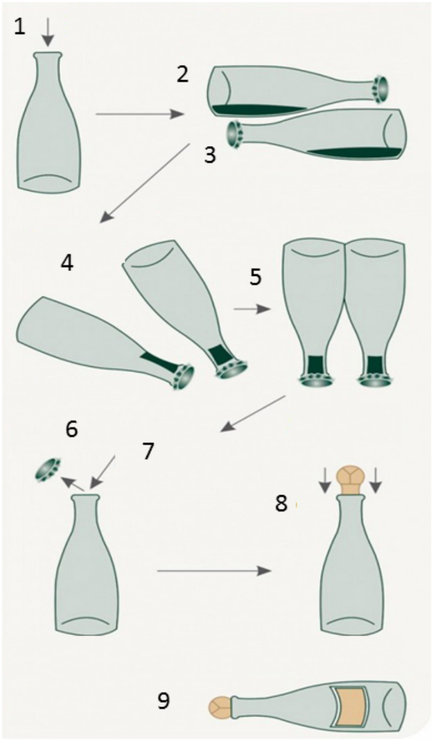


Figura 5. *Etapas de elaboración del vino espumoso, método tradicional-Champenoise (2ª etapa), partiendo de un vino base ya elaborado.*

2.2.1. Elaboración del vino base

La elaboración del vino base es la misma utilizada para la elaboración de vinos tranquilos explicado en el apartado anterior (2.1.). La única diferencia es que la recolección de la uva en el viñedo se realiza de forma más temprana que para la elaboración de vinos tranquilos (35), debido a que se pretende conseguir vinos base con una elevada acidez para transmitir sensaciones de frescura en boca por lo que, cabe destacar que, durante el proceso de vinificación del vino base es habitual que no se realice la fermentación maloláctica. Por último, los vinos base deberán estar previamente clarificados y estabilizados antes de ser embotellados junto con la adición del licor de tiraje con el fin de evitar posibles precipitaciones de cristales de tartárico o de proteínas (36), pues una vez en botella ya no se le podrá realizar ningún tratamiento.

2.2.2. Tiraje

El tiraje consiste en la adición de una solución llamada licor de tiraje la cual está formada por levaduras y azúcar para propiciar la segunda fermentación en botella. Es importante añadir proporciones adecuadas y controladas de la mezcla del licor de tiraje para después obtener las presiones deseadas de dióxido de carbono cuando las botellas son cerradas mediante un tapón obturador y una chapa (tapón corona). Además, durante el embotellado también se puede añadir un poquito de bentonita para facilitar la precipitación de las levaduras después de terminar la segunda fermentación (37). Hay que tener en cuenta la elección del tipo de levadura las cuales podrán ser *Saccharomcyces* o no-*Saccharomyces*, siempre que sean resistentes principalmente a bajas temperaturas, al medio hidroalcohólico y a la presión de CO_2 (38).

2.2.3. Rima

Después del embotellado y tiraje del vino base, este queda preparado para llevar a cabo la crianza en botella. Para ello, las botellas que contienen el vino base junto con el licor de tiraje se colocan en posición horizontal o en fase rima en las zonas de crianzas, habitualmente una zona oscura y tranquila de la bodega, habilitada para que transcurra a baja temperatura (14-16 ºC) y humedad controlada. En esta etapa se producirá la segunda fermentación en botella, con el objetivo de producir gas carbónico (que conllevará a la formación de la espuma del vino espumoso acabado) y su integración durante su envejecimiento sobre lías. A lo largo de la etapa de crianza, las levaduras permanecen en contacto con el vino. Es una fase muy importante ya que las levaduras transmiten al vino sus componentes dándole complejidad y untuosidad. De hecho, durante esta etapa se produce el fenómeno de autólisis (muerte de las levaduras por saturación del carbónico y por la propia intoxicación que les provoca el alcohol que ellas mismas producen) de manera que los restos de las levaduras formaran las lías, las cuales aportan características organolépticas de gran complejidad a lo largo del tiempo de crianza, hasta que se depositen de forma natural en el fondo de la botella.

El tiempo establecido por legislación europea para la crianza sobre lías de un espumoso deberá de ser como mínimo de noventa días. Sin embargo, la legislación española establece que el periodo mínimo de la crianza sobre lías será de nueve meses desde el tiraje hasta el degüelle. Además, el reglamento de la DO Cava determina periodos mínimos de crianza de quince y treinta meses, a las categorías de Reserva y Gran Reserva respectivamente.

Por otra parte, el reglamento de la DO Champagne exige periodos de crianza con lías más largo que los anteriores con un mínimo de 12 meses. Sin embargo, en general, este periodo es casi siempre más largo ya que, en Champagnes para las cosechas sin añada el promedio es de 2 a 3 años, mientras que para los Champagnes con añada *(millésimes*) esta crianza esta entre los 4 y 10 años.

2.2.4. Punta y removido

Después de terminar el periodo de crianza sobre lías, y de producirse la progresiva precipitación en el fondo de la botella formando el poso de levaduras, es cuando se lleva a cabo el proceso de removido, *remuage* o clarificación. Este proceso consiste en la eliminación del poso de levaduras sometiendo las botellas a un proceso de removido para conseguir que todo el sedimento se dirija al cuello de la misma.

Tradicionalmente este proceso se ha realizado de forma manual, colocando las botellas sobre pupitres y girándolas cada día un octavo de vuelta durante quince días hasta que lentamente y de forma controlada quedan las botellas de punta, con el fin de concentrar los sedimentos en el cuello de la botella. Aunque actualmente todavía hay bodegas que lo sigue realizando de forma manual, este proceso ha sido sustituido por sistemas automatizados que realizan los movimientos y que permiten manipular a la vez y de forma más rápida un conjunto de botellas. Por tanto, al final del removido las botellas quedan en punta de forma vertical sobre el cuello, listas para la siguiente etapa, el degüelle.

2.2.5. Degüelle

El proceso de degüelle es la operación final para la eliminación de las materias solidas o lías depositada en el obturador y chapa, en el cuello de la botella. Actualmente la eliminación se realiza mediante la congelación del cuello de la botella. Para ello se introduce esta parte (4-5 cm) en un baño criogénico, se invierte y se le quita la chapa. Con la propia presión interna generada por el carbónico formado durante la segunda fermentación en botella, el depósito congelado sale expulsado, quedando el vino perfectamente brillante, sin restos de sedimento alguno después de realizar esta operación. Durante este proceso se producen perdidas de vino en las botellas, las cuales son rellenadas nuevamente a su volumen inicial mediante la adición del licor de expedición. Este licor puede estar constituido por el propio vino espumoso, o bien por una mezcla de sacarosa, mosto de uva natural o rectificado, vino base, o una mezcla de todo ello. Con esta adición se consigue el toque final de dulzor o sequedad que se quiera para el vino espumoso final. Por último, se cierran las botellas con el tapón de corcho elegido y se sujeta con una especie de alambre llamado bozal o morrión.

2.2.6. Envejecimiento en botella en ausencia de lías

Tras el taponado final, los vinos espumosos realizan un periodo de maduración, alrededor de 15 días o un mes posterior al degüelle, donde se recuperan y estabilizan del choque producido en el destapado del degüelle. A partir de este momento comienzan a descender las cualidades organolépticas relacionadas con los atributos de frescura y juventud, dependiendo de las condiciones de conservación. Sin embargo, a lo largo de los años se ha comprobado que los vinos espumosos pueden ser envejecidos en botella durante meses o incluso durante años antes de su consumo. Durante este envejecimiento los aromas y sabores van evolucionando, las notas más frescas van disminuyendo y se van convirtiendo en florales. Con el paso del tiempo, siguen apareciendo aromas con matices de frutos secos, tostados e incluso notas oxidativas. Por otro lado, durante este envejecimiento pueden producirse fenómenos de pardeamiento, afectando negativamente al color de los vinos espumosos (39). Tanto los aromas como el color se pueden ver influenciados por transmisión de oxígeno a través del corcho a lo largo de tiempo. Además a través del corcho también se produce una pérdida de CO_2 (40), lo cual ocasiona una depreciación de la calidad de la espuma (41).

3. Embotellado y almacenamiento

3.1. Proceso de embotellado

Después del proceso de vinificación y crianza en madera, llega la última etapa de embotellado y almacenamiento del vino (en botella) hasta su consumo.

El embotellado de los vinos consiste en el fraccionamiento del producto para su posible distribución y venta en el mercado. Además, es fundamental su correcto transporte y almacenamiento para conservar las propiedades organolépticas del vino dentro de la botella. En el caso de los vinos con crianza en botella, la función del embotellado debe permitir la correcta maduración y evolución del vino hasta alcanzar un punto óptimo para su consumo.

En el proceso del embotellado, es muy usual proteger al vino de las posibles oxidaciones mediante la adición de productos protectores, siendo el anhídrido sulfuroso (SO_2) el más utilizado en la industria enológica debido a sus propiedades antioxidantes, antisépticas y desinfectantes. El SO_2 juega un papel muy importante en la prevención de la oxidación de compuestos presentes en el vino, incluyendo compuestos fenólicos responsables del color y de los compuestos volátiles. Por ello se emplea justo antes del embotellado, para conseguir una mejor conservación durante su periodo de almacenamiento.

El éxito de la conservación del vino dentro de la botella depende de unas buenas condiciones de almacenado de las botellas. Sin embargo, antes de esto hay que tener en cuenta ciertos factores que influirán en su conservación, como son la elección del tipo de cierre y del tipo de botella que lo contiene.

3.1.1. Tipos de cierres de botella

El tipo de cierre debe seleccionarse de acuerdo con el tiempo de consumo esperado. Los cierres deben asegurar que el contenido no gotee de la botella y que el contenido no sea alterado por el oxígeno. Actualmente, los productores de vino tienen varias opciones para taponar botellas de vino, como tapones de rosca, tapones de corcho natural o sintético, aglomerado, tapones de plástico o tapones de vidrio (figura 6).

Figura 6. *Diferentes tipos de tapones (vidrio, rosca, corcho, aglomerado) empleados para el taponado de vino.*

Se pueden producir varios cambios en el vino después del embotellado, algunos de ellos deseados y esperados como aumento de complejidad, redondez, evolución agradable y deseada. De todos modos, también pueden ocurrir cambios inesperados derivados de los tapones debido a la oxidación o reducción del vino (42). Algunos de estos cambios inesperados pueden modificar la calidad de los vinos y, en el peor de los casos, estos vinos podrían considerarse productos defectuosos e incluso imbebibles.

Uno de los factores que más influye en el embotellado y almacenamiento del vino es el oxígeno. El oxígeno queda atrapado en el espacio superior de la botella después del embotellado, pero también está presente en el vino disuelto y también penetra a través del cierre combinado con la temperatura y la luz, modificando el estado oxidativo del vino durante el almacenamiento (43). Así, los enólogos tienen la opción de modificar y controlar la evolución de su vino después del embotellado seleccionando el tipo de cierre. El flujo de oxígeno que puede pasar a través del cierre de una botella de vino se denomina OTR (Oxygen Transmission Rate o tasa de transmisión de oxígeno en 24 horas). Este parámetro depende del espesor del material y del gradiente de presión parcial entre la atmósfera del ambiente externo y el espacio de cabeza de la botella (44). Esta entrada de oxígeno suele ser más lenta que la tasa de consumo de oxígeno del vino, por lo que, después del consumo del exceso inicial de oxígeno, las concentraciones de oxígeno disuelto y en el espacio de cabeza suelen ser muy bajas (a menudo microgramos por litro) (45). Otro indicador común en enología es el porcentaje total de oxígeno (PTO) que puede variar en un rango de aproximadamente 1 y 9 mg/L. Este parámetro consiste en la suma de dos componentes, el oxígeno disuelto del vino y la concentración en el espacio de cabeza (46).

Se pueden encontrar diferentes rangos de OTR, detallando la tasa de transmisión de oxígeno aproximada para cada tipo de cierre. Los tapones de rosca Saranex y los tapones de rosca Saran-Tin son las opciones de cierre con valores de OTR más bajos con 0,0006 y 0,0008 mL de O_2 por día, respectivamente (mediciones AWRI) (47). Los corchos técnicos de microaglomerado con una OTR muy baja podrían obtener valores similares a los tapones de rosca cercanos a 0,0006 y 0,0007 mL de O_2 por día, los corchos naturales aumentan levemente la permeabilidad entre 0,0002 y 0,006 mL de O_2 por día. Finalmente, los cierres sintéticos extruidos muestran una mayor permeabilidad alrededor de 0.0019-0.0030 mL de O_2 por día.

3.1.2. *Tipos de botella*

Otro factor muy importante en la etapa de embotellado del vino es la elección del recipiente en el que se almacenará el vino hasta su consumo. Este envase o embalaje debe tener en cuenta la protección y conservación del producto. En el caso del vino existen diferentes tipos de envases como: el tetra brick que es un cartón para bebidas compuesto por diferentes capas de polietileno, papel y aluminio; el bag in box que consta de una bolsa de polietileno y un grifo con válvula para dosificarlo; recipientes de plástico PET (tereftalato de polietileno); latas de aluminio; y finalmente, el envase más utilizado en el mundo del vino, la botella de vidrio. El vidrio es un producto mineral obtenido por fusión y que solidifica sin cristalizar. También es un material inerte, y desde el punto de vista medioambiental es favorable porque es un material totalmente reciclable.

Hoy en día se utilizan botellas de vidrio de diferentes formas (Burdeos, Borgoña, Champagne, Rhin, Jerez, Oporto) con diferentes capacidades, y de diferente color y tonalidad de vidrio (pedernal, ámbar, verde, azul, etc.). La elección del color de la botella es de gran importancia para la conservación del vino durante el almacenamiento o la crianza (figura 7).

Figura 7. *Diferentes tonalidades de color de botella de vidrio.*

Esto se debe a que la luz incidente en las botellas penetra a través del vidrio produciendo reacciones de oxidación-reducción en el vino y consecuentemente afectando sus cualidades organolépticas. Las botellas de vino de vidrio sólo transmiten longitudes de onda superiores a 300 nm (48). Las botellas transparentes estándar (pedernal) generalmente transmiten más del 80 % de la radiación UV visible por encima de 360 nm, mientras que las botellas transparentes con protección UV adicional, que se fabrican agregando una especie absorbente de UV al vidrio o recubriendo las botellas transparentes con una película que contiene un especie de este tipo, transmite menos radiación UV (49). Las botellas verdes transmiten considerablemente menos luz que las transparentes, particularmente en la región por debajo de 520 nm, mientras que las botellas ámbar transmiten muy poca radiación por debajo de 520 nm. Para las botellas de colores más oscuros, las botellas pesadas, que tienen vidrio más grueso, transmiten un poco menos de luz que las más claras.

3.2. Envejecimiento en botella

El vino es un medio en constante evolución por lo que el envejecimiento se produce hasta el momento de su consumo. Por tanto, después del embotellado, el vino sigue una evolución natural. En esta etapa el color, el sabor y el aroma evolucionan dando lugar a importantes cambios, influenciados por el ambiente reductor en el que se encuentran dentro de la botella. La etapa de envejecimiento en botella puede ser beneficiosa o perjudicial para la calidad del vino ya que, dependiendo de la tipología de vino, algunos mejoran en esta etapa, dando lugar a vinos más complejos y elegantes, y otros empeoran, perdiendo su frescura. La capacidad de un vino para envejecer está estrechamente relacionada con su composición.

3.2.1. Etapas de evolución en botella

El envejecimiento o crianza en botella se puede representar con una curva de evolución a lo largo del tiempo (figura 8), cuya duración depende de las características del vino y principalmente de su calidad. Existen vinos con gran calidad evolutiva que se caracterizan por un largo periodo de envejecimiento, mientras que también existen vinos comunes que necesitan poco tiempo de conservación para obtener su momento óptimo de consumo. Durante la crianza en botella se pueden distinguir tres fases (50).

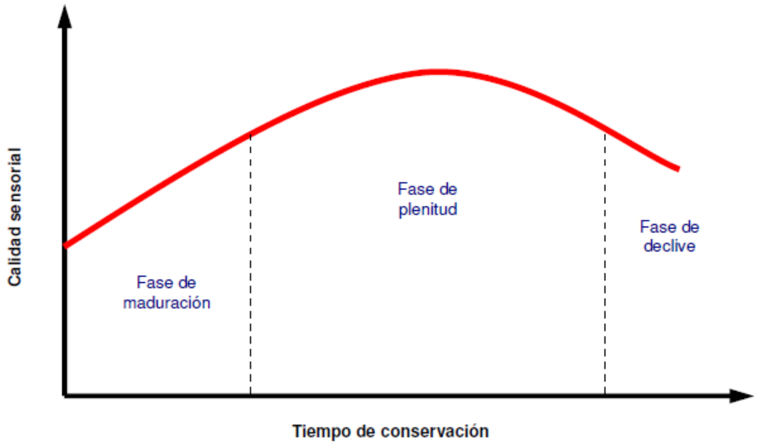


Figura 8. *Evolución en botella de la calidad sensorial de un vino.*

1) <u>Fase de maduración</u>. Esta fase comienza tras la etapa del embotellado del vino. El vino pasa por una serie de oscilaciones al principio debido a que debe de recuperarse del proceso brusco de embotellado. Entonces comienza a desarrollar unos fenómenos en condiciones de reducción, que como consecuencia incrementan la calidad organoléptica del vino.

2) Fase de plenitud. Este punto es cuando el vino alcanza su máxima calidad y coincide con el máximo de la curva.

3) Fase de declive. Tras alcanzar el vino su máxima calidad, esta comienza a descender. Dependiendo de las características del vino y de las condiciones de conservación la duración de este periodo será mayor o menor.

Por tanto, dependiendo del tiempo, el vino evolucionara de forma diferente, mostrando en cada fase de las anteriores un periodo de mayor o menor duración. Por una parte, los vinos menos complejos, presentaran una fase de declive muy temprana ya que mejorarían muy poco en botella. Por otra parte, vinos con cierta tolerancia a la crianza mejorarían durante un tiempo comenzando posteriormente la fase declive. Y por último los vinos con alta calidad evolutiva se mantendrían en la fase de plenitud largo tiempo, siendo su fase declive muy lenta.

3.2.2. Fenómenos producidos durante el envejecimiento en botella

Durante el envejecimiento en botella, se producen una gran cantidad de cambios y reacciones de oxidación-reducción en el vino debido a los cambios producidos en el potencial redox. Estas reacciones afectan principalmene a la evolución de la materia colorante y de la composición aromática del vino.

- **Evolución de la materia colorante durante la conservación en botella**

El color del vino se debe fundamentalmente a su contenido en antocianos extraídos del hollejo. Durante el almacenamiento en botella, los antocianos se van combinando gradualmente con otros compuestos fenólicos extraídos también de la uva. La concentración de antocianos junto con, el acetaldehído, el pH, la temperatura y la presencia de oxígeno y de dióxido de azufre son algunos de los factores que influyen en el desarrollo de las reacciones que implican a los compuestos fenólicos durante el envejecimiento en botella (51). Esas reacciones son las responsables de la aparcición de nuevos pigmentos (52). En general, en los vinos tintos el color se va modificando desde los tonos púrpuras o violáceos hacia tonos más rojos y anaranjados, y en los vinos blancos desde los tonos verdes hacia los amarillos y anarajados.

Durante el primer año de permanencia en botella es el periodo donde el color cambia más rápidamente (53). Esos cambios están causados fundamentalmente por reacciones de polimerización de los monómeros de antocianos formando estructuras oligoméricas más estables, tanto en presencia como en ausencia de oxígeno. Estas reacciones de polimerización se dan durante el almacenamiento de los vinos en botellas de vidrio, en un ambiente esencialmente anaerobio, donde la influencia de la temperatura es más importante que la propia concentración de oxígeno (54, 55).

La composición polifenólica del vino responsable del color, está determinada por la calidad de la uva y por el proceso de vinificación empleado (56). Por tanto, dependiendo de la tecnología aplicada, esta podrá modificar la composicion fenolica, la estabilidad y la evolucion durante el proceso de conservacion en botella.

En general, el indice de polifenoles totates (IPT) y los taninos del vino evolucionan de manera irregular durante el proceso de envejecimiento, sin embargo los antocianos sufren un descenso más constante y continuado a lo largo del tiempo aunque, en última instancia, todas esta evolución dependerá de la variedad de uva y de las características iniciales del vino en el momento de su embotellado (57, 58).

- **Evolución del aroma durante la conservación en botella**

El aroma de un vino recién vinificado está formado, por una parte, por los compuestos provenientes de la uva que constituyen los aromas primarios y, por otra parte, por los producidos mediante reacciones químicas durante la fermentación, los llamados aromas secundarios. Más tarde, durante el envejecimiento y la conservación en botella, aparecen los llamados aromas terciarios, los cuales pueden ser aromas oxidativos o reductivos (59).

Dependiendo de las condiciones de conservación, tendrán lugar diferentes reacciones químicas las cuales podrán modificar la composición volátil de los vinos dependiendo de la composición del vino, el tipo de almacenamiento, el tiempo de permanencia en botella, la ausencia o la cantidad de oxígeno transmitido al vino y la temperatura (60).

La conservación en botella, normalmente trae como consecuencia un descenso en la concentración de ciertos compuestos aromáticos responsables de proporcionar a los vinos notas frescas relacionadas con las frutas y flores, como es el caso de algunos acetatos y ésteres etílicos (61).

Sin embargo, a medida que van descendiendo las concentraciones de los aromas primarios y secundarios, van a ir apareciendo nuevos compuestos relacionados con los aromas terciarios responsables del envejecimiento y evolución en botella de los vinos (62, 63).

Los cambios producidos en la composición aromática del vino se verán reflejados también en las sensaciones que el consumidor percibe en el vino final y por tanto en la percepción de su calidad.

Evaluar la capacidad de envejecimiento y preveer la evolución que va a tener un vino durante su estancia en botella es algo que hoy en dia los profesionales del vino prententen determianr pero que todavía no está definido claramente el proceso a seguir (64). Actualmente, la combinación de la fase visual y gustativa, junto con la parte aromática que engloba las notas olfativas son los factores en los que se basan los catadores profesionales a la hora de valorar el potencial de envejecimiento de los vinos (64).

3.2.3. Factores que intervienen en la crianza en botella

Existen diferentes factores que pueden afectar a la calidad del vino durante el envejecimiento en botella (3).

Por una parte, los factores externos que rodean a la botella como la temperatura, la humedad y la luz. Es muy importante mantener el vino almacenado a temperatura y humedad constante, pues las grandes variaciones afectan negativamente provocando una rápida e incorrecta evolución. La temperatura no deberá de ser superior a los 20 ºC para asegurar una buena conservación. Respecto a la humedad, no debe ser ni excesiva, porque existe el riesgo de que aparezcan mohos sobre la superficie de las botellas, ni tampoco debe ser demasiado baja porque el corcho se resecaría provocando pérdidas de vino y oxidaciones. Respecto al efecto que la luz puede provocar durante la crianza en botella, este se puede traducir en una evolución acelerada del vino, pero también en el desarrollo del defecto llamado ‘gusto de luz’ o ’enfermedad de la luz’.

Por otra parte, están los factores del envasado de la botella, como son el taponado, el color y volumen de la botella, y la cantidad de sulfuroso añadido. Como ya se ha explicado anteriormente, la elección del tipo de cierre es fundamental para la conservación del vino y evitar la formación de aromas indeseables. La calidad del tapón está condicionada por la transmisión de oxígeno y el estado de reducción del vino. También el color de botellas y su capacidad serán claves para su conservación. Los vinos tienden a desarrollar un mejor envejecimiento en botellas de mayor capacidad y con vidrios de color oscuro, los cuales protegen más de las radiaciones. Además, hay que tener en cuenta la presencia de sulfuroso en el vino para protegerlo de las oxidaciones ya que emplearlo a bajas concentraciones puede suponer un riesgo de oxidación prematura. Sin embargo, a concentraciones elevadas también supone un riesgo para la aparición de aromas de reducción indeseables.

3.2.4. Riesgos que aparecen en la crianza en botella

Teniendo en cuanta los factores anteriores, los principales riesgos que puede presentar un vino envejecido en botella en condiciones inadecuadas son por una parte la oxidación del vino provocando la aparición de los aromas terciarios relacionados con notas rancias, achocolatados, aromas caídos y sobremaduros con notasa manzana asada o flores marchitas. Sin embargo, y de forma contraria, durante esta etapa también pueden producirse los aromas de reducción o el defecto ‘gusto de luz’. Estos suelen aparecer debido a un fuerte descenso del potencial de redox al mantener al vino al abrigo del aire, provocando la aparición de aromas desagradables relacionados con olores de col hervida, ajo, cebolla o gas, entre otros.

4. Calidad y composición del vino

4.1. Calidad del vino

Como ya se ha explicado anteriormente, el vino es obtenido a partir de la uva mediante la fermentación alcohólica donde el azúcar es convertido en alcohol por acción de las levaduras, junto a muchísimas otras reacciones más. Sin embargo, desde un punto de vista químico, el vino es una matriz compleja formada por un gran número de moléculas, las cuales sufren grandes cambios a lo largo del tiempo. Es por ello que una vez elaborado, la etapa de envejecimiento en botella es crucial para que se dé una correcta evolución del vino y es clave para conseguir un producto de calidad (43, 65-70).

La calidad del vino se considera una variable compleja y multidimensional que está formada tanto por dimensiones intrínsecas como por dimensiones extrínsecas (71). Por un lado, las dimensiones extrínsecas son propiedades externas al vino, es decir que no son físicamente parte del vino, como la región, la añada, el diseño de la etiqueta, el enólogo, peso de la botella, etc. Por otro lado, están las dimensiones intrínsecas que son las relacionadas con el vino en sí. Estas propiedades están vinculadas tanto a las propiedades físico-químicas como a las organolépticas (72, 73). Estas variables físico-químicas son diferentes de un vino a otro, y depende de multitud de factores.

El análisis de la composición físico-química del vino es un método ideal de evaluación fiable y objetivo pero que debe de estar íntimamente relacionado con el análisis organoléptico para poder evaluar el concepto de la calidad. Por lo tanto para la obtención de un vino de calidad hay que controlar tanto las propiedades físico-químicas enológicas como las sensoriales (74).

4.2. Composición físico-química de un vino

Entre los muchos los componentes químicos que se encuentran en un vino los mayoritarios son: agua, azúcares, alcoholes, ácidos orgánicos, proteínas, sales minerales, compuestos fenólicos y compuestos aromáticos (75-77). Los dos últimos son considerados como los parámetros más importantes con los que evaluar la calidad de la uva, del mosto y del vino terminado (78-80).

Agua. Es el compuesto mayoritario del vino, el cual procede directamente de la uva. Sus proporciones varían dependiendo del grado alcohólico, de entre un 80-90 % del total. Ayuda a disolver las sales, minerales y microelementos que la vid ha tomado del suelo durante el ciclo vegetativo.

Azúcares y grado alcohólico. Los principales azúcares que constituyen el vino son la glucosa y fructosa los cuales proceden directamente de la uva y del mosto. Durante la fermentación alcohólica estos son transformados en etanol el cual representa entre un 10-15 % de la composición del vino acabado, siendo el segundo componente más abundante desde el punto de vista cuantitativo. Además del etanol, se producen otros muchos compuestos en menores cantidades pero con alto impacto en la calidad del vino. Este es el caso del glicerol, componente más abundante después del etanol (4-10 g/L en vinos). Este compuesto contribuye en el sabor dulce del vino además de otorgarle mayor cuerpo y volumen en boca (81, 82). Cabe destacar que también se generan otros alcoholes, aunque a concentraciones mucho más pequeñas, por debajo a 1 g/L, como el propanol, metanol, isobutanol, sorbitol o feniletanol, entre otros. Estos alcoholes son en muchos casos precursores de la formación de otros compuestos aromáticos como los ésteres, que aportaran aromas a los vinos (83).

Acidez y pH. Los principales ácidos que constituyen el vino se pueden clasificar en dos grupos. Por una parte, están los que constituyen la acidez fija y por otro los que constituyen la acidez volátil. Así, la primera está formada por los ácidos tartárico, málico y cítrico, los cuales provienen directamente de la uva, junto con los ácidos succínico y láctico que son generados durante la fermentación. La presencia de estos ácidos en el vino permite la conservación del color, el sabor y los aromas en el vino. Por lo que a la acidez volátil respecta, está constituida por los ácidos volátiles de la serie acética que principalmente son el acético, propiónico,y butírico, todos ellos formados durante la fermentación. Es muy importante controlar los valores de esta acidez volátil por debajo de los parámetros establecidos, pues un aumento de esta provocaría la aparición del defecto de picado o avinagrado en el vino. Sin embargo, a pesar de realizar esta diferenciación de grupos (fijos y volátiles), la acidez del mosto o del vino se expresa como la suma de todos de los ácidos presentes en él, y se expresa en acidez total como gramos por litro (g/l) de tartárico ya que es el ácido principal, presentándose habitualmente entre 5.5 y 8.5 g/L. En general, los vinos espumosos presentan mayores valores de acidez total, seguida de los vinos blancos y finalmente, encontraríamos los vinos tintos.

El pH del vino es uno de los parámetros más importantes para la calidad. El pH refleja la cantidad y la fuerza de los ácidos presentes en el vino, es decir la concentración de los iones hidrógeno. Afectando a los parámetros organolépticos, como color, acidez y amargor. Los valores medios de pH en un vino van entre 3-4.

Aunque la acidez total y el pH presentan cierta correlación, ni expresan lo mismo ni son proporcionales. Cada uno de los ácidos que constituyen la acidez total aportan cantidades diferentes de iones hidrogeno lo cual con llevará a una contribución diferente en términos relativos al pH. Siendo el ácido tartárico el que más contribuye y más cambios genera en el pH. El potasio es otro de los elementos que hace que la relación entre acidez total y pH no sea proporcional. Las sales de bitartrato de potasio se forman a partir de ácido tartárico y modifican la concentración de ácidos en el vino. Así, los mostos que presentan cantidades más elevadas de potasio darán lugar a vinos con niveles de pH más altos.

Por tanto controlar los parámetros de acidez y pH es muy importante para la calidad, ya que permiten mantener la acidez y frescura de los vinos, controlar la estabilidad y conservar los componentes relacionados con el aroma y el color (2, 84).

Sales minerales. Las sales minerales y las de algunos ácidos pueden transmitir al vino un gusto salado característico. El vino contiene alrededor de 2 a 4 g/L de sales minerales, cuya función principal es aportar estabilidad a los vinos y potenciar sabores y determinadas sensaciones como el frescor. Sus principales componentes en sales y compuestos minerales son:

Aniones: Fosfato, Sulfato, Cloruro, Sulfito, Tartrato, Malato, Lactato.
Cationes: Potasio, Sodio, Magnesio, Calcio, Hierro, Aluminio, Cobre.
Oligoelementos: Flúor, Silicio, Yodo, Bromo, Boro, Zinc, Magnesio, Plomo, Cobalto, Cromo.

Proteínas y aminoácidos. Otros compuestos químicos que se pueden encontrar en el vino, aunque en bajas concentraciones, son aminoácidos libres y proteínas.

Los aminoácidos son moléculas orgánicas constituidas con un grupo amino (-NH_2) en uno de los extremos de la molécula y un grupo carboxilo (-COOH) en el otro extremo, y son la base de las proteínas. Las proteínas son macromoléculas formadas por la unión de aminoácidos formando cadenas lineales. La unión de varios aminoácidos da lugar a péptidos y la unión de dichos péptidos en cadenas da lugar a proteínas.

Respecto a las proteínas, es de gran importancia su estabilidad ya que estas estructuras pueden afectar a la calidad del vino blanco debido a la desnaturalización de estas proteínas como resultado de un incorrecto almacenamiento (85). La aparición de sedimentos amorfos o floculados resultante es, en la mayoría de los casos, inaceptable para el consumidor.

Una de las fuentes más importantes de proteínas son las paredes celulares de levaduras muertas o de las lías denominadas manoproteínas. Estas manoproteinas son responsables de aportar cualidades sensoriales positivas y mejoras en los vinos criados sobre lías, así como en la mejora de la estabilidad tartárica y proteica de los vinos (86-88). Además, en los vinos espumosos elaborados por el método tradicional, las manoproteínas liberadas durante el proceso autolítico de las levaduras han sido relacionadas con la mejora en la calidad y la estabilidad de la espuma (89, 90).

Por otro lado, los aminoácidos que se identifican en el vino tienen distintos orígenes, algunos provienen directamente de la uva, otros son metabolizados por las levaduras durante la fermentación, y los restantes son producidos por degradación enzimática de las proteínas presentes en la uva (91).

La importancia de los aminoácidos libres (arginina, prolina, alanina, cisteína, metionina, glutamina, tirosina, entre otros) que son aportados directamente de la uva presentan la principal función de ser la fuente de nitrógeno para las levaduras durante la fermentación y, como consecuencia, son precursores de muchos de los compuestos aromáticos que se formarán durante dicha fermentación teniendo un considerable impacto en las características organolépticas del vino (92-94). Además, en la elaboración de vinos espumosos la composición en aminoácidos libres tiene gran importancia ya que influyen en la calidad de la espuma (95).

Vitaminas. El vino contiene muchas vitaminas las cuales son imprescindibles para ciertas funciones de las levaduras. Entre las vitaminas hidrosolubles en cantidades pequeñas, encontramos: Tiamina (B1), Riboflavina (B2), Nicotinamida (B3), Ácido Pantoténico (B5), Piricloxina (B6), Mesoinositol (B7), Biotina (B8), Cobalamina (B12), Ácido Fólico (B9), Cianocobalamina (B12) y Ácido Ascórbico (vitamina C).

Compuestos fenólicos. El color y la estabilidad del color de los vinos están directamente relacionados con el contenido de compuestos fenólicos que lo constituyen. Estos compuestos y las reacciones entre los distintos compuestos fenólicos son los responsables de otorgar el color al vino, y de la constante evolución cromática a lo largo del tiempo.

En los vinos tintos normalmente los polifenoles están presentes como flavonoides y no flavonoides. Dentro de los flavonoides, compuestos responsables del color, los que mayor influencia tienen sobre el color del vino tinto y su estabilidad son los antocianos y los taninos. Como ya se explicó en apartados anteriores, los antocianos y taninos son compuestos que principalmente se extraen de la uva, y pasan a formar parte de la composición de los vinos.

La cromaticidad de los vinos se asocia más con la presencia de antocianos y de los pigmentos derivados de ellos, mientras que los taninos son compuestos responsables de la astringencia y de la sensación de estructura o cuerpo del vino, debido a su interacción con las proteínas de la saliva (96). Ambos compuestos (antocianos y taninos) intervienen en numerosas reacciones de condensación durante la maduración y el envejecimiento del vino, por lo que tienen gran influencia en la capacidad para mantener el color a lo largo del tiempo. Por lo tanto, la calidad de un vino se verá afectada por la composición fenólica la cual influirá directamente sobre el color y su astringencia final.

Compuestos aromáticos. El aroma del vino es uno de los factores más importantes a la hora de valorar la calidad. El aroma del vino es el resultado de una mezcla compleja de cientos de moléculas, volátiles y no volátiles (los precursores), pertenecientes a diferentes familias químicas, las cuales resultan de un gran número de reacciones químicas y enzimáticas. La formación de los compuestos aromáticos se produce de forma continua y comienza ya en el propio grano de uva con la síntesis de precursores de aroma y de moléculas de carácter varietal. Luego, durante la elaboración del vino y con las fermentaciones alcohólica y maloláctica siguen generándose nuevos compuestos aromáticos dependientes del tipo de levaduras y bacterias utilizadas en cada caso y de las condiciones de fermentación. Finalmente, durante el almacenamiento y envejecimiento del vino tanto en barrica como en botella siguen dándose reacciones químicas responsables de la desaparición de unos compuestos aromáticos y la aparición de otros (67, 78, 97).

Desde un punto de vista químico, el aroma está formado por centenares de compuestos orgánicos con una gran heterogeneidad en su estructura química, que pueden estar presentes a concentraciones muy distintas (desde ng/L hasta g/L) pero todos ellos presentan una característica común y es que son volátiles (98).

Sin embargo, para que una sustancia volátil sea aromática es necesario que esta interaccione con los receptores olfativos que se encuentran en la nariz humana. Por ello, de entre todos los compuestos volátiles sólo una pequeña parte tiene importancia desde el punto de vista del aroma. Además, para que un compuesto aromático pueda ser detectado sensorialmente ha de presentar una concentración superior a su umbral olfativo o límite sensorial. Este umbral de detección sensorial es diferente para cada compuesto y, además depende de la matriz en la que se encuentre por lo que cada compuesto necesitará estar a una cierta concentración para poder manifestar su poder odorante en cada vino.

Para poder determinar cómo un olor participa en el aroma general del vino y si será percibido por la nariz humana se ha definido el denominado valor de la actividad olfativa (OAV, odor activity value) (78). El OAV se define como la relación entre la concentración de una determinada molécula volátil y su umbral de detección (ecuación 1). En general, un OAV mayor o igual a la unidad indica un olor activo, que contribuye a la percepción del aroma del vino.

$$OAV = CC/LS$$

CC es la concentración del compuesto en el vino
LS es el umbral de detección del compuesto en el vino

Tal y como ya se ha explicado, los aromas del vino se pueden clasificar en tres grupos según su origen: los aromas varietales o primarios cuando procedan de la uva, los aromas fermentativos o secundarios cuando se generan en la etapa de vinificación y los aromas de envejecimiento o terciarios cuando son generados durante la durante la etapa de crianza (98, 99).

<u>Aromas Primarios:</u>

El aroma primario o varietal del vino tiene su origen en la uva (100-103) los cuales pueden ser liberados durante los procesos a los que se somete la uva desde el viñedo hasta el comienzo de la fermentación alcohólica. Estos aromas son compuestos complejos, que pasan de la uva al vino sin transformación. Como ya se ha comentado previamente en el apartado 1.2., los compuestos aromáticos varietales se encuentran principalmente en los hollejos de la uva (104), por lo que para ser transmitidos al vino es muy importante el proceso de la maceración.

Estos compuestos se pueden encontrar como sustancias aromáticas libres, o precursores de aromas combinados, que no son aromáticos en sí mismos, pero que liberan aromas por hidrólisis ácida o enzimática.

Los compuestos varietales se pueden agrupar por familias dependiendo sus estructuras químicas y composición. Como se había mencionado en el apartado 1.2., dentro de las familias más importantes están los monoterpenos, los terpenos polioxigenados, alcoholes, aldehídos, compuestos C6, y fenoles volátiles, ente otros, aportando caracteres que hacen recordar en el vino a ciertas flores, o a frutas, y dando también en ocasiones notas herbáceas.

Aunque los aromas varietales representan entre el 1 y el 6 % de concentración de la composición volátil de los vinos (105), estos compuestos aromáticos provenientes de la uva y característicos de la variedad son determinantes en la calidad y en la tipicidad de cada uno de los diferentes tipos de vinos (106).

Aromas Secundarios:

El aroma secundario o también llamado aroma fermentativo se forma durante las fermentaciones, alcohólica y maloláctica, en el proceso de vinificación (107, 108) como consecuencia principal del metabolismo de las levaduras y bacterias con las condiciones en las que ésta se desarrolle (109). En esta etapa el vino se enriquece en gran cantidad de aromas con respecto al mosto. Otros factores como el pH, la temperatura y la composición del mosto determinan la aparición de los compuestos volátiles generados por la levadura durante la fermentación. Sin embargo, los factores más importantes en la generación de aromas fermentativos son el tipo de cepa de levadura empleada (110), la variedad de uva, y la composición del mosto en general del cual se parte (106, 111).

Los compuestos aromáticos generados en la etapa fermentativa pueden agruparse en diferentes familias siendo las más importantes los ésteres, los alcoholes, los acetatos, y los ácidos grasos. La familia de los ésteres es considerada la más importante de los aromas secundarios tanto a nivel cuantitativo como cualitativo. La mayoría de los ésteres del vino son ésteres etílicos de ácidos grasos. En general, los compuestos que forman dicha familia son los responsables principales de aportar a los vinos los aromas relacionados con las frutas y las flores (112), contribuyendo de forma positiva en la calidad general de los vinos. Los alcoholes es otra de las familias importante de los compuestos volátiles formados durante la fermentación alcohólica. Son característicos por aportar aromas floras y frutales, pero también notas herbáceas. Los ácidos grasos volátiles son otra familia que también se producen durante la fermentación. Estos pueden presentar estructuras de cadena corta (C2-C4), media (C6-C10) y larga (C12-C18), o ramificados y se ha comprobado que a medida que aumenta la longitud de su cadena, la volatilidad disminuye y el olor cambia de jabonoso a rancio (113). Como la mayoría de los compuestos aromáticos, cuando los ácidos están a altas concentraciones presentan aromas desagradables sin embargo su concentración en el vino no suele sobrepasar su umbral de percepción. Cabe destacar que estos ácidos son necesarios para un buen equilibrio del aroma fermentativo debido a su influencia en la formación de ésteres (114). Por último, está la familia de los acetatos los cuales, en general, aportan aromas agradables a los vinos, aunque existe una excepción con el comportamiento del acetato de etilo ya que, cuando este se encuentra a altas concentraciones en el vino, puede disminuir la calidad del vino por aportar aromas negativos relacionados con disolventes o pegamento (115).

Aromas Terciarios:

Los aromas terciarios, aromas de evolución o *bouquet* son generados durante el almacenamiento (116-119) y la crianza de los vinos (120, 121), y aportan complejidad aromática.

Los aromas terciarios suelen ser originados mediante reacciones complejas de oxidación-reducción (122, 123) que generan la formación de nuevos compuestos y/o transforman los existentes (124, 125). Estos aromas se van formando poco a poco durante la crianza en barrica y en botella, alcanzando su momento óptimo a la largo del tiempo según el tipo de vino.

Por lo general, la evolución de aroma durante el envejecimiento del vino consiste en la pérdida de los aromas característicos varietales y de fermentación para dejar paso a la aparición de notas de crianza. Es decir, el vino se empobrece en compuestos relacionados con notas aromáticas más frescas y asociadas con aromas florales y frutales (126, 127), y se van produciendo la aparición de notas tostadas, miel, caramelo, balsámicas, frutas sobremaduras y frutos secos, ente otros (46, 84, 124, 125).

Los aromas de evolución/oxidación se pueden agrupan en diferentes familias según su composición y estructura química. Dentro de las más relevantes se encuentran los alquenales, los aldehídos Strecker, las lactonas, los furanos, y ciertas cetonas, entre otras. Dependiendo de las concentraciones que presenten y del tipo de matriz en las que encuentren, estos compuestos pueden pasar de aportar complejidad agradable al vino a pasar a provocar defectos cuando su concentración supera un cierto nivel. Es decir, hay ciertos compuestos que en un principio aportan unas determinadas notas agradables a los vinos, pero sin embargo cuando aumentan su concentración pueden aportar aromas desagradables.

Por tanto, la calidad aromática del vino adquirida durante su crianza y almacenamiento no sólo está representada por los aromas agradables, sino que además debe evitar la aparición de aromas defectuosos. Y es que los principales aromas defectuosos pueden aparecer durante las etapas de vinificación, pero sobre todo aparecen durante el envejecimiento y/o conservación de los vinos por reacciones de oxidación/reducción.

Los aromas oxidativos y/o de evolución suelen ser generados con la exposición excesiva de oxígeno, provocando la pérdida de compuestos aromáticos y la evolución de compuestos que pueden generar aromas indeseables. Por el contrario, cuando los vinos son almacenados o conservados en atmosferas anaeróbicas, donde existe muy poca exposición de oxígeno aparecen los aromas reductivos del vino, lo cual afectará a la calidad final del producto. Estos aromas reductivos están principalmente relacionados con los compuestos azufrados volátiles de bajo peso molecular (128-130) los cuales aportan aromas desagradables muy característicos como olores que recuerdan a huevo podrido, col, ajo, gas o putrefacción. Este defecto se considera un problema producido durante el envejecimiento en botella. Las principales familias aromáticas que agrupan a los diferentes compuestos de reducción son los tioles, los sulfuros y los disulfuros.

Con todo lo expuesto hasta ahora, resulta evidente que a lo largo de los procesos de vinificación y conservación es necesario no sólo conseguir un buen equilibro entre los diferentes aromas primarios, secundarios y terciarios en un vino, sino que hay que evitar también la formación y aparición de aromas defectuosos, con el fin de conseguir las mejores cualidades y características organolépticas del vino.

4.3. Calidad sensorial

La evaluación de la calidad sensorial se puede definir como el método utilizado para medir, analizar e interpretar las respuestas a los productos según la percepción. Se divide en tres fases: la fase visual (que se realiza mediante la vista), la fase olfativa (que se realiza mediante el olfato) y la fase gustativa (que se realiza mediante el gusto) (131).

En el vino, a través de la fase visual se determina la calidad y cantidad de color, mediante los parámetros de luminosidad, tonalidad e intensidad. El color es lo primero que el consumidor o catador percibe de un vino, por lo que es muy importante en la impresión organoléptica percibida. El color aporta información sobre el cuerpo (concentración fenólica), la evolución y el estado del vino, parámetros característicos de su edad y su carácter (74).

Mediante la fase olfativa se evalúa el aroma del vino. Esta evaluación es uno de los procesos más difíciles y complejos a desarrollar en proceso de la cata. Las percepciones olfativas, vía retronasal o directa, dan una idea más específica de los criterios importantes para evaluar la calidad del vino y de ahí que haya que diferenciar entre la manera de evaluar la calidad aromática. Así, el término *flavor* se utiliza para describir conjuntamente la percepción nasal directa y la realizada por vía retronasal (132) mientras que el término *aroma* es utilizado para describir las sensaciones odorantes percibida en el vino, principalmente los aromas primarios y secundarios. Respecto al término *bouquet*, éste se emplea para referirse al aroma del vino formado durante el envejecimiento, es decir, a los aromas terciarios.

Por último, la fase gustativa determina el sabor del vino. Existen diferentes componentes del vino que determinaran las diferentes sensaciones percibidas en boca como son, el alcohol cuyo contenido en exceso produce quemazón y ardor en boca; los ácidos que en general contribuyen en el frescor de los vinos; el azúcar que determina la sensación de dulzor y sequedad; el amargo y la astringencia, que aportan sensación seca y áspera en la boca. Todas estas sensaciones son percibidas en diferentes zonas de la lengua, y su combinación provoca la denominada textura del vino (133).

5. Reaprovechamiento de subproductos de bodega

Actualmente uno de los temas que más preocupan a cualquier tipo de industria es adoptar las condiciones necesarias para revertir el efecto que tiene el funcionamiento de esa industria sobre el cambio climático. Para ello, lo más importante es emplear una conciencia ambiental en toda acción industrial y social con el fin de mantener un medio ambiente sostenible. De ahí la importancia de una buena gestión de residuos la cual consiste, primero, en intentar prevenir su generación y, segundo, en conseguir una gestión sostenible mediante el empleo de los procesos de reutilización, reciclaje, recuperación y revalorización.

Tanto en la industria vitivinícola como en la corchera, se generan grandes cantidades de residuos sólidos durante el desarrollo de su actividad, llegando a cantidades de hasta el 20 %

p/p (peso/peso) en la industria enológica y alrededor del 25 % de la materia prima en la industria corchera, que llega a producir anualmente 300 mil toneladas de corcho.

Los subproductos o residuos generados en la industria vinícola y corchera son revalorizados en su empleo como fertilizantes de terrenos para el crecimiento de plantas, o como sustrato/combustible para la producción de biomasa. Respecto a los corchos, ya sea en forma de tapones, fragmentos, granulados o polvo (figura 9), son residuos de gran interés ya que han dado muy buenos resultados cuando han sido empleados como agente clarificante en los vinos (134) e incluso como material de retención de contaminantes del agua (135, 136).

Figura 9. *Aspecto de los tapones, granulados y polvo de corcho, obtenidos como subproductos del corcho.*

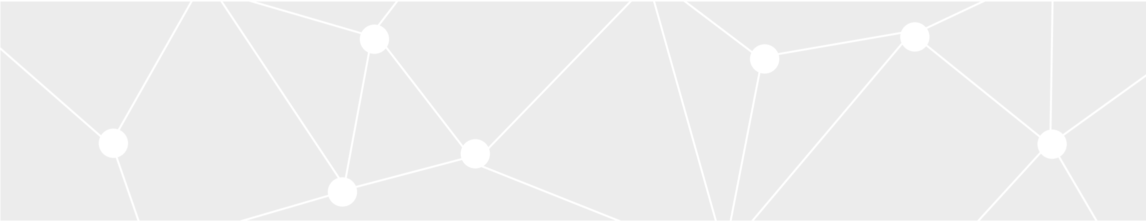
Por otra parte, también hay que tener en cuenta que estos subproductos están constituidos por sustancia bioactivas y nutracéuticas, las cuales promueven una actividad valiosa en la prevención de reacciones oxidantes, problemas cardiovasculares y procesos inflamatorios. Además, la composición fenólica de las plantas, de la *Vitis vinifera* o del *Quercus suber L.,* está relacionada con actividades biológicas antioxidantes, anticancerígenas y antitumorales (137, 138). Esto ha generado la reutilización de residuos agroindustriales ricos en ingredientes vegetales saludables, siendo aplicados en industrias farmacéuticas, cosméticas y alimentarias (139).

Otras propiedades muy características de estos subproductos es su contenido en componentes aromáticos. Es conocido que los corchos pueden aportar aromas negativos al vino durante la etapa del embotellado como consecuencia del defecto al corcho (140, 141). Sin embargo, el corcho y sus subproductos también poseen aromas positivos los cuales presentan notas de vainilla, coco, ahumado, especiado, miel, clavel, almendras, caramelo, cítrico o menta, entre otros. Estos aromas pueden ser de especial interés ya que no sólo se usan en el desarrollo de cosméticos y fragancias sino que también podrían emplearse en la industria alimentaria como esencia en la preparación de tortas, dulces, caramelos y helados, como aditivos aromáticos (142).

Las principales familias aromáticas que presentan los corchos y subproductos son las vainillinas y derivados, fenoles volátiles, aldehídos, alcoholes, terpenos, lactonas, ácidos grasos y furanos. La familia de las vainillinas es de las más importantes tanto cualitativa como cuantitativamente. La vainillina es muy característica por proporcionar un aroma muy intenso y agradable con notas dulces y florales. Además, como se ha visto en otros estudios (143, 144), la vainillina es un componente bioactivo natural presente en el corcho, que muestra una importante actividad antioxidante. Este compuesto se emplea actualmente en muchos campos industriales diferentes, como en la producción de fragancias, en uso alimentario como en repostería (145), o en cosmética (146).

Por tanto, el reaprovechamiento de los subproductos del corcho permite por una parte obtener compuestos bioactivos relacionados con los efectos beneficiosos para la salud, y por otra parte obtener los compuestos aromáticos, los cuales pueden ser reutilizados por las industrias alimentaria, agrícola, cosmética o farmacéutica.

Material y métodos



1. Tipologías de muestras

1.1. Uvas

Para el estudio del capítulo 1, se emplearon uvas de la variedad Syrah procedentes de un viñedo ubicado en Raimat, Lleida (Cataluña, Noreste de España) cosechadas durante las añadas de 2016, 2017 y 2018. Las vides fueron plantadas en 2002 a una altitud de 299 metros sobre el nivel del mar, injertadas sobre un patrón 1103-Paulsen y adiestradas sobre un cordón bilateral con riego por goteo. Las vides se dispusieron con un espaciado de 3 × 2 metros entre hileras y dentro de hileras, respectivamente. El viñedo se manejó de acuerdo con las prácticas de viticultura estándar para el cultivar y la región.

1.2. Vinos tranquilos

Para diferentes estudios de los capítulos 2 y 3, los vinos tranquilos empleados fueron vinos blancos monovarietales de la variedad Albariño representativos de la Denominación de Origen Rías Baixas (2017-2018), vinos rosados de Garnacha (2017-2018) y vinos tintos de Tempranillo (2006, 2007, 2008, 2009, 2010, 2015 y 2016), ambos de la Denominación de Origen Calificada Rioja. Las elaboraciones de los vinos fueron realizadas de forma convencional como se ha explicado en el apartado 2.1 de la introducción.

1.3. Vinos espumosos

En los estudios de los capítulos 2 y 3, los vinos espumosos empleados fueron Cavas y Champagnes. El Cava fue elaborado con las variedades de uva Chardonnay y Xarel.lo pertenecientes a la zona geográfica del Penedés (Cataluña) y a la Denominación de Origen Cava, mientras que el Champagne fue elaborado con las variedades Pinot Noir y Chardonnay. Ambos espumosos fueron elaborados mediante el método *Champenoise* o método tradicional explicado previamente en el apartado 2.2 de la introducción.

1.4. Corchos y granulados de corcho

Para el estudio del capítulo 4, diferentes tapones de corcho, de iguales dimensiones, pero distinta porosidad, y sus respectivos granulados con diferente tamaño, fueron macerados en solución hidroalcoholica (13 % de etanol y el pH ajustado a 3,7) en frascos de vidrio a diferentes tiempos para su posterior análisis.

2. Parámetros estudiados y técnicas analíticas empleadas

Los parámetros analíticos básicos se determinaron siguiendo el compendio de los métodos internacionales, establecido por la Organización Internacional de la Viña y el Vino (OIV) (147).

Grado alcohólico y la acidez volátil se determinaron mediante un sistema de espectroscopia infrarroja (FTIR) (WineScanTM de FOSS, Hilleroed, Dinamarca), calibrado internamente según los métodos de referencia de la OIV (OIV-MA-AS312-01B y OIV-MA-AS313-01).

Acidez. Obtenida mediante la acidez total titulable (OIV-MA-AS313 01) expresada como g/L de ácido tartárico y el pH (OIV-MA-AS313-15), los cuales se midieron mediante potenciometría con un titulador automático (TitroMatic Hach de Crison®) previamente calibrado con disoluciones patrón.

Dado que el contenido de ácidos orgánicos también influye en la acidez total de los vinos, también se determinaron los ácidos orgánicos L-málico (OIV-MA-AS313-11), L-láctico (OIV-MA-AS313-07), acético (OIV-MA-AS313-04), cítrico (OIV-MA-AS313-09) y tartárico (OIV-MA-AS313-10) obtenidos mediante Kits de BioSystems S.A. mediante reacción enzimática basados en los métodos propuestos por la OIV.

Azúcares, expresados como la suma glucosa y fructosa (OIV-MA-AS311-02) la cual se determinó al igual que los ácidos orgánicos mediante reacción enzimática con Kits de BioSystems S.A. basados en los métodos propuestos por la OIV.

Nitrógeno Fácilmente Asimilable (YAN), se determinó utilizando la metodología enzimática desarrollada por Bio-Sistems SA (148) según Zoecklein et al. (1999), y el compendio de métodos internacionales de análisis de vinos y mosto (147).

Sulfuroso, fue obtenido a través del contenido de azufre libre y total, el cual se analizó mediante el método oficial de referencia de la OIV, el método Paul (OIV-MA-AS323-04A). El dióxido de azufre de un vino o mosto se libera, después de acidular, por arrastre con una corriente de aire y se oxida a ácido sulfúrico haciéndolo borbotear a través de una solución diluida y neutra de peróxido de hidrógeno (agua oxigenada). El ácido sulfúrico formado se determina con una solución estandarizada de hidróxido de sodio. La liberación en frío (20 ºC) asegura la extracción del dióxido de azufre libre y en caliente (100 ºC) la del dióxido de azufre total.

Además, en alguno de los vinos espumosos también se determinaron otros parámetros característicos de este tipo de vino:

Espumabilidad mediante el método de Mosalux descrito por Maujean (149). Este método consiste en medir los siguientes tres parámetros para determinarla calidad de la espuma. HM = altura máxima de espuma, expresada en milímetros, es la altura máxima alcanzada por la espuma después de la inyección de CO_2, representa la capacidad de la disolución para producir espuma. HS = altura a la cual se estabiliza la espuma durante la inyección de CO_2, expresado en milímetros, representa la capacidad de la disolución para producir una espuma estable. TS = tiempo de estabilidad de la espuma, expresado en segundos, es el tiempo que transcurre desde la interrupción del flujo de gas y la desaparición total de la espuma.

Proteínas, se determinó el contenido total mediante un espectrofotómetro UV-visible (BioDrop _Lite, de Thermo Fisher Scientific, Waltham, MA, EE. UU) y la composición proteica mediante electroforesis en gel de poliacrilamida con dodecilsulfato de sodio (SDS-PAGE). La cuantificación de proteínas con espectrofotometría es un método que usa la luz ultravioleta y la espectroscopía visible para determinar de manera rápida la concentración de proteínas utilizando solo 1 a 2 µL de muestra. Esta técnica calcula la concentración de proteínas basándose en la absorción de la muestra en 280 nm y el coeficiente de extinción específico de la proteína y su longitud de onda. Por otra parte, mediante la técnica de SDS-PAGE se pueden llegar a cuantificar las proteínas dependiendo de su peso molecular. La poliacrilamida está formada por dos polímeros diferentes, acrilamida y bisacrilamida. Estos dos se combinan entre sí y forman la red que delimitará la movilidad de las proteínas. La electroforesis es el proceso de separación de moléculas cuando se aplica un campo eléctrico, el cual provoca el movimiento de las moléculas hacía un electrodo u otro en función de su carga la cual, a su vez, depende de su masa molecular. De ahí que las aplicaciones estén asociadas al cálculo de pesos molecular (proteína y DNA), la caracterización molecular o para comprobar la pureza.

OTR. La tasa de transferencia de oxígeno a través de un corcho fue determinada por equipo MOCON (figura 10). Esta técnica se basa en la normativa de los envases plásticos ASTM-F107-14, al medir el oxígeno que se transfiere a través de un cuello de botella sellado a una placa metálica y al medir entrada y salida de gases por técnicas barométricas.

Figura 10*. Equipamiento MOCON del laboratorio de cierres de VITEC, para la medición de transferencia de oxígeno (OTR). Los distintos materiales son adheridos mediante un pegamento especial al cuello de una botella donde se realiza la medición como si de un cierre de botella se tratara.*

Por último, otros parámetros más complejos en su determinación e instrumentación analítica fueron determinados:

Fenoles. Índice Folin-Ciocalteu: Los fenoles totales se determinaron mediante la determinación del índice de Folin-Ciocalteu (150) siguiendo el método usual de la OIV (OIV-MA-AS2-10). . Este método se basa en una reacción de óxido-reducción entre el reactivo de Folin y los fenoles presentes en la muestra. El reactivo de Folin Ciocalteu está formado por una mezcla de ácido fosfotúngstico y ácido fosfomolíbdico que, al reaccionar con los compuestos fenólicos, se reduce a una mezcla compleja de color azulado adecuada para la determinación espectrofotométrica por medida de la absorbancia a 765 nm. el índice fenólico total (IPT), y el contenido de antocianos y taninos se midieron mediante espectrofotometría siguiendo los métodos propuestos por la OIV (OIV-MA-AS2-07B). Para ello se empleó un espectrofotómetro Helios-α (Thermo Fisher Scientific, Waltham, MA EE. UU.).

Color. Características cromáticas y la intensidad de color (IC): Para caracterizar el color de los vinos, se determinó el valor de las absorbancias a 420, 520 y 620 nm en cubeta de 1 cm mediante espectrofotometría UV-vis. Las muestras se filtraron previamente por 0.45 µm antes de realizar las medidas de absorbancia. La intensidad colorante se obtuvo con la fórmula (ecuación 2):

$$IC = A420 + A520 + A620$$

Los parámetros CIELab, L* (luminosidad), a* (rojo-verde), b* (amarillo-azul) (figura 11), se determinaron mediante el software MSCV® siguiendo las recomendaciones de la Commission Internationale de L'Eclariage (CIE); observador estándar (campo visual de 10º) e iluminante estándar D65 como referencias. Las diferencias de color (ΔE*ab) se calcularon como la distancia euclídea entre dos puntos del espacio 3D definido por L*, a* y b* (ecuación 3) (151).

$$\Delta E^*ab= [(L^*_1-L^*_2)^2 + (a^*_1-a^*_2)^2 + (b^*_1-b^*_2)^2]^{1/2}$$

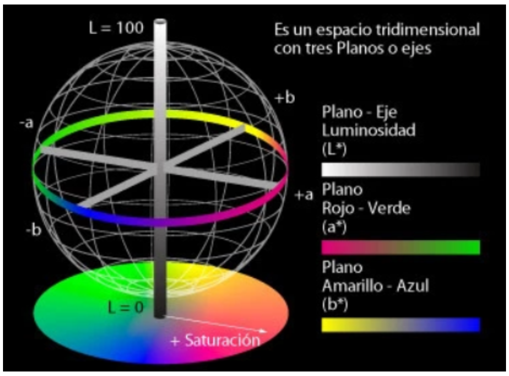


Figura 11. *Representación en espacio tridimensional de las coordenadas CIELab.*

Actividad antioxidante. Este parámetro se determinó en las muestras utilizadas en el capítulo 4 de la tesis, mediante el método de la ABTS. Este método se fundamenta en la cuantificación de la decoloración del radical ABTS+, debido a su reducción a ABTS por la acción de antioxidantes. El radical catiónico ABTS+ es un cromóforo verde azulado que absorbe a una longitud de onda de 734 nm y se genera por una reacción de oxidación del ABTS (2,2'-azino-bis- (3-etil benzotiazolin-6-sulfonato de amonio) con persulfato de potasio. Para ello se empleó un espectrofotómetro Helios-α (Thermo Fisher Scientific, Waltham, MA EE. UU.). De esta manera el grado de decoloración como porcentaje de inhibición del radical ABTS+ está determinado en función a la concentración.

Estabilidad tartárica. Cuando fue necesario, la estabilidad tartárica del vino se determinó mediante un analizador CheckStab (Alfa 2016 iLife, Delta Acque, Firenze, Italia) equipado con tecnología ISS. Este sistema se basa en dos métodos analíticos para monitorear la estabilidad de KHT, la prueba de minicontacto (MC) y las mediciones de temperatura de saturación (ST), basadas en la evaluación de conductividad. La prueba de minicontacto es la determinación de la conductividad por frío, con la adición de bitartrato de potasio ("crémor tártaro"). La prueba MC permite evaluar el nivel de inestabilidad del vino. Mientras que la temperatura de saturación expresa el valor de temperatura más baja en el que el bitartrato de potasio añadido se disuelve en el vino.

Metales. El contenido de metales en vino, y de micronutrientes y macronutrientes en uvas se determinó mediante un espectrómetro de emisión atómica de plasma acoplado inductivamente, serie iCAP 7000 (Thermo Fisher Scientific, Waltham, Massachusetts, EE. UU.). La espectroscopia de emisión atómica con plasma de acoplamiento inductivo (ICP-OES), es una técnica de análisis multi elemental capaz de determinar y cuantificar la mayoría de los elementos de la tabla periódica, a excepción de C, N, O, H, F, gases nobles, y otros elementos poco frecuentes. Las muestras son introducidas en forma líquida tratadas previamente, y son transformadas mediante un nebulizador en un aerosol y excitadas mediante un plasma de argón. Las emisiones de los átomos excitados se recogen mediante un sistema óptico basado en un policromador, obteniendo espectros de emisión para las líneas seleccionadas en cada elemento.

Aminoácidos y vitaminas. El contenido de aminoácidos se determinó por cromatografía líquida de alta presión con detector de diodos (HPLC-DAD), Agilent 1200 Series (Agilent Technologies, Palo Alto, CA, EE UU). Por su parte, la vitamina B12 o riboflavina, se determinó mediante cromatografía líquida de ultra alta presión con detector de fluorescencia (UPLC-FLD), Agilent 1260 Series (Agilent Technologies, Palo Alto, EE. UU.).

La cromatografía líquida de alta presión es una técnica idónea para este tipo de determinación ya que permite separar los componentes de una mezcla compleja como el vino mediante la selección de una fase estacionaria y una móvil adecuadas. Así, la fase móvil actúa de portador de la muestra a través de la fase estacionaria alojada dentro de la columna cromatográfica, de manera que los analitos sufren distintos tipos de interacciones con esta fase estacionaria. Estas interacciones químicas, determinan la separación de los distintos analitos de la muestra los cuales van eluyendo de la columna a distintos tiempos a la vez que van siendo detectados si se selecciona un detector adecuado a la naturaleza de los compuestos a determinar. Por ello para la determinación de vitaminas, en concertó de la vitamina B12 o riboflavina se emplea un detector de fluorescencia mientras que para el análisis de aminoácidos y de la composición fenólica, como se verá a continuación, se emplea un detector de diodos. Para ambas determinaciones, todas las muestras de vino se filtraron usando filtros de 0,45 micras de tamaño de poro y se inyectaron directamente en el cromatógrafo líquido modular.

Composición fenólica, antocianinas y pigmentos derivados. La identificación y cuantificación de los compuestos fenólicos se realizó mediante HPLC-DAD, igual que se hizo para los análisis de aminoácidos. Las muestras de vino se filtraron por filtros de 0,45 micras de tamaño de poro y se inyectaron directamente un cromatógrafo líquido modular

Composición aromática. Los compuestos volátiles se determinaron mediante cromatografía de gases con detector de masas (GC-MS) en el caso de aromas varietales, fermentativos, oxidativos y aromas de corcho, y con detector fotométrico de llama (GC-FPD) en el caso de los aromas de reducción. Con respecto al tratamiento de muestra, para determinar los aromas varietales, oxidativos y los del corcho se realizó una extracción en fase solida (SPE) previa e inyección directa de líquido mediante jeringa. Para la determinación de los aromas fermentativos y de reducción la técnica empleada para separarlos y concentrarlos fue la microextracción directa en fase solida (SPME) mediante fibras específicas para cada tipo de aromas a analizar. La separación de los distintos analitos determinados en los distintos estudios, así como su identificación se realizaron usando un cromatógrafo de gases 7890A (Agilent Technologies, Palo Alto, EE. UU.) (figura 12). La técnica de cromatografía de gases tiene especial importancia en esta tesis debido a que ha sido empleada en todos los estudios que la configuran.

Figura 12. *Equipo Cromatógrafo de gases 7890A acoplado al espectrómetro de masas 5975C.*

La cromatografía de gases tiene el mismo fundamento físico-químico que la de líquidos, pero en este caso se consigue separar compuestos que pueden volatilizarse, y la fase móvil es un gas inerte cuya función es transportar a la muestra a través de la fase estacionaria que, en este caso se encuentra alojado en una columna de un diámetro capilar pero de longitud entre 25-50 metros. La separación de los compuestos se realiza en base a las interacciones de los distintos analitos con la fase estacionaria y ayudados por la temperatura que se va aplicando en el horno donde se encuentra la columna cromatográfica. Una vez los compuestos de interés van eludiendo de la columna pasan a través de un sistema de detección que permite su detección, identificación y cuantificación.

Respecto a los detectores empleados en los diferentes estudios de esta tesis, como se ha comentado más arriba, por una parte, se empleó un detector fotométrico de llama (FPD) para la detección y cuantificación de los compuestos volátiles azufrados responsables de generar los aromas de reducción. Este detector (FPD), utiliza una llama de hidrógeno para excitar a un estado energético superior los electrones de fragmentos de moléculas que contengan átomos de azufre. Cuando los electrones retornan a su estado fundamental emiten energía que se puede identificar con las líneas características de sus espectros; las líneas analíticas de interés son seleccionadas por medio de un filtro (392 nm para determinar azufre) y la intensidad de la radiación emitida es medida por medio de un fotomultiplicador.

Por otra parte, para la identificación y cuantificación de la mayoría de los aromas analizados a lo largo de la tesis (varietales, fermentativos, oxidativos y derivados del corcho) se empleó un espectrómetro de masas (Agilent serie 5975C) acoplado al cromatógrafo. La espectrometría de masas (MS) es una de las técnicas analíticas más completas que existen y que consiste en la ionización de la muestra mediante diferentes métodos. El sistema de ionización más frecuente es el de impacto electrónico que bombardea las moléculas con electrones de una cierta energía, de manera que las moléculas se rompen dando lugar a distintos iones. Posteriormente, estos iones se separan de acuerdo a su masa y carga para poder ser detectados e identificados con un sistema adecuado para tratar la información bidimensional obtenida: por un lado, la abundancia de cada ion y por el otro la relación masa/carga de cada ion obtenido. Esta técnica se caracteriza por su alta capacidad de identificación de forma prácticamente inequívoca, ya que proporciona información estructural sobre las moléculas analizadas lo que da lugar a un espectro característico para cada una de ellas.

3. Análisis sensorial

Los análisis sensoriales llevados a cabo en los distintos estudios realizados a lo largo de la presente tesis fueron realizados por un panel de cata de VITEC (Centro Tecnológico del Vino) acreditado según la norma ISO 8586: 2010, y en una sala de catas estandarizada (ISO 8589: 2007) (figura 13).

Figura 13. *Sala de cata acreditada por ISO 8589: 2007, donde se realizaron todos los análisis sensoriales de la tesis. La imagen de la izquierda es la sala de cata con luz led blanca, mientras que la imagen de la derecha es la sala de cata con luz led roja empleada para realizar el análisis sensorial.*

Todas las catas fueron realizadas utilizando el análisis descriptivo cuantitativo (QDA). Este método se basa en cuantificar las intensidades percibidas de las distintas características sensoriales evaluadas en un producto, en este caso el vino. El análisis sensorial descriptivo cuantitativo (QDA) realizado mediante la cata, es el medio más razonable para evaluar la calidad de los vinos.

La cata o análisis sensorial es un proceso que pasa por las tres fases anteriormente descritas y, el resultado es la combinación de los resultados obtenidos en cada una de ellas. Así, se empieza con la fase visual con el fin de evaluar el color, y la presencia y calidad del carbónico en el caso de vinos espumosos. A esta fase le la fase gustativa y aromática donde se valoraron las sensaciones gustativas de aquellas sustancias no volátiles percibidas en boca y el aroma de las sustancias volátiles percibidas por el olfato ya fuera por vía nasal o retronasal. El análisis sensorial realizado contempló los diferentes factores que engloban estas tres fases con el fin de poder definir la calidad de los vinos evaluados.

Las fichas de cata fueron confeccionadas para plasmar los resultados obtenidos en la cata. En ellas, se fue anotando las sensaciones y atributos percibidos, así como su intensidad mediante las diferentes puntuaciones. Dado que en cada estudio se requirió el análisis sensorial de compuestos distintos, las fichas de cata fueron diseñadas según el estudio y las muestras a analizar en cada caso. Finalmente, los datos obtenidos se procesaron con el software FIZZ (V.2.47B Biosystems, Barcelona, España).

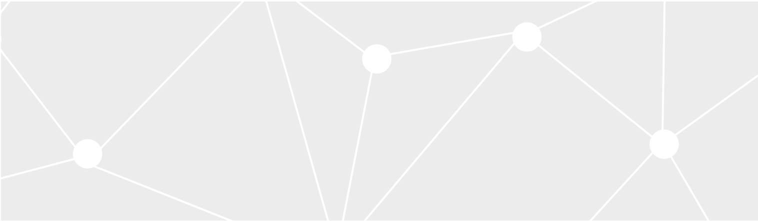
4. Análisis estadístico

Los análisis estadísticos para realizar el tratamiento de los datos obtenido en los diferentes estudios que componen la tesis fueron:

- Análisis de varianza simple y de dos vías (ANOVA) utilizando el programa StatGraphics Centurion XVI (Manugistics Inc., Pockville, MD, EE. UU.). Se utilizó el procedimiento de Tukey y las diferencias en *p*-valores <0,05 se consideraron significativas.

- Análisis de componente principales (PCA) con el fin de visualizar la estructura del conjunto de datos experimentales se aplicó un análisis multivariante, utilizando el paquete XLSTAT 2016.01 para el paquete Microsoft Excel (Manugistics Inc., Pockville, MD, EE. UU.).

Resultados



CAPÍTULO 1

CALIDAD DE LA UVA

Tratamientos con bioestimulantes y su repercusión en la composición química de la uva

Justificación y objetivo

La fertilización de la vid es muy importante para obtener un estado óptimo de calidad de la uva, pero para que este tratamiento sea sostenible es necesario que no suponga una contaminación ambiental, ni tampoco un gran coste económico.

Una fertilización nitrogenada equilibrada es fundamental para el crecimiento y desarrollo de la vid. El nitrógeno de la uva sirve para el desarrollo de la actividad de las levaduras durante el proceso de fermentación contribuyendo a la obtención de un vino de calidad. Durante la vinificación, el nitrógeno está relacionado con la formación de metabolitos secundarios, como aminoácidos y compuestos volátiles que influyen en la percepción aromática del vino. Además, el aporte de aminoácidos también puede influir en el color y el potencial de envejecimiento del mismo.

Actualmente, se está extendiendo el uso de los denominados bioestimulantes como complementos nutricionales para las plantas, los cuales incluyen diferente composición de aminoácidos. Sin embargo, la respuesta fisiológica de la vid a estos nuevos tratamientos y su repercusión en la calidad de la uva es todavía muy desconocida. Por ello, en el estudio de este primer capítulo se planteó profundizar en el conocimiento y repercusión del uso de estos nuevos tratamientos bioestimulantes. El estudio se llevó a cabo mediante ensayos de fertirrigación. Para ello se utilizaron formulaciones basadas en un elicitor activador de las defensas de las plantas (la menadiona sodio bisulfito) complementados por macro (N, Ca) y microelementos (Mn, B y Zn). Además, se compararon formulados de aminoácidos de origen animal frente a formulados de origen vegetal con la intención de comparar su eficacia y posible sustitución.

Objetivos específicos

El principal objetivo del artículo 1 fue evaluar el efecto de los fertilizantes constituidos con aminoácidos de origen animal y de origen vegetal en la calidad de las uvas de Syrah y especialmente en su composición aromática.

Publicaciones asociadas

- **Artículo 1:** *Effects of fertigation by elicitors enriched in amino acids from vegetal and animal origins on Syrah plant gas exchange and grape quality.*

Artículo 1

Effects of fertigation by elicitors enriched in amino acids from vegetal and animal origins on Syrah plant gas exchange and grape quality.

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Effects of fertigation by elicitors enriched in amino acids from vegetal and animal origins on Syrah plant gas exchange and grape quality

Rafael Roda[a], Laura Martín[a], Ana María Mislata[a,b], Francisco Javier Castaño[a], Miquel Puxeu[a], Raúl Ferrer-Gallego[a,*]

[a] *Wine Technology Centre (VITEC), Ctra. de Porrera km 1, 43730, Falset, Tarragona, Spain*
[b] *Sensometria Instrumental (i-Sens), Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Tarragona 43007, Spain*

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ABSTRACT

Nutrition management of vines is a key factor that regulates production and determines grape quality. Currently, one of the main objectives of fertilization is to apply eco-friendly and balanced nutrition. In this case, fertigation is the most efficient strategy. The objective of this work was to assess the effect of innovative fertilizers on Syrah grapevines. The tested products contained an elicitor, macro- and microelements and amino acids from animal and vegetal origins. Fertilizers were applied by fertigation under field conditions in 2016, 2017 and 2018. The influence of the treatments on the grapevine gas exchange, element assimilation and oenological composition of must was evaluated. Additionally, the amino acid contents and volatile composition were determined. The results suggest that application of fertigation does not directly influence the water status of Syrah vines under the conditions of the study but can improve the gas exchange balance and N assimilation. Moreover, the leaf assimilation of Zn, Ca and, to a lesser extent, B were improved at the bloom stage, and the assimilation of Mn was improved at veraison. Small changes were observed in the basic parameters of musts, and an increase in yeast assimilable nitrogen was detected in treated vines. The aromatic profile of grape musts was enhanced in treated vines, and the elicitors enriched in vegetal amino acids could be a sustainable alternative for improving certain vegetative and grape quality parameters of Syrah.

1. Introduction

Grapevine is one of the most economically important fruit crops (Zhang et al., 2016). Genetic factors (i.e., grape variety and clonal selection) (Portu et al., 2018; Cornelis van Leeuwen, Roby, Alonso-Villaverde, & Gindro, 2013), climatic conditions and viticultural practices, such as irrigation (Canoura, Kelly, & Ojeda, 2018; L. Zhang, Marguerit, Rossdeutsch, Ollat, & Gambetta, 2016), soil and canopy management, fertilization (Bell & Henschke, 2005; Canfora et al., 2018; T. Garde-Cerdán et al., 2014; Vilanova, Fandiño, Frutos-Puerto, & Cancela, 2019), and control of pests and diseases have a huge influence on the plant growth, grape development and must composition.

Grapevine cultivation requires a balanced fertilization. Unbalanced fertilization (excess or deficiency in certain nutrients) results in high cost in terms of environmental contamination, economy, and production and ultimately can affect wine quality and value. For example, a severe nutritional deficiency of iron can cause a drastic reduction in the quality of the grape musts as a result of a reduction in the contents of sugars and anthocyanins and induce an increase in total acidity (Casanova-Gascón, Martín-Ramos, Martí-Dalmau, & Badía-Villas, 2018). Micronutrients essential for grapevine include boron (B), which plays an important role in sugar transport, root elongation, plant hormone regulation and generative growth of plants (Marschner, 1995), and zinc (Zn), which is an integral component or activator of a number of enzymes, whose deficiency causes a reduction in the instantaneous transpiration efficiency of the leaves (Sabir & Sari, 2019). Nitrogen plays a key role in the vine vigor, fermentation process and final wine quality (Bell & Henschke, 2005; Canoura et al., 2018). In winemaking, nitrogen is indirectly related to formation of the secondary metabolites, such as amino acids, phenolic compounds and volatile composition (Bell & Henschke, 2005; Portu, López, Baroja, Santamaría, & Garde-Cerdán, 2016; Vilanova et al., 2019). All these factors have an impact on sensory perception of aroma, flavor and mouthfeel of wine (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

The improvement of amino acid composition and grape phenolic content is the major concern in red winemaking industry because these are the key factors that determine fermentation, wine color, wine ageing potential and mouthfeel properties (Ribéreau-Gayon et al.,

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[*] Corresponding author.
E-mail address: raul.ferrer@vitec.wine (R. Ferrer-Gallego).

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2006). In recent years, a number of studies reported foliar fertilization treatments with various nitrogen compounds (Gastón Gutiérrez-Gamboa, Garde-Cerdán, Gonzalo-Diago, Moreno-Simunovic, & Martínez-Gil, 2017; Rubio-Bretón, Gutiérrez-Gamboa, Pérez-álvarez, Santamaría, & Garde-Cerdán, 2018). These treatments have substantial impact on the must amino acids contents (T. Garde-Cerdán et al., 2014; G. Gutiérrez-Gamboa, Portu, Santamaría, López, & Garde-Cerdán, 2017) and a lesser impact on phenolic composition (Portu, López-Alfaro, Gómez-Alonso, López, & Garde-Cerdán, 2015; Portu, Santamaría, López, & Garde-Cerdán, 2017). However, the method of application may influence these results. The effect of a type of nitrogen application (soil and/or leaves) on the biochemical composition of *Vitis vinifera* L. cv. Chardonnay and Syrah grape berries was recently studied by Canoura et al. (2018). The results showed that foliar application increased the amino acid contents to a greater extent than that observed in the case of soil application; however, the combination of both methods was the most effective (Canoura et al., 2018). These results demonstrate that the type of fertilization and the method of application have an effect on the chemical composition of grapes. At the same time, commercial nitrogen foliar fertilizers have appeared on the market and their composition includes amino acids with nutritional purposes. A current goal for the manufacture of these products is to replace animal amino acids with amino acids from a vegetal origin. There is a lack of information on assimilation of amino acids by the grapevine depending on their origin (animal or vegetal).

Application of elicitors is another interesting alternative for improvement of grape phenolic content (Apolinar-Valiente et al., 2018; Paladines-Quezada, Moreno-Olivares, Fernández-Fernández, Bautista-Ortín, & Gil-Muñoz, 2019). Elicitors provide plants with greater defensive capacity and better response to stress situations caused by biotic and abiotic factors. Plant defense mechanisms include activation of secondary biosynthetic pathways such as the formation of phenolic compounds. Initially, elicitors were tested to increase plant resistance to pathogens; however, certain previous studies have investigated the impact of the foliar pre-harvest treatment with the elicitors methyl jasmonate, chitosan, and yeast extract on amino acid and phenolic contents and on grape volatile composition (Teresa Garde-Cerdán, Gutiérrez-Gamboa, Baroja, Rubio-Bretón, & Pérez-Álvarez, 2018; Gil-Muñoz, Fernández-Fernández, Crespo-Villegas, & Garde-Cerdán, 2017; Portu et al., 2016).

The molecules with eliciting activity include menadione sodium bisulfite (MSB), a vitamin reported to function as an activator of plant defense against several pathogens (Boubakri et al., 2016; Zhang, Jain, Fleites, Rayside, & Gabriel, 2018). Very little is known about the effect of MSB elicitor on grapevines, their physiology, activation of defense response, and subsequent biochemical composition of grapes. Considering the lack of information, the aim of the present study was to evaluate the nutritional assimilation, physiological gas exchange and grape composition of cv. Syrah in response to various fertigation treatments with amino acids and the elicitor MSB.

2. Materials and methods

2.1. Experimental conditions

The experiment was carried out in 2016, 2017 and 2018 in a *Vitis vinifera* cv. Syrah commercial vineyard located in Raimat, Lleida (Catalonia, North East Spain). Vines were planted in 2002 at an altitude of 299 m a.s.l., grafted onto a 1103-Paulsen rootstock and trained on a bilateral cordon with drip irrigation. Vines were arranged with 3 × 2 m spacing between-row and within-row, respectively. The vineyard was managed according to the standard viticulture practices for the cultivar and region. Weather conditions were recorded by a meteorological station installed in the experimental field. The accumulated annual rainfall was 346 mm in 2016, 317 mm in 2017 and 485 mm in 2018. The minimum temperature was −5.6 °C, −8.3 °C and − 6.3 °C, respectively, and the maximum temperature was 37.2 °C, 38.7 °C and 36.5 °C, respectively. The average annual temperature in the three vintages was approximately 14.0 °C (Supplementary Material S1). The relative humidity was 71% in 2016, 69% in 2017 and 73% in 2018. The soil was classified according to the American Soil Taxonomy as typical clay-loam on the grounds of average granulometry with a few gross elements.

The fertilizer products were formulated according to the manufacturer protocol (AgroStock S.A.) and contained MSB as an elicitor, macro- and microelements (N, Ca, Mn, B and Zn) and amino acids (see Supplementary Material, S2). The composition and the nutrient richness of the fertilizers and application treatments are shown in the Supplementary Material S2. Briefly, two treatments by fertigation were compared with a control treatment: (T1) elicitor with amino acids from animal origin, (T2) elicitor with amino acids from vegetal origin. A randomized block in the vineyard was assigned for application to include four replicates which contained five or seven vines per line (Supplementary Material S2). Following the manufacturer's instructions, irrigation was applied through a drip system and T treatments were applied by fertigation at 25 L/Ha. The treatments (five times per vintage) were carried out weekly, three times before flowering and two times before veraison. Control plants were not treated with fertilizer products and were only irrigated.

2.2. Physiological measurements

Measurements of leaf water potential (Ψ_{leaf}) and gas exchange were performed in the treated (T1 and T2) and untreated plants (C). The plant water status was estimated by the Ψ_{leaf} parameter and measured with a Scholander chamber (Soil Moisture Equipment Corp., USA). Net leaf photosynthesis (A_N), stomatal conductance (gs) and transpiration (E) rates were determined simultaneously in leaves with a portable infrared gas analysis system (IRGA) (LCI Portable Phothosyntesis System, ADC BioScientific Ltd., Hoddesdon, England). Leaf temperature and internal CO_2 (Ci) partial pressure were calculated automatically by the internal software of the device. Intrinsic water use efficiency (WUEi) was determined by the ratio of (A_N/gs) which provides the cost of water for the CO_2 assimilation (Tomás et al., 2014). The IRGA was equipped with a clamp-on leaf cuvette that exposed 6.25 cm^2 of leaf area and PAR (photosynthetic active radiation) was always above the saturation levels. All measurements were performed in sunny and adult leaves located in the middle part of the cane from 9.00 to 11.00 a.m. just before midday depression as described by other authors (Cartechini & Palliotti, 1995). Physiological determinations were performed at two different phenological stages, including pea-size and veraison, in two vines per each replicate (n = 8). The measurements were performed at pea-size (July) and at post-veraison (September).

2.3. Determination of the elements by inductively coupled plasma optical emission spectrometry (ICP-OES)

Macro- and micronutrients were quantified in leaves and berries. In the case of leaves, petioles were separated, rinsed with distilled water, oven-dried (Dry-Big; J.P. Selecta) at 100 °C until constant weight and manually crushed. For determination of macronutrients (K, P, Ca, S, and Mg) and micronutrients (Fe, Cu, B, Mn, and Zn), dry ashing (calcination at 450–600 °C) was carried out in a muffle furnace (Nabertherm LT 9/11/B140). Approximately 1.0 g sample was transferred to a porcelain crucible and then incinerated in a muffle furnace adjusted to reach the temperature of 450 °C at a rate of 50 °C/h for 12 h. The obtained ash was solubilized at the 1:1 ration in 65% HNO_3 (de PanReac AppliChem). The residue was filtered through a 0.22 μm syringe filter; the sample was diluted to 100 mL with distilled water in polypropylene tubes and analyzed by ICP-OES. All analyses were carried out on an iCAP 7000 Series inductively coupled plasma optical emission spectrometer (Thermo Scientific). Total N (N-organic + N-

NH_4^+) was measured by the Kjeldahl method after mineralization of 0.5 g of the crushed sample in 12 mL concentrated H_2SO_4 with a catalyst mixture (selenium tablets with 3.5 g K_2SO_4 + 3.5 mg Se) at 420 °C for 60 min using a DT 220 Digestor (FOSS). Subsequently, NH_3 was distilled, collected in 1% H_3BO_3 solution and automatically titrated with 0.01 N HCl by a Kjeltec 8400 analyzer (FOSS). For must analysis, a representative sample of the grapes from each of the field replicates was used to elaborate the corresponding grape juice (n = 4). An aliquot from approximately 500 mL of the juice was diluted, filtered by a 0.22 μm syringe filter, and directly analyzed in duplicate by the ICP-OES equipment as described above.

2.4. *Oenological parameters of musts*

The total polyphenol index (TPI) was measured after dilution with water (1:10) by directly measuring the absorbance at 280 nm in a 1 mm path length quartz cell. The value of the TPI for each sample was calculated as absorbance multiplied by the dilution. All basic oenological parameters (pH, total titratable acidity and alcoholic strength) were determined according to the OIV methods (OIV, 2016). Anthocyanins and tannins were measured according to the methodology described by Ribéreau-Gayon and Stonestreet (1965, 1966), respectively) (Ribéreau-Gayon & Stonestreet, 1965, 1966). Yeast assimilable nitrogen (YAN) was calculated using the enzymatic methodology developed by Bio-Sistems S.A. according to Zoecklein, Fugelsang, Gump, and Nury (1999) and the compendium of international methods of wine and must analysis (OIV, 2016; Zoecklein et al., 1999) as follows: YAN = ammonium content (mg/L) × 0.78 + free α-amino nitrogen (FAN).

2.5. *Primary amino acid analysis by HPLC-DAD*

Analysis of amino acid composition was carried out according to the procedure of Henderson, Robert, Brian, and Cliff (2000) (Henderson et al., 2000). The primary amino acids were analyzed by an Agilent UPLC 1260 Series (Palo Alto, USA) equipped with a DAD detector and an autosampler. The homogenized grape juice samples were filtered through a 0.2 μm Millex syringe-driven filter unit (Millipore Corporation, Bedford, MA, USA) and submitted to an automatic precolumn derivatization with *o*-phthaldialdehyde (OPA Reagent, Agilent) before chromatographic analysis. The injected volume of the derived sample was 5 μL; an Agilent Zorbax Eclipse Plus C18 (4.6 × 150 mm, 3.5 μm) column thermostated at 40 °C was used. Solvents were HPLC grade; eluent A: 40 mM Na_2HPO_4, pH 7.8, and eluent B: ACN: MeOH: water (45:45:10, *v*/v/v). Gradient conditions were as follows: 0–1.9 min, 0% B; 1.9–18.1 min, 57% B; 18.1–18.6, 100% B; 18.6–22.3 min, 100% B; 22.3–23.2 min, 0% B and 23.2–26 min, 0% B. Flow rate was 1.5 mL/min and detection was performed at λ = 338 nm. The identification of amino acids was performed by comparison of their retention times with those of pure reference standards. The pure reference compounds and internal standards were supplied by Sigma Aldrich. Quantification of amino acids was performed with standard calibration curves (see supplementary material S3). All samples for analysis were injected in duplicate (n = 8).

2.6. *Volatile compound analysis by GC–MS*

The volatile compounds of the grapes were extracted by solid phase extraction (SPE) and analyzed by GC–MS as described in Vilanova et al. with minor modifications (Vilanova & Martínez, 2007). Briefly, approximately 500 g of grapes were manually crushed to obtain approximately 100 mL of must, which was centrifuged (15 min at 6000 rpm). Then, 100 μL of 2-octanol was added as an internal standard and the mixture was passed through an SPE cartridge (Agilent Bond Elut ENV, 500 mg, 6 mL). The cartridges have been preconditioned and the compounds were eluted with pentane-dichloromethane (50/50) and dried (final volume 200 μL). A GC 7890A (Agilent Technologies) system equipped with a 5975C inert MSD mass spectrometer was used. The column was DB-WAX IU (60 m × 0.25 mm × 0.25 μm). A constant flow of 2.1 mL/min of He was used as a carrier gas. The injected volume was 5 μL in the splitless mode at 17.33 psi pressure (septum purge flow was 15 mL/min and splitless time was 1 min). The injector temperature was maintained at 180 °C for 1 min and then heated up to 260 °C at 250 °C/min. The temperature of the oven (60 °C) was maintained for 15 min and then increased up to 220 °C at 3 °C/min for 25 min. The mass spectrometer operated at 70 eV. The analysis was performed in the scan mode (*m/z* 10–1000). The volatile compounds were identified by their retention times and the mass fragments were compared with those of the pure standard compounds. The quantification was carried out using the method of internal standard patterns by the response factor of 2-octanol and expressed as equivalents of 2-octanol in μg/L. All analyses were performed in triplicate.

2.7. *Statistical analysis*

Significant differences (p value ≤ 0.05) were determined by one-way analysis of variance (ANOVA) by the Tukey (HSD) test using the XLSTAT package for Excel software.

3. Results and discussion

3.1. *Effect of elicitor fertigation on the physiological parameters of vines*

In this study, the physiology of Syrah grapevines was monitored during three vintages (2016, 2017 and 2018) at two phenological stages, including pea-size and post-veraison (Table 1). Under our conditions, vines differed in the water status measured as the Ψ_{leaf} depending on the vintage and phenological stage. On the one hand, results of Table 1 (related to E, gs and A_N) are consisted with the data of the literature since the phenology (post-veraison) generally decreases carbon fluxes in all three years compared to that obtained by the measurements at pea-size (Douthe et al., 2018; Escalona, Flexas, Bota, & Medrano, 2003). Low values of Ψ_{leaf} (< -1.1 MPa) are considered a moderate to weak water deficit (van Leeuwen, Trégoat, Choné, Bois, & Gaudillère, 2009) and were observed at post-veraison in 2016; however, in 2017 and 2018, a similar deficit was observed at the pea size stage (Table 1). No significant effect on the gas exchange (E and gs) was found at post-veraison due to a moderate water deficit level (2016) compared with that in the vintages that were not water-deficient (2017 and 2018).

At pea size, significant differences between the years were found in the majority of the measured physiological parameters. The data indicate that concomitant to a decrease in the Ψ_{leaf} in 2017 and 2018 (< -1.1 MPa), gas exchange (E, A_N and gs) was decreased resulting in the higher WUEi values (Table 1). At leaf scale, under moderate water deficit, an improved WUEi is generally reported because the photosynthesis is moderated by stomatal closure or mesophyll conductance (Medrano et al., 2015). However, according to a recent review, the WUE at the whole-plant level decreases during drought (Douthe et al., 2018). The whole plant-level water and carbon fluxes were not measured in this work. In general, treated plants (T1 and T2) showed a slight increase in the A_N compared with that in the untreated vines (C) in the three vintages at both phenological stages (Table 1). The results suggest that the application of fertigation does not directly influence the water status of Syrah vines under the conditions of the study but is able to improve the gas exchange balance. Little is known about the effect of elicitors on water and carbon fluxes. Foliar application of chitosan and MSB has been reported to decrease net photosynthesis (Romanazzi & Feliziani, 2016; Zhang et al., 2018). This effect was not observed when MSB was applied by fertigation in our study. Similarly, a low supplementation of N has been shown to reduce single leaf photosynthesis in a Pinot Noir cultivar, while low P and K supplementation did not have this effect (Schreiner, Lee, & Skinkis, 2013). The results of

Table 1

Physiological parameters of Syrah vines at pea-size and post-veraison phenological stages in Control (C) and treated (T1 and T2) plants. Leaf water potential (Ψ_{leaf}), transpiration (E), stomatal conductance (gs), net photosynthesis (A_N) and intrinsic water use efficiency (WUEi).

	2016[a]			2017[b]			2018[c]		
	C	T1	T2	C	T1	T2	C	T1	T2
Pea-size									
Ψ_{leaf} (MPa)	−0.88 ± 0.08 a	−0.91 ± 0.09 a	−0.95 ± 0.08 a	−1.10 ± 0.07 a	−1.19 ± 0.12 a	−1.19 ± 0.15 a	−1.28 ± 0.22 a	−1.35 ± 0.26 a	−1.33 ± 0.14 a
E (mmoles/m^2s)	6.57 ± 1.67 a	6.64 ± 1.14 a	7.24 ± 1.62 a	4.25 ± 1.28 a	5.08 ± 1.30 a	4.31 ± 1.37 a	4.18 ± 1.02 a	5.12 ± 1.24 a	4.24 ± 1.11 a
gs (moles/m^2s)	0.43 ± 0.16 a	0.37 ± 0.06 a	0.58 ± 0.28 a	0.14 ± 0.04 a	0.20 ± 0.08 a	0.15 ± 0.06 a	0.15 ± 0.07 a	0.18 ± 0.06 a	0.14 ± 0.04 a
A_N (μmoles/m^2s)	14.84 ± 2.06 a	14.39 ± 1.23a	15.23 ± 1.72 a	9.65 ± 2.42 a	11.57 ± 3.11 a	10.20 ± 2.65 a	5.42 ± 1.54 b	7.59 ± 1.09 a	6.83 ± 1.85 ab
WUEi (A_N/gs)	37.02 ± 8.70 a	39.77 ± 6.37 a	32.06 ± 14.01 a	68.29 ± 6.63 a	61.74 ± 9.30 a	70.26 ± 11.49 a	40.92 ± 18.24 a	45.10 ± 12.20 a	50.03 ± 6.58 a

	2016[d]			2017[e]			2018[f]		
	C	T1	T2	C	T1	T2	C	T1	T2
Post-veraison									
Ψ_{leaf} (MPa)	−1.17 ± 0.16 a	−1.18 ± 0.12 a	−1.29 ± 0.11 a	−0.94 ± 0.12 a	−0.92 ± 0.27 a	−0.92 ± 0.25 a	−0.99 ± 0.13 a	−0.89 ± 0.14 a	−0.86 ± 0.14 a
E (mmoles/m^2s)	4.16 ± 1.40 a	4.55 ± 0.99 a	3.84 ± 1.29 a	3.79 ± 1.25 a	4.15 ± 1.06 a	4.09 ± 2.17 a	3.99 ± 1.40 a	4.07 ± 0.89 a	3.54 ± 1.40 a
gs (moles/m^2s)	0.23 ± 0.12 a	0.22 ± 0.07 a	0.17 ± 0.06 a	0.21 ± 0.10 a	0.19 ± 0.16 a	0.22 ± 0.12 a	0.19 ± 0.07 a	0.20 ± 0.06 a	0.18 ± 0.11 a
A_N (μmoles/m^2s)	10.65 ± 1.95 a	11.89 ± 1.26 a	10.56 ± 2.13a	8.25 ± 2.83 a	9.01 ± 3.57 a	8.80 ± 4.39 a	6.77 ± 2.03 a	7.15 ± 2.63 a	7.14 ± 2.50 a
WUEi (A_N/gs)	54.63 ± 18.29 a	56.71 ± 11.90 a	64.93 ± 14.79 a	46.35 ± 10.06 a	53.32 ± 18.23 a	44.03 ± 17.23 a	37.36 ± 9.01 a	35.19 ± 7.38 a	45.37 ± 15.10 a

Different letters in rows indicate significant ($p \leq 0.05$) differences by Tukey (HSD) test for each vintage individually. The mean values and their standard deviation (n = 8).

