UNIVERSIDAD DE LA REPÚBLICA
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EVALUACIÓN DE ALTERNATIVAS TECNOLÓGICAS SUSTENTABLES PARA LA ELABORACIÓN DE VINOS TINTOS DIFERENCIADOS

por

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…El mundo es eso -reveló- un montón de gente, un mar de fueguitos…
extracto de El Mundo

Eduardo Galeano (El libro de los abrazos)

A mi mamá, a Alfonsina y a Olga,
al fuego que enciende, al que alumbra y al que abriga…

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…Tothom intenta realitzar alguna cosa gran, sense adornar-se que la vida es composa de coses petites… / …Todo el mundo intenta realizar algo grande, sin darse cuenta de que la vida se compone de cosas pequeñas...

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RESUMEN

La variabilidad climática durante el ciclo de la vid y sobre todo durante la maduración afecta el potencial enológico de la uva y la calidad del vino. El incremento de la variación climática interanual debido al cambio climático hace que sea imprescindible la adopción de medidas de gestión de la vinificación para elaborar vinos de calidad. La producción de vinos tintos con cuerpo y de color intenso requiere cosechar las uvas en un estado avanzado de madurez y una intensa extracción de compuestos fenólicos durante la vinificación. Las uvas con madurez avanzada presentan altas concentraciones de azúcares y elevado pH originando vinos alcohólicos y con problemas de estabilidad. Este proyecto doctoral se enfocó en estudiar alternativas de vinificación que permitan regular el contenido de alcohol y el pH de los vinos sin afectar su concentración fenólica, como herramienta para mitigar el efecto de la variabilidad climática interanual. En particular, se evaluó la sustitución de mosto de uva madura por mosto de uvas inmaduras y la maceración pre-fermentativa en caliente en los cultivares Pinot noir y Tannat en las vendimias 2016, 2017 y 2018. Adicionalmente se realizó otro ensayo donde se evaluó el agregado y la sustitución con agua o mosto de uva blanca como alternativas para la reducción del contenido de alcohol de los vinos. Se determinó el potencial enológico de las uvas cosechadas y la composición general, color, composición fenólica y en polisacáridos de los vinos. También se realizaron evaluaciones sensoriales de los vinos. A través de las técnicas de vinificación propuestas se logró elaborar vinos con menor contenido de alcohol y pH sin afectar negativamente su color ni su concentración fenólica y en polisacáridos de los vinos. El efecto de estas técnicas dependió fuertemente del cultivar y del potencial enológico de la uva en cada vendimia. Los resultados obtenidos sugieren que estas técnicas de vinificación pueden minimizar los efectos de la variabilidad climática interanual sobre la composición de los vinos siendo herramientas que contribuyen a mejorar la sustentabilidad del sector vitivinícola nacional.

Palabras clave: vino de bajo grado alcohólico, sustitución de mostos, termovinificación, Tannat, Pinot noir

EVALUATION OF SUSTAINABLE TECHNOLOGICAL ALTERNATIVES TO PRODUCE DIFFERENTIATED RED WINES

SUMMARY

The climatic variability during the grapevine cycle and especially during the ripening affects the oenological potential of the grape and the quality of the wine. The increase in interannual climate variation due to climate change makes it essential to adopt winemaking management measures to produce quality wines. The production of red wines with body and intense color requires harvesting the grapes in an advanced state of maturity and an intense extraction of phenolic compounds during winemaking. Grapes with advanced maturity have high concentrations of sugars and high pH, causing alcoholic wines and stability problems. This doctoral project focused on studying winemaking alternatives that allow regulating the alcohol content and the pH of the wines without affecting their phenolic concentration, as a tool to mitigate the effect of interannual climate variability. In particular, the substitution of ripe grape must for immature grape must, and the pre-fermentation hot maceration in the Pinot noir and Tannat cultivars in the 2016, 2017 and 2018 harvests were evaluated. Additionally, another trial was carried out where the aggregate and replacement with water or white grape must as alternatives for reducing the alcohol content of the wines. The oenological potential of the grapes harvested, and the general composition, color, phenolic composition, and polysaccharides of the wines were determined. Sensory evaluations of the wines were also carried out. Through the winemaking techniques proposed, it was possible to produce wines with lower alcohol content and pH without negatively affecting their color or phenolic concentration and in polysaccharides of the wines. The effect of these techniques depended strongly on the cultivation and the oenological potential of the grapes in each harvest. The results obtained suggest that these winemaking techniques can minimize the effects of interannual climate variability on the composition of the wines, being tools that contribute to improving the sustainability of the national wine sector.

Keywords: low alcohol wine, must replace, thermovinification, Tannat, Pinot noir

1. INTRODUCCIÓN

1.1. FUNDAMENTOS Y ANTECEDENTES

Uruguay cuenta con 6018 hectáreas de viñedo, de las cuales el 20 % corresponden a variedades blancos y el 80% a tintas. En total se produjeron 101.359.039 kg de uva en la vendimia 2019 (22% de uva blanca y 78 % de uva tinta) lo que determinó una elaboración de 74.500.152 l de vino (16 % de vino blanco, 33 % de vino rosado y clarete y 33 % de vino tinto). En el año 2018, el 80% de la producción se comercializó en el mercado interno en tanto que el restante 20 % se exportó tanto envasado (2%) como a granel (18%) (INAVI, 2019).

El clima es uno de los componentes mayoritarios del Terroir que determina el potencial enológico de la uva y la tipicidad del vino. Uruguay se divide en seis regiones agroclimáticas determinadas a partir de índices bioclimáticos adaptadas al cultivo de la vid (Figura 1) (Ferrer, 2007, Ferrer et al., 2007). La zona sur, principal región vitivinícola del país, está influenciada por la penetración de la brisa del río de La Plata que tiene un importante efecto en la temperatura (Fourment et al., 2014) y, por ende, en la fisiología de la vid y la maduración de las uvas (Sadras et al., 2012, Bonada y Sadras, 2015). Las altas temperaturas durante el período de maduración provocan una mayor acumulación de azúcares en la baya y una degradación de la acidez (Sadras et al., 2013), debido al consumo de ácido málico (Jackson y Lombard, 1993), y alteran la síntesis de polifenoles (Mori et al., 2007, Nicholas et al., 2011). El estrés térmico durante el período de maduración causa la degradación e inhibición de la acumulación de antocianos (Mori et al., 2007). En la zona sur, la brisa marítima puede atenuar hasta 4ºC la temperatura en momentos de riesgo de estrés térmico durante la maduración lo que repercute sobre todo en la composición secundaria de la uva Tannat (Fourment et al., 2017). Además, las precipitaciones, según la etapa del ciclo del cultivo, tienen un efecto negativo o positivo en los contenidos de azúcares y de ácidos (Hunter y Bonnardot, 2011) y en la sanidad de la uva (Ferrer et al., 2017).

La adaptación del cultivo de la vid al cambio climático implica conocer los factores ambientales locales que impactan en la fisiología de la vid, y que determinan, por ende, la composición de la uva y del vino.

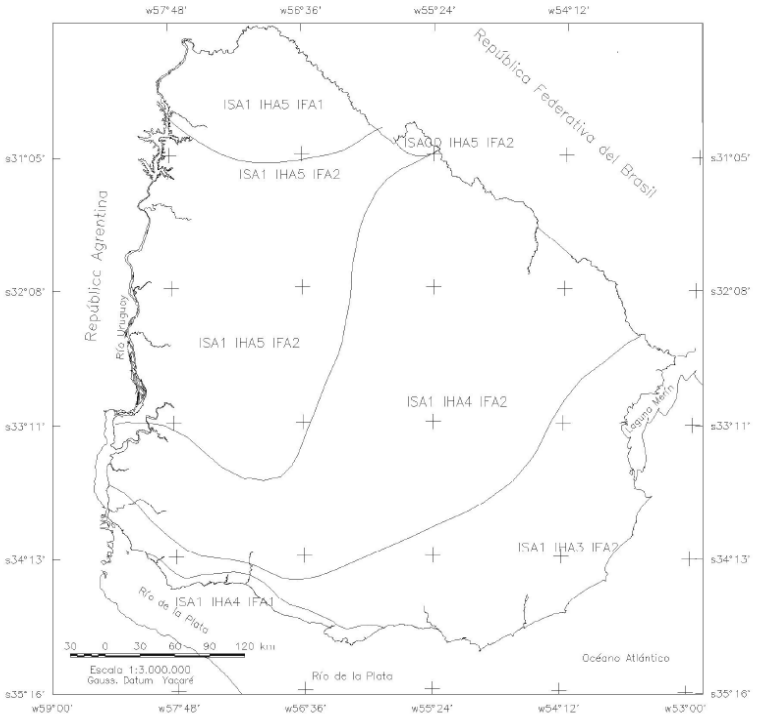

Figura 1 Clasificación climática multicriterio (Ferrer, 2007)

El potencial enológico de las uvas tintas está determinado en gran medida por la concentración en compuestos fenólicos los cuales son también responsables de las principales características sensoriales de los vinos. Dentro de este grupo de compuestos, los antocianos son las principales responsables del color de los vinos tintos jóvenes (Boulton, 2001). Estos pigmentos son sintetizados por el metabolismo secundario de la vid y se acumulan en los hollejos de uva durante la maduración (González-Neves et al., 2004a y b, Llaudy et al., 2008). La concentración de antocianos del vino está determinada principalmente por el cultivar (González-Neves et al., 2005 y 2016, Mattivi et al., 2009, Ortega-Regules et al., 2006), el nivel de maduración de la uva y las técnicas de maceración utilizadas durante la vinificación (Cheynier et al., 2006, González-Neves et al., 2016). Sin embargo, el color del vino

depende no solo de la concentración de antocianos sino también del pH, la presencia de copigmentos y las reacciones de adición y condensación con otros polifenoles de uvas o compuestos formados durante la fermentación alcohólica (Boulton, 2001, He et al., 2012). Otro grupo importante de compuestos fenólicos son las proantocianidinas, también conocidas como taninos condensados. Los contenidos de las proantocianidinas del vino están fuertemente relacionados con las sensaciones de amargor y astringencia (Vivas et al., 2004). Estos compuestos son polímeros de flavan-3-oles presentes en los hollejos y las semillas de las uvas, y su composición depende de su origen. Las proantocianidinas de semillas están compuestas de (+)-catequina, (-)-epicatequina y (-)-epicatequina-3-galato, mientras que las proantocianidinas de hollejos también contienen (-)-epigalocatequina y tienen una proporción mucho menor de (-)-epicatequina-3-galato (Souquet et al., 1996). Además, el grado medio de polimerización (mDP) de las proantocianidinas es menor en las semillas que en hollejos. En consecuencia, los hollejos liberan procianidinas y prodelfinidinas de alto grado de polimerización, mientras que las semillas solo liberan procianidinas de bajo grado de polimerización y alto grado de galloilación. Se ha reportado que las proantocianidinas con mayor grado de polimerización y un mayor porcentaje de galloilación causan una mayor sensación de astringencia (Souquet et al., 2000, Vivas et al., 2004).

Otros compuestos determinantes de las características sensoriales del vino son los polisacáridos. Estos compuestos forman parte de las paredes celulares de las células vegetales (Vidal et al., 2001) y de los microorganismos involucrados en el proceso de vinificación (levaduras y bacterias) (González-Royo et al., 2013). Las enfermedades causadas por hongos en las uvas pueden aumentar el contenido de polisacáridos del vino (Francolí et al., 1999). Si bien existen varios tipos de polisacáridos de uva, muchos de ellos se degradan o precipitan enzimáticamente durante la fermentación alcohólica, por lo que el vino contiene cantidades apreciables de arabinogalactano (AGP) y de ramnogalacturonano de tipo II (RG-II) (Vidal et al., 2003, Guadalupe y Ayesterán, 2007). Otra fuente importante de polisacáridos en el vino son las levaduras, que pueden liberar cantidades

significativas de manoproteínas (MP) en las etapas finales de la fermentación alcohólica (Escot et al., 2001).

La madurez de la uva también tiene un efecto indirecto sobre la solubilización de los polifenoles y de los polisacáridos. En particular, un mayor contenido de etanol favorece la extracción de polifenoles durante la maceración (Gonzalez-Manzano et al., 2004, Canals et al., 2005) pero disminuyen la concentración de polisacáridos por precipitación (Guadalupe y Ayesterán, 2007).

En la medida que se conocen los impactos de la variabilidad climática sobre el potencial enológico de las uvas y la calidad del vino, es posible evaluar medidas de gestión de la vinificación que permitan mitigar las diferencias en la calidad del vino año a año.

Existen tecnologías de vinificación que pueden ser adaptados en relación con el potencial enológico de la uva. Dentro de las técnicas que modifican la extracción de los compuestos fenólicos se encuentran:

- La extracción diferida de antocianos (Bosso et al., 2004, González-Neves et al., 2015a).
- La maceración extendida (Canals et al., 2005, Cheynier et al., 2006, Fulcrand et al., 2006, González-Neves et al., 2015a).
- La maceración pre-fermentativa en frio (Casassa, 2007, Heredia et al., 2010, Piccardo y González-Neves, 2013, Favre et al., 2014, González-Neves et al., 2015b, Piccardo, 2015).
- La maceración post-fermentativa en caliente (Blouin et al., 2000, Casassa, 2007, Piccardo y González-Neves, 2013, Piccardo, 2015).
- La ***maceración pre-fermentativa en caliente*****.** Esta técnica consiste en calentar los racimos enteros o estrujados antes de la fermentación alcohólica, con el objetivo de obtener vinos más coloreados (El Darra et al., 2013, Boulet, 2003). El calor altera los tejidos de los hollejos transfiriendo sus componentes al mosto. Las temperaturas a las que se eleva el mosto durante la etapa pre-fermentativa varían entre 40 y 80 ºC, a la vez que la duración de la maceración depende de la temperatura (entre 12 y 24 h) (Atanackovic et al., 2012). Durante el calentamiento y la posterior maceración, el incremento en la extracción de

antocianos determina que se alcancen valores elevados de intensidad colorante, estando este fenómeno determinado por el tiempo y la temperatura de maceración (Andrade-Neves et al., 2014, Piccardo y González-Neves, 2013, Piccardo, 2015). Al terminar la fermentación alcohólica los vinos resultan con más color que los elaborados por sistemas tradicionales (El Darra et al., 2013, Atanackovic et al., 2012, Piccardo y González-Neves, 2013, Piccardo, 2015).

Dentro de las prácticas propuestas para modificar la composición básica del vino se encuentran:

- La cosecha anticipada de las uvas (Bovo et al., 2016, Schmidtke et al., 2012).
- La introducción de nuevos cultivares y modificación de las condiciones y técnicas de cultivo (Schultz, 2000).
- El uso de levaduras nativas con una menor producción de etanol (Mestre et al. 2019).
- El empleo de glucosa oxidasa (EC 1.1.3.4) (Pickering et al., 1998).
- La reducción parcial de la concentración de azúcares del mosto y la desalcoholización parcial o total del vino utilizando tecnologías de membrana (Gómez-Plaza et al., 1999, Takács et al., 2007, García-Martín et al., 2010 y 2009, Gil et al., 2013).
- La ***mezcla de mostos o vinos obtenidos de uvas con diferente grado de madurez***. La sustitución de mostos de uvas cosechadas en madurez tecnológica por mostos o vinos de uvas inmaduras permiten disminuir la concentración de azúcares del mosto y obtener vinos menos alcohólicos (Kontoudakis et al., 2011, Rolle et al., 2017). Durante la maduración no solo la concentración de azúcares se incrementa, sino que también ocurre una disminución en la acidez del mosto ocasionando cambios en el pH, a la vez que se incrementa la concentración de compuestos fenólicos y aromáticos. La cosecha anticipada de la uva origina vinos con bajo contenido de alcohol, pero con elevada acidez, amargos y con aromas herbáceos. Ésta se debe principalmente a que las uvas cosechadas no han llegado a una composición fenólica y aromática adecuada. Por tanto, la extracción de mosto de uvas inmaduras y su maceración con hollejos y semillas provenientes

de uvas cosechadas en madurez tecnológica puede ser una alternativa sustentable para disminuir la graduación alcohólica del vino, manteniendo su composición fenólica.

- El ***agregado de agua y ácidos minerales al jugo de uva antes del inicio de la fermentación alcohólica***. Esto reduce la concentración de azúcares y el pH, pero tiene un efecto negativo general sobre la calidad del vino porque diluye todos los demás compuestos, y aunque esta práctica está autorizada en algunos países, está estrictamente prohibida en otros (OIV, 2018).

En general las técnicas que modificar la composición básica del vino se focalizan en reducir el contenido de alcohol del vino. En el contexto actual, muchos países miembros de la Organización Mundial de la Salud (OMS) han adoptado políticas públicas para regular el consumo problemático de alcohol. Si bien varias investigaciones sugieren que el consumo moderado de vino tinto puede presentar efectos beneficiosos para la salud debido a sus contenidos importantes en compuestos bioactivos (antocianos y otros polifenoles, como los taninos) (González-Neves et al. 2014, Fulcrand et al. 2006), el vino es una bebida alcohólica. Se ha demostrado que el consumo excesivo de vividas alcohólicas ejerce un efecto directo en el sistema nervioso central, ocasionando cambios en sus funciones y afectando negativamente la salud del individuo y su comportamiento social (OMS, 2010). El consumo de bebidas alcohólicas ocupa el tercer lugar entre los principales factores de riesgo de mala salud en el mundo (OMS, 2010). El consumo excesivo del alcohol es uno de los cuatro factores de riesgo de enfermedades no transmisibles importantes que son susceptibles de modificación y prevención (Peruga, 2001). También se ha reportado que contribuye a aumentar la morbilidad relacionada con enfermedades transmisibles (Espada et al. 2008).

En general, la aplicación de estas técnicas permite la elaboración de vinos diferenciados, asegurando la sustentabilidad de los sistemas productivos y del sector vitivinícola en su conjunto y contribuyendo a mitigar los efectos de la variabilidad climática interanual sobre la composición del vino.

1.2. RELEVANCIA DEL PROBLEMA A ABORDAR

La composición de la uva en cosecha está determinada por las condiciones climáticas de la temporada dado su efecto sobre la fisiología y el desarrollo de la vid. En el contexto de cambio climático, para el Uruguay se prevé una mayor variabilidad interanual de las precipitaciones, en particular durante el período de maduración de la uva, así como un aumento de la temperatura media. Esta situación condiciona el potencial enológico de las uvas y favorece la aparición de enfermedades fúngicas que determinan pudriciones a nivel de racimos, dificultando la producción de vinos de calidad en diferentes vendimias (Ferrer et al., 2018) Esto constituye una limitante para los vitivinicultores a la hora de producir uva y elaborar vinos de calidad a partir de diferentes vendimias.

Asimismo, las tendencias actuales han llevado a que los vinos tintos con cuerpo y de color intenso sean muy apreciados por los consumidores. La producción de este tipo de vino requiere una intensa extracción de compuestos fenólicos durante la vinificación. Sin embargo, una extracción excesiva, podría causar un exceso de amargor, astringencia y aromas herbáceos, especialmente cuando las uvas no están lo suficientemente maduras ya que la madurez tiene una gran influencia en los aromas (Boido et al., 2003) y la composición fenólica de los vinos tintos (Ó-Marques et al., 2005). Las uvas con una madurez avanzada presentan concentraciones altas de azúcares y pH (Fulcrand et al., 2006) ya que la pulpa madura más rápido que los hollejos y semillas (Llaudy et al., 2008).

La vinificación de uva con una madurez avanzada o sobremaduras trae consigo dos problemas importantes en la elaboración de vinos. Por un lado, los vinos presentan altos valores de pH lo que ocasiona una menor efectividad del anhidrido sulfuroso y una mayor susceptibilidad del vino al desarrollo de microorganismos indeseados, a la vez que puede ocasionar una disminución en la intensidad del color. En estas condiciones, se hace imprescindible la corrección del pH del vino, lo que incrementa los costos de elaboración y disminuye la rentabilidad. Por otro lado, los vinos elaborados con uvas en madurez avanzada presentan alto contenido de alcohol. La aplicación de políticas públicas y la concientización por la salud humana han

llevado a una disminución en el consumo de bebidas con altos niveles de alcohol. Si bien varias investigaciones han informado que el consumo moderado de vino tinto puede tener efectos beneficiosos para la salud debido a su contenido significativo de compuestos bioactivos (Yu et al., 2005, Actis-Goretta et al., 2002), el vino es una de las bebidas con un contenido de alcohol importante.

En estas circunstancias, a nivel técnico se presentan dos opciones para definir el momento de cosecha. Por un lado, se puede cosechar uvas con un contenido adecuado de azúcares y pH, pero con una maduración inadecuada de hollejos y semillas, lo que probablemente resultarán vinos mal coloreados, amargos, astringentes y herbáceos. Por otro lado, se puede cosechar uvas sobremaduras y asumir que presentaran un contenido de azúcares y pH elevados (Beech, 1979).

La exportación de vino es una alternativa comercial imprescindible para la sustentabilidad del sector vitivinícola nacional. Sin embargo, la variabilidad interanual del potencial enológico de las uvas genera dificultades a la hora de cumplir con las características cualitativas que deben presentar los vinos para su exportación. Esto se debe a la dificultad para producir vinos con una calidad similar a partir de vendimias con diferente potencial enológico.

En general, las tecnologías utilizadas en bodega que promueven la concentración de compuestos fenólicos en los vinos determinan un incremento en su contenido de alcohol y pH. En este sentido, se hace necesario evaluar las técnicas de gestión de la vinificación que permitan reducir el contenido de alcohol y el pH del vino manteniendo o incrementando su concentración en compuestos fenólicos, como una herramienta que contribuya a la resolución del problema planteado y que permita mejorar la sustentabilidad del sector vitivinícola nacional, tratando de mitigar los efectos de la variabilidad climática.

Como hipótesis de esta investigación se plantea que, a través de técnicas alternativas de gestión de la vinificación, tales como la sustitución de mostos y la maceración pre-fermentativa en caliente, es posible regular el contenido de alcohol y el pH de los vinos manteniendo o incrementando su concentración en compuestos fenólicos en función del potencial enológico de la uva en cada vendimia.

1.3. OBJETIVO GENERAL Y ESPECÍFICOS

El objetivo general de este proyecto fue evaluar técnicas de gestión de la vinificación que permitan regular el contenido de alcohol y el pH manteniendo o incrementando el color y la concentración de compuestos fenólicos y polisacáridos de los vinos como herramienta para mitigar el efecto de la variabilidad climática interanual.

Para cumplir con el objetivo general se plantearon los siguientes objetivos específicos:

1. Determinar la efectividad de la sustitución de mosto de uva madura por mosto de uva con menor nivel de madurez y la maceración pre-fermentativa en caliente para obtener vinos tintos con menor contenido de alcohol y pH y mayor color y concentración de compuestos fenólicos y polisacáridos.
2. Estudiar la eficiencia de la sustitución de mostos de uvas con diferente grado de maduración para elaborar vinos tintos con menor contenido de alcohol en diferentes vendimias.
3. Evaluar el efecto de la maceración pre-fermentativa en caliente sobre la composición fenólica y el color de vinos tintos al descube y durante su conservación.
4. Determinar el impacto de la sustitución de mosto de uvas con diferente grado de maduración y la maceración pre-fermentativa en caliente en el color de los vinos tintos Tannat producidos en diferentes vendimias.
5. Evaluar otras alternativas de vinificación que permitan reducir el contenido de alcohol de los vinos.

1.4. ESQUEMA GENERAL DE LA TESIS

Las investigaciones realizadas en esta tesis se desarrollaron sobre uvas Tannat y Pinot noir cosechadas durante las vendimias 2016, 2017 y 2018 de viñedos comerciales localizados en la zona sur de Uruguay. Las vinificaciones fueron realizadas en la bodega experimental de Facultad de Agronomía (Udelar, Uruguay). Adicionalmente se realizó un ensayo sobre uvas Macabeo, Merlot y Tempranillo

cosechadas manualmente de un viñedo comercial ubicado en Els Guiamets, AOC Montsant, (Tarragona, España), durante la vendimia 2017. Las vinificaciones se llevaron a cabo en la bodega experimental Mas dels Frares, de la Universitat Rovira i Virgili, (Tarragona, España).

Sobre los mostos se determinó la composición básica y su potencial enológico, mientras que en los vinos se determinó su composición básica, color, concentración de diferentes familias fenólicas y polisacáridos. Todos los vinos fueron analizados entre los 2 y 6 meses del descube, mientras que en los vinos elaborados a partir del cultivar Tannat se también fueron evaluados durante su conservación (3 años para los elaborados en el 2016, 2 años para los 2017 y 1 año para los 2018). La riqueza fenólica de la uva se determinó en el laboratorio de la Bodega Experimental de la Facultad de Agronomía (Udelar, Uruguay). Los análisis de composición general del vino se realizaron en el Instituto Nacional de Vitivinicultura (Las Piedras, Canelones, Uruguay). Los análisis espectrofotométricos de los vinos se realizaron en el laboratorio de la Bodega Experimental de la Facultad de Agronomía (Udelar, Uruguay) y el laboratorio del grupo de investigación en Tecnología Enológica del Departamento de Bioquímica y Biotecnología de la Universitat Rovira i Virgili (Tarragona, España). Las técnicas de determinación de compuestos fenólicos por HPLC-DAD fueron realizadas durante 3 pasantías (2016, 2017 y 2018 de 45 días cada una) en la Facultat d'Enologia de la Universitat Rovira i Virgili (Tarragona, España).

1.5. PRESENTACIÓN DE LOS CAPÍTULOS

La tesis se presenta en seis capítulos, además del presente. En los capítulos 2, 3, 4, 5 y 6 se presentan las investigaciones realizadas para cumplir con los objetivos específicos mencionados. Cada capítulo se corresponde con un objetivo específico en orden cronológico. En estos capítulos se exponen los antecedentes y revisión general de la literatura vinculada con la investigación, objetivos, diseño experimental, resultados, discusión y conclusiones. El capítulo 7 corresponde a las conclusiones generales y perspectivas. La descripción de cada capítulo se detalla a continuación:

- El **Capítulo 2** se centra en evaluar la efectividad de la sustitución de mosto de uva madura por mosto de uva inmadura y la maceración pre-fermentativa en caliente para reducir el contenido de alcohol y el pH de vinos tintos Pinot noir y Tannat elaborados en la vendimia 2016, manteniendo su color, composición fenólica y en polisacáridos y sus características sensoriales. En cada cultivar se evaluaron los siguientes tratamientos: vino control elaborado con uvas cosechadas en madurez tecnológica seguido por una maceración tradicional (CW-TM), vino reducido en alcohol elaborado por sustitución de mostos de uvas con diferente grado de maduración seguidos por una maceración tradicional (RAW-TM), vino control elaborado con uvas cosechadas en madurez tecnológica seguidos por una maceración pre-fermentativa en caliente (CW-HM) y vino reducido en alcohol elaborado por sustitución de mostos de uvas con diferente grado de maduración seguido por una maceración pre-fermentativa en caliente (RAW-HM). Las vinificaciones se realizaron por triplicado. Las determinaciones analíticas de los vinos se realizaron a los dos meses del embotellado (seis meses del descube).
- El **Capítulo 3** se centra en evaluar la eficiencia de la sustitución de mosto de uva madura por mosto de uva inmadura para elaborar vinos tintos Tannat y Pinot noir con menor contenido de alcohol y pH en diferentes vendimias. En cada cultivar, durante las vendimias 2016, 2017 y 2018, se evaluaron los siguientes tratamientos: mosto testigo (MT) y mosto reducido en azúcares a través de la sustitución de mosto de uvas con diferente grado de maduración (MRA). Las vinificaciones se realizaron por triplicado. Para todos los vinos, las determinaciones analíticas se realizaron a los 2 meses del descube.
- El **Capítulo 4** se enfoca en determinar el efecto de la maceración pre-fermentativa en caliente sobre la composición fenólica y el color de vinos tintos Tannat al descube y durante su conservación. Esta investigación se centró sobre el cultivar Tannat dada la relevancia del cultivar para el país y a los problemas relacionados con la extractibilidad de los antocianos y la estabilidad del color que presentan sus vinos. Para ello, durante la vendimia 2016 se evaluaron los siguientes tratamientos: vinificación tradicional (VT) y maceración pre-

fermentativa en caliente (MPC). Las vinificaciones se realizaron por triplicado. Las determinaciones analíticas de los vinos se realizaron a los dos meses y se repitieron a los 1 y 2 años.

- El **Capítulo 5** busca determinar el impacto de la sustitución de mosto de uvas con diferente grado de maduración y la maceración pre-fermentativa en caliente en el color de los vinos tintos Tannat producidos en diferentes vendimias. Para ello se evaluaron los siguientes tratamientos: vino control elaborado con uvas cosechadas en madurez tecnológica seguido por una maceración tradicional (OM-TM), vino reducido en alcohol elaborado por sustitución de mostos de uvas con diferente grado de maduración seguidos por una maceración tradicional (MR-TM), vino control elaborado con uvas cosechadas en madurez tecnológica seguido por una maceración pre-fermentativa en caliente (OM-HM), y vino reducido en alcohol elaborado por sustitución de mosto de uvas con diferente grado de maduración seguido por una maceración pre-fermentativa en caliente (MR-HM). Las vinificaciones se realizaron por triplicado. Las determinaciones analíticas de los vinos se realizaron a los dos meses del embotellado (seis meses del descube).
- El **Capítulo 6** se evalúan otras alternativas de vinificación que permitan reducir el contenido de alcohol de los vinos. Esta investigación se llevo a cabo en la vendimia 2017 sobre los cultivares Merlot y Tempranillo en el marco de la segunda pasantía realizada en la Facultat d'Enología de la Universitat Rovira i Virgili. En cada cultivar se evaluaron los siguientes tratamientos: vino control obtenido de uvas cosechadas en madurez tecnológica, vino control obtenido de uvas sobremaduras, adición de agua acidificada en mosto de uvas sobremaduras, sustitución de mosto de uvas sobremaduras por agua acidificada, adición de mosto acidificado al mosto de uva sobremadura y sustitución de mosto de uvas subremaduras por mosto acidificado. Las vinificaciones se realizaron por triplicado. Las determinaciones analíticas se realizaron al mes del descube.
- El **Capítulo 7** se presenta una síntesis de los principales resultados obtenidos, presentando las conclusiones y perspectivas de las investigaciones realizadas.

2. INFLUENCE OF THE USE OF UNRIPE GRAPES TO REDUCE ETHANOL CONTENT AND PH ON THE COLOR, POLYPHENOL AND POLYSACCHARIDE COMPOSITION OF CONVENTIONAL AND HOT MACERATED PINOT NOIR AND TANNAT WINES*

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2.1. ABSTRACT

The aim of this research was to determine the effectiveness of the substitution of grape juice of grapes with different maturation degree and prefermentative hot maceration to obtain Pinot Noir and Tannat red wines with lower alcohol content and Ph and higher color and phenolic compound concentrations. Immature grape juice was extracted of grapes harvested in veraison and kept at 4 °C until its use. In technological maturity, the grapes harvested were destemmed, crushed and distributed in 12 containers per cultivar. Six were controls while in the other six 3 L of the original grape juice were substituted by 3 L of unripe grape juice. Next, three containers from each experimental group were traditionally macerated, while the other three were submitted to a prefermentative hot maceration (one hour at 60–70 °C). All treatments performed a fermentative maceration of 7 days. Wines produced from substituted musts had lower alcohol content (14% off) and pH (9% off) and higher titratable acidity (48% more), but no other important changes in wine components were detected. Prefermentative hot maceration increased wine color intensity (50%), total phenolic compounds (66%), total anthocyanin (42%), proanthocyanidin (65%) and polysaccharide (95%) concentrations. The joint consideration of both techniques is an interesting tool to simultaneously mitigate the problems caused by climate change with respect to the maturity of the grape and improve the color of the wine and its concentration in polysaccharides and phenolic compounds.

Keywords: Ethanol reduction, pH reduction, Unripe grapes, Pre-fermentative hot maceration, Wine color, Wine composition

2.2. INTRODUCTION

Nowadays, strongly colored full-bodied red wines are highly appreciated by the market. However, producing this kind of wine requires an intense extraction of phenolic compounds during winemaking which, in the case of over-extraction, could cause an excess of bitterness, astringency and herbaceous aromas, especially when the grapes are not ripe enough. Indeed, grape maturity strongly influences in the aroma [1] and the phenolic composition of red wines [2, 3]. For all these reasons, winemakers are very interested in harvesting the grapes as ripe as possible. Nevertheless, grapes with a very high phenolic maturity frequently present high sugar and low acid concentrations [4, 5]. Grape pulp usually ripens faster than skins and seeds and results in high pH and sugar concentrations in the must [6]. Under these circumstances, winemakers can choose between two options. On the one hand, they can harvest grapes with an adequate sugar content and pH but inadequate skin and seed maturation, which will probably result in poorly colored, bitter, astringent and herbaceous wines. Or on the other, they can wait for complete phenolic maturity and assume that will lead to grape with very high pH and sugar content [7]. Moreover, global warming has been exacerbating this problem in recent years [8–10].

Recent social policies implemented in many countries have highlighted the need to regulate the consumption of alcoholic beverages in the short term. As a result of the application of these measures along with concerns regarding the health and social behavior of the population, there has been a drop in the consumption of beverages with high levels of alcohol. Wine is one of the most popular alcoholic beverages. Several investigations have reported that the moderate consumption of red wine may have beneficial effects on health due to its significant content of bioactive compounds [11, 12]. The bioactive compounds in wine are stilbenes, anthocyanins, tannins and other polyphenols which are important due to their technological and sensory characteristics [4, 13]. These compounds have an antioxidant capacity and participate in numerous biochemical processes that are potentially positive for the organism. Reducing the alcohol content of wine but maintaining or even increasing its bioactive components is, therefore, one of the wine industry’s objectives [14].

Several techniques have been proposed for making wines with a lower alcohol content without altering their phenolic composition, such as harvesting the grapes at an early stage of ripening [15, 16], adding water and mineral acids to the grape juice before fermentation begins [17–21], introducing new cultivars and modifying culture techniques [22], using glucose oxidase (EC 1.1.3.4) [23], using yeast with a low ethanol yield [24], and applying physical techniques to partially reduce the sugar concentration in grape juice or the alcohol in wine [25–29]. Kontoudakis et al. [30] have proposed substituting a proportion of grape juice from very ripe grapes with wine produced by unripe grapes from clusters thinning as a way of simultaneously reducing wine ethanol content and pH. More recently, the impact of this procedure on the on polysaccharide and tannin composition [17] and on wine volatile composition and sensory properties [18] has been studied in comparison with the addition of water reaching similar conclusions. This procedure has the advantage of not needing any specific equipment such as membrane techniques or the spinning cone column and does not violate the regulations of many countries such as the addition of water. In addition, immature grapes that are discarded during the thinning of the vineyards represent for the agri-food industry an important source of bioactive compounds which have anti-browning effect and antioxidant activity that are easy to produce, turning this agricultural waste into value-added products [31]. Additionally, combining one of these techniques of alcohol reduction with a hot prefermentative maceration could be an alternative to produce lower alcohol wines with increased phenolic content. It has been reported that the highest extraction of polyphenols and anthocyanins is achieved during hot pre-fermentative maceration, and their concentrations are maintained until the end of the winemaking process [32–37].

As a result of the application of this prefermentative winemaking technique, the wines have a high concentration of phenolic compounds, anthocyanins and color intensity. This means that, per unit of consumption, the bioactive compound contributions of these wines could be greater than those produced by traditional techniques [38].

The objective of this research is to determine the effectiveness of the substitution of ripe grape juice for grape juice with a lower level of maturity and pre-fermentative maceration to obtain Pinot noir and Tannat red wines with lower alcohol content and pH and greater color and phenolic concentration.

2.3. MATERIALS AND MÉTHODS

2.3.1. Chemicals and equipment

Methanol, acetonitrile, formic acid, and acetic acid were of HPLC grade (> 99%) and purchased from Panreac (Barcelona, Spain). Acetaldehyde (> 99.5%), phloroglucinol (> 99%), ascorbic acid (> 99%), sodium acetate (> 99%), and ammonium formate (> 99%) were purchased from Sigma-Aldrich (Madrid, Spain). Absolute ethanol and hydrochloric acid (37%) were purchased from Panreac. Malvidin-3-O-glucoside chloride (≥ 95%), proanthocyanidin dimer B2 (≥ 90%), (+)-catechin (≥ 99%), (−)-epicatechin (≥ 99%), (−)-epigallocatechin (≥ 98%), and (−)-epicatechin-3-O-gallate (≥ 97.5%) were purchased from Extrasynthese (Genay, France). A pullulan calibration kit Shodex P-82 (P-5, MW = 5.9 kDa; P-10, MW = 11.8 kDa; P-20, MW = 22.8 kDa; P-50, MW = 47.5 kDa; P-100, MW = 112 kDa; P-200, MW = 212 kDa; P-400, MW = 404 kDa; P-800, MW = 788 kDa) was obtained from Waters (Barcelona, Spain), while a pullulan 1.3 kDa and four dextrans BioChemika (12, 25, 50, and 80 kDa) were obtained from Fluka (St. Louis, MO, USA). A Winescan TM Autosampler 79,000 infrared analyzer (Foss, USA) and the Foss Integrator software version 154 (Foss, Denmark) were used to determine the alcohol content, total acidity and pH of the wines. The polysaccharides used as external standards for quantification were pectins from citrus fruit (≥ 90%) and dextrans synthesized by Leuconostoc mesenteroides (≥ 99.9%) purchased from Sigma-Aldrich (St. Louis, MO, USA). The HPLC analyses were performed using an Agilent 1200 series liquid chromatograph equipped with a G1362A refractive index detector (RID), a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1316A column oven, and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). All the spectrophotometric measurements were performed using a

Helios Alpha UV–Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA, USA).

2.3.2. Grapes and wines

The study was carried out using grapes from Pinot Noir (Vitis International Variety Catalogue number VIVC 9279) [39] and Tannat (Vitis International Variety Catalogue number VIVC 12257) [39] cultivars (*Vitis vinifera* L.) from the 2016 vintage. Both cultivars were manually harvested from a commercial vineyard located in the department of Canelones in the south of Uruguay.

At the beginning of veraison, 100 kg of Pinot Noir and 100 kg of Tannat grapes were harvested to obtain a grape juice with a very low sugar concentration and high acidity. The grapes were crushed (Alfa 60 R crusher, Italcom, Piazzola Sul Brenta, Italy) and lightly pressed in a manual press to obtain 50 L of an unripe grape juice. The grape juice was immediately sulphited with 100 mg/L of $K_2S_2O_5$ (which equals approximately 50 mg/L of SO_2), settled overnight, packed in a 50 L polyethylene container and conserved at 4 °C until use. When the grapes reached technological maturity, 120 kg of grapes from both cultivars were manually collected and randomly distributed into twelve lots of 10 kg. The grapes were destemmed and crushed (Alfa 60 R crusher, Italcom, Piazzola Sul Brenta, Italy) and the grape juice and the pomace obtained was sulfited with 100 mg/L of $K_2S_2O_5$ and distributed among twelve polyethylene containers (10 L capacity each) per cultivar. The must containers were randomly divided into two groups of six containers each. Six were considered to be controls (control wines—CW) while the other six (reduced alcohol wines—RAW) were bled of 3 L of the original grape juice, which was substituted by 3 L of the juice from the unripe grapes with the aim of decreasing sugar content and pH. This substitution represents around 31% of the weight of the destemmed and crushed grapes.

Next, three containers from each experimental group (CW and RAW) were traditionally macerated (TM), while the other three were submitted to a prefermentative hot maceration (HM) for one hour at a temperature between 60 and 70 °C. The heating was carried out by transferring the pomace to 11 L stainless steel

containers that were submerged in a hot water bath (80–90 °C). During the warming, the pomace was homogenized manually. At the end of the heat treatment, the stainless-steel tanks were submerged in a cold-water bath to cool them to ambient temperature (around 26 °C). After that the grape juice was transferred back to the original 10 L polyethylene containers. Thus, four experimental groups for each cultivar were obtained: control wine with traditional maceration (CW-TM), reduced alcohol wine with traditional maceration (RAW-HM), control wine with prefermentative hot maceration (CW-HM), and reduced alcohol wine with prefermentative hot maceration (RAW-TM).

All the containers were inoculated with 200 mg/L of active dry yeast (*Saccharomyces cerevisiae* ex bayanus Natuferm 804; Oenobiotech, Paris, France) and fermented in contact with skins and seeds. During maceration, all the containers were manually pumped over once daily, followed by a manual punching down the cap to favor polyphenol extraction. The fermentation temperature ranged between 25 and 29 °C. After 7 days of maceration, the free run wine was extracted by gravity and the resting pomace was lightly pressed in a manual press. The free-run wine and the lightly pressed wine from each tank were blended and kept in 5 L vessels at room temperature (18 ± 2 °C). The alcoholic fermentation was completed when the daily measurements of musts density was less than 998 g/L in three consecutive days. Wines were preserved in polyethylene containers of 5 L of capacity at laboratory room temperature (18 ± 2 °C) and once spontaneous malolactic fermentation was finished (around 35 days later), all the wines were stabilized with 100 mg/L of $K_2S_2O_5$ and 300 mg/L of lysozyme (Delvo®Zyme, Delft, The Netherlands). Finally, the wines were bottled and stored in a dark cellar at laboratory ambient temperature until analysis. The analyses started 2 months after bottling and ended 3 weeks later.

2.3.3. Standard grape juice and wine analysis

The analytical methods recommended by the International Organization of Vine and Wine [40] were used to determine the sugar concentration, pH and titratable acidity of the grape juices. The alcohol content, total acidity and pH of the

wines were determined using a Winescan TM Autosampler 79,000 infrared analyzer (Foss, USA) and the Foss Integrator software version 154 (Foss, Denmark).

The total anthocyanin content of the grapes, their extractability and total phenolic index were determined following the method proposed by González-Neves et al. [41]. The total anthocyanin content of the wines was estimated in accordance with the spectrophotometric method proposed by Niketic-Aleksic & Hrazdrina [42]. The total phenolic index (TPI) was estimated by measuring the absorbance at 280 nm [43].

2.3.4. Color parameters

The color parameters were determined directly on the wine samples placed in a 1 mm optical path cuvette. Color intensity (CI) was estimated using the method described by Glories [44]. The CIELAB coordinates, lightness (L*), chroma (C*), hue (H*), red-greenness (a*), and yellow-blueness (b*) were determined following the method described by Ayala et al. [45] and data processing was performed using MSCV software [46].

2.3.5. HPLC anthocyanin analysis

Reversed-phase HPLC analyses of the anthocyanins were carried out by injecting 40 μL of wine into an Agilent 1200 series liquid chromatograph (HPLC-DAD) and using an Agilent Zorbax Eclipse XDBC18, 4.6 × 250 mm, 5 μm column (Agilent Technologies). The solvents used were 10% aqueous formic acid (solvent A) and a mixture of 45% methanol, 45% water, and 10% formic acid (solvent B) in accordance with the method described by Valls [47]. Chromatograms were recorded at 530 nm, and anthocyanin standard curves were made using malvidin-3-O-glucoside chloride. Compounds were identified considering the relative retention times between the compounds and by recording their UV spectra with the diode array detector and comparing these with the UV spectra reported by Valls [47]. The five anthocyanidin-3-monoglucosides of wine (delphinidin, cyanidin, peonidin, petunidin, and malvidin) and their respective acetylated and p-coumaroylated anthocyanins were quantified.

2.3.6. Wine proanthocyanidin analysis

Acid-catalyzed depolymerization of proanthocyanidin in the presence of an excess of phloroglucinol was used to analyze the content of the proanthocyanidins, their monomeric composition, and their mDP, as described by Kennedy and Jones [48]. A 10 mL sample of wine was evaporated under a low-pressure vacuum (Univapo 100 ECH, Uni Equip, Germany). Subsequently, it was resuspended in 6 mL of distilled water and then applied to Sep Pak Plus tC18 Environmental cartridges (Waters, Milford, MA, USA) that had previously been activated with 10 mL of methanol and 15 mL of water. The samples were washed with 15 mL of distilled water, and then the proanthocyanidins were eluted with 12 mL of methanol, immediately evaporated under a vacuum, and redissolved in 2 mL of methanol. Finally, 100 L of this sample was reacted with a 100 μL phloroglucinol solution (0.2 N HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid) at 50 °C for 20 min. The reaction was stopped by adding 1000 μL of 40 mM aqueous sodium acetate.

Reversed-phase HPLC analysis (Agilent series 1200 HPLC-DAD) was carried out with an Agilent Zorbax Eclipse XDBC18, 4.6 × 250 mm, 5 μm column (Agilent Technologies) as described below, and the injection volume was 30 μL. The solvents used were 1% aqueous acetic acid (solvent A) and methanol (solvent B) at a flow rate of 1 mL/min. Elution was performed with a gradient starting at 5% B for 10 min, a linear gradient from 5 to 20% B in 20 min, and a linear gradient from 20 to 40% B in 25 min. The column was then washed with 90% B for 10 min and re-equilibrated with 5% B for 5 min before the next injection. The monomers (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-O-gallate were detected to 280 nm and identified by comparing their retention times with those of the pure compounds. The phloroglucinol adducts of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and (−)-epicatechin-3-O-gallate were identified by their retention time (described in the literature) and confirmed through an HPLC–MS analysis. Analyses were performed with the Agilent 1200 series HPLC using an Agilent 6210 time-offlight (TOF) mass spectrometer equipped with an electrospray ionization system (ESI). Elution was carried out under the same HPLC analysis conditions described above.

The capillary voltage was 3.5 kV. Nitrogen was used both as a dry gas at a flow rate of 12 L/min at 350 °C and as a nebulizer gas at 60 psi. Spectra were recorded in positive ionization mode between m/z 50 and 2400. This assay was also carried out without the addition of phloroglucinol to measure the flavan-3-ol monomers that are naturally present in wine. The number of terminal subunits was considered to be the difference between the total monomers measured in normal conditions (with phloroglucinol) and thus obtained when the analysis was performed without phloroglucinol addition. The number of extension subunits was considered as the addition of all the phloroglucinol adducts. The mDP was calculated by adding the terminal and extension subunits (in moles) and dividing by the terminal subunits. Because acid catalysis with phloroglucinol is not completely efficient, the real yield of the reaction was measured using a pure B2 proanthocyanidin dimer [(−)-epicatechin-(4,8)-(−)-epicatechin]. This yield was used to calculate the total proanthocyanidin concentration of the wine.

2.3.7. Polysaccharide analysis

The wine samples were processed using the methodology described by Ayestarán et al. [49]. Briefly, 10 mL of wine was centrifuged (8500 rpm, 7029xg, 20 min) with a Biofuge Primo centrifuge (Heraeus, Hanau, Germany), and the supernatant was concentrated to a final volume of 2 mL using a vacuum evaporator (Univapo 100ECH, Uniequip, Martinsried, Germany). Total soluble polysaccharides were precipitated by adding 10 mL of cold acidified ethanol (0.3 M HCl in absolute ethanol) and then kept for 24 h at 4 °C. Next, the samples were centrifuged (8500 rpm, 7029×g 10 min, 4 °C), the supernatants were discarded, and the pellets were washed four times with cold ethanol to remove the interfering materials. Finally, the precipitates were dissolved in 1 mL of ultrapure water, frozen to − 80 °C, and freeze-dried using an Alpha 1–4 (Martin Christ, Osterode am Harz, Germany) freeze drier. To determine the molecular distribution and quantify the polysaccharides obtained from the wines, the soluble fractions were analyzed by high-resolution size exclusion chromatography (HRSEC) using a refraction index detector (RID). The lyophilized samples were resuspended in 1 mL of 30 mM ammonium formate and filtered

through a 0.45 μm pore size nylon membrane, after which 100 μL was injected into the column. Separation was carried out at 20 °C using two Shodex OHpak SB-803 HQ and SB-804 HQ columns connected in series (300 mm × 8 mm i.d.; Showa Denko, Japan). The mobile phase consisted of an aqueous solution of ammonium formate (30 mM), applied with a constant flow of 0.6 mL/min for 60 min, and a RID cell at a temperature of 35 °C. The molecular weight distribution of the wine fractions was followed by calibration with pullulan and dextran standards of different molecular weights (see above). The polysaccharides were quantified on the basis of the peak area for each fraction, using the external standard method with pectin and dextran commercial standards. The calibration curve was obtained by injecting standard solutions, under the same conditions as for the samples analyzed, in the range between 0 and 2 g/L.

2.3.8. Sensory analysis

All the wines were tasted by a group of twelve assessors (6 male and 6 female) ages ranging from 22 to 56 years old. Assessors were recruited among winemakers and professors from Faculty of Agronomy (University of the Republic, Uruguay) and Higher School of Viticulture (Professional Technical Education Board, Uruguay) and selected according to their availability to participate in the study. All the assessors had extensive previous experience in sensory evaluation of wines as part of their regular jobs. Assessors attended a 20-min previous training session; therefore, they could homogenize criteria of evaluations.

Sensory evaluation was carried out under laboratory conditions in the Enology Laboratory of the Faculty of Agronomy (University of the Republic, Uruguay). Pinot noir and Tannat wines were evaluated after 6 months of their respective bottling. Assessors evaluated twelve wines per cultivar (four treatments for three repetitions) blindly and randomly. For each wine, assessors evaluated eight sensorial attributes on a continuous scale from 1 to 10: color intensity, quotient between the red and the yellow components of the color, fruitiness, acidity, astringency, bitterness and mouthfeel.

The values indicate the intensity of the sensation for each attribute. Two trial were performed for each cultivar, the first compare the control wines (CW) with the reduced-alcohol wines (RAW), while the second compare wines from traditional maceration (TM) with those from prefermentative hot maceration (HM).

2.3.9. Statistical analysis

All the data are expressed as the arithmetic average ± standard deviation of three replicates. One-factor ANOVA test was performed to compare the composition of the musts of the grapes of the first and the second harvest of each cultivar. Two-factor ANOVA was carried out to compare the composition of the different wines depending on whether or not alcohol reduction was applied and on the winemaking technique (traditional or hot maceration). ANOVA tests were carried out with XLSTAT (version 2017) software, and multiple comparisons between samples was performed using the Tukey test. Principal component analysis (PCA) was applied to determine the association between all the variables (color, anthocyanin, proanthocyanidin and polysaccharide composition) and the treatments evaluated (CW-TM, RAW-TM, CW-HM, RAW-HM) for each cultivar.

2.4. RESULTS AND DISCUSION

2.4.1. General composition of the grapes and wines

Table 1 shows the sugar concentration, titratable acidity and pH of the grape juices of both cultivars from the unripe grapes harvested during veraison (1st harvest) and from the grapes harvested at technological maturity (2nd harvest). As expected, the sugar concentration and pH increased throughout the maturation time, whereas titratable acidity decreased.

The indicators of grape phenolic maturity were not determined in grapes from the 1st harvest because only the skins and seeds of mature grape contributed with phenolic compounds during maceration. Tannat grapes would be expected to have a higher potential than Pinot Noir grapes for total phenolic compounds (TPI) and potential anthocyanins (ApH1). However, the extractability (EA%) was higher in Tannat grapes than in Pinot Noir grapes, which indicates that the proportion of

anthocyanins released to the wine during maceration in respect to the total content of anthocyanins will be lower with Tannat than with Pinot Noir grapes. This data is in agreement with the characteristic described for Tannat in Uruguay [50].

Table 1. Composition of the grape juices of Pinot Noir and Tannat harvested at the two different ripening stages

Cultivar	Ripening stage	Sugar content (g/L)	Titrable acidity (g/L)	pH	TPI	ApH1 (mg/L)	EA%
Pinot Noir	1st harvest	129 ± 0 b	12.25 ± 0.09 b	3.10 ± 0.05 b	–	–	–
	2nd harvest	243 ± 1 a	3.62 ± 0.18 a	3.52 ± 0.10 a	33.5 ± 3.7	939 ± 18	30.8 ± 1.8
Tannat	1st harvest	175 ± 2 b	8.43 ± 0.12 b	3.12 ± 0.04 b	–	–	–
	2nd harvest	243 ± 3 a	4.51 ± 0.07 a	3.31 ± 0.03 a	47.5 ± 0.5	2258 ± 145	51.6 ± 2.6

All data are expressed as the average values of 4 replicates ± standard deviation. Different letters indicate the existence of statistical differences ($p \leq 0.05$). Titratable acidity is expressed in g of sulfuric acid/L. ApH1 corresponds to the potential anthocyanins grape content. TPI corresponds to the grape total phenolic index. EA% corresponds to the anthocyanin extractability and is expressed in percentage.

Table 2 shows the ethanol content, total acidity, pH, total anthocyanin concentration (measured by spectrophotometry), and total phenolic index (TPI) of the different wines made from Pinot Noir and Tannat. With both cultivars, the ethanol content and pH of the wines made with must obtained from grapes harvested at technological maturity (CW) were significantly higher than those of wines made from blended musts (RAW), whereas titratable acidity was significantly lower. Specifically, the ethanol content of the wines decreased 21% in the case of Pinot Noir and 10% in the case of Tannat; titratable acidity increased 72% in the case of Pinot Noir and 25% in the case of Tannat, and finally pH was reduced 0.42 units in the case of Pinot Noir and 0.26 in the case of Tannat. These results were expected since the substitution of the grape juice with that from unripe grapes determined a decrease in sugar content and an increase in acidity. This data is in accordance with the works of Kontoudakis et al. [30] and Schelezki et al. [17, 18] in which they applied a comparable technique. In contrast, no significant differences in ethanol or titratable acidity were found between wines that underwent traditional maceration (TM) and those that underwent prefermentative hot maceration (HM) in any of the studied cultivars. The pH also showed similar values in TM and HM wines in the

case of Tannat but was significant higher in HM wine than in TM wine in the case of Pinot Noir probably because hot maceration can boost the extraction of potassium from the skins [51]. As it is known, potassium can neutralize part of the tartaric acid which results in a decrease in the titratable acidity and an increase in the pH of the wines.

Table 2. General analytic parameters of Pinot Noir and Tannat wines

Cultivar	Parameter	Initial grape juice	Winemaking technique		Alcohol reduction
			TM	HM	
Pinot Noir	Ethanol content (% v/v)	CW	14.3 ± 0.1 α. A	14.7 ± 0.2 α. A	**14.5 ± 0.2 a**
		RAW	11.5 ± 0.1 β. A	11.5 ± 0.2 β. A	**11.5 ± 0.2 b**
		Winemaking technique	**12.9 ± 1.6 a**	**13.1 ± 1.7 a**	***p* interaction value = 0.1202**
	Titratable Acidity (g/L)	CW	3.11 ± 0.04 β. A	3.11 ± 0.01 β. A	**3.11 ± 0.03 b**
		RAW	5.42 ± 0.07 α. A	5.28 ± 0.16 α. A	**5.35 ± 0.16 a**
		Winemaking technique	**4.26 ± 1.26 a**	**4.20 ± 1.38 a**	***p* interaction value = 0.6103**
	pH	CW	3.67 ± 0.02 α. B	3.86 ± 0.02 α. A	**3.82 ± 0.05 a**
		RAW	3.41 ± 0.01 β. A	3.38 ± 0.02 β. A	**3.40 ± 0.02 b**
		Winemaking technique	**3.58 ± 0.21 a**	**3.63 ± 0.25 a**	***p* interaction value = 0.0118**
	Total anthocyanins (mg/L)	CW	209 ± 8 α. B	311 ± 21 α. A	**260 ± 18 a**
		RAW	189 ± 21 α. B	292 ± 28 α. A	**241 ± 24 a**
		Winemaking technique	**199 ± 57 a**	**302 ± 61 b**	***p* interaction value = 0.9068**
	TPI	CW	32.0 ± 0.5 α B	61.7 ± 1.5 α. A	**46.8 ± 16.3 a**
		RAW	32.7 ± 0.2 α. B	62.2 ± 0.5 α. A	**47.4 ± 16.2 a**
		Winemaking technique	**32.3 ± 0.5 a**	**62.0 ± 1.1 b**	***p* interaction value = 0.8495**
Tannat	Ethanol content (% v/v)	CW	14.6 ± 0.2 α. A	14.8 ± 0.1 α. A	**14.7 ± 0.2 a**
		RAW	13.1 ± 0.2 β. A	13.3 ± 0.1 β. A	**13.2 ± 0.2 b**
		Winemaking technique	**13.9 ± 0.8 a**	**14.1 ± 0.8 a**	***p* interaction value = 0.8258**
	Titratable Acidity (g/L)	CW	3.88 ± 0.19 α. A	3.76 ± 0.03 β. A	**3.82 ± 0.12 b**
		RAW	4.51 ± 0.25 α. A	5.06 ± 0.01 α. A	**4.79 ± 0.56 a**
		Winemaking technique	**4.20 ± 0.70 a**	**4.41 ± 0.71 a**	***p* interaction value = 0.2643**
	pH	CW	4.01 ± 0.13 α. A	4.09 ± 0.01 α. A	**4.05 ± 0.05 a**
		RAW	3.85 ± 0.06 β. A	3.73 ± 0.01 β. A	**3.79 ± 0.12 b**
		Winemaking technique	**3.93 ± 0.14 a**	**3.91 ± 0.19 a**	***p* interaction value = 0.0958**
	Total anthocyanins (mg/L)	CW	796 ± 35 α. B	1054 ± 67 α. A	**925 ± 147 a**
		RAW	794 ± 44 α. B	1038 ± 26 α. A	**916 ± 125 a**
		Winemaking technique	**795 ± 40 b**	**1046 ± 46 a**	***p* interaction value = 0.8430**
	TPI	CW	69.9 ± 3.6 α. B	95.5 ± 4.4 α. A	**82.7 ± 14.3 a**
		RAW	63.6 ± 1.6 α. B	92.5 ± 0.7 α. A	**78.0 ± 15.8 a**
		Winemaking technique	**66.8 ± 3.6 b**	**94.0 ± 3.3 a**	***p* interaction value = 0.2623**

The data in bold in rows correspond to the average of both winemaking techniques (TM and HM). The data in in bold in columns correspond to the average of control and reduced alcohol wines (CW and RAW) All data are expressed as the average values of 3 replicates ± standard deviation. Different Greek letters indicate the existence of statistical differences ($p < 0.05$) between control and reduced alcohol wines. Different Capital Roman letters indicate the existence of statistical differences ($p < 0.05$) between traditional and hot macerations. CW control wine, RAW reduced alcohol wines, TM traditional maceration, HM hot maceration. Titratable acidity is expressed in g of sulfuric acid/L. TPI total phenolic index

No differences were observed in the total anthocyanin concentration and total phenolic index (TPI) between CW and RAW wines in both cultivars. As expected, the replacement of the grape juice did not modify the anthocyanin concentration and total polyphenol index. These results agree with those obtained in the works carried out by Kontoudakis et al. [30] and Rolle et al. [52], applying different techniques of substitution of must which are comparable to the one evaluated in this research. In contrast, the anthocyanin concentration and TPI in wines from HM were significantly higher than in TM wines. These results are in agreement with previous studies [35, 37, 38, 53] and confirm that this technique is a useful tool for improving polyphenol extraction. The different increases in TPI by hot preferential maceration may be related to the different extractability of polyphenols in the grapes of both varieties. Tannat grapes had significantly higher values of EA%, which means that these grapes had cellular structures that made it difficult to extract anthocyanins. In proportional terms, the Tannat wines produced by hot prefermentative maceration had an increase of 41% in the TPI, while the Pinot Noir wines produced by this winemaking technique increased by 92%.

2.4.2. Wine color

The color parameters of the wines from both cultivars and both treatments are shown in Table 3. In the case of Pinot Noir, RAW wines have a deeper red color because CI and C* were significantly higher and L* significantly lower than in CW wines. Tannat wines showed a similar behavior, although the differences were not significant in all the color parameters. These results can be explained by the lower pH of RAW wines. As it is well known, when the pH decreases, the balance between the different forms of the anthocyanins is displaced towards the red form, the flavylium cation [54, 55]. Once again, these results agree with those obtained by Kontoudakis et al. [30] in a similar trial. The hue (H*) had a different behavior depending on the cultivar. In the case of Pinot Noir there was an increase in H* in the RAW wines, but in the case of Tannat, no significant differences were observed.

Moreover, pre-fermentative hot maceration produced wines with a deeper color. Specifically, CI was significantly higher and L* significantly lower in HM wines from both cultivars. In the case of the Pinot Noir wines, C* was significantly higher in the HM wine, but this difference was not significant in the case of the Tannat wines. These results can be explained by the increased extraction of phenolic compounds, particularly anthocyanins, during the pre-fermentative heating step, as described above (Table 2). Other authors have described similar results [35, 56]. In the case of the Pinot Noir, the color of the HM wine was significantly more bluish than the TM wine, but this trend was not observed in the Tannat wines.

Table 3. Color parameters of Pinot Noir and Tannat wines

Cultivar	Parameter	Initial grape juice	Winemaking technique		Alcohol reduction
			TM	HM	
Pinot Noir	CI	CW	11.0 ± 0.4 β. B	20.1 ± 0.4 β. A	**15.6 ± 4.8 a**
		RAW	13.0 ± 0.3 α. B	21.5 ± 0.6 α. A	**17.3 ± 4.4 a**
		Winemaking technique	**12.0 ± 1.1 b**	**20.8 ± 0.8 a**	***p* interaction value = 0.0706**
	*L**	CW	70.9 ± 0.9 α. A	50.9 ± 0.8 α. B	**60.9 ± 10.4 a**
		RAW	63.2 ± 0.8 β. A	47.8 ± 1.1 β. B	**55.2 ± 8.4 a**
		Winemaking technique	**67.0 ± 4.1 a**	**49.0 ± 2.2 b**	***p* interaction value = 0.0001**
	*C**	CW	20.9 ± 0.8 β. B	24.8 ± 0.9 β. A	**22.8 ± 2.3 b**
		RAW	31.2 ± 0.3 α. B	35.2 ± 1.9 α. A	**33.2 ± 2.4 a**
		Winemaking technique	**25.9 ± 5.5 a**	**30.2 ± 5.6 a**	***p* interaction value = 0.8543**
	*H**	CW	17.5 ± 1.8 β. B	5.21 ± 0.9 β. A	**11.4 ± 6.6 b**
		RAW	354.9 ± 0.8 α. A	357.2 ± 1.8 α. A	**356.0 ± 1.8 a**
		Winemaking technique	**11.3 ± 6.7 a**	**4.1 ± 1.9 a**	***p* interaction value = 0.0001**
Tannat	CI	CW	26.7 ± 0.9 β. B	34.7 ± 1.5 β. A	**30.7 ± 4.3 a**
		RAW	29.9 ± 2.1 α. B	37.7 ± 1.6 α. A	**33.8 ± 4.4 a**
		Winemaking technique	**28.3 ± 2.3 b**	**36.2 ± 2.1 a**	***p* interaction value = 0.9090**
	*L**	CW	38.6 ± 1.3 α. A	27.2 ± 1.6 α. B	**32.9 ± 6.2 a**
		RAW	34.8 ± 1.7 β. A	25.3 ± 1.1 α. B	**26.3 ± 5.2 a**
		Winemaking technique	**36.7 ± 2.4 a**	**26.3 ± 1.7 b**	***p* interaction value = 0.1349**
	*C**	CW	42.4 ± 0.5 α. A	43.8 ± 2.2 β. A	**43.1 ± 1.2 b**
		RAW	45.6 ± 4.2 α. A	48.2 ± 1.6 α. A	**46.9 ± 2.3 a**
		Winemaking technique	**44.7 ± 3.0 a**	**45.3 ± 3.6 a**	***p* interaction value = 0.0672**
	*H**	CW	346.6 ± 1.0 α. A	348.1 ± 0.8 α. A	**347.3 ± 1.2 a**
		RAW	348.7 ± 1.8 α. A	348.9 ± 2.3 α. A	**348.8 ± 2.0 a**
		Winemaking technique	**347.6 ± 1.5 a**	**348.5 ± 2.0 a**	***p* interaction value = 0.3188**

The data in bold in rows correspond to the average of both winemaking techniques (TM and HM). The data in in bold in columns correspond to the average of control and reduced alcohol wines (CW and RAW) All data are expressed as the average values of 3 replicates ± standard deviation. Different Greek letters indicate the existence of statistical differences ($p < 0.05$) between control and reduced alcohol wines. Different Capital Roman letters indicate the existence of statistical differences ($p < 0.05$) between traditional and hot macerations. CW control wine, RAW reduced alcohol wines, TM traditional maceration, HM hot maceration, CI color intensity, *L** lightness, *C** chroma, *H** hue.

2.4.3. Anthocyanin composition of the wines

Table 4 shows the anthocyanin composition of the Pinot Noir and Tannat wines. The total anthocyanin concentrations determined by HPLC-DAD were lower than those measured by spectrophotometry. This is to be expected because spectrophotometric analysis includes the contribution from other pigments in the measurement, and therefore, overestimates the total anthocyanin concentration, whereas the HPLC-DAD analysis only was used for quantifying free anthocyanins. The Pinot Noir wines had lower anthocyanin concentrations and contained only the five common anthocyanin-3-O-monoglucosides (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside and malvidin-3-O-glucoside) in all winemaking conditions. The absence of acylated anthocyanins in Pinot Noir wines is well known [57–59]. Indeed, this feature could serve as a taxonomic marker in differentiating Pinot Noir wines from wines produced with other cultivars [60]. In contrast, Tannat wines had a high proportion of nonacylated glucosides and low acylated anthocyanin (acetylated and coumaroylated), as has been previously reported [61].

The total anthocyanin concentration estimated by HPLCDAD does not tally with the changes observed in the anthocyanin concentration estimated by spectrophotometry (Table 2). This different behavior may be because spectrophotometry detects red pigments other than free anthocyanins. The total anthocyanin concentration of the RAW wines from Pinot Noir was significantly lower than for the CW wines, especially in the case of the hot macerated wines. These data also suggest that the lower pH brought about by the substitution of the grape juice favored the formation of these red pigments at high temperature. Nevertheless, in Tannat wines, the changes in total anthocyanins caused by alcohol reduction can be considered somewhat erratic because a significant increase was observed in the RAW wine from traditional maceration whereas the opposite was observed in the RAW wine from hot maceration. This trend was overall similar in monoglucosides, acetylated and coumaroylated anthocyanins. It should be considered that the replacement by green grape juice would not necessarily imply

losses of anthocyanins since this operation is carried out before the maceration. However, the discarding of part of the juice of ripe grapes during the substitution, could imply a loss of anthocyanins since these are easily extracted from the skins during the crushed and the short time of contact with the must. So, the fraction of grape juice removed could contain a considerable amount of anthocyanins [52]. Also, these results could also be linked to the favored release of copigments during fermentation, which protects anthocyanins from oxidation [62].

Cultivar	Parameter	Initial grape juice	Winemaking technique		Alcohol reduction
			TM	HM	
Pinot Noir	Total anthocyanins (mg/L)	CW	78.1 ± 2.8 α. A	79.7 ± 6.7 α. A	**78.9 ± 4.9 a**
		RAW	50.2 ± 4.9 β. A	30.7 ± 4.3 β. B	**41.5 ± 10.4 b**
		Winemaking technique	**64.1 ± 15.8 a**	**56.2 ± 26.2 b**	***p* interaction value = 0.0108**
	Anthocyanidin-3-monoglucosides (mg/L)	CW	78.1 ± 2.8 α. A	79.7 ± 6.7 α. A	**78.9 ± 4.9 a**
		RAW	50.2 ± 4.9 β. A	30.7 ± 4.3 β. B	**41.5 ± 10.4 b**
		Winemaking technique	**64.1 ± 15.8 a**	**56.2 ± 26.2 b**	***p* interaction value = 0.0108**
	Acetylated anthocyanins (mg/L)	CW	nd	nd	–
		RAW	nd	nd	–
		Winemaking technique	nd	nd	–
	p-Coumarylated anthocyanins (mg/L)	CW	nd	nd	–
		RAW	nd	nd	–
		Winemaking technique	nd	nd	–
Tannat	Total anthocyanins (mg/L)	CW	396.6 ± 6.5 β. B	528.1 ± 17.7 α. A	**462.5 ± 73.0 a**
		RAW	427.7 ± 3.8 α. B	469.7 ± 22.1 β. A	**448.7 ± 27.1 a**
		Winemaking technique	**412.6 ± 17.9 b**	**498.9 ± 36.1 a**	***p* interaction value = 0.0008**
	Anthocyanidin-3-monoglucosides (mg/L)	CW	296.5 ± 6.5 β. B	384.8 ± 17.7 α. A	**340.7 ± 49.9 a**
		RAW	330.0 ± 0.4 α. A	347.4 ± 15.7 β. A	**338.7 ± 13.8 a**
		Winemaking technique	**313.2 ± 19.1 b**	**366.2 ± 25.4 a**	***p* interaction value = 0.0012**
	Acetylated anthocyanins (mg/L)	CW	83.8 ± 2.1 α. B	113.1 ± 2.5 α. A	**98.5 ± 16.2 a**
		RAW	77.5 ± 2.5 α. B	99.5 ± 6.3 β. A	**88.5 ± 12.8 a**
		Winemaking technique	**80.6 ± 4.2 b**	**106.3 ± 8.6 a**	***p* interaction value = 0.1424**
	p-Coumarylated anthocyanins (mg/L)	CW	16.3 ± 1.0 β. B	30.1 ± 2.5 α. A	**23.2 ± 7.7 a**
		RAW	20.2 ± 1.5 α. A	22.7 ± 2.0 β. A	**18.2 ± 2.1 a**
		Winemaking technique	**18.2 ± 2.4 b**	**26.4 ± 4.5 a**	***p* interaction value = 0.0006**

Table 4. Anthocyanin quantification by HPLC-DAD of Pinot Noir y Tannat wines

The data in bold in rows correspond to the average of both winemaking techniques (TM and HM). The data in in bold in columns correspond to the average of control and reduced alcohol wines (CW and RAW) All data are expressed as the average values of 3 replicates ± standard deviation. Different Greek letters indicate the existence of statistical differences ($p < 0.05$) between control and reduced alcohol wines. Different Capital Roman letters indicate the existence of statistical differences ($p < 0.05$) between traditional and hot macerations. CW control wine, RAW reduced alcohol wines, TM traditional maceration, HM hot maceration.

The effect of prefermentative hot maceration on the total anthocyanin concentration estimated by HPLC-DAD was not clear because no significant differences were found in the CW wines and a significant decrease was even observed in the RAW wines. As commented above (Table 2), hot maceration produced a significant increase in the total anthocyanin concentration estimated by spectrophotometry, which contradicts these new results. Once again, this different behavior is probably related to the fact that most of the anthocyanins were transformed into new red pigments that are not detected by HPLC-DAD. In contrast, the total anthocyanin concentration estimated by HPLC-DAD for the Tannat wines increased significantly when hot maceration was applied, and this trend was in general observed in monoglucosides and acylated anthocyanins.

2.4.4. Wine proanthocyanidins

The results of the analysis of wine proanthocyanidins obtained by acid depolymerization in the presence of excess phloroglucinol are shown in Table 5. The total proanthocyanidin concentration of Pinot Noir and Tannat wines was not affected by the alcohol reduction. The substitution of mature grape juice with that obtained from unripe grapes did not affect the proanthocyanidin concentration or composition in either cultivar. In fact, the mean degree of polymerization (mDP) and the proportions of prodelphinidins and galloylation were similar in both CW and RAW wines. These results agree with those reported by Kontoudakis et al. [30] for a comparable method of alcohol reduction and confirm that this procedure does not alter the tannin composition of the wines.

Table 5. Proanthocyanidin analysis by phloroglucinolysis of pinot noir and Tannat wines

Cultivar	Parameter	Initial grape juice	Winemaking technique		AR effect
			TM	HM	
Pinot Noir	Total PA (mg/L)	CW	278 ± 11 α. B	525.8 ± 50.8 α. A	**402.0 ± 139.3 a**
		RAW	271.1 ± 9.3 α. B	531.6 ± 78.8 α. A	**401.4 ± 151.2 a**
		WT effect	**275.8 ± 11.3 b**	**528.6 ± 59.5 a**	***p* interaction value = 0.8130**
	mDP	CW	3.79 ± 0.02 α. B	4.38 ± 0.35 α. A	**4.09 ± 0.39 a**
		RAW	3.56 ± 0.01 α. A	3.54 ± 0.02 α. A	**3.55 ± 0.10 b**
		WT effect	**3.68 ± 0.14 a**	**3.96 ± 0.53 a**	***p* interaction value = 0.0350**
	%PD	CW	8.59 ± 0.91 α. A	9.91 ± 0.98 α. A	**9.25 ± 1.34 a**
		RAW	11.09 ± 0.97 α. A	9.89 ± 0.33 α. A	**10.45 ± 0.92 a**
		WT effect	**9.84 ± 1.65 a**	**9.90 ± 0.90 a**	***p* interaction value = 0.0663**
	%Gal	CW	11.35 ± 0.52 α. B	5.58 ± 0.44 α. A	**8.46 ± 3.20 a**
		RAW	10.54 ± 0.38 α. B	5.76 ± 1.02 α. A	**8.15 ± 2.70 a**
		WT effect	**10.91 ± 0.65 a**	**5.67 ± 0.71 b**	***p* interaction value = 0.2351**
Tannat	Total PA (mg/L)	CW	652.3 ± 24.6 α. B	906.5 ± 73.2 α. A	**779.3 ± 147.9 a**
		RAW	657.1 ± 67.3 α. B	897.4 ± 76.1 α. A	**777.2 ± 146.5 a**
		WT effect	**655.6 ± 46.7 b**	**902.9 ± 67.0 a**	***p* interaction value = 0.8572**
	mDP	CW	5.36 ± 0.83 α. A	5.68 ± 0.19 α. A	**5.80 ± 0.67 a**
		RAW	5.06 ± 0.66 α. A	5.75 ± 0.32 α. A	**5.40 ± 0.60 a**
		WT effect	**5.49 ± 0.90 a**	**5.42 ± 0.24 a**	***p* interaction value = 0.2414**
	%PD	CW	16.7 ± 2.9 α. A	21.5 ± 2.1 α. A	**19.1 ± 3.3 a**
		RAW	22.12 ± 2.8 α. A	24.9 ± 2.9 α. A	**23.5 ± 2.9 a**
		WT effect	**19.4 ± 3.7 a**	**23.1 ± 2.9 a**	***p* interaction value = 0.5038**
	%Gal	CW	4.16 ± 0.14 α. A	3.13 ± 0.27 α. B	**3.66 ± 0.62 a**
		RAW	4.21 ± 0.42 α. A	3.07 ± 0.28 α. B	**3.64 ± 0.70 a**
		WT effect	**4.20 ± 0.29 a**	**3.10 ± 0.25 b**	***p* interaction value = 0.8219**

The data in bold in rows correspond to the average of both winemaking techniques (TM and HM). The data in in bold in columns correspond to the average of control and reduced alcohol wines (CW and RAW) All data are expressed as the average values of 3 replicates ± standard deviation. Different Greek letters indicate the existence of statistical differences ($p < 0.05$) between control and reduced alcohol wines. Different Capital Roman letters indicate the existence of statistical differences ($p < 0.05$) between traditional and hot macerations. CW control wine, RAW reduced alcohol wines, TM traditional maceration, HM hot maceration. AR effect of alcohol reduction, WT effect of winemaking technique, PA proanthocyanidins, mDP mean degree of polymerization, PD percentage ofprodelphinidins, %GAL percentage of galloylation

The total proanthocyanidin concentration was significantly higher in all the wines produced by pre-fermentative hot maceration regardless of the cultivar and the alcohol level. These results confirmed those available in the literature [35]. The results obtained by phloroglucinolysis should be considered with caution in the case of hot macerated wines since this treatment can cause oxidation and consequently decrease the effectiveness of the depolymerization of proanthocyanidins. In any case, the mDP and the proportion of prodelphinidins (%PD) were similar among the HM

and TM wines, the only exception was the Pinot Noir control wine in which the mDP of the HM wine was slightly but significantly higher than in the corresponding TM wine. In contrast, the percentage of galloylation (%GAL) was significantly lower in all the HM wines than the TM wines regardless of the cultivar and whether or not the mature grape juice had been replaced by the unripe grape juice. Since the %GAL is lower in skin tannins than in seed tannins [63], these data suggest that HM increased the extraction of skin tannins. However, skin tannins have a greater mDP [64] and prodelphinidins [65], although the differences were not significant. This lower %GAL of the HM wine proanthocyanidins is very interesting because it has been proven that a lower %GAL implies lower astringency [66, 67]. Therefore, although HM wines have a significantly higher concentration of proanthocyanidins than TM wines, they have a lower proportion of seed proanthocyanidins which are theoretically more astringent.

2.4.5. Polysaccharide concentration of the wines

Table 6 shows the polysaccharide concentration of the different wines. In general, the alcohol reduction strategy does not affect the total polysaccharide concentration regardless of the cultivar or maceration technique, the only exception was the TM wine of Pinot Noir in which the total polysaccharide concentration of RAW wine was significantly lower than in the CW wine. This difference was mainly due to the medium and low molecular weight fractions (MMW and LMW). These data suggest that the polysaccharide concentration of the unripe grape juice from Pinot Noir was lower than the well-ripened grape juice, whereas in the case of the Tannat wines it seems that both grape juices have similar concentrations. However, when prefermentative hot maceration was applied, the difference between the total polysaccharide concentrations of the CW and RAW wines disappears, probably because this technique favors the extraction of polysaccharides from grape skins, thereby minimizing the differences. The effect of prefermentative hot maceration on the total polysaccharide extraction from skins becomes evident when comparing TM wines with HM wines from both cultivars. All the HM wines have significantly higher concentrations of total polysaccharides than the corresponding TM wines, and

these differences were significant in all molecular weight fractions with the only exception of Pinot noir HMW. It, therefore, seems clear that prefermentative hot maceration favors polysaccharide extraction from skins. Similar results were obtained by Doco, Williams & Cheynier [68] in wines obtained with flash thermo-treatment.

Table 6. Polysaccharide Analysis by HRSEC of Pinot Noir and Tannat Wines

Cultivar	Parameter	Initial grape juice	Winemaking technique		AR effect
			TM	HM	
Pinot Noir	Total polysaccharides (mg/L)	CW	730.6 ± 7.5 α. B	1090.0 ± 72.0 α. A	**910.3 ± 202.1 a**
		RAW	513.5 ± 13.0 β. B	1036.4 ± 76.6 α. A	**775.0 ± 290.6 a**
		WT effect	**622.1 ± 119.3 b**	**1063.2 ± 72.7 a**	***p* interaction value = 0.0287**
	HMW polysaccharides (mg/L)	CW	168.9 ± 1.6 α. A	191.6 ± 17.6 α. A	**180.1 ± 16.6 a**
		RAW	149.6 ± 15.5 α. A	170.8 ± 20.6 α. A	**160.2 ± 20.1 a**
		WT effect	**159.2 ± 14.3 a**	**181.2 ± 20.4 a**	***p* interaction value = 0.9228**
	MMW polysaccharides (mg/L)	CW	481.7 ± 10.4 α. B	802.5 ± 54 0.1 α. A	**642.1 ± 179.2 a**
		RAW	328.8 ± 11.5 β. B	723.0 ± 51.9 α. A	**525.9 ± 218.5 a**
		WT effect	**405.6 ± 84.4 b**	**762.8 ± 64.4 a**	***p* interaction value = 0.1357**
	LMW polysaccharides (mg/L)	CW	80.2 ± 5.3 α. B	96.0 ± 4.1 β. A	**88.1 ± 9.9 a**
		RAW	35.0 ± 4.1 β. B	141.8 ± 6.9 α. A	**88.4 ± 58.8 a**
		WT effect	**57.6 ± 25.2 b**	**119 ± 25.6 a**	***p* interaction value = 0.0001**
Tannat	Total polysaccharides (mg/L)	CW	556.2 ± 12.5 α. B	1229.1 ± 152.9 α. A	**892.7 ± 381.0 a**
		RAW	558.6 ± 28.8 α. B	1218.4 ± 150.8 α. A	**888.5 ± 374.0 a**
		WT effect	**557.4 ± 20.7 b**	**1223.7 ± 135.5 a**	***p* interaction value = 0.9191**
	HMW polysaccharides (mg/L)	CW	73.6 ± 5.1 α. B	86.5 ± 4.2 α. A	**80.0 ± 8.5 a**
		RAW	70.5 ± 1.0 α. B	95.1 ± 9.1 α. A	**80.8 ± 14.7 a**
		WT effect	**72.0 ± 4.9 b**	**90.8 ± 7.9 a**	***p* interaction value = 0.1247**
	MMW polysaccharides (mg/L)	CW	397.4 ± 5.1 α. B	1001.2 ± 135.1 α. A	**699.6 ± 341.6 a**
		RAW	424.8 ± 37.8 α. B	1003.3 ± 118.8 α. A	**714.2 ± 326.5 a**
		WT effect	**411.0 ± 28.5 b**	**1002.5 ± 113.7 a**	***p* interaction value = 0.8200**
	LMW polysaccharides (mg/L)	CW	85.3 ± 2.5 α. B	141.4 ± 12.8 α. A	**113.4 ± 31.9 a**
		RAW	63.3 ± 9.3 α. B	119.9 ± 25.4 α. A	**91.6 ± 35.4 a**
		WT effect	**74.3 ± 13.5 b**	**130.7 ± 21.5 a**	***p* interaction value = 0.9792**

The data in bold in rows correspond to the average of both winemaking techniques (TM and HM). The data in bold in columns correspond to the average of control and reduced alcohol wines (CW and RAW). All data are expressed as the average values of 3 replicates ± standard deviation. Different Greek letters indicate the existence of statistical differences ($p < 0.05$) between control and reduced alcohol wines. Different Capital Roman letters indicate the existence of statistical differences ($p < 0.05$) between traditional and hot macerations. CW control wine, RAW reduced alcohol wines, TM traditional maceration; HM hot maceration. AR effect of alcohol reduction WT effect of winemaking technique. HMW high molecular weight, MMW medium molecular weight, LMW low molecular weight

2.4.6. Sensorial attributes of the wines

Figure 1 shows the influence of the alcohol reduction process on the sensory attributes of Pinot Noir (Fig. 1a) and Tannat (Fig. 1b) wines represented as two spider-web diagrams. The results are presented as the average of 12 values for each sensory attribute, regardless of the maceration winemaking technique.

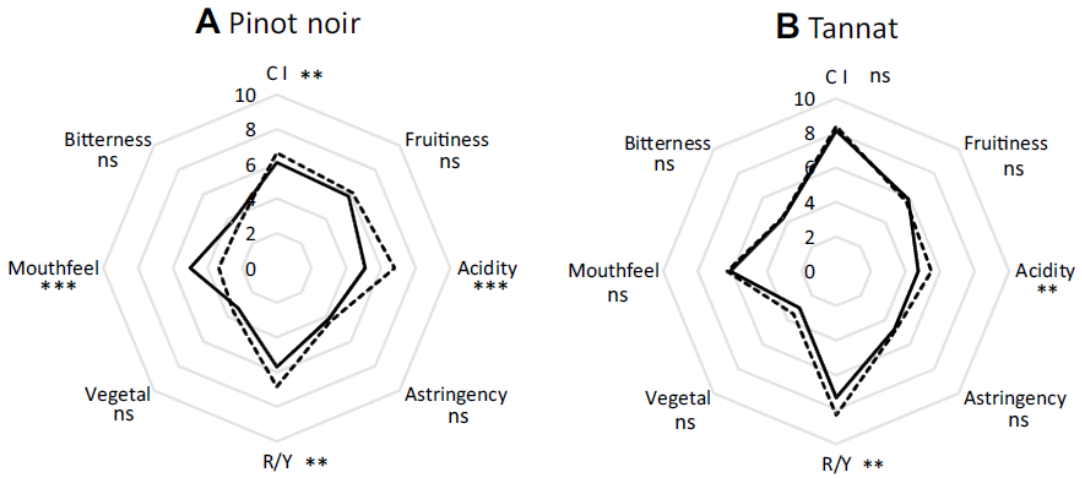

Fig. 1 Influence of alcohol reduction on the sensory attributes of Pinot Noir and Tannat Wines. All data are expressed as the average values of 3 replicates ± standard deviation. "—" Average value of both control wines; "---" Average value of both reduced alcohol wines. CI color intensity, R/Y quotient between red and yellow component. ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$

In general terms, the tasters found that the color intensity (CI) of the RAW Pinot Noir wines was significantly higher than the CW wines. However, they did not find any differences in the CI of RAW and CW in the case of the Tannat wines. In contrast, the RAW wines were more bluish (R/Y) than the CW wines in both cultivars. RAW wines from both cultivars were more acidic than the corresponding CW wines. In the case of the Pinot Noir wines, testers reported that the mouthfeel of the CW wines scored higher than the RAW wines. Finally, no differences in the other attributes were pointed out by the tasters. It is necessary to highlight that RAW wines did not present significant differences in astringent, bitter or vegetal notes although the used juice of unripe grapes was not treated with charcoal and bentonite as in the work of Kountoudakis et al. [30].

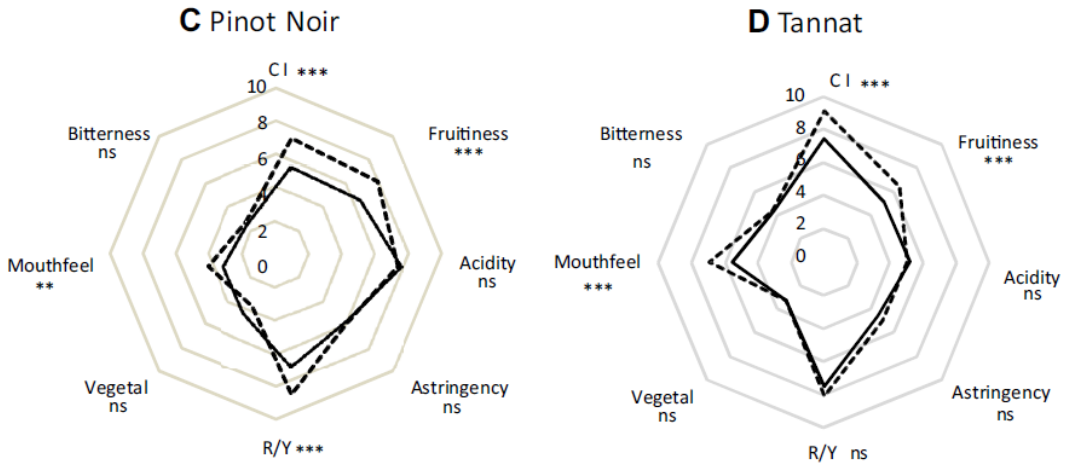

Fig. 2 Influence of type of maceration on the sensory attributes of Pinot Noir and Tannat Wines. All data are expressed as the average values of 3 replicates ± standard deviation. "—" Average value of both control wines; "---" Average value of both reduced alcohol wines. CI color intensity, R/Y quotient between red and yellow component. ***p < 0.005, **p < 0.01, *p < 0.05

Figure 2 compares the sensory attributes of the wines according to the maceration technique of the two initial must compositions. In general, the pre-fermentative maceration increases color intensity, the red-to-yellow ratio (significant only in Pinot Noir) and the fruit notes, achieving wines that are more equilibrated than those produced by traditional maceration techniques. According to the analytical results, from a sensorial point of view the prefermentative hot maceration does not increase the astringency or the bitterness of the wines.

2.4.7. Principal component analysis

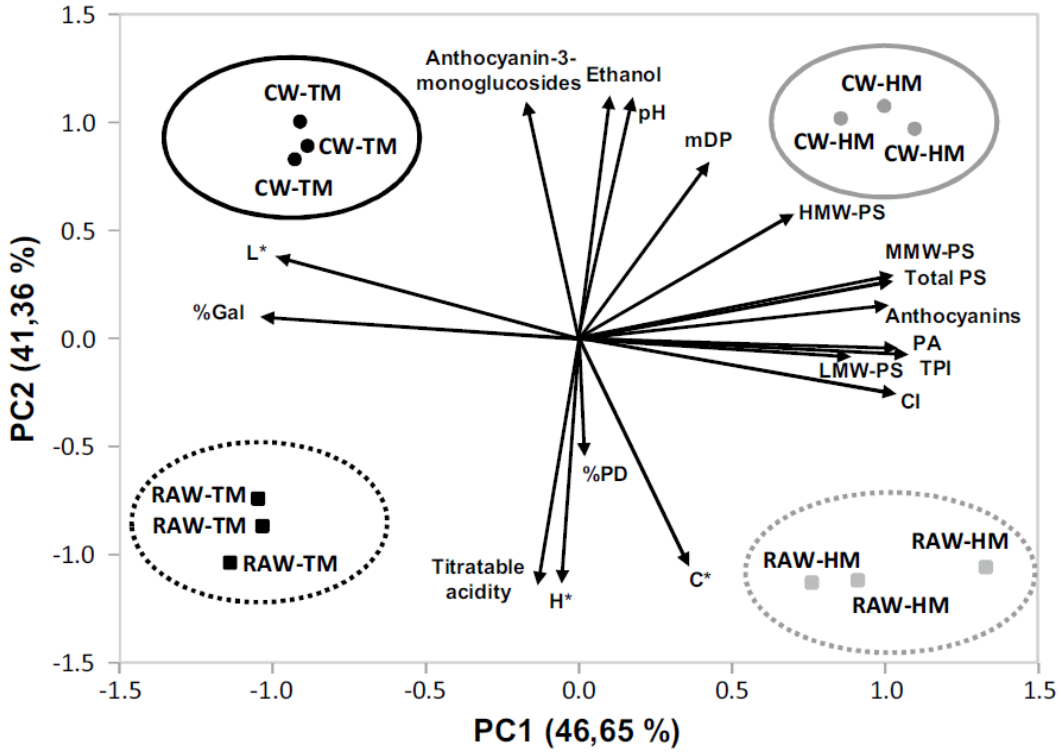

Fig. 3 Plot of varimax-rotated principal components analysis for Pinot Noir wines. All data are expressed as the average values of 3 replicates ± standard deviation. CW-TM control wine vinified by traditional maceration, RAW-TM reduced alcohol wine vinified by traditional maceration, CW-HM control wine vinified by hot maceration, RAW-HM reduce alcohol wine vinified by hot maceration, TPI total phenolic index, CI color intensity: L* Lightness; C* Chroma; H*: Hue; PA proanthocyanidins, mDP mean degree of polymerization, %PD percentage of prodelphinidins, %GAL percentage of galloylation, Total PS total polysaccharide, HMW-PS high molecular weight polysaccharide, MMW-PS medium molecular weight polysaccharide, LMW-PS low molecular weight polysaccharide

To better understand the influence of the ethanol and pH reduction using unripe grapes and the prefermentative hot maceration on the wine composition, a principal component analysis (PCA) was performed on the wines from both cultivars. Figures 3 and 4 shows the plot of the varimax-rotated PCA for Pinot Noir and Tannat wines, respectively. In both cases the PCA enables separation of the four experimental group wines with an explained aggregate variance for the two

components of over 70%. The loadings are presented as arrows, the length and direction of which indicate the contribution made by the two components.

In both varieties, the control wines (CW) were separated from the reduced-alcohol wines (RAW) by PC2. In addition, the traditionally macerated wines (TM) were also separated from the prefermentative hot macerated wines (HM) by PC1.

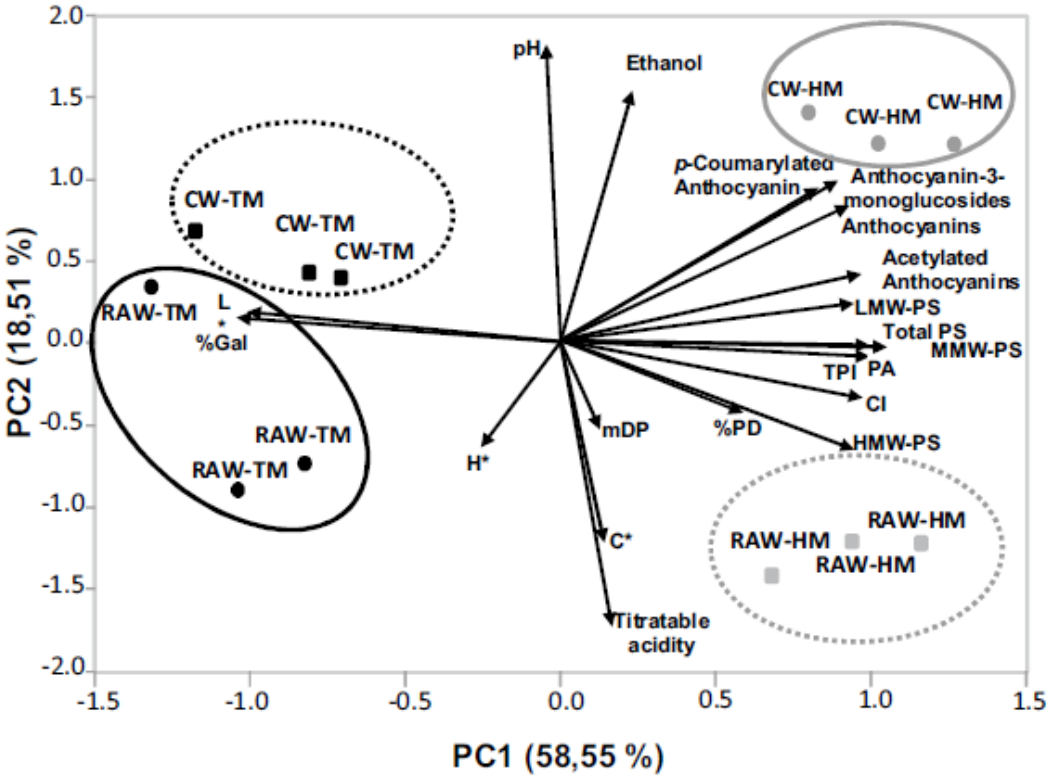

Fig. 4 Plot of varimax-rotated principal components analysis for Tannat wines. All data are expressed as the average values of 3 replicates ± standard deviation. CW-TM control wine vinified by traditional maceration, RAW-TM reduced alcohol wine vinified by traditional maceration, CW-HM control wine vinified by hot maceration, RAW-HM reduce alcohol wine vinified by hot maceration TPI total phenolic index, CI color intensity: L* lightness, C* chroma, H* hue, PA proanthocyanidins, mDP mean degree of polymerization, %PD percentage of prodelphinidins, %GAL percentage of galloylation, Total PS total polysaccharide, HMW-PS high molecular weight polysaccharide, MMW-PS medium molecular weight polysaccharide, LMW-PS low molecular weight polysaccharide

As expected, in wines from both varieties the arrows corresponding to ethanol and pH point upwards, whereas those for titratable acidity (TA) point downwards. These data are completely understandable since the aim of using unripe grapes was to reduce ethanol and pH and increase TA. This simply confirms graphically that the technique is useful for this purpose.

In addition, the arrows for total anthocyanins, total phenolic index (TPI), color intensity (CI), proanthocyanidins (PA), and total polysaccharides (PS) point to the right, while those for lightness (L*) and the percentage of galloylation of proanthocyanidins (% GAL) point to the left. This confirms that the wines obtained by hot maceration have a deeper color and higher total phenolic compounds and anthocyanin, proanthocyanidin and polysaccharide concentrations. Moreover, it indicates that hot maceration has mainly increased the extraction of skin proanthocyanidins, since the %GAL points towards the traditional macerated wines.

2.5. CONCLUSIONS

It can be concluded that must substitution and pre-fermentative hot maceration really do have a considerable influence on the color, chemical composition, and sensory characteristics of wines of both varieties. Must substitution decrease the alcohol concentration and pH of the wines and increased the total acidity. No important changes in other wine components were detected when this technique was applied; the only exception is the significant positive effect on wine color due to the higher proportion of the flavylium form of anthocyanins favour by the low pH.

Pre-fermentative hot maceration, on the other hand, increased wine color, total phenolic compounds, and total anthocyanin, proanthocyanidin and polysaccharide concentrations, and produced wines that could be better support the aging process.

Overall, the data demonstrate that the combination of these two techniques—the use of the unripe grape juice and prefermentative hot maceration—is an interesting tool for simultaneously mitigating the problems that climate change is

causing as regards grape maturity and improving wine color and phenolic and polysaccharides concentration.

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3. REDUCCIÓN DEL CONTENIDO DE ALCOHOL Y PH DE VINOS TINTOS PINOT NOIR Y TANNAT EMPLEANDO UVAS CON DIFERENTES NIVELES DE MADURACIÓN *

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3.1. RESUMEN

El objetivo de esta investigación fue determinar la efectividad de la mezcla de mostos de uvas con diferente grado de maduración para la obtención de vinos Pinot noir y Tannat con menor contenido de alcohol y pH. En envero, se extrajo mosto de uvas inmaduras que se conservó a 4◦C hasta su uso. En madurez tecnológica, las uvas cosechadas fueron despalilladas, estrujadas y distribuidas en seis recipientes. Tres fueron vinificados de forma tradicional, mientras que en los otros tres se realizó una sustitución de 3 L del mosto de uva madura por 3 L del mosto de uvas inmaduras. Ambos tratamientos realizaron una maceración fermentativa de 7 días. Se determinó la composición básica del vino, color, concentración de fenoles totales, antocianos, catequinas y proantocianidinas a los dos meses del descube. La sustitución de mosto permitió elaborar vinos Pinot noir y Tannat con menor contenido de alcohol y pH, sin reducir las concentraciones de polifenoles totales, catequinas o antocianos. El efecto sobre la concentración de proantocianidinas fue variable de acuerdo con el cultivar y año de vendimia. Los vinos Tannat reducidos en alcohol presentaron mayor intensidad de color, menor tono e igual cromaticidad que los vinos testigos.

3.2. ABSTRACT

The aim of this research was to determine the effectiveness of the mixture of the must of grapes with different degree of maturation to obtain Pinot Noir and Tannat wines with lower alcohol content and pH. In veraison, immature grape must was extracted and kept at 4 ° C until its use. In technological maturity, the grapes harvested were destemmed, crushed and distributed in six containers. Three of them were vinified in a traditional way, while in the other three, 3 L of the mature grape must was substituted for 3 L of the immature grape must. Both treatments performed a fermentative maceration of 7 days. The basic wine composition, color, concentration of total phenols, anthocyanins, catechins and proanthocyanidins were determined two months after devatting. The substitution of must allowed the production of Pino noir and Tannat wines with lower alcohol content and pH without reducing the concentrations of total polyphenols, catechins or anthocyanins. The effect on the concentration of proanthocyanidins was variable according to the cultivar and year of harvest. The Tannat wines reduced in alcohol presented greater intensity of color, lower hue and similar chromaticity than the control wines.

3.3. INTRODUCCIÓN

Los vinos tintos con mucho cuerpo y de color intenso son muy apreciados por el mercado. Su producción requiere una intensa extracción de compuestos fenólicos durante la vinificación, proceso afectado por la madurez de la uva [1,2] y razón por la que se busca cosecharla lo más madura posible. Las uvas con madurez muy avanzada presentan altas concentraciones de azúcares y baja acidez [3,4]. Frente a estas circunstancias se pueden tomar dos decisiones diferentes de cosecha. Por un lado, cosechar uvas con un contenido de azúcares y pH adecuados, pero con una maduración inadecuada de hollejos y semillas que probablemente originan vinos mal coloreados, amargos, astringentes y herbáceos. Por otro lado, se puede esperar la madurez fenólica completa y suponer que sus vinos tendrán el inconveniente de un pH y un contenido de alcohol elevados [5]. Adicionalmente, el calentamiento global está acentuando esta tendencia en los últimos años [6,7].

Las recientes políticas sociales llevadas a cabo por muchos países han puesto de manifiesto la necesidad de regular el consumo de bebidas alcohólicas a corto plazo. Como resultado de la aplicación de estas medidas sumadas a las preocupaciones relacionadas con la salud y los comportamientos sociales de la población, hay una disminución en el consumo de bebidas con altos niveles de alcohol. El vino es una de las bebidas alcohólicas más consumidas. Varias investigaciones han informado que el consumo moderado de vino tinto puede tener efectos beneficiosos para la salud debido a los contenidos significativos de compuestos bioactivos [8]. Los compuestos bioactivos del vino son los estilbenos, antocianos, taninos y otros polifenoles cuya relevancia está determinada por sus características tecnológicas, sensoriales y nutricionales [3,9]. Estos compuestos tienen capacidad antioxidante y participan en numerosos procesos bioquímicos potencialmente positivos para el organismo. Reducir el contenido de alcohol sin modificar el resto de los componentes del vino es uno de los objetivos actuales perseguidos por la industria [10].

Varias técnicas de vinificación han sido propuestas para elaborar vinos con menor contenido de alcohol, sin alterar su composición fenólica. Entre ellas destacan: la cosecha de uvas en una etapa temprana de maduración [11], el agregado de agua y ácidos minerales al mosto antes del inicio de la fermentación [12], la introducción de nuevos cultivares y la modificación de las técnicas de manejo del viñedo [13], el uso de

glucosa oxidasa (EC 1.1.3.4) [14], el uso de levaduras con un menor rendimiento en producción de etanol [15] y la aplicación de técnicas físicas para reducir parcialmente la concentración de azúcares en el mosto o el alcohol en el vino [16-18]. En ese sentido, la sustitución de un parte del mosto de uvas muy maduras por mosto de uvas verdes previamente fermentado [19] o por mosto previamente tratado por osmosis inversa [20] han sido procedimientos propuestos reducir simultáneamente el contenido de etanol y pH de vino.

El objetivo de esta investigación fue determinar la efectividad de la mezcla de mostos de uvas con diferente grado de maduración para obtener vinos tintos Pinot noir y Tannat con menor contenido de alcohol y pH, sin modificar su color y composición fenólica.

3.4. MATERIALES Y METODOS

3.4.1. Cosecha y vinificaciones

Los vinos se elaboraron durante la vendimia 2016, 2017 y 2018 con uvas del cultivar Pinot noir (*Vitis vinifera* L., Vitis International Variety Cataloque number: VIVC 9279) [21] y Tannat (*Vitis vinifera* L., Vitis International Variety Catalogue number: VIVC 12257) [21] cosechadas de viñedos comerciales ubicados en Canelón Chico, Canelones, Uruguay.

Al comienzo del envero, se cosecharon 100 kg de uva de cada cultivar para obtener un mosto con baja concentración de azúcares y alta acidez. Las uvas se despalillaron, estrujaron (Alfa 60 R, Italcom, Piazzola Sul Brenta, Italia) y se prensaron ligeramente en una prensa manual hasta obtener 50 L de mosto inmaduro. Este mosto se sulfito con 100 mg/L de $K_2S_2O_2$, se sedimentó durante la noche, se envasó en un recipiente de 50 L de capacidad y se conservó a 4 ° C hasta su uso. Cuando las uvas alcanzan la madurez tecnológica, se cosecharon 120 kg de uvas de ambos cultivares y se distribuyeron aleatoriamente en seis lotes de 10 kg cada uno por cultivar. Las uvas fueron despalilladas y estrujadas (Alfa 60 R, Italcom, Piazzola Sul Brenta, Italia) y el mosto se sulfito con 100 mg/L de $K_2S_2O_2$. Los contenedores de mostos se dividieron aleatoriamente en dos grupos de tres contenedores cada uno. Tres contenedores se consideraron como testigos (mosto testigo - MT) mientras que en los otros tres

contenedores se sustituyeron 3 l de mosto original por 3 l del mosto inmaduro con baja concentración de azúcares y pH (mosto reducidos en azúcares – MRA).

Los mostos de ambos tratamientos fueron sometidos a una maceración fermentativa de 7 días. Todos los tanques se inocularon con 200 mg/L de levadura seca activa (*Saccharomyces cerevisiae* ex bayanus Natuferm 804; Oenobiotech, Paris, Francia). Durante la maceración, se realizó un remontaje diario a fin de favorecer la extracción de polifenoles. La temperatura de fermentación estuvo comprendida entre 25 y 29 °C. Después de 7 días de maceración, el vino se extrajo por gravedad y los orujos se prensaron ligeramente en una prensa manual. El vino se mantuvo en recipientes de 5 L de capacidad a temperatura ambiente. Una vez finalizada la fermentación maloláctica espontánea, todos los vinos se estabilizaron con 100 mg/l de $K_2S_2O_2$ y 300 mg/l de lisozima (Delvo®Zyme, Delft, Países Bajos). Finalmente, los vinos fueron embotellados y almacenados hasta su análisis. Los análisis se realizaron a los 2 meses del descube.

3.4.2. Análisis de la composición general de las uvas y los vinos elaborados

Los métodos analíticos recomendados por la Organización Internacional de la Viña y el Vino [22] se utilizaron para determinar la concentración de azúcares, el pH y la acidez titulable de los mostos. El contenido total de antocianos de las uvas, su extractibilidad y riqueza fenólica se determinó de acuerdo con el método de Glories y Augusten [23] modificado por González-Neves et al. [24].

El contenido de etanol, la acidez titulable y el pH de los vinos se determinaron a los dos meses del descube utilizando el analizador de infrarrojos Winescan TM Autosampler 79000 (Foss, EE. UU.) y el software Foss Integrator versión 154 (Foss, Dinamarca).

3.4.3. Determinación de los parámetros cromáticos

Los parámetros de color se determinaron directamente en las muestras de vino colocadas en una cubeta de 1 mm de recorrido óptico. La intensidad colorante (IC) se estimó utilizando el método descrito por Glories [25]. Las coordenadas CIELAB, luminosidad (L*), cromaticidad (C*) y tono (H*), se determinaron de acuerdo con el método de Ayala et al. [26] y el procesamiento de datos se realizó utilizando el software MSCV [27].

3.4.4. Análisis espectrofotométricos de la composición fenólica de los vinos y parámetros relacionados

Los análisis espectrofotométricos de los vinos se realizaron a los dos meses del descube.

La composición polifenólica se evaluó utilizando índices clásicos espectrofotométricos. Los polifenoles totales se determinaron usando el Folin-Ciocalteu, de acuerdo con Singleton y Rossi [28], y sus contenidos en los vinos se expresan en mg de ácido gálico por litro. La concentración de antocianos fue analizada por Ribéreau-Gayon y Stonestreet [29] y se expresa en mg de malvidin-3-glucósido equivalente (EMG) por litro. Las catequinas se cuantificaron usando el método de Swain y Hillis [30] y sus concentraciones se expresan en mg de D-catequina por litro. Las proantocianidinas se determinaron según Ribéreau-Gayon y Stonestreet [29] y sus contenidos se expresan en mg de cloruro de cianidina por litro de vino.

3.4.5. Análisis estadistico

Todos los datos se expresan como el promedio aritmético ± desviación estándar de tres repeticiones. Las diferencias estadísticas entre los tratamientos fueron determinadas aplicando análisis de varianza multivariados. Los análisis estadísticos se realizaron utilizando el software Infostat versión 2015 [31].

3.5. RESULTADOS Y DISCUSIÓN

3.5.1. Composición de los mostos

La Tabla 1 muestra la concentración de azúcares, la acidez titulable y el pH de los mostos Pinot noir y Tannat cosechados a principios del envero (1er cosecha) y en madurez tecnológica (2da cosecha) durante las vendimias 2016, 2017 y 2018. Como era de esperar, la concentración de azúcares y el pH aumentaron durante la maduración de las uvas, mientras que la acidez titulable disminuyó.

Tabla 1. Composición básica de las uvas según cultivar y momento de cosecha

Cultivar	Año de vendimia	Momento de cosecha	Azúcares (g/L)	Acidez titulable (gH_2SO_4/L)	pH	A280	ApH1	EA (%)
Pinot Noir	2016	1er cosecha	129 ± 1 b	12.25 ± 0.09 a	3.10 ± 0.05 b	-	-	-
		2da cosecha	243 ± 1 a	3.62 ± 0.18 b	3.52 ± 0.10 a	33.5 ± 3.7	939 ± 18	30.8 ± 1.8
	2017	1er cosecha	171 ± 1 b	5,11 ± 0,04 a	3,29 ± 0,01 b	-	-	-
		2da cosecha	224 ± 21 a	4,86 ± 0,03 b	3,45 ± 0,02 a	53,4 ± 3,3	212 ± 2,3	26,0 ± 1,8
	2018	1er cosecha	175 ± 2 b	4,48 ± 0,10 a	3,22 ± 0,01 b	-	-	-
		2da cosecha	245 ± 3 a	3,94 ± 0,17 b	3,59 ± 0,04 a	58,9 ± 3,8	434,5 ± 41,3	25 ± 1,2
Tannat	2016	1er cosecha	175 ± 2 b	8.43 ± 0.12 a	3.12 ± 0.04 b	-	-	-
		2da cosecha	243 ± 3 a	4.51 ± 0.07 b	3.31 ± 0.03 a	47.5 ± 0.5	2258 ± 145	51.6 ± 2.6
	2017	1er cosecha	182 ± 2 b	5,98 ± 0,05 a	3,10 ± 0,02 b	-	-	-
		2da cosecha	193 ± 1 a	5,48 ± 0,07 b	3,23 ± 0,03 a	61,5 ±1,5	949 ± 3,0	49,2 ± 0,6
	2018	1er cosecha	191 ± 1 b	4,19 ± 0,27 a	3,14 ± 0,03 b	-	-	-
		2da cosecha	263 ± 6 a	3,63 ±0,05 b	3,27 ± 0,01 a	81,6 ± 3,4	2334,5 ± 19,3	47,0 ± 1,1

Medias con distinta letra indican diferencias significativas ($p<0.05$). A280: riqueza fenólica de la uva, ApH1: potencial en antocianos totales, EA (%): es índice de extractibilidad de los antocianos.

Los parámetros indicadores del potencial fenólico de la uva se determinaron en cada año, solamente en las uvas de la 2dª cosecha ya que solo los hollejos y semillas de las uvas cosechadas en madurez tecnológica aportaron compuestos fenólicos durante la maceración. Como se esperaba, las uvas Tannat presentaron un mayor potencial en compuestos fenólicos totales (A280) y antocianos totales (ApH1) que las uvas Pinot Noir. Sin embargo, el índice de extractabilidad de los antocianos (EA%) fue mayor en las uvas Tannat que en las uvas Pinot Noir, lo que indica que la proporción de antocianos que se liberarán durante la maceración será menor en las uvas Tannat que en las uvas Pinot Noir. Estos resultados están de acuerdo con las características descritas para estas variedades en Uruguay [24].

Asimismo, el potencial enológico de las uvas fue diferente de acuerdo con las condiciones de maduración de cada año. Tanto las uvas Tannat como Pinot noir presentaron mayor concentración de azúcares, potencial en compuestos fenólicos totales y antocianos totales, y menor índice de extractibilidad de los antocianos en la vendimia 2018. En contrapartida, el menor potencial enológico de las uvas se registró en la vendimia 2017 para ambos cultivares. Investigaciones realizadas en nuestras condiciones climáticas por Ferrer et al. [32] demuestran que los compuestos relacionados con la calidad de la uva se ven favorecidos por la acumulación de

temperaturas durante las primeras etapas del ciclo de cultivo, mientras que las altas temperatura y la disponibilidad hídrica durante la maduración los afectan negativamente. Estas condiciones son muy variables entre años, lo cual explicaría las diferencias registradas en el potencial enológico de las uvas entre vendimias.

3.5.2. Composición básica de los vinos

La tabla 2 muestra el contenido de etanol, la acidez total y el pH de los vinos Pinot noir y Tannat elaborados durante las vendimias 2016, 2017 y 2018.

En ambos cultivares, el contenido de etanol y el pH de los vinos elaborados con mosto testigo (MT) fue significativamente superior al de los vinos elaborados a partir de mostos reducidos en azúcares (MRA) con la única excepción del Tannat 2017 donde no se observaron diferencias significativas en el pH. La acidez total fue menor para los vinos testigo de ambos cultivares, si bien no se observaron diferencias significativas entre los tratamientos en todas las vinificaciones.

Estos resultados eran esperables ya que la sustitución de mosto de uva en madurez tecnológica por mosto de uvas inmaduras implica una disminución en el contenido de azúcares y un aumento de la acidez. Por ende, los vinos elaborados con esta técnica de vinificación presentan vinos con menor contenido de alcohol y pH. Estos resultados están de acuerdo con los obtenidos por Kontoudakis et al. [19] y Rolle et al. [20] en el que han aplicado una técnica de sustitución de mosto de uvas maduras.

Tabla 2. Composición básica de los vinos según cultivar y año

Cultivar	Año de vendimia	Composición del mosto	Alcohol (%v/v)	Acidez Total (gH_2SO_4/L)	pH
Pinot noir	2016	MT	14,3 ± 0,1 a	3,11 ± 0,04 b	3,77 ± 0,02 a
		MRA	13,5 ± 0,1 b	5,42 ± 0,06 a	3,38 ± 0,01 b
	2017	MT	13,2 ± 0,1 a	2,60 ± 0,04 a	3,91 ± 0,02 a
		MRA	12,2 ± 0,1 b	2,65 ± 0,05 a	3,84 ± 0,03 b
	2018	MT	15,7 ± 0,2 a	3,09 ± 0,18 b	3,90 ± 0,02 a
		MRA	13,7 ± 0,1 b	3,30 ± 0,03 a	3,68 ± 0,02 b
Tannat	2016	MT	14,6 ± 0,2 a	3,88 ± 0,21 b	4,01 ± 0,06 a
		MRA	13,1 ± 0,1 b	4,51 ± 0,84 a	3,85 ± 0,04 b
	2017	MT	11,5 ± 0,2 a	3,02 ± 0,01 a	3,82 ± 0,01 a
		MRA	10,9 ± 0,2 b	3,07 ± 0,04 a	3,83 ± 0,04 a
	2018	MT	16,1 ± 0,1 a	3,93 ± 0,05 a	3,93 ± 0,01 a
		MRA	13,8 ± 0,1 b	4,04 ± 0,02 a	3,75 ± 0,02 b

Medias con distinta letra indican diferencias significativas ($p<0.05$). MT: vino elaborado con mosto testigo; MRA: vino elaborado con mosto reducido en azúcares.

3.5.3. Composición fenólica de los vinos

La concentración de fenoles totales, antocianos, catequinas y proantocianidinas para los vinos Pinot noir y Tannat elaborados durante la vendimia 2016, 2017 y 2018 se observa en la Tabla 3.

En general, la concentración de compuestos fenólicos totales de los vinos Tannat y Pinot noir no fue afectada por la composición inicial del mosto, con la única excepción del Tannat 2016 donde la diferencia entre los tratamientos fue de un 9.3 %.

Para Pinot noir, la concentración de antocianos fue significativamente mayor para los vinos MRA (10.1%) elaborados en la vendimia 2016, en cuanto a las restantes vendimias las diferencias entre los tratamientos no fueron significativas. Resultados similares se observaron en los vinos del cultivar Tannat, donde las diferencias entre los tratamientos fueron significativas solo en la vendimia 2017 (10.7 %). Con la aplicación de esta técnica de reducción de la concentración de alcohol podría esperarse una menor concentración de antocianos ya que se eliminó

una porción de mosto. Sin embargo, el reemplazo por mosto de uva verde no implicaría necesariamente pérdidas de antocianos ya que esta operación se realiza antes de la maceración. Por otra parte, el descarte de parte del mosto de uvas maduras durante la sustitución podría implicar una pérdida de antocianos ya que estos se extraen fácilmente de los hollejos durante el proceso de estrujado durante el corto tiempo de contacto con el mosto, por lo que la fracción de mosto eliminada podría contener una cantidad considerable de antocianos [33]. La similar o mayor concentración de antocianos de los vinos MRA en relación con los vinos MT demuestran que este efecto no fue relevante. Asimismo, estos resultados también podrían estar vinculados a la liberación favorecida de copigmentos durante la fermentación, que protege a los antocianos contra la oxidación [34].

Tabla 3. Composición fenólica de los vinos según cultivar y año de vendimia.

Cultivar	Año de vendimia	Composición del mosto	Polifenoles totales (mg/L)	Antocianos (mg/L)	Catequinas (mg/L)	Proantocianidinas (mg/L)
Pinot noir	2016	MT	1201 ± 49 a	283 ± 13 b	742 ± 38 b	1830 ± 68 b
		MRA	1201 ± 36 a	315 ± 16 a	840 ± 64 a	2075 ± 110 a
	2017	MT	644 ± 57 a	151 ± 7 a	183 ± 24 a	529 ± 49 a
		MRA	681 ± 10 a	160 ± 2 a	176 ± 46 a	423 ± 39 b
	2018	MT	829 ± 31 a	207 ± 7 a	536 ± 10 a	883 ± 29 a
		MRA	795 ± 37 a	190 ± 9 a	504 ± 32 a	930 ± 68 a
Tannat	2016	MT	2376 ± 126 a	1031 ± 52 a	1408 ± 31 a	3711 ± 107 a
		MRA	2155 ± 89 b	1010 ± 67 a	1328 ± 78 a	3454 ± 113 b
	2017	MT	1311 ± 20 a	523,8 ± 6 b	1058 ± 43 a	1965 ± 42 b
		MRA	1367 ± 07 a	586,1 ± 26 a	1125 ± 54 a	2142 ± 15 a
	2018	MT	1773 ± 42 a	941 ± 32 a	1351 ± 182 a	2700 ± 57 a
		MRA	1718 ± 43 a	932 ± 28 a	1411 ± 71 a	2609 ± 109 a

Medias con distinta letra indican diferencias significativas ($p<0.05$). MT: vino elaborado con mosto testigo; MRA: vino elaborado con mosto reducido en azúcares.

La concentración de catequinas no difirió entre los vinos de los distintos tratamientos con la excepción de los vinos Pinot noir elaborados durante la vendimia 2016. Estos resultados concuerdan con los reportados en otros estudios [20, 35].

El efecto de reducción del contenido de alcohol sobre la concentración de proantocianidinas no fue claro. Tanto para Pinot noir como Tannat, se observaron valores significativamente mayores de proantocianidinas en los vinos MRA en una vendimia, menores en otra y sin diferencia con los vinos MT en otra. Sin embargo, Rolle et al. [20] encontraron que en los vinos en los que se redujo el contenido de alcohol sustituyendo mosto de uva madura por mosto de la misma uva tratado por osmosis inversa, los flavonoles de alto peso molecular disminuyeron. La menor concentración de etanol podría dificultar la extracción de flavonoles polimerizados de las uvas durante la fermentación [33]. Por otra parte, el trabajo realizado por Gil et al. [18], donde la desalcoholización de los vinos tintos elaborados con uvas Cabernet Sauvignon, Garnacha y Cariñena se hizo utilizando tecnologías de membrana, no se observaron efecto sobre la concentración de proantocianidinas ni en su grado medio de polimerización.

3.5.4. Color de los vinos

Los parámetros cromáticos de los vinos Tannat y Pinot noir elaborados durante las vendimias 2016, 2017 y 2018 se muestran en la Tabla 4.

El color de los vinos MT y MRA presentó diferencias asociadas al cultivar y al año de vendimia. Como es de esperar, en todas las elaboraciones, los vinos con mayor intensidad colorante fueron los que tuvieron menor luminosidad.

El efecto de la reducción de alcohol sobre el color de los vinos Pinot noir no fue claro. Para la vendimia 2016, los vinos MRA presentaron mayor intensidad colorante respecto de los MT, lo que se explica por una mayor concentración de antocianos. Sin embargo, en las vendimias 2017 y 2018 se observó el efecto contrario, a iguales concentración de antocianos entre tratamientos. Las diferencias en la cromaticidad (C*) y el tono (H*) entre los vinos MRA y MT fue variable según el año de vendimia.

Sin embargo, los vinos MRA elaborados a partir del cultivar Tannat presentaron una intensidad colorante significativamente superior a los MT. La cromaticidad y el tono de los vinos MRA fueron iguales o superiores a los MT. Estos resultados pueden estar asociados a las diferencias en el pH de los vinos. Como es

sabido, cuando el pH disminuye, el equilibrio entre las diferentes formas de los antocianos se desplaza hacia la forma roja de las moléculas, el catión flavilio [36]. Otro factor que puede incidir es la contribución de los copigmentos al color del vino. La copigmentación es un fenómeno importante, que ocurre en los vinos tintos jóvenes. El etanol tiene un papel disociador en los complejos de copigmentación como consecuencia del debilitamiento de las interacciones hidrofóbicas [37]. Sin embargo, la solubilidad de algunos copigmentos podría aumentar con la mayor producción de etanol, compensando el efecto disruptivo. La matriz del vino influye en el efecto del etanol sobre la copigmentación y el color, particularmente la proporción entre antocianos y copigmentos relacionados [34].

Tabla 4. Color de los vinos según cultivar y año de vendimia.

Cultivar	Año de vendimia	Composición del mosto	Intensidad colorante	L*	C*	H*
Pinot noir	2016	MT	11,0 ± 0,4 b	70,9 ± 0,9 a	20,7 ± 0,8 b	17,5 ± 1,08 b
		MRA	13,0 ± 0,3 a	63,2 ± 0,8 b	31,2 ± 0,3 a	355,0 ± 0,7 a
	2017	MT	6,4 ± 0,2 a	72,3 ± 1,4 a	25,2 ± 1,4 a	27,2 ± 1,5 a
		MRA	5,9 ± 0,4 b	73,2 ± 5,2 a	16,9 ± 1,9 b	24,8 ± 1,2 b
	2018	MT	4,6 ± 0,1 a	76,7 ± 0,7 a	23,9 ± 0,4 a	26,6 ± 0,1 a
		MRA	4,0 ± 0,1 b	79,2 ± 1,1 a	22,7 ± 0,7 a	20,7 ± 1,3 b
Tannat	2016	MT	26,7 ± 0,9 b	38,6 ± 1,3 a	43,8 ± 0,5 a	348,1 ± 1,0 a
		MRA	29,9 ± 2,1 a	34,8 ± 1,7 b	45,6 ± 4,2 a	348,9 ± 1,8 a
	2017	MT	12,7 ± 0,5 b	67,3 ± 1,1 a	22,0± 0,5 b	14,8 ± 0,7 a
		MRA	14,2 ± 0,2 a	63,8 ± 0,5 b	25,4 ± 0,7 a	12,7 ± 1,6 b
	2018	MT	19,6 ± 0,7 a	31,7 ± 1,0 a	55,3 ± 0,1 a	12,4 ± 0,6 a
		MRA	19,3 ± 0,6 a	32,,6 ± 0,8 a	56,7 ± 0,3 a	11,0 ± 0,2 b

Medias con distinta letra indican diferencias significativas ($p<0.05$). L*: luminosidad; C*: cromaticidad; H*: tono; MT: vino elaborado con mosto testigo; MRA: vino elaborado con mosto reducido en azúcares.

Se debe considerar que los cultivares Tannat y Pinot noir presentan diferencias considerables en su composición fenólica, lo cual podría explicar las diferencias observadas en los resultados. Tannat se caracteriza por tener contenidos muy elevados de pigmentos (antocianos) y taninos, en cuanto Pinot noir presenta una gran

riqueza polifenólica (sobre todo en semillas), pero constituida casi exclusivamente por taninos, con muy bajos contenidos de antocianos.

3.5.5. Análisis multivariado de la varianza

Los análisis multivariados de la varianza muestran el efecto de cada factor en los diferentes componentes de los vinos (Tabla 5). Se verifica una incidencia muy importante del año de vendimia, la composición del mosto y su interacción sobre el contenido de alcohol, el pH y la acidez titulable de los vinos Pinot noir. Para el caso de los vinos Tannat, la mayor incidencia de estos factores se observó en el contenido de alcohol y en el pH, la acidez titulable fue afectada por el año de vendimia. Estos resultados parecen lógicos ya que la composición del mosto en cosecha (concentración de azúcares, pH y acidez titulable) fue muy diferente entre añadas (Tabla 1). Además, los tratamientos donde se realizó la sustitución de mosto modifican la composición de este y en consecuencia del vino.

El año de vendimia influyó fuertemente en la concentración de todos los compuestos fenólicos. Sin embargo, el efecto de la composición del mosto fue menos significativo. La interacción año de vendimia - composición del mosto fue más significativa para los vinos Pinot noir que para los vinos Tannat. Varios autores han demostrado que la composición fenólica de la uva y, por lo tanto, del vino está determinada por las condiciones de maduración de cada año en particular [24, 32]. La composición del mosto solo influyó significativamente en la concentración de polifenoles totales. Como se discutió anteriormente, el contenido de etanol y el pH son factores que contribuyen a la extracción durante la maceración fermentativa.

El año de vendimia tuvo un fuerte impacto sobre todos los parámetros cromáticos de los vinos de ambos cultivares. Por su parte la composición del mosto tuvo un impacto diferente de acuerdo con el cultivar. Para los vinos Tannat, la composición del mosto tuvo un impacto muy relevante en la intensidad colorante, la luminosidad (L*) y cromaticidad (C*), en cuanto al tono (H*) fue menos afectado. Para los vinos Pinot noir el mayor impacto se observó en el tono (H*) y en la intensidad colorante. La interacción año de vendimia - composición del mosto fue altamente significativa para todos los parámetros cromáticos determinados en los

vinos Pinot noir, en cambio que para los vinos Tannat solo fue relevante en la luminosidad e intensidad del color. El color del vino tinto es el resultado de la concentración de pigmentos, sus interacciones con otros compuestos y las condiciones fisicoquímicas del medio en el que se encuentran. Por lo tanto, cualquier modificación de estos factores determina un cambio en el color del vino.

Tabla 5. Análisis multivariado de la varianza

Cultivar	Parámetros analíticos	Año de vendimia	Composición del mosto	Año de vendimia x Composición del mosto
Pinot noir	**Alcohol**	757,97 ***	1848,09 ***	142,18 ***
	Acidez total	1196,57 ***	940,21 ***	687,74 ***
	pH	752,53 ***	1258,73 ***	195,58 ***
	Polifenoles totales	546,32 ***	1,44	0,51
	Antocianos	629,62 ***	6,18 *	17,28 ***
	Catequinas	717,39 ***	2,31	9,20 ***
	Proantocianidinas	1573,83 ***	8,08 **	20,86 ***
	Intensidad colorante	1435,56 ***	7,77 **	48,05 ***
	L*	66,88 ***	3,39 *	17,09 ***
	C*	61,11 ***	0,87	230,39 ***
	H*	930,77 ***	630,50 ***	233,88 ***
Tannat	**Alcohol**	1711,41 ***	699,08 ***	79,76 ***
	Acidez total	36,33 ***	4,94 *	2,46
	pH	9,54 ***	24,14 ***	7,67 **
	Polifenoles totales	477,14 ***	9,02 **	10,77 ***
	Antocianos	456,07 ***	0,63	3,75 *
	Catequinas	38,53 ***	0,27	2,45
	Proantocianidinas	586,31 ***	2,4	11,89 ***
	Intensidad colorante	638,70 ***	18,96 ***	8,81 **
	L*	3045,80 ***	3097 ***	16,33 ***
	C*	1026,31 ***	13,79 ***	1,07
	H*	1865,48 ***	5,72 *	5,31 *

Se indican los valores de F y su significación estadística ($p < 0.001$ = ***; $p < 0.01$= **; $p < 0.1$= *)

3.6. CONCLUSIONES

Se puede concluir que la técnica de sustitución de una proporción de mosto de uva madura por mosto de uva inmadura permitió elaborar vinos Tannat y Pinot noir con menor contenido de alcohol y el pH.

La sustitución de mosto no redujo la concentración de polifenoles totales, catequinas y antocianos en los vinos de ambos cultivares. El efecto sobre la

concentración de proantocianidinas fue variable de acuerdo con el cultivar y con el año de vendimia.

El efecto de la sustitución de mosto sobre el color dependió del cultivar. Los vinos Tannat elaborados por este tratamiento tuvieron una mayor intensidad de color con menor tono e igual cromaticidad que los vinos testigos. En los vinos Pinot noir las tendencias no fueron claras.

El año de vendimia tuvo un impacto muy importante en la composición y el color de los vinos en tanto que la composición del mosto impactó más fuertemente en la composición básica y el color con un efecto diverso según el cultivar.

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4. EVALUACIÓN DE LA COMPOSICIÓN Y CALIDAD DEL COLOR DE VINOS TINTOS TANNAT ELABORADOS POR MACERACIÓN PRE-FERMENTATIVA EN CALIENTE *

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4.1. RESUMEN

Durante la vendimia 2016 se elaboraron vinos tintos Tannat por maceración pre-fermentativa en caliente (una hora de calentamiento a 60-70°C seguido de una maceración fermentativa de 7 días) y maceración tradicional (maceración fermentativa de 7 días) con el objetivo de evaluar su concentración en compuestos fenólicos y color durante la conservación. Las vinificaciones se realizaron por triplicado en recipientes de 10 litros de capacidad. Se determinó la composición básica del vino, color, concentración de fenoles totales, antocianos, catequinas, proantocianidinas y los índices de ionización, copigmentados y PVPP. Los vinos con maceración pre-fermentativa en caliente presentaron mayor concentración de antocianos y taninos, mayor porcentaje de antocianos ionizados, co-pigmentados y condensados. Adicionalmente, presentaron mayor proporcion de delfinidina, petunidina y peonidina al descube. El calentamiento degrada las enzimas polifenoloxidasas por lo que estas formas antociánicas pudieron haber sido preservadas de la oxidación. Las concentraciones de las diferentes formas antociánicas disminuyeron durante la conservación de los vinos de ambos tratamientos, sin observarse diferencias en el perfil antocianico después de dos años. Los vinos elaborados por maceración pre-fermentativa en caliente presentaron mayor intensidad colorante durante todo el período de evaluación sugiriendo una mayor estabilidad del color.

4.2. ABSTRACT

During the 2016 vintage, Tannat red wines were elaborated by pre-fermentative hot maceration (one hour of heating at 60-70 °C followed by a fermentative maceration of 7 days) and traditional maceration (fermentative maceration of 7 days) with the aim of evaluate its concentration in phenolic compounds and color during conservation. The vinifications were made by triplicate in containers of 10 liters of capacity. The basic wine composition, color, concentration of total phenols, anthocyanins, catechins, proanthocyanidins and the ionization, copigmented and PVPP indices were determined. The wines with pre-fermentative hot maceration showed higher concentration of anthocyanins and tannins, higher percentage of ionized anthocyanins, co-pigmented and condensed. Additionally, they presented a greater proportion of delphinidin, petunidin and peonidin at devatting. The heating degrades the polyphenoloxidases enzymes reason why these anthocyanin forms could have been preserved of the oxidation. The concentrations of the different anthocyanin forms decreased during the conservation of the wines of both treatments, without observing differences in the anthocyanin profile after two years. The wines elaborated by pre-fermentative hot maceration showed greater color intensity during the entire evaluation period, suggesting a greater color stability.

4.3. INTRODUCCIÓN

Tannat es la variedad más implantada en Uruguay debido a su adaptación a las condiciones eco-fisiológicas del país y a la tipicidad de sus vinos. Las uvas de este cultivar se caracterizan por presentar una elevada riqueza fenólica y antocianica y una baja extractibilidad de los antocianos (altos valores de EA%) [1]. Sus vinos presentan un perfil antociánico característico, con menores proporciones de malvidina y glucósidos acetilados, respecto a los vinos elaborados a partir de los cultivares Cabernet-Sauvignon y Merlot. En consecuencia, la estabilidad del color de los vinos Tannat elaborados por maceraciones tradicionales es menor a la de los vinos de otras variedades [1,2,3]. Esta dificultad relativa a la extracción y composición de los antocianos pueden ser compensadas con operaciones que promuevan su solubilización y estabilización desde el inicio de la maceración a fin de aprovechar el potencial enológico de las uvas y producir vinos con mejor color y más estables en el tiempo [1,4,5].

Numerosos estudios se han centrado en la extracción y la estabilización química del color. Algunas técnicas propuestas buscan romper las barreras celulares e incrementar la extracción de los antocianos favoreciendo sus interacciones con otros constituyentes del vino [5,6]. Otras, proponen el uso de agregados externos (taninos o manoproteinas entre otros) para estabilizar el color [7,8].

En este sentido, la maceración pre-fermentativa en caliente potencia la extracción de los compuestos fenólicos con el objetivo de obtener vinos tintos con mayor color. En el calentamiento pre-fermentativo de los racimos enteros o estrujados [9,10] se alteran los tejidos de los hollejos y se transfieren sus componentes (principalmente antocianos y taninos) al mosto. Las temperaturas a las que se calienta el mosto varían entre 40 y 80 ºC y su duración entre 1 y 24 h [5, 11]. Después del tratamiento térmico, los mostos pueden continuar o no con una maceración fermentativa dependiendo del objetivo buscado. En consecuencia, la extracción de los antocianos incrementa la intensidad colorante del mosto desde el inicio de la maceración [12] favoreciendo su reacción tanto con compuestos procedentes de la fermentación alcohólica como con otros compuestos fenólicos. Adicionalmente, la copigmentación se ve favorecida mejorando la calidad del color del vino a través de un incremento en el espectro visible (efecto hipercromico) y un incremento en el máximo de absorbancia (efecto batocrómico) [13]. Sin embargo, la copigmentación disminuye si el aumento de la temperatura es lento ya que estos

pigmentos son exotérmicos y su equilibrio tiende a desplazarse hacia la forma chalcona [14]. Al terminar la fermentación alcohólica los vinos resultan con más color que los elaborados por sistemas tradicionales y estando dotados de una mayor estabilidad [9,11].

Esta investigación tiene como objetivo evaluar el efecto de la maceración pre-fermentativa en caliente sobre la composición fenólica y el color de vinos tintos Tannat al descube y durante su conservación.

4.4. MATERIALES Y METODOS

4.4.1. Cosecha y vinificaciones

Los vinos se elaboraron durante la vendimia 2016 con uvas del cultivar Tannat (*Vitis vinifera* L., Vitis International Variety Catalogue number: VIVC 12257) [15] cosechadas de un viñedo comercial ubicado en Canelón Chico, Canelones, Uruguay. La cosecha se realizó cuando las uvas alcanzaron la madurez tecnológica (Tabla 1).

Tabla 1. Composición inicial de la uva.

Azúcares (mg/L)	Acidez titulable (mg H_2SO_4/L)	pH	A280	ApH1 (mg/L)	EA (%)
243 ± 3	4.51 ± 0.07	3.31 ± 0.03	47.5 ± 0.5	2258 ± 145	51.6 ± 2.6

A280: riqueza fenólica de la uva; ApH1: potencial en antocianos totales, EA: extractibilidad de los antocianos.

Una vez cosechadas, las uvas fueron estrujadas, despalilladas y distribuidas al azar entre las distintas unidades experimentales.

Las vinificaciones se realizaron en recipientes de 10 litros de capacidad donde se encubaron 8 kg de uva. Tres recipientes fueron vinificados de manera tradicional (7 días de maceración fermentativa, VT) mientras que los otros tres fueron sometidos a una maceración pre-fermentativa en caliente (60-70°C) durante una hora, seguida de una maceración fermentativa de 7 días (MPC). El calentamiento se llevó a cabo transfiriendo el mosto, hollejos y semillas a recipientes de acero inoxidable de 11 L de capacidad que se sumergieron en un baño de agua caliente (80-90 °C). Durante el calentamiento, el mosto se homogeneizó manualmente. Al final del tratamiento térmico, los recipientes de

acero inoxidable se sumergieron en un baño de agua fría para refrigerarlos a temperatura ambiente (alrededor de 26 ºC). Posteriormente, el mosto, hollejos y semillas se transfirieron a los recipientes originales donde se continuó con una maceración fermentativa de 7 días.

Todos los tanques se inocularon con 200 mg/L de levadura seca activa (*Saccharomyces cerevisiae* ex *bayanus* Natuferm 804; Oenobiotech, Paris, Francia). Durante la maceración, se realizó un remontaje diario a fin de favorecer la extracción de polifenoles. La temperatura de fermentación estuvo comprendida entre 25 y 29 °C. Después de 7 días de maceración, el vino se decantó por gravedad y los orujos se prensaron ligeramente en una prensa manual. El vino se mantuvo en recipientes de 5 L de capacidad a temperatura ambiente. Una vez finalizada la fermentación maloláctica espontánea, todos los vinos se estabilizaron con 100 mg/l de $K_2S_2O_2$ y 300 mg/l de lisozima (Delvo®Zyme, Delft, Países Bajos). Finalmente, los vinos fueron embotellados y almacenados hasta el análisis. Los análisis se realizaron a los 2 meses del descube y se repitieron a los 1 y 2 años.

4.4.2. Análisis de la composición general de las uvas y los vinos elaborados

Los métodos analíticos recomendados por la Organización Internacional de la Viña y el Vino [16] se utilizaron para determinar la concentración de azúcares, el pH y la acidez titulable de los mostos. El contenido total de antocianos de las uvas, su extractibilidad y riqueza fenólica se determinó de acuerdo con el método de Glories y Augustien [17] modificado por González-Neves et al. [1].

El contenido de etanol, la acidez titulable y el pH de los vinos se determinaron a los dos meses del descube utilizando el analizador de infrarrojos Winescan TM Autosampler 79000 (Foss, EE. UU.) y el software Foss Integrator versión 154 (Foss, Dinamarca).

4.4.3. Determinación de los parámetros cromáticos

Los parámetros de color se determinaron directamente en las muestras de vino colocadas en una cubeta de 1 mm de recorrido óptico. La intensidad colorante (IC) se estimó utilizando el método descrito por Glories [18]. Las coordenadas CIELAB, luminosidad (L*), cromaticidad (C*) y tono (H*), se determinaron de acuerdo con el

método de Ayala et al. [19] y el procesamiento de datos se realizó utilizando el software MSCV [20].

4.4.4. Análisis espectrofotométricos de la composición fenólica de los vinos y parámetros relacionados

La composición polifenólica se evaluó utilizando índices espectrofotométricos clásicos. Los polifenoles totales se determinaron usando el Folin-Ciocalteu, de acuerdo con Singleton y Rossi [21], y sus contenidos en los vinos se expresan en mg de ácido gálico por litro. La concentración de antocianos fue analizada por Ribéreau-Gayon y Stonestreet [22] y se expresan en mg de malvidin-3-glucósido equivalente (EMG) por litro. Las catequinas se cuantificaron usando el método de Swain y Hillis [23] y sus concentraciones se expresan en mg de D-catequina por litro. Las proantocianidinas se determinaron según Ribéreau-Gayon y Stonestreet [22] y sus contenidos se expresan en mg de cloruro de cianidina por litro de vino. El índice de ionización (que indica la proporción de antocianos que presentan color rojo al pH del vino) y el índice de PVPP (que indica la proporción de antocianos combinados con proantocianidinas) se determinaron de acuerdo con Glories [18]. El índice de copigmentación se midió de acuerdo con Boulton [24].

4.4.5. Determinación de antocianos por HPLC

Los análisis de HPLC en fase reversa de los antocianos se llevaron a cabo inyectando 40 μL de vino en un cromatógrafo líquido Agilent serie 1200 (HPLC-DAD) y utilizando una columna Agilent Zorbax Eclipse XDBC18, 4,6 x 250 mm, 5 μm (Agilent Technologies). Los disolventes utilizados fueron ácido fórmico acuoso al 10% (disolvente A) y una mezcla de metanol al 45%, agua al 45% y ácido fórmico al 10% (disolvente B) de acuerdo con el método descrito por Valls [25]. Los cromatogramas se registraron a 520 nm, y las curvas estándar de antocianina se realizaron usando malvidin-3-O-glucósido cloruro. Los compuestos se identificaron registrando sus espectros UV con el detector de matriz de diodos y comparándolos con los espectros UV reportados en la literatura. Se cuantificaron los cinco antocianidin-3-monoglucósidos del vino

(delfinidina, cianidina, peonidina, petunidina y malvidina) y sus respectivas antocianidinas acetiladas y p-cumarilados.

4.4.6. Análisis estadístico

Todos los datos se expresan como el promedio aritmético ± desviación estándar de tres repeticiones. Las diferencias estadísticas entre los tratamientos fueron determinadas aplicando análisis de varianza. Los análisis estadísticos se realizaron utilizando el software Infostat versión 2015 [26].

4.5. RESULTADOS Y DISCUSIÓN

4.5.1. Composición básica de los vinos

La Tabla 2 muestra el contenido de etanol, la acidez titulable y el pH de los vinos Tannat elaborados por vinificación tradicional (VT) y maceración pre-fermentativa en caliente (MPC).

Los vinos MPC no mostraron diferencias significativas en su composición básica respecto a los vinos VT. Varias investigaciones han reportado un incremento en el contenido de alcohol y una disminución del pH en los vinos elaborados por maceración pre-fermentativa en caliente [27]. Este efecto no se observó en nuestra investigación.

Tabla 2. Composición básica de los vinos

	Alcohol (% v/v)	**Acidez titulable (g/L H_2SO_4)**	**pH**
VT	14,6 ± 0,2 a	3,88 ± 0,19 a	4,01 ± 0,13 a
MPC	14,8 ± 0,1 a	3,76 ± 0,03 a	4,09 ± 0,01 a

Medias con distinta letra indican diferencias significativas ($p<0.05$). VT: vinificación tradicional; MPC: maceración pre-fermentativa en caliente.

4.5.2. Composición fenólica de los vinos y parámetros relacionados

La Tabla 3 muestra la composición fenólica de los vinos VT y MPC a los dos meses del descube (0), 1 y 2 años.

Las concentraciones de polifenoles totales, antocianos, catequinas y proantocianidinas de los vinos MPC a los dos meses del descube y durante su

conservación fueron significativamente mayores respecto a las de los vinos VT. Estos resultados concuerdan con los reportados por otros autores quienes observaron una mayor de los compuestos fenólicos en los vinos que fueron sometidos a un tratamiento térmico pre-fermentativo del mosto [9, 11, 27]. Esta investigación demuestra que las diferencias en la composición fenólica de los vinos se mantienen hasta los dos años posteriores a la primera determinación analítica.

Durante el primer año de conservación, la concentración de polifenoles totales de los vinos VT y MPC disminuye significativamente, estabilizándose hacia el segundo año. Por su parte, la concentración de antocianos de los vinos de ambos tratamientos disminuyó significativamente durante todo el período de evaluación. Estos compuestos experimentan fenómenos de precipitación que pueden ocurrir tanto en los vinos jóvenes como en los envejecidos. En los vinos jóvenes se relaciona con el estado coloidal de la materia colorante y con el tamaño molecular, y depende del contenido alcohólico del vino y de la temperatura de la conservación [28]. En los vinos envejecidos está más relacionado con las reacciones de estos pigmentos con otros constituyentes del vino. La copigmentación [29]; la formación de piranoantocianos [30], la reacción directas tanino-antocianos [31] o la reacción tanino-antociano mediada por el acetaldehído [32] pueden estabilizar a los antocianos, determinando una disminución en su concentración original y un cambio en el color del vino.

La concentración de catequinas aumento significativamente durante la conservación de los vinos de ambos tratamientos en cambio las proantocianidinas disminuyeron, aunque la disminución en la concentración de éstas últimas no fue significativa. Los taninos sufren reacciones de ruptura durante la conservación que generan moléculas de bajo peso molecular [30,33], lo cual podría estar explicando el incremento en la concentración de catequinas observado.

La Tabla 4 muestra el índice de ionización, copigmentación y PVPP determinados a los dos meses del descube para los vinos de VT y MPC. Los vinos de MPC presentaron índices más altos de ionización, copigmentación y PVPP. Estos resultados son consistentes con los obtenidos en la composición fenólica de los vinos (Tabla 3) y reafirman la hipótesis de que la maceración pre-fermentativa en caliente favorece las interacciones entre los antocianos y otros constituyentes del vino

promoviendo la copigmentación y la formación de aductos entre antocianos y taninos.

Tabla 3. Composición fenólica de los vinos

		Polifenoles totales (mg/L)	Antocianos (mg/L)	Catequinas (mg/L)	Proantocianidinas (mg/L)
VT	0	2377 ± 127 a,B	1031 ± 52 a,B	1189 ± 101 c,B	3711 ± 107 a,B
	1er año	2084 ± 123 b,B	566 ± 33 b,B	1408 ± 103 b,B	3472 ± 199 a,B
	2do año	2007 ± 64 b,B	348 ± 16 c,B	1741 ± 28 a,B	3345 ± 139 a,B
MPC	0	2639 ± 126 a,A	1260 ± 148 a,A	1862 ± 141 c,B	4677 ± 113 a,A
	1er año	2554 ± 165 b,A	712 ± 79 b,A	2125 ± 123 b,A	4564 ± 165 a,A
	2do año	2486 ± 149 b,A	438 ± 15 c,A	2454 ± 139 a,A	4435 ± 112 a,A

Letras minúsculas indican diferencias significativas entre los momentos de análisis para un mismo tratamiento ($p<0.05$). Letras mayúsculas indican diferencias significativas entre los tratamientos para un momento de análisis ($p<0.05$). VT: vinificación tradicional; MPC: maceración pre-fermentativa en caliente.

Tabla 4. Composición fenólica de los vinos

	Indice de Ionización (%)	Índice de Copigmentación (%)	Índice de PVPP (%)
VT	25,6 ± 2,8 b	12,2 ± 2,0 b	39,4 ± 4,7 b
MPC	42,2 ± 6,2 a	20,7 ± 0,7 a	51,0 ± 8,8 a

Medias con distinta letra indican diferencias significativas ($p<0.05$). VT: vinificación tradicional; MPC: maceración pre-fermentativa en caliente.

4.5.3. Composición antociánica de los vinos

La concentración de las diferentes formas antociánicas de los vinos VT y MPC a dos meses del descube y a los dos años se observa en la Figura 1a y b respectivamente.

A los dos meses del descube, los vinos MPC presentaron mayor concentración de todas las formas antociánicas respecto a los vinos VT, con excepción de la cianidina-3-O-glucósido donde no se encontraron diferencias significativas (Figura 1a). Estos resultados concuerdan con las concentraciones totales de antocianos obtenidas por espectrofotometría y son explicados por una mayor extracción de estos durante el tratamiento térmico pre-fermentativo. Para los vinos de ambos

tratamientos, la concentración de todas las formas antociánicas disminuyó a los dos años respecto a su concentración original (Figura 1b). En este sentido, la ausencia de diferencias significativas entre los tratamientos sugiere que los vinos MPC tuvieron una mayor disminución en la concentración de estos pigmentos lo cual podría estar relacionado con una mayor formación de pigmentos derivados.

La Figura 2a y b muestra los perfiles antociánicos de los vinos VT y MPC a los dos meses del descube y a los dos años respectivamente.

Los perfiles antociánicos de los vinos VT y MPC fueron significativamente diferentes a los dos meses del descube (Figura 2a). Los vinos MPC presentaron mayor porcentaje de delfinidina, petunidina y peonidina y menor de malvidina respecto a los vinos VT. La malvidina es más resistente a la degradación térmica que otras antocianidinas [11], por lo que no debería ser degradada durante el tratamiento térmico. Por otra parte, se ha demostrado que el calentamiento pre-fermentativo por encima de 60ºC degrada las enzimas polifenoloxidasas, responsables de la oxidación de los compuestos fenólicos en las primeras etapas de la vinificación [12,28]. Debido a que los hidroxilos adyacentes de los grupos O-difenol son sensibles a la oxidación, el malvidin-3-O-glucósido y peonidin-3-O-glucósido que no poseen grupos hidroxilo en posición orto son comparativamente más resistentes a la oxidación que la cianidina-3-O-glucósido [34]. Podría pensarse que el aumento en las proporciones de delfinidina, petunidina y peonidina se debe al hecho de que estas formas fueron preservadas de la oxidación enzimática durante la vinificación.

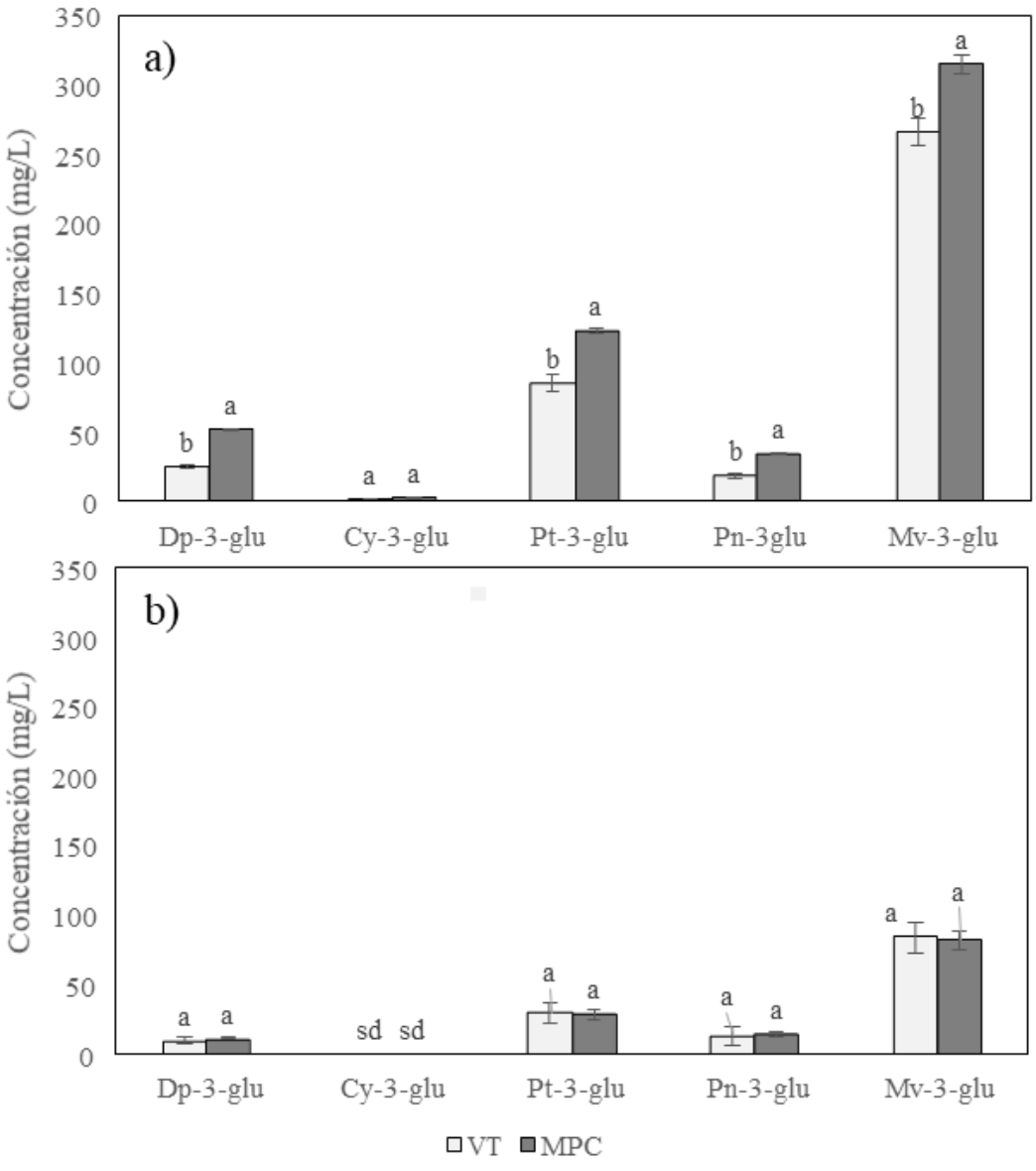

Figura 1. Concentración de antocianos a dos meses del descube (a) y a los dos años (b). Medias con distinta letra indican diferencias significativas ($p<0.05$). VT: vinificación tradicional; MPC: maceración pre-fermentativa en caliente; Dp-3-glu: Delfinidina-3-glucósido; Cy-3-glu: Cianidina-3-glucósido; Pt-3-glu: Petunidina-3-glucósido; Pn-3-glu: Peonidina-3-glucósido; Mv-3-glu: Malvidina-3-glucósido.

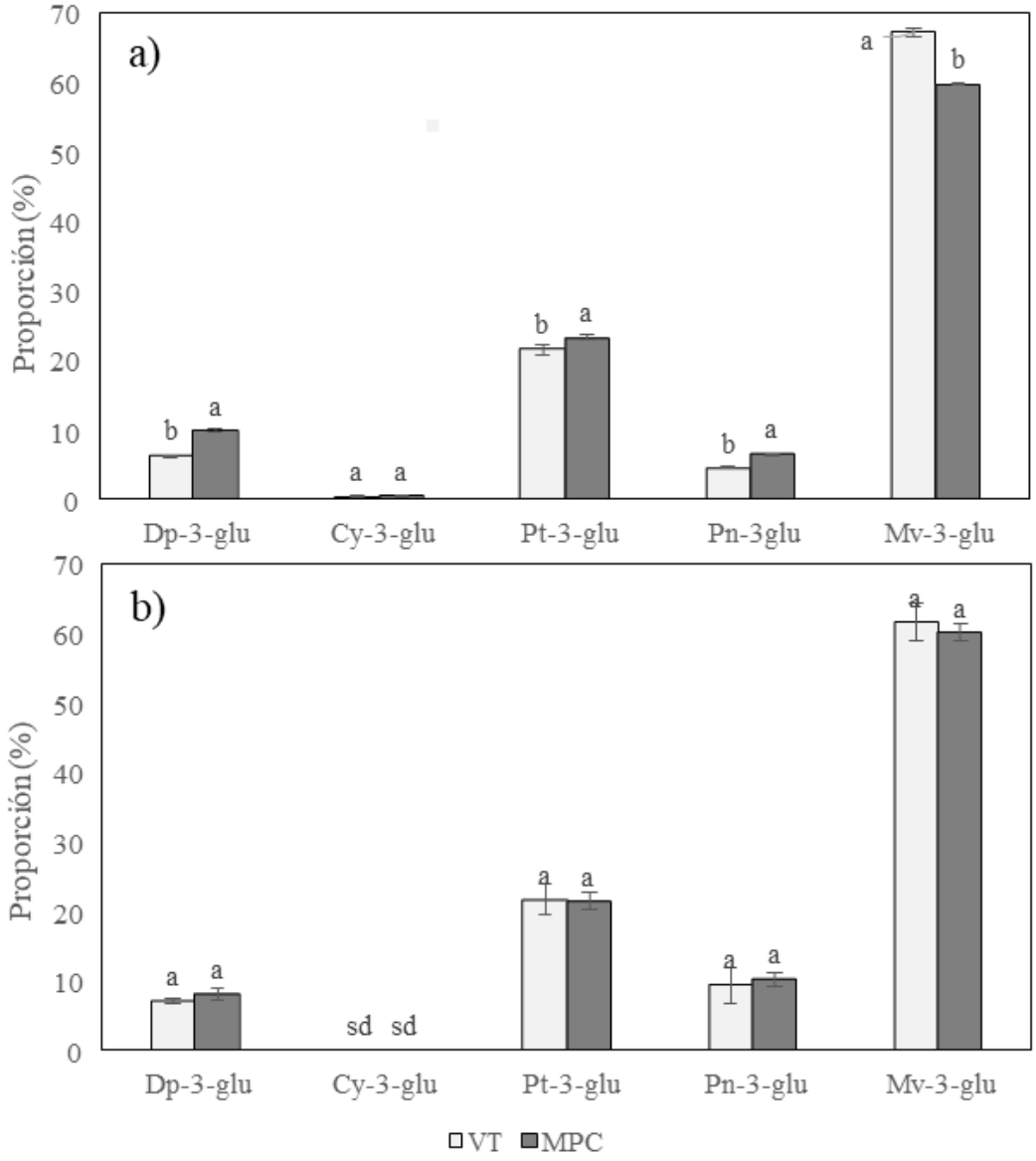

Figura 2. Proporción de antocianos a dos meses del descube (a) y a los dos años (b). Medias con distinta letra indican diferencias significativas ($p<0.05$). VT: vinificación tradicional; MPC: maceración pre-fermentativa en caliente; Dp-3-glu: Delfinidina-3-glucósido; Cy-3-glu: Cianidina-3-glucósido; Pt-3-glu: Petunidina-3-glucósido; Pn-3-glu: Peonidina-3-glucósido; Mv-3-glu: Malvidina-3-glucósido.

A los dos años no se observaron diferencias en los perfiles antociánicos de los vinos de ambos tratamientos (Figura 2b). Es posible que delfinidina, petunidina y peonidina, que se encontraban en mayor proporción en los vinos MPC al descube, hayan sido las más afectadas durante la conservación respecto a las otras formas

antocíanicas. En este sentido, estos pigmentos son más susceptibles al proceso de oxidación no enzimática que ocurre durante la conservación del vino [34].

4.5.4. Color de los vinos

Los parámetros de color de los vinos VT y MPC se muestran en la Tabla 5.

Tabla 5. Color de los vinos

		Intensidad colorante	L*	C*	H*
VT	0	26,7 ± 0,9 a,B	38,6 ± 1,3 a,A	42,4 ± 0,5 b,A	346,5 ± 1,0 a,A
	1er año	18,4 ± 0,4 b,B	33,3 ± 2,2 b,A	48,9 ± 0,4 a,A	13,6 ± 1,3 c,A
	2do año	17,9 ± 0,1 b,B	34,3 ± 1,7 b,A	48,5 ± 0,1 a,A	23,4 ± 1,5 b,A
MPC	0	34,7 ± 1,5 a,A	27,6 ± 1,6 c,B	43,8 ± 2,2 b,A	348,1 ± 0,8 a,A
	1er año	27,2 ± 1,1 b,A	20,4 ± 1,7 b,B	49,7 ± 0,4 a,A	9,1 ± 3,8 c,A
	2do año	23,0 ± 0,4 c,A	22,6 ± 0,8 b,B	47,7 ± 1,3 a,A	21,3 ± 2,3 b,A

Letras minúsculas indican diferencias significativas entre los momentos de análisis para un mismo tratamiento ($p<0.05$). Letras mayúsculas indican diferencias significativas entre los tratamientos para un momento de análisis ($p<0.05$). VT: vinificación tradicional; MPC: maceración pre-fermentativa en caliente.

A los dos meses del descube, los vinos MPC presentaron un color más intenso que los VT. Específicamente, la intensidad colorante (IC) fue significativamente más alta y la luminosidad (L*) significativamente más baja. Estos resultados son explicados fundamentalmente por la mayor concentración de antocianos y mayores valores de los índices de ionización, copigmentación y PVPP de los vinos MPC. La cromaticidad (C*) y el tono (H*) fue mayor en los vinos MPC, pero estas diferencias no fueron significativas.

Si bien la intensidad colorante disminuyó significativamente durante la conservación de los vinos de ambos tratamientos, los vinos MPC mantuvieron los mayores valores luego de dos años de evaluación. En este sentido, el aumento en la extracción de antocianos desde las primeras etapas de la maceración y el aumento en la extracción de taninos permite una mayor asociación de estas moléculas, lo que se ha reportado como un factor determinante para mejorar la estabilización del color

[24, 25]. La luminosidad de los vinos disminuyó únicamente durante el primer año de conservación, siendo los vinos MPC los más oscuros. Este resultado no resulta muy lógico ya que la disminución en la intensidad del color implicaría un incremento en la luminosidad y no una disminución como fue observado en nuestros resultados. La cromaticidad se incrementó en ambos vinos durante el primer año, sin observarse diferencias significativas entre los tratamientos. Por otra parte, los vinos pasaron de tonalidades (H*) rojo-azuladas al descube a tonalidades más rojo-amarillentas a los dos años. Esta evolución del color es la esperada ya que la mayoría de los pigmentos derivados poseen color amarillo-naranja y contribuyen al cambio de color durante el envejecimiento del vino tinto [34].

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4.6. CONCLUSIONES

La maceración prefermentativa en caliente permitió elaborar vinos Tannat con mayor concentración de compuestos fenólicos e intensidad colorante al descube y hasta los dos años de conservación.

En particular, los vinos elaborados con esta técnica presentaron mayor concentración de compuestos fenólicos totales, antocianos, catequinas y proantocianidinas al descube, 1 y 2 años de conservación. Al descube, el perfil antocianico de los vinos fue modificado por la maceración pre-fermentativa en caliente, en donde se preservan las formas antociánicas más susceptibles a la oxidación enzimática. Sin embargo, estas formas antociánicas son degradadas durante la conservación del vino. La intensidad del color de los vinos MPC fue significativamente mayor al descube y durante la conservación, sugiriendo un color más estable. Estos resultados están de acuerdo con los perfiles antociánicos de los vinos y las mayores concentraciones de antocianos y taninos que podrían favorecer la formación de pigmentos derivados. Sin embargo, los parámetros cromáticos relacionados a la calidad del color no fueron afectados por esta técnica de vinificación.

Futuras investigaciones deben centrarse en determinar el efecto de la maceración pre-fermentativa en caliente sobre la evolución del perfil antociánico de los vinos y la formación de pigmentos poliméricos durante la conservación del vino.

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5. IMPACT OF MUST REPLACEMENT AND HOT PRE-FERMENTATIVE MACERATION ON THE COLOR OF URUGUAYAN TANNAT RED WINES *

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5.1. ABSTRACT

This research aimed to evaluate the impact of different options for winemaking on the color composition of Uruguayan Tannat red wines. The techniques evaluated were the substitution of ripe grape juice with immature grape juice and the heating of the crushed grapes before fermentation, called must replacement and hot pre-fermentative maceration, respectively. These procedures were proposed to reduce the alcohol content and increase the phenolic composition of the wine, according to the expected effects of climate change and current trends in consumer preferences. The investigation was made over three consecutive years (2016, 2017, and 2018). Both winemaking techniques allow the enhancement of the chromatic characteristics of wines via the modification of the phenolic composition. Additionally, such techniques allow the overcoming of the well-known limitations in the extractability of anthocyanins presented by the Tannat cultivar. Hot pre-fermentative maceration increases the proportion of the most oxidizable molecules delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, and petunidin-3-O-glucoside, suggesting heat inactivation of polyphenoloxidases enzymes. Must replacement and hot pre-fermentative maceration are technological alternatives that could significantly improve the intensity and chromatic characteristics of red wines.

Keywords: Tannat; must replacement; hot pre-fermentative maceration; wine color; wine composition

5.2. INTRODUCCIÓN

The color of red wine is generally the first sensory property to be appreciated by consumers [1]. The limpidity and intensity of the wine color are responsible for the consumer's first opinion, which can also condition the sensory perception of other wine qualities, such as the aroma, taste, or mouthfeel [1,2]. Wines with little color, the presence of precipitates in the bottle, or with unexpected hue relative to their age can be a reason for an initial rejection [3].

Anthocyanins are the primary pigment responsible for the color of grapes and young red wines [4]. These compounds are synthesized by the secondary metabolism of the vine and are accumulated in grape skins during maturation [5]. In *Vitis vinifera* cultivars, grape anthocyanins are delphinidin, cyanidin, petunidin, peonidin, and malvidin monoglucosides, as well as acylated derivatives with acetic, p-coumaric, and caffeic acids. The composition of wine anthocyanins is determined by the cultivar [6–8], the grape maturity state and the extractability of its components [9,10], and the maceration procedures used in winemaking [11–13]. The climatic conditions and therefore the year of each harvest are factors of great importance [9,10,14,15]. In traditional winemaking, only 40% of the anthocyanins of the grapes are transferred to the wine [4,16]. The limited extraction of anthocyanins is mainly due to the lack of permeability of cell walls and cytoplasmic membranes [17,18], because these compounds are in the skin, in the upper cellular layers of the hypodermis. The composition of cell walls is genetically determined and modifies the changes in the hardness of skin and seed tissues along with ripening. The extraction of anthocyanins and proanthocyanidins during winemaking depends on the grape variety [19,20]. The simultaneous development of maceration and alcoholic fermentation influence the extraction of polyphenols, because the ethanol content determines the disintegration of the vacuolar membranes and the walls of the skin cell [15]. Anthocyanins are compounds easily soluble in water and therefore are dissolved from the beginning of the maceration, independent of the ethanol concentrations [21].

However, wine color not only depends on the anthocyanin concentration [4,22]. Anthocyanins undergo structural transformations depending on the pH of the medium. They present a red color in an acid medium, