Estrategias vitivinícolas de adaptación al cambio climático en una zona de clima cálido

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MEMORIA que presenta el Graduado en Enología D. **Pau Sancho Galán** para optar al grado de Doctor por la Universidad de Cádiz.

Fdo.: Pau Sancho Galán

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EL Dr. VÍCTOR MANUEL PALACIOS MACÍAS, CATEDRÁTICO DE UNIVERSIDAD DEL ÁREA DE TECNOLOGÍA DE ALIMENTOS, Y LA Dra. ANA C. JIMÉNEZ CANTIZANO, PROFESORA AYUDANTE DOCTORA DEL ÁREA DE PRODUCCIÓN VEGETAL

INFORMAN:

Que la presente Tesis Doctoral titulada "**Estrategias vitivinícolas de adaptación al cambio climático en una zona de clima cálido"** constituye la Memoria que presenta **D. Pau Sancho Galán** para aspirar al grado de Doctor en el programa de Recursos Agroalimentarios. Ha sido dirigida y se considera que cumple los requisitos exigidos por la legislación vigente para optar al grado de Doctor por la Universidad de Cádiz.

Y para que así conste, expedimos y firmamos la presente en Puerto real a abril de 2022.

Fdo.: Dr. Víctor M. Palacios Macías

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Directora del Departamento de Ingeniería Química y Tecnología de Alimentos
Universidad de Cádiz

A l'Ernest, per tot.

I know that you cannot live on hope alone,

but without it, life is not worth living

Harvey Milk

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Juro solemnemente que esto es una travesura…

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…travesura realizada.

Índice

1. Resumen

La vid (*Vitis vinifera* L.) es una de las especies de cultivo más antiguas y extendidas a nivel mundial, lo que ha contribuido a que presente una amplia diversidad genética y fenotípica. A nivel mundial se estima que hay 7,3 millones de hectáreas de superficie de viñedo, siendo España el país con mayor superficie de cultivo. Sin embargo, las predicciones y simulaciones climáticas pronostican que el cambio climático será uno de los grandes desafíos para la producción de vino en un futuro próximo, dado que la calidad de la uva y el rendimiento de la vid dependen de una compleja interacción entre la temperatura, la disponibilidad de agua, el material vegetal y las técnicas vitivinícolas empleadas. De forma general, el cambio climático está provocando un adelanto generalizado de la vendimia, un crecimiento acelerado de la vid y un exceso de maduración de las uvas, que conlleva a la producción de mosto con elevados grados alcohólicos potenciales, pH más elevados, menor acidez e importantes carencias nutricionales.

Con la intención de minimizar los efectos que puedan provocar sobre la superficie vitícola el aumento de las temperaturas y la disminución de las precipitaciones, resulta de gran interés el estudio de alternativas vitivinícolas que permitan disponer de herramientas a corto plazo para hacer frente a los cambios que el calentamiento global va a provocar en la industria vitivinícola. Por este motivo, en esta Tesis Doctoral se aúnan trabajos de investigación relacionados, por un lado, con la búsqueda de variedades autóctonas o variantes somáticas de vid de una zona de clima cálido que puedan estar mejor adaptadas a las condiciones agroclimáticas asociadas al cambio climático, y por otro, a la aplicación y recuperación de técnicas ancestrales enológicas de la zona ("asoleo" y la fermentación con pieles), que puedan contribuir en este nuevo escenario climático, a una mejora de la calidad y la producción de vinos.

Los principales resultados de esta Tesis Doctoral muestran la existencia de variedades autóctonas y variantes somáticas en el Marco de Jerez, que presentan características de adaptación frente a las condiciones impuestas por el cambio climático. Por otro lado, tanto la fermentación en presencia de hollejos como la elaboración de vinos blancos secos a partir de uva asoleada, pueden ser consideradas como técnicas de resiliencia muy indicadas cuando se registran altas temperaturas durante la fase de maduración. A su vez, la recuperación de estas técnicas consideradas ancestrales para la elaboración de vinos en la zona del Marco de Jerez, pueden ser un estímulo para la producción de nuevos vinos que se adaptan a las nuevas tendencias de los consumidores actuales.

2. Abstract

Grapevine (*Vitis vinifera* L.) is one of the oldest and most widespread crop species in the world, which has contributed to its wide genetic and phenotypic diversity. It is estimated that there are 7.3 million hectares of vineyards worldwide, with Spain being the country with the largest cultivated area in the world. However, climate predictions and simulations forecast that climate change will be one of the major challenges for wine production in the near future, as grape quality and vine yields depend on a complex interaction between temperature, water availability, plant material and viticultural techniques employed. In general, climate change is leading to a generalised earlier harvest, accelerated vine growth and over-ripening of grapes, leading to the production of grape must with high potential alcoholic strength, higher pH, lower acidity and important nutritional deficiencies.

With the intention of reducing the effects that rising temperatures and decreasing rainfall may have on the vineyard surface, it is of great interest to study viticultural alternatives that will provide short-term tools to deal with the changes that global warming will cause in the wine industry. For this reason, this Ph.D Thesis combines research work related, on the one hand, to the search for autochthonous varieties or somatic variants of vines from a warm climate area that may be better adapted to the agro-climatic conditions associated with climate change, and on the other hand, to the application and recovery of ancestral oenological techniques from the area ("asoleo" and fermentation with grape skins), which may contribute in this new climate scenario improving wine quality and production.

The main results of this Ph.D Thesis show the existence of autochthonous varieties and somatic variants in the Marco de Jerez region, which present characteristics of adaptation to the conditions imposed by climate change. On the other hand, both fermentation in the presence of skins and the production of dry white wines from sun-dried grapes can be considered as resilience techniques that are highly recommended when high temperatures are registered during grape ripening phase. In turn, the recovery of these techniques considered as ancestral for winemaking in the Marco de Jerez area can be a stimulus for the production of new wines that meet today's consumers new trends.

3. Introducción

La vid (*Vitis vinifera L.*) es una de las especies de cultivo más antigua [1-2] y extendida a nivel mundial [3], lo que ha contribuido a que presente una amplia diversidad genética y fenotípica [4-5]. Dicha diversidad se conserva fundamentalmente en bancos de germoplasma [6] que conservan *ex situ* accesiones del género *Vitis* como fuente de recursos genéticos necesarios para la mejora de las variedades cultivadas [7].

Actualmente, según las previsiones de la Organización Internacional de la Viña y el Vino [8], la superficie de viñedo a nivel mundial asciende a 7,3 millones de hectáreas (mha) y se producen unos 260 millones de hectolitros de vino. En la Unión Europa (UE), este cultivo ocupa 3,3 mha y España ocupa el liderazgo mundial con 961000 ha, seguido de Francia (797000 ha), China (785000 ha) e Italia (719000 ha) [8]. Estas superficies y producciones, ponen de manifiesto la repercusión económica que tiene el cultivo de la vid en algunos países europeos. Sin embargo, las predicciones y simulaciones climáticas pronostican que el cambio climático será uno de los grandes desafíos para la producción de vino en un futuro próximo, dado que la calidad de la uva y el rendimiento de la vid dependen de una compleja interacción entre la temperatura, la disponibilidad de agua, el material vegetal y las técnicas vitivinícolas empleadas [9]. Los cambios observados en 27 regiones vitivinícolas de alta calidad de todo el mundo han mostrado un aumento de la temperatura media durante la fase de crecimiento desde 1950 hasta el año 2000 de 1,3 °C mientras que, en Europa, el aumento fue de 1,7 °C desde 1950 hasta 2004 [10-12]. Según el "Intergovernmental Panel on Climate Change" [13] se prevé un aumento de temperatura de 1,8 a 4 °C sobre los valores actuales para finales del siglo XXI. Por otro lado, considerando la media del modelo HadCM3, las temperaturas previstas para las regiones productoras de vino de alta calidad aumentarán en 2,04 °C en el periodo comprendido entre 2000 y 2049 [12]. Este aumento de temperatura provocará una mayor evapotranspiración y un mayor riesgo de sequía para los cultivos [14]. En este sentido, diversos modelos climáticos han ido confirmando que, de cumplirse las previsiones, podría disminuir la producción de uva hasta un 80% en algunas regiones vitivinícolas del mundo [15-17]. Este hecho afectaría de forma directa al sector y provocaría una pérdida de rentabilidad que podría llevar al abandono del cultivo en algunas regiones vitivinícolas.

Ante estos acontecimientos, el sector vitivinícola debe afrontar grandes retos a medio y largo plazo. Uno de ellos es la sostenibilidad medioambiental ante los posibles efectos del cambio climático o emergencia climática. Por ello, sería necesario identificar y aplicar prácticas sostenibles que ayuden al sector a mitigar y adaptarse a los nuevos escenarios.

3.1. El cambio climático en el sector vitivinícola

Los principales factores asociados al cambio climático con influencia directa sobre el cultivo de la vid son, por un lado, el marcado incremento de la temperatura y de las

concentraciones de dióxido de carbono [18], y por otro, una reducción de la pluviometría [19]. La temperatura es un regulador importante que afecta tanto la fenología de la vid como a la composición fisicoquímica de la uva [11, 20], y su previsible aumento puede provocar cambios bioquímicos y alteraciones fisiológicas. Las altas temperaturas pueden influir negativamente en el desarrollo de la vid, afectando al periodo de floración, pudiendo llegar a reducirse hasta un 25% el número de flores por inflorescencia, provocándose así una pérdida de rendimiento para la vendimia [21]. Además, podrían adelantar y acelerar el proceso de maduración, produciendo un aumento de sólidos solubles en los mostos y, disminuir fuertemente el contenido de antocianinas [22], ácidos grasos y ácidos orgánicos como el ácido málico [23]. Por ello, la idea que el aumento de la temperatura puede reducir la calidad de las uvas y los vinos está ampliamente aceptada [11, 24].

En cuanto, al dióxido de carbono, se estima que aumentará en torno al 20%, para el año 2050 [25]. Un mayor contenido de dióxido de carbono deriva en un aumento de la fotosíntesis de las plantas e implica un mayor crecimiento, tal y como se ha comprobado en la vid [26]. El aumento de dióxido de carbono que se está produciendo puede aumentar el crecimiento y la producción de la vid, lo que podría tener un impacto negativo en la calidad de la uva y, por ende, en la del vino que se elabore [27]. Por otro lado, una disminución de las precipitaciones unida a las restricciones en el riego del cultivo de vid de algunas regiones vitícolas, pueden acentuar el problema [28]. El estrés hídrico inducido a largo plazo en la vid cultivada en el campo, conduce a una disminución progresiva de la conductancia estomática, acompañada de una disminución en la asimilación de CO_2 (40%) [29].

De manera general, el cambio climático está provocando un adelanto generalizado de la vendimia de 10-24 días en los últimos 30-50 años [30], un crecimiento acelerado de la vid y un exceso de maduración en las uvas, que conllevan a la producción de mostos con altos grados alcohólicos potenciales, pH más elevados, con menor acidez y con importantes carencias nutricionales que se traducen generalmente en bajos niveles en nitrógeno fácilmente asimilable (NFA) [10, 31-33]. Si bien es cierto, las consecuencias de muchas variables del cambio climático no están bien estudiadas y podrían ser muy dependientes, tanto de la variedad de uva como de la zona donde se cultive [34].

3.2. Estrategias vitivinícolas para hacer frente al cambio climático

Con el fin de minimizar los posibles efectos asociados al calentamiento global, la Organización Internacional de la Viña y el Vino (https://www.oiv.int) [35] como organización intergubernamental de carácter científico-técnico y de reconocido prestigio, está liderando e impulsado diferentes estudios para desarrollar estrategias de adaptación del sector vitivinícola frente al cambio climático. Actualmente, esta entidad tiene aprobado un plan estratégico a desarrollar entre 2020-24, en el que contempla objetivos para atender a los distintos desafíos a los que se enfrenta el sector vitivinícola internacional y a la voluntad de incorporar los Objetivos de Desarrollo Sostenible (ODS)

incluidos en la Agenda 2030 de las Naciones Unidas. Entre estos objetivos se incluyen: hacer frente al cambio climático con medidas de mitigación y adaptación, mejorar la eficacia ambiental y preservar los recursos naturales. Para la consecución de estos, se plantean una serie de acciones como: evaluar el impacto del cambio climático en la producción vitivinícola a distintas escalas (local, regional y mundial) sin olvidar los *terroirs*, promover la creación de bases de datos abiertas, armonizadas y sistemáticas; estudiar y evaluar las innovaciones, estrategias y oportunidades de adaptación y mitigación, así como su aceptación teniendo en cuenta la especificidad y la capacidad de cada país; estudiar y recopilar información relativa a la adaptación de los métodos de producción vitícolas y enológicos para hacer frente al cambio climático y promover la diversificación intra e intervarietal en los viñedos comerciales.

Por otro lado, se han propuesto distintas estrategias de adaptación al cambio climático por parte de la comunidad científica internacional como: la selección y mejora de nuevos cultivares y portainjertos [36-39], el cultivo de variedades de ciclo largo o maduración tardía [40], empleo de sistemas de riego de emergencia [41], protección contra el calor extremo y las quemaduras solares, gestión del suelo, cambios en los sistemas de formación [42], estrategias de base microbiológica/biotecnológica [43] y la reubicación de los viñedos a zonas de mayor altitud [44]. Entre todas estas propuestas, según Duchêne et al. [45], las posibles respuestas hacia el futuro calentamiento proyectado en los viñedos, incluyen: aceptar cambios en la tipicidad de los vinos y alterar su producción en consecuencia, para así producir otras tipologías de vinos; adaptar variedades para mantener una tipicidad constante; y trasladar el cultivo de la vid a áreas que actualmente son más frías. Por ello, junto a la selección y mejora de las variedades de vid cómo de estrategias de adaptación desde el punto de vista vitícola [46-48], también deben establecerse estudios que incluyan nuevos procesos enológicos en zonas especialmente cálidas, ya sea mediante la incorporación de productos, para paliar desequilibrios en la maduración de la uva [49-50], o mediante la búsqueda de nuevos procesos de vinificación [51]. Estos estudios permitirían adoptar estrategias a corto plazo, con las variedades que actualmente se cultivan, dado que hay que tener en cuenta que la idoneidad varietal de la vid está fuertemente ligada a las condiciones ambientales regionales y los productores tienden a seleccionar las variedades que mejor se adaptan a estas condiciones [52]. Dentro de las regiones vitivinícolas denominadas como regiones de clima cálido, se cultivan variedades que podrían tener mejor adaptación a las nuevas condiciones de calentamiento global.

En 2016, se presentó una propuesta de resolución del Grupo de recursos Genéticos de la Vid de la OIV (GENET), para fomentar el desarrollo de programas de mejora y tecnología genética en las distintas zonas vitivinícolas del mundo, que ayuden a seleccionar variedades que puedan hacer frente al desafío del cambio climático en distintas condiciones. Además, a través del proyecto VITI GENET-PROTECT 565AB (OIV) se pretende facilitar el intercambio de material vegetal y germoplasma para la investigación y el comercio de nuevos cultivares.

A nivel mundial, según el "*Vitis* International Variety Catalogue" (*V*IVC, www.vivc.de) [53] existen actualmente 130 colecciones de variedades de vid distribuidas en 45 países. En España, existen 13 bancos de germoplasma de vid [54], destacando por el número de accesiones conservadas las colecciones del Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), ubicada en Alcalá de Henares (Finca El Encín), y del Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaria y de la Producción Ecológica (IFAPA) ubicada en Jerez de la Frontera (Centro Rancho de la Merced) (Figura 1). Estas colecciones conservan gran parte del patrimonio genético de la vid y han sido sujeto de distintas investigaciones científicas desde hace años [55-60]. Entre el material vegetal conservado en estas colecciones, las variedades autóctonas de vid pueden considerarse un valioso recurso genético por su adaptación a determinados ambientes [61]. Cada variedad tiene un genotipo específico, una morfología y un contenido en sus metabolitos secundarios que la hacen única [62]. Todos estos elementos explican la adaptación de las distintas variedades a diferentes climas o ambientes, y las propiedades fisicoquímicas que tienen sus bayas [10, 52, 63-64]. Muchas de estas variedades han sido identificadas con marcadores de repetición de secuencia simple (SSR) o microsatélites y descriptores ampelográficos [6, 65-70]. Sin embargo, son pocos los estudios que incluyen datos sobre las composiciones fisicoquímicas de los mostos obtenidos a partir de los frutos de estas variedades, siendo esta información decisiva para determinar el potencial enológico y la capacidad de adaptación de estas variedades a las nuevas condiciones climáticas.

Figura 1. Banco de germoplasma del IFAPA Centro Rancho de la Merced.

En cuanto, a la mejora de las variedades de vinificación, tradicionalmente, se ha llevado a cabo, mediante la técnica de la selección clonal [71], técnica que permite mantener la identidad que caracteriza a la variedad. La variabilidad intravarietal observada en la vid se debe a la variación somática que se acumula en las variedades que se reproducen por multiplicación vegetativa. Así pues, las mutaciones somáticas

han conducido a la adaptación de la vid y a su evolución bajo condiciones ambientales y de cultivo cambiantes, siendo éstas una fuente de caracteres de innovación [72]. Esta variación se convirtió en la base de la selección clonal de las variedades europeas, comenzando en Alemania en el siglo XIX y continuando en otros países europeos como Francia, Italia y España en la segunda mitad del siglo XX [71]. Inicialmente, el objetivo básico de la selección clonal era obtener plantas sanas y altamente productivas [71], no obstante, en los últimos años se han iniciado programas de hibridación para la obtención de nuevas variedades resistentes a enfermedades criptogámicas [73] y resistencia a los efectos del cambio climático [74]. Esta última técnica de mejora podría desarrollarse más en los próximos años, ya que la nueva normativa que regula las variedades que se pueden emplear para la plantación de un viñedo en Europa destinado a la producción de uva para vinificación permite el uso de híbridos interespecíficos. Sin embargo, para mantener la tipicidad de los vinos de las regiones vitivinícolas acogidas a menciones de calidad, que tienen restringidas las variedades que pueden cultivar, por el carácter diferenciador que les aporta, se hace necesario explorar de la variabilidad genética intravarietal con el fin de seleccionar los genotipos mejor adaptados a las nuevas condiciones de cultivo. En este sentido, para seleccionar genotipos adaptados a condiciones de altas temperaturas podría considerarse el estudio y la selección de variedades autóctonas de zonas de clima cálido.

La región vitivinícola del Marco de Jerez es la región más meridional de Europa, y se caracteriza por presentar un clima cálido, con una temperatura media anual de 17,3 °C, con inviernos muy suaves en los que rara vez hiela y veranos muy calurosos, con temperaturas frecuentemente por encima de los 40 °C. La zona disfruta de un promedio anual de horas de sol efectivo muy alto, entre 3.000 y 3.200 [75]. La variedad de vid, más cultivada en el Marco de Jerez, es la Palomino Fino, variedad autóctona de esta región [53], y su cultivo se conoce desde el siglo XVI [76]. Esta variedad se cultiva en otras regiones vitivinícolas y está entre las 40 variedades de vid más cultivadas en el mundo, ocupando el puesto 36. Destacar que su cultivo se desarrolla en un 70% en zonas de clima muy cálido, un 20% en zonas de clima cálido y un 10% en zonas de clima templado [77]. Esto justificaría su adaptación a condiciones de clima cálido.

Históricamente, en la región del Marco de Jerez se cultivaban un mayor número de variedades de vid. Simón de Rojas Clemente y Rubio describió a principio del siglo XIX, 43 variedades de vid cultivadas en los viñedos del suroeste de Andalucía [78]. A mediados del siglo XX, Fernández de Bobadilla citó entre las vides replantadas: Palomino Fino, Pedro Ximenez, Cañocazo y Albillo como variedades clásicas; a Garrido, Perruno, Mantuo y Beba como secundarias y como variedades especiales a Moscatel y Tintilla de Rota [79]. Por otro lado, en la última mitad del siglo XX se introdujo material vegetal clonal para las nuevas plantaciones de Palomino Fino atendiendo a criterios de productividad, que provocaron una pérdida de recursos genéticos para esta variedad. La larga historia del cultivo de Palomino Fino ha dado lugar a un elevado número de clones, lo que representa una importante fuente de diversidad genética. El primer

programa de selección clonal de Palomino Fino fue iniciado por Fernández de Bobadilla [79] y continuado por García de Luján et al., donde se seleccionaron un total de 28 clones [80]. Estos clones están certificados, sin embargo, para la mayoría de las nuevas plantaciones se han utilizado solo dos de estos clones (Palomino 84 y Palomino California). Por lo tanto, actualmente se hace necesario identificar nuevas variantes somáticas o clones que puedan satisfacer las necesidades actuales de los viticultores. Además, para poder realizar estudios de comportamiento que permitan seleccionar nuevo material vegetal, es necesario cultivarlos en la misma parcela y condiciones de cultivo. Los estudios de identificación y caracterización de nuevo material vegetal de vid son necesarios para poder inscribirlo en el Registro de Variedades Comerciales [81].

Por otro lado, junto a la búsqueda de estrategias de adaptación desde el punto de vista vitícola, se hace necesario el estudio de nuevas herramientas tecnológicas que permitan una adaptación al cambio climático a corto plazo, ya sea mediante la adición de productos naturales para paliar los desequilibrios en la maduración de la uva [82-83], o mediante la búsqueda de nuevos procesos de vinificación [84]. En este sentido, una de las estrategias podría ser la elaboración de nuevos vinos blancos a partir de uvas sobremaduradas. La sobremaduración de la uva es una técnica que varía en función de las condiciones climáticas y del producto a obtener, así como de la localización geográfica y de la variedad de uva empleada [85]. En China, India y Turquía, la sobremaduración se emplea en la producción de pasas [86]. Sin embargo, en la mayoría de los países cálidos y secos, esta técnica se ha utilizado para la producción de ciertos vinos dulces y fortificados, tal y como es el caso de la región de Jerez. La sobremaduración de la uva al sol, o asoleo, es una técnica ancestral que permite una modificación natural de la composición de la uva y conduce a la producción de nuevos tipos de vinos. Esta técnica, podría utilizarse como estrategia de adaptación o resiliencia a las condiciones establecidas por el cambio climático en una zona de clima cálido.

El asoleo, bajo las condiciones climatológicas particulares de la zona de producción, provoca una deshidratación parcial de las bayas, normalmente acompañada de una ligera pasificación según sea la duración de la exposición al sol, sin llegar a la pasificación total que imposibilitaría la extracción del mosto por prensado, manteniendo en todo caso la integridad de la piel del fruto y sus condiciones sanitarias [87-88]. No obstante, la práctica del asoleo conlleva modificaciones importantes en la merma de peso de la uva, que puede oscilar entre un 25% y un 60% de pérdida de peso respecto al peso inicial de la baya. Esta pérdida puede depender de la madurez inicial de la uva y el vino a obtener y también en función de la duración y las condiciones en las que se desarrolle dicho asoleo [87, 89]. Además, implica cambios en algunas características físicas de las uvas como la disminución en el tamaño de las bayas y la aparición de una cutícula cerosa externa, y químicas como el aumento de la concentración de azúcares y disminución de la acidez [86]. Todos estos cambios pueden provocar modificaciones en las características sensoriales de los vinos, como cambios en el color y aparición de diferentes notas aromáticas [89].

Antiguamente, los racimos se extendían al sol en capachos para llevar a cabo el asoleo, actualmente se utilizan largas tiras de malla de plástico alimentario en lugares especialmente destinados para ello por su orientación y ligera pendiente (Figura 2).

Figura 2. Asoleo en un viñedo de a DO Jerez-Xérès-Sherry.

Además, en algunos casos, los racimos son cubiertos mediante un plástico transparente con objeto de evitar la humedad durante la noche o los daños asociados a precipitaciones [90-92]. En condiciones climáticas apropiadas, los tiempos de secado son más cortos, pudiendo durar entre 5 y 10 días con temperaturas diurnas que pueden superar los 40 °C y nocturnas por debajo de los 18 °C [93,94].

Tradicionalmente, el asoleo en Andalucía se ha llevado a cabo mayoritariamente en la provincia de Córdoba y con la intención de elaborar vinos dulces de la variedad Pedro Ximénez [95]. Sin embargo, según Viñega et al., [96] el asoleo es una de las etapas más importantes en la elaboración de algunos vinos de Andalucía, aportándole una posibilidad de diversificación vitivinícola que puede establecerse en la región con la finalidad de mejorar la calidad de sus vinos. Existen diferentes referencias que aluden al uso de esta práctica en la provincia de Cádiz [97-98]. En los últimos años, algunos elaboradores de vinos tranquilos de la provincia de Cádiz han empezado a emplear el asoleo de la uva Palomino Fino para elaborar vinos de añada con alta graduación alcohólica sin necesidad de alcoholizar, mejorando así la calidad organoléptica de los vinos obtenidos y contribuyendo a la diversificación de la producción en el Marco de Jerez mediante una técnica sostenible que evita la compra y adición de alcohol externo. Esta estrategia de adaptación permitiría seguir produciendo vino en las regiones vinícolas tradicionales, la diversificación de su producción y el desarrollo de nuevas oportunidades de negocio. Además, respondería a las expectativas de los consumidores actuales, ávidos de nuevos conceptos enológicos para recuperar técnicas históricas y

fusionarlas en nuevos productos [99-100]. Más concretamente, los vinos generosos de Jerez están considerados como uno de los productos más apreciados en el mundo de la enología [101] y su diversidad es, sin duda, uno de los rasgos distintivos de la identidad de estos vinos, donde sólo tres variedades de uva dan lugar a distintos vinos que difieren claramente en cuanto a color, aroma, sabor y textura en función de su proceso de elaboración [102].

Por otro lado, existen técnicas de elaboración de vinos blancos que deberían ser estudiadas para analizar su respuesta como estrategia de adaptación al cambio climático desde el punto enológico, tales como la fermentación de vinos blancos en presencia de hollejos, o fermentación pelicular. En general, los protocolos de elaboración de vinos blancos se basan en la fermentación del mosto de uva en ausencia de las partes sólidas [103]. Sin embargo, es una práctica habitual en algunas vinificaciones, las maceraciones peliculares prefermentativas para enriquecer el mosto en compuestos aromáticos procedentes del hollejo, mejorando la tipicidad sensorial varietal [104-109]. Varios estudios han demostrado las modificaciones que se producen en la composición de los vinos por la presencia de las partes solidas durante la fermentación [110]. Los resultados de estos estudios demuestran que la presencia de los hollejos durante su fermentación alcohólica da lugar a la aparición de aromas herbáceos o a un aumento del amargor en los vinos debido a un incremento de la concentración de terpenos y compuestos fenólicos [111]. Asimismo, otros efectos que aparecen son una mayor susceptibilidad al pardeamiento [112], una absorción prolongada de potasio que favorece la precipitación de bitartrato de potasio y un descenso de la acidez total [113], y olores desagradables junto con una mayor astringencia y amargor [114]. También se ha constatado un aumento de los compuestos varietales libres, una intensificación de los caracteres frescos y afrutados y una modificación del cuerpo del vino, lo que se percibe como positivo por el análisis sensorial en un panel de cata [115].

Sin embargo, en la actualidad son pocas las bodegas y enólogos que han optado por la elaboración de vinos blancos con maceración de partes sólidas más allá de la fase prefermentativa, ya que requieren un control más exhaustivo del proceso de elaboración para evitar efectos indeseables. Por ello, este tipo de vino no ha sido estudiado en profundidad. Históricamente, el vino *Kvevri* (Georgia) es un ejemplo de vino fermentado con presencia de pieles de forma espontánea [116]. Los vinos blancos *Kvevri*, como era de esperar, tienen un mayor contenido en polifenoles que los vinos blancos tradicionales, alcanzando en algunos casos casi las mismas concentraciones que los vinos tintos [117]. Estos vinos han adquirido fama internacional en los últimos 10 años, formando parte de una nueva tendencia entre los consumidores, y presentándose como una mezcla perfecta de innovación y prácticas históricas [114]. Sin embargo, a pesar del éxito que está teniendo este tipo de vinos, las investigaciones que se han llevado a cabo en relación con su elaboración se centran bien en los procesos de oxidación de la materia polifenólica [118], o desde un punto de vista sensorial de los vinos finales en comparación con otros tratamientos de maceración con hollejos [119].

Actualmente, los nuevos consumidores demandan vinos más particulares y exclusivos que se distingan del resto por sus características organolépticas distintivas [100]. En general, todos los estudios publicados en relación con la maceración de las partes sólidas con el mosto de uva blanca durante la fermentación han concluido que es necesario controlar el proceso para minimizar la aparición de todos aquellos atributos indeseables desde el punto de vista sensorial, como el aumento de la astringencia o la pérdida de acidez y frescura [120]. No obstante, no se ha encontrado literatura en relación al efecto de la presencia de partes sólidas durante el trascurso de la fermentación desde el punto de vista de la cinética fermentativa.

Dados los anteriores precedentes, se plantea esta Tesis Doctoral, con la intención de contribuir al conocimiento generado sobre estrategias vitivinícolas de adaptación al cambio climático. Para ello se plantean diferentes alternativas que implican el estudio de variedades autóctonas y variantes somáticas de la variedad Palomino Fino en una zona de clima cálido, así como la elaboración de nuevas tipologías de vinos mediante la fermentación en presencia de hollejos y/o a partir de uva sobremadura.

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4. Objetivos

En vista de los aspectos expuestos anteriormente, parte de los componentes del grupo de investigación AGR-203 "Ingeniería y Tecnología de Alimentos" de la Universidad de Cádiz desarrollan desde 2018 la línea de investigación "Estrategias vitivinícolas de adaptación al cambio climático en una zona de clima cálido" con el objetivo de buscar herramientas de adaptación o resiliencia para que el sector vitivinícola pueda a hacer frente a las nuevas condiciones impuestas por el cambio climático.

De este modo, el trabajo que se presenta en esta Tesis Doctoral, pretende ofrecer resultados en relación al potencial de variedades autóctonas andaluzas y variantes somáticas de la variedad 'Palomino Fino' cultivadas en una zona de clima cálido. A su vez, buscar estrategias de resiliencia para hacer frente a corto plazo al aumento de temperaturas y alteraciones en el régimen de precipitaciones, basadas en la elaboración de nuevas tipologías de vinos en zonas de clima cálido.

En base a la bibliografía existente y la problemática actual, se plantean las siguientes hipótesis:

1. Potenciar el uso y la expansión del cultivo de variedades autóctonas y variantes somáticas adaptadas a zonas de clima cálido, como herramientas de adaptación a los efectos asociados al cambio climático.
2. Caracterizar variedades autóctonas o variantes somáticas de la variedad Palomino Fino, para disponer de estudios de identidad mediante el empleo de marcadores genéticos y morfológicos y del potencial enológico que faciliten su inclusión en el Registro Oficial de variedades autorizadas para la elaboración de vinos.
3. Aportar herramientas tecnológicas que permitan la obtención de nuevas tipologías de vinos para paliar los efectos de desequilibrio nutricional de los mostos asociados al cambio climático.
4. Rescatar prácticas ancestrales, como el asoleo de la uva para la producción de nuevas tipologías de vinos y contribuir a la diversificación de la producción de las regiones vitivinícolas.

Para responder a las hipótesis planteadas se propone como objetivo principal de esta tesis el estudio de estrategias vitivinícolas de adaptación al cambio climático en una zona de clima cálido. Dicho objetivo se puede desglosar en los siguientes objetivos específicos:

- Identificación genética, caracterización morfológica y fisicoquímica de los mostos de variedades autóctonas, así como de variantes somáticas de la variedad Palomino Fino cultivadas en una zona de clima cálido como es el Marco de Jerez.

- Estudio y evaluación a escala de laboratorio de la fermentación en presencia de hollejos durante la elaboración de vinos de la variedad Palomino Fino.
- Estudio y evaluación a escala de laboratorio de los efectos del asoleo de la uva y/o la presencia de hollejos sobre la cinética y desarrollo de las levaduras durante el proceso fermentación de los mostos de la variedad Palomino Fino.
- Estudio y evaluación a escala de laboratorio de los efectos del asoleo de la uva y/o la presencia de hollejos sobre la composición fisicoquímica y las características sensoriales de los vinos obtenidos con la variedad Palomino Fino.

Los resultados obtenidos en esta Tesis Doctoral se presentan mediante un compendio de seis publicaciones científicas indexadas en el *Journal Citation Reports* del siguiente modo:

- Publicación 1: Genetical, Morphological and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (*Vitis vinifera L.*).
- Publicación 2: Identification and Characterization of White Grape Varieties Autochthonous of a Warm Climate Region (Andalusia, Spain).
- Publicación 3: Preliminary Study of Somatic Variants of Palomino Fino (*Vitis vinifera* L.) Grown in a Warm Climate Region (Andalusia, Spain).
- Publicación 4: Influence of the Presence of Grape Skins during WhiteWine Alcoholic Fermentation.
- Publicación 5: Effect of Grape Over-Ripening and Its Skin Presence on White Wine Alcoholic Fermentation in a Warm Climate Zone.
- Publicación 6: Volatile Composition and Sensory Characterisation of Dry White Wines Made with Overripe Grapes by Means of Two Different Techniques.

5. Resultados

5.1. Identificación genética, caracterización morfológica y fisicoquímica de los mostos de variedades autóctonas, así como de variantes somáticas de la variedad Palomino Fino cultivadas en una zona de clima cálido como es el Marco de Jerez.

Los resultados presentados en este capítulo se han publicado en:

1) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Genetical, Morphological and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (*Vitis vinifera* L.). *Agronomy* **2019**, *9*, 563; doi:10.3390/agronomy9090563.
2) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Identification and Characterization of White Grape Varieties Autochthonous of a Warm Climate Region (Andalusia, Spain). *Agronomy* **2020**, *10*, 205; doi:10.3390/agronomy9090563.
3) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Preliminary Study of Somatic Variants of Palomino Fino (*Vitis vinifera* L.) Grown in a Warm Climate Region (Andalusia, Spain). *Agronomy* **2020,** *10*, 654; doi:10.3390/agronomy10050654.

1) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Genetical, Morphological and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (*Vitis vinifera* L.). *Agronomy* **2019**, *9*, 563; doi:10.3390/agronomy9090563.

Abstract

'Uva Rey' is considered an Andalusian (Spain) ancient autochthonous cultivar with hard white grapes used for the production of wine and raisins and also for raw consumption. Currently, this cultivar is not included in the official register of Spanish grapevine varieties and there is neither a description nor a characterization that could facilitate its insertion in this register. In order to study this genetic resource, a genetic and morphological characterization of 'Uva Rey' has been carried out in comparison with 'Palomino Fino', the main cultivar in Andalusia (Spain). Additionally, grape must physicochemical characterization and grape berry texture profile analyses were performed. Genetically, 'Uva Rey' was synonymous with the cultivar 'De Rey'. 'Uva Rey' grape must physicochemical results showed a lower sugar concentration and a higher malic acid content compared to 'Palomino Fino' must, while the analysis of the grape berry texture profile proved to be more consistent and cohesive. These results can be attributed to the longer phenological cycle presented by 'Uva Rey'. All these facts could lead to consideration of 'Uva Rey' as a cultivar for the production of white wines in warm climate regions.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most ancient and important fruit crops worldwide [1]. Around 12,500 cultivars have been registered in the *Vitis* International Variety Catalogue [2]. However, based on their DNA profiles, the number of grapevine varieties is estimated at around 5000, many of them closely related [3,4].

Nowadays, 7.4 mHa of the Earth area is covered by grapevines, with Spain being the first country in terms of cultivated land extension. Spanish vineyards cover thousands of hectares and produce approximately 44.4 mHL of wine per year [5]. For that reason, viticulture could be considered as one of the most important socioeconomic sectors in the Spanish agro-industrial network. Grapevine cultivation throughout the country, and the significance over time, have led to a grapevine heritage of great magnitude. Spain's varietal heritage had continuously increased from its origin until the arrival of diseases and pathogens from America (mildews and *Phylloxera*) [6]. According to García de los Salmones [7], the first *Phylloxera* outbreak in Spain was detected in Malaga (Andalusia) in 1876. From that moment on, this pathogen spread throughout the

whole country and destroyed more than 1,000,000 hectares, which caused serious damages to the Spanish native germplasm [8]. In order to preserve the maximum number of *Vitis vinifera* genetic diversity, a number of germplasm banks were created. 'El Encín', the most important germplasm bank in Spain, was established in 1914 in Alcalá de Henares (Madrid, Spain) [9]. Later on, the currently germplasm bank known as 'Rancho de la Merced', was created in 1940, with the first collection of grapevines in Jerez de la Frontera (Andalusia, Spain) [10].

From then on, the prospection, collection and conservation of different grapevine cultivars as a genetic resource have been the subject of numerous studies that intend to preserve those cultivars considered as autochthonous [7–11]. For the identification of that genetic material, molecular characterization using Simple Sequence Repeats (SSR) markers [12], ampelographic [4] and physicochemical [13] techniques have been used. Grapevine genotypes are highly heterozygous and the relevance of near-homozygous lines was not considered until recently due to the need to generate high quality reference sequences [14], and has been maintained in cultivated plants through vegetative propagation [15].

Modern wine industries only use a limited number of *Vitis vinifera* cultivars [16]. In Spain, by virtue of the Spanish Royal-Decree-Law RD 1338/2018, only those varieties that have been properly registered can be planted [17]. However, there is a current trend towards the production of genuine and characteristic wines [18]. Currently, the changing climate is expected to impose new challenges to varietal selection. Since grapevine varietal suitability is strongly linked to regional environmental conditions, growers are prone to select varieties that are best suited to these changing agroclimatic factors [19].

As a result, autochthonous cultivars, such as 'Uva Rey' would require to be identified and characterized, since they were already used for wine making in the 19th century in southwestern regions in Andalusia [20]. Roxas Clemente [21] included this variety in Tribe III of the First Section and indicated that it was cultivated under different denominations in different districts within Cadiz and Seville provinces in Andalusia. Regarding its grapes, this author described them as very large, round, somewhat golden and with a long cycle. With regards to its winemaking potential, Abela [22] confirmed that this grape variety was able to produce fine wines with plenty of mouth-feel and acidity.

The main objective of this research work is to complete the characterization of the cultivar 'Uva Rey' as currently kept in a specific vineyard located in Andalusia (Spain). For this purpose, the genetic identification, the ampelographic characterization, the grape berry texture profile analysis and the physicochemical characterization of the grape musts have been carried out.

Materials and Methods

Plant Material and Experimental Design.

A total of 10 plants of 'Uva Rey' from a vineyard in the town of Chiclana de la Frontera municipal district (Andalusia, Spain) were selected (lat. 36°27′30.6″ N; long. 6°05′46.2″ W; 69 m above sea level). In addition, 'Palomino Fino' was used as a reference cultivar for all the studies, as it is the most widespread variety in the southwest of Andalusia [23]. Both cultivars were 15 years old and had been grown with the same vine spacing (2.30 × 1.15 m) as well as trained according to the 'Vara y Pulgar' (stick and thumb) system. Additional Figures S1a–c and S2a–c show the temperature, humidity, radiation and rainfall during the period from July (veraison) to September (harvest) for 2016 and 2017 respectively. For the genetic characterization of the cultivar, four varieties: 'Cabernet Sauvignon', 'Chardonnay', 'Muscat a Petits Grains Blancs' and 'Pinot Noir' were included as reference to compare their genotype databases and confirm the new cultivar accession identity (Table 1).

The morphological description and the texture profile analysis (TPA) of the berries as well as the grape must characterization were carried out for 'Uva Rey' and 'Palomino Fino' cultivars from the same vineyard and in two consecutive years (2016 and 2017) in order to study the vintage effect on the different cultivars. Both cultivars were grown at the same vineyard and under the same agroclimatic conditions, the cultural practices and were harvested in the same period (first week in September). In order to minimize variability due to grapevine sampling, Santesteban et al. [24] criterion was applied. For this purpose, the trunk cross sectional area (TCSA) of a total of 50 vines were measured at 30 cm height using a digital Verner calliper Maurer 93,110 (Padova, Italy). Of all the vines measured, 10 were selected and marked as their TCSA value was the closest to the TCSA average ± 10%.

Microsatellite Analysis

Two young fresh leaves from each accession were collected at the vineyard and kept at –80 °C until analysis. DNA extraction was carried out using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Varietal identification was performed using 22 nuclear microsatellite loci. The first set of 20 microsatellite loci located in the 19 linkage groups of grapevine genome (VMC1B11 (GeneBank, Accession Number BV681754) [25], VMC4F3-1 [26]; VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD28, VVMD32 [27,28]; VVS2 [29]; VV1B01, VVIH54, VVIN16, VVIN73, VVIP31, VVIP 60, VVIQ52, VVIV37, VVIV67 [30]) were analysed as described by Vargas et al. [31], using two multiplex Polimerase Chain Reactions (PCR). An additional set of two microsatellite loci [VrZAG62 and VRZAG79) [32] were analyed following the conditions described in detail by Jiménez-Cantizano et al. [33], in order to complete the list of loci authorized by the International Organisation of Vine and Wine (OIV). PCR

amplifications were performed using a 9700 thermocycler and the amplified products were separated by capillary electrophoresis using an automated sequencer ABI Prism 3130 (Applied Biosystems, Foster City, CA, USA). Fluorescent labelled fragments (6-FAM, VIC, PET and NED) were detected and sized using GeneMapper v. 3.7 and fragment lengths were assessed with the help of internal standards GeneScan-500 LIZTM (Applied Biosystems, Foster City, CA, USA). The microsatellite genotypes obtained after the analysis were compared with the genetic profiles provided by Lacombe et al. [34] and the data contained in the microsatellite databases *Vitis* International Variety Catalogue [35], Rancho de la Merced Germplasm Bank genotype database [36] and the *Vitis* Germplasm Bank at Finca el Encín [26,37,38]. The SSR profiles obtained were compared using the microsatellite toolkit v. 9.0 software [39].

Morphological Characterization

For the morphological analysis, Benito et al. [40] criterion was followed. A total of 10 young shoots, young and mature leaves, flowers, bunches and berries from each accession were analysed using 34 descriptors from the Organisation Internationale de la Vigne et du Vin descriptor list [41]. Each accession from two different vintages was described by five ampelographers and the modal value was selected as the final description.

Physicochemical Characterization of Grape Berries and Musts

Grapevine berries (n = 50) were evaluated using a texture-meter (Lloyd Material Testing Machine, West Sussex, UK) fitted with a 2 mm cylindrical flat probe at 1 mm/s. The results regarding consistency, firmness, work of penetration (WoP) and cohesiveness were calculated as the average values for 50 berries.

Once harvested, 5 kg of berries of each cultivar (500 g from each vine) were destemmed, grounded and pressed. pH determinations were carried out using a Crisson-2001 digital pH-meter (Loveland, CO, USA). Sugar concentration (°Bé) was determined using a calibrated Dujardin-Salleron hydrometer (Laboratories Dujardin-Salleron, Arcueil Cedex, France). Total acidity (TA) was calculated according to the official methods of analysis [42]. Ripening index (RI) was calculated following the equation proposed by Hidalgo [43]. Yeast Assimilable Nitrogen (YAN) was determined according to Aerny [44]. Citric, tartaric and malic acids were assessed following the methodology proposed by Sancho-Galán et al. [45]. Organic acids concentrations were obtained by ionic chromatography using a Metrohm 930 compact IC Flex ionic chromatographer equipped with a conductimetric detector on a Metrosep Organic Acids column-250/7.8 (Herisau, Switzerland). Organic acids separation was performed using as eluent H_2SO_4 0.4 mM in a 12% acetone solution with an isocratic 0.4 mL/min flow. All the physicochemical measurements were destructive analysis and were conducted in triplicate to ensure statistical significance.

Statistical Analysis

Means and standard deviations were calculated using Microsoft Office Excel 2016 for Windows 10. Significant differences were evaluated by two-way ANOVA and Bonferroni's multiple range (BSD) test; $p < 0.05$ was considered significant (GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego, CA, USA).

Results

Microsatellite Analysis

The allele profiles obtained for 'Uva Rey' and the five reference cultivars at 22 microsatellite loci are shown in Table 1. The genotype obtained for 'Uva Rey' was compared with the Rancho de la Merced Germplasm Bank genotype database [14,36], the *Vitis* Germplasm Bank at the Finca El Encín [31,37,38] and European databases [34,35]. 'Uva Rey' showed the same genotype as 'Mantuo de Pilas' kept in Rancho de la Merced Germplasm Bank at 22 SSR loci and 'De Rey' at Finca El Encín at 20 SSR loci.

Table 1. Genetic profiles of 'Uva Rey' and reference cultivars at 22 microsatellite loci. Alleles sizes are given in base pairs.

Locus	'Uva Rey'		'Palomino Fino'		'Cabernet Sauvignon'		'Chardonnay'		'Muscat a Petits Grains Blancs'		'Pinot Noir'	
VVIB01	307	307	291	307	291	291	289	295	291	295	289	295
VMC1b11	184	188	184	188	184	184	166	184	184	188	166	172
VMC4F31	184	190	176	206	174	178	174	180	168	206	174	180
VVMD5	224	232	226	238	228	238	232	236	226	324	226	236
VVMD7	244	246	236	246	236	236	236	240	323	246	236	240
VVMD21	243	249	243	249	249	257	249	249	249	265	249	249
VVMD24	209	209	209	209	209	217	209	217	213	217	215	217
VVMD25	238	252	240	240	238	246	238	252	240	246	238	246
VVMD27	180	182	186	194	176	190	182	190	180	194	186	190
VVMD28	246	248	238	250	236	238	220	230	248	270	220	238
VVMD32	270	270	254	256	238	238	238	270	262	270	238	270
VVIH54	166	168	166	166	166	182	164	168	166	166	164	168
VVIN16	151	153	151	151	153	153	151	151	149	149	151	159
VVIN73	264	264	256	264	264	268	264	266	264	264	264	266
VVIP31	176	190	188	190	188	188	180	184	184	188	180	180
VVIP60	318	326	318	322	306	314	318	322	318	318	318	320
VVIQ52	85	89	85	85	83	89	83	89	83	83	89	89
VVS2	131	142	131	144	137	151	135	142	131	131	135	151
VVIV37	161	161	163	167	163	163	153	163	163	165	153	163
VVIV67	372	375	364	366	364	372	364	372	364	375	364	372
VrZAG62	187	193	187	193	187	193	187	195	185	195	187	193
VrZAG79	242	248	250	260	246	246	242	244	250	254	238	244
Variety	'De Rey'											

Morphological Characterization

Modal values for the ampelographic descriptions of 'Uva Rey' cultivar corresponding to years 2016 and 2017 are shown in Table 2 compared to the reference cultivar 'Palomino Fino'.

Table 2. Ampelographic description of 'Uva Rey' and 'Palomino Fino' cultivars using the International Organisation of Vine and Wine (OIV) descriptors.

Code	Descriptor	'Uva Rey'	'Palomino Fino'
OIV 001	Young shoot: opening of the shoot tip. 1 closed, 3 half open, 5 fully open.	5	5
OIV 003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip. 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	3	5
OIV 004	Young shoot: density of prostrate hairs on the shoot tip. 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	7	5
OIV 006	Shoot: attitude (before tying). 1 erect, 3 semi-erect, 5 horizontal, 7 semi-drooping, 9 drooping.	3	3
OIV 007	Shoot: colour of the dorsal side of internodes. 1 green, 2 green and red, 3 red.	1	2
OIV 008	Shoot: colour of the ventral side of internodes. 1 green, 2 green and red, 3 red.	1	2
OIV 015-1	Shoot: distribution of anthocyanin coloration on the bud scales. 1 absent, 2 basal, 3 up to 3/4 of bud scale, 4 almost on the whole bud scale.	1	3
OIV 016	Shoot: number of consecutive tendrils. 1 two or less, 2 three or more.	1	1
OIV 051	Young leaf: colour of upper side of blade (4th leaf). 1 green, 2 yellow, 3 bronze, 4 copper-reddish.	3	3
OIV 053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf). 1 none or very low, 3 low, 5, medium, 7 high, 9 very high.	9	5
OIV 065	Mature leaf: size of blade. 1 very small, 3, small, 5 medium, 7 large, 9 very large.	7	7
OIV 067	Mature leaf: shape of blade. 1 cordate, 3 wedge-shaped, 3 pentagonal, 4 circular, 5 kidney-shaped.	3	3

OIV 068	Mature leaf: number of lobes. 1 one, 2 three, 3 five, 4 seven, 5 more than seven.	3	3
OIV 070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade. 1 absent, 2 only at the petiolar point, 3 up to the 1st bifurcation, 4 up to the 2nd bifurcation, 5 beyond the 2nd bifurcation.	1	3
OIV 072	Mature leaf: goffering of blade. 1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong.	7	5
OIV 074	Mature leaf: profile of blade in cross section. 1 flat, 2 V-shaped, 3 involute, 4 revolute, 5 twisted.	5	4
OIV 075	Mature leaf: blistering of upper side of blade. 1 absent or very weak, 2 weak, 3 medium, 4 strong, 9 very strong.	5	3
OIV 076	Mature leaf: shape of teeth. 1 both sides concave, 2 both sides straight, 3 both sides convex, 4 one side concave on side convex, 5 mixture between both sides straight and both sides convex.	2	3
OIV 079	Mature leaf: degree of opening/overlapping of petiole sinus. 1 very wide open, 3 open, 5 closed, 7 overlapped, 9 strongly overlapped.	3	5
OIV 080	Mature leaf: shape of base petiole sinus. 1 U-shaped, 2 brace-shaped, 3 V-shaped.	3	3
OIV 081-1	Mature leaf: teeth in the petiole sinus. 1 none, 9 present.	1	1
OIV 081-2	Mature leaf: petiole sinus base limited by vein. 1 not limited, 3 on one side, 3 on both sides.	1	1
OIV 083-2	Mature leaf: teeth in the upper lateral sinuses. 1 none, 9 present.	1	1
OIV 084	Mature leaf: density of prostrate hairs between main veins on lower side of blade. 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	7	7
OIV 087	Mature leaf: density of erect hairs on main veins on lower side of blade. 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	9	1
OIV 151	Flower: sexual organs. 1 fully developed stamens and no gynoecium, 2 fully developed stamens and reduced gynoecium, 3 fully developed stamens and fully developed gynoecium, 4 reflexed stamens and fully developed gynoecium.	3	3

OIV 202	Bunch: length (peduncle excluded). 1 very short, 3 short, 5 medium, 7 long, 9 very long.	5	7
OIV 203	Bunch: width. 1 very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide.	5	5
OIV 204	Bunch: density. 1 very loose, 3 loose, 5 medium, 7 dense, 9 very dense.	5	5
OIV 206	Bunch: length of peduncle of primary bunch. 1 very short, 3 short, 5 medium, 7 long, 9 very long.	3	1
OIV 220	Berry: length. 1 very short, 3 short, 5 medium, 7 long, 9 very long.	5	3
OIV 221	Berry: width. 1very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide.	5	3
OIV 223	Berry: shape. 1 obloid, 2 globose, 3 broad ellipsoid, 4 narrow ellipsoid, 5 cylindrical, 6 obtuse ovoid, 7 ovoid, 8 obovoid, 9 horn shaped, 10 finger shaped.	7	2
OIV 225	Berry: colour of skin. 1 green yellow, 2 rose, 3 red, 4, grey, 5 dark red violet, 6 blue black.	1	1

34 descriptors were studied, eight of which correspond to shoots, 17 to leaves, one to inflorescence, four to bunches and four to berries. In regard to the density of prostate hairs between the main veins on lower side of blade (OIV 053), 'Uva Rey' showed very high density while 'Palomino Fino' prostate hair density was medium. Also, the density of erect hairs on the main veins on the lower side of tthe blade (OIV 087) was high for 'Uva Rey' and non-existent or low for 'Palomino Fino' cultivar. Finally, grape berries were green yellow in both cases (OIV 225), but their shapes differed (OIV 223), being ovoid for 'Uva Rey' and globose for 'Palomino Fino'.

Physicochemical Characterization of Grapes and Musts.

'Uva Rey' and 'Palomino Fino' grape must physicochemical characterizations and berry texture profile analyses (TPA) from two vintages (2016 and 2017) are displayed in Table 3.

Table 3. 'Uva Rey' and 'Palomino Fino' grape berry texture profile analysis (TPA) and must characterization.

	2016		2017	
	'Palomino Fino'	**'Uva Rey'**	**'Palomino Fino'**	**'Uva Rey**
Physicochemical Parameters				
pH	3.93 ± 0.01 [a]	3.87 ± 0.07 [a]	4.02 ± 0.03 [a]	3.97 ± 0.02 [a]
Total Acidity (g/L TH_2)	3.74 ± 0.05 [a]	3.51 ± 0.07 [a]	3.15 ± 0.08 [b]	3.25 ± 0.21 [b]
Sugar (°Bé)	12.85 ± 0.01 [a]	8.45 ± 0.02 [b]	11.70 ± 0.02 [c]	7.40 ± 0.06 [d]
Ripening Index (RI)	3.44 ± 0.02 [a]	2.41 ± 0.01 [b]	3.71 ± 0.02 [a]	2.28 ± 0.01 [b]
YAN (mg/L)	200.00 ± 2.00 [a]	140.00 ± 2.00 [b]	161.00 ± 6.00 [c]	140.00 ± 3.00 [b]
Tartaric Acid (g/L)	3.140 ± 0.050 [a]	2.720 ± 0.008 [b]	2. 470 ± 0.100 [b]	2.600 ± 0.200 [b]
Citric Acid (g/L)	0.030 ± 0.005 [a]	0.100 ± 0.001 [b]	0.030 ± 0.010 [a]	0.150 ± 0.002 [c]
Malic acid (g/L)	0.420 ± 0.020 [a]	0.650 ± 0.003 [b]	0.100 ± 0.020 [c]	0.600 ± 0.010 [d]
TPA				
Consistency (Nmm)	89.58 ± 1.59 [a]	138.24 ± 8.47 [b]	93.66 ± 2.27 [a]	152.42 ± 11.18 [c]
Hardness (Nmm)	237.57 ± 4.58 [a]	239.20 ± 7.56 [a]	237.29 ± 5.18 [a]	245.05 ± 12.08 [a]
WoP (Nmm)	260.47 ± 12.87 [a]	351.35 ± 14.98 [b]	280.13 ± 16.70 [a]	409.93 ± 23.70 [c]
Cohesiveness	0.21 ± 0.02 [a]	0.41 ± 0.02 [b]	0.23 ± 0.02 [a]	0.40 ± 0.03 [b]

Different superscript letters mean statistically significant differences between samples at p-adjust < 0.05 obtained by two-way ANOVA and Bonferroni's multiple range (BSD) test. Results are the means ± SD of three repetitions.

The main differences between 'Uva Rey' and 'Palomino Fino' cultivars grape musts were related to the physicochemical parameters sugar (°Bé), YAN (mg/L), malic acid (g/L) and TPA consistency (Nmm) and cohesiveness. The pH values obtained for both cultivars as well as for the two vintages were all similar. However, both cultivars exhibited very similar acidity in both vintages, with slightly higher values in 2017 (ANOVA p-adjust < 0.05). Regarding grape sugar content, it was significantly higher in 'Palomino Fino' grapes than in 'Uva Rey' from the two vintages studied (ANOVA p-adjust < 0.05). Again, greater sugar values (°Bé) as well as total acidity were measured in 2016 grapes from both cultivars (Table 3). Consequently, Ripening Index (RI) values obtained were significantly greater in 'Palomino Fino' than in 'Uva Rey'. However, very different content levels in both cultivars were obtained for YAN, where 'Palomino Fino' showed significantly higher concentrations of YAN than 'Uva Rey' (ANOVA p-adjust < 0.05), which yielded the same content level in the two vintages under study (Table 3).

Regarding organic acids content, it could be observed that tartaric acid represents over 75% of their total acidity. It can be seen that this particular acid content follows the same trend as the total acidity of the grapes. With respect to citric acid concentration, it was significantly lower in 'Palomino Fino' than in 'Uva Rey' cultivar and did not exceed 150 mg/L in either case. However, 'Uva Rey' showed a significantly higher content of malic acid than 'Palomino Fino' in both of the vintages studied (ANOVA p-adjust < 0.05).

With respect to the results obtained from the TPA, 'Uva Rey' obtained higher values for consistency, WoP and cohesiveness than 'Palomino Fino' in both vintages (ANOVA p-adjust < 0.05). However, no differences were observed between cultivars or vintages with regards to grape berry hardness.

Discussion

To identify grapevine cultivars, nuclear microsatellite markers are the most widely used tool, as was demonstrated by the European projects GENRES 081 and GrapeGen06. Regardless of the high degree of heterozygosity existing in the grapevine, the genotype with six microsatellite loci (VVMD5, VVMD7, VVMD27, VVS2, VrZAG62 and VrZAG79) is enough to establish the identity of a variety [6], with the exception of the peculiar case of closely related varieties [36] which requires analysis of more loci. For this reason, as a result of the GrapeGen06 project, an international consensus was established to increase the number of microsatellite loci to 20, located in different binding groups for correct identification. In this study, the analysis was extended to 22 microsatellite loci. It is very important to use the same microsatellite loci in different studies in order to be able to compare genotypes later. The identification of 'Uva Rey' genotype allowed us to confirm the synonyms of this cultivar with both 'De Rey' and 'Mantúo de Pilas', which have already been registered in the *Vitis* International Variety Catalogue (VIVC) at seven loci SSR [35]. The genetic profile for 15 additional loci is presented in this study and the synonymy between 'De Rey' and 'Uva Rey' is confirmed for the first time with the analysis at 22 microsatellite loci. Along with the cultivar genetic identification and, according to the recommendation for the adequate characterisation of *Vitis* genetic material, an ampelographic description was carried out [46]. Such morphological description has been the method previously used by different countries to have a particular cultivar included in the official lists [46]. The phenotype obtained for the cultivar 'Uva Rey' showed some differences with 'Mantuo de Pilas' as described by García de Luján et al. [47]. Some differences were found in OIV 007, OIV 008, OIV 051, OIV 053, OIV 070, OIV 074, OIV 075, OIV 087, OIV 202 and OIV 221 descriptors. It is worth mentioning, the differences in erect hairs density on main veins on lower side of blade in mature leaves (OIV 087). 'Uva Rey' showed a very high density unlike 'Mantuo de Pilas' with a very low one. Similar phenotypic differences have been found between other cultivars such as 'Garnacha' and 'Garnacha Peluda' [48], both considered as somatic variants.

Due to the high temperatures associated to the current global warming, the period during which the minimal temperatures required for the physiological activities of vines is reached is longer than it used to be, and hence, there is an increment in metabolic rates that have an impact on metabolite accumulation [49,50]. In the last 10–30 years, some major changes have been observed in grape development and ripening patterns, such as premature budbreak, flowering and fruit maturity due to agroclimatic changes [51]

The differences between the two cultivars with regards to pH and total acidity can be attributed to climate variations between the two years studied, as such differences can be found in both cultivars (Figures S1 and S2). RI values confirm the above-mentioned differences between cultivars (ANOVA p-adjust < 0.05), with significant differences between both cultivars regardless of the vintage analysed. The variations of these parameters associated to grape ripening processes may be related with each cultivar's phenological stages. 'Uva Rey' is, unlike 'Palomino Fino' a long cycle cultivar [52]. For this reason, grape ripening stages are not reached at the same time.

Organic acids content in each cultivar could be due to their phenological cycle differences [52]. With regard to tartaric acid content, the values remained similar except for 'Palomino Fino' cultivar in the 2016 year. During the grape ripening process, the production of malic acid decreases [53] since this carboxylic acid is also used by the plant at this stage for energy production [54]. In this way, the different malic acid content levels in each cultivar could be explained by their aforementioned asynchronous phenological cycles. Such difference in malic acid content levels could be relevant to prospective winemaking process, where malolactic fermentation (MLF) could result in wines with a greater microbiological stability and sensory complexity [55]. Some authors argue that higher weather temperatures due to global warming may lead to grape musts with a higher pH, which in turn may promote oxidation reactions [51,56]. In this sense, grapevine cultivars with similar characteristics to those presented by 'Mantúo de Pilas' could lead to the production of wines through oxidative ageing.

The YAN values that have been observed in 'Palomino Fino' musts were higher than those observed in 'Uva Rey' for both vintages. Such differences between the two cultivars may be related to the variations observed in their ripening processes, since YAN content increases in grape berries when ripening [57]. In any case, YAN values remained at a sufficient level for a proper alcoholic fermentation (AF) [58]. Yeast assimilable nitrogen (YAN) is a fundamental element for the correct AF of grape musts; since nitrogen is essential for the completion of some yeasts, its presence is compulsory for yeasts to develop in normal conditions during this biological process [59].

According to the TPA, the two vintages of 'Uva Rey' in the study had a higher consistency, hardness, WoP and cohesiveness. It should be noted that cohesiveness depends on the strength of the pulp internal bonds of the grape berries. This parameter is highly related to the OIV 235 descriptor [41], which is employed for the sensory evaluation of grapes during their ripening process. The results obtained from the TPA could be explained by the lack of synchrony between both cultivars phenological cycles.

'Uva Rey' berries, with a longer cycle, were less ripe and therefore presented a greater turgidity at the time of analysis. Such superior berry turgidity plus its higher consistency and WoP could contribute to protect grape berries from dehydration under Andalusian warm weather conditions (SW Spain). When these results are compared to those obtained by Giacosa et al. [60], it can be observed that 'Palomino Fino' presents similar cohesiveness to 'Perle von Csaba' cultivar (Hungarian white vinification grape). Nonetheless, 'Uva Rey' showed a higher degree of similarity with the cultivar 'Sultanina' (a Turkish white table grape). In view of its grape berry TPA, 'Uva Rey' could be considered as a cultivar with a greater resistance than 'Palomino Fino', mainly because of its greater pulp cohesiveness and consistency. These results might be influenced by the phenological cycle differences observed between the two cultivars studied, where the higher values correspond to less ripe berries. In this sense, these phenotypical traits could increase the cultivar's resistance to drought and to high temperatures, which would make it a more appropriate cultivar for warm dry areas and for global warming conditions.

Conclusions

Microsatellite analysis confirmed that 'Uva Rey' is a synonym of 'De Rey' cultivar and a somatic variant of 'Mantuo de Pilas'. With respect to the physicochemical grape must characterization, major differences were found in YAN and malic acid concentration. The TPA showed that 'Uva Rey' grape berries are more cohesive and consistent than 'Palomino Fino' ones. In this sense, 'Uva Rey' can be stated as an autochthonous grapevine cultivar with a long phenological cycle. This study recognizes Uva Rey as a somatic variant of 'Mantuo de Pilas' and as such, supports any actions towards its recovery. According to the results obtained from the different analysis that have been completed on 'Uva Rey' grape berries and musts from two consecutive vintages, this autochthonous cultivar should be further studied and included in the Spanish official register to allow is cultivation.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4395/9/9/563/s1, Figure S1. (a) Temperature (°C) (T^a_max, T^a_min, T^a_avg), (b) humidity (%) (H_max, H_min, H_avg) and (c) radiation (W/m^2) and rainfall (L/m^2) between July and September 2016. Figure S2. (a) Temperature (°C) (T^a_max, T^a_min, T^a_avg), (b) humidity (%) (H_max, H_min, H_avg) and (c) radiation (W/m^2) and rainfall (L/m^2) between July and September 2017.

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Supplementary Materials

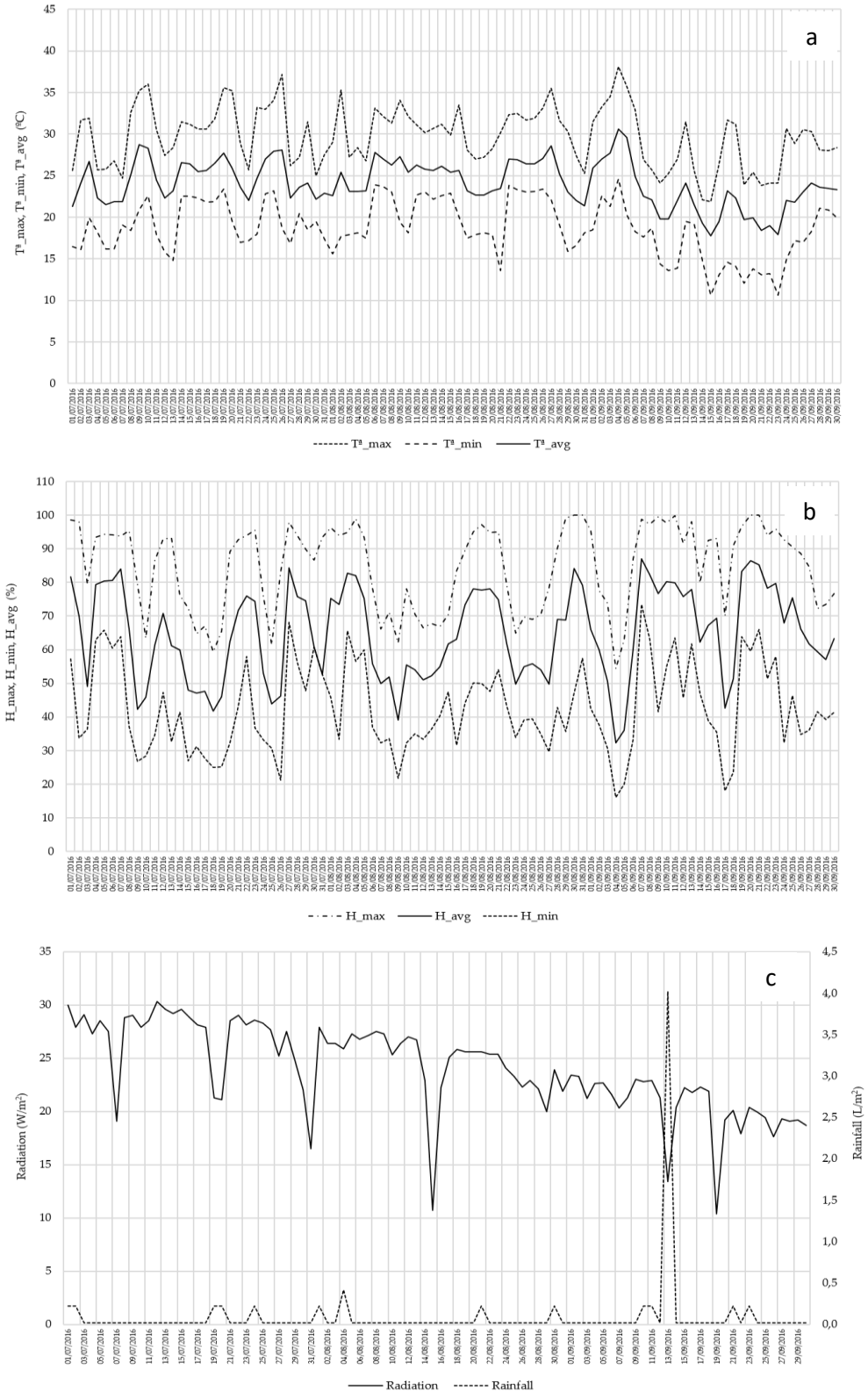

Figure S1. a) Temperature (ºC) (Tª_max, Tª_min, Tª_avg), b) Humidity (%) (H_max, H_min, H_avg) and c) Radiation (W/m2) and Rainfall(L/m2) between July – September 2016.

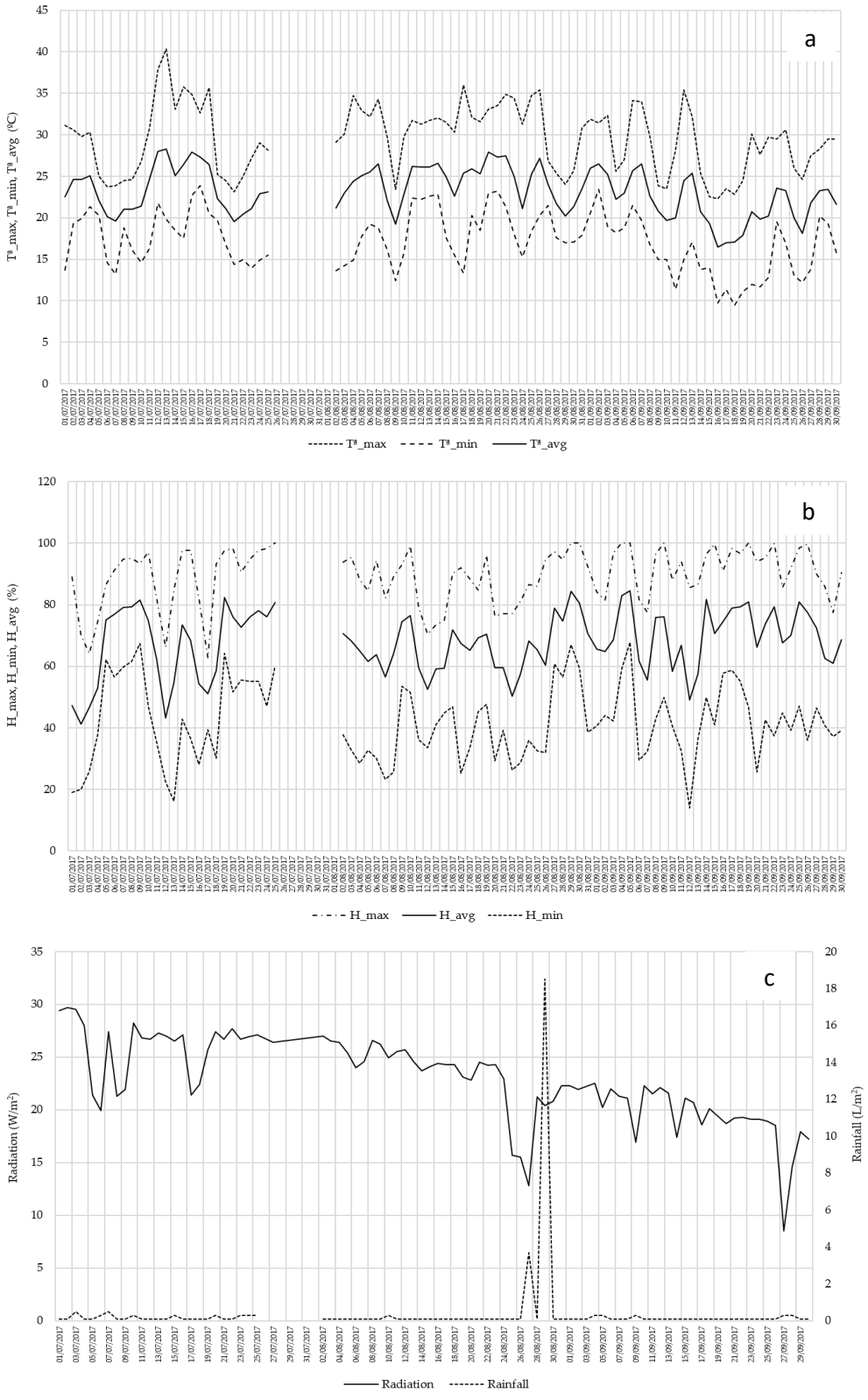

Figure S2. a) Temperature (ºC) (Tª_max, Tª_min, Tª_avg), b) Humidity (%) (H_max, H_min, H_avg) and c) Radiation (W/m2) and Rainfall(L/m2) between July – September 2017.

2) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Identification and Characterization of White Grape Varieties Autochthonous of a Warm Climate Region (Andalusia, Spain). *Agronomy* **2020**, *10*, 205; doi:10.3390/agronomy9090563.

Abstract

The high sensitivity of one of the most important crops in the world, such as vine (*Vitis vinifera* L.), to particular changes caused by the phenomena associated with global warming, is encouraging the wine industry to place value on grape varieties that are autochthonous to each production area. These are generally conserved in germplasm banks and may pose a useful tool to counteract the effects of climate change. In order to determine the actual resource that such varieties constitute, this research has carried out a genetic identification, a morphological characterization, and an analysis of the grape musts obtained from four autochthonous varieties (Cañocazo, Castellano, Mantúo de Pilas, and Palomino Fino). This genetic analysis has allowed the identification of autochthonous varieties with different genotypes. However, all of them had similar phenotypic characteristics in terms of high hair density in adult leaves. With respect to the physicochemical composition of the musts, significant differences have been observed between the autochthonous varieties, with respect to the control variety of Palomino Fino. Nevertheless, all of them have exhibited an adequate physicochemical composition to produce quality white wines. For all of the above reasons, these local varieties should be considered suitable for cultivation in areas with warmer and drier climates, such as Andalusia (Spain).

Introduction

The so-called area known as Marco de Jerez, located in the south of the Iberian Peninsula, is one of the most important wine-growing regions in Spain, which reached its fullness and international recognition during the 19th century [1]. However, the wines produced in this area have evolved throughout history because of different biological and political circumstances. From a viticultural point of view, the invasion of *phylloxera* in the area in 1894 caused the loss of a large part of the Jerez vineyards, which had to be replanted [2]. This led to a significant loss of vine varieties. Clemente and Rubio [3], at the beginning of the 19th century, described 43 vine varieties that were cultivated in the Marco de Jerez vineyards before the *phylloxera* outbreak. After the replanting of the vineyards to deal with the plague, the number of varieties cultivated dropped significantly. Fernández de Bobadilla [4] quotes among other replanted vines: Palomino Fino, Pedro Ximénez, Cañocazo, and Albillo as classic varieties, Garrido, Perruno,

Mantúo, and Beba as secondary varieties, and Moscatel and Tintilla de Rota as special varieties. Subsequently, severe regulations were approved and the vine varieties that were authorized for wine production were restricted [5]. Likewise, in the second half of the 20th century, based on productivity criteria, clone plant material was introduced in the new Palomino Fino plantations. This caused a loss of genetic resources from this variety. Consequently, only three white grape varieties are currently grown in Marco de Jerez for the production of Sherry wines: Palomino Fino, Pedro Ximénez, and Moscatel, although the last two are grown at a very small scale [6]. For this reason, in order to preserve the biodiversity in the *Vitis vinifera* species, and to safeguard the different autochthonous varieties from each zone, grapevine germplasm collections, or banks, were created [7–11].

At present, these autochthonous vine varieties, which have been conserved in the germplasm banks, can be considered a valuable genetic resource for addressing one of the most important challenges that the global wine sector faces: global warming [10]. Each variety has its own specific genotype, morphology, and content in its secondary metabolites that make it unique [12]. All of these elements explain the adaptation of vine varieties to different climates, or environments, and the physicochemical properties that their berries have [13–16]. There are numerous works related to the genetic and morphological characterization of autochthonous vine varieties using Simple Sequence Repeat (SSR), or microsatellites markers and ampelographic descriptors [17–19]. However, these studies do not include data on the physicochemical compositions of their musts, which are decisive to determine their oenological potential and the adaptation capacity of these varieties to the climatic conditions in the area. In addition, the studies on the identification and characterization of the agronomic and oenological behavior of a particular vine variety are an essential requirement when it comes to applying for its inclusion in the register of authorized varieties.

For all these reasons, the main objective of this work focuses on the identification and characterization of white autochthonous grape varieties grown in a warm climate region (Andalusia, Spain). Its morphological and molecular characterization could contribute to the detection of new synonyms, homonyms, or false attributions. On the other hand, the analysis of their musts could contribute to producing new white wines in warm climate areas.

Materials and Methods

Grapevine Material

Three autochthonous vine varieties have been analyzed: Cañocazo (CÑ), Castellano (CS) and Mantúo de Pilas (MP). Palomino Fino (PF) has been employed as the control variety, since it is the most commonly cultivated autochthonous variety in the Marco de Jerez region (Andalusia, Spain) [20]. A total of ten plants from each variety were selected for the study (2016–2017), following Santesteban et al. [21] criteria, in order to minimize

the intrinsic variability of samplings. For this purpose, the trunk cross sectional area (TCSA) of 40 vines was measured at a 30 cm height using a digital Vernier Caliper Maurer 93110 (Padova, Italy). The 10 plants marked as subjects were selected for presenting a TCSA value close to the mean ± 10%. All these varieties were planted on the same date and were located on the same plot (latitude 36° 41' 10'' N; longitude 6º 08' 10'' W; 20 m above sea level), in the municipality of Jerez de la Frontera (Cadiz, Spain). The plot has a limestone soil, a plantation surface of 2.30 × 1.15 m, and a double Guyot training system. No fertilization or irrigation treatments were applied during the studied years, and different conventional phytosanitary products were applied to obtain ripe and sound grapes. Supplemental Figures S1a-c and S2a-c show the historical temperature, humidity, precipitation, and solar radiation during the period between the veraison and the harvest date (from July to September) in the two years studied respectively.

Only for genetic characterization (SSR analysis), four other reference varieties planted in the same plot (Cabernet Sauvignon (CSV), Chardonnay (CH), Muscat a Petits Grains Blancs (MPGB), and Pinot Noir (PN)) were included in order to compare the genotypes obtained with those in the databases, to confirm the identity of the variety analyzed.

Simple Sequence Repeat (SSR) Analysis

A total of 22 nuclear microsatellite loci were employed to perform the varietal identification following the methodology proposed in recently published papers [22]. A DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was employed to carry out the DNA extraction. PCR amplifications were performed using a 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA), and the amplified products were separated by capillary electrophoresis, using an automated sequencer ABI PRISM 3130 (Applied Biosystems, Foster City, CA, USA). Fluorescent labelled fragments (6-FAM, VIC, PET, and NED) were detected and sized using GeneMapper v. 3.7, and fragment lengths were assessed with the help of internal standards GeneScan-500 LIZTM (Applied Biosystems, Foster City, CA, USA). The comparison of the SSR obtained was performed using a microsatellite toolkit v. 9.0 software [23]. Lastly, the microsatellite genotypes obtained after the analysis were compared to the genetic profiles given by Lacombe et al. [24], and to the data contained in several genetic databases [25–28] and scientific research.

Ampelographic Characterization

Three autochthonous varieties (Cañocazo (CÑ), Castellano (CS), and Mantúo de Pilas (MP)), and the control variety Palomino Fino (PF) were characterized ampelographically. At least ten young shoots, young and mature leaves, flowers, bunches, and berries from each variety were described using Benito et al. [29] criteria, and 36 descriptors, according to the International Organization of Vine and Wine's descriptor list [17] (14 priority descriptors for primary descriptions plus 22 additional

descriptors). Eight of those descriptors correspond to the branches, 19 to the leaves, one to the inflorescence, four to the bunches, and four to the berries. Samples from two consecutive crops of all the varieties were described by five ampelographers with varied expertise knowledge. The modal value for each descriptor was selected as the final descriptor.

Physicochemical Analysis

For the physicochemical characterization, 5 kg of berries of each variety (500 g per plant) were harvested on the date recommended by the winery. All the samples were harvested at the same time since the varieties were planted on the same plot and were therefore processed when the control variety Palomino Fino was harvested. The sugar content (°Bé), total acidity (TA), pH, tartaric acid, malic acid, glycerine, oxidative index, yeast assimilable nitrogen (YAN), and the concentration of cations potassium, calcium, magnesium, iron, copper, and sodium were determined in the musts of the four varieties that were studied for two consecutive years. All of the analyses were carried out in triplicate.

°Bé was determined using a calibrated Dujardin–Salleron hydrometer (Laboratories Dujardin-Salleron, Arcueil Cedex, France). Total acidity (TA) was assessed following the International Organization of Vine and Wine (OIV) reference method [30]. The pH was measured using a digital pH-meter CRISON-2001 (Crison, Barcelona, Spain), equipped with a combined electrode with automatic temperature compensation. Organic Acids (tartaric and malic acid) were assessed using an ionic chromatograph (Metrohm 930 Compact IC Flex) with a conductivity detector, following the conditions proposed by Sancho-Galán et al. [31]. Yeast Assimilable Nitrogen (YAN) was determined according to the described formal method [32]. The glycerine content of the samples was determined by means of an enzymatic kit (Biosystems, Barcelona, Spain) and the colorimetric measurement of the enzymatic reaction in a HITACHI UV-Vis spectrophotometer (Pacisa y Giralt S.L, Madrid, Spain). All the samples were previously filtered through a 0.45 μm nylon syringe filter (FILTER-LAB, Barcelona, Spain) for chromatographic and spectrophotometric analysis. To calculate the oxidative index, the musts were measured at a wavelength of 420 nm. The musts were then incubated at 45 °C for 5 days, and after this time, the absorbance was again determined at 420 nm. The oxidative index was calculated according to the equation $((Abs_{end} - Abs_{beginning}) / Abs_{beggining}) \times 100$ and expressed as a percentage.

To determine the cation content in the musts, 20 mL of the samples were first incinerated in a Carbolite ELF 11/148 furnace (Sigma Aldrich, Saint Louis, United States) at 500 °C for two hours. Once the ashes were obtained, they were digested acidically using nitric acid, following the protocol proposed by the Association Française de Normalisation (AFNOR) [33]. All the cations were determined by atomic emission spectroscopy by inductively coupled plasma in an Iris Intrepid ICP-AES (Thermo Scientific, Waltham, United States).

Statistical Analysis

Means and standard deviations were calculated and significant differences were evaluated by one-way ANOVA and Bonferroni's multiple range (BSD) test with a *p*-adjust < 0.05 (GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego, CA) statistical package. Principal component analysis (PCA) was performed using the statistical computer package SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

Results

Simple Sequence Repeat (SSR) Analysis

The genotypes obtained after the analysis of the autochthonous varieties and reference varieties with 22 microsatellites loci are shown in Table 1. The three microsatellite profiles obtained for each one of the autochthonous varieties have been matched with known varieties or "prime names" according to the *Vitis* International Variety Catalogue (*VIVC*). In addition, the different genotypes have been compared to the genotypes published in the databases that are kept at Rancho de la Merced Germplasm Bank [7,26], the El Encín Germplasm Bank [27,28], and other European databases [24], in order to establish new synonyms.

Table 1. Genetic profiles of the autochthonous and reference varieties at 22 microsatellite loci. Allele sizes are given in base pairs.

	Autochthonous variety								**Reference variety**							
Variety code	**CÑ**		**CS**		**MP**		**PF**		**CSV**		**CH**		**MPGB**		**PN**	
Microsatellite locus																
VVIB01	291	307	291	307	307	307	291	307	291	291	289	295	291	295	289	295
VMC1b11	184	188	184	184	184	188	184	188	184	184	166	184	184	188	166	172
VMC4F31	188	206	168	176	184	190	176	206	174	178	174	180	168	206	174	180
VVMD5	232	234	220	224	224	232	226	238	228	236	232	236	226	234	226	236
VVMD7	240	246	236	246	244	246	236	246	236	236	236	240	232	246	236	240
VVMD21	249	255	243	265	243	249	243	249	249	257	249	249	249	265	249	249
VVMD24	209	209	209	211	209	209	209	209	209	217	209	219	213	217	215	217
VVMD25	240	252	252	252	238	252	240	240	238	246	238	252	240	246	238	246
VVMD27	186	194	182	182	182	182	186	194	176	190	182	190	180	194	186	190
VVMD28	236	250	246	246	246	248	238	250	236	238	220	230	248	270	220	238
VVMD32	254	270	270	270	270	270	254	256	238	238	238	270	262	270	238	270
VVIH54	166	166	166	168	166	168	166	166	166	182	164	168	166	166	164	168
VVIN16	153	153	151	151	151	153	151	151	153	153	151	151	149	149	151	159
VVIN73	264	264	264	264	264	264	264	264	264	268	264	266	264	264	264	266
VVIP31	180	190	176	176	176	190	188	190	190	190	180	184	184	188	180	180
VVIP60	318	326	322	322	318	326	318	322	306	314	318	322	318	318	318	320
VVIQ52	85	89	85	89	85	89	85	85	83	89	83	89	83	83	89	89
VVS2	142	144	142	142	131	142	131	144	137	151	135	142	131	131	135	151
VVIV37	163	177	163	167	161	161	163	167	163	163	153	163	163	165	153	163
VVIV67	358	372	366	375	372	375	364	366	364	372	364	372	364	375	364	372
VrZAG62	187	203	187	193	187	193	187	193	187	193	187	195	185	195	187	193
VrZAG79	236	246	236	258	242	248	250	260	246	246	242	244	250	254	238	244

CÑ: Cañocazo. CS: Castellano. MP: Mantúo de Pilas. PF: Palomino Fino. CSV: Cabernet Sauvignon. CH: Chardonnay. MPGB: Muscat a Petits Grains Blancs. PN: Pinot Noir.

Ampelographic Characterization

The results of the morphological description are shown in Table 2. All the varieties presented different phenotypes. The main morphological differences in the leaves were observed in the variety Mantúo de Pilas, which presented a very high density of prostate hairs between main veins on the lower side of the blade in young leaves (OIV 053), and a very high density in prostate hairs on the main veins on the lower side of the blade in adult leaves (OIV 086). OIV 233 descriptor refers to the shape of the berry, and was the most discriminating descriptor among the four varieties characterized, with different shapes being observed for each of the varieties.

Table 2. Modal values for the International Organization of Vine and Wine (OIV) ampelographic descriptors observed in the four varieties analyzed during two consecutive years.

Code	Descriptor	CÑ	CS	MP	PF
OIV 001	Young shoot: opening of the shoot tip; 1 closed, 3 half open, 5 fully open.	5	5	5	5
OIV 003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	1	7	1	5
OIV 004	Young shoot: density of prostrate hairs on the shoot tip; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	5	7	5
OIV 006	Shoot: attitude (before tying); 1 erect, 3 semi-erect, 5 horizontal, 7 semi-drooping, 9 drooping.	1	3	1	3
OIV 007	Shoot: color of the dorsal side of internodes; 1 green, 2 green and red, 3 red.	1	2	1	2
OIV 008	Shoot: color of the ventral side of internodes; 1 green, 2 green and red, 3 red.	1	2	1	2
OIV 015-2	Shoot: intensity of anthocyanin coloration on the bud scales; 1 none or very weak, 3 weak, 5 medium, 7 strong, 9 very strong.	1	5	1	3
OIV 016	Shoot: number of consecutive tendrils; 1 two or less, 2 three or more.	1	1	1	1
OIV 051	Young leaf: color of upper side of blade (4th leaf); 1 green, 2 yellow, 3 bronze, 4 copper-reddish.	1	3	3	3

OIV 053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf); 1 none or very low, 3 low, 5, medium, 7 high, 9 very high.	7	7	9	5
OIV 065	Mature leaf: size of blade; 1 very small, 3, small, 5 medium, 7 large, 9 very large.	5	5	5	7
OIV 067	Mature leaf: shape of blade; 1 cordate, 3 wedge-shaped, 3 pentagonal, 4 circular, 5 kidney-shaped.	3	3	3	3
OIV 068	Mature leaf: number of lobes; 1 one, 2 three, 3 five, 4 seven, 5 more than seven.	3	3	3	3
OIV 070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade; 1 absent, 2 only at the petiolar point, 3 up to the 1st bifurcation, 4 up to the 2nd bifurcation, 5 beyond the 2nd bifurcation.	1	1	1	3
OIV 074	Mature leaf: profile of blade in cross section; 1 flat, 2 V-shaped, 3 involute, 4 revolute, 5 twisted.	5	5	3	4
OIV 075	Mature leaf: blistering of upper side of blade; 1 absent or very weak, 2 weak, 3 medium, 4 strong, 9 very strong.	5	3	5	3
OIV 076	Mature leaf: shape of teeth; 1 both sides concave, 2 both sides straight, 3 both sides convex, 4 one side concave on side convex, 5 mixture between both sides straight and both sides convex.	3	3	2	3
OIV 079	Mature leaf: degree of opening/overlapping of petiole sinus; 1 very wide open, 3 open, 5 closed, 7 overlapped, 9 strongly overlapped.	7	7	3	5
OIV 080	Mature leaf: shape of base petiole sinus; 1 U-shaped, 2 brace-shaped, 3 V-shaped.	3	3	3	3
OIV 081-1	Mature leaf: teeth in the petiole sinus; 1 none, 9 present.	1	2	1	1
OIV 081-2	Mature leaf: petiole sinus base limited by vein; 1 not limited, 3 on one side, 3 on both sides.	1	1	1	1
OIV 083-1	Mature leaf: shape of the base of upper lateral sinuses; 1 U-shaped, 2 brace-shaped, 3 V-shaped.	3	3	1	1
OIV 083-2	Mature leaf: teeth in the upper lateral sinuses; 1 none, 9 present.	1	1	1	1

OIV 084	Mature leaf: density of prostrate hairs between main veins on lower side of blade; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	7	5	7
OIV 085	Mature leaf: density of erect hairs between main veins on lower side of blade; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	5	5	5
OIV 086	Mature leaf: density of prostrate hairs on main veins on lower side of blade; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	5	9	5
OIV 087	Mature leaf: density of erect hairs on main veins on lower side of blade; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	5	3	1
OIV 151	Flower: sexual organs; 1 fully developed stamens and no gynoecium, 2 fully developed stamens and reduced gynoecium, 3 fully developed stamens and fully developed gynoecium, 4 reflexed stamens and fully developed gynoecium.	3	3	3	3
OIV 202	Bunch: length (peduncle excluded); 1 very short, 3 short, 5 medium, 7 long, 9 very long.	7	7	7	7
OIV 203	Bunch: width; 1 very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide.	5	5	5	5
OIV 204	Bunch: density; 1 very loose, 3 loose, 5 medium, 7 dense, 9 very dense.	3	5	5	5
OIV 206	Bunch: length of peduncle of primary bunch; 1 very short, 3 short, 5 medium, 7 long, 9 very long.	1	1	1	1
OIV 220	Berry: length; 1 very short, 3 short, 5 medium, 7 long, 9 very long.	5	5	5	3
OIV 221	Berry: width; 1 very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide.	5	5	5	3
OIV 223	Berry: shape; 1 obloid, 2 globose, 3 broad ellipsoid, 4 narrow ellipsoid, 5 cylindric, 6 obtuse ovoid, 7 ovoid, 8 obovoid, 9 horn shaped, 10 finger shaped.	1	7	5	2
OIV 225	Berry: color of skin; 1 green yellow, 2 rose, 3 red, 4 grey, 5 dark red violet, 6 blue black.	1	1	1	1

CÑ: Cañocazo. CS: Castellano. MP: Mantúo de Pilas. PF: Palomino Fino.

Grape Must Physicochemical Characterization

Table 3 shows the results (mean value ± standard deviation) of the physicochemical analyses carried out on the musts from the four varieties studied over two consecutive years. The autochthonous varieties showed differences in sugar concentration in the two years studied.

Table 3. Autochthonous varieties and Palomino Fino grape must characterization during two consecutive years (2016 and 2017).

Physicochemical parameters	**CÑ**	**CS**	MP	PF
		2016		
°Bé	10.88 ± 0.02 [a]	11.54 ± 0.01 [b]	9.12 ± 0.02 [c]	11.10 ± 0.01 [d]
pH	3.72 ± 0.02 [a]	3.94 ± 0.01 [b]	3.93 ± 0.07 [b]	3.77 ± 0.01 [a]
TA ($g \cdot L^{-1}$ TH_2)	3.79 ± 0.06 [a]	3.47 ± 0.04 [b]	3.51 ± 0.07 [b]	3.24 ± 0.06 [c]
Ripening Index (RI)	2.87 ± 0.02 [a]	3.32 ± 0.06 [b]	3.51 ± 0.01 [c]	3.42 ± 0.04 [b]
Tartaric acid (g/L)	2.80 ± 0.03 [a]	2.96 ± 0.01 [b]	2.88 ± 0.05 [a]	2.37 ± 0.04 [c]
Malic acid(g/L)	1.08 ± 0.08 [a]	0.80 ± 0.03 [b]	1.20 ± 0.10 [c]	0.24 ± 0.00 [d]
Glycerin (g/L)	0.26 ± 0.00 [a]	0.35 ± 0.00 [b]	0.33 ± 0.00 [b]	0.19 ± 0.01 [d]
Oxidative index (%)	10.45 ± 0.64 [a]	29.97 ± 1.05 [b]	21.18 ± 0.71 [c]	56.32 ± 1.57 [d]
YAN (mg/L)	184.73 ± 1,07 [a]	188.50 ± 1.54 [a,c]	143.57 ± 2.50 [b]	192.57 ± 2.14 [c]
Calcium (mg/L)	362.33 ± 1.00 [a]	158.70 ± 1.00 [b]	146.07 ± 1.00 [c]	167.95 ± 1.00 [b]
Magnesium (mg/L)	151.61 ± 0.30 [a]	82.72 ± 0.20 [b]	80.72 ± 0.40 [b]	75.47 ± 0.20 [c]
Sodium (mg/L)	13.16 ± 0.20 [a]	9.24 ± 0.11 [b]	6.06 ± 0.40 [c]	13.82 ± 0.50 [d]
Potassium (mg/L)	3228.55 ± 4.00 [a]	2369.66 ± 9.01 [b]	1749.59 ± 9.00 [c]	2308.84 ± 12.00 [b]
Iron (mg/L)	3.79 ± 0.01 [a]	6.21 ± 0.04 [b]	6.25 ± 0.10 [c]	8.20 ± 0.20 [d]
Copper (mg/L)	0.79 ± 0.01 [a]	1.20 ± 0.02 [b]	1.19 ± 0.04 [b]	3.95 ± 0.03 [c]
		2017		
°Bé	9.05 ± 0.02 [a]	9.40 ± 0.01 [b]	8.65 ± 0.01 [c]	10.65 ± 0.01 [d]
pH	3.76 ± 0.01 [a]	3.92 ± 0.01 [b]	3.90 ± 0.01 [b]	3.89 ± 0.01 [b]
TA ($g \cdot L^{-1}$ TH_2)	3.82 ± 0.02 [a]	3.56 ± 0.01 [b]	3.53 ± 0.01 [b]	3.46 ± 0.01 [b]
Ripening Index (RI)	2.37 ± 0.02 [a]	2.64 ± 0.02 [b]	2.45 ± 0.10 [a]	3.07 ± 0.04 [c]
Tartaric acid (g/L)	2.80 ± 0.01 [a,c]	2.90 ± 0.01 [b]	2.68 ± 0.01 [a,b]	2.58 ± 0.01 [c]

Malic acid(g/L)	0.37	±	0.01 [a]	0.34	±	0.01 [b]	0.46	±	0.01 [c]	0.31	±	0.01 [d]
Glycerin (g/L)	0.03	±	0.01 [a]	1.30	±	0.01 [b]	0.21	±	0.01 [c]	0.03	±	0.01 [a]
Oxidative index (%)	9.46	±	0.10 [a]	27.49	±	0.97 [b]	19.98	±	0.89 [c]	50.24	±	1.42 [d]
YAN (mg/L)	168.24	±	0.98 [a]	175.73	±	1.24 [b]	140.30	±	2.80 [c]	184.24	±	1.70 [d]
Calcium (mg/L)	371.22	±	0.98 [a]	159.22	±	1.14 [b]	154.28	±	1.03 [b]	179.91	±	2.05 [c]
Magnesium (mg/L)	148.72	±	0.41 [a]	78.74	±	0.15 [b]	79.70	±	1.22 [b]	71.52	±	0.18 [c]
Sodium (mg/L)	13.89	±	0.18 [a]	9.01	±	0.09 [b]	6.37	±	0.34 [c]	12.87	±	0.38 [d]
Potassium (mg/L)	3105.28	±	7.06 [a]	2472.02	±	6.97 [b]	1821.46	±	11.06 [c]	2340.51	±	8.85 [d]
Iron (mg/L)	4.02	±	0.01 [a]	6.23	±	0.01 [b]	6.21	±	0.08 [b]	7.98	±	0.07 [c]
Copper (mg/L)	0.82	±	0.02 [a]	0.99	±	0.03 [b]	1.27	±	0.03 [c]	4.11	±	0.14 [d]

Different superscript letters mean statistically significant differences between samples at p-adjust < 0.05 obtained by one-way ANOVA and Bonferroni's multiple range (BSD) test. Results are the means ± SD of three repetitions. CÑ: Cañocazo. CS: Castellano. MP: Mantúo de Pilas. PF: Palomino Fino.

The pH values obtained for the four varieties studied were high and very similar for the two years of the study, with slightly higher values observed for the Castellano and Mantúo de Pilas varieties, regardless of the year. In terms of organic acid content, tartaric acid represented more than 70% of the total acidity of the musts from all the varieties. Their values did not vary significantly from one year to the next regardless of the degree of ripeness, but their concentration was always higher in the autochthonous varieties than in the control variety (Palomino Fino). On the other hand, the malic acid content varied from one year to the next, especially in the autochthonous varieties (CÑ, CS, and MP). In the case of these varieties, malic acid content was higher in 2016, when higher Baume degrees and ripening index were reached. As for the oxidative index, or tendency of the musts to enzymatic oxidation, the musts of the varieties analyzed presented differences, and their values did not generally differ between the two years studied. In both years, Palomino Fino grape musts presented a tendency to oxidation higher than the rest of the autochthonous varieties, being the lowest results showed by Cañocazo. Yeast assimilable nitrogen (YAN) content differed between the varieties studied, reaching higher values in 2016. As with the oxidative index, the Palomino Fino grape must had the highest YAN values in both years. Regarding the concentration of the different cations analyzed, the different varieties showed differences in cation content, maintaining these differences during the two years of study. Potassium was the predominant cation, followed by calcium, and magnesium.

The results of the principal component analysis (PCA) (Figure 1) based on the physicochemical data of the different varieties, showed two factors that explain 76.7% of

the total variance. Factor 1 (F1), representing 44.7% of the variance, correlates positively with the total acidity, tartaric, and malic acid, and with the main cations in musts (potassium, calcium, and magnesium), and negatively with the pH, the oxidative index, and the metallic cations iron and copper. Factor 2 (F2), which explains 32.02% of the variance, correlates positively with density, YAN, and cations potassium and sodium, and negatively with pH, tartaric acid, and malic acid. As can be seen, the representation of the values leads to a segregation of the different varieties independently of the year of study. Of the two factors obtained, F2 is the one that discriminates the most between the varieties. F1 is higher in all the autochthonous varieties studied, with respect to Palomino Fino, highlighting Cañocazo. The varieties Mantúo de Pilas and Castellano presented similar values of F1 and values closer to Palomino Fino. On the other hand, Palomino Fino has the highest F2 values, followed by Castellano, Cañocazo, and finally Mantúo de Pilas. This corresponds to the values of the ripening index (°Bé/total acidity) that were calculated for the different varieties (Table 3).

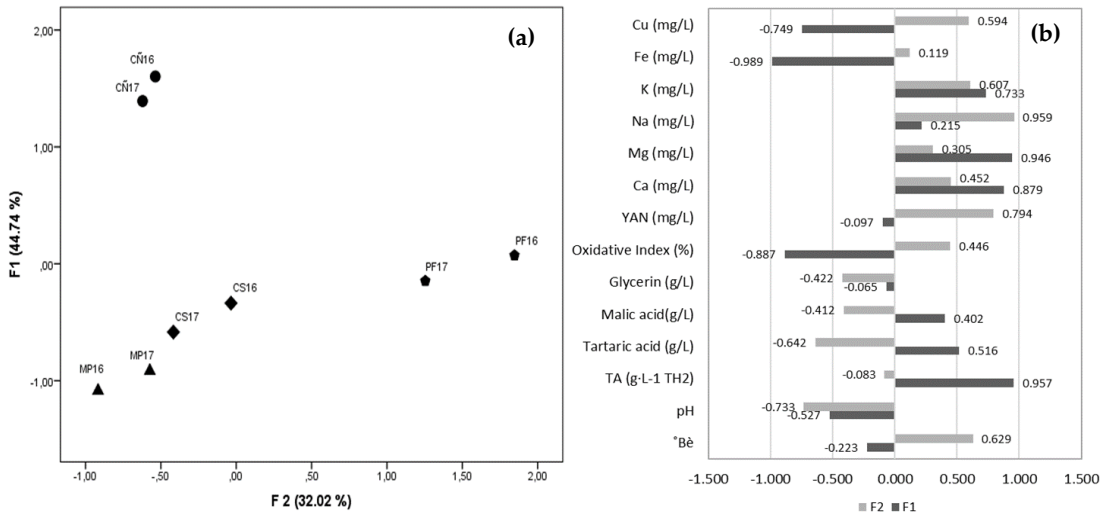

Figure 1. Principal component analysis (a) and its loading factors (b) of Cañocazo [CÑ], Castellano [CS], Mantúo de Pilas [MP], and Palomino Fino [PF] grape musts physicochemical analysis during two consecutive years (2016 and 2017).

Discussion

The use of SSR molecular markers is recommended for the genetic identification of vine varieties [34]. The analysis of several microsatellites allows a unique fingerprint to be obtained for each variety [35]. However, it is very important that the same set of microsatellites is used in this work in order to allow comparison of the genotypes obtained with those in other databases. There is also an international consensus that six microsatellite loci are the minimum number that can be used to discriminate between two varieties [25]. In the case of closely related varieties, the number of these should be increased [36]. In this study, a set of 22 microsatellite loci has been analyzed, consisting

of the six recommended by the OIV, and agreed upon as a result of the GENRES 081 project (VVMD5, VVMD7, VVMD27, VVS2, VrZAG62, and VrZAG79). This has been extended to 22 with those proposed by the European GrapeGen06 project. After comparing the genotypes obtained for the autochthonous varieties with the *Vitis* International Variety Catalogue (*VI*VC) database, two synonyms that were previously described in this database have been confirmed for Castellano and Mantúo de Pilas. The genotype presented by the variety Castellano corresponds to that of Manteudo for the nine microsatellite loci (*VI*VC), and 20 loci, according to Lacombe et al. [24]. This variety is registered as a white variety autochthonous to Portugal, and is conserved in Spain under this name only at Finca El Encín (Holding Institution Code: ESP080). In this study, the genotype is extended by 13 additional loci. The variety Mantúo de Pilas has presented the same genotype as the variety De Rey; thus, confirming the synonymy described by Sancho-Galán et al. [22] for the 22 microsatellite loci studied.

Alongside the genetic characterization, a morphological characterization was carried out in order to obtain a complete description of the vine material [37]. The varieties Cañocazo and Palomino Fino have shown a similar phenotype to the one described by García de Lujan et al. [38], while the variety Castellano is similar to that described by Serrano et al. [39]. However, the variety Mantúo de Pilas analyzed in this work, differs slightly with respect to its phenotype in some of the descriptors, when compared to those described for Uva Rey, which had been confirmed as synonyms through genetic identification [22]. These differences could be attributed to environmental conditions, since the two varieties that have been compared are planted in plots of land at different geographical locations. All of the autochthonous varieties presented a medium to high density of hairiness on adult leaves (OIV 084, OIV 085, OIV 086, and OIV 087), similar to Palomino Fino. Non-glandular vine hairs or trichomes play a functional role in the plant since they modulate evapotranspiration by restricting air movement around the stomata pores [40]. Thus, all the varieties that were studied could be considered as autochthonous white varieties adapted to warm climate areas because of their higher hair density.

In recent years, we have seen changes in vineyard development, such as premature budding and flowering as a result of higher temperatures, and changes in the rainfall regime associated with global climate warming [41]. These changes have the potential to affect the concentration of the different secondary metabolites in berries since they are mostly influenced by the physiological activity of the vines during the grape ripening stage (from July to September in Marco de Jerez region). Latterly, the grape ripening stage has been affected by climate change, and the vegetative period in which the plant carries out physiological activities has been lengthened [41]. This has increased metabolic rates in vineyards, and therefore affected the secondary metabolites content in berries and consequently in their musts [42,43]. After studying two consecutive years, it has become clear how global warming could affect autochthonous grape musts composition, particularly with regard to the balance between sugar content and malic

acid concentration; an increase in sugar content is accompanied by a decrease in malic acid concentration in berries during the ripening stage [44]. The accumulation of sugars in berries takes place because of the mobilization of reserves in stems and roots, and of sugars from leaf photosynthesis [45]; the malic acid is then consumed in the grain cell by respiratory combustion as a substrate energy source [46]. Some studies have shown that malic acid consumption is enhanced when the weather is warmer during the ripening stage [46,47]. This is why low levels of malic acid in grape musts are common in warm climate areas [48,49]. Analyzing the evolution of temperatures during the years 2016 and 2017 (Figures S1S2), it may be thought that in the year with the hottest ripening period (2016), musts with a higher °Bé and lower malic acid content than in 2017 should be obtained. This is true for the Palomino Fino variety (Table 3); however, with the other autochthonous varieties the opposite phenomenon occurs. This lack of correlation between these two parameters in autochthonous varieties might be due to a ripening problem because of the high temperatures. The temperatures reached in 2016 were very high, punctually (Figure S1), and this, together with a low rainfall, could have led to a biological and metabolic interruption in the grape cells, as well as an increase in the final density of the grapes, mainly due to the phenomena of water evaporation. Nonetheless, the year 2017 (Figure S2), with relatively lower temperatures, was more favorable to the biological activity of the autochthonous varieties, and grape musts with lower malic acid content were obtained.

Between varieties, Castellano and Palomino Fino showed a higher concentration of sugars. This may be due to the fact that these varieties have a shorter phenological cycle than Mantúo de Pilas and Cañocazo [38]; therefore, they ripen earlier, causing a lower concentration of sugars in berries. The choice of the variety, according to the climate, is a matter of great importance in order to obtain ripe grapes with a balanced composition. According to Hidalgo-Togores [45], in warm climates, varieties with a late cycle should be used so that the grapes mature when the climate is more favorable. In this sense, it is reasonable to think that the autochthonous varieties, especially Mantúo de Pilas and Cañocazo, should be harvested later than Palomino Fino.

In addition to the organic acids, YAN content and the oxidative index of grape musts could also be considered as characteristics of the variety, although they are subject to fluctuations, depending on the year and the environmental conditions during the ripening period. The differences found in YAN values between the varieties could also be due to differences in the degree of ripeness as the YAN content increases during grape ripening [50]. In spite of that, the time lag in the ripening cycle between the varieties, all of them reached levels higher than 140 mg/L; thus, ensuring the proper development of alcoholic fermentation [51].

With respect to the grape musts oxidative index, Palomino Fino—from both years—shows a greater tendency to oxidize. This fact could be because this variety generally presents a very high content in polyphenolic compounds susceptible of being oxidized [52]. A greater presence of iron and copper, which are powerful catalysts for this

reaction, may also contribute to the oxidation of polyphenols by chemical means [53]. The quantification of the remaining cations is of major importance for wines since they may exert physiological effects on the consumer or hinder technological processes, such as wine stabilization [45]. With regard to the other cations found in the samples, it was observed that potassium represents almost the entire concentration of those cations, since it is the most important ionic compound present in grapes, and plays a major role in the enzymatic reactions and processes of grapes [45,54]. It is important to determine calcium and magnesium content, since the former, similar to potassium, may cause precipitation problems in wines (calcium tartrate). However, the concentration of both cations is highly influenced by the geographical area of origin of the grapes and the composition of soil [55]. Finally, sodium content is significantly below the limit established by the OIV, and this cation does not pose a problem for wine production or consumption [29].

The PCA, together with all of the physicochemical variables analyzed, corroborates the results and the differences determined between the varieties in this research. On the one hand, F1 (Figure 1), establishes that autochthonous varieties studied could have a higher acidity potential than Palomino Fino, regardless of the year or ripeness level; being Cañocazo particularly noteworthy. This fact, combined with warm climate conditions, constitutes an advantage when it comes to making white wines with an improved sweetness/acidity balance. The main cations found in grape musts (potassium, magnesium, and calcium), with positive loading factors (Figure 1), also contribute significantly to F1, while metals (iron and copper), with negative values, also make a considerable contribution (Table 3). Therefore, the high acidity factor of Cañocazo is due partly to the fact that its must has significantly higher levels of potassium, magnesium, and calcium and lower levels of iron and copper than the other varieties. F2, which is positively correlated with grape must density, can clearly discriminate between all the varieties that have been studied, regardless of the year (Figure 1). The lower values of F2 in the autochthonous varieties would corroborate that they have been harvested earlier and require longer ripening periods, especially Mantúo de Pilas, since it has longer cycles than Palomino Fino.

Conclusions

Molecular analysis with 22 SSR *loci* allowed the identification of autochthonous varieties with different genotypes. However, all of them showed similar phenotypic characteristics in terms of high hair density on adult leaves, which could be of interest as a mechanism to regulate grapevine evapotranspiration, and therefore adapt to an increase in temperature as a consequence of global warming. With regard to the physicochemical composition of the musts, after multivariate analysis of the results, different behaviors have been observed among the autochthonous varieties, with respect to the control variety Palomino Fino. It should be highlighted that Mantúo de Pilas and Cañocazo had a longer phenological cycle and, as a result, a higher acidity, thereby

allowing for the production of quality wines in hot climate areas. As a result of all the above, these autochthonous varieties could be considered suitable for cultivation in areas with warmer and drier climates, a trend that has been observed in many winemaking regions as a consequence of the climate change. In order to promote their cultivation, it would be necessary to apply for their inclusion in the Official Register of Authorized Varieties.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: (a) Temperature (°C) (T_max, T_min, T_avg), (b) humidity (%) (H_max, H_min, H_avg), and (c) radiation (W/m^2) and rainfall (L/m^2) among July and September 2016. Figure S2: (a) Temperature (°C) (T_max, T_min, T_avg), (b) humidity (%) (H_max, H_min, H_avg), and (c) radiation (W/m^2) and rainfall (L/m^2) among July and September 2017.

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Supplementary Materials

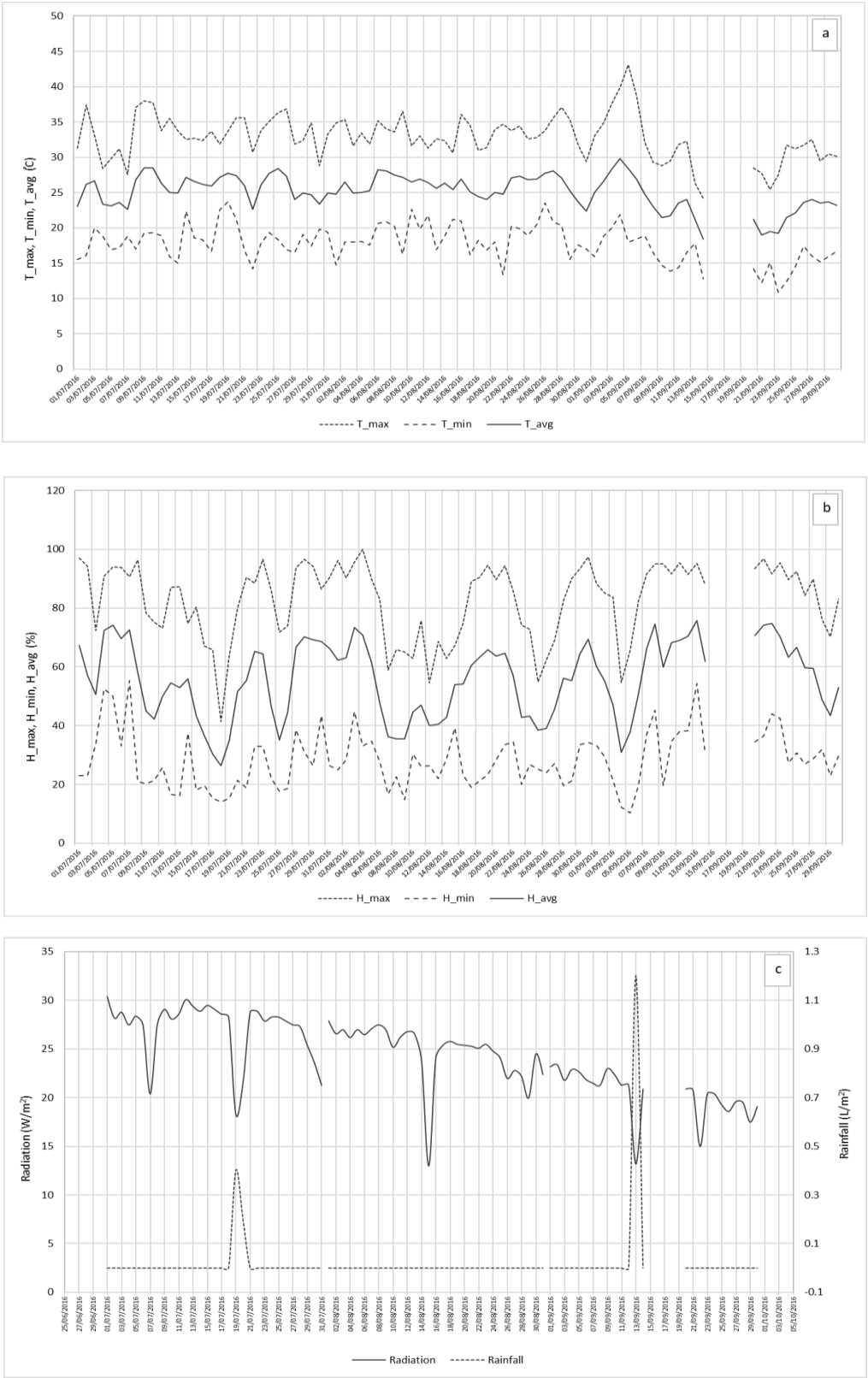

Figure S1a-c. (a) Temperature (°C) (T_max, T_min, T_avg), (b) humidity (%) (H_max, H_min, H_avg) and (c) radiation (W/m²) and rainfall (L/m²) among July and September 2016.

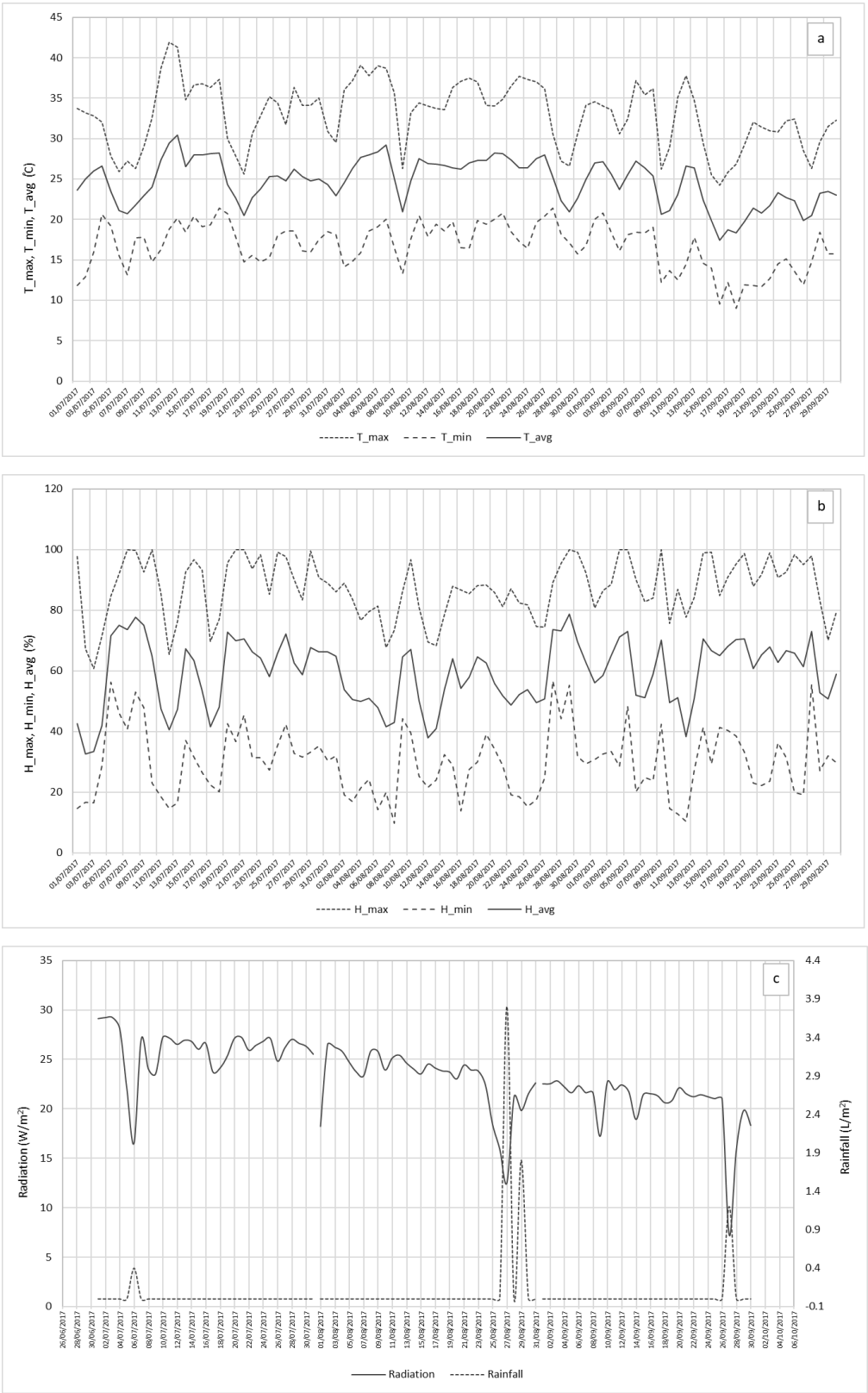

Figure S2a-c. (a) Temperature (°C) (T_max, T_min, T_avg), (b) humidity (%) (H_max, H_min, H_avg) and (c) radiation (W/m²) and rainfall (L/m²) among July and September 2017.

3) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Preliminary Study of Somatic Variants of Palomino Fino (*Vitis vinifera* L.) Grown in a Warm Climate Region (Andalusia, Spain). *Agronomy* **2020,** *10*, 654; doi:10.3390/agronomy10050654

Abstract

Vegetative propagation of *Vitis vinifera* cultivars over hundreds of years has led to the accumulation of a large number of somatic variants of the same grapevine variety. These variants are now considered a working tool to cope with changing environmental conditions as a result of, among others, global warming. In this work, three somatic variants of the major grapevine variety of the South West (SW) of Andalusia (Spain), Palomino Fino, have been genetically and morphologically characterized, as well as their grape musts from two different vintages. The genetic analysis at 22 microsatellite loci confirmed the identity of the three somatic variants that presented the same genotype as Palomino Fino, while the morphological study showed differences between the three somatic variants and Palomino Fino, highlighting the somatic variant Palomino Pelusón. Regarding the physicochemical analysis of its musts, differences were also observed between the somatic variants and Palomino Fino. As a result of all of the above, the use of grapes from somatic variants can be a viable and natural alternative for the production of quality wines in warm climate areas. On the other hand, promoting the cultivation of the somatic variants could contribute to preventing the loss of Palomino Fino intraspecific variability.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest and most widely cultivated fruit crops in the world [1], used mainly for wine and spirit making [2]. This species presents a wide genetic and phenotypic diversity mainly due to the history of vine cultivation [3] and vegetative propagation, which has allowed the conservation of different cultivars for centuries [4]. Grapevine was one of the first fruit species domesticated, and its vegetative propagation has been practiced since ancient times [5]. During many cycles of vegetative propagation, mutations have appeared spontaneously, some of them leading to phenotypic differences giving rise to different somatic variants or clones [6].

The somatic variations have led to grapevine adaptation and to its evolution under changing environmental and cultivation conditions, this being a source of novel traits [7]. This variation became the base of grapevine clonal selection, starting in Germany in the nineteenth century and continuing in some other European countries such as France, Italy and Spain in the second half of the twentieth century [8]. Initially, the basic aim of

clonal selection was to get healthy and highly productive plants [8]. However, the aim of obtaining highly productive plants alongside the trend to cultivate only certain varieties has contributed to the disappearance of many local cultivars [9]. Recently, this trend has started to change, and some wineries, grape growers and consumers have been looking for new local products considering grape quality as a relevant goal to the detriment of yield [10].

Currently, clonal selection has been postulated as one of the working tools to face the adaptation of grapevine varieties to new conditions and environments [11] due to climate change. In the medium to short term, the International Organization of Vine and Wine (OIV) is undertaking the selection and improvement of new varieties for adaptation to climate change [12]. Since 2016, different resolutions of the Genetic Resources and Vine Selection group (GENET) and several research projects have aimed to facilitate the exchange of plant material and germplasm to improve the research and trade in new grapevine varieties. The conservation of this plant material is not a recent development, since the work of prospection, collection and conservation of different vine varieties has been the subject of numerous scientific studies over the years [13–15]. More specifically, in an area with a warm climate such as SW Andalusia (Spain), the germplasm bank at Rancho de la Merced preserves different somatic variants of the main grapevine variety in Andalusia, Palomino Fino [16].

Palomino Fino is considered an autochthonous grapevine variety [17], and its cultivation in this region has been known since the sixteenth century [18], becoming the eighth most cultivated in Spain between 1990–2012 [19,20]. Actually, this grapevine variety is predominant in the Marco de Jerez for the production of Sherry wines. The long history of Palomino Fino cultivation has led to a high number of clones, which represents an important genetic source. The first clonal selection programme of Palomino Fino was started by Fernández de Bobadilla [21] and continued by García de Luján et al., selecting 28 clones that are currently preserved in the Rancho de la Merced germplasm bank [22]. These clones have been used for new grapevine plantations since the end of the 20th century. Therefore, it is currently very difficult to find new somatic variants or clones that can meet the current needs of wine makers. In addition, in order to carry out behavioural studies of this new plant material, it needs to be preserved in the same plot and growing conditions. These studies are necessary in order to select new plant material better adapted to the changing climate conditions we are facing.

In this sense, the main objective of this work focuses on the characterization of three different somatic variants of Palomino Fino (Palomino Gacho, Palomino de Jerez and Palomino Pelusón) by means of molecular markers, morphological description and physicochemical analysis of grape musts. Their morphological description and grape must analysis could contribute to the detection of traits that could contribute to the production of new white wines in warm climate areas from varieties that could also be better adapted to warm climates.

Materials and Methods

Experimental Design and Grapevine Samples

Three different somatic variants of the grapevine variety Palomino Fino were chosen for the analysis: Palomino Gacho (PG), Palomino Pelusón (PP) and Palomino de Jerez (PJ). Palomino Fino (PF) was employed as the control. All samples were selected from the same vineyard and plot (latitude 36°34′29.7″ N and longitude 5°49′53.5″ W; 150 m above sea level), located in the municipality of San José del Valle (Cádiz, Spain). Vines were 15 years old and were planted with a SW orientation over a limestone soil and with a 2.4 × 1.2 framework vertically trellised, allowing a plant density of 3472 plants per hectare. No irrigation or fertilization treatments were applied during the studied years, and different conventional phytosanitary products were applied to ensure correct grape development. However, during the year 2017, an outbreak of different fungal diseases affected more than 70% of the plots in the Jerez-Xérès-Sherry zone [23], making it impossible to carry out the study during 2017, having to postpone it to 2018.

In order to minimize the intrinsic variability of sampling different vines in the same plot, Santesteban et al.'s [24] criteria were followed. For that reason, 40 vines of each clone's trunk cross sectional area (TCSA) were measured at 30 cm height using a digital Vernier Caliper 93,110 (Maurer, Padova, Italy). Of all the vines measured, 10 were selected and marked as their TCSA value was the closest to the average ±10%.

Additionally, and only for the genetic characterization, four reference varieties (Cabernet Sauvignon (CS), Chardonnay (CH), Muscat a Petits Grains Blancs (MPGB) and Pinot Noir (PN)) were included as reference varieties to compare the genotype obtained in the analysis and those published in databases in order to confirm the identity of the grapevine variety analysed. Those varieties came from a plot previously described in recently published papers [25].

Genetic Analysis

A total of 22 microsatellite loci were employed to perform the genetic analysis following the methodology established in recently published papers [26]. Young fresh leaves from each somatic variant and from the reference varieties were collected at the vineyard. A DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was employed to extract the DNA. DNA amplifications were carried out using a 9700 thermal cycler (Applied Biosystensm Foster City, CA, USA), and the amplified products were separated by capillary electrophoresis using an automated sequencer ABI Prism 3130 (Applied Biosystensm Foster City, CA, USA). The four fluorescent labelled fragments (6-FAM, VIC, PET and NED) were detected and measured using GeneMapper v. 3.7 (Applied Biosystensm Foster City, CA, USA), and the fragments were assessed using international standards GeneScan-500 LIZTM (Applied Biosystensm Foster City, CA, USA). The microsatellite genotypes obtained after the analysis were compared with the genetic

profiles provided by the databases *Vitis* International Variety Catalogue (*V*IVC) [27] and the Rancho de la Merced Germplasm Bank database [28].

Ampelographic Description

A total of 58 descriptors from the International Organization of Vine and Wine (OIV) Descriptor List [29] were evaluated. To this end, five different ampelographers with different knowledge and expertise described a total of 10 shoots, leaves, bunches and berries from each somatic variant following Benito et al.'s [30] criterion during the years 2016 and 2018. The modal value was selected as the final descriptor.

Grape Musts Physicochemical Characterization

For the physicochemical characterization, the sampling conditions were the same proposed in recently published papers [25]. pH, sugar concentration (°Bé), total acidity, tartaric acid, malic acid and yeast assimilable nitrogen (YAN) were determined in the must of the three Palomino somatic variants studied and Palomino Fino. The analyses were performed in triplicate during the years 2016 and 2018 in order to ensure statistical significance. pH was measured using a digital pH-meter CRISON-2001 (Crison, Barcelona, Spain) equipped with a combined electrode with automatic temperature compensation. Sugar concentration was assessed using a calibrated Dujardin–Salleron hydrometer (Laboratories Dujardin–Salleron, Arcueil Cedex, France). Total acidity was determined following the OIV reference method [31]. The Ripening Index was calculated following the equation given by Hidalgo [32]. The concentration of tartaric and malic acid was determined using an ionic exchange chromatograph (Metrohm 930 Compact IC Flex, Herisau, Switzerland) with a conductivity detector on a Metrosep Organic Acids column-250/7.8 (Metrosep, Herisau, Switzerland) following the conditions given by Sancho-Galán et al. [33]. Yeast assimilable nitrogen (YAN) was determined according to the formal method [34].

Statistical Analysis

Data means and standard deviations were calculated, and significant differences were evaluated by two-way ANOVA and Bonferroni's multiple range (BSD) test with a *p*-adjust <0.05 (GraphPad Prism v. 6.01 for Windows, GraphPad Software, San Diego, CA, USA). A hierarchical clustering analysis (HCA) using Ward's method and the Euclidean square distance was performed using the statistical software SPSS 24.0 (SPSS Inc., Chicago, IL, USA).

Results

Genetic Analysis

The allele profiles obtained for the three somatic variants studied, Palomino Fino and the reference varieties at 22 microsatellite loci are shown in Table 1. All Palomino accessions analysed presented the same genotype. It was compared with the published genotype at the Rancho de la Merced Germplasm Bank genotype database [28] and international databases [27].

Table 1. Genetic profiles of different Palomino variants at 22 microsatellite loci. Allele sizes are given in base pairs.

Grapevine Variety Code	PF, PJ, PG, PP		CS		CH		MPGB		PN	
Microsatellite Locus										
VVIB01	291	307	291	291	289	295	291	295	289	295
VMC1b11	184	188	184	184	166	184	184	188	166	172
VMC4F31	176	206	174	178	174	180	168	206	174	180
VVMD5	226	238	228	236	232	236	226	234	226	236
VVMD7	236	246	236	236	236	240	232	246	236	240
VVMD21	243	249	249	257	249	249	249	265	249	249
VVMD24	209	209	209	217	209	219	213	217	215	217
VVMD25	240	240	238	246	238	252	240	246	238	246
VVMD27	186	194	176	190	182	190	180	194	186	190
VVMD28	238	250	236	238	220	230	248	270	220	238
VVMD32	254	256	238	238	238	270	262	270	238	270
VVIH54	166	166	166	182	164	168	166	166	164	168
VVIN16	151	151	153	153	151	151	149	149	151	159
VVIN73	256	264	264	268	264	266	264	264	264	266
VVIP31	188	190	190	190	180	184	184	188	180	180
VVIP60	318	322	306	314	318	322	318	318	318	320
VVIQ52	85	85	83	89	83	89	83	83	89	89
VVS2	131	144	137	151	135	142	131	131	135	151
VVIV37	163	167	163	163	153	163	163	165	153	163
VVIV67	364	366	364	372	364	372	364	375	364	372
VrZAG62	187	193	187	193	187	195	185	195	187	193
VrZAG79	250	260	246	246	242	244	250	254	238	244

PF: Palomino Fino. PJ: Palomino de Jerez. PG: Palomino Gacho. PP: Palomino Pelusón. CS: Cabernet Sauvignon. CH: Chardonnay. MPGB: Muscat a Petits Grains Blancs. PN: Pinot Noir.

Ampelographical Description

Table S1 shows the modal values obtained after the morphological description of the three somatic variants compared to the control for the years 2016 and 2018. All the somatic variants and Palomino Fino presented different phenotypes. In order to sum up

the information displayed in Table S1, Table 2 and Figure 1 show the number of differences between the analysed Palomino variants. Table 1 shows Palomino Pelusón as the somatic variant with a greater number of differences with respect to the rest of the studied Palomino variants. This is due to the fact that this somatic variant had higher scores in all the hair-related descriptors (OIV 004, OIV 013, OIV 051, OIV 054, OIV 084, OIV 085, OIV 086, OIV 087 and OIV 088). Additionally, Palomino Fino showed differences with the rest of the somatic variants regarding three of the six analysed descriptors concerning the bunch (OIV 202, OIV 203 and OIV 502).

Table 2. Number of different descriptors between the different somatic variants (Palomino de Jerez, Palomino Gacho and Palomino Pelusón) and the control Palomino Fino**.**

	Palomino Fino	Palomino de Jerez	Palomino Gacho	Palomino Pelusón
Palomino Fino	X			
Palomino de Jerez	10	X		
Palomino Gacho	8	10	X	
Palomino Pelusón	19	17	17	X

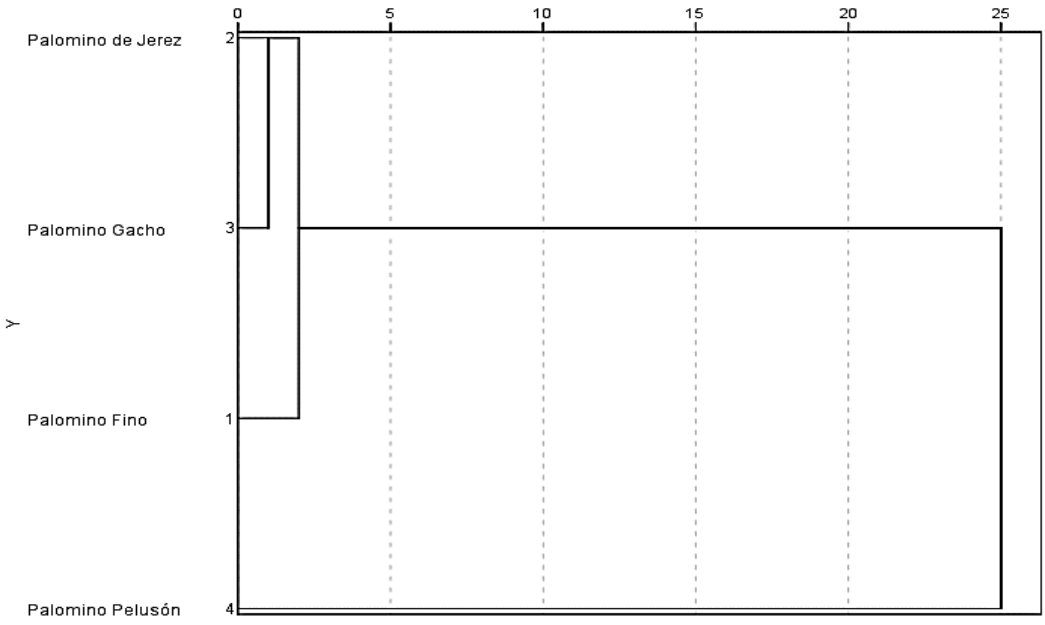

Figure 1. Dendrogram representing the differences among the different variants studied based on ampelographic characterization employing an average link between groups and re-scaled distance cluster combination.

According to the 58 descriptors studied, the HCA analysis (Figure 1) shows how Palomino Pelusón is the most different somatic variant regarding its morphological

traits. Furthermore, the rest of the variants analysed are displayed in one group, Palomino Fino being the most different type among this second group.

Grape Must Physicochemical Characterization

Table 3 shows the result of the grape must physicochemical characterization at harvest from the four Palomino variants analysed during 2016. The pH values ranged from 3.80 for Palomino Fino to 3.53 for the somatic variants Palomino Gacho and Palomino de Jerez. Palomino Fino showed significant differences with the rest of the somatic variants (p-adjust < 0.05). Regarding sugar concentration, the results varied between 12.85°Bé and 10.35°Bé for Palomino Fino and Palomino de Jerez, respectively, with significant differences between all the studied variants. In this way, it can be seen how Palomino Fino was the ripest variant at the time of harvest. In relation to total acidity, this parameter showed values ranging from 4.851 to 3.151 g/L for Palomino Gacho and Palomino Fino, respectively. In this way, it can be seen that Palomino Fino, with the highest sugar content, had the lowest total acidity content, but not the inverse case since the highest values of total acidity were observed for the somatic variant Palomino Gacho and not for Palomino de Jerez. The Ripening Index, calculated from the values of sugar concentration and total acidity, showed the highest value for the Palomino Fino (4.07), while the somatic variant Palomino de Jerez showed the lowest value on the Ripening Index (2.48). The content of the two main organic acids present in the grape must, tartaric acid and malic acid, showed significant differences between all the cultivars studied (p-adjust > 0.05). On the one hand, tartaric acid content ranged between 4.002 and 2.340 g/L for the Palomino de Jerez and Palomino Fino variants, respectively. However, the malic acid content showed a completely opposite behaviour, showing its maximum content in the control Palomino Fino (0.622 g/L) and its lowest concentration in the somatic variant Palomino Gacho (0.104 g/L). Finally, the YAN content ranged from 247.54 mg/L for Palomino Gacho to 189.27 mg/L for Palomino Pelusón, the values of the former significantly higher than in the other somatic variants studied.

Table 3. Palomino Fino (control) and somatic variants (Palomino Gacho, Pelusón and de Jerez) grape must physicochemical characterization at harvest during the year 2016.

	Palomino Fino	Palomino Gacho	Palomino Pelusón	Palomino de Jerez
pH	3.87 ± 0.01 [a]	3.53 ± 0.01 [b]	3.61 ± 0.03 [b]	3.53 ± 0.03 [b]
Baumé	12.85 ± 0.00 [a]	11.98 ± 0.09 [b]	11.10 ± 0.01 [c]	10.35 ± 0.10 [d]
Total Acidity (g/L)	3.15 ± 0.05 [a]	4.58 ± 0.10 [b]	3.32 ± 0.06 [a]	4.17 ± 0.06 [c]
Ripening Index	4.07 ± 0.02 [a]	2.61 ± 0.08 [b]	3.34 ± 0.07 [c]	2.48 ± 0.01 [b]
Tartaric Acid (g/L)	2.340 ± 0.050 [a]	2.460 ± 0.062 [b]	2.663 ± 0.041 [c]	4.002 ± 0.055 [d]
Malic Acid (g/L)	0.622 ± 0.064 [a]	0.104 ± 0.006 [b]	0.264 ± 0.040 [c]	0.200 ± 0.009 [d]
YAN (mg/L)	200.16 ± 2.13 [a]	247.54 ± 2.61 [b]	189.27 ± 1.54 [a]	196.47 ± 5.69 [a]

Different superscript letters mean statistically significant differences between samples at p-adjust < 0.05 obtained by two-way ANOVA and Bonferroni's multiple range (BSD) test. Results are the means ± SD of three repetitions.

Given the significant differences observed in the physicochemical composition of the different grape musts at the time of harvest, it was decided to extend the analysis with different sampling points. During 2018, the control and the three somatic variants were studied during the final ripening stages, sampling every 7 days. Technological ripening parameters such as total acidity and sugar concentration were monitored, as well as tartaric and malic acid concentration given their involvement in some metabolic processes during grape ripening. Figure 2a,b shows the evolution of the above-mentioned parameters during the end of the ripening process until harvest.

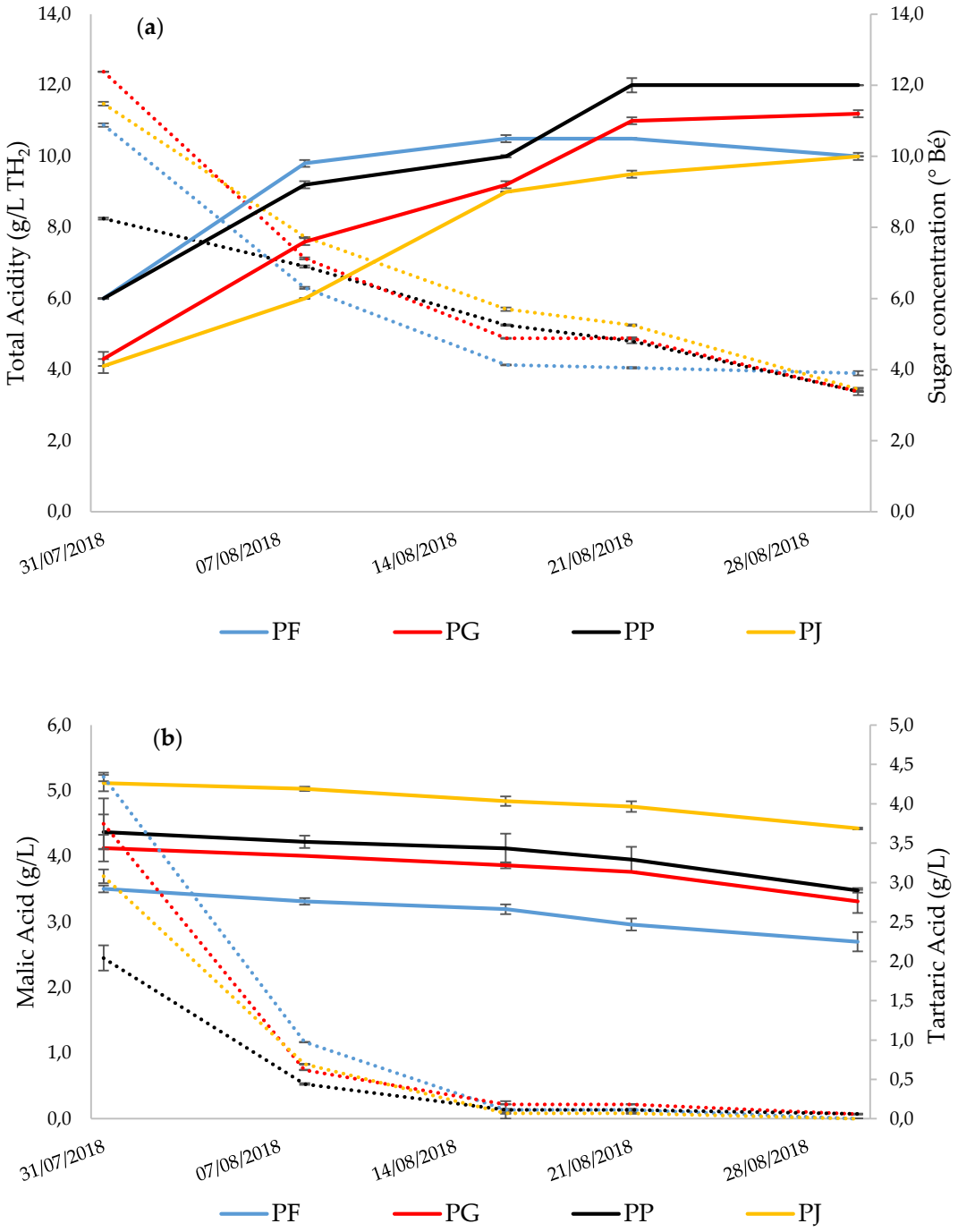

Figure 2. (**a**,**b**) Evolution of total acidity (left axis, dotted line) and sugar concentration (right axis, solid line) (**a**) and malic (left axis, dotted line) and tartaric acid (right axis, solid line) (**b**) during ripening final stages of Palomino Fino (PF), Palomino Gacho (PG), Palomino Pelusón (PP) and Palomino de Jerez (PJ).

In general, for all the accessions studied, Figure 2a shows a considerable gradual decrease in total acidity during the ripening process. Each cultivar studied starts the ripening process with different acidity values, Palomino Gacho being the somatic variant

with the highest total acidity value (12.38 g/L TH_2) and Palomino Pelusón the somatic variant with a significantly lower value (8.25 g/L TH_2) (p-adjust < 0.05). Noting the evolution of this parameter, it can be seen how Palomino Fino undergoes a rapid decrease in total acidity between the 01 August 2018 and 17 August 2018, and then stabilizes until the date of harvest. In the remaining cases, the evolution of the different somatic variants between 17 August 2018 and 31 August 2018 is not so pronounced, with similar final total acidity values and no significant differences between all the Palomino variants studied. With regard to sugar concentration, the most significant increase during the ripening process is observed during the first 16 days of sampling. From this moment on, two different trends can be observed: on the one hand, Palomino Fino and Palomino de Jerez show a similar concentration of sugar until harvest. On the other hand, Palomino Gacho and Palomino Pelusón continue increasing their sugar concentration until 21 August 2018, stabilizing at that time until the harvest date. At the end of the ripening process, sugar concentration of somatic variants and the control ranged from 10° Bé for Palomino Fino and Palomino de Jerez to 12° for Palomino Pelusón.

Figure 2b shows how the tartaric acid content decreases gradually as the grapes ripen. The initial concentration of tartaric acid is different for each somatic variant studied, highlighting the significantly low value of Palomino Fino (3.504 g/L of TH_2) and the significantly high value (p-adjust < 0.05) of the somatic variant Palomino de Jerez (5.114 g/L TH_2). Tartaric acid content shows a linear and constant decrease during the grapes' ripening process, being more pronounced near the end of the ripening. Malic acid content (Figure 2b) shows that both the somatic variants and the control experience a similar decrease of this acid during ripening. In the first phase of the ripening process (until 08 August 2018), there is a sharp drop in the concentration of this analyte, which decreases until 16 August 2018. From then on, the concentration of malic acid remains stable (close to zero) and no significant differences between the different somatic variants and the control can be observed.

Discussion

Simple sequence repeat (SSR) markers are one of the most widely used tools in genetic identification of grapevine varieties [35], but it is important to use the same set of microsatellites in every work to be able to compare the results with those published in different databases. In spite of the high heterozygosity that vine has, a set of six microsatellites is enough to discriminate between two grapevine varieties [36]. However, if the grapevine varieties are highly related, it is compulsory to extend the number of microsatellites. In this study, a set of 22 microsatellite loci comprised of the six proposed by the OIV and the consensus established within the European research projects GENRES 081 and GrapeGen06 were used. In this sense, the analysis of this number of microsatellites allows us to create a unique genetic fingerprint [37]. The use of these 22

microsatellite loci did not allow finding genetic differences between the different somatic variants studied. Similar results in which no genotypic differences were observed at 20 microsatellite loci but morphological differences were observed were obtained by Jimenez-Cantizano et al. [38]. However, clear phenotypical differences were observed over time. This result could be due to mutation or epi-mutation events that take place in single cells that belong to specific grapevine meristem cell layers [38]. In this way, grapevine genetic profile was not affected by somatic variation. However, the genetic profile of Palomino Fino was presented at 22 microsatellite loci, 14 of which are not listed on the *Vitis* International Variety Catalogue (VIVC) [27]. Furthermore, Palomino de Jerez and Palomino Pelusón are listed on the *V*IVC database as synonyms of Palomino Fino, but Palomino Gacho is not. However, this database does include the accession Listán Gacho as a synonym for Palomino Fino. Jiménez-Cantizano [28] analysed both accessions, preserved in the germplasm bank of Rancho de la Merced (Cádiz, Spain), with 20 SSR loci and presented the same genotype. Phenotypic analysis showed slight differences for the OIV descriptors 202, 204, 206, 502 and 506. Therefore, Palomino Gacho could be considered a synonym for Listán Gacho, and both somatic variants of Palomino Fino.

Despite that vegetative propagation is used in vineyards to multiply plant material and produce descendants identical to the original parent, spontaneous phenotypical variation can occur on some shoots as a result of somatic mutations [39]. In this sense, in order to complete grapevine characterization, and following the recommendations established for an adequate characterization of *Vitis* plant material [40], a complete ampelographic description was carried out. Of the 58 descriptors analysed, 14 corresponded to the primary ones proposed by the OIV to discriminate between varieties [29], and the additional 34 were analysed in order to look for differences between the somatic variants. In these first 14 descriptors' set, differences in 50% were found (OIV 004, OIV 051, OIV 076, OIV 079, OIV 084, OIV 087, OIV 203). These differences in the different organs within the same genotype constitute an interesting genetic resource that could be transferred through classical breeding or genetic engineering in the creation of new cultivars [41]. In this sense, the genetic erosion that the *Vitis vinifera* species is undergoing could be diminished, and the transfer of interesting traits between parents and descendants could also be possible. One of these characteristics of interest could be the one shown by the somatic variant Palomino Pelusón. This somatic variant showed a greater intensity in the expression of those characteristics that imply the presence and density of hairiness (Table S1). High density of erect and/or prostate hairs in any organ can be a trait that could make a grapevine variety better adapted to a warm climate zone. Non-glandular vine hairs or trichomes play a functional role in the plant since they modulate evapotranspiration by restricting air flow between the stomatal pores [42].

Currently, the conditions imposed by global warming are substantially affecting the ripening phase of the grape, as well as other previous processes such as plant bud break

and flowering [43]. Thus, in recent years, differences have been observed in the metabolic rates of the vine, and therefore in the production and accumulation of metabolites [44,45]. The high temperatures and consequent high evaporation of water from the plant during the months of fruit ripening make this process difficult [46]. This fact, together with the decrease and irregularity of rainfall, makes the obtention of quality grapes for wine making a difficult task for wine makers. Given this trend, one of the possible solutions to solve the problems being experienced could be the study of the physicochemical composition of musts of different somatic variants. The results observed during 2016 show a general trend between the different somatic variants studied and Palomino Fino. The latter showed a higher maturity at the time of harvest and analysis (higher sugar concentration, lower total acidity value and consequently a significantly higher maturity index (p-adjust < 0.05)). This fact shows that the control had a lack of synchrony with the somatic variants analysed. Despite this lack of synchrony, the YAN content in all cases was higher than the minimum value required to carry out fermentation [47].

Thus, the analysis of different parameters of interest during ripening has shown that differences between somatic variants and the control were observed during the two years of study, confirming in a preliminary way that the differences are inter-annual and that they are specific to each somatic variant. Regarding sugar concentration in the second phase of the study, the evolution observed between the first and the ninth day could be due to the effect of the high temperatures of those days, exceeding 40 °C (Figure S1). Temperature has a direct influence on sugar content [48]. An increase in temperature leads to increased transpiration and a greater transfer of sugars to the fruit [30]. In addition, in areas with high temperatures and a great amount of sunlight present, photosynthesis is encouraged, increasing CO2 fixation and its conversion into sugars that are transported to the fruit [49]. Furthermore, grapevine production is also a very influential factor in the ripening process, largely determining the final state of ripeness of the vines [50]. This fact could explain the differences in the evolution of some of the somatic variants studied, since the control Palomino Fino presented the highest production of all of them (497 g/bunch), and the process of accumulation of sugars stopped on the 17th day, while Palomino Pelusón experienced the opposite effect, being the somatic variant with the lowest production of grapes (269 g/bunch) and able to accumulate sugars until the end of the ripening process. In this sense, it is clear that Palomino Fino, unlike the other somatic variants, was selected for its high yield. However, in the case of varieties employed for the production of Sherry wines, it would be advisable to select those that have a longer phenological cycle and mature later, thus allowing wines with a higher alcohol content to be obtained and minimizing the addition of alcohol involved in the production of these kinds of wines.

As far as the evolution of total acidity is concerned, the differences described (Figure 2a) are considered normal, since during ripening, different physical-chemical processes take place that lead to a reduction in the acid fraction of the berries and, therefore, to a

decrease in total acidity and an increase in pH [51]. The great decrease in the acid fraction of the must may be due to the high temperatures observed in the first phase of ripening (Figure S1(a–c)), which produces an increase in the respiratory combustion phenomena of malic acid [52,53]. Theoretically, the best weather conditions for optimum ripening of sherry grapes include sunny but not excessively hot weather [54]. If temperatures exceed 38 °C for 4–6 consecutive days, fruit ripening stops and the musts obtained under these conditions have high pH values and low sugar and acid content [55].

When selecting the appropriate cultivars for the production of wines according to the parameters studied in this section, it is essential to take into account that warm regions such as SW Andalusia (Spain) tend to have high values of sugars and low values of acidity, which is a problem when producing table wines, which are too soft [56]. Therefore, it would be interesting to select those grapevine varieties or somatic variants with greater acidity values in case of early vintages (PG, PP, PJ).

Tartaric and malic acids (Figure 2b) represent 70–90% of the acid fraction of the grapes [57], showing the most important differences when comparing the behaviour of the somatic variants and Palomino Fino [58]. The tartaric acid content of each cultivar is due to differences in adaptation to the environment or possible somatic differences [58]. As in this case all the accessions were planted in the same plot, we can assume that these differences in tartaric content between accessions were determined by somatic differences between them. The decrease in tartaric acid content during ripening is mainly due to its salification and the formation of tartrate salts [59], as well as dilution processes caused by the increase in berry size, accentuating this effect in the moments close to the harvest [53]. The tartaric acid content is hardly influenced by the effect of high temperatures, as its concentration does not change much in the first nine days of the ripening final stages. This is due to the fact that tartaric acid is not a substrate for the respiratory combustion of the grain and remains practically constant during the ripening process [60].

As for the malic acid content, the drastic decrease in malic acid concentration does match the period of high temperatures in the first week of August. This is because the high temperatures favour its combustion in the grape cells. Malic acid combustion occurs during ripening, when the plant switches from using carbohydrates as an energy source to using organic acids (including malic acid) [53]. This, in addition to the decrease in malic acid synthesis during the ripening process, results in a significant decrease in malic acid concentration in the fruit [61]. It is important to take into account the strong influence of temperature on respiratory processes, which increase whenever temperature rises and vice versa [52]. For this reason, in periods when there are significant increases in temperature, lower concentrations of malic acid are obtained in wines, which mean lower total acidity and a higher pH. It should also be noted that the Palomino Fino usually has low levels of malic acid [58]. Therefore, in general terms, it could be said that the decrease in total acidity that occurred in the different accessions

was mainly due to the combustion of malic acid in the early stages of maturation and the salification and dilution of tartaric acid in its final stages.

Conclusions

Genetic analysis at 22 loci microsatellites confirmed the identity of the three somatic variants that presented the same genotype as Palomino Fino. However, the morphological analysis of the plants did show differences between the different variants studied. The greater presence of hairs in the different organs of Palomino Pelusón may give it a greater adaptation in hot climate areas. After the physicochemical analysis, it was observed that there was a lag in the phenological cycles of the variants studied, the somatic variants having a longer phenological cycle than the control variety Palomino Fino; this fact is beneficial for the production of white wines in early vintages in warm climate areas. As a result of all the above, the use of grapes from somatic variants can be a viable and natural alternative for the production of quality wines in hot climate areas, as well as for preventing the genetic erosion of the *Vitis vinifera* species. In addition, promoting the cultivation of the somatic variants could contribute to preventing the loss of the intraspecific variability of Palomino Fino.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4395/10/5/654/s1, Figure S1 (a) Humidity (%) (H_max, H_min, H_avg), (b) Temperature (°C) (T_max, T_min, T_avg) and **(c)** radiation (W/m^2) and rainfall (L/m^2) among July and September 2018. Table S1. Ampelographic description of Palomino Fino (PF), Palomino de Jerez (PJ), Palomino Gacho (PG) and Palomino Pelusón (PP) using the International Organization of Vine and Wine (OIV) descriptors.

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Supplementary materials

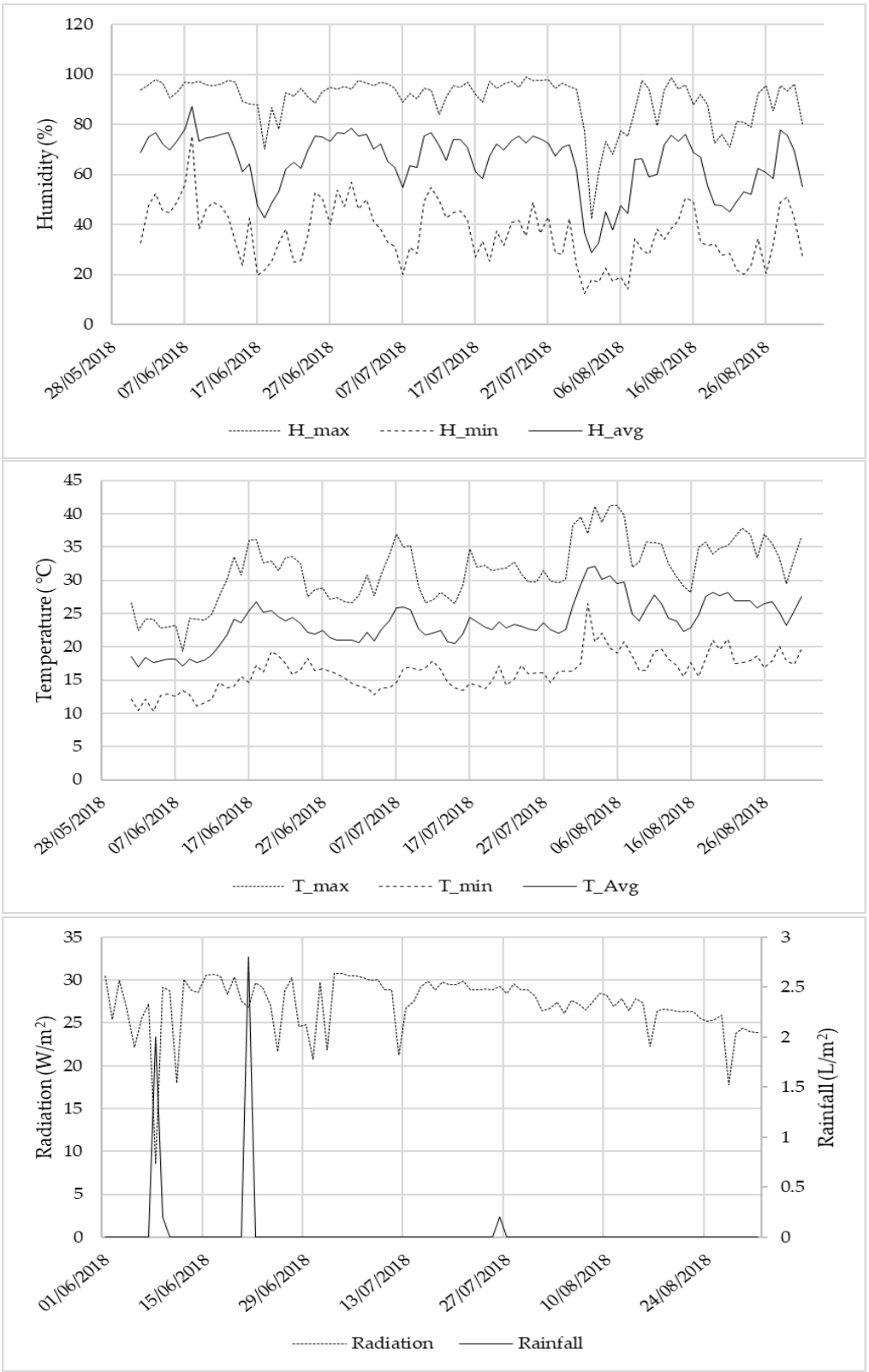

Figure S1a-c. (a) Humidity (%) (H_max, H_min, H_avg), **(b)** Temperature (°C) (T_max, T_min, T_avg) and **(c)** radiation (W/m²) and rainfall (L/m²) among July and September 2018.

Table S1. Ampelographic description of Palomino Fino (PF), Palomino de Jerez (PJ), Palomino Gacho (PG) and Palomino Pelusón (PP) using the International Organisation of Vine and Wine (OIV) descriptors.

Organ	Code	Description	Scale	Variety			
				PF	PJ	PG	PP
Young shoot	OIV 001	Opening of the shoot tip	1 closed, 3 half open, 5 fully open	7	7	7	7
	OIV 002	Distribution of anthocyanin coloration on prostrate hairs of the shoot tip	1 absent, 2 piping, 3 overall	2	2	2	1
	OIV 003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5	5	5	1
	OIV 004	Density of prostrate hairs on the shoot tip	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5	5	5	9
	OIV 005	Density of erect hairs on the shoot tip	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1	1	1
Shoot	OIV 006	Attitude	1 erect, 3 semi-erect, 5 horizontal, 7 semi-dropping, 9 drooping	3	3	3	3
	OIV 007	Colour of the dorsal side of internodes	1 green, 2 green and red, 3 red	2	2	2	2
	OIV 008	Colour of the ventral side of internodes	1 green, 2 green and red, 3 red	2	2	2	2
	OIV 009	Colour of the dorsal side of nodes	1 green, 2 green and red, 3 red	2	1	2	2
	OIV 010	Colour of the ventral side of nodes	1 green, 2 green and red, 3 red	2	1	1	2

	OIV 011	Density of erect hairs nodes	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1	1	1
	OIV 012	Density of erect hairs internodes	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1	1	1
	OIV 013	Density of prostrate hairs on nodes	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	3	3	3	7
	OIV 014	Density of prostrate hairs on internodes	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5	5	5	5
	OIV 015-1	Distribution of anthocyanin coloration on the bud scales	1 absent, 2 basal, 3 up to 3/4 of bud scale, 4 almost on the whole bud scale	3	3	3	3
	OIV 015-2	Intensity of anthocyanin coloration on the bud scales	1 none or very weak, 3 weak, 5 medium, 7 strong, 9 very strong	5	5	5	5
	OIV 016	Number of consecutive tendrils	1 two or less, 2 three or more	1	1	1	1
	OIV 017	Length of tendrils	1 very short, 3 short, 5 medium, 7 long, 9 very long	3	3	3	3
Young leaf	OIV 051	Colour of upper side of blade (4th leaf)	1 green, 2 yellow, 3 bronze, 4 copper-reddish	4	2	2	2
	OIV 053	Density of prostrate hairs between main veins on lower side of the blade (4th leaf)	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5	5	5	9

	OIV 054	Density of erect hairs between main veins on lower side of blade (4th leaf)	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1	1	3
	OIV 055	Density of prostrate hairs on main veins on lower side of blade (4th leaf)	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5	5	5	5
	OIV 056	Density of erect hairs on main veins on lower side of blade (4th leaf)	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1	1	1
Mature leaf	OIV 065	Size of blade	1 very small, 3 small, 5 medium, 7 large, 9 very large	7	7	7	7
	OIV 067	Shape of blade	1 cordate, 2 wedge-shaped, 3 pentagonal, 4 circular, 5 kidney-shaped	3	3	3	3
	OIV 068	Number of lobes	1 one (entire leaf), 2 three, 3 five, 4 seven, 5 more than seven	3	3	3	3
	OIV 069	Colour of the upper side of blade	3 pale green, 5 medium green, 7 dark green	7	7	7	7
	OIV 070	Area of anthocyanin coloration of main veins on upper side of blade	1 absent, 2 only at the petiolar point, 3 up to the first bifurcation, 4 up to the second bifurcation, 5 beyond the second bifurcation	3	3	3	3
	OIV 071	Area of anthocyanin coloration of main veins on lower side of blade	1 absent, 2 only at the petiolar point, 3 up to the first bifurcation, 4 up to the second bifurcation, 5 beyond the second bifurcation	2	1	2	1

OIV 072	Goffering of blade	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong	5	5	5	5
OIV 073	Undulation of blade between main or lateral veins	1 absent, 9 present	9	9	9	9
OIV 074	Profile of blade in cross section	1 flat, 2 V-shaped, 3 involute, 4 revolute, 5 twisted	5	5	5	5
OIV 075	Blistering of upper side of blade	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong	3	3	3	3
OIV 076	Shape of teeth	1 both sides concave, 2 both sides straight, 3 both sides convex, 4 one side concave, one side convex, 5 mixture between both sides straight and both sides convex	3	3	2	2
OIV 077	Size of teeth in relation to blade size	1 very small, 3 small, 5 medium, 7 large, 9 very large	5	5	5	5
OIV 078	Length of teeth compared with their width	1 very short, 3 short, 5 medium, 7 long, 9 very long	5	5	5	5
OIV 079	Degree of opening/overlapping of petiole sinus	1 veri wide open, 3 open, 5 closed, 7 overlapped, 9 strongly overlapped	5	5	3	5
OIV 080	Shape of base of petiole sinus	1 U-shaped, 2 bace-shaped, 3 V-shaped.	3	3	3	3
OIV 081-1	Teeth in the petiole sinus	1 none, 9 present	1	1	1	1

OIV 081-2	Petiole sinus base limited by vein	1 not limited, 2 on one side, 3 on both sides	1	1	1	1
OIV 082	Degree of opening/overlapping of upper lateral sinuses	1 open, 2 closed, 3 slightly overlapped, 4 strongly overlapped, 5 absence of sinus	2	2	2	2
OIV 083-1	Shape of the base of upper lateral sinuses	1 U-shaped, 2 bace-shaped, 3 V-shaped.	1	1	1	1
OIV 083-2	Teeth in the upper lateral sinuses	1 none, 9 present	1	1	1	1
OIV 084	Density of prostrate hairs between main veins on lower side of blade	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	7	5	7	9
OIV 085	Density of erect hairs between main veins on lower side of blade	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5	5	5	7
OIV 086	Density of prostrate hairs on main veins on lower side of blade	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5	5	7	9
OIV 087	Density of erect hairs on main veins on lower side of blade	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1	1	3
OIV 088	Prostrate hairs on main veins on upper side of blade	1 absent, 9 present	1	1	1	9
OIV 089	Erect hairs on main veins on upper side of blade	1 absent, 9 present	1	1	1	1
OIV 090	Density of prostrate hairs on petiole	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	3	5	5	5

	OIV 091	Density of erect hairs on petiole	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1	1	1
	OIV 093	Length of petiole compared to length of middle vein	1 much shorter, 3 slightly shorter, 5 equal, 7 slightly longer, 9 much longer.	1	1	1	1
Bunch	OIV 202	Length (peduncle excluded)	1 very short, 3 short, 5 medium, 7 long, 9 very long	9	7	7	7
	OIV 203	Width	1 very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide	9	7	5	5
	OIV 204	Density	1 very loose, 3 loose, 5 medium, 7 dense, 9 very dense	5	3	5	7
	OIV 208	Shape	1 cylindrical, 2 conical, 3 funnel-shaped	2	2	2	1
	OIV 209	Number of wings of the primary bunch	1 absent, 2 one-two wings, 3 three-four wings, 4 five-six wings, 5 more than six wings.	3	2	2	1
	OIV 502	Single bunch weight (g)	1 very low, 3 low, 5 medium, 7 high, 9 very high	5	3	3	3
Berry	OIV 220	Length	1 very short, 3 short, 5 medium, 7 long, 9 very long	3	3	5	3
	OIV 221	Width	1 very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide	5	5	5	3

	OIV 222	Uniformity of size	1 not uniform, 2 uniform	2	1	2	2
	OIV 223	Shape	1 obloid, 2 globose, 3 broad ellipsoid, 4 narrow ellipsoid, 5 cylindric, 6 obtuse ovoid, 7 ovoid, 8 obovoid, 9 horn shaped, 10 finger shape	1	1	2	1
	OIV 225	Colour of skin	1 green yellow, 2 rose, 3 red, 4 grey, 5 dark red violet, 6 blue black	1	1	1	1
	OIV 226	Uniformity of skin colour	1 not uniform, 2 uniform	2	2	2	2
	OIV 229	Hilum	1 little visible, 2 visible	2	2	2	2
	OIV 236	Intensity of flesh anthocyanin coloration	1 none, 2 muscat, 3 foxy, 4 herbaceous, 5 other flavour than muscat, foxy or herbaceous	1	1	1	1
	OIV 241	Formation of seeds	1 none, 2 rudimentary, 3 complete	3	3	3	3
	OIV 503	Single berry weight (g)	1 very low, 3 low, 5 medium, 7 high, 9 very high	3	3	3	3

Palomino Fino (PF), Palomino Gacho (PG), Palomino Pelusón (PP) and Palomino de Jerez (PJ).

5.2. Estudio y evaluación a escala de laboratorio de la fermentación en presencia de hollejos durante la elaboración de vinos de la variedad Palomino Fino.

Los resultados presentados en este capítulo se han publicado en:

4) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Influence of the Presence of Grape Skins during White Wine Alcoholic Fermentation. *Agronomy* **2021,** *11*, 452; doi:10.3390/agronomy11030452

Abstract

The production of white wines with the presence of grape skins is a historical technique used in different regions with winemaking tradition. However, the current trend is to maintain the presence of grape skins during white wine making only during the pre-fermentation phase in order to enrich and give greater complexity to the sensory profile of the wines. Given these precedents, this study is the first to consider the effect of the presence of different grape skins doses throughout the alcoholic fermentation process. To this end, the effect of 5 different doses of grape skins (20, 40, 60, 80 and 100%) has been studied with respect to a control (0%) during alcoholic fermentation, the physicochemical composition of the final wines and a preliminary sensory analysis. The presence of grape skins has led to an increase in viable biomass and speed of fermentation with respect to the control. However, no differences have been observed in terms of the consumption of nitrogenous sources by yeasts. The wines produced have not shown great differences in their physicochemical composition, except for the volatile acidity. In addition, the preliminary sensory analysis showed differences between the different grape skins doses studied, where the wine produced with 20% grape skins has been the best evaluated by the tasting panel. In this sense, the production of wines with a 20% grape skins presence during the entire alcoholic fermentation is presented as a viable technique that would allow the diversification of the production of white wines and meet the trends and expectations of current wine consumers.

Introduction

Vine (*Vitis vinifera* L.) is one of the most widely cultivated species worldwide [1]. Currently, 7.4 million hectares of land are covered by this crop, producing 27.3 million of grape tones. Spain represents 13% of this area, being the country with the largest area of vineyards [2]. Of this amount of grapes, those destined for wine production produce a quantity of 292 million hL, of which Spain represents 15.2%. However, at national level, white wine production represents only 26.51% [3].

In general, the protocols for making white wines are based on fermenting the grape must in the absence of the solid parts [4]. However, for some winemaking processes, oenological practices that encourage the extraction of different compounds that will influence the chemical composition of the wine and its sensory properties, such as terpenes and aromatic precursors (amino acids, fatty acids, etc.) from the skins to the must are introduced [5]. In this sense, the aromatic complexity of the white wine depends on factors such as the grapevine variety used in the production (primary and varietal aromas), the aromas produced during fermentation (secondary) [6] and the evolution of these aromas during ageing (tertiary) [7–9].

Some white wines are currently made by allowing contact of Grape Skins (GS) with the grape must before fermentation in order to increase the extraction of compounds that influence the intensity of varietal aromas [10]. Several studies have demonstrated the modification of wines by the presence of GS [11], in which it has been shown that the presence of GS in a pre-fermentative stage results in the appearance of herbaceous aromas or an increase in bitterness due to an increase in the concentration of terpenes and phenolic compounds [12]. Also, the detrimental effects of grape must contact with its solid parts are a higher browning susceptibility [13], extended up-take of potassium that enhance potassium bi-tartrate precipitation and a drop in titratable acidity [14] and off-odors alongside with a higher astringency and bitterness [15]. Increases in free varietal com-pounds, an intensification of fresh and fruity characters, and a modification in the body and acidity of the wine have also been found, which is perceived as positive by sensory analysis in a tasting panel [16].

However, currently few wineries and winemakers have opted to make white wines with maceration of solid parts beyond the pre-fermentative stage, as they require more exhaustive control over the winemaking process in order to avoid undesirable effects. This means that this type of wine has not been studied in depth. Historically, *Kvevri* wine (Georgia) is an example of wine fermented with the presence of skins spontaneously [17]. *Kvevri* white wines, as might be expected, have a higher polyphenol content than traditional white wines, in some cases reaching almost the same concentrations found in red wines [18]. These wines have become internationally famous over the last 10 years, forming part of a new trend among consumers, and presenting themselves as a perfect blend of innovation and historical practices [15]. This type of wine, also known as orange wine, skin contact wine or skin contact white, depending on the competent regulation [19] would meet the expectations of today's consumers, eager for these oenological concepts in order to recover historical techniques and merge them into new products [20,21]. However, despite the success that this type of wine is having, the research that has been carried out in relation to the production of these wines is focused either on the processes of oxidation of the polyphenolic matter [22], or from a sensory point of view of the final wines in comparison with other GS-must maceration treatments [23].

Currently, new consumers are demanding more particular and exclusive wines that stand out from the rest because of their distinctive organoleptic characteristics [24]. In

general, all the studies published in relation to long-term maceration have concluded that it is necessary to control the process in order to minimise the appearance of all those at-tributes that are undesirable from a sensory point of view, such as increased astringency, or loss of acidity and freshness [25]. However, to the best of our knowledge, there are no precedents in the scientific literature that study the effect of the presence of GS in the alcoholic fermentation kinetics. Thus, this research presents, for the first time, the effect of the presence of different concentrations of GS during the alcoholic fermentation of a grape must from the warm climate autochthonous variety 'Palomino Fino'. Hence, this research could be beneficial to diversify the production of white wines in a warm climate region innovating, at the same time, by recovering historical winemaking techniques such as GS fermentation.

Materials and Methods

Materials

Raw material used was 'Palomino Fino' grapes. Grapes were harvested manually in a vine plot located at 36° 64' 29.7'' N, 5° 49' 53.5'' W in the municipality of San José del Valle (Cadiz, Spain) at 150 MSL. No irrigation or fertilization treatments were applied during the studied year, and different conventional phytosanitary products were applied to ensure correct grape development.

Grapes were destemmed manually and the whole grapes were crushed in a vertical press. The grape must was homogenised in a stainless steel tank of 100 L and its acidity was corrected employing tartaric acid (Agrovin, Ciudad Real, Spain). In order to avoid possible grape must oxidation, 80 mg/L of potassium metabisuphite was added (Agrovin, Ciudad Real, Spain). The grape must obtained from the press showed the following characteristics after correction: pH = 3.74, sugar concentration = 12 °Bé, density = 1.0862 g/cm3 and a total acidity of 4.24 g/L of tartaric acid and 0.30 g/L of malic acid. Once all the pre-fermentation corrections had been carried out, the must was distributed in glass made 5-litre tanks. To each tank, displayed in duplicate, a proportion of Grape Skins (GS) was added, ranging from 0 % (control) to 100 % (skin contact wine), studying also the intermediate fractions of 20, 40, 60 and 80 % of GS calculated by volume. After the addition of the different proportions of GS, an inoculum of Saccharomyces cerevisiae active dry yeast, Lalvin 71B (Lallemand, Barcelona, Spain) was added at a concentration of 10 g/hL. The alcoholic fermentation was carried out at 18 °C. After the alcoholic fermentation (21 days), the resulting wines were finned employing gelatin (4 g/hL, Agrovin, Ciudad Real, Spain) and, after 24 hours bentonite (40 g/hL, Agrovin, Ciudad Real, Spain). After 72 h wines were filtered using a plate filter and bottled employing nitrogen as inert gas. Cork was employed to close the bottles.

Analytical Methodology

For grape must physicochemical characterisation, pH was measured using a digital pH-meter CRISON-2001 (Crison, Barcelona, Spain), equipped with a combined electrode with automatic temperature compensation. Sugar concentration (°Bé) was determined using a calibrated Dujardin–Salleron hydrometer (Laboratories Dujardin-Salleron, Arcueil Cedex, France). Total acidity (TA) was assessed following the International Organization of Vine and Wine (OIV) reference method [26]. Density was determined by direct measurement with a DMA 5000M densimeter (Anton-Paar, Graz, Austria). During fermentation, viable biomass, density and Free Amino Nitrogen (FAN) were assessed. To determine the viable biomass populations counts were performed using an optical Nikon microscope with 400 X magnificence, using methylene blue staining method in a Merck Neubauer chamber (Madrid, Spain) as reported in previous publications [27]. The relative density of samples was carried out according to the methodology proposed by Amores-Arrocha et al. [28]. FAN content was determined according to Abernathy et al. [29]. For wine characterization, alcohol content, total acidity and volatile acidity were determined following the officially approved methods of wine analysis [23]. Residual sugars were determined assessed by means of the dinitrosalicylic acid (DNS) method according to Gonçalves et al. [30]. Malic and lactic acid was determined using an ionic exchange cromatograph (Metrohm 930 Compact IC Flex, Herisau, Switzerland) with a conductivity detector on a Metrosep Organic Acids column-250/7.8 (Metrosep, Herisau, Switzerland) following the conditions given by Sancho-Galán et al. [27]. Color was determined by following the recommendations of the International Commission of L'Eclairage, which establishes the CIELAB parameters that better define the color of wine and allow for more precise diferentiation [31–33]. Total Polyphenolic Index (TPI) was determined by measuring the absorbance of wine at 280 nm wavelength in quartz cuvettes with 1 mm light path in a spectrophotometer Genesis UV-VisTM 10s (ThermoScientific, Whaltman, United States).

Sensory Analysis.

The sensory analysis of the different wines produced was carried out with the aim of finding differences between the different proportions of skins present during fermentation. 5 days after the bottling of the wines, a 20-member panel, experienced with wine tasting methodology, carried out the sensory analysis. 50 mL of wine were served to each taster in standard tasting glasses [34]. Each taster was provided with a specific tasting file with scores to be evaluated on a 10-point scale where the olfactory and taste attributes were selected according to Jackson [35].

Statistical Analysis.

Means and standard deviations were calculated and significant differences were evaluated by two-way ANOVA and Bonferroni's multiple range (BSD) test with a $p < 0.05$ (GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego, CA, United States) statistical package.

Results and Discussion

Grape Skin Effect on Yeast Populations During Alcoholic Fermentation

Figure 1 shows the evolution of the viable yeast population during the alcoholic fermentation process. As can be seen, the population of viable yeasts does not correlate with the proportion of skins present in the tank. In addition, the control has a longer lag phase compared to the samples with skins, so that the exponential growth phase starts 24 hours later than the rest of the samples. This exponential phase continued until the 9th day for all samples when the maximum population of viable yeast is reached. However, the mini-mum population was obtained for the control wine (6.7×10^6 CFU/mL) and the maximum for those who fermented with the presence of 60% of their skins (1.07×10^7 CFU/mL). During this phase the control sample, 20, 40 and 100 % GS showed a similar trend with a similar population decline in some cases. For the 60 and 80% GS samples the trend was different; these samples that presented a greater number of viable populations, showing a more pronounced decrease in CFU/mL than the rest, with no significant differences be-tween them in any case from day 11 on. At all times, the control sample showed a significantly smaller population than the rest, both in the decline phase and in the cell death phase.

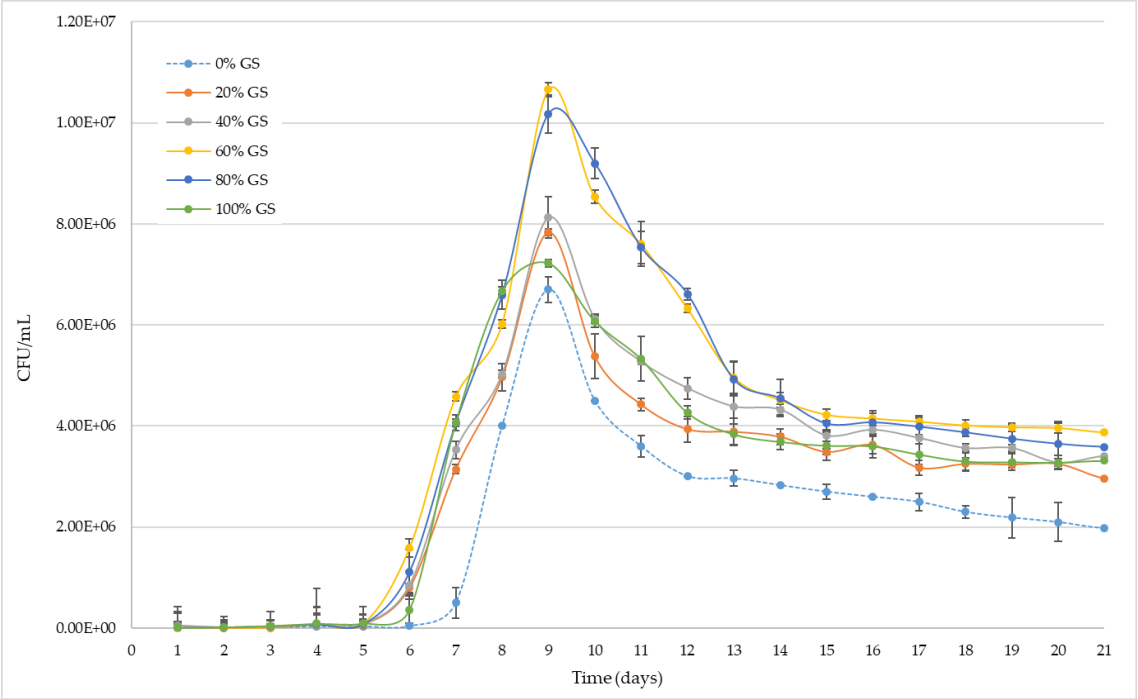

Figure 1. Viable biomass development during alcoholic fermentation of 'Palomino Fino' grape must with different GS doses. CFU: Colony Forming Unit.

The results observed could be due to three main factors; on the one hand, the presence of a higher proportion of GS in each fermenter could contribute to a greater and faster development of yeast cells. This is probably due to the fact that the exocarp of the grape skin, and to a lesser extent the endocarp, contains nitrogenised compounds that could be a source of nitrogen for the fermentation process, together with other co-factors necessary for the correct development of the cells, such as vitamins [36,37]. However, Figure 1 shows that the 80 and 100 % GS tanks do not present the largest amount of viable biomass, and therefore there is no correlation between the dose of GS present in each fermentation tank with the total yeast population. In this sense, the presence of skins in the fermentation tanks and therefore of short and medium chain fatty acids and their possible precursors [38], as well as their esters could act as inhibitors of the alcoholic fermentation process [39], increasing its toxicity with its solubility and therefore with the amount of ethanol present in the medium [40,41]. Finally, the addition of GS can lead to an increase in the presence of polyphenols in the fermentation medium [42]. In this way, the presence of such bioactive compounds can lead to an inhibitory effect on the activity of the membrane enzyme H+ -ATPase during the initial stages of the alcoholic fermentation [43]. In any case, the activating effect of the presence of the GS outweighs the inhibiting effect of it at the start of fermentation and in the exponential yeast growth phase, being the yeast population achieved higher than that observed by the control for all samples.

Grape Skin Effect on Alcoholic Fermentation Kinetics.

The influence of the presence of different doses of GS on grape must alcoholic fermentation kinetics is shown in Figure 2. As occurred with viable biomass, there is no correlation between the dose of GS present in the fermenter and a greater fermentation speed.

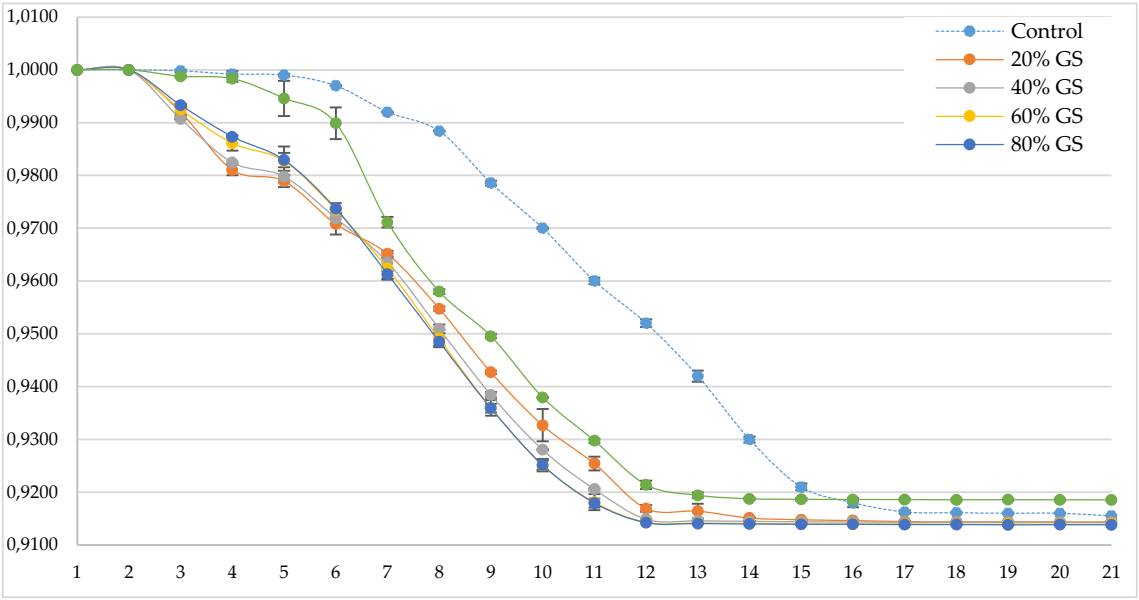

Figure 2. Relative density (adimensional, Y axis) during alcoholic fermentation (days, X axis) of 'Palomino Fino' grape-must with different GS doses.

At the beginning of the alcoholic fermentation, and coinciding with the dormancy phase of yeast, it can be seen that during the first 48 hours the samples do not differ significantly from each other. However, from the third day onwards, all the samples except the control and the 100% GS sample show a small but significant decrease (ANOVA $p < 0.05$) in their relative density. Coinciding with the exponential phase of yeast's development (day 6), all samples showed a decrease in its relative density. During the course of the alcoholic fermentation, it is observed that the contribution of GS does not have a significant effect on the fermentation speed for the samples between 40 and 80 % GS, however, if the slopes between day 3 and day 13 of the fermentation are analyzed, a lower fermentation speed is observed for the control and 100% samples. The results obtained in the analysis of the relative density of the different samples show that the samples with the greatest amount of viable biomass (Figure 1) have shown a higher rate of fermentation. As expected, more viable biomass leads to a greater consumption of fermentable must sugars and, consequently, a greater decrease in density values. However, none of the samples presented problems in the final phase of alcoholic fermentation and all the wines were able to carry out this process without problems, maintaining the significant differences previously observed for the control and 100% GS (ANOVA $p < 0.05$), being the density less than 0.92 g/cm^3 in all cases.

As on the previous occasion, the fact of not supplying GS to the fermentation tank could mean a lower availability of nutrients and/or co-factors as minerals or vitamins for a rapid development of the yeast at the beginning of the alcoholic fermentation [36,37] and, therefore, more time for yeast cells to start consuming sugars. In this case, the possible inhibitory effect of a high proportion of skins can also be seen, since the sample with 100% GS again shows a certain delay in the consumption of sugars with respect to the rest of the samples with GS. However, in this case, the speed of fermentation is similar to the other samples from day 7 until the end of fermentation.

Grape Skin influence on Free Amino Nitrogen (FAN) during Alcoholic Fermentation.

Figure 3 shows the evolution of FAN (mg/L) during alcoholic fermentation. As can be seen, the initial FAN content after 24 hours of inoculum addition and the different GS proportions did not vary significantly between the different samples, being the values observed between 172 and 180 mg FAN/L for the control and the sample with 100% GS respectively.

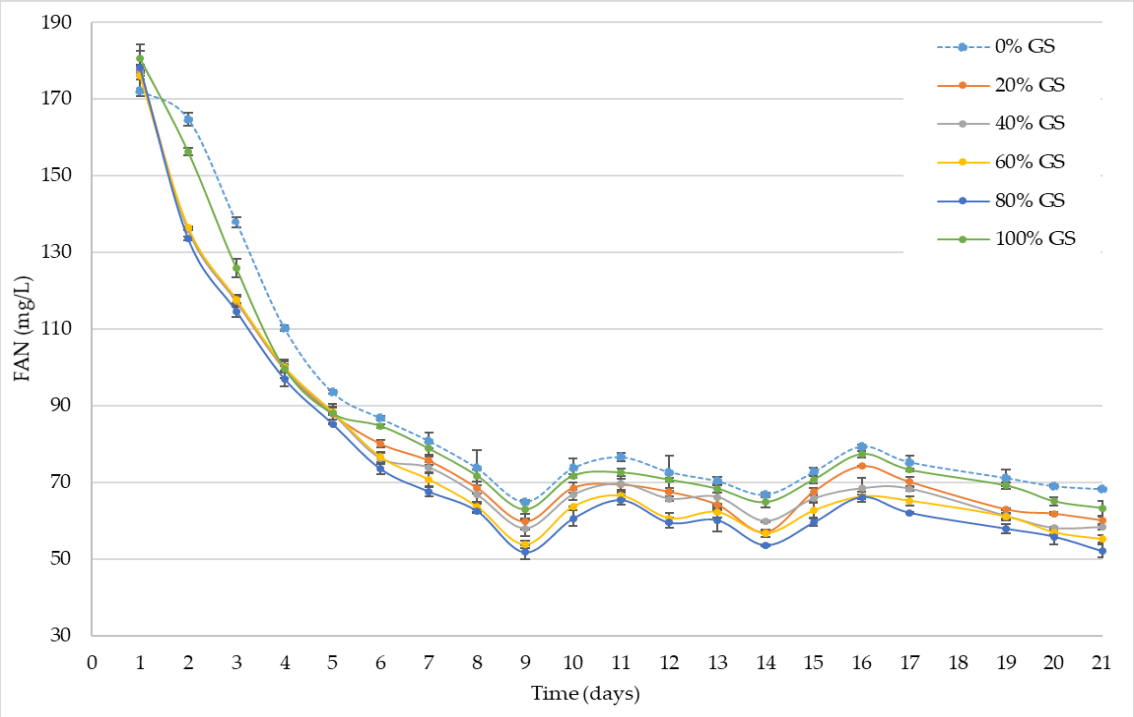

Figure 3. Evolution of FAN (mg N/L) in 'Palomino Fino' grape musts with different GS proportions during alcoholic fermentation.

In contrast to what was observed in the study of viable biomass (Figure 1) and the study of fermentation kinetics (Figure 2), the consumption of FAN begins within the first 24 hours after yeast inoculation, with around 25% of the total FAN content being consumed in 20, 40, 60 and 80% GS doses. After 48 hours, the control and 100 % GS samples begin to differ significantly (ANOVA $p < 0.05$) with the rest of the samples, as

was the case for the analysis of viable biomass and fermentation kinetics. These differences remain until day 7 of fermentation (start of the exponential growth phase of the yeast, Figure 1). The maximum FAN consumption (average decrease of 66% with respect to the initial value), and therefore its lower value in the medium, was observed on day 9, coinciding with the maximum viable biomass value in the medium (Figure 1). From this moment of fermentation on, all the samples showed a very similar behavior, with average values of FAN between 57 and 68 mg FAN/L. At the end of the alcoholic fermentation, all the samples showed FAN values between 50 and 70 mg FAN/L. Significant differences were observed between all samples with the exception of the 20 and 40 % GS samples (ANOVA $p < 0.05$).

Nitrogen compounds are essential for the development and metabolism of fermentation yeasts. Regarding the nutrients used by yeasts during fermentation of grape must, nitrogen is quantitatively the second most important after carbon. The nitrogen fraction in grape musts is composed of amino acids, ammonium ions, peptides and proteins [44]. More specifically, the Free Amino Nitrogen (FAN) determined in this research by means of nynhydrin based assay, reflects the nitrogen content available to yeasts during fermentation as the nitrogen of the amino acids and ammonium, as well as the various small pep-tides, is quantified as outlined by the 1977 European Brewery Convention Methods of Analysis [29]. On this occasion, initial values of FAN greater than 140 mg FAN/L were observed in all cases, being this value the minimum stipulated for the correct development of yeasts and the completion of alcoholic fermentation [45]. Once the lowest FAN value was obtained at the time of maximum viable biomass value, concentrations fluctuated for all samples until the end of fermentation. This fluctuation could be due to the process of autolysis by the lees, which would release amino acids as well as other substances into the fermentation medium [46–49], thus, affecting FAN values. Finally, the low FAN values available in the different samples at the end of fermentation ensure that wines have correct microbiological stability, while minimising other problems such as the accumulation of harmful compounds such as ethyl carbamate [50].

Final Wines Physicochemical Characterisation.

The results of wines physicochemical analysis are shown in Table 1. In relation to wines total acidity, the values ranged between 4.52 g/L and 3.78 g/L of tartaric acid for the control sample and 100% GS respectively, appearing only significant differences between the control and the rest of the samples (ANOVA $p < 0.05$). However, although no significant differences were observed between the different samples with GS, a negative correlation is observed between the dose of GS and the values of total acidity ($r = -0.9295$). These results could be due to the release of buffering ions from GS. The decrease in TA was also seen by Olejar [51] in 'Sauvignon Blanc' wines and suggest that

there is a release of basic materials [52] or potassium resulting in potassium bitartrate precipitation [53].

Table 1. Physicochemical composition and colour analysis of 'Palomino Fino' final wines made with different GS concentrations.

	Control	20% GS	40% GS	60% GS	80% GS	100% GS
TA (g/L)	4.52 ± 0.02^{a}	4.12 ± 0.0^{b}	4.05 ± 0.02^{b}	3.91 ± 0.02^{b}	3.85 ± 0.03^{b}	3.78 ± 0.02^{b}
VA (g/L)	0.15 ± 0.01^{a}	0.24 ± 0.02^{b}	0.33 ± 0.03^{c}	0.42 ± 0.01^{d}	0.62 ± 0.05^{e}	0.72 ± 0.04^{f}
% Alc.	11.73 ± 0.11^{a}	11.92 ± 0.12^{a}	11.85 ± 0.12^{a}	12.05 ± 0.17^{a}	11.98 ± 0.10^{a}	11.81 ± 0.02^{a}
RS (g/L)	2.32 ± 0.14^{a}	1.87 ± 0.03^{b}	1.72 ± 0.02^{c}	1.68 ± 0.04^{c}	1.70 ± 0.02^{c}	2.02 ± 0.06^{d}
Malic Acid (mg/L)	329.90 ± 0.80^{a}	440.52 ± 0.20^{b}	463.18 ± 1.00^{c}	472.32 ± 1.20^{d}	480.70 ± 0.20^{e}	487.20 ± 0.20^{f}
Lactic Acid (mg/L)	104.86 ± 1.00^{a}	70.02 ± 1.60^{b}	92.94 ± 2.20^{c}	136.30 ± 0.20^{d}	136.68 ± 0.14^{d}	161.08 ± 0.20^{e}
L*	96.48 ± 0.34^{a}	98.20 ± 0.23^{a}	98.38 ± 0.3^{a}	98.79 ± 0.25^{a}	98.49 ± 0.48^{a}	98.30 ± 0.15^{a}
a*	−0.50 ± 0.07^{a}	−0.57 ± 0.02^{b}	−0.60 ± 0.02^{c}	−0.63 ± 0.03^{b}	−0.58 ± 0.02^{b}	−0.60 ± 0.06^{b}
b*	14.16 ± 0.67^{a}	7.39 ± 0.47^{b}	6.84 ± 0.63^{b}	6.07 ± 0.00^{b}	5.99 ± 0.00^{b}	6.82 ± 0.32^{b}
H*	92.05 ± 0.38^{a}	94.4 ± 0.47^{a}	95.02 ± 0.57^{a}	95.98 ± 0.31^{a}	95.63 ± 0.13^{a}	94.80 ± 0.31^{a}
C*	14.17 ± 0.67^{a}	7.41 ± 0.47^{b}	6.87 ± 0.63^{b}	6.15 ± 0.24^{b}	6.02 ± 0.01^{b}	6.85 ± 0.32^{b}
Abs 420 nm	0.20 ± 0.01^{a}	0.11 ± 0.01^{b}	0.10 ± 0.01^{b}	0.08 ± 0.01^{c}	0.09 ± 0.01$^{b, c}$	0.10 ± 0.01^{c}
TPI	0.470^{a} ± 0.068	0.544 ± 0.024^{a}	0.696 ± 0.090^{b}	0.864 ± 0.081^{c}	0.887 ± 0.039^{c}	0.901 ± 0.075^{c}

TA: Total Acidity (g/L tartaric acid), VA: Volatile Acidity (g/L acetic acid), RS: Residual Sugars. CIELab coordinates: L* (lightness), a* (red/green), b* (yellow/blue, H* (hue) and C* (chroma). TPI: Total Polyphenol Index. Different superscript letters mean a significant difference between the samples (ANOVA $p < 0.05$) determined by two-way ANOVA applying a Bonferroni Multiple Range (BSD) Test.

VA values ranged from 0.15 g/L to 0.72 g/L of acetic acid for the control and 100% GS sample respectively. However, in this case significant differences were observed for all samples (ANOVA $p < 0.05$). In this case, unlike total acidity, the contribution of the different GS doses and the volatile acidity values showed a positive correlation ($r = 0.9889$). The increase in volatile acidity values could be attributed to an increase in the concentration of volatile acids, esters and alcohols as their concentration depends on the contact time be-tween GS and grape must [54]. With regard to the alcoholic strength, no significant differences were found between the different samples analysed, with values ranging from 11.73 and 12.05 % for the control and 60% GS respectively. Thus, it is verified that the contribution of the GS does not significantly affect the alcohol content of final wines. The results of residual sugar concentration (2.32 g/L for control and 1.68 g/L for 80% GS) indicated that the fermentation was completed [55] and was carried out until the fermentable sugars present in the medium were exhausted. Although significant differences appear between the different samples, these are almost imperceptible from an oenological point of view.

Regarding the two organic acids specifically analyzed, the concentration of malic ac-id ranged between 329.90 mg/L and 497.70 mg/L for the control and the 80% GS sample respectively, showing significant differences between all samples with respect to the control. However, its content in the control sample did not show significant differences with respect to the initial malic acid content in grape must. After comparing the results with the initial malic acid concentration, it is observed that the contribution of GS implies a higher concentration of this acid, following a linear correlation ($r = 0.8302$). On the other hand, the lactic acid content in the final wines showed a similar trend to that of malic acid, with the difference that no lactic acid was detected in the initial grape must. The values of this acid ranged from 70.02 mg/L for 20% GS to 161.08 mg/L for 100% GS. Thus, it can be observed that simultaneously to the alcoholic fermentation process, a malolactic fermentation process could have been carried out, increasing the presence of lactic acid in final wines. However, this hypothesis also agrees with the results observed regarding the volatile acidity of the wine, given that the lactic acid bacteria can present a catabolic metabolism of sugars present in the medium during the fermentation phase, and also can coexist with the inoculum of Saccharomyces cerevisiae, consequently increasing the values of acetic acid in the medium [56,57]. Nevertheless, despite the increase in volatile acidity that could have been caused by lactic acid bacteria, the increase in volatile acidity could also be mainly due to the growth of oxidative yeasts and/or bacteria.

The mean values and standard deviation of the CIELab, L* (Lightness), a* (red/green), b* (yellow/blue), H* (Hue), C* (Chroma) and Abs at 420 nm for the different samples are also shown in Table 1. As can be seen, the presence of GS has a significant effect (ANOVA $p < 0.05$) on the coordinates a*, b*, C* and Abs at 420 nm. In comparison with the control wine, when studying the behavior of a*, it was observed that all the wines showed a shift towards the characteristic coordinates of greenish tones, and more

pronounced in the wines made with 60% of GS. On the other hand, with respect to parameter b*, the control wine was the one with the highest value, being in the three-dimensional space of the yellow colour. Also, a negative correlation was observed for the samples comprised between 20 and 80 % GS ($r = -0.9630$). It is necessary to indicate that none of the wines showed any alteration either in the values of luminosity (L*) or in the tonality or shade (H*). In that sense, the presence of the skins during the alcoholic fermentation could be exerting a screen effect against possible oxidation effects during the alcoholic fermentation. In addition, it is necessary to indicate the significant decrease (ANOVA $p < 0.05$) observed in the Abs 420 nm values in all the wines produced in the presence of grape skins, reaching reductions of 40% (for the 60% GS wine) in this parameter compared to the control. Finally, in order to observe whether the presence of different doses of skins implied an in-crease in the polyphenolic content of the wine, TPI values (Abs $\lambda = 280$ nm) were analysed. The results ranged between 0.470 and 0.901 for the control and the 100% GS sample respectively, showing significant differences and a linear correlation ($r = 0.9164$) between GS dose and IPT TPI content. These differences are due to the release of the polyphenolic compounds present in GS during the alcoholic fermentation [42]. Therefore, it can be stated that the polyphenols present in the medium can induce significant changes in the yeast metabolism and thus in their fermentation kinetics [42].

Sensory Analysis

Subsequently to the analysis of the various influences exerted by the presence of GS during the alcoholic fermentation process, a preliminary sensory analysis was carried out in order to explore the viability of this technique in diversifying white wine production in an area with a warm climate. Figure 4 shows the average values of all the attributes (olfactory and gustatory, as well as an overall evaluation) evaluated during wine preliminary sensory analysis.

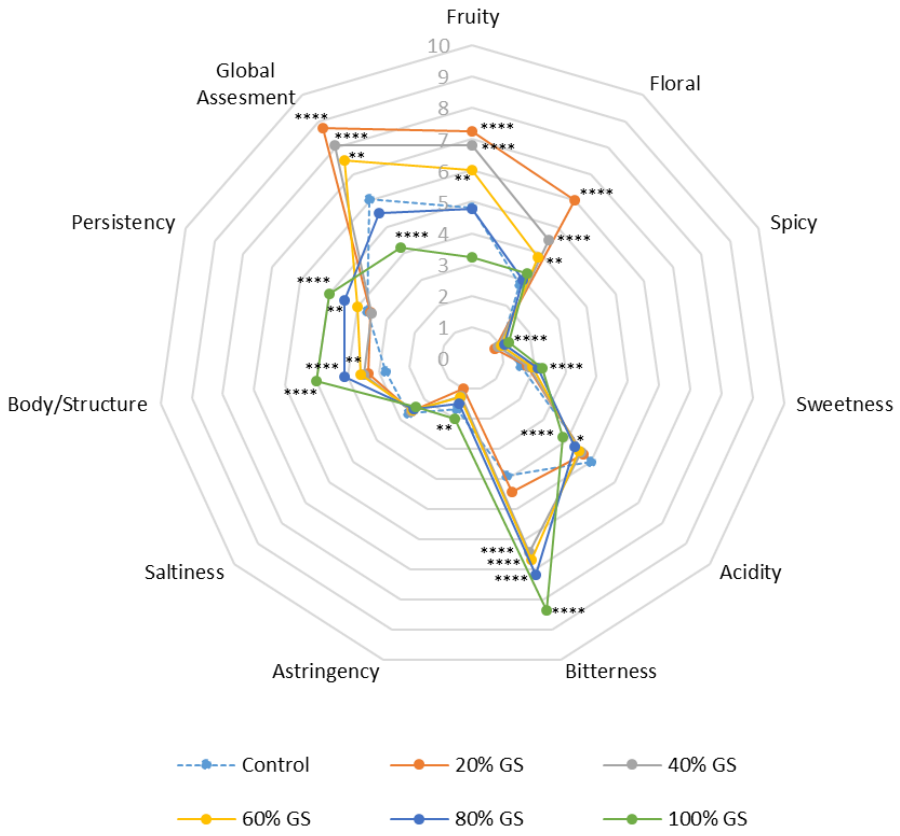

Figure 4. GS effect on visual and olfactory evaluation of 'Palomino Fino' wines. Stars indicate level of significance for two-way ANOVA according to Bonferroni's multiple range test (BSD) (* $p < 0.1$, ** $p < 0.01$and **** $p < 0.0001$).

With regard to the fruity character, there were differences between the samples with GS and the control wine. The wines that showed the fruitiest character were those made with 20% of skins, followed by those made with 40 and 60%. The wines made with 80% of GS had a similar score to the control wine, while the wines made with 100% of skins had the lowest values for this attribute. Similar results were obtained for the "floral" attribute, with significant differences (ANOVA, $p < 0.0005$), mainly, in 20% GS, followed by 40% and 60% GS wines. In relation to the spicy (nose) and sweetness (mouth) characteristics, all the samples showed similar behaviour, obtaining very low ratings for all the doses and the control, except for the wines made with 100% GS. All the samples showed average values in acidity, with the control wine showing the highest values, while the lowest values were obtained in the wine made with 100% GS. These results are related to those obtained in the analysis of the TA (Table 1), the contribution of cations that can precipitate with the free acids of the wine by the grape skins, could be the cause of this decrease in the acidity of the wines [49], in both physicochemical and sensory ways. With regard to bitterness, a positive correlation was observed ($r = 0.9724$) in the different samples, with the lowest values in the control and the highest in the wine made with 100% GS. The increase in bitterness in the wines could be due to a greater extraction of the polyphenolic com-pounds present in GS, which could alter the sensory profile of

the wine, making it more bitter [22,53,58,59]. As regards the wine astringency and salinity, low values were obtained in all cases, with only a slight increase in astringency for wines made with 100% GS. As regards the body/structure attribute of the wines, a linear correlation (r = 0.9477) was observed with the increase in the percentage of grape skins for all wines. In this sense, the control sample presented lower values in these results, while the wines made with 100% GS presented a greater body/structure. This fact, as with the bitterness attribute, may be due to a greater presence of polyphenols and other compounds extracted in excess during winemaking [60,61] as seen in Table 1. However, in the case of persistence, a linear increase was only observed with the dose of skins in this attribute from 60% GS on (r = 0.9983). Probably, these results could be due to the fact that it is from this concentration of GS that the polyphenols start to become noticeable from a sensory point of view. Finally, the wines were evaluated globally by the tasters, with a significantly higher preference for the wine made with 20% GS in the first place, followed by those made with 40 and 60% GS respectively. The wines that showed the minimum overall score by the tasting panel were those made with 100%, 80% GS control.

Conclusions

In conclusion, the contribution of different GS doses during the production of white wine has led to an increase in the total population of viable biomass and an increase in the speed of fermentation compared to control wine produced without GS. However, the contribution of different GS doses has not caused a significant increase in the free amino nitrogen content at the beginning of fermentation nor has it led to major differences in its consumption during alcoholic fermentation. The final wines made with GS presence have not shown great physicochemical differences between them or with respect to control from an oenological point of view, with the exception of volatile acidity, which has shown significant differences depending on the different proportion of grape skins. However, after a preliminary sensory analysis, great differences were observed between them, with the wine made with 20% GS being the one that received the best evaluation by the tasting panel. Thus, the production of wines with a 20% GS presence during the entire alcoholic fermentation is presented as a viable technique that would allow the diversification of the production of white wines and meet the trends and expectations of current wine consumers.

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5.3. Estudio y evaluación a escala de laboratorio de los efectos del asoleo de la uva y/o la presencia de hollejos sobre la cinética y desarrollo de las levaduras durante el proceso fermentación de los mostos de la variedad Palomino Fino

Los resultados presentados en este capítulo se han publicado en:

5) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Effect of Grape Over-Ripening and Its Skin Presence on White Wine Alcoholic Fermentation in a Warm Climate Zone. *Foods* **2021**, *10*, 1583; doi:10.3390/foods10071583.

Abstract

The current trend of rising temperatures and sun irradiation associated to climate change is pushing traditional grape-producing areas with a warm climate towards a very accelerated ripening, leading to earlier harvesting dates and grape must with an unbalanced composition. However, this climatic trend could be exploited to produce other types of wine. In this sense, the increase in temperature could be used to produce wines with overripe grapes. In this regard, the aim of this research work is to evaluate the influence of different degrees and techniques of grape over-ripening to produce wines with the presence or absence of its skins during alcoholic fermentation. To this end, a physicochemical characterization of grape musts and wines obtained from overripe grapes and the monitoring of their fermentation has been performed. Over-ripening grapes by sun-drying has been established as a viable technique viability, producing musts and wines with unique physicochemical and sensory characteristics. In view of the above, it is considered that the production of wines from overripe grapes and in the presence or absence of grape skins is a viable approach to make new white wines taking advantage of the conditions imposed by climate change in a warm climate zone and meet the trends and expectations of current wine consumers.

Introduction

Grapevines are one of the most important crops worldwide, with vineyard area of 7.4 million hectares and a production of 77.8 million tons of grapes [1]. According to the Food and Agriculture Organization (FAO), in 2018 [2], approximately 37% of world grape production was used for wine production in 71 countries, the 50.7% being produced in three European countries (Italy, France and Spain). These data evidence that the wine industry contributes to the economy and reputation of many countries all over the world. Nowadays, grapevine (*Vitis vinifera* L.) cultivation is primarily located in the Mediterranean basin [3] and other temperate climate regions between the latitudes of 30° and 50° in the northern hemisphere and 40° and 50° in the southern hemisphere [4], although grape-vines have been grown outside these limits, in the tropics, for a long time

[5]. In overall terms, climate change is gradually modifying the established cultivation limits. More specifically, it is causing a generalized advance of the grape harvest by 10–24 days over the last 30–50 years [6–8] and an accelerated vine growth and over-ripening of the grapes, leading to the production of musts with high potential alcoholic strength [9,10], higher pH [11,12], lower acidity [13] and significant nutritional deficiencies, generally resulting in low levels of free amino nitrogen (FAN) [13–15]. The effects associated with climate change on grape quality pose important challenges for the winemaking process and the production of quality wines—more particularly, all the factors associated with the expression of varietal aromas, chemical and microbiological stability and sensory balance [7]. Therefore, quality wine production could be affected in those areas that have a warm cli-mate.

To the best of our knowledge, most adaptation actions against climate change focus on the growth and development of different grape varieties [16–21]. Among these, the use of later ripening cultivars [22], new and better adapted cultivars [23] and the relocation of vineyards to higher altitudes zones [17] stand out. These adaptation measures would imply a long-term solution and changes in the regulations of the denominations of origin. Therefore, it is considered necessary to continue studying new strategies that will make possible to continue producing quality wines in wine-growing regions, where wine has an important social and economic significance. In this sense, strategies regarding the re-search of new or better adapted rootstocks [24], irrigation emergency systems [25], protection against extreme heat and sunburns, soil management, changes in training systems [26] and microbiological/biotechnological based strategies [27] are currently investigated all across the world. Regions that already have an eminently warm climate are the ones that are most interested in the search for strategies to adapt to climate change.

For this reason, alongside with the search for adaptation strategies from a viticultural point of view [28–30], it is necessary to study new winemaking processes as a strategy for adapting to climate change in particularly warm areas, either by the addition of natural products, to alleviate imbalances in the ripening of the grapes [31,32], or through the search for new winemaking processes [33]. In this sense, one of the strategies to adapt to climate change associated effects could be the elaboration of new white wines from over-ripe grapes. Grape over-ripening is a technique that varies according to climatic conditions and the product to be obtained, as well as the geographical location and the grape variety employed [34]. In China, India and Turkey, it is a method focused on raisin production [35]. However, in most hot and dry countries, this technique has been used for the production of certain sweet and fortified wines.

In this sense, grape over-ripening is a technique that can take advantage of the conditions established by climate change in a warm climate zone (high radiation and temperatures). Thereby, grape over-ripening by means of sunlight techniques allow a natural modification of grape composition and lead to the production of new types of wines. In addition, this adaptation strategy would allow to keep producing wine in

traditional winemaking regions, the diversification of its production and the development of new business opportunities. In addition, it would meet the expectations of today's consumers, eager for oenological concepts in order to recover historical techniques and merge them into new products [35,36].

In view of the above, the aim of this research is to evaluate new white wine production processes that will allow new wines to be made and maintain the continued production of quality white wines in an eminently warm area under the effects of climate change. In this research paper, the results of the production of new dry white wine typologies from the autochthonous grapevine cultivar 'Palomino Fino', over-ripped by means of sun-drying and in climatic chamber and also fermented with or without the presence of its skins, are presented.

Materials and Methods

Raw Material

'Palomino Fino' grapes were harvested manually from a vine plot located at 36° 64' 29.7" N, 5° 49' 53.5" W, at 150 m above sea level, during the two years of study (2018 and 2019). No fertilization or irrigation treatments were applied in the vine plot during the studied years and conventional phytosanitary products were applied to ensure a proper grape development. A control without over-ripening and two different over-ripening techniques were applied to the grapes. On one side, for the sun-drying (SD) technique, grapes were spread out under the sun in a single layer for 48 and 96 h (hence, SD48h and SD96h). On the other side, climatic chamber drying was performed in a drying chamber (CH) (Ibercex ASL, Madrid, Spain), at 35 ± 1 °C and 10% of relative humidity for 48 and 96 h (hence, CH48h and CH96h), in order to compare natural over-ripening versus chamber over-ripening in controlled conditions of temperature and humidity. Temperature and humidity were controlled employing data loggers (LOG-210 Labprocess, Barcelona, Spain) during the whole process.

Once overripe, grapes were destemmed manually and the whole grapes crushed in a vertical press (MECAMAQ M030, Mollerussa, Spain) at a pressure equal to 2 bars. Grape musts were acidified using tartaric acid (Agrovin, Ciudad Real, Spain) and 80 mg/L of potassium metabisulphite (Agrovin, Ciudad Real, Spain). After all the pre-fermentation corrections had been carried out, the different grape musts were distributed in glass-made 5-liter tanks. To each tank, displayed in duplicate, an optimal dose of 20% grape skins (GS) calculated by volume was added according with previously published results [33], in order to study their effect on white winemaking. For its fermentation, a Saccharomyces cerevisiae pre-ferment of Lalvin 71B® (Lallemand, Barcelona, Spain) was employed. For each vintage studied, the experiment included 10 different fermentations (control without over-ripening, sun-dried and climatic chamber 48 and 96 h) without GS and the same layout with the presence of 20% of GS. The fermentation was carried out under controlled conditions at 18 °C. As soon as the

alcoholic fermentation was completed, wines where finned employing gelatin and bentonite at 4 g/hL and 40 g/hL, respectively. After 72 h, wines were filtered by means of a plate filter, bottled, employing nitrogen as inert gas, and corked.

Methodology

Grape must physicochemical characterization (pH, total acidity and °Bé) were performed according to the International Organization of Vine and Wine (OIV) procedures [37]. Free amino nitrogen (FAN) quantification was carried out according to the methodology proposed by Abernathy et al. [38].

Alcoholic fermentation was controlled by a daily measurement of its viable biomass, density and FAN. Viable biomass counts were carried out employing an optical Nikon Microscope using the methylene blue staining method in a Neubauer chamber (Merck, Madrid, Spain). Density was determined in a DMA 5000 M densimeter (Anton Paar, Graz, Austria). Wine analytical measurements (total acidity, volatile acidity and alcoholic strength) were carried out following the methodology stablished by OIV [37]. Residual sugars were determined by means of the dinitrosalicylic acid (DNS) method, according to Gonçalves et al. [39]. CIELab parameters were determined following the recommendations of the International Commission of l'Eclairage [40–42]. Absorbance at 420 nm and total polyphenolic index (TPI) were determined using a spectrophotometer Genesis UV-VisTM 10 s (ThermoScientific, Whaltman, TX, USA), by means of measuring its absorbance at 280 nm wavelength in quartz cuvettes.

Statistical Analysis

Significant differences between samples were evaluated by two-way ANOVA and Bonferroni multiple range (BSD) test with a $p < 0.05$ (GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego, CA, USA) statistical package.

Results and Discussion

Over-Ripening Effects in Grape Must Physicochemical Composition

The results of the physicochemical composition of 'Palomino Fino' grape musts after the different grape over-ripening treatments and times during two vintages are shown in Table 1.

Table 1. Grape must physicochemical composition after grape over-ripening treatments.

	Control	SD48h	SD96h	CH48h	CH96h
			2018		
pH	3.470 ± 0.014 [a]	3.440 ± 0.014 [b]	3.420 ± 0.028 [c]	3.300 ± 0.014 [d]	3.200 ± 0.014 [e]
TA (g/L)	3.630 ± 0.117 [a]	3.640 ± 0.175 [a]	3.920 ± 0.058 [b]	4.106 ± 0.058 [c]	4.996 ± 0.058 [d]
FAN (mg/L)	145.600 ± 0.000 [a]	183.40 ± 1.980 [b]	208.600 ± 5.940 [c]	189.000 ± 1.980 [b]	246.400 ± 3.960 [d]
°Bé	11.300 ± 0.140 [a]	12.800 ± 0.140 [b]	13.500 ± 0.140 [c]	12.800 ± 0.000 [b]	15.000 ± 0.140 [d]
			2019		
pH	3.360 ± 0.021 [a]	3.290 ± 0.042 [b]	3.230 ± 0.035 [c]	3.280 ± 0.078 [b]	3.230 ± 0.070 [c]
TA	3.620 ± 0.080 [a]	4.310 ± 0.053 [b]	5.525 ± 0.053 [c]	5.063 ± 0.043 [d]	5.780 ± 0.070 [e]
FAN (mg/L)	162.500 ± 2.256 [a]	200.230 ± 1.978 [b]	224.600 ± 1.450 [c]	207.650 ± 2.465 [b]	265.130 ± 3.472 [d]
°Bé	12.180 ± 0.020 [a]	12.770 ± 0.040 [b]	13.910 ± 0.090 [c]	14.210 ± 0.060 [c,d]	15.680 ± 0.030 [e]

Control: without over-ripening. SD48h: sun-dried grapes during 48 h. SD96h: sun-dried grapes during 96 h. CH48h: climatic chamber drying during 48 h. CH96h: climatic chamber drying during 96 h. FAN: free amino nitrogen. TA: total acidity (g/L tartaric acid). °Bé: Baumé degrees. Different superscript letters mean a significant difference between samples (ANOVA $p < 0.05$) determined by two-way ANOVA applying a Bonferroni multiple range (BSD) test.

For the two vintages studied, the pH values ranged from 3.20 (2018, CH96h) to 3.47 (2018, control). Comparing the different over-ripening treatments, all samples were significantly different from the control (ANOVA $p < 0.05$) during the two vintages studied. The pH values decreased with the hours of over-ripening treatment, this decrease being more pronounced in the case of chamber over-ripening. This may be due to the fact that chamber drying is presented as a continuous process during the treatment, whereas sun drying is paused at night. Closely related to pH, the total acidity values of the samples showed a similar trend, increasing with the treatment hours, from 3.62 g/L for control (2019) to 5.78 g/L of tartaric acid for CH96h (2019), this increase being more remarkable and significantly different (ANOVA $p < 0.05$) than the rest of the samples for climatic chamber drying (both, 48 and 96 h). The increase in total acidity values depends on the difference between the acid concentration phenomenon due to the evaporation of the vegetation water present in the grapes [43] and the metabolism of malic acid by respiratory combustion [44]. As can be seen in Table 1, the acidity increases that occurred in the sun-dried grapes as well as in the chamber dried grapes during the 2018 campaign are much lower than those corresponding to the 2019 campaign, probably due to a higher consumption of malic acid. However, in all cases, the pH variation has been much smaller than in the case of acidity, possibly due to the difficulty of altering a buffered medium such as wine [45]. The FAN content shows a similar behavior for the two vintages studied, increasing with the time of over-ripening and being significantly higher (ANOVA $p < 0.05$) in the case of over-ripening in climatic chamber. Grape musts from the 2019 vintage showed a higher FAN content compared to 2018, possibly due to concentration phenomena. Increasing the FAN content in musts has benefits such as improving yeast cell growth at the beginning and during alcoholic

fermentation [46,47], as well as an increased survival of yeasts at the end of alcoholic fermentation [48]. This FAN increase is positive in order to improve the fermentative potential of grape musts with deficiencies that could lead to stuck or sluggish fermentations due to its high sugar content [49].

Effect of Over-Ripening and Grape Skin (GS) Presence during Alcoholic Fermentation

Figures 1a–1d show the evolution of the viable yeast population during the alcoholic fermentation process during the vintages 2018 and 2019 with GS (b,d) and control (a,c), respectively.

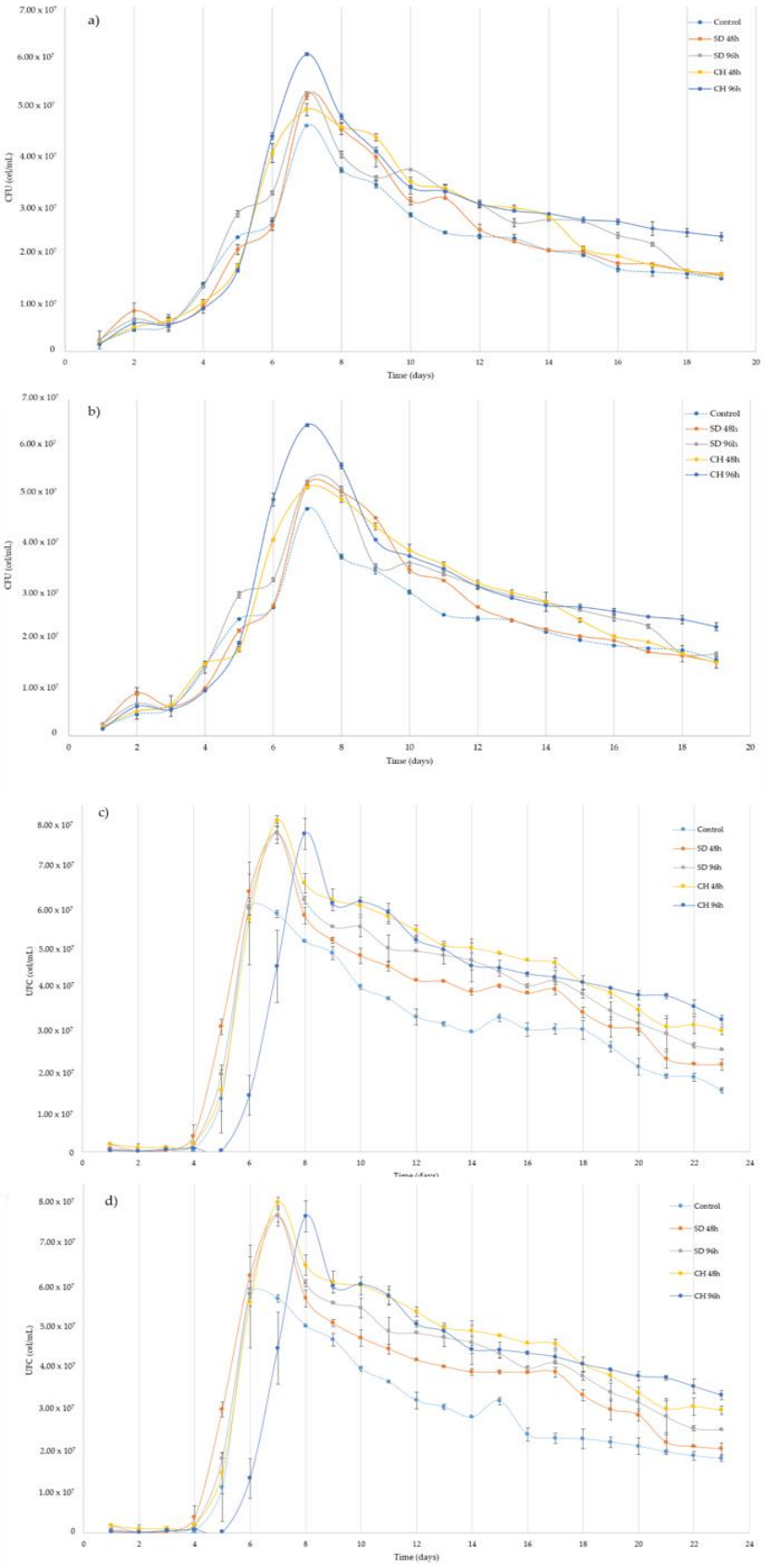

Figure 1. (**a**–**d**) Viable biomass development during grape must alcoholic fermentation without (**a**,**c**) and with (**b**,**d**) the presence of grape skins during two vintages (2018, **a**,**b,** and

2019, **c**,**d**). Control: without over-ripening. SD 48 h: sun-dried grapes during 48 h. SD 96 h: sun-dried grapes during 96 h. CH 48 h: climatic chamber drying during 48 h. CH96h: climatic chamber drying during 96 h.

Different grape over-ripening treatments, as well as fermentation with or without the presence of skins, do not affect the yeast lag phase times during the alcoholic fermentation. Year-on-year, the lag phase in the 2019 vintage is slightly longer, possibly due to the higher concentration of present sugars in the medium and, therefore, a greater osmotic shock and difficulty for the yeasts to adapt to the fermentation medium. For all the cases studied, the exponential phase begins 72–96 h after the yeast inoculation, reaching the maximum population after 7 days for all cases in the 2018 vintage and between days 6 and 8 for the 2019 vintage, depending on the time and over-ripening technique used. Regarding the maximum populations reached, in all cases of study the populations are significantly higher than those presented by the control (ANOVA $p < 0.05$), the CH96h samples being the ones with a greater yeast population in the 2018 vintage and the CH48h samples in the 2019 vintage. Once the maximum populations were reached, a progressive decrease in yeast populations was observed for both over-ripening techniques, regardless of the presence or absence of skins (ANOVA $p < 0.05$). It can be observed that the final yeast populations show a higher survival in the case of a higher concentration of sugars at the beginning of fermentation, thus confirming the positive correlation between yeast survival time and the concentration of sugars present in the medium [50]. Similarly, these results coincide with those that state that musts rich in sugars carry out a large part of the alcoholic fermentation with their yeasts in the decline phase [51]. Alcoholic fermentation is extended by 19 days for the 2018 vintage and 23 days for the 2019 vintage, possibly due to a higher initial concentration of sugars in the latter case.

Comparing the presence or absence of 20% of GS during alcoholic fermentation, a slight increase in yeast populations was observed in the cases where the musts had GS, without affecting the different stages of yeast growth or the time necessary to carry out fermentation. In this sense, control wines with GS presence showed a 27.7% and 25.1% higher population for the 2018 and 2019 vintages, respectively. In the same way and coinciding with the results of recently published research papers, it is confirmed that the presence of a certain amount of GS is able to sponsor a greater growth and survival of yeasts [33]. This may possibly be due to the presence of nitrogen compounds and other growth cofactors necessary for yeast growth found in grape skins [52,53]. Thus, the presence of a 20% of GS ensures a higher population of viable yeasts in the fermenters, regardless of the grape over-ripening time and technique.

Over-Ripening and GS Presence Effect on the Alcoholic Fermentation kinetics

Figures 2a–2d show the evolution of the relative density in the different alcoholic fermentation processes during the vintages 2018 and 2019 with GS (b,d) and control (a,c).

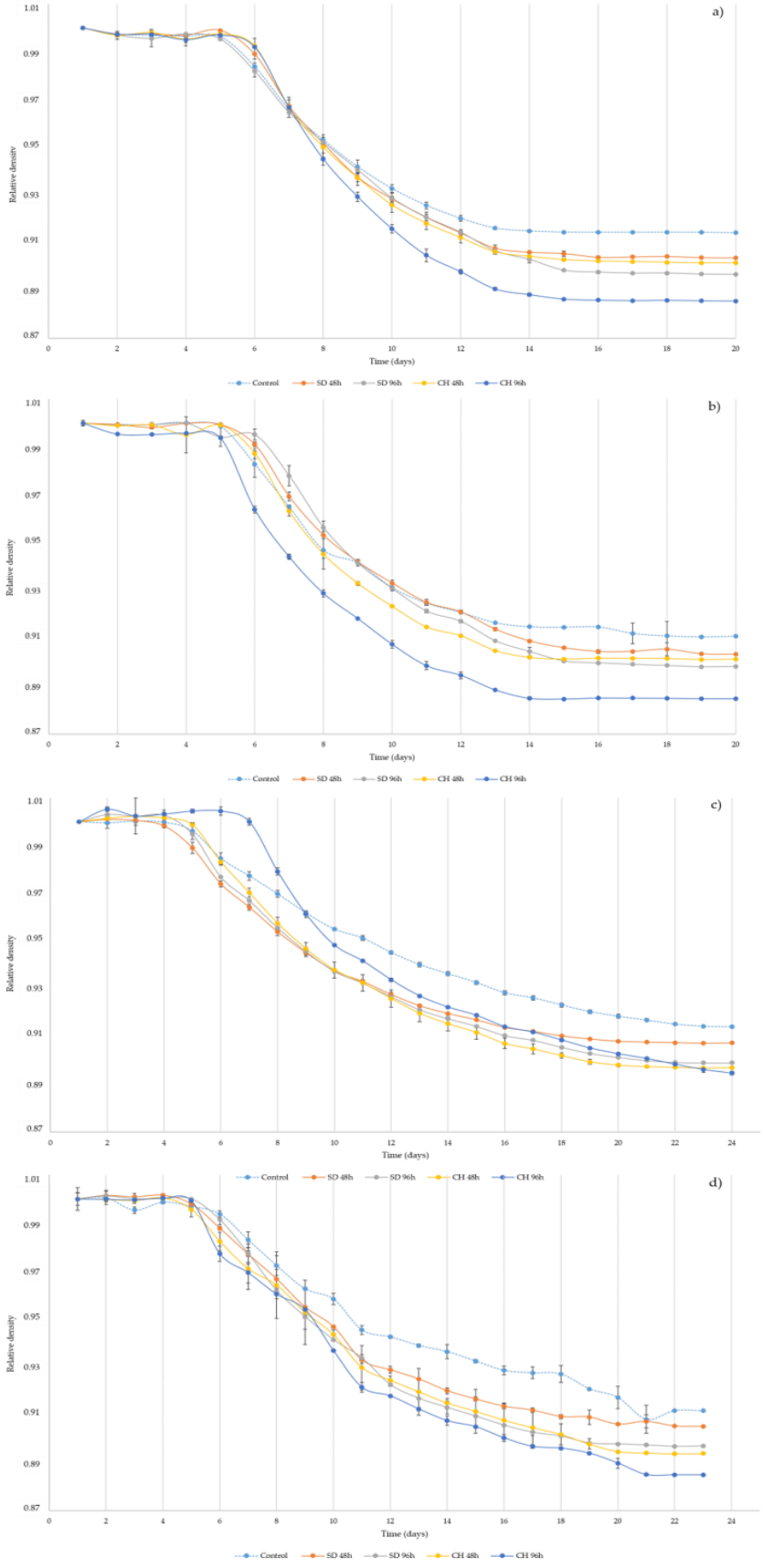

Figure 2. (**a**–**d**) Relative density evolution during grape must alcoholic fermentation without (**a**,**c**) and with (**b**,**d**) the presence of grape skins during two vintages (2018, **a**,**b**, and 2019,

c,**d**). Control: without over-ripening. SD48h: sun-dried grapes during 48 h. SD96h: sun-dried grapes during 96 h. CH48h: climatic chamber drying during 48 h. CH96h: climatic chamber drying during 96 h.

At the beginning, fermentation is slow with no significant variations in the density of the different samples until the fifth day of fermentation, regardless of the over-ripening technique, the time of over-ripening and the presence or absence of GS in the fermentation medium. Once the exponential yeast growth starts, the decrease in density starts to accelerate. During the days when the yeast has a higher reproduction rate, a greater decrease in relative density is observed. Year-on-year, samples from the 2018 vintage (Figures 2a and 2b) show a higher fermentation rate than those from the 2019 vintage (Figures 2c and 2d). As mentioned before, higher sugar concentrations could again be responsible for the slightly slower beginning of fermentation, especially in the case of sample CH96h 2019 (Figure 2c). However, the concentration of compounds by means of water evaporation in grapes during the over-ripening process implies a higher concentration of compounds necessary for yeasts, such as nitrogenized compounds (Table 1), and, therefore, its extensive development (Figure 2c) [54]. As expected, a higher viable biomass implies, in all cases, a higher fermentation speed given the higher consumption of sugars by yeasts. In all cases, it is observed that control samples, made with grapes without over-ripening, are the first to show a slowdown in their fermentation speed, showing a significantly higher relative density at the end of fermentation (ANOVA $p < 0.05$). This fact is supported by the observations in Figure 1, where it can be seen that a lower viable biomass implies a slower fermentation rate in the final phase of alcoholic fermentation. As for the contribution of 20% GS (Figures 2b and 2d), this practice implies an extra contribution of nutrients and/or co-factors, such as minerals and vitamins from the grape skins (Figures 2b and 2d) [52,53]. However, significant differences (ANOVA $p < 0.05$) were only observed for the CH96h samples, where the fermentation rate was higher. Nevertheless, for the rest of the samples, no major differences in fermentation kinetics were detected, coinciding with the recent results of the research group, a higher proportion of GS being necessary to significantly accelerate the fermentation process [33]. Finally, none of the samples during the two vintages studied presented problems in the final phase of fermentation, which could be carried out correctly until the end.

Over-Ripening and GS Presence Effect in Free Amino Nitrogen (FAN) during Alcoholic Fermentation

Nitrogenized compounds are necessary for the proper development of yeasts and alcoholic fermentation, nitrogen being the most important compound in fermentation, after carbon. FAN represents those nitrogenous compounds that are available to yeast, i.e., amino acids and ammonium, as well as some small peptides [38]. Figures 3a–3d show the evolution of FAN content during alcoholic fermentation for the different over-

ripening times and techniques, as well as for the presence or absence of skins in the fermentation medium.

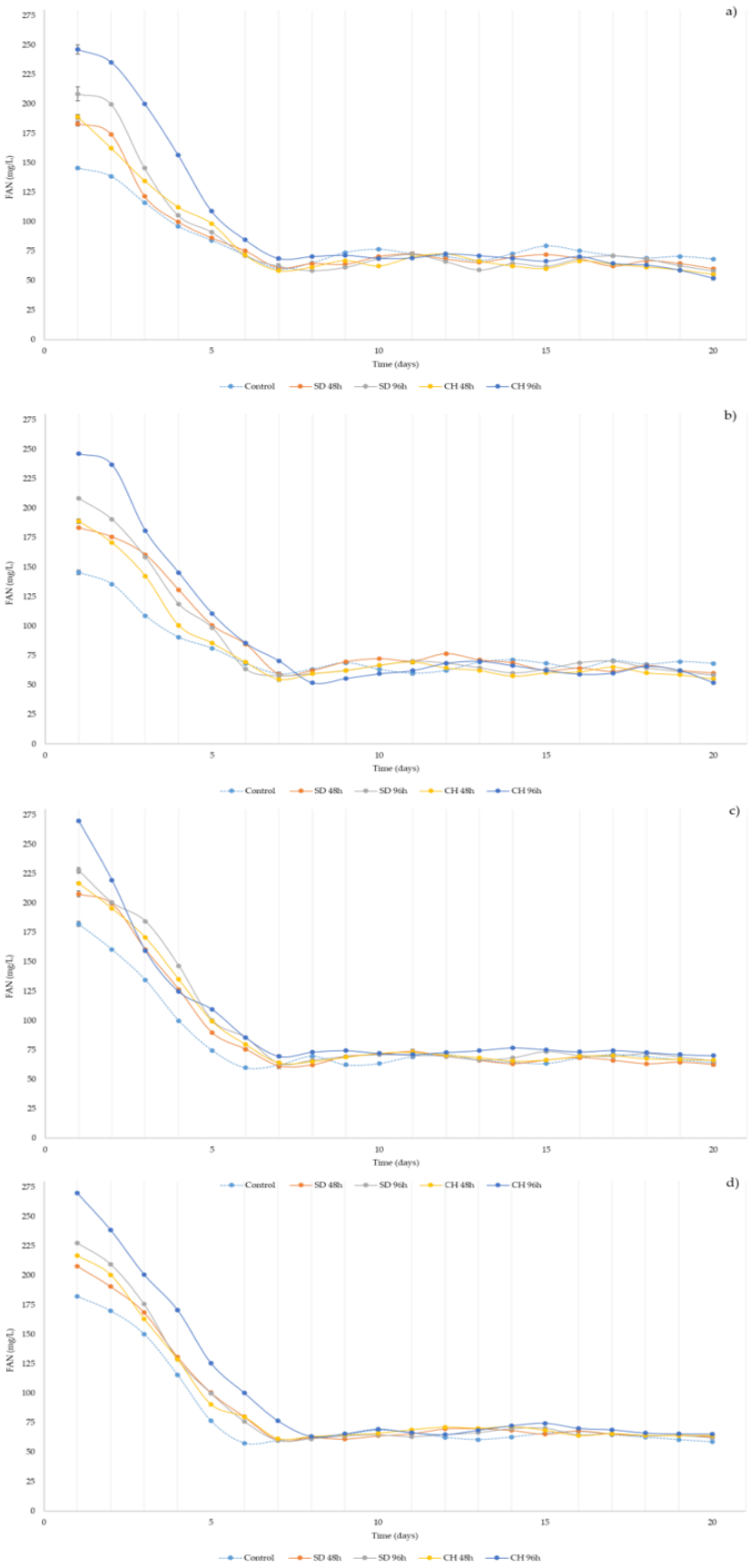

Figure 3. (**a**–**d**) Free amino nitrogen (FAN) concentration during grape must alcoholic fermentation without (**a**,**c**) and with (**b**,**d**) the presence of grape skins during two vintages (2018, **a**,**b,** and 2019, **c**,**d**). Control: without over-ripening. SD48h: sun-dried grapes

during 48 h. SD96h: sun-dried grapes during 96 h. CH48h: climatic chamber drying during 48 h. CH96h: climatic chamber drying during 96 h.

Grape over-ripening implies an increase in FAN concentration, with maximum values for the CH96h samples and minimum values for the control samples that did not receive any over-ripening treatment during the two years of study. At the beginning of fermentation, all samples showed significant differences among them (ANOVA $p < 0.05$). Unlike what was observed for viable biomass (Figure 1) and relative density (Figure 2), FAN content starts to decrease after the first 24 h from the inoculation of the yeasts in the fermentation medium. This is due to the consumption of substances nitrogenized by the yeasts for their survival and reproduction. The minimum values of FAN were obtained between days 6 and 7 of fermentation, coinciding, in all cases, with the highest values of viable biomass present in the fermentation tanks and the moment when yeast began to consume more sugars, thus, a higher fermentation activity. Once the minimum FAN content was reached, it was observed that the concentrations of this parameter showed an oscillatory nature until the end of fermentation, with significant differences being observed only occasionally and in some cases in the overripe samples, with and without skins, with respect to the control (ANOVA $p < 0.05$). In this case, the addition of 20% of skins to the fermentation medium led to a less pronounced decrease in the FAN content in all the samples for the two vintages studied, which could be due to the extra release of nitrogenized compounds from GS into the fermentation medium.

For all the samples and during the two vintages analyzed, FAN content was above 140 mg/L in grape musts, which is the limit defined for a correct alcoholic fermentation by the yeasts [55]. Once most of the FAN was consumed, the oscillatory nature observed in all samples on an inter-annual basis may have been due to the autolysis process of the dead yeasts, which release, into the environment, compounds considered to be part of the FAN, such as some amino acids [56–59]. The final FAN values in the samples were significantly higher for the 2018 vintage, compared to 2019; nevertheless, the final values in all samples and for both vintages ensured that wines were stable from a microbiological point of view, as well as minimizing the occurrence of other problems, such as the accumulation of harmful compounds in the wine, such as ethyl carbamate [32,33].

Effect of Over-Ripening and the Presence of GS on the Physicochemical Composition of Final Wines

Table 2 shows the physicochemical characterization of the final wines made from overripe grapes and in the absence or presence of GS.

Table 2. Wine physicochemical and color characterization.

	2018				
	Control	**SD48h**	**SD96h**	**CH48h**	**CH96h**
	Without GS				
TA (g/L)	4.629 ± 0.027 [a]	4.763 ± 0.067 [a]	4.905 ± 0.080 [a]	5.102 ± 0.241 [b]	5.554 ± 0.013 [b]
VA (g/L)	0.162 ± 0.012 [a]	0.184 ± 0.031 [a]	0.400 ± 0.024 [b]	0.231 ± 0.012 [a]	0.366 ± 0.021 [b]
% Alc.	11.854 ± 0.182 [a]	13.430 ± 0.060 [a]	14.633 ± 0.159 [a,b]	13.656 ± 0.398 [a]	16.584 ± 0.016 [c]
RS (g/L)	1.418 ± 0.285 [a]	1.922 ± 0.330 [b]	2.220 ± 0.509 [b]	1.733 ± 0.107 [a,b]	2.998 ± 0.264 [c]
TPI	7.990 ± 0.141 [a]	6.540 ± 0.170 [b]	6.090 ± 0.269 [b]	6.450 ± 0.891 [b]	8.160 ± 0.085 [a]
Abs 420	0.074 ± 0.015 [a]	0.093 ± 0.008 [a]	0.093 ± 0.001 [a]	0.109 ± 0.008 [b]	0.110 ± 0.002 [b]
L*	96.834 ± 0.923 [a]	98.279 ± 0.057 [a]	98.664 ± 0.173 [a]	98.372 ± 0.071 [a]	98.247 ± 0.127 [a]
a*	0.160 ± 0.119 [a]	0.420 ± 0.020 [b]	0.470 ± 0.033 [b]	0.600 ± 0.064 [c]	0.430 ± 0.023 [b]
b*	10.484 ± 2.874 [a]	5.120 ± 0.572 [b]	4.860 ± 0.246 [b]	5.683 ± 0.492 [b]	6.168 ± 0.009 [b]
H*	91.019 ± 0.931 [a]	94.671 ± 0.301 [a]	95.526 ± 0.664 [a]	96.021 ± 0.115 [a]	93.970 ± 0.217 [a]
	With 20% GS				
TA (g/L)	4.413 ± 0.102 [a]	4.569 ± 0.140 [a,b]	4.769 ± 0.097 [b]	4.958 ± 0.305 [b,c]	5.068 ± 0.198 [c]
VA (g/L)	0.361 ± 0.068 [a]	0.412 ± 0.006 [a,c]	0.580 ± 0.100 [b]	0.428 ± 0.168 [c,d]	0.551 ± 0.136 [b,d]
% Alc.	11.970 ± 0.256 [a]	13.569 ± 0.147 [a,b]	14.896 ± 0.253 [a,b]	13.852 ± 0.539 [a,b]	16.489 ± 0.187 [c]
RS (g/L)	1.257 ± 0.149 [a]	1.567 ± 0.698 [a]	2.541 ± 0.410 [b]	1.710 ± 0.205 [a]	3.056 ± 0.423 [b]
TPI	8.690 ± 0.157 [a]	7.214 ± 0.099 [a]	7.724 ± 0.301 [a]	7.158 ± 0.249 [a]	8.879 ± 3.265 [a]
Abs 420	0.158 ± 0.008 [a]	0.087 ± 0.005 [b]	0.048 ± 0.001 [c]	0.087 ± 0.003 [b]	0.099 ± 0.001 [b]
L*	98.698 ± 0.587 [a]	100.025 ± 0.147 [a]	101.259 ± 0.257 [a]	99.995 ± 0.009 [a]	102.025 ± 0.298 [a]
a*	0.153 ± 0.111 [a]	0.411 ± 0.015 [b]	0.468 ± 0.019 [b,c]	0.530 ± 0.054 [c]	0.384 ± 0.069 [b]
b*	5.699 ± 0.547 [a]	3.568 ± 0.413 [b]	2.567 ± 0.154 [b]	2.541 ± 0.056 [b]	2.354 ± 0.016 [b]
H*	93.545 ± 0.931 [a]	97.541 ± 0.149 [a]	98.035 ± 0.761 [a]	98.221 ± 0.431 [a]	96.028 ± 0.199 [a]
	2019				
	Control	**SD 48 h**	**SD 96 h**	**CH 48 h**	**CH 96 h**
	Without GS				
TA (g/L)	5.570 ± 0.098 [a]	5.810 ± 0.104 [a]	6.480 ± 0.057 [b]	6.320 ± 0.421 [b]	6.460 ± 0.268 [b]
VA (g/L)	0.189 ± 0.030 [a]	0.214 ± 0.012 [a]	0.256 ± 0.036 [b]	0.296 ± 0.016 [b]	0.489 ± 0.080 [c]
% Alc.	10.756 ± 0.430 [a]	12.380 ± 0.320 [a,d]	14.299 ± 0.190 [b]	13.420 ± 0.598 [b,d]	16.240 ± 0.480 [c]
RS (g/L)	1.356 ± 0.018 [a]	1.976 ± 0.143 [b]	1.447 ± 0.169 [a,b]	1.238 ± 0.188 [a]	4.813 ± 0.268 [c]
TPI	6.513 ± 0.091 [a]	4.976 ± 0.100 [b]	3.790 ± 0.082 [c]	5.713 ± 0.712 [b]	10.268 ± 0.55 [d]
Abs 420	0.040 ± 0.010 [a]	0.051 ± 0.010 [a,d]	0.062 ± 0.001 [b,d]	0.073 ± 0.010 [b]	0.110 ± 0.01 [c]
L*	97.563 ± 1.235 [a]	98.593 ± 0.147 [a]	97.305 ± 0.846 [a]	95.168 ± 0.992 [a]	96.436 ± 0.589 [a]
a*	0.5 ± 0.006 [a]	0.769 ± 0.110 [b]	0.782 ± 0.015 [b]	0.988 ± 0.036 [c]	0.846 ± 0.087 [b]
b*	10.312 ± 0.653 [a]	11.241 ± 0.216 [a]	11.983 ± 0.549 [a]	10.673 ± 0.630 [a]	14.297 ± 0.55 [b]
H*	97.536 ± 2.541 [a]	98.631 ± 0.964 [a]	97.995 ± 0.966 [a]	104.531 ± 2.174 [a]	93.631 ± 0.501 [a]
	With 20% GS				
TA (g/L)	5.170 ± 0.070 [a]	5.460 ± 0.070 [b]	6.118 ± 0.050 [c]	6.025 ± 0.560 [c]	6.165 ± 0.150 [c]
VA (g/L)	0.230 ± 0.030 [a]	0.240 ± 0.010 [a,b]	0.280 ± 0.010 [b]	0.340 ± 0.010 [c]	0.620 ± 0.030 [d]
% Alc.	10.860 ± 0.910 [a]	12.430 ± 0.440 [b]	14.390 ± 0.260 [c]	13.390 ± 1.470 [b,c]	16.480 ± 0.360 [d]
RS (g/L)	1.200 ± 0.050 [a]	1.450 ± 0.090 [a,b]	1.640 ± 0.001 [a,b]	1.770 ± 0.140 [b]	4.510 ± 0.080 [c]
TPI	4.320 ± 0.160 [a]	5.620 ± 0.100 [b]	7.260 ± 0.070 [c]	6.350 ± 0.880 [b,c]	10.974 ± 0.550 [d]
Abs 420	0.140 ± 0.010 [a]	0.058 ± 0.010 [b]	0.063 ± 0.001 [b]	0.071 ± 0.010 [b]	0.780 ± 0.010 [b]
L*	100.220 ± 0.430 [a]	100.110 ± 0.050 [a]	100.190 ± 0.000 [a]	97.990 ± 2.020 [a]	98.900 ± 0.140 [a]
a*	0.511 ± 0.130 [a]	0.862 ± 0.090 [b]	0.852 ± 0.010 [b]	1.053 ± 0.250 [c]	0.911 ± 0.030 [b,c]
b*	3.330 ± 0.300 [a]	4.280 ± 0.640 [a,b]	4.780 ± 0.010 [a,b]	3.660 ± 0.490 [a]	9.900 ± 0.550 [b]
H*	100.290 ± 1.810 [a]	101.480 ± 1.220 [a]	100.100 ± 0.130 [a]	106.480 ± 5.660 [a]	95.270 ± 0.490 [a]

SD48h: sun-dried grapes during 48h. SD96h: sun-dried grapes during 96h. CH48h: climatic chamber drying during 48 h. CH96h: climatic chamber drying during 96 h. TA: total acidity

(g/L tartaric acid). VA: volatile acidity (g/L acetic acid). RS: residual sugars. TPI: total polyphenolic index. CIELab coordinates: L* (lightness), a* (red/green), b* (yellow/blue), H* (hue) and C* (chroma). Different superscript letters mean a significant difference between the samples (ANOVA $p < 0.05$) determined by two-way ANOVA applying a Bonferroni multiple range (BSD) test.

As for total acidity, wines from the 2019 vintage showed higher values of total acidity than those made in the 2018 vintage, as was also the case for grape musts. More specifically, it can be seen that the results fluctuate in one way or another depending on the over-ripening technique and its time of application. Thus, the wines made by overripe grapes in a climatic chamber for 96 h had the highest total acidity values, in all cases, and were significantly different (ANOVA $p < 0.05$) compared to the control, which presented the lowest values, in all cases. On the other hand, the presence of GS in the fermentation medium caused a slight decrease in wine acidity, which could be due to the release of minerals, such as potassium, by GS [60], resulting in tartaric precipitation. Such decrease has been previously observed by Olejar et al. [61] in white wines made from 'Sauvignon Blanc'. In general, the increase of total acidity in wines made from over-ripened grapes is mainly due to an increase in the concentration, explained by the decrease in the amount of water [62]. As for volatile acidity, there is a clear tendency in this parameter to increase with both the over-ripening time and technique, being higher in climatic chamber over-ripening than in sun-drying. In general, the proportion of acetic acid produced during fermentation increases proportionally with the initial sugar concentration. The minimum value of volatile acidity corresponds to the control wine in all cases and the highest value for the CH96h wine, with significant differences between them (ANOVA $p < 0.05$) during the two vintages studied. As for the presence of grape skins, it is observed that their contribution implies an increase in the volatile acidity of the final wines. This volatile acidity increase in wines made in the presence of GS could be due to an increase in the concentration of volatile acid esters and alcohols, whose concentration in wines depends on the maceration time [63]. Acetic acid is the main component of volatile acidity and has a major influence on wine quality [64]. Acetic acid concentration in dry wines normally ranges between 0.1 and 0.5 g/L and its threshold of perception varies in the range of 0.7–1.0 g/L [65]. In winemaking from sound grapes, this acid is produced by yeasts during alcoholic fermentation, in different concentrations depending on the species and strain of yeast used [66], although it can also be synthesized by lactic and acetic acid bacteria [58]. On the other hand, under the osmotic stress conditions to which yeasts are subjected in musts with high sugar concentration, they express genes that regulate glycolysis and the pentose phosphate pathway, thereby increasing the synthesis of fermentation by-products, such as glycerol and acetic acid [67]. However, there is also the possibility that some of the acetic acid is of accidental origin, due to the presence of lactic acid bacteria in the grape must in the grapes [58].

Logically, as with the sugar concentration in the initial musts (Table 1), wines made from overripe grapes showed higher alcohol content values than the control. Specifically, the wines made from musts with higher sugar content provided wines with higher alcohol content. This is the case of CH96h, which showed significantly higher values (ANOVA $p < 0.05$) than the rest of the wines. In this case, the addition of 20% of GS did not influence the alcohol content of final wines, coinciding with the results recently published [33]. During the alcoholic fermentation metabolic pathway, sugar in grape must is transformed into ethanol and other by-products by fermenting yeasts. However, yeasts are not tolerant to high alcoholic strength. In this sense, yeasts tolerance to ethanol can be observed in the results of residual sugar analysis. Those wines that have shown a higher alcoholic content have also presented higher residual sugar values at the end of fermentation. Thus, for the two vintages studied, wines made from overripe grapes for 96h in the climatic chamber showed significantly higher residual sugar values (ANOVA $p < 0.05$) than the control and some samples. From a certain alcoholic strength onwards, cell viability starts to decrease, due to the stress to which the yeast is subjected by ethanol. This decrease in cell viability starts to be observed from 13% *v*/*v* in *Saccharomyces cerevisiae* [67]. Ethanol tolerance is a factor that can lead to incomplete fermentation. The toxicity of ethanol arises due to its ability to interact with membranes, altering their fluidity and, as a consequence, all metabolic functions of the cell [68]. As for the polyphenolic content present in final wines (TPI), the ones from the 2018 vintage have higher values than those from the 2019 vintage, which may be due to the greater polyphenolic maturity of the grapes at the time of harvest. However, the over-ripening process of these grapes implies an increase in the TPI values, with the maximum values again being observed for the CH96h samples. White grapes over-ripening causes a series of changes in the phenolic composition of wine, resulting in browning as a result of the formation of brown pigments due to the Maillard reaction [69]. This process is favored by large quantities of sugars, the high temperatures reached by the grapes during over-ripening and the polymerization of phenolic compounds [70]. However, some oxidative phenomena may occur due to the high temperatures and the degree of insolation reached during the over-ripening process, leading to a reduction of the polyphenol content in grapes skins and must [71–73]; consequently, the decrease in TPI values for SD48h and SD96h in both vintages when fermented without GS with respect to the control can be explained. On the other hand, wines made with 20% of GS show slightly higher data than wines made conventionally, which shows a transfer of compounds present in the GS during alcoholic fermentation [74].

The absorbance at 420 nm of a wine gives an indication of the yellow color that white wines should have. This wine quality control parameter is associated with the presence of yellowish-brown pigments and, therefore, measures the rate of oxidation of polyphenols in white wine [69]. The absorbance at 420 nm values for the different samples shows significant differences from a statistical point of view, but they are almost negligible from an oenological point of view. It can be seen that the wines made by

chamber over-ripened grapes have higher values than those made by sun-drying. However, it is noteworthy that, in wines made in the presence of GS, the behavior of the values has been the opposite. Control wines showed significantly higher browning values than those made from overripe grapes (ANOVA $p < 0.05$), regardless the technique employed. In this sense, the presence of skins during winemaking could exert a protective effect against possible oxidation effects during alcoholic fermentation [33]. For wine color, CIELab is the L*a*b* color space, which is one of the most popular color spaces for measuring samples. In CIELab, L* indicates brightness or lightness, varying in value between 0, which indicates black, or minimum brightness, and 100 which relates to white, or maximum brightness. On the other hand, a* and b* are the chromatic coordinates [75]. In terms of brightness, all wines showed very similar values, with no significant differences between them. All the values are close to 100, which indicates that they are bright and luminous wines. In regard to the chromatic coordinates, significant differences were observed between some wines, mainly between the control wine and the rest, and also between the wines with different over-ripening techniques and/or time. If we look at Table 2, all the wines show values close to zero for a* and positive values for b*, which means that all of them, to a greater or lesser extent, have a yellow-greenish color. Finally, as for the hue angle, no significant differences were observed between the different wines, all of which were close to 100, indicating a high hue.

Conclusions

In conclusion, grape over-ripening increases the sugar content and other organic compounds, such as acids and FAN, in the initial grape musts. The start of fermentation was more difficult the higher the grape over-ripening degree was and, therefore, the greater the concentration of sugars in its must. In terms of fermentation kinetics, over-ripening did not influence yeast lag phase; however, the presence of 20% of GS sponsored a higher yeast population. Final wines showed significant differences depending on the time and technique of over-ripening used. From a physicochemical point of view, the presence of GS slightly increases the volatile acidity and TPI, but on the other hand decreases the degree of browning. Nevertheless, in all cases, it has been possible to produce dry white wines without problems at any stage of fermentation or deviations in their basic oenological or chromatic parameters. Comparing the two techniques, it has been observed that sun-drying is capable of producing significant modifications in wines, diversifying production and having lower requirements in terms of facilities, investment and energy, than the drying procedure in a climatic chamber. With the intention of further deepening this research, it would be advisable to carry out this study for more vintages, in order to check the reproducibility of the technique, as well as to try it with other white wine varieties. In view of the above, it is considered that the production of wines from overripe grapes and in the presence or absence of GS is a viable approach to make new white wines taking advantage of the conditions imposed

by climate change in a warm climate zone and meet the trends and expectations of current wine consumers.

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6) Pau Sancho-Galán, Antonio Amores-Arrocha, Víctor Palacios and Ana Jiménez-Cantizano. Volatile Composition and Sensory Characterisation of Dry White Wines Made with Overripe Grapes by Means of Two Different Techniques. *Foods* **2022**, *11*, 509; doi:10.3390/foods11040509

Abstract

Grape over-ripening is a technique that has historically been used for the production of white wines in southern Spain. However, this technique is still widely used for the production of sweet wines. In this study and after recently proving the feasibility of making dry white wines from overripe grapes with and without the presence of grape skin in a warm climate zone, the senso-ry characterisation and analysis of the major and minor volatile compounds in dry white wines made from overripe grapes are presented for the first time. Two over-ripening techniques (sun-drying and climatic chamber drying) were studied for two different periods of time (48 and 96 h), as has the presence of grape skins during alcoholic fermentation. Grape over-ripening im-plies modifications in the composition of both the major and minor volatile compounds in wines. In terms of sensory analysis, wines with a similar profile were obtained year-on-year. The results of the preference test show that the wines made from grapes that had been over-ripened in the sun for 96 h were preferred by the tasting panel for both vintages. Thus, grape over-ripening under the sun could be considered as a resilience and adaptation technique for increased temperature conditions during the ripening season caused by the effects of climate change.

Introduction

Viticulture is a key socioeconomic and cultural sector in many countries and regions worldwide, with a high economic impact in the network of all relevant industry branches of the supply and distribution chains [1]. Geographically, grapevines are historically cultivated on six out of seven continents, between latitudes 4 and 51 in the northern hemi-sphere and between latitudes 6 and 45 in the southern hemisphere and across a large diversity of climates (oceanic, temperate, continental, Mediterranean, etc.), with the majority occurring in temperate climate regions [2]. However, climate change is exerting an increasingly profound influence on vine phenology and grape composition, and ultimately affects winemaking, wine microbiology, and chemistry and sensory aspects [3]. Observed changes in 27 premium viticultural regions across the globe have shown an increase in the average growing season temperature of 1.3 °C from 1950 to 2000, while in Europe, an increase of 1.7 °C was observed from 1950 to 2004 [4–6]. According to HadCM3 model average, the predicted temperatures for high-quality wine producing regions will increase by 2.04 °C within the period from 2000 to 2049 [5].

Understanding the changing suitability of regions for viticulture under climate change will help to us develop adaptation strategies in traditional winegrowing regions [7]. In order to maintain profitability and to ensure long-term future, producers will be required to adapt to changing climatic characteristics. Some of the guidelines for feasible adaptation strategies in the short term have been taken up in the Clim4Vitis action and include, among others, crop cultural measures [8–10], protection against extreme heat and sunburn [11–13], irrigation [14,15], pest and disease control [16,17], and soil management [18,19]. On the other hand, there are also long-term adaptation strategies such as changes in training systems [20,21], varietal/clonal and rootstock selection [22,23], or vineyard relocation [24–26]. The adoption of timely, cost-effective, and suitable adaptation strategies may significantly contribute to risk reduction, thereby decreasing the susceptibility of the sector and enhancing its resilience under a changing climate [27].

Given the foregoing precedents, quality wine production could be affected in those areas that already have a warm climate [28]. In this sense, another strategy to adapt to climate change-associated effects could be the application of traditional methodologies for the production of new types of wines other than traditional ones, taking advantage of the new conditions that have been imposed due to climate change, e.g., the production of dry white wines from overripe grapes [28]. Grape over-ripening is a method used in the production of raisins in countries such as India and China [29], but, in turn, it is also used in the hottest and driest countries of the world for the production of certain sweet and fortified wines [28]. In Andalusia (Southern Spain), special sweet wines are obtained using grapes that have been dried by direct exposure to the sun. While grape sugar enrichment can be achieved through the over-ripening of grapes on vines by twisting their stems without cutting them off, the traditional system used in Andalusia (Southern Spain) is the so-called asoleo technique, which consists of drying grape bunches in sun for several days in order to partially dry or raisin the grapes [30]. When subjected to hours of intense sunshine, grapes gradually lose water, resulting in a significant increase in the sugar concentration and a variation in the aromatic profile of the grapes [31]. However, this traditional system is susceptible to climatological variations that can alter the final product, in particular, rains during this period can cause the grapes to rot. It is useful to devise an alternative s over-ripening system that allows for greater control of the process [3235] but that has no negative influence on the sensorial properties of final product. In recent years, a possible alternative to the traditional sun drying technique has appeared. Forced convection with hot air inside drying chambers is being used for the drying of horticultural products [36]. Climatic chambers for grape raisining [33-34] al-low for the temperature and humidity to be controlled y, reduces the length of the required drying time, and makes the process independent of external meteorological conditions [35]. Nevertheless, grape over-ripening, regardless of the technique used, allows for natural modifications in the grape composition and leads to the production of new types of wines [28].

Present tendencies in wine consumption focus on well-structured wines that are full-bodied in the mouth [35]. Wine flavour is a combined perception of taste and aroma, the latter being the most responsible for the global perception of wines [36,37]. Wine aroma compounds can be grouped according to their origin: varietal aromas found in grapes, fermentative aromas from alcoholic and malolactic fermentation, and aging aromas obtained during aging or storage [38]. Their presence or absence in a particular wine de-pends on several factors, such as the environment (climate and soil), ripeness and grape variety, winemaking conditions, and wine aging [39–41]. Many of the volatile compounds that are generated during alcoholic fermentation are produced via the metabolic activity of Saccharomyces cerevisiae and quantitatively account for the biggest fraction of the total aroma composition of wine [38,42–44]. The extent to which these compounds persist from the grapes through to the finished wine is influenced by the winemaking conditions and the aging process [45]. The volatile fraction of wine is determined by several hundreds of chemically different compounds. Alcohols, aldehydes, esters, acids, monoterpenes, and other minor compounds usually constitute the volatile fraction of this product.

New wine consumers are demanding more particular and exclusive wines that stand out from the rest because of their distinctive organoleptic characteristics [45]. Diversifying and innovating white wine production in a warm climate region by recovering historical winemaking techniques such as grape over-ripening and Grape Skin (GS) fermentation can be a way to bring together the search for new winemaking procedures as strategies to cope with the effects associated with climate change [28]. Thus, in this study and after recently proving the feasibility of making dry white wines from overripe grapes with and without the presence of GS in a warm climate zone [28], the sensory characterisation and analysis of the major and minor volatile compounds in dry white wines made from over-ripe grapes using two techniques, sun and climatic chamber over-ripening, are presented for the first time.

Materials and Methods

'Palomino Fino' grapes, an autochthonous cultivar from a warm climate region [46], were harvested from a privately owned vine plot located at 36°64′29.7″ N, 5°49′53.5″ W at 150 m ASL in San José del Valle (Cadiz, Spain). Neither fertilisation nor irrigation treatments were applied in the vine plot during the two years of study. The experimental layout that was followed was similar to those that have been recently published [28,47]. Two different over-ripening techniques were studied: on one side, Sun-Drying (SD), and on the other side, Climatic Chamber drying (CH) at 35 ± 1 °C and with 10% relative humidity, in an Ibercex ASL climatic chamber (Madrid, Spain) located in the Institute of Viticulture and Agri-food Research (IVAGRO) of Cadiz University, in order to compare the over-ripening behaviour under controlled conditions. In both cases, two times were studied, 48 and 96 h, resulting in four different samples and a control without over-

ripening in duplicate for each vintage studied (2018 and 2019). Grape ripeness after the grape over-ripening procedures was expressed as °Bé: Control: 11.300 ± 0.140, SD48h: 12.800 ± 0.140, SD96h: 13.500 ± 0.140, CH48h: 12.800 ± 0.000, and CH96h: 15.000 ± 0.140 for the 2018 vintage. Regarding the 2019 vintage, the °Bé values were as follows: Control: 12.180 ± 0.020, SD48h: 12.770 ± 0.040, SD96h: 13.910 ± 0.090, CH48h: 14.210 ± 0.060, and CH96h: 15.680 ± 0.030. Additionally, the grape composition required for this experiment and can be found in Sancho-Galán et al., 2021 [28]. In this sense, for each vintage, the experiment included 10 different fermentations (control without over-ripening, SD, and CH 48 and 96 h each, in duplicate) without GS and the same exact layout with the presence of 20% GS in order to study their effect on white winemaking. The different grape musts obtained were acidified with tartaric acid, and 80 mg/L of potassium metabisulphite was added as an antioxidant (Agrovin, Ciudad Real, Spain). For grape must fermentation, Lalvin 71B® (Lallemand, Barcelona, Spain) was employed as a pre-ferment. Alcoholic Fermentation (AF) was carried out under controlled conditions at 18 °C in 5 L glass-made tanks, and as soon as it was completed, the wines were fined with 4 g/hL of gelatin and 40 g/hL of bentonite. After 72 h, the final wines were filtered, bottled, and corked.

Analysis of Volatile Compounds

The methodology and equipment employed to determine the major volatile compounds were the same as those proposed by Amores-Arrocha et al. [48] and Sancho-Galán et al. [49]. Gas chromatography with flame ionisation detection (GC-FID, HP 5890 Series II) on a Carbowax 20 M column (L 50 m, ID 0.25 mm, PD 0.25 μm) was employed to determine major volatile compounds. The injector and detector temperatures were 175 °C and 225 °C, respectively, using hydrogen (1 mL/min) as a carrier gas. The oven temperature was 35 °C for the first 5 min, with a ramp of 5 °C/min until the temperature reached 100 °C. A direct injection of 5 μL of distilled sample was employed. Acetaldehyde, ethyl acetate, methanol, 2-propanol, and 2-methyl-1-propanol were determined using 4-methyl-2-pentanol (Sigma-Aldrich Química, S.A., Madrid, Spain) as an internal standard to determine retention times and calibration curves.

Free minor volatile compounds were identified and quantified by semi-quantitative GC-MS analysis after the Solid Phase Extraction (SPE) of the different samples following the method described by Di Stefano [50]. The compound 1-Heptanol was used as an internal standard. The GC-MS methodology and specifications were the same as those reported in Amores-Arrocha et al. [48]. A GC-MS model Voyager® (Termoquest, Milan, Italy) was used with a Supelcowax-10 column (L 60 m, ID 0.32 mm, PD 0.5 μm). The operation conditions were as follows: injector and detector temperature, 300 °C; oven temperature, 40 °C for 5 min followed by a 2 °C/min ramp and 200 °C for 5 min; sample volume, 2 μL in splitless mode (40s); He as carrier gas at a 1 mL/min flow. The MS conditions were as follows: electronic impact mode (EI +) at 70 eV; initial temperature,

220 °C; interface temperature, 320 °C, scan index, 1 scan/s; mass acquisition range 45–400 *m*/*z*. Semi-quantitative analyses were carried out by assuming a response equal to one.

Sensory Analysis

A sensory analysis of the different wines produced during the two vintages was performed in order to determine the differences between the over-ripening technique, its time, and the presence or absence of GS. The wines were tasted 5 days after bottling by a 20-member panel comprising 12 women (30–54 years old) and 8 men (32–56 years old) who were experienced with wine tasting methodology. Informed consent was obtained from all the subjects involved in the study. An amount of 50 mL of wine was served to each taster in standard tasting glasses [51] at room temperature (22 ± 2 °C). Each of the glasses was randomly coded with a three-digit combination code and covered by a glass cover to prevent any of the volatile compounds from evaporating before the sensory analysis began. Additionally, the wines were presented to each panelist in a randomized order, and sample replicates were also assessed. Each panel member was provided with a specific tasting file comprising the olfactory and taste attributes selected according to Jackson [52], with scores to be evaluated on a 0- to 10-point scale, with 0 points representing the lowest score and 10 points representing the highest score. In accordance with the UNE-ISO-8587 standard [53], a preference test was carried out on the wines that were tasted in order to study the existence of significant differences in the different wines according to their elaboration methodology. To this end, the wines were grouped by vintage and by the presence or absence of GS in the fermentative medium, resulting in two preference tests of five wines each per vintage. The 20 tasters scored the wines from 1 to 5 according to their preference. The results of the preference analysis were calculated using Page's preference test in accordance with the above-mentioned rule.

Statistical Analysis

Means and standard deviations were calculated, and significant differences were evaluated by a two-way ANOVA and Bonferroni's Multiple Range (BSD) test with a $p < 0.05$ using GraphPad Prism 6.01 (GraphPad Software, San Diego, CA, USA). The statistical analysis was performed on the volatile compounds obtained after wine analysis as well as on the results determined by the tasting panel.

Results and Discussion

Figures 1–4 a-b show the effect of the applied over-ripening treatment and its duration and the presence or absence of GS on the profile of volatile compounds sorted by families during the two vintages studied (2018 and 2019). The statistical analysis and significant differences are reported as Supplementary Materials (Tables S1–S4).

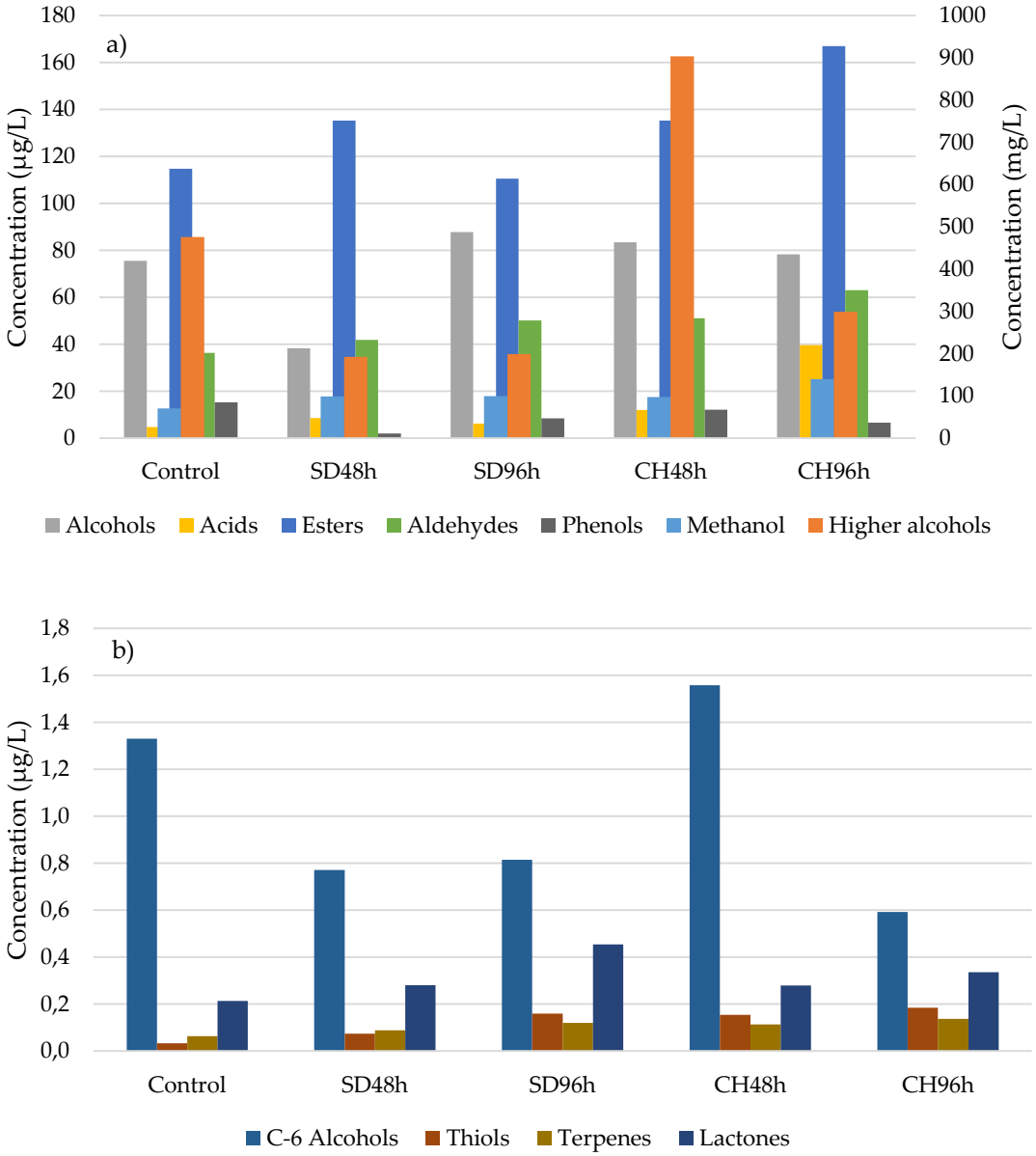

Figure 1. **(a)** Major and minor volatile compounds present in samples (n = 3) fermented without GS during 2018 vintage. Methanol and higher alcohols (mg/L, right axis). Alcohols, acids, esters, aldehydes and phenols (µg/L, left axis). **(b)** Minor volatile compounds present in samples (n = 3) fermented without GS during 2018 vintage. SD48h: sun-drying 48 h; SD96h: sun-drying 96 h; CH48h: climatic chamber drying 48 h; CH96h: climatic chamber drying 96 h.

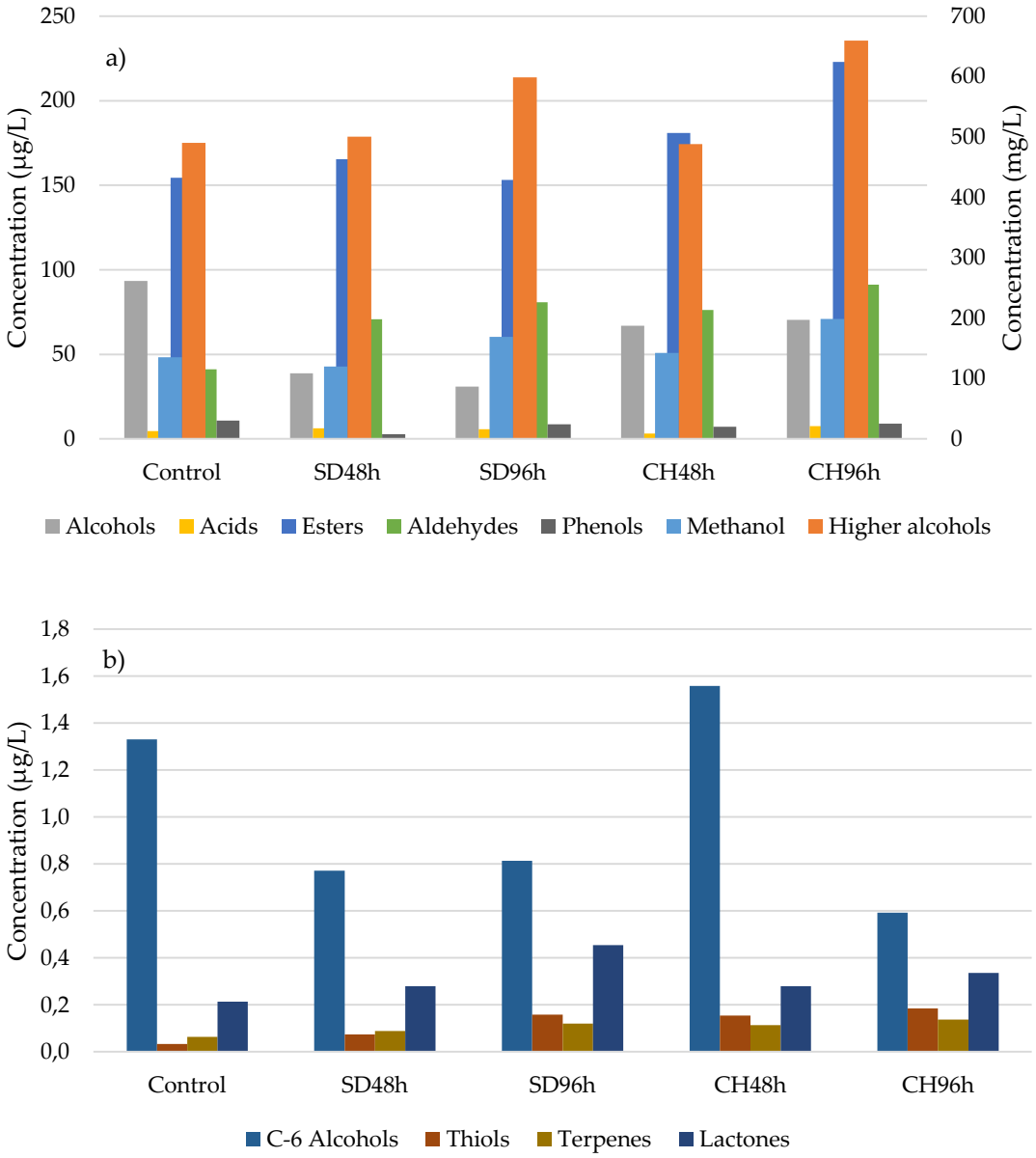

Figure 2. Major and minor volatile compounds present in samples (*n* = 3) fermented with GS during 2018 vintage. Methanol and higher alcohols (mg/L, right axis). Alcohols, acids, esters, aldehydes and phenols (µg/L, left axis). **(b)** Minor volatile compounds present in samples (n = 3) fermented with GS during 2018 vintage. SD48h: sun-drying 48 h; SD96h: sun-drying 96 h; CH48h: climatic chamber drying 48 h; CH96h: climatic chamber drying 96 h.

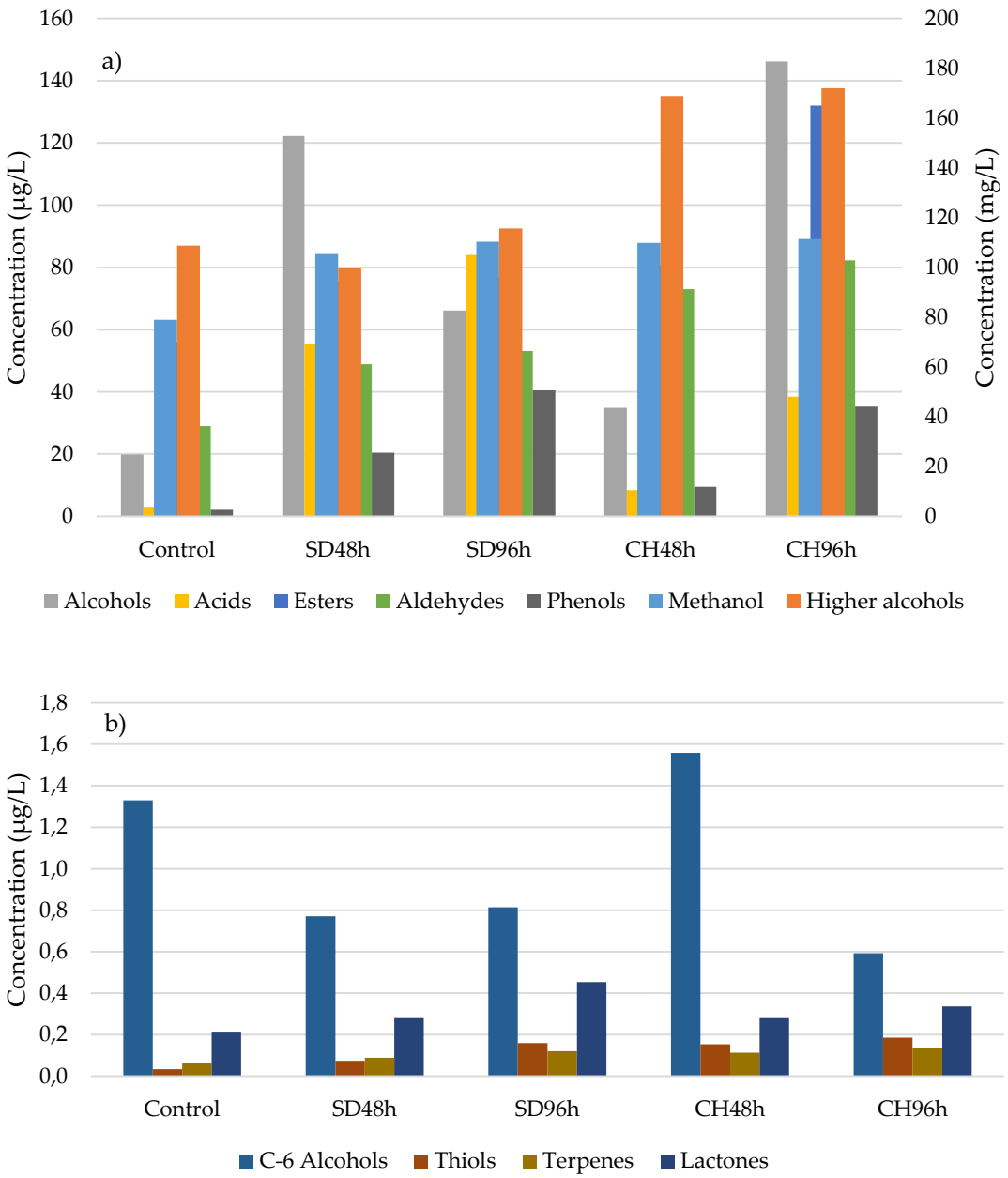

Figure 3. Major and minor volatile compounds present in samples (n = 3) fermented without GS during 2019 vintage. Methanol and higher alcohols (mg/L, right axis). Alcohols, acids, esters, aldehydes and phenols (µg/L, left axis). **(b)** Minor volatile compounds present in samples (n = 3) fermented without GS during 2019 vintage. SD48h: sun-drying 48 h; SD96h: sun-drying 96 h; CH48h: climatic chamber drying 48 h; CH96h: climatic chamber drying 96 h.

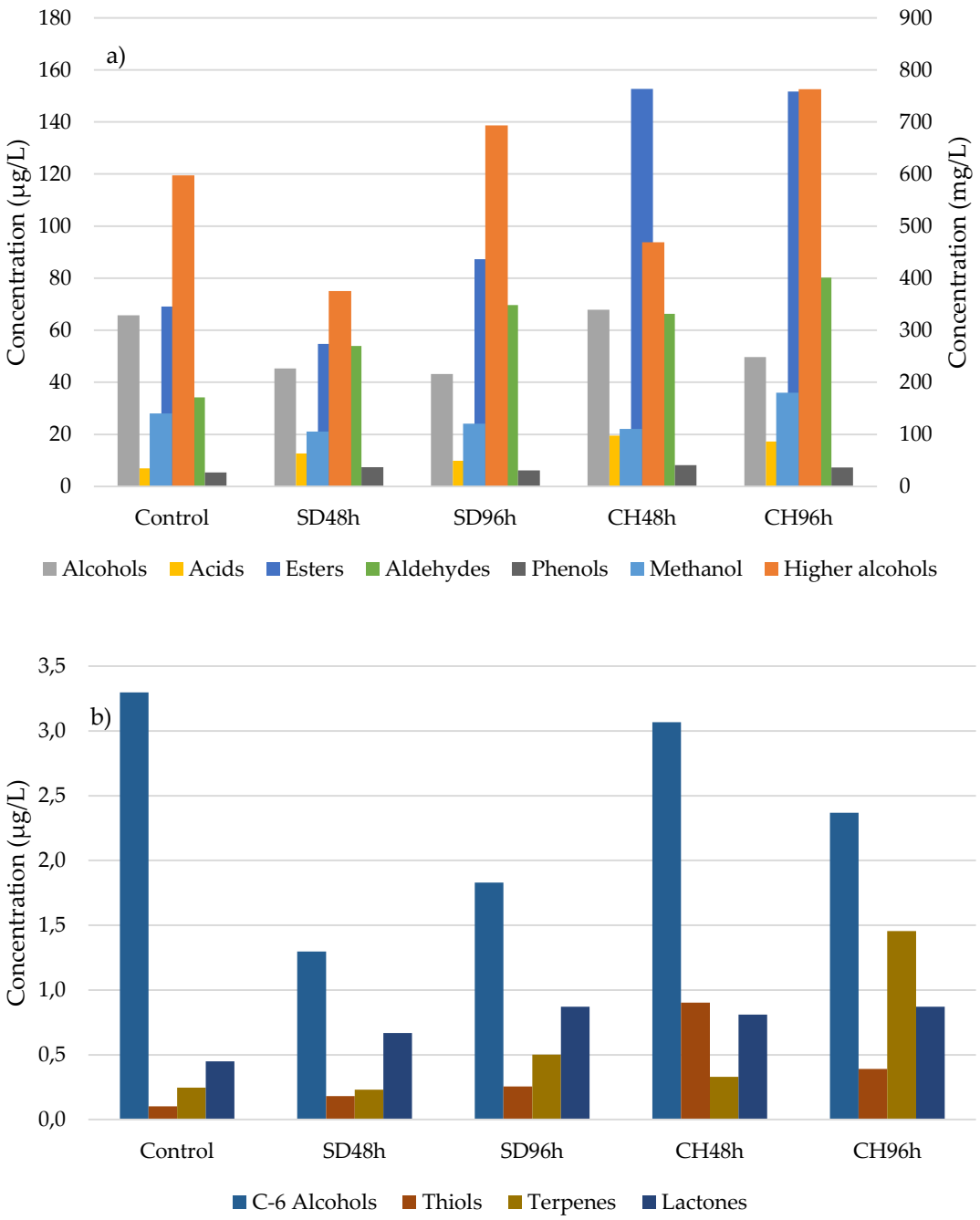

Figure 4. Major and minor volatile compounds present in samples (n = 3) fermented with GS during 2019 vintage. Methanol and higher alcohols (mg/L, right axis). Alcohols, acids, esters, aldehydes and phenols (µg/L, left axis). **(b)** Minor volatile compounds present in samples (n = 3) fermented with GS during 2019 vintage. SD48h: sun-drying 48 h; SD96h: sun-drying 96 h; CH48h: climatic chamber drying 48 h; CH96h: climatic chamber drying 96 h.

Methanol and Major Alcohols

The methanol content observed in the two studied vintages and the different overripening and winemaking methodologies shows a predominant trend towards this analyte having higher concentrations in the wines made with the CH96h grapes (Figures 1-4). After analysing the data, no correlation was observed between the different over-ripening treatments applied to the grapes, but there was a correlation with the hours of application: the methanol content was higher in the wines made with grapes that had been over-ripened for 96 h. However, it can be observed that the presence of grape skins in the fermentation medium produced an increase in the methanol concentration compared to wines produced without GS. This may be due to the contribution of the pectins from the skins, which are metabolized by the yeasts during the FAL, thus producing ethanol through enzymatic hydrolysis [48,49].

As for the major alcohols, this family of compounds represents the main part of the volatile compounds identified in the wines during the two vintages. Both 2-methyl-1-propanol and 2-propanol were identified in all of the wines produced, regardless of their methodology. In no case was there any correlation between the type of over-ripening treatment applied and the concentration of these secondary metabolites of the fermenting yeasts. Again, the presence of skins in the medium sponsored a higher concentration of major alcohols. This could be due to the Ehrlich catabolic pathway, where amino acids act as the precursors of volatile compounds [48]. This fact can be explained by the presence of skins, which implies an increase in the concentration of Free Amino Nitrogen (FAN) and therefore amino acids [28]. Despite that, the over-ripening procedures, which have also been shown to increase the amino acid concentration, did not lead to an increase in the major alcohol content in any of the cases studied. This second fact could be justified by the metabolic regulation of the yeast, where the absence of metabolic cofactors in the oxidised state such as NAD^+ prevents the transformation of the intermediate aldehyde into a higher alcohol [54].

Volatile Alcohols and Acids

A total of 11 volatile minor alcohols were detected and showed fluctuations within the different samples and vintages, with no correlations being observed. This is mainly marked by fluctuations in the 2-phenylethanol and 2-nonanol contents. As for the volatile acid content, the same trend happened as with the alcohols: no clear trend was able to be observed between the different over-ripening treatments, application times, and vintages. However, for all of the cases studied, the concentration is significantly lower for the control wine (ANOVA $p < 0.05$; Tables S1–S4)). This could be due to the fact that grape over-ripening implies an increase in their compounds due to water evaporation. This increase in fatty acids, among other compounds, can lead to an increase in the volatile acid content because an increase in the latter in grape musts can imply a lower degree yeast synthesis [55,56].

Esters

In all cases, the ester concentrations were found to be less than 1% of the total volatile compounds. It has been observed that regardless of the absence or presence of GS, wines with higher concentrations of these compounds were those that were made from grapes that had been over-ripened in a climatic chamber, specifically those that were subjected to this process for a longer period of time (96 h) (Figures 1-4). Regarding the contribution of GS to the ester concentration, it was observed that their presence in the fermentation medium caused an increase in the concentration of these compounds. Ethyl acetate is the main compound that was observed, and the behaviour observed for the different samples during the two vintages was dependent on this compound to large extent.

Esters are compounds that are formed during the alcoholic fermentation of wines and play a fundamental role in wine aroma. They are of particular interest because they contribute to the series of fruity aromas [57]. The synthesis of these compounds, similar to volatile acids, is conditioned by the presence of fatty acids in the medium, which, together with alcohol, are the substrate for esterification reactions [48]. Thus, the higher the presence of fatty acids in the musts made from overripe grapes could explain the behaviour observed in the esters.

Aldehydes

The aldehyde concentration increased significantly in all cases (ANOVA $p < 0.05$; Tables S1–S4) with respect to the control, and within each over-ripening treatment, it increased with the duration of the treatment as well as in the presence of GS (Figures 1-4). The behaviour observed for this family of compounds is mainly due to acetaldehyde, which is the major compound in this group. However, in all cases, it is present in concentrations lower than 100 mg/L, so its contribution to the sensory profile of the wine is noticeable [48]. However, other compounds, such as benzeneacetaldehyde or valeraldehyde, can contribute to wines with nutty or floral notes due to their low perception thresholds [58].

C6-Alcohols

The compounds 1-Hexanol and (*Z*)-3-hexen1-ol appeared in a higher quantity in those wines that had been fermented in the presence of skins; however, no trend or correlation was observed in their concentration with respect to the over-ripening treatment or the time applied to the grapes in either of the two vintages studied. These compounds involve aromas of fresh herbs and vegetables [58,59], and their occurrence in greater quantities will depend on the presence of their precursors, linoleic and α-linoleic acids [60], in grape musts. Thus, the presence of skins and therefore the

contribution of fatty acids [47] could explain the trend observed in the study samples over the two years.

Phenols and Minor Compounds

The phenol content did not show any trend in relation to the over-ripening technique or its time and/or the presence of GS in the fermentation medium in any of the samples studied. These compounds, which may play an important role in the aromatic notes of the spice family, were found to have a high phenol content [61] that would have originated during AF via the decarboxylation of hydroxycinnamic acids by *Saccharomyces cerevisiae*.

As for the minor compounds, thiols (1-Propanol-3-metilthiol), terpenes (Linalool), and lactones (2,3-dihydro-benzofuranone) were detected. Regarding thiols, in all cases, the values were lower than 1 μg/L depending on the vintage and grape over-ripening time and methodology. An upward trend was observed for this compound with the hours of over-ripening, with the observed differences being significant in some cases (ANOVA $p < 0.05$; Tables S1–S4). Despite this increase, this compound of fermentative origin is related to the cysteine precursor content present in the musts that are degraded by yeasts [62]. The only terpene detected in the wine was Linalool, which showed higher concentrations in those wines fermented in the presence of grape skins, its concentration increasing with the hours that the grapes spent in the over-ripening process. This family of compounds gives floral notes to the wines [63], are of varietal origin, and are mainly found in the grape skins [64]; this would explain its higher presence in wines fermented in the presence of GS. Finally, the only lactone detected showed the same behaviour as terpenes. It could have originated during the wine alcoholic fermentation process and could have formed part of the aroma of the wine [65,66].

Sensory Analysis

Figures 5–8 show the results of the sensory analysis of all the wines made during the two vintages

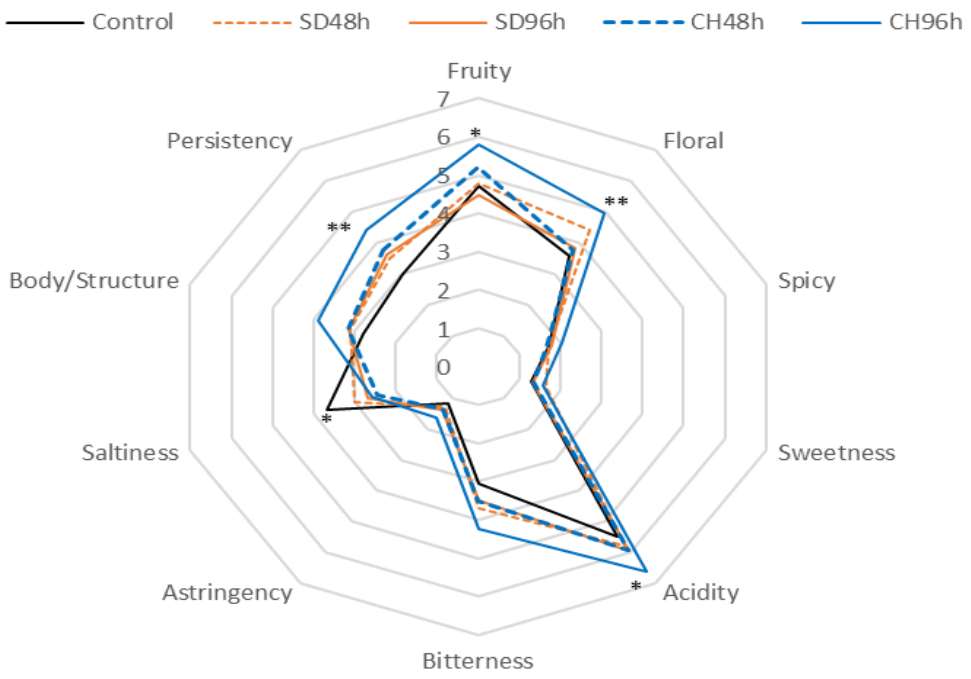

Figure 5. General attributes (aroma, taste, and mouth-feel properties) of the sensory analysis of wines fermented without GS during 2018 vintage. * Indicates level of significance for two-way ANOVA (BSD test) (* $p < 0.05$, ** $p < 0.01$). SD48h: sun-dried grapes during 48h. SD96h: sun-dried grapes during 96h. CH48h: climatic chamber drying during 48h. CH96h: climatic chamber drying during 96h.

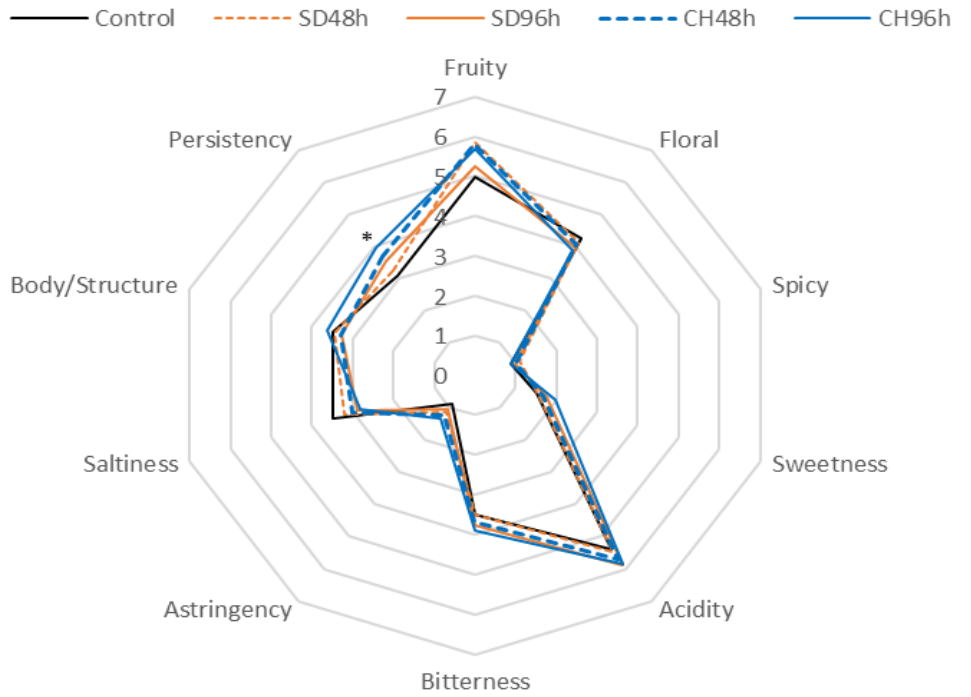

Figure 6. General attributes (aroma, taste, and mouth-feel properties) of the sensory analysis of wines fermented without GS during 2019 vintage. * Indicates level of significance for two-way ANOVA (BSD test) (* $p < 0.05$). SD48h: sun-dried grapes during 48h. SD96h: sun-dried

grapes during 96h. CH48h: climatic chamber drying during 48h. CH96h: climatic chamber drying during 96h.

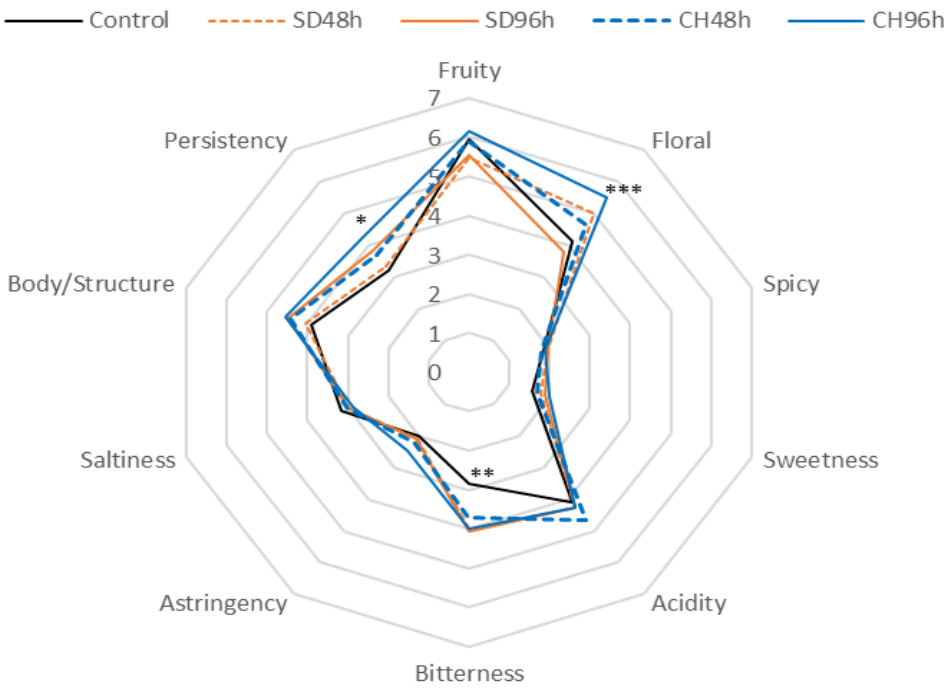

Figure 7. General attributes (aroma, taste, and mouth-feel properties) of the sensory analysis of wines fermented with GS during 2018 vintage. * Indicates level of significance for two-way ANOVA (BSD test) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). SD48h: sun-dried grapes during 48h. SD96h: sun-dried grapes during 96h. CH48h: climatic chamber drying during 48h. CH96h: climatic chamber drying during 96h.

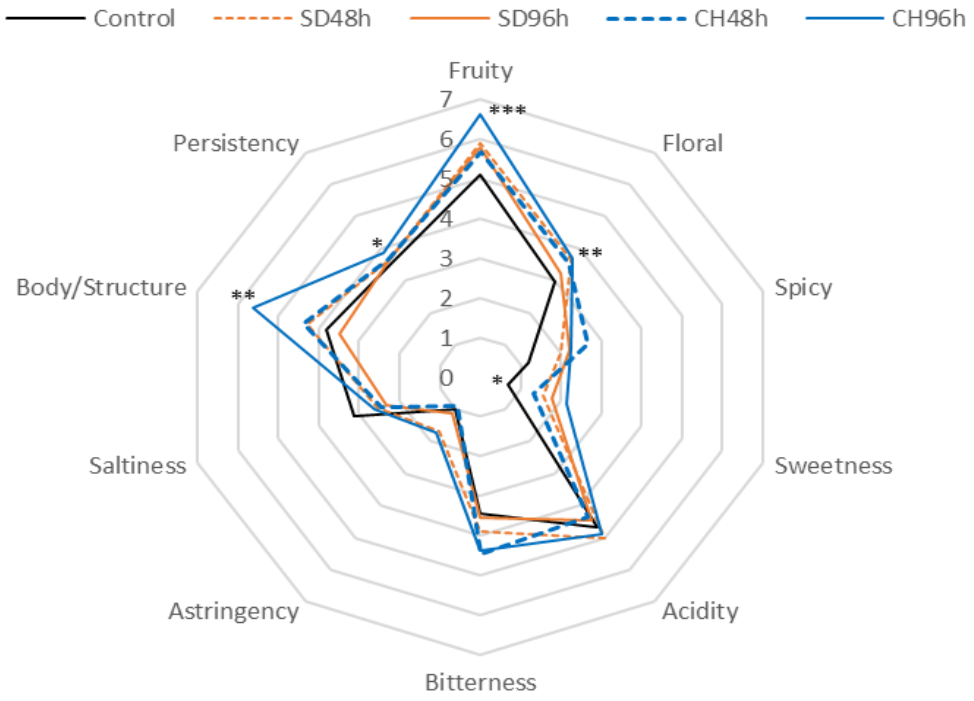

Figure 8. General attributes (aroma, taste, and mouth-feel properties) of the sensory analysis of wines fermented with GS during 2019 vintage. * Indicates level of significance for two-way ANOVA (BSD test) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). SD48h: sun-dried grapes during 48h. SD96h: sun-dried grapes during 96h. CH48h: climatic chamber drying during 48h. CH96h: climatic chamber drying during 96h.

In general terms, all of the wines showed an olfactory profile in which fruit and floral notes stand out, and in terms of taste, acidity and in some cases bitter notes are prominent. As for the wines made in the 2018 vintage, in both cases, a similar sensory profile is shown regardless GS were present in the fermentation tank. For wines made in absence of GS (Figures 5 and 6), the CH96h wine showed significant differences (ANOVA $p < 0.05$) with respect to the control wine in the olfactory phase in terms of fruity and floral notes; the same differences in taste were observed in terms of persistence. The several parameters that were evaluated showed higher values in all cases in wines made with over-ripened grapes than in the control, probably due to the concentration effect exerted by the evaporation of the water from the grapes during grape over-ripening process [67]. This concentration can lead to an increase in those compounds that provide greater acidity and structure to the wine. In the same way, the wines made during 2019 without GS (Figure 6) behaved very similarly to those made in 2018, thus showing no effect of the vintage factor on the sensory profile of the wine. Once again, the different attributes that were evaluated showed higher scores in those wines made from overripe grapes, regardless of the over-ripening technique and time. However, in the latter case, significant differences were only observed in the persistence attribute with respect to the control.

As for the wines made with presence the presence of GS, similar behaviour was observed during the two vintages studied. Again, the wines showed a predominant floral aroma and acidity on the palate. With regard to the elaboration of GS presence in 2019, the differences found in the fruity and floral notes and in the body/structure and persistence of the CH96h wine with respect to the control wine stand out. It should also be noted, although not significantly, that this same wine presented higher sweetness values due to its final residual sugar content [28].

When comparing the presence and absence of skins within the same vintage, the presence of GS makes the wines more intense in terms of fruit and floral notes. This fact may be due to the increase in terpenes observed in Figures 5–8 (and also in Tables S1–S4). The wines made with GS showed average acidity values, but lower than those produced conventionally, this fact was observed in previous research and may be due to the release of the Ca^{2+} and K^{+} cations by GS that help the precipitation of tartaric acid, resulting in a lower acid perception in wine sensory analysis [68]. The bitterness values were low in all of the analysed cases (3 out of 10 points); however, wines in the presence of GS had higher values, possibly due to the extraction of polyphenolic compounds, which can increase these perception values as well as in the body/structure and therefore the persistence of the wines [69–72].

Finally, the analysis of the preference test (UNE-ISO-8587) results showed significant differences in the tasters' preferences for the two vintages according to the *F* values obtained through the Page test and are displayed on Table 1.

Table 1. *F* test values obtained after the preference test analysis for the two vintages.

	2018		**2019**	
	Without GS	With GS	Without GS	With GS
F test	354.6	392.6	385.6	397.0

For both vintages and with either the presence or absence of GS in the fermentation medium, the results of the preference test were significant in all cases, thus significantly indicating the preferences of the tasting panel. The best evaluated wine was the one made from grapes that had been over-ripened under the sun for 96 h (SD96h). According to the scores given by the tasting panel, those wines made with GS were significantly preferred to those made without GS, also during the two studied vintages. Thus, the CH96h wine, despite obtaining higher scores in terms of sensory perception of fruity and floral notes (Figures 5–8), was not the preferred wine, probably because it presented higher sensations of bitterness in the tasting phase, thus devaluing its preference over the rest of the wines.

Conclusions

In conclusion, grape over-ripening implies modifications regarding major and minor volatile compounds in wines. As expected, there are differences between grapes that have been over-ripened naturally or in the sun versus those that have been over-ripened in a climatic chamber under controlled conditions. In both cases, it was observed that the application time significantly affects the content of the volatile compounds, regardless of the ripening technique used. In addition, the presence of grape skins during alcoholic fermentation also produces differences in wines, resulting in wines with higher ester concentrations, which translates into wines with more floral and fruity notes.

In terms of sensory analysis, the wines were obtained with a very similar profile from one year to the other. In general terms, the wines made with overripe grapes had a significantly different sensory profile compared to the control wine. The wines that were obtained were dominated by fruity and floral notes. In addition, the results of the preference test showed that wines made from grapes that had been over-ripened under the sun for 96 h (SD96h) were preferred by the tasting panel during the two vintages studied. Based on the results obtained in this research work, it could be considered that the production of white wines from overripe grapes would help to diversify the production of quality white wines in a warm climate area. In turn, over-ripening under the sun could be considered as a resilience and adaptation technique for the increased temperature conditions during the ripening season caused by the effects of climate change.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/foods11040509/s1, Table S1: Volatile compound concentration (μg/L) in wines elaborated without GS during 2018 vintage, Table S12: Volatile compound concentration (μg/L) in wines elaborated with GS presence during 2018 vintage, Table S3: Volatile compound concentration (μg/L) in wines elaborated without GS during 2019 vintage, Table S4: Volatile compound concentration (μg/L) in wines elaborated with GS presence during 2019 vintage.

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Supplementary Materials

Table S1. Volatile compound concentration (μg/L) in wines elaborated without GS during 2018 vintage.

	Control	SD48h	SD96h	CH48h	CH96h
Methanol	7059.800 ± 10.249[a]	9863.100 ± 12.961[b]	9985.600 ± 12.389[b]	9756.200 ± 5.976[b]	13989.600 ± 12.679[c]
% Methanol	12.53	16.40	14.22	16.58	17.43
Higher alcohols					
2-Methyl-1-Propanol	775.000 ± 0.106[a,b]	123.300 ± 0.679[a]	82.200 ± 0.016[a,b]	65.200 ± 0.849[a,b]	50.900 ± 0.596[b]
2-Propanol	46852.000 ± 29.890[a]	19120.500 ± 3.668[b]	19834.100 ± 3.566[b]	8968.700 ± 0.835[b]	29864.700 ± 8.866[a,b]
Total	47627.000 ± 29.996	19243.800 ± 4.347	19916.300 ± 3.583	9033.200 ± 1.684	29915.600 ± 9.462
% Higher alcohols	87.02	83.23	85.40	82.92	82.13
Alcohols					
3-Penten-2-ol	0.097 ± 0.005[a]	0.750 ± 0.087[b]	2.336 ± 0.319[c]	0.074 ± 0.004[a]	0.128 ± 0.013[a]
1-Nonanol	0.178 ± 0.014[a]	0.146 ± 0.011[a]	0.155 ± 0.010[a]	0.303 ± 0.008[b]	0.190 ± 0.018[a]
Benzyl alcohol	0.262 ± 0.020[a]	0.124 ± 0.004[b]	0.273 ± 0.009[a]	0.484 ± 0.013[c]	0.292 ± 0.013[a]
2-Phenylethanol	71.272 ± 2.618[a]	36.804 ± 0.067[b]	76.758 ± 2.461[a]	72.502 ± 1.078[a]	70.321 ± 2.013[a]
3-Ethoxy-1-Propanol	0.036 ± 0.001[a,b]	0.050 ± 0.002[a]	0.026 ± 0.004[b,c]	0.016 ± 0.002[c]	0.130 ± 0.011[d]
2-Nonanol	1.439 ± 0.082[a]	0.000 ± 0.000[b]	6.167 ± 4.796[c]	7.667 ± 0.697[d]	6.040 ± 1.894[c]
DL-2,3-Butanediol	1.143 ± 0.150[a]	0.013 ± 0.001[b]	1.102 ± 0.156[a]	0.821 ± 0.116[c]	0.022 ± 0.001[b]
3-Ethyl-2-Pentanol	0.599 ± 0.055[a,d]	0.177 ± 0.007[b]	0.555 ± 0.002[a]	0.780 ± 0.006[c]	0.681 ± 0.040[d,c]
3-Methyl-1-Pentanol	0.245 ± 0.021[a,d]	0.029 ± 0.000[b]	0.124 ± 0.009[c]	0.287 ± 0.007[a]	0.219 ± 0.012[d]
4-Methyl-1-Pentanol	0.219 ± 0.022[a,c]	0.219 ± 0.025[a,c]	0.186 ± 0.005[a]	0.288 ± 0.004[b]	0.238 ± 0.011[c]
1-Octanol	0.074 ± 0.007[a]	0.049 ± 0.007[b]	0.094 ± 0.003[a]	0.155 ± 0.003[c]	0.138 ± 0.003[c]
Total	75.564 ± 2.997	38.349 ± 0.209	87.775 ± 7.774	83.376 ± 1.938	78.377 ± 4.028
% Alcohols	0.13	0.06	0.13	0.14	0.10
Acids					
Heptanoic acid	0.396 ± 0.028[a]	0.128 ± 0.003[b]	0.353 ± 0.002[a]	0.368 ± 0.001[a]	0.222 ± 0.020[c]
2-Hexenoic acid	0.212 ± 0.017[a]	0.064 ± 0.002[b]	0.099 ± 0.007[c]	0.200 ± 0.011[a]	0.231 ± 0.027[a]
3-Methylbutanoic acid	0.303 ± 0.032[a]	0.046 ± 0.007[b]	0.237 ± 0.020[c]	0.165 ± 0.003[d]	0.200 ± 0.022[c,d]

Hexanoic acid	2.739 ± 0.197[a]	0.597 ± 0.084[b]	4.413 ± 0.324[c]	3.289 ± 0.098[a]	2.910 ± 0.287[a]
Benzoic acid	0.116 ± 0.006[a]	0.039 ± 0.005[b]	0.058 ± 0.001[b]	0.132 ± 0.002[a]	0.079 ± 0.011[c]
n-Decanoic acid	0.290 ± 0.041[a]	7.320 ± 0.438[b]	0.358 ± 0.051[a]	7.054 ± 0.829[b]	35.369 ± 3.933[c]
Butanoic acid	0.695 ± 0.047[a,d]	0.349 ± 0.006[b]	0.722 ± 0.017[a]	0.858 ± 0.010[c]	0.585 ± 0.045[d]
Total	4.751 ± 0.368	8.543 ± 0.545	6.241 ± 0.423	12.067 ± 0.950	39.595 ± 4.344
% Acids	0.02	0.00	0.01	0.02	0.05
Esters					
Ethyl 9-decenoate	0.377 ± 0.032[a]	0.139 ± 0.003[b]	0.295 ± 0.024[a,c]	0.674 ± 0.012[d]	0.236 ± 0.008[b,c]
Ethyl decanoate	0.725 ± 0.053[a]	0.305 ± 0.014[b]	0.935 ± 0.032[a,d]	1.635 ± 0.038[c]	1.132 ± 0.098[d]
Ethyl-2-phenyl acetate	0.078 ± 0.004[a]	0.502 ± 0.055[b]	1.205 ± 0.139[c]	0.081 ± 0.011[a]	1.385 ± 0.188[c]
Diethyl succinate	0.474 ± 0.024[a]	0.316 ± 0.010[b,d]	0.530 ± 0.015[a]	0.790 ± 0.000[c]	0.433 ± 0.034[a,d]
Ethyl-3-hydroxy butanoate	0.791 ± 0.063[a]	0.315 ± 0.001[b]	0.439 ± 0.023[c]	1.801 ± 0.029[d]	0.866 ± 0.033[a,b]
Phenetyl acetate	0.481 ± 0.014[a]	0.220 ± 0.018[b]	1.181 ± 0.078[c]	0.780 ± 0.022[d]	0.805 ± 0.000[d]
Ethyl octanoate	0.258 ± 0.020[a]	0.040 ± 0.006[b]	0.258 ± 0.010[a]	0.277 ± 0.004[a]	0.289 ± 0.008[a]
Ethyl octadecanoate	0.102 ± 0.009[a]	0.021 ± 0.003[b]	0.124 ± 0.002[c]	0.101 ± 0.000[a]	0.039 ± 0.001[b]
Isoamil laurate	0.168 ± 0.010[a]	0.044 ± 0.006[b]	0.107 ± 0.009[c]	0.250 ± 0.002[d]	0.086 ± 0.012[c]
2-propenyl benzoate	0.162 ± 0.010[a]	0.088 ± 0.003[b]	0.115 ± 0.010[b]	0.186 ± 0.001[a]	0.166 ± 0.012[a]
Ethyl octanoate	0.712 ± 0.057[a]	0.324 ± 0.001[b]	0.374 ± 0.053[b]	0.754 ± 0.090[a]	1.182 ± 0.064[c]
Isoamyl acetate	59.939 ± 1.847[a]	78.085 ± 2.202[b]	48.711 ± 0.893[a,c]	46.195 ± 0.066[c]	59.892 ± 0.312[a]
Ethyl nonanoate	0.054 ± 0.003[a]	0.046 ± 0.006[a]	0.080 ± 0.001[b]	0.092 ± 0.003[b,c]	0.097 ± 0.007[c]
Ethyl lactacte	0.117 ± 0.006[a]	0.137 ± 0.001[a]	0.134 ± 0.011[a]	0.129 ± 0.001[a]	0.178 ± 0.004[b]
Isoamyl lactate	0.045 ± 0.002[a]	0.030 ± 0.004[a]	0.092 ± 0.001[b]	0.115 ± 0.002[b]	0.161 ± 0.002[c]
Ethyl acetate	50.234 ± 3.468[a]	54.589 ± 2.479[a]	55.946 ± 3.478[a]	81.379 ± 22.289[b]	100.025 ± 8.364[c]
Total	114.717 ± 5.623	135.202 ± 4.813	110.524 ± 4.778	135.239 ± 12.570	166.971 ± 9.145
% Esters	0.20	0.22	0.16	0.23	0.21
Aldehydes					
Valeraldehyde	0.017 ± 0.002[a]	0.077 ± 0.006[b]	0.037 ± 0.005[c]	0.075 ± 0.001[b]	0.021 ± 0.003[a]
Benzeneacetaldehyde	0.089 ± 0.004[a]	0.137 ± 0.009[b]	0.107 ± 0.004[a]	0.174 ± 0.003[c]	0.171 ± 0.004[c]
2-Methyl valeraldehyde	0.721 ± 0.049[a]	0.254 ± 0.021[b]	0.208 ± 0.022[b]	0.614 ± 0.029[a,c]	0.515 ± 0.059[c]

Acetaldehyde	35.580 ± 1.389[a]	41.398 ± 1.579[b]	49.810 ± 1.678[c]	50.213 ± 2.623[c]	62.314 ± 1.498[d]
Total	36.406 ± 1.444	41.866 ± 1.616	50.162 ± 1.709	51.076 ± 2.656	63.021 ± 1.563
% Aldehydes	0.06	0.07	0.07	0.09	0.08
C6 Alcohols					
1-Hexanol	0.279 ± 0.010[a]	0.274 ± 0.002[a]	0.285 ± 0.028[a]	0.304 ± 0.000[a,b]	0.353 ± 0.012[b]
(*Z*)-3-hexen-1-ol	1.050 ± 0.065[a]	0.497 ± 0.014[b]	0.528 ± 0.065[b]	1.254 ± 0.023[c]	0.239 ± 0.016[d]
Total	1.330 ± 0.075	0.771 ± 0.016	0.814 ± 0.092	1.558 ± 0.023	0.592 ± 0.028
% C6 Alcohols	0.00	0.00	0.00	0.00	0.00
Thiols					
3-Methylthiol-1-Propanol	0.033 ± 0.004[a]	0.074 ± 0.004[a]	0.159 ± 0.015[b]	0.154 ± 0.003[b]	0.185 ± 0.007[b]
% Thiols	0.00	0.00	0.00	0.00	0.00
Phenols					
Guaiacol	0.080 ± 0.009[a]	0.036 ± 0.005[a,c]	0.343 ± 0.005[b]	0.000 ± 0.000[c]	0.286 ± 0.033[d]
Acetovanillone	0.190 ± 0.011[a,c]	0.029 ± 0.004[b]	0.158 ± 0.001[a]	0.214 ± 0.010[c]	0.110 ± 0.016[d]
4-Hydroxy-Benzeneethanol	14.450 ± 0.965[a]	1.492 ± 0.211[b]	6.888 ± 0.107[c]	10.737 ± 0.106[d]	5.330 ± 0.754[c]
4-vinylguayacol	0.371 ± 0.034[a]	0.275 ± 0.016[a]	0.991 ± 0.044[b]	1.015 ± 0.017[b]	0.781 ± 0.073[c]
2,6-Dimethoxyphenol	0.164 ± 0.018[a]	0.120 ± 0.014[b]	0.130 ± 0.009[b]	0.169 ± 0.009[a]	0.113 ± 0.008[b]
Total	15.254 ± 1.336	1.952 ± 0.250	8.510 ± 0.167	12.135 ± 0.141	6.620 ± 0.884
% Phenols	0.03	0.00	0.01	0.02	0.01
Terpenes					
Linalool	0.064 ± 0.002[a]	0.089 ± 0.006[b]	0.120 ± 0.006[c,d,e]	0.113 ± 0.001[d]	0.137 ± 0.001[e]
% Terpenes	0.00	0.00	0.00	0.00	0.00
Lactones					
2,3-Dihydro-Benzofuran	0.214 ± 0.011[a]	0.280 ± 0.018[a,c]	0.454 ± 0.016[b]	0.279 ± 0.020[a,c]	0.336 ± 0.003[c]
% Lactones	0.00	0.00	0.00	0.00	0.00

SD48h: sun-dried grapes during 48h. SD96h: sun-dried grapes during 96h. CH48h: climatic chamber drying during 48h. CH96h: climatic chamber drying during 96h. Different letters in superscript mean significant differences ($p < 0.05$) between samples determined by a two-way ANOVA according to Bonferroni's multiple range (BSD) test.

Table S2. Volatile compound concentration (μg/L) in wines elaborated with GS presence during 2018 vintage.

	Control	SD48h	SD96h	CH48h	CH96h
Methanol	13527.900 ± 5.900[a]	11998.200 ± 5.182[a]	16894.600 ± 2.826[a]	14230.100 ± 3.617[a]	19856.300 ± 3.140[a]
% Methanol	22.01	38.06	45.55	60.19	39.61
Higher alcohols					
2-Methyl-1-Propanol	41.000 ± 0.041[a]	0.441 ± 0.028[a]	0.063 ± 0.001[b]	0.429 ± 0.005[a]	0.406 ± 0.043[a]
2-Propanol	48996.300 ± 29.634[a]	50046.5 ± 18.468[a]	59897.537 ± 58.945[b]	48798.700 ± 46.923[a]	65936.594 ± 109.459[b]
Total	49037.300 ± 29.675	50046.900 ± 18.496	59897.600 ± 58.946	48799.100 ± 46.928	65937.000 ± 109.502
% Higher alcohols	77.48	61.05	53.69	38.21	59.67
Alcohols					
3-Penten-2-ol	0.633 ± 0.086[a,c,d]	1.550 ± 0.046[b]	0.086 ± 0.003[a,d]	0.899 ± 0.058[c]	0.049 ± 0.040[d]
1-Nonanol	0.590 ± 0.014[a,c]	0.044 ± 0.064[b]	0.708 ± 0.006[a]	0.261 ± 0.080[c,b]	0.639 ± 0.056[a]
Benzyl alcohol	1.031 ± 0.016[a]	0.126 ± 0.023[b]	1.289 ± 0.026[a]	0.382 ± 0.028[b]	1.230 ± 0.078[a]
2-Phenylethanol	77.853 ± 2.225[a]	29.600 ± 2.695[b,c]	17.865 ± 2.362[b]	58.256 ± 1.204[c]	57.302 ± 3.435[c]
3-Ethoxy-1-Propanol	0.190 ± 0.016[a]	0.125 ± 0.015[a]	0.065 ± 0.004[b]	0.060 ± 0.019[b]	0.063 ± 0.031[b]
2-Nonanol	3.824 ± 1.348[a,b]	3.279 ± 0.060[a]	4.379 ± 0.106[b]	1.532 ± 0.075[c]	4.236 ± 0.108[b]
DL-2,3-Butanediol	6.715 ± 0.293[a]	3.497 ± 0.037[b]	4.705 ± 0.114[a,b]	4.249 ± 0.047[a,b]	4.735 ± 0.272[a,b]
3-Ethyl-2-Pentanol	1.362 ± 0.004[a]	0.257 ± 0.073[b]	1.166 ± 0.024[a]	0.746 ± 0.091[a,b]	1.306 ± 0.094[a]
3-Methyl-1-Pentanol	0.437 ± 0.021[a]	0.277 ± 0.018[a,b]	0.171 ± 0.022[b]	0.264 ± 0.022[a,b]	0.252 ± 0.010[a,b]
4-Methyl-1-Pentanol	0.531 ± 0.023[a]	0.102 ± 0.020[b]	0.282 ± 0.003[a,b]	0.239 ± 0.025[b,c]	0.378 ± 0.018[a,c]
1-Octanol	0.273 ± 0.003[a]	0.037 ± 0.012[b]	0.169 ± 0.018[a,b]	0.110 ± 0.016[b]	0.287 ± 0.011[a]
Total	93.439 ± 4.048	38.893 ± 3.063	30.884 ± 2.688	66.999 ± 1.664	70.478 ± 4.154
% Alcohols	0.2	0.1	0.1	0.3	0.1
Acids					
Heptanoic acid	0.105 ± 0.081[a]	1.254 ± 0.036[b]	1.092 ± 0.011[b]	0.365 ± 0.101[b,c]	0.872 ± 0.071[b,c]
2-Hexenoic acid	0.249 ± 0.073[a]	0.993 ± 0.036[b]	1.211 ± 0.024[b]	0.081 ± 0.091[a]	0.968 ± 0.218[b]
3-Methylbutanoic acid	0.103 ± 0.014[a]	0.974 ± 0.084[b]	0.280 ± 0.013[a]	0.179 ± 0.017[a]	0.313 ± 0.012[a]
Hexanoic acid	0.925 ± 0.063[a]	2.323 ± 0.262[a,b]	1.290 ± 0.021[a]	1.220 ± 0.079[a]	3.592 ± 0.056[b]
Benzoic acid	0.102 ± 0.002[a]	0.096 ± 0.017[a]	0.107 ± 0.003[a]	0.142 ± 0.021[a]	0.143 ± 0.015[a]

n-Decanoic acid	1.346 ± 0.158[a]	0.384 ± 0.006[b]	0.646 ± 0.016[b]	0.432 ± 0.007[b]	0.369 ± 0.032[b]
Butanoic acid	1.819 ± 0.062[a]	0.280 ± 0.017[b]	1.097 ± 0.002[a,b]	0.703 ± 0.021[b]	1.258 ± 0.669[a]
Total	4.649 ± 0.453	6.304 ± 0.458	5.723 ± 0.089	3.122 ± 0.338	7.515 ± 1.073
% Acids	0.00	0.00	0.00	0.00	0.00
Esters					
Ethyl 9-decenoate	1.264 ± 0.027[a]	0.124 ± 0.003[b]	0.209 ± 0.014[b]	0.449 ± 0.004[b]	0.477 ± 0.003[b]
Ethyl decanoate	2.239 ± 0.256[a,c]	0.195 ± 0.050[b]	3.874 ± 0.069[a]	1.230 ± 0.063[c,b]	3.305 ± 0.442[a]
Ethyl-2-phenyl acetate	0.254 ± 0.013[a]	0.780 ± 0.069[a]	2.807 ± 0.028[b]	0.131 ± 0.086[a]	0.135 ± 0.061[a]
Diethyl succinate	1.950 ± 0.238[a]	0.532 ± 0.011[b]	1.527 ± 0.002[a]	1.561 ± 0.014[a]	1.903 ± 0.096[a]
Ethyl-3-hydroxy butanoate	0.840 ± 0.086[a,b]	0.236 ± 0.035[a]	1.755 ± 0.024[b]	0.727 ± 0.043[a,b]	2.361 ± 0.303[b]
Phenetyl acetate	1.172 ± 0.127[a,c]	0.165 ± 0.098[b]	1.055 ± 0.040[a,b]	0.547 ± 0.022[a,b]	2.065 ± 0.859[c]
Ethyl octanoate	0.086 ± 0.005[a]	0.097 ± 0.018[a]	0.345 ± 0.008[b]	0.225 ± 0.022[a,b]	0.344 ± 0.015[b]
Ethyl octadecanoate	0.436 ± 0.038[a]	0.105 ± 0.004[b]	0.288 ± 0.007[a,b]	0.127 ± 0.005[b]	0.153 ± 0.003[b]
Isoamil laurate	0.375 ± 0.039[a]	0.113 ± 0.006[b]	0.112 ± 0.003[b]	0.184 ± 0.007[b]	0.129 ± 0.005[b]
2-propenyl benzoate	0.110 ± 0.003[a]	0.470 ± 0.013[b]	0.107 ± 0.003[a]	0.227 ± 0.017[a]	0.274 ± 0.012[a,b]
Ethyl octanoate	0.518 ± 0.006[a,b]	0.315 ± 0.071[a]	0.546 ± 0.038[a,b]	1.061 ± 0.089[c]	1.005 ± 0.062[b,c]
Isoamyl acetate	63.693 ± 6.299[a,b]	119.644 ± 6.936[a]	80.223 ± 1.688[a,b]	103.628 ± 8.668[a,b]	48.684 ± 6.091[b]
Ethyl nonanoate	0.188 ± 0.013[a]	0.027 ± 0.011[b]	0.197 ± 0.001[a]	0.088 ± 0.014[b]	0.185 ± 0.100[a]
Ethyl lactacte	1.324 ± 0.015[a]	0.272 ± 0.075[b]	0.514 ± 0.006[b]	0.406 ± 0.094[b]	0.564 ± 0.066[b]
Isoamyl lactate	0.292 ± 0.008[a]	0.023 ± 0.034[b]	0.358 ± 0.004[a]	0.090 ± 0.043[b]	0.335 ± 0.300[a]
Ethyl acetate	79.650 ± 6.167[a,b]	42.398 ± 3.429[a]	59.210 ± 0.235[a,c]	110.258 ± 3.863[b,c]	119.087 ± 3.354[b]
Total	154.391 ± 13.342	165.497 ± 10.863	153.127 ± 2.169	181.006 ± 11.773	220.939 ± 13.054
% Esters	0.25	0.52	0.41	0.93	0.36
Aldehydes					
Valeraldehyde	0.605 ± 0.069[a]	0.249 ± 0.088[b,c]	0.385 ± 0.002[a,c]	0.033 ± 0.001[b]	0.696 ± 0.096[a]
Benzeneacetaldehyde	0.613 ± 0.021[a]	0.460 ± 0.094[a]	0.498 ± 0.009[a]	0.101 ± 0.012[b]	0.435 ± 0.043[a]
2-Methyl valeraldehyde	0.432 ± 0.034[a]	0.177 ± 0.026[b,c]	0.061 ± 0.004[b]	0.336 ± 0.033[a,c]	0.458 ± 0.023[a]
Acetaldehyde	39.874 ± 3.783[a]	69.879 ± 2.424[a,b]	79.846 ± 5.798[a,b]	75.812 ± 3.032[a,b]	89.645 ± 2.633[b]
Total	41.523 ± 3.906	70.765 ± 2.633	80.791 ± 5.814	76.282 ± 3.078	91.233 ± 2.794

% Aldehydes	0.07	0.22	0.22	0.32	0.18
C6 Alcohols					
1-Hexanol	0.465 ± 0.026^{a}	0.644 ± 0.022^{a,b}	0.352 ± 0.034^{a}	0.921 ± 0.027^{b}	0.644 ± 0.019^{a,b}
(*Z*)-3-hexen-1-ol	1.043 ± 0.050^{a,b}	0.414 ± 0.060^{a}	1.055 ± 0.028^{a,b}	0.914 ± 0.075^{a,b}	1.493 ± 0.052^{b}
Total	1.508 ± 0.050	1.058 ± 0.060	1.407 ± 0.028	1.835 ± 0.075	2.137 ± 0.052
% C6 Alcohols	0.00	0.00	0.00	0.01	0.00
Thiols					
3-Methylthiol-1-propanol	0.040 ± 0.039^{a}	0.180 ± 0.032^{b}	0.234 ± 0.004^{a,b}	0.131 ± 0.040^{b}	0.319 ± 0.028^{a,b}
% Thiols	0.00	0.00	0.00	0.00	0.00
Phenols					
Guaiacol	1.039 ± 0.047^{a}	0.163 ± 0.041^{b}	0.448 ± 0.004^{b}	0.273 ± 0.051^{b}	0.422 ± 0.036^{b}
Acetovanillone	0.417 ± 0.046^{a}	0.058 ± 0.013^{b}	0.237 ± 0.006^{a,b}	0.142 ± 0.016^{b}	0.263 ± 0.011^{a}
4-Hydroxy-Benzeneethanol	7.470 ± 0.560^{a}	1.925 ± 0.318^{b}	2.274 ± 0.055^{b,c}	4.545 ± 0.398^{a,b}	5.582 ± 0.279^{a,c}
4-vinylguayacol	1.212 ± 0.074^{a}	0.608 ± 0.075^{a}	5.092 ± 0.048^{b}	2.053 ± 0.094^{a}	2.260 ± 0.066^{a}
2,6-Dimethoxyphenol	0.762 ± 0.074^{a}	0.124 ± 0.054^{b}	0.540 ± 0.005^{a,c}	0.192 ± 0.067^{b,c}	0.497 ± 0.047^{a,c}
Total	10.900 ± 0.802	2.877 ± 0.500	8.592 ± 0.117	7.205 ± 0.625	9.024 ± 0.087
% Phenols	0.02	0.01	0.02	0.03	0.02
Terpenes					
Linalool	0.104 ± 0.030^{a}	0.163 ± 0.024^{a}	0.307 ± 0.006^{a,b}	0.199 ± 0.014^{b}	0.468 ± 0.006^{c}
% Terpenes	0.00	0.00	0.00	0.00	0.00
Lactones					
2,3-Dihydro-Benzofuran	0.201 ± 0.010^{a}	0.378 ± 0.012^{a}	1.215 ± 0.029^{b}	0.578 ± 0.052^{a,c}	1.039 ± 0.086^{b,c}
% Lactones	0.00	0.00	0.00	0.00	0.00

SD48h: sun-dried grapes during 48h. SD96h: sun-dried grapes during 96h. CH48h: climatic chamber drying during 48h. CH96h: climatic chamber drying during 96h. Different letters in superscript mean significant differences ($p < 0.05$) between samples determined by a two-way ANOVA according to Bonferroni's multiple range (BSD) test.

Table 3. Volatile compound concentration (μg/L) in wines elaborated without GS during 2019 vintage.

	Control	SD48h	SD96h	CH48h	CH96h
Methanol	7896.500 ± 4.727[a]	10539.800 ± 15.931[b]	11035.000 ± 2.483[b]	10987.400 ± 2.898[b]	11138.900 ± 3.271[b]
% Methanol	41.81	50.07	47.99	39.13	38.69
Higher alcohols					
2-Methyl-1-Propanol	6.000 ± 0.001[a]	1.000 ± 0.021[b]	1.200 ± 0.002[b]	1.700 ± 0.001[b]	2.700 ± 0.004[c]
2-Propanol	10873.800 ± 104.082[a]	9999.870 ± 84.318[a]	11563.680 ± 82.221[a]	16879.450 ± 72.788[b]	17201.030 ± 82.163[b]
Total	10879.800 ± 104.082	10000.870 ± 84.318	11564.880 ± 82.223	16881.150 ± 72.789	17203.730 ± 82.167
% Higher alcohols	57.60	47.51	50.30	60.12	59.76
Alcohols					
3-Penten-2-ol	0.021 ± 0.003[a]	0.120 ± 0.017[a]	2.977 ± 0.420[b]	0.013 ± 0.001[a]	0.082 ± 0.008[a]
1-Nonanol	0.188 ± 0.011[a]	54.282 ± 7.249[b]	15.019 ± 2.044[c]	0.829 ± 0.053[a]	3.845 ± 0.432[a]
Benzyl alcohol	0.153 ± 0.020[a]	1.206 ± 15.134[b]	9.869 ± 1.310[a]	1.676 ± 0.115[a]	9.315 ± 1.050[a]
2-Phenylethanol	17.540 ± 0.106[a]	45.350 ± 2.309[b]	18.492 ± 0.989[a]	17.829 ± 1.095[a]	68.447 ± 4.344[c]
3-Ethoxy-1-Propanol	0.049 ± 0.000[a]	0.180 ± 0.025[b]	0.035 ± 0.005[a]	0.096 ± 0.009[c]	0.153 ± 0.005[b]
2-Nonanol	1.067 ± 0.031[a]	1.609 ± 0.293[a]	1.754 ± 0.940[a]	5.267 ± 0.658[b]	3.148 ± 0.199[b]
DL-2,3-Butanediol	0.371 ± 0.010[a]	4.047 ± 0.858[b]	7.858 ± 0.569[c]	7.291 ± 0.567[c]	4.692 ± 0.371[b]
3-Ethyl-2-Pentanol	0.275 ± 0.009[a]	10.020 ± 1.007[b]	6.664 ± 0.852[c]	1.159 ± 0.031[a]	2.595 ± 0.163[a]
3-Methyl-1-Pentanol	0.075 ± 0.003[a]	1.787 ± 0.139[b]	2.392 ± 0.322[c]	0.207 ± 0.006[a]	0.604 ± 0.040[a]
4-Methyl-1-Pentanol	0.106 ± 0.002[a]	3.872 ± 0.394[b]	1.120 ± 0.128[c]	0.308 ± 0.011[a]	0.962 ± 0.071[a]
1-Octanol	0.031 ± 0.001[a]	0.283 ± 0.040[b]	0.120 ± 0.007[a]	0.269 ± 0.016[b]	0.293 ± 0.005[b]
Total	19.875 ± 0.196	122.757 ± 27.645	66.180 ± 52.578	34.944 ± 3.562	146.136 ± 15.687
% Alcohols	0.11	1.09	0.29	0.12	0.51
Acids					
Heptanoic acid	0.384 ± 0.036[a]	4.177 ± 0.693[b]	0.143 ± 0.020[a]	1.029 ± 0.013[a]	3.935 ± 0.448[b]
2-Hexenoic acid	0.255 ± 0.009[a]	16.218 ± 0.957[b]	9.086 ± 1.266[c]	0.789 ± 0.025[a]	10.821 ± 1.463[c]
3-Methylbutanoic acid	0.111 ± 0.003[a]	7.411 ± 0.608[b]	10.046 ± 1.378[c]	0.455 ± 0.006[a]	2.516 ± 0.219[d]
Hexanoic acid	1.316 ± 0.004[a]	11.832 ± 6.081[b]	1.885 ± 0.267[a]	2.320 ± 0.118[a,c]	4.722 ± 0.611[c]
Benzoic acid	0.082 ± 0.002[a]	3.605 ± 0.059[b]	4.823 ± 0.671[c]	0.303 ± 0.012[a]	1.515 ± 0.167[d]

n-Decanoic acid	0.487 ± 0.013[a]	3.439 ± 0.486[a,c]	48.619 ± 6.736[b]	2.565 ± 0.276[a,c]	11.780 ± 0.787[c]
Butanoic acid	0.388 ± 0.029[a]	8.785 ± 0.678[b]	9.421 ± 1.277[b]	1.010 ± 0.035[a]	3.197 ± 0.361[c]
Total	3.024 ± 0.096	55.468 ± 9.564	84.023 ± 11.614	8.471 ± 0.485	38.486 ± 4.057
% Acids	0.02	0.26	0.37	0.03	0.13
Esters					
Ethyl 9-decenoate	0.227 ± 0.021[a]	2.572 ± 0.364[b]	3.652 ± 0.028[c]	2.061 ± 0.153[b]	3.242 ± 0.118[c]
Ethyl decanoate	0.554 ± 0.015[a]	2.760 ± 0.237[b]	1.076 ± 0.086[c]	5.218 ± 0.382[d]	5.160 ± 2.759[d]
Ethyl-2-phenyl acetate	1.166 ± 0.134[a]	1.772 ± 0.373[b]	1.273 ± 0.285[a]	0.183 ± 0.006[c]	1.918 ± 0.113[b]
Diethyl succinate	0.249 ± 0.020[a]	0.164 ± 0.023[a]	0.075 ± 0.011[a]	0.223 ± 0.013[a]	1.163 ± 0.241[b]
Ethyl-3-hydroxy butanoate	0.422 ± 0.014[a]	4.974 ± 0.032[b]	2.015 ± 0.052[a]	3.658 ± 0.026[b]	11.141 ± 0.758[c]
Phenetyl acetate	0.092 ± 0.003[a]	1.445 ± 0.086[b,c]	1.853 ± 0.060[b]	3.238 ± 0.240[d]	1.138 ± 0.031[c]
Ethyl octanoate	0.121 ± 0.006[a]	2.679 ± 0.099[b]	8.425 ± 1.171[c]	0.523 ± 0.041[a]	3.421 ± 0.398[b]
Ethyl octadecanoate	0.069 ± 0.005[a]	4.123 ± 0.558[b]	4.129 ± 0.573[b]	0.133 ± 0.007[a]	3.262 ± 0.422[c]
Isoamil laurate	0.053 ± 0.006[a]	0.230 ± 0.033[a]	4.178 ± 0.580[b]	0.202 ± 0.015[a]	1.044 ± 0.114[a]
2-propenyl benzoate	0.059 ± 0.001[a]	0.269 ± 0.038[a]	4.516 ± 0.635[b]	0.306 ± 0.009[a]	4.001 ± 0.535[b]
Ethyl octanoate	0.683 ± 0.015[a]	4.718 ± 0.059[b]	7.027 ± 0.016[c]	3.004 ± 0.425[d]	6.050 ± 0.484[b,c]
Isoamyl acetate	6.283 ± 0.795[a]	2.252 ± 0.100[b,c]	3.311 ± 0.172[b]	1.747 ± 0.147[c]	2.701 ± 0.045[b]
Ethyl nonanoate	0.088 ± 0.004[a]	4.434 ± 0.080[b]	1.942 ± 0.249[c]	0.340 ± 0.019[a]	0.826 ± 0.057[a]
Ethyl lactacte	0.065 ± 0.008[a]	0.856 ± 0.098[b]	0.713 ± 0.097[b]	0.109 ± 0.015[a]	0.771 ± 0.073[b]
Isoamyl lactate	0.053 ± 0.002[a]	2.670 ± 0.080[b]	0.009 ± 0.001[a]	0.436 ± 0.031[a]	2.011 ± 0.205[c]
Ethyl acetate	45.821 ± 1.760[a,b]	39.869 ± 7.059[a]	32.358 ± 0.206[a,b]	59.374 ± 3.096[b]	84.127 ± 3.495[c]
Total	56.004 ± 2.808	75.786 ± 9.317	76.553 ± 3.918	80.753 ± 4.626	131.976 ± 9.847
% Esters	0.30	0.36	0.35	0.29	0.46
Aldehydes					
Valeraldehyde	0.060 ± 0.005[a]	0.694 ± 0.071[b]	0.075 ± 0.011[a,c]	0.209 ± 0.018[c]	0.130 ± 0.008[c]
Benzeneacetaldehyde	0.110 ± 0.003[a]	0.822 ± 0.084[b]	0.098 ± 0.014[a]	0.719 ± 0.057[b]	0.808 ± 0.055[b]
2-Methyl valeraldehyde	0.115 ± 0.007[a]	2.249 ± 0.061[b]	3.097 ± 0.404[c]	0.367 ± 0.028[a]	1.074 ± 0.051[d]
Acetaldehyde	28.743 ± 3.031[a]	45.168 ± 2.775[b]	49.896 ± 5.095[b]	71.685 ± 2.430[c]	80.268 ± 2.743[c]
Total	29.027 ± 3.046	48.933 ± 2.991	53.166 ± 5.524	72.980 ± 2.532	82.281 ± 2.849

% Aldehydes	0.15	0.23	0.23	0.26	0.29
C6 Alcohols					
1-Hexanol	0.084 ± 0.006^{a}	1.277 ± 0.034^{b}	0.587 ± 0.034^{c}	0.243 ± 0.006^{a}	0.070 ± 0.010^{a}
(Z)-3-hexen-1-ol	0.607 ± 0.012^{a}	1.294 ± 8.200^{b}	1.719 ± 2.278^{c}	1.518 ± 0.032^{a}	1.749 ± 0.030^{a}
Total	0.690 ± 0.012	2.571 ± 8.200	2.306 ± 2.278	1.762 ± 0.032	1.819 ± 0.040
% C6 Alcohols	0.00	0.01	0.01	0.01	0.01
Thiols					
3-Methylthiol-1-Propanol	0.030 ± 0.001^{a}	0.080 ± 0.011^{a}	0.638 ± 0.090^{b}	0.171 ± 0.007^{a}	0.927 ± 0.101^{c}
% Thiols	0.00	0.00	0.00	0.00	0.00
Phenols					
Guaiacol	0.181 ± 0.016^{a}	1.661 ± 0.235^{b}	1.856 ± 0.478^{b}	0.234 ± 0.019^{c}	0.686 ± 0.094^{d}
Acetovanillone	0.051 ± 0.004^{a}	0.255 ± 0.036^{a}	3.365 ± 0.463^{b}	1.029 ± 0.131^{c}	1.663 ± 0.185^{c}
4-Hydroxy-Benzeneethanol	1.870 ± 0.056^{a}	13.972 ± 1.976^{b}	17.334 ± 1.146^{c}	15.834 ± 0.381^{d}	22.121 ± 1.279^{e}
4-vinylguayacol	0.179 ± 0.000^{a}	3.603 ± 0.061^{b}	11.544 ± 1.611^{c}	1.712 ± 0.048^{a}	8.117 ± 0.943^{c}
2,6-Dimethoxyphenol	0.071 ± 0.002^{a}	0.913 ± 0.129^{a}	6.705 ± 0.918^{b}	0.712 ± 0.052^{a}	2.702 ± 0.331^{c}
Total	2.352 ± 0.078	20.404 ± 6.937	40.805 ± 23.616	9.522 ± 0.632	35.289 ± 38.832
% Phenols	0.01	0.10	0.18	0.03	0.12
Terpenes					
Linalool	0.070 ± 0.002^{a}	0.085 ± 0.012^{a}	0.523 ± 0.074^{b}	0.349 ± 0.025^{a}	1.105 ± 0.173^{c}
% Terpenes	0.00	0.00	0.00	0.00	0.02
Lactones					
2,3-Dihydro-Benzofuran	0.308 ± 0.022^{a}	0.358 ± 0.056^{a}	0.596 ± 0.057^{b}	0.794 ± 0.100^{c}	1.060 ± 0.188^{c}
% Lactones	0.00	0.01	0.09	0.00	0.02

SD48h: sun-dried grapes during 48h. SD96h: sun-dried grapes during 96h. CH48h: climatic chamber drying during 48h. CH96h: climatic chamber drying during 96h. Different letters in superscript mean significant differences ($p < 0.05$) between samples determined by a two-way ANOVA according to Bonferroni's multiple range (BSD) test.

Table S4. Volatile compound concentration (μg/L) in wines elaborated with GS presence during 2019 vintage.

	Control	SD48h	SD96h	CH48h	CH96h
Methanol	14001.600 ± 4.148[a]	10552.700 ± 4.567[b]	12054.900 ± 10.005[c]	11025.900 ± 2.718[d]	18000.300 ± 3.239[c]
% Methanol	18.94	21.87	14.77	18.93	19.03
Higher alcohols					
2-Methyl-1-Propanol	98.530 ± 0.002[a]	1.100 ± 0.000[b]	2.500 ± 0.001[b]	3.900 ± 0.012[b]	8.700 ± 0.006[b]
2-Propanol	59636.300 ± 91.316[a]	37524.200 ± 64.925[b]	69374.800 ± 100.264[c]	46890.200 ± 89.988[d]	76277.800 ± 81.357[e]
Total	59734.830 ± 91.317	37525.300 ± 64.925	69327.300 ± 100.265	46894.100 ± 90.000	76286.500 ± 81.363
% Higher alcohols	80.81	77.77	84.96	80.52	80.64
Alcohols					
3-Penten-2-ol	3.984 ± 0.001[a]	0.043 ± 0.000[b]	0.029 ± 0.001[b]	0.193 ± 0.003[b]	0.054 ± 0.004[b]
1-Nonanol	1.134 ± 0.030[a]	0.435 ± 0.033[b]	0.592 ± 0.024[c]	2.198 ± 0.024[d]	0.865 ± 0.066[e]
Benzyl alcohol	0.516 ± 0.053[a]	0.613 ± 0.087[a]	1.051 ± 0.042[b]	0.528 ± 0.092[a]	1.800 ± 0.054[c]
2-Phenylethanol	38.883 ± 0.654[a]	31.506 ± 0.758[b]	36.604 ± 0.524[a]	54.934 ± 4.136[c]	30.391 ± 0.608[b]
3-Ethoxy-1-Propanol	2.880 ± 0.010[a]	0.014 ± 0.002[b]	0.040 ± 0.002[b]	1.090 ± 0.027[c]	0.042 ± 0.006[b]
2-Nonanol	1.485 ± 0.117[a]	8.812 ± 0.542[b]	1.759 ± 0.094[a]	2.765 ± 0.071[c]	6.502 ± 0.162[d]
DL-2,3-Butanediol	2.286 ± 0.038[a]	2.751 ± 0.039[a]	0.812 ± 0.030[b]	2.319 ± 0.049[a]	7.010 ± 0.194[c]
3-Ethyl-2-Pentanol	9.619 ± 0.072[a]	0.624 ± 0.052[b]	1.351 ± 0.058[c]	2.063 ± 0.057[d]	1.823 ± 0.020[c,d]
3-Methyl-1-Pentanol	1.604 ± 0.027[a]	0.190 ± 0.006[b]	0.355 ± 0.021[b]	0.535 ± 0.043[b]	0.421 ± 0.021[b]
4-Methyl-1-Pentanol	2.291 ± 0.019[a]	0.180 ± 0.011[b]	0.360 ± 0.015[c]	0.554 ± 0.045[d]	0.495 ± 0.010[c,d]
1-Octanol	1.077 ± 0.006[a]	0.139 ± 0.012[b]	0.242 ± 0.005[b]	0.676 ± 0.069[b]	0.294 ± 0.016[b]
Total	65.761 ± 1.026	45.307 ± 1.541	43.196 ± 0.816	67.855 ± 4.616	49.698 ± 1.161
% Alcohols	0.09	0.09	0.05	0.12	0.05
Acids					
Heptanoic acid	0.868 ± 0.001[a]	0.966 ± 0.064[b]	0.800 ± 0.001[a]	1.170 ± 0.034[c]	1.129 ± 0.124[c]
2-Hexenoic acid	0.378 ± 0.012[a]	0.900 ± 0.066[b]	0.240 ± 0.010[a]	1.593 ± 0.064[c]	2.074 ± 0.056[d]
3-Methylbutanoic acid	1.314 ± 0.029[a]	3.271 ± 0.045[b]	0.533 ± 0.023[c]	1.015 ± 0.099[d]	0.651 ± 0.000[c]
Hexanoic acid	2.753 ± 0.125[a]	4.148 ± 0.154[b]	5.814 ± 0.100[c]	3.931 ± 0.043[b]	9.058 ± 0.056[d]
Benzoic acid	0.138 ± 0.006[a]	0.183 ± 0.003[b]	0.096 ± 0.004[c]	0.511 ± 0.049[d]	0.276 ± 0.019[e]

n-Decanoic acid	0.535 ± 0.099[a]	1.978 ± 0.076[b]	2.123 ± 0.080[b]	6.980 ± 0.099[c]	2.092 ± 0.029[b]
Butanoic acid	0.968 ± 0.013[a]	1.233 ± 0.076[a]	0.257 ± 0.011[b]	4.317 ± 0.055[c]	1.954 ± 0.076[d]
Total	6.953 ± 0.285	12.680 ± 0.484	9.862 ± 0.228	19.515 ± 0.442	17.235 ± 0.360
% Acids	0.01	0.03	0.01	0.03	0.02
Esters					
Ethyl 9-decenoate	1.276 ± 0.008[a]	1.071 ± 0.076[a]	0.179 ± 0.006[b]	5.218 ± 0.051[c]	0.557 ± 0.019[d]
Ethyl decanoate	0.802 ± 0.168[a]	1.793 ± 0.133[b]	3.262 ± 0.135[c]	5.230 ± 0.164[d]	5.297 ± 0.092[d]
Ethyl-2-phenyl acetate	0.556 ± 0.041[a]	0.135 ± 0.012[b]	3.829 ± 0.032[c]	1.181 ± 0.041[d]	0.106 ± 0.015[d]
Diethyl succinate	0.762 ± 0.004[a]	0.188 ± 0.010[b]	0.250 ± 0.003[b]	0.416 ± 0.021[c]	2.102 ± 0.066[d]
Ethyl-3-hydroxy butanoate	0.431 ± 0.107[a]	1.409 ± 0.083[b]	1.907 ± 0.085[c]	7.001 ± 0.114[d]	3.079 ± 0.048[e]
Phenetyl acetate	0.357 ± 0.022[a]	1.473 ± 0.014[b]	0.472 ± 0.018[a]	8.420 ± 0.255[c]	3.232 ± 0.103[d]
Ethyl octanoate	0.815 ± 0.031[a]	0.311 ± 0.018[b]	0.469 ± 0.025[c]	1.136 ± 0.014[d]	0.542 ± 0.038[c]
Ethyl octadecanoate	0.474 ± 0.010[a]	0.358 ± 0.022[b]	0.217 ± 0.008[c]	0.405 ± 0.045[d]	0.588 ± 0.020[e]
Isoamil laurate	0.010 ± 0.005[a]	0.073 ± 0.010[a,d]	0.116 ± 0.004[b,d]	1.274 ± 0.065[c]	0.179 ± 0.003[b]
2-propenyl benzoate	0.684 ± 0.002[a]	0.430 ± 0.015[b]	0.073 ± 0.001[c]	0.754 ± 0.067[d]	0.213 ± 0.009[e]
Ethyl octanoate	0.250 ± 0.012[a]	0.411 ± 0.043[b]	0.405 ± 0.009[b]	2.304 ± 0.084[c]	0.576 ± 0.023[d]
Isoamyl acetate	0.170 ± 0.022[a]	0.516 ± 0.031[b]	5.488 ± 0.338[c]	1.854 ± 0.020[d]	4.247 ± 0.056[e]
Ethyl nonanoate	0.618 ± 0.031[a]	0.338 ± 0.019[b]	0.429 ± 0.025[c]	0.991 ± 0.083[d]	0.352 ± 0.018[b]
Ethyl lactacte	0.435 ± 0.018[a]	0.097 ± 0.012[b]	0.106 ± 0.014[b]	0.141 ± 0.002[c]	0.211 ± 0.001[d]
Isoamyl lactate	0.464 ± 0.001[a]	0.203 ± 0.021[b]	0.360 ± 0.001[c]	1.217 ± 0.035[d]	0.551 ± 0.014[e]
Ethyl acetate	40.951 ± 3.441[a]	45.889 ± 2.435[a]	69.681 ± 0.451[b]	115.186 ± 0.226[c]	129.874 ± 3.460[d]
Total	69.054 ± 10.323	54.696 ± 5.954	87.244 ± 1.157	152.729 ± 1.287	151.705 ± 0.945
% Esters	0.09	0.11	0.11	0.26	0.16
Aldehydes					
Valeraldehyde	0.970 ± 0.017[a]	0.315 ± 0.002[b]	0.261 ± 0.013[b]	0.547 ± 0.050[c]	0.096 ± 0.014[d]
Benzeneacetaldehyde	0.763 ± 0.017[a]	0.452 ± 0.012[b]	0.394 ± 0.013[b]	1.838 ± 0.017[c]	0.824 ± 0.017[a]
2-Methyl valeraldehyde	0.481 ± 0.009[a]	0.209 ± 0.004[b]	0.085 ± 0.007[c]	0.802 ± 0.076[d]	0.508 ± 0.046[a]
Acetaldehyde	31.970 ± 2.659[a]	53.036 ± 2.137[b]	68.981 ± 1.440[c]	63.101 ± 0.557[d]	78.793 ± 2.716[e]
Total	34.184 ± 2.702	54.012 ± 2.154	69.721 ± 1.474	66.288 ± 0.700	80.222 ± 2.792

% Aldehydes	0.05	0.11	0.09	0.11	0.08
C6 Alcohols					
1-Hexanol	0.110 ± 0.005^{a}	0.082 ± 0.003^{a}	0.548 ± 0.004^{b}	0.386 ± 0.006^{c}	0.215 ± 0.036^{d}
(*Z*)-3-hexen-1-ol	3.189 ± 0.002^{a}	1.215 ± 0.053^{b}	1.281 ± 0.001^{b}	2.852 ± 0.065^{c}	1.982 ± 0.079^{d}
Total	3.299 ± 0.002	1.297 ± 0.053	1.829 ± 0.001	3.067 ± 0.065	2.368 ± 0.079
% C6 Alcohols	0.00	0.00	0.00	0.00	0.00
Thiols					
3-Methylthiol-1-propanol	0.101 ± 0.006^{a}	0.181 ± 0.010^{b}	0.256 ± 0.005^{c}	0.902 ± 0.042^{d}	0.390 ± 0.017^{e}
% Thiols	0.00	0.00	0.00	0.00	0.00
Phenols					
Guaiacol	0.364 ± 0.002^{a}	0.588 ± 0.023^{b}	0.429 ± 0.002^{c}	0.362 ± 0.011^{a}	0.393 ± 0.025^{a,c}
Acetovanillone	0.653 ± 0.007^{a}	0.080 ± 0.001^{b}	0.143 ± 0.006^{c}	0.453 ± 0.048^{d}	0.355 ± 0.015^{e}
4-Hydroxy-benzeneethanol	3.415 ± 0.087^{a}	4.622 ± 0.173^{b}	4.511 ± 0.070^{b}	4.971 ± 0.048^{c}	3.838 ± 0.024^{d}
4-vinylguayacol	0.852 ± 0.116^{a}	1.255 ± 0.092^{b}	0.907 ± 0.093^{a}	1.244 ± 0.034^{b}	2.137 ± 0.089^{c}
2,6-Dimethoxyphenol	0.138 ± 0.008^{a}	0.888 ± 0.088^{b}	0.178 ± 0.007^{a}	1.128 ± 0.020^{c}	0.618 ± 0.026^{d}
Total	5.421 ± 0.221	7.433 ± 0.377	6.168 ± 0.177	8.159 ± 0.161	7.340 ± 0.179
% Phenols	0.01	0.02	0.01	0.01	0.01
Terpenes					
Linalool	0.247 ± 0.019^{a}	0.231 ± 0.020^{a}	0.501 ± 0.016^{b}	0.329 ± 0.015^{c}	1.455 ± 0.117^{d}
% Terpenes	0.00	0.00	0.01	0.00	0.01
Lactones					
2,3-Dihydro-Benzofuran	0.450 ± 0.004^{a}	0.669 ± 0.040^{b}	0.870 ± 0.045^{c}	0.811 ± 0.097^{c}	0.872 ± 0.011^{c}
% Lactones	0.00	0.00	0.00	0.00	0.00

SD48h: sun-dried grapes during 48h. SD96h: sun-dried grapes during 96h. CH48h: climatic chamber drying during 48h. CH96h: climatic chamber drying during 96h. Different letters in superscript mean significant differences ($p < 0.05$) between samples determined by a two-way ANOVA according to Bonferroni's multiple range (BSD) test.

6. Discusión conjunta de resultados.

6.1. Identificación genética de variedades de vid

Para la identificación de variedades de vid, los marcadores moleculares conocidos como microsatélites o Simple Sequence Repeat (SSR) son los de mayor diversidad y fiabilidad, tal y como han demostrado distintos proyectos europeos como el GENRES 081 o el GrapeGen06 [1]. Independientemente del alto grado de heterozigosidad existente en la especie *Vitis vinifera*, el genotipo de una variedad puede ser establecido mediante el empleo de seis *loci* de microsatélites (VVMD5, VVMD7, VVMD27, VVS2, VrZAG62, VrZAG79) [2], a excepción de aquellas variedades que se encuentren altamente emparentadas, en las que se requiere aumentar el número de *loci* [3]. Por este motivo, se estableció un consenso internacional para incrementar el número de *loci* de microsatélites hasta 20 *loci*, localizados en distintos grupos de ligamiento para la correcta identificación de las variedades de vid [4-7]. En esta Tesis Doctoral, se han empleado un total de 22 *loci* de microsatélites para incluir los 6 autorizados por la OIV entre los 20 *loci* consensuados y poder comparar los genotipos obtenidos con los publicados en las bases de datos internacionales disponibles [5-8].

En la Tabla 1 se muestran los genotipos obtenidos para las cinco variedades de vid autóctonas y las cuatro variedades de referencia analizadas, para poder adaptar los tamaños de los alelos obtenidos a los publicados en las bases de datos comparadas.

Tabla 1. Perfil genético de las cinco accesiones de variedades autóctonas y cuatro de variedades de referencia caracterizadas con 22 *loci* de microsatélites nucleares. El tamaño de los alelos se expresa en pares de bases.

	Variedades autóctonas										***Variedades de referencia***							
Código de la variedad	**UR**		**CÑ**		**CS**		**MP**		**PF**		**CSV**		**CH**		**MPGB**		**PN**	
Microsatélite *locus*																		
VVIB01	307	307	291	307	291	307	307	307	291	307	291	291	289	295	291	295	289	295
VMC1b11	184	188	184	188	184	184	184	188	184	188	184	184	166	184	184	188	166	172
VMC4F31	184	190	188	206	168	176	184	190	176	206	174	178	174	180	168	206	174	180
VVMD5	224	232	232	234	220	224	224	232	226	238	228	236	232	236	226	234	226	236
VVMD7	244	246	240	246	236	246	244	246	236	246	236	236	236	240	232	246	236	240
VVMD21	243	249	249	255	243	265	243	249	243	249	249	257	249	249	249	265	249	249
VVMD24	209	209	209	209	209	211	209	209	209	209	209	217	209	219	213	217	215	217

VVMD25	238	252	240	252	252	252	238	252	240	240	238	246	238	252	240	246	238	246
VVMD27	180	182	186	194	182	182	182	182	186	194	176	190	182	190	180	194	186	190
VVMD28	246	248	236	250	246	246	246	248	238	250	236	238	220	230	248	270	220	238
VVMD32	270	270	254	270	270	270	270	270	254	256	238	238	238	270	262	270	238	270
VVIH54	166	168	166	166	166	168	166	168	166	166	166	182	164	168	166	166	164	168
VVIN16	151	153	153	153	151	151	151	153	151	151	153	153	151	151	149	149	151	159
VVIN73	264	264	264	264	264	264	264	264	264	264	264	268	264	266	264	264	264	266
VVIP31	176	190	180	190	176	176	176	190	188	190	190	190	180	184	184	188	180	180
VVIP60	318	326	318	326	322	322	318	326	318	322	306	314	318	322	318	318	318	320
VVIQ52	85	89	85	89	85	89	85	89	85	85	83	89	83	89	83	83	89	89
VVS2	131	142	142	144	142	142	131	142	131	144	137	151	135	142	131	131	135	151
VVIV37	161	161	163	177	163	167	161	161	163	167	163	163	153	163	163	165	153	163
VVIV67	372	375	358	372	366	375	372	375	364	366	364	372	364	372	364	375	364	372
VrZAG62	187	193	187	203	187	193	187	193	187	193	187	193	187	195	185	195	187	193
VrZAG79	242	248	236	246	236	258	242	248	250	260	246	246	242	244	250	254	238	244

UR: Uva Rey; CÑ: Cañocazo; CS: Castellano; MP: Mantúo de Pilas; PF: Palomino Fino; CSV: Cabernet Sauvignon; CH: Chardonnay; MPGB: Muscat a Petits Grains Blancs; PN: Pinot Noir.

La identificación del genotipo de la variedad Uva Rey ha permitido confirmar su sinonimia con las variedades conocidas como De Rey y Mantúo de Pilas, las cuales se encuentran registradas en el *Vitis* International Variety Catalogue (*V*IVC) con un perfil genético para nueve *loci* de SSR [8-9]. Por primera vez, para esta variedad, se aporta el genotipo para un total de 22 *loci* de microsatélites (13 adicionales a los publicados). Respecto a la variedad Castellano, esta presentó el mismo genotipo que Manteaudo para 9 *loci* de microsatélites según el *V*IVC [8], y para 20 *loci* según Lacombe et al. [10]. Por tanto, en este estudio se habría una nueva sinominia de Manteaudo.

En cuanto al estudio de la variabilidad intravarietal de la variedad de vid Palomino Fino, el empleo de 22 *loci* de microsatélites no ha permitido encontrar diferencias en cuanto al perfil genético obtenido para las distintas variantes somáticas estudiadas. Resultados similares se obtuvieron por Jiménez-Cantizano et al. [11] para seis *loci* de microsatélite recomentados por la OIV. Por ello, las diferencias fenotípicas documentadas pueden deberse a resultados de mutaciones somáticas que tienen lugar en células individuales en los meristemas de vid [12] y se trasmiten por multiplicación vegetativa [11]. El genotipo obtenido para la variedad Palomino Fino, fue confirmado

con el publicado en la base de datos *V*IVC [8] para 9 *loci* de microsatélites. En esta base de datos Palomino de Jerez y Palomino Pelusón se encuentran listados como sinónimos de Palomino Fino, pero no se recoge el nombre de la variante somática Palomino Gacho.

6.2. Caracterización morfológica de variedades de vid

De acuerdo con las recomendaciones establecidas para una correcta y completa identificación del material vegetal del género *Vitis*, posterior a una caracterización genética, debe llevarse a cabo la descripción ampelográfica de dicho material [13]. Los resultados obtenidos en esta Tesis Doctoral indican que la accessión de vid Uva Rey caracterizada presenta diferencias para 10 descriptores morfológicos con respecto a la variedad Mantúo de Pilas descrita por García de Luján et al. [14]. La diferencia más destacable entre estas accesiones, hace referencia a la densidad de pelos erguidos sobre los nervios principales del envés del limbo (OIV 087), donde Uva Rey presenta una alta densidad, todo lo contrario que Mantuo de Pilas. Diferencias fenotípicas similares se han publicado para otras variedades como Garnacha y Garnacha Peluda [12] y Zalema y Zalema Peluda [14] consideradas ambas como variantes somáticas. En cuanto a las variedades de uva Cañocazo y Palomino Fino, ambas mostraron un fenotipo similar al descrito por García de Luján et al. [15], mientras que Castellano mostró un alto grado de similitud con la descrita previamente por Serrano et al. [16]. No obstante, las accesiones Mantúo de Pilas y Uva Rey analizadas en esta Tesis Doctoral, difieren ligeramente en su fenotipo. Algunas diferencias fenotípicas se podrían atribuir a las condiciones ambientales dado que ambas accesiones se cultivan en parcelas con distinta localización. En general, para todas las variedades autóctonas estudiadas, se observó un alto grado de densidad en cuanto a los descriptores que describen la vellosidad en las hojas adultas (OIV 084, OIV 085, OIV 086 y OIV 087). Esta vellosidad, o tricomas no glandulares, presentan un rol funcional en las hojas, dado que restringen el movimiento del aire alrededor de los poros estomatales de las hojas, y por lo tanto pueden modular los procesos de evapotranspiración [17]. De este modo, estas variedades autóctonas podrían considerarse mejor adaptadas a las condiciones de clima cálido dada su alta densidad en cuanto a la vellosidad.

De los 68 descriptores utilizados en la descripción de las distintas variantes somáticas de la variedad Palomino Fino caracterizadas en esta Tesis Doctoral, 14 corresponden a los descriptores primarios propuestos por la OIV para discriminar entre variedades, y además se amplió con descriptores adicionales con la intención de encontrar diferencias fenotípicas entre las distintas variantes somáticas. En la Tabla 2 y Figura 3 se muestran las principales diferencias fenotípicas encontradas entre las distintas variantes somáticas. Para el primer set de los 14 descriptores primarios propuestos por la OIV, se encontraron diferencias morfológicas para 7 de ellos. Estas variaciones fenotípicas deben considerarse como un recurso fitogenético de interés a conservar con el objetivo de usarse en nuevos programas de selección clonal [18-19].

Igualmente, entre los distintos Palominos, una de las características morfológicas consideradas de interés en relación a la adaptación al cambio climático es la mayor densidad de vellosidad [17] observada en la variante somática Palomino Pelusón.

Tabla 2. Número de descriptores diferentes entre las variantes somáticas caracterizadas y la variedad Palomino Fino.

	Palomino Fino	**Palomino de Jerez**	**Palomino Gacho**	**Palomino Pelusón**
Palomino Fino	X			
Palomino de Jerez	10	X		
Palomino Gacho	8	10	X	
Palomino Pelusón	19	17	17	X

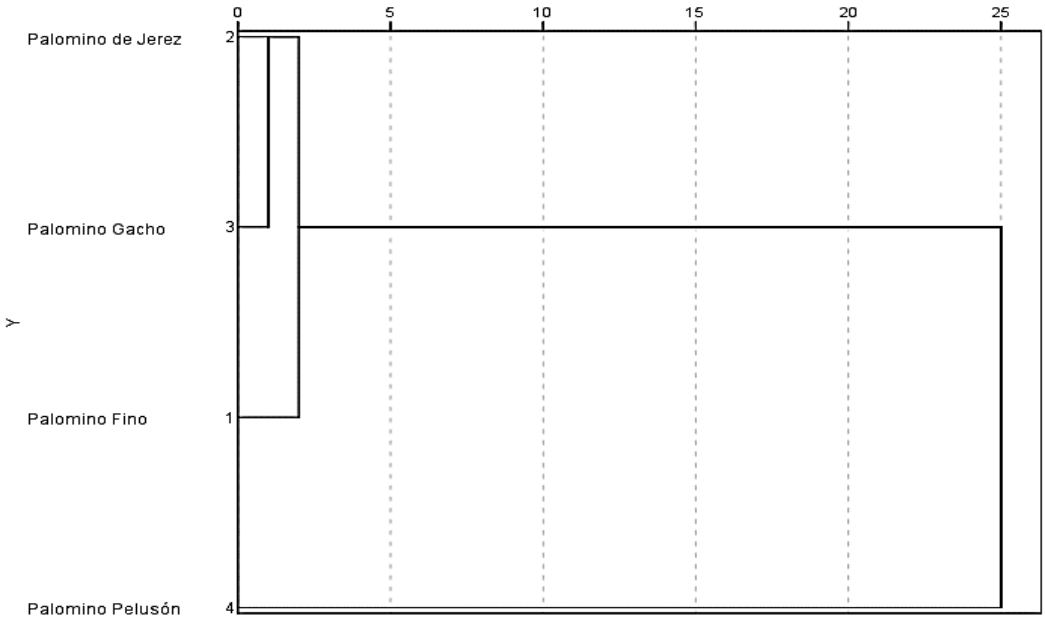

Figura 3. Dendrograma de representación de las diferencias entre las distintas variantes somáticas basado en las diferencias ampelográficas detectadas.

6.3. Caracterización fisicoquímica de los mostos procedentes de variedades autóctonas.

En el trascurso de los últimos años se vienen observando cambios durante las fases de desarrollo vegetativo de la vid, así como en el periodo de maduración de las bayas de uva. Entre los cambios asociados a los efectos del cambio climático, destacan el adelanteo del desborre y la floración como resultado de un aumento de las temperaturas y los cambios en el régimen de precipitaciones [20]. Estos cambios tienen el potencial suficiente como para afectar a los distintos metabolitos secundarios presentes en el mosto de uva, dado que la concentración de éstos depende en gran parte de la actividad fisiológica de la vid durante el periodo de maduración [21]. De este modo, durante el proceso de selección de los cultivares adecuados, se hace esencial considerar que en las zonas de clima cálido como Andalucía la tendencia general es obtener mostos de uva con un elevado contenido en azúcares y una baja acidez [22].

Tras el análisis del contenido en metabolitos secundarios en las distintas variedades de uva y variantes somáticas estudiadas, se observa como el ciclo fenológico de éstas es un factor predominante e influye en la concentración de azúcares y ácidos orgánicos. Así pues, los resultados de esta Tesis Doctoral muestran como las accesiones Uva Rey, Mantúo de Pilas y Cañocazo presentan un ciclo fenológico más largo que Castellano o Palomino Fino. En cuanto al contenido en nitrógeno fácilmente asimilable, los mostos de todas las variedades mostraron el contenido considerado como mínimo para poder llevar a cabo una vinificación sin problemas [23].

Las diferencias observadas en los mostos de las distintas variedades de vid se ven confirmadas mediante el análisis de componentes principales (PCA) llevado a cabo. Así pues, se ha podido observar como las variedades de vid autóctonas presentan un mayor potencial en cuanto a la acidez si se compara con Palomino Fino, independientemente del año de estudio. Este hecho, combinado con las condiciones de clima cálido de la región, puede constituir una ventaja para la elaboración de vinos blancos sobre todo en lo que respecta a obtener un adecuado equilibrio dulce/acidez. No obstante, los resultados de la PCA también indican que las variedades de vid estudiadas en esta Tesis Doctoral fueron vendimiadas de forma prematura y requieren un mayor tiempo de maduración, en especial la variedad Mantúo de Pilas. Por lo que refiere a las distintas variantes somáticas estudiadas, se observa una tendencia similar a la mostrada por las variedades autóctonas. De este modo, la variedad Palomino Fino mostró mayores índices de maduración si se compara con sus variantes somáticas Palomino Gacho, Palomino Pelusón y Palomino de Jerez.

De este modo, el estudio de las variantes somáticas de la variedad Palomino Fino, así como de las variedades autóctonas, ha permitido determinar la viabilidad para su cultivo en zonas de clima cálido. De este modo, su empleo puede establecerse como una alternativa viable y natural para la producción de vinos blancos de calidad en una región de clima cálido, a la vez que permitirá contribuir a conservar la diversidad genética de estas variedades. No obstante, con la intención de promover su cultivo en la región andaluza, en las Denominaciones de Origen del Marco de Jerez y en otras regiones vitivinícolas con climas parecidos, se hace necesario solicitar su inclusión en el registro oficial de variedades autorizadas.

6.4. Elaboración de vinos blancos en presencia de hollejos

La elaboración de vinos blancos secos con la variedad de uva Palomino Fino en presencia de diferentes proporciones de hollejos ha permitido obtener los siguientes resultados. Por un lado, en relación a la evolución de las poblaciones viables de levaduras *Saccharomyces cerevisiae*, la presencia de una mayor proporción de hollejos durante el proceso fermentativo contribuyó a un incremento de las poblaciones de levaduras, así como a un aumento de la velocidad de desarrollo de éstas. Probablemente, la presencia de compuestos nitrogenados contenidos en el exocarpo del

grano de uva, junto con otros cofactores necesarios para el correcto desarrollo de las levaduras [24], podrían jugar un papel de gran interés en el seno fermentativo, comportándose como fuente de compuestos nitrogenados. No obstante, la adición de una dosis de hollejos igual o superior al 80% p/v no asegura una mayor proporción de levaduras viables durante la fermentación alcohólica. Posiblemente, la presencia de ácidos grasos de cadena corta y sus posibles precursores, así como sus ésteres, pueden ejercer un efecto inhibidor en el proceso de fermentación alcohólica. Asimismo, la mayor presencia de sustancias polifenólicas, aportadas por los hollejos durante la fermentación, puede llegar también a causar un efecto inhibidor de la enzima H^+ATPasa durante la fase inicial de la fermentación alcohólica [25].

En relación a la proporción de biomasa viable, los incrementos de población de levaduras durante la fermentación alcohólica en presencia de hollejos, generaron un mayor consumo de azúcares que conlleva a una disminución más pronunciada de la densidad. No obstante, cabe destacar que, pese al efecto inhibidor comentado anteriormente, no se han observado problemas en los finales de fermentación en todos los casos estudiados. La ausencia de hollejos en el medio fermentativo puede implicar una menor disponibilidad de nutrientes y/o cofactores como minerales o vitaminas, necesarios para el rápido desarrollo de las levaduras al inicio de la fermentación alcohólica [26], siendo preciso un tiempo mayor de latencia o de adaptación de las levaduras.

Por otro lado, el estudio del contenido en Nitrógeno Fácilmente Asimilable (NFA) mostró, en todos los casos, niveles superiores a los mínimos teóricos establecidos para un correcto desarrollo de la fermentación alcohólica (140 mg/L) [23]. No obstante, se observó que la presencia de hollejos con las proporciones estudiadas (20, 40, 60, 80 y 100 %), no implicó un aumento significativo de los valores de NFA iniciales en el mosto de uva. Durante la fermentación alcohólica, disminuyó el contenido en NFA hasta valores normales que aseguran una correcta estabilidad química y microbiológica de los vinos finales [27].

Respecto a la evaluación de la composición fisicoquímica de los vinos elaborados con las distintas proporciones de hollejos, cabe indicar que la acidez total presentó una correlación negativa con la proporción de hollejos, probablemente debido a la liberación de potasio al medio, que conduce a la formación de precipitados de sales tartáricas [28-29]. Sin embargo, un comportamiento contrario fue observado para la acidez volátil, que mostró una tendencia directamente proporcional entre su concentración y la presencia de hollejos, probablemente motivada por una mayor presencia de ésteres, alcoholes y ácidos volátiles [30]. El contenido final en azúcares residuales indicó que en todos los casos la fermentación alcohólica se completó, sin mostrar diferencias significativas en los grados alcohólicos finales obtenidos. Con respecto a las características cromáticas de los vinos elaborados, los resultados obtenidos indican la liberación de materia polifenólica al medio durante el trascurso de la fermentación, siendo mayor cuanto más elevada es la proporción de hollejos en el medio fermentativo.

Por último, el análisis sensorial de los vinos elaborados mostró como la presencia de hollejos en el medio disminuyó la sensación ácida en la fase gustativa, al mismo tiempo, que se experimentó un aumento de las características florales y frutales de los vinos en la fase olfativa. Tras la evaluación global de los vinos, el elaborado en presencia de un 20% de hollejos fue el mejor puntuado por los jueces. De este modo, y con los resultados obtenidos en esta Tesis Doctoral, la elaboración de vinos blancos en presencia de hollejos se presenta como una técnica de elaboración de vinos de carácter viable y que, además, permitiría diversificar la elaboración de vinos blancos en regiones de clima cálido, contribuyendo así a satisfacer las necesidades que presentan los nuevos consumidores de vino.

6.5. Elaboración de vinos blancos a partir de uvas sobremaduradas

Con el fin de comprobar la viabilidad enológica del proceso de sobremaduración de la uva para la elaboración de vinos blancos secos en una región de clima cálido, se llevaron a cabo distintos ensayos en condiciones de secado natural (asoleo) y en cámara climática, para ver sus efectos sobre la composición del vino. En la vinificación con la uva sobremadurada se incluyeron también ensayos de fermentación con hollejos (20%) para ver su efecto. Los resultados obtenidos en esta Tesis Doctoral muestran que el proceso de sobremaduración de la uva es capaz de generar un incremento del contenido en parámetros como la acidez total, azúcares totales o nitrógeno fácilmente asimilable (Tabla 3), observándose un mayor aumento de estos valores en los mostos obtenidos a partir de uva sobremadurada en cámara climática. Este hecho se debe a que la sobremaduración en cámara climática es un proceso continuo de tratamiento y, a diferencia del proceso llevado a cabo de forma natural, no se ve influenciado por las horas de luz naturales. Los aumentos en la concentración de dichos parámetros con respecto al control se deben, principalmente, a la pérdida por evaporación del contenido en agua de vegetación presente en la uva [31].

Tabla 3. Composición fisicoquímica de los mostos tras la sobremaduración de la uva durante las dos añadas de estudio.

	Control	SD 48h	SD 96h	CH 48h	CH 96h
			2018		
pH	3.470 ± 0.014 [a]	3.440 ± 0.014 [b]	3.420 ± 0.028 [c]	3.300 ± 0.014 [d]	3.200 ± 0.014 [e]
A.T (g/L)	3.630 ± 0.117 [a]	3.640 ± 0.175 [a]	3.920 ± 0.058 [b]	4.106 ± 0.058 [c]	4.996 ± 0.058 [d]
NFA (mg/L)	145.600 ± 0.000 [a]	183.400 ± 1.980 [b]	208.600 ± 5.940 [c]	189.000 ± 1.980 [b]	246.400 ± 3.960 [d]
°Bé	11.300 ± 0.140 [a]	12.800 ± 0.140 [b]	13.500 ± 0.140 [c]	12.800 ± 0.000 [b]	15.000 ± 0.140 [d]
			2019		
pH	3.360 ± 0.021 [a]	3.290 ± 0.042 [b]	3.230 ± 0.035 [c]	3.280 ± 0.078 [b]	3.230 ± 0.070 [c]
A.T (g/L)	3.620 ± 0.080 [a]	4.310 ± 0.053 [b]	5.525 ± 0.053 [c]	5.063 ± 0.043 [d]	5.780 ± 0.070 [e]
NFA (mg/L)	162.500 ± 2.256 [a]	200.230 ± 1.978 [b]	224.600 ± 1.450 [c]	207.650 ± 2.465 [b]	265.130 ± 3.472 [d]
°Bé	12.180 ± 0.020 [a]	12.770 ± 0.040 [b]	13.910 ± 0.090 [c]	14.210 ± 0.060 [d,c]	15.680 ± 0.030 [e]

Control: uva sin sobremadurar; SD 48h: sobremaduración natural durante 48 h; SD 96h: sobremaduración natural durante 96 h; CH 48h: sobremaduración en cámara climática durante 48 h; CH 96h: sobremaduración en cámara climática durante 96 h. A.T: acidez total (g/L tde ácido tartárico); NFA: Nitrógeno Fácilmente Asimilable. Letras distintas significan diferencias significativas entre las muestras (ANOVA $p < 0.05$) determinado por un ANOVA bidireccional mediante Bonferroni multiple range (BSD) test.

Al estudiar la cinética del proceso de fermentación alcohólica, se observó que la presencia de un mayor contenido en azúcares en el medio provocó un aumento del tiempo de la fase de latencia en todos los casos. Por otro lado, la presencia de hollejos no ha afectado a las distintas fases del desarrollo de las poblaciones de levaduras, aunque si se observó una incidencia positiva sobre la biomasa viable y sobre las poblaciones máximas alcanzadas. Posiblemente, este hecho sea debido, como se ha comentado anteriormente, a una mayor presencia de compuestos nitrogenados y cofactores de interés para el desarrollo de las levaduras, y que se encuentran presentes de manera especial en los hollejos de la uva [24,26]. Esto derivó en una mayor velocidad de consumo de los azúcares, disminuyendo los tiempos de fermentación.

Independiente de la técnica de sobremaduración empleada (asoleo o cámara climática), su tiempo y/o la presencia o ausencia de hollejos en el medio fermentativo, no se observaron problemas en la fase final de la fermentación alcohólica. Respecto al consumo de los compuestos nitrogenados presentes en el medio, en todos los casos se observó una coincidencia en el tiempo entre la mínima concentración en el medio con el momento en el que se alcanzaron los mayores niveles de biomasa viable. Por otro lado, se observó que los descensos hacia las concentraciones mínimas en NFA fueron menos pronunciados en los casos con presencia de hollejos durante la fermentación alcohólica. Posiblemente, la presencia de los hollejos ejerció un aporte adicional y progresivo de compuestos nitrogenados al medio durante el proceso de fermentación alcohólica.

En cuanto a la composición fisicoquímica de los vinos finales (Tabla 4), los valores de acidez total y volátil fluctuaron en función de la técnica de sobremaduración

empleada y el tiempo, obteniéndose los mayores valores en los vinos elaborados con uva sobremadurada durante 96 horas en cámara climática. La presencia de hollejos en el medio fermentativo afectó del mismo modo que se ha visto en el apartado anterior, es decir, generando una disminución de los valores de acidez total y aumentando los de la acidez volátil. En cuanto al grado alcohólico de los vinos elaborados, a mayor concentración del contenido en azúcares presentes en el mosto, se obtuvo un mayor grado alcohólico en los vinos finales, no observándose ningún efecto debido a la presencia o ausencia de hollejos en el medio fermentativo. Por último, en relación al estudio de las características cromáticas, los vinos elaborados a partir de uvas sobremaduras han presentado una mayor intensidad de color desplazándose hacia el espacio cromático correspondiente al amarillo, siendo superiores estos desplazamientos en los vinos elaborados a partir de uvas sobremaduradas en cámara climática. No obstante, la presencia de hollejos en el medio fermentativo ha podido ejercer un efecto protector del vino frente a oxidaciones al igual que se observó en el apartado anterior.

Tabla 4. Composición fisicoquímica de los vinos obtenidos a partir de uvas sobremaduras durante las dos añadas de estudio.

	2018				
	Control	**SD48h**	**SD96h**	**CH48h**	**CH96h**
	Sin Hollejos				
A.T (g/L)	4.629 ± 0.027 ^{a}	4.763 ± 0.067 ^{a}	4.905 ± 0.080 ^{a}	5.102 ± 0.241 ^{b}	5.554 ± 0.013 ^{b}
A.V (g/L)	0.162 ± 0.012 ^{a}	0.184 ± 0.031 ^{a}	0.400 ± 0.024 ^{b}	0.231 ± 0.012 ^{a}	0.366 ± 0.021 ^{b}
% Alc.	11.854 ± 0.182 ^{a}	13.430 ± 0.060 ^{a}	14.633 ± 0.159 ^{a,b}	13.656 ± 0.398 ^{a}	16.584 ± 0.016 ^{c}
A.R (g/L)	1.418 ± 0.285 ^{a}	1.922 ± 0.330 ^{b}	2.220 ± 0.509 ^{b}	1.733 ± 0.107 ^{a,b}	2.998 ± 0.264 ^{c}
IPT	7.990 ± 0.141 ^{a}	6.540 ± 0.170 ^{b}	6.090 ± 0.269 ^{b}	6.450 ± 0.891 ^{b}	8.160 ± 0.085 ^{a}
Abs 420	0.074 ± 0.015 ^{a}	0.093 ± 0.008 ^{a}	0.093 ± 0.001 ^{a}	0.109 ± 0.008 ^{b}	0.110 ± 0.002 ^{b}
	Con 20 % hollejos				
A.T (g/L)	4.413 ± 0.102 ^{a}	4.569 ± 0.140 ^{a,b}	4.769 ± 0.097 ^{b}	4.958 ± 0.305 ^{b,c}	5.068 ± 0.198 ^{c}
A.V (g/L)	0.361 ± 0.068 ^{a}	0.412 ± 0.006 ^{a,c}	0.580 ± 0.100 ^{b}	0.428 ± 0.168 ^{c,d}	0.551 ± 0.136 ^{b,d}
% Alc.	11.970 ± 0.256 ^{a}	13.569 ± 0.147 ^{a,b}	14.896 ± 0.253 ^{a,b}	13.852 ± 0.539 ^{a,b}	16.489 ± 0.187 ^{c}
A.R (g/L)	1.257 ± 0.149 ^{a}	1.567 ± 0.698 ^{a}	2.541 ± 0.410 ^{b}	1.710 ± 0.205 ^{a}	3.056 ± 0.423 ^{b}
IPT	8.690 ± 0.157 ^{a}	7.214 ± 0.099 ^{a}	7.724 ± 0.301 ^{a}	7.158 ± 0.249 ^{a}	8.879 ± 3.265 ^{a}
Abs 420	0.158 ± 0.008 ^{a}	0.087 ± 0.005 ^{b}	0.048 ± 0.001 ^{c}	0.087 ± 0.003 ^{b}	0.099 ± 0.001 ^{b}
	2019				
	Control	**SD 48h**	**SD 96h**	**CH 48h**	**CH 96h**
	Sin hollejos				
A.T (g/L)	5.570 ± 0.098 ^{a}	5.810 ± 0.104 ^{a}	6.480 ± 0.057 ^{b}	6.320 ± 0.421 ^{b}	6.460 ± 0.268 ^{b}
A.V (g/L)	0.189 ± 0.030 ^{a}	0.214 ± 0.012 ^{a}	0.256 ± 0.036 ^{b}	0.296 ± 0.016 ^{b}	0.489 ± 0.080 ^{c}
% Alc.	10.756 ± 0.430 ^{a}	12.380 ± 0.320 ^{a,d}	14.299 ± 0.190 ^{b}	13.420 ± 0.598 ^{b,d}	16.240 ± 0.480 ^{c}
A.R (g/L)	1.356 ± 0.018 ^{a}	1.976 ± 0.143 ^{b}	1.447 ± 0.169 ^{a,b}	1.238 ± 0.188 ^{a}	4.813 ± 0.268 ^{c}
IPT	6.513 ± 0.091 ^{a}	4.976 ± 0.100 ^{b}	3.790 ± 0.082 ^{c}	5.713 ± 0.712 ^{b}	10.268 ± 0.55 ^{d}
Abs 420	0.040 ± 0.010 ^{a}	0.051 ± 0.010 ^{a,d}	0.062 ± 0.001 ^{b,d}	0.073 ± 0.010 ^{b}	0.110 ± 0.01 ^{c}
	Con 20 % hollejos				
A.T (g/L)	5.170 ± 0.070 ^{a}	5.460 ± 0.070 ^{b}	6.118 ± 0.050 ^{c}	6.025 ± 0.560 ^{c}	6.165 ± 0.150 ^{c}
A.V (g/L)	0.230 ± 0.030 ^{a}	0.240 ± 0.010 ^{a,b}	0.280 ± 0.010 ^{b}	0.340 ± 0.010 ^{c}	0.620 ± 0.030 ^{d}
% Alc.	10.860 ± 0.910 ^{a}	12.430 ± 0.440 ^{b}	14.390 ± 0.260 ^{c}	13.390 ± 1.470 ^{b,c}	16.480 ± 0.360 ^{d}
A.R (g/L)	1.200 ± 0.050 ^{a}	1.450 ± 0.090 ^{a,b}	1.640 ± 0.001 ^{a,b}	1.770 ± 0.140 ^{b}	4.510 ± 0.080 ^{c}
IPT	4.320 ± 0.160 ^{a}	5.620 ± 0.100 ^{b}	7.260 ± 0.070 ^{c}	6.350 ± 0.880 ^{b,c}	10.974 ± 0.550 ^{d}
Abs 420	0.140 ± 0.010 ^{a}	0.058 ± 0.010 ^{b}	0.063 ± 0.001 ^{b}	0.071 ± 0.010 ^{b}	0.780 ± 0.010 ^{b}

Control: uva sin sobremadurar; SD 48h: sobremaduración natural durante 48 h; SD 96h: sobremaduración natural durante 96 h; CH 48h: sobremaduración en cámara climática durante 48 h; CH 96h: sobremaduración en cámara climática durante 96 h. A.T: acidez total (g/L tde ácido tartárico); NFA: Nitrógeno Fácilmente Asimilable. Letras distintas significan diferencias significativas entre las muestras (ANOVA $p < 0.05$) determinado por un ANOVA bidireccional mediante Bonferroni multiple range (BSD) test.

En vista a los resultados obtenidos, se puede considerar que el asoleo y la fermentación en presencia de hollejos pueden ser una herramienta tecnológica viable para la elaboración de vinos blancos. A su vez, la recuperación de una técnica ancestral

como el asoleo permitiría aprovechar las condiciones impuestas por el cambio climático, dar encuentro con las tendencias de los actuales consumidores de vino y diversificar la producción en una zona de clima cálido como el Marco de Jerez.

6.6. Caracterización volátil y sensorial de los vinos elaborados a partir de uvas sobremaduras

Por otro lado, los resultados del análisis de los compuestos volátiles de los distintos vinos elaborados durante las dos añadas de estudio, indican como la técnica de la sobremaduración modifica significativamente el perfil de todos los compuestos volátiles de los vinos con respecto al control, a excepción de los alcoholes mayoritarios y los fenoles. Cabe resaltar que el efecto producido por el tiempo de sobremaduración de las uvas, influyó positivamente en el contenido de algunos compuestos volátiles y familias de compuestos como el metanol, ésteres, aldehídos y tioles. Respecto a la presencia de hollejos, se ha podido constatar como la presencia de éstos en el medio fermentativo incrementó los niveles de metanol, alcoholes mayoritarios, alcoholes C6, terpenos y lactonas.

Por último, el análisis sensorial realizado reveló que todos los vinos elaborados con uva sobremadura presentaron con respecto al control, un perfil sensorial donde destacaron principalmente las notas afrutadas y florales en la fase olfativa, así como la acidez y el amargor en la fase gustativa. Para todos los casos, los vinos elaborados a partir de uvas sobremaduradas mostraron una mayor intensidad olfativa, en comparación con el vino control, posiblemente debido al efecto de concentración provocado por la evaporación del agua de las bayas [32]. Comparando los resultados entre los vinos elaborados en presencia o no de hollejos, se observó como las notas afrutadas y florales presentan una mayor intensidad en el primer grupo de vinos. Este último hecho concuerda con los resultados obtenidos tras la determinación de las distintas familias de compuestos volátiles, donde se observó un aumento en la concentración de terpenos para aquellos vinos elaborados en presencia de hollejos.

De forma adicional, para conocer el grado de preferencia de estos vinos por los jueces, se realizó un test de ordenación como ensayo complementario al análisis sensorial descriptivo. El resultado mostrado por este test reveló como el vino elaborado con uvas sobremaduradas de forma natural durante 96 horas fué el preferido de forma significativa; y paralelamente, como los vinos elaborados en presencia de hollejos han sido preferidos durante las dos añadas de estudio. En este sentido, se podría indicar que la sobremaduración de las uvas de forma natural, mediante asoleo, podría ser considerada como una estrategia de resiliencia y adaptación a las condiciones de aumento de temperaturas durante la época de maduración de la uva.

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7. Conclusiones

Las principales conclusiones que se pueden extraer de la presente Tesis Doctoral, se han clasificado según las publicaciones y los capítulos correspondientes.

Con respecto a las publicaciones (Capítulo 5.1):

- Genetical, Morphological and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (*Vitis vinifera* L.). *Agronomy* **2019**, *9*, 563, doi:10.3390/agronomy9090563.
- Identification and Characterization of White Grape Varieties Autochthonous of a Warm Climate Region (Andalusia, Spain). *Agronomy* **2020**, *10*, 205, doi:10.3390/agronomy10020205.
- Preliminary Study of Somatic Variants of Palomino Fino (*Vitis vinifera* L.) Grown in a Warm Climate Region (Andalusia, Spain). *Agronomy* **2020**, *10*, 654, doi:10.3390/agronomy10050654.

El análisis molecular de cinco accesiones de variedades autóctonas de una zona de clima cálido, ha permitido su identificación con 4 genotipos diferentes mediante marcadores tipo Simple Sequence Repeats (SSR).

Tras comparar con diferentes bases de datos, se ha confirmado la sinonimia de Uva Rey y Mantúo de Pilas, publicándose por primera vez su genotipo con 22 *loci* de microsatélites. Además, se ha identificado una nueva sinonimia entre Castellano y Manteaudo.

La descripción morfológica de las diferentes variedades autóctonas y variantes somáticas de la variedad Palomino Fino, pone de manifiesto que todas las variedades muestran una mayor densidad de pelos erguidos en el envés de las hojas adultas respecto a Palomino Fino, característica que puede ser considerada de interés por su capacidad de modular los procesos de evapotranspiración. Por lo tanto, estas accesiones pueden presentar una mejor adaptación a una zona de clima cálido y, su cultivo se podría considerar como una estrategia de adaptación al cambio climático.

El análisis fisicoquímico de los mostos de las distintas variedades autóctonas, así como del estudio de la evolución de su composición durante la maduración en las variantes somáticas (Palomino de Jerez, Palomino Gacho y Palomino Pelusón) ha mostrado diferencias notables con respecto a la variedad Palomino Fino. El estudio de la composición de los mostos indica que Uva Rey o Mantúo de Pilas, Cañocazo y las tres variantes somáticas estudiadas presentan un ciclo fenológico más largo que Palomino Fino, lo que deriva en una maduración mucho más tardía y mejor adaptada para la producción de vinos blancos en una zona de clima cálido. Estas variedades deberían de ser incluidas en los listados de variedades autorizadas según las normativas que regulan su cultivo, contribuyendose al aumento la diversidad varietal y enológica de las regiones vitivinícolas de clima cálido.

Con respecto a las publicaciones:

- Influence of the Presence of Grape Skins during White Wine Alcoholic Fermentation. *Agronomy* **2021**, *11*, 452, doi:10.3390/agronomy11030452. (Capítulo 5.2.)
- Effect of Grape Over-Ripening and Its Skin Presence on White Wine Alcoholic Fermentation in a Warm Climate Zone. *Foods* **2021**, *10*, 1583, doi:10.3390/foods10071583. (Capítulo 5.3.)
- Volatile Composition and Sensory Characterisation of Dry White Wines Made with Overripe Grapes by Means of two Different Techniques. *Foods* **2022**, *11*, 509, doi:10.3390/foods11040509. (Capítulo 5.4.)

La presencia de hollejos durante la fermentación alcohólica produce un aumento significativo de la biomasa viable de levaduras y una mejora en la cinética fermentativa, no teniendo efecto ni correlacion alguna con los contenidos en nitrógeno fácilmente asimilable.

Los vinos resultantes no han mostrado grandes diferencias en su composición fisicoquímica con respecto al control. En todos los casos, el vino elaborado en presencia de un 20% de hollejos ha sido el mejor evaluado sensorialmente por el panel de cata.

En lo referente al proceso de sobremaduración, independientemente de las técnicas empleada, se ha observado un aumento en el contenido en azúcares, ácidos orgánicos y nitrógeno fácilmente asimilable en los mostos, que ha derivado en un retraso en el inicio de la fermentación alcohólica por parte de las levaduras.

Los vinos finales elaborados a partir de uvas sobremaduradas variaron significativamente en su composición fisicoquímica en función del tiempo y la técnica de sobremaduración empleada, así como de la presencia o ausencia de hollejos durante la fermentación.

Desde el punto de vista sensorial, la sobremaduración de la uva implica cambios en las concentraciones de los compuestos volátiles, tanto mayoritarios como minoritarios. Asimismo, la presencia de hollejos en el medio fermentativo origina un aumento en la producción de ésteres, lo que se traduce en una mayor percepción de notas florales y afrutadas en los vinos.

Los resultados del test de preferencia mostraron que el vino elaborado con uvas sobremaduradas durante 96 horas de forma natural en las dos añadas fueron los mejor valorados por el panel de cata.

Por tanto, la elaboración de vinos blancos a partir de uvas sobremaduradas y en presencia o no de hollejos, pueden considerarse como una herramienta de adaptación al cambio climático y de diversificación de la producción de vinos blancos de calidad en una zona de clima cálido. No obstante, se considera necesario continuar con los estudios durante más años con la intención de comprobar la reproducibilidad de las técnicas

empleadas, así como explorar estos mismos procedimientos con las variedades autóctonas y variantes somáticas identificadas.

8. Anexos

Informe con el factor de impacto y cuartil del *Journal Citation Reports* (SCIE)

Anexo 1

Sancho-Galán, P.; Amores-Arrocha, A.; Palacios, V.; Jiménez-Cantizano, A. Genetical, Morphological and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (Vitis vinifera L.). *Agronomy* **2019**, *9*, 563, doi:10.3390/agronomy9090563.

- Nombre de la revista: Agronomy
- Editorial: Multidisciplinary Digital Publishing Institute (MDPI).
- eISSN: 2073-4395
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    - Posición 18 de 91 (**Q1**) en la categoría "**AGRONOMY**"
    - Posición 65 de 234 (**Q2**) en la categoría "**PLANT SCIENCES**"

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Modelo de consentimiento de coautores para inclusión de trabajos en la modalidad de tesis por compendio de publicaciones.

Los investigadores/as:

Dr. Antonio Amores Arrocha, Dr. Víctor Manuel Palacios Macías y Dra. Ana Concepción Jiménez Cantizano como coautores/as del artículo:

- Genetical, Morphological and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (*Vitis vinifera* L.). *Agronomy* **2019**, *9*, 563, doi:10.3390/agronomy9090563.

De acuerdo con lo establecido en el **Artículo 23.4 del Reglamento UCA/CG06/2012, de 27 de junio de 2012**, por el que se regula la ordenación de los estudios de doctorado en la Universidad de Cádiz (BOUCA nº 208).

Manifiestan su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Pau Sancho Galán, titulada "Estrategias vitivinícolas de adaptación al cambio climático en una zona de clima cálido" y **expresan su renuncia** a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

Además, declaran que D. Pau Sancho Galán ha realizado las siguientes aportaciones a los mencionados artículos:

- Los estudios experimentales incluidos en el artículo, así como la contribución a la recopilación de la bibliografía y su análisis.
- La colaboración de manera intensiva en la interpretación y análisis de los datos extraídos de la investigación, así como también en la escritura de dicha publicación y en la preparación de figuras y tablas.

En Puerto Real, a 11 de febrero de 2022.

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Genetical, Morphological and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (*Vitis vinifera* L.)

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Abstract: 'Uva Rey' is considered an Andalusian (Spain) ancient autochthonous cultivar with hard white grapes used for the production of wine and raisins and also for raw consumption. Currently, this cultivar is not included in the official register of Spanish grapevine varieties and there is neither a description nor a characterization that could facilitate its insertion in this register. In order to study this genetic resource, a genetic and morphological characterization of 'Uva Rey' has been carried out in comparison with 'Palomino Fino', the main cultivar in Andalusia (Spain). Additionally, grape must physicochemical characterization and grape berry texture profile analyses were performed. Genetically, 'Uva Rey' was synonymous with the cultivar 'De Rey'. 'Uva Rey' grape must physicochemical results showed a lower sugar concentration and a higher malic acid content compared to 'Palomino Fino' must, while the analysis of the grape berry texture profile proved to be more consistent and cohesive. These results can be attributed to the longer phenological cycle presented by 'Uva Rey'. All these facts could lead to consideration of 'Uva Rey' as a cultivar for the production of white wines in warm climate regions.

Keywords: *Vitis vinifera*; autochthonous cultivar; 'Uva Rey'

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most ancient and important fruit crops worldwide [1]. Around 12,500 cultivars have been registered in the *Vitis* International Variety Catalogue [2]. However, based on their DNA profiles, the number of grapevine varieties is estimated at around 5000, many of them closely related [3,4].

Nowadays, 7.4 mHa of the Earth area is covered by grapevines, with Spain being the first country in terms of cultivated land extension. Spanish vineyards cover thousands of hectares and produce approximately 44.4 mHL of wine per year [5]. For that reason, viticulture could be considered as one of the most important socioeconomic sectors in the Spanish agro-industrial network. Grapevine cultivation throughout the country, and the significance over time, have led to a grapevine heritage of great magnitude. Spain's varietal heritage had continuously increased from its origin until the arrival of diseases and pathogens from America (mildews and *Phylloxera*) [6]. According to García de los Salmones [7], the first *Phylloxera* outbreak in Spain was detected in Malaga (Andalusia) in 1876. From that moment on, this pathogen spread throughout the whole country and destroyed more than 1,000,000 ha, which caused serious damages to the Spanish native germplasm [8]. In order to preserve the maximum number of *Vitis vinifera* genetic diversity, a number of germplasm banks were created. 'El Encín', the most important germplasm bank in Spain, was established in 1914 in Alcalá de Henares

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Anexo 2

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- La colaboración de manera intensiva en la interpretación y análisis de los datos extraídos de la investigación, así como también en la escritura de dicha publicación y en la preparación de figuras y tablas.

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Identification and Characterization of White Grape Varieties Autochthonous of a Warm Climate Region (Andalusia, Spain)

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Abstract: The high sensitivity of one of the most important crops in the world, such as vine (*Vitis vinifera* L.), to particular changes caused by the phenomena associated with global warming, is encouraging the wine industry to place value on grape varieties that are autochthonous to each production area. These are generally conserved in germplasm banks and may pose a useful tool to counteract the effects of climate change. In order to determine the actual resource that such varieties constitute, this research has carried out a genetic identification, a morphological characterization, and an analysis of the grape musts obtained from four autochthonous varieties (Cañocazo, Castellano, Mantúo de Pilas, and Palomino Fino). This genetic analysis has allowed the identification of autochthonous varieties with different genotypes. However, all of them had similar phenotypic characteristics in terms of high hair density in adult leaves. With respect to the physicochemical composition of the musts, significant differences have been observed between the autochthonous varieties, with respect to the control variety of Palomino Fino. Nevertheless, all of them have exhibited an adequate physicochemical composition to produce quality white wines. For all of the above reasons, these local varieties should be considered suitable for cultivation in areas with warmer and drier climates, such as Andalusia (Spain).

Keywords: *Vitis vinifera*; autochthonous variety; simple sequence repeat analysis; warm climate

1. Introduction

The so-called area known as Marco de Jerez, located in the south of the Iberian Peninsula, is one of the most important wine-growing regions in Spain, which reached its fullness and international recognition during the 19th century [1]. However, the wines produced in this area have evolved throughout history because of different biological and political circumstances. From a viticultural point of view, the invasion of phylloxera in the area in 1894 caused the loss of a large part of the Jerez vineyards, which had to be replanted [2]. This led to a significant loss of vine varieties. Clemente and Rubio [3], at the beginning of the 19th century, described 43 vine varieties that were cultivated in the Marco de Jerez vineyards before the phylloxera outbreak. After the replanting of the vineyards to deal with the plague, the number of varieties cultivated dropped significantly. Fernández de Bobadilla [4] quotes among other replanted vines: Palomino Fino, Pedro Ximénez, Cañocazo, and Albillo as classic varieties, Garrido, Perruno, Mantúo, and Beba as secondary varieties, and Moscatel and Tintilla de Rota as special varieties. Subsequently, severe regulations were approved and the vine varieties that were authorized for wine production were restricted [5]. Likewise, in the second half

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Anexo 3

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Preliminary Study of Somatic Variants of Palomino Fino (*Vitis vinifera* L.) Grown in a Warm Climate Region (Andalusia, Spain)

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Abstract: Vegetative propagation of *Vitis vinifera* cultivars over hundreds of years has led to the accumulation of a large number of somatic variants of the same grapevine variety. These variants are now considered a working tool to cope with changing environmental conditions as a result of, among others, global warming. In this work, three somatic variants of the major grapevine variety of the South West (SW) of Andalusia (Spain), Palomino Fino, have been genetically and morphologically characterized, as well as their grape musts from two different vintages. The genetic analysis at 22 microsatellite loci confirmed the identity of the three somatic variants that presented the same genotype as Palomino Fino, while the morphological study showed differences between the three somatic variants and Palomino Fino, highlighting the somatic variant Palomino Pelusón. Regarding the physicochemical analysis of its musts, differences were also observed between the somatic variants and Palomino Fino. As a result of all of the above, the use of grapes from somatic variants can be a viable and natural alternative for the production of quality wines in warm climate areas. On the other hand, promoting the cultivation of the somatic variants could contribute to preventing the loss of Palomino Fino intraspecific variability.

Keywords: *Vitis vinifera*; Palomino Fino; somatic variants; simple sequence repeat analysis; warm climate

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest and most widely cultivated fruit crops in the world [1], used mainly for wine and spirit making [2]. This species presents a wide genetic and phenotypic diversity mainly due to the history of vine cultivation [3] and vegetative propagation, which has allowed the conservation of different cultivars for centuries [4]. Grapevine was one of the first fruit species domesticated, and its vegetative propagation has been practiced since ancient times [5]. During many cycles of vegetative propagation, mutations have appeared spontaneously, some of them leading to phenotypic differences giving rise to different somatic variants or clones [6].

The somatic variations have led to grapevine adaptation and to its evolution under changing environmental and cultivation conditions, this being a source of novel traits [7]. This variation became the base of grapevine clonal selection, starting in Germany in the nineteenth century and continuing in some other European countries such as France, Italy and Spain in the second half of the twentieth century [8]. Initially, the basic aim of clonal selection was to get healthy and highly productive plants [8]. However, the aim of obtaining highly productive plants alongside the trend to cultivate only certain varieties has contributed to the disappearance of many local cultivars [9]. Recently, this trend has

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Anexo 4

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Influence of the Presence of Grape Skins during White Wine Alcoholic Fermentation

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Abstract: The production of white wines with the presence of grape skins is a historical technique used in different regions with winemaking tradition. However, the current trend is to maintain the presence of grape skins during white wine making only during the pre-fermentation phase in order to enrich and give greater complexity to the sensory profile of the wines. Given these precedents, this study is the first to consider the effect of the presence of different grape skins doses throughout the alcoholic fermentation process. To this end, the effect of 5 different doses of grape skins (20, 40, 60, 80 and 100%) has been studied with respect to a control (0%) during alcoholic fermentation, the physicochemical composition of the final wines and a preliminary sensory analysis. The presence of grape skins has led to an increase in viable biomass and speed of fermentation with respect to the control. However, no differences have been observed in terms of the consumption of nitrogenous sources by yeasts. The wines produced have not shown great differences in their physicochemical composition, except for the volatile acidity. In addition, the preliminary sensory analysis showed differences between the different grape skins doses studied, where the wine produced with 20% grape skins has been the best evaluated by the tasting panel. In this sense, the production of wines with a 20% grape skins presence during the entire alcoholic fermentation is presented as a viable technique that would allow the diversification of the production of white wines and meet the trends and expectations of current wine consumers.

Keywords: white wine; grape skins; alcoholic fermentation; Palomino Fino

Citation: Sancho-Galán, P.; Amores-Arrocha, A.; Jiménez-Cantizano, A.; Palacios, V. Influence of the Presence of Grape Skins during White Wine Alcoholic Fermentation. *Agronomy* **2021**, *11*, 452. https://doi.org/10.3390/agronomy11030452

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1. Introduction

Vine (*Vitis vinifera* L.) is one of the most widely cultivated species worldwide [1]. Currently, 7.4 million hectares of land are covered by this crop, producing 27.3 million of grape tonnes. Spain represents 13% of this area, being the country with the largest area of vineyards [2]. Of this amount of grapes, those destined for wine production produce a quantity of 292 million hL, of which Spain represents 15.2%. However, at national level, white wine production represents only 26.51% [3].

In general, the protocols for making white wines are based on fermenting the grape must in the absence of the solid parts [4]. However, for some winemaking processes, oenological practices that encourage the extraction of different compounds that will influence the chemical composition of the wine and its sensory properties, such as terpenes and aromatic precursors (amino acids, fatty acids, etc.) from the skins to the must are introduced to the must [5]. In this sense, the aromatic complexity of the white wine depends on factors such as the grapevine variety used in the production (primary and varietal aromas), the aromas produced during fermentation (secondary) [6] and the evolution of these aromas during ageing (tertiary) [7–9].

Some white wines are currently made by allowing contact of grape skins (GS) with the grape must before fermentation in order to increase the extraction of compounds that influence the intensity of varietal aromas [10]. Several studies have demonstrated

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Informe con el factor de impacto y cuartil del *Journal Citation Reports* (SCIE)

Anexo 5

Sancho-Galán, P.; Amores-Arrocha, A.; Palacios, V.; Jiménez-Cantizano, A. Effect of Grape Over-Ripening and Its Skin Presence on White Wine Alcoholic Fermentation in a Warm Climate Zone. *Foods* **2021**, *10*, 1583, doi:10.3390/foods10071583

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Modelo de consentimiento de coautores para inclusión de trabajos en la modalidad de tesis por compendio de publicaciones.

Los investigadores/as:
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Manifiestan su conformidad para la presentación de la citada publicación como parte de la tesis doctoral de D. Pau Sancho Galán, titulada "Estrategias vitivinícolas de adaptación al cambio climático en una zona de clima cálido" y **expresan su renuncia** a presentar la citada publicación como parte de otra tesis doctoral en cualquier otra universidad.

Además, declaran que D. Pau Sancho Galán ha realizado las siguientes aportaciones a los mencionados artículos:

- Los estudios experimentales incluidos en el artículo, así como la contribución a la recopilación de la bibliografía y su análisis.
- La colaboración de manera intensiva en la interpretación y análisis de los datos extraídos de la investigación, así como también en la escritura de dicha publicación y en la preparación de figuras y tablas.

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Effect of Grape Over-Ripening and Its Skin Presence on White Wine Alcoholic Fermentation in a Warm Climate Zone

Pau Sancho-Galán, Antonio Amores-Arrocha *, Víctor Palacios and Ana Jiménez-Cantizano

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Abstract: The current trend of rising temperatures and sun irradiation associated to climate change is pushing traditional grape-producing areas with a warm climate towards a very accelerated ripening, leading to earlier harvesting dates and grape must with an unbalanced composition. However, this climatic trend could be exploited to produce other types of wine. In this sense, the increase in temperature could be used to produce wines with overripe grapes. In this regard, the aim of this research work is to evaluate the influence of different degrees and techniques of grape over-ripening to produce wines with the presence or absence of its skins during alcoholic fermentation. To this end, a physicochemical characterization of grape musts and wines obtained from overripe grapes and the monitoring of their fermentation has been performed. Over-ripening grapes by sun-drying has been established as a viable technique viability, producing musts and wines with unique physicochemical and sensory characteristics. In view of the above, it is considered that the production of wines from overripe grapes and in the presence or absence of grape skins is a viable approach to make new white wines taking advantage of the conditions imposed by climate change in a warm climate zone and meet the trends and expectations of current wine consumers.

Keywords: over-ripening; alcoholic fermentation; white wine; warm climate; yeast; viticulture; climate change

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1. Introduction

Grapevines are one of the most important crops worldwide, with vineyard area of 7.4 million hectares and a production of 77.8 million tons of grapes [1]. According to the Food and Agriculture Organization (FAO), in 2018 [2], approximately 37% of world grape production was used for wine production in 71 countries, the 50.7% being produced in three European countries (Italy, France and Spain). These data evidence that the wine industry contributes to the economy and reputation of many countries all over the world. Nowadays, grapevine (*Vitis vinifera* L.) cultivation is primarily located in the Mediterranean basin [3] and other temperate climate regions between the latitudes of 30° and 50° in the northern hemisphere and 40° and 50° in the southern hemisphere [4], although grapevines have been grown outside these limits, in the tropics, for a long time [5]. In overall terms, climate change is gradually modifying the established cultivation limits. More specifically, it is causing a generalized advance of the grape harvest by 10–24 days over the last 30–50 years [6–8] and an accelerated vine growth and over-ripening of the grapes, leading to the production of musts with high potential alcoholic strength [9,10], higher pH [11,12], lower acidity [13] and significant nutritional deficiencies, generally resulting in low levels of free amino nitrogen (FAN) [13–15]. The effects associated with climate change on grape quality pose important challenges for the winemaking process and the production of quality wines—more particularly, all the factors associated with the expression of varietal aromas, chemical and microbiological stability and sensory balance [7]. Therefore, quality wine production could be affected in those areas that have a warm climate.

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Anexo 5

Sancho-Galán, P.; Amores-Arrocha, A.; Palacios, V.; Jiménez-Cantizano, A. Volatile Composition and Sensory Characterisation of Dry White Wines Made with Overripe Grapes by Means of two Different Techniques. *Foods* **2022**, *11*, 509, doi:10.3390/foods11040509

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Volatile Composition and Sensory Characterisation of Dry White Wines Made with Overripe Grapes by Means of Two Different Techniques

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Abstract: Grape over-ripening is a technique that has historically been used for the production of white wines in southern Spain. However, this technique is still widely used for the production of sweet wines. In this study and after recently proving the feasibility of making dry white wines from overripe grapes with and without the presence of grape skin in a warm climate zone, the sensory characterisation and analysis of the major and minor volatile compounds in dry white wines made from overripe grapes are presented for the first time. Two over-ripening techniques (sun-drying and climatic chamber drying) were studied for two different periods of time (48 and 96 h), as has the presence of grape skins during alcoholic fermentation. Grape over-ripening implies modifications in the composition of both the major and minor volatile compounds in wines. In terms of sensory analysis, wines with a similar profile were obtained year-on-year. The results of the preference test show that the wines made from grapes that had been over-ripened in the sun for 96 h were preferred by the tasting panel for both vintages. Thus, grape over-ripening under the sun could be considered as a resilience and adaptation technique for increased temperature conditions during the ripening season caused by the effects of climate change.

Keywords: grape over-ripening; alcoholic fermentation; warm climate; climate change; wine aroma; volatile compounds; gas chromatography

Citation: Sancho-Galán, P.; Amores-Arrocha, A.; Palacios, V.; Jiménez-Cantizano, A. Volatile Composition and Sensory Characterisation of Dry White Wines Made with Overripe Grapes by Means of Two Different Techniques. *Foods* **2022**, *11*, 509. https://doi.org/10.3390/foods11040509

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1. Introduction

Viticulture is a key socioeconomic and cultural sector in many countries and regions worldwide, with a high economic impact in the network of all relevant industry branches of the supply and distribution chains [1]. Geographically, grapevines are historically cultivated on six out of seven continents, between latitudes 4 and 51 in the northern hemisphere and between latitudes 6 and 45 in the southern hemisphere and across a large diversity of climates (oceanic, temperate, continental, Mediterranean, etc.), with the majority occurring in temperate climate regions [2]. However, climate change is exerting an increasingly profound influence on vine phenology and grape composition, and ultimately affects winemaking, wine microbiology, and chemistry and sensory aspects [3]. Observed changes in 27 premium viticultural regions across the globe have shown an increase in the average growing season temperature of 1.3 °C from 1950 to 2000, while in Europe, an increase of 1.7 °C was observed from 1950 to 2004 [4–6]. According to HadCM3 model average, the predicted temperatures for high-quality wine producing regions will increase by 2.04 °C within the period from 2000 to 2049 [5]. Understanding the changing suitability of regions for viticulture under climate change will help to us develop adaptation strategies in traditional winegrowing regions [7]. In order to maintain profitability

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