The measurements were performed.

[a] July 27 (from 11.00 to 12.00 a.m).

[b] July 11 (from 11.00 to 12.00 a.m).

[c] August 7 (from 11:15 to 12:30 p.m).

[d] September 7 (from 11.00 to 12.00 a.m).

[e] September 5 (from 10.00 to 12.00 a.m).

[f] September 18 (from 13:30 to 14:30 p.m).

Table 2
Concentration of macroelements (nitrogen and calcium in %) and microelements (zinc, manganese and boron in ppm) determined in leaf petiole of Syrah vines at bloom (I phenological stage) and at veraison (M phenological stage) in Control (C) and treated (T1 and T2) plants.

Bloom	2016			2017				2018			
	Control	T1	T2		Control	T1	T2		Control	T1	T2
N*	0.88 ± 0.25	0.88 ± 0.17	0.86 ± 0.09	N*	0.67 ± 0.06	0.68 ± 0.06	0.72 ± 0.06	N*	0.78 ± 0.05	0.89 ± 0.09	0.92 ± 0.16
Ca*	1.87 ± 0.27	2.19 ± 0.43b	2.01 ± 0.17	Ca*	2.20 ± 0.24	2.20 ± 0.15	2.20 ± 0.12	Ca*	1.84 ± 0.17	1.94 ± 0.09	1.95 ± 0.20
Zn	32.00 ± 2.45	31.00 ± 0.81	34.75 ± 6.50	Zn*	21.25 ± 4.43	24.00 ± 3.74	20.00 ± 1.82	Zn*	27.20 ± 6.04	26.13 ± 3.96	27.30 ± 3.41
Mn*	22.00 ± 4.83	29.25 ± 6.13	28.00 ± 1.41	Mn*	35.75 ± 3.50	34.00 ± 5.41	38.00 ± 2.45	Mn*	36.79 ± 1.55	41.56 ± 5.77	40.44 ± 6.21
B*	64.25 ± 7.63	62.25 ± 2.06	62.75 ± 3.59	B	42.00 ± 2.71	41.75 ± 2.98	41.50 ± 1.91	B*	**29.16 ± 2.46****	32.72 ± 0.87	**33.49 ± 2.30****

Veraison	2016			2017				2018			
	Control	T1	T2		Control	T1	T2		Control	T1	T2
N	0.56 ± 0.03	0.55 ± 0.05	0.56 ± 0.02	N	0.50 ± 0.01	0.51 ± 0.03	0.54 ± 0.03	N	0.73 ± 0.04	0.76 ± 0.02	0.80 ± 0.08
Ca	3.40 ± 0.23	3.39 ± 0.21a	3.28 ± 0.03	Ca	2.92 ± 0.24	2.89 ± 0.16	2.76 ± 0.03	Ca	3.35 ± 0.17	3.37 ± 0.24	3.45 ± 0.31
Zn	31.25 ± 3.86	38.00 ± 5.41	29.75 ± 3.59	Z	33.50 ± 3.87	35.75 ± 4.35	42.50 ± 16.17	Zn	41.25 ± 3.30	41.00 ± 2.94	38.75 ± 4.43
Mn)	74.25 ± 16.88	91.00 ± 27.31	87.00 ± 30.94	Mn)	85.50 ± 7.77	91.75 ± 15.45	93.00 ± 18.03	Mn)	109.50 ± 13.23	109.75 ± 13.30	107.00 ± 24.86
B	43.00 ± 3.91	46.25 ± 5.18	42.25 ± 2.63	B	41.00 ± 1.41	42.00 ± 1.41	38.75 ± 2.99	B	47.50 ± 3.00	49.25 ± 2.36	48.00 ± 1.83

* indicate significant ($p \leq .05$) differences between seasons (bloom and veraison) in all treatments for the same vintage. ** (in bold) indicate significant ($p \leq .05$) differences by Tukey (HSD) test between treatments for the same vintage. The mean value and standard deviation (n = 4).

the tests of plant physiology are consisted with the nutrient status and assimilation (see section below).

3.2. Effect of elicitor fertigation on the nutrient assimilation by the plants

The results obtained at the bloom and veraison phenological stages in the three monitored vintages are shown in Table 2. In general, concentrations of all microelements (Mn, Zn, and B) in the grapevine petioles were approximately optimal or within the upper normal values according to the phenological stage of the plants (Pradubsuk & Davenport, 2011; Romero, García-Escudero, & Martín, 2010). In the case of macroelements, the values of N were lower than the standard values at the bloom stage (standard value 0.9%) except that in the case of T2 2018 (0.92%). However, at veraison, samples showed optimum (standard values are from 0.42 to 0.51%) or even higher values (0.73–0.80% in 2018). Apparently, fertilization improves the N assimilation in 2017 and 2018 (higher values than that in the control groups at both stages); however, the differences were not significant. In the case of Mn, a significant and cumulative effect of years was detected (minimum in control vines in 2016 at bloom (22.00 ppm) and maximum in T1 vines in 2018 at veraison (109.75 ppm)). Consistently, in 2016, 2017 and 2018, the percentage of N was significantly higher at bloom than that at veraison while the levels of Ca and Mn were significant higher at veraison than that at bloom. This type of variation and evolution of the assimilation of nutrients throughout the complete growth season according to the phenological stage has been well documented in other *V. vinifera* L. cultivars (Christensen, 1984; Dominguez, García-Escudero, Romero, Benito, & Martín, 2015; Romero Laibarra et al., 2005). Particularly, the same seasonal trends were identified previously including patterns of macronutrient decrease for N, P and K, while Ca and Mg are increased through the season (Romero et al., 2010). Pradubsuk and Davenport (2011) reported that petioles had the highest concentration of B at bloom and Mn at harvest. Our results are in agreement with the data on Mn, which was always higher at veraison; however, in the case of B, variability was very high.

Small significant differences were observed depending on the treatments. At the bloom stage in 2016, an increase in the percentage of Ca over and close to the optimum (> 2.02%) was observed in the treated T1 and T2 plants (2.19% and 2.01%, respectively) but not in the control plants (1.87%). At the veraison stage, higher Mn levels were detected in the treated plants (T1 and T2) than that in the control plants and the results were consistent over the three studied years (except T2 in 2018). Moreover, a slight increase in Zn was regularly observed in the treated plants and this increase was important at veraison in the case of T1 and T2 in 2016 and 2017, respectively. In vineyard, microsite differences in soil pH are likely to contribute to variable nutrient availability around the root system as demonstrated previously by Pradubsuk & Davenport, 2011. In summary, the tested treatments with new fertilizers with the MSB elicitor plus vegetal or animal amino acids showed a similar effect on the nutrient assimilation by Syrah. The treatment appears to improve the leaf assimilation of Zn and Ca although the differences were not significant. The fertigation apparently improves the assimilation of N and Mn during the plant development and, to a lesser extent, B at the bloom stage (with significant differences in 2018).

3.3. Effect of elicitor fertigation on the grape quality

The quality of the grape musts was monitored throughout the whole maturation period (Supplementary Material S4). In general, a higher decrease in the weight of berries was observed in the control vines. Moreover, treated vines generally reached the higher levels of anthocyanins and tannins along the maturation period, although a clear pattern was not observed. Small effect of the treatments on the pH, TTA and alcoholic strength was observed. Table 3 shows the oenological parameters of the grape musts from 2016 to 2018 at harvest time. No

Table 3
Must enological parameters at harvest from the fertigation treatments (T1 and T2) and control.

	2016			2017			2018		
	Control	T1	T2	Control	T1	T2	Control	T1	T2
ASv[a]	12.6 ± 0.9	12.8 ± 1.2	12.6 ± 0.3	14.0 ± 0.5	14.3 ± 0.3	13.9 ± 0.4	14.3 ± 0.8	14.6 ± 0.2	14.1 ± 0.8
TTA [b]	5.55 ± 0.5	5.93 ± 1.3	5.30 ± 0.5	**4.3 ± 0.3***	**3.8 ± 0.1***	4.0 ± 0.2	4.03 ± 0.1	4.05 ± 0.1	4.06 ± 0.1
pH	3.33 ± 0.1	3.34 ± 0.0b	3.36 ± 0.1	3.6 ± 0.0	3.6 ± 0.0	3.6 ± 0.0	3.62 ± 0.1	3.62 ± 0.1	3.61 ± 0.03
Total anthocyanins [c]	1118 ± 230	1067 ± 101	991 ± 145	1250 ± 126	1316 ± 239	1252 ± 306	1123 ± 72	1218 ± 79	1146 ± 88
Removable anthocyanins	641 ± 136	574 ± 40	556 ± 56	733 ± 87	764 ± 50	791 ± 85	661 ± 41	702 ± 42	684 ± 74
Tannins (g/L)	3.4 ± 0.6	3.7 ± 0.5	3.2 ± 0.5	4.1 ± 0.7	3.9 ± 0.3	3.9 ± 0.3	4.8 ± 0.6	5.1 ± 0.3	5.2 ± 0.3
TPI [d]	42.0 ± 4.8	39.0 ± 3.3	36.4 ± 3.3	53.2 ± 4.1	51.4 ± 4.1	54.0 ± 5.0	49.3 ± 2.9	49.2 ± 3.3	48.3 ± 2.6
FAN [e]	62.9 ± 13.3	77.2 ± 6.3	68.7 ± 1.7	83.0 ± 12.1	88.2 ± 2.3	94.4 ± 3.1	123.0 ± 13.0	125.5 ± 18.2	117.5 ± 7.9
NH_4^+ (mg/L)	32.8 ± 7.4	37.8 ± 5.1	38.8 ± 7.3	35.8 ± 5.9	42.0 ± 4.6	46.75 ± 6.6	52.0 ± 5.4	50.0 ± 10.0	52.8 ± 8.3
YAN [f]	88.4 ± 18.2	106.7 ± 7.6	99.0 ± 5.8	110.8 ± 17.1	121.0 ± 5.8	130.8 ± 10.9	163.6 ± 16.7	166.6 ± 25.9	156.5 ± 13.9

* indicate significant ($p \leq .05$) differences by Tukey (HSD) test. The mean value and standard deviation (n = 4).

[a] Alcoholic strength by volume (% *v*/v).

[b] Titratable Total Acidity as g/L of tartaric acid.

[c] Total and removable anthocyanins in mg/L

[d] Total polyphenol index.

[e] Free amino nitrogen in mg/L.

[f] Yeast asimilable nitrogen in mg/L.

significant differences were observed in these parameters between the control and treatment groups except TTA in 2017. Various studies have described an effect of nitrogen foliar fertilization on pH and total acidity; however, the trends are not always consistent (Portu, Santamaría, & López, 2017). Total and removable anthocyanins and the polyphenol index concentration (TPI) of the Syrah berries were not significantly influenced by the treatments, which is in agreement with the results of Canoura et al. (2018). With regard to the nitrogen forms, all samples were clearly above the stated threshold concentration for the completion of fermentation under normal sugar concentration (Bell & Henschke, 2005) and there were no significant differences between the treatments (Table 3). However, T1 and T2 fertigation treatments produced an increase in YAN in 2016 and 2017 and in T1 in 2018. In this regard, Garde-Cerdán et al. (2014) reported that phenylalanine and urea applications may enhance the YAN contents. The effect of the treatments on the nutrient composition of grapes was also investigated (data not shown). The contents of B, Ca, Mn and Zn were analyzed but no significant differences between the control and treated vines were observed. In summary, the basic parameters of musts and therefore the Syrah grape quality were not influenced by the treatments. Non-volatile compounds were not influenced by fertigation treatments in Albariño grapes (Vilanova et al., 2019). On the other hand, a recent review showed that certain biostimulants, such as elicitors, have become an interesting strategy to improve the grape quality by foliar application (G. Gutiérrez-Gamboa, Romanazzi, Garde-Cerdán, & Pérez-Álvarez, 2019).

3.4. Effect of elicitor fertigation on the amino acid composition of grapes

The results of amino acid composition of grapes are shown in Table 4. All tested amino acids were within the standard range of the corresponding concentrations (Bell & Henschke, 2005). A tendency to increase the individual contents of several amino acids was observed in treated vines of all vintages. It should be noted that an increase in all individual amino acids was observed in 2016 in T1 and T2 treatments. In 2017, the increase was remarkable in the T2 treatment for all individual amino acids and induced a significant increase in the total content (958 mg/L). In 2018, the fertigation appears to have a small effect on the treated vines likely due to the greater rainfall in the area in this vintage at the time of the treatments which may be associated with dilution of the fertilizers into the soil (Supplementary Marterial S1). Individually, arginine represents the major constituent amino acid in all samples reaching the highest value in 2017 in the case of the vegetal amino acid elicitor treatment (412 mg/L). The results are in agreement with the studies of other authors who demonstrated that application of foliar nitrogen fertilizers may change the amino acid composition and has a certain oenological interest (Garde-Cerdán et al., 2014; Gutiérrez-Gamboa, Garde-Cerdán, et al., 2017). Recently, Canoura et al., demonstrated that a double treatment (soil and foliar) has a considerably higher impact on the amino acid concentration than a single foliar application. On the other hand, it is known that elicitors play an important role in the defense against pathogens by increasing secondary metabolites. The effect of the elicitors has been investigated as an alternative to chemical pesticides, to a lesser extent, on the amino acid composition of the grapes (Gutiérrez-Gamboa, Portu, Santamaría, López, & Garde-Cerdán, 2017). According to these authors, the foliar application of an elicitor methyl jasmonate increased the concentrations of methionine and phenylalanine. However, a decrease in total amino acid composition was reported previously in the case of foliar application of chitosan and yeast extract elicitors (Gutiérrez-Gamboa, Portu, et al., 2017). The studies reported in the literature and our results apparently indicate that the amino acids can be better assimilated and transported to the berry when the elicitors are applied. Our results showed a proper assimilation of the elicitors enriched with amino acids from vegetal origin. These results suggest a possibility of replacement of the amino acids from the animal origin with amino acids from the vegetal origin as a sustainable alternative.

3.5. Effect of elicitor fertigation on the aromatic composition of grapes

Up to twenty-five varietal compounds mainly related to fruit and flower aroma descriptors were determined. The compounds were grouped into six families according their chemical structures including aldehydes, C6-compounds, alcohols, monoterpenes, oxygenated polyterpenes and volatile phenols. In general, the musts obtained from the treated grapes had similar or higher levels of the volatile compounds (Table 5). The differences between the treatment and control groups were the highest in the case of aldehydes and C6 compounds and the differences in two of the three studied vintages were significant. The total concentrations of the C6 compounds were higher in T1 than that in T2. This pattern was also observed in the case of the total content of aldehydes in 2016 and 2018. Monoterpenes and polyoxygenate terpenes also showed significant differences in one of the three vintages (2016 and 2018, respectively). Four of the six studied monoterpenes (linalool, terpinen-4-ol, epoxy-linalool and geraniol) showed significant differences in 2016. These monoterpenes are commonly related to

Table 4
Mean values of the individual amino acid concentration (mg/L) in must from control and plants treated with elicitors from animal (T1) and vegetal (T2) origin.

	2016			2017			2018		
	Control	T1	T2	Control	T1	T2	Control	T1	T2
Aspartic acid	4.5a	5.3a	5.9a	22.6a	22.7a	21.0a	4.1a	5.2a	3.7a
Asparagine	2.0b	3.4a	2.9a	3.2a	2.0a	3.1a	3.6a	3.6a	3.8a
Glutamic acid	6.9a	6.9a	7.1a	31.5a	32.4a	33.6a	6.3ab	9.6b	5.6b
Glutamine	47.1a	58.3a	57.1a	108.9a	102.7a	122.5a	40.3a	46.2a	37.5a
Serine	29.1b	32.1a	33.0a	48.3a	48.1a	50.6a	14.9a	18.3a	17.0a
Histidine	8.4b	9.8ab	10.7a	20.1b	19.6b	31.0a	18.2a	20.4a	17.5a
Glycine	2.4b	2.8a	2.5b	3.5a	1.9b	3.9a	1.5a	2.0a	1.7a
Threonine	19.3b	24.8a	24.5a	30.6a	28.0a	37.3a	18.6a	24.7a	20.5a
Arginine	88.9b	124.4a	142.7a	305.1ab	194.4b	412.0a	338.5a	338.0a	345.7a
Alanine	25.4a	30.7a	29.5a	69.0ab	62.2b	93.2a	18.8a	23.3a	19.2a
Tyrosine	3.1a	3.5a	2.5a	8.6a	7.4a	9.1a	6.7a	7.3a	5.6a
Valine	13.5b	14.1ab	16.0a	18.2a	18.6a	17.6a	18.7a	15.3ab	14.3b
Methionine	31.2a	35.1a	34.3a	62.6ab	60.8b	72.7a	31.7b	42.6a	28.9b
Phenylalanine	5.4a	6.0a	6.0a	9.3a	6.9b	8. ab	6.4b	11.3a	5.5b
Isoleucine	5.8b	6.7a	6.6ab	7.6b	8.1b	10.0a	5.2a	3.7a	4.0a
Leucine	11.8b	13.6ab	13.8a	20.9ab	20.0b	26.1a	9.5a	7.3a	6.1a
Lysine	2.1a	2.6a	2.6a	3.5b	3.1b	5.5a	22.0a	20.7a	19.0a
Total	307.0b	380.4a	397.4a	773.5ab	639.1b	958.037a	565.2a	599.9a	556.3a

Different letters in rows indicate significant ($p \leq .05$) differences by Tukey (HSD) test for each vintage individually (n = 4).

Table 5
Concentration of the aromatic compounds determined in Syrah musts from Control (C) and treated grapes (T1 and T2).

	2016			2017			2018		
	C	T1	T2	C	T1	T2	C	T1	T2
Benzaldehyde	319 ± 91 a	385 ± 243 a	260 ± 147 a	66 ± 36 a	56 ± 8 a	117 ± 23 a	12 ± 1 a	32 ± 17 a	12 ± 2 a
2-hexenal	22 ± 15 a	63 ± 53 a	31 ± 11 a	37 ± 7 a	32 ± 18 a	42 ± 6 a	22 ± 6 a	64 ± 19 b	36 ± 15 ab
ALDEHYDES	341 ± 103 a	449 ± 283 a	291 ± 157 a	103 ± 29 ab	88 ± 20 a	159 ± 29 b	34 ± 7 a	96 ± 36 b	49 ± 16 ab
Trans-3-hexen-1-ol	106 ± 12 a	375 ± 365 a	198 ± 134 a	126 ± 42 a	182 ± 9 a	213 ± 47 a	129 ± 42 a	202 ± 12 b	171 ± 1 ab
Cis-3-hexen-1-ol	13 ± 5 a	11 ± 5 a	7 ± 2 a	11 ± 2 a	12 ± 3 a	16 ± 12 a	7 ± 1 a	11 ± 4 a	12 ± 4 a
1-hexanol	317 ± 110 a	404 ± 82 a	298 ± 146 a	168 ± 46 a	466 ± 17 a	371 ± 244 a	345 ± 184 a	372 ± 44 a	271 ± 11 a
C6 COMPOUNDS	436 ± 124 a	790 ± 361 a	503 ± 24 a	305 ± 8 ab	660 ± 30 b	599 ± 244 a	481 ± 220 a	584 ± 52 a	454 ± 11 a
1-octanol	21 ± 13 a	51 ± 14 a	69 ± 67 a	45 ± 33 a	46 ± 27 a	77 ± 15 a	49 ± 13 a	33 ± 16 a	36 ± 6 a
1-octen-3-ol	20 ± 6 a	37 ± 13 a	53 ± 51 a	13 ± 8 a	10 ± 4 a	10 ± 5 a	38 ± 2 a	29 ± 8 a	27 ± 4 a
Benzyl alcohol	152 ± 82 a	247 ± 126 a	272 ± 71 a	522 ± 133 a	589 ± 102 a	635 ± 112 a	292 ± 67 a	428 ± 58 ab	571 ± 70 b
Phenylethyl alcohol	73 ± 6 b	113 ± 29 a	103 ± 49 a	62 ± 12 a	73 ± 27 a	82 ± 13 a	200 ± 22 a	164 ± 32 a	174 ± 58 a
ALCOHOLS	269 ± 107 a	447 ± 170 a	497 ± 231 a	641 ± 99 a	719 ± 97 a	803 ± 120 a	579 ± 99 a	655 ± 42 a	807 ± 133 a
Linalool	54 ± 29 b	130 ± 43 a	180 ± 183 a	78 ± 68 a	93 ± 40 a	125 ± 6 a	52 ± 9 a	62 ± 26 a	38 ± 8 a
Terpinen-4-ol	20 ± 9 b	102 ± 37 a	155 ± 124 a	61 ± 39 a	116 ± 69 a	95 ± 52 a	57 ± 6 a	52 ± 17 a	37 ± 3 a
Epoxylinalool	279 ± 13 b	447 ± 146 a	435 ± 201 a	830 ± 412 a	665 ± 181 a	1017 ± 220 a	401 ± 167 a	548 ± 79 a	662 ± 65 a
b-citronellol	250 ± 8 a	383 ± 132 a	398 ± 202 a	692 ± 296 a	685 ± 279 a	892 ± 50 a	860 ± 514 a	837 ± 186 a	1227 ± 463 a
Geraniol	52 ± 14 b	95 ± 41 ab	146 ± 139 a	79 ± 4 a	96 ± 27 a	87 ± 18 a	40 ± 6 a	70 ± 43 a	62 ± 54 a
a-terpineol	6 ± 4 a	9 ± 4 a	7 ± 6 a	7 ± 3 a	7 ± 1 a	7 ± 0 a	4 ± 1 a	5 ± 1 a	9 ± 2 b
MONOTERPENS	661 ± 54 a	1165 ± 390 a	1322 ± 838 a	1746 ± 778 a	1660 ± 451 a	2224 ± 214 a	1414 ± 649 a	1573 ± 250 a	2036 ± 376 a
Cis-linalool oxide	3 ± 1 a	4 ± 3 a	3 ± 2 a	0 ± 0 a	0 ± 0 a	1 ± 1 a	0.3 ± 1 a	0.3 ± 1 a	1 ± 1 a
Trans-linalool oxide	3 ± 1 a	9 ± 7 a	4 ± 3 a	2 ± 0 a	1 ± 0 a	2 ± 2 a	1 ± 1 a	2 ± 1 a	4 ± 2 a
Trans-pyran linalool oxide	361 ± 22 a	528 ± 166 a	569 ± 291 a	693 ± 287 a	721 ± 295 a	834 ± 100 a	609 ± 58 a	733 ± 109 ab	918 ± 64 b
2,6-dimethyl-3,7-octadiene-2,6-diol	23 ± 11 a	46 ± 8 a	65 ± 55 a	24 ± 6 a	24 ± 18 a	28 ± 8 a	30 ± 0 a	47 ± 21 a	40 ± 27 a
2,6-dimethyl-1,7-octadiene-3,6-diol	3 ± 2 a	4 ± 2 a	5 ± 3 a	2 ± 0 b	0 ± 0 a	0 ± 0 a	2 ± 1 a	1 ± 1 a	2 ± 1 a
3,7-dimethyl-1,7-octanediol	8 ± 2 a	20 ± 12 a	10 ± 9 a	2 ± 1 a	11 ± 15 a	5 ± 3 a	23 ± 3 a	24 ± 22 a	20 ± 8 a
8-hidroxylinalool	98 ± 1 a	134 ± 53 a	131 ± 22 a	133 ± 138 a	42 ± 16 a	116 ± 24 a	1 ± 0 a	1 ± 1 a	1 ± 1 a
POLYOXYGENATED TERPENES	500 ± 20 a	745 ± 162 a	787 ± 368 a	858 ± 412 a	799 ± 278 a	986 ± 105 a	666 ± 61 a	810 ± 140 ab	986 ± 90 b
Eugenol	10 ± 3 b	56 ± 59 a	12 ± b	48 ± 71 a	34 ± 48 a	15 ± 22 a	1 ± 0 a	3 ± 2 a	2 ± 2 a
Methyl salicilate	1 ± 1 a	0.5 ± 1 a	2 ± 2 a	7 ± 1 a	7 ± 1 a	10 ± 1 a	1 ± 0 a	1 ± 1 a	2 ± 0 a
Ethyl salicilate	2 ± 2 a	1 ± 2 a	6 ± 4 a	0.3 ± 1 a	0.3 ± 1 a	0.3 ± 1 a	1 ± 0 a	1 ± 1 a	2 ± 0 a
VOLATIL PHENOLS	13 ± 5 a	57 ± 58 a	19 ± 12 a	56 ± 73 a	41 ± 46 a	25 ± 23 a	4 ± 0	5 ± 5 a	5 ± 3 a

The different letters in the rows indicate significant differences ($p \leq 0.05$) between the treatments of the same vintage by Tukey (HSD) test.
The mean value and standard deviation (n = 3).

tropical fruit aromas. In the case of polyoxygenated terpenes, *trans*-pyran linalool oxide was most abundant in the treated vines (with significant differences in the 2018 vintage). This family of the compounds is characteristic for herbaceous, floral and fruity aroma descriptors. The highest differences between the control and treatment groups were observed in 2016. Curiously, the lowest concentration of all varietal volatile compounds was observed in this vintage. Moreover, it should be noted that higher concentrations were obtained in the T2 treatment for the great number of the compounds in the three studied vintages. In general, treatments with elicitors enriched in amino acids from animal and vegetal origin apparently enhances the aromatic profile of musts. Thus, the results on the use of amino acids from vegetal origin (T2) open a sustainable alternative in the use of fertilizers to improve the aromatic quality of grapes.

4. Conclusions

In this work, elicitors enriched with animal and vegetal amino acids were compared in fertigation treatments during the production of three consecutive vintages. The climatic conditions clearly influenced the treatment efficiency. Small differences in the physiological stage and grape quality between the treated and untreated vines were observed. In general, the treatments improved the assimilation of micro- and macronutrients and may improve the gas exchange balance of the vines. The amino acid content tended to increase in treated vines, and a similar trend was observed in the case of the volatile compounds of musts. Similar results were obtained when animal or vegetal amino acids were used, indicating that the elicitors enriched with vegetal amino acids can be suitable and sustainable alternatives for vineyard fertilization.

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Appendix A. Supplementary data

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References

Apolinar-Valiente, R., Ruiz-García, Y., Williams, P., Gil-Muñoz, R., Gómez-Plaza, E., & Doco, T. (2018). Preharvest application of elicitors to Monastrell grapes: Impact on wine polysaccharide and oligosaccharide composition. *Journal of Agricultural and Food Chemistry, 66*(42), 11151–11157.

Bell, S. J., & Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research, 11*(3), 242–295.

Boubakri, H., Gargouri, M., Mliki, A., Brini, F., Chong, J., & Jbara, M. (2016). Vitamins for enhancing plant resistance. *Planta, 244*(3), 529–543.

Canfora, L., Vendramin, E., Felici, B., Tarricone, L., Florio, A., & Benedetti, A. (2018). Vineyard microbiome variations during different fertilisation practices revealed by 16s rRNA gene sequencing. *Applied Soil Ecology, 125*, 71–80 December.

Canoura, C., Kelly, M. T., & Ojeda, H. (2018). Effect of irrigation and timing and type of nitrogen application on the biochemical composition of Vitis vinifera L. cv. Chardonnay and Syrah grapeberries. *Food Chemistry, 241*(February 2016), 171–181.

Cartechini, A., & Palliotti, A. (1995). Effect of shading on vine morphology and productivity and Leaf gas exchange characteristics in grapevines in the field. *American Journal of Enology and Viticulture, 46*(2), 227–234.

Casanova-Gascón, J., Martín-Ramos, P., Martí-Dalmau, C., & Badía-Villas, D. (2018). Nutrients assimilation and chlorophyll contents for different grapevine varieties in calcareous soils in the Somontano DO (Spain). *Beverages, 4*(4) (90–90).

Christensen, P. (1984). Nutrient level comparisons of leaf petioles and blades in twenty-six grape cultivars over three years (1979 through 1981). *American Journal of Enology and Viticulture, 35*(3), 124–133.

Dominguez, N., García-Escudero, E., Romero, I., Benito, A., & Martín, I. (2015). Leaf blade and petiole nutritional evolution and variability throughout the crop season for Vitis Vinifera L. cv. Graciano. *Spanish Journal of Agricultural Research, 13*(3).

Douthe, C., Medrano, H., Tortosa, I., Escalona, J. M., Hernández-Montes, E., & Pou, A. (2018). Whole-plant water use in field grown grapevine: Seasonal and environmental effects on water and carbon balance. *Frontiers in Plant Science, 9* (1540–1540).

Escalona, J. M., Flexas, J., Bota, J., & Medrano, H. (2003). Distribution of leaf photosynthesis and transpiration within grapevine canopies under different drought conditions. *Vitis, 42*(2), 57–64.

Garde-Cerdán, T., Gutiérrez-Gamboa, G., Baroja, E., Rubio-Bretón, P., & Pérez-Álvarez, E. P. (2018). Influence of methyl jasmonate foliar application to vineyard on grape volatile composition over three consecutive vintages. *Food Research International, 112*(January), 274–283.

Garde-Cerdán, T., López, R., Portu, J., González-Arenzana, L., López-Alfaro, I., & Santamaría, P. (2014). Study of the effects of proline, phenylalanine, and urea foliar application to Tempranillo vineyards on grape amino acid content. Comparison with commercial nitrogen fertilisers. *Food Chemistry, 163*, 136–141.

Gil-Muñoz, R., Fernández-Fernández, J. I., Crespo-Villegas, O., & Garde-Cerdán, T. (2017). Elicitors used as a tool to increase stilbenes in grapes and wines. *Food Research International, 98*, 34–39.

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Gonzalo-Diago, A., Moreno-Simunovic, Y., & Martínez-Gil, A. M. (2017). Effect of different foliar nitrogen applications on the must amino acids and glutathione composition in cabernet sauvignon vineyard. *LWT - Food Science and Technology, 75*, 147–154.

Gutiérrez-Gamboa, G., Portu, J., Santamaría, P., López, R., & Garde-Cerdán, T. (2017). Effects on grape amino acid concentration through foliar application of three different elicitors. *Food Research International, 99*(April), 688–692.

Gutiérrez-Gamboa, G., Romanazzi, G., Garde-Cerdán, T., & Pérez-Álvarez, E. P. (2019). A review of the use of biostimulants in the vineyard for improved grape and wine quality: Effects on prevention of grapevine diseases. *Journal of the Science of Food and Agriculture, 99*(3), 1001–1009.

Henderson, J. W., Robert, D. R., Brian, A. B., & Cliff, W. (2000). Rapid, accurate, sensitive and reproducible HPLC analysis of amino acids. *Agilent Technologies* (application note, publication no: 5980-1193. In).

van Leeuwen, C., Roby, J.-P., Alonso-Villaverde, V., & Gindro, K. (2013). Impact of clonal variability in *Vitis vinifera* cabernet franc on grape composition, wine quality, leaf blade Stilbene content, and downy mildew resistance. *Journal of Agricultural and Food Chemistry, 61*(1), 19–24.

van Leeuwen, C., Trégoat, O., Choné, X., Bois, B. D. P., & Gaudillère, J. (2009). Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? *OENO One, 43*(3).

Marschner, H. (1995). Preface to first edition. In H. Marschner (Ed.). *Mineral nutrition of higher plants* (pp. v–vi). (2nd ed.). London: Academic press.

Medrano, H., Tomás, M., Martorell, S., Pou, A., Fuentes, S., Flexas, J., & Bota, J. (2015). Improving water use efficiency of vineyards in semi-arid regions. A review. *Agronomy for Sustainable Development, 35*, 499–517.

OIV (2016). *Compendium of international methods of wine and must analysis. Vol. 2*, OIV-MA-ANNEX-C2.

Paladines-Quezada, D. F., Moreno-Olivares, J. D., Fernández-Fernández, J. I., Bautista-Ortín, A. B., & Gil-Muñoz, R. (2019). Influence of methyl jasmonate and benzothiadiazole on the composition of grape skin cell walls and wines. *Food Chemistry, 277*, 691–697.

Portu, J., López, R., Baroja, E., Santamaría, P., & Garde-Cerdán, T. (2016). Improvement of grape and wine phenolic content by foliar application to grapevine of three different elicitors: Methyl jasmonate, chitosan, and yeast extract. *Food Chemistry, 201*, 213–221.

Portu, J., López, R., Ewald, P., Santamaría, P., Winterhalter, P., & Garde-Cerdán, T. (2018). Evaluation of Grenache, Graciano and Tempranillo grape stilbene content after field applications of elicitors and nitrogen compounds. *Journal of the Science of Food and Agriculture, 98*(5), 1856–1862.

Portu, J., López-Alfaro, I., Gómez-Alonso, S., López, R., & Garde-Cerdán, T. (2015). Changes on grape phenolic composition induced by grapevine foliar applications of phenylalanine and urea. *Food Chemistry, 180*, 171–180.

Portu, J., Santamaría, P., & López, R. (2017). Effects on grape amino acid concentration through foliar application of three different elicitors. *Food Research International,* 0–1 June.

Portu, J., Santamaría, P., López, R., & Garde-Cerdán, T. (2017). Phenolic composition of Tempranillo grapes following foliar applications of phenylalanine and urea: A two-year study. *Scientia Horticulturae, 219*, 191–199.

Pradubsuk, S., & Davenport, J. R. (2011). *Seasonal distribution of micronutrients in mature 'Concord' grape: Boron, iron, manganese, copper, and zinc. Vol. 136 (1)*, 69.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2006). *Handbook of enology. Vol. 2*.

Ribéreau-Gayon, P., & Stonestreet, E. (1965). Determination of anthocyanins in red wine. *Bulletin de la Société Chimique de France, 9*, 2649–2652.

Ribéreau-Gayon, P., & Stonestreet, E. (1966). Dosage des tanins dans le vin rouge et détermination de leur structure. *Chimie Analytique, 48*(4), 188–196.

Romanazzi, G., & Feliziani, E. (2016). Use of chitosan to control postharvest decay of temperate fruit: Effectiveness and mechanisms of action. *Chitosan in the Preservation of Agricultural Commodities,* 155–177.

Romero, I., García-Escudero, E., & Martín, I. (2010). Effects of leaf position on blade and petiole mineral nutrient concentration of Tempranillo grapevine (*Vitis vinifera* L.). *American Journal of Enology and Viticulture, 61*(4), 544–550.

Romero Laibarra, I., Martín Rueda, I., Lorenzo Iñigo, I., López Llaría, D., Ibáñez Pascual, S., Arroyo de Grandes, M. C., & García-Escudero Domínguez, E. (2005). Diagnóstico nutricional de Vitis vinifera L cv Tempranillo en la D. O. Ca. Rioja. Niveles Críticos Prelimminares. *Zubía Monográfico, 16-17*(2004–2005), 41–58.

Rubio-Bretón, P., Gutiérrez-Gamboa, G., Pérez-álvarez, E. P., Santamaría, P., & Garde-Cerdán, T. (2018). Foliar application of several nitrogen sources as fertilisers to Tempranillo grapevines: Effect on wine volatile composition. *South African Journal of*

Enology and Viticulture, 39(2), 235–245.

Sabir, A., & Sari, G. (2019). Zinc pulverization alleviates the adverse effect of water deficit on plant growth, yield and nutrient acquisition in grapevines (*Vitis vinifera* L.). *Scientia Horticulturae, 244*(September 2018), 61–67.

Schreiner, R. P., Lee, J., & Skinkis, P. A. (2013). N, P, and K supply to pinot noir grapevines: Impact on vine nutrient status, growth, physiology, and yield. *American Journal of Enology and Viticulture, 64*(1), 26–38.

Tomás, M., Medrano, H., Escalona, J. M., Martorell, S., Pou, A., Ribas-Carb, M., & Flexas, J. (July 2014). Variability of water use efficiency in grapevines. *Journal Environmental and Experimental Botany, 103*, 148–157.

Vilanova, M., Fandiño, M., Frutos-Puerto, S., & Cancela, J. J. (2019). Assessment fertigation effects on chemical composition of Vitis vinifera L. cv. Albariño. *Food Chemistry, 278*(September 2018), 636–643.

Vilanova, M., & Martínez, C. (2007). First study of determination of aromatic compounds of red wine from Vitis vinifera cv. Castañal grown in Galicia (NW Spain). *European Food Research and Technology, 224*(4), 431–436.

Zhang, C., Jia, H., Zeng, J., Perraiz, T., Xie, Z., Zhu, X., & Wang, C. (2016). Fertilization of grapevine based on gene expression. *The Plant Genome, 9*(3) (0–0).

Zhang, L., Marguerit, E., Rossdeutsch, L., Ollat, N., & Gambetta, G. A. (2016). The influence of grapevine rootstocks on scion growth and drought resistance. *Theoretical and Experimental Plant Physiology, 28*(2), 143–157.

Zhang, S., Jain, M., Fleites, L. A., Rayside, P. A., & Gabriel, D. W. (2018). Identification and characterization of Menadione and Benzethonium chloride as potential treatments of Pierce's disease of grapevines. *Phytopathology, 109*(2), 233–239.

Zoecklein, B., Fugelsang, K. C., Gump, B., & Nury, F. S. (1999). *Wine analysis and production.* Springer US.

CAPÍTULO 2

FERMENTACIÓN Y PRÁCTICAS ENOLÓGICAS

El uso de levaduras no-Saccharomyces y la aplicación de columnas de intercambio catiónico

Justificación y objetivo

Las prácticas enológicas que se desarrollan durante el proceso de fermentación del vino y las técnicas modernas y actuales empleadas en las bodegas más innovadoras durante toda la vinificación tienen una repercusión importante en la calidad final de los vinos elaborados, independientemente del tipo de vino, ya sean vinos tranquilos, espumosos, jóvenes o destinados al envejecimiento.

El uso de levaduras no-*Saccharomyces* durante las primeras fases de la fermentación es una tendencia que se está extendiendo progresivamente y empieza a ser una herramienta habitual usada por los enólogos para buscar la diferenciación y la tipicidad de sus vinos. La adición de levaduras no-*Saccharomyces* en las primeras etapas de la fermentación y su participación en este proceso puede aumentar la calidad del aroma del vino debido fundamentalmente a la formación de productos metabólicos que forman parte de su fracción volátil, como terpenoides, ésteres, alcoholes superiores, glicerol, acetaldehído y ácidos orgánicos. Estos compuestos también pueden contribuir indirectamente en la mejora de otras cualidades organolépticas como el color, el sabor, la textura, o incluso la espumabilidad. Sin embargo, la mayoría de estos efectos todavía deben de ser aclarados y se necesita profundizar en el estudio de la adición de estos microorganismos considerando distintos factores y casuísticas que nos lleven a un mejor entendimiento de las repercusiones que pueden llegar a tener en la calidad de los vinos. Por ello, en este capítulo nos propusimos estudiar el efecto de las levaduras *Saccharomyces* y no-*Saccharomyces* empleadas durante la primera fermentación en la elaboración de vino espumoso.

En este sentido y considerando la vinificación el proceso más importante de todos los que tienen lugar durante la elaboración del vino, también nos propusimos llevar a cabo ensayos que contemplasen el uso de tecnologías innovadoras. La técnica de la acidificación y estabilización del vino mediante el uso de columnas de intercambio catiónico es una herramienta que puede ser de gran utilidad para obtener vinos más equilibrados y competitivos en los mercados extranjeros. Es sabido que en muchas zonas vitícolas españolas, debido al cambio climático, existe una descompensación y desequilibrio durante el proceso de la maduración de la uva. En muchas ocasiones no se consigue una acidez adecuada lo cual repercute directamente en la calidad final del vino. Una práctica habitual para corregirla ha sido la adición de ácido tartárico. Sin embargo, esta adición puede llevar consigo un problema en la estabilidad del vino ya que puede producir un incremento en la aparición de sedimentos en los vinos embotellados debidos a la precipitación de las sales de birtartrato. Así pues, para evitar esta precipitación en botella y conseguir la estabilidad del vino se suelen emplear diferentes métodos físicos como el tratamiento por frio, aunque supone un gran coste económico y un largo periodo de tiempo. Por ello, con el fin de evitar la falta de acidez y de estabilidad en el vino, desde hace unos años, se han empezado a utilizar tratamientos con resinas de intercambio catiónico para reducir el nivel de cationes, bajar el pH y limitar el riesgo de formación de tartrato. Sin embargo, todavía existe un desconocimiento del efecto *a posteriori* de estos tratamientos sobre la composición química, aromática y sobre la estabilidad durante la etapa de crianza del vino.

Por todo ello, en este capítulo se investigó el efecto de ciertas técnicas y procesos desarrollados en la etapa de la vinificación sobre la calidad final del vino. Por una parte se estudió el efecto de las levaduras *Saccharomyces* y no-*Saccharomyces* empleadas durante la primera fermentación en la elaboración de vino espumoso. Por otra parte, se evaluó el efecto de la práctica de acidificación y estabilización del vino mediante el uso de resinas de intercambio catiónico sobre la calidad final de un vino tinto después de la etapa de crianza.

Objetivos específicos

El objetivo del artículo 2 fue comparar el uso de levaduras *Saccharomyces* y no-*Saccharomyces* en la fermentación del vino base, en términos de calidad aromática, contenido proteico y capacidad espumante del cava.

El objetivo del artículo 3 fue evaluar el efecto de las resinas de intercambio catiónico sobre las características enológicas del vino y su estabilidad durante el período de crianza, prestando especial atención a la composición volátil.

Publicaciones asociadas

- **Artículo 2**: *Effect of the addition of non-Saccharomyces at first alcoholic fermentation on the enological characteristics of Cava wines.*
- **Artículo 3**: *Preliminary study of the effect of cation-exchange resin treatment on the aging of Tempranillo red wines.*

Artículo 2

Effect of the addition of non-Saccharomyces at first alcoholic fermentation on the enological characteristics of Cava wines.

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Article

Effect of the Addition of Non-*Saccharomyces* at First Alcoholic Fermentation on the Enological Characteristics of Cava Wines

Ana María Mislata [1,2], Miquel Puxeu [1], Immaculada Andorrà [1], Noelia Espligares [1], Sergi de Lamo [1], Montserrat Mestres [2] and Raúl Ferrer-Gallego [1,*]

[1] VITEC—Centro Tecnológico del Vino, Ctra. Porrera Km.1, 43730 Falset, 43007 Tarragona, Spain; anamaria.mislata@vitec.wine (A.M.M.); miquel.puxeu@vitec.wine (M.P.); imma.andorra@vitec.wine (I.A.); noelia.espligares@vitec.wine (N.E.); sergi.delamo@vitec.wine (S.d.L.)
[2] Instrumental Sensometry (i-Sens), Department of Analytical Chemistry and Organic Chemistry, Campus Sescelades, Universitat Rovira i Virgili, 43007 Tarragona, Spain; montserrat.mestres@urv.cat
* Correspondence: raul.ferrer@vitec.wine

Abstract: Background: Cava is considered to be a high-quality wine internationally. Hence, it has undergone consistent improvement and/or the preservation of its aromatic qualities, bouquet, color, and foamability, throughout its elaboration and aging. Methods: This study investigates the use of different *Saccharomyces* and non-*Saccharomyces* yeasts strains (*Torulaspora delbrueckii* and *Metschnikowia pulcherrima*) in Chardonnay and Xarel.lo cava wines. The usual enological parameters, the volatile composition, the protein contents, and foamability were determined, and sensory analyses were also performed for all of the vinifications (both before tirage and after 18 months of aging on the lees). Results: the protein and foamability results show that there is a direct relationship between both parameters, with better foam persistence achieved in some non-*Saccharomyces* fermentation. *M. pulcherrima* base wines showed a high protein content, improving foamability and foaming persistence. In addition, the results of the aromatic composition and the sensory analysis showed that the use of *T. delbrueckii* at first fermentation produced interesting cavas from an aromatic perspective. These cavas showed the highest values of ethyl isovalerate (120–126 µg/L), providing aromatic fruity notes, especially fresh green apple. Conclusions: the use of non-*Saccharomyces* yeasts in the base wine fermentation can be an alternative to produce cavas with differentiated aromatic characteristics and interesting foaming ability.

Keywords: non-*Saccharomyces*; yeast; cava; foamability; protein; aroma; volatile

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1. Introduction

In the last century, *Saccharomyces* was the only genus of yeasts used in wine cellars and, although grapes have a great diversity of species and yeasts strains, the dominance of *Saccharomyces cerevisiae* during alcoholic fermentation was expected and desired. Traditionally, the use of *Saccharomyces* yeasts together with non-*Saccharomyces* species during alcoholic fermentation influences the final composition of the wine. Thus, the contribution to the final quality of the wine of some yeast strains that acted during fermentation can be negative, because in some cases increases in ethyl acetate, volatile acidity, and acetaldehyde are observed, among others [1,2]. Conversely, they may present a positive contribution, increasing some desirable metabolites, such as acetate esters [3], which generally provide greater organoleptic complexity. Different genera and species of non-*Saccharomyces* yeasts are now emerging to improve the winemaking process and the quality and differentiation of wines. Thus, many research topics are based on the use of non-*Saccharomyces* species to improve wine quality and aromatic profile or to modulate the composition of the wine [4,5].

Among the non-*Saccharomyces* yeasts, the most studied genera are *Candida*, *Metschnikowia*, *Pichia*, *Torulaspora*, *Starmerella*, and *Lachancea*, because they help improve the organoleptic characteristics of wines, such as color, aroma, and sensory characteristics.

It is described that, when non-*Saccharomyces* yeasts are added in the early stages of grape must fermentation, the aroma wine quality improves because of the resulting metabolic products, such as terpenoids, esters, higher alcohols, glycerol, acetaldehyde, and organic acids [4,5]. Comitini et al. [6] reviewed the use of different non-*Saccharomyces* yeasts in winemaking and, in addition to this aromatic improvement, they noted other benefits, such as a reduction in the undesirable microflora, alcohol degree, and the amounts of sulfur dioxide, hydrogen sulfide, acetaldehyde and copper [6]. Therefore, because non-*Saccharomyces* yeasts produce wines with many distinctive characteristics, there are progressively more commercially available for different yeast species, such as *T. delbrueckii*, *M. pulcherrima*, and *P. kluyveri*.

The literature currently shows that many studies that have used non-*Saccharomyces* yeasts during alcoholic fermentation to produce different types of wines. However, few studies have examined the effect of these yeasts on the quality of sparkling wines, such as cava. The most characteristic note of these appreciated wines is their bubbles, generated in a second fermentation, which provides many other distinctive organoleptic properties. This second fermentation is not easy because, once the first fermentation is complete, the yeasts face a hostile environment due to high alcohol content (around 11%), high pressure (5–6 bars), lack of nutrients, low pH, and the presence of SO_2. Furthermore, the yeast must be able to ferment at low temperatures and must have both a good flocculation and autolytic ability. As the *Saccharomyces* genus meets all of these requirements, it has been the most commonly used yeast for this purpose. However, recently, other species have been also studied, such as *Schizosaccharomyces pombe* [1], which shows a better adaptation to these specific circumstances, allowing the transformation of malic acid into ethanol, significantly reducing the levels of biogenic amines, and presenting the ability to ferment the sparkling base wine to dryness without producing aromatic defects [7–9].

T. delbrueckii is another non-*Saccharomyces* yeast that is able to carry out the second fermentation with interesting results because it enhances the sensory profile of the sparkling wine obtained compared to that provided by *S. cerevisiae.* This is due to a higher production of esters with subsequent high scores for some positive aromatic descriptors [10].

Finally, it should be noted that some non-*Saccharomyces* yeasts have been also used to obtain base wine for sparkling wine production by the sequential inoculation of non-*Saccharomyces* and *S. cerevisiae*. Gonzalez-Royo et al. [11] studied the influence of *T. delbrueckii* and *M. pulcherrima* when used for the first fermentation and the results showed different interesting characteristics. Thus, when *T. delbrueckii* was used, the volatility acidity of the base wine decreased, the glycerol amount increased, and positive effects on foaming properties were found. These results agree with those found by Medina-Trujillo et al., who also detected improvements in foam and effervescence [12]. Regarding *M. pulcherrima*, the results showed that this yeast produces high amounts of β-glucosidase, reduces the volatile acidity, and implies an increase in medium chain fatty acids, esters, terpenols, and glycerol. Therefore, when it is inoculated in the first fermentation, it can improve the aroma profile of the base wine obtained, in addition to the foaming characteristics [12].

Thus, because non-*Saccharomyces* yeast can modify the amounts of amino acids, ammonia, glycerol, volatile aromatic compounds, and proteins, which results in a possible improvement in the wine's flavor and foaming capacity, it is unsurprising that there is growing interest in the use of these yeasts to produce sparkling wine.

The aim of this work was to compare the use of both *Saccharomyces* and non-*Saccharomyces* yeasts in the fermentation of base wine, in terms of the aromatic quality, protein content, and foaming capacity of cava.

2. Materials and Methods

2.1. Winemaking Process

Chardonnay (CH) and Xarel.lo (XA) grape varieties were used for the production of the studied cavas. For each grape variety, 100 kg of grapes harvested in 2018 was destemmed, crushed and distributed into 50 L stainless steel fermenters. The basic chemical composition of the musts was as follows: for CH, brix grade 16.9; nitrogen assimilable by yeast 297 mg/L; ammonium 122 mg/L; pH 3.41; total acidity 8.23 g/L expressed as tartaric acid; and malic acid 5.7 mg/L, and for XA, brix grade 16.3; nitrogen assimilable by yeast 193 mg/L; pH 3.15; total acidity 6.45 g/L expressed as tartaric acid; and malic acid 1.9 mg/L. During grape processing, 40 mg/L of SO_2 (Sulphur 18, Agrovin S.A., Ciudad Real, Spain) was added to prevent oxidation and for microbiological control. The vinifications were carried out in duplicate for each variety on a pilot scale in the experimental VITEC winery. For each vinification, 26 L of must obtained from 40 kg of varietal grapes was used (65% yield). The cold settling was carried out with active pectolytic enzymes (Endozym Éclair, AEB Iberica S.A.U., Barcelona, Spain) at 11 °C overnight and the alcoholic fermentation was carried out at a controlled and constant temperature (17 °C) in cold water baths. To carry out the alcoholic fermentation to obtain the base wine, five different commercial yeasts were used: three different strains from *S. cerevisiae*, Y1 (IOC 18-2007, Institut Oenologique de Champagne, Epernay Cedex, France) indigenous yeast selected from the Champagne vineyards, Y2 (Viacell Rhône 4600, Lallemand SAS, Blagnac Cedex, France) yeast selected by the Inter-Rhône Technical Service in the northern Cotes du Rhône region, and Y3 (Sensy Yseotm Lalvin, Lallemand SAS, Blagnac Cedex, France) indigenous yeasts selected by natural crossing, and two non-*Saccharomyces* yeasts, Y4 (Flavia MP346, Lallemand SAS, Blagnac Cedex, France) pure culture of *Metschnikowia pulcherrima* and selected from nature by the University of Santiago de Chile (USACH), and Y5 (Level Biodiva, Lallemand SAS, Blagnac Cedex, France) pure culture of *Torulaspora delbrueckii*. The addition of each of the yeasts was carried out as recommended by the manufacturer. The course of the fermentations was monitored by the consumption of sugar, and it was considered complete when the residual sugar concentrations were below 0.5 g/L. In all cases, fermentation began 24–48 h after inoculation and lasted about 10 days. After fermentation, base wines were sulphited to reach 30 mg/L of free SO_2. After 24 h, wines were racked and clarified with the addition of 40 g/hL bentonite (Bentogran, AEB Iberica S.A.U., Barcelona, Spain). After that, and to carry out the second fermentation, base wines were bottled in glass bottles of 750 mL and, finally, cavas were obtained. For this, *S. bayanus* (Uvaferm PMA, Lallemand SAS, Blagnac Cedex, France) was added in the tirage solution. Tirage also contained 40 g/L adjuvant (Adjuvant 92, Station Oenotechnic of Champagne, France) and 20 g/L sugar (glucose). Finally, after disgorgement, cavas were aged on their lees for 18 months. Analyses were made in base wines (BW) just before tirage, and in cavas after 18 months of aging on lees (18M).

2.2. Enological Analysis

The enological parameters of samples were quantified by applying the methods recommended by the International Organization of Vine and Wine (OIV) [13]. These methods were the color intensity (CI) and the chromatic characteristics (measured by Helios-α spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) (OIV-MA-AS2-07B); total acidity (OIV-MA-AS313-01) and pH (OIV-MA-AS313-15) (measured potentiometry by using an automatic titrator, TitroMAtic Hach by Crison®, Barcelona, Spain); the content of glucose-fructose (OIV-MA-AS311-02) (measured by enzymatic reaction with a Y15 BioSystems device and Control Wine®, BioSystems S.A., Barcelona, Spain); the content of malic acid (OIV-MA-AS313-10) (also measured by enzymatic reaction).

The values of the alcoholic strength and the volatile acidity were obtained using a Fourier transform infrared spectroscopy (FTIR) system (WineScan™ by FOSS, Hilleroed, Denmark), internally calibrated according to OIV [13].

2.3. Protein Analysis

2.3.1. Total Protein Concentration Determined by UV Spectrophotometric Method

Protein quantification was performed using a UV-visible spectrophotometer (BioDrop µLite, from Thermo Fisher Scientific, Waltham, MA, USA) for micro sample volumes. For measurement and quantification, all of the protein powder extracted from 16 mL of wine sample according to the preparation described by Silvestri et al. [14] was used. Thus, 40 µL of 0.16 M TRIS-HCL (pH 6.8) was added to the protein powder in a 1.5 mL tube and then placed in an ultrasound bath for 60 min at 50 °C. The measurement of the absorbance of the proteins was carried out at 280 nm using 3 µL of sample. Previously, 3 µL of 0.16 M TRIS-HCL (pH 6.8) was used as a blank.

2.3.2. Wine Protein Composition Evaluated by SDS-PAGE

Samples of base wines were analyzed by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Figure S1). A previous sample extraction treatment was carried out, which was used both for its quantification by UV and for gel electrophoresis, as described by Silvestri et al. [14]. The proteins of the extracted wine samples were separated using SDS-PAGE (15% resolving gel and 5% stacking gel) in a vertical electrophoresis unit with an applied voltage of 100 V. The marker used was Precision Plus Protein™ Dual color Standards (Bio-Rad, Hercules, CA, USA). The gels were stained with Coomassie Brilliant Blue R-250 (0.05%, *w*/*v*) in methanol/acetic acid/water (25:10:65, *v*/*v*/*v*) and decolorized in the same solution without the colorant.

2.4. Foamability

The foamability of the different cavas was measured using the Mosalux procedure [15]. Briefly, before making the first measurement, it was necessary to regulate CO_2 flow at 7 L/h and the pressure at 1 atm for 15 min. Then, the gas injection was stopped. To measure the foamability of the cavas, 100 mL of sample was used, which was previously filtered through a membrane with a porosity of 0.65 µm and warmed to 18 °C for 12 h. The sample was then placed in the Mosalux cylinder and CO_2 was injected through the glass frit. The maximum height reached by the foam (maximum) was first measured and then, when the foam sample showed stable persistence, the height was measured in a steady state (minimum). Both measurements were performed in duplicate for each sample.

2.5. GC-MS Analysis

The volatile compounds of base wines (BW) and cavas (18 M) were extracted using SPME (DVB/CAR/PDMS) and analyzed using a GC 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an inert mass spectrometer 5975C MSD (Electronic Shock Source Triple Axis Detector) according to the method developed by Torrens et al. [16]. The chromatographic conditions were those previously optimized and described [17]. The results of the volatile compounds were semi-quantitative data and expressed as µg/L in relation to the response provided by the internal standard (2-octanol). All analyses were performed in duplicate.

2.6. Sensory Analysis

The quantitative descriptive analysis (QDA) was performed by a trained tasting panel (by ISO 8586: 2012) in a sensory standardization room (ISO 8589: 2007). A total of ten cavas were tasted after 18 months of aging in the bottle on lees (18M). The analysis was carried out over two sessions, one session to taste Chardonnay cavas and the other session to taste Xarel.lo cavas. Prior to the sensory analysis, seven descriptors were selected to guide to the panelists when performing the evaluation. These were: fresh, candied, and dried fruit, floral, cake shop, honey, and evolution. The perceived aroma intensity was rated by each panelist from 0 to 5 and the data obtained were processed with the FIZZ software (V.2.47B Biosystems, Barcelona, Spain). A specific tasting sheet was designed according to the aforementioned olfactory descriptors.

2.7. Statistical Analysis

A simple analysis of variance (ANOVA) was carried out using the StatGraphics Centurion XVI (Manugistics Inc., Rockville, MD, USA) program. Tukey's procedure was used and the differences at p-values < 0.05 were considered significant.

3. Results and Discussion

3.1. Enological Analysis

The values of glucose and fructose, malic acid, total acidity, and pH were determined for both Chardonnay and Xarel.lo of all base wines (BW) were analyzed before tirage (Table S1). In addition, the alcoholic strength, volatile acidity, and color characteristics were analyzed of all base wines and also of all cavas after 18 months of aging on lees (18M) (Table 1).

Table 1. Basic analysis of Chardonnay (CH) and Xarel.lo (XA) base wines (BW) and cavas after 18 months of aging on lees (18M), with the addition of five different *S. cerevisiae* (Y1, Y2, Y3) and non-*Saccharomyces* (Y4 and Y5) yeasts. Different letters indicate significant differences between samples with different yeasts for each grape variety, and in each of the analysis times. Units: alcoholic strength (%vol) and volatile acidity (g/L). Samples (n = 2).

			CHARDONNAY			
	Alcoholic Strength	**Volatile Acidity**	**Abs. 420 nm**	**Abs. 520 nm**	**Abs. 620 nm**	**Color Intensity**
BW						
Y1	10.1 a ± 0.0	0.40 c ± 0.00	0.099 a ± 0.005	0.036 ab ± 0.002	0.011 ab ± 0.003	0.15 a ± 0.01
Y2	9.9 a ± 0.3	0.38 bc ± 0.00	0.179 a ± 0.098	0.047 b ± 0.002	0.013 b ± 0.001	0.24 a ± 0.10
Y3	10.1 a ± 0.0	0.52 d ± 0.01	0.090 a ± 0.012	0.023 a ± 0.006	0.006 a ± 0.002	0.12 a ± 0.02
Y4	10.5 a ± 0.6	0.33 a ± 0.01	0.118 a ± 0.011	0.035 ab ± 0.006	0.012 ab ± 0.000	0.17 a ± 0.02
Y5	9.7 a ± 0.0	0.37 b ± 0.01	0.122 a ± 0.023	0.038 ab ± 0.008	0.013 b ± 0.001	0.17 a ± 0.03
18M						
Y1	11.3 c ± 0.0	0.54 b ± 0.02	0.146 e ± 0.001	0.065 c ± 0.007	0.029 c ± 0.001	0.24 c ± 0.01
Y2	11.2 b ± 0.0	0.48 ab ± 0.04	0.123 d ± 0.001	0.036 b ± 0.001	0.019 abc ± 0.001	0.18 b ± 0.00
Y3	11.5 e ± 0.0	0.72 c ± 0.01	0.110 b ± 0.000	0.023 a ± 0.001	0.007 a ± 0.001	0.14 a ± 0.01
Y4	11.1 a ± 0.0	0.44 a ± 0.01	0.106 a ± 0.001	0.037 b ± 0.001	0.022 bc ± 0.001	0.16 b ± 0.01
Y5	11.3 c ± 0.0	0.41 a ± 0.01	0.119 c ± 0.001	0.030 ab ± 0.001	0.015 ab ± 0.007	0.16 b ± 0.01
			XAREL.LO			
	Alcoholic Strength	**Volatile Acidity**	**Abs. 420 nm**	**Abs. 520 nm**	**Abs. 620 nm**	**Color Intensity**
BW						
Y1	9.9 a ± 0.0	0.26 a ± 0.02	0.075 a ± 0.013	0.016 ab ± 0.001	0.004 a ± 0.001	0.09 a ± 0.01
Y2	9.9 a ± 0.0	0.26 a ± 0.00	0.064 a ± 0.006	0.015 a ± 0.002	0.005 a ± 0.002	0.08 a ± 0.01
Y3	11.7 d ± 0.0	0.31 b ± 0.00	0.065 a ± 0.001	0.023 b ± 0.001	0.006 a ± 0.001	0.09 a ± 0.00
Y4	10.0 b ± 0.0	0.25 a ± 0.00	0.067 a ± 0.005	0.017 ab ±0.004	0.005 a ± 0.001	0.09 a ± 0.01
Y5	10.1 c ± 0.0	0.29 ab ± 0.01	0.081 a ± 0.001	0.019 ab ± 0.001	0.004 a ± 0.001	0.10 a ± 0.00
18M						
Y1	11.1 b ± 0.0	0.30 b ± 0.01	0.075 bc ± 0.000	0.017 b ± 0.001	0.008 c ± 0.000	0.10 c ± 0.01
Y2	11.4 e ± 0.0	0.29 b ± 0.01	0.074 b ± 0.001	0.018 b ± 0.001	0.003 a ± 0.001	0.09 b ± 0.00
Y3	11.3 d ± 0.0	0.35 c ± 0.01	0.065 a ± 0.001	0.010 a ± 0.000	0.002 a ± 0.000	0.08 a ± 0.1
Y4	11.0 a ± 0.0	0.24 a ± 0.00	0.078 c ± 0.001	0.016 b ± 0.001	0.005 b ± 0.000	0.10 c ± 0.1
Y5	11.2 c ± 0.0	0.30 b ± 0.01	0.087 d ± 0.001	0.027 c ± 0.000	0.015 d ± 0.001	0.13 d ± 0.01

All of the base wines (BW) had glucose and fructose values below 0.5 mg/L, which indicated that they were completely dry base wines. In the case of CH wines, they showed a total acidity between 7.4 and 8.4 g/L. The amount of malic acid varied between 5.3 and 5.5 mg/L, and pH values were around 3.5 units. The base wines made from XA showed lower values than those of CH. The total acidity concentration ranged from 5.3 to 6.3 g/L, malic acid values from 1.2 to 1.6 mg/L, and pH from 3.1 to 3.2.

Regarding the effects of the different yeasts on the wines, the results showed that the CH base wines did not present significant differences in terms of their alcoholic strength and color intensity values, regardless of the yeast used. However, the volatile acidity parameter showed significant differences for all of the yeasts used, with Y3 yeast providing the highest concentration, and non-*Saccharomyces* yeasts (Y4 and Y5) provided the lowest concentrations. This behavior is consistent with previous studies that also found that some non-*Saccharomyces*, such as *S. pombe*, *T. delbrueckii*, and *L. thermotolerans*, produced lower concentrations of volatile acidity than some *S. cerevisiae* (approximately 0.1 g/L) [4,11]. The XA base wines also presented a similar trend, with wines fermented with Y3 presenting the highest volatile acidity, and the lowest concentration was found in wines fermented with the addition of *M. pulcherrima* (Y4). Wine fermented with the addition of *T. delbrueckii* (Y5) also showed low values on the volatile acidity content.

In addition, these same parameters were analyzed in the cavas after 18 months of aging on the lees (18M). All of them presented significant differences. In the CH variety, the cavas elaborated with Y3 presented the highest values of alcoholic degree and volatile acidity, while the cavas with *M. Pulcherrima* presented the lowest values, as already happened in BW. Additionally, the cavas elaborated with addition to *T. delbrueckii* presented very low concentrations of volatile acidity. In the XA variety, as in CH, the cavas with the addition of Y3 presented the highest values of alcoholic strength and volatile acidity. However, the cavas with Y4 present the lowest values of both parameters. Finally, regarding color intensity, few differences were observed. The highest value of color intensity was obtained in CH cava with Y1.

Previous studies [18,19] already pointed out that the use of yeasts other than *Saccharomyces* can reduce the alcoholic content of wine, which is in agreement with our data. Furthermore, it is well known that volatile acidity is one of the important parameters that influence the quality of wine or cava and also depends to a great extent on the type of yeast that undergoes alcoholic fermentation. As has been observed in recent studies [20] there are different non-*Saccharomyces* yeast strains related to producing a low content of volatile acidity, as occurs in our study, with the yeast *M. Pulcherrima* (Y4), which shows that they may have or generate desirable oenological properties in wines and cavas.

3.2. Protein Analysis

3.2.1. Total Protein Concentration Determined by UV-Visible Spectrophotometry

The results obtained sowed that the quantity of protein was more influenced by variety than by the yeast strain added. Specifically, whereas the total concentrations ranged from 78.2 to 44.5 mg/L in the CH samples, these ranged from 36.3 to 21.3 mg/L in the XA base wines. This relationship between the variety and the amount of protein was previously verified by Cilindre et al. who found a higher content of protein in Chardonnay than in Pinot Meunier base wines [21].

In addition, Dambrouck et al. also observed that a large portion of the wine protein came directly from grapes, which in turn was influenced by the variety; however, it should be taken into account that other proteins were produced by yeasts during the fermentation process [22]. This last consideration explains why, in the present study, non-*Saccharomyces* yeasts provided similar or even higher protein values than some *Saccharomyces* yeasts for the two grape varieties. The protein content obtained with *M. pulcherrima* (Y4) in XA and CH was as high as the highest value obtained by one of *the S. cerevisiae* (Figure 1).

3.2.2. Wine Protein Composition Evaluated by SDS-PAGE

Proteins released by yeast have been reported as exerting a positive effect on the foaming capacity of sparkling wines [11]. Soluble protein fractions of CH and XA base wines were evaluated to study their relationship with foam characteristics. For CH variety, the MW values ranged between 15.9 and 97.7 KDa, and between 18.2 and 92.2 KDa for the XA variety (Table 2). In general, SDS-PAGE did not show notable differences in the distribution and intensity of protein bands between proteins extracted with *Saccharomyces* (Y1, Y2, Y3) and non-*Saccharomyces* yeasts (Y4, Y5), as was observed in previous studies [23].

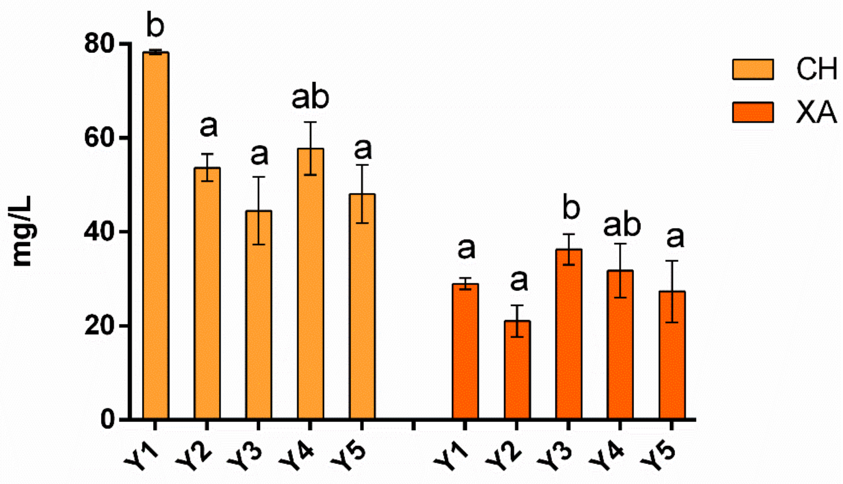

Figure 1. Total protein content base wines of Chardonnay (CH) and Xarel.lo (XA) elaborated with the addition of different *S. cerevisiae* (Y1, Y2, Y3) and non-*Saccharomyces* yeasts, *M. pulcherrima* (Y4) and *T. delbrueckii* (Y5). Different letters indicate significant differences between samples with different yeasts for each variety, Chardonnay (CH) and Xarel.lo (XA). Samples (n = 2).

Table 2. Analysis of the protein profile by SDS-PAGE and quantification in terms of the percentage of protein bands fractions (%) by detecting their molecular weights (MW), of the base wine of Chardonnay (CH) and Xarel.lo (XA) elaborated with different yeast strains: *Saccharomyces cerevisiae* (Y1, Y2, Y3) and non-Saccharomyces, *M. pulcherrima* (Y4) and *T. delbrueckii* (Y5).

CH Y1 (Bands)	V12	V13	V14	V15	V16	V17		
MW (Kda)	96.1	59.4	28.6	26.1	22.8	19.4		
%	12.8	2.7	9.1	7.4	63	5.1		
CH Y2 (Bands)	V30	V19	V20	V21	V22	V23		
MW (Kda)	97.7	64.3	27.2	24.9	22.1	18.6		
%	10.1	2.7	11.6	25.2	48.4	2		
CH Y3 (Bands)	V19	V20	V21	V22	V23	V24	V25	V26
MW (Kda)	66.7	57.4	47.2	40.4	32.7	26.4	21	18
%	14.6	12.8	5.7	5.7	7.2	7.2	32.2	2.2
CH Y4 (Bands)	V11	V12	V13	V14	V15	V16	V17	V18
MW (Kda)	74.8	53.9	36.3	28.2	23.4	20.2	18.7	15.9
%	28.8	4.5	2.4	12.2	42.9	3.7	4.3	1.2
CH Y5 (Bands)	V1	V2	V3	V4	V5	V6	V7	V8
MW (Kda)	68.5	48.7	35.9	26.8	23.1	20.3	18	16.2
%	20.2	2.9	5.7	25.1	30	3.7	3.2	9.3
XA Y1 (Bands)	V29	V30	V31	V32				
MW (Kda)	68.4	26.9	21.4	18.2				
%	27.6	33.9	26.5	12.1				
XA Y2 (Bands)	V22	V23	V24	V25				
MW (Kda)	73.2	29.5	27.6	25.5				
%	43.9	26.8	19.5	9.8				
XA Y3 (Bands)	V11	V12	V13	V14	V15			
MW (Kda)	92.1	32.9	28.9	25.3	22.9			
%	10.5	14.4	3.2	70.4	1.5			
XA Y4 (Bands)	V1	V2	V3	V4	V5			
MW (Kda)	91.6	31.7	29.5	27.6	23.9			
%	24.9	3.3	38.2	15.4	18.3			
XA Y5 (Bands)	V25	V26	V27	V28	V29			
MW (Kda)	92.2	31.5	28.7	25	20.8			
%	28.1	50.2	11.3	6.1	4.3			
10% <						< 80%		

As shown in Table 2, Chardonnay samples showed the most intense bands around molecular weights between 21 and 24 KDa. When observing the yeast effect, CH base wines fermented with the addition of Y1 presented up to 63% of their total content with an MW of 22.8 KDa. A similar trend was observed in wines with the addition of Y2, which showed 48.4% protein of 22.1 KDa and 25% of 24.9 KDa of MW. The wine with the addition of Y3 showed differences with respect to the previous wines, because although it presented 30% protein with MW of 21 KDa, this sample also presented around 30% of the proteins with higher molecular weights of 66.7 and 57.4 KDa. Wines fermented with the addition of non-*Saccharomyces* yeasts (Y4 and Y5) presented the highest content in proteins with high MW (28.8% of 74.8 KDa in the case of Y4 and 20.2% of 68.5 KDa in the case of Y5). Regarding base wines produced by Xarel.lo, we can observe that the Y1 and Y2 wines presented the highest MW percentages at 68.4 and 26.9 KDa values for Y1, and 73.2 and 29.5 KDa values for Y2. Y3 wine showed up to 70% of its proteins with MW of 25.3 KDa. However, and as happened in the CH variety, the wines with the Y4 and Y5 yeasts presented between 30% and 50% protein with MW around 30 KDa, and between 20% and 30% protein with the highest MW, 91.6 and 92.2 KDa, respectively. Therefore, it can be highlighted that the Chardonnay variety presented a greater number of bands (protein fractions) compared to the Xarel.lo variety, which could be influenced by the different quantity of proteins extracted from the grape. González-Royo et al. [11] observed that wines elaborated with sequential inoculation with *T. delbrueckii* and *M. pulcherrima* had higher values of lower molecular weight (LMW, molecular weight < 60 kDa) compared to the control wines (wines without addition of non-*Saccharomyces* yeasts), which related to the improved foam parameters observed in wines produced with non-*Saccharomyces* yeasts. In our study, this trend was observed in XA cavas produced from base wines of Y4 (*M. pulcherrima*), which presented higher values of LMW (molecular weight <60 kDa). This fact could explain the height of foam stability (HS) value observed in Y4 (Figure 2).

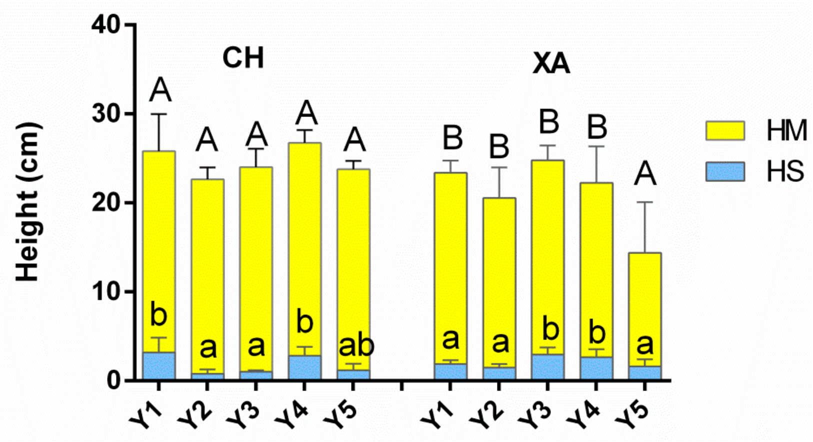

Figure 2. Foamability measurements of the different Chardonnay (CH) and Xarel.lo (XA) base wines made with different yeasts strains, *Saccharomyces cerevisiae* (Y1, Y2, Y3) and non-*Saccharomyces*, *M. pulcherrima* (Y4) and *T. delbrueckii* (Y5), using the Mosalux procedure. HM indicates the maximum height reached by the foam and HS the height in a steady state. Different letters indicate significant differences between samples with different yeasts. Uppercase letters for HM and lowercase letters for HS. Samples (n = 2).

3.3. *Foamability*

Figure 2 shows the values of the parameters HM (maximum foam height) and HS (height of foam stability) for each of the studied cavas (CH and XA). In general, as can be seen, CH cava presented slightly higher HM values than XA, as was observed in the previous studies by Andrés-Lacueva et al. [24] and Vanrell et al. [25], in which Chardonnay cavas showed higher HM values than Macabeo, Xarel.lo, and Parellada cava wines. However, the HS values were similar in all varieties.

By comparison, when the possible influence of the different yeasts on foamability was studied, it was observed that the highest HM and HS measurements were obtained when using Y1 and Y4 for the Chardonnay variety and Y3 and Y4 for the Xarel.lo variety. As can be seen, the samples that provided the highest HM values coincided with those with the highest values of protein content, so it can be concluded that there is a direct relationship between both parameters. These results agree with previous studies which reported that proteins and mannoproteins released by yeasts exert a positive effect on the foam [26], and that an increase in the amount of protein could lead to an increase in foamability [26] and lower velocities of foam dissipation [27]. Therefore, in this study, the results indicate that the wines examined with the addition of *M. pulcherrima* at first fermentation presented good foamability. Furthermore, this may be related to the high protein content, which can stabilize the bubble film due to its surface properties [26].

3.4. Volatile Composition

Among the different volatile compounds, a total of 18 were selected because they represent the four most abundant families (esters, acetates, alcohols, and fatty acids) of wines after carrying out the alcoholic fermentation. As shown in Table 3, these were analyzed in base wines (BW) and cavas, after 18 months of aging bottled on lees (18M).

Table 3 shows the total volatile concentration of the base wines and cavas. In general, and as expected, there was an aromatic decrease between the analysis times, before tirage in base wines (BW) and after 18 months of aging on lees (18M) as previously observed in other studies [28]. When observing BW values, a higher content of volatiles was found with the XA variety. However, when comparing the different yeasts, both varieties of wine showed the same trend. Thus, the volatile content was similar regardless of the yeast used, except when Y3 was used, which presented significant differences with respect to the other yeasts and provided the lowest volatile concentration.

Regarding the values after 18 months of aging on the lees, the aromatic content values were similar between varieties; thus, considering its higher volatile contents in base wine, XA suffered a greater decrease. When observing each of the varieties independently, greater significant differences were observed between the five yeasts used with respect to BW. In addition, the yeast *T. delbrueckii* (Y5) stood out in both varieties, with which high values of aroma concentrations were obtained, similar and/or higher than those obtained by some *Saccharomyces* yeasts.

To better study the results obtained, the different chemical families were separately studied. Thus, in relation to esters, in general these showed the same trend as the one described above for total aromas (Table 3). This behavior is due to the fact that the esters are the most abundant chemicals found in wine aroma and, therefore, are those that contribute the most to the total aroma. Furthermore, this family is of great importance because it provides the two important descriptors, fruit and flowers [29,30]. The base wines with non-*Saccharomyces* yeasts (Y4 and Y5) presented the highest concentrations of esters in CH, and values equal to or higher than the other *Saccharomyces* yeasts in XA. After 18 months of aging on the lees, higher amounts of esters were found when using Y2 and Y5 and, when dealing with XA cavas, also Y1. With reference to the different esters found, the highest concentrations of ethyl butyrate, ethyl isovalerate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate were observed in Y2 and Y5 in CH at 18 M (Table S2). Y5 also showed the highest value of ethyl isovalerate in XA cavas. These compounds are directly related to the fruity notes of pineapple, apple, pear, anise, and flowers (Table S2). Furthermore, in general, all of these compounds presented concentrations well above their sensory limit (Table S2), with the exception of ethyl butyrate, which only exceeded it in some cases. Therefore, non-*Saccharomyces* yeasts, such as *T. delbrueckii* (Y5), presented and conserved the same or higher concentrations of esters than some *Saccharomyces* yeasts.

Table 3. Families of volatile compounds (µg/L) from Chardonnay and Xarel.lo base wines (BW) and cavas, after 18 months of aging on the lees (18M), with the addition of five different yeasts in the alcoholic fermentation (Y1, Y2, Y3, Y4, Y5). Different letters indicate significant differences between samples with different yeasts for each grape variety, and in each of the analysis times. Samples (n = 2).

	CHARDONNAY					XAREL.LO				
Sample BW	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
ESTERS	26,557 b ± 387	29,560 b ± 311	17,351 a ± 209	29,375 b ± 357	29,256 b ± 825	30,854 ± 1266	32,710 b ± 1173	21,101 a ± 101	29,373 ab ± 2103	30,109 b ± 3834
Ethyl butyrate	164 b ± 2	208 b ± 8	88 a ± 11	210 b ± 17	201 b ± 16	599 ab ± 94	669 b ± 127	329 a ± 2	391 ab ± 26	411 ab ± 81
Ethyl isovalerate	267 b ± 4	233 b ± 0	111 a ± 19	265 b ± 35	241 b ± 8	279 b ± 13	278 ab ± 15	161 a ± 1	179 ab ± 19	270 ab ± 60
Ethyl hexanoate	5143 b ± 251	5272 b ± 134	2182 a ± 217	5326 b ± 704	5265 b ± 160	7149 b ± 244	7988 b ± 100	3755 a ± 14	6730 ab ± 336	7171 b ± 1283
Ethyl octanoate	14,015 b ± 172	15,199 b ± 56	8683 a ± 1498	15,457 b ± 41	15,060 b ± 192	14,242 b ± 464	15,093 b ± 738	9426 a ± 15	13,970 b ± 815	13,765 b ± 1901
Ethyl decanoate	6825 ab ± 286	8481 b ± 222	6027 a ± 399	7903 ab ± 1034	8321 b ± 463	7939 a ± 412	8065 a ± 634	7003 a ± 67	7602 a ± 78	7895 a ± 477
Ethyl dodecanoate	123 a ± 16	152 ab ± 5	243 b ± 47	198 ab ± 36	153 ab ± 4	621 a ± 42	595 a ± 79	413 a ± 3	468 a ± 119	556 a ± 35
Diethyl succinate	16 a ± 1	14 a ± 2	18 a ± 1	14 a ± 2	14 a ± 1	24 ab ± 3	21 a ± 4	13 a ± 0	34 b ± 4	36 b ± 3834
ACETATES	13,319 b ± 229	13,029 b ± 274	9219 a ± 319	14,189 b ± 1565	12,988 b ± 426	17,177 a ± 1237	16,816 a ± 1096	14,721 a ± 685	13,728 a ± 1091	16,104 a ± 783
Ethyl acetate	2809 a ± 45	3060 a ± 107	2902 a ± 67	2928 a ± 151	3016 a ± 150	4819 b ± 273	4492 b ± 49	4801 b ± 67	3195.3 a ± 250	4234 b ± 254
Isoamyl acetate	10,270 b ± 185	9741 b ± 158	6190 a ± 249	11,043 b ± 1412	9745 b ± 264	12,137 b ± 934	12,063 b ± 101	9724 a ± 621	10,342.3 ab ± 826	11,603 b ± 517
Hexyl acetate	81 bc ± 0	88 c ± 2	43 a ± 0	71 b ± 1	75 b ± 5	62 b ± 9	65 b ± 3	41 a ± 0	53.4 ab ± 4	58 ab ± 3
2-phenylethyl acetate	159 c ± 1	140 b ± 7	83 a ± 2	146 bc ± 1	151 bc ± 7	159 a ± 20	195 a ± 31	154 a ± 3	137 a ± 11	208 a ± 10
ALCOHOLS	3703 ab ± 59	3217 a ± 150	3823 ab ± 9	3944 b ± 132	3682 ab ± 339	7611 a ± 4	7671 a ± 147	8166 a ± 189	8282 a ± 711	7965 a ± 341
Isoamyl alcohol	3147 ab ± 54	2705 a ± 139	3291 ab ± 9	3408 b ± 120	3125 ab ± 306	6609 a ± 55	6592 a ± 137	7014 b ± 176	7146 b ± 623	6914 ab ± 280
Isobutanol	117 b ± 1	105 a ± 2	103 a ± 2	95 a ± 1	101 a ± 5	78 ab ± 1	81 ab ± 8	64 a ± 1	100 c ± 1	85 bc ± 5
Benzyl alcohol	2 a ± 0	2 cd ± 0	2 b ± 0	2 c ± 0	3 d ± 0	5 a ± 0	6 ab ± 0	5 a ± 0	6 ab ± 0	7 b ± 0
2-phenylethyl alcohol	437 a ± 6	405 a ± 16	426 a ± 1	439 a ± 11	452 a ± 29	919 a ± 50	991 a ± 19	1082 b ± 14	1030 b ± 86	959 a ± 56
FATTY ACIDS	924 b ± 19	1016 b ± 18	543 a ± 3	963 b ± 12	1032 b ± 55	1362 b ± 31	1626 b ± 131	819 a ± 4	1353 b ± 110	1640 b ± 102
Hexanoic acid	169 b ± 10	168 b ± 2	90 a ± 0	151 b ± 1	172 b ± 5	223 b ± 2	260 bc ± 8	117 a ± 1	223 b ± 21	282 c ± 22
Octanoic acid	511 b ± 3	550 b ± 12	273 a ± 2	516 b ± 15	561 b ± 29	786 b ± 17	930 b ± 69	461 a ± 4	790 b ± 62	944 b ± 56
Decanoic acid	243 b ± 6	299 c ± 3	180 a ± 1	296 c ± 4	299 c ± 19	353 ab ± 16	435 b ± 54	242 a ± 0	339 ab ± 37	414 b ± 24
TOTAL AROMAS	44,503 b ± 538	46,824 b ± 138	30,937 a ± 2410	48,471 b ± 1327	46,957 b ± 5	57,004 ab ± 2538	58,823 b ± 2547	44,808 a ± 601	52,737 ab ± 4015	55,819 ab ± 5061

	CHARDONNAY					XAREL.LO				
Sample 18M	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
ESTERS	11,917 a ± 140	15,320 c ± 13	10,801 a ± 107	11,867 a ± 438	13,842 b ± 487	13,274 d ± 118	13,837 d ± 192	9072 a ± 297	10,390 b ± 134	11,931 c ± 12
Ethyl butyrate	305 b ± 3	437 d ± 6	258 a ± 14	283 ab ± 3	370 c ± 2.	417 d ± 0	423 d ± 4	237 a ± 0	328 b ± 1	387 c ± 7
Ethyl isovalerate	64 a ± 1	113 bc ± 2	71 a ± 0	110 b ± 4	126 c ± 7	67 b ± 0	89 c ± 2	51 a ± 1	64 b ± 1	120 d ± 3
Ethyl hexanoate	3452 a ± 89	4261 c ± 39	3240 a ± 71	3271 a ± 42	3792 b ± 47	4110 cd ± 49	4353 d ± 65	2505 a ± 103	3625 b ± 66	3875 bc ± 45

Table 3. *Cont.*

	CHARDONNAY					XAREL.LO				
Sample 18M	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Ethyl octanoate	6105 ab ± 19	7805 c ± 35	5351 a ± 34	6044 a ± 347	7013 bc ± 390	6761 d ± 11	7549 e ± 238	4562 a ± 120	5370 b ± 29	6121 c ± 48
Ethyl decanoate	1938 a ± 27	2649 d ± 13	1824 a ± 13	2112 b ± 41	2475 c ± 40	1854 c ± 154	1353 b ± 20	1660 bc ± 70	943 a ± 35	1369 b ± 4
Ethyl dodecanoate	19 b ± 0	18 a ± 0	20 c ± 0	25 d ± 0	53 e ± 0	38 b ± 1	37 b ± 0	20 a ± 0	37 b ± 2	37 b ± 0
Diethyl succinate	33 c ± 1	36 d ± 0	37 d ± 1	21 b ± 0	12 a ± 0	25 a ± 0	33 b ± 1	36 b ± 2	24 a ± 1	23 a ± 0
ACETATES	9613 b ± 2	12,472 d ± 28	13,010 e ± 268	9086 a ± 90	10,955 c ± 701	9724 b ± 85	10,018 bc ± 172	8491 a ± 96	8132 a ± 38	10,387 c ± 31
Ethyl acetate	5715 b ± 68	7168 b ± 23	8496 c ± 170	4554 a ± 15	5853 b± 24	5703 b ± 6	5490 b ± 75	5432 b ± 35	4500 a ± 16	5402 b ± 37
Isoamyl acetate	3776 a ± 67	5136 c ± 42	4399 b ± 92	4430 b ± 73	4961 c ± 37	3931 c ± 78	4405 d ± 95	2978 a ± 61	3535 b ± 21	4828 e ± 5
Hexyl acetate	104 bc ± 2	143 d ± 2	95 b ± 5	67 a ± 1	117 c ± 9	51 b ± 1	67 c ± 1	28 a ± 0	51 b ± 0	97 d ± 1
2-phenylethyl acetate	17 a ± 1	24 ab ± 6	20 a ± 0	34 b ± 0	23 ab ± 0	39 a ± 0	56 d ± 1	52 c ± 0	46 b ± 0	59 e ± 0
ALCOHOLS	5437 b ± 88	6353 c ± 7	7507 d ± 180	4964 a ± 24	5621 b ± 39	6153 b ± 16	6063 b ± 105	6175 b ± 78	6066 b ± 14	5657 a ± 25
Isoamyl alcohol	4879 b ± 77	5780 c ± 9	4806 b ± 15	4465 a ± 21	5110 b ± 18	5402 c ± 16	5241 bc ± 94	4960 a ± 45	5089 ab ± 7	4960 a ± 24
Isobutanol	114 ab ± 5	146 cd ± 2	168 d ± 13	97 a ± 0	134 bc ± 1	135 bc ± 0	132 b ± 1	99 a ± 5	147 cd ± 4	149 d ± 2
Benzyl alcohol	2 a ± 0	4 c ± 0	6 e ± 0	5 d ± 0	3 b ± 0	4 b ± 0	4 c ± 0	4 a ± 0	5 d ± 0	4 a ± 0
2-phenylethyl alcohol	441 b ± 6	424 ab ± 0	527 c ± 15	396 ab ± 3	374 a ± 3	611 b ± 0	686 c ± 10	1111 e ± 30	826 d ± 19	544 a ± 1
FATTY ACIDS	845 b ± 3	988 d ± 2	625 a ± 14	877 c ± 4	853 bc ± 0	875 b ± 5	1068 d ± 4	709 a ± 10	945 c ± 14	971 c ± 9
Hexanoic acid	214 c ± 1	243 d ± 1	150 a ± 0	201 b ± 3	210 c ± 0	223 b ± 3	259 c ± 2	144 a ± 3	231 b ± 7	236 b ± 4
Octanoic acid	497 b ± 3	589 d ± 0	352 a ± 7	525 c ± 1	509 b ± 0	511 b ± 0	628 e ± 11	404 a ± 2	554 c ± 2	584 d ± 0
Decanoic acid	133 a ± 1	156 b ± 1	122 a ± 6	151 b ± 1	133 a ± 0	141 a ± 2	181 c ± 5	160 b ± 5	160 b ± 5	151 ab ± 5
TOTAL AROMAS	27,811 a ± 47	35,134 c ± 9	31,943 b ± 569	26,794 a ± 556	31,271 b ± 596	30,026 d ± 225	30,987 d ± 89	24,446 a ± 481	25,534 b ± 200	28,946 c ± 15

Regarding acetates, their highest concentration in CH base wine was shown with yeast Y4 but, in contrast, in XA this yeast provided the lowest concentration. In general, the main compounds that showed significant differences were isoamyl acetate, hexyl acetate, and 2-phenylethyl acetate (Table 3), which provide aromas of banana, pear, and green tea, respectively (Table S2) [31,32]. Regarding the 18M analysis time, the highest concentrations of acetates were observed in the Chardonnay variety with the yeasts Y2, Y3, and Y5. It should be noted that, for cavas with Y2 and Y5 yeasts, the main compounds responsible for these high concentrations were isoamyl acetate and hexyl acetate. In the case of cava with yeast Y3, the increase was due to the ethyl acetate. This compound should be carefully considered because it provides positive aromas such as sweet fruits when it appears at low concentrations, but its presence at high concentrations implies undesirable aromas, such as glue or solvent. In this case, the cava with the Y3 yeast presented a high concentration of this compound, with a concentration of 8496 μg/L (Table 3), and was the only sample to exceed the sensory limit (LS of ethyl acetate 7500 μg/L) [33]. This indicates that there are some species of *S. cerevisiae* which provide certain aromatic deviations in cavas, unlike other non-*Saccharomyces* yeasts which do not produce them. The Xarel.lo cavas presented the highest concentrations of acetates with the three yeasts Y1, Y2, and Y5. These high values were mainly due to the isoamyl acetate compound, with values well above its sensory limit (670 μg/L) [32]. This could give the cavas a marked tropical aromatic profile because banana is their main aromatic descriptor. In addition, in cavas with Y2 and Y5, higher concentrations of isoamyl acetate and 2-phenylethyl acetate were observed. All of these results show that the yeast *T. delbrueckii* (Y5) is also capable of providing a more marked tropical character in cavas, unlike others in which only *Saccharomyces* yeasts were used.

Regarding aromatic alcohols, a different trend between the two grape varieties was observed. Whereas Xarel.lo presented a slight decrease in alcohol concentrations during aging, the Chardonnay variety showed a slight increase in the concentration of alcohol. However, despite these different trends, after 18 months of aging on lees, it was observed that both varieties present similar values of total alcohols, with a concentration range between 4964 and 6353 μg/L. In reference to the analyses of alcohols in Chardonnay BW, all of the base wines presented similar values, obtaining the highest value with yeast Y4 and the lowest concentration with Y2. For the Xarel.lo variety, the high concentrations of alcohols produced by Y3 and Y4 yeasts stand out. These high values were mainly due to two of the studied compounds: isoamyl alcohol and 2-phenylethyl alcohol. These compounds provide floral aromas of roses, pollen, and perfume [1,34]. Regarding the 18M time, in the Chardonnay variety, the cava obtained with Y2 had the highest concentration followed by Y5, Y3, and Y1. This is mainly due to the obtained concentrations of the isoamyl alcohol compound (Table 3). In XA, all of the cavas generally presented similar values, with the exception of the cava with yeast Y5, which presented a slightly lower value. Therefore, with respect to the chemical family of alcohols, it could be noted that the yeast *M. pulcherrima* (Y4) implied the highest alcohol content of BW but, after aging on lees, these values were generally similar to those obtained by the *Saccharomyces* yeasts, as observed in the XA variety.

Finally, in the family of fatty acids, the cava obtained with the Y3 yeast presented the lowest concentrations of fatty acids (in all studied cases), with significant differences compared to the other yeasts. In Chardonnay, four base wines presented similar values (Y1, Y2, Y4, Y5). However, for the Xarel.lo variety, the highest concentrations of fatty acids obtained when using Y2 and Y5 stood out. It should be noted that *T. delbrueckii* (Y5) presented the highest fatty acid values. These high values were mainly due to the concentrations obtained for hexanoic acid and octanoic acid (Table 3). These compounds give aromas of soap, cheese, and yogurt (Table S2). By comparison, after 18 months of aging on the lees, the cava with the Y2 yeast presented the highest concentrations of fatty acids in both varieties. However, it could be noted that non-*Saccharomyces* yeasts (Y4 and Y5) presented similar or higher values than those produced by other *Saccharomyces* yeasts (Y1 and Y3).

3.5. Sensory Analysis

Figure 3 shows the results of sensory analysis of the cavas after 18 months of aging in the bottle on lees. The Chardonnay variety showed the highest values of the fresh fruit descriptor when the Y2 and Y5 yeasts were used. This behavior may be directly related to the high concentrations of esters and acetates that were observed in the analytical analysis. Furthermore, it was observed how cava obtained with Y2 and Y4 presented the highest scores for bakery and floral descriptors, in addition to Y5 for the latter. These aromatic descriptors are related to the high concentrations of fatty acids and alcohols, which are related to lactic and rose aromas, respectively. In comparison, it was shown that the cava obtained with the yeast Y3 was the most evolved sensorial and also showed the highest scores of candied fruit and evolution descriptors, which could be due to its high content of ethyl acetate. In the Xarel.lo variety, it was observed that the cavas with the highest fresh fruit descriptors were those made with Y4 and Y5 yeasts, followed by those obtained with Y2. This can be directly related to the high concentrations of esters and acetates found in gas chromatography analysis. As happened with the Chardonnay variety, Xarel.lo cava obtained with Y3 was the most sensorially evolved. Therefore, it can be emphasized that non-*Saccharomyces* yeasts provided more fruity and fresh aromatic profiles, especially in the case of *T. delbrueckii*, and much less evolved than most *Saccharomyces* yeasts.

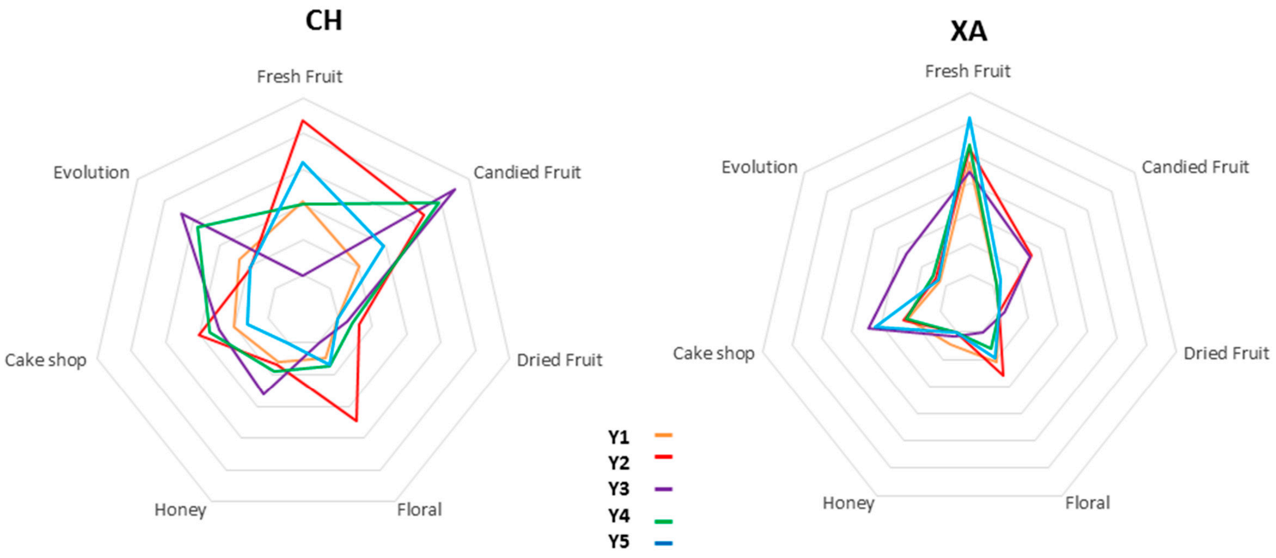

Figure 3. Aromatic profiles of the cavas obtained after 18 months of aging on lees, of the Chardonnay (CH) and Xarel.lo (XA) varieties made with five different yeasts strains (Y1, Y2, Y3, Y4, Y5). Results obtained by the mean and standard deviation of the scores given by the tasters.

4. Conclusions

The use of different *Saccharomyces* and non-*Saccharomyces* yeasts strains for the production of cavas allows products with certain distinctive chemical–physical and sensory attributes to be obtained. In addition, the different aromatic profiles of the wines not only appear after the moment of vinification with the addition of different yeasts, but certain aromatic, taste, and visual distinctions with respect to foamability also appear, after a long time of aging on the lees. In this study, it was possible to corroborate that the use of non-*Saccharomyces* yeasts allowed cavas with a similar or even better organoleptic quality to be obtained than those made with *Saccharomyces*. This was the case of the base wine made with *M. pulcherrima* yeast (Y4), which provided cavas with better persistence in foaminess. Moreover, the cavas made with *T. delbrueckii* preserved a higher concentration of aromas after 18 months of aging on the lees, with the subsequently more complex sensory properties, than those obtained with respect to *S. cerevisiae*. These results open the door to new studies that allow for longer aging periods to be evaluated, testing the behavior of other varieties, and verifying the effect of the combination of yeasts other than *Saccharomyces*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fermentation7020064/s1, Figure S1. SDS-PAGE of the proteins of Chardonnay (CH) and Xarel.lo (XA) base wines elaborated with different yeasts strains, *Saccharomyces cerevisiae* (Y1, Y2, Y3) and non-*Saccharomyces*, *M. pulcherrima* (Y4) and *T. delbrueckii* (Y5).

Author Contributions: A.M.M.: participated on the analysis of samples, manuscript redaction and editing. M.P.: performed and designed the experiment. I.A.: participated in the manuscript. N.E.: participated in the analysis of samples. S.d.L.: performed and designed the experiment, and also contributed to obtaining the funding resources. M.M.: participated in the redaction and revision of the manuscript, R.F.-G.: participated in the sample analysis, redaction, revision and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Ivit, N.N.; Kemp, B. The Impact of Non-*Saccharomyces* Yeast on Traditional Method Sparkling Wine. *Fermentation* **2018**, *4*, 73. [CrossRef]
2. Plata, C.; Millán, C.; Mauricio, J.C.; Ortega, J.M. Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts. *Food Microbiol.* **2003**, *20*, 217–224. [CrossRef]
3. Rojas, V.; Gil, J.V.; Piñaga, F.; Manzanares, P. Acetate ester formation in wine by mixed cultures in laboratory fermentations. *Int. J. Food Microbiol.* **2003**, *86*, 181–188. [CrossRef]
4. Benito, S. The impacts of *Schizosaccharomyces* on winemaking. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 4291–4312. [CrossRef]
5. Morata, A.; Escott, C.; Bañuelos, M.A.; Loira, I.; Del Fresno, J.M.; González, C.; Suárez-Lepe, J.A. Contribution of non-*saccharomyces* yeasts to wine freshness. A review. *Biomolecules* **2020**, *10*, 34. [CrossRef]
6. Comitini, F.; Capece, A.; Ciani, M.; Romano, P. New insights on the use of wine yeasts. *Curr. Opin. Food Sci.* **2017**, *13*, 44–49. [CrossRef]
7. Benito, Á.; Jeffares, D.; Palomero, F.; Calderón, F.; Bai, F.-Y.; Bähler, J.; Benito, S. Selected *Schizosaccharomyces* pombe Strains Have Characteristics That Are Beneficial for Winemaking. *PLoS ONE* **2016**, *11*, e0151102. [CrossRef]
8. Ivit, N.N.; Loira, I.; Morata, A.; Benito, S.; Palomero, F.; Suárez-Lepe, J. Making natural sparkling wines with non-*Saccharomyces* yeasts. *Eur. Food Res. Technol.* **2018**, *244*. [CrossRef]
9. Loira, I.; Morata, A.; Comuzzo, P.; Callejo, M.J.; Gonzalez, C.; Calderón, F.; Suárez-Lepe, J. Use of *Schizosaccharomyces* pombe and Torulaspora delbrueckii strains in mixed and sequential fermentations to improve red wine sensory quality. *Food Res. Int.* **2015**, *76*. [CrossRef]
10. Canonico, L.; Comitini, F.; Ciani, M. Torulaspora delbrueckii for secondary fermentation in sparkling wine production. *Food Microbiol.* **2018**, *74*, 100–106. [CrossRef]
11. González-Royo, E.; Pascual, O.; Kontoudakis, N.; Esteruelas, M.; Esteve-Zarzoso, B.; Mas, A.; Canals, J.-M.; Zamora, F. Oenological consequences of sequential inoculation with non-*Saccharomyces* yeasts (Torulaspora delbrueckii or Metschnikowia pulcherrima) and *Saccharomyces* cerevisiae in base wine for sparkling wine production. *Eur. Food Res. Technol.* **2014**, *240*. [CrossRef]
12. Medina-Trujillo, L.; González-Royo, E.; Sieczkowski, N.; Heras, J.; Canals, J.M.; Zamora, F. Effect of sequential inoculation (Torulaspora delbrueckii/*Saccharomyces* cerevisiae) in the first fermentation on the foaming properties of sparkling wine. *Eur. Food Res. Technol.* **2017**, *243*, 681–688. [CrossRef]
13. OIV. International Organisation of Vine and Wine. Compendium of International Methods of Wine and Must Analysis. In Paris, France. 2020. Volumes 1 and 2. Available online: http://www.oiv.int (accessed on 7 May 2020).
14. Silvestri, A.C.; Sabatier, J.; Ducruet, J. A new method of protein extraction from red wines. *J. Int. Sci. Vigne Vin.* **2013**, *47*, 213–220. [CrossRef]
15. Maujean, A.; Poinsaut, P.; Dantan, H.; Brissonnet, F.; Cossiez, E. Etude de la tenue et de la qualité de mousse des vins effervescents. II: Mise au point d'une technique de mesure de la moussabilité de la tenue et de la stabilité de la mousse des vins effervescents. *Bull. OIV* **1990**, *63*, 405–427.

16. Torrens, J.; Riu-Aumatell, M.; López-Tamames, E.; Buxaderas, S. Volatile Compounds of Red and White Wines by Headspace-Solid-Phase Microextraction Using Different Fibers. *J. Chromatogr. Sci.* **2004**, *42*, 310–316. [CrossRef] [PubMed]
17. Mislata, A.M.; Puxeu, M.; Tomás, E.; Nart, E.; Ferrer-Gallego, R. Influence of the oxidation in the aromatic composition and sensory profile of Rioja red aged wines. *Eur. Food Res. Technol.* **2020**, *246*, 1167–1181. [CrossRef]
18. Benito, S.; Hofmann, T.; Laier, M.; Lochbühler, B.; Schüttler, A.; Ebert, K.; Fritsch, S.; Röcker, J.; Rauhut, D. Effect on quality and composition of Riesling wines fermented by sequential inoculation with non-*Saccharomyces* and *Saccharomyces* cerevisiae. *Eur. Food Res. Technol.* **2015**, *241*, 707–717. [CrossRef]
19. Binati, R.L.; Lemos Junior, W.J.F.; Luzzini, G.; Slaghenaufi, D.; Ugliano, M.; Torriani, S. Contribution of non-*Saccharomyces* yeasts to wine volatile and sensory diversity: A study on Lachancea thermotolerans, Metschnikowia spp. and Starmerella bacillaris strains isolated in Italy. *Int. J. Food Microbiol.* **2020**, *318*. [CrossRef] [PubMed]
20. Padilla, B.; Gil, J.V.; Manzanares, P. Past and future of non-*Saccharomyces* yeasts: From spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front. Microbiol.* **2016**, *7*. [CrossRef]
21. Cilindre, C.; Liger-Belair, G.; Villaume, S.; Jeandet, P.; Marchal, R. Foaming properties of various Champagne wines depending on several parameters: Grape variety, aging, protein and CO_2 content. *Anal. Chim. Acta* **2010**, *660*, 164–170. [CrossRef]
22. Dambrouck, T.; Marchal, R.; Marchal-Delahaut, L.; Parmentier, M.; Maujean, A.; Jeandet, P. Immunodetection of proteins from grapes and yeast in a white wine. *J. Agric. Food Chem.* **2003**, *51*, 2727–2732. [CrossRef]
23. Mostert, T.T.; Divol, B. Investigating the proteins released by yeasts in synthetic wine fermentations. *Int. J. Food Microbiol.* **2014**, *171*, 108–118. [CrossRef]
24. Andrés-Lacueva, C.; Lamuela-Raventós, R.M.; Buxaderas, S.; de la Torre-Boronat, M.d.C. Influence of Variety and Aging on Foaming Properties of Cava (Sparkling Wine). 2. *J. Agric. Food Chem.* **1997**, *45*, 2520–2525. [CrossRef]
25. Vanrell, G.; Canals, R.; Esteruelas, M.; Fort, F.; Canals, J.M.; Zamora, F. Influence of the use of bentonite as a riddling agent on foam quality and protein fraction of sparkling wines (Cava). *Food Chem.* **2007**, *104*, 148–155. [CrossRef]
26. Martínez-Rodríguez, A.; Carrascosa, A.; Barcenilla, J.; Pozo-Bayón, M.; Polo, M. Autolytic capacity and foam analysis as additional criteria for the selection of yeast strains for sparkling wine production. *Food Microbiol.* **2001**, *18*, 183–191. [CrossRef]
27. Condé, B.C.; Bouchard, E.; Culbert, J.A.; Wilkinson, K.L.; Fuentes, S.; Howell, K.S. Soluble Protein and Amino Acid Content Affects the Foam Quality of Sparkling Wine. *J. Agric. Food Chem.* **2017**, *65*, 9110–9119. [CrossRef]
28. Ancín-Azpilicueta, C.; González-Marco, A.; Jiḿenez-Moreno, N. Evolution of esters in aged Chardonnay wines obtained with different vinification methods. *J. Sci. Food Agric.* **2009**, *89*, 2446–2451. [CrossRef]
29. Ferreira, V.; López, R.; Cacho, J.F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667. [CrossRef]
30. Tao, Y.-S. Active volatiles of cabernet sauvignon wine from Changli County. *Health* **2009**, *01*, 176–182. [CrossRef]
31. Gómez-Míguez, M.J.; Cacho, J.F.; Ferreira, V.; Vicario, I.M.; Heredia, F.J. Volatile components of Zalema white wines. *Food Chem.* **2007**, *100*, 1464–1473. [CrossRef]
32. Peinado, R.A.; Moreno, J.; Bueno, J.E.; Moreno, J.A.; Mauricio, J.C. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chem.* **2004**, *84*, 585–590. [CrossRef]
33. Guth, H. Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032. [CrossRef]
34. Lambrechts, M.; Pretorius, I. Yeast and its importance to wine aroma—A review. *S. Afr. J. Enol Vitic* **2000**, *21*. [CrossRef]

Artículo 3

Preliminary study of the effect of cation-exchange resin treatment on the aging of Tempranillo red wines.

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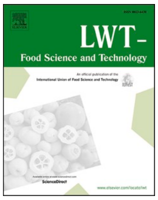

Preliminary study of the effect of cation-exchange resin treatment on the aging of tempranillo red wines

A.M. Mislata [a,b], M. Puxeu [a], E. Nart [a], S. de Lamo [a], R. Ferrer-Gallego [a,*]

[a] *VITEC- Centro Tecnológico Del Vino, Ctra. Porrera Km.1, 43730, Falset (Tarragona), Spain*
[b] *Sensometria Instrumental (i-Sens), Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira I Virgili, Tarragona, 43007, Spain*

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ABSTRACT

This research reports the effect of the cation exchange treatment in Tempranillo wines during the aging period in oak barrels. The control wine and the mixtures with different percentages (5, 10 and 20%) of wine treated by a cation exchange resin (permeate wine) were evaluated. In general, a greater acidity, color and tartaric stability, and a lower mineral content were observed in the treated wines, especially in those with 20% of permeate wine. In addition, during the entire aging time, the decrease in potassium and magnesium cations stood out. A higher concentration of aromatic compounds such as hexyl acetate, isobutanol, 2-phenylethyl alcohol, ethyl isovalerate and diethyl succinate was observed in treated wines after six months of aging. The two last compounds reached the highest concentrations when the percentage of permeated increased and reached values up to 23 μg/L for ethyl isovalerate and up to 3186 μg/L for diethyl succinate. In addition, the perception thresholds of these esters were exceeded in all wines. The use of cation exchange resins seems to be an effective industrial alternative to produce balanced red aged wines in a climate change scenario.

1. Introduction

Vineyards and consequently wine industry are sensitive to the climate change. The difference between grape maturity on wine alcohol, phenolics, and aromatics are becoming increasingly evident (Bindon, Varela, Kennedy, Holt, & Herderich, 2013; Ferrer-Gallego, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, 2012; Jones & Davis, 2000; Mira de Orduña, 2010). The increases in berry sugar content and pH, and the losses of acidity of grapes are worrying in some varieties and particularly in those cultivated in the Mediterranean regions, especially in Spain. Controlling acidity and pH of wines is essential to ensure the wine quality and its stability along time. Several factors may influence the content of grape acids. Tartaric acid, the most abundant acid in grapes, is clearly influenced by different variables like the climatic and ripeness conditions, the soil composition, and others (Cosme, Vilela, & Jordão, 2017). Often, high temperatures during ripeness lead to lower levels of tartaric acid in grape juice and therefore, the acidity has to be increased by winemaking practices to produce balanced wines (Mira de Orduña, 2010). Nowadays, the addition of organic acids (e.g. citric and tartaric) is becoming a common practice for some wineries to improve wine quality (e.g. color and freshness) and also the microbial stability (Bdiri et al., 2020). However, some adverse effects derived from this practice should be taken into account. As consequence of the tartaric acid addition, an increment in the appearance of sediments in bottled wines can occur (Gómez Benítez, Palacios Macías, Szekely Gorostiaga, Veas López and Pérez Rodríguez, 2003), mainly formed by potassium hydrogen tartrate (KHT) and at lesser extent by calcium tartrate (CaT). This precipitation, associated to the tartrate salt crystals, may involve some harmful wine chemical changes regarding polyphenols and/or polysaccharides which lead to a loss in the organoleptic properties and wine stability (Vernhet et al., 1999).

Overtime, many chemicals and physical methodologies have been used to preserve the wine tartaric stability (Waters, Pellerin, & Brillouet, 1994). Among them, the use of additives (metatartaric acid, mannoproteins, and carboxymethylcellulose) and physical treatments (cold stabilitzation, electrodialysis and ion-exchange resins) have been proposed (Cabrita, Garcia, & Catarino, 2016; El Rayess & Mietton-Peuchot, 2016; Lasanta & Gómez, 2012). Among the physical treatments, one of the most used methods over time is cold stability, as it is a technique that achieves a very good overall quality of the wines, mainly in the stability of white wines. However, its main drawbacks are the high energy consumption and its long duration, assuming a high economic cost. In

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* Corresponding author.
E-mail address: raul.ferrer@vitec.wine (R. Ferrer-Gallego).

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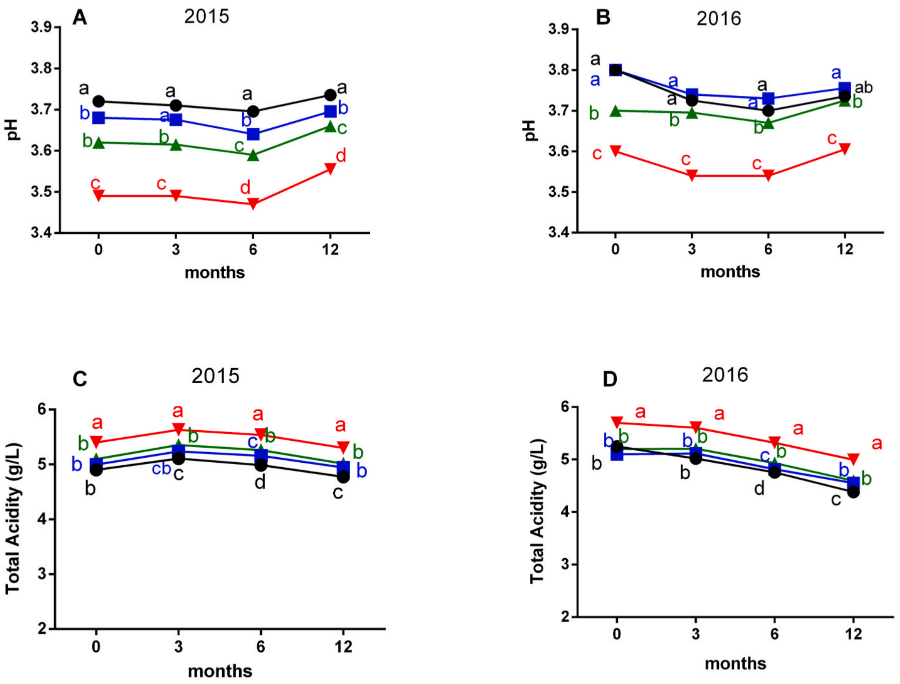

Fig. 1. Comparative pH (A and B) and total acidity (C and D) of control wine (●) and wines blended with cation-exchange treated wine at different percentages (5% ( ), 10% ( ) and 20% ( ) and aging times (0, 3, 6 and 12 months) in 2015 and 2016. *Samples (n = 2).*

Table 1
Mean values of the organic acids (g/L) in control wine (CW) and wines blended with different percentage (5%; W5, 10%; W10 and 20%, W20) of cation-exchange treated wine, at 0, 3, 6 and 12 months for 2015 and 2016.

		2015				2016			
		Tartaric	Malic	Lactic	Acetic	Tartaric	Malic	Lactic	Acetic
0 months	CW	1.61 ab	0.11a	1.81a	0.70a	2.46a	0.09a	1.83a	0.60a
	W5	1.62a	0.15a	1.81a	0.71a	2.19b	0.09a	1.86a	0.66a
	W10	1.59 ab	0.17a	1.78 ab	0.72a	2.26 ab	0.09a	1.79a	0.61a
	W20	1.56b	0.19a	1.74b	0.73a	2.34 ab	0.09a	1.78a	0.56a
	SDpooled	*0.02*	*0.00*	*0.04*	*0.02*	*0.05*	*0.00*	*0.04*	*0.02*
3 months	CW	1.48b	0.06a	1.72a	0.75a	1.92 ab	0.10a	1.70 ab	0.59c
	W5	1.53a	0.06a	1.65a	0.77a	1.79b	0.07a	1.80b	0.73a
	W10	1.56a	0.07a	1.64a	0.76a	1.80b	0.07a	1.81b	0.67b
	W20	1.53a	0.07a	1.61a	0.71a	2.11a	0.05a	1.67a	0.61c
	SDpooled	*0.06*	*0.02*	*0.05*	*0.04*	*0.08*	*0.02*	*0.03*	*0.01*
6 months	CW	1.48b	0.31a	1.87a	0.73a	1.70b	0.06a	1.68a	0.62b
	W5	1.57a	0.32a	1.89a	0.77a	1.82b	0.05a	1.79a	0.71a
	W10	1.55a	0.30a	1.78a	0.77a	1.89 ab	0.06a	1.75a	0.66 ab
	W20	1.57a	0.30a	1.81a	0.74a	2.08a	0.06a	1.80a	0.63b
	SDpooled	*0.06*	*0.01*	*0.12*	*0.01*	*0.06*	*0.02*	*0.04*	*0.01*
12 months	CW	1.40b	0.10a	1.61a	0.80a	1.67b	0.10a	1.58a	0.64b
	W5	1.48 ab	0.25b	1.61a	0.80a	1.70 ab	0.10a	1.63a	0.77a
	W10	1.44 ab	0.27a	1.66a	0.80a	1.75 ab	0.26a	1.63a	0.69 ab
	W20	1.57a	0.10b	1.62a	0.80a	1.89a	0.10a	1.63a	0.62b
	SDpooled	*0.04*	*0.01*	*0.04*	*0.01*	*0.05*	*0.00*	*0.02*	*0.03*

Different letters in columns indicate significant differences by HSD Tukey test ($p \leq 0.05$) between the control and treated samples at each of the aging times (0, 3, 6 and 12 months). *Samples (n = 2).*

addition, there are other inconveniences such as the loss of color that occurs together with the precipitation of tartrate sales during the stability of red wines (Gómez Benítez et al., 2003). Another method today is the use of ion exchange resins. This technique is considered much more economical compared to cold stability (Lasanta & Gómez, 2012). In addition, the chemical-physical advantages it produces in treated wines should be taken into account, such as low pH, improved color and organoleptic preservation of the wines (Lasanta, Caro, & Pérez, 2013; Mira, Leite, Ricardo-da-Silva, & Curvelo-Garcia, 2006).

The ion-exchange resin treatments involve passing wine through a cartridge containing cationic or anionic resin in order to reduce the level of cations, lower pH and to limit the risk of the formation of tartrate. Cationic resins in acid cycle are the most used and the only authorized resins in the EU for wines (EU, 2009). The 442/2012 and 443/2012 resolution of the OIV and the 144/2013 EU regulation accept and authorize the use of the cation exchange as an alternative method in the acidification of grape juices and wines (EU, 2013). A common practice is blending the treated wine (passed through the resin) and the untreated wine in order to obtain an equilibrated and stable final wine. Usually, rates between 10 and 20% of the treated wine are used (Lasanta et al., 2013; Ponce, Mirabal-Gallardo, Versari, & Laurie, 2018). The treatments by ion-exchange resins modify widely the chemical composition of red

Table 2
Mean values of the cation content (mg/L) in control wines (CW) and wines with the addition of 5, 10 and 20% of resin treated wine (W5, W10, W20) at 0, 3, 6 and 12 months after aging in barrels, in both vintages (2015 and 2016).

	2015	K$^+$	Mg^{2+}	Ca^{2+}	Na$^+$	Fe$^{2+/3+}$	Cu^{2+}
0 months	CW	1012a	102a	33a	10.0a	0.75a	0.14a
	W5	994a	99a	33a	11.0a	0.74a	0.14a
	W10	931a	94 ab	30 ab	10.0a	0.71 ab	0.14a
	W20	833b	83b	27b	10.0a	0.65b	0.13a
	SDpooled	*26*	*3*	*1*	*0.0*	*0.02*	*0.01*
3 months	CW	1028a	110a	32a	6.5a	0.74a	0.12a
	W5	1015a	99 ab	32a	8.5a	0.71 ab	0.13a
	W10	971b	95 ab	31a	8.0a	0.69b	0.13a
	W20	908c	86b	30a	7.0a	0.74a	0.13a
	SDpooled	*8*	*4*	*1*	*0.5*	*0.01*	*0.01*
6 months	CW	1147a	110a	35a	10.0a	0.71a	0.12b
	W5	1109 ab	104 ab	36a	9.0 ab	0.73a	0.13 ab
	W10	1060 ab	98b	34a	9.0 ab	0.71a	0.13 ab
	W20	969b	87c	35a	8.5b	0.70a	0.14a
	SDpooled	*36*	*2*	*1*	*0.4*	*0.02*	*0.01*
12 months	CW	1235a	113a	35a	12.5a	0.75b	0.13a
	W5	1239a	111a	37a	14.0a	1.20a	0.15a
	W10	1118b	104b	37a	13.5a	0.85 ab	0.15a
	W20	1069b	93c	37a	12.5a	0.70b	0.14a
	SDpooled	*28*	*1*	*1*	*0.6*	*0.09*	*0.01*
	2016	**K$^+$**	**Mg^{2+}**	**Ca^{2+}**	**Na$^+$**	**Fe$^{2+/3+}$**	**Cu^{2+}**
0 months	CW	1164a	87a	47a	15.0a	0.70a	0.17a
	W5	1089 ab	82 ab	38b	15.0a	0.65 ab	0.17b
	W10	1028bc	78bc	39b	14.5a	0.63bc	0.17b
	W20	930c	70c	38b	13.0b	0.58c	0.17b
	SDpooled	*28*	*2*	*1*	*0.4*	*0.02*	*0.01*
3 months	CW	1074 ab	88a	38a	14.0a	0.76a	0.16 ab
	W5	1099a	85a	35a	12.5a	0.68a	0.13c
	W10	1027 ab	84a	38a	11.5a	0.71a	0.14bc
	W20	984b	79a	39a	11.0a	0.66a	0.17a
	SDpooled	*24*	*6*	*3*	*0.9*	*0.03*	*0.01*
6 months	CW	1110a	93a	43a	15.0a	0.72a	0.17a
	W5	1149a	84b	34b	13.5 ab	0.69bc	0.15a
	W10	1111a	81b	36b	13.0 ab	0.71 ab	0.16a
	W20	1011a	71c	37b	12.5b	0.67c	0.17a
	SDpooled	*42*	*1*	*1*	*0.5*	*0.01*	*0.01*
12 months	CW	1119b	95a	39 ab	18.5a	0.70a	0.16a
	W5	1290a	90b	34c	18.0a	0.70a	0.14 ab
	W10	1140b	87b	35bc	16.5 ab	0.75a	0.15 ab
	W20	1025b	76c	40a	15.5b	0.80a	0.14b
	SDpooled	*33*	*1*	*1*	*0.6*	*0.08*	*0.01*

Different letters in columns indicate significant differences by HSD Tukey test ($p \leq 0.05$) between the control and treated samples at each aging time (0, 3, 6 and 12 months). *Samples* ($n = 2$).

wines. Recently, Ponce et al. (2018) have shown differences in the pH, metal content and tartaric stability depending on the resin activation and procedures (Ponce et al., 2018). Other authors confirmed the use of the cation exchange resins as an interesting tool in red winemaking to improve the tartaric stability even though this treatment involved decreases of the anthocyanin and tannin content — compounds closely related to the wine aging ability (Ibeas, Correia, & Jordão, 2015; Lasanta et al., 2013; Mira et al., 2006). Therefore, assessing the effect of cation exchange resin on the aging ability of red wine is needed. However, literature regarding the effect of the resin treatments in wine aging is scarce sparsely. For this, the aim of this study was to evaluate the effect of the cation exchange resin on the oenological characteristics of wine during the aging period, paying special attention on the volatile composition.

2. Material and methods

2.1. *Chemicals*

The organic acids (succinic, malic, lactic, citric, tartaric and acetic) analyzed by the enzymatic method were from BioSystems (Barcelona, Spain). The 2-octanol ($\geq$96%) used as reagent in the analysis of aromas and malvidin 3-*O*-glucoside for the analysis of anthocyanins were from Sigma-Aldrich (Merck Life Science, Barcelona, Spain) analytical quality. Solvents such as HCl and HNO_3 were of highest purity from Panreac (AppliChem, Barcelona, Spain). Also, the potassium bitartrate used for the taratric stability analysis was supplied by Delta Acque (Firenze, Italy).

2.2. *Samples and treatment conditions*

The red wines used in this study were produced in 2015 and 2016 vintage in Rioja D.O.Ca region from cv. Tempranillo red grapes. Wines were produced by standard winemaking procedure. Grapes were collected manually in boxes and transported in a refrigerated truck. Grape juices were sulfited with 20 mg/L of potassium metabisulfite (Laffort, Guipuzcoa, Spain) and fermented at controlled temperature ranged from 22 to 25 °C. Wines were treated at industrial scale by a cation-exchange resin equipment supplied by Agrovin S.L. (freeK$^+$ system®, Barcelona, Spain). Resins were regenerated with HCl solution (6 M). The untreated or control wine (CW) was blended with different percentages of the treated wine. Thus, 25 L (at 5% (W5)), 50 L (at 10% (W10)) and 100L (at 20% (W20)) of treated wine was added to 475 L, 450 L and 400 L, respectively, of the untreated wine. Finally, wines were aging in 225 L oak barrels (by duplicate) and analyzed during the aging period. The measurements were made at 0 (one week after the blending)

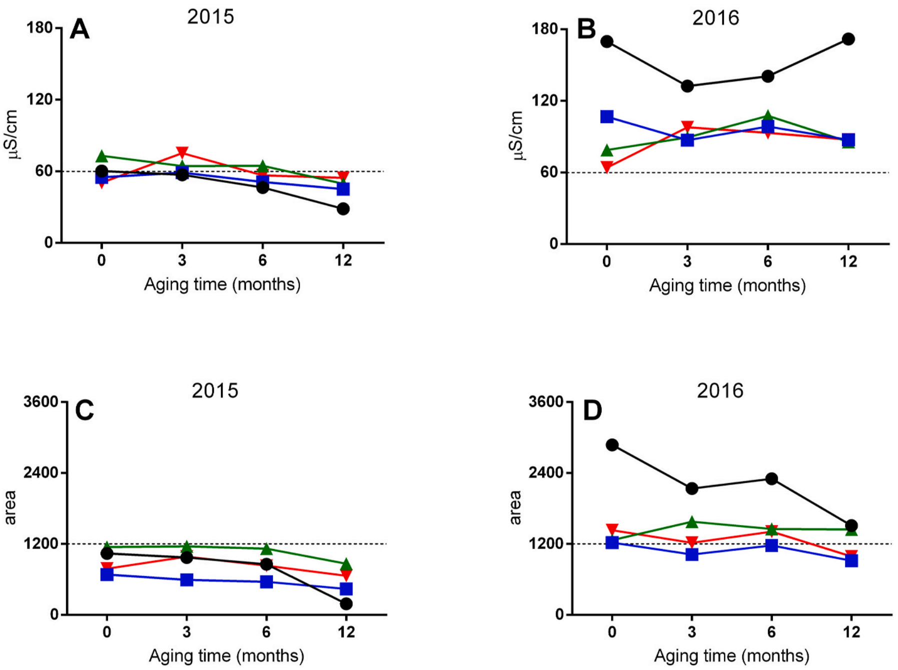

Fig. 2. Evolution of the MC test (A and B) and ISS value (C and D) of control wine (●) and wines blended with cation-exchange treated wine at different percentages (5% ( ), 10% ( ) and 20% ( ) and aging times (0, 3, 6 and 12 months) in 2015 and 2016. *Samples (n = 2).*

3, 6 and 12 months after the treatment.

2.3. Enological and colorimetric analysis

The basic analytical parameters of wines were determined following the compendium of the international methods, established by the International Organization of Vine and Wine (OIV, 2016). Titratable acidity and pH were measured by potentiometry with an automatic titrator (TitroMatic Hach from Crison®); turbidity with a HACH TL2310 turbidimeter; the content of organic acids (L-malic, L-lactic, acetic, citric, tartaric and acetic) was determined using methods established by BioSystems S.A. using Y15 BioSystems equipment and Control Wine® (BioSystems S.A., Barcelona, Spain) for enzymatic reaction; and total phenols were determined using the Folin-Ciocalteu assay (Method OIV-MA-AS2-10) with some modifications. Briefly, 100 μL of sample, 500 μL of Folin- Ciocalteu reagent and 2 mL of a sodium carbonate solution (1.88 M) were mixed, with final volume — 10 mL with water. The solution was stocked for 30 min for the reaction to take place and stabilize. Finally, the absorbance was measured at 750 nm by a Helios-α spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). To determine the total polyphenol index (TPI), Wine samples were diluted 10-fold with water and the absorbance was measured directly at 280 nm in 1 mm path length quartz cell. For the colorimetric measurements, 10 mm path length plastic cells were used. The *CIELAB* color parameters and the color differences between controls and treated wines ($\Delta E^{*}ab = ((\Delta L^{*})^{2} + (\Delta a^{*})^{2} + (\Delta b^{*})^{2})^{1/2}$) were calculated.

2.4. Anthocyanins and derivative pigments

The content of anthocyanins was determined by the method commonly used in cellars developed by Glories and co-workers (Saint-Cricq, Vivas, & Glories, 1998) and by HPLC-DAD. Analyses were performed in an Agilent 1200 Series HPLC series liquid chromatograph (Agilent Technologies, Waldbronn, Germany). Wine samples were diluted 2-fold with acidified water (pH 1, HCl) and filtered through a 0.22 μm Millex syringe-driven filter unit (Millipore Corporation, Bedford, MA, USA) before HPLC-DAD-MS analysis. A Zorbax Eclipse Plus C18 reversed-phase, 5 μm, 150 mm × 4.6 mm column (Agilent Technologies, Barcelona, Spain) (thermostatted at 35 °C) was used. The HPLC-DAD method was carried out as described in the study of Alcalde-Eon, Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2006 (Alcalde-Eon et al., 2006). Twenty-eight anthocyanins and derivative pigments (five anthocyanin 3-*O*-glucosides, two anthocyanin diglucosides, five anthocyanin 3-*O*-acetyl-glucosides, five anthocyanin 3-*O*-coumaroyl-glucosides, two anthocyanidin aglycones, flavanol-anthocyanin direct condensation products, the A-type vitisin and B-type of malvidin 3-*O*-glucoside and six pyranoanthocyanins) were quantified and were grouped into five families. Quantification was performed by HPLC/DAD at 520 nm using calibration curves of the corresponding anthocyanin 3-*O*-glucoside (delphinidin, cyanidin, petunidin, peonidin and malvidin 3-*O*-glucoside), purchased from Sigma Aldrich.

2.5. Mineral analysis

Agilent certified reference material was used as metal standards solutions. 2 mL of wine samples were filtered through 0.22 μm syringe filter and diluted 4-fold with nitric acid solution (25 g of HNO_3 in 1000 mL of water). The analyses were carried out on iCAP 7000 Series, inductively coupled plasma optical emission spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, US).

2.6. Tartaric stability measurements

The stability of the wine was determined using a CheckStab analyzer (Alfa 2016 iLife, Delta Acque, Firenze, Italy) equipped with ISS technology. This system is based on two analytical methods to monitor the stability of KHT, the mini-contact test (MC) and the saturation temperature (ST) measurements, based on the conductivity evaluation. The mini-contact test is the determination of conductivity by cold, with the addition of potassium bitartrate ("cream of tartar"). The MC test allows the evaluation of the level of wine instability. The test is useful in the initial phase of the process to demonstrate the degree of tartaric instability under limit conditions; for red wines, the method requires that the

Table 3
Mean values of the phenolic composition and color characteristics studied in control wines (CW) and in wines with addition of 5, 10 and 20% of resin treated wine (W5, W10, W20) at 0, 3, 6 and 12 months later from barrel aging, in both vintages (2015 and 2016).

	2015	Phenolic Composition			Color Characteristic				
		Anthocyanins (mg/L)	Tannins (g/L)	TPI^a	Color Intensity	Δ*E*ab*	*a**	*b**	*L**
0 months	CW	522a	2.5a	62.5a	12.5c	–	33.6d	2.5a	66.3c
	W5	520a	3.0a	59.1a	12.8b	1.7a	35.1c	2.0a	65.6b
	W10	492a	3.5a	61.4a	13.0a	1.4a	36.1b	1.9 ab	65.2 ab
	W20	486a	3.7a	60.6a	13.0a	2.4a	37.9a	1.0b	65.0a
	SDpooled	*11*	*0.5*	*1.4*	*0.0*	*0.3*	*0.2*	*0.2*	*0.1*
3 months	CW	505a	3.4a	66.4a	11.8c	–	30.0c	5.6a	68.3c
	W5	470b	3.4a	65.5a	12.0a	1.3b	31.2b	5.4b	67.8a
	W10	458bc	3.4a	64.7a	11.9b	0.2c	31.4b	5.3b	68.1b
	W20	448c	3.3a	65.5a	12.0a	1.8a	33.0a	4.8c	67.7a
	SDpooled	*4*	*0.1*	*0.6*	*0.0*	*0.1*	*0.1*	*0.0*	*0.1*
6 months	CW	442a	3.5a	62.9c	11.7a	–	28.9b	7.1a	69.0a
	W5	400b	3.1b	65.4a	11.4c	0.7b	28.7b	7.3b	69.6c
	W10	393b	3.2b	64.9 ab	11.5b	1.1a	29.7a	6.9bc	69.3b
	W20	392b	3.4 ab	63.6b	11.3d	0.8b	30.0a	6.7c	69.8c
	SDpooled	*2*	*0.1*	*0.4*	*0.0*	*0.1*	*15.0*	*3.4*	*0.1*
12 months	CW	576a	3.2 ab	61.1a	10.1b	–	24.5c	9.5a	73.0b
	W5	555b	3.4a	59.8a	10.2 ab	1.0b	25.5b	9.0b	72.8 ab
	W10	554b	2.9b	59.8a	10.2a	0.3c	25.5b	9.1b	72.7 ab
	W20	511c	3.2 ab	60.7a	10.3a	1.6a	26.9a	8.7c	72.5a
	SDpooled	*1*	*0.1*	*1.3*	*0.0*	*0.1*	*0.2*	*0.1*	*0.1*
	2016	Anthocyanins (mg/L)	Tannins (g/L)	TPI^a	Color Intensity	Δ*E*ab*	*a**	*b**	*L**
0 months	CW	934a	3.7a	70.1a	19.0a	–	46.7c	1.6a	55.3c
	W5	904a	3.1a	70.1a	19.5a	10.0a	51.9b	−6.4b	52.1a
	W10	914a	3.7a	70.8a	18.3a	1.4a	51.4b	−7.1b	54.2b
	W20	878a	3.2a	67.8a	18.7a	3.8a	54.7a	−8.2b	53.8b
	SDpooled	*16*	*0.3*	*1.1*	*0.4*	*1.9*	*0.5*	*1.9*	*0.2*
3 months	CW	828a	3.4a	75.5a	14.5c	–	41.4b	−2.7a	61.2c
	W5	785b	3.0a	74.7a	18.6a	10.4b	49.0a	−3.7 ab	54.2a
	W10	804 ab	3.1a	74.9a	17.9b	6.5a	49.2a	−5.2bc	54.8 ab
	W20	783b	2.8a	71.5b	18.0b	6.4b	51.1a	−6.1c	55.1b
	SDpooled	*8*	*0.2*	*0.5*	*0.1*	*0.3*	*0.7*	*0.5*	*0.2*
6 months	CW	700a	3.3a	63.4c	14.7b	–	41.4b	−2.4 ab	60.8b
	W5	631c	3.3a	71.4a	15.8a	2.3 ab	42.4b	−1.7a	58.8a
	W10	651b	3.4a	72.3a	15.6a	1.5b	42.6b	−1.5a	59.3a
	W20	612d	3.5a	67.2b	15.6a	3.2a	44.7a	−2.9b	59.1a
	SDpooled	*3*	*0.1*	*0.4*	*0.1*	*0.3*	*0.4*	*0.3*	*0.3*
12 months	CW	878a	3.2bc	65.5a	11.9b	–	33.2a	1.7a	67.1b
	W5	863a	3.1c	67.4a	13.3a	3.8a	35.2a	1.2b	64.0a
	W10	825b	3.4a	67.8a	12.8 ab	2.1a	35.0a	1.4 ab	65.0 ab
	W20	800b	3.3 ab	64.6a	12.1b	2.1a	35.3a	0.2c	66.2 ab
	SDpooled	*7*	*0.0*	*2.3*	*0.3*	*0.9*	*0.6*	*0.1*	*0.6*

Different letters in columns indicate significant differences by HSD Tukey test ($p \leq 0.05$) between treatments for each aging time. Anthocyanins expressed in malvidin 3-glucoside equivalents and tannins expressed in epicatechin equivalents. ^{a}Total Polyphenol Index. *Samples* ($n = 6$). Δ*E*ab* was calculated in control *vs* sample inside each vintage and aging point.

wine remain at −4 °C for 3 h after the addition of fine-grained "cream of tartar" (pure hydrogen tartrate) at a dose of 2 g/100 mL. A red wine can be considered stable if the decrease in conductivity is less than 60 μS, slightly unstable for values between 60 and 80 μS, 90–100 μS very unstable, 100–110 μS too unstable, and values higher than 110 μS totally unstable. The saturation temperature expresses the lowest temperature value at which the added potassium bitartrate dissolves in the wine. The determination of the saturation temperature occurs in the temperature range between - 4 °C and 32 °C. Two curves are obtained whose comparison allows the complete study of the instability of a wine. The ST value alone cannot confirm the stability of the wine, since the parameter does not change after the addition of the cream of tartar. To obtain an accurate stability, the ISS value should also be calculated, since this parameter considers both conductometric curves. The first important parameter is represented by the intersection of the two curves (one curve represents the conductivity with KHT and the other curve the conductivity without KHT) which corresponds to the ST (saturation temperature) of the wine. However, the second and most important parameter is the ISS, represented by the area between the two curves before their intersection. Thus, the more stable a wine is, the curves will be closer to being practically touching, considering it a stable wine with ISS values lesser than 1200 area units. In general, the mini-contact test tends to overestimate the tartaric instability of red wines compared to the saturation temperature. Hence, some wines can be unstable with this MC test and stable with the ST test. That is why both tests are used to obtain as much information as possible about the wine, but the ISS is used as a reference to determine if a wine is stable or unstable.

Sample Preparation. For the MC and ST analysis, 100 mL of wine previously filtered by 1.2 μm (Minisart Sirynge filter of nylon, NML hydrophilic, Sartorius, Goettingen, Germany) were placed in a 200 mL glass beaker into the CheckStab equipment, then 2 g of potassium bitartrate (Enolab, DeltaAcque, Italy) were added to samples and shaken by means of an automatic stirrer for 3 h until reaching −4 °C for MC analysis and 1 h, approximately, to obtain the ISS data.

2.7. GC-MS analysis

The analysis of the fermentative aromas was carried out according to the method used by Torrens et al. (Torrens, Riu-Aumatell, López-Tamames, & Buxaderas, 2004). The wine samples were analyzed directly, without prior treatment, by means of solid-phase microextraction (SPME). A GC 7890A gas chromatograph (Agilent

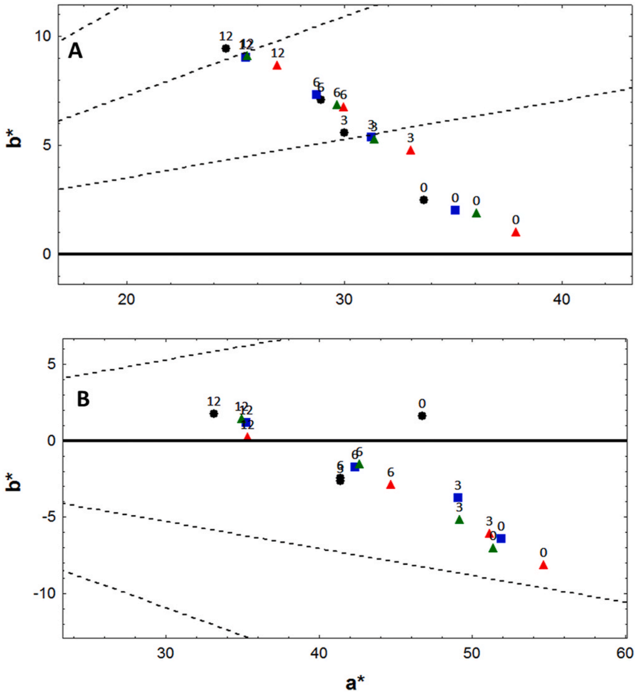

Fig. 3. Changes on the (*a*b**)-plane of *CIELAB* color space for control wine ( ) and wines blended with cation-exchange treated wine at different percentages (5% ( ); 10% ( ); and 20% ( )) and aging times (0, 3, 6 and 12 months) in 2015 (A) and 2016 (B). *Samples (n = 2)*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Technologies) equipped with an inert 5975C mass spectrometer MSD (electron shock source triple-axis detector) was used. Separation was done with a DB-WAX IU column (60 m × 0.25 mm x 0.25 μm, Agilent). And the extraction was carried out with fiber type (DVB/CAR/PDMS) in splitless mode at 270 °C. The analyses conditions and the characteristics of gas chromatography together with detection by mass spectrometry (GC-MS) are described elsewhere in Mislata et al. (Mislata, Puxeu, Tomás, Nart, & Ferrer-Gallego, 2020). The quantification was carried out using the internal standard (IS) method. The results of the volatile compounds were expressed as semi-quantitative data expressed in μg/L as a response with respect to the internal standard using 2-octanol. All analyzes were done in duplicate.

2.8. *Statistical analysis*

A simple analysis of variance (ANOVA) was carried out using the StatGraphics Centurion XVI (Manugistics Inc., Pockville, MD, USA) program. The Tukey's procedure was used and the differences at *p-values* < 0.05 were considered significant. A principal component analysis (PCA) was carried out to study and visualize the possible relationships and/or trends between the different aromatic compounds and the studied wines at different aging times, using the XLSTAT package for the Excel software.

3. Results and discussion

3.1. *Effects of the cation-exchange treatment in the oenological parameters*

The treatment with cation-exchange resin reduced significantly the pH of the studied wines, as stated by other authors (Ibeas et al., 2015; Walker, Morris, Threlfall, & Main, 2004). The pH value of the treated wines was 2.22 in 2015 and 2.29 in 2016 (table S1) while the pH of the

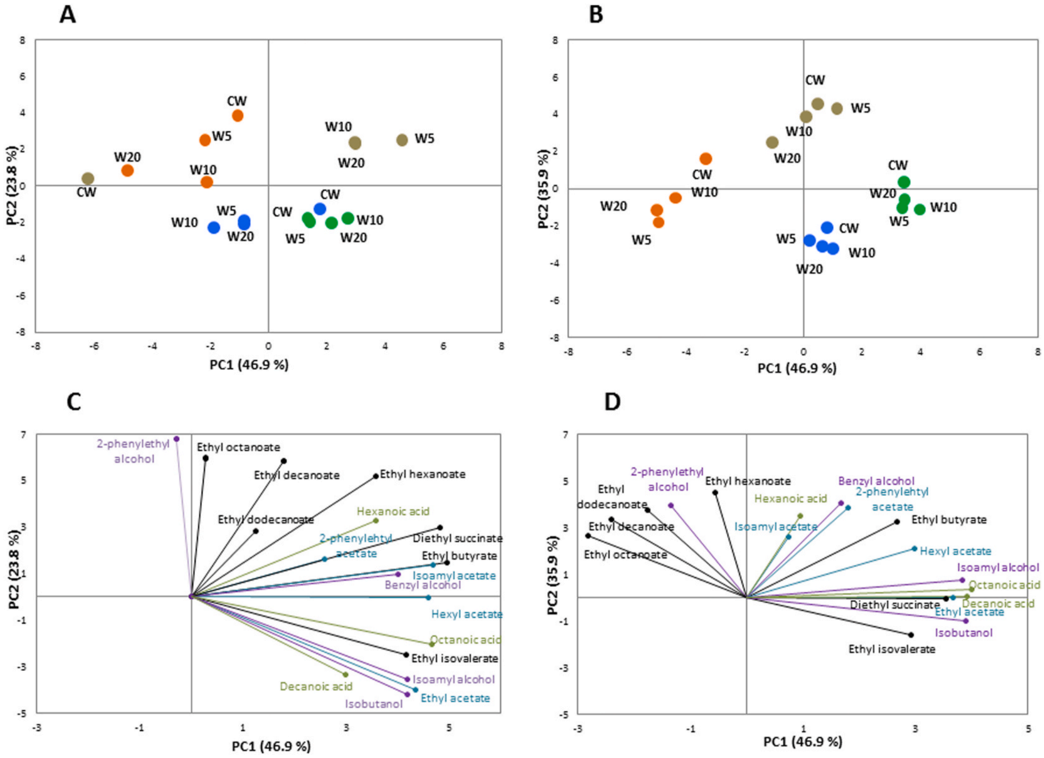

Fig. 4. Projection of the samples and loadings on the plane defined by the first and second principal components of the aromatic compounds obtained from the control wines (CW) and wines treated in different proportions (5%; W5, 10%; W10 and 20%; W20) with ion exchange resins for the studied vintages 2015 (A and C) and 2016 (B and D) at different aging times in oak barrels (zero months ( ), three months ( ), six months ( ) and twelve months ( ). *Samples (n = 2)*.

Table 4

Families of volatile compounds (μg/L) studied in control wines (CW) and in wines with addition of 5, 10 and 20% of resin treated wine (W5, W10, W20) at 0, 3, 6 and 12 months later from barrel aging, in both vintages (2015 and 2016).

2015	0 months						3 months					
	Control	5%	10%	20%	SDpooled		Control	5%	10%	20%	SDpooled	
Ethyl butyrate	69 aA	889 aC	85 aB	86 aC	±	17	59 aA	55 aA	51 aA	44 aA	±	7
Ethyl isovalerate	15 aA	20 aB	21 aB	23 aB	±	4	13 aA	12 aA	11 aA	10 aA	±	2
Ethyl hexanoate	1255 aA	1617 aC	1553 aB	1585 aA	±	299	1313 aA	1214 aB	1132 aA	963 aA	±	170
Ethyl octanoate	6571 aA	8673 aC	8419 aC	8797 aA	±	1726	8969 aA	8517 aBC	6782 aB	6275 aA	±	1394
Ethyl decanoate	2729 aA	3948 aC	3578 aB	3622 aA	±	928	4194 aA	3347 aB	2559 aAB	2260 aA	±	615
Ethyl dodecaonate	169 aA	227 aB	210 aA	187 aA	±	68	353 bA	205 aAB	140 aA	118 aA	±	33
Diethyl succinate	2576 aA	3210 aA	3081 aA	3098 aA	±	489	3173 aA	3087 aA	2837 aA	2753 aA	±	259
ESTERS	**13385 aA**	**17785 aC**	**16946 aB**	**17395 aA**	±	**3490**	**18074 aA**	**16437 aC**	**13512 aA**	**12422 aA**	**±**	**2329**
Ethyl acetate	3536 aA	4238 aB	4205 aAB	4298 aB	±	508	3711 aA	3529 aA	3450 aA	2994 aA	±	298
Isoamyl acetate	926 aA	1141 aD	1096 aB	1106 aA	±	205	779 aA	877 aB	513 aA	594 aA	±	215
Hexyl acetate	29 aA	38 aB	38 aBC	39 aC	±	7	27 aA	25 aA	27 aA	20 aA	±	3
2-phenylethyl acetate	117 aA	159 aC	151 aC	149 aB	±	34	139 aA	133 aB	109 aAB	107 aAB	±	13
ACETATES	**4609 aA**	**5577 aBC**	**5491 aBC**	**5592 aB**	±	**751**	**4656 aA**	**4565 aA**	**4099 aA**	**3716 aA**	**±**	**450**
Isoamyl alcohol	2932 aA	3689 aB	3514 aB	3595 aAB	±	578	2986 aA	2871 aA	2709 aA	2445 aA	±	273
Isobutanol	207 aA	287 aB	250 aB	240 aAB	±	72	139 aA	137 aA	141 aA	112 aA	±	19
Benzyl alcohol	8 aA	10 aC	10 aB	10 aA	±	2	8 aA	8 aAB	7 aA	7 aA	±	1
2-phenylethyl alcohol	2777 aA	3311 aB	3283 aA	3285 aB	±	436	3442 aA	3367 aB	3276 aA	3110 aAB	±	204
ALCOHOLS	**5923 aA**	**7298 aB**	**7058 aA**	**7130 aA**	±	**1066**	**6576 aA**	**6383 aA**	**6133 aA**	**5675 aA**	**±**	**488**
Hexanoic acid	91 aA	127 aB	120 aC	115 aA	±	27	112 bA	98 abA	76 aA	73 aA	±	9
Octanoic acid	141 aA	280 aA	191 aB	174 aAB	±	68	161 aA	160 aA	114 aA	115 aA	±	19
Decanoic acid	19 aA	35 aA	33 aB	27 aAB	±	11	28 aA	28 aA	16 aA	16 aA	±	10
FATTY ACIDS	**252 aA**	**442 aA**	**344 aB**	**317 aAB**	**±**	**97**	**301 aA**	**286 aA**	**206 aA**	**204 aA**	**±**	**35**
TOTAL AROMAS	**24169 aA**	**31102 aC**	**29839 aB**	**30434 aA**	±	**5373**	**29607 aA**	**27671 aB**	**23950 aA**	**22018 aA**	**±**	**3133**
2015	**6 months**						**12 months**					
	Control	**5%**	**10%**	**20%**	**SDpooled**		**Control**	**5%**	**10%**	**20%**	**SDpooled**	
Ethyl butyrate	70 aA	70 aB	73 aB	70 aBC	±	1	68 bA	60 abAB	57 aA	60 abAB	±	2
Ethyl isovalerate	17 aA	17 abB	19 bcB	21 cB	±	0	20 aA	20 aB	18 aB	23 aB	±	1
Ethyl hexanoate	1208 aA	1166 aAB	1209 aA	1101 aA	±	33	1205 aA	1065 aA	972 aA	1016 aA	±	68
Ethyl octanoate	4786 aA	4680 aA	4971 aA	4579 aA	±	230	7914 aA	7136 aB	6608 aB	6892 aA	±	**467**
Ethyl decanoate	2717 bA	2229 aA	2258 abA	1994 aA	±	119	3609 cA	2729 abA	2312 aA	2801 bA	±	118
Ethyl dodecaonate	282 bA	133 aA	117 aA	105 aA	±	17	351 bA	213 aB	142 aA	145 aA	±	21
Diethyl succinate	2601 aA	2928 abA	3152 bA	3218 bA	±	131	2960 aA	3006 aA	2952 aA	3186 aA	±	73
ESTERS	**11800 cA**	**11089 aA**	**11682 bcA**	**11224 abA**	**±**	**135**	**16128 bA**	**14230 abB**	**13061 aA**	**14124 abA**	**±**	**656**
Ethyl acetate	4409 aA	4548 aB	4937 aB	4832 aB	±	299	4523 aA	4382 aB	4148 aAB	4382 aB	±	101
Isoamyl acetate	1072 aA	1021 aC	1018 aB	957 aA	±	55	860 aA	712 aA	661 aA	674 aA	±	53
Hexyl acetate	34 aA	35 aB	45 bC	33 aBC	±	1	29 aA	26 aA	29 aAB	27 aBA	±	1
2-phenylethyl acetate	146 aA	145 aBC	140 aC	151 aB	±	5	106 aA	92 aA	90 aA	92 aA	±	8
ACETATES	**6140 aA**	**5974 aC**	**5661 aC**	**5749 aAB**	**±**	**336**	**5519 bA**	**5212 abB**	**4929 aAB**	**5176 abAB**	**±**	**121**
Isoamyl alcohol	3313 aA	3581 aB	3897 aB	3713 aB	±	152	4007 aA	3839 aB	3616 aB	3692 aB	±	243
Isobutanol	256 aA	270 abB	289 cB	274 bcB	±	4	319 aA	269 aB	257 aB	267 aAB	±	26
Benzyl alcohol	10 aA	10 aBC	11 aB	10 aA	±	1	7 aA	7 aA	7 aA	7 aA	±	1
2-phenylethyl alcohol	2414 aA	2667 abA	2912 bA	2941 bAB	±	116	2741 aA	2737 aA	2712 aA	2725 aA	±	48
ALCOHOLS	**7108 bA**	**6938 abAB**	**5993 aA**	**6528 abA**	**±**	**260**	**7075 aA**	**6853 aAB**	**6592 aA**	**6691 aA**	**±**	**294**
Hexanoic acid	95 aA	103 aAB	104 aBC	109 aA	±	5	106 aA	93 aA	89 aAB	90 aA	±	11
Octanoic acid	240 aA	228 aA	219 aB	242 aC	±	15	237 aA	183 aA	172 aAB	184 aBC	±	21
Decanoic acid	50 bA	38 abA	33 aB	38 abB	±	4	67 bA	39 aA	33 aB	36 aB	±	6
FATTY ACIDS	**357 aA**	**389 aA**	**385 aB**	**368 aAB**	**±**	**21**	**410 aA**	**315 aA**	**295 aAB**	**310 aAB**	**±**	**38**
TOTAL AROMAS	**25406 aA**	**24391 aA**	**23722 aAB**	**23869 aA**	**±**	**595**	**29131 bA**	**26611 abB**	**24876 aAB**	**26301 abA**	**±**	**938**
2016	0 months						3 months					
	Control	5%	10%	20%	SDpooled		Control	5%	10%	20%	SDpooled	
Ethyl butyrate	74 aA	73 aB	71 aA	70 aA	±	13	64 bA	48 aA	55 abA	53 abA	±	3
Ethyl isovalerate	8 aAB	9 aB	9 aB	10 aAB	±	2	6 aA	5 aA	6 aA	6 aA	±	0

(continued on next page)

Table 4 (*continued*)

2015	0 months						3 months					
	Control	5%	10%	20%		SDpooled	Control	5%	10%	20%		SDpooled
Ethyl hexanoate	17312 aB	1869 aC	1782 aC	1660 aB	±	161	1556 aAB	1292 aAB	1401 aB	1360 aAB	±	92
Ethyl octanoate	9505 aA	9808 aB	10189 aC	9980 aC	±	1028	9790 aA	8531 aAB	9662 aBC	9132 aC	±	872
Ethyl decanoate	4958 aA	5180 aA	5042 aB	4728 aB	±	598	4934 aA	4111 aA	4506 aAB	4190 aB	±	794
Ethyl dodecaonate	469 aA	482 aB	482 aB	417 aA	±	87	338 aA	302 aAB	340 aAB	289 aA	±	83
Diethyl succinate	1741 aB	2139 aB	1948 aB	1776 aAB	±	111	1260 aA	1231 aA	1212 aA	624 aA	±	365
ESTERS	**18486 aA**	**19562 aB**	**19524 aC**	**18641 aC**	**±**	**1907**	**17949 aA**	**15519 aAB**	**17182 aBC**	**15654 aBC**	**±**	**1766**
Ethyl acetate	2730 aAB	3532 aB	3241 aB	2832 aAB	±	250	2362 aA	2339 aA	2250 aA	2105 aA	±	156
Isoamyl acetate	1297 aA	670 aA	965 aB	1109 aA	±	175	1010 aA	679 aA	888 aB	836 aA	±	220
Hexyl acetate	26 aA	23 aAB	25 aA	25 aA	±	5	22 aA	17 aA	20 aB	19 aA	±	2
2-phenylethyl acetate	202 aB	146 aB	159 aC	167 aB	±	21	168 aAB	112 aAB	128 aB	134 aAB	±	7
ACETATES	**4255 aAB**	**4371 aBC**	**4389 aB**	**4132 aAB**	**±**	**424**	**3562 aA**	**3147 aA**	**3286 aA**	**3094 aA**	**±**	**219**
Isoamyl alcohol	3624 aA	3948 aC	3719 aB	3410 aAB	±	221	3372 bA	2861 aA	3007 abA	2918 abA	±	121
Isobutanol	218 aAB	243 aB	205 aB	181 aA	±	26	148 aA	134 aA	140 aA	143 aA	±	18
Benzyl alcohol	16 aC	19 aC	16 aC	13 aA	±	1	11 aA	10 aA	9 aA	9 aA	±	1
2-phenylethyl alcohol	3678 aAB	3929 aB	3728 aB	3598 aA	±	100	3762 aB	3491 aAB	3429 aAB	3438 aA	±	182
ALCOHOLS	**7537 aA**	**8139 aC**	**7670 aB**	**7202 aA**	**±**	**283**	**7293 aA**	**6495 aA**	**6586 aA**	**6510 aA**	**±**	**287**
Hexanoic acid	162 aA	175 aB	160 aB	139 aA	±	26	115 aA	95 aA	104 aA	99 aA	±	10
Octanoic acid	280 aAB	287 aAB	251 aB	211 aAB	±	62	171 aA	137 aA	145 aA	136 aA	±	15
Decanoic acid	64 aA	59 aA	49 aA	38 aAB	±	32	23 aA	17 aA	23 aA	17 aA	±	8
FATTY ACIDS	**506 aA**	**521 aA**	**460 aB**	**388 aAB**	**±**	**119**	**309 aA**	**249 aA**	**272 aA**	**252 aA**	**±**	**31**
TOTAL AROMAS	**30786 aA**	**32593 aB**	**32043 aB**	**30363 aA**	**±**	**2591**	**29114 aA**	**25411 aA**	**27326 aAB**	**25511 aA**	**±**	**1989**
2016	**6 months**						**12 months**					
	Control	**5%**	**10%**	**20%**	**SDpooled**		**Control**	**5%**	**10%**	**20%**	**SDpooled**	
Ethyl butyrate	69 aA	66 aAB	68 aA	72 aA	±	4	587 aA	60 aAB	59 aA	57 aA	±	1
Ethyl isovalerate	10 aAB	9 aB	10 abB	12 bB	±	1	112 aB	11 aB	14 aC	13 aB	±	1
Ethyl hexanoate	1369 aAB	1356 aB	1252 aAB	1226 aAB	±	70	1149 aA	1062 aA	1057 aA	1044 aA	±	27
Ethyl octanoate	6367 bA	6072 abA	5902 abA	5556 aA	±	142	8118 aA	7889 aAB	7832 aAB	7401 aB	±	379
Ethyl decanoate	3047 bA	3007 bA	2957 bA	2408 aA	±	130	3599 aA	3498 aA	3352 aAB	3411 aAB	±	151
Ethyl dodecaonate	209 aA	225 aA	210 aA	187 aA	±	15	244 aA	267 aAB	238 aA	230 aA	±	10
Diethyl succinate	1979 aB	210 cB	2095 bB	2040 bAB	±	85	1964 aB	2065 aB	2027 aB	2192 aB	±	70
ESTERS	**12839 aA**	**13051 aA**	**12495 aA**	**11502 aA**	**±**	**410**	**15145 aA**	**14853 aAB**	**14581 aAB**	**14349 aAB**	**±**	**447**
Ethyl acetate	3388 aB	3693 aB	3473 aB	3385 aB	±	190	2829 aAB	3416 bB	3094 abB	3228 bB	±	86
Isoamyl acetate	1324 cA	803 aA	913 abB	1113 bA	±	51	861 bA	460 aA	567 aA	519 aA	±	56
Hexyl acetate	29 bA	27 aB	26 aA	28 bA	±	2	22 aA	19 aAB	19 aA	20 aA	±	2
2-phenylethyl acetate	190 cB	140 aB	147 abBC	163 bAB	±	5	122 aA	93 aA	97 aA	107 aA	±	10
ACETATES	**4662 aB**	**4932 aC**	**4559 aB**	**4689 aB**	**±**	**242**	**3833 aAB**	**3988 aB**	**3779 aA**	**3875 aAB**	**±**	**128**
Isoamyl alcohol	3967 aA	4121 aC	4015 aB	3845 aB	±	176	3737 bA	3512 aB	3757 bB	3507 aAB	±	54
Isobutanol	276 aB	297 bC	283 bC	293 bB	±	11	271 aAB	238 aB	275 aC	246 aB	±	20
Benzyl alcohol	14 bB	14 bB	13 abB	12 aA	±	0	10 aA	11 aA	10 aA	10 aA	±	1
2-phenylethyl alcohol	3200 aAB	3245 aA	3160 aA	3114 aA	±	129	3068 aA	3235 aA	3158 aA	3304 aA	±	149
ALCOHOLS	**7678 aA**	**7457 aBC**	**7471 aB**	**7264 aA**	**±**	**308**	**7087 aA**	**6996 aAB**	**7200 aAB**	**7067 aA**	**±**	**195**
Hexanoic acid	124 aA	131 aAB	126 aAB	127 aA	±	5	117 aA	107 aA	103 aA	104 aA	±	6
Octanoic acid	328 aB	332 aB	339 aB	334 aB	±	20	255 aAB	234 aAB	259 aB	262 aAB	±	11
Decanoic acid	66 aA	65 aA	66 aA	72 aB	±	3	57 aA	55 aA	59 aA	57 aAB	±	7
FATTY ACIDS	**528 aA**	**518 aA**	**532 aB**	**533 aB**	**±**	**27**	**429 aA**	**396 aA**	**421 aAB**	**424 aAB**	**±**	**22**
TOTAL AROMAS	**25708 aA**	**25958 aA**	**25058 aA**	**23989 aA**	**±**	**965**	**26495 aA**	**26233 aAB**	**25981 aA**	**25716 aA**	**±**	**659**

Different lower case letters indicate significant differences by HSD Tukey test ($p \leq 0.05$) between treatments for each aging time. Different capital letters indicate significant differences according to the Tukey HSD test ($p \leq 0.05$) between the aging times for each of the treatments. *Samples* ($n = 2$).

control wines was 3.72 and 3.80, respectively (Fig. 1A and B). Before the aging period, the pH value in W20 decreased about 0.2 pH units (from 3.80 to 3.60 in 2016; and from 3.72 to 3.49 in 2015). W10 showed significant differences in both vintages, and W5 also showed significant differences in 2015. These patterns were maintained along the aging period. As far as the total acidity is concerned, before the aging period (0 months) no significant differences were observed between CW, W5 and W10. W20 was clearly higher in all cases. Differences between treated wines seemed to be higher after six and twelve months of aging (Fig. 1C and D).

The effect of resin treatment in the content of the organic acids is reported in Table 1. In general, no significant differences in organic acids concentration were observed between wine control and blends, as reported by other authors in young treated wines (Ibeas et al., 2015; Walker et al., 2004). However, a slight increment in the tartaric acid which took place during aging period in wines, that contained 20% of blend (W20), may be responsible one of the differences showed in total acidity. Significant differences in tartaric acid were found in W20 for 3, 6 and 12 months for 2015, and after 6 and 12 months for 2016, which likely contributed on the differences observed for the total acidity and promoting more acidic wines from a sensory point of view. This fact could be explained for the release of cations exchange and the subsequent formation of acid tartaric during the aging time. The low presence of malic acid (0.09–0.19 g/L) at the beginning (0 months) indicated that the malolactic fermentation was performed before the aging period. No obvious trends were found in acetic and lactic acid content. However, acetic acid showed significant differences in the 2016 vintage at the times of 3, 6 and 12 months, obtaining the highest acetic acid value after 3 months in all cases. The low content in acetic at the end of the study indicates a correct evolution of the wine during the aging period.

3.2. Effects of the cation-exchange treatment in the mineral composition

The effect of the resin treatment in minerals content of wines is reported in Table 2. It is well known that this is an efficient technique to remove K^+ in red wines (Ibeas et al., 2015). In this study, a reduction of more than 95% was observed between controls and treated wines. This decrease was from 1012.5 to 1164.5 to 25.0 mg/L (table S1) for 2015 and 2016 wines, respectively. In general, a decrease in minerals was observed when the percentage of treated wine increased. In the case of potassium, significant differences were observed at all studied times between CW and W20. In calcium, some significant differences were observed at the beginning of the study between control and blended wines, although these differences seemed to decrease after the aging period in oak (no significant differences were observed in wines after 3 months for 2015 and nor in 2016 for all times). These patterns may be explained by the precipitation of KHT and Ca_2T in the oak barrel during the aging period. A decrease in magnesium was also observed at the beginning of the study and was maintained during the aging period. The content in iron decreased at the beginning of the study (0 months) and this decrease seemed to be maintained in the wine of 2016 but not in that of 2015. The resins can adsorb metallic ions such as magnesium, iron, potassium, calcium, sodium and copper (Benítez, Castro, & Barroso, 2002). The reduction in iron could involve some phenolic oxidative stability since red wine oxidation is catalyzed by this metal (Danilewicz, 2018). In this study, copper showed little variations between treatments at different times. These results agree with the previous observations reported by Ibeas et al. (2015) where the used resin did not show any affinity to copper. Copper may have an important impact on the rate of oxygen consumption in wine (Kontoudakis & Clark, 2020). In 2016 wines, a significant reduction in potassium (−8.5%), magnesium (−20.0%), sodium (−16.2%) and copper (−12.5%) was observed in W20 at the end of the experiment (12 months). In 2015, this reduction was mainly observed in potassium (−13.4%) and magnesium (−17.6%).

Moreover, it could be outlined that in both vintages, the potassium and the magnesium content seemed to increase during the considered period until 12 months indicating transference of these metals to wine during aging, as was observed in other studies (Kaya, Bruno de Sousa, Curvelo-Garcia, Ricardo-da-Silva, & Catarino, 2017).

3.3. Effects of the cation-exchange treatment in tartrate stability

A red wine can be considered stable if the decrease in conductivity is lesser than 60 μS/cm as mentioned above. All wines in 2015 were generally quite stable (Fig. 2A) and all of them tend to the stability along time and were stable after 12 months of aging. In the same way, the saturation temperature picture (Fig. 2C) indicated that all samples were stable and also showed a slight trend of better stability throughout the aging months.

The control wine was much more unstable compared to the samples treated at all times.

At the beginning, wines with higher percentage of blend were clearly more stable. In 2016 wines (initially more unstable), the resin treatment had a great impact than in 2015 wines. After twelve months of aging, up to 50% of decrease was observed in the MC values. It was observed a clear pattern, being the treated wines more stable than controls, but no clear pattern was observed regarding the percentage of permeate wine added.

Therefore, in general, proton exchange technology treatment increased the stability towards tartaric acid precipitations in the tested red wines, according to the patterns observed by other authors (Ibeas et al., 2015; Lasanta et al., 2013), and seems to be more efficient in wines with higher instability. In other type of wines (Sherry wines), this technology also showed great results regarding the tartrate stability (Gomez Benítez, Palacios Macías, Sánchez Pazo, & Pérez Rodríguez, 2002).

3.4. Effects of the cation-exchange treatment in the phenolic composition and color

The influence of the treatment with cation-exchange resin in the phenolic composition and color characteristics are shown in Table 3. The resin treatment did not affect the stability of the tannins during the aging period. No significant differences were observed in the tannin concentration and TPI at the end of the study, and little differences were observed during the aging period in oak barrel. Other authors' stated slight decreases in the tannin content of red wines after a resin treatment, from 1 to 3% in wines with up to 25% of permeate wine (Ibeas et al., 2015; Lasanta et al., 2013). Significant differences were observed in the case of anthocyanins that decreased by increasing the percentage of the treated wine (about 522 *vs* 317 mg/L in 2015, and 934 *vs* 589 mg/L in 2016, Table 3). The high loss of anthocyanins may be due to pigment retention on the resin surface (Mira et al., 2006). Treated wines showed lower content of anthocyanin monoglucosides than untreated, especially in 2016 wines with a high content in these compounds (table S2). Acetates, coumarates and pyranoanthocyanins, more stable forms of anthocyanins (Sarni-Manchado, Fulcrand, Souquet, Cheynier, & Moutounet, 1996) were not affected by treatments. In this way, this strategy may be interesting in view of the color stability during the aging period. In general, significant higher a^* values and lower b^* values were obtained (Table 3). These differences were clearly observed at the beginning of the study and were lower as the aging period increased (Fig. 3). Wines blended with higher content of treated wine (W20) had a more purple or red-bluish color. This can be explained by the acidity of wines that was higher as the percentage of treated wine increased. The relative amount of each structural form of anthocyanins, which coexist in equilibrium, is a function of the pH of the medium (Brouillard, 1982, pp. 1–40). At an acidic pH, the predominant form is the protonated form (flavilium cation AH^+). This could also explain the mentioned higher retention by the resin surface of anthocyanins in the treated wines.

Among all treated wines, no clear effect in L^* could be observed and in general, changes were not significant. However, control wines showed significant higher values of lightness.

The color differences between treated and untreated wines were detectable by the human eye. In general, the human eye is able to discriminate between two colors when $\Delta E^*ab > 1.0$ CIELAB unit (Gonnet, 1998).

3.5. Effects of the cation-exchange treatment on the volatile composition

Four aromatic families; esters, acetates, alcohols and fatty acids, were evaluated to show the influence of the ion exchange treatment effect on the aromatic composition of wines during the aging period. In general, an aromatic loss in Tempranillo wines during the aging period in the barrel was observed by other authors, mainly in the esters family (Castro-Vázquez et al., 2011).

For this study, a total of 18 volatile compounds corresponding to 7 esters, 4 acetates, 4 alcohols and 3 fatty acids were analyzed. A PCA was carried out for each of the vintages studied in order to observe trends of the studied wines during their aging period (Fig. 4). The proportion of the explained variance, accumulated by the two main components (PC1 and PC2), was greater than 70% in the 2015 harvest and greater than 80% in 2016.

In general, wines were distributed in the same way in both vintages, forming four groups according to the aging time (0, 3, 6 and 12 months). The samples in time 0M and 3M were located in general, in the upper part of the PCA (except W5-3M, W10-3M and W20-3M 2016 that were located below of the PCA), leaving the 3M on the left side and the 0M on the right side of the PCA. The 0M was strongly associated with esters in general, in addition to certain alcohols such as 2-phenylethyl alcohol and benzyl alcohol, and with 2-phenylacetate and isoamyl acetate. This pattern indicates a possible tendency for wines after three months of aging to present less esters and alcohol than wines at 0M. Contrary to the 0M wines, 6M and 12M wines were located in the lower part of the PCA. The 6M wines were located on the positive side of PC1, mainly related to diethyl succinate and ethyl isovalerate, and with the octanoic and decanoic fatty acids.

Furthermore, to deepen, the effect of the different treatments on the wines was studied at each of the analysis times in both vintages. At the beginning (0 months), none of the compounds showed significant differences in any of the two vintages studied, indicating that blended wines with different proportions of treated wines (5, 10 and 20%) just after being treated by the resins did not suffer any aromatic loss with respect to the control wines.

After three months of aging, the treated wines W5, W10 and W20 kept the same aromatic composition as the control (CW). Only two compounds showed significant differences in each of the vintages. In the 2015 vintage, ethyl dodecanoate and hexanoic acid presented a lower concentration in the treated wines, with gradually lower concentrations as the percentages increase. In the 2016 vintage, the compounds with significant differences were ethyl butyrate and isoamyl alcohol, with the lowest concentration in W5. All these compounds are responsible for providing aromatic fruity and floral notes to the wines (Ferreira, López, & Cacho, 2000; Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004). However, they did not exceed their sensory limits (1500, 3000, 400 and 60.000 μg/L) in any of the wines studied. Table 4 .

After the six months of aging, it should be noted that five compounds presented higher concentrations in the treated wines compared to controls. Within esters, ethyl isovalerate and diethyl succinate reached higher concentrations as the percentage of treated wines increased in both vintages. In all wines, the concentrations exceeded their sensory limits, 3 and 1200 μg/L respectively. These compounds are characterized for having fruity aromatic descriptors, in cases of apple and melon, respectively. Regarding the family of acetates, hexyl acetate was the compound that stood out for having the highest concentration in W10 wine from the 2015 vintage, and for presenting the same concentration in W20 as in CW in the 2016 vintage. Although the sensory limit (670 μg/L) was not exceeded in any of the studied wines, this compound is characterized by providing aromas related to fruits, such as pear. From alcohols, isobutanol showed the highest concentrations in the treated wines in both vintages, and 2-phenylethyl alcohol also increased as the percentage of treated wines was added in 2015. Both compounds at high concentration produce characteristic floral aromas, particularly notes of roses as the last one (Genisheva, Graña, Oliveira, & Vilanova de la Torre, 2013). After 12 months of aging, the treated wines showed similar concentrations than controls in both vintages. As can be seen in the 2015 vintage, the total content of esters, acetates and alcohols have significant differences, presenting the lowest concentration of all of them in the treated wine at 10%. However, the wines treated at 5% and 20%; although they have slightly lower values than the control wine, did not show significant differences between them. Regarding the 2016 vintage, none of the total aromatic families present significant differences between treatments and the controls. In esters, there is a decreasing trend (from CW, W5, and W10 to W20). However, other important families, such as acetates, alcohols and fatty acids showed concentrations very similar in treated wines and controls. These results suggest that the ion exchange treatment did not negatively affect the aromatic profile of Tempranillo wines after a large aging period in oak barrel. Highlighting the wines treated at 20%, which did not show significant differences in their total aromatic composition after the aging period, indicating a correct aromatic conservation after aging.

4. Conclusions

In this study, Tempranillo wines blended with different percentages of wine previously treated by cation exchange resin, generally conserved their chemical and sensory properties during the aging period. In general, the treated wines maintained similar acidity, color, tannin content, mineral composition and aromatics than controls. Wines blended with 20% of permeate wine, showed lower pH, greater acidity and a greater purple coloration after the aging period. Furthermore, treated wines showed generally lower concentration of potassium and calcium ions which gave the wines a greater tartaric stability. Regarding the aromatic composition, although the treated wines presented similar concentrations to the controls at all times after six months of aging, W20 and W10 presented higher concentrations of some of the compounds responsible for the aromatic descriptors of fruit and flowers of the wines. Therefore, it could be said that wines aged in oak barrels with a previous cation exchange treatment conserve or even improve their physicochemical characteristics. Future studies should be made in order to evaluate sensory parameters and the effects of this technology after a long aging period in bottle.

Ethics declarations

Compliance with Ethics Requirements:

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

CRediT authorship contribution statement

A.M. Mislata: participated on the analysis of samples, manuscript redaction and edition. **M. Puxeu:** performed and designed the experiment. **E. Nart:** performed and designed the experiment. **S. de Lamo:** performed and designed the experiment, also contributed to obtain the funding resources. **R. Ferrer-Gallego:** participated on the sample analysis, redaction, revision and edition of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110669.

References

Alcalde-Eon, C., Escribano-Bailón, M. T., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2006). Changes in the detailed pigment composition of red wine during maturity and ageing: A comprehensive study. *Analytica Chimica Acta, 563*(1), 238–254.

Bdiri, M., Perreaultc, V., Mikhaylinc, S., Larcheta, C., Hellalb, F., Bazinetc, L., et al. (2020). Identification of phenolic compounds and their fouling mechanisms in ion exchange membranes used at an industrial scale for wine tartaric stabilization by electrodialysis. *Separation and Purification Technology, 233*, 115995.

Benítez, P., Castro, R., & Barroso, C. G. (2002). Removal of iron, copper and manganese from white wines through ion exchange techniques: Effects on their organoleptic characteristics and susceptibility to browning. *Analytica Chimica Acta, 458*(1), 197–202.

Bindon, K., Varela, C., Kennedy, J., Holt, H., & Herderich, M. (2013). Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry, 138*(2–3), 1696–1705.

Brouillard, R. (1982). Chapter 1-chemical structure of anthocyanins. In P. Markakis (Ed.), *Anthocyanins as food colors* (Academic Press).

Cabrita, M. J., Garcia, R., & Catarino, S. (2016). Recent developments in wine tartaric stabilization Recent Advances in Wine. *Stabilization and Conservation Technologies*, 49–63.

Castro-Vázquez, L., Alañón, M. E., Calvo, E., Cejudo, M. J., Díaz-Maroto, M. C., & Pérez-Coello, M. S. (2011). Volatile compounds as markers of ageing in Tempranillo red wines from La Mancha D.O. stored in oak wood barrels. *Journal of Chromatography A, 1218*(30), 4910–4917.

Commission Implementing Regulation (EU) No 144/2013 of 19 February 2013 (2013).

Commission Regulation (Ec) N 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (Ec) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions (2009).

Cosme, F., Vilela, A., & Jordão, A. M. (2017). The role of tartaric acid in grapes and wines. *Advances in Chemistry Research, 40*, 198–216.

Danilewicz, J. C. (2018). [Fe(III)]:[Fe(II)] ratio and redox status of red wines: Relation to so-called "reduction potential". *American Journal of Enology and Viticulture, 69*(2), 141.

El Rayess, Y., & Mietton-Peuchot, M. (2016). Membrane Technologies in wine industry: An overview. *Critical Reviews in Food Science and Nutrition, 56*(12), 2005–2020.

Ferreira, V., López, R., & Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture, 80*(11), 1659–1667.

Ferrer-Gallego, R., Hernández-Hierro, J. M., Rivas-Gonzalo, J. C., & Escribano-Bailón, M. T. (2012). Influence of climatic conditions on the phenolic composition of *Vitis vinifera* L. cv. *Graciano. Analytica Chimica Acta, 732*, 73–77.

Genisheva, Z., Graña, M., Oliveira, J. M., & Vilanova de la Torre, M.d. M. (2013). *Determination of odorants in varietal wines from international grape cultivars (Vitis vinifera) grown in NW Spain*.

Gomez Benítez, J., Palacios Macías, V., Sánchez Pazo, J., & Pérez Rodríguez, L. (2002). Industrial development of proton exchange for tartrate stabilization of sherry wines. *European Food Research and Technology, 214*(5), 418–422.

Gómez Benítez, J., Palacios Macías, V. M., Szekely Gorostiaga, P., Veas López, R., & Pérez Rodríguez, L. (2003). Comparison of electrodialysis and cold treatment on an industrial scale for tartrate stabilization of sherry wines. *Journal of Food Engineering, 58*(4), 373–378.

Gonnet, J. F. (1998). Colour effects of co-pigmentation of anthocyanins revisited - 1. A colorimetric definition using the CIELAB scale. *Food Chemistry, 63*(3), 409–415.

Ibeas, V., Correia, A. C., & Jordão, A. M. (2015). Wine tartrate stabilization by different levels of cation exchange resin treatments: Impact on chemical composition, phenolic profile and organoleptic properties of red wines. *Food Research International, 69*, 364–372.

Jones, G. V., & Davis, R. E. (2000). Climate influences on grapevine phenology, grape composition, and wine production and quality for bordeaux, France. *American Journal of Enology and Viticulture, 51*(3), 249–261.

Kaya, A. D., Bruno de Sousa, R., Curvelo-Garcia, A. S., Ricardo-da-Silva, J. M., & Catarino, S. (2017). Effect of wood aging on wine mineral composition and 87Sr/86Sr isotopic ratio. *Journal of Agricultural and Food Chemistry, 65*(23), 4766–4776.

Kontoudakis, N., & Clark, A. C. (2020). Sulfide-binding to Cu(II) in wine: Impact on oxygen consumption rates. *Food Chemistry, 316*, 126352.

Lasanta, C., Caro, I., & Pérez, L. (2013). The influence of cation exchange treatment on the final characteristics of red wines. *Food Chemistry, 138*(2–3), 1072–1078.

Lasanta, C., & Gómez, J. (2012). Tartrate stabilization of wines. *Trends in Food Science & Technology, 28*(1), 52–59.

Mira, H., Leite, P., Ricardo-da-Silva, J. M., & Curvelo-Garcia, A. S. (2006). Use of ion exchange resins for tartrate wine stabilization. *OENO One, 40*(4), 223–246.

Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International, 43*(7), 1844–1855.

Mislata, A. M., Puxeu, M., Tomás, E., Nart, E., & Ferrer-Gallego, R. (2020). Influence of the oxidation in the aromatic composition and sensory profile of Rioja red aged wines. *European Food Research and Technology, 246*, 1167–1181.

Oiv. (2016). *Compendium of international methods of wine and must analysis*.

Peinado, R. A., Moreno, J., Bueno, J. E., Moreno, J. A., & Mauricio, J. C. (2004). Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chemistry, 84*(4), 585–590.

Ponce, F., Mirabal-Gallardo, Y., Versari, A., & Laurie, V. F. (2018). The use of cation exchange resins in wines: Effects on pH, tartrate stability, and metal content. *Ciencia e Investigacian Agraria, 45*(1), 82–92.

Saint-Cricq, N., Vivas, N., & Glories, Y. (1998). Maturité phénolique: Definition et côntrole. [Phenolic maturity: Definition and control]. *Revue Français d'Oenologie, 173*, 22–25.

Sarni-Manchado, P., Fulcrand, H., Souquet, J. M., Cheynier, V., & Moutounet, M. (1996). Stability and color of unreported wine anthocyanin-derived pigments. *Journal of Food Science, 61*(5), 938–941.

Torrens, J., Riu-Aumatell, M., López-Tamames, E., & Buxaderas, S. (2004). Volatile compounds of red and white wines by headspace-solid-phase microextraction using different fibers. *Journal of Chromatographic Science, 42*(6), 310–316.

Vernhet, A., Dupre, K., Boulange-Petermann, L., Cheynier, V., Pellerin, P., & Moutounet, M. (1999). Composition of tartrate precipitates deposited on stainless steel tanks during the cold stabilization of wines. Part II. Red wines. *American Journal of Enology and Viticulture, 50*(4), 398–403.

Walker, T., Morris, J., Threlfall, R., & Main, G. (2004). Quality, sensory and cost comparison for pH reduction of Syrah wine using ion exchange or tartaric acid. *Journal of Food Quality, 27*(6), 483–496.

Waters, E. J., Pellerin, P., & Brillouet, J. M. (1994). A Saccharomyces mannoprotein that protects wine from protein haze. *Carbohydrate Polymers, 23*(3), 185–191.

CAPÍTULO 3

EMBOTELLADO Y ALMACENAMIENTO

Factores de interés para la conservación de la calidad del vino

Justificación y objetivo

La calidad de los vinos blancos y rosados embotellados disminuye progresivamente con el tiempo debido a varias reacciones químicas relacionadas con el envejecimiento y a una gran cantidad de factores que pueden acelerar este deterioro. Entre ellos, los más importantes son la temperatura, la humedad, el envasado, el tipo de cierre, el color de la botella y la exposición a la luz.

Es bien sabido que la exposición a la luz del vino embotellado provoca cambios de color y la aparición de olores indeseables ya que la luz UV-visible acelera el consumo de oxígeno, lo que afecta directamente a la composición del vino. Los aromas generados durante este proceso de deterioro son sobre todo compuestos azufrados volátiles los cuales son responsables de aportar a los vinos aromas desagradables descritos como olor a col, ajo, huevos podridos o gas. De entre las distintas longitudes de onda que conforman la radiación UV-visible, aquellas que se encuentran por debajo de 520 nm son las más perjudiciales para los vinos embotellados puesto que catalizan las reacciones fotoquímicas.

El efecto de la radiación luminosa sobre la calidad del vino es un tema que ha sido muy estudiado, pero prácticamente siempre utilizando lámparas UV-visible. La aparición de la tecnología LED ha supuesto un cambio importante en lo que a eficiencia lumínica se refiere ya que estas lámparas distribuyen mejor la luz, tienen una vida útil más larga y una mejor conversión energética, lo que se traduce en una fuente de energía lumínica más sostenible. Estas ventajas no han pasado desapercibidas para el sector enológico que ve en este tipo de energía una buena alternativa a las lámparas tradicionales.

Una manera de minimizar los efectos los efectos de la irradiación de la luz sobre los cambios en la composición volátil del vino es cambiar el color de las botellas de manera que la industria vitivinícola lo ha adaptado tanto para satisfacer a los consumidores como para preservar las características del vino. Actualmente se utiliza un amplio espectro de colores que van desde las tradicionales botellas transparentes hasta las botellas de azul ártico, hoja muerta, ámbar, verde oscuro, verde antiguo, pedernal o incluso de loza. Por tanto, para cada color de la botella se debe determinar qué longitudes de onda atraviesan el cristal y pueden influir en los procesos de fotodegradación.

Además del color del vidrio de la botella hay que tener en cuenta el tipo de cierre empleado y los diferentes materiales que se pueden utilizar (chapa corona, rosca, corcho sintético, corcho natural). Cada tipo de cierre puede presentar diferentes permeabilidades o velocidades de transferencia de oxígeno (OTR) al vino. Por lo tanto, dependiendo del tipo de cierre se pueden provocar cambios significativos en la composición química y sensorial de los vinos después de ciertos periodos de almacenamiento. Por último, también hay que tener en cuenta el efecto de la conservación y almacenamiento de los vinos tintos durante largos periodos de crianza en botella, siempre y cuando estos tengan suficiente capacidad de envejecimiento. Hay que tener en cuenta que a lo largo de la crianza se produce una evolución en el perfil aromático del vino, provocada principalmente por numerosas reacciones químicas que dan lugar a nuevos aromas. Las reacciones de oxidación son responsables de cambios importantes en la composición química de los vinos. Estas reacciones implican un aumento de la cantidad de ciertos compuestos, la formación de otros nuevos, y también la disminución de los aromas frescos y varietales, como los frutales y florales. Durante la etapa de envejecimiento el vino está expuesto en menor o mayor medida al oxígeno provocando un deterioro en el vino. Este deterioro oxidativo incluye la pérdida de los aromas fermentativos y la aparición de algunos compuestos oxidativos indeseables que a menudo producen efectos negativos sobre su calidad final. Sin embargo, la presencia de los compuestos oxidativos y sus descriptores sensoriales relacionados no siempre tiene que ser un indicador negativo del vino. Dependiendo del estilo de vino, la aparición de estos compuestos evolutivos le puede otorgar una mayor complejidad y calidad aromática.

En el capítulo 3 se estudiaron los factores externos que afectan al vino durante su periodo de conservación (crianza en botella y almacenamiento). Así como el efecto de la incidencia de la exposición lumínica, los factores relacionados con el color de botella y el tipo de cierre, también fueron evaluados.

Objetivos específicos

El objetivo del artículo 4 fue evaluar el uso de nuevas luces LED como alternativa a las lámparas para evitar el deterioro de los vinos embotellados. Este efecto se evaluó mediante la determinación de la fotodegradación de la riboflavina, la cisteína y la metionina, la formación de compuestos volátiles azufrados y los cambios de color a lo largo del tiempo de exposición a la luz.

El objetivo del artículo 5 fue estudiar el efecto de diferentes tipos de tapones y colores de botella en vinos Albariño sobre su composición aromática, color y calidad sensorial a lo largo de diferentes tiempos de almacenamiento con y sin exposición a la luz UV-visible.

El objetivo del artículo 6 fue estudiar la influencia de la permeabilidad del oxígeno a través del tapón de corcho y la dosis de sulfuroso en la calidad y composición aromática del Champagne.

El objetivo principal del artículo 7 fue estudiar el perfil aromático de vinos envejecidos de Tempranillo de alta calidad para profundizar en el conocimiento de su composición aromática y establecer posibles marcadores de evolución.

Publicaciones asociadas

- **Artículo 4***: Influence of different types of LEDs lights on the formation of volatile sulfur compounds in white and rosé wines.*
- **Artículo 5:** *Effect of light exposure, bottle color and type of closure on the aromatic composition and quality of Albariño wines.*
- **Artículo 6:** *Influence of the cork stoppers and sulphur dioxide dose in the final quality of Champagne wines*
- **Artículo 7:** *Influence of the oxidation in the aromatic composition and sensory profile of Rioja red aged wines*

Artículo 4

Influence of different types of LEDs lights on the formation of volatile sulfur compounds in white and rosé wines.

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Influence of different types of LEDs lights on the formation of volatile sulfur compounds in white and rosé wines

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Corresponding Author:	Raul FERRER GALLEGO VITEC Falset, SPAIN
First Author:	Ana María Mislata
Order of Authors:	Ana María Mislata
	Miquel Puxeu
	Montse Nadal
	Sergi de Lamo
	Montserrat Mestres
	Raul FERRER GALLEGO
Abstract:	The effect of LEDs light on the formation of volatile sulfur compounds (VSCs) and the final sensory quality of white and rosé wines was evaluated. Thus, different commercial wines were exposed for ten days to three types of lights. All wine samples were analyzed throughout the exposure period to determine the usual oenological parameters together with some other chemical characteristics (color evolution; riboflavin, cysteine and methionine photodegradation), VSC amounts and sensory characteristics. The results showed that the wines exposed to ultraviolet light suffered greater degradation of the aromatic precursors, mainly riboflavin, and had higher concentrations of VSCs. Regarding LED lights, these produced minimal degradative effects. So that we can consider this type of light as an alternative to reduce the economic impact that currently occurs due to the photodegradation of bottled wines.

Influence of different types of LEDs lights on the formation of volatile sulfur compounds in white and rosé wines

Authors: A.M. Mislata [1, 2], M. Puxeu[1], M. Nadal[1], S. de Lamo[1], M. Mestres[2] and R. Ferrer-Gallego[1]*

[1]VITEC- Centro Tecnológico del Vino, Ctra. Porrera Km.1, 43730 Falset (Tarragona), Spain
[2]Sensometria Instrumental (i-Sens), Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Tarragona, 43007, Spain

** Raúl Ferrer-Gallego e-mail: raul.ferrer@vitec.wine*

Abstract

The effect of LEDs light on the formation of volatile sulfur compounds (VSCs) and the final sensory quality of white and rosé wines was evaluated. Thus, different commercial wines were exposed for ten days to three types of lights. All wine samples were analyzed throughout the exposure period to determine the usual oenological parameters together with some other chemical characteristics (color evolution; riboflavin, cysteine and methionine photodegradation), VSC amounts and sensory characteristics. The results showed that the wines exposed to ultraviolet light suffered greater degradation of the aromatic precursors, mainly riboflavin, and had higher concentrations of VSCs. Regarding LED lights, these produced minimal degradative effects. So that we can consider this type of light as an alternative to reduce the economic impact that currently occurs due to the photodegradation of bottled wines.

Keywords: amino acids, wine, riboflavin, aroma, LED ligths, sensory analysis

1. Introduction

Sustainable development is currently one of the main goals of the European Union Commission as well power consumption has become one of the most considerable environmental issues. Light-Emitting Diodes (LED), ubiquitous in modern electronic devices and lighting, offer a high series of advantages, especially from a sustainability point of view. From an oenological standpoint, the recent emergence of high-power LEDs brings new opportunities to modernize wineries as this type of lighting offers an exceptional flux range, better light distribution efficiency, versatility, longer shelf life, and best power conversion, among others (Mills, 2004). Thus, the replacement of existing lighting in wineries with LED technology can generate significant savings of up 60% when an optimal control and automation system is used (Vela, 2017).

On the other hand, the effect of light radiation on the quality of wine is a recurring theme. There are studies from the 70s in which already it was observed that the quality of Champagnes sold in supermarkets was lower than those sold in specialized stores and that this was due to UV-visible radiation exposure (A. Maujean, Haye, M., Feuillat, M., 1978). Later it was found that was happening was a photochemical transformation that promoted an unwanted change in the wine properties which was called Goût de Lumière (D'Auria, Emanuele, Mauriello, & Racioppi, 2003) and it has been suggested that the most damaging wavelengths in bottled wines are those below 520 nm (Clark, Dias, Smith, Ghiggino, & Scollary, 2011; A. Maujean, Haye, M., Feuillat, M., 1978). This change occurs in color, odor and flavour and depends on several factors. The most important ones are; i) the chemical composition of wines, ii) the irradiation conditions, and iii) the exposure time (Grant-Preece, Barril, Schmidtke, Scollary, & Clark, 2017). Among the different compounds degradable by light, one of the most studied is riboflavin, which is generated in significant quantities during alcoholic fermentation (Mattivi, Monetti, Vrhovsek, Tonon, & Andrés-Lacueva, 2000; Santos, García-Ramírez, & Revuelta, 1995). This molecule has a chemical structure that absorbs radiation between 300 and 510 nm with maximum absorbance at 370 and 442 nm. Therefore, if wine is irradiated with fluorescent lamps that provides mercury emissions with maxima around 313, 365, 405, 436, 546 and 578 nm, and phosphor emissions with maxima round 480 and 580 nm (Spikes, 1981), this molecule will be irretrievably affected. However, new LED technology allows designing lights that minimize or even remove the emission of some wavelengths so it could minimize the risk of wine degradation.

Apart from riboflavin, many other molecules can be degraded due to light exposure. Some of them have been related to Fenton reaction which consumes free SO_2 and promotes the oxidation of some compounds such as tartaric acid to glyoxylic acid, a known precursor of the xanthylium cation pigments. The formation of this compound has been correlated with an increased level of browning, so these chemical degradations, induced by UV-visible radiation, usually imply loss of wine quality (Clark, 2008; Clark, Prenzler, & Scollary, 2007; George, Clark, Prenzler, & Scollary, 2006; Maury, Clark, & Scollary, 2010). In addition to these color changes, also UV-visible light exposure has been found to accelerate oxygen consumption. Thus, it directly affects the aromatic composition of wine (Grant-Preece, Barril, Schmidtke, Scollary, & Clark, 2017; Haye, 1977). The aromas generated under these conditions are mainly volatile sulfur compounds (VSCs) due to the photosensitivity of some sulfur-amino acids, such as methionine or cysteine (Daniela Fracassetti, Tirelli, Limbo, Mastro, Pellegrino, & Ragg, 2020; Kinzurik, Herbst-Johnstone, Gardner, & Fedrizzi, 2016). VSCs have a significant impact on the wine aroma as sulfides, disulfides and some thiols. They are the key contributors to some undesirable wine flavors described as rotten eggs, cabbage or cooked onions.

Currently, the photo-oxidation of wine is in the framework for wine researchers (D. Fracassetti, Di Canito, Bodon, Messina, Vigentini, Foschino, et al., 2021; Daniela Fracassetti, Tirelli, Limbo, Mastro, Pellegrino, & Ragg, 2020; Lan, Li, Yang, Li, Yuan, & Guo, 2021). However, little information regarding the influence of the currently used LEDs on the quality of the wine has been reported. This research aims to evaluate the use of new LED lights to avoid bottled wines spoilage by determining the photo-degradation of riboflavin, cysteine and methionine, the formation of VSCs and changes in color throughout the time of light exposure.

2. Material and Methods

2.1. Chemicals and reagents

Thiophene, 2-propanethiol and methyl ethyl sulfide were used as internal standards for the analysis of aromas. Hydrogen sulfide, methanol, ethanol, dimethyl sulfide, diethyl sulfide, dimethyl disulfide and diethyl disulfide were used as sulfur volatile standards. The other standards used were riboflavin, cysteine and methionine. All of them were supplied by Sigma-Aldrich (Darmstadt, Germany) with a purity ≥ 90%. HCl and HNO_3 solvents were of high quality and were supplied by Panreac (AppliChem, Barcelona, Spain).

2.2. Samples and experimental design

Six commercial white wines representative from Albariño variety (Rias Baixas Apellation of Origin) and three rosé wines from Garnacha variety (Rioja Apellation of Origin) from 2017 and 2018 chosen randomly were evaluated. All bottles of wine were subjected to direct light incidence by using three different types of lamps. Light A (LA) was achieved by using an ultraviolet light lamp similar to the current one used in wineries, warehouses and supermarkets. Light B (LB) as a type of LED which minimized the 400-450 nm radiation and light C (LC) as LED lamp that completely eliminated the radiation emitted into the 400-450nm region. To carry out the experiment, a black wooden box was designed into which the bottles were introduced in a horizontal position and the tested lamps were located in the upper part (Figure S1A and B). A box for each type of light was built and, during the experiment, the three boxes containing the wine samples were kept in darkness in a controlled temperature room (20 ± 2 °C). All controls were stored in darkness also in a controlled temperature room (20 ± 2 °C). The exposure was carried out for 10 days. Samples were taken at different analysis times (3, 6, 24, 48, 120 and 240 hours). All treatments were performed in duplicate.

2.3. Oenological parameters

Titratable acidity and pH were potentiometrically measured with an automatic titrator (TitroMatic Hach from Crison®) and turbidity with a HACH TL2310 turbidimeter. Free and total sulfur content, alcoholic degree and volatile acidity were determined by using an infrared spectroscopy (FTIR) system (WineScanTM by FOSS, Hilleroed, Denmark), internally calibrated according to OIV reference methods.

Colorimetric measurements were performed by using a Helios-α spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). CIELAB color parameters and color differences between samples in darkness and samples exposed to light ($\Delta E * ab = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$) were calculated.

2.4. Analysis of aromatic precursors by UPLC

2.4.1. Riboflavin

For the determination and quantification of riboflavin (vitamin B12) the method described by (Andrés-Lacueva, Mattivi, & Tonon, 1998) was followed with slight modifications. Thus, all wine samples were passed through a 0.2 µm syringe filter (Millipore Corporation, Bedford, MA, USA) and directly injected into the ultra-performance liquid chromatograph (UPLC 1260 Series, Agilent, Palo Alto, USA) with a fluorescence detector. The solvents used were: solvent A, 0.05 M NaH_2PO_4 buffer at pH 3.0 with H_3PO_4 and solvent B, acetonitrile. The calibration lines used for quantitation were carried out by preparing standard solutions under dim light, amber glass bottles and vials. The concentration range was from 5 to 200 µg/L. The different riboflavin standard solutions were prepared by diluting a concentrated solution (10.000 µg/L) with suitable amounts of a mixture of 20 % solvent B and 80 % solvent A. Then each one was filtered and injected into the UPLC. Both the standard and samples were analyzed in duplicate.

2.4.2. Amino Acids

Methionine and cysteine were determined following a procedure previously optimized and validated (Roda, Martín, Mislata, Castaño, Puxeu, & Ferrer-Gallego, 2019). Wine samples were filtered using a 0.2 µm Millex syringe filter (Millipore Corporation, Bedford, MA, USA) and subjected to automatic pre-column derivatization with *o*-phthaldialdehyde (OPA Reagent, Agilent) prior to the injection to the UPLC (Agilent UPLC 1260 Series (Palo Alto, USA) equipped with DAD detector and autosampler. The solvents used were, solvent A: 40 mM Na_2HPO_4 at pH 7.8, and solvent B: ACN: MeOH: water (45:45:10, v/v/v). Detection was performed at $\lambda = 338$ nm. The identification of the amino acids was carried out by comparing their retention times with those of the pure reference standards. Standard calibration curves were used to quantify amino acids, whose concentration ranges were from 0.1 to 5 mg/L for cysteine and from 1 to 50 mg/L for methionine. The different standard amino acid solutions were prepared by diluting a concentrated solution (1000 mg/L) in a synthetic wine solution (130 mL of pure ethanol with 5.5 g of tartaric acid in one liter of Milli-Q water). Then each was filtered and injected into the UPLC. Both the standard and the samples were run in duplicate. All samples for analysis were injected in duplicate (n = 2).

2.5. Analysis of aromatic sulfur compounds by GC-FPD

The analysis of aromas was carried out according to the method developed by M. Mestres et al. 1999 (Mestres, Martí, Busto, & Guasch, 1999), with some minor modifications. To avoid loss of volatile compounds during sample handling, the wines were refrigerated at -4 ºC for 24 hours in a cold room. Once a wine bottle was opened, the sample to be analyzed was prepared by using a dry ice bath under a stream of nitrogen gas to provide an inert atmosphere. Specifically, 10 ml of wine together with, 100 µL of 2-propanal to eliminate interference caused by the sulfur content of wine, and 100 µL of a mixture composed of 1000 µg/L of each internal standard were placed into a 20 mL glass vial which contained 2.7g of NaCl and 0.15g of ethylenediaminetetraacetic acid (EDTA).

The volatile sulfur compounds were extracted and concentrated using the headspace-solid phase microextraction (HS-SPME) technique with Carboxen/PDMS fibers of 85 µm. The samples were equilibrated for 20 min at 35ºC with shaking and, afterwards, the fiber was exposed to the headspace for 30 minutes at the same temperature in the

GC auto sampler. The fiber desorption was carried out into a TDU-thermal desorption unit coupled to a cold equipment, making a temperature ramp from -60 ºC to 260 ºC, which allowed the retention of extremely volatile sulfur compounds. After 120 seconds, the unit was heated up to 260 ºC and the sample was injected into a GC 7890A gas chromatograph (Agilent Technologies) equipped with a flame photometric detector (FPD) (G3348B, Agilent Technologies). The separation was carried out using an HP-1 column (30 m x 0.320 mm x 4.00 µm, Agilent), with helium as carrier gas at a flow rate of 2.09 mL/min. The oven temperature ramp was: 35 ºC to 260 ºC in 8 min. The detector temperature was 200 ºC and it was fed with 75 mL/min of hydrogen, and 86 mL/min of synthetic air.

The quantification was carried out by means of calibration curves of standards. With the use of the standards, the retention times of each of the compounds were identified and determined. The results of the volatile compounds were expressed as quantitative data expressed in µg/L as a response in consideration to the internal standard. All analyzes were done in duplicate.

2.6. Sensory analysis

The quantitative descriptive analysis (QDA) was performed by a tasting panel accredited by ISO 8586: 2010, and in a sensory standardization room (ISO 8589: 2007). Due to the pandemic situation arising from COVID 2019, the sensory panel was limited to four expert tasters.

The sensory analysis was carried out in 9 sessions of 3 blocks made up of 7 wines each session, where the panelists did not know the identification of the sample in each of the glasses, nor the type of light, nor the exposure time of the same.

Therefore, a total of 189 wines were tasted, grouped into 9 references by type of wine, and in turn by type of light and by the time they were exposed. The analysis was based on the olfactory phase. The aromatic descriptors considered were fresh fruit, candied/dried fruit, spicy, floral, pastry, evolution, reduction and 'light taste'. All the data obtained were processed with the FIZZ software (Biosystems, V.2.47B). And a specific tasting sheet was designed according to the aforementioned olfactory descriptors.

2.7. Statistical analysis

A simple analysis of variance (ANOVA) was carried out and the data were presented as the mean ± standard deviation. The Tukey procedure was used and differences in p values <0.05 were considered significant. StatGraphics Centurion XVI (Manugistics Inc., Pockville, MD, USA) was the program used.

3. Results

3.1. Oenological analysis

The oenological parameters of the different samples were determined in duplicate after 0, 3, 6, 24, 48, 120 and 240 hours of light exposure. Table 1 shows the mean values obtained for each sample at the beginning of the experiment (samples in darkness or 0 hours of exposure), and after 240 hours (10 days) exposed to the three types of light. As can be seen, the values found at the beginning of the experiment are comparable to those found after 10 days to light exposure, except when dealing with SO_2 and color. Wines presented values of total SO_2 lower than 100 mg/L avoiding problems related to greater wine reduction when working at high SO_2 amounts (Lopes, Silva, Pons, Tominaga, Lavigne, Saucier, et al., 2009; Ugliano, 2013). Some changes on the free and total SO_2 were found in samples exposed to lights. In general, free sulfur was more affected in samples exposed to LB after 10 days of exposure. According to other authors (Lan, Li, Yang, Li, Yuan, & Guo, 2021), the exposure to light accelerated the consumption of free SO_2 compared to controls (in darkness). Furthermore, UV irradiation resulted in a greater decrease in free SO_2 than white light after 160 days of storage. This fact raises the possibility of thinking that although after 10 days LB was the light that most affected free SO_2, it could be that if we increased this exposure time it was LA (UV light) that increased the loss of free sulfur in the wines.

Regarding color, in general the absorbance of the wines at 420 nm remained stable in the wines exposed to lights for 10 days compared to 0h. As Diaz et al 2021 (Díaz, Castro, Ubeda, Loyola, & Laurie, 2021) observed until the concentration of free SO_2 was not remarkably low, the absorbance at 420 nm of the wines remained without differences between the wines kept in the light and those kept in the dark. In the Cielab coordinates, it was mainly the *b** coordinate that presented the most significant differences in the wines exposed to LA after 10 days compared to 0h. Table 1 shows how the *b** values are lower in the wines exposed to LA (UV light), which indicates a greater loss of yellow color or a greater browning of the wines. As observed by Lan et al 2021 (Lan, Li, Yang, Li, Yuan, & Guo, 2021), the exposure to ultraviolet light affected the color parameters more compared to the control (in the dark). However, when calculating AE**ab*, all the results obtained were less than 1.0 CIELAB unit (table 1). However, the wines exposed to LA return values very close to 1 so that, if we consider the dispersion, they will exceed it, although not in all cases Therefore, it is probable that if the experiment was extended along time, color differences will be appeared and detected with the naked eye by the human eye in LA samples (Gonnet, 1998).

3.2. Analysis of aromatic precursors by UPLC

3.2.1. Riboflavin

The mean riboflavin values of the samples that remained in the dark (time 0h) presented concentrations between 138-182 µg/L in white wines, and between 152-178 µg/L in rosé wines (table S1). These values indicate that the studied wines presented high concentrations of riboflavin, since in all cases they exceeded 100 µg/L. Although the concentration values agree with others found in literature, these are above 100 µg/L so, according to previous studies, the sample wines are at risk of triggering the appearance of volatile sulfur compounds in wine (D. Fracassetti, Limbo, Pellegrino, & Tirelli, 2019; Mattivi, Monetti, Vrhovsek, Tonon, & Andrés-Lacueva, 2000). In general, rosé wines presented more abrupt degradation of riboflavin than white wines throughout the time of exposure to the lights. Furthermore, this greater degradation of riboflavin was observed in all the wines exposed mainly to light LA followed by LB (figure 1). However, it is worth noting the behavior of the wines with exposure to LC, because the riboflavin concentrations practically did not vary throughout the exposure time. This behavior is probably due to wavelengths in the 370 to 450 nm range. Wavelengths at which LA light emits and LC light completely removes, can probably excite riboflavin to the short-lived singlet state and then to the triplet state, which then participates in photo-oxidation reactions through various mechanisms (Grant-Preece, Barril, Schmidtke, Scollary, & Clark, 2017).

In general, as figure 1 shows, white wines (W1-W6) presented different degrees of affectation. After the last exposure time (240 hours) with LA, riboflavin losses of up to 44 % (W1 and W6) were observed. This decrease in riboflavin concentration occurred progressively over time of exposure. Even after only 24 hours of exposure, the wines showed losses of 17 and 13 % (W1 and W6, respectively). Regarding the values obtained in the samples exposed with LB light, in general, the wines showed a trend similar to that observed with LA light but with less degradation of riboflavin in all cases. With this light (LB), the wines reached a degradation of 35-38 % after the last exposure time (240h) (W1 and W6, respectively). Even after 48 hours of exposure, they presented losses of 11 and 16 %. In general, both lights, LA and LB, behaved similarly in the degradation of riboflavin in white wines. Even in the W4 and W5 wines in which there was hardly any degradation with any of the lights over time. The latter could be due to the green color of the bottle of these two wines, which unlike the rest of the wines had bottles of clear color. As already observed in other studies, the color of the bottle can be a crucial factor influencing the photodegradation of the wine, as different wavelengths of light will be transmitted depending on the color. Exposing wine to light at wavelengths close to 370 or 442 nm is particularly effective in inducing riboflavin degradation and the formation of volatile sulfur compounds, especially when using transparent glass bottles (Dias, Smith, Ghiggino, & Scollary, 2012; D. Fracassetti, Gabrielli, Encinas, Manara, Pellegrino, & Tirelli, 2017).

In the case of rosé wines, as shown in figure 1, the pronounced degradations produced with exposure to light LA over the time in all wines (W7-W9) stand out, reaching losses of up to 85 % (W8) after 10 days of exposure. Even after 24 hours of exposure, this decrease was already around 30 % of degradation. And only after 6 hours a loss of 10 % of riboflavin was produced. As for rosé wines exposed to LB light, they showed a similar trend to that observed with LA light, but with less degradation of riboflavin over time. After the last time of exposure, they showed degradation of around 45 %, half of the degradation produced with LA.

Finally, it should be emphasized again that in general none of the white and rosé wines exposed to LC light showed riboflavin degradation over time.

3.2.2. Methionine and Cysteine

Amino acids such as cysteine and methionine represent the most important form of total nitrogen in musts and wines. In addition to being the most important precursors of certain volatile sulfur compounds (Bekker, Wilkes, & Smith, 2018), due to their polyfunctional character, amino acids have great chemical reactivity compared to carbonyl compounds, in particular with sugars, according to the Maillard reaction (Marchand, De Revel, & Bertrand, 2000). This Strecker degradation of methionine and cysteine to aldehydes by α-dicarbonyl compounds formed during fermentation or oxidation contributes to the evolution of aroma in bottled wine (Ugliano, 2013).

The initial content of cysteine ranged between 0.35 and 1.79 mg/L in white wines and between 0.56 and 0.86 mg/L in rosé wines (table S2), values that are within the usual ones (Bekker, Wilkes, & Smith, 2018). As can be seen in figure 2, on the whole, the wines suffered decreases in cysteine after exposure to light, mainly with LA light, reaching decreases of around 30-45 % in white wines, and even up to 55 % in rosé wines (W9). In the case of exposure to LB light, these decreases in cysteine were slightly less than those produced with LA, reaching values of around 20-35% in white wines and up to 50 % in rosé wines (W9). However, the cysteine decreases of the samples with exposure to LC light were between 10-35%, up to three times less than the losses obtained with LA.

This degradation of the amino acid cysteine observed in all the wines studied may have as a consequence the formation of VSCs through Strecker degradation of sulfur amino acids, as already observed by Pripis et al 2000 (Pripi-Nicolau, De Revel, Bertrand, & Maujean, 2000). Cysteine can react with the α-dicarbonyl compound in wine to form hydrogen sulfide, methanethiol, and other volatile compounds (Marchand, De Revel, & Bertrand, 2000)

The initial concentrations of methionine in the wines ranged between 3.6 and 6.0 mg/L in white wines and between 4.8 and 8.1 mg/L in rosé wines (table S2), values similar to those found in literature (D. Fracassetti, Limbo, Pellegrino, & Tirelli, 2019; Grant-Preece, Barril, Schmidtke, Scollary, & Clark, 2017). Furthermore, the range of concentrations in wine was around between 1 and 37 mg/L (Bekker, Wilkes, & Smith, 2018). Figure 2 shows how in general, after 10 days of exposure, the wines exposed to LA light presented further degradation of methionine, reaching losses of around 10-30% in white wines and up to 40% in rosé wines. (W9). In the case of samples exposed to LB light, generally this decrease in methionine was slightly less than with LA, but decreases in methionine were achieved in the same range (around 10-30%). However, it should be noted that W1, W4, W5, W6, and W7 wines exposed to LC light, did not exceed 10% loss of methionine in the last exposure time.

These decreases in methionine may be due to the presence of riboflavin, which acts as a photosensitizer in its degradation. Fracassetti et al 2019 (D. Fracassetti, Limbo, Pellegrino, & Tirelli, 2019) observed that methionine proved to be stable in a hydroalcoholic acid solution without riboflavin since no degradation occurred in the model wine enriched with methionine (3 mg/L) and exposed to light for two hours. In contrast, the presence of riboflavin caused methionine degradation of up to 27%, depending on the initial concentrations of the two compounds. Furthermore, when riboflavin and methionine concentrations were high, then the amounts of degraded methionine increased as well, which demonstrated the influence of the methionine concentration on its own degradation.

3.3. Analysis of volatile sulfur composition

The analysis of volatile sulfur compounds (VSCs) was carried out as these can be generated by the degradation of riboflavin and the amino acids, methionine, and cysteine, later in post-bottling (Bekker, Day, Holt, Wilkes, & Smith, 2016; D. Fracassetti, Limbo, Pellegrino, & Tirelli, 2019). In total six compounds were detected and quantified, which were grouped in three aromatic families based on their chemical structures: thiols, sulfides and disulfides.

The thiol family consisted of hydrogen sulfide (H_2S), methanethiol (MeSH), and ethanethiol (EtSH). Although, as in other studies (Bekker, Day, Holt, Wilkes, & Smith, 2016; Siebert, Solomon, Pollnitz, & Jeffery, 2010), EtSH was not detected in any of the samples analyzed. Regarding H_2S and MeSH, these are the main compounds responsible for the formation of "reducing" aromas after bottling (Ugliano, 2013; Ugliano, Dieval, Siebert, Kwiatkowski, Aagaard, Vidal, et al., 2012; Ugliano, Kolouchova, & Henschke, 2011). As shown in figure 3, in general both compounds, increased their concentrations throughout the light exposure time but this increase was much higher when dealing with the exposure to light A, followed by light B, and to a lesser extent, light C.

Hydrogen sulfide is a characteristic compound for providing wines with unpleasant aromas of rotten eggs, decomposing algae, or wastewater when its concentration is higher than its odor threshold (OT) of 1.6 µg/L in vinica matrix (Siebert, Solomon, Pollnitz, & Jeffery, 2010). As can be seen in figure 3a, all the wines except for W4 exceeded the OT concentration of H_2S just after 6 hours of exposure with both LA and LB light. In the case of exposure to LC light, this sensory limit was not or slightly exceeded. In general, it should be noted that rosé wines (W7-W9) presented the highest concentrations of this compound after exposure to all lights. In general, the highest concentrations of H_2S were obtained in the wines exposed to LA. In some cases, these wines exposed to LA came to present twice the concentration of H_2S than that obtained with LB light, and even three times higher than that obtained with LC light.

After the last exposure time (240 hours) with light LA, in general, the samples continued to show an increase in the concentration of H_2S compared to the 6 hours of exposure. However, after 10 days (240 hours) of exposure to LB light, only rosé wines were affected. The same happened with LC light, which mainly affected rosé wines but to a lesser extent than with previous lights, being half the concentration obtained with LB.

Another of the thiols studied was methanethiol (MeSH). This compound is characteristic for providing wines with aromas related to descriptors such as putrefaction and cooked cabbage. In addition, it should be noted that this compound presents very low OT values (0.3 µg/L) which makes it play an important role in the perception of 'reducing' aromas in wine (D. Fracassetti & Vigentini, 2018; Nguyen

Dang-Dung, 2012). As can be seen in figure 3.b, only after 6 hours of exposure to LA light, all wines (except W4 and W6) exceeded the OT concentration of MeSH. However, only two white wines (W3 and W5) and two rosés (W8 and W9) exceeded the OT with exposure to LB light. And in the case of exposing the samples in LC, only two wines (W3 and W9) slightly exceeded the sensory limit. After the last analysis time (240 hours), the samples exposed to LA light increased their MeSH concentrations with respect to the previous time, mainly highlighting the large increase produced in W9 (5.5 µg / L), since it was practically the double that obtained after 6 hours. In the case of exposure to LB light, generally the wines that were already affected after 6 hours, followed an increase in concentration. And finally, in general, the wines exposed to LC light did not show great changes in the concentrations of MeSH throughout the exposure time.

As seen in other studies, cysteine and methionine are the main sources of H_2S and MeSH formation (Bekker, Wilkes, & Smith, 2018; Perpète, Duthoit, De Maeyer, Imray, Lawton, Stavropoulos, et al., 2006). But in addition, riboflavin plays a key role in the induction of methionine degradation (D. Fracassetti, Limbo, Pellegrino, & Tirelli, 2019). This fact could explain the relationship observed between the higher degradations of these amino acids (figure 2) and the higher concentrations of these aromatic compounds (figure 3) obtained in the studied wines and exposed to LA light, mainly in the case observed in W9.

Therefore, exposure to LA light favored the appearance of thiols in the samples, mainly in rosé wines. The LB light affected to a lesser extent than the LA, but emphasizing the increase of H_2S mainly in rosé wines. And especially, it should be noted that the LC light hardly affected the samples in the formation of thiols, except in rosé wines where the concentration of H_2S increased, although to a lesser extent compared to the other lights.

The next family studied was the sulfides. This family consisted of dimethyl sulfide (Me_2S) and diethyl sulfide (Et_2S) (figure 4). As shown in figure 4, and as was the case in the thiol family, in general, both compounds, Me_2S and Et_2S, increased their concentrations throughout the exposure time, in all wines. In general, this increase was much greater with the exposure of light LA, followed by light LB and to a lesser extent with light LC.

Dimethyl sulfide (Me_2S) is a compound that is associated with cabbage, asparagus, corn, or vegetable flavors when present in high concentrations, since it has an odor threshold of 25 µg/L (Mestres, Busto, & Guasch, 2000; Siebert, Solomon, Pollnitz, & Jeffery, 2010; Ullrich, Neef, & Schmarr, 2018). Factors that influence Me_2S formation in wines after bottling include grape variety, viticulture and winemaking processes, and wine pH value. These factors likely affect the Me_2S precursor compounds (Bekker, Day, Holt, Wilkes, & Smith, 2016; Escudero, Campo, Fariña, Cacho, & Ferreira, 2007; Ugliano, 2013). As shown in figure 4a, in general rosé wines and some white wines (W4 and W6) presented the highest concentrations of the compound Me_2S under the exposure of all the lights, presenting very high values after 6 hours of exposure to the light LA. Also, it should be noted that W9 was the only wine that exceeded the OT of this compound. However, in the particular case of the wines exposed to both LB and LC light, none of them exceeded the OT, at this time. After 10 days of exposure with LA light, all rosé wines exceeded the OT, in addition to white wine W6. In the case of exposure to LB light, on the whole, the wines behaved similarly to those exposed to LA light. However, it should be noted that none of the samples exposed to LC light presented values higher than OT for Me_2S.

As is already known, riboflavin and methionine can be precursors of Me_2S (D'Auria, Emanuele, Mauriello, & Racioppi, 2003; A. Maujean, Haye, Feuillat, Thomas, & Petit, 1978). Therefore, when observing figures 1 and 2, the highest degradations of riboflavin and methionine are obtained in samples from W6 to W9, which also coincide with the highest concentrations of Me_2S obtained (Figure 4a).

The second compound analyzed within the sulfide family was **diethyl sulfide**. This compound is characteristic because it has garlic as the main aromatic descriptor. In addition, it has a great reducing influence on wines since it has a very low sensory limit, OT 0.93 µg/L (Mestres, Busto, & Guasch, 2000; Siebert, Solomon, Pollnitz, & Jeffery, 2010; Ullrich, Neef, & Schmarr, 2018). In figure 4.b it can be seen how the white wine W2, and all the rosés (W7-W9), in general, presented the highest concentrations of Et_2S after being exposed to all the lights. After the 6-hour exposure time to LA light, three of the study wines (W2, W7 and W9) presented Et_2S values higher than their OT. In the case of exposure with the LB and LC lights, only two wines (W2 and W9) exceeded the OT value. After 10 days of exposure to LA light, in general, all the wines exceeded the OT of Et_2S. In the case of the exposure with the LB and LC lights, it was the rosé wines (W7-W9) and the W2 white wine, which exceeded the OT of Et_2S (with the exception of the W8 with LC). However, it should be noted that exposure of the wines to LC light over time did not dramatically increase the concentrations of the Et_2S compound.

Therefore, exposure to LA light favored the appearance of sulfides in the samples, mainly in rosé wines. The LB light affected less than the LA light. And the LC light, in general, did not favor the formation of sulfides in the wines over time.

And finally, there is the family of disulfides, in which the compounds dimethyl disulfide (Me_2S_2) and diethyl disulfide (Et_2S_2) were evaluated. As in the previous families (thiols and sulfides), both compounds were increasing their concentrations throughout the exposure time to the lights. In addition, generally this increase was greater with the exposure of light LA, followed by light LB and to a lesser extent with light LC.

Dimethyl disulfide is a characteristic compound for providing aromas related to cooked cabbage, sulfurous or onion, and for having a high odor threshold (29 µg/L) (Siebert et al 2010; Ullrich et al 2017). As shown in figure 5a, in general all wines (except W5) were affected by LA light, followed by LB, over time. After 6 hours of exposure to LA light, all wines exceeded the OT of Me_2S_2 (except W4 and W5). This could be due to the fact that the W4 and W5 wines had the green bottle color, which can act as a protector in the formation of the Me_2S_2 compound. However, with LB light exposure, only rosé wines (W7 and W8) exceeded OT. It should be noted that exposure to LC light, in general, did not affect the increase in the concentration of Me_2S_2, since none of the wines exceeded the OT. After the last analysis time (240 hours), all samples exceeded the sensory limit of Me_2S_2 with exposure to LA light. In the case of exposure to LB, the highest increases in Me_2S_2 occurred in the same wines as with exposure to LA, but with lower concentrations. And in the case of exposure with LC light, it should be noted that in general none of the wines exceeded the OT of Me_2S_2 (except W7, which slightly exceeded it).

Maujean and Seguin, 1983a, b (A. Maujean, Haye, Feuillat, Thomas, & Petit, 1978), observed that two MeSH molecules could produce dimethyl disulfide. This mechanism has been investigated in white wines that are responsible for an unpleasant aroma, generally called 'lumière'. In this study, when comparing the values obtained from the degradation of riboflavin (figure 1) and those obtained from the compound of Me_2S_2 (figure 5a), it can be observed that the wines with the highest degradation of riboflavin were those with the highest concentration of Me_2S_2, in whites wines W1, W3, W6 and in all rosé wines (W7-W9).

The second disulfide analyzed was diethyl disulfide (Et_2S_2). This compound gives wines aromas associated with bad smells or onion notes when concentrations are higher than 4.3 µg/L (OT) (Mestres, Busto, & Guasch, 2000; Siebert, Solomon, Pollnitz, & Jeffery, 2010; Ullrich, Neef, & Schmarr, 2018). As can be seen in figure 5.b, in general, the samples that presented higher concentrations were rosé wines and W2 white wine, with exposure to LA and followed by exposure to LB. However, it should be noted that exposure of the samples to LC light did not affect the increase in Et_2S_2. After 6 hours of exposure with LA, almost all wines (except W1, W4, and W5) exceeded the OT. In the case of exposure to LB, it was the same wines as those with LA that exceeded the OT of Et_2S_2, presenting similar concentrations. However, with exposure to LC none of the samples presented concentrations higher than OT. After 10 days of exposure to LA, all the wines presented values higher than OT (except W1). The same happened with the exposure to LB, where most of the wines exceeded the OT (except W1 and W3). However, it should be noted that in exposure to LC, none of the wines presented higher OT values (except W7).

Therefore, both exposures to LA light and LB light favored the appearance of disulfides in the samples, mainly after 10 exposure times. However, it should be noted that the LC light hardly affected the samples in the formation of both disulfides.

3.4. Sensory analysis

Finally, the chemical analysis of the volatile composition of the wines was completed by sensory analysis. The results of the sensory analysis seem to confirm the patterns observed in the chemical analysis of aromatic compounds (figure S2). In general, the wines showed a greater appearance of reduction and 'lumiere' aromas after the longest exposure time, mainly with exposure to LA light and LB light, although less intense with the latter (LB). However, the wines exposed to LC light showed hardly any reduction or ‘lumiere’ notes. In addition, in the analysis, it was observed how throughout the exposure time the fresh fruit and the floral notes, are decreasing, presenting a greater intensity of the same all the controls in general.

4. Conclusions

Our findings highlighted that both the degradation of methionine and to a greater extent the degradation of riboflavin resulted in the formation of high levels of VSCs. As expected, the photodegradation of riboflavin was greatly affected by the light emitted in the region of 400-450 nm (LA), reaching losses of up to 80 % after 10 days of exposure. However, it should be noted that wines exposed to new LEDs, eliminated by this wavelength region (LC), showed conservation of riboflavin concentration throughout the exposure time. Consequently, the wines exposed to LA presented higher concentrations of thiols, sulfides, and disulfides, exceeding the sensory limits in most cases. Wines with LB behaved similarly but presenting less intense riboflavin degradation than with LA, and the formation of VSCs but in lower concentrations than with LA. However, LC was the light with which the wines were preserved over time without presenting high concentrations of VSCs. Demonstrating that simply by eliminating this wavelength range (400-450), new LEDs sources ca completely avoid the appearance of unpleasant aromas in wines related to VSCs.

Figure captions

Figure 1. Riboflavin degradation in % of all wines (W1-W9) throughout the time of exposure (0-240 hours) to the different study lights (LA, LB and LC).

Figure 2. Degradation of methionine and cysteine in percentage of all wines (W1-W9) throughout the exposure time (0-6-240 hours) at different LEDs lights (LA, LB and LC).

Figure 3. Aromatic family of thiols, hydrogen sulfide (a) and methanethiol (b) concentrations (µg/L) obtained in all wines (W1-W9) throughout the exposure time (0-6-240 hours) with each of the study lights (LA, LB and LC). Samples (n = 2).

Figure 4. Aromatic family of sulfides, dimethyl sulfide (a) and diethyl sulfide (b) concentrations (µg/L) obtained in all wines (W1-W9) throughout the exposure time (0-6-240 hours) with each of the study lights (LA, LB and LC). Samples (n = 2).

Figure 5. Aromatic family of disulfides, dimethyl disulfide (a) and diethyl disulfide (b) concentrations (µg/L) obtained in all wines (W1-W9) throughout the exposure time (0-6-240 hours) with each of the study lights (LA, LB and LC). Samples (n = 2).

Figure S1. a) Experimental design of the black wooden box where the wines were exposed to the three study lights. b) Spotlights which were located in the upper part of the black box. c) Wine bottles exposed to light, and protected with aluminum foil for protection until analysis.

Figure S2. Aromatic profiles of W1-W9 wines obtained with exposure to the three types of lights (LA, LB and LC) throughout the exposure time (0 - 240 hours). Results obtained by the mean of the scores given by the tasters.

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References

Andrés-Lacueva, C., Mattivi, F., & Tonon, D. (1998). Determination of riboflavin, flavin mononucleotide and flavin–adenine dinucleotide in wine and other beverages by high-performance liquid chromatography with fluorescence detection. *Journal of Chromatography A, 823*(1), 355-363.

Bekker, M. Z., Day, M. P., Holt, H., Wilkes, E., & Smith, P. A. (2016). Effect of oxygen exposure during fermentation on volatile sulfur compounds in Shiraz wine and a comparison of strategies for remediation of reductive character. *Australian Journal of Grape and Wine Research, 22*(1), 24-35.

Bekker, M. Z., Wilkes, E. N., & Smith, P. A. (2018). Evaluation of putative precursors of key 'reductive' compounds in wines post-bottling. *Food Chem, 245*, 676-686.

Clark, A. C. (2008). The production of yellow pigments from (+)-catechin and dihydroxyfumaric acid in a model wine system. *European food research and technology = Zeitschrift fur Lebensmittel-Untersuchung und -Forschung. A, 226*(4), 925-931.

Clark, A. C., Prenzler, P. D., & Scollary, G. R. (2007). Impact of the condition of storage of tartaric acid solutions on the production and stability of glyoxylic acid. *Food Chem, 102*(3), 905-916.

D'Auria, M., Emanuele, L., Mauriello, G., & Racioppi, R. (2003). On the origin of "Goût de Lumiere" in champagne. *Journal of Photochemistry and Photobiology A: Chemistry, 158*(1), 21-26.

Dias, D. A., Smith, T. A., Ghiggino, K. P., & Scollary, G. R. (2012). The role of light, temperature and wine bottle colour on pigment enhancement in white wine. *Food Chem, 135*(4), 2934-2941.

Díaz, I., Castro, R. I., Ubeda, C., Loyola, R., & Laurie, V. F. (2021). Combined effects of sulfur dioxide, glutathione and light exposure on the conservation of bottled Sauvignon blanc. *Food Chem, 356*.

Escudero, A., Campo, E., Fariña, L., Cacho, J., & Ferreira, V. (2007). Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *Journal of Agricultural and Food Chemistry, 55*(11), 4501-4510.

Fracassetti, D., Di Canito, A., Bodon, R., Messina, N., Vigentini, I., Foschino, R., & Tirelli, A. (2021). Light-struck taste in white wine: Reaction mechanisms, preventive strategies and future perspectives to preserve wine quality. *Trends in Food Science and Technology, 112*, 547-558.

Fracassetti, D., Gabrielli, M., Encinas, J., Manara, M., Pellegrino, I., & Tirelli, A. (2017). Approaches to prevent the light-struck taste in white wine. *Australian Journal of Grape and Wine Research, 23*(3), 329-333.

Fracassetti, D., Limbo, S., Pellegrino, L., & Tirelli, A. (2019). Light-induced reactions of methionine and riboflavin in model wine: Effects of hydrolysable tannins and sulfur dioxide. *Food Chem, 298*.

Fracassetti, D., Tirelli, A., Limbo, S., Mastro, M., Pellegrino, L., & Ragg, E. M. (2020). Investigating the Role of Antioxidant Compounds in Riboflavin-Mediated Photo-Oxidation of Methionine: A 1H-NMR Approach. *ACS Omega, 5*(40), 26220-26229.

Fracassetti, D., & Vigentini, I. (2018). Occurrence and Analysis of Sulfur Compounds in Wine. In *Grapes and Wines - Advances in Production, Processing, Analysis and Valorization*, (pp. 225-251): INTECH, Open Access book.

George, N., Clark, A. C., Prenzler, P. D., & Scollary, G. R. (2006). Factors influencing the production and stability of xanthylium cation pigments in a model white wine system. *Australian Journal of Grape and Wine Research, 12*(1), 57-68.

Gonnet, J. F. (1998). Colour effects of co-pigmentation of anthocyanins revisited - 1. A colorimetric definition using the CIELAB scale. *Food Chem, 63*(3), 409-415.

Grant-Preece, P., Barril, C., Schmidtke, L. M., Scollary, G. R., & Clark, A. C. (2017). Light-induced changes in bottled white wine and underlying photochemical mechanisms. *Critical Reviews in Food Science and Nutrition, 57*(4), 743-754.

Haye, B., Maujean, A., Jacquemin, C., Feuillat, M. (1977). Contribution a l'étude des "Gouts de lumière" dans le vin de Champagne. I.- Asepects analytiques - dosage des mercaptans et des thiols dans les vins.-. *Connaissance Vigne et Vin, 11*(3), 243-254.

Kinzurik, M. I., Herbst-Johnstone, M., Gardner, R. C., & Fedrizzi, B. (2016). Hydrogen sulfide production during yeast fermentation causes the accumulation of ethanethiol, S-ethyl thioacetate and diethyl disulfide. *Food Chem, 209*, 341-347.

Lan, H., Li, S., Yang, J., Li, J., Yuan, C., & Guo, A. (2021). Effects of light exposure on chemical and sensory properties of storing Meili Rosé wine in colored bottles. *Food Chem, 345*, 128855.

Lopes, P., Silva, M. A., Pons, A., Tominaga, T., Lavigne, V., Saucier, C., Darriet, P., Teissedre, P. L., & Dubourdieu, D. (2009). Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon Blanc wine during bottle storage. *J Agric Food Chem, 57*(21), 10261-10270.

Marchand, S., De Revel, G., & Bertrand, A. (2000). Approaches to wine aroma: Release of aroma compounds from reactions between cysteine and carbonyl compounds in wine. *Journal of Agricultural and Food Chemistry, 48*(10), 4890-4895.

Mattivi, F., Monetti, A., Vrhovsek, U., Tonon, D., & Andrés-Lacueva, C. (2000). High-performance liquid chromatographic determination of the riboflavin concentration in white wines for predicting their resistance to light. *J Chromatogr A, 888*(1-2), 121-127.

Maujean, A., Haye, M., Feuillat, M., Thomas, J. C., & Petit, D. (1978). Contribution à l'étude des « goûts de lumière » dans le vin de champagne. II. Influence de la lumière sur le potentiel d'oxydoreduction. Corrélation avec la teneur en thiols du vin. *OENO One, 12*(4), 277-290.

Maujean, A., Haye, M., Feuillat, M. (1978). Contribution a l'étude des "Gouts de lumière" dans le vin de Champagne. II.- Influence de la lumière sur le potentiel d'oxydoreduction. Correlation avec la teneur en thiols du vin. *Connaissance Vigne et Vin, 12*(4), 277-290.

Maury, C., Clark, A. C., & Scollary, G. R. (2010). Determination of the impact of bottle colour and phenolic concentration on pigment development in white wine stored under external conditions. *Anal Chim Acta, 660*(1-2), 81-86.

Mestres, M., Busto, O., & Guasch, J. (2000). Analysis of organic sulfur compounds in wine aroma. *Journal of Chromatography A, 881*(1-2), 569-581.

Mestres, M., Martí, M. P., Busto, O., & Guasch, J. (1999). Simultaneous analysis of thiols, sulphides and disulphides in wine aroma by headspace solid-phase microextraction-gas chromatography. *Journal of Chromatography A, 849*(1), 293-297.

Mills, A. (2004). Lighting: The progress & promise of LEDs. *III-Vs Review, 17*(4), 39-41.

Nguyen Dang-Dung, N. L. a. A. K. P. (2012). Application of an Automated Headspace Solid Phase Micro-Extraction for the GC-MS Detection and Quantification of Reductive Sulfur Compounds in Wines. In D. B. Salih (Ed.), *Gas Chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications*): InTech.

Perpète, P., Duthoit, O., De Maeyer, S., Imray, L., Lawton, A. I., Stavropoulos, K. E., Gitonga, V. W., Hewlins, M. J., & Dickinson, J. R. (2006). Methionine catabolism in Saccharomyces cerevisiae. *FEMS Yeast Res, 6*(1), 48-56.

Pripi-Nicolau, L., De Revel, G., Bertrand, A., & Maujean, A. (2000). Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. *Journal of Agricultural and Food Chemistry, 48*(9), 3761-3766.

Roda, R., Martín, L., Mislata, A. M., Castaño, F. J., Puxeu, M., & Ferrer-Gallego, R. (2019). Effects of fertigation by elicitors enriched in amino acids from vegetal and animal origins on Syrah plant gas exchange and grape quality. *Food Research International, 125*.

Santos, M. A., García-Ramírez, J. J., & Revuelta, J. L. (1995). Riboflavin biosynthesis in Saccharomyces cerevisiae. Cloning, characterization, and expression of the RIB5 gene encoding riboflavin synthase. *J Biol Chem, 270*(1), 437-444.

Siebert, T. E., Solomon, M. R., Pollnitz, A. P., & Jeffery, D. W. (2010). Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection. *Journal of Agricultural and Food Chemistry, 58*(17), 9454-9462.

Spikes, J. D. (1981). Photodegradation of Foods and Beverages. In K. C. Smith (Ed.), *Photochemical and Photobiological Reviews: Volume 6*, (pp. 39-85). Boston, MA: Springer US.

Ugliano, M. (2013). Oxygen contribution to wine aroma evolution during bottle aging. *Journal of Agricultural and Food Chemistry, 61*(26), 6125-6136.

Ugliano, M., Dieval, J. B., Siebert, T. E., Kwiatkowski, M., Aagaard, O., Vidal, S., & Waters, E. J. (2012). Oxygen consumption and development of volatile sulfur compounds during bottle aging of two Shiraz wines. influence of pre- and postbottling controlled oxygen exposure. *Journal of Agricultural and Food Chemistry, 60*(35), 8561-8570.

Ugliano, M., Kolouchova, R., & Henschke, P. A. (2011). Occurrence of hydrogen sulfide in wine and in fermentation: Influence of yeast strain and supplementation of yeast available nitrogen. *Journal of Industrial Microbiology and Biotechnology, 38*(3), 423-429.

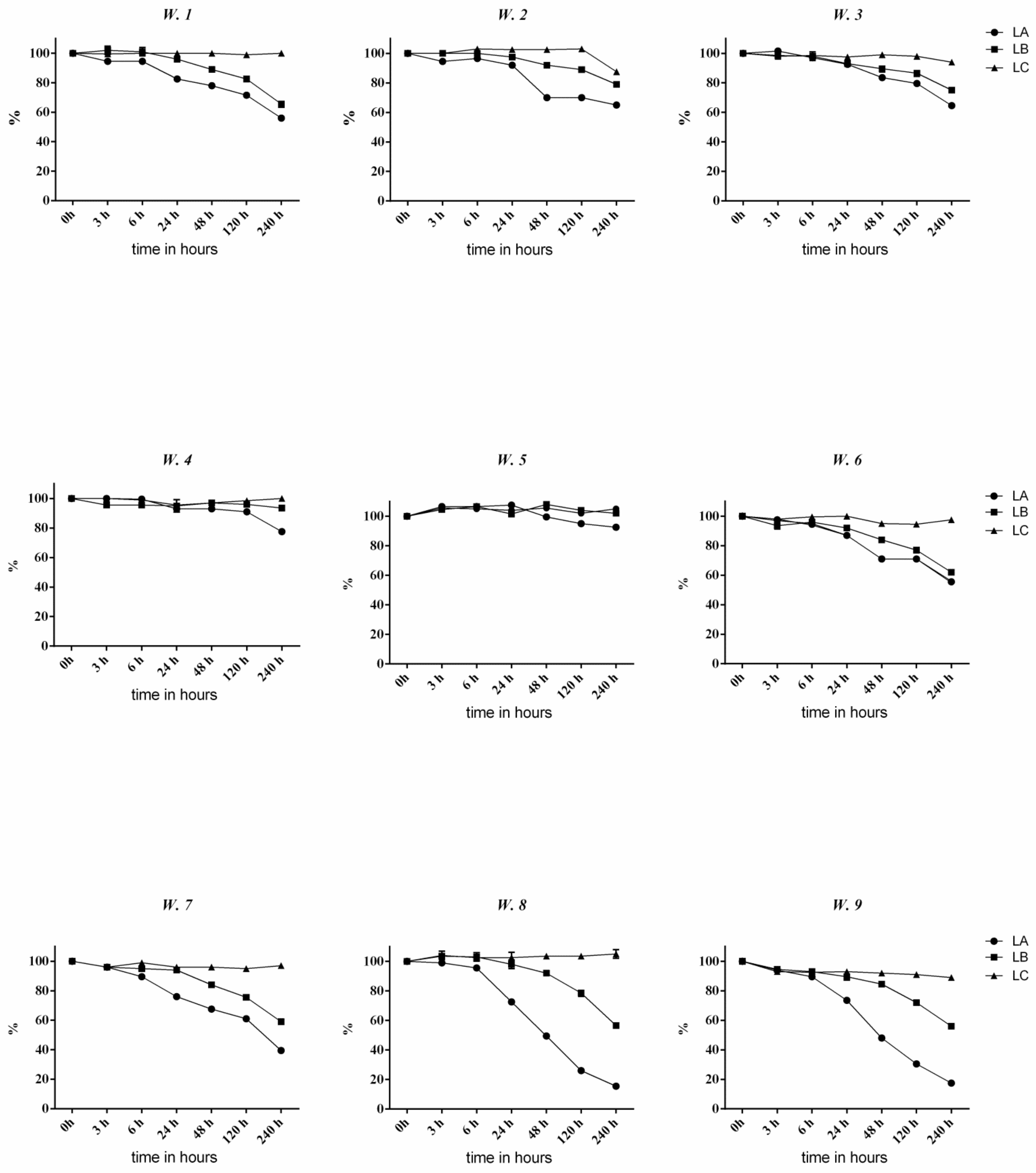
Ullrich, S., Neef, S. K., & Schmarr, H. G. (2018). Headspace solid-phase microextraction and gas chromatographic analysis of low-molecular-weight sulfur volatiles with pulsed flame photometric detection and quantification by a stable isotope dilution assay. *Journal of Separation Science, 41*(4), 899-909.

Vela, R., Mazarrón, F.R., Fuentes-Pila, J., Baptista, F., Silva, L.L., García, J.L. (2017). Improved energy efficiency in wineries using data from audits. *Ciência Téc. Vitiv., 32*(1), 62-71.

Table 1. Basic analysis of all wines (W1-W9), without exposure to light (time 0 hours) and after the last exposure time (240 hours) with each of the study lights (LA, LB and LC). The asterisk (*) indicates significant differences in the samples with exposure to each of the lights (240 hours) compared to the samples in darkness (0 hours). Free sulfur (SO_2 F, mg/L), total sulfur (SO_2 T, mg/L), total acidity (TA, g/L expressed as tartaric acidity), alcoholic strength (AG, %vol), and volatile acidity (VA, g/L). Luminosity (*L*), *a* and *b* coordinates CIELAB. Samples (n = 2).

	SO2 F	**SO2 T**	**pH**	**TA**	**AG**	**VA**	**420nm**	***L***	***a***	***b***	***AE ab***
W1 0h	18 ± 0.0	95 ± 0.7	3.31 ± 0.0	6.3 ± 0.1	12.6 ± 0.0	0.4 ± 0.0	0.085 ± 0.0	99.1 ± 0.1	(-1.1) ± 0.0	6.6 ± 0.0	-
W2 0h	16 ± 0.7	96 ± 0.7	3.25 ± 0.0	6.3 ± 0.0	12.5 ± 0.0	0.4 ± 0.0	0.101 ± 0.0	98.2 ± 0.1	(-0.5) ± 0.1	6.7 ± 0.1	-
W3 0h	11 ± 0.0	82 ± 0.7	3.28 ± 0.0	6.1 ± 0.1	12.8 ± 0.0	0.5 ± 0.0	0.086 ± 0.0	98.7 ± 0.1	(-0.9) ± 0.0	5.7 ± 0.3	-
W4 0h	15 ± 0.7	89 ± 0.7	3.25 ± 0.0	6.5 ± 0.0	12.4 ± 0.0	0.4 ± 0.0	0.106 ± 0.0	98.2 ± 0.1	(-1.2) ± 0.0	7.5 ± 0.1	-
W5 0h	17 ± 0.0	91 ± 0.7	3.30 ± 0.0	5.9 ± 0.1	12.6 ± 0.0	0.4 ± 0.0	0.085 ± 0.0	98.7 ± 0.1	(-0.8) ± 0.0	6.1 ± 0.1	-
W6 0h	18 ± 0.0	79 ± 0.7	3.08 ± 0.0	6.1 ± 0.0	12.6 ± 0.0	0.4 ± 0.0	0.112 ± 0.0	97.0 ± 0.1	0.9 ± 0.0	6.9 ± 0.0	-
W7 0h	11 ± 0.7	60 ± 0.0	3.11 ± 0.0	5.5 ± 0.1	12.4 ± 0.0	0.3 ± 0.0	0.117 ± 0.0	96.7 ± 0.1	1.0 ± 0.0	7.9 ± 0.1	-
W8 0h	19 ± 1.4	81 ± 0.7	3.10 ± 0.0	5.9 ± 0.1	12.6 ± 0.0	0.3 ± 0.0	0.111 ± 0.0	96.9 ± 0.1	1.8 ± 0.0	7.5 ± 0.1	-
W9 0h	20 ± 1.4	87 ± 0.0	3.10 ± 0.0	6.0 ± 0.1	12.8 ± 0.0	0.3 ± 0.0	0.111 ± 0.0	97.0 ± 0.1	1.6 ± 0.0	7.4 ± 0.1	-
W1 LA 240h	18 ± 1.4	96 ± 4.2	3.31 ± 0.0	6.4 ± 0.0	12.6 ± 0.0	0.4 ± 0.0	0.082 ± 0.0	99.0 ± 0.2	(-1.1) ± 0.1	6.0 ± 0.3	0.6 ± 0.3
W2 LA 240h	20 ± 0.0	100 ± 0.7	3.25 ± 0.0	6.4 ± 0.1	12.5 ± 0.0	0.4 ± 0.0	0.090 ± 0.0	98.6 ± 0.1	(-1.1) ± 0.1 *	6.0 ± 0.1 *	1.0 ± 0.3
W3 LA 240h	12 ± 2.1	86 ± 2.1	3.29 ± 0.0	6.1 ± 0.0	12.8 ± 0.0	0.4 ± 0.0	0.079 ± 0.0 *	98.6 ± 0.2	(-1.0) ± 0.3	5.6 ± 0.1	0.2 ± 0.2
W4 LA 240h	14 ± 0.0 *	86 ± 1.4	3.25 ± 0.0	6.5 ± 0.1	12.4 ± 0.0	0.4 ± 0.0	0.103 ± 0.0	98.4 ± 0.1	(-1.1) ± 0.0	7.2 ± 0.0	0.4 ± 0.2
W5 LA 240h	18 ± 0.7	93 ± 0.7	3.29 ± 0.0	5.8 ± 0.0	12.6 ± 0.0	0.4 ± 0.0	0.084 ± 0.0	98.5 ± 0.1	(-1.0) ± 0.1	5.8 ± 0.0 *	0.4 ± 0.3
W6 LA 240h	18 ± 0.0	77 ± 1.4	3.08 ± 0.0	6.1 ± 0.0	12.6 ± 0.0	0.4 ± 0.0	0.103 ± 0.0	97.6 ± 0.2	0.9 ± 0.1	6.6 ± 0.4	0.7 ± 0.2
W7 LA 240h	11 ± 0.0	60 ± 0.7	3.09 ± 0.0	5.5 ± 0.0	12.4 ± 0.0	0.3 ± 0.0	0.106 ± 0.0	97.0 ± 0.0	0.9 ± 0.0	7.2 ± 0.0	0.8 ± 0.4
W8 LA 240h	21 ± 0.7	82 ± 1.4	3.10 ± 0.0	5.9 ± 0.0	12.6 ± 0.0	0.3 ± 0.0	0.106 ± 0.0	96.9 ± 0.1	2.0 ± 0.1	6.6 ± 0.1 *	0.9 ± 0.4
W9 LA 240h	20 ± 0.7	82 ± 1.4	3.09 ± 0.0	6.0 ± 0.0	12.8 ± 0.0	0.3 ± 0.0	0.103 ± 0.0 *	97.0 ± 0.1	1.9 ± 0.0	6.5 ± 0.2 *	0.9 ± 0.3
W1 LB 240h	18 ± 0.0	94 ± 0.7	3.30 ± 0.0	6.4 ± 0.1	12.6 ± 0.0	0.4 ± 0.0	0.083 ± 0.0	99.2 ± 0.0	(-1.0) ± 0.0	6.2 ± 0.0	0.2 ± 0.0
W2 LB 240h	17 ± 3.5	95 ± 7.1	3.28 ± 0.0	6.3 ± 0.1	12.6 ± 0.0	0.4 ± 0.0	0.092 ± 0.0	98.6 ± 0.1	(-0.9) ± 0.1	6.2 ± 0.1	0.3 ± 0.2
W3 LB 240h	12 ± 2.1	87 ± 2.1	3.29 ± 0.0	6.1 ± 0.0	12.7 ± 0.0	0.4 ± 0.0	0.082 ± 0.0	98.8 ± 0.0	(-0.9) ± 0.2	5.8 ± 0.1	0.3 ± 0.1
W4 LB 240h	14 ± 0.0 *	88 ± 1.4	3.25 ± 0.0	6.5 ± 0.0	12.4 ± 0.0	0.4 ± 0.0	0.106 ± 0.0	98.3 ± 0.1	(-0.9) ± 0.3	7.4 ± 0.1	0.2 ± 0.2
W5 LB 240h	15 ± 0.0 *	97 ± 1.4	3.30 ± 0.0	5.8 ± 0.0	12.3 ± 0.0	0.4 ± 0.0	0.088 ± 0.0	98.1 ± 0.1	(-1.2) ± 0.2	5.6 ± 0.0 *	0.4 ± 0.1
W6 LB 240h	19 ± 0.0	79 ± 0.7	3.08 ± 0.0	6.1 ± 0.0	12.6 ± 0.0	0.4 ± 0.0	0.097 ± 0.0	97.7 ± 0.1 *	0.7 ± 0.1	6.4 ± 0.1	0.4 ± 0.1
W7 LB 240h	11 ± 0.7	60 ± 2.1	3.10 ± 0.0	5.5 ± 0.0	12.4 ± 0.0	0.3 ± 0.0	0.117 ± 0.0	96.4 ± 0.4	1.0 ± 0.0	7.6 ± 0.2	0.7 ± 0.1
W8 LB 240h	21 ± 1.4	80 ± 0.7	3.09 ± 0.0	5.9 ± 0.0	12.6 ± 0.0	0.3 ± 0.0	0.113 ± 0.0	96.7 ± 0.1	1.8 ± 0.1	7.2 ± 0.1	0.7 ± 0.1
W9 LB 240h	14 ± 0.7 *	79 ± 0.0*	3.10 ± 0.0	6.0 ± 0.0	12.7 ± 0.2	0.3 ± 0.0	0.112 ± 0.0	96.7 ± 0.1	2.1 ± 0.2 *	7.2 ± 0.1	0.8 ± 0.2
W1 LC 240h	19 ± 0.0	98 ± 0.7	3.30 ± 0.0	6.4 ± 0.0	12.6 ± 0.0	0.4 ± 0.0	0.086 ± 0.0	99.0 ± 0.1	(-1.1) ± 0.1	6.4 ± 0.0	0.4 ± 0.1

W2 LC 240h	16 ± 0.7	98 ± 2.1	3.25 ± 0.0	6.3 ± 0.0	12.5 ± 0.0	0.4 ± 0.0	0.096 ± 0.0	98.4 ± 0.1	(-1.0) ± 0.2 *	6.4 ± 0.2	0.3 ± 0.2
W3 LC 240h	13 ± 1.4	85 ± 3.5	3.29 ± 0.0	6.1 ± 0.0	12.7 ± 0.0	0.4 ± 0.0	0.083 ± 0.0	98.7 ± 0.1	(-0.8) ± 0.0	5.8 ± 0.1	0.2 ± 0.2
W4 LC 240h	14 ± 0.0*	91 ± 1.4	3.24 ± 0.0	6.5 ± 0.0	12.4 ± 0.0	0.4 ± 0.0	0.103 ± 0.0	98.4 ± 0.1	(-1.0) ± 0.2	7.3 ± 0.1	0.2 ± 0.2
W5 LC 240h	16 ± 0.0	90 ± 2.8	3.30 ± 0.0	5.9 ± 0.1	12.6 ± 0.0	0.4 ± 0.0	0.086 ± 0.0	98.4 ± 0.1	(-1.0) ± 0.1	5.8 ± 0.0 *	0.4 ± 0.5
W6 LC 240h	18 ± 0.7	82 ± 0.7	3.08 ± 0.0	6.1 ± 0.1	12.6 ± 0.0	0.3 ± 0.0	0.102 ± 0.0	97.5 ± 0.1	0.6 ± 0.2	6.6 ± 0.1	0.3 ± 0.0
W7 LC 240h	12 ± 0.7	61 ± 2.1	3.09 ± 0.0	5.5 ± 0.0	12.4 ± 0.0	0.3 ± 0.0	0.115 ± 0.0	96.7 ± 0.1	0.9 ± 0.2	8.1 ± 0.3	0.6 ± 0.2
W8 LC 240h	18 ± 0.0	82 ± 0.0	3.10 ± 0.0	5.9 ± 0.0	12.6 ± 0.0	0.3 ± 0.0	0.113 ± 0.0	96.9 ± 0.1	1.6 ± 0.2	7.6 ± 0.1	0.4 ± 0.3
W9 LC 240h	20 ± 2.1	86 ± 2.1	3.09 ± 0.0	6.0 ± 0.0	12.8 ± 0.0	0.3 ± 0.0	0.113 ± 0.0	96.8 ± 0.1	1.7 ± 0.1	7.4 ± 0.1	0.5 ± 0.1


W. 1
W. 2
W. 3
W. 4
W. 5
W. 6
W. 7
W. 8
W. 9
● LA
■ LB
▲ LC
%
0
20
40
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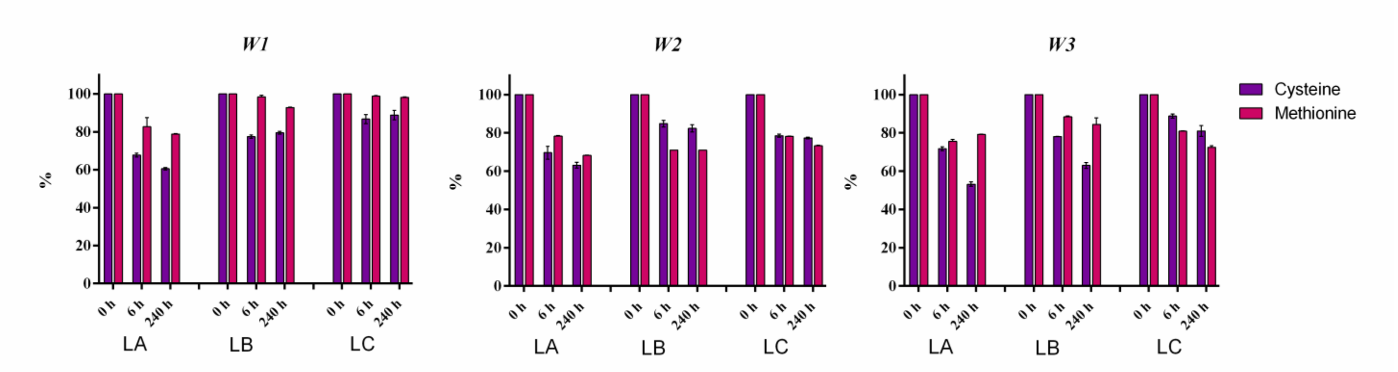
80
100
0h
3 h
6 h
24 h
48 h
120 h
240 h
time in hours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LA
LB
LC
Cysteine
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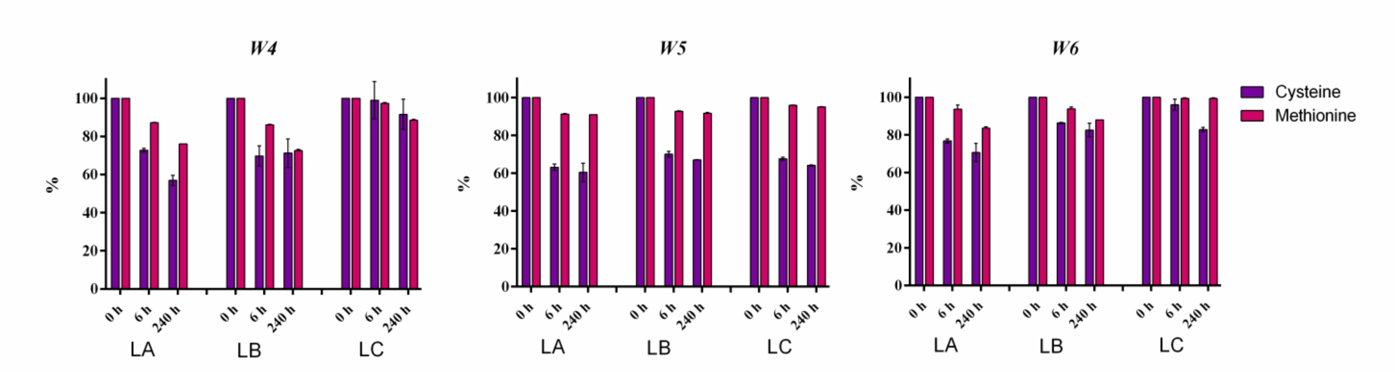
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LA
LB
LC
Cysteine
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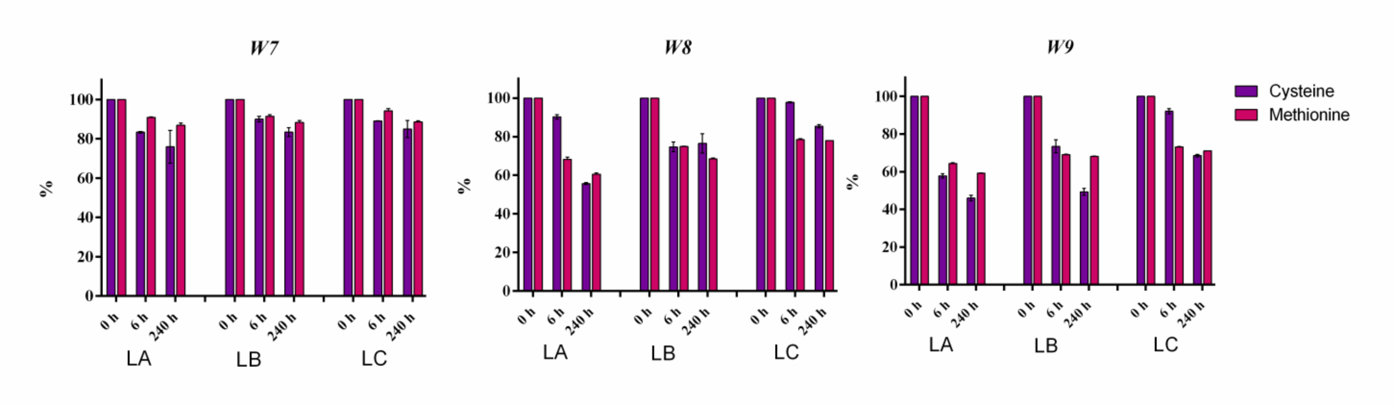
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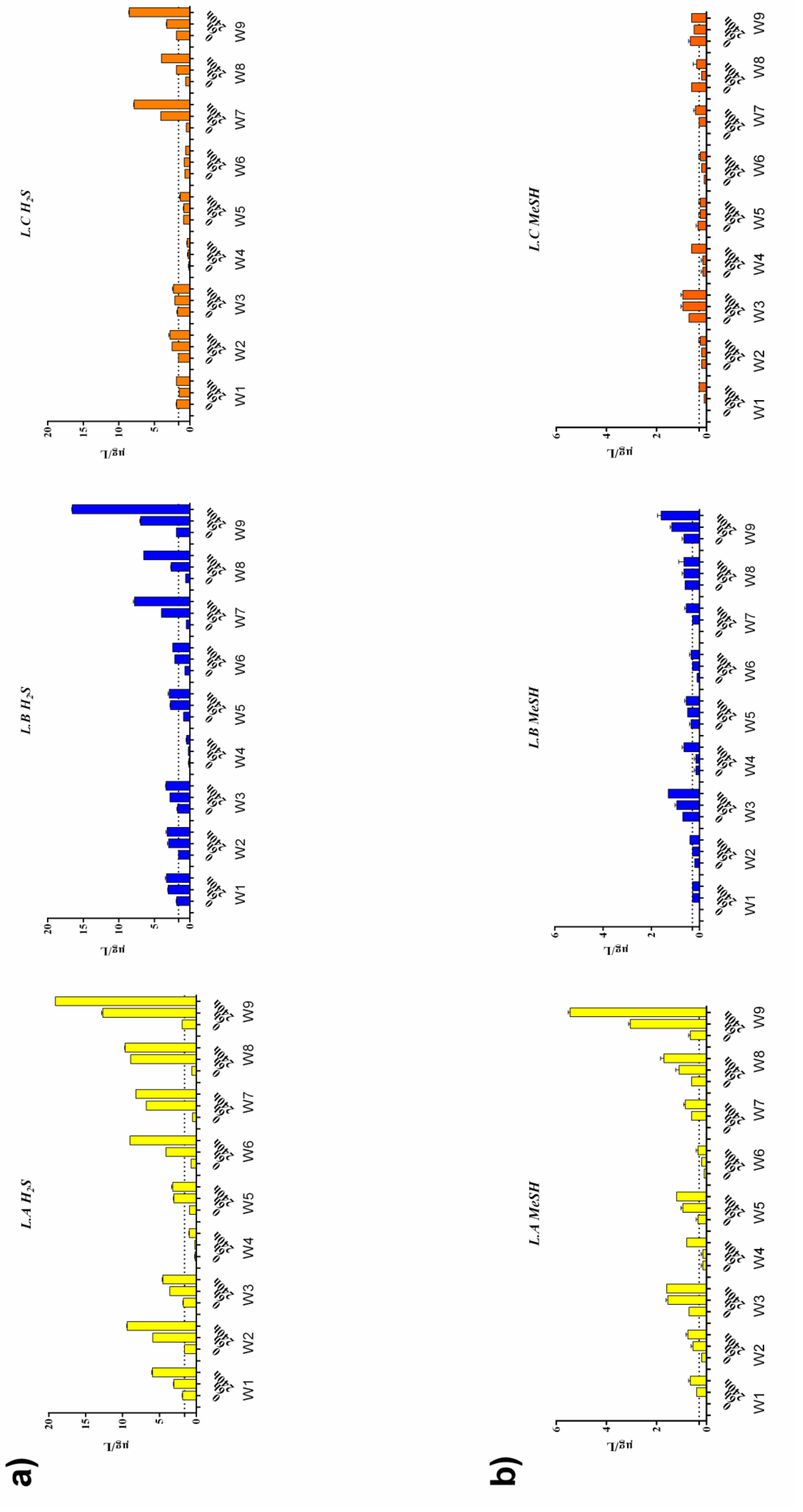
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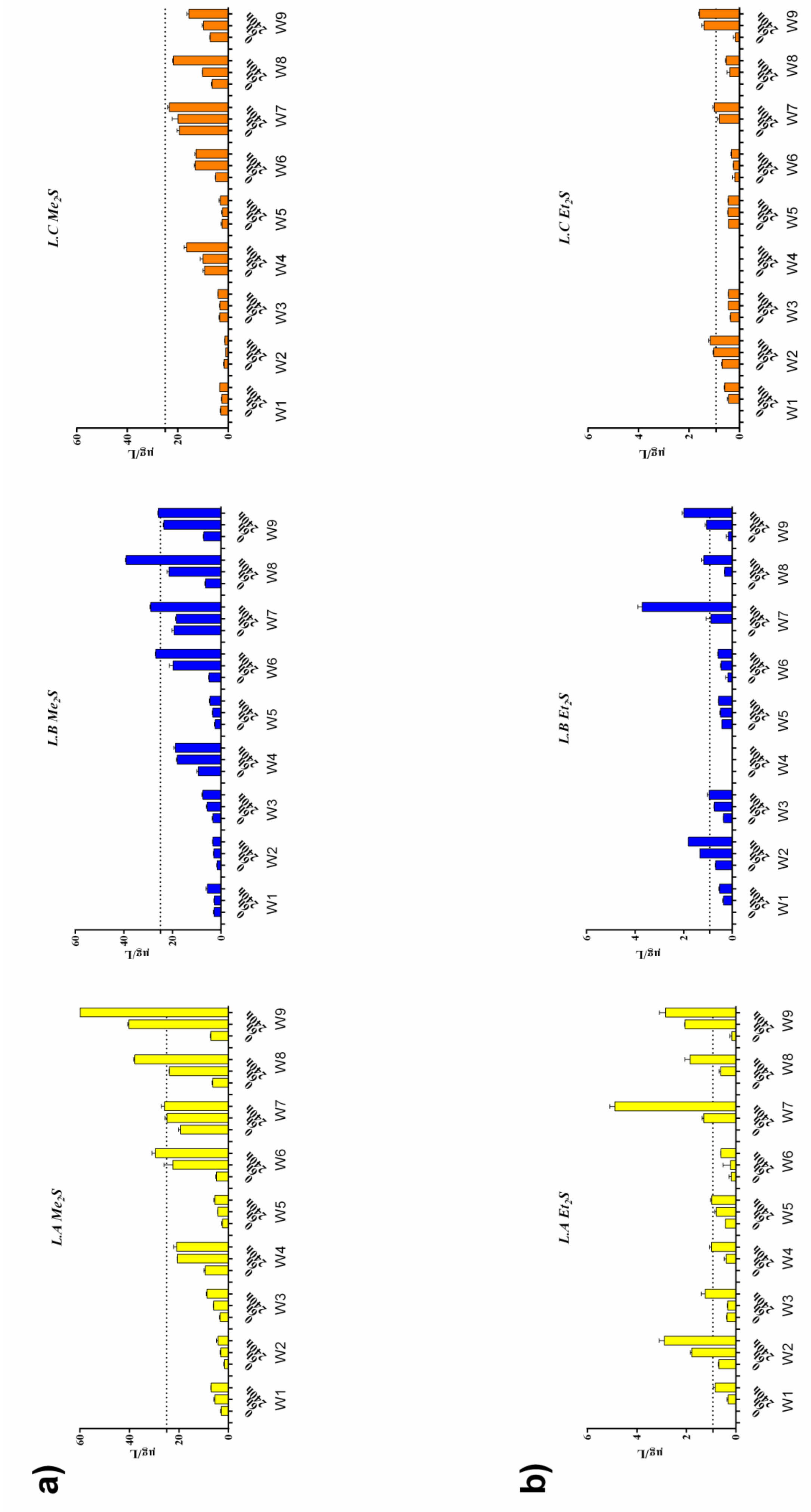
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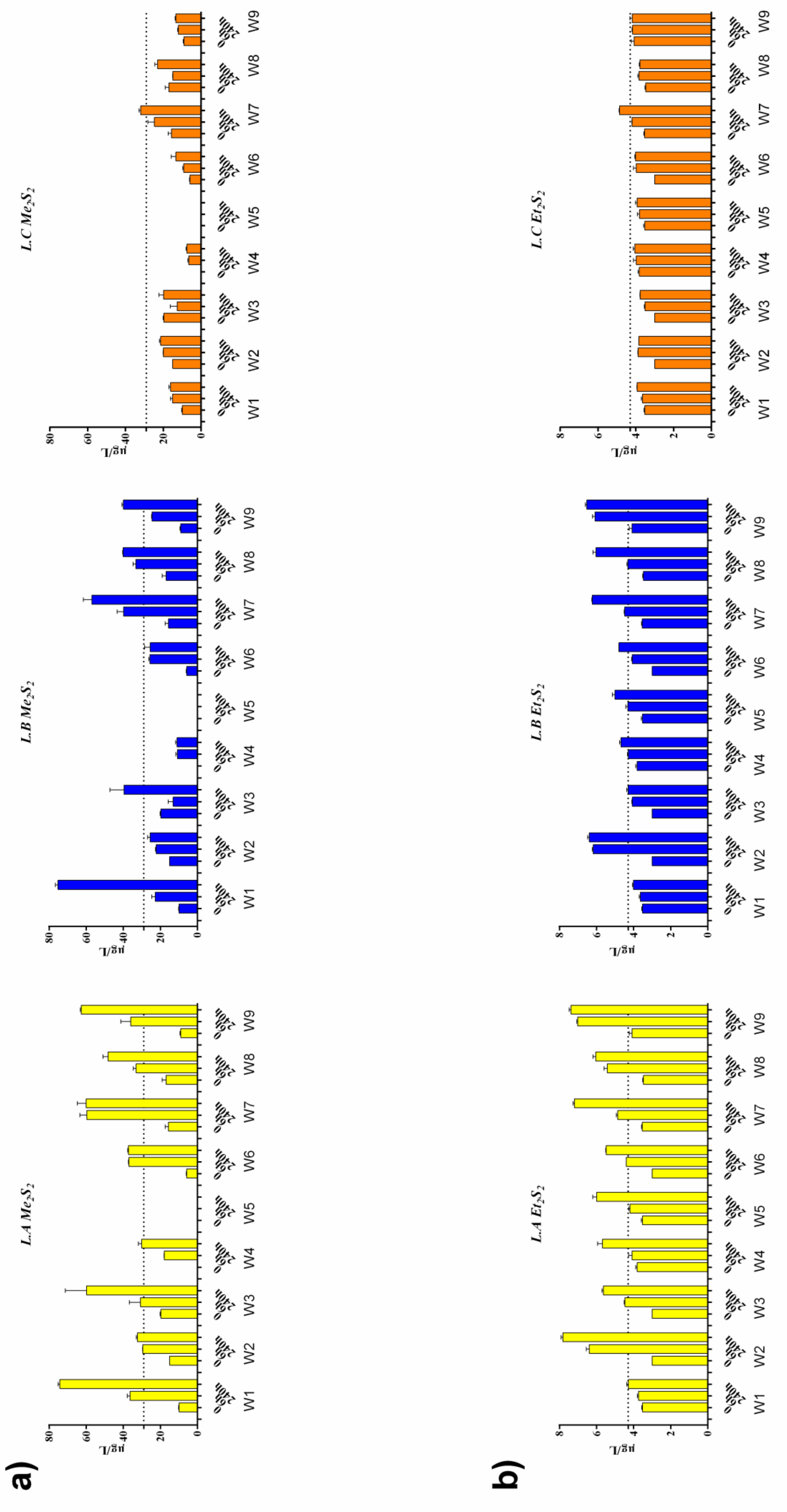
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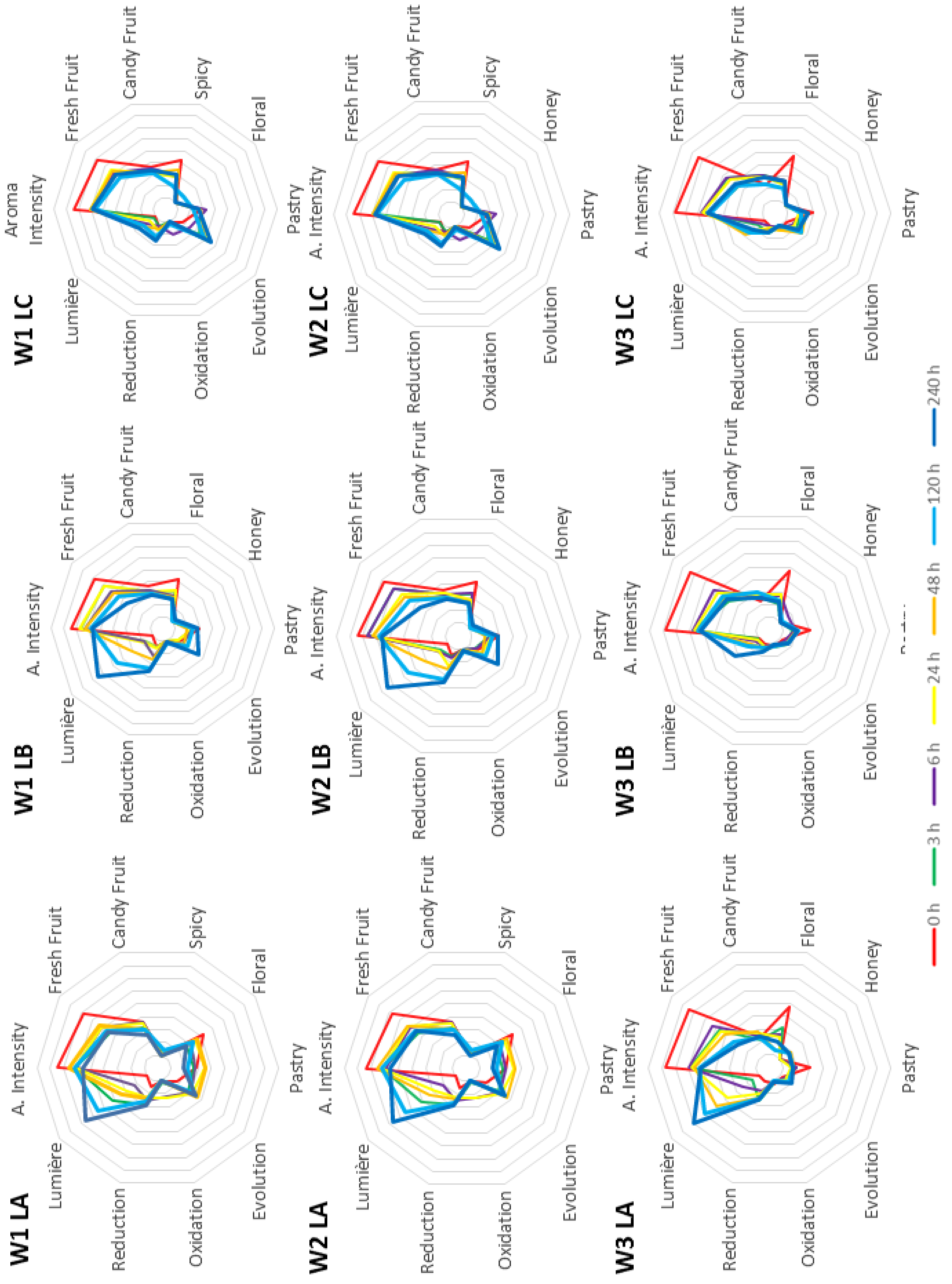
b)
L.A Et2S2
L.B Et2S2
L.C Et2S2
μg/L
0
20
40
60
80
2
4
6
8
0
6h
24h
W1
W2
W3
W4
W5
W6
W7
W8
W9

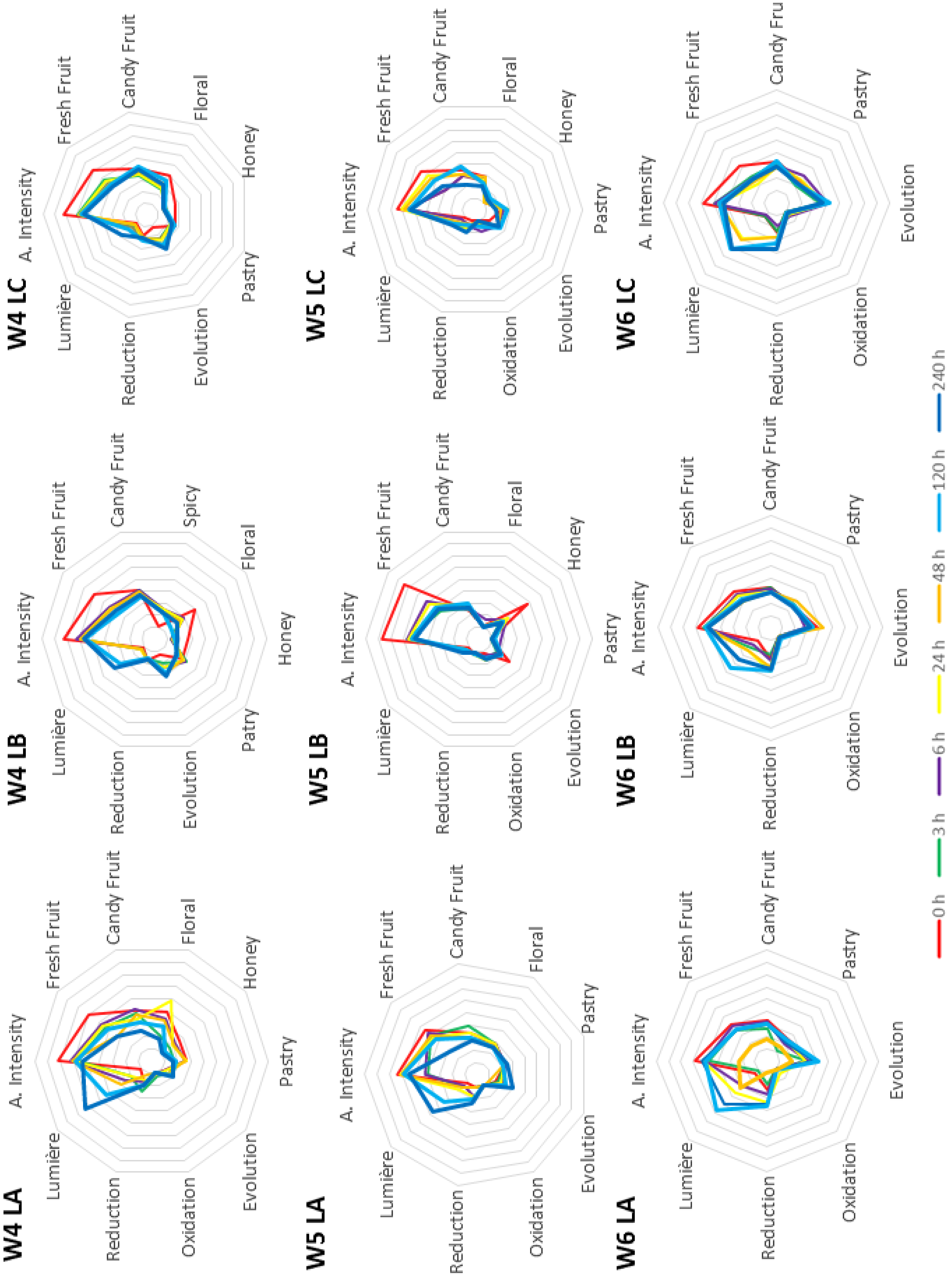
Table S1. Riboflavin values (mg/L) obtained with the three types of lights (LA, LB and LC) in all wines (W1-W9) throughout the exposure time (0-240 hours). Different letters indicate significant differences in the same wine between the different exposure times in each of the study lights. Samples (n=2).

LIGHTS	TIMES	W1	W2	W3	W4	W5	W6	W7	W8	W9
LA	**0 hours**	182 e ± 3	171 e ± 2	161 f ± 1	169 c ± 1	138 bc ± 3	154 e ± 1	178 g ± 1	152 f ± 2	168 g ± 2
	3 hours	173 d ± 4	162 cd ± 2	162 f ± 1	171 c ± 1	147 d ± 3	150 de± 0	171 f ± 1	150 f ± 0	158 f± 2
	6 hours	172 d ± 4	165 d ± 0	156 e ± 1	169 c ± 0	147 cd ± 3	146 d ± 3	159 e ± 1	145 e ± 1	150 e ± 1
	24 hours	151 c ± 0	157 c ± 1	149 d ± 0	157 b ± 2	147 e ± 1	134 c ± 1	136 d ± 1	110 d ± 1	123 d ± 2
	48 hours	142 c ± 1	120 b ± 1	134 c ± 1	157 b ± 2	137 ab ± 1	110 b ± 1	120 c ± 0	75 c ± 1	81 c ± 1
	120 hours	131 b ± 1	120 b ± 1	128 b ± 0	154 b ± 3	139 cd ± 2	110 b ± 1	109 b ± 1	39 b ± 1	51 b ± 1
	240 hours	103 a ± 0	112 a ± 2	103 a ± 3	132 a ± 2	128 a ± 3	86 a ± 1	71 a ± 2	24 a ± 2	30 a ± 1
LB	**0 hours**	182 e ± 3	171 c ± 2	161 e ± 1	169 b ± 1	138 a ± 3	154 f ± 1	178 e ± 1	152 de ± 2	168 f ± 2
	3 hours	186 e ± 1	154 b ± 2	158 e ± 3	162 ab ± 2	144 abc ± 3	144 d± 1	171 d ± 2	157 e ± 2	159 e ± 1
	6 hours	184 e ± 1	171 c ± 1	158 de ± 4	162 ab ± 1	147 bc ± 2	148 e ± 0	169 d ± 1	155 e ± 2	157 de ± 2
	24 hours	175 c± 1	167 b ± 2	149 bc ± 1	161 ab ± 6	140 c± 0	141 c ± 1	167 c ± 1	149 c ± 2	151 c ± 2
	48 hours	163 d ± 1	158 c ± 1	144 cd ± 2	164 ab ± 1	149 ab ± 1	129 d ± 1	150 d ± 1	139 d ± 1	142 d ± 2
	120 hours	151 b ± 1	152 b ± 1	139 b ± 2	162 ab ± 1	144 abc ± 1	119 b ± 1	135 b ± 0	119 b ± 1	122 b ± 1
	240 hours	119 a ± 1	135 a ± 1	120 a ± 0	158 a ± 2	141 ab ± 0	95 a ± 1	104 a ± 1	86 a ± 1	94 a ± 1
LC	**0 hours**	182 ab ± 3	171 b ± 2	161 b ± 1	169 b ± 1	138 a ± 3	154 b ± 1	178 c ± 1	152 a ± 2	168 c ± 2
	3 hours	184 ab ± 1	171 b ± 1	157 b ± 1	169 b ± 1	145 b ± 0	151 b ± 1	171 ab ± 2	158 ab ± 2	157 b ± 2
	6 hours	188 b ± 1	175 c ± 1	158 b ± 0	167 b ± 1	145 b ± 1	154 b ± 0	176 bc ± 1	155 ab ± 1	156 b ± 1
	24 hours	184 ab ± 1	175 bc ± 1	157 b ± 0	161 a ± 2	143 b ± 1	154 b ± 1	171 ab ± 1	155 ab ± 3	157 b ± 0
	48 hours	183 ab ± 1	175 bc ± 0	159 b ± 1	164 ab ± 1	145 b ± 1	146 a ± 1	171 ab ± 1	157 ab ± 1	156 b ± 1
	120 hours	181 a ± 0	176 c ± 1	157 b ± 1	166 b ± 1	140 ab ± 1	146 a ± 1	169 a ± 1	157 ab ± 2	153 ab ± 1
	240 hours	184 ab ± 1	150 a ± 0	151 a ± 1	169 b ± 1	145 b ± 1	150 b ± 1	172 ab ± 1	159 b ± 2	150 a ± 0

Table S2. Cysteine and methionine values (mg/L) obtained with the three types of lights (LA, LB and LC) in all wines (W1-W9) throughout the exposure time (0- 6-240 hours). Different letters indicate significant differences in the same wine between the different exposure times in each of the study lights. Samples (n=2).

		LA			**LB**			**LC**		
		0 hours	**6 hours**	**240 hours**	**0 hours**	**6 hours**	**240 hours**	**0 hours**	**6 hours**	**240 hours**
W1	Cysteine	0.95 c ± 0.00	0.65 b ± 0.00	0.58 a ± 0.01	0.95 b ± 0.00	0.74 a ± 0.01	0.76 a ± 0.01	0.95 b ± 0.00	0.83 a ± 0.02	0.85 a ± 0.02
	Methionine	4.2 b ± 0.0	3.5 a ± 0.2	3.3 a ± 0.0	4.2 b ± 0.0	4.1 b ± 0.1	3.9 a ± 0.0	4.2 b ± 0.0	4.2 a ± 0.0	4.1 a ± 0.0
W2	Cysteine	0.67 b ± 0.00	0.46 a ± 0.02	0.42 a ± 0.01	0.67 b ± 0.00	0.56 a ± 0.01	0.55 a ± 0.02	0.67 b ± 0.00	0.52 a ± 0.01	0.5 a ± 0.01
	Methionine	4.4 c ± 0.0	3.4 b ± 0.0	3.0 a ± 0.0	4.4 b ± 0.0	3.1 a ± 0.0	3.1 a ± 0.0	4.4 c ± 0.0	3.4 b ± 0.0	3.2 a ± 0.0
W3	Cysteine	1.04 c ± 0.02	0.75 b ± 0.02	0.55 a ± 0.00	1.04 c ± 0.02	0.81 b ± 0.01	0.66 a ± 0.01	1.04 b ± 0.02	0.92 a ± 0.02	0.84 a ± 0.02
	Methionine	3.6 b ± 0.0	2.8 a ± 0.0	2.9 a ± 0.0	3.6 b ± 0.0	3.2 a ± 0.0	3.1 a ± 0.1	3.6 c ± 0.0	2.9 b ± 0.0	2.6 a ± 0.1
W4	Cysteine	0.35 c ± 0.02	0.26 b ± 0.01	0.20 a ± 0.00	0.35 b ± 0.02	0.25 a ± 0.00	0.25 a ± 0.01	0.35 a ± 0.02	0.35 a ± 0.01	0.32 a ± 0.01
	Methionine	4.6 c ± 0.0	4.0 b ± 0.0	3.5 a ± 0.0	4.6 c ± 0.0	3.9 b ± 0.0	3.3 a ± 0.0	4.6 c ± 0.0	4.4 b ± 0.0	4.0 a ± 0.0
W5	Cysteine	1.79 b ± 0.01	1.13 a ± 0.04	1.08 a ± 0.10	1.79 b ± 0.01	1.25 a ± 0.04	1.20 a ± 0.01	1.79 c ± 0.01	1.22 b ± 0.02	1.16 a ± 0.00
	Methionine	6.0 b ± 0.0	5.5 a ± 0.1	5.5 a ± 0.0	6.0 c ± 0.0	5.6 b ± 0.0	5.5 a ± 0.0	6.0 b ± 0.0	5.8 a ± 0.0	5.7 a ± 0.0
W6	Cysteine	0.66 b ± 0.01	0.51 a ± 0.02	0.47 a ± 0.03	0.66 b ± 0.01	0.57 a ± 0.01	0.55 a ± 0.03	0.66 b ± 0.01	0.64 b ± 0.01	0.55 a ± 0.02
	Methionine	5.4 c ± 0.0	5.1 b ± 0.1	4.5 a ± 0.1	5.4 c ± 0.0	5.1 b ± 0.1	4.8 a ± 0.0	5.4 a ± 0.0	5.4 a ± 0.0	5.4 a ± 0.1
W7	Cysteine	0.56 b ± 0.01	0.47 ab ± 0.01	0.43 a ± 0.04	0.56 b ± 0.01	0.50 ab ± 0.00	0.47 a ± 0.02	0.56 a ± 0.01	0.50 a ± 0.01	0.48 a ± 0.04
	Methionine	8.1 c ± 0.0	7.4 b ± 0.0	7.1 a ± 0.0	8.1 c ± 0.0	7.4 b ± 0.0	7.2 a ± 0.0	8.1 c ± 0.0	7.7 b ± 0.1	7.2 a ± 0.0
W8	Cysteine	0.83 c ± 0.02	0.75 b ± 0.01	0.46 a ± 0.01	0.83 b ± 0.02	0.63 a ± 0.03	0.64 a ± 0.06	0.83 b ± 0.02	0.82 b ± 0.02	0.71 a ± 0.01
	Methionine	4.8 c ± 0.0	3.2 b ± 0.0	2.9 a ± 0.0	4.8 c ± 0.0	3.6 b ± 0.0	3.3 a ± 0.0	4.8 b ± 0.0	3.7 a ± 0.0	3.7 a ± 0.0
W9	Cysteine	0.86 c ± 0.01	0.50 b ± 0.02	0.40 a ± 0.01	0.86 c ± 0.01	0.63 b ± 0.04	0.43 a ± 0.02	0.86 c ± 0.01	0.79 b ± 0.02	0.59 a ± 0.01
	Methionine	4.8 c ± 0.0	3.1 b ± 0.0	2.8 a ± 0.0	4.8 b ± 0.0	3.3 a ± 0.0	3.3 a ± 0.0	4.8 b ± 0.0	3.5 a ± 0.0	3.4 a ± 0.0


W1 LC
Aroma Intensity
Fresh Fruit
Candy Fruit
Spicy
Floral
Evolution
Oxidation
Reduction
Lumière
W1 LB
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Evolution
Oxidation
Reduction
Lumière
W1 LA
A. Intensity
Fresh Fruit
Candy Fruit
Spicy
Floral
Evolution
Oxidation
Reduction
Lumière
W2 LC
Pastry
A. Intensity
Fresh Fruit
Candy Fruit
Spicy
Honey
Pastry
Evolution
Oxidation
Reduction
Lumière
W2 LB
Pastry
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Evolution
Oxidation
Reduction
Lumière
W2 LA
Pastry
A. Intensity
Fresh Fruit
Candy Fruit
Spicy
Floral
Evolution
Oxidation
Reduction
Lumière
W3 LC
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Pastry
Evolution
Oxidation
Reduction
Lumière
W3 LB
Pastry
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Evolution
Oxidation
Reduction
Lumière
W3 LA
Pastry
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Pastry
Evolution
Oxidation
Reduction
Lumière
0 h
3 h
6 h
24 h
48 h
120 h
240 h


W4 LA
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Pastry
Evolution
Oxidation
Reduction
Lumière
W4 LB
A. Intensity
Fresh Fruit
Candy Fruit
Spicy
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Honey
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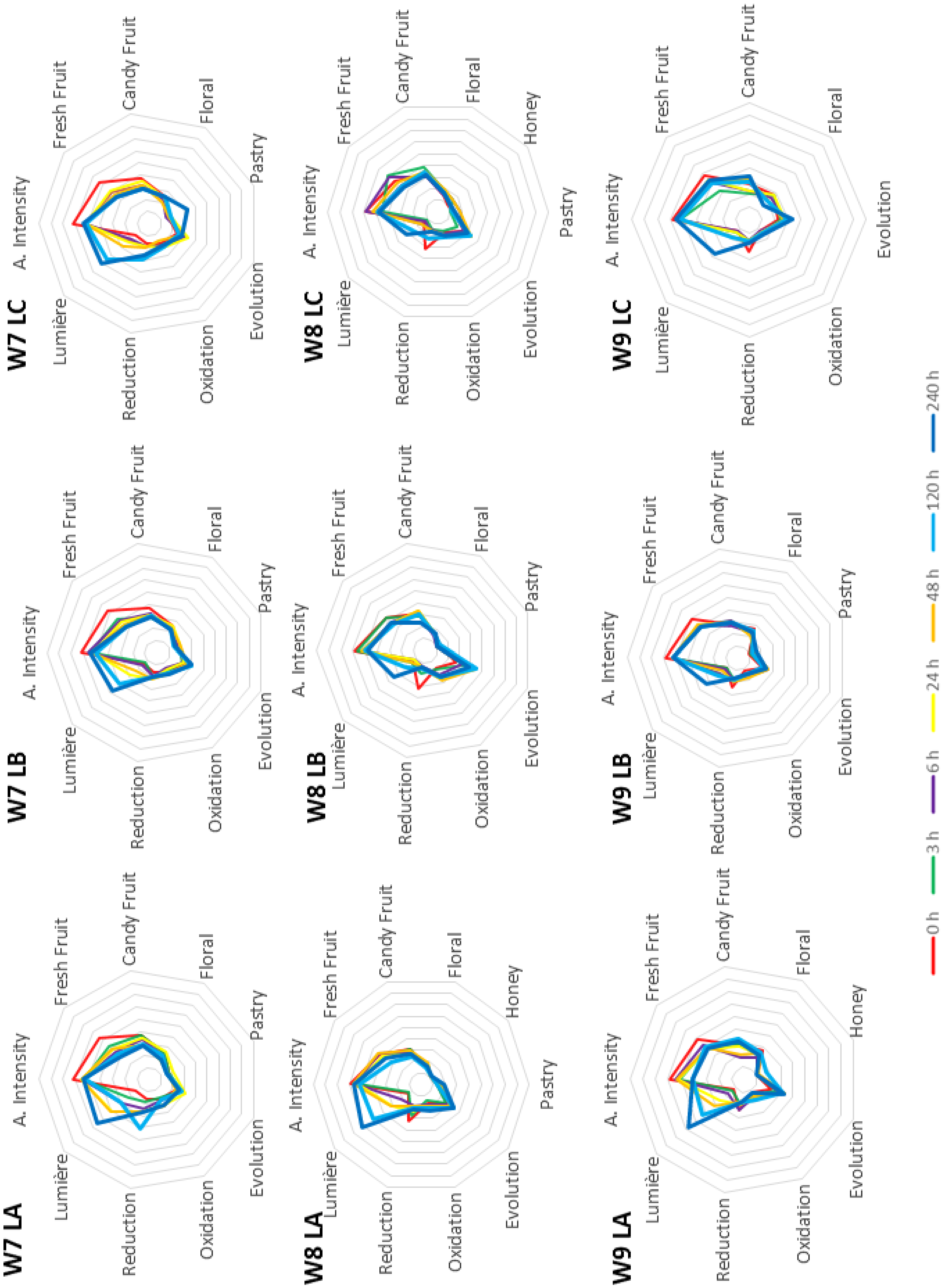
Lumière
W4 LC
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Pastry
Evolution
Reduction
Lumière
W5 LA
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Pastry
Evolution
Oxidation
Reduction
Lumière
W5 LB
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Pastry
Evolution
Oxidation
Reduction
Lumière
W5 LC
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Pastry
Evolution
Oxidation
Reduction
Lumière
W6 LA
A. Intensity
Fresh Fruit
Candy Fruit
Pastry
Evolution
Oxidation
Reduction
Lumière
W6 LB
A. Intensity
Fresh Fruit
Candy Fruit
Pastry
Evolution
Oxidation
Reduction
Lumière
W6 LC
A. Intensity
Fresh Fruit
Candy Fruit
Pastry
Evolution
Oxidation
Reduction
Lumière
0 h
3 h
6 h
24 h
48 h
120 h
240 h


W7 LA
W7 LB
W7 LC
W8 LA
W8 LB
W8 LC
W9 LA
W9 LB
W9 LC
A. Intensity
Fresh Fruit
Candy Fruit
Floral
Honey
Pastry
Evolution
Oxidation
Reduction
Lumière
0 h
3 h
6 h
24 h
48 h
120 h
240 h

Artículo 5

Effect of light exposure, bottle color and type of closure on the aromatic composition and quality of Albariño wines.

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Effect of light exposure, bottle color and type of closure on the aromatic composition and quality of Albariño wines

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Corresponding Author:	RAUL FERRER GALLEGO VITEC, Wine Technology Centre Falset, Not US or Canada SPAIN
First Author:	Ana María Mislata
Order of Authors:	Ana María Mislata
	Miquel Puxeu
	Enric Nart
	Sergi de Lamo
	Montserrat Mestres
	Raul Ferrer-Gallego
Abstract:	The quality of white wines is rapidly affected by the storage conditions. The type of closure and the color of bottle combined with a light exposure can cause an important loss of quality. In this study changes on the chemical aromatic profile, color parameters and sensory perception of Albariño wines exposed to light were evaluated. The study was carried out using different colors of glass bottle (clear, amber and green) and different types of closures (screw cap, natural cork and synthetic cork). Chemical and sensory analyses were performed at 3, 6, and 12 months after bottling. The results showed that Albariño wines were firstly influenced by the time of storage, although other studied factors also influenced their final quality. Thus, amber bottle and screw cap seems to better preserve the fruity and floral fermentative aromas of wines exposed to light. Contrarily, the wines bottled in clear glass and stopped with cork increased their content in oxidative aromas which clearly affected their sensory quality.

Effect of light exposure, bottle color and type of closure on the aromatic composition and quality of Albariño wines

Abstract: The quality of white wines is rapidly affected by the storage conditions. The type of closure and the color of bottle combined with a light exposure can cause an important loss of quality. In this study changes on the chemical aromatic profile, color parameters and sensory perception of Albariño wines exposed to light were evaluated. The study was carried out using different colors of glass bottle (clear, amber and green) and different types of closures (screw cap, natural cork and synthetic cork). Chemical and sensory analyses were performed at 3, 6, and 12 months after bottling. The results showed that Albariño wines were firstly influenced by the time of storage, although other studied factors also influenced their final quality. Thus, amber bottle and screw cap seems to better preserve the fruity and floral fermentative aromas of wines exposed to light. Contrarily, the wines bottled in clear glass and stopped with cork increased their content in oxidative aromas which clearly affected their sensory quality.

Keywords: light exposure; white wine; volatile compounds; sensory analysis; Albariño, color.

1. Introduction

Albariño is a recognized old grape variety widespread in the NW of Spain and North of Portugal. Wines made from Albariño variety are aromatic, balanced, fresh and, intense in fruits and floral aromas (1). Albariño is the most important white grapevine cultivar for the economy of Galicia (Spain). Like most young white wines, wines produced from Albariño are being made for consumption within twelve months after production. However, sometimes white wines are stored in shops beyond this period of time, entailing drastic consequences on their quality. One of the current concerns in wine market is the loss of the so-called typicity (2, 3). The loss of the typical volatile compounds associated with a specific grape variety and/or the appearance of oxidation volatiles over time involve a loss of the final quality of white wines. The consumption of white wines later than recommended by experts may lead to some disappointments in consumers with the subsequent economical devaluation of them.

The quality of bottled white wine is easily damaged over time due to several chemical reactions related to aging (4) and a great number of factors can accelerate this spoilage. Among them, the most important ones are temperature, humidity, packaging, type of closure, color of bottle and light exposure. Exposure to light of bottled wine causes color changes and the appearance of undesirable odors (5-8). On the other hand, the color of the bottle influences the wine photodegradation since the different light wavelengths pass through the bottle glass depending on its color (4, 9). Thus, it has been reported that the quality and composition of Chardonnay and Sauvignon blanc wines varied in bottled wines depending on the color and the light exposure (4, 9). Moreover, Hopfer and co-workers (2012) observed significantly changes in sensory and chemical composition of Chardonnay wines after a storage period of 3 months in bottles closed with different stoppers (10). Recently, Diaz et al. (2021) found that Sauvignon blanc wines exposed to light during 3 months showed less fruity and floral aromas and higher nuances of solvent, earthy and honey aromas (8). These results about the effects of light irradiation on the changes in the levels of wine volatiles currently concern wineries and wine researchers (7, 11, 12) so the color of bottles has also been adapted for both to satisfy consumers and preserve wine characteristics (14). A high spectrum of colors are used in wine industry, from the traditional clear bottles to artic blue, dead leaf, amber, dark green, antique green, flint, or even faience bottles. Although each one of them is known to behave differently (7, 9, 15, 16), more information regarding changes in wine characteristics stored under different conditions is necessary.

On the other hand, packaging is a key factor which can prolong or shorten the wine shelf life. However, achieving an appropriate packaging system that minimizes risks to the wine quality during the storage period and avoids economic losses is a difficult task. Consumer preferences and marketing strategies should be also considered to enhance sales but without leading a wine quality detriment. This is why different types of closures such as screw cap or synthetic corks have been considered in order to preserve wine quality although, some of them, have not been well accepted by consumers (13).

In this study, Albariño white wines were evaluated over a long period of light irradiation (12 months). Changes on their aromatic composition, enological parameters, color and sensory quality of Albariño wines exposed to light with different types of closures and color of bottle were studied.

2. Materials and Methods

2.1. Experimental Design

The commercial white wines used in this study were produced in Galicia (Rias Baixas Appellation of Controlled Origin) from Albariño grapevine cultivar. It was bottled using three different colors of bottles (clear, amber and green) and each kind of bottle was stopped with three different bottle stoppers (screw cap, natural cork and synthetic cork). Each different combination bottle-closure was carried out in duplicate. All of them were stored for one year under a controlled temperature of 18-20 °C, 24 bottles were kept in the dark (control wines) and 24 bottles were stored under light exposure. The light used in the experiment was an ultraviolet lamp like those used traditionally in warehouses, and supermarkets. Analyses were made after three, six and twelve months of storage by duplicate.

2.2. Enological Basic Parameters and Colorimetric Measurements

The enological parameters analyzed were: total and free sulfur dioxide, alcoholic strength, volatile acidity, titratable acidity, pH, chromatic characteristics and CIELAB values. Total and free sulfur dioxide were analyzed by the benchmark method OIV-MA-AS323-04A (resolution oeno 377/2009). The values of the alcoholic strength, volatile acidity, titratable acidity and pH were obtained using a Fourier transform infrared spectroscopy (FTIR) system (WineScanTM by FOSS, Hilleroed, Denmark), internally calibrated as recommend the International Organization of Vine and Wine OIV (14). The chromatic characteristics of wines were measured according to OIV-MA-AS2-07B by a Helios-α-spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The CIELAB color parameters were calculated by the MSCV® software (15) following the recommendations of the Commission Internationale de L'Eclariage (CIE); standard observer (10º visual field) and standard illuminant D65 as references.

2.3. GC-MS Analysis

Both fermentative and oxidative aromas were analyzed by gas chromatography by using a GC 7890A (Agilent Technologies) equipped with a mass spectrometer 5975C inert MSD (Triple-Axis Detector with electron impact source). The separation was performed with a DB-WAX IU (60 m x 0.25 mm × 0.25 µm, Agilent) and a VF-200 ms (30 m x 0.25 mm × 0.25 µm, Agilent), respectively. Samples preparation and compounds extraction, identification and quantification were carried out according to Mislata *et al.* (16). All analyses were carried out in duplicate.

2.5. Sensory Analysis

Sensory properties of the studied wines were evaluated by six professional tasters selected and trained following the normative ISO 8586:2012. A quantitative descriptive analysis (QDA) was carried out in a normalized room (ISO 8589:2007). The evaluated sensory descriptors were: color evolution, color intensity, aromatic intensity, fruit, tropical fruit, citric, dried fruit, greenness, damp vegetal, floral, smoked, cake, evolution, reduction, mushroom, volume, acidity, burning, dryness, unctuosity, bitterness, permanence and global punctuation. Attributes were rated from 1 (absence) to 5 (maximum intensity) and the data obtained were processed with the FIZZ software (V.2.47B Biosystems, Barcelona, Spain). The significance of the results was assessed by Tukey test ($p < 0.05$) performed by means of XLSTAT tool of the EXCEL software.

3. Results and Discussion

3.1. Basic Enological Parameters

The basic oenological parameters were determined in all the wine samples prepared with the different bottle color and type of closure after 3, 6 and 12 months of storage.

In general, all the wines had free sulfur values below 25 mg/L and total sulfur values below 105 mg/L throughout the 12 months of the study, which indicates that they were protected white wines but without excess of sulfur content. The alcoholic degree was 12.3-12.4% vol. The parameters related to acidity did not show significant differences over time. The total tartaric acidity presented values between 6.0 and 6.2 g/L and the pH values were between 3.34 and 3.36. The volatile acidity did not present significant differences throughout the storage time (from 0 to 12 months), and the values did not exceed 0.48 g/L. This indicates adequate storage conditions that ensured a correct evolution of the wines throughout the study.

Regarding the color evaluation, the absorbance at 420 nm and the CieLab coordinates were determined (figure 1). As can be seen in figure 1.A, in general, all wines have an increase in absorbance (420 nm), which implies a greater degree of visual browning over time, especially in wines with amber and green bottles. Furthermore, in figure 1.B it is observed how after 12 months of storage with exposure to light, the wines conserved a green hue, since all the values of the coordinate a* were negative. However, it should be noted that wines with a clear bottle present lower b* coordinate values, which indicates a lower yellow coloration than that observed with absorbance at 420nm.

These results are similar to those obtained by other authors (9, 17) who had greater losses of polyphenols in white wines that presented bottles with clear glasses and, consequently, a lower degree of visual browning. This behavior was related to increased formation of xanthilium pigments and catechin-like phenolic compounds in dark bottles. In our study, all wines showed an increase in the degree of visual browning (abs 420 nm) but this was more severe for wines bottled in amber and green glasses.

3.2. Total Aromatic Composition, Storage and Light Exposure

The effect of the storage time on the total aromatic composition of Albariño wines can be observed in figure 2. In general, results showed a drastic loss in the total fermentative aromas (as the sum of esters, alcohols, acetates and fatty acids) during the storage period (fig. 2.A). Conversely, the total oxidative aromas (as the sum of Streckers aldehydes, furans, alkenals, ketones and lactones) started to appear after 6 months of storage and increased considerably until 12 months (figure 2.B). The development of new aromatic compounds linked to the oxidation process together with the important loss of fresher and fruity notes could indicate a loss of quality and typicity of these wines. These results could also indicate the optimum time for consumption; that could be established between 3 months (there was still no significant loos of freshness) and 6 months (the beginning of the oxidation notes). At

12 months an obvious oxidation of Albariño wines was observed indicating that the best time for their consumption had already finished.

Considering the color of the bottles and the type of closures, a drastic decrease in fermentative aromas was observed after three months of light exposure (green bars) in all studied conditions (figure 3). In general, the screw caps better preserved the fermentative aromas for all bottles. After 12 months clear and green bottles showed differences for all type of closures while amber bottles showed no differences for screw cap. In addition, at this time, the loss of the fermentative aromas was more than 50% in most cases. This great reduction in fermentative aromas could indicate a loss of aromatic quality independently of the type of closure and the color of the bottle. It seems that amber bottles stopped with screw cap could be a good option to preserve fermentative aromas during in the medium/long term (figure 4). However, it should be noted that this type of closures is not the most accepted by the consumer and it has even been reported that consumers expect to pay significantly less for a bottle with a screw cap (13, 18, 19), which may be self-defeating in a traditional wine, such as Albariño. Considering that natural cork is better valued by consumers (20) and regarding figure 3, this type of closure may be interesting in short-term (3 months) to stopper Albariño wines but, contrary, this cork entails a great loss of fermentative aromas (more than 60 %, see figure 4) in prolonged storage (12 months).

Regarding oxidative aromas, these increased considerably between 6 months and 12 months of storage (figure 5) regardless of the bottle color being natural corks those that provided the highest values. In addition, more oxidative aromas were generated when the bottles were exposed to light and these increased over time of exposition. The highest content was observed in clear bottles stopped with corks, probably related to a higher oxygen contribution (21). In this study, the oxygen transmission rate (OTRs) have not been evaluated but it is well known that natural cork have highest OTRs values.

3.3. Individual Aromatic Composition, Storage and Light Exposure

The fruity and floral character of Albariño wines has been already reported by Vilanova *et al.* (22). Ethyl esters, alcohols, acetates and fatty acids are important studied families that contribute to their pleasant sensations. In this study, a total of 18 fermentative volatile compounds were studied. Specifically 6 esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl isovaleriate and diethyl succinate), 4 alcohols (isoamyl alcohol, isobutanol, benzyl alcohol and 2-phenylethyl alcohol), 4 acetates (ethyl acetate, isoamyl acetate, hexyl acetate and 2-phenylethyl acetate), and 3 fatty acids (hexanoic acid, octanoic acid and decanoic acid) were determined. Among them, ethyl isovalerate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate and isoamyl acetate reached the corresponding odour threshold (OT) established in wine (figure 6).

When looking at each compound separately, a similar trend was found for the color of the bottles. It seems that after 3 months there is a significant drop in all compounds except ethyl octanoate, which shows a greater decrease after 6 months. Another different behavior is observed for ethyl isovaletate (figure 6.A), a compound with a characteristic aroma related to fruits such as green apple (23). In this case, it can be seen a great increase of this compound after 12 months of storage regardless the bottle color. This increase in concentration was slightly higher in samples exposed to light with an amber bottle followed by a green bottle with a screw cap. Ethyl hexanoate (figure 6.B), in addition to a green apple aroma, it can also provide aniseed notes to wines (24). Regardless of the bottle color used after 6 months, this compound was practically not affected until 12 months. Like ethyl isovaleriate, the samples exposed to light after 12 months with an amber bottle and screw cap were the wines that presented the highest concentration of this compound. Therefore, these two compounds (ethyl isovalerate and ethyl hexanoate) had a greater aromatic contribution in Albariño wines preserved in amber bottle and screw cap after 12 months of light exposure.

As previously mentioned, ethyl octanoate (figure 6.C) was the only compound that remained with similar concentrations along 6 months, but then suffered a large decrease at 12 months. This compound gives to wines aromatic notes of sweets, pineapple and pear (25). As can be seen in figure 6.C, after 3 months the samples exposed to light showed a slight decrease in concentration compared to the controls. However, at 6 months, the samples with the screw cap were the ones that best conserved the ethyl octanoate after the light exposure, highlighting the bottles with amber color. Ethyl decanoate (figure 6.D) is a compound that began to decrease progressively at 3 months, mainly in the control samples, and more sharply after 6 months in all samples. Ethyl decanoate is a compound that, like the previous esters, provides pleasant fruity aromas mainly related to grapes (25). As can be seen in figure 6.D, it seems that the wines that presented a clear and green bottle with a screw cap better conserved the concentrations of this compound at all times. In the case of the control wine with amber bottle and with a cork stopper, this compound was preserved in a better way. However, it should be noted that at 6 months the wines with a screw cap and exposure to light were those with the highest concentrations of ethyl decanoate. Therefore, these two compounds (ethyl octanoate and ethyl deanoate) had a greater aromatic contribution in Albariño wines preserved in amber bottle and screw cap after 6 months of exposure.

Ethyl dodecanoate (figure 6.E) is a compound whose behavior stood out for presenting a large increase at three months, being higher in control wines. At this point (3 months), the concentrations exceeded the sensory limit well above the sensory limit in all cases, and thereafter fell drastically. And finally, isoamyl acetate (figure 6.F), which is a compound of great aromatic interest because it contributes with banana notes to wines (25). As can be seen in figure 6.F, this compound undergoes a great decrease at 6 months; however, they increase slightly at 12 months. All the control wines presented a higher concentration than the wines exposed to light after 3 and 12 months.

The other group of volatile compounds studied was those related to the oxidation processes. Wine oxidation is mainly due to the entry of oxygen through the closure of the bottle. In this process, determined by the presence of oxygen, odorous compounds are formed, such as 2-phenylacetaldehyde, 3-methylbutanal, benzaldehyde and furfural that are considered volatile oxidation markers (26, 27). They are higher aldehydes that are formed by oxidation of their corresponding alcohols. Its aromatic notes of nuts, dried herbs, honey, cooked vegetables and cooked potatoes progressively mask the fruity varietal aroma and cause a characteristic wood aroma in the most extreme cases.

Among the different volatile compounds related to oxidation process, the ones found in the wine samples were: 2-phenylacetaldehyde, methional, 2-methylpropanal, 3-methylbutanal, 2-hexenal, 2-octenal, 3-metil-2,4-nonandiona and ɣ-nonalactone. Other important compounds in the oxidative character of wine (e.g. 2-heptenal, 2-nonenal and sotolon) were not found. Figure 7 shows volatiles that obtained values above their OT. Figure 7.A shows the values obtained for 3-metil-2,4-nonandiona which has OT value about 0.016 µg/L (28) so this compound could have a remarkable effect in the aromatic oxidative notes of wines. Some aromatic notes of this compound are associated to cooked fruit, anise, dry parsley or prune passes (29). It should be noted that after 6 months this compound appeared in all samples exposed to light but did not appear in some controls. The second important oxidative compound in the studied wines was 2-phenylacetaldehyde that reached the OT value in almost all samples after 6 months of light exposure. This compound neither appeared in some control samples. The associated aromatic notes are described as past flowers and honey (28).

At lesser extent, other Strecker aldehydes, such us 2-methylpropanal, 3-methybutanal and methional could contribute to the aromatic notes of Albariño after a long oxidative period (figure 7C, D and E). The first of them did not appear until after 6 months of storage. Usually, the aromatic notes associated to these compounds (fusel, rancid, malty or baked potatoes) appear in evolved and aged wines.

3.3. Sensory Analysis

A principal component analysis (PCA) was performed with the sensory attributes that were significantly different after the ANOVA analysis performed between samples exposed to light after 12 months (figure 8). The main proportion of the accumulated explained variance by the two components was greater than 80%. As shown in figure 8, the sensory attributes with the highest incidence on the horizontal axis (which explains 58.8% of the variability) were aromatic intensity, permanence, tropical fruit and floral, located to the right of PC1, in addition to wet damp vegetal, mushrooms and evolution, located on the left side of PC1.

In general, the results showed differences between the samples according to the type of closure and bottle color. On the one hand, the wines corked by the natural cork were more evolved than the synthetic cork and screw cap, and with a higher content of aromas related to mushrooms and wet vegetables. On the other hand, reducing aromas were detected in clear bottles capped with a screw cap. And finally the wines with amber and green bottle color and with a screw cap and synthetic were associated with fresher, fruity and floral aromas. However, the wines with a screw cap and amber bottle were the ones that stand out among the rest, due to their greater permanence and aromatic intensity.

4. Conclusions

In this study, it was observed that Albariño white wine presented a great influence regarding its aromatic composition due to the time of storage. Moreover, the light exposure, the type of closure and, finally the color of the bottle clearly affects its chemical composition and sensory perception. This study checks the importance of making a correct choice of bottle color and type of closure in order to achieve a better preservation of the organoleptic qualities during the wine bottle storage both in the cellar and during its commercialization stage. In this study, the results showed that the wines exposed to light better preserve the fermentative aromas when were bottled in amber color and stopped with a screw cap. On the contrary, wines with a clear bottle and cork stopper increase oxidative aromas. However, the colored bottles (amber and green) showed a slightly higher visual browning than the clear bottles. In addition, the sensory analysis showed that wines with a cork stopper were the most evolved, while the amber and screw cap bottled wine was one of the wines with the greatest fruit and floral characteristics.

Future studies should be done in order to evaluate other type of wines, considering the OTR values or even expand the aromatic research regarding the formation of reductive aromas.

Figure captions

Figure 1. A) Absorbance values at 420 nm of Albariño wines with different bottle color (clear, amber and green) and type of closure (screep cap (SC), cork (C) and synthetic (S)) exposed to light at 3 (green bar), 6 (yellow bar) and 12 (orange bar) months. B) Coodenadas CieLab values, a* and b*, of Albariño wines after 12 months of exposure to light. Clear bottle in gray color, amber bottle in orange and green

bottle in black. Screew Cap represented with circles, cork with squares and synthetic cap with triangles.

Figure 2. Time effect on the aromatic composition of Albariño wines. Total fermentative aromas (a) and total oxidative aromas at three months (3M), six months (6M) and twelve months (12M) of storage.

Figure 3. Comparison of total fermentative aromas of Albariño wines with different bottle colors (clear, amber and green) and types of closures after 3 months (green bars), 6 months (yellow bars) and 12 months (orange bars) of light exposure. Control wines without light exposure are represented in light colors, respectively

Figure 4. Loss of total fermentative aroma content over time of Albariño wines exposed to light respect to the corresponding controls for different bottle colors (clear bottles in grey lines, amber bottles in brown lines and green bottles in green lines) and type of closures (screw cap in circles, cork in squares and synthetic cork in triangles).

Figure 5. Comparison of total oxidative aromas of Albariño wines bottled with different bottle colors (clear, amber and green) and types of closures after 6 months (yellow bars) and 12 months (orange bars) of light exposure. Control wines without light exposure are represented in light colors, respectively.

Figure 6. Individual fermentative aromas of Albariño wines (A: ethyl isovalerate; B: ethyl hexanoate; C: ethyl octanoate; D: ethyl decanoate; E: ethyl dodecanoate; F: isoamyl acetate) bottled with different bottle colors (clear, amber and green) and types of closures (screw cap in circles, cork in squares and synthetic cork in

triangles) after 12 months of light exposure (dotted line). Control wines without light exposure are represented continuous lines. The red lines indicate the perception threshold for each of the compounds.

Figure 7. Individual oxidative aromas of Albariño wines (A: 3-methyl-2,4-nonanedione, B: 2-phenylacetaldehyde, C: methional, D: 2-methylpropanal; E: 3-methylbutanal) bottled with different bottle colors (clear, amber and green) and types of closures (screw cap in circles, cork in squares and synthetic cork in triangles) after 6 months (yellow bars) and 12 months (orange bars) of light exposure. Control wines without light exposure are represented in light colors, respectively.

Figure 8. Principal Component Analysis (PCA) performed by sensory attributes that resulted significantly different after the ANOVA analysis made between samples exposed to light after 12 months.

Author Contributions: A.M. Mislata: participated on the analysis of samples, manuscript redaction and edition. M. Puxeu: performed and designed the experiment. E.N: designed and analyzed the sensory analysis. S.d.L: contributed to obtain the funding resources. M. Mestres: participated on the redaction and revision of the manuscript and R. Ferrer-Gallego: participated on the sample analysis, redaction, revision and edition of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest

References

1. Clark AC, Dias DA, Smith TA, Ghiggino KP, Scollary GR. Iron(III) Tartrate as a Potential Precursor of Light-Induced Oxidative Degradation of White Wine: Studies in a Model Wine System. Journal of Agricultural and Food Chemistry. 2011;59(8):3575-81.

2. Philipp C, Eder P, Sari S, Hussain N, Patzl-Fischerleitner E, Eder R. Aromatypicity of Austrian Pinot Blanc Wines. Molecules (Basel, Switzerland). 2020;25(23).

3. Souza Gonzaga L, Capone DL, Bastian SEP, Jeffery DW. Defining wine typicity: sensory characterisation and consumer perspectives. Australian Journal of Grape and Wine Research. 2021;27(2):246-56.

4. Dias DA, Clark AC, Smith TA, Ghiggino KP, Scollary GR. Wine bottle colour and oxidative spoilage: Whole bottle light exposure experiments under controlled and uncontrolled temperature conditions. Food Chemistry. 2013;138(4):2451-9.

5. Clark AC, Prenzler PD, Scollary GR. Impact of the condition of storage of tartaric acid solutions on the production and stability of glyoxylic acid. Food Chemistry. 2007;102(3):905-16.

6. Mattivi F, Monetti A, Vrhovsek U, Tonon D, Andrés-Lacueva C. High-performance liquid chromatographic determination of the riboflavin concentration in white wines for predicting their resistance to light. Journal of chromatography A. 2000;888(1-2):121-7.

7. Arapitsas P, Dalledonne S, Scholz M, Catapano A, Carlin S, Mattivi F. White wine light-strike fault: A comparison between flint and green glass bottles under the typical supermarket conditions. Food Packaging and Shelf Life. 2020;24:100492.

8. Díaz I, Castro RI, Ubeda C, Loyola R, Laurie VF. Combined effects of sulfur dioxide, glutathione and light exposure on the conservation of bottled Sauvignon blanc. Food Chemistry. 2021;356:129689.

9. Maury C, Clark AC, Scollary GR. Determination of the impact of bottle colour and phenolic concentration on pigment development in white wine stored under external conditions. Analytica chimica acta. 2010;660(1-2):81-6.

10. Hopfer H, Ebeler SE, Heymann H. The combined effects of storage temperature and packaging type on the sensory and chemical properties of chardonnay. Journal of Agricultural and Food Chemistry. 2012;60(43):10743-54.

11. Kim SH, Jung HJ, Lee JH. Changes in the levels of headspace volatiles, including acetaldehyde and formaldehyde, in red and white wine following light irradiation. Journal of Food Science. 2021;86(3):834-41.

12. Lan H, Li S, Yang J, Li J, Yuan C, Guo A. Effects of light exposure on chemical and sensory properties of storing Meili Rosé wine in colored bottles. Food Chemistry. 2021;345:128854.

13. Agnoli L, Outreville JF. Consumer Wine Closure Preferences: The Role of Gender, Price, and Regional Preferences. Journal of Wine Economics. 2020;15(4):412-21.

14. OIV. International Organisation of Vine and Wine. Compendium of International Methods of Wine and Must Analysis. Available online: http://www.oiv.int (accessed on 7 May 2020). In Paris, France, 2020. Vol. 1 & 2.

15. Pérez-Magariño S, González-San José ML. Prediction of red and rosé wine CIELab parameters from simple absorbance measurements. Journal of the Science of Food and Agriculture. 2002;82(11):1319-24.

16. Mislata AM, Puxeu M, Tomás E, Nart E, Ferrer-Gallego R. Influence of the oxidation in the aromatic composition and sensory profile of Rioja red aged wines. European Food Research and Technology. 2020;246(6):1167-81.

17. Benítez P, Castro R, García Barroso C. Changes in the Polyphenolic and Volatile Contents of "Fino" Sherry Wine Exposed to Ultraviolet and Visible Radiation during Storage. Journal of Agricultural and Food Chemistry. 2003;51(22):6482-7.

18. Barber N, Taylor DC, Dodd T. The importance of wine bottle closures in retail purchase decisions of consumers. Journal of Hospitality and Leisure Marketing. 2009;18(6):597-614.

19. Marin AB, Durham CA. Effects of wine bottle closure type on consumer purchase intent and price expectation. American Journal of Enology and Viticulture. 2007;58(2):192-201.

20. Echave J, Barral M, Fraga-Corral M, Prieto MA, Simal-Gandara J. Bottle Aging and Storage of Wines: A Review. Molecules (Basel, Switzerland). 2021;26(3).

21. Ghidossi R, Poupot C, Thibon C, Pons A, Darriet P, Riquier L, et al. The influence of packaging on wine conservation. Food Control. 2012;23(2):302-11.

22. Vilanova M, Genisheva Z, Masa A, Oliveira JM. Correlation between volatile composition and sensory properties in Spanish Albariño wines. Microchemical Journal. 2010;95(2):240-6.

23. Francis IL, Newton JL. Determining wine aroma from compositional data. Australian Journal of Grape and Wine Research. 2005;11(2):114-26.

24. Ferreira V, López R, Cacho JF. Quantitative determination of the odorants of young red wines from different grape varieties. Journal of the Science of Food and Agriculture. 2000;80(11):1659-67.

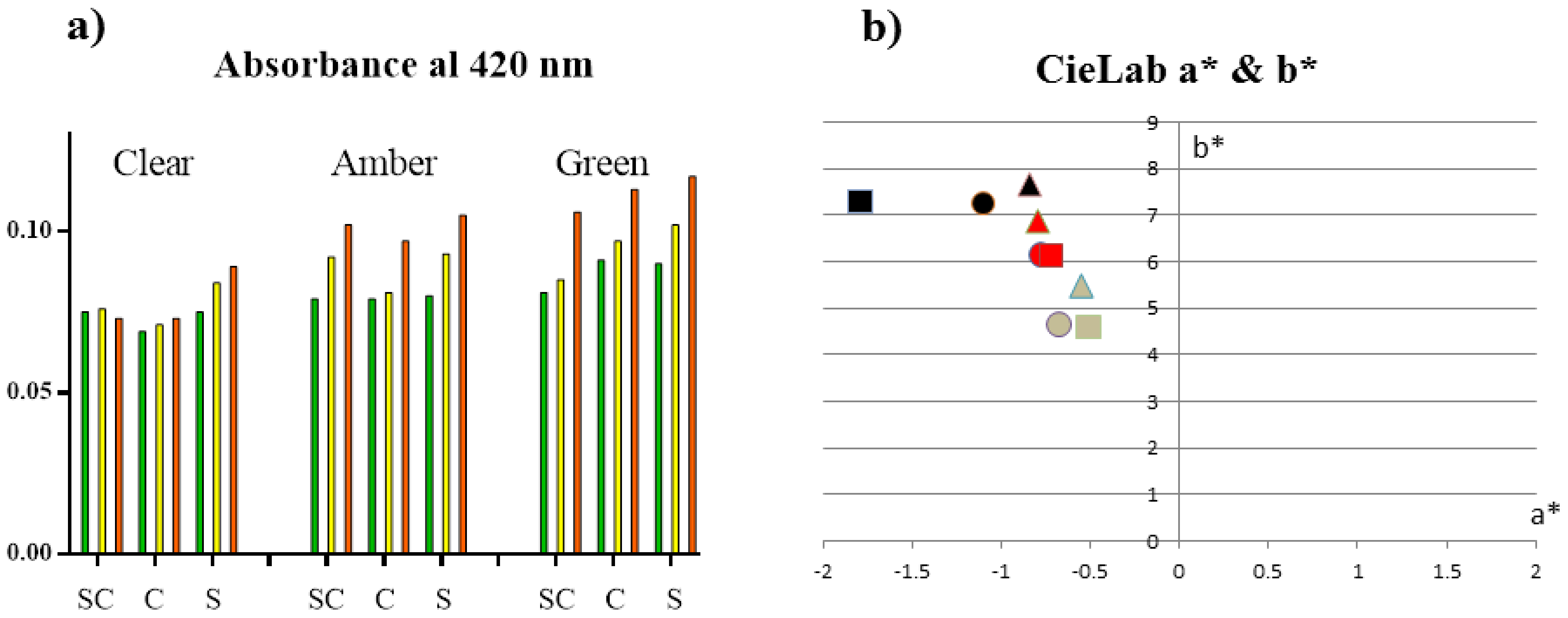
25. Peinado RA, Moreno J, Bueno JE, Moreno JA, Mauricio JC. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. Food Chemistry. 2004;84(4):585-90.

26. Escudero A, Asensio E, Cacho J, Ferreira V. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. Food Chemistry. 2002;77(3):325-31.

27. Silva Ferreira AC, Hogg T, Guedes de Pinho P. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. J Agric Food Chem. 2003;51(5):1377-81.

28. Mayr CM, Capone DL, Pardon KH, Black CA, Pomeroy D, Francis IL. Quantitative Analysis by GC-MS/MS of 18 Aroma Compounds Related to Oxidative Off-Flavor in Wines. Journal of Agricultural and Food Chemistry. 2015;63(13):3394-401.

29. Pons A, Lavigne V, Darriet P, Dubourdieu D. Role of 3-methyl-2,4-nonanedione in the flavor of aged red wines. Journal of Agricultural and Food Chemistry. 2013;61(30):7373-80.


a)
Absorbance al 420 nm
Clear
Amber
Green
0.10
0.05
0.00
SC
C
S
SC
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b)
CieLab a* & b*
b*
a*
9
8
7
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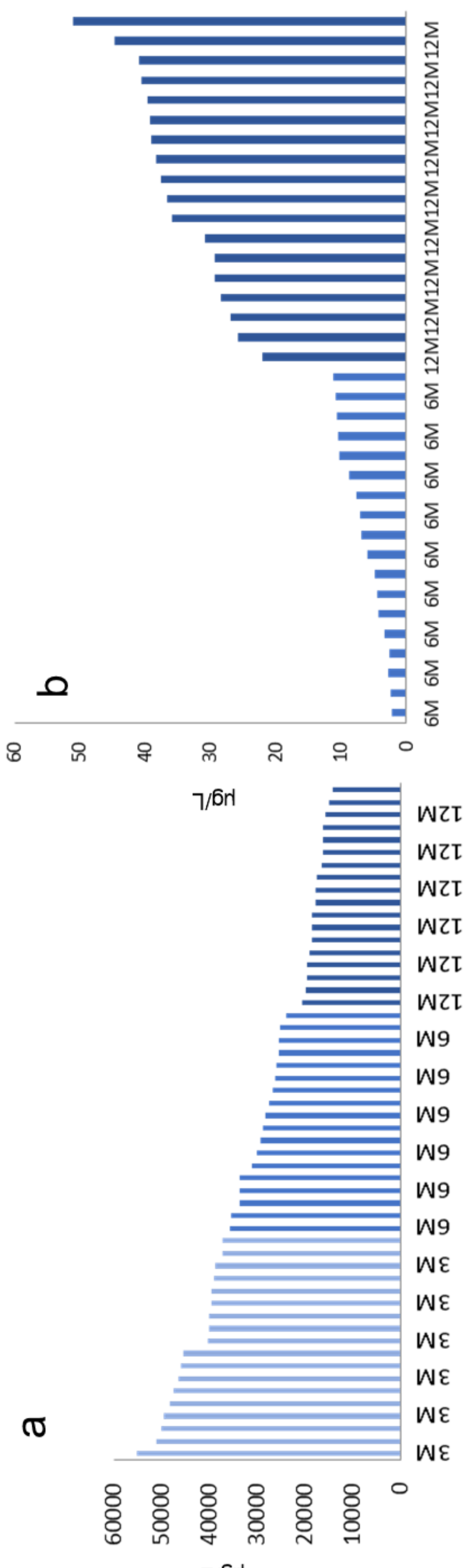
b
μg/L
60
50
40
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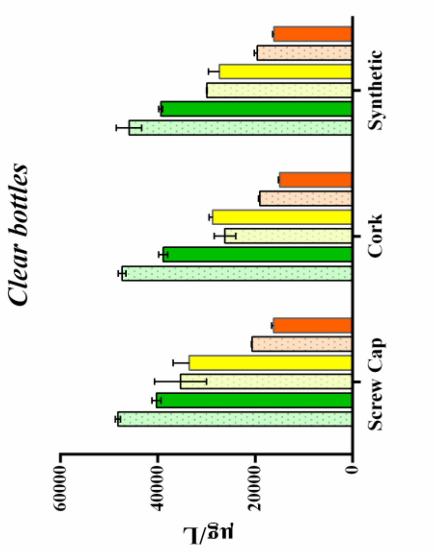
Clear bottles
μg/L
60000
40000
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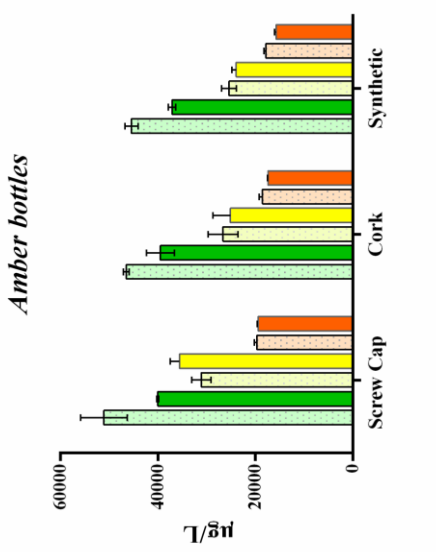
Screw Cap
Cork
Synthetic


Amber bottles
μg/L
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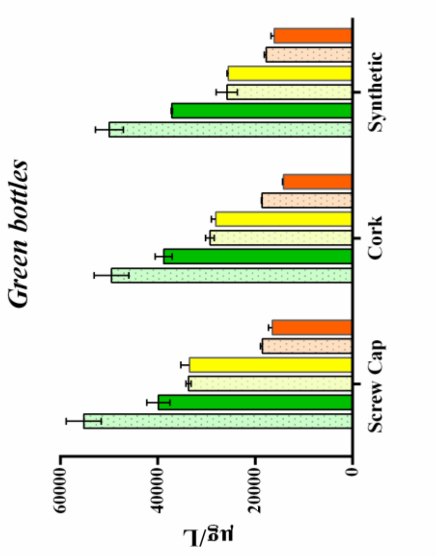
Screw Cap
Cork
Synthetic


Green bottles
μg/L
60000
40000
20000
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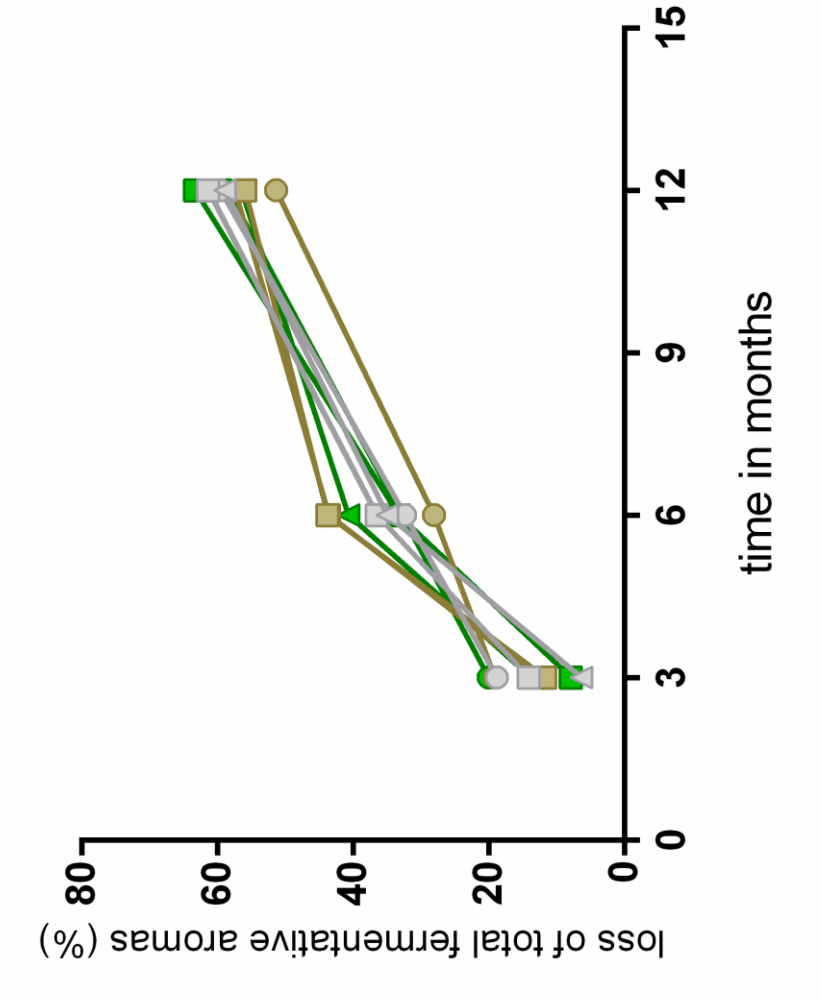
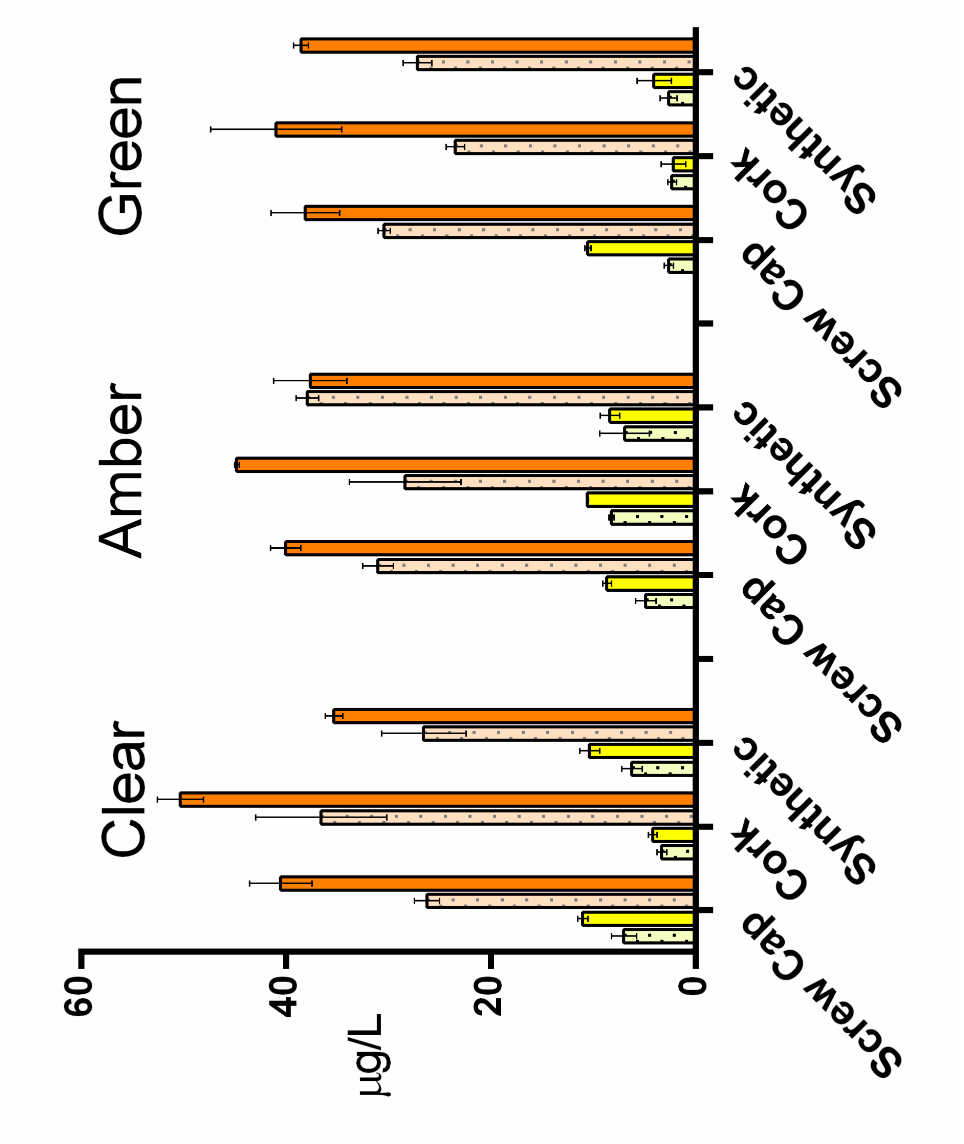
Screw Cap
Cork
Synthetic


loss of total fermentative aromas (%)
0
20
40
60
80
time in months
0
3
6
9
12
15


Clear
Amber
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Screw Cap
Cork
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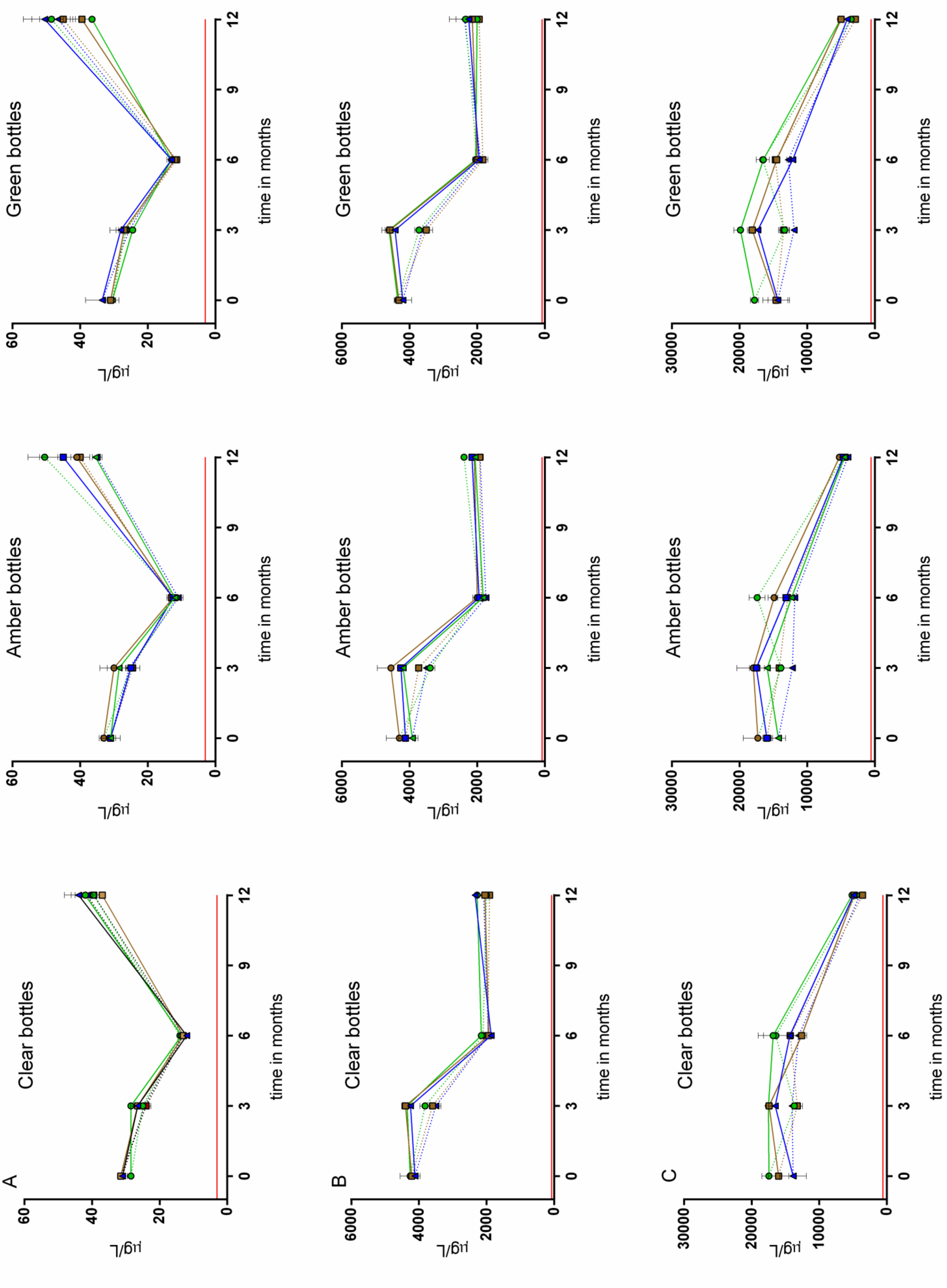
A
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Clear bottles
Amber bottles
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time in months
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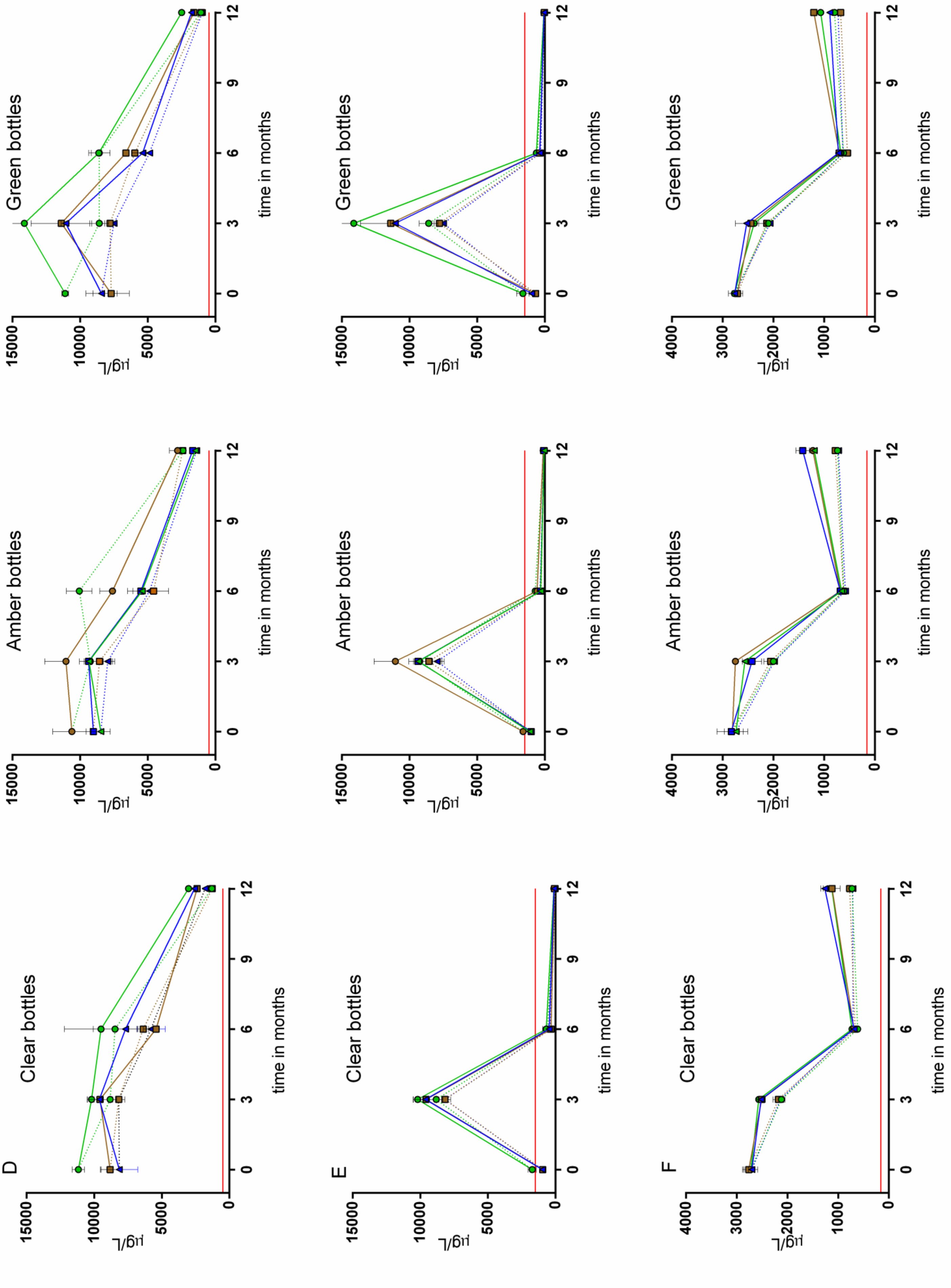
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Clear bottles
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time in months
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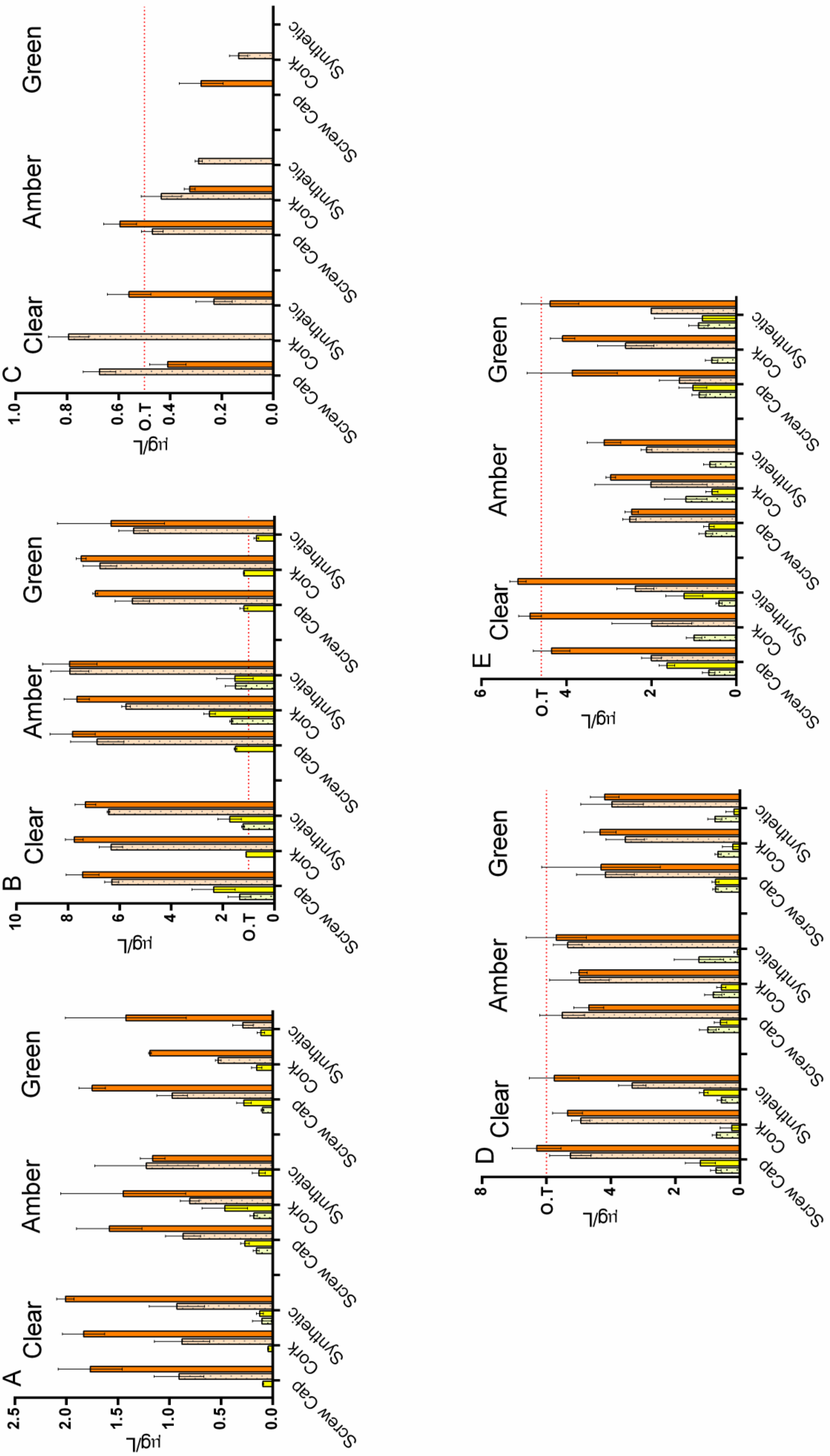
A
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Clear
Amber
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Screw Cap
Cork
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µg/L
O.T
0.0
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4
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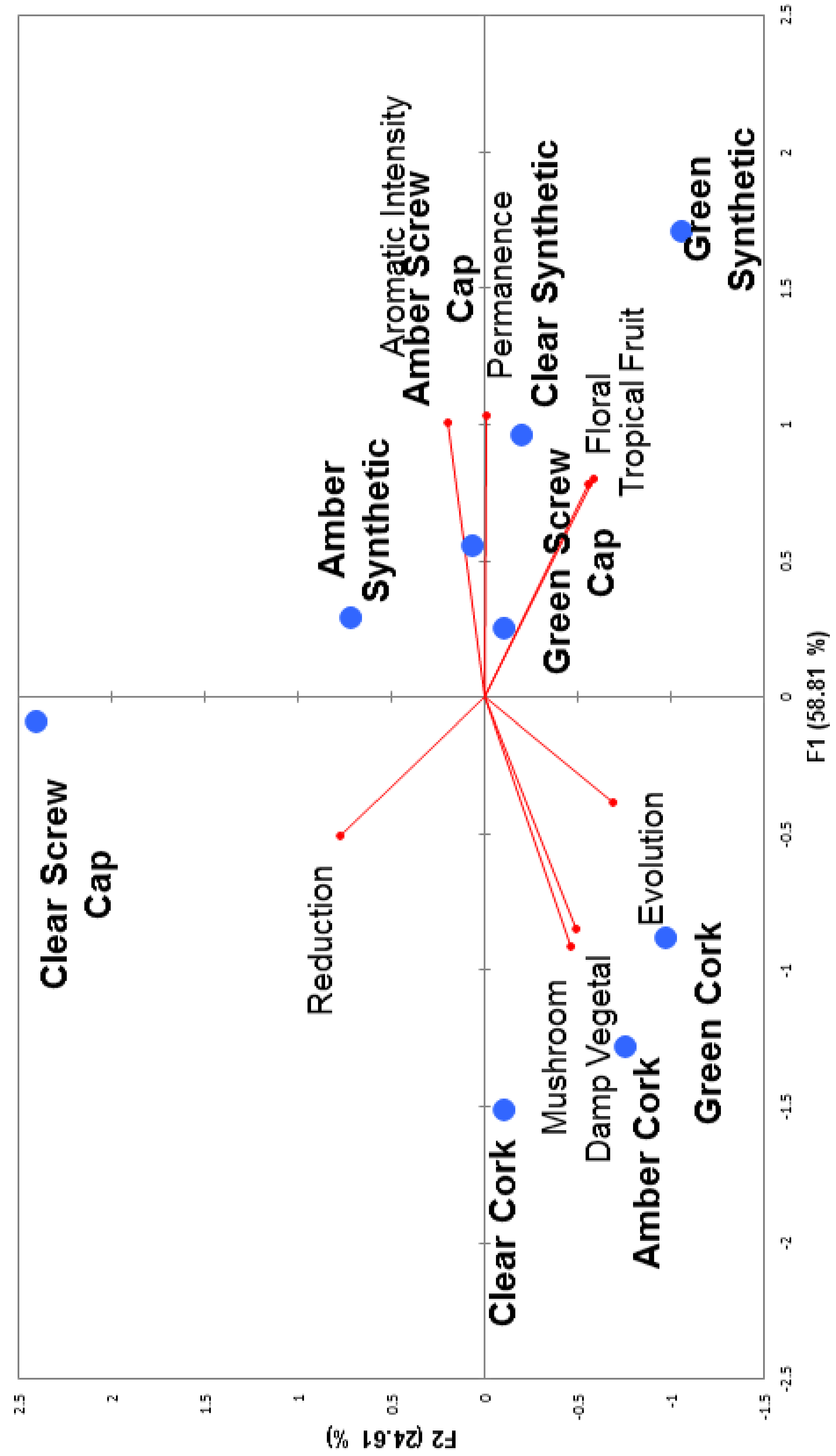
Clear Screw Cap
Reduction
Mushroom
Damp Vegetal
Clear Cork
Amber Cork
Green Cork
Evolution
Amber Synthetic
Aromatic Intensity
Amber Screw Cap
Permanence
Clear Synthetic
Green Screw Cap
Floral
Tropical Fruit
Green Synthetic
F1 (58.81 %)
F2 (24.61 %)
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5

Artículo 6

Influence of the cork stoppers and sulphur dioxide dose in the final quality of Champagne wines

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Influence of the cork stoppers and sulfur dioxide dose in the final quality of Champagne wines

Authors: Mislata, Ana María[1,2]; Rodríguez, Michelle[2]; Puxeu, Miquel[1]; Nadal, Montse[1]; Mestres, Montserrat[2]; de Lamo, Sergi[1]; and Ferrer-Gallego, Raúl*[1].

[1] VITEC- Centro Tecnológico del Vino, Ctra. Porrera Km.1, Falset, 43730 Tarragona, Spain

[2] Sensometria Instrumental (i-Sens), Department of Analytical Chemistry and Organic Chemistry Universitat Rovira i Virgili, 43007 Tarragona, Spain.

***corresponding author e-mail:** raul.ferrer@vitec.wine

Keywords: Champagne, volatile compounds, aroma, sulfur dioxide, oxygen, cork.

ABSTRACT

Storage post-bottling of Champagne is a critical stage as its quality can be affected by the evolution of volatile compounds. In this study, five types of corks with different oxygen permeabilities and three different doses of sulfur dioxide (0 mg/L, 10 mg/L and 20 mg/L) were studied at different storage times: 2 weeks after bottling (0M), three months (3M), six months (6M) and twelve months (12M). It was observed that Champagne at a dose of 20 mg/L of SO_2 affects its chromatic characteristics. The fermentation compounds did not have significant differences over time; oxidative compounds at 12M and doses 0 mg/L SO_2 had higher concentration being 2-methylpropanal and ɣ-nonalactone the main components of oxidative aromas, however, at doses 10 mg/L and 20 mg/L of SO_2 the oxidative compounds were less evolved. The reduction compounds began to be detected after three months of storage and it

increases with time, so the Champagne at 20 mg/L of SO_2 after 12M showed the highest concentration of reductive compounds but did not exceed their Odor Threshold (OT) except for H_2S. The sensory evaluation carried out at 12M scored Champagne with the highest doses of SO_2 and corks with less permeability with reductive aromas and less evolved. However, the corks with higher permeability were the best qualified at the three SO_2 doses because it preserved notes of fresh fruit, more complex and did not present acetaldehyde and reductive aromas.

1. INTRODUCTION

Champagne is the most consumed sparkling wine on the World (OIV, 2020). It is obtained by the so-called traditional method that involves a second fermentation into the bottle. This process supplies carbon dioxide, effervescence and foam to the wine. To do this, sugars, yeast and nutrients are added to wine and then bottles are stored in the cellars for some months (9 months at least). During this time flavor of the Champagnes is developed. At this point, yeast autolysis take place getting complexity and toasty and pastry aromatic notes. It is well known that aging time of Champagnes may improve its organoleptic qualities. During this aging period, the practice of riddling (remuage in French) is carried out. Finally, the lees are eliminated by the disgorging procedure (Liger-Belair, 2013). Then a dosage to replace the wine losses during the disgorging is made by the same wine or by special liquor. The dosage must not increase the alcoholic strength of the sparkling wine by more than 0.5 % vol. (Buxaderas & López-Tamames, 2012). After disgorging the Champagne is corking and prepared for its commercialization. However, before consumption, Champagne may suffer chemical reactions involving a loss of quality (Martínez-García et al., 2021). Some authors have reported changes in color and aroma composition of sparkling wines at post bottling. Significant oxygen enrichment occurs during disgorging and the uncontrolled conditions of storage, such as temperature or light exposure, may devalue the final quality (Benucci, 2020).

Volatile composition plays an important role in the organoleptic properties of sparkling wines and consequently in consumer acceptability (Caliari, Panceri, Rosier, & Bordignon-Luiz, 2015). Several volatiles increase their concentration during aging and can be used as aging markers (Ubeda et al., 2019). The addition of sulfur dioxide (SO_2) to sparkling wines is traditionally used for preventing oxidation and to protect wine oxidation (Webber et al., 2017). However, wine industry tends to reduce the use of this antioxidant due to the reported adverse reactions in humans, including intolerances and allergies (Vally, Misso, & Madan, 2009). For this reason, reducing the amount of SO_2 in sparkling wines is a decisive strategy for the wine industry and one of the current topics on the oenological science. Actually, the demand of wine with low amounts of sulfites is better accepted for consumers, who prefers less processed and more organic wines (Costanigro, Appleby, & Menke, 2014). Some alternatives are being sought to supply this demand by new preservative agents or stabilization techniques (Ferrer-Gallego, 2018). Scientific results evidence the possibility to reduce significantly the SO_2 addition at different stages of winemaking, such as bottling (Valera, Sainz, Mas, & Torija, 2017; Webber et al., 2017). The reduction of this antioxidant in Champagne can contribute to a better acceptability for consumers.

Cork stoppers have been used since ancient times thanks to their properties; sealing capacity, inertness towards the wine, durability along storage time and possibility of removal with adequate effort (Pereira, 2007). In general, the cork stoppers used in Champagne are cylindrical assembled by agglomerated or micro-agglomerated cork and natural cork discs. A well-suited closure should scarcely interact with the wine and should correctly regulate the oxygen exchange with the external atmosphere, ensuring its excellent conservation and the gradual evolution during the aging in bottle. Cork is an extraordinary material for the closure of wine bottles, its characteristics allow an adequate balance of oxygen transfer (Oliveira, Knapic, & Pereira, 2015; Sánchez-González & Pérez-Terrazas, 2018). However, corks that allow a high transfer of oxygen can be harmful in insufficiently sulfited wines. The adjustment of the SO_2 dose in Champagne according to the cork used is scarce and can contribute to avoid significant economic losses for wineries related to the loss of quality. Therefore, the objective of this work was studying the influence of cork stoppers with different Oxygen Transmission Rate (OTR) in Champagnes sulfited at different doses. For this, the evolution of both volatile composition and sensory characteristics were evaluated over time.

2. MATERIAL AND METHODS

2.1. Wine samples

Champagnes made from Chardonnay and Pinot Noir varieties were disgorged in VITEC (Wine Technology Center) facilities. Sulfur dioxide content was adjusted at 10 mg/L and 20 mg/L of free SO_2 by means of sulfur dioxide aqueous solution (Sulphur 18, Agrovin S.A, Spain). Champagne without any addition of sulfur dioxide was also evaluated. Finally, bottles were corking with five corks (C1, C2, C3, C4, C5) with different oxygen permeability (figure 1), and by a crown tap as control. Wines were stored in a controlled room temperature 15 ºC ± 1. The oenological and volatile compounds analysis was carried out throughout different storage times, after 15 days of bottling (0 M), and after 3, 6 and 12 months (3M, 6M, 12M). Treatments were performed by triplicate.

2.2. Reagents

All reagents used were of analytical quality (Sigma-Aldrich); 2-octanol (≥ 96%), 3-hydroxy-4, 5-dimethylfuran-2(5H)-one (sotolon) (≥ 97%), methional (≥ 97%), 2-phenylacetaldehyde (≥ 95%), 2-methylpropanal (≥ 99%), 3-methylbutanal (≥ 97%), (E)-2-hexenal (≥ 95.%), (E)-2-heptenal (≥ 95%), (E)-2-octenal (≥ 95%), (E)-2-nonenal (≥ 95%), (E) 2-propanal, 2-methyl-2, 4-nonanedione, ɣ-nonalactone (≥ 98%), 2-methylpentanal (98%), thiophene, (E)-2-propanethiol, methylethylsuphide, ethyledeniaminetetracetic (EDTA) and PFBHA: O-(2,3,4,5,6- pentafluorobenzyl) hydroxylamine hydrochloride (> 99%) (derivatizing product), Thiophene (≥ 98%), 2-propanethiol (≥ 98%), and Methylethylsuphide (≥ 98%). The following products and solvents were also used in the previous preparation of the samples to analyze the oxidative aromas; ammonium sulphate (for analysis of ACS, ISO, by Panreac), ethanol absolute, and dichloromethane anhydrous (> 99.8%) by Sigma-Aldrich.

2.3. Oenological analysis

All basic oenological parameters of Champagne were carried out according to the methods recommended by International Organization of Vine and Wine (OIV) pH, total acidity (TTA), volatile acid and alcohol content was done with FTIR (WinescanTM SO_2 by Foss, Hilleroed, Denmark) internally calibrated according to OIV. Total pressure, CO_2 Pressure and dissolved CO_2 with laser spectroscopy (L. sensor CO_2). Color intensity and chromatic characteristics coordinates (CIELAB) were measured by spectrophotometer (Thermo Fisher Scientific,Waltham, MA, USA). Also, Malic acid (OIV-MA-AS313-11), acetic acid (OIV-MA-AS313-04) and lactic acid were carried out with OIV Methods. SO_2 free and SO_2 total determinate by OIV method (OIV-MA-AS323-04A)

2.4. Analysis of the volatile composition related to fermentation aromas by GC-MS

The analysis of the fermentative aromas was carried out according to (Mislata, Tomás and Ferrer, 2020). Samples were extracted by solid phase microextraction (SPME) and then were separated and analyzed by gas chromatography coupled with mass spectrometry detection (GC-MS). 10 mL of sample were placed in 20 mL headspace vials together with 2.7g of NaCl and 100 µl of 2-octanol (1.000 ppm) as internal standard.

A gas chromatograph GC 7890 (Agilent Technologies) equipped with a mass spectrometer 5975C inert MSD (Triple-axis detector with electron impact source) was used. The separation was carried out with a column DB-WAX IU (60 m x 0.25mm x 0.25µm, Agilent). Helium was used as carried gas with 2 mL/min of constant flow. Finished the extraction time, the fiber (DVB/CAR/PDMS) was exposed for 2.5 minutes in splitless mode at 270 ºC. The oven temperature ramp was started at 40 ºC which was maintained for 2 minutes and was increased at 5 ºC/min up to 225 ºC and maintained for 15 min. The ionization source temperature was 220 ºC and the interface remained at 280ºC. It worked with the source of electron impact (EI), the mass analyzer operating in Scan and SIM (single-ion monitoring) mode. The volatile compounds were identified by comparison of the mass spectra, with the help of the NIST library. The quantification was carried out by internal standard method (IS). The individual volatile compounds were quantified with the factor of 2-octanol in µg/L. All analyses were carried out in triplicate and were quantified 18 individual compounds.

2.5. Analysis of the volatile composition related oxidative aromas by GC-MS

For the isolation of oxidative volatile analytes was carried out according to (Mislata, Tomás and Ferrer, 2020). The sample preparation is made up of the derivatization and formation of the corresponding oxime. In 20 mL of wine 20µL of 2-metilpentanal (1.000 ppm) were added. The solution was equilibrated using a magnetic stirring for 15 min at 700 rpm. Then, 1 mL of an aqueous solution of and PFBHA: O-(2,3,4,5,6- pentafluorobenzyl) hydroxylamine hydrochloride was added as derivatization reagent, previously this solution was prepared in methanol/water 50/50% (v/v) and stirred during 15 min. After that 3 g of ammonium sulfate were added and the samples were immediately passed through a solid phase extraction (SPE) cartridge from Agilent (Bond Elut ENV, 500 mg and 6 mL). The cartridge has been previously conditioned with 5 mL of dichloromethane to conditionate, 5 mL of methanol to clean up, and 5 ml of a model wine like solution (15% (v/v) ethanol, 5 g/L tartaric acid, adjusted at pH 3.3 with NaOH). Then, once the sample has been added to the cartridge, 6 mL of 0.05 M of sulfuric acid solution were added to eliminate the excess of the derivatization reagent. Finally, the analytes were eluted with dichloromethane (12 mL), collected and dried using SpeedVac (Thermo Fischer) concentrator vacuum at 35ºC. The final volume of samples was 200 µl.

GC analysis was performed on a GC 7890 A (Agilent Technologies) system equipped with a mass spectrometer 5975 inert MSD (with Triple-Axis detector) and with a VF-200 ms column (30 m x

0.25 mm x 0.25 µm, Agilent). A constant flow of 2.1 mL/min of helium (He) was used as carried gas. Five microliters of sample were injected in splitless mode with 16.95 psi pressure (septum purge flow 3 mL/min and splitless time 1 min). The injector temperature was maintained at 180 ºC for 1 min and then heated up 260 ºC at 250 ºC/minute. The temperature of the oven (40 ºC) was maintained for 1 min and then increased up to 220 ºC at 10 ºC/min, and finally up to 270 ºC (100 ºC/min). Following each analytical series, the temperature was held to 270 ºC, washing for 7.24 min at 25 psi. The transfer line temperature was 240ºC and 1.5 mL/min flow of the carrier gas (He) The mass spectrometer operated at 70 eV electron ionization mode. The analysis was performed in scan mode (m/z 50-350). The compounds were identified by their retention times and mass fragments. The quantification was carried out using the method of internal standard patterns. They were prepared in individual ethanol solutions at 1000 ppm. The mixed solution containing all compounds were prepared to elaborate the standard calibration curves of each compound. All analyses were carried out in triplicate and were quantified nine individual compounds.

2.6. Analysis of volatile composition related to reduction aromas by GC-FPD

This analysis was carried out with modification according to the method used by (Mestres, Busto and Guasch, 2000). Before handling the samples, the wine was refrigerated for 24 hours in a cold room at -4ºC, that permitted the sample to kept very cold to be handled, this manage prevent the loss of volatile compounds at room temperature. During the preparation of the sample, the wine was placed in a dry ice bath under stream of nitrogen gas to provide an inert atmosphere. Under this atmosphere, in a headspace which contained 2.7 g of NaCl and 0.15 g of ethyledeniaminetetracetic (EDTA) were added 10 ml of wine together with 100 µL of 2-propanal and 100 µL of a mix of internal standards (Thiophene, 2-propanethiol and methylethylsuphide) with a concentration of 100 µg/L.

The samples were analyzed by solid phase microextraction (SPME) and a GC 7890A gas chromatograph (Agilent Technologies) equipped with a FPD detector (Flame photometric detector, Agilent Technologies) was used. The extraction of the sulfur compounds was carried out with fiber type (Carboxen/PDMS) of 85 µm. Before extraction, the samples were incubated for 20 minutes at 35ºC with shaking, automatically in the GC. The fiber injection was carried out in the TDU (Therma desorption unit) port coupled to a cold equipment, making a temperature ramp from -60ºC to 260ºC, this ramp allowed the retention of extremely volatile sulfur compounds. The separation was carried out using a HP-1 Column (30 m x 0.320 mm x 4.00 µm, Agilent

Technologies), with helium as carrier gas at a flow rate of 2.09 ml/min. This oven performed a temperature ramp from 35ºC to 260ºC for 8 min. The detector was set at 200 °C and the flame was fed with a flow of 75 ml/min of hydrogen and 86 mL/min of synthetic air. The quantification was carried out by means of calibration lines with reference standards of the compounds to be determined, and the addition of internal standard (IS). The results of the volatile compounds were expressed as quantitative data in µg/L as a response with respect to the internal standard. All analyzes were done in triplicate. Seven compounds were quantified of three different families.

2.7. Statistical analysis

Principal Component Analysis (PCA) was carried out using Xlstat 2021 statistical software (Microsoft Ibérica, Barcelona, Spain) add-on for Microssoft Excel package (Microsoft Ibérica, Barcelona, Spain) and significant differences between time, corks and dose were determined by one-way ANOVA using the Tukey´s HDS (Honestly significant difference) test, at 95% of confidence level. GraphPad Prism 9.1.0 (GraphPad, California, United States) were used to represent the graphics for results.

2.8. Oxygen Permeability

Permability of all cork stoppers used were studied by MOCON system. The oxygen transmission rate (OTR) is a value commonly measured in different industries to evaluate oxygen flow through all types of packaging. OTR is globally accepted and is recommended in different standards such as the ASTM (F1307-02). The Mocon® Ox-tran® method (Mocon Inc., Minneapolis, MN, USA) were used by measuring oxygen transmission rate in food films and cork. The stabilization of the measurement takes time and only a limited number of samples can be analyzed, these it is the main disadvantage of this methodology offset by high accuracy.

Studied cork stoppers were used to close glass champagne bottles, after that, bottle neck were cut and stuck in a copper plating with a pair of tubes, one enters the carrier gas, and other drags the oxygen stored in head space to the detector. OTR it is expressed in cubic centimeters of oxygen each closure and day (cc3/cork x day). As a carrier gas formigas were used, being a mixture of nitrogen and hydrogen, respectively. Temperature set point in all the test were 23ºC, relative humidity 40,7%, permeant concentration 21% of oxygen, test was done in continuous mode with 30 minutes of conditioning.

2.9. Pressure and CO_2 analysis

Total Pressure, Total Carbon Dioxide pressure and dissolved carbon dioxide were analyzed by non-destructive methodology L-Pro Gases Sensing (FT System L-Pro Division, Mestrino PD, Italy). L-Pro sensor is the electronic evolution of the traditional mechanical aphrometer, designed for the wine sector providing the results in a non-destructive way and based in laser spectroscopy.

3. RESULTS AND DISCUSSION

Figure 2 shows a principal component analysis (PCA) performed with all the analytical parameters evaluated: thirteen basic oenological parameters, two color parameters, Cielab coordinates and volatile composition. When dealing with this last parameter, 34 compounds were analyzed which were divided into three chemical families: fermentative family, oxidative family and reductive family. The results were obtained considering all the studied corks (Control, C1-C5), all the doses of sulfur used in bottling (0, 10 and 20 mg/L) and all analysis times from 15 days after bottling (time 0 months) to 12 months. As can be seen in Figure 2, the time factor is the variable with the greatest weight in the distribution of the samples since these are distributed in the PCA in four well-differentiated groups, 0M, 3M, 6M and 12M.

3.1. Oenological analysis

The results obtained of the oenological parameters from 0M to 12M with their mean and standard deviation (SD) are shown in Table S1. All the Champagnes presents glucose and fructose values below 1.3 g/L, considerate in the category of brut nature (0-3 g/L) (McMahon et al., 2017). The volatile acidity and acetic acid remained below 0.46 g/L throughout all the analysis times, which indicates that the champagnes present a correct evolution over time, which is crucial for sparkling wines quality (Tufariello et al., 2021). Total acidity concentration ranged from 8.44 g/L to 8.61 g/L, and the average pH of the Champagnes was 2.88, similar as the study of (Ubeda et al., 2019a) who evaluated the evolution of sparkling wines during aging. Both parameters showed the high and suitable acidity that the samples maintained throughout the conservation time in bottle. The lactic acid had no significant different, presenting values around 1.57 g/L. Malic acid showed values between 2.50 g/L (0M) to 2.20 g/L (12M). Alcohol content ranged from 11.93% to 12.31% (v/v) and free SO_2 presented values < 10 mg/L. The storage conditions after bottling significantly reduced the total SO_2 from 72.6 mg/L to 62.39 mg/L (Table S1), what agrees with the results presented by (Torrens et al., 2010) and that could be related to the evolution of reductive compounds. Finally, it was observed an increase of color at Abs420nm that implies that the color yellow raised over the time of storage as already reported by (Benucci, 2020). Clarity (L*) did not significantly present difference, but a* and b* color has more evolved over the time of storage.

3.2. Volatile composition

3.2.1. Fermentative compounds

Among the different volatile compounds related to fermentation aromas, a total of 18 compounds were selected that represented the four most abundant families: esters, acetates, alcohols, and fatty acids. As shown in Table S2-S5, these compounds were analyzed in champagne with the five different corks and crown cap as control, at all its doses of SO_2 used in bottling, after the four analysis times, 0, 3, 6 and 12 months of storage. Figure 3 shows the evolution of the samples with the different types of corks and doses of sulfur over time per families.

Esters are the main family due to their greater contribution in compounds during the fermentation of wines, and mainly due to their aromatic contribution, since they have pleasant aromatic descriptors related to flowers and fruits (Ferreira, López and Cacho, 2000). As can be seen in figure 3a, in general the esters show a decreasing trend in concentration from 0M to 12M, although it seems that after 6M there is a slight increase. In addition, the Champagnes that were bottled with the highest dose of sulfur (20 mg/L) presented better preservation of the esters over time. Ethyl octanoate was the compound that evolved and kept it concentration during time of storage, been the compound with major concentration (Table S5), above their detection thresholds in all times (Table S6) followed by ethyl hexanoate. Ethyl octanoate at 6M presented a slight decrease in all corks, however after 12M there was a slight recovery of it. C3 and C4 were the corks at 10 mg/L of sulfur dose that presented more concentration of esters, ethyl octanoate especially at 12M. C4 at 20 mg/L was the cork that had major concentration of ethyl octanoate and ethyl decanoate with no significant different between times of storage. These compounds are characteristic for providing aromatic notes of pineapple, pear and grape (Ferreira, López and Cacho, 2000; Peinado et al., 2004).

The acetates family is characterized for presenting aromatic compounds related to fruit as pear, apple, cherry, or banana. However, when their concentration is too high, the aroma can change and even become unpleasant (terminal threshold). This is the case of ethyl acetate that present pleasant sweet aromas at low-normal concentrations but at higher concentrations it can provide unpleasant aromas related to solvents and glue (Guth, 1997). As can be seen in figure 3b, in general the acetates show the same trend over time regardless of the dose of sulfur used. From 0M to 3M they increase, at 6M they decrease and at 12M they increase again, all slightly.

In the case of ethyl acetate, at 0M and 3M exceed the odor threshold, except in 6M that decrease below it, except C4 at 20 mg/L. At 12M this compound had a slight gain of concentration, this increment makes a significantly difference between the first months and corks. Isoamyl acetate is the second compound of this family that the concentration was above the sensorial limit, despite the concentration decrease in the course of time. Isoamyl acetate usually derived from yeast metabolism during alcohol fermentation and contributes to a banana-like note (Cotea et al., 2021). As shows in figure 3b. C4 had the highest concentration of acetates regarding the other corks. Ethyl acetate and isoamyl acetate have a great effect on the quality of the aroma of sparkling wines (Mamede, Cardello and Pastore, 2005).

About alcohols, this family is aromatically important for providing floral aromas to wines (Peinado et al., 2004). As observed in figure 3c. alcohols had the same behavior over the time at the three doses. In general, they decreased slightly to 6M and increased again to 12M obtaining concentrations like those of 0M and 3M. At 3 months C4 had the highest concentration of alcohol at the three doses, but at 6M decrease and was C3 that presented the highest value. Isoamyl alcohol is the compound that had a significant evolution over the time. At 0 mg/L after 12 months of storage C2 had the highest concentration of isoamyl alcohol above the other corks and control.

However, at 3M with 10 mg/L of SO_2, the composition of alcohols increased and C4 was the cork with the highest concentrations of alcohols due to the high values of isoamyl alcohol and Isobutanol (Table S3). The same trend occurred in 6M but with lower concentrations. After 12M the alcohols increased slightly, being C1 and C3 the corks with the highest concentration of isoamyl alcohol and 2-phenylethyl alcohol. Some authors have observed that at 12 months of aging in contact with lees, 2-phenylethanol did not change significantly (Ubeda et al., 2019b), in this study during there are no significant difference between corks and doses during 0M-3M, but at 6M there are difference between corks and doses, the same at 12M. 2-phenylethanol and benzyl alcohol increase their amount during aging and related with yeast metabolism (Torrens et al., 2010). Isoamyl alcohol showed concentrations above its odor threshold (Table S6).

Finally, the family of fatty acids which is made up of compounds that are produced in the lipid metabolism of yeast and the fermentation conditions has a relevant role for the formation of this volatile compounds (Pérez Olivero and Pérez Trujillo, 2011). In this study were analyzed hexanoic, octanoic and decanoic acids. These compounds are responsible for the rancid and cheesy aromatic notes of wine (Caliari et al., 2015). As can be seen in figure 3d, in general the fatty acids behaved in the same way over time regardless of the doses of sulfides used in bottling, showing a slight increase after 3M and a quite pronounced drop in concentration at 12M.

In general, decanoic acid had the lowest concentration in contrast with hexanoic and octanoic acid at 12M (Table S5). Same behavior of these compounds was reported before (Ubeda et al., 2019a; Cotea et al., 2021) during aging with lees. However, it should be noted that none of the three compounds exceeded their sensory limits in any of the cases studied.

3.2.2. Oxidative compounds

A total of 9 compounds related with oxidation were identified in samples of Champagne, belonging to the families of alkenals, acetates, ketones, and lactones respectively as show in Table S2-S5. Figure 4 shows the evolution of the samples with the different types of corks and doses of sulfur over time.

Alkenals are long-chained aldehydes as (E)-Hexanal, (E)-octanal and (E)-nonenal and these usually increase their concentration in wine exposed to oxygen (Mayr et al., 2015). In addition, they are characteristic compounds because they add aromas of apple, walnut, sawdust, or fat to wines. All these compounds were identified and quantified in this study. As shown in Figure 4a, alkenals showed the same tendency at all doses of sulfur, showing an increase in their concentration over time. It should be noted the large increase produced from 6M to 12M. In general, the samples without addition of SO_2 in bottling presented higher values than the samples with addition of sulfites.

At a dose of 0 mg/L of SO_2, it was observed that at 3M and 6M the control and C5 had the highest concentrations of the three compounds (Table S3 and Table S4). However, at 12M, the C2 cork obtained the major concentration of alkenals above their OT. This behavior of the C2 cork was also observed at doses at 10 and 20 mg/L of SO_2. In general, this large increase in alkenals observed over time was influenced by the high concentrations octanal. There was significant difference between the variables under study. In addition, it should be noted that the C4 cork presented the lowest concentration in the sulfur doses of the study. Some aldehydes such as 2-methylpropanal, 3 methyl butanal, 2-phenylacetaldehyde and methional have a great and important impact on the sensory profile of aged wine. Methional, based in studies, is the key odorant to contribute to the oxidative off-flavors in wine like cooked vegetables (Escudero, Cacho and Ferreira, 2000) or cooked potatoes-like (Mayr et al., 2015)

As shown in figure 4b the concentration of aldehydes began to increase drastically from 0M to 3M, at 6M stay or decrease to 12M. It should be noted that at 12M the samples with doses of 20m/L of sulfur presented lower concentrations of aldehydes compared to the other doses.

At 0 mg/L of SO_2 the cork C3 had the highest concentration of 2-phenylacetaldehide between the rest corks at 0M and 6M (Table S2-S4). Over the time at 3M this compound decreased and the values of 2-methylpropanal, 3-methylbutanal increased and appeared at 3M methional over it odor thresholds. The concentration of this compounds decreased at 12M been C4 the cork that presented the main concentration of 2-methylpropanal; and C2 increased the concentration of 3-methylbutanal, 2-phenylacetaldehide and methional. While at dose 10 mg/L of SO2, corks C1, C3 and C4 at 3M got the highest concentration of aldehydes, and at this time of storage methional was detected with values above its odor threshold. At 6M the concentrations of 2-phenylacetaldehyde and 3-methylbutanal decreased and showed significantly differences between time and corks at 12M. C2 presented values above the rest of corks due to the high concentration of 3-methylbutanal (18.2 µg/L) at 12M. At 20mg / L dose of SO2, C4 had the highest aldehyde concentrations compared to the other corks up to 6M. 2-methylpropanal was the main compound responsible for the increase of aldehydes with this cork, at 12M it decreased significantly. At 12M, the C1 and C5 corks presented the highest aldehyde values being 2-methylpropanal the compound with highest concentration at 12M.

Finally, there are the families of ketones and lactones which are produced during fermentation, but few appear to have sensory significance (Jackson, 2020). The presence of 3-methyl-2,4-nonandione (ketone) and ɣ-nonalactone (lactone) are characteristic as oxidation markers, specially of red wines, that appeared on the first stage of the oxidation. They are linked to aromatic notes of cooked fruit, anise, dry parsley, prune passes and fig passes, peach, coconut, respectively (Mislata, Tomás and Ferrer, 2020).

As can be seen in figure 4c, Champagnes at dose 0 mg/L SO2 had initial concentration of 3-methyl-2,4-nonandione that exceeded the OT. Over the time, the corks at this dose had different behavior. At 3M, the concentration of this compound got the highest value of all the study times, where at this time only two corks (C1 and C4) had the highest concentration of this ketone (Table S3). C4 with 0 mg/L of SO_2 maintained a high concentration of 3-methyl-2,4-nonandione until 12M. At 10 mg/L of SO_2 the dynamic of dispersion between corks was different than the concentration of sulfur explained before, over the time until 12M the concentrations of the compounds decrease getting an average of 1.5 µg/L. With sulfur at 20 mg/L, the corks (including the control) increased the concentration compound at 3M, then started to decrease at 6M except C2 with an average of 2.8 µg /L, but at 12M decreased and the rest corks got a slight increase and the average at this time was the same as the dose explained before, 1.5 µg /L of 3-methyl-2,4-nonandione. In the case of ɣ-nonalactone the OT is 60 µg/L, but in this study none of the samples exceeded this value. As figure 4d shows, at dose 0 mg/L the initial concentration was over 10 µg/L in all corks. Over the time, this compound increased or maintained their values until 12M depending on the cork. C1 (24 µg/L) and C4 (27.4 µg/L) had the highest value of ɣ-nonalactone. At dose 10 mg/L SO2, C1 increased over the time and it concentration was the greatest over the rest corks. As observed in figure 4d at dose 20 mg/L, C1 has the lowest concentration of ɣ-nonalactone. The control and C3 decreased their concentrations at 3M while the other corks increased their values to 6 M, keeping the concentration constant until 12M. C2 (17.4 µg/L) and C4 (16.5 µg/L) had the highest value of ɣ-nonalactone compared to other corks. It should be noted that in general champagnes with a dose of SO_2 in bottling keep lowest concentration of ketones and lactones low.

3.2.3. Reductive compounds

The volatile sulfur compounds in wine can have a significant impact on wine aroma attributes and wine quality. Reduction is a term often used in winemaking to identify unpleasant aroma properties as reminiscent of rotten egg, cabbage, garlic and putrefaction, this maybe caused of different low molecular wight sulfur compounds. (Ugliano, 2013). The sulfur compounds found in wines are classified in five different families according to their structure: thiols, sulfide, polysulfides, and thioesters compounds (Mestres, Busto and Guasch, 2000). In this study, seven compounds were identified which were grouped into three families: thiols, sulfides, and disulfide.

The family of thiols usually is assumed to be primarily responsible for the formation of reductive aromas post-bottling. Some authors suggested that the high concentration of cysteine in the wine could increase the accumulation of hydrogen sulfide (H2S) in the wine after bottling (Ugliano, 2013). Also, the production of excess H2S can potentially lead to the formation of other sulfur-containing compounds such as methanethiol (MeSH) and ethanethiol (EtSH) (Siebert et al., 2010; Bekker, Wilkes and Smith, 2018). In general, figure 5 shows how the reduction aromas increase over time, mainly after 6M.

As is shown in figure 5, in all doses at 0M and 3M has no presence of thiols. However, from 6M there was a great growth in the concentration of thiols up to 12M. H2S was the main compound of thiols detected in this study with values above its OT. It should be noted that at higher doses of sulfur added in the bottling of champagnes, higher values of thiols were produced, mainly at 12M. The crown cap (control) had the highest concentration of thiols as can be seen in figure 5a, corresponding at 0 mg/L of sulfur.

H_2S and MeSH were detected since 6M, where all corks, including control, had presence of H2S (Table S4). At 0 mg/L C1 (0.9 µg/L) and C3 (1.0 µg/L) did not exceed the OT of H2S (TableS8). At 6M Control (14.0 µg/L) and C2 (9.2 µg/L) had the highest value of this compound of H2S. MeSH was detected but did not exceed it OT. At 12M, the Champagne with the corks C1 (14.5 µg/L) exceeded the OT, and the control (19.7 µg/L) increased it concentration of H2S. However, C3 was the cork with less amount of this compound. At 6M, MeSH was only detected in control and C1 but, did not exceeded the OT, except C3 (0.5 µg/L). EtSH was not detected at this dose. H_2S had major concentration at dose 10 mg/L. Control (17.8 µg/L), C2 (10.4 µg/L) and C3 (10.9 µg/L) presented the highest values of this compound at 6M, all corks exceeded the OT. Control and C1 had presence of MeSH over it limit perception. Until 12M H_2S increased significantly in champagnes with C1 (15.1 µg/L) and C4 (14.1µg/L), at this time all corks exceed the OT. Over time, control and C1 decreased it concentration of MeSH. Despite, at this time increased the amount of MeSH in C2-C5.

At dose 20 mg/L of SO_2, the corks started to elevate their concentration of thiols from 6M, mainly the closures control (19.5 µg/L H2S - 3.9 µg/L MeSH), followed by C1 (9.3 µg/L H_2S – 3.1 µg/L MeSH). All corks exceeded their OT of H_2S, except MeSH that was only detected in the corks mentioned before. At this time EtSH was detected at small amount in C1 (0.4 µg/L). The low oxygen exposure conditions can reach higher concentrations of H_2S (Ugliano et al., 2011). According to the results Control and C1 got the highest concentration of H_2S, those stoppers had very low permeability.

The compounds have significant difference between corks and doses. The degradation of amino acids, cysteine for example, by microorganisms can lead to the presence of sulfides and disulfides. In this study we evaluated dimethyl sulfide (Me_2S), diethyl sulfide (ET_2S), dimethyl disulfide (Me_2S_2) and diethyl disulfide (Et_2S_2). These compounds can increase over the aging time and storage temperature increase, and they can influence the aroma complexity of aged wine (Bekker, Wilkes and Smith, 2018; Fracassetti and Vigentini, 2018). In addition, these compounds are characteristic for providing unpleasant aromas to wines such as asparagus, garlic, cooked cabbage and onion, respectively (Siebert et al., 2010).

As can be seen in figure 5b sulfides, specially Me2S, started to appear in the Champagnes over 3M in all doses. Et_2S was detected at 12 M. None of the samples exceeded the odor threshold of these compounds. The main compound of sulfide was Me2S at 12M, where C3 with 0 mg/L and 10 mg/L of SO_2 and C4 at 20 mg/L of SO_2 raised the highest concentration of this compounds over the rest corks (Table S5). In the case of disulfides (Figure 5c), dimethyl disulfide and diethyl disulfide appeared since 3M of storage, and they maintain their concentration until 6M. Over the time, at 12M the amount of Me_2S_2 increased in all Champagne but did not exceed their limit of perception. At 12M, C1 at 0 mg/L and 10 mg/L of SO_2 had more concentration of Me2S2, and C3 and C5 at 20 mg/L of SO_2. The low degree of oxygen exposure associated with wine stoppers (natural or synthetic corks) can promote the expression of ‘reductive’ off-odors. The selection of closures with adequate oxygen permeability can allow effective control of both reduction and oxidation, ultimately delivering wines with improved sensory characteristics (Ugliano, 2013).

3.4. Multivariate analysis at 12 months

Figure 6 shows the scores and loadings of Principal Component Analysis (PCA), corresponding to the oxidative and reductive families and color at 12M. For oxidative aromas at 12M the PCA explain the (87.69%) of variance as it shows in figure 6a. C2 at all doses, C1, C4 and C5 at 0 mg/L SO_2 evolved over time presenting higher concentrations of oxidative families, and they grouped in PC1 (43.34%). As already observed in the analytical results C2 at 12M presented high concentrations of E-(2)-hexanal as the main compound at the studied doses, followed by E-(2)-nonenal. C1 presented the same compounds of alkenals and 2-methylpropanal as aldehyde. The rest corks that are explained in PC2 (16.44%), with doses of SO_2 were less evolved.

Figure 6b the reduction aromas at 12M, where the PCA explain 87.71%. C1, C3, C4 and C5 with 20 mg/L of SO_2 had more concentrations of sulfides and disulfides. Same as C3 and C4 at 10 mg/L of SO_2. C1 at 20 mg/L of SO_2 had more concentration of thiols. This result shows that at more addition of SO_2 over the time, produce more concentration of reduction compounds. At 12M the Champagnes were evaluated by a sensorial panel. In general, the champagnes without doses of

SO_2, showed a greater olfactory evolution compared to champagnes with dose of SO_2. C2 were more evolved and had more complex-elegant evolution than the others, C5 was describe as a fresh wine, more intense and expressive. C4 was evaluated has better evolute with vegetable and ripe mature notes that brings great complexity, that is a descriptor of 3-metil-2,4-nonadina and ɣ-nonalactone. In general, the champagnes at without dose were very evolved with acetaldehyde notes but wines better expressive, C1 and C3 got the lowest rate because presented more oxidation notes and less elegant.

The champagnes with doses were less evolved; at 10 mg/L in general did not present acetaldehyde-oxidation notes, it means that role of SO_2 was relevant over time, to avoid an increase in oxidative compounds. C2 and C4 were the better evaluated because of its evolution over time, had vegetal and dry flowers notes. C5 was rated as fresh wines, presented fresh fruit notes. C1 and C3 were the worst evaluated, presented more oxidation and rancid notes. At 20 mg/L SO_2, in general were evaluated as much less evolved wines than the series before. The acetaldehydes-oxidation notes were less marked, also presented reductive notes: sulfur, phosphorous, garlic. Control was the Champagne with more reductive notes, C2 was more complex, more evolute but elegant. C5 were better evaluated because of it freshness and fresh fruit notes, (Ugliano, 2013) mention that the high exposure of oxygen did not affect the increase concentration of DMS, for example, in effect can reduce it concentration, it means that low degree of oxygen exposure can promote the expression of reductive aromas. As mention in the section before, the samples with SO_2 at 12M, H2S was the compound that exceeded the OT (1.6 µg/L). The results of color at 12M its shows in figure 6c, where the PCA explain 92.80 % of the variables. The addition of SO_2 had influence in color over time. It means that at more doses of SO_2, less evolution in color. PC1 (74.65%) explain the samples without SO_2 and at 10 mg/L of SO_2 had less clarity (L*).

4. CONCLUSIONS

In this study differences and evolution over time of the volatile compounds were observed; fermentative compounds got slight decrease, but not affected significantly over time. The oxidative compounds present higher concentration in Champagne without SO_2 doses, although they were also observed at doses 10 and 20 mg/L SO_2, their compounds were less evolved. The reduction compounds in all the doses under study began to increase since 3M, they did not exceed their OT except for H2S. At 12M it was observed that Champagnes with 20 mg/L of SO_2 presented a higher concentration of reductive compounds and were also detected by the sensorial panel in Champagnes with corks that had less permeability. The Champagnes with corks that had more permeability were better rated by the sensorial panel at 12M because presented fresh fruit notes, were less evolved and did not had reductive odors with doses of SO_2. Time and SO_2 are main factors to promote the production of volatile compounds, specifically at more doses of SO_2 more production of reduction compounds will appear, and less evolution of oxidative compounds.

As future perspectives it would be of interest to continue with this study at 18 and 24 months of post-bottling storage and observe their evolution of aromatic compounds over these times. The application of these treatments under study in cava and rosé analyze their volatile compounds can contribute for the research and oenological knowledge.

Figure captions

Figure 1. Permeability values per cork and control in cm O_2/ day.

Figure 2. Principal component analysis of oenological and volatile compounds.

Figure 3. Evolution of fermentative families per doses, storage time and per each studied cork and control: control (yellow), C1 (blue), C2 (grey), C3(light blue), C4 (red), C5 (green).

Figure 4. Evolution of oxidatives families per doses, storage time and for each studied corks and control: control (yellow), C1 (blue), C2 (grey), C3(light blue), C4 (red), C5 (green)

Figure 5. Evolution of reductive families per doses, storage time and for each studied corks and control: control (yellow), C1 (blue), C2 (grey), C3(light blue), C4 (red), C5 (green)

Figure 6. Score and loadings of oxidative and reductive families and color with CIELAB coordinates at 12M.

5. REFERENCES

Bekker, M. Z., Wilkes, E. N. and Smith, P. A. (2018) 'Evaluation of putative precursors of key "reductive" compounds in wines post-bottling', Food Chemistry. Elsevier, 245(June 2017), pp. 676–686. doi: 10.1016/j.foodchem.2017.10.123.

Benucci, I. (2020). LWT - Food Science and Technology Impact of post-bottling storage conditions on colour and sensory profile of a rosé sparkling wine. LWT - Food Science and Technology, 118(February 2019), 108732-108732. doi: 10.1016/j.lwt.2019.108732

Buxaderas, S., & López-Tamames, E. (2012). Sparkling Wines: Features and Trends from Tradition (Vol. 66).

Caliari, V., Panceri, C. P., Rosier, J. P., & Bordignon-Luiz, M. T. (2015). Effect of the Traditional, Charmat and Asti method production on the volatile composition of Moscato Giallo sparkling wines. LWT - Food Science and Technology, 61(2), 393-400. doi: https://doi.org/10.1016/j.lwt.2014.11.039

Costanigro, M., Appleby, C., & Menke, S. D. (2014). The wine headache: Consumer perceptions of sulfites and willingness to pay for non-sulfited wines. Food Quality and Preference, 31, 81-89. doi: http://dx.doi.org/10.1016/j.foodqual.2013.08.002

Cotea, V. V. et al. (2021) 'Influence of Different Commercial Yeasts on Volatile Fraction of Sparkling Wines', Foods 2, 10. doi: 10.1016/S0140-6736(01)76267-8.

Escudero, A. et al. (2007) 'Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines', Journal of Agricultural and Food Chemistry, 55(11), pp. 4501–4510. doi: 10.1021/jf0636418.

Escudero, A., Cacho, J. and Ferreira, V. (2000) 'Isolation and identification of odorants generated in wine during its oxidation: A gas chromatography-olfactometric study', European Food Research and Technology, 211(2), pp. 105–110. doi: 10.1007/s002179900128.

Ferreira, V., López, R. and Cacho, J. F. (2000) 'Quantitative determination of the odorants of young red wines from different grape varieties', Journal of the Science of Food and Agriculture, 80(11), pp. 1659–1667. doi: 10.1002/1097-0010(20000901)80:11<1659::AID-JSFA693>3.0.CO;2-6.

Ferrer-Gallego, R., Puxeu, M., Martín, L., Nart, E., Hidalgo, C. and Andorrà, I. (2018). Microbiological, Physical, and Chemical Procedures to Elaborate High-Quality SO2-Free Wines. In

A. Manuel Jordão, Cosme, F. , (Ed.), Grapes and Wines - Advances in Production, Processing, Analysis and Valorization. London, U.K: IntechOpen Limited.

Fracassetti, D. and Vigentini, I. (2018) 'Occurrence and Analysis of Sulfur Compounds in Wine', Grapes and Wines - Advances in Production, Processing, Analysis and Valorization. doi: 10.5772/intechopen.72823.

Gómez-Míguez, M. J. et al. (2007) 'Volatile components of Zalema white wines', Food Chemistry, pp. 1464–1473. doi: 10.1016/j.foodchem.2005.11.045.

Grant-Preece, P. et al. (2017) 'Light-induced changes in bottled white wine and underlying photochemical mechanisms', Critical Reviews in Food Science and Nutrition, 57(4), pp. 743–754. doi: 10.1080/10408398.2014.919246.

Guth, H. (1997) 'Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties', Journal of Agricultural and Food Chemistry, 45(8), pp. 3027–3032. doi: 10.1021/jf970280a.

Liger-Belair, G. (2013). The Making of Champagne (pp. 19-30): Princeton University Press.

Malakar, S., Paul, S. K. and Jolvis Pou, K. R. (2019) 'Biotechnological interventions in beverage production', in Biotechnological Progress and Beverage Consumption: Volume 19: The Science of Beverages. Elsevier, pp. 1–37. doi: 10.1016/B978-0-12-816678-9.00001-1.

Mamede, M. E. O., Cardello, H. M. A. B. and Pastore, G. M. (2005) 'Evaluation of an aroma similar to that of sparkling wine: Sensory and gas chromatography analyses of fermented grape musts', Food Chemistry, 89(1), pp. 63–68. doi: 10.1016/j.foodchem.2004.02.012.

Martínez-García, R., Moreno, J., Bellincontro, A., Centioni, L., Puig-Pujol, A., Peinado, R. A., . . . García-Martínez, T. (2021). Using an electronic nose and volatilome analysis to differentiate sparkling wines obtained under different conditions of temperature, ageing time and yeast formats. Food Chem, 334, 127574. doi: 10.1016/j.foodchem.2020.127574

Mayr, C. M. et al. (2015) 'Quantitative Analysis by GC-MS/MS of 18 Aroma Compounds Related to Oxidative Off-Flavor in Wines', Journal of Agricultural and Food Chemistry, 63(13), pp. 3394–3401. doi: 10.1021/jf505803u.

McMahon, K. M. et al. (2017) 'Trained and consumer panel evaluation of sparkling wines sweetened to brut or demi sec residual sugar levels with three different sugars', Food Research International, pp. 173–185. doi: 10.1016/j.foodres.2017.05.020.

Mestres, M., Busto, O. and Guasch, J. (2000) 'Analysis of organic sulfur compounds in wine aroma', Journal of Chromatography A, 881(1–2), pp. 569–581. doi: 10.1016/S0021-9673(00)00220-X.

Mislata, A. M., Tomás, M. P. E. and Ferrer, E. N. R. (2020) 'Influence of the oxidation in the aromatic composition and sensory profile of Rioja red aged wines', European Food Research and Technology. Springer Berlin Heidelberg, 2(0123456789). doi: 10.1007/s00217-020-03473-4.

OIV. (2020). OIV Focus: The global spakling wine market (pp. 1-21).

Oliveira, V., Knapic, S., & Pereira, H. (2015). Classification modeling based on surface porosity for the grading of natural cork stoppers for quality wines. Food and Bioproducts Processing, 93(January), 69-76. doi: 10.1016/j.fbp.2013.11.004

Peinado, R. A. et al. (2004) 'Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration', Food Chemistry, 84(4), pp. 585–590. doi: 10.1016/S0308-8146(03)00282-6.

Pereira, H. (2007). Wine and cork. Cork, 305-327. doi: 10.1016/b978-044452967-1/50016-9

Pérez Olivero, S. J. and Pérez Trujillo, J. P. (2011) 'A new method for the determination of short-chain fatty acids from the aliphatic series in wines by headspace solid-phase microextraction-gas chromatography-ion trap mass spectrometry', Analytica Chimica Acta, 696(1–2), pp. 59–66. doi: 10.1016/j.aca.2011.03.063.

Pons, A. et al. (2013) 'Role of 3-methyl-2,4-nonanedione in the flavor of aged red wines', Journal of Agricultural and Food Chemistry, 61(30), pp. 7373–7380. doi: 10.1021/jf400348h.

Sánchez-González, M., & Pérez-Terrazas, D. (2018). Assessing the percentage of cork that a stopper should have from a mechanical perspective. Food Packaging and Shelf Life, 18(October), 212-220. doi: 10.1016/j.fpsl.2018.10.009

Torrens, J. et al. (2010) 'Assessment of volatlle and sensory profiles between base and sparkling wines', Journal of Agricultural and Food Chemistry, 58(4), pp. 2455–2461. doi: 10.1021/jf9035518.

Tufariello, M. et al. (2021) 'Non-targeted metabolomic approach as a tool to evaluate the chemical profile of sparkling wines fermented with autochthonous yeast strains', Food Control. Elsevier Ltd, 126(December 2020), p. 108099. doi: 10.1016/j.foodcont.2021.108099.

Ubeda, C., Kania-Zelada, I., del Barrio-Galán, R., Medel-Marabolí, M., Gil, M., & Peña-Neira, Á. (2019). Study of the changes in volatile compounds, aroma and sensory attributes during the production process of sparkling wine by traditional method. Food Research International, 119(June 2018), 554-563. doi: 10.1016/j.foodres.2018.10.032

Ugliano, M. et al. (2011) 'Evolution of 3-mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen', Journal of Agricultural and Food Chemistry, 59(6), pp. 2564–2572. doi: 10.1021/jf1043585.

Ugliano, M. (2013) 'Oxygen contribution to wine aroma evolution during bottle aging', Journal of Agricultural and Food Chemistry, 61(26), pp. 6125–6136. doi: 10.1021/jf400810v.

Valera, M. J., Sainz, F., Mas, A., & Torija, M. J. (2017). International Journal of Food Microbiology. International Journal of Food Microbiology, 246, 1-4. doi: 10.1016/j.ijfoodmicro.2017.01.022

Vally, H., Misso, N. L. A., & Madan, V. (2009). Clinical effects of sulphite additives. Clinical & Experimental Allergy, 39(11), 1643-1651. doi: 10.1111/j.1365-2222.2009.03362.x

Bekker, M. Z., Wilkes, E. N. and Smith, P. A. (2018) 'Evaluation of putative precursors of key "reductive" compounds in wines post-bottling', Food Chemistry. Elsevier, 245(June 2017), pp. 676–686. doi: 10.1016/j.foodchem.2017.10.123.

Benucci, I. (2020) 'LWT - Food Science and Technology Impact of post-bottling storage conditions on colour and sensory pro fi le of a rosé sparkling wine', LWT - Food Science and Technology. Elsevier, 118(February 2019), p. 108732. doi: 10.1016/j.lwt.2019.108732.

Buxaderas, S. and López-Tamames, E. (2012) Sparkling Wines: Features and Trends from Tradition, Advances in Food and Nutrition Research. doi: 10.1016/B978-0-12-394597-6.00001-X.

Caliari, V. et al. (2015) 'Effect of the traditional, charmat and asti method production on the volatile composition of moscato giallo sparkling wines', LWT - Food Science and Technology. Elsevier Ltd, 61(2), pp. 393–400. doi: 10.1016/j.lwt.2014.11.039.

Cotea, V. V. et al. (2021) 'Influence of Different Commercial Yeasts on Volatile Fraction of Sparkling Wines', Foods 2, 10. doi: 10.1016/S0140-6736(01)76267-8.

Escudero, A. et al. (2007) 'Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines', Journal of Agricultural and Food Chemistry, 55(11), pp. 4501–4510. doi: 10.1021/jf0636418.

Escudero, A., Cacho, J. and Ferreira, V. (2000) 'Isolation and identification of odorants generated in wine during its oxidation: A gas chromatography-olfactometric study', European Food Research and Technology, 211(2), pp. 105–110. doi: 10.1007/s002179900128.

Ferreira, V., López, R. and Cacho, J. F. (2000) 'Quantitative determination of the odorants of young red wines from different grape varieties', Journal of the Science of Food and Agriculture, 80(11), pp. 1659–1667. doi: 10.1002/1097-0010(20000901)80:11<1659::AID-JSFA693>3.0.CO;2-6.

Fracassetti, D. and Vigentini, I. (2018) 'Occurrence and Analysis of Sulfur Compounds in Wine', Grapes and Wines - Advances in Production, Processing, Analysis and Valorization. doi: 10.5772/intechopen.72823.

Gómez-Míguez, M. J. et al. (2007) 'Volatile components of Zalema white wines', Food Chemistry, pp. 1464–1473. doi: 10.1016/j.foodchem.2005.11.045.

Grant-Preece, P. et al. (2017) 'Light-induced changes in bottled white wine and underlying photochemical mechanisms', Critical Reviews in Food Science and Nutrition, 57(4), pp. 743–754. doi: 10.1080/10408398.2014.919246.

Guth, H. (1997) 'Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties', Journal of Agricultural and Food Chemistry, 45(8), pp. 3027–3032. doi: 10.1021/jf970280a.

International Organisation of Vine and Wine (2020) 'The Global Sparkling Wine Market', Focus OIV, (April), pp. 1–21.

Jackson, R. S. (2020) Chemical constituents of grapes and wine, Wine Science. doi: 10.1016/b978-0-12-816118-0.00006-4.

Liger-Belair, G. (2013) 'The Making of Champagne', in Uncorked: The Science of Champagne-Revised edition. Princeton University Press, pp. 19–30. Available at: https://ebookcentral-proquest-com.sabidi.urv.cat/lib/urv/reader.action?docID=1169553 (Accessed: 23 April 2021).

Malakar, S., Paul, S. K. and Jolvis Pou, K. R. (2019) 'Biotechnological interventions in beverage production', in Biotechnological Progress and Beverage Consumption: Volume 19: The Science of Beverages. Elsevier, pp. 1–37. doi: 10.1016/B978-0-12-816678-9.00001-1.

Mamede, M. E. O., Cardello, H. M. A. B. and Pastore, G. M. (2005) 'Evaluation of an aroma similar to that of sparkling wine: Sensory and gas chromatography analyses of fermented grape musts', Food Chemistry, 89(1), pp. 63–68. doi: 10.1016/j.foodchem.2004.02.012.

Mayr, C. M. et al. (2015) 'Quantitative Analysis by GC-MS/MS of 18 Aroma Compounds Related to Oxidative Off-Flavor in Wines', Journal of Agricultural and Food Chemistry, 63(13), pp. 3394–3401. doi: 10.1021/jf505803u.

McMahon, K. M. et al. (2017) 'Trained and consumer panel evaluation of sparkling wines sweetened to brut or demi sec residual sugar levels with three different sugars', Food Research International, pp. 173–185. doi: 10.1016/j.foodres.2017.05.020.

Mestres, M., Busto, O. and Guasch, J. (2000) 'Analysis of organic sulfur compounds in wine aroma', Journal of Chromatography A, 881(1–2), pp. 569–581. doi: 10.1016/S0021-9673(00)00220-X.

Mislata, A. M., Tomás, M. P. E. and Ferrer, E. N. R. (2020) 'Influence of the oxidation in the aromatic composition and sensory profile of Rioja red aged wines', European Food Research and Technology. Springer Berlin Heidelberg, 2(0123456789). doi: 10.1007/s00217-020-03473-4.

Oliveira, V., Knapic, S. and Pereira, H. (2015) 'Classification modeling based on surface porosity for the grading of natural cork stoppers for quality wines', Food and Bioproducts Processing. Institution of Chemical Engineers, 93(January), pp. 69–76. doi: 10.1016/j.fbp.2013.11.004.

Peinado, R. A. et al. (2004) 'Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration', Food Chemistry, 84(4), pp. 585–590. doi: 10.1016/S0308-8146(03)00282-6.

Pereira, H. (2007) 'Wine and cork', Cork, pp. 305–327. doi: 10.1016/b978-044452967-1/50016-9.

Pérez Olivero, S. J. and Pérez Trujillo, J. P. (2011) 'A new method for the determination of short-chain fatty acids from the aliphatic series in wines by headspace solid-phase microextraction-gas chromatography-ion trap mass spectrometry', Analytica Chimica Acta, 696(1–2), pp. 59–66. doi: 10.1016/j.aca.2011.03.063.

Pons, A. et al. (2013) 'Role of 3-methyl-2,4-nonanedione in the flavor of aged red wines', Journal of Agricultural and Food Chemistry, 61(30), pp. 7373–7380. doi: 10.1021/jf400348h.

Sánchez-González, M. and Pérez-Terrazas, D. (2018) 'Assessing the percentage of cork that a stopper should have from a mechanical perspective', Food Packaging and Shelf Life. Elsevier, 18(October), pp. 212–220. doi: 10.1016/j.fpsl.2018.10.009.

Siebert, T. E. et al. (2010) 'Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection', Journal of Agricultural and Food Chemistry, 58(17), pp. 9454–9462. doi: 10.1021/jf102008r.

Torrens, J. et al. (2010) 'Assessment of volatlle and sensory profiles between base and sparkling wines', Journal of Agricultural and Food Chemistry, 58(4), pp. 2455–2461. doi: 10.1021/jf9035518.

Tufariello, M. et al. (2021) 'Non-targeted metabolomic approach as a tool to evaluate the chemical profile of sparkling wines fermented with autochthonous yeast strains', Food Control. Elsevier Ltd, 126(December 2020), p. 108099. doi: 10.1016/j.foodcont.2021.108099.

Ubeda, C. et al. (2019a) 'Study of the changes in volatile compounds, aroma and sensory attributes during the production process of sparkling wine by traditional method', Food Research International. Elsevier, 119(June 2018), pp. 554–563. doi: 10.1016/j.foodres.2018.10.032.

Ubeda, C. et al. (2019b) 'Study of the changes in volatile compounds, aroma and sensory attributes during the production process of sparkling wine by traditional method', Food Research International. Elsevier, 119(October 2018), pp. 554–563. doi: 10.1016/j.foodres.2018.10.032.

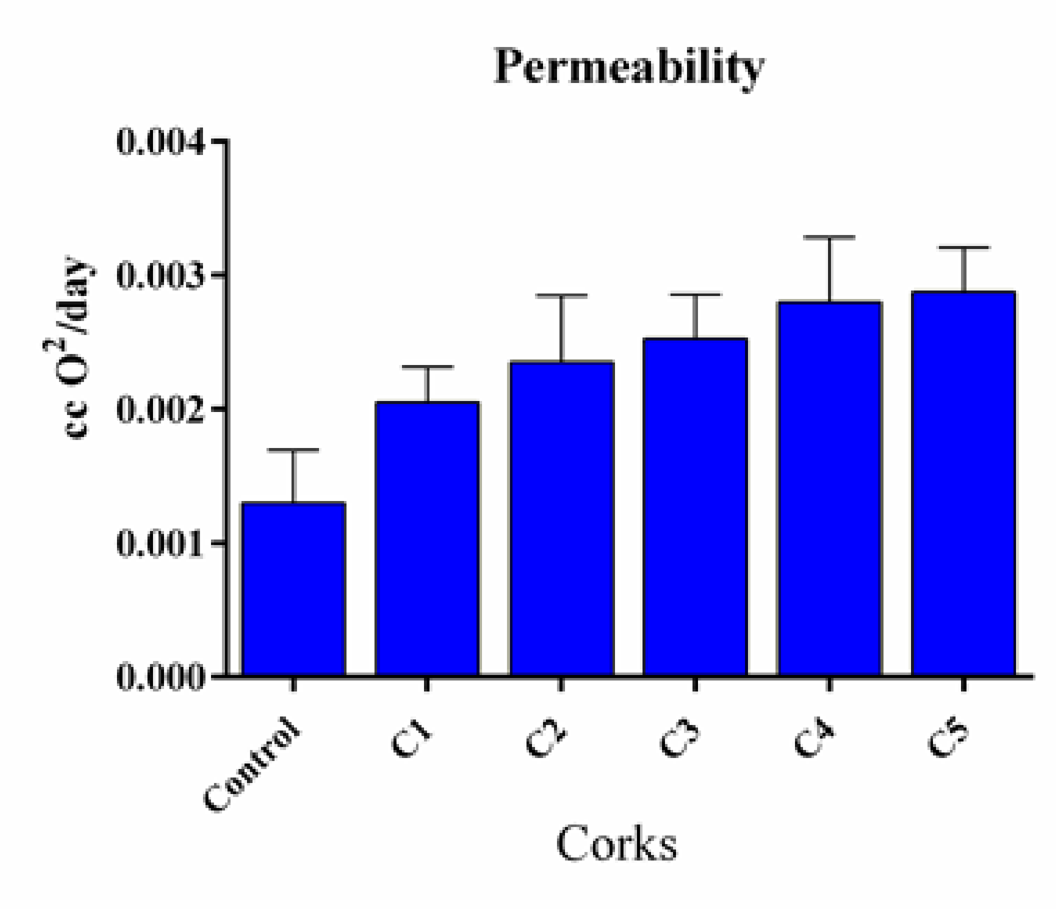
Ugliano, M. et al. (2011) 'Evolution of 3-mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen', Journal of Agricultural and Food Chemistry, 59(6), pp. 2564–2572. doi: 10.1021/jf1043585.

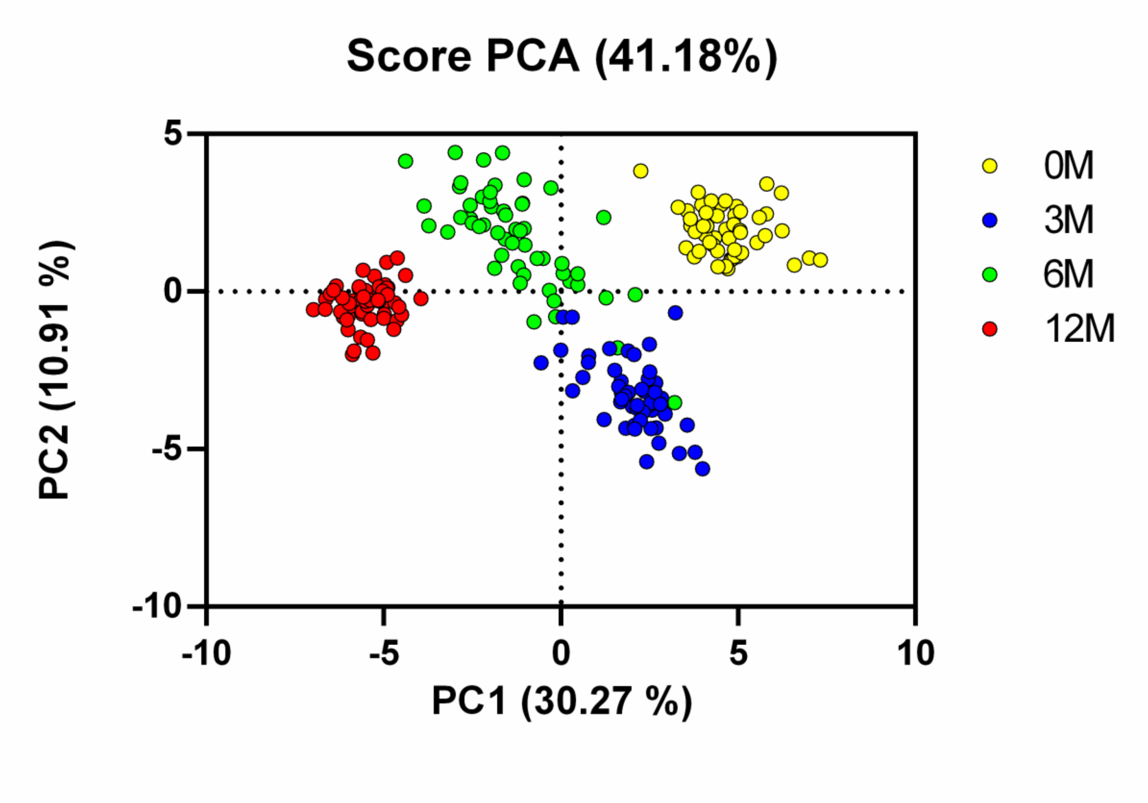
Ugliano, M. (2013) ‘Oxygen contribution to wine aroma evolution during bottle aging’, Journal of Agricultural and Food Chemistry, 61(26), pp. 6125–6136. doi: 10.1021/jf400810v.

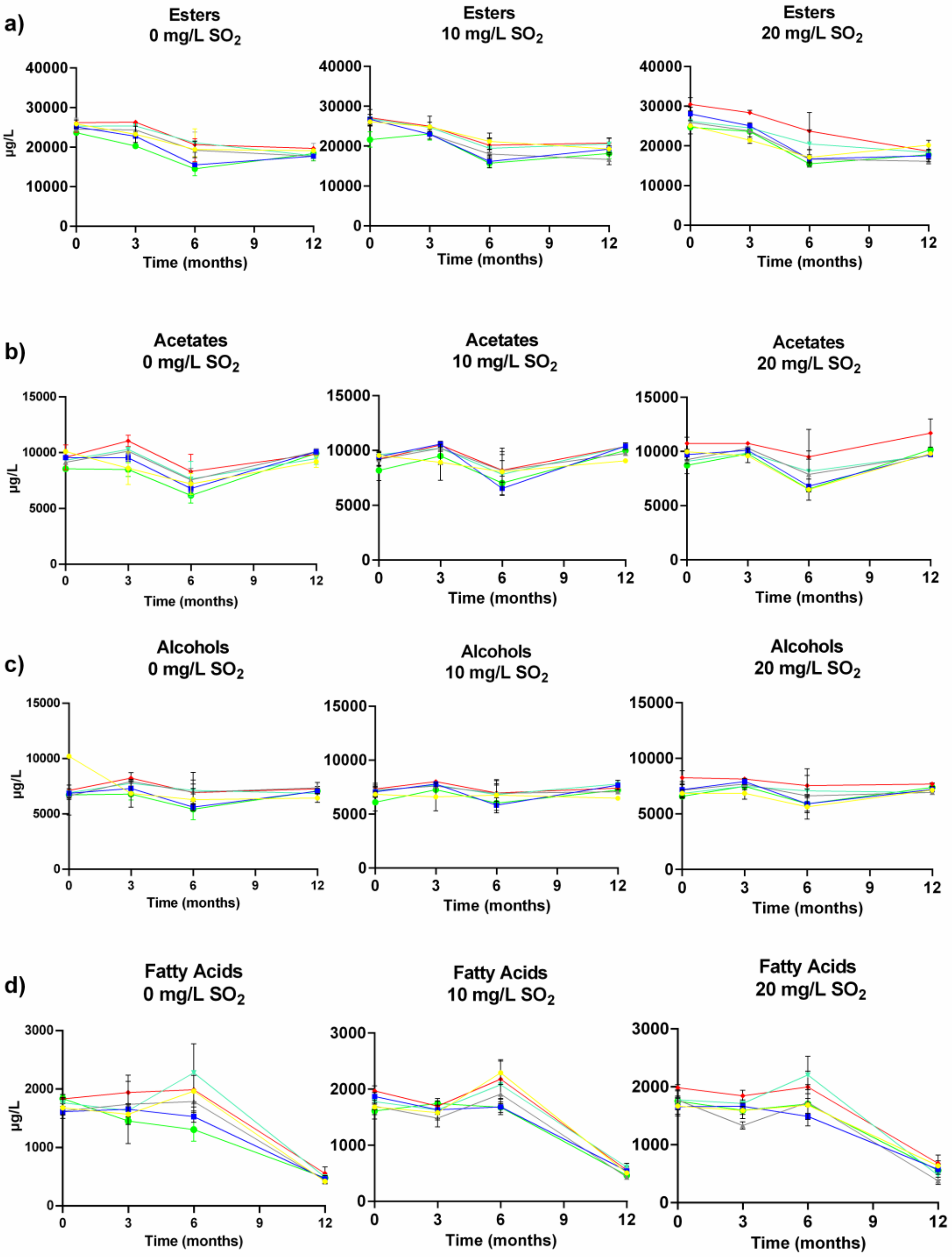
Valera, M. J. et al. (2017) ‘International Journal of Food Microbiology’, International Journal of Food Microbiology. Elsevier B.V., 246, pp. 1–4. doi: 10.1016/j.ijfoodmicro.2017.01.022.

Webber, V. et al. (2017) ‘Effect of glutathione during bottle storage of sparkling wine’, Food Chemistry. Elsevier Ltd, 216, pp. 254–259. doi: 10.1016/j.foodchem.2016.08.042.

Yong-Sheng Tao, H. L. (2009) ‘Active volatiles of cabernet sauvignon wine from Changli County’, Health, 01(03), pp. 176–182. doi: 10.4236/health.2009.13028.

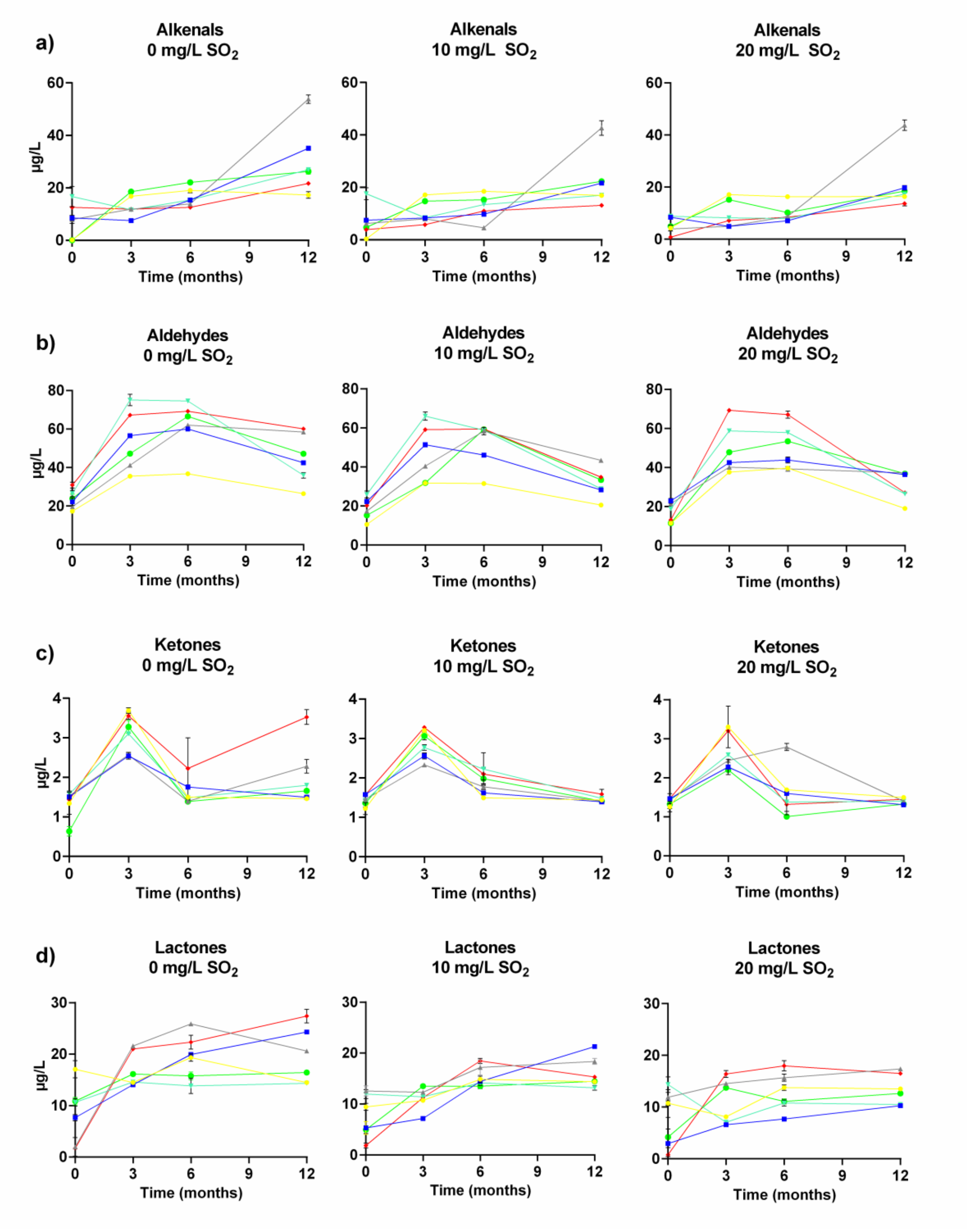

Permeability
0.004
0.003
0.002
0.001
0.000
cc O2/day
Control
C1
C2
C3
C4
C5
Corks

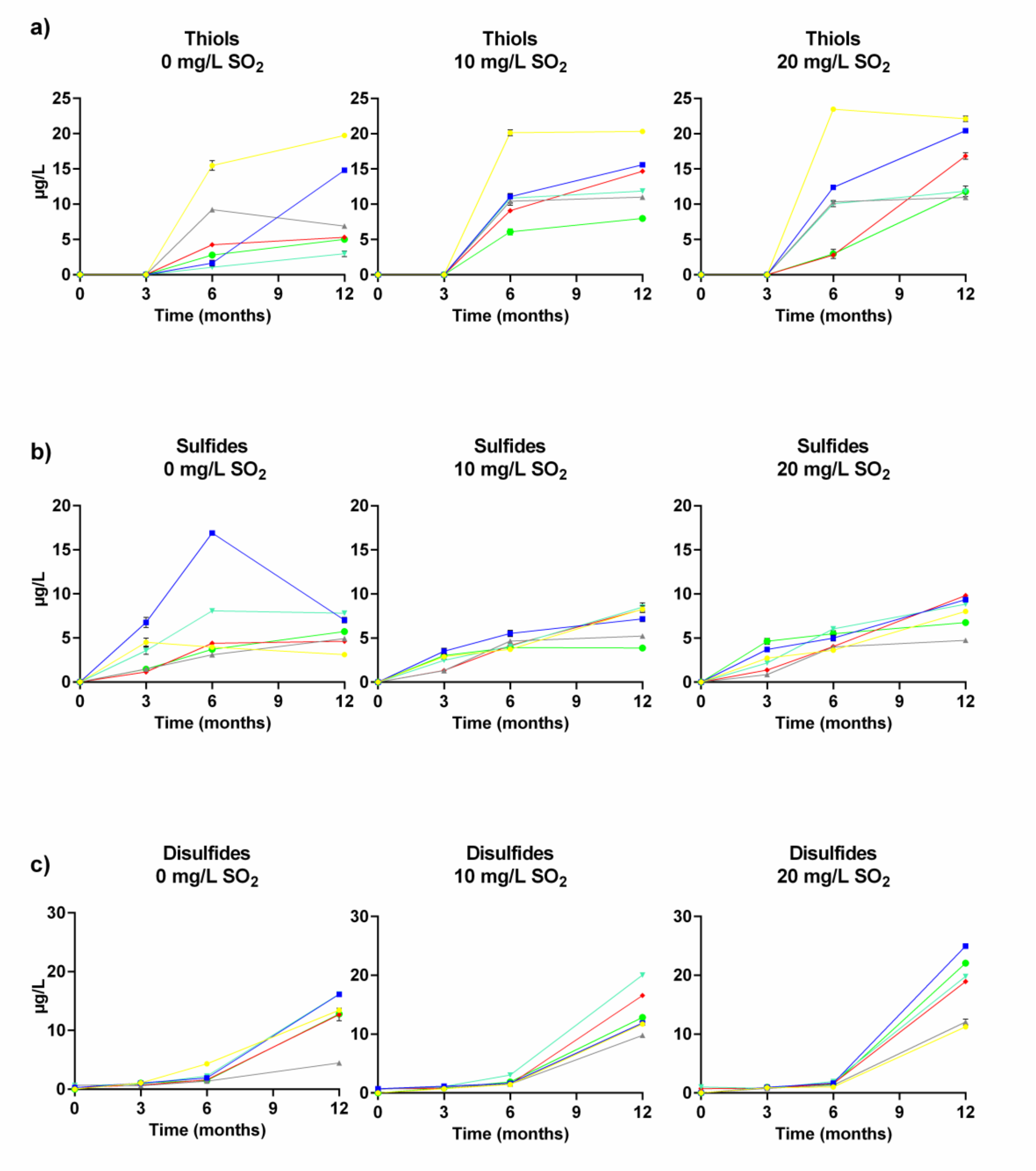

Score PCA (41.18%)
PC2 (10.91 %)
PC1 (30.27 %)
5
0
-5
-10
-10
-5
0
5
10
0M
3M
6M
12M


a)
Esters 0 mg/L SO_2
Esters 10 mg/L SO_2
Esters 20 mg/L SO_2
40000
30000
20000
10000
0
µg/L
0
3
6
9
12
Time (months)
b)
Acetates 0 mg/L SO_2
Acetates 10 mg/L SO_2
Acetates 20 mg/L SO_2
15000
10000
5000
c)
Alcohols 0 mg/L SO_2
Alcohols 10 mg/L SO_2
Alcohols 20 mg/L SO_2
d)
Fatty Acids 0 mg/L SO_2
Fatty Acids 10 mg/L SO_2
Fatty Acids 20 mg/L SO_2
3000
2000
1000

723

724


a)
Alkenals 0 mg/L SO2
Alkenals 10 mg/L SO2
Alkenals 20 mg/L SO2
μg/L
0
20
40
60
Time (months)
0
3
6
9
12
b)
Aldehydes 0 mg/L SO2
Aldehydes 10 mg/L SO2
Aldehydes 20 mg/L SO2
μg/L
0
20
40
60
80
Time (months)
c)
Ketones 0 mg/L SO2
Ketones 10 mg/L SO2
Ketones 20 mg/L SO2
μg/L
0
1
2
3
4
Time (months)
d)
Lactones 0 mg/L SO2
Lactones 10 mg/L SO2
Lactones 20 mg/L SO2
μg/L
0
10
20
30
Time (months)


a)
Thiols
0 mg/L SO2
Thiols
10 mg/L SO2
Thiols
20 mg/L SO2
μg/L
0
5
10
15
20
25
0
3
6
9
12
Time (months)
b)
Sulfides
0 mg/L SO2
Sulfides
10 mg/L SO2
Sulfides
20 mg/L SO2
μg/L
0
5
10
15
20
Time (months)
c)
Disulfides
0 mg/L SO2
Disulfides
10 mg/L SO2
Disulfides
20 mg/L SO2
μg/L
0
10
20
30
Time (months)

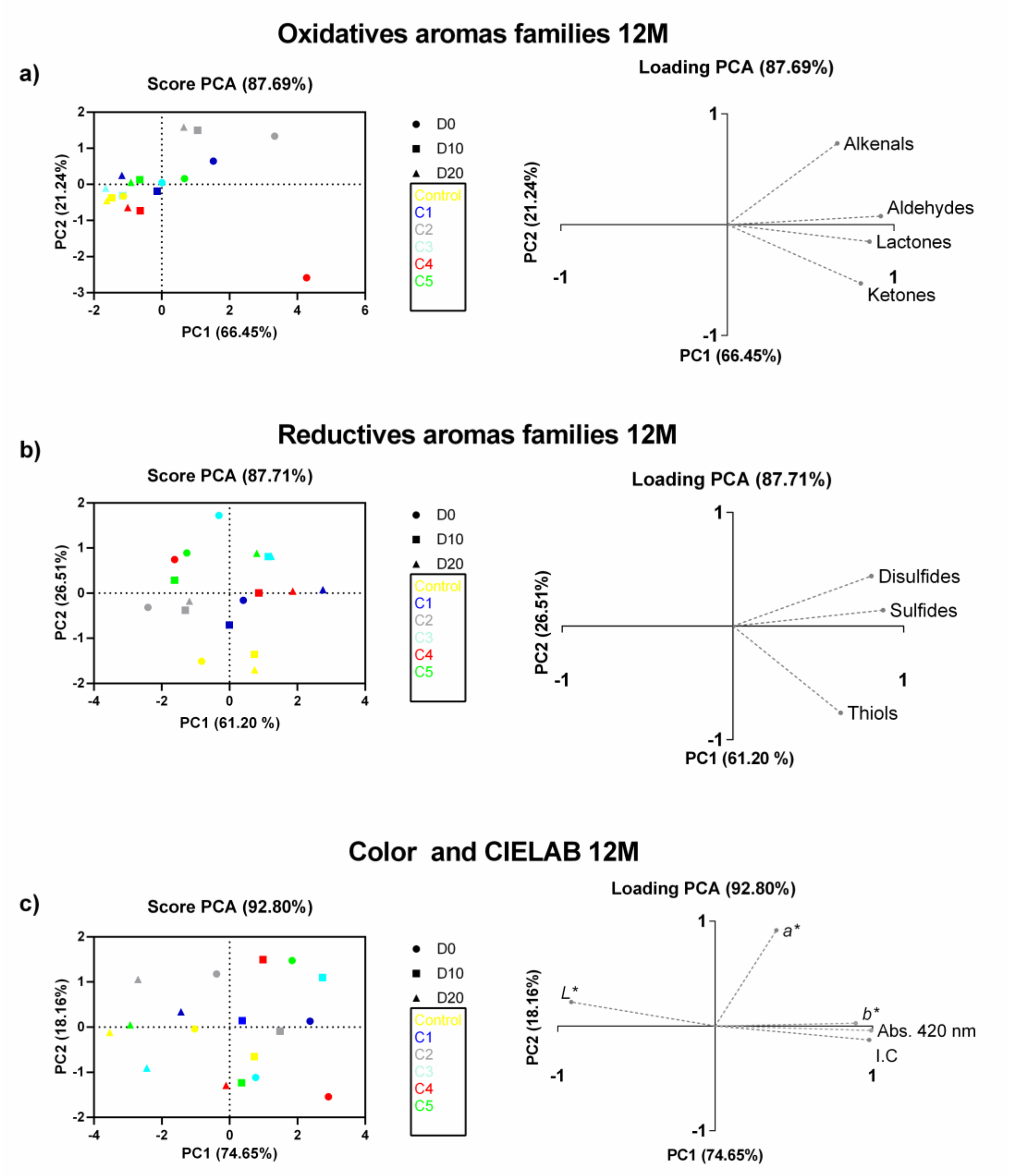

Oxidatives aromas families 12M
a)
Score PCA (87.69%)
PC2 (21.24%)
PC1 (66.45%)
2
1
0
-1
-2
-3
-2
0
2
4
6
● D0
■ D10
▲ D20
Control
C1
C2
C3
C4
C5
Loading PCA (87.69%)
Alkenals
Aldehydes
Lactones
Ketones
1
-1
Reductives aromas families 12M
b)
Score PCA (87.71%)
PC2 (26.51%)
PC1 (61.20 %)
-4
-2
0
2
4
Loading PCA (87.71%)
Disulfides
Sulfides
Thiols
Color and CIELAB 12M
c)
Score PCA (92.80%)
PC2 (18.16%)
PC1 (74.65%)
Loading PCA (92.80%)
a*
L*
b*
Abs. 420 nm
I.C

Table S 1. Oenological parameters of Champagne from 0M to 12M.

Time	**0M**	**3M**	**6M**	**12M**
Parameters				
pH	2.89 ±0.01 a	2.87±0.01 b	2.87± 0.01 b	2.87±0.01 b
G+F	1.3±0.08 a	1.2±0.15 b	1.3±0.16 ab	1.3±0.17 a
Malic acid	2.50±0.20 a	2.38±0.06 b	2.26±0.08 c	2.20±0.08 d
Lactic acid	1.59±0.13 a	1.55±0.05 a	1.59±0.04 a	1.59±0.07 a
Acetic acid	0.37±0.032 b	0.33±0.05 c	0.36±0.01 b	0.40±0.06 a
Alcohol content	11.93±0.26 d	12.15±0.07 c	12.23±0.03 b	12.31±0.05 a
Volatil acid	0.45±0.015 b	0.43±0.01 c	0.46±0.01 a	0.44±0.01 b
TTA	8.47±0.06 b	8.44±0.06 c	8.61±0.03 a	8.59±0.05 a
Total SO$_2$	72.61 ± 10.43 a	69.93 ± 9.60 a	61.04 ± 8.98 b	62.39 ± 10.78 b
Total pressure	7.68±0.25 b	8.06±0.35 a	7.74±0.30 b	7.68±0.27 b
CO$_2$ pressure	5.35±0.15 a	4.09±0.28 c	4.88±0.27 b	4.96±0.15 b
Dissolved CO$_2$	8.81±0.24 a	6.70±0.43 c	8.04±0.46 b	8.18±0.29 b
Abs. 420 nm	0.09±0.01 c	0.09±0.01 c	0.09±0.01b	0.10±0.01 a
I.C.	0.13±0.01c	0.13±0.01 c	0.13±0.01 b	0.14±0.01 a
L*	97.70±0.70 a	97.75±0.80 a	97.83±0.19 a	97.80±0.20 a
a*	0.09±0.25 ab	-0.13±1.48 b	-0.01±0.23 ab	0.36±0.11 a
b*	6.2±0.27 b	6.2±1.61 b	6.7±0.28 a	7.0±0.25 a

Table S2. Families of volatile compounds (µg/L) from Champagne with the five corks and crown tap (Control, C1, C2, C3, C4, C5) with the doses of SO_2 under study (0, 10, 20 mg/L) Different letters indicate significant differences between corks and doses after 2 weeks of bottling (0M). Samples (n = 3).

	0 MONTH																	
Fermentatives compounds	**Control**			**C1**			**C2**			**C3**			**C4**			**C5**		
	0 mg/L	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**
ESTERS																		
Ethyl butyrate	726 ± 49 a	537 ± 73 c	612 ± 68 abc	634 ± 20 abc	597 ± 27 abc	519 ± 67 c	597 ± 26 abc	533 ± 12 c	588 ± 34 abc	594 ± 48 abc	593 ± 22 abc	545 ± 41 bc	615 ± 103 abc	622 ± 72 abc	721 ± 110 ab	567 ± 28 abc	541 ± 66 c	553 ± 59 abc
Ethyl isovaleriate	221 ± 15 ab	161 ± 18 b	182 ± 22 b	181 ± 41 b	173 ± 5 b	150 ± 14 b	174 ± 11 b	158 ± 6 b	166 ± 15 b	174 ± 16 b	173 ± 8 b	156 ± 8 b	273 ± 82 a	173 ± 17 b	209 ± 21 ab	166 ± 6 b	175 ± 18 b	170 ± 12 b
Ethyl hexanoate	5395 ± 995 ab	5489 ± 366 ab	5339 ± 364 ab	5352 ± 735 ab	5619 ± 613 ab	5920 ± 840 ab	5477 ± 374 ab	5963 ± 605 ab	5904 ± 466 ab	5468 ± 114 ab	5977 ± 482 ab	5795 ± 537 ab	5698 ± 165 ab	5704 ± 169 ab	6527 ± 109 a	5183 ± 516 ab	4624 ± 815 b	5430 ± 79 ab
Ethyl octanoate	13019 ± 570 bcde	13174 ± 643 ab	13074 ± 78 bcd	12499 ± 161 cde	12993 ± 184 bcde	14870 ± 883 ab	12864 ± 200 bcde	14143 ± 378 abc	13149 ± 301 bcd	13327 ± 613 bcd	14124 ± 429 abc	13605 ± 330 bcd	13362 ± 835 bcd	13932 ± 1601 abc	15820 ± 917 a	11631 ± 91 de	10856 ± 1308 e	12425 ± 953 cde
Ethyl decanoate	5668 ± 534 ab	6016 ± 700 ab	5119 ± 457 ab	5739 ± 568 ab	5885 ± 456 ab	5864 ± 91 ab	4752 ± 747 b	5186 ± 216 ab	5322 ± 208 ab	4995 ± 15 ab	5224 ± 324 ab	5365 ± 203 ab	5517 ± 570 ab	5922 ± 710 ab	6416 ± 943 a	5270 ± 609 ab	4722 ± 293 b	5307 ± 725 ab
Ethyl dodecaonat	83 ± 20 a	98 ± 20 a	87 ± 29 a	79 ± 20 a	83 ± 6 a	89 ± 5 a	64 ± 12 a	80 ± 14 a	60 ± 12 a	58 ± 15 a	69 ± 10 a	77 ± 17 a	56 ± 21 a	64 ± 15 a	73 ± 27 a	86 ± 19 a	62 ± 14 a	95 ± 28 a
Diethyl succinate	754 ± 98 a	643 ± 17 a	655 ± 66 a	627 ± 59 a	673 ± 37 a	651 ± 81 a	624 ± 16 a	653 ± 76 a	680 ± 33 a	673 ± 44 a	684 ± 46 a	665 ± 74 a	655 ± 15 a	687 ± 10 a	695 ± 8 a	749 ± 23 a	652 ± 62 a	690 ± 24 a
ACETATOS																		
Ethyl acetate	8857 ± 99 ab	8052 ± 355 abc	8559 ± 154 abc	8278 ± 126 abc	8128 ± 130 abc	8426 ± 367 abc	7870 ± 97 abc	7931 ± 582 abc	7953 ± 318 abc	8045 ± 224 abc	8351 ± 129 abc	7892 ± 61 abc	8252 ± 912 abc	8012 ± 498 abc	9009 ± 634 a	7528 ± 172 bc	7248 ± 883 c	7727 ± 659 abc
Isoamyl acetate	1195 ± 137 bcde	1434 ± 216 ab	1350 ± 101 ab	1221 ± 65 bcd	1252 ± 406 bcd	1237 ± 129 bcd	1215 ± 34 bcde	1199 ± 60 bcde	1233 ± 62 bcd	1214 ± 54 bcde	1207 ± 41 bcde	1145 ± 115 bcde	1278 ± 251 bc	1152 ± 140 bcde	1667 ± 97 a	971 ± 29 cde	889 ± 66 e	939 ± 107 de
Hexyl acetate	35 ± 4 ab	27 ± 2 bc	32 ± 4 ab	32 ± 3 ab	33 ± 5 ab	19.7 ± 3 c	30 ± 2 bc	24 ± 2 bc	29 ± 3 bc	29 ± 3 bc	30 ± 3 abc	26 ± 3 bc	34 ± 7 ab	33 ± 3 ab	41 ± 8 a	26 ± 1 bc	24.7 ± 3 bc	25 ± 2 bc
2-phenylehtyl acetate	23 ± 3 a	25 ± 3 a	25 ± 3 a	23 ± 3 a	23 ± 3 a	25 ± 1 a	25 ± 1 a	28 ± 6 a	26 ± 1 a	26 ± 4 a	28 ± 3 a	26 ± 3 a	26 ± 3 a	27 ± 1 a	29 ± 1 a	29 ± 2 a	24 ± 4 a	28 ± 3 a
ALCOHOLS																		
Isoamyl alcohol	5910 ± 298 ab	5498 ± 246 b	5612 ± 293 ab	5694 ± 270 ab	5809 ± 406 ab	5985 ± 620 ab	5511 ± 333 b	5867 ± 426 ab	5804 ± 454 ab	5675 ± 333 ab	5807 ± 362 ab	5554 ± 315 b	5844 ± 440 ab	5987 ± 360 ab	6837 ± 583 a	5438 ± 432 b	4962 ± 686 b	5320 ± 154 b
Isobutanol	181 ± 18 ab	180 ± 33 ab	223 ±20 a	160 ± 27 b	177 ± 4 ab	152 ± 9 b	163 ± 12 ab	158 ± 9 b	179 ± 16 ab	176 ± 9 ab	186 ± 4 ab	164 ± 13 ab	186 ± 34 ab	171 ± 14 ab	188 ± 15 ab	186 ± 19 ab	166 ± 37 ab	179 ± 21 ab
Benzil alcohol	8 ± 2 a	8 ± 0.1 a	9 ± 1 a	9 ± 2 a	7 ± 1 a	8 ± 2 a	7 ± 1 a	9 ± 1 a	9 ± 1 a	8 ± 1 a	7 ± 1 a	8.3 ± 1 a	8 ± 2 a	9 ± 1 a	9 ± 2 a	8 ± 1 a	7 ± 1 a	9 ± 2 a
2-phenylethyl alcohol	4113 ± 5319 a	1149 ± 96 a	1010 ± 23 a	1004 ± 127 a	1057 ± 31 a	1032 ± 105 a	952 ± 42 a	1197 ± 188 a	1140 ± 83 a	1062 ± 37 a	1120 ± 101 a	1119 ± 145 a	1096 ± 63 a	1168 ± 94 a	1217 ± 59 a	1100 ± 69 a	956 ± 83 a	1074 ± 42 a
FATTY ACIDS																		
Hexanoic acid	365± 29 a	352 ± 21 a	363 ± 36 a	331 ± 27 a	373 ± 22 a	323 ± 26 a	328 ± 6 a	364 ± 49 a	361 ± 23 a	373 ± 31 a	356 ± 22 a	357 ± 34 a	382 ± 25 a	378 ± 16 a	403 ± 13 a	403 ± 52 a	332 ± 33 a	384 ± 27 a
Octanoic acid	977 ± 102 ab	956 ± 28 ab	968 ± 84 ab	913 ± 35 b	1067 ± 35 ab	948 ± 93 ab	946 ± 42 ab	951 ± 90 ab	1025 ± 39 ab	1012 ± 51 ab	1005 ± 59 ab	1011 ± 89 ab	1050 ± 61 ab	1145 ± 96 a	1141 ± 41 a	1013 ± 13 ab	919 ± 84 b	977 ± 20 ab
Decanoic acid	336 ± 55 b	374 ± 11 ab	335 ± 17 b	369 ± 25 ab	430 ± 19 ab	386 ± 46 ab	354 ± 32 ab	380 ± 27 ab	385 ± 14 ab	382 ± 20 ab	415 ± 69 ab	407 ± 48 ab	398 ± 14 ab	446 ± 31 a	440 ± 17 a	419 ± 13 ab	360 ± 36 ab	392 ± 18 ab

Table S2. Families of volatile compounds (μg/L) from Champagne with the five corks and crown tap (Control, C1, C2, C3, C4, C5) with the doses of SO_2 under study (0, 10, 20 mg/L) Different letters indicate significant differences between corks and doses after 2 weeks of bottling (0M). Samples (n = 3). (continue)

	0 MONTH																	
Oxidatives compounds	**Control**			**C1**			**C2**			**C3**			**C4**			**C5**		
	0 mg/L	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**
ALKENALS																		
HEXANAL	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.31 ± 0.3 cd	0.0 ± 0.0 d	0.3 ± 0.5 bcd	0.0 ± 0.0 d	0.9 ± 0.3 a	0.9 ± 0.3 ab	0.3 ± 0.3 cd	0.35 ± 0.1 bcd	0.98 ± 0.1 a	0.7 ± 0.3 abc	0.2 ± 0.1 cd	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
OCTANAL	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	2.5 ± 1.3 b	7.5 ± 0.7 a	2.2 ± 1 b	2.5 ± 0.5 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
NONENAL	0.0 ± 0.0 hi	0.34 ± 0.1 ghi	4.3 ± 1.1 ef	8.5 ± 1 bc	7.2 ± 0.7 bcde	8.4 ± 0.8 bc	7.6 ± 1.0 bcd	6.2 ± 0.6 cde	2.9 ± 0.4 fgh	13.3 ± 2.7 a	9.8 ± 1.3 b	6.2 ± 0.7 cde	9.0 ± 1.0 bc	3.2 ± 0.4 fg	0.6 ± 0.5 ghi	0.0 ± 0.0 i	4.71 ± 0.4 def	4.9 ± 0.9 def
ALDEHYDES																		
2-methylpropanal	4.3 ± 0.9 fgh	3.4 ± 0.3 h	4.1 ± 0.1 gf	4.6 ± 0.3 defg	4.5 ± 0.2 defgh	5.5 ± 0.2 bcd	5.4 ± 0.3 bcdef	6.1 ± 0.2 b	5.4 ± 0.1 bcde	4.7 ± 0.3 defg	5.9 ± 0.2 bc	5.9 ± 0.1 bc	7.5 ± 0.5 a	4.6 ± 0.0 defg	4.4 ± 0.3 efgh	5.4 ± 0.4 bcde	4.9 ± 0.3 cdefg	4.6 ± 0.3 defg
3-methylbutanal	2.6 ± 0.4 j	2.6 ± 0.2 j	3.7 ± 0.3 fghij	4.6 ± 0.3 defgh	5.5 ± 0.4 cd	6.8 ± 0.4 b	4.2 ± 0.1 efghi	3.6 ± 0.1 ghij	4.8 ± 0.3 defg	6.6 ± 0.3 bc	7.6 ± 0.5 b	5.2 ± 0.4 de	7.4 ± 0.2 b	4.8 ± 0.1 def	3.0 ± 0.1 j	9.9 ± 1.2 a	3.5 ± 0.2 hij	3.0 ± 0.1 ij
2-phenylacetaldehide	10.3 ± 1.0 cde	4.4 ± 0.2 gh	3.7 ± 0.0 h	13 ± 2.0 bc	12.3 ± 0.9 c	10.5 ± 0.9 cde	10.1 ± 0.5 cde	7.6 ± 0.5 ef	11.3 ± 1.0 cd	15.3 ± 1.2 ab	12.4 ± 1.9 bc	7.6 ± 0.5 ef	16.0 ± 1.1 a	10.8 ± 0.9 cd	5.8 ± 0.4 fgh	8.5 ± 0.9 def	6.8 ± 0.3 fg	3.8 ± 0.4 h
KETONE																		
3-metil-2,4-nonandiona	1.3 ± 0.3 ab	1.2 ± 0.2 b	1.3 ± 0.1 ab	1.5 ± 0.0 ab	1.6 ± 0.0 a	1.5 ± 0.1 ab	1.5 ± 0.1 ab	1.4 ± 0.0 ab	1.5 ± 0.1 ab	1.6 ± 0.1 a	1.4 ± 0.0 ab	1.4 ± 0.1 ab	1.4 ± 0.1 ab	1.6 ± 0.0 a	1.5 ± 0.0 ab	0.64 ± 0.1 c	1.36 ± 0.1 ab	1.3 ± 0.1 ab
LACTONE																		
γ-nonalactone	17.1 ± 1.7 a	9.4 ± 0.7 cde	10.7 ± 0.5 bcd	7.6 ± 0.6 def	5.3 ± 1.3 efg	2.9 ± 2.8 g	1.98 ± 1.8 g	12.6 ± 1.0 abc	11.9 ± 1.5 bcd	10.6 ± .7 bcd	11.9 ± 0.9 bcd	14.3 ± 1.5 ab	1.7 ± 0.4 g	1.8 ± 0.5 g	0.7 ± 1.4 g	10.8 ± 0.7 bcd	4.9 ± 0.7 efg	4.2 ± 3.8 fg
Reductive compounds																		
DISULFIDES																		
Et_2S_2	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.7 ± 0.1 b	0.63 ± 0.1 b	0.0 ± 0.0 d	0.64 ± 0.1 b	0.7 ± 0.1 b	1.0 ± 0.1 a	0.3 ± 0.1 c	0.74 ± 0.1 b	0.74 ± 0.1 b	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d

Table S3. Families of volatile compounds (µg/L) from Champagne with the five corks and crown tap (Control, C1, C2, C3, C4, C5) with the doses of SO_2 under study (0, 10, 20 mg/L) Different letters indicate significant differences between corks and doses after 3M of bottling. Samples (n = 3).

	3 MONTHS																	
Fermentatives compounds	**Control**			**C1**			**C2**			**C3**			**C4**			**C5**		
	0 mg/L	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**
ESTERS																		
Ethyl butyrate	355 ± 67 d	455 ± 73 bcd	503± 46 abc	407 ± 24 cd	554 ± 44 ab	476 ± 38 abcd	479 ± 14 abc	488 ± 47 abc	493 ± 57 abc	474 ± 7 abcd	502 ± 7 abc	449 ± 28 bcd	593 ± 26 a	562 ± 46 ab	536 ± 12 ab	385 ± 26 cd	488 ± 33 abc	477 ± 29 abc
Ethyl isovaleriate	100 ± 21 f	130 ± 21 cdef	145 ± 14 abcd	120 ± 6 def	167 ± 11 ab	134 ± 12 bcdef	130 ± 6 cdef	137 ± 7 abcde	149 ± 9 abcd	135 ± 5 bcde	142 ± 5 abcd	125 ± 3 cdef	169 ± 6 a	167 ± 18 ab	155 ± 8 abc	108 ± 4 ef	146 ± 8 abcd	141 ± 9 abcde
Ethyl hexanoate	4632 ± 1046 b	5170 ± 1059 ab	5237 ± 515 ab	5237 ± 222 ab	6326 ± 170 a	5923 ± 235 ab	5760 ± 21 ab	5650 ± 86 ab	5760 ± 176 ab	5966 ± 355 ab	5856 ± 188 ab	5648 ± 229 ab	6505 ± 339 a	5805 ± 315 ab	6248 ± 120 a	4786 ± 305 b	5569 ± 520 ab	5516 ± 159 ab
Ethyl octanoate	13869 ± 612 b	14434 ± 186 ab	11314 ± 889 c	12560 ± 460 bc	14604 ± 267 ab	13980 ± 108 b	13525 ± 857 b	12647 ± 823 bc	13138 ± 1068 bc	14277 ± 179 ab	13623 ± 319 b	13751 ± 509 b	14450 ± 264 ab	13522 ± 1236 b	16217 ± 468 a	11106 ± 381 c	12596 ± 1302 bc	12960 ± 204 bc
Ethyl decanoate	3584 ± 275 a	4034 ± 30 a	3621 ± 646 a	3541 ± 170 a	4193 ± 1054 a	3886 ± 574 a	3769 ± 974 a	3504 ± 539 a	3694 ± 701 a	3872 ± 204 a	3915 ± 536 a	3863 ± 578 a	3884 ± 209 a	4325 ± 979 a	4528 ± 765 a	3263 ± 280 a	3556 ± 330 a	3826 ± 698 a
Ethyl dodecaonat	155 ± 18 a	84 ± 10 bc	105 ± 15 ab	84 ± 5 bc	67 ± 23 bc	69 ± 18 bc	69 ± 29 bc	51 ± 15 c	60 ± 23 bc	75 ± 19 bc	62 ± 2 bc	56 ± 8 bc	54 ± 6 bc	65 ± 24 bc	58 ± 11 bc	98 ± 11 bc	64 ± 13 bc	81 ± 25 bc
Diethyl succinate	544 ± 102 b	528 ± 131 b	504 ± 24 b	790 ± 76 a	602 ± 68 ab	591 ± 56 ab	614 ± 76 ab	498 ± 24 b	497 ± 53 b	551 ± 97 b	576 ± 8 ab	623 ± 76 ab	659 ± 79 ab	542 ± 19 b	609 ± 20 ab	520 ± 29 b	640 ± 53 ab	618 ± 110 ab
ACETATOS																		
Ethyl acetate	8128 ± 1540 ab	7981 ± 1513 ab	8490 ± 607 ab	8777 ± 322 ab	9387 ± 220 ab	9115 ± 237 ab	9140 ± 75 ab	9168 ± 92 ab	9251 ± 22 ab	9305 ± 144 ab	9143 ± 301 ab	8932 ± 401 ab	9819 ± 460 a	9342 ± 102 ab	9578 ± 32 ab	7800 ± 617 b	8606 ± 745 ab	8909 ± 173 ab
Isoamyl acetate	443 ± 88 g	895 ± 134 cde	1062 ± 18 abc	711 ± 33 ef	1151 ± 43 a	945 ± 89 bcd	940 ± 9 bcd	994 ± 150 abcd	1002 ± 15 abcd	943 ± 36 bcd	1006 ± 21 abcd	878 ± 147 cde	1179 ± 87 a	1101 ± 48 ab	1120 ± 10 ab	654 ± 8 f	846 ± 23 def	830 ± 27 def
Hexyl acetate	18 ± 2 f	22 ± 2 def	28 ± 2 abcde	22 ± 2 ef	29 ± 2 abc	24 ± 1 cdef	26 ± 1 bcde	28 ± 3 abcde	27 ± 1 abcde	26 ± 1 bcde	27 ± 1 abcde	28 ± 1 abcd	34 ± 1 a	31 ± 4 ab	27 ± 2 abcde	20 ± 3 f	24 ± 3 cdef	24 ± 1 cdef
2-phenylehtyl acetate	21 ± 2 abc	18 ± 4 c	20 ± 2 bc	28 ± 4 a	17 ± 2 c	22 ± 1 abc	21 ± 2 abc	19 ± 1 bc	18 ± 2 c	19 ± 2 c	20 ± 1 bc	21 ± 3 abc	26 ± 5 ab	19 ± 1 bc	20 ± 1 bc	20 ± 1 bc	22 ± 3 abc	22 ± 3 abc
ALCOHOLS																		
Isoamyl alcohol	5779 ± 1146 a	5601 ± 1106 a	5861 ± 547 a	6191 ± 275 a	6751 ± 201 a	6768 ± 230 a	6750 ± 8 a	6583 ± 128 bc	6678 ± 38 a	6730 ± 165 a	6616 ± 209 a	6361 ± 227 a	7009 ± 295 a	6911 ± 172 a	6959 ± 72 a	5777 ± 492 a	6099 ± 670 a	6426 ± 211 a
Isobutanol	146 ± 15 c	164 ± 28 bc	176 ± 5 bc	155 ± 13 bc	190 ± 11 bc	185 ± 6 bc	183 ± 8 bc	164 ± 9 bc	164 ± 19 bc	183 ± 4 bc	186 ± 5 bc	172 ± 14 bc	204 ± 15 ab	247 ± 51 a	195 ± 7 bc	156 ± 8 bc	185 ± 7 bc	179 ± 5 bc
Benzil alcohol	8 ± 0 a	6 ± 1 ab	7 ± 1 a	6 ± 1 ab	7 ± 1 a	6 ± 1 abc	6 ± 1 ab	6 ± 1 ab	6 ± 1 a	5 ± 0 abc	3 ± 0 cd	5 ± 2 abc	6 ± 0.0 ab	3 ± 1 d	4 ± 1 bcd	6 ± 1 ab	7 ± 0 a	7 ± 0 a
2-phenylethyl alcohol	928 ± 101 a	819 ± 163 a	807 ± 60 a	955 ± 376 a	834 ± 16 a	948 ± 33 a	990 ± 64 a	841 ± 19 a	839 ± 70 a	850 ± 80 a	893 ± 12 a	949 ± 91 a	1027 ± 193 a	844 ± 9 a	978 ± 18 a	850 ± 33 a	964 ± 50 a	870 ± 94 a
FATTY ACIDS																		
Hexanoic acid	322 ± 39 a	321 ± 57 a	321 ± 3 a	351 ± 95 a	346 ± 22 a	355 ± 24 a	366 ± 26 a	309 ± 4 a	304 ± 22 a	339 ± 46 a	347 ± 6 a	360 ± 29 a	410 ± 31 a	354 ± 11 a	360 ± 16 a	306 ± 15 a	361 ± 22 a	357 ± 67 a
Octanoic acid	885 ± 122 ab	909 ± 174 ab	891 ± 3 ab	948 ± 362 ab	905 ± 87 ab	955 ± 51 ab	999 ± 27 ab	865 ± 27 ab	772 ± 23 b	932 ± 79 ab	921 ± 82 ab	993 ± 41 ab	1136 ± 130 a	949 ± 15 ab	1065 ± 47 ab	817 ± 31 ab	989 ± 41 ab	908 ± 91 ab
Decanoic acid	360 ± 14 ab	350 ± 26 ab	380 ± 55 ab	353 ± 135 ab	381 ± 22 ab	353 ± 23 ab	373 ± 41 ab	308 ± 13 ab	256 ± 20 b	361 ± 22 ab	360 ± 76 ab	358 ± 10 ab	394 ± 36 ab	394 ± 68 ab	418 ± 37 a	331 ± 44 ab	395 ± 23 ab	329 17 ab

Table S3. Families of volatile compounds (μg/L) from Champagne with the five corks and crown tap (Control, C1, C2, C3, C4, C5) with the doses of SO_2 under study (0, 10, 20 mg/L) Different letters indicate significant differences between corks and doses after 3months of bottling (3M). Samples (n = 3). (continue)

Oxidatives compounds	3 MONTHS																	
	Control			C1			C2			C3			C4			C5		
	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L
ALKENALS																		
Hexanal	0.9 ± 0.0 efg	1.8 ± 0.1 d	2.9 ± 0.0 b	0.6 ± 0.0 fghi	1.0 ± 0.1 e	0.5 ± 0.0 ghij	0.5 ± 0.0 hij	0.4 ± 0.0 hij	0.2 ± 0.0 j	2.5 ± 0.2 c	1.9 ± 0.2 d	1.0 ± 0.1 ef	0.3 ± 0.0 ij	0.6 ± 0.0 fgh	2.5 ± 0.4 c	2.1 ± 0.1 cd	0.3 ± 0.0 ij	5.5 ± 0.2 a
Octanal	11.2 ± 0.3 a	10.4 ± 0.0 b	8.4 ± 0.3 c	4.2 ± 0.1 f	5.5 ± 0.2 e	3.2 ± 0.0 g	2.6 ± 0.1 h	1.5 ± 0.0 ij	1.3 ± 0.0 j	5.7 ± 0.1 e	4.3 ± 0.1 f	4.4 ± 0.1 f	3.4 ± 0.1 g	1.8 ± 0.0 i	1.6 ± 0.1 ij	11.4 ± 0.2 a	8.6 ± 0.1 c	7.0 ± 0.1 d
Nonenal	4.7 ± 0.0 f	5.0 ± 0.1 e	5.8 ± 0.1 d	2.7 ± 0.0 i	1.8 ± 0.1 k	1.3 ± 0.0 l	8.8 ± 0.0 a	6.1 ± 0.1 c	3.4 ± 0.0 g	3.4 ± 0.0 g	2.2 ± 0 j	2.9 ± 0.1 i	8.3 ± 0.0 b	3.3 ± 0.1 g	3.1 ± 0.1 h	5.0 ± 0.1 e	5.9 ± 0.0 cd	2.7 ± 0.0 i
ALDEHYDES																		
2-methylpropanal	4.3 ± 0.9 fgh	3.4 ± 0.3 h	4.1 ± 0.1 gf	4.6 ± 0.3 defg	4.5 ± 0.2 defgh	5.5 ± 0.2 bcd	5.4 ± 0.3 bcdef	6.1 ± 0.2 b	5.4 ± 0.1 bcde	4.7 ± 0.3 defg	5.9 ± 0.2 bc	5.9 ± 0.1 bc	7.5 ± 0.5 a	4.6 ± 0.0 defg	4.4 ± 0.3 efgh	5.4 ± 0.4 bcde	4.9 ± 0.3 cdefg	4.6 ± 0.3 defg
3-methylbutanal	2.6 ± 0.4 j	2.6 ± 0.2 j	3.7 ± 0.3 fghij	4.6 ± 0.3 defgh	5.5 ± 0.4 cd	6.8 ± 0.4 b	4.2 ± 0.1 efghi	3.6 ± 0.1 ghij	4.8 ± 0.3 defg	6.6 ± 0.3 bc	7.6 ± 0.5 b	5.2 ± 0.4 de	7.4 ± 0.2 b	4.8 ± 0.1 def	3.0 ± 0.1 j	9.9 ± 1.2 a	3.5 ± 0.2 hij	3.0 ± 0.1 ij
2-phenylacetaldehide	10.3 ± 1.0 cde	4.4 ± 0.2 gh	3.7 ± 0.0 h	13 ± 2.0 bc	12.3 ± 0.9 c	10.5 ± 0.9 cde	10.1 ± 0.5 cde	7.6 ± 0.5 ef	11.3 ± 1.0 cd	15.3 ± 1.2 ab	12.4 ± 1.9 bc	7.6 ± 0.5 ef	16.0 ± 1.1 a	10.8 ± 0.9 cd	5.8 ± 0.4 fgh	8.5 ± 0.9 def	6.8 ± 0.3 fg	3.8 ± 0.4 h
Methional	8.3 ± 0 abcde	5.6 ± 0.1 bcde	2.3 ± 0.1 de	14.6 ± 0.1 a	13.4 ± 0.1 ab	12.6 ± 0.1 abc	1.6 ± 0.1 e	1.2 ± 0.0 e	0.9 ± 0.1 e	6.3 ± 0.0 bcde	8.3 ± 10.7 abcde	4.0 ± 0.1 de	0.8 ± 0.0 e	0.7 ± 0.0 e	0.9 ± 0.0 e	9.4 ± 0 abcd	4.2 ± 0.0 de	5.1 ± 0.1 cde
KETONE																		
3-metil-2,4-nonandiona	3.7 ± 0.1 a	3.2 ± 0.0 bcd	3.3 ± 0.5 abc	2.5 ± 0.1 ef	2.7 ± 0.1 ef	2.3 ± 0.1 f	2.6 ± 0.1 ef	2.3 ± 0.0 ef	2.4 ± 0.1 ef	3.1 ± 0.0 bcd	2.7 ± 0.1 de	2.6 ± 0.1 ef	3.5 ± 0.1 ab	3.3 ± 0.0 abc	3.2 ± 0.1 bcd	3.3 ± 0.2 abc	3.1 ± 0.1 cd	2.2 ± 0.1 f
LACTONE																		
ɣ-nonalactone	14.5 ± 0.1 d	10.7 ± 0.0 i	8.1 ± 0 j	14.1 ± 0 de	7.2 ± 0.1 k	6.6 ± 0.1 l	21.6 ± 0.2 a	12.3 ± 0.1	14.5 ± 0.1 d	14.6 ± 0.1 d	11.4 ± 0.0 h	7.1 ± 0.1 kl	21.0 ± 0.1 b	11.3 ± 0.1 h	16.4 ± 0.7 c	16.1 ± 0.1 c	13.5 ± 0.0 f	13.7 ± 0.1 ef
Reductive compounds																		
SULFIDES																		
Me_2S	4.5 ± 0.5 bc	2.9 ± 0.1 def	2.7 ± 0.2 ef	6.8 ± 0.6 a	3.5 ± 0.3 de	3.7 ± 0.2 cd	1.5 ± 0.3 gh	1.3 ± 0.2 h	0.9 ± 0.1 h	3.6 ± 0.4 de	2.5 ± 0.2 f	2.2 ± 0.2 fg	1.1 ± 0.1 h	1.3 ± 0.1 h	1.4 ± 0.0 gh	1.5 ± 0.1 gh	3.0 ± 0.4 def	4.6 ± 0.3 b
DISULFIDES																		
Me_2S_2	0.5 ± 0.5 a	0.6 ± 0.0 a	0.3 ± 0.1 bc	0.3 ± 0.1 b	0.2 ± 0.0 bcd	0.2 ± 0 def	0.1 ± 0.1 f	0.2 ± 0.0 bcde	0.2 ± 0.0 bcdef	0.1 ± 0.0 ef	0.2 ± 0.0 f	0.1 ± 0.0 ef	0.1 ± 0.0 def	0.1 ± 0.0 def	0.2 ± 0.0 def	0.3 ± 0.0 b	0.2 ± 0.0 bcdef	0.2 ± 0.1 cdef
Et_2S_2	0.6 ± 0 ef	0.1 ± 0.0 g	0.6 ± 0.0 ef	0.7 ± 0.1 bcd	0.9 ± 0.0 a	0.8 ± 0 abc	0.5 ± 0 f	0.7 ± 0.0 bcd	0.6 ± 0.0 ef	0.7 ± 0.0 cd	0.8 ± 0.0 ab	0.6 ± 0.0 de	0.5 ± 0.0 f	0.6 ± 0.0 ef	0.6 ± 0.0 ef	0.7 ± 0.0 cd	0.6 ± 0.0 de	0.8 ± 0.0 bc

Table S4. Families of volatile compounds (µg/L) from Champagne with the five corks and crown tap (Control, C1, C2, C3, C4, C5) with the doses of SO_2 under study (0, 10, 20 mg/L) Different letters indicate significant differences between corks and doses after 6M of bottling. Samples (n = 3).

	6 MONTHS																	
Fermentatives compounds	**Control**			**C1**			**C2**			**C3**			**C4**			**C5**		
	0 mg/L	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**
ESTERS																		
Ethyl butyrate	456 ± 35 a	523 ± 61 a	419 ± 55 ab	449 ± 39 a	430 ± 20 ab	454 ± 7 a	493 ± 43 a	509 ± 57 a	455 ± 83 a	449 ± 79 a	476 ± 49 a	512 ± 90 a	493 ± 59 a	480 ± 102 a	498 ± 156 a	207 ± 144 b	471 ± 64 a	417 ± 8 ab
Ethyl isovaleriate	140 ± 14 a	164 ± 21 a	129 ± 21 a	139 ± 6 a	133 ± 6 a	147 ± 2 a	158 ± 17 a	159 ± 21 a	149 ± 27 a	147 ± 25 a	158 ± 18 a	170 ± 33 a	165 ± 21 a	158 ± 34 a	176 ± 42 a	128 ± 13 a	148 ± 25 a	126 ± 0 a
Ethyl hexanoate	5290 ± 869 a	5276 ± 709 a	4775 ± 928 a	4407 ± 476 a	4656 ± 830 a	4756 ± 792 a	5831 ± 998 a	5506 ± 579 a	5142 ± 258 a	5964 ± 1277 a	5762 ± 872 a	6078 ± 1313 a	5740 ± 1262 a	5615 ± 522 a	6005 ± 1442 a	4384 ± 910 a	4593 ± 118 a	4588 ± 611 a
Ethyl octanoate	9495 ± 1643 ab	10679 ± 1782 ab	8586 ± 1414 b	8209 ± 58 b	8334 ± 1041 b	9099 ± 550 ab	10412 ± 1859 ab	9502 ± 708 ab	9002 ± 556 ab	11147 ± 2317ab	10213 ± 1515 ab	10718 ± 2312 ab	10876 ± 1831 ab	10453 ± 1157 ab	13336 ± 2991 a	7874 ± 1276 b	8346 ± 389 b	8147 ± 523 b
Ethyl decanoate	3953 ± 463 ab	4476 ± 491 a	3150 ± 435 bcde	2268 ± 396 efg	2558 ± 281 defg	2227 ± 285 efg	2283 ± 20 efg	2269 ± 159 efg	1867 ± 180 g	3355 ± 208 bcd	2790 ± 206 cdefg	2942 ± 215 cdef	3272 ± 50 bcd	3450 ± 4 bcd	3630 ± 86 abc	1893 ± 298 g	2118 ± 656 fg	3 ± 355 fg
Ethyl dodecaonat	21 ± 11 ab	28 ± 9 a	19 ± 7 ab	12 ± 2 b	11 ± 2 b	8 ± 2 b	10 ± 0 b	12 ± 1 b	8 ± 0 b	11 ± 3 b	9 ± 0 b	12 ± 3 b	12 ± 1 b	15 ± 5 b	13 ± 0 b	12 ± 2 b	11 ± 3 b	9 ± 3 b
Diethyl succinate	61 ± 10 a	51 ± 3 abc	37 ± 10 cde	30 ± 1 de	42 ± 3 bcde	25 ± 5 e	34 ± 5 cde	47 ± 2 abcd	50 ± 5 abc	47 ± 5 abcd	42 ± 4 bcde	43 ± 6 bcde	45 ± 7 abcd	58 ± 3 ab	58 ± 9 ab	35 ± 6 cde	42 ± 10 bcde	42 ± 2 bcde
ACETATOS																		
Ethyl acetate	6355 ± 862 a	7090 ± 1874 a	5748 ± 871 a	5965 ± 225 a	5770 ± 535 a	6025 ± 122 a	6697± 973 a	7307 ± 1354 a	7089 ± 1299 a	6835 ± 1437 a	6978 ± 1159 a	7243 ± 1712 a	7461 ± 1454 a	7314 ± 1521 a	8667 ± 2433 a	5594 ± 638 a	6328 ± 512 a	5793 ± 249 a
Isoamyl acetate	812 ± 75 ab	916 ± 263 a	695 ± 106 ab	793 ± 40 ab	725 ± 43 ab	738 ± 8 ab	813 ± 69 ab	820 ± 93 ab	754 ± 112 ab	768 ± 136 ab	802 ± 86 ab	884 ± 150 a	812 ± 87 ab	845 ± 170 ab	800 ± 97 ab	542 ± 38 b	669 ± 102 ab	702 ± 7 ab
Hexyl acetate	20 ± 0 a	24 ± 6 a	18 ± 3 a	21 ± 1 a	18 ± 1 a	18 ± 0 a	22 ± 2 a	22 ± 3 a	18 ± 4 a	19 ± 3 a	21 ± 3 a	22 ± 4 a	23 ± 3 a	19 ± 5 a	22. ± 5 a	17 ± 2 a	19 ± 4 a	16 ± 1 a
2-phenylehtyl acetate	22 ± 0 ab	25 ± 10 a	17 ± 2 abc	19 ± 0 abc	19 ± 2 abc	17 ± 0 bc	18 ± 2 abc	19 ± 1 abc	17 ± 0 abc	20 ± 3 abc	20 ± 1 abc	21 ± 3 abc	18 ± 0 abc	19 ± 3 abc	19 ± 2 abc	13 ± 1 c	17 ± 2 bc	19 ± 2 abc
ALCOHOLS																		
Isoamyl alcohol	5108 ± 743 a	5402 ± 1243 a	4612 ± 850 a	4551 ± 176 a	4582 ± 632 a	4723 ± 457 a	5543 ± 862 a	5422 ± 545 a	5203 ± 580 a	5546 ± 1158 a	5377 ± 770 a	5644 ± 1167 a	5589 ± 826 a	5448 ± 825 a	6202 ± 1260 a	4381 ± 713 a	4822 ± 102 a	4679 ± 589 a
Isobutanol	109 ± 32 a	125 ± 32 a	78 ± 20 a	108 ± 6 a	148 ± 0 a	207 ± 112 a	234 ± 100 a	206 ± 48 a	165 ± 52 a	147 ± 29 a	113 ± 11 a	115 ± 13 a	128 ± 14 a	177 ± 76 a	191 ± 82 a	195 ± 75 a	131 ± 38 a	110 ± 0 a
Benzil alcohol	6 ± 1 ab	6 ± 0 ab	5 ± 1 ab	5 ± 0 ab	6 ± 0 ab	5 ± 0 ab	6 ± 1 ab	6 ± 1 ab	6 ± 0 ab	7 ± 2 a	7 ± 1 ab	7 ± 1 ab	7 ± 0 ab	7 ± 1 ab	7 ± 1 ab	4 ± 0 b	6 ± 0 ab	6 ± 0 ab
2-phenylethyl alcohol	1074 ± 230 ab	1228 ± 158 ab	941 ± 239 ab	955 ± 83 ab	1082 ± 67 ab	966 ± 93 ab	1191 ± 132 ab	1295 ± 154 ab	1248 ± 3 ab	1437 ± 433 a	1305 ± 229 ab	1308 ± 209 ab	1203 ± 35 ab	1321 ± 249 ab	1148 ± 159 ab	845 ± 142 b	1060 ± 11 ab	1106 ± 190 ab
FATTY ACIDS																		
Hexanoic acid	435 ± 69 ab	512 ± 44 ab	385 ± 77 ab	413 ± 21 ab	444 ± 37 ab	411 ± 35 ab	480 ± 57 ab	521 ± 52 ab	473 ± 28 ab	563 ± 138 a	505 ± 77 ab	543 ± 72 a	471 ± 11 ab	516 ± 111 ab	462 ± 74 ab	336 ± 47 b	450 ± 19 ab	454 ± 86 ab
Octanoic acid	1132 ± 185 abc	1323 ± 148 ab	989 ± 232 abc	925 ± 52 abc	1016 ± 98 abc	899 ± 40 bc	1086 ± 121 abc	1171 ± 111 abc	1066 ± 48 abc	1374 ± 310 a	1266 ± 129 ab	1340 ± 213 ab	1204 ± 12 abc	1307 ± 121 ab	1209 ± 175 abc	784 ± 132 c	1017 ± 54 abc	1035 ± 150 abc
Decanoic acid	397 ± 16 ab	457 ± 18 a	310 ± 47 c	191 ± 5 d	223 ± 4 d	178 ± 24 d	219 ± 11 d	221 ± 8 d	192 ± 8 d	342 ± 47 bc	308 ± 0 c	321 ± 37 c	314 ± 10 c	355 ± 21 bc	324 ± 25 c	185 ± 17 d	211 ± 22 d	215 ± 12 d

Table S4. **Families of volatile compounds (μg/L) from Champagne with the five corks and crown tap (Control, C1, C2, C3, C4, C5) with the doses of SO_2 under study (0, 10, 20 mg/L) Different letters indicate significant differences between corks and doses after 6M of bottling. Samples (n = 3). (continue)**

	6 MONTHS																	
Oxidatives compounds	**Control**			**C1**			**C2**			**C3**			**C4**			**C5**		
	0 mg/L	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**
ALKENALS																		
Hexanal	2.0 ± 0.0 bc	1.5 ± 0.3 cdefg	1.1 ± 0.2 fghi	1.4 ± 0.1 defgh	0.7 ± 0.1 ijk	0.3 ± 0.0 k	1.0 ± 0.0 ghi	0.40 ± 0.0 jk	0.4 ± 0.2 jk	2.5 ± 0.1 ab	2.4 ± 0.1 ab	1.6 ± 0.0 cde	0.9 ± 0.1 hij	1.1 ± 0.3 efghi	2.2 ± 0.4 ab	2.5 ± 0.3 a	1.7 ± 0.0 cd	1.5 ± 0.1 cdef
Octanal	9.6 ± 0.9 bc	10.3 ± 0.1 b	9.4 ± 0.3 c	7.1 ± 0.2 e	2.7 ± 0.3 gh	2.2 ± 0.3 hi	3.3 ± 0.1 g	2.2 ± 0.2 hi	1.8 ± 0.1 i	6.9 ± 0.2 e	4.2 ± 0.3 f	4.2 ± 0.1 f	3.4 ± 0.2 fg	2.1 ± 0.0 hi	1.9 ± 0.0 hi	11.3 ± 0.1 a	8.3 ± 0.4 d	7.0 ± 0.1 e
Nonenal	7.4 ± 0.0 c	6.6 ± 0.3 d	5.9 ± 0.1 fg	6.8 ± 0.0 d	6.4 ± 0.1 def	4.7 ± 0.0 h	9.6 ± 0.2 a	2.0 ± 0.0 i	6.5 ± 0.4 de	6.0 ± 0.3 ef	6.8 ± 0.3 d	2.1 ± 0.0 i	8.3 ± 0.1 b	7.8 ± 0.2 bc	4.4 ± 0.1 h	8.3 ± 0.0 b	5.4 ± 0.0 g	1.7 ± 0.0 i
ALDEHYDES																		
2-methylpropanal	18.6 ± 0.0 h	13.6 ± 0.2 i	13.3 ± 0.5 i	25.0 ± 0.1 e	22.4 ± 0.1 fg	21.3 ± 0.5 g	40.6 ± 0.5 a	34.4 ± 1.2 b	23.7 ± 1.2 ef	39.2 ± 0.4 a	25.0 ± 0.4 e	27.9 ± 0.7 d	40.3 ± 0.5 a	32.5 ± 0.4 c	32.3 ± 1.0 c	22.0 ± 0.4 fg	22.1 ± 0.5 fg	14.8 ± 0.6 i
3-methylbutanal	6.0 ± 0.3 l	3.1 ± 0.6 m	5.4 ± 0.2 l	15.1 ± 0.4 ef	12.9 ± 0.4 hi	13.3 ± 0.7 ghi	14.2 ± 0.6 fgh	12.8 ± 0.4 i	10.2 ± 0.0 j	20.9 ± 0.5 a	17.6 ± 0.2 bc	17.2 ± 0.2 cd	20.6 ± 0.2 a	18.9 ± 0.4 b	16.1 ± 0.3 de	14.6 ± 0.6 fg	7.9 ± 0.3 k	9.8 ± 1.1 j
2-phenylacetaldehide	1.4 ± 0.1 kl	2.1 ± 0.3 jkl	12.1 ± 0.5 b	9.9 ± 0.1 c	1.2 ± 0.1 l	1.2 ± 0.2 l	2.7 ± 0.1 j	8.0 ± 0.3 d	3.2 ± 0.0 ij	6.2 ± 0.2 ef	5.3 ± 0.1 fg	7.1 ± 0.0 de	2.6 ± 0.1 jk	4.5 ± 0.1 gh	16.1 ± 0.6 a	4.0 ± 0.1 hi	8.0 ± 0.6 d	10.2 ± 1.2 c
Methional	10.8 ± 0.2 ef	12.6 ± 0.6 d	8.9 ± 0.1 ghi	10.0 ± 0.2 efg	9.5 ± 0.3 fgh	8.0 ± 0.1 i	4.5 ± 0.2 jk	3.1 ± 0.0 lm	2.2 ± 0.0 m	8.3 ± 0.0 hi	10.9 ± 0.6 e	5.8 ± 0.6 j	5.7 ± 0.3 j	3.6 ± 0.4 kl	2.6 ± 0.0 lm	26.0 ± 0.4 a	21.2 ± 1.1 b	18.7 ± 0.6 c
KETONE																		
3-metil-2,4-nonandiona	1.5 ± .0.1 cde	1.5 ± 0.0 cde	1.7 ± 0.0 bcde	1.8 ± 0.0 bcd	1.6 ± 0.0 bcde	1.6 ± 0.0 bcde	1.4 ± 0.1 cde	1.8 ± 0.1 bcd	2.8 ± 0.1 a	1.5 ± 0.1 cde	2.2 ± 0.4 ab	1.4 ± 0.2 de	2.2 ± 0.8 ab	2.1 ± 0.2 abc	1.3 ± 0.3 de	1.4 ± 0.0 cde	2.0 ± 0.1 bcd	1.0 ± 0.0 e
LACTONE																		
ɣ-nonalactone	19.4 ± 0.7 cd	14.8 ± 0.6 fg	13.8 ± 0.5 g	19.9 ± 0.1 c	14.4 ± 0.2 g	7.7 ± 0.2 i	25.9 ± 0.2 a	17.2 ± 1.5 def	15.7 ± 0.7 efg	13.8 ± 1.5 g	14.2 ± 0.5 g	10.8 ± 0.6 h	22.3 ± 1.3 b	18.5 ± 0.5 cd	18.0 ± 1.0 cde	15.8 ± 0.7 efg	13.5 ± 0.5 g	11.1 ± 0.5 h
Reductive compounds																		
THIOLS																		
H_2S	14.0 ± 0.5 c	17.8 ± 0.2 b	19.5 ± 0.2 a	0.9 ± 0.1 k	8.9 ± 0.1 g	9.3 ± 0.1 efg	9.2 ± 0.2 fg	10.4 ± 0.6 d	10.3 ± 0.2 de	1.0 ± 0.2 k	10.9 ± 0.7 d	10.1 ± 0.4 def	4.2 ± 0.1 i	9.1 ± 0.3 fg	2.8 ± 0.2 j	2.8 ± 0.2 j	6.1 ± 0.5 h	3.0 ± 0.6 j
MeSH	1.5 ± 0.1 d	2.4 ± 0.2 c	3.9 ± 0.3 a	0.7 ± 0.2 e	2.2 ± 0.0 c	3.1 ± 0.1 b	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0 f
SULFITES																		
Me2S	4.0 ± 0.1 hij	3.7 ± 0.1 ij	3.6 ± 0.2 j	16.9 ± 0.2 a	5.5 ± 0.3 d	5.0 ± 0.3 ef	3.1 ± 0.1 k	4.7 ± 0.1 fg	4.0 ± 0.1 hij	8.1 ± 0.0 b	4.1 ± 0.1 hij	6.0 ± 0.1 c	4.4 ± 0.0 gh	4.2 ± 0.0 ghi	4.0 ± 0.1 hij	3.7 ± 0.0 ij	3.9 ± 0.1 hij	5.5 ± 0.3 de
DISULFIDES																		
Me_2S_2	2.5 ± 0.0 a	1.0 ± 0.3 defg	0.7 ± 0.2 g	1.3 ± 0.0 bc	1.0 ± 0.0 cdef	1.0 ± 0.0 def	0.8 ± 0.1 fg	0.9 ± 0.0 defg	1.0 ± 0.1 defg	1.2 ± 0.1 bcd	2.3 ± 0.1 a	1.1 ± 0.0 cde	0.9 ± 0.0 defg	0.9 ± 0.0 efg	1.0 ± 0.0 defg	1.0 ± 0.0 def	1.4 ± 0.0 b	0.8 ± 0.1 fg
Et_2S_2	1.8 ± 0.1 a	0.5 ± 0.1 fghi	0.3 ± 0.0 i	0.6 ± 0.1 defg	0.6 ± 0.1 defg	0.7 ± 0.1 cde	0.6 ± 0.1 efg	0.5 ± 0.0 efgh	0.4 ± 0.0 hi	1.1 ± 0.0 b	0.8 ± 0.1 cd	0.9 ± 0.1 c	0.6 ± 0.1 defg	0.6 ± 0.0 defg	0.6 ± 0.1 defg	0.5 ± 0.0 efghi	0.5 ± 0.0 ghi	0.6 ± 0.0 def

Table S5. Families of volatile compounds (μg/L) from Champagne with the five corks and crown tap (Control, C1, C2, C3, C4, C5) with the doses of SO_2 under study (0, 10, 20 mg/L) Different letters indicate significant differences between corks and doses after 12M bottling. Samples (n = 3).

	12 MONTHS																	
Fermentatives compounds	Control			C1			C2			C3			C4			C5		
	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L	0 mg/L	10 mg/L	20 mg/L
ESTERS																		
Ethyl butyrate	355 ± 20 a	371 ± 2 a	320 ± 2 a	304 ± 23 a	358 ± 7 a	329 ± 1 a	336 ± 20 a	308 ± 11 a	327 ± 49 a	337 ± 33 a	332 ± 3 a	343 ± 5 a	341 ± 36 a	338 ± 53 a	343 ± 66 a	312 ± 23 a	355 ± 20 a	381 ± 19 a
Ethyl isovaleriate	147 ± 8 ab	147 ± 7 ab	141 ± 6 ab	124 ± 13 b	139 ± 7 ab	126 ± 7 ab	132 ± 4 ab	121 ± 3 b	126 ± 20 ab	132 ± 5 ab	131 ± 4 ab	134 ± 6 ab	137 ± 15 ab	131 ± 20 ab	139 ± 21 ab	137 ± 8 ab	150 ± 11 ab	160 ± 3 a
Ethyl hexanoate	5589 ± 351 abc	5861 ± 192 a	5486 ± 88 abc	5099 ± 73 c	5926 ± 27 a	5489 ± 93 abc	5631 ± 292 abc	5067 ± 44 c	5142 ± 222 bc	5524 ± 445 abc	5845 ± 23 ab	5635 ± 44 abc	5719 ± 93 abc	5875 ± 352 a	5987 ± 532 a	5279 ± 162 abc	5681 ± 118 abc	5691 ± 5 abc
Ethyl octanoate	10818 ± 595 abc	10748 ± 93 abc	11961 ± 429 abc	11274 ± 767 abc	12002 ± 590 abc	10809 ± 1140 abc	11011 ± 331 abc	10574 ± 1153 bc	9995 ± 808 c	10866 ± 181 abc	13009 ± 997 ab	11156 ± 465 abc	12390 ± 1206 abc	13297 ± 1474 a	10690 ± 1355 abc	11521 ± 706 abc	11108 ± 641 abc	10784 ± 864 abc
Ethyl decanoate	2139 ± 768 ab	2105 ± 636 abc	2188 ± 867 a	891 ± 290 cd	769 ± 314 d	724 ± 273 d	529 ± 114 d	541 ± 130 d	480 ± 131 d	941 ± 332 bcd	1152 ± 395 abcd	1037 ± 335 abcd	1044 ± 231 abcd	1100 ± 197 abcd	1436 ± 378 abcd	1067 ± 340 abcd	816 ± 236 d	720 ± 295 d
Ethyl dodecaonat	40 ± 27 a	35 ± 23 ab	30 ± 22 abc	12 ± 7 abc	4 ± 2 bc	7 ± 4 abc	0 ± 0 c	3 ± 0 bc	1 ± 1 bc	5 ± 2 bc	10 ± 7 abc	3 ± 2 bc	4 ± 4 bc	4 ± 4 bc	11 ± 9 abc	19 ± 15 abc	14 ± 9 abc	7 ± 5 abc
Diethyl succinate	16 ± 11 cd	13 ± 0 d	42 ± 5 ab	25 ± 4 bcd	39 ± 10 ab	36 ± 0 abc	33 ± 5 abcd	38 ± 3 abc	30 ± 10 bcd	27 ± 0 bcd	53 ± 2 a	28 ± 3 bcd	34 ± 12 abcd	38 ± 8 abc	32 ± 5 abcd	41 ± 8 ab	30 ± 10 bcd	29 ± 11 bcd
ACETATES																		
Ethyl acetate	8705 ± 490 cd	8539 ± 133 d	9415 ± 131 bcd	9764 ± 336 bcd	10021 ± 474 abc	9237 ± 53 bcd	9765 ± 403 bcd	9464 ± 29 bcd	9348 ± 286 bcd	9004 ± 500 bcd	10170 ± 52 ab	9191 ± 65 bcd	9531 ± 105 bcd	9994 ± 120 abc	11310 ± 1520 a	9633 ± 4 bcd	9602 ± 47 bcd	9649 ± 250 bcd
Isoamyl acetate	463 ± 31 a	490 ± 3 a	377 ± 64 a	299 ± 149 a	346 ± 174 a	478 ± 8 a	303 ± 135 a	243 ± 123 a	311 ± 170 a	440 ± 18 a	168 ± 3 a	454 ± 20 a	321 ± 189 a	343 ± 198 a	368 ± 214 a	296 ± 157 a	365 ± 98 a	480 ± 6 a
Hexyl acetate	17 ± 1 a	15 ± 3 a	12 ± 1 ab	13 ± 2 ab	15 ± 1 a	14 ± 0 ab	15 ± 0 a	14 ± 0 ab	14 ± 3 a	16 ± 1 a	13 ± 1 ab	15 ± 1 a	15 ± 1 a	15 ± 2 a	7 ± 7 b	11 ± 1 ab	15 ± 2 a	16 ± 1 a
2-phenylehtyl acetate	9 ± 3 a	8 ± 3 a	8 ± 2 a	6 ± 0 a	6 ± 1 a	6 ± 1 a	5 ± 0 a	5 ± 0 a	5 ± 1 a	5 ± 0 a	6 ± 1 a	5 ± 1 a	5 ± 1 a	6 ± 1 a	6 ± 2 a	7 ± 2 a	6 ± 1 a	6 ± 2 a
ALCOHOLS																		
Isoamyl alcohol	6028 ± 358 d	6032 ± 49 d	6511 ± 90 abcd	6525 ± 188 abcd	7057 ± 455 ab	6546 ± 12 abcd	6824 ± 417 abc	6500 ± 59 abcd	6459 ± 137 bcd	6349 ± 406 cd	7123 ± 77 a	6413 ± 111 bcd	6671 ± 118 abcd	6828 ± 20 abc	6961 ± 68 abc	6512 ± 32 abcd	6674 ± 13 abcd	6760 ± 202 abc
Isobutanol	115 ± 21 bc	102 ± 3 c	128 ± 4 abc	127 ± 6 abc	145 ± 6 ab	141 ± 3 ab	140 ± 12 ab	1300 ± 3 abc	122 ± 9 bc	127 ± 7 abc	141 ± 2 ab	129 ± 2 abc	134 ± 4 ab	131 ± 7 abc	155 ± 5 a	127 ± 11 abc	129 ± 9 abc	127 ± 24 abc
Benzil alcohol	2 ± 0 b	2 ± 0 b	3 ± 0 ab	2 ± 0 b	3 ± 0 ab	3 ± 0 ab	2 ± 0 b	3 ± 0 ab	2 ± 0 b	2 ± 0 b	3 ± 0 ab	2 ± 0 ab	3 ± 1 ab	3 ± 0 ab	4 ± 1 a	3 ± 0 ab	2 ± 0 ab	3 ± 1 ab
2-phenylethyl alcohol	304 ± 27 b	339 ± 38 ab	496 ± 15 ab	399 ± 54 ab	480 ± 9 ab	511 ± 52 ab	407 ± 58 ab	432 ± 43 ab	352 ± 64 ab	375 ± 7 ab	515 ± 67 ab	379 ± 35 ab	462 ± 106 ab	449 ± 52 ab	557 ± 148 a	444 ± 45 ab	409 ± 57 ab	495 ± 178 ab
FATTY ACIDS																		
Hexanoic acid	144 ± 11 b	169 ± 13 ab	201 ± 11 ab	174 ± 21 ab	207 ± 2 ab	216 ± 34 ab	176 ± 25 ab	168 ± 10 ab	181 ± 24 ab	175 ± 3 ab	212 ± 19 ab	165 ± 10 ab	190 ± 35 ab	189 ± 16 ab	218 ± 35 a	176 ± 11 ab	183 ± 7 ab	214 ± 59 ab
Octanoic acid	227 ± 31 cd	277 ± 16 abcd	361 ± 22 ab	258 ± 32 bcd	301 ± 5 abcd	326 ± 45 abcd	234 ± 31 bcd	239 ± 14 bcd	206 ± 34 d	281 ± 6 abcd	348 ± 41 abc	272 ± 14 abcd	322 ± 69 abcd	320 ± 34 abcd	393 ± 84 a	264 ± 30 abcd	248 ± 9 bcd	310 ± 96 abcd
Decanoic acid	51 ± 9 abcd	59 ± 11 abc	73 ± 9 a	32 ± 1 def	32 ± 3 def	32 ± 7 def	25 ± 0 ef	24 ± 2 ef	21 ± 4 f	37 ± 4 cdef	49 ± 8 abcde	38 ± 2 cdef	43 ± 7 bcdef	47 ± 6 bcde	67 ± 24 ab	41 ± 8 cdef	30 ± 1 def	32 ± 10 def

Tabla S5. Families of volatile compounds (µg/L) from Champagne with the five corks and crown tap (Control, C1, C2, C3, C4, C5) with the doses of SO_2 under study (0, 10, 20 mg/L) Different letters indicate significant differences between corks and doses after 12M bottling. Samples (n = 3). (Continue)

	12 MONTHS																	
Oxidatives compounds	**Control**			**C1**			**C2**			**C3**			**C4**			**C5**		
	0 mg/L	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**	**0 mg/L**	**10 mg/L**	**20 mg/L**
ALKENALS																		
Hexanal	3.2 ± 0.3 fgh	1.8 ± 0.0 h	1.8 ± 0.0 gh	19.0 ± 0.74 c	10.2 ± 0.5 d	9.6 ± 0.4 d	42.3 ± 1.5 a	33.4 ± 2.5 b	34.5 ± 1.9 b	10.2 ± 0.1 d	4.3 ± 0.1 efgh	4.9 ± 0.4 ef	10.0 ± 0.0 d	3.8 ± 0.6 efgh	4.9 ± 0.7 ef	6.4 ± 0.6 e	5.7 ± 0.0 ef	4.5 ± 0.1 efg
Octanal	9.6 ± 0.9 bc	10.3 ± 0.1 b	9.4 ± 0.3 c	7.1 ± 0.3 e	2.7 ± 0.3 gh	2.2 ± 0.3 hi	3.4 ± 0.2 fg	2.1 ± 0.4 hi	1.9 ± 0.0 hi	6.9 ± 0.2 e	4.2 ± 0.3 f	4.2 ± 0.1 f	3.3 ± 0.1 g	2.2 ± 0.2 hi	1.8 ± 0.1 i	11.3 ± 0.1 a	8.3 ± 0.3 d	7.0 ± 0.1 e
Nonenal	4.4 ± 0.1 i	4.8 ± 0.1 hi	5.2 ± 0.2 h	8.9 ± 0.2 b	8.7 ± 0.3 bc	7.9 ± 0.2 ef	8.1 ± 0.0 de	7.2 ± 0.2 g	7.4 ± 0.1 fg	9.7 ± 0.0 a	8.5 ± 0.3 bcde	8.2 ± 0.2 cde	8.4 ± 0.0 bcde	7.1 ± 0.2 g	7.0 ± 0.1 g	8.5 ± 0.3 bcd	8.3 ± 0.0 cde	7.0 ± 0.2 g
ALDEHYDES																		
2-methylpropanal	15.7 ± 0.2 ef	13.1 ± 0.4 ij	12.0 ± 0.2 jk	23.7 ± 0.3 b	16.5 ± 0.1 e	20.9 ± 0.1 c	21.2 ± 0.4 c	15.9 ± 0.0 ef	15.3 ± 0.1 fg	18.4 ± 1.2 d	15.4 ± 0.0 fg	13.8 ± 0.2 hi	32.0 ± 0.1 a	18.1 ± 0.3 d	14.6 ± 0.3 gh	20.6 ± 0.2 c	14.4 ± 0.2 gh	11.4 ± 0.3 k
3-methylbutanal	3.3 ± 0.4 l	2.6 ± 0.0 m	2.6 ± 0.0 m	9.4 ± 0.1 fg	6.3 ± 0.0 h	9.9 ± 0.1 f	21.3 ± 0.2 a	18.2 ± 0.3 b	16.5 ± 0.1 c	6.3 ± 0.1 h	4.7 ± 0.1 j	4.5 ± 0.2 j	13.8 ± 0.1 d	5.2 ± 0.0 i	3.9 ± 0.2 k	14.3 ± 0.3 d	9.4 ± 0.2 g	10.8 ± 0.1 e
2-phenylacetaldehide	1.3 ± 0.0 i	1.6 ± 0.3 hi	1.3 ± 0.0 i	3.9 ± 0.1 e	1.1 ± 0.1 i	1.3 ± 0.1 i	6.9 ± 0.3 c	4.4 ± 0.1 e	2.0 ± 0.5 gh	4.0 ± 0.0 e	2.3 ± 0.1 g	3.1 ± 0.1 f	9.0 ± 0.3 a	8.3 ± 0.1 b	6.4 ± 0.1 cd	6.7 ± 0.2 cd	6.2 ± 0.2 d	6.4 ± 0.0 cd
Methional	6.1 ± 0.1 d	3.3 ± 0.1 i	3.2 ± 0.4 i	5.4 ± 0.3 e	4.3 ± 0.2 gh	4.2 ± 0.1 h	9.1 ± 0.0 a	4.8 ± 0.2 fg	3.3 ± 0.3 i	7.3 ± 0.0 c	6.5 ± 0.0 d	5.1 ± 0.0 ef	5.4 ± 0.1 e	3.1 ± 0.0 i	2.3± 0.0 j	5.5 ± 0.1 e	3.4 ± 0.1 i	8.1 ± 0.1 b
KETONE																		
3-metil-2,4-nonandiona	1.5 ± 0.0 def	1.4 ± 0.0 ef	1.5 ± 0.0 def	1.5 ± 0.0 def	1.4 ± 0.0 ef	1.3 ± 0.0 f	2.3 ± 0.1 b	1.4 ± 0.1 ef	1.4 ± 0.0 ef	1.8 ± 0.0 c	1.5 ± 0.0 def	1.4 ± 0.0 ef	3.5 ± 0.2 a	1.6 ± 0.2 cde	1.4 ± 0.0 def	1.7 ± 0.0 cd	1.4 ± 0.0 ef	1.3 ± 0.0 f
LACTONE																		
ɣ-nonalactone	14.5 ± 0.2 gh	14.4 ± 0.2 gh	13.5 ± 0.3 hi	24.3 ± 0.3 b	21.3 ± 0.1 c	10.3 ± 0.3 j	20.6 ± 0.4 c	18.4 ± 0.6 d	17.4 ± 0.4 de	14.3 ± 0.0 gh	13.2 ± 0.5 hi	10.5 ± 0.3 j	27.4 ± 1.3 a	15.3 ± 0.4 fg	16.5 ± 0.1 ef	16.4 ± 0.4 ef	14.5 ± 0.4 gh	12.6 ± 0.0 i
Reductive compounds																		
THIOLS																		
H_2S	19.7 ± 0.2 bc	20.3 ± 0.2 b	22.1 ± 0.4 a	14.5 ± 0.3 ef	15.1 ± 0.1 de	18.9 ± 0.1 c	6.6 ± 0.2 i	10.7 ± 0.3 g	10.6 ± .0 g	2.5 ± 0.4 k	11.3 ± 0.2 g	11.0 ± 0.8 g	5.2 ± 0.0 j	14.1 ± 0.1 f	16.0 ± 0.4 d	5.0 ± 0.0 j	7.8 ± 0.2 h	10.5 ± 0.2 g
MeSH	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 i	0.3 ± 0.0 fg	0.5 ± 0.1 cde	1.2 ± 0.1 a	0.3 ± 0.0 fg	0.3 ± 0.0 efg	0.4 ± 0.0 def	0.5 ± 0.0 cd	0.6 ± 0.0 c	0.9 ± 0.0 b	0.1 ± 0.0 hi	0.6 ± 0.0 c	0.9 ± 0.1 b	0.0 ± 0.0 i	0.2 ± 0.0 gh	0.9 ± 0.1 b
EtSH	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.4 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
SULFIDES																		
Me_2S	2.2 ± 0.2 i	7.8 ± 0.0 c	7.2 ± 0.3 d	7.0 ± 0.3 de	6.5 ± 0.3 e	8.5 ± 0.1 b	4.9 ± 0.0 g	5.2 ± 0.0 fg	4.7 ± 0.1 g	7.8 ± 0.3 c	8.5 ± 0.5 b	8.8 ± 0.1 b	4.6 ± 0.2 g	8.3 ± 0.1 bc	9.8 ± 0.1 a	5.7 ± 0.0 f	3.9 ± 0.1 h	6.8 ± 0.1 de
Et2S	0.9 ± 0.0 a	0.4 ± 0.3 b	0.9 ± 0.0 a	0.0 ± 0.0 c	0.7 ± 0.0 a	0.8 ± 0.0 a	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
DISULFIDES																		
Me_2S_2	12.6 ± 0.1 f	11.1 ± 0.4 hi	10.3 ± 0.2 i	15.4 ± 0.1 e	11.3 ± 0.3 ghi	23.9 ± 0.1 a	3.7 ± 0.1 k	9.2 ± 0.3 j	11.4 ± 0.5 gh	15.1 ± 0.1 e	19.0 ± 0.2 c	18.9 ± 0.1 c	11.9 ± 1.0 fgh	15.7 ± 0.2 e	17.9 ± 0.1 d	12.2 ± 0.2 fg	12.3 ± 0.2 fg	0.2 ± 0.1 cdef
Et_2S_2	0.8 ± 0.0 bc	0.6 ± 0.0 e	0.9 ± 0.0 b	0.7 ± 0.0 cd	0.6 ± 0.0 e	1.1 ± 0.0 a	0.8 ± 0.0 bc	0.6 ± 0.0 e	0.7 ± 0.0 de	1.0 ± 0.0 a	1.1 ± 0.0 a	0.9 ± 0.1 b	0.7 ± 0.0 cd	0.9 ± 0.0 b	1.1 ± 0.1 a	0.6 ± 0.0 e	0.6 ± 0.5 e	0.8 ± 0.0 bc

Artículo 7

Influence of the oxidation in the aromatic composition and sensory profile of Rioja red aged wines

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ORIGINAL PAPER

Influence of the oxidation in the aromatic composition and sensory profile of Rioja red aged wines

A. M. Mislata[1,2] · M. Puxeu[1] · E. Tomás[3] · E. Nart[1] · R. Ferrer-Gallego[1]

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Abstract

Rioja is one of the most important Spanish wine regions, and commonly produces wines with good aging potential. The quality of Rioja aged wines is well appreciated by tasters and consumers in the international wine market. In this study, the influence of the aromatic composition and oxidation on their sensory profile of different high-quality long-aged wines produced in Rioja was evaluated. The aromatic compounds that can participate actively on the aromatic profile of this type of wines have been identified. In this way, 3-methyl-2,4-nonanedione and 2-phenylacetaldehyde were the most sensory active compounds. At lesser extent, 3-methylbutanal, (*E*)-2-nonenal, and (*E*)-2-octenal were also important volatile compounds in this type of wines. Surprisingly, sotolon was only detected as active compounds in the oldest wines with lower price. Contrary, methional mainly appeared as active compound in the youngest samples of wines with higher price. 2-methylpropanal was a non-active compound in wines correctly aged and stored. This work contributes to an improved understanding about the key odorants in Rioja high-quality old wines.

Keywords Aroma · Gas chromatography · Sensory analysis · Tempranillo · Volatile compounds · Wine

Introduction

The quality of red wines is intimately related to their aging capacity. Along the aging period, an evolution on the wine aromatic profile takes place, mainly caused by numerous chemical reactions resulting in new aromas [1, 2]. Among all of them are the secondary or fermentative aromas which usually appear as a consequence of the alcoholic and malolactic fermentations. These aromas depend on the fermentation conditions and the type of yeasts and lactic bacteria used. They tend to give a sweet olfactory sensation related to baking, lactic, and amylic aromas [3]. The fermentative aromas are usually classified into different families and the main ones are alcohols, ethyl esters, acetates, and fatty acids [4]. Finally, the oxidation or tertiary aromas are those that have been acquired during the aging period, in barrels or bottle. They are mainly produced from the oxidation reactions and can be also released from wood. Their olfactory sensations are commonly associated to woody, balsamic, confectionery fruit, animal notes, and empyreumatic aromas [5–7]. In addition, tertiary aromas can be classified in different families, such as furans, Strecker aldehydes, alkenes, ketones, lactones, etc.[8].

Within the aromatic compounds involved in the wine evolution process, aldehydes such as 2-phenylacetaldehyde, methional, 2-methylpropanal, and 3-methylbutanal have a great impact on the sensory profile of aged wines [8]. They are commonly related to honey, dead flowers, boiled potatoes, and rancid wines; they are considered as key odorants of the wine oxidation. In this way, sotolon (3-hydroxy-4, 5-dimethylfuran-2 (5H) -one) also has a great interest and presents characteristic aromatic notes of curry, spices, caramel or toasted. This compound has been identified in aged red and white wines, such as old Sherry wines [7, 9, 10]. In addition, 3-methyl-2, 4-nonanedione, and γ-nonalactone are

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✉ R. Ferrer-Gallego
raul.ferrer@vitec.wine

[1] VITEC- Centro Tecnológico del Vino, Ctra. Porrera Km.1, Falset, 43730 Tarragona, Spain

[2] Sensometria Instrumental (I-Sens), Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira I Virgili, 43007 Tarragona, Spain

[3] Bodegas Roda, Avda. Vizcaya, 5. Barrio de la Estación, 26200 Haro, La Rioja, Spain

characteristic oxidation markers of red wines, that appeared prematurely (on the first stage of the oxidation). They are linked to aromatic notes of rotten and cooked fruits [11–14].

The oxidation reactions are responsible for important changes in the chemical composition of wines. Previous studies have shown that these reactions involve an increase on the amount of certain compounds [15], the formation of new ones, and also the decrease in the fresh and varietal aromas, such as fruity and floral aromas [16]. It has already been shown in other studies that certain aldehydes, such as methional and phenylacetaldehyde, are favored to appear under high temperature conditions and with the presence of high levels of oxygen in wine. Therefore, these molecules are considered markers in oxidized wines [17]. More recent studies showed that Strecker aldehydes were formed during alcoholic fermentation, and especially in wines made without addition of sulfites. These aldehydes form reversible adducts with the SO_2 molecules (complexed forms), that are released during the storage of the bottled wine, producing an increase in the aldehydes free forms when the SO_2 is depleted [18, 19]. This oxidative deterioration includes the loss of the fermentative aromas and the appearance of some undesirable oxidative compounds that often produce negative effects on the final quality of wines. There is also the formation of certain alkenales (*E*-2-octenal and *E*-2-nonenal) from the oxidative degradation of certain unsaturated fatty acids [9, 20]. Some carbonyls such as aldehydes appear as a consequence of oxidation reactions. They are volatile aromatic compounds, with low concentrations and high chemical reactivity, whose interactions with the complex matrix of the wine makes them difficult to determine. Therefore, it is necessary to carry out a previous step of derivatization of the compounds for their possible identification and quantification by means of GC–MS. As already known from previous studies [21], some carbonyls such as alkenals, aldehydes, and some ketones do not have a specific fragmentation pattern in electron impact mass spectrometry (EI-MS). The derivatization of the carbonyls groups consists of the formation of oximes with higher detection sensitivity in EI-MS [22].

Tempranillo, the most important Spanish variety cultivated in Rioja, produces wines with good aging potential. It has a large number of aromatic precursors, such as volatile phenols, unsaturated fatty acid derivatives ((*E*)-hexenal and (*E*)-2-heptenal), norisoprenoids (β-damascenone), and vanillin [23]. During the aging period, these compounds are transformed into the above-mentioned oxidative aromas. Some aldehydes, alkenals, and ketones, such as 2-phenylacetaldehyde, 2-methylpropanal, (*E*)-2-octenal (*E*)-2-nonenal, and 3-methyl-2, 4-nonanedione are the main responsible compounds on the oxidation of Tempranillo wines [2, 9]. The presence of the oxidative compounds and their related sensory descriptors does not always have to be a negative wine indicator. There exist different types of wines in which these oxidation attributes are a quality requirement, such as in Sherry or Porto wines. In these wines, strong notes of curry, toasted, raisins or even leather and ink are considered positive aromas [24]. In this way, Rioja aged wines are commonly produced with Tempranillo, and sometimes blended with Graciano and Garnacha (native varieties that are traditionally blended in this region). These type of wines show aromatic oxidative notes that are very appreciated by tasters and consumers. However, the influence of the aromatic composition and its oxidation on the sensory quality of this type of wines remains of current interest to researchers and wineries [25]. In this work, the aromatic composition of Rioja old red wines was compared to study the influence of the key odorants on the sensory profile of this type of wines with different price. Moreover, changes on the aromatic composition after a semi-forced oxidation period were studied to evaluate differences in the quality of Rioja red wines after an adequate or forced aging.

Material and methods

Wine samples

Wines were representative of Rioja Qualified Denomination of Origin and were classified into two different series depending on their price and blend. The samples were collected from five commercial wines with a cost price ranged from 20–30 euros (W1) and five corresponds to wines between 30–50 euros (W2). W1 were Tempranillo wines blended with Garnacha and Graciano (3–11%) and W2 were mainly Tempranillo wines. Wines were aged between 12 and 16 month in French oak barrels and elaborated in 2006, 2007, 2008, 2009, and 2010 vintages. Wines were also evaluated after a semi-forced oxidation process. Thus, an aliquot of wine (300 mL) was collected directly from each bottle just after its opening (t0). The rest of the wine (450 mL) was maintained for 1 month inside each bottle until analyses were carried out (t1). Bottles were stored in darkness and at controlled temperature conditions (23 ± 2 °C) during this time. The same oxidation conditions were established for all samples. For the analysis, two bottles were used by type of sample, and the analysis of each of the samples was carried out in duplicate ($n = 4$).

Reagents

All reagents used were of analytical quality (Sigma-Aldrich); 2-octanol (≥ 96%), 3-hydroxy-4, 5-dimethylfuran-2(5H)-one (sotolon) (≥ 97%), methional (≥ 97%), 2-phenylacetaldehyde (≥ 95%), 2-methylpropanal (≥ 99%), 3-methylbutanal (≥ 97%), (*E*)-2-hexenal (≥ 95.%), (*E*)-2-heptenal (≥ 95%), (*E*)-2-octenal (≥ 95%), (*E*)-2-nonenal (≥ 95%), 2-methyl-2, 4-nonanedione, γ-nonalactone (≥ 98%), 2-methylpentanal (98%) (as internal standard), and PFBHA: O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (> 99%) (derivatizing product). The following products and solvents were also used in the previous preparation of the samples to analyze the oxidative aromas; ammonium sulphate (for analysis of ACS, ISO, by Panreac), ethanol absolute, and dichloromethane anhydrous (> 99.8%) by Sigma-Aldrich.

Oenological analysis

All basic oenological parameters of wines were carried out according to the OIV methods. The color intensity (CI), the total phenolic index (TPI), the total anthocyanins, and the total tannin content were measured by spectrophotometry, while the total acidity was measured by potentiometry using an automatic titrator (TitroMAtic Hach by Crison®). The acetic acid content was analysed by enzymatic reaction [26].

Analysis of individual volatile compounds

The analysis of the fermentative aromas was carried out according to Torrens and co-workers [27]. Briefly, samples were extracted by solid phase micro extraction (SPME) and then were separated and analyzed by gas chromatography coupled with mass spectrometry detection (GC–MS). Samples (10 mL) were placed in 20 mL headspace vials together with 2.7 g NaCl and 100 µL of 2-octanol (1.000 ppm) as internal standard.

A gas chromatograph GC 7890A (Agilent Technologies) equipped with a mass spectrometer 5975C inert MSD (triple-axis detector with electron impact source) was used. The separation was carried out with a column DB-WAX IU (60 m × 0.25 mm × 0.25 µm, Agilent). Helium was used as a carrier gas with 2 mL/min of constant flow. At the end of the extraction time, the fiber (DVB/CAR/PDMS) was exposed for 2.5 min in splitless mode at 270 °C. The oven temperature ramp was started at 40 °C which was maintained for 2 min and was increased at 5 °C/min up to 225 °C and maintained for 15 min. The ionization source temperature was 220 °C and the interface remained at 280 °C. It worked with the source of electron impact (EI) and the mass analyzer operating in Scan and SIM (single-ion monitoring) mode. The volatile compounds were identified by comparison of their mass spectra, with the help of the NIST library. The quantification was carried out by the internal standard method (IS). The individual volatile compounds (table S1, supplementary material) were quantified with the factor of response of the 2-octanol, and expressed as equivalents of 2-octanol in µg/L. All analyses were carried out in duplicate.

Analysis of the oxidative aromas

The isolation of the oxidative volatile analytes was carried out according to Zapata and co-workers [28]. A previous preparation of the sample, consisting of the derivatization and formation of the corresponding oxime was performed. Twenty microlitres of 2-metilpentanal (1.000 ppm) as internal standard were added to 20 mL of wine. The solution was equilibrated using a magnetic stirring for 15 min at 700 rpm. Then, 1 mL of an aqueous solution of *O*-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine hydrochloride was added as derivatization reagent. This solution was prepared in methanol/water 50/50% (v/v) and stirred during 15 min [29]. After that, 3 g of ammonium sulfate were added and the samples were immediately passed through a solid-phase extraction (SPE) cartridge purchased to Agilent (Bond Elut ENV, 500 mg and 6 mL). The cartridges have been previously conditioned with 5 mL of dichloromethane, 5 mL of methanol, and 5 mL of a model wine-like solution (12% (v/v) ethanol, 5 g/L tartaric acid, adjusted at pH 3.5 with NaOH). Then, once the sample has been added to the cartridge, 6 mL of 0.05 M sulphuric acid solution were added to eliminate the excess of the derivatization reagent. Finally, the analytes were eluted with dichloromethane (12 mL), collected, and dried using a SpeedVac (Thermo Fischer) concentrator vacuum at 35 °C. The final volume of samples was 200 µL.

GC analysis was performed on a GC 7890A (Agilent Technologies) system equipped with a mass spectrometer 5975C inert MSD (with Triple-Axis Detector) and with a VF-200 ms column (30 m × 0.25 mm × 0.25 µm, Agilent). A constant flow of 2.1 mL/min of helium was used as carrier gas. Five microliters of sample was injected in splitless mode with 16.95 psi pressure (septum purge flow 3 mL/min and splitless time 1 min). The injector temperature was maintained at 180 °C for 1 min and then heated up to 260 °C at 250 °C / minute. The temperature of the oven (40 °C) was maintained for 1 min and then increased up to 220 °C at (10 °C/min), and finally up to 270 °C (100 °C/min). After each analytical series, the temperature was held to 270 °C, washing for 7.24 min to a 25 psi. The transfer line temperature was 240 °C and 1.5 mL/min flow of the carrier gas (He). The mass spectrometer operated at 70 eV electron ionization modes. The analysis was performed in scan mode (*m/z* 50−350). The compounds were identified by their retention times and mass fragments. The quantification was carried out using the method of internal standard patterns. They were prepared in individual ethanol solutions at 1000 ppm. Mixed solutions containing all compounds were prepared to elaborate the standard calibration curves of each compound (table S2, supplementary material). All analyses were carried out in duplicate. The compounds analysed were grouped in five different families (furans, Strecker aldehydes, alkenales, ketones, and lactones) and quantified individually by its corresponding calibration curve using 2-methylpentanal as internal standard.

Sensory analysis

The quantitative descriptive analysis (QDA) was performed by a trained tasting panel (by the ISO 8586:2012) in a sensory standardize room (ISO 8589:2007). The tasting panel consisted of five professional tasters, three men and two women, aged between 28 and 40 years old. The analysis was randomly conducted, and panellists did not know the sample identification. Red fruit, black fruit, sweet fruit, balsamic, spicy, smooked/toasted, empyreumatic, nuts, chemical, acetic/acetate, dry vegetal, dead flowers, and rancid notes were considered as aromatic descriptors. The intensity of the global aroma was considered as pleasant perception. The aroma intensity perceived was rated from 0 to 5 and the obtained data were processed with the FIZZ software (Biosystems, V.2.47B). A specific tasting sheet was designed according to the mentioned olfactory descriptors.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine the significant differences by the Tukey (HSD) test using the XLSTAT package for Excel software. A principal component analysis (PCA) was carried out to easily explore and visualize the relationships and patterns between the aromatic composition and the sensory data, using the XLSTAT package for Excel software.

Results and discussion

Oenological parameters of wines

The alcohol content of the studied wines was quite similar, around 14% (v/v). The oenological characteristics of the studied wines at different vintages are shown in Table 1. In general, the anthocyanins and tannin content, CI and TPI were higher in the youngest wines, as expected. The W1 wine series showed higher significant TPI values in 2009 and 2010 vintages and the lowest value in 2008. The TPI and tannin values of W2 series were slightly higher than W1. This fact could indicate a higher aging potential of the wines

Table 1 Blends and basic enological parameters of the studied wines

	2006	2007	2008	2009	2010
Blend	Graciano (3%)	Graciano (3%) Garnacha (8%)	Graciano (4%) Garnacha (6%)	Graciano (11%)	Graciano (10%)
W1					
CI	9.9 ± 0.1 a	9.8 ± 0.3 a	9.6 ± 1.0 a	10.1 ± 0.2 a	10.2 ± 0.1 a
Alcohol	14.0 ± 0.2 b	14.0 ± 0.2 b	13.5 ± 0.2 c	14.5 ± 0.2 a	14.0 ± 0.2 b
TPI	56.7 ± 0.3 ab	55.6 ± 1.3 ab	52.2 ± 0.5 b	57.6 ± 0.1 a	58.3 ± 0.1 a
Anthocyanins	85 ± 1 c	111 ± 3 bc	128 ± 1 b	128 ± 1 b	187 ± 1 a
Tannins	2.9 ± 0.1 a	3.0 ± 0.0 a	3.0 ± 0.1 a	4.0 ± 0.5 a	3.1 ± 0.5 a
pH	3.8 ± 0.0 a	3.6 ± 0.0 a	3.6 ± 0.0 b	3.6 ± 0.0 b	3.7 ± 0.0 a
TTA*	4.4 ± 0.0 a	4.5 ± 0.0 a	4.7 ± 0.0 a	4.4 ± 0.1 a	4.5 ± 0.3 a
Acetic acid	0.79 ± 0.01 abc	0.76 ± 0.01 bc	0.82 ± 0.04 a	0.75 ± 0.00 c	0.79 ± 0.01 ab
Blend	–	–	–	Graciano (5%)	Graciano (2%)
W2					
CI	11.7 ± 0.2 a	11.8 ± 0.0 a	10.7 ± 1.1 a	11.6 ± 0.5 a	12.5 ± 0.3 a
Alcohol	14.0 ± 0.2 b	14.5 ± 0.2 a	14.0 ± 0.2 b	14.5 ± 0.2 a	14.0 ± 0.2 b
TPI	57.1 ± 6.2 a	61.6 ± 1.0 a	55.9 ± 0.2 a	59.8 ± 4.5 a	63.3 ± 1.2 a
Anthocyanins	139 ± 1 bc	160 ± 2 bc	135 ± 7 c	183 ± 7 b	243 ± 1 a
Tannins	3.5 ± 0.1 a	3.3 ± 0.2 ab	2.8 ± 0.0 b	3.3 ± 0.0 ab	3.5 ± 0.1 a
pH	3.7 ± 0.0 a	3.6 ± 0.0 b	3.6 ± 0.0 b	3.6 ± 0.0 b	3.7 ± 0.0 a
TTA*	4.5 ± 0.0 a	4.7 ± 0.0 a	4.7 ± 0.0 a	4.6 ± 0.1 a	4.7 ± 0.1 a
Acetic acid	0.87 ± 0.04 a	0.86 ± 0.00 a	0.94 ± 0.05 a	0.83 ± 0.01 a	0.83 ± 0.00 a

Color intensity (CI), alcoholic strength (% v/v), total polyphenol index (TPI), anthocyanins (mg/L), tannins (g/L), pH, titratable total acidity (TTA), and acetic acid content (g/L). Different letters indicate significant differences between the different vintages among the same group of samples (W1 or W2) (by Tukey HSD test ($p < 0.05$). The mean value and SD (standard deviation).Samples (n = 2). *expressed as g/L of tartaric acid from the W2 series. Regarding anthocyanins of W1, significant differences were also observed, and the lowest values corresponded to 2006 and 2007, in contrast to 2010, which showed the highest value (187 mg/L). As expected, older wines showed lower values in anthocyanins. It is well known that these compounds decrease along the wine aging [30]. For W2, significant differences were found in both anthocyanins and tannins, showing the wines of 2010 the highest values. It should be note that wines of 2008 showed the lowest values in both parameters. The enological parameters of 2008 with lower values on alcohol, CI, TPI, anthocyanins and tannins, and higher values on TTA and acetic acid may be due to the climatic conditions on the region in this vintage (abundant precipitation from April to June and cool temperatures in summer), which favored a slow and irregular ripening of Tempranillo grapes [31]. In general, differences on TTA and acetic acid were not observed, and all wines presented low acetic acid levels, indicating an appropriate evolution during the bottle aging and a correct storage. The chemical composition of W1 and W2 wines was quite similar independently of their price and blend.

Aromatic composition of wines

Figure 1 shows the total content of the fermentative and oxidative aromas before and after the oxidation period (the individual compounds analyzed are shown in tables S1 and S2). As occurred on the chemical composition, from an aromatic point of view, wines W1 and W2 showed little differences. Higher concentrations on the oxidative compounds in the oldest vintages (2006–2007) were found. On the other hand, wines generally showed a significant decrease on the total fermentative aromas after the oxidation conditions (t1). In contrast, the total oxidative aromas showed a significant increase after this period. This trend was already observed by Balboa-Lagunero et al. 2011, which showed a decrease in the concentrations of some aromatic compounds such as 2, 3-butanedione, 3-hexenol, β-citronellol or geraniol, as well as the large increase in other compounds related to the evolution of wines such as 2-methylpropanal, 3-methylbutanal, methional, or phenylacetaldehyde, after oxidation processes [9]. The oxidative conditions of this study produced a loss of ca. 40% of the fermentative aromas and an average increase of approximately 85% of the oxidative aromas reflects a clear evolution on the aromatic profile.

Fermentative aromas

A total of 18 compounds were determined and grouped by families according to their chemical structures (table S1). Table S1 also shows the families of the fermentative compounds with the corresponding descriptors and thresholds [32–36]. For W1 wine series, in general, a significant decrease in the esters content after the oxidation conditions (47% of average loss) was found (Fig. 2), presenting significant differences in the vintages 2007, 2009, and 2010. In the same way, fatty acids declined significantly in this period (52% of average loss), presenting significant differences in all vintages. On the other hand, acetates and alcohols were the families that are less decreased (19% and 12%, respectively). The same pattern was observed for W2 with a significant decrease in esters (ca. 57%) and fatty acids (ca. 61%), with significant differences in all vintages, and lower decreases in acetates and alcohols (27% and 22%, respectively), showing significant differences only in some of the vintages. This large loss of esters and fatty acids is due to the great oxidation produced under the oxidation conditions used. Esters are commonly related to fruit aromas and highly affected by the oxidation processes. Ancín-Azpilicueta and co-workers observed a decrease on their concentration in Chardonnay wines after a long aging period, either in barrels or in bottles [37]. In our case, significant differences were

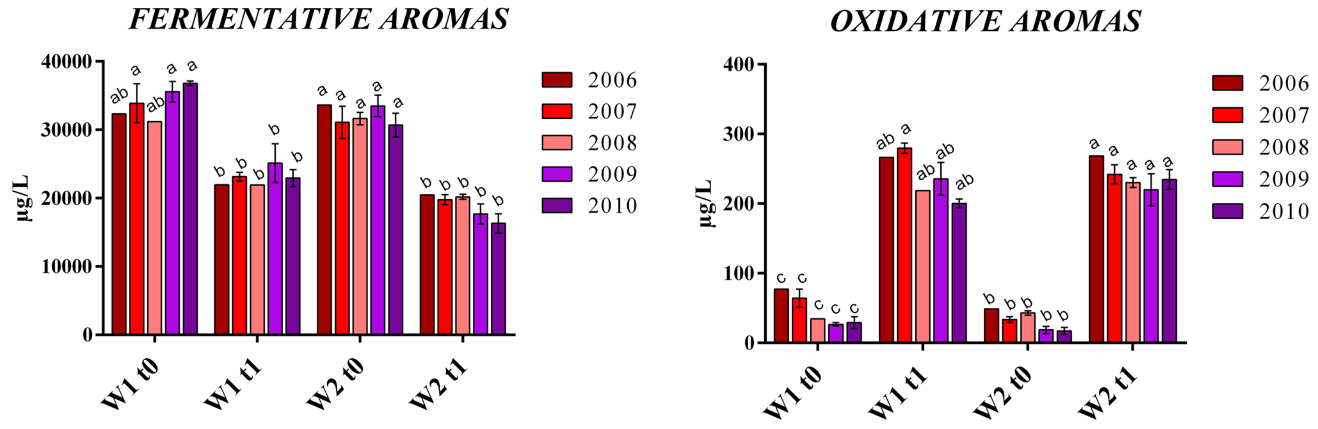

Fig. 1 Total concentrations of the fermentative and oxidative aromas before (t0) and after (t1) the oxidation conditions for W1 and W2 wine series. Different letters indicate significant differences between samples for each wine series

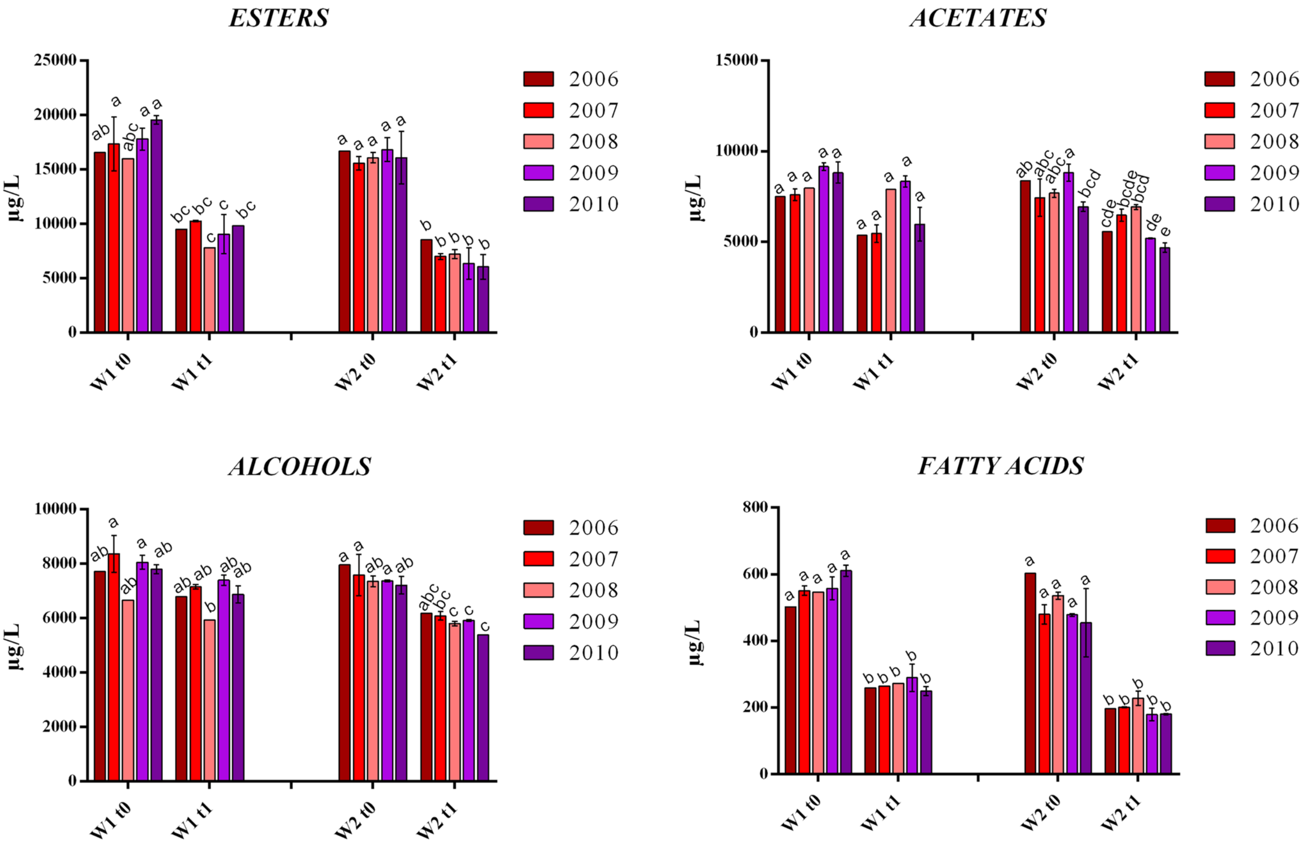

Fig. 2 Total concentration of the fermentative aromas before (t0) and after (t1) the experimental oxidation conditions; esters, acetates, alcohols, and fatty acids. Different letters indicate significant differences between samples for each wine series

found in ethyl butyrate, ethyl hexanoate, ethyl decanoate, and ethyl isovaleriate in all studied vintages after the semi-oxidation process (Table 2). The concentrations of ethyl decanoate and ethyl isovaleriate decreased even under their detection thresholds. These compounds are very characteristic because they are related to descriptors of aromatic fruits such as grapes and apples, respectively [32, 35]. Futhermore, ethyl hexanoate and ethyl octanoate maintained values above their detection thresholds before (t0) and after (t1) the oxidation process in both W1 and W2, despite the loss of concentration of approximately 50% (from t0 to t1). Regarding fatty acids, hexanoic and octanoic compounds presented significant differences in the two different series of wines and in all vintages studied. These compounds are related to soapy and stale notes [35]; however, their concentrations did not exceed the detection thresholds neither before nor after the oxidation process. As it was shown in previous studies [38], the concentration of fatty acids, especially hexanoic and octanoic acids, decreases significantly during the period of storage, as occurs in our study.

In general, a lower decrease in alcohols and acetates than esters was found in both series of wines. There are certain acetates as is the case of the ethyl acetate that increases its concentration during the wine oxidation. This could be related to a perception of sweet aromas when this compound is found at low concentrations, and to glue notes when it is in high levels. In contrast, the isoamyl acetate which has a characteristic banana aroma decreases along the wine evolution [39]. In our study, isoamyl acetate was the only compound with aromatic contribution before (t0) and after (t1) the oxidation process applied. This compound is characteristic for providing aromatic banana notes, and for maintaining them throughout the evolution of wines [34]. On the other hand, a notable decrease was observed in the 2-phenylethyl acetate content, showing significant differences in most of the studied wines. Ethyl acetate, isoamyl acetate, and hexyl acetate also showed a considerable decrease after the oxidation period with significant differences in some samples of W2.

Regarding individual alcohols, the benzyl alcohol content decreased significantly in all studied cases. Isoamyl alcohol showed significant differences after the oxidation period in all studied vintages of W2. In the case of isobutanol, some significant differences were also observed for W1 (2009 and 2010) and W2 (2009). None of the alcohols studied here exceeded their detection thresholds neither before (t0) nor after (t1) the oxidation process applied in bottles, which

Table 2 Concentrations of the individual fermentative compounds before (t0) and after (t1) the experimental oxidation conditions

	t0					t1				
	2006	2007	2008	2009	2010	2006	2007	2008	2009	2010
W1										
Ethyl esters										
Ethyl butyrate	180 ± 23 a	156 ± 24 a	183 ± 18 a	184 ± 13 a	167 ± 9 a	56 ± 5 b	61 ± 3 b	57 ± 2 b	61 ± 2 b	52 ± 5 b
Ethyl hexanoate	2661 ± 235 a	2811 ± 228 a	2661 ± 66 a	2860 ± 53 a	2673 ± 73 a	1223 ± 100 b	1472 ± 99 b	1148 ± 35 b	1435 ± 84 b	1361 ± 144 b
Ethyl octanoate	7441 ± 758 ab	8364 ± 1484 ab	7874 ± 145 ab	8796 ± 651 ab	10,197 ± 256 a	4919 ± 61 b	5677 ± 57 b	4272 ± 84 b	4732 ± 142 b	5344 ± 216 b
Ethyl decanoate	2554 ± 502 a	2463 ± 431 a	2204 ± 106 a	2441 ± 257 a	2676 ± 53 a	595 ± 33 b	498 ± 47 b	373 ± 91 b	401 ± 110 b	571 ± 82 b
Ethyl dodecanoate	35 ± 9 a	38 ± 8 a	43 ± 7 a	39 ± 19 a	40 ± 21 a	32 ± 6 a	26 ± 3 a	21 ± 4 a	24 ± 1.4 a	27 ± 1 a
Ethyl isovaleriate	83 ± 4 a	93 ± 4 a	87 ± 2 a	94 ± 5.4 a	104 ± 6 a	2 ± 0.5 b	2 ± 0.1 b	2 ± 0.8 b	4 ± 3 b	5 ± 4 b
Diethyl succinate	3619 ± 215 a	3425 ± 338 a	2935 ± 102 ab	3383 ± 16 a	3696 ± 141 a	2688 ± 55 ab	2531 ± 40 b	1948 ± 70 b	2405 ± 190 b	2481 ± 164 b
Acetates										
Ethyl acetate	6841 ± 122 a	6959 ± 168 a	7060 ± 107 a	8070 ± 291 a	7608 ± 577 a	5072 ± 154 a	5105 ± 460 a	7435 ± 102 a	7647 ± 394 a	5590 ± 735 a
Isoamyl acetate	474 ± 95 a	464 ± 131 a	747 ± 97 a	859 ± 91 a	956 ± 12 a	235 ± 33 a	268 ± 17 a	361 ± 51 a	625 ± 465 a	284 ± 169 a
Hexyl acetate	54 ± 7 abcde	57 ± 9.8 abcd	59 ± 3 abc	71 ± 3 ab	74 ± 2 a	32 ± 6 bde	38 ± 2 bcde	46 ± 8 abcde	37 ± 1 bcde	29 ± 9 be
2-phenylethyl acetate	132 ± 8 ab	129 ± 16 b	109 ± 11 bc	157 ± 9 ab	186 ± 10 a	46 ± 0.8 c	52 ± 0.1 c	64 ± 2 c	51 ± 3 c	73 ± 14 c
Alcohols										
Isoamyl alcohol	3841 ± 58 a	4280 ± 655 a	3449 ± 55 a	3904 ± 41 a	3678 ± 40 a	3210 ± 87 a	3363 ± 156 a	2869 ± 91 a	3595 ± 269 a	3109 ± 105 a
Isobutanol	361 ± 41 ab	330 ± 56 ab	306 ± 18 ab	436 ± 12 a	453 ± 74 a	136 ± 12 b	207 ± 19 b	189 ± 27 b	250 ± 18 b	194 ± 12 b
Benzyl alcohol	31 ± 5 a	20 ± 4 bc	21 ± 1 abc	19 ± 0.2 bc	22 ± 2 ab	11 ± 0.6 cd	10 ± 0.4 d	8 ± 0.6 d	7 ± 0.4 d	8 ± 0.7 d
2-phenylethyl alcohol	3492 ± 84 ab	3733 ± 83 a	2885 ± 71 bc	3692 ± 203 ab	3643 ± 197 ab	3434 ± 41	3575 ± 51 ab	2866 ± 27 b	3544 ± 91 ab	3558 ± 189 ab
Fatty acids										
Hexanoic acid	209 ± 8 a	229 ± 2 a	227 ± 4 a	229 ± 11 a	223 ± 2 a	92 ± 4 b	98 ± 0.6 b	107 ± 14 b	105 ± 17 b	87 ± 3 b
Octanoic acid	279 ± 9 a	304 ± 11 a	299 ± 13 a	311 ± 20 a	353 ± 17 a	151 ± 6 b	152 ± 2 b	154 ± 6 b	168 ± 24 b	146 ± 10 b
Decanoic acid	15 ± 4 b	18 ± 6 b	22 ± 4 ab	17 ± 4 b	35 ± 3 a	17 ± 5 b	15 ± 1 b	12 ± 1 b	17 ± 0.5 b	16 ± 1 b
W2										
Ethyl esters										
Ethyl butyrate	161 ± 9 ab	150 ± 8 b	180 ± 13 a	171 ± 8 ab	98 ± 1 c	55 ± 3 d	50 ± 2 d	46 ± 1 d	49 ± 4 d	45 ± 3 d
Ethyl hexanoate	2539 ± 11 ab	2576 ± 42 ab	2783 ± 3 a	2576 ± 17 ab	2427 ± 2 b	1145 ± 10 c	1089 ± 53 c	1069 ± 15 c	1025 ± 111 c	922 ± 182 c
Ethyl octanoate	7590 ± 127 ab	6776 ± 564 ab	7402 ± 218 ab	7875 ± 716 a	8300 ± 5 a	4369 ± 59 bc	3096 ± 77 c	3743 ± 275 c	2712 ± 121 c	2465 ± 906 c
Ethyl decanoate	2248 ± 240 a	2451 ± 349 a	2312 ± 300 a	2446 ± 139 a	3053 ± 11 a	470 ± 1 b	260 ± 8 b	278 ± 47 b	226 ± 114 b	214 ± 85 b
Ethyl dodecanoate	33 ± 7 bcd	46 ± 10 ab	42 ± 3 bc	47 ± 3 ab	63 ± 2 a	25 ± 2 bcd	22 ± 3 d	23 ± 2 cd	16 ± 4 d	13 ± 4 d
Ethyl isovaleriate	105 ± 8 a	79 ± 10 b	88 ± 5 ab	76 ± 1 bc	61 ± 1 c	2 ± 0.1 d	2 ± 0.1 d	2 ± 0.4 d	2 ± 0.3 d	2 ± 0.1 d
Diethyl succinate	4009 ± 105 a	3493 ± 457 a	3264 ± 29 a	3634 ± 201 a	2070 ± 239 a	2479 ± 88 a	2482 ± 112 a	2075 ± 110 a	2339 ± 21 a	2403 ± 43 a
Acetates										
Ethyl acetate	7442 ± 133 ab	6681 ± 765 abc	6845 ± 62 abc	7812 ± 360 a	6447 ± 51 abc	5368 ± 14 cd	6009 ± 300 bcd	6350 ± 186 bc	4905 ± 16 de	4435 ± 247 e
Isoamyl acetate	747 ± 99 ab	582 ± 244 ab	642 ± 149 ab	806 ± 93 a	370 ± 182 ab	148 ± 9 b	389 ± 27 ab	467 ± 44 ab	243 ± 15 b	197 ± 10 b

Table 2 (continued)

	t0					t1				
	2006	2007	2008	2009	2010	2006	2007	2008	2009	2010
Hexyl acetate	52 ± 5 bc	60 ± 4 ab	70 ± 3 a	61 ± 6 ab	35 ± 3 de	18 ± 1 f	34 ± 3 e	47 ± 2 cd	20 ± 0.8 f	17 ± 0.3 f
2-phenylethyl acetate	134 ± 11 ab	125 ± 13 ab	132 ± 7 ab	138 ± 7 a	96 ± 20 bc	43 ± 2 d	54 ± 5 d	64 ± 8 cd	41 ± 2 d	35 ± 2 d
Alcohols										
Isoamyl alcohol	4163 ± 88 a	3830 ± 264 ab	3801 ± 114 ab	3710 ± 11 ab	3471 ± 179 bc	2903 ± 3 cd	2733 ± 6 de	2538 ± 20 de	2596 ± 50 de	2245 ± 50 e
Isobutanol	304 ± 22 abc	357 ± 27 ab	373 ± 37 a	382 ± 17 a	207 ± 13 bc	141 ± 21 c	203 ± 70 bc	249 ± 23 abc	159 ± 41 c	144 ± 44 c
Benzyl alcohol	39 ± 6 a	22 ± 2 b	18 ± 0.8 bc	17 ± 0.1 cd	14 ± 2 d	13 ± 0.01 d	8 ± 0.01 e	7 ± 0.3 e	6 ± 0.3 e	6 ± 0.01 e
2-phenylethyl alcohol	3449 ± 157 a	3375 ± 459 a	3154 ± 126 a	3262 ± 5 a	3514 ± 128 a	3116 ± 6 a	3137 ± 90 a	3010 ± 90 a	3153 ± 7 a	2989 ± 3 a
Fatty acids										
Hexanoic acid	223 ± 1 ab	205 ± 4 ab	227 ± 5 a	196 ± 0.3 ab	176 ± 28 b	73 ± 1 c	78 ± 1 c	89 ± 8 c	69 ± 6 c	69 ± 2 c
Octanoic acid	349 ± 3 a	259 ± 21 a	294 ± 5 a	265 ± 4 a	261 ± 67 a	113 ± 1 b	114 ± 2 b	130 ± 12 b	101 ± 11 b	103 ± 1 b
Decanoic acid	31 ± 1 a	15 ± 4 ab	16 ± 2 ab	17 ± 0.8 ab	18 ± 8 ab	10 ± 0.1 b	9 ± 0.4 b	10 ± 1 b	9 ± 2 b	8 ± 1 b

Different letters indicate significant differences between the vintages and oxidation conditions (t0 and t1) among the same group of samples (W1 or W2) (by Tukey HSD test ($p < 0.05$). The mean value and SD (standard deviation)

indicates that they do not undergo major changes with aging in the organoleptic properties, as occurred in previous works [38].

Oxidative aromas

A total of eleven volatile compounds related to the oxidation were determined in wine samples (table S2). All of them were analyzed under natural oxidation conditions after their period of aging in the bottle (t0), as well as after the semi-forced oxidation condition imposed. In this way, it is intended to study the possible differences in relation to the aromatic composition that a Tempranillo wine can suffer when it evolves naturally or when forced oxidation occurs, to find differentiating oxidative markers. These compounds were grouped according to their chemical structures. Figure 3 shows the total content of the oxidative aromas; furans, Strecker aldehydes, alkenals, ketones, and lactones. In both series of wines (W1 and W2), a significant increase in all the studies families was found at t1. In the case of furans, there was an important increase found (only 2006 and 2007 samples had some content at t0). It should be noted that furans clearly appeared after the oxidation period (in general, no furans were found at t0 in the youngest wines). As in the case of furans, aldehydes suffered a significant increase of approximately 90% in both wine series. The concentration of ketones also increased significantly (ca. 82%). On the other hand, alkenales and lactones showed moderate increases (ca. 64% and 62%, respectively). The families of the furans, aldehydes, and ketones presented significant differences in all the vintages, whereas in alkenales and lactones, significant differences were only observed for some vintages.

To better understand the importance of the aromatics on the sensory perception, the individual compounds were evaluated taking into account their odor threshold (OT). Figure 4 shows the content and the odor threshold of the studied individual oxidative compounds before (t0) and after (t1) the oxidation conditions. Regarding sotolon, significant differences were observed in all vintages (between t0 and t1); it can be observed that wine samples at t0 did not present this aroma, except for the 2006 and 2007 vintages in W1. However, after the oxidation conditions (t1), sotolon was found in all samples noticeably upper its odor threshold. As reported by other studies, this is a characteristic compound with spicy and caramel notes that appears in oxidized wines, especially in fortified wines such as Port, Madeira, and Sherry wines [10, 24, 40, 41].

Among the studied aldehydes, it should be noted that methional only appeared in some W2 wines. 2-methylpropanal did not contribute to the aroma of this type of wines since before the oxidation conditions (t0), none of the wine samples reached the OT. In general, 3-methylbutanal and 2-phenylacetaldehyde showed a slight contribution before

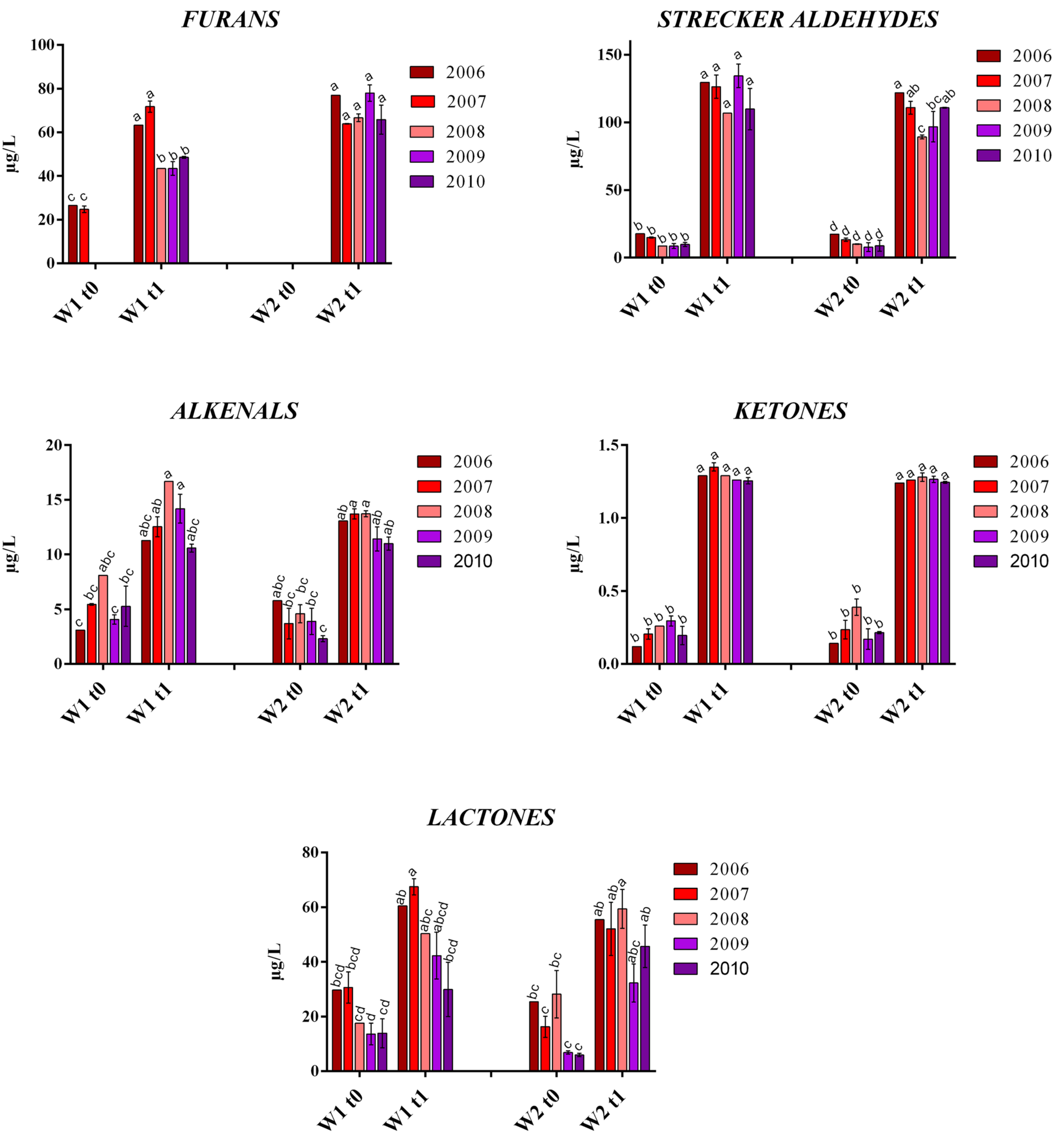

Fig. 3 Total concentration of the oxidative aromas before (t0) and after (t1) the experimental oxidation conditions; furans, Strecker aldehydes, alkenals, ketones, and lactones. Different letters indicate significant differences between samples for each wine series

the oxidation period which seemed to be more remarkable in the oldest wines, which slightly exceeded the OT value. This could indicate that both compounds are present in wines after long aging periods. They may provide sweet aromatic notes, such as honey and flowers, and especially 2-phenylacetaldehyde which have been considered an oxidation wine marker [16, 17]. These three Strecker aldehydes suffered an important increase after the oxidation conditions (t1) reaching values well above their respective OT, with significant differences in all the studied cases. Furthermore, the presence of 2-methylpropanal after the oxidation conditions (t1) could indicate its importance as oxidation marker on the studied conditions. This compound is characterized by providing malty aromatic notes [8]. On the other hand, the

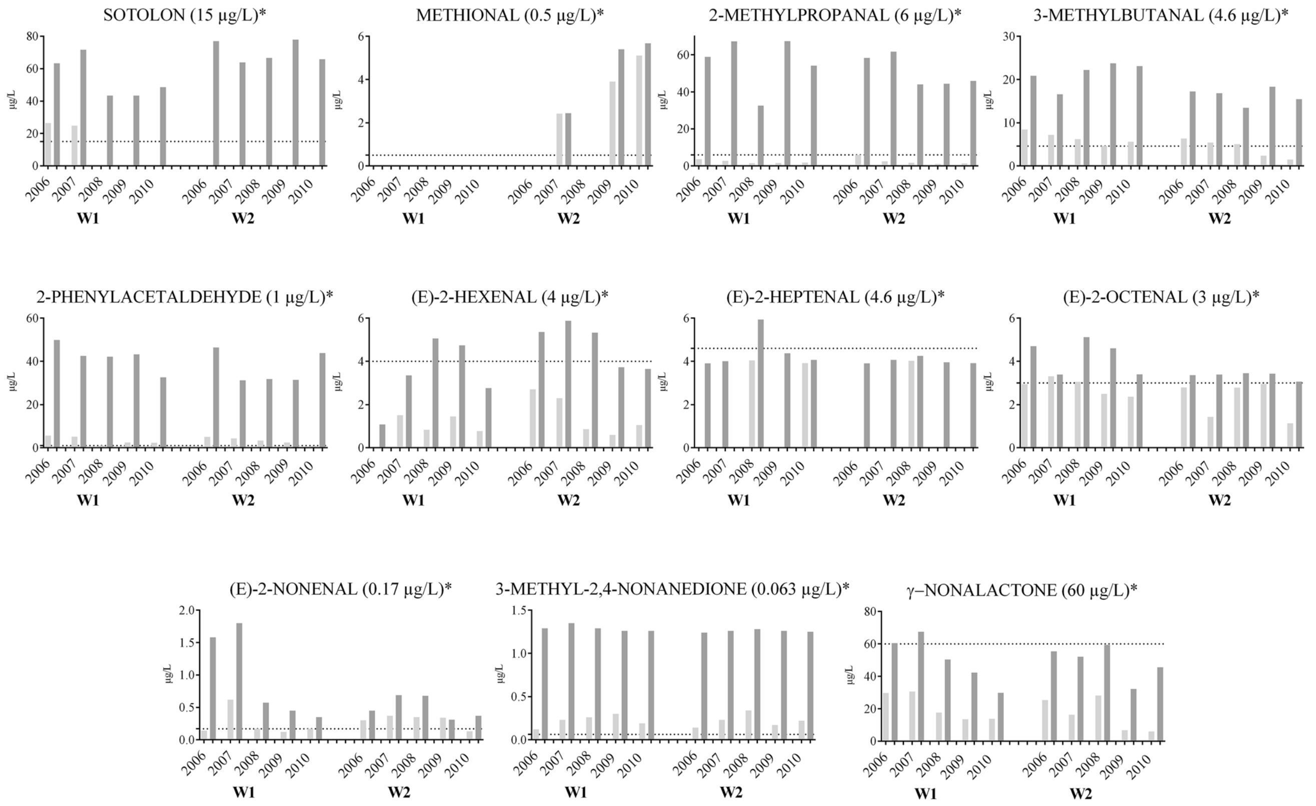

Fig. 4 Total concentration and the odor threshold (OT) of the individual oxidative compounds before (t0 in light grey) and after (t1 in grey) the experimental oxidation conditions. Different letters indicate significant differences between samples for each wine series. The *OT were established according to the literature (Mayr et al. 2015; Pons et al. 2013)

content on methional seems to be more related to the intrinsic wine characteristics than to the rapid oxidation.

Within the alkenals, it could be said that (*E*)-2-hexenal, (*E*)-2-heptenal and (*E*)-2-octenal did not contribute aromatically from the Tempranillo aged wines studied in t0. In the case of the (*E*)-2-nonenal, this compound showed a slight contribution before the forced oxidation with some values upper to the OT (samples W1 2007, and W2 2006, 2007, 2008, and 2009). This compound presented a remarkable increment after the oxidation conditions in W1 2006 and 2007 samples (with significant differences in all vintages, except in 2010), showing values well above the OT. This is a product of the oxidative degradation of unsaturated fatty acids that may contribute with sawdust notes on the aromatic profile of wines [9]. Among ketones, the 3-methyl-2,4-nonanedione showed significant differences in all vintages. It exceeds the content, overcomes the odor threshold markedly at t0, and reached values 20 times upper the OT at t1. This is a very characteristic aromatic compound that provides mint notes at lower concentrations. However, at high concentrations, as observed in our study after the oxidation (t1), its aromatic notes comprises of ripe fruit, ripe prunes and anise [14]. In the case of γ-nonalactone, this compound only exceeded OT after oxidation conditions (as occurred in some of the studied alkenals). Its values were higher in the older vintages, as reported other authors [12, 14]. In these studies, high concentrations of γ-nonalactone were directly related to red wine oxidation process. These authors also related the production of this compound during the aging period with peach and dried-fruit notes.

Odor activity values

As previously seen, the detection thresholds for wine aromatic compounds vary from very low concentrations (ie 0.06 µg/L for 3-methyl-2,4-nonanedione [2]) to very high concentrations (ie that is, 5 mg/L for ethyl acetate [42]). However, the value of the smell activity (OAV) of each compound allows us to determine how a smell participates in the overall aroma of the wine and whether it will be perceived by the human nose [43]. Therefore, to determine how each oxidative compound studied in the global aroma of wine participates, the OAV is defined as the ratio between the concentration of a certain volatile molecule and its detection threshold. In general, the OAV greater than or equal to the unit indicates an active odor, which contributes to the perception of the aroma of the wine.

The contribution to the overall aromatic perception of Rioja wines can be observed before (t0) and after (t1) the oxidation conditions (Table 3). Visually, we can appreciate the most important active key odors with OAV ≥ 1. At t0, 3-methyl-2,4-nonanedione was the only volatile compound which reached OAVs upper than the unity for all samples and obtained a high value at this time (OAV = 21.0). Also, 2-phenylacetaldehyde reached the unity in many cases, obtaining OAV values ranged from 1 to 5.6. In general, 3-methylbutanal and (*E*)-2-nonenal also obtained values upper than the unity. At t1, 3-methyl-2,4-nonanedione and 2-phenylacetaldehyde continue to be the most active compounds reaching important OAVs (ranged from 31 to 84 units). It should be noted that sotolon, an important volatile compound related to the wine oxidation was not detected in the majority of the samples at t0. However, this compound was highly affected by the oxidation conditions. This pattern was also strongly found in 2-methylpropanal and at lesser extent in (*E*)-2-octenal and γ-nonalactone.

It seems that the studied aromas could be related to pleasant aromatic notes and associated to the wine quality at low concentrations but when these compounds reach certain levels, they could be associated to an incorrect wine evolution. In this way, a sensory analysis was made to evaluate how the stated changes on the volatile composition during the oxidation process affected the aromatic profile of wines.

Sensory analysis: principal component analysis

To observe trends from the sensory data, a PCA was carried out by the evaluated olfactory attributes (Fig. 5). The proportion of the explained variance accumulated by the two main components was greater than 60%. As shown in Fig. 5, the sensory attributes with the highest incidence on the horizontal axis (which explains 45% of the variability) were the aromatic intensity, red fruit, black fruit, and balsamic, on the right of the PC1. On the other hand, rancid, acetates, and sweet fruit attributes was located on the left of the PC1.

In general, wines before the oxidation conditions (t0) were related with "fresh" attributes and located at right of the PCA, independently of their price. In contrast, wines after the oxidation period were related with “evolution” attributes and located at left of the plot. In addition, it was observed that at t0, the youngest wines (2010 and 2009 vintages) were placed on the right and down of the PC1 and the oldest wines (2006 and 2007 vintages) were located more to the left and in the upper. This observed pattern (from right to the left and from bottom to top) in t0 samples was mainly due to a loss on the red fruit attribute and a higher contribution of the nuts and chemical attributes as the wines aged in bottle. This result proves that the price of wine is not directly related to its initial volatile composition of the wines as well as after being subjected to forced oxidation conditions.

However, this pattern was not observed in t1 samples. These samples were intimately related to the attributes; sweet fruit, rancid, and acetates. It should be noted that the W2_10 sample (t1) was placed near to the samples of 2006 (t0). This may indicate that 2006 wines before the oxidation (t0) showed similar aromatic attributes to that of 2010 wines after the oxidation (t1). Hence, it seems that wines with a long aging period, around 10 years in bottle (wines were tasted in 2016), may present a similar aromatic profile than wines 4 years younger which have suffered a forced oxidation in bottle.

Conclusions

The aromatic profile of representative long-aged Rioja wines has been characterized by GC–MS and sensory analysis. The aromatic compounds that participate actively on the aromatic profile of Tempranillo long-aged wines have been identified. 3-methyl-2,4-nonanedione and 2-phenylacetaldehyde were identified as the most important sensory active compounds. At lesser extent 3-methylbutanal, (*E*)-2-nonenal, and (*E*)-2-octenal were also important volatile compounds in this type of wines. Surprisingly, sotolon was only detected as active compounds in the oldest wines (2006 and 2007) with lower price. Contrary, methional mainly appeared as active compound in the youngest samples of wines with higher price. 2-methylpropanal was a non-active compound in wines correctly aged and stored. The results showed a drastic change on the volatile composition of the studied wines after the oxidation conditions with a loss of 40% on the fermentative aromas and an increase of 85% of the oxidative aromas. The oxidation compounds reached the odor thresholds and odor activity values upper the unity after the oxidation process. The aromatic profiles of this type of wines were similar independently of their price, blend, and oxidation exposition. The aromatic compounds that can participate actively on the aromatic profile of Rioja high-quality old wines have been identified, contributing to an improvement in the understanding about the key odorants in this type of wines.

Table 3 Odorant active values (OAV) of the individual oxidative compounds before (t0) and after (t1) the experimental oxidation conditions for W1 and W2 wine series

	W1					**W2**				
t0	2006	2007	2008	2009	2010	2006	2007	2008	2009	2010
Methional	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**4.8**	**nd**	**7.8**	**10.2**
2-heptenal	**nd**	**0.9**	**0.9**	**nd**	**0.9**	**nd**	**nd**	**0.9**	**nd**	**nd**
2-hexenal	**nd**	**0.4**	**0.2**	**0.4**	**0.2**	**0.7**	**0.6**	**0.2**	**0.1**	**0.3**
γ-nonalactone	**1.0**	**1.0**	**0.6**	**0.5**	**0.5**	**0.8**	**0.5**	**0.9**	**0.2**	**0.2**
2-octenal	**1.0**	**1.1**	**1.0**	**0.8**	**0.8**	**0.9**	**0.5**	**0.9**	**1.0**	**0.4**
2-nonenal	**0.8**	**3.6**	**1.0**	**0.7**	**1.0**	**1.8**	**2.2**	**2.1**	**2.0**	**0.8**
Sotolon	**1.8**	**1.6**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
3-methylbutanal	**1.8**	**1.6**	**1.3**	**1.0**	**1.2**	**1.4**	**1.2**	**1.1**	**0.5**	**0.3**
2-methylpropanal	**0.6**	**0.5**	**0.2**	**0.3**	**0.3**	**1.0**	**0.4**	**0.3**	**0.2**	**0.2**
2-phenylacetaldehyde	**5.6**	**5.1**	**1.0**	**2.4**	**2.4**	**5.0**	**4.3**	**3.3**	**2.4**	**0.9**
3-methyl-2,4-nonanedione	**7.0**	**14.0**	**16.0**	**19.0**	**12.0**	**9.0**	**14.0**	**21.0**	**11.0**	**14.0**
t1										
Methional	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**4.9**	**nd**	**10.8**	**11.4**
2-heptenal	**0.8**	**nd**	**1.3**	**1.0**	**0.9**	**0.8**	**0.9**	**0.9**	**0.9**	**0.9**
2-hexenal	**0.3**	**0.8**	**1.3**	**1.2**	**0.7**	**1.3**	**1.5**	**1.3**	**0.9**	**0.9**
γ-nonalactone	**2.0**	**2.2**	**1.7**	**1.4**	**1.0**	**1.8**	**1.7**	**2.0**	**1.1**	**1.5**
2-octenal	**1.6**	**1.1**	**1.7**	**1.5**	**1.1**	**1.1**	**1.1**	**1.2**	**1.1**	**1.0**
2-nonenal	**9.3**	**10.6**	**3.4**	**2.6**	**2.1**	**2.6**	**4.1**	**4.0**	**1.8**	**2.2**
Sotolon	**4.2**	**4.8**	**2.9**	**2.9**	**3.2**	**5.1**	**4.3**	**4.4**	**5.2**	**4.4**
3-methylbutanal	**4.5**	**3.6**	**4.8**	**5.2**	**5.0**	**3.7**	**3.7**	**2.9**	**4.0**	**3.4**
2-methylpropanal	**9.8**	**11.2**	**5.4**	**11.2**	**9.0**	**9.7**	**10.3**	**7.3**	**7.4**	**7.7**
2-phenylacetaldehyde	**49.9**	**42.6**	**42.1**	**43.3**	**32.6**	**46.4**	**31.2**	**31.8**	**31.4**	**43.9**
3-methyl-2,4-nonanedione	**80.0**	**84.0**	**81.0**	**79.0**	**79.0**	**78.0**	**79.0**	**80.0**	**79.0**	**78.0**

OAV 0.1 < < 85

nd (not detected)

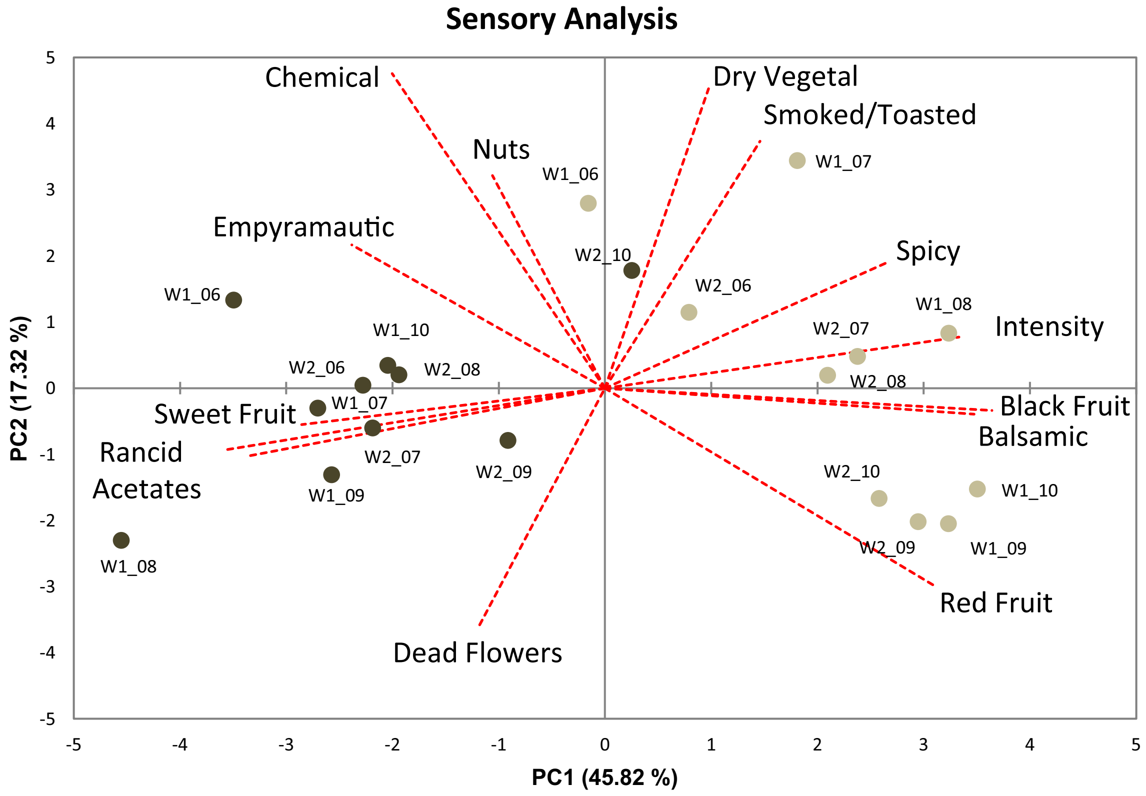

Fig. 5 Principal component analysis of the sensory data obtained from the studied wines (W1 and W2) before (in red) and after (in black) the experimental oxidation conditions. Numbers at the end of the samples indicates vintages

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

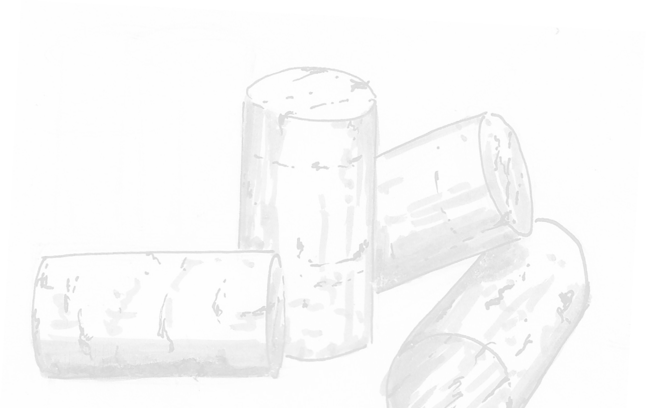
Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

References

1. Loscos N, Hernández-Orte P, Cacho J, Ferreira V (2010) Evolution of the aroma composition of wines supplemented with grape flavour precursors from different varietals during accelerated wine ageing. Food Chem 120(1):205–216. https://doi.org/10.1016/j.foodchem.2009.10.008
2. Pons A, Lavigne V, Darriet P, Dubourdieu D (2013) Role of 3-methyl-2,4-nonanedione in the flavor of aged red wines. J Agric Food Chem 61(30):7373–7380. https://doi.org/10.1021/jf400348h
3. Hernández-Orte P, Lapeña AC, Escudero A, Astrain J, Baron C, Pardo I, Polo L, Ferrer S, Cacho J, Ferreira V (2009) Effect of micro-oxygenation on the evolution of aromatic compounds in wines: malolactic fermentation and ageing in wood. LWT Food Sci Technol 42(1):391–401. https://doi.org/10.1016/j.lwt.2008.05.020
4. Vilanova M, Genisheva Z, Graña M, Oliveira J (2013) Determination of odorants in varietal wines from international grape cultivars (*Vitis vinífera*) grown in NW Spain. South Afr J Enol Viticult 34(2):212–222. https://doi.org/10.21548/34-2-1097
5. Cacho J (2009) Genesis e importancia sensorial de los componentes aromaticos delvino. In, Logroño.
6. Escudero A, Cacho J, Ferreira V (2000) Isolation and identification of odorants generated in wine during its oxidation: a gas chromatography-olfactometric study. Eur Food Res Technol 211(2):105–110. https://doi.org/10.1007/s002179900128
7. Ferreira AC, Hogg T, Guedes de Pinho P (2003) Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. J Agric Food Chem 51(5):1377–1381. https://doi.org/10.1021/jf025847o
8. Mayr CM, Capone DL, Pardon KH, Black CA, Pomeroy D, Francis IL (2015) Quantitative analysis by GC-MS/MS of 18 aroma compounds related to oxidative off-flavor in wines. J Agric Food Chem 63(13):3394–3401. https://doi.org/10.1021/jf505803u
9. Balboa-Lagunero T, Arroyo T, Cabellos JM, Aznar M (2011) Sensory and olfactometric profiles of red wines after natural and forced oxidation processes. Am J Enol Vitic 62(4):527–535. https://doi.org/10.5344/ajev.2011.10080
10. Ferreira AC, Barbe J-C, Bertrand A (2003) 3-Hydroxy-4,5-dimethyl-2(5H)-furanone: a key odorant of the typical aroma of oxidative aged port wine. J Agric Food Chem 51(15):4356–4363. https://doi.org/10.1021/jf0342932

11. Dubourdieu D, Pons A, Lavigne V (2010) Identificación de nuevos marcadores de envejecimiento. Enólogos Andaluces.
12. Allamy L, Darriet P, Pons A (2018) Molecular interpretation of dried-fruit aromas in Merlot and Cabernet Sauvignon musts and young wines: impact of over-ripening. Food Chem 266:245–253. https://doi.org/10.1016/j.foodchem.2018.06.022
13. Escudero A, Campo E, Fariña L, Cacho J, Ferreira V (2007) Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. J Agric Food Chem 55(11):4501–4510. https://doi.org/10.1021/jf0636418
14. Pons A, Lavigne V, Eric F, Darriet P, Dubourdieu D (2008) Identification of volatile compounds responsible for prune aroma in prematurely aged red wines. J Agric Food Chem 56(13):5285–5290. https://doi.org/10.1021/jf073513z
15. Han G, Webb MR, Waterhouse AL (2019) Acetaldehyde reactions during wine bottle storage. Food Chem 290:208–215. https://doi.org/10.1016/j.foodchem.2019.03.137
16. Ferreira AC, De Pinho PG, Rodrigues P, Hogg T (2002) Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters. J Agric Food Chem 50(21):5919–5924. https://doi.org/10.1021/jf0115847
17. Bueno M, Culleré L, Cacho J, Ferreira V (2010) Chemical and sensory characterization of oxidative behavior in different wines. Food Res Int 43(5):1423–1428. https://doi.org/10.1016/j.foodres.2010.04.003
18. Bueno M, Carrascón V, Ferreira V (2016) Release and formation of oxidation-related aldehydes during wine oxidation. J Agric Food Chem 64(3):608–617. https://doi.org/10.1021/acs.jafc.5b04634
19. Bueno M, Zapata J, Ferreira V (2014) Simultaneous determination of free and bonded forms of odor-active carbonyls in wine using a headspace solid phase microextraction strategy. J Chromatogr A 1369:33–42. https://doi.org/10.1016/j.chroma.2014.10.004
20. Ferreira V, Escudero A, Lopez R, Cacho J (1998) Analytical characterization of the flavor of oxygen-spoiled wines through the gas chromatography-ion-trap mass spectrometry of ultratrace odorants: optimization of conditions. J Chromatogr Sci 36(7):331–339. https://doi.org/10.1093/chromsci/36.7.331
21. Pons A, Lavigne V, Darriet P, Dubourdieu D (2011) Determination of 3-methyl-2,4-nonanedione in red wines using methanol chemical ionization ion trap mass spectrometry. J Chromatogr A 1218(39):7023–7030. https://doi.org/10.1016/j.chroma.2011.08.017
22. Ferreira V, Culleré L, López R, Cacho J (2004) Determination of important odor-active aldehydes of wine through gas chromatography-mass spectrometry of their *O*-(2,3,4,5,6-pentafluorobenzyl) oximes formed directly in the solid phase extraction cartridge used for selective isolation. J Chromatogr A 1028(2):339–345. https://doi.org/10.1016/j.chroma.2003.11.104
23. López R, Ezpeleta E, Sánchez I, Cacho J, Ferreira V (2004) Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry. Food Chem 88(1):95–103. https://doi.org/10.1016/j.foodchem.2004.01.025
24. Collin S, Nizet S, Bouuaert T, Despatures PM (2012) Main odorants in Jura flor-sherry wines. Relative contributions of sotolon, abhexon, and theaspirane-derived compounds. J Agric Food Chem 60(1):380–387. https://doi.org/10.1021/jf203832c
25. Alamo-Sanza Md, Nevares I, Martínez-Gil A, Rubio-Bretón P, Garde-Cerdán T (2019) Impact of long bottle aging (10 years) on volatile composition of red wines micro-oxygenated with oak alternatives. LWT Food Sci Technol 101:395–403. https://doi.org/10.1016/j.lwt.2018.11.049
26. Compendium of international methods of wine and must analysis. (2016)
27. Torrens J, Riu-Aumatell M, López-Tamames E, Buxaderas S (2004) Volatile compounds of red and white wines by headspace-solid-phase microextraction using different fibers. J Chromatogr Sci 42:310–316. https://doi.org/10.1093/chromsci/42.6.310
28. Zapata J, Mateo-Vivaracho L, Cacho J, Ferreira V (2010) Comparison of extraction techniques and mass spectrometric ionization modes in the analysis of wine volatile carbonyls. Anal Chim Acta 660(1):197–205. https://doi.org/10.1016/j.aca.2009.09.041
29. Tessini C, Müller N, Mardones C, Meier D, Berg A, von Baer D (2012) Chromatographic approaches for determination of low-molecular mass aldehydes in bio-oil. J Chromatogr A 1219:154–160. https://doi.org/10.1016/j.chroma.2011.10.093
30. García-Puente E, Alcalde-Eon C, Santos-Buelga C, Rivas-Gonzalo JC, Escribano-Bailón MT (2006) Behaviour and characterisation of the colour during red wine making and maturation. Anal Chim Acta 563:215–222. https://doi.org/10.1016/j.aca.2005.11.044
31. Ferrer-Gallego R, Hernández-Hierro JM, Rivas-Gonzalo JC, Escribano-Bailón MT (2012) Influence of climatic conditions on the phenolic composition of Vitis vinifera L. cv Graciano. Anal Chim Acta 732:73–77. https://doi.org/10.1016/j.aca.2011.12.072
32. Ferreira V, López R, Cacho JF (2000) Quantitative determination of the odorants of young red wines from different grape varieties. J Sci Food Agric 80(11):1659–1667. https://doi.org/10.1002/1097-0010(20000901)80:11%3c1659:aid-jsfa693%3e3.0.co;2-6
33. Francis IL, Newton JL (2005) Determining wine aroma from compositional data. Aust J Grape Wine Res 11(2):114–126. https://doi.org/10.1111/j.1755-0238.2005.tb00283.x
34. Gómez-Míguez MJ, Cacho JF, Ferreira V, Vicario IM, Heredia FJ (2007) Volatile components of Zalema white wines. Food Chem 100(4):1464–1473. https://doi.org/10.1016/j.foodchem.2005.11.045
35. Peinado RA, Moreno J, Bueno JE, Moreno JA, Mauricio JC (2004) Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. Food Chem 84(4):585–590. https://doi.org/10.1016/S0308-8146(03)00282-6
36. Yong-Sheng T, Li H (2009) Active volatiles of cabernet sauvignon wine from Changli County. Health 1(3):176–182
37. Ancín-Azpilicueta C, González-Marco A, Jiḿenez-Moreno N (2009) Evolution of esters in aged Chardonnay wines obtained with different vinification methods. J Sci Food Agric 89(14):2446–2451. https://doi.org/10.1002/jsfa.3744
38. Câmara JS, Alves MA, Marques JC (2006) Changes in volatile composition of Madeira wines during their oxidative ageing. Anal Chim Acta 563(1):188–197. https://doi.org/10.1016/j.aca.2005.10.031
39. Pérez-Magariño S, Bueno-Herrera M, López de la Cuesta P, González-Lázaro M, Martínez-Lapuente L, Guadalupe Z, Ayestarán B (2018) Volatile composition, foam characteristics and sensory properties of Tempranillo red sparkling wines elaborated using different techniques to obtain the base wines. Eur Food Res Technol. https://doi.org/10.1007/s00217-018-3209-y
40. Lavigne V, Pons A, Darriet P, Dubourdieu D (2008) Changes in the sotolon content of dry white wines during barrel and bottle aging. J Agric Food Chem 56(8):2688–2693. https://doi.org/10.1021/jf072336z
41. Gaspar JM, Pereira V, Marqués J (2018) Odor detection threshold (ODT) and odor rejection threshold (ORT) determination of sotolon in Madeira wine: a preliminary study. AIMS Agriculture and Food 3(3):172–180
42. Pino JA, Queris O (2010) Analysis of volatile compounds of pineapple wine using solid-phase microextraction techniques. Food Chem 122(4):1241–1246. https://doi.org/10.1016/j.foodchem.2010.03.033

43. Guth H (1997) Quantitation and sensory studies of character impact odorants of different white wine varieties. J Agric Food Chem 45(8):3027–3032. https://doi.org/10.1021/jf970280a



CAPÍTULO 4

SUBPRODUCTOS ENOLÓGICOS

El corcho y su posible reaprovechamiento en la industria agroalimentaria

Justificación y objetivo

Hoy en día, para cualquier tipo de industria son muy importantes los conceptos de reaprovechamiento, reciclaje y reutilización, con el fin de conseguir una sostenibilidad global mediante el cuidado del medio ambiente.

En el sector enológico, el 90 % de los vinos embotellados son taponados con corchos. Esto implica que se produzcan anualmente alrededor de 300 mil toneladas de corcho, de las cuales alrededor del 25 % acaban como residuos.

Por tanto, ante esta gran cantidad de residuos, tanto la industria enológica como la corchera consideran necesarias acciones de reaprovechamiento, reutilización y/o reciclado. Los fragmentos, granulados y el polvo de corcho representan una gran cantidad de residuos que deben ser devueltos al sistema productivo. Los residuos del corcho han sido empleados en el sector agrario como medio de crecimiento de plantas, incluso en el sector enológico como agente clarificante, sin embargo, es interesante explorar nuevas posibilidades y usos con mayor potencial económico. Los compuestos bioactivos y aromáticos que contiene estos subproductos pueden ser de gran interés para la industria agroalimentaria e incluso cosmética o farmaceútica.

Por ello, el capítulo 4 estudia el potencial de reaprovechamiento de los subproductos del corcho en la industria agroalimentaria, mediante el estudio de sus compuestos fenólicos, su composición aromática y su potencial antioxidante.

Objetivos específicos

El principal objetivo del artículo 8 fue estudiar los compuestos aromáticos, fenólicos y bioactivos de los tapones y subproductos del corcho con el fin de poder ser reaprovechados en la industria agroalimentaria.

Publicaciones asociadas

- **Artículo 8**: *Aromatic Potential and Bioactivity of Cork Stoppers and Cork By-Products*

Artículo 8

Aromatic Potential and Bioactivity of Cork Stoppers and Cork By-Products

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foods

Article

Aromatic Potential and Bioactivity of Cork Stoppers and Cork By-Products

Ana Maria Mislata [1,2], Miquel Puxeu [1] and Raul Ferrer-Gallego [1,*]

[1] Centro Tecnológico del Vino—VITEC, Carretera de Porrera Km. 1, 43730 Falset, Spain; anamaria.mislata@vitec.wine (A.M.M.); miquel.puxeu@vitec.wine (M.P.)
[2] Sensometria Instrumental (i-Sens), Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, 43007 Tarragona, Spain
* Correspondence: raul.ferrer@vitec.wine

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Abstract: The characterization of natural waste sources is the first step on the reutilization process, circular economy, and global sustainability. In this work, the aromatic composition and bioactive compounds related to beneficial health effects from cork stoppers and cork by-products were assessed in order to add value to these wastes. Twenty-three aromatic compounds with industrial interest were quantified by gas chromatography coupled mass spectrometry GC–MS in both samples. Vanillins and volatile phenols were the most abundant aromatic families. Other aromatic compounds, such as aldehydes, lactones, terpenols, and alcohols, were also determined. Furthermore, the phenolic composition and the antioxidant activity were also evaluated. Overall, extracts showed high aromatic and antioxidant potential to be further used in different industrial fields. The recovery of these valuable compounds from cork stoppers and cork by-products helps to reuse them in agricultural, cosmetic, pharmaceutical, or food industries.

Keywords: cork; volatile compounds; antioxidant activity; polyphenols; aroma; waste

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1. Introduction

Nowadays, a key issue in any field of research is the fight to curb climate change. This environmental awareness is of great importance in every society in order to achieve a sustainable environment considering the current human actions. The re-use and material recycling is a priority for waste management in the European Union (Directive 2008/98/EC on Waste). The characterization of natural waste sources is the first step on the reutilization process and the global sustainability. Bioactive compounds related to beneficial health effects and aromatics from by-products can be explored by food, agricultural, cosmetic, or pharmaceutical industries. Fragments, granulates, and powder from cork represent a large waste stream from cork processing [1,2]. They have been commonly used as combustion fuel, although they have also been employed in agriculture. Composted residues from cork were used as plant growth media to suppress plant diseases [3], and hydrological properties of substrates based on industrial cork residue have also been reported [4]. Recently, their adsorption properties, such as fining agent in wines, were stated [5], and some works showed that cork wastes are cost-effective and green alternatives to the retention of contaminants from water [6–9]. However, to the best of our knowledge, few works were reported regarding their revalorization for the food industry. According to statistical reports of the International Organization of Vine and Wine (OIV), the annual world wine production is around 275 million hectoliters (292 MhL in 2018) [10], and 90% of bottle wines are stopped by corks. In cork stoppers production (about 300 thousand tons of cork are produced annually), the cork waste represents around 25% of the raw material. Different cork wastes can be found depending on their characteristics, density, moisture, granulometry, size, ash content, and tannin concentration [1,11,12].

It is well known that the phenolic composition of plants is related to antioxidative, anticarcinogenic, and antitumor biological activities [13,14]. Specially, polyphenols from *Quercus suber* L. have been associated with beneficial health effects linked to hydrolysable tannins and phenolic compounds with low molecular weight [15]. Recent research valorized cork powder and granulates from a phenolic point of view [12], however, little research focused on the recovery of valuable aromatic compounds. On the other hand, a great number of works are related to the off-flavors or cork-taint compounds in wine [16–19]. However, the characterization of the positive aromas of cork stoppers and cork by-products is scarce. In this work, the aromatic composition of different wine cork stoppers and granulates was determined, enhancing the added value of their re-use in other industrial fields. The tannin concentration and the antioxidant activity of the cork extracts were also compared and discussed.

2. Materials and Methods

2.1. Samples and Extraction

Different cork stoppers and their respective cork granulate (A, B, and C), from which the corks were made, were evaluated in this study. The granulate cork size ranged from 6.1 to 14.3 mm for A samples (high size), from 1.6 to 4.4 mm (medium size) for B samples, and from 0.85 to 1.9 mm (low size) for C samples (Figure 1 and Table S1). The cork stoppers dimensions were 30 × 50 mm for all samples. To determine the migration of the phenolic compounds from granulates and cork samples to model solution, a liquid–liquid extraction with ethyl acetate was made according to Azevedo et al. [20]. Briefly, 30 g of each sample (cork stoppers and granulates) was weighted in glass jars and macerated in 1 L of hydro alcoholic solution. The model solution contained 13% of ethanol, 5 g/L of tartaric acid, and the pH was adjusted to 3.7 with sodium hydroxide (1 *N*). Solutions were stored at controlled temperature (20–22 °C) until the analyses were performed. Different times of maceration were evaluated in this study (3, 5, and 15 days).

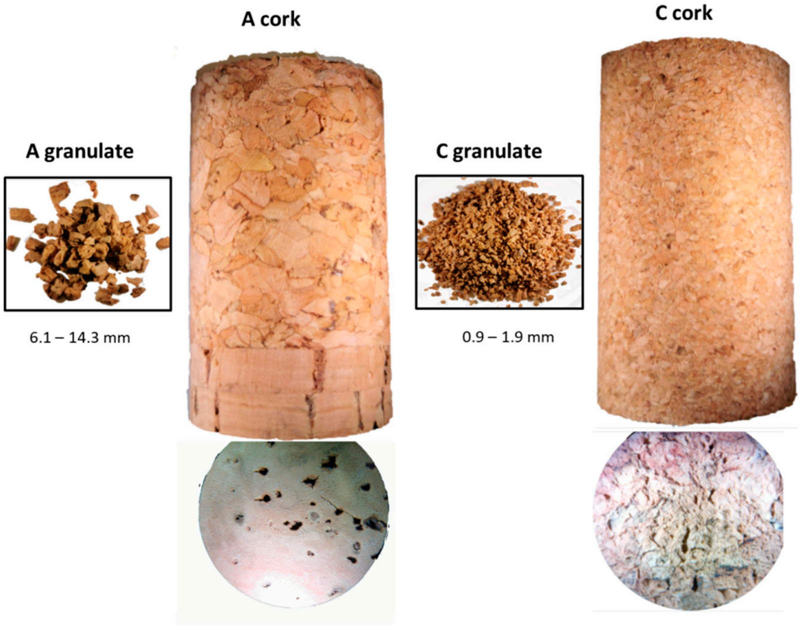

Figure 1. Representative image of the studied granulates and cork stoppers (A, high particle size and C, low particle size).

2.2. Reagents

Protocatechuic acid and gallic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Folin–Ciocalteu reagent, sodium carbonate anhydrous, ethyl alcohol 96%, and acetic acid glacial were purchased from PanReac AppliChem, Barcelona, Spain. All the aromatic standards were supplied by Sigma (Sigma Aldrich, Merck Life Science, Barcelona, Spain). Vanillin 99%, guaicol 98%, eugenol 98%, benzaldehyde ≥ 99%, nonenal 97%, phenylacetaldehyde ≥ 90%, phenylethyl alcohol ≥ 98%, benzyl alcohol ≥ 99%, camphor 96%, borneol 97%, 4-terpineol ≥ 96%, α-terpineol 90%, γ-nonalactone ≥ 98%, nonanoic acid ≥ 97%, vanillic acid ≥ 97%, octanoic acid ≥ 99%, dodecanoic acid 98%, benceneacetic acid ≥ 99%, furfural 99%. Dichlorometane anhydrous 99.8%, pentane anhydrous ≥ 99%, acetonitrile anhydrous ≥ 99.8%, phosphate buffered saline (PBS), potassium peroxodisulfate ≥ 99%, 6-hidroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid 97% (Trolox), and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were also purchased from Sigma Aldrich, Merck Life Science, Barcelona, Spain.

2.3. GC–MS Analysis

A volume of 100 μL of 2-octanol, as internal standard, was added to 100 mL of extract. Afterward, samples were separated by a SPE (solid phase extraction) cartridge (Bond Elut ENV, 500 mg and 6 mL, Agilent Tech., Santa Clara, California, USA). The cartridges were previously conditioned with 5 mL of dichloromethane, 5 mL of ethanol, and 5 mL of hydroalcoholic solution (12%). Analytes were eluted with pentane-dichloromethane (50:50, *v*/*v*), then dried using a concentrator vacuum (Savant™ SPD131DDA, Thermo Fisher Scientific, Barcelona, Spain). Finally, samples were redissolved in 200 μL of dichloromethane.

GC analysis was performed on a GC 7890A (Agilent Tech., Santa Clara, California) system equipped with a mass spectrometer 5975C inert MSD (with Triple-Axis Detector). The column was a DB-5 (30 m × 0.25 mm × 0.25 μm, Agilent Tech.). A constant flow of 2.1 mL/min of He was used as carrier gas. Five microliters of sample was injected in splitless mode with 17.33 psi pressure (septum purge flow 15 mL/min and splitless time 1 min.). The injector temperature was maintained at 225 °C for 1 min and then heated up to 250 °C at 5 °C/min. The temperature of the oven (40 °C) was maintained for 1 min and then increased up to 260 °C at (20 °C/min.) for 25 min. The mass spectrometer operated at 70 eV (electron ionization) modes. The analysis was performed in Scan mode (m/z 10−1000). The compounds were identified by retention times and mass fragments, to compare with those of pure standard compounds. The quantification was carried out using internal standard patterns.

2.4. Total Phenolic Content

The phenolic composition was determined by the Folin–Ciocalteu assay and HPLC–DAD/MS analysis. Total phenols (TP) were determined using the Folin–Ciocalteu assay [21] with some modifications. Briefly, 100 μL of sample, 500 μL of Folin–Ciocalteu reagent, and 2 mL of a solution of sodium carbonate (20% *w*/*v*) were mixed, final volume 10 mL with water. The solution was stocked for 30 min for the reaction to take place and stabilize and finally, the absorbance was measured at 750 nm.

Chromatographic analyses were carried out in an Agilent 1200 series (Agilent Technologies, Palo Alto, CA, USA) coupled with DAD and MS detectors. A volume of 50 mL of each extract was washed 3 times with 20 mL of ethyl acetate. The organic phases were pooled and evaporated, re-dissolved in 1 mL of water/methanol (50:50) and then identified and quantified by HPLC–DAD/MS. A Zorbax Eclipse Plus C18 column (3.5 μm, 150 × 4.6 mm) was used. The chromatographic conditions were used according to Azevedo et al. (2014) [20]. Briefly, solvent A was 0.1% of acetic acid in water, solvent B was acetic acid, acetonitrile, and water (1:20:79, *v*/*v*/*v*). The gradient was from 80% to 20% of solvent A over 55 min, from 20% to 10% of A from 55 to 70 min, and from 10% to 0% of A from 70 to 90 min. The flow rate was 0.3 mL/min, and the sample injection 20 μL. Gallic acid and protocatechuic acid were identified by the retention time and UV–VIS spectra. The compounds were quantified with phenolic standards using peak area data of resolved peaks at 280 nm. The corresponding calibration curves were made up for gallic acid ($r^2 = 0.999$) and protocatechuic acid ($r^2 = 0.999$). The identity of the phenolic compounds was confirmed by mass spectrometry. A TSQ Quantum™ Access MAX (Thermo Fisher Scientific, Waltham, MA USA) equipped with an HESI

(Heated Electrospray Ionization) source which was operated in the negative ionization mode between *m*/*z* 80 and 800 was used. The HESI spray voltage was set at 3.5 kV, and the capillary temperature was maintained at 350 °C. Nitrogen was used for nebulization and desolvation (sheath gas 60 arb. and auxiliary gas 20 arb.). The vaporizer temperature was maintained at 350 °C. Argon was used as the collision gas for collision-induced dissociation.

2.5. Antioxidant Activity Assay

The ABTS method allows determining the antioxidant activity through the discoloration of the cationic ABTS$^+$ radical produced by the oxidation of ABTS with potassium persulphate. This assay was performed according to the procedure described by Re. et al. (1999) [22] with slight modifications. A stock solution of 7 mM ABTS in water was prepared. To form the radical cation, a solution of potassium persulfate (2.45 mM) was prepared, using the ABTS stock solution as solvent. This solution was stored at 4 °C in the absence of light to complete the reaction. To prepare the working reagent, the solution was diluted with phosphate buffered saline (PBS) at pH 7.4, until an absorbance around 0.7 at 734 nm was obtained. Trolox or samples were added, and the decrease on the absorbance at 734 nm was measured, since the coloration disappears when the radical is reduced by antioxidants. Blank was made by adding 2 mL of reagent in a cuvette, and its absorbance was measured at 734 nm. Subsequently, 50 μL of diluted sample was added and vortexed for 30 s. After 4 min of incubation at room temperature, the absorbance was measured again at 734 nm, results were expressed in μmol/L of Trolox reagent. Samples were analyzed in several concentrations, making five dilutions of each sample by duplicate.

2.6. Statistics

Xlstat 2016.01 statistical software (Microsoft Ibèrica, Barcelona, Spain) add-on for Microsoft Excel package (Microsoft Ibèrica, Barcelona, Spain) was used for data processing. Significant differences between granulates and cork samples were determined by one-way ANOVA using the Tukey's HSD (honestly significant difference) test, at 95% of confidence level.

3. Results and Discussion

3.1. Aromatic Characterization

A total of 23 aromatic compounds were determined in granulates and cork samples and grouped according their chemical structures. Vanillins and derivatives, volatile phenols, aldehydes, alcohols, terpenes, lactones, fatty acids, and furans were found. A lot of these volatiles are closely related to certain pleasant aromatic descriptors. Table 1 shows the minimum and maximum content of the individual compounds found. The most important compound in the analyzed samples was vanillin (up to 170 μg/g), and to a lesser extent 4-vinylguaicol (23 μg/g), acetovanillone (14 μg/g), and dodecanoic acid (6.3 μg/g). All these volatiles have very pleasant aromas, such as vanilla, coconut, or wood, highly used in culinary industry and cosmetics.

Table 1. Aromatic compounds, families, descriptors, and minimum and maximum content found in the studied granulates and cork macerates.

Aromatic Compound	Aromatic Descriptor	Content (μg/g)
Vainillins		
Vanillin	vanilla	9–170
Acetovanillone	vanilla	0.6–14
Volatile phenols		
Guaicol	wood, smoked, sweet, medicine	0.03–5.0
4-vinylguaicol	wood, spice cloves, curry	0.5–23
Eugenol	spice cloves, honey	0.01–0.3

Isoeugenol	carmination	0.06–2.4
Cerulignol	spicy	0.04–2.2
Aldehydes		
Benzaldehyde	almonds, sweet, caramel	0.02–0.21
Nonenal	wax, citrus	0.03–0.47
Phenylacetaldehyde	green, grass, honey	0.05–4.5
Alcohols		
Phenylethyl alcohol	flowers, honey, pollen	0.01–2.26
Benzyl alcohol	roses, almond	0.05–0.13
Terpenols		
Camphor	mint	0–0.23
Borneol	pine tree	0–0.2
4-terpineol	spices, wood, soil	0.02–0.14
α-terpineol	flowers, lilac, sweet	0–0.2
Lactones		
γ-nonalactone	coconut, peach	0.03–0.11
Fatty acids		
Nonanoic acid	wax, dry, fatty	0.12–0.67
Vanillic acid	vanilla	0.07–0.86
Octanoic acid	coconut, lactic, rancid, cheese, sweat	0.14–3.38
Dodecanoic acid	coconut, fatty, metallic	0–6.3
Benceneacetic acid	honey, fruity, sour	0–3.0
Furans		
Furfural	caramel, candy	0–0.19

Figure 2(**a**) shows the sum of the total aromatic content of granulates and corks. In this figure, we can observe that the granulate samples extracted about 75% more than the cork stopper samples. Curiously, the highest amount of aromas were extracted in A granulate, corresponding to the largest particle size, followed by B granulate (medium size), and finally C granulate (smallest size). This could be due to the differences on the weight-volume relationship of granulates and their porosity, since the natural cork is a heterogeneous material with structural differences [23]. In this case, the smaller size of particles did not contribute to an increase of the extraction, likely because of differences in the volume of lenticels and dense matter [24]. Regarding cork stoppers, significant differences were not observed, A and B extracted a similar amount of aromas, slightly higher than that of C. Granulate samples (A and B) obtained the highest concentrations of total aromas after 5 days of maceration (Figure 2b), while for corks the maximum content was generally obtained at the end of the assay (after 15 days of extraction). In granulate samples, the highest amount of aromatic compounds may be reached earlier than in cork samples, likely because the characteristics of the samples and size facilitate the extraction of volatiles. In the studied samples, it seems that the bigger the granulates, the faster the extraction rate of the volatiles. The A granulates that correspond with the highest granulate size, mean value around 9.2 mm, obtained the highest amount of volatiles reaching levels over 200 μg/g. This increment in A granulate at 5 days is mainly due to a high extraction of vanillins and volatile phenols, and to a lesser extent to terpenols and fatty acids (Figure 3).

Considering the time of extraction in cork stoppers, only in the case of A corks was the maximum value of volatiles reached in 5 days of maceration, since for B and C corks, the maximum values were reached after 15 days of maceration (Figure 2).

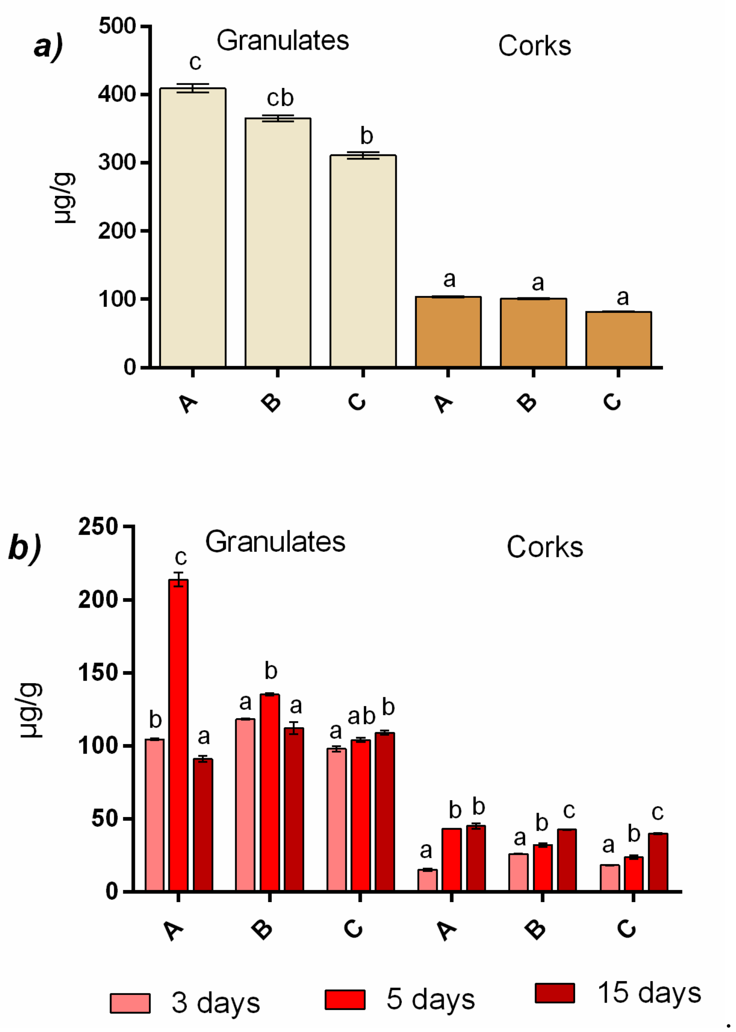

Figure 2. Total aromatic compounds (mean ± SD) of granulates and corks (**a**) and total aromatic compounds of granulates and corks at different time of extraction (**b**). Different particle size from cork stoppers and their respective granulates (A; high, B; medium, C; small). Different letters indicate significant differences, $p < 0.05$.

Figure 3 shows the total aromatic composition of the extracts per studied family. Vanillins are the most important family. In this study, this compound reached values from 9.3 µg/g (in C corks) to 167.9 µg/g (in A granulates, Table S2). Therefore, vanillin has a great impact on the overall aroma of cork extracts. This compound is very characteristic for providing a very intense and pleasant aroma with sweet and floral notes, which is currently used in many different industrial fields, such as in the production of fragrances, in food use as in baking [25], or in cosmetics [11]. In addition, as was seen in other studies [26,27], vanillin is a natural bioactive component present in corks, which showed an important antioxidant activity. Another important aromatic family of compounds studied in the food industry were volatile phenols [28,29]. As shown in Figure 3, the maximum content of these aromatic compounds was extracted after 5 days of maceration in A and B granulates. This pattern seems to be also reproduced in A and B cork samples. It may be due to the high concentration of vinylguaiacol (values up to 23 µg/g) and, to a lesser extent, to guaiacol content (5 µg/g). Both compounds are characteristic for having aromas of spicy notes, specifically clove, wood, and smoked [30,31].

Regarding aldehydes, it was observed that the granulates extracted the highest concentrations after 15 days of maceration with values up to five times higher with respect to the corks. The main compounds were phenylacetaldehyde with maximum values of 4.5 µg/g and nonenal with values of

0.47 μg/g. These compounds are characteristic for providing fresh and intense aromas even at low concentrations, such as green grass, citrus, and wax [32]. It should be noted that the odor threshold for phenylacetaldehyde in hydroalcoholic solution was established in 5 μg/L [33], and the maximum content found in this work corresponded to 135 μg/L (30 g of cork in 1 L). In this way, the maximum nonenal amount in these samples was 14.1 μg/L higher than the established odor threshold determined in water (0.065 μg/L) and in hydroalcoholic solution (0.17 μg/L) [34].

Lactones exhibited a behavior similar to that of aldehydes. The aroma of lactones is of interest for commercial aromatization of food [35]. Here, γ-nonalactone is the compound that represents this family. This compound is better extracted from granulates than from corks after 15 days of maceration. Despite presenting low concentrations (0.11 μg/g) in the extracts, its concentration is higher than the odor threshold in water (0.03 μg/g) [35]. This is a characteristic compound for providing pleasant aromatic descriptors, such as coconut, peach, and sweet cream butter notes [36,37]. This aroma is commonly used in the development of cosmetics and fragrances. In addition, they could also be used in the food industry as an essence in the preparation of cakes, sweets, candies, and ice cream, as margarine or aroma [36].

Similarly, the terpenols had a higher concentration in the macerates of granulates than in corks, especially after 15 days of extraction. The contribution of the individual terpenols seems to be similar, since little differences were observed among them. Their concentrations ranged from 0.05 (A cork after 3 days) to 0.62 μg/g (A granulate after 5 days) (table S2). Terpenes are aromatic compounds commonly synthesized in plants, trees, and vegetables. They are usually the main constituents of essential oils of most plants, offering a wide variety of pleasant scents, from flowery to fruity, to woody, or balsamic notes [38]. Hence, cork has a wide range of terpenoid variety within the family of terpenols formed in this study by the following determined compounds: camphor, borneol, α-terpineol, and 4-terpineol. All of them have interesting aromatic descriptors such as mint, pine, spices, and flowers, respectively. In addition, today they are used in cosmetics, especially in the elaboration of anti-aging creams, because they possess bioactive properties [39]. They may act as elastase inhibitors, preventing the structural degradation of elastin fibers in the dermal matrix [2,40,41], and play an important role as constituents of flavors for spicing foods, sweets, beverages, and baked foods [42]. Furfural, an important aromatic additive in food and beverages [43], also showed the highest concentrations after 15 days of maceration, being higher in granulates (5.7 μg/L) than in corks (1.5 μg/L). The odor threshold of this compounds is also lower (1 μg/L) [44] than the concentrations found in the studied samples, as occurred in other compounds.

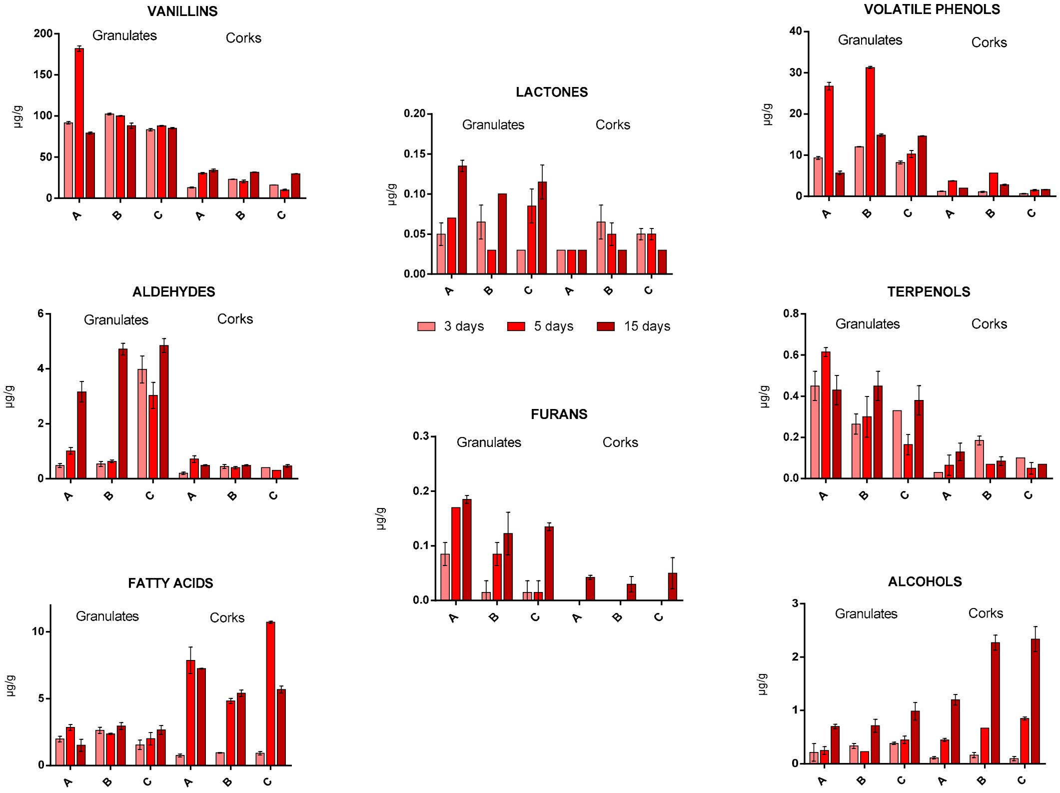

Figure 3. Composition per family of the studied aromatic compounds. Different particle size from cork stoppers and their respective granulates (A; high, B; medium, C; small).

On the other hand, alcohols and fatty acids showed higher concentrations in macerated cork stoppers than in granulates (Figure 3). With regard to alcohols, granulates and corks showed a large increase in concentration after 15 days of maceration, reaching values of up to 0.98 µg/g and 2.32 µg/g, respectively. Among them, the phenylethyl alcohol stands out for presenting the highest concentrations (Table S2), and for having interesting aromatic descriptors, such as flowers. For this reason, this compound is commonly used in the production of perfumes as well as flavoring in the food industry. It is also used in the cosmetic industry for the preparation of creams and soaps, acting as a preservative due to its stability in basic conditions. Another important property of this compound is its antimicrobial activity [45]. Fatty acids showed a large increase in concentration after 5 and 15 days of maceration in cork stoppers. This family reached a value of 10.74 µg/g. It should be noted that their concentrations were three times higher than in the case of granulates. The main fatty acids determined were vanillic, bezenacetic, decanoic, and octanoic, highlighting the latter for having the highest concentrations. All of them contributed to providing very pleasant aromatic notes such as vanilla, honey, lactic, and coconut, respectively.

The great increase in the concentration of phenylethyl alcohol and octanoic acid in the macerates of corks with respect to granulates could be due to the presence of this compound in glues used for the manufacture of agglomerated corks [46]. Their use is due to their aromatic, antimicrobial, and antibiotic properties.

In summary, the families studied provide the aromatic profile of the granules and cork stoppers, which are rich in very pleasant aromas at the sensory level. This aromatic composition extracted from cork could have a second shelf life in different types of industries. On the one hand, the cosmetic and pharmaceutical industries could use these extracted compounds as ingredients in the manufacture of products such as sunscreens, wrinkle products, fragrances, or even soaps. On the other, they could be also used in processed food as flavoring additive, and it should be noted their bioactive properties and beneficial health effects, such as antioxidant and antimicrobial activities.

3.2. Phenolic Composition

Many studies have used the Folin–Ciocalteu method to determine polyphenols in plant extracts [47,48]. The combination of HPLC–DAD/MS analysis with this methodology helps the determination of the compounds, from the individual to the polymeric polyphenols. Figure 4 shows the Folin–Ciocalteu index (Figure 4a,b) and the content in the phenolic compounds in the studied cork samples determined by HPLC (Figure 4c,d).

In general, higher polyphenol content was obtained for granulates than for corks. A and B granulates obtained the highest value after three days of maceration (Figure 4a). As shown in Figure 4c,d, in general, a higher concentration of phenolics was extracted after 15 days of maceration. In the case of granulates, it is observed that A and B granulates had concentrations above 150 µg/g after 3 and 5 days of maceration, and increased to concentrations around 500 µg/g after 15 days. The highest concentration was obtained in A granulate (513.5 µg/g), with larger particle size. In C granulate (smaller size), the extraction was more constant, around 350–400 µg/g for all maceration times. After 15 days of maceration, the phenolic composition in cork stoppers increased in all cases from 10 µg/g to 65 µg/gin the C sample. Other authors observed differences in the phenolic extraction depending on the type of cork stopper, granulate, and powder [12,20,49]. Further research should be done to optimize time of extraction and methods. Furthermore, the use of new promising technologies may be interesting in order to optimize the process in a real scale [50].

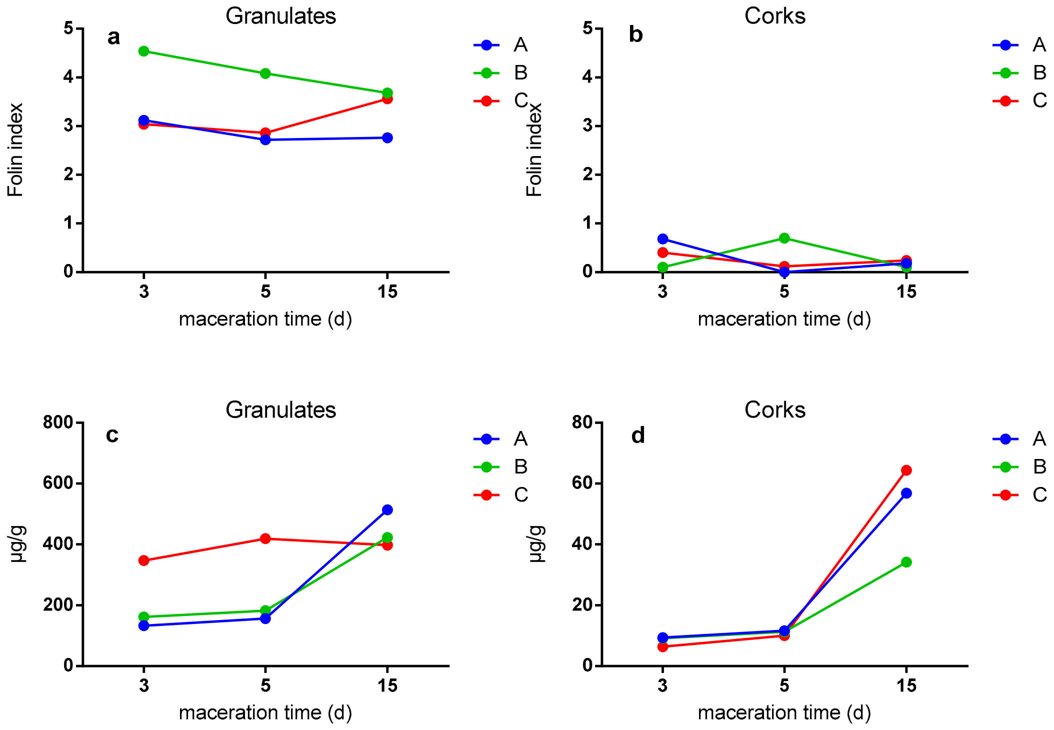

Figure 4. Folin–Ciocalteu index (**a** and **b**) and phenolic content determined by HPLC (**c** and **d**; note difference in *y*-axis scales). Particle size from cork stoppers and their respective granulates, A; high, B; medium, C; small.

Considering the individual content of the phenolic compounds , gallic acid obtained the highest concentrations with values between 60.6 and 180.9 µg/g, followed by protocatechuic acid with values between 41.3 and 161.6 µg/g, and finally the protocatechuic aldehyde with values between 26.3 and

118.6 µg/g. As stated in previous studies [51,52], gallic acid and protocatechuic acid are phenolic compounds with abundant presence in cork extractive fractions.

3.2. Antioxidant Activity

Previous studies have already shown that the cork has bioactive compounds with antioxidant activity [2,53]. Touati et al. studied cork extracts in methanol solution and water using the ABTS method, demonstrating its high antioxidant activity, and Fernandes et al. characterized the great antioxidant activity of cork extracts, which was directly related to the phenolic composition [15,54].

Figure 5 shows the antioxidant activity in µmol/L of Trolox of the granules and corks throughout the maceration time. In general, it is observed that granulate samples obtained higher antioxidant activity than corks (up to one hundred times). Figure 5A shows that B and C granulates had higher antioxidant activity compared with the larger A granulate. Likewise, the three types of granulates obtained similar antioxidant activity after all maceration times, as reported Azevedo and co-workers, who observed no significant differences in wine model solution at different times when bottling with different cork stoppers [20]. With respect to the corks (Figure 5B), in general, the same trend is observed in all types of corks. In this case, the antioxidant activity increases by increasing the maceration time. The differences observed between samples after 15 days of maceration may result from the higher amount of simpler phenolic compounds at this time (Figure 4d). The maceration of A cork (larger granulate) presented the highest concentration at all times, especially after 15 days of maceration. On the one hand, this trend is not explained from the obtained data, so this could be due to hydrolyzable tannins that are powerful antioxidant agents and were not determined in this study [15].

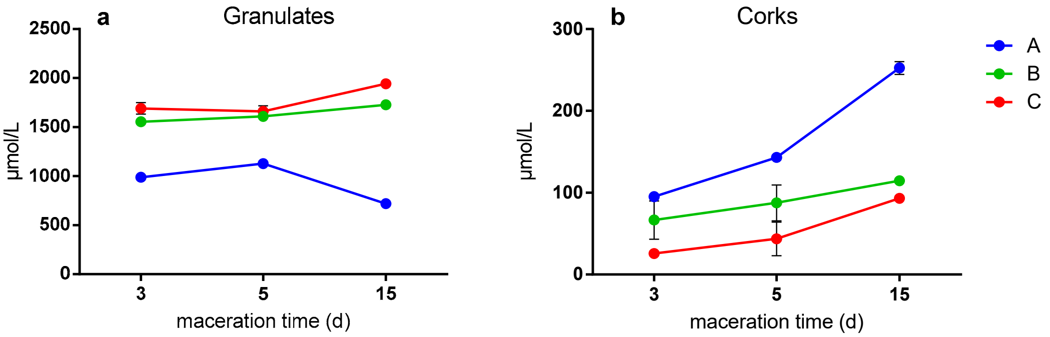

Figure 5. Antioxidant activity of granulates (**a**) and cork (**b**) samples determined by ABTS method (expressed in µmol/L of Trolox reagent), note difference in *y*-axis scales. Particle size from cork stoppers and their respective granulates, A; high, B; medium, C; small.

On the other hand, the high antioxidant capacity of the cork samples studied, especially in the case of granulates, could be directly related to phenolic acids, in particular the high concentrations of gallic acid and protocacatechuic acid [26,54]. In addition, this antioxidant capacity could also be due to the aromatic composition of the corks studied, especially to the high concentrations of vanillin (170 µg/g), which is considered a natural bioactive component of cork [15,55]. According to Azevedo et al., the amounts of gallic acid, protocatechuic acid, protocatechuic aldehyde, and vanillin in cork samples appear to be crucial for the antioxidant activity [20].

4. Conclusions

Overall, this work highlights valuable aromatic compounds (vanillins, volatile phenols, aldehydes, alcohols, terpenols, lactones, fatty acids, and furans) found in cork by-products and cork stoppers to be reused as flavoring agents and antioxidants in the food industry. The most important family of compounds was vanillins with a high content of vanillin and acetovanillone, and 4-vinylguaicol was the most abundant volatile phenol. Furthermore, the phenolic composition of corks and their by-products may provide interesting antioxidant properties with increasing interest on the industry for their health benefits. Granulates showed higher potential than corks, however, corks used daily (e.g., wine stoppers) have to be considered for this purpose. Further research should be done in order to optimize waste management and extraction procedures, among others.

Supplementary Materials: Table S1: Size of cork granulates (mm) used in this study (*n* = 20), Table S2: Individual aromatic composition in the studied granulates and corks at 3, 5, and 15 days of maceration.

Author Contributions: A.M.M performed the experiment and participated on the manuscript redaction and edition. M.P. designed the experiment and reviewed the manuscript. R.F.G. participated on the experiment analysis, redaction and edition. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest

References

1. Gil, L. Cork powder waste: An overview. *Biomass Bioenerg.* **1997**, *13*, 59–61.
2. Carriço, C.; Ribeiro, H.M.; Marto, J. Converting cork by-products to ecofriendly cork bioactive ingredients: Novel pharmaceutical and cosmetics applications. *Ind. Crop. Prod.* **2018**, *125*, 72–84.
3. Borrero, C.; Castillo, S.; Casanova, E.; Segarra, G.; Trillas, M.I.; Castaño, R.; Avilés, M. Capacity of composts made from agriculture industry residues to suppress different plant diseases. *Act. Hortic.* **2013**, *1013*, 459–464.
4. Carmona, E.; Ordovás, J.; Moreno, M.T.; Avilés, M.; Aguado, M.T.; Ortega, M.C. Hydrological Properties of Cork Container Media. *HortScience* **2003**, *38*, 1235–1241.
5. Filipe-Ribeiro, L.; Cosme, F.; Nunes, F.M. A Simple Method To Improve Cork Powder Waste Adsorption Properties: Valorization as a New Sustainable Wine Fining Agent. *ACS Sustain. Chem. Eng.* **2019**, *7*, 1105–1112.
6. Silva, B.; Martins, M.; Rosca, M.; Rocha, V.; Lago, A.; Neves, I.C.; Tavares, T. Waste-based biosorbents as cost-effective alternatives to commercial adsorbents for the retention of fluoxetine from water. *Sep. Purif. Technol.* **2020**, *235*, 116139.
7. Pintor, A.M.A.; Vieira, B.R.C.; Boaventura, R.A.R.; Botelho, C.M.S. Removal of antimony from water by iron-coated cork granulates. *Sep. Purif. Technol.* **2020**, *233*, 116020.
8. Pirozzi, C.; Pontoni, L.; Fabbricino, M.; Bogush, A.; Campos, L.C. Effect of organic matter release from natural cork used on bisphenol a removal from aqueous solution. *J. Clean. Prod.* **2020**, *244*, 118675.
9. Olivella, M.À.; Jové, P.; Oliveras, A. The use of cork waste as a biosorbent for persistent organic pollutants-Study of adsorption/desorption of polycyclic aromatic hydrocarbons. *J. Environ. Sci. Health., Part A* **2011**, *46*, 824–832.
10. OIV. *2019 Statistical Report on World Vitiviniculture*; International Organization of Vine and Wine: Paris, France, 2019; p 13.
11. Aroso, I.M.; Araújo, A.R.; Pires, R.A.; Reis, R.L. Cork: Current Technological Developments and Future Perspectives for this Natural, Renewable, and Sustainable Material. *ACS Sustain. Chem. Eng.* **2017**, *5*, 11130–11146.
12. Reis, S.F.; Lopes, P.; Roseira, I.; Cabral, M.; Mateus, N.; Freitas, V. Recovery of added value compounds from cork industry by-products. *Ind. Crop. Prod.* **2019**, *140*, 111599.
13. Mark, R.; Lyu, X.; Lee, J.J.L.; Parra-Saldívar, R.; Chen, W.N. Sustainable production of natural phenolics for functional food applications. *J. Funct. Foods* **2019**, *57*, 233–254.
14. Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds - Nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.* **2000**, *80*, 1094–1117.

15. Fernandes, A.; Fernandes, I.; Cruz, L.; Mateus, N.; Cabral, M.; de Freitas, V. Antioxidant and Biological Properties of Bioactive Phenolic Compounds from Quercus suber L. *J. Agric. Food. Chem.* **2009**, *57*, 11154–11160.
16. Fontana, A.R. Analytical methods for determination of cork-taint compounds in wine. *TrAC, Trends Anal. Chem.* **2012**, *37*, 135–147.
17. Garcia, A.R.; Lopes, L.F.; Barros, R.B.D.; Ilharco, L.M. The problem of 2,4,6-trichloroanisole in cork planks studied by attenuated total reflection infrared spectroscopy: Proof of concept. *J. Agric. Food. Chem.* **2015**, *63*, 128–135.
18. Mayr, C.M.; Capone, D.L.; Pardon, K.H.; Black, C.A.; Pomeroy, D.; Francis, I.L. Quantitative Analysis by GC-MS/MS of 18 Aroma Compounds Related to Oxidative Off-Flavor in Wines. *J. Agric. Food. Chem.* **2015**, *63*, 3394–3401.
19. Tarasov, A.; Rauhut, D.; Jung, R. “Cork taint” responsible compounds. Determination of haloanisoles and halophenols in cork matrix: A review. *Talanta* **2017**, *175*, 82–92.
20. Azevedo, J.; Fernandes, I.; Lopes, P.; Roseira, I.; Cabral, M.; Mateus, N.; Freitas, V. Migration of phenolic compounds from different cork stoppers to wine model solutions: antioxidant and biological relevance. *Eur. Food Res. Technol.* **2014**, *239*, 951–960.
21. OIV. *Compendium of International Methods of Analysis*; International Organization of Vine and Wine: Paris, France, 2019
22. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio. Med.* **1999**, *26*, 1231–1237.
23. Oliveira, V.; Knapic, S.; Pereira, H. Natural variability of surface porosity of wine cork stoppers of different commercial classes. *J. Int. Sci. Vigne Vin.* **2012**, *46*, 331–340.
24. Crouvisier-Urion, K.; Chanut, J.; Lagorce, A.; Winckler, P.; Wang, Z.; Verboven, P.; Nicolai, B.; Lherminier, J.; Ferret, E.; Gougeon, R.D., et al. Four hundred years of cork imaging: New advances in the characterization of the cork structure. *Sci. Rep.* **2019**, *9*, 19682.
25. Ciriminna, R.; Fidalgo, A.; Meneguzzo, F.; Parrino, F.; Ilharco, L.M.; Pagliaro, M. Vanillin: The Case for Greener Production Driven by Sustainability Megatrend. *ChemistryOpen* **2019**, *8*, 660–667.
26. Rodrigues, F.; Pimentel, F.B.; Oliveira, M.B.P.P. Olive by-products: Challenge application in cosmetic industry. *Ind. Crop. Prod.* **2015**, *70*, 116–124.
27. Silva, S.P., Sabino, M. A., Fernandes, E.M., Correlo, V.M., Boesel, L.F., Reis, R.L. Cork: properties, capabilities and applications. *Int. Mater. Rev.* **2005**, *50*, 345–365.
28. Salgado, J.M.; Rodríguez-Solana, R.; Curiel, J.A.; Rivas, B.d.l.; Muñoz, R.; Domínguez, J.M. Production of vinyl derivatives from alkaline hydrolysates of corn cobs by recombinant Escherichia coli containing the phenolic acid decarboxylase from Lactobacillus plantarum CECT 748T. *Bioresour. Technol.* **2012**, *117*, 274–285.
29. Sharma, U.K.; Sharma, N.; Salwan, R.; Kumar, R.; Kasana, R.C.; Sinha, A.K. Efficient synthesis of hydroxystyrenes via biocatalytic decarboxylation/deacetylation of substituted cinnamic acids by newly isolated Pantoea agglomerans strains. *J. Sci. Food Agric.* **2012**, *92*, 610–617.
30. Culleré, L.; Cacho, J.; Ferreira, V. Comparative study of the aromatic profile of different kinds of wine cork stoppers. *Food Chem.* **2009**, *112*, 381–387.
31. Francis, I.L.; Newton, J.L. Determining wine aroma from compositional data. *Aus. J. Grape Wine R.* **2005**, *11*, 114–126.
32. Rocha, S.; Delgadillo, I.; Ferrer Correia, A.J. GC−MS Study of Volatiles of Normal and Microbiologically Attacked Cork from Quercus suber L. *J. Agric. Food. Chem.* **1996**, *44*, 865–871.
33. Ferreira, V.; López, R.; Cacho, J.F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80*, 1659–1667.
34. Chatonnet, P.; Dubourdieu, D. Identification of substances responsible for the ‘sawdust’ aroma in oak wood. *J. Sci. Food Agric.* **1998**, *76*, 179–188.
35. Belitz, H.-D.; Grosch, W.; Schieberle, P. *Food Chemistry*, 4th ed.; Springer-Verlag: Berlin Heidelberg, Germany, 2009.
36. Dadalı, C.; Elmacı, Y. Optimization of Headspace-Solid Phase Microextraction (HS-SPME) technique for the analysis of volatile compounds of margarine. *J. Food Sci. Technol.* **2019**, *56*, 4834–4843.
37. Mosandl, A.; Günther, C. Stereoisomeric Flavor Compounds. 20. Structure and Properties of γ-Lactone Enantiomers. *J. Agric. Food. Chem.* **1989**, *37*, 413–418.

38. Lorenzo, C.; Eugenio, A. Use of Terpenoids as Natural Flavouring Compounds in Food Industry. *Recent Pat. Food. Nutr.* **2011**, *3*, 9–16.
39. Brahmkshatriya, P.P.; Brahmkshatriya, P.S. Terpenes: Chemistry, biological role, and therapeutic applications. In *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes*; Springer, Berlin, Heidelberg, Germany, 2013; pp. 2665–2691.
40. Karim, A.A.; Azlan, A.; Ismail, A.; Hashim, P.; Abd Gani, S.S.; Zainudin, B.H.; Abdullah, N.A. Phenolic composition, antioxidant, anti-wrinkles and tyrosinase inhibitory activities of cocoa pod extract. *BMC Complem. Altern. M.* **2014**, *14*, 381.
41. Mukherjee, P.K.; Maity, N.; Nema, N.K.; Sarkar, B.K. Bioactive compounds from natural resources against skin aging. *Phytomedicine* **2011**, *19*, 64–73. Eberhard
42. Breitmaier, E. *Terpenes: Flavors, Fragrances, Pharmaca, Pheromones*, Wiley-VCH Verlag GmbH & Co. KGaA:Weinheim, Germany, 2006; pp. 1–214.
43. Hidalgo-Carrillo, J.; Marinas, A.; Urbano, F.J. Chemistry of Furfural and Furanic Derivatives. In *Furfural*; World Scientific (Europe): London, UK, 2017; Vol. 2, pp. 1–30.
44. Gómez-Míguez, M.J.; Cacho, J.F.; Ferreira, V.; Vicario, I.M.; Heredia, F.J. Volatile components of Zalema white wines. *Food Chem.* **2007**, *100*, 1464–1473.
45. Ren, G.; Xue, P.; Sun, X.; Zhao, G. Determination of the volatile and polyphenol constituents and the antimicrobial, antioxidant, and tyrosinase inhibitory activities of the bioactive compounds from the by-product of Rosa rugosa Thunb. var. plena Regal tea. *BMC Complem. Altern. M.* **2018**, *18*, 307.
46. Crouvisier-Urion, K.; Bellat, J.P.; Gougeon, R.D.; Karbowiak, T. Mechanical properties of agglomerated cork stoppers for sparkling wines: Influence of adhesive and cork particle size. *Compos. Struct.* **2018**, *203*, 789–796.
47. Blainski, A.; Lopes, G.C.; De Mello, J.C.P. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from limonium brasiliense L. *Molecules* **2013**, *18*, 6852–6865.
48. Cicco, N.; Lanorte, M.T.; Paraggio, M.; Viggiano, M.; Lattanzio, V. A reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining phenolics of plant methanol extracts. *Microchem. J.* **2009**, *91*, 107–110.
49. Fernandes, A.; Sousa, A.; Mateus, N.; Cabral, M.; de Freitas, V. Analysis of phenolic compounds in cork from Quercus suber L. by HPLC–DAD/ESI–MS. *Food Chem.* **2011**, *125*, 1398–1405.
50. Albanese, L.; Bonetti, A.; D'Acqui, L.P.; Meneguzzo, F.; Zabini, F. Affordable production of antioxidant aqueous solutions by hydrodynamic cavitation processing of silver fir (*Abies alba* Mill.) needles. *Foods* **2019**, *8*, 65.
51. Santos, S.A.O.; Pinto, P.C.R.O.; Silvestre, A.J.D.; Neto, C.P. Chemical composition and antioxidant activity of phenolic extracts of cork from *Quercus suber* L. *Ind. Crop. Prod.* **2010**, *31*, 521–526.
52. Santos, S.A.O.; Villaverde, J.J.; Sousa, A.F.; Coelho, J.F.J.; Neto, C.P.; Silvestre, A.J.D. Phenolic composition and antioxidant activity of industrial cork by-products. *Ind. Crop. Prod.* **2013**, *47*, 262–269.
53. Igueld, S.B., Hatem, A., Trabelsi-Ayadi, M., Cherif, J. K. Study of Physicochemicals Characteristics and Antioxidant Capacity of Cork Oak Acorns (*Quercus Suber* L.) grown in Three Regions in Tunisia. *J. App. Pharm. Sci.* **2015**, *5*, 26–32.
54. Touati, R.; Santos, S.A.O.; Rocha, S.M.; Belhamel, K.; Silvestre, A.J.D. The potential of cork from Quercus suber L. grown in Algeria as a source of bioactive lipophilic and phenolic compounds. *Ind. Crop. Prod.* **2015**, *76*, 936–945.
55. Anand, A.; Khurana, R.; Wahal, N.; Mahajan, S.; Mehta, M.; Satija, S.; Sharma, N.; Vyas, M.; Khurana, N. Vanillin: A comprehensive review of pharmacological activities. *Plant Arch.* **2019**, *19*, 1000–1004.

Discusión general

En esta tesis doctoral se ha estudiado el efecto de diferentes procesos productivos sobre la calidad y composición química del vino. Para ello se ha tenido en cuenta tanto la materia prima (la uva) como los diferentes procesos empleados para su vinificación. Para dar una visión más global del estudio también se estudiaron las etapas de embotellado, crianza y almacenamiento. Se ha realizado un estudio exhaustivo de la composición físico-química de diferentes tipos de vino, fundamentalmente sobre la composición aromática y fenólica. Además, se ha evaluado su impacto en la estabilidad y en las características organolépticas.

El primer estudio se realizó alineándonos con las investigaciones más actuales y de gran importancia ecológica que tratan sobre el desarrollo y empleo de nuevos tratamientos en campo como alternativas sostenibles que no alteren la calidad final de la uva. Concretamente, en el estudio del **artículo 1** se observó como el uso de nuevos fertilizantes en viñedo elaborados con elicitores a base de aminoácidos de origen vegetal presentaban mejores resultados sobre la calidad final de la uva que los elaborados con aminoácidos de origen animal. Esta mejora de la calidad de la uva se vio reflejada al obtener una uva más rica en el contenido de aminoácidos y en compuestos aromáticos. Los resultados indicaron que los tratamientos con elicitores enriquecidos tanto con aminoácidos de origen animal y vegetal conseguían mejorar la asimilación y el transporte de los aminoácidos a las bayas y potenciaban la calidad del perfil aromático de los mostos, especialmente en el caso de los aminoácidos de origen vegetal. Los resultados obtenidos sugieren una posibilidad de sustitución de los aminoácidos de origen animal por aminoácidos de origen vegetal como alternativa sostenible.

A continuación, el siguiente capítulo se llevó a cabo teniendo en cuenta que, durante la etapa de vinificación es necesario un control de los diferentes procesos tecnológicos empleados en bodega lo cual implica el realizar una correcta elección de productos enológicos, condiciones de trabajo y manejo de las técnicas, ya que todas ellas repercutirán en la calidad final del vino. Uno de los procesos clave en la vinificación es la fermentación y la elección de la levadura. Actualmente hay muchas investigaciones sobre el uso de levaduras no-*Saccharomyces* debido a la posible obtención de un vino con características químicas y organolépticas diferenciadoras a los elaborados únicamente con *Saccharomyces.* En el **artículo 2** de esta tesis, se observó cómo los vinos espumosos elaborados con adición de levadura no-*Saccharomyces* presentaban características espumantes y aromáticas de gran calidad. Los resultados relacionados con el contenido de proteínas y la espumabilidad mostraron una relación directa entre ambos parámetros, con una mejor persistencia de la espuma lograda en algunas fermentaciones con no-*Saccharomyces*. Los vinos base elaborados con *M. pulcherrima* mostraron un alto contenido proteico, mejorando la espumabilidad y persistencia de la espuma. Además, los resultados de la composición aromática y el análisis sensorial mostraron que el uso de *T.*

delbrueckii en la primera fermentación puede producir cavas más aromáticos, proporcionando notas aromáticas afrutadas, especialmente de manzana verde fresca.

Otro proceso muy importante durante la vinificación es la estabilización y acidificación de los vinos. Actualmente se buscan nuevas tendencias para la estabilización y acidificación de los vinos las cuales sean más innovadoras, más sostenibles medioambientalmente, con menor consumo energético, más económicas, con un bajo consumo de tiempo en su aplicación y con la finalidad de no alterar la calidad del vino final. Una de las técnicas empleadas para la estabilización y acidificación de los vinos son las resinas de intercambio catiónico tal y como se mostró en el **artículo 3** de esta tesis. Concretamente, en este estudio se comprobó que, los vinos tratados con vino permeado (pasados por las resinas de intercambio catiónico) especialmente en aquellos con un 20 % de vino tratado, presentaron una mayor acidez, color y estabilidad tartárica y un menor contenido de minerales respecto a los vinos sin tratar. Además, durante todo el tiempo de envejecimiento en barrica destacó la disminución de cationes de potasio y magnesio y sobre todo la conservación o aumento de ciertos compuestos aromáticos como el acetato de hexilo, isobutanol, alcohol 2-feniletílico, isovalerato de etilo y succinato de dietilo, principalmente en los vinos tratados y después de seis meses de envejecimiento.

El capítulo 3 se encuadra en los procesos que tienen lugar después de la vinificación y que son las etapas del embotellado, envejecimiento y almacenamiento del vino en botella hasta llegar al consumidor. En general, a lo largo de estas etapas los vinos pueden sufrir grandes cambios en su composición físico-química, composición aromática y organoléptica, los cuales pueden provocar en este periodo un mayor afinamiento y una mejor complejidad del vino, o por el contrario pueden aparecer defectos organolépticos por una incorrecta evolución. Por ello, dentro de los factores influyentes a tener en cuenta encontramos, por un lado, los que rodean y protegen directamente al vino, el tipo de botella y el cierre, y por otro lado, los que externamente pueden influir sobre la calidad del vino, como la exposición a la luz, la temperatura o el oxígeno. Hoy en día, el factor exposición a la luz todavía constituye uno de los grandes retos a los que se enfrenta el sector enológico. Los estudios enfocados por encontrar nuevas tecnologías LED que no impliquen un deterioro visual y aromático de los vinos embotellados son fundamentales para seguir avanzando en este campo de estudio. Los resultados del **artículo 4** de la tesis, mostraron que comparando las nuevas tecnologías LED con las lámparas UV-visible tradicionales, los vinos expuestos a la luz usual (UV-visible) sufrían una mayor degradación de los precursores aromáticos, tanto de la metionina y de la cisteína pero principalmente de la riboflavina y presentaban mayores concentraciones de compuestos volátiles azufrados responsables de aportar aromas desagradables a los vinos. En general, las nuevas tecnologías LED probadas en este estudio producían efectos de degradación mínimos.

Por tanto, se podrían considerar como una alternativa de interés capaz de reducir el impacto económico que se produce actualmente por la fotodegradación de los vinos.

Otro de los factores clave en la conservación del vino embotellado es la elección del tipo de cierre y del color de la botella. Una incorrecta elección de ambos y su combinación con la exposición a la luz puede provocar una importante pérdida de calidad en el vino. Los resultados del **artículo 5** demostraron que estos factores influencian claramente la calidad de los vinos de la variedad Albariño. Las botellas de color ámbar y el tapón de rosca mostraron buenos resultados y parecen conservan mejor los aromas fermentativos afrutados y florales en los vinos expuestos a la luz pero, en cambio, los vinos embotellados en vidrio transparente y taponados con corcho natural aumentaron el nivel de aromas oxidativos y fueron los más evolucionados sensorialmente. Por otra parte, dentro de los diferentes tipos de cierres de botellas está el tapón de corcho como el cierre más empleado en el embotellado del vino. Uno de los factores clave del tapón de corcho es su permeabilidad la cual permite que cierta cantidad de oxigeno se transmita al vino a través de la porosidad del tapón de corcho durante el periodo de su almacenamiento y que se mide con el parámetro denominado velocidad de transmisión de oxigeno (OTR, oxygen transfer rate). Con estas distintas transferencias de oxígeno, el vino va evolucionando de manera distinta dependiendo del tipo de vino y del tipo de corcho, lo que implica una mejor o peor conservación a lo largo del tiempo. Los resultados del **artículo 6** mostraron la influencia de diferentes tipos de corchos con distintas OTRs en la calidad y composición del Champagne durante un año de almacenamiento. La dosis de sulfuroso añadida en el momento de embotellado después del tiraje también se mostró un factor de gran influencia sobre la composición aromática del Champagne. Concretamente, a mayor tiempo de almacenamiento y sin adición de sulfuroso (0 mg/L) los Champagnes presentaron mayores concentraciones de los compuestos oxidativos, presentando los mayores valores de 2-metilpropanal y ɣ-nonalactona. Por otro lado, los compuestos de reducción comenzaron a detectarse después de tres meses de almacenamiento y aumentaron con el paso del tiempo principalmente en los Champagnes con las mayores dosis de sulfuroso (20 mg/L). Respecto a los tapones, se observó que aquellos que presentaron valores de OTR intermedios mantenían un mejor equilibrio entre los aromas oxidativos y de reducción a lo largo tiempo de almacenamiento en botella conservando notas aromáticas más frescas y frutales.

Independientemente de los factores que rodean al vino embotellado durante su almacenamiento, es muy importante tener en cuenta el tipo o estilo de vino que se ha elaborado a la hora de llevar a cabo el desarrollo de su envejecimiento en botella. Los vinos tintos elaborados para largas crianzas pueden desarrollar una mayor complejidad organoléptica durante la etapa de envejecimiento. Contrariamente, una incorrecta evolución puede llevar a producir cambios en el perfil aromático de los vinos tintos durante el período de crianza afectando sin duda a su calidad final. Así pues, tal y como muestran los resultados del **artículo 7**, la composición volátil de los vinos de Tempranillo, estudiados después del proceso de oxidación semi-forzada, produjeron una pérdida del 40 % de los aromas fermentativos y un aumento del 85 % de los aromas oxidativos. Los compuestos de oxidación alcanzaron los umbrales de percepción con los valores de actividad de olor (OAV, odor activity value) por encima de la unidad después del proceso de oxidación semi-forzada indicando su contribución en la percepción aromática del vino. Principalmente se determinaron como los marcadores de oxidación semi-forzada el 2-metilpropanal y el sotolon. La 3-metil-2,4-nonanediona, el 2-fenilacetaldehído y en menor medida el 3-metilbutanal y el (E)-2-nonenal fueron determinados como compuestos volátiles de gran importancia en el perfil aromático de vinos de alta calidad de Tempranillo con larga crianza en botella.

Finalmente, para tener una visión realmente global de todo el proceso de producción también se debería de plantear un reaprovechamiento de los subproductos derivados. En el caso del residuo procedente de los corchos, cabe resaltar su importancia debido a las propiedades bioactivas y aromáticas, las cuales podrían ser reutilizadas en la industria. Los resultados obtenidos en el **artículo 8** mostraron que los subproductos de corcho, tanto en forma de tapones como de granulados, presentaban gran cantidad de compuestos aromáticos agradables a nivel sensorial, siendo las vainillinas y los fenoles volátiles las familias mayoritarias. Esta composición aromática extraída del corcho podría tener una nueva aplicación en diferentes tipos de industrias lo cual proporcionaría una segunda vida útil al corcho. Las industrias alimentaria, cosmética y farmacéutica podrían utilizar estos compuestos extraídos como ingredientes en la fabricación de productos como protectores solares, productos antiarrugas, fragancias o incluso jabones. La alta capacidad antioxidante de las muestras de corcho estudiadas, especialmente en el caso de granulados, podría estar directamente relacionada con los ácidos fenólicos, en particular las altas concentraciones de ácido gálico y ácido protocatéquico. Esta capacidad antioxidante también puede proceder de los compuestos aromáticos, especialmente de las altas concentraciones de vainillina, la cual ha demostrado ser un componente bioactivo natural del corcho.

Conclusiones

Las principales conclusiones que se derivan de esta tesis son**:**

- El empleo de bioestimulantes (elicitor MSB (menadiona sodio bisulfito)) enriquecidos con aminoácidos mejoran la asimilación de micro y macronutrientes de la planta de vid, favorecen su desarrollo y aumentan el potencial aromático de la uva.
- Los bioestimulantes enriquecidos con aminoácidos vegetales pueden ser una alternativa adecuada y sostenible para la fertilización del viñedo.
- El uso de *M. pulcherrima* y *T. delbrueckii* en la fermentación del vino base para la elaboración de cava es una alternativa de interés para obtener perfiles aromáticos diferenciadores y mejorar la calidad de la espuma.
- Los vinos elaborados mediante mezclas de vinos tratados con columnas de intercambio catiónico conservan o incluso mejoran sus características físico-químicas, aromáticas y sensoriales durante el período de crianza. Especialmente, las mezclas de vinos con 10 % y 20 % de vino permeado presentaron concentraciones más altas en algunos de los compuestos responsables de los descriptores aromáticos relacionados con aromas de frutas y flores.
- Las luces LED son una alternativa sostenible para reducir el impacto económico derivado de la pérdida de calidad de los vinos por la fotodegradación.
- Las nuevas luces LED sin emisión del rango de longitud de onda entre 400-450, son capaces de evitar por completo la aparición de aromas desagradables del vino, relacionados con compuestos volátiles azufrados.
- El tiempo de conservación en botella, la exposición a la luz, el tipo de cierre y finalmente el color de la botella son factores con una gran influencia en la composición aromática en los vinos de Albariño.
- Los vinos embotellados de Albariño y expuestos a la luz conservan mejor sus cualidades a lo largo del tiempo en botella de color ámbar y con cierre de rosca.
- El tiempo de almacenamiento y la adición de SO_2 en el momento del embotellado son los factores a considerar para conservar la calidad aromática del Champagne durante su almacenamiento después del tiraje. Dosis más altas de SO_2 (20 mg/L) favorecieron la formación de compuestos de reducción mientras que en muestras sin adición de sulfuroso se favoreció la formación de compuestos oxidativos.
- La permeabilidad de los tapones de corcho tiene una gran influencia en la composición aromática durante la conservación del Champagne a lo largo del tiempo de almacenamiento después del tiraje.

- La composición aromática de los vinos con largas crianza de Tempranillo nos aporta información de interés sobre su correcta evolución gracias a la detección de diferentes marcadores de oxidación.
- Los subproductos del corcho en forma de tapones y sus granulados poseen un valioso contenido de compuestos fenólicos y aromáticos de interés para la industria.

Referencias

1. Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D. Handbook of Enology, 2nd edJohn Wiley & Sons: Chichester, 2006. pp 1-441.
2. Conde C, Silva P, Fontes N, Dias A, Tavares R, Sousa M, et al. Biochemical changes throughout Grape Berry development and fruit and wine quality. Global Science Books, 2007. pp 1-22.
3. Hidalgo Togores José. Tratado De Enología, Segunda edición.; Mundi-Prensa: Madrid, 2011; Vol. Tomo I.
4. Pinelo M, Arnous A, Meyer AS. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. Trends in Food Science and Technology. 2006; 17(11): 579-90.
5. Lund ST, Bohlmann J. The molecular basis for wine grape quality - A volatile subject. Science. 2006; 311(5762): 804-5.
6. Waterhouse AL. Wine phenolics. Annals of the New York Academy of Sciences 2002. p. 21-36.
7. Crozier A, Clifford MN, Ashihara H. Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Die, 2007. pp 1-372.
8. Kennedy JA, Matthews MA, Waterhouse AL. Changes in grape seed polyphenols during fruit ripening. Phytochemistry. 2000;55(1):77-85.
9. Vidal S, Francis L, Guyot S, Marnet N, Kwiatkowski M, Gawel R, et al. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. Journal of the Science of Food and Agriculture. 2003;83(6):564-73.
10. Togores José Hidalgo. *La Calidad Del Vino Desde El Viñedo*; Mundi-Prensa: España, 2008.
11. Ramey D, Bertrand A, Ough C, Singleton VL, Sanders E. Effects of Skin Contact Temperature on Chardonnay Must and Wine Composition. American Journal of Enology and Viticulture. 1986;37:99-106.
12. Fenoll J, Manso A, Hellin P, Ruiz-García L, Flores P. Changes in the aromatic composition of the Vitis vinifera grape Muscat Hamburg during ripening. Food Chemistry. 2009; 114: 420-8.
13. Luan F, Wüst M. Differential incorporation of 1-deoxy-D-xylulose into (3S)-linalool and geraniol in grape berry exocarp and mesocarp. Phytochemistry. 2002;60(5):451-9.
14. Ribéreau-Gayon P, Boidron JN, Terrier A. Aroma of Muscat Grape Varieties. Journal of Agricultural and Food Chemistry. 1975;23(6):1042-7.
15. Park SK, Morrison JC, Adams DO, Noble AC. Distribution of free and glycosidically bound monoterpenes in the skin and mesocarp of Muscat of Alexandria grapes during development. Journal of Agricultural and Food Chemistry. 1991;39(3):514-8.
16. Razungles AJ, Babic I, Sapis JC, Bayonove CL. Particular Behavior of Epoxy Xanthophylls during Veraison and Maturation of Grape. Journal of Agricultural and Food Chemistry. 1996;44(12):3821-5.

17. Winterhalter P, Sefton MA, Williams PJ. Volatile C₁₃-Norisoprenoid Compounds in Riesling Wine Are Generated From Multiple Precursors. American Journal of Enology and Viticulture. 1990;41(4):277-83.

18. Delteil DF, M; Guilloux-Benatier, M; Sapis, JC. Los vinos blancos secos. En Flanzy, C. Enología: Fundamentos científicos y tecnológicos. AMV Ediciones y Ediciones Mundi-Prensa M, 2000. pp 443-453.

19. Ribéreau-Gayon Pascal; Ribéreau-Gayon Pascal. Handbook of Enology, 2nd ed. /.; John Wiley & Sons: Chichester, 2006. pp 1-497.

20. Balcones EC, MM. Principales levaduras de interés enológico. Alimentos, Equipos y Tecnologia, 1996. pp 83-7.

21. Soufleros EH, Bertrand A. Rôle de la « souche de levure » dans la production des substances volatiles au cours de la fermentation du jus de raisin. OENO One. 1979;13(3):181-98.

22. Benito, S. The Impacts of Schizosaccharomyces on Winemaking. Applied Microbiology and Biotechnology 2019, 103 (11), 4291–4312.

23. Morata A, Escott C, Bañuelos M, Loira I, Fresno J, Gonzalez C, et al. Contribution of Non-Saccharomyces Yeasts to Wine Freshness. A Review. Biomolecules. 2019;10:34.

24. Suárez-Lepe. Impacto de levaduras y bacterias en los aromas vínicos fermentativos. I Encuentro International de Ciencias Sensoriales y de la Percepción; Barcelona y Sant Sadurní d’Anoia: Rubes Editorial SL, 2002. pp 43-45.

25. Davis CR, Wibowo D, Eschenbruch R, Lee TH, Fleet GH. Practical Implications of Malolactic Fermentation: A Review. American Journal of Enology and Viticulture. 1985;36(4):290.

26. Fauvet JG, A. . La vinificación en rosado. En Flanzy, C. Enología: Fundamentos científicos y tecnológicos. Madrid, 2000. pp 454-61 .

27. Escudier JM, M; Batlle, JL; Brugirard, A; Dubernet, M; Saint- Pierre, B; Vernhet, A. . Clarificación, estabilización de los vinos. Madrid, 2000. pp 558-607.

28. Feuillat M. Stabilisation des vins par le froid. Vignes et vins,. 1980;291:12-20.

29. Blouin J. Les techniques de stabilisation tartrique des vins par le froid. J Int Sci Vigne Vin. 1982;16:1:63-77.

30. Marchal R, Laigre, M., Jeandet, P., Robillard, B., Legras, V. Utilisation de CMC* pour la stabilisation tartrique des vins blancs. Revue des Œnologues n° 133, 2009. pp 22-23 .

31. Contreras J, Tomé, J. Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J.M., Zamora, F. Effectiveness of Carboxymethyl Cellulose (CMC) on Tartaric Stabilization of Cava Base Wine. Macrowine 2010; Torino (Italia).

32. Bosso A, Panero L, Petrozziello M, Sollazzo M, Asproudi A, Motta S, et al. Use of polyaspartate as inhibitor of tartaric precipitations in wines. Food Chemistry. 2015; 185:1-6.

33. Bonorden WR, Nagel CW, Powers JR. The adjustment of high pH/high titratable acidity wines by ion exchange. American journal of enology and viticulture. 1986;37(2):143-8.

34. Hubicki Z, Jakowicz A, Łodyga A. Application of the ion-exchange method to remove metallic ions from waters and sewages. In: Dąbrowski A, editor. Studies in Surface Science and Catalysis. 120: Elsevier; 1999. pp 497-531.

35. Jones JE, Kerslake FL, Close DC, Dambergs RG. Viticulture for sparkling wine production: A review. American Journal of Enology and Viticulture. 2014;65(4):407-16.

36. Kemp B, Alexandre H, Robillard B, Marchal R. Effect of production phase on bottle-fermented sparkling wine quality. Journal of Agricultural and Food Chemistry. 2015;63(1):19-38.

37. Martínez-Rodriguez AJ, Polo MC. Effect of the addition of bentonite to the tirage solution on the nitrogen composition and sensory quality of sparkling wines. Food Chemistry. 2003;81(3):383-8.

38. Martínez-Rodríguez A, Carrascosa AV, Barcenilla JM, Angeles Pozo-Bayón M, Carmen Polo M. Autolytic capacity and foam analysis as additional criteria for the selection of yeast strains for sparkling wine production. Food Microbiology. 2001;18(2):183-91.

39. Serra-Cayuela A, Aguilera-Curiel MA, Riu-Aumatell M, Buxaderas S, López-Tamames E. Browning during biological aging and commercial storage of Cava sparkling wine and the use of 5-HMF as a quality marker. Food Research International. 2013;53(1):226-31.

40. Liger-Belair G, Villaume S. Losses of dissolved CO2 through the cork stopper during champagne aging: Toward a multiparameter modeling. Journal of Agricultural and Food Chemistry. 2011;59(8):4051-6.

41. Cilindre C, Liger-Belair G, Villaume S, Jeandet P, Marchal R. Foaming properties of various Champagne wines depending on several parameters: Grape variety, aging, protein and CO2 content. Analytica Chimica Acta. 2010;660(1-2):164-70.

42. Gabrielli M, Fracassetti D, Romanini E, Colangelo D, Tirelli A, Lambri M. Oxygen-induced faults in bottled white wine: A review of technological and chemical characteristics. Food Chemistry. 2021; pp 348.

43. Godden P, Francis L, Field J, Gishen M, Coulter A, Valente P, et al. Wine bottle closures: Physical characteristics and effect on composition and sensory properties of a Semillon wine I. Performance up to 20 months post-bottling. Australian Journal of Grape and Wine Research. 2001;7(2):64-105.

44. Crouvisier-Urion K, Bellat JP, Gougeon RD, Karbowiak T. Gas transfer through wine closures: A critical review. Trends in Food Science and Technology. 2018;78:255-69.

45. Dimkou E, Ugliano M, Dieval JB, Vidal S, Aagaard O, Rauhut D, et al. Impact of headspace oxygen and closure on sulfur dioxide, color, and hydrogen sulfide levels in a Riesling wine. American Journal of Enology and Viticulture. 2011;62(3):261-9.

46. Ugliano M. Oxygen contribution to wine aroma evolution during bottle aging. Journal of Agricultural and Food Chemistry. 2013;61(26):6125-36.

47. Godden P, Lattey K, Francis I, Gishen M, Cowey G, Holdstock M, et al. Towards offering wine to the consumer in optimal condition - The wine, the closures and other packaging variables: A review of AWRI research examining the changes that occur in wine after bottling. Wine Industry Journal. 2005;20.

48. Clark AC, Dias DA, Smith TA, Ghiggino KP, Scollary GR. Iron(III) tartrate as a potential precursor of light-induced oxidative degradation of white wine: Studies in a model wine system. Journal of Agricultural and Food Chemistry. 2011;59(8):3575-81.

49. Hartley A. The Effect of Ultraviolet Light on Wine Quality. Wrap. Material change for a better envirenment, 2008. 1-84405-386-5. pp 4-20.

50. Zamora Marín Fernando. *Elaboración Y Crianza Del Vino Tinto : Aspectos Científicos Y Prácticos*; A. Madrid Vicente: Madrid, 2003.

51. Dallas C, Ricardo-Da-Silva JM, Laureano O. Degradation of oligomeric procyanidins and anthocyanins in a Tinta Roriz red wine during maturation. Vitis - Journal of Grapevine Research. 1995;34(1):51-6.

52. Bakker J, Timberlake CF. Isolation, Identification, and Characterization of New Color-Stable Anthocyanins Occurring in Some Red Wines. Journal of Agricultural and Food Chemistry. 1997;45(1):35-43.

53. Somers T, Evans M. Evolution of red wines I. Ambient influences on colour composition during early maturation. Vitis: Journal of Grapevine Research. 2015;25:31. pp 31-39.

54. Gao L, Girard B, Mazza G, Reynolds A. Changes in Anthocyanins and Color Characteristics of Pinot Noir Wines During Different Vinification Processes. Journal of Agricultural and Food Chemistry, 1997;45(6), 2003–2008.

55. Scrimgeour N, Nordestgaard S, Lloyd N, Wilkes E. Exploring the effects of elevated storage temperature on wine composition. 2015;21:713.

56. Auw JM, Blanco V, O'Keefe SF, Sims CA. Effect of processing on the phenolics and color of Cabernet Sauvignon, Chambourcin, and noble wines and juices. American Journal of Enology and Viticulture. 1996;47(3):279-86.

57. Gómez-Plaza E, Gil-Muoz R, López-Roca JM, Martínez-Cutillas A, Fernández-Fernández JI. Maintenance of colour composition of a red wine during storage. Influence of prefermentative practices, maceration time and storage. LWT - Food Science and Technology. 2002;35(1):46-53.

58. Monagas M, Gómez-Cordovés C, Bartolomé B. Evolution of the phenolic content of red wines from Vitis vinifera L. during ageing in bottle. Food Chemistry. 2006;95(3):405-12.

59. Rapp A, Mandery H. Wine aroma. Experientia. 1986;42(8):873-84.

60. Ramey DD, Ough CS. Volatile Ester Hydrolysis or Formation During Storage of Model Solutions and Wines. Journal of Agricultural and Food Chemistry. 1980;28(5):928-34.

61. Chisholm MG, Guiher LA, Zaczkiewicz SM. Aroma characteristics of aged Vidal blanc wine. American journal of enology and viticulture. 1995;46(1):56-62.

62. Simpson RF. Aroma composition of bottle aged white wine. Vitis: Journal of Grapevine Research. 2016;18:148-148.

63. Vázquez-Pateiro I, Arias-González U, Mirás-Avalos JM, Falqué E. Evolution of the aroma of treixadura wines during bottle aging. Foods. 2020;9(10).

64. Langlois J, Ballester J, Campo E, Dacremont C, Peyron D. Combining Olfactory and Gustatory Clues in the Judgment of Aging Potential of Red Wine by Wine Professionals. American Journal of Enology and Viticulture. 2010;61(1):15.

65. Alcalde-Eon C, Escribano-Bailón MT, Santos-Buelga C, Rivas-Gonzalo JC. Changes in the detailed pigment composition of red wine during maturity and ageing: A comprehensive study. Analytica Chimica Acta. 2006;563(1-2 SPEC. ISS.):238-54.

66. Antalick G, Perello MC, de Revel G. Esters in wines: New insight through the establishment of a database of french wines. American Journal of Enology and Viticulture. 2014;65(3):293-304.

67. Coetzee C, Van Wyngaard E, Šuklje K, Silva Ferreira AC, Du Toit WJ. Chemical and Sensory Study on the Evolution of Aromatic and Nonaromatic Compounds during the Progressive Oxidative Storage of a Sauvignon blanc Wine. Journal of Agricultural and Food Chemistry. 2016;64(42):7979-93.

68. Gambuti A, Rinaldi A, Ugliano M, Moio L. Evolution of phenolic compounds and astringency during aging of red wine: Effect of oxygen exposure before and after bottling. Journal of Agricultural and Food Chemistry. 2013;61(8):1618-27.

69. Monagas M, Gómez-Cordovés C, Bartolomé B. Evolution of polyphenols in red wines from Vitis vinifera L. during aging in the bottle: I. Anthocyanins and pyranoanthocyanins. European Food Research and Technology. 2005;220(5-6):607-14.

70. Recamales AF, Sayago A, González-Miret ML, Hernanz D. The effect of time and storage conditions on the phenolic composition and colour of white wine. Food Research International. 2006;39(2):220-9.

71. Charters S, Pettigrew S. The dimensions of wine quality. Food Quality and Preference. 2007;18(7):997-1007.

72. Sáenz-Navajas MP, Ballester J, Pêcher C, Peyron D, Valentin D. Sensory drivers of intrinsic quality of red wines. Effect of culture and level of expertise. Food Research International. 2013;54(2):1506-18.

73. Sáenz-Navajas MP, Ballester J, Peyron D, Valentin D. Extrinsic attributes responsible for red wine quality perception: A cross-cultural study between France and Spain. Food Quality and Preference. 2014;35:70-85.

74. Guerrero RF, Cantos-Villar E. Demonstrating the efficiency of sulphur dioxide replacements in wine: A parameter review. Trends in Food Science and Technology. 2015;42(1):27-43.

75. Arrhenius SP, McCloskey LP, Sylvan M. Chemical markers for aroma of Vitis vinifera Var. Chardonnay regional wines. Journal of Agricultural and Food Chemistry. 1996;44(4):1085-90.

76. Jackson D, Lombard P. Environmental and Management Practices Affecting Grape Composition and Wine Quality - A Review. American Journal of Enology and Viticulture. 1993;44:409-30.

77. Lambrechts M, Pretorius I. Yeast and its importance to wine aroma-a review. South African Journal of Enology and Viticulture. 2000;21 (1): 3560.

78. Guth H. Quantitation and Sensory Studies of Character Impact Odorants of Different White Wine Varieties. Journal of Agricultural and Food Chemistry. 1997;45(8):3027-32.

79. Harbertson JF, Spayd S. Measuring phenolics in the winery. American Journal of Enology and Viticulture. 2006;57(3):280-8.

80. Sáenz-Navajas MP, Avizcuri JM, Ballester J, Fernández-Zurbano P, Ferreira V, Peyron D, et al. Sensory-active compounds influencing wine experts' and consumers' perception of red wine intrinsic quality. LWT - Food Science and Technology. 2015;60(1):400-11.

81. Remize F, Sablayrolles JM, Dequin S. Re-assessment of the influence of yeast strain and environmental factors on glycerol production in wine. Journal of Applied Microbiology. 2000;88(3):371-8.

82. Zuzuarregui A, Carrasco P, Palacios A, Julien A, Del Olmo M. Analysis of the expression of some stress induced genes in several commercial wine yeast strains at the beginning of vinification. Journal of Applied Microbiology. 2005;98(2):299-307.

83. Lambrechts M, Pretorius I. Yeast and its importance to wine aroma. South African Journal of Enology and Viticulture. 2000;21:97-129.

84. Coetzee C, Du Toit WJ. Sauvignon blanc wine: Contribution of Ageing and Oxygen on Aromatic and Non-aromatic Compounds and Sensory Composition: A Review. South African Journal of Enology and Viticulture. 2015;36(3):347-65.

85. Ferreira RB, Piçarra-Pereira MA, Monteiro S, Loureiro VlB, Teixeira AR. The wine proteins. Trends in Food Science & Technology. 2001;12(7):230-9.

86. Gonzalez-Ramos D, Cebollero E, Gonzalez R. A recombinant Saccharomyces cerevisiae strain overproducing mannoproteins stabilizes wine against protein haze. Applied and Environmental Microbiology. 2008;74(17):5533-40.

87. Moine V, Dubourdieu D. Role yeast mannoproteins with regard to tartaric stabilisation of wines. Bull OIV. 2002;75:471-82.

88. Waters EJ, Alexander G, Muhlack R, Pocock KF, Colby C, O'Neill BK, et al. Preventing protein haze in bottled white wine. Australian Journal of Grape and Wine Research. 2005;11(2):215-25.

89. Coelho E, Reis A, Domingues MRM, Rocha SM, Coimbra MA. Synergistic effect of high and low molecular weight molecules in the foamability and foam stability of sparkling wines. Journal of Agricultural and Food Chemistry. 2011;59(7):3168-79.

90. Núñez YP, Carrascosa AV, González R, Polo MC, Martínez-Rodríguez A. Isolation and characterization of a thermally extracted yeast cell wall fraction potentially useful for improving the foaming properties of sparkling wines. Journal of Agricultural and Food Chemistry. 2006;54(20):7898-903.

91. Rapp A, Versini G. Influence of nitrogen compounds in grapes on aroma compounds of wines. Developments in Food Science. Elsevier. 1995; 45(1):1659-94.

92. Callejón RM, Troncoso AM, Morales ML. Determination of amino acids in grape-derived products: a review. Talanta. 2010;81(4-5):1143-52.

93. Garde-Cerdán T, Ancín-Azpilicueta C. Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. LWT - Food Science and Technology. 2008;41(3):501-10.

94. Hernández-Orte P, Ibarz MJ, Cacho J, Ferreira V. Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake and aroma generation during alcoholic fermentation. Food Chemistry. 2006;98(2):300-10.

95. Moreno-Arribas V, Pueyo E, Nieto FJ, Martín-Álvarez PJ, Polo MC. Influence of the polysaccharides and the nitrogen compounds on foaming properties of sparkling wines. Food Chemistry. 2000;70(3):309-17.

96. Cheynier V, Dueñas-Paton M, Salas E, Maury C, Souquet JM, Sarni-Manchado P, et al. Structure and properties of wine pigments and tannins. American Journal of Enology and Viticulture. 2006;57(3):298-305.

97. Ferreira V, Bueno M, Franco-Luesma E, Culleré L, Fernández-Zurbano P. Key changes in wine aroma active compounds during bottle storage of Spanish red wines under different oxygen levels. Journal of Agricultural and Food Chemistry. 2014;62(41):10015-27.

98. Styger G, Prior B, Bauer FF. Wine flavor and aroma. Journal of Industrial Microbiology and Biotechnology. 2011;38(9):1145-59.

99. Moreno-Arribas, M. V.; Moreno-Arribas, M. V.; Polo, C.; Polo, M. C.; Polo, C. *Wine Chemistry and Biochemistry*; Springer: New York, NY, 2009. pp 1-735.

100. Boido E, Lloret A, Medina K, Farñia L, Carrau F, Versini G, et al. Aroma composition of Vitis vinifera cv. Tannat: The typical red wine from Uruguay. Journal of Agricultural and Food Chemistry. 2003;51(18):5408-13.

101. Hashizume K, Samuta T. Green Odorants of Grape Cluster Stem and Their Ability to Cause a Wine Stemmy Flavor. Journal of Agricultural and Food Chemistry. 1997;45(4):1333-7.

102. Lanaridis P, Salaha MJ, Tzourou I, Tsoutsouras E, Karagiannis S. Volatile compounds in grapes and wines from two Muscat varieties cultivated in Greek Islands. Journal International des Sciences de la Vigne et du Vin. 2002;36(1):39-47.

103. Mallouchos A, Skandamis P, Loukatos P, Komaitis M, Koutinas A, Kanellaki M. Volatile compounds of wines produced by cells immobilized on grape skins. Journal of Agricultural and Food Chemistry. 2003;51(10):3060-6.

104. Gómez E, Laencina J, Martinez A. Vinification Effects on Changes in Volatile Compounds of Wine. Journal of Food Science. 1994;59(2):406-9.

105. Coelho E, Coimbra MA, Nogueira JMF, Rocha SM. Quantification approach for assessment of sparkling wine volatiles from different soils, ripening stages, and varieties by stir bar sorptive extraction with liquid desorption. Analytica Chimica Acta. 2009;635(2):214-21.

106. Caliari V, Burin VM, Rosier JP, BordignonLuiz MT. Aromatic profile of Brazilian sparkling wines produced with classical and innovative grape varieties. Food Research International. 2014;62:965-73.

107. Aleixandre JL, Padilla AI, Navarro LL, Suria A, García MJ, Álvarez I. Optimization of making barrel-fermented dry muscatel wines. Journal of Agricultural and Food Chemistry. 2003;51(7):1889-93.

108. Frivik SK, Ebeler SE. Influence of sulfur dioxide on the formation of aldehydes in white wine. American Journal of Enology and Viticulture. 2003;54(1):31-8.

109. Gerbaux V, Vincent B, Bertrand A. Influence of maceration temperature and enzymes on the content of volatile phenols in Pinot noir wines. American Journal of Enology and Viticulture. 2002;53(2):131-7.

110. Torrens J, Urpí P, Riu-Aumatell M, Vichi S, López-Tamames E, Buxaderas S. Different commercial yeast strains affecting the volatile and sensory profile of cava base wine. International Journal of Food Microbiology. 2008;124(1):48-57.

111. Webber V, Dutra SV, Spinelli FR, Marcon ÂR, Carnieli GJ, Vanderlinde R. Effect of glutathione addition in sparkling wine. Food Chemistry. 2014;159:391-8.

112. Ferreira V, Fernández P, Peña C, Escudero A, Cacho JF. Investigation on the role played by fermentation esters in the aroma of young Spanish wines by multivariate analysis. Journal of the Science of Food and Agriculture. 1995;67(3):381-92.

113. Francis IL, Newton JL. Determining wine aroma from compositional data. Australian Journal of Grape and Wine Research. 2005;11(2):114-26.

114. Ferreira V, Fernandez P, Pena C, Escudero A, Cacho JF. Investigation on the role played by fermentation esters in the aroma of young Spanish wines by multivariate analysis. Journal of the science of food and agriculture. 1995;67(3):381-92.

115. Simpson R. Some important aroma components of white wine. Food technology in Australia, 1979. pp 516-522.

116. Benítez P, Castro R, García Barroso C. Changes in the Polyphenolic and Volatile Contents of “Fino” Sherry Wine Exposed to Ultraviolet and Visible Radiation during Storage. Journal of Agricultural and Food Chemistry. 2003;51(22):6482-7.

117. Cutzach I, Chatonnet P, Dubourdieu D. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. Journal of Agricultural and Food Chemistry. 1999;47(7):2837-46.

118. Cutzach I, Chatonnet P, Dubourdieu D. Influence of storage conditions on the formation of some volatile compounds in white fortified wines (Vins doux Naturels) during the aging process. Journal of Agricultural and Food Chemistry. 2000;48(6):2340-5.

119. Pérez-Coello M, Hidalgo MC. Volatile Compounds and Wine Aging. Springer. 2009. pp 295-311.

120. Jiménez Moreno N, Torrea Goñi D, Ancín Azpilicueta C. Changes in Amine Concentrations during Aging of Red Wine in Oak Barrels. Journal of Agricultural and Food Chemistry. 2003;51(19):5732-7.

121. Tominaga T, Guimbertau G, Dubourdieu D. Role of certain volatile thiols in the bouquet of aged Champagne wines. Journal of Agricultural and Food Chemistry. 2003;51(4):1016-20.

122. Carnacini A, Antonelli A, Galletti GC, Natali N, Farris GA. Quantitative Changes of Some Volatile Components in Vernaccia di Oristano (a Sardinian Sherry-like Wine) during Maturation. Journal of Agricultural and Food Chemistry. 1997;45(6):2225-8.

123. Martin B, Etievant PX, Le Quere JL, Schlich P. More clues about sensory impact of sotolon in some flor sherry wines. Journal of Agricultural and Food Chemistry. 1992;40(3):475-8.

124. Escudero A, Asensio E, Cacho J, Ferreira V. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. Food Chemistry. 2002;77(3):325-31.

125. Silva Ferreira AC, De Pinho PG, Rodrigues P, Hogg T. Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters. Journal of Agricultural and Food Chemistry. 2002;50(21):5919-24.

126. Torrens J, Riu-Aumatell M, Vichi S, López-Tamames E, Buxaderas S. Assessment of Volatile and Sensory Profiles between Base and Sparkling Wines. Journal of Agricultural and Food Chemistry. 2010;58(4):2455-61.

127. Welke JE, Zanus M, Lazzarotto M, Pulgati FH, Zini CA. Main differences between volatiles of sparkling and base wines accessed through comprehensive two dimensional gas chromatography with time-of-flight mass spectrometric detection and chemometric tools. Food Chemistry. 2014;164:427-37.

128. Lopes P, Silva MA, Pons A, Tominaga T, Lavigne V, Saucier C, et al. Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon Blanc wine during bottle storage. J Agric Food Chem. 2009;57(21):10261-70.

129. Ugliano M, Dieval J-B, Siebert TE, Kwiatkowski M, Aagaard O, Vidal S, et al. Oxygen Consumption and Development of Volatile Sulfur Compounds during Bottle Aging of Two Shiraz Wines. Influence of Pre- and Postbottling Controlled Oxygen Exposure. Journal of Agricultural and Food Chemistry. 2012;60(35):8561-70.

130. Ugliano M, Kwiatkowski M, Vidal S, Capone D, Siebert T, Dieval JB, et al. Evolution of 3-mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. J Agric Food Chem. 2011;59(6):2564-72.

131. Herbert Stone RB, Heather Thomas. Sensory Evaluation Practices. 4th ed. Elsevier. San Diego, 2012. pp 1-446.

132. Hadley K, Orlandi RR, Fong KJ. Basic anatomy and physiology of olfaction and taste. Otolaryngologic clinics of North America. 2004;37(6):1115-26.

133. Almanza-Merchán PJ. Sensory evaluation of the wine grape Isabella (Vitis labrusca L.) hand crafted. Ciencia y Agricultura. 2015;Vol. 12:71-81.

134. Filipe-Ribeiro L, Cosme F, Nunes FM. A Simple Method to Improve Cork Powder Waste Adsorption Properties: Valorization as a New Sustainable Wine Fining Agent. ACS Sustainable Chemistry and Engineering. 2019;7(1):1105-12.

135. Olivella MÀ, Jové P, Oliveras A. The use of cork waste as a biosorbent for persistent organic pollutants-Study of adsorption/desorption of polycyclic aromatic hydrocarbons.

Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering. 2011;46(8):824-32.

136. Silva B, Martins M, Rosca M, Rocha V, Lago A, Neves IC, et al. Waste-based biosorbents as cost-effective alternatives to commercial adsorbents for the retention of fluoxetine from water. Separation and Purification Technology. 2020;235.

137. Mark R, Lyu X, Lee JJL, Parra-Saldívar R, Chen WN. Sustainable production of natural phenolics for functional food applications. Journal of Functional Foods. 2019;57:233-54.

138. Santos-Buelga C, Scalbert A. Proanthocyanidins and tannin-like compounds - Nature, occurrence, dietary intake and effects on nutrition and health. Journal of the Science of Food and Agriculture. 2000;80(7):1094-117.

139. Teixeira A, Baenas N, Dominguez-Perles R, Barros A, Rosa E, Moreno DA, et al. Natural bioactive compounds from winery by-products as health promoters: A review. International Journal of Molecular Sciences. 2014;15(9):15638-78.

140. Garcia AR, Lopes LF, Barros RBD, Ilharco LM. The problem of 2,4,6-trichloroanisole in cork planks studied by attenuated total reflection infrared spectroscopy: Proof of concept. Journal of Agricultural and Food Chemistry. 2015;63(1):128-35.

141. Tarasov A, Rauhut D, Jung R. “Cork taint” responsible compounds. Determination of haloanisoles and halophenols in cork matrix: A review. Talanta. 2017;175:82-92.

142. Dadalı C, Elmacı Y. Optimization of Headspace-Solid Phase Microextraction (HS-SPME) technique for the analysis of volatile compounds of margarine. Journal of food science and technology. 2019;56(11):4834-43.

143. Rodrigues F, Pimentel FB, Oliveira MPP. Olive by-products: Challenge application in cosmetic industry. Industrial Crops and Products. 2015;70:116-24.

144. Silva SP, Sabino MA, Fernandas EM, Correlo VM, Boesel LF, Reis RL. Cork: Properties, capabilities and applications. International Materials Reviews. 2005;50(6):345-65.

145. Ciriminna R, Fidalgo A, Meneguzzo F, Parrino F, Ilharco LM, Pagliaro M. Vanillin: The Case for Greener Production Driven by Sustainability Megatrend. ChemistryOpen. 2019;8(6):660-7.

146. Aroso IM, Araújo AR, Pires RA, Reis RL. Cork: Current Technological Developments and Future Perspectives for this Natural, Renewable, and Sustainable Material. ACS Sustainable Chemistry and Engineering. 2017;5(12):11130-46.

147. OIV. International Organisation of Vine and Wine. Compendium of International Methods of Wine and Must Analysis. Available online: http://www.oiv.int (accessed on 7 May 2020). In Paris, France, 2020. Vol. 1 & 2.

148. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS. Volatile Acidity. In: Zoecklein BW, Fugelsang KC, Gump BH, Nury FS, editors. Wine Analysis and Production. Boston, MA: Springer US; 1995. pp 192-8.

149. Maujean A, Poinsaut P, Dantan H, Brissonnet F, Cossiez E. Etude de la tenue et de la qualité de mousse des vins effervescents. II: Mise au point d'une technique de mesure de la moussabilité de la tenue et de la stabilité de la mousse des vins effervescents. Bull OIV 1990;63:405-27.

150. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture. 1965;16(3):144-58.

151. Martínez JA, Melgosa M, Pérez MM, Hita E, Negueruela AI. Note. Visual and Instrumental Color Evaluation in Red Wines. Food Science and Technology International. 2001;7(5):439-44.

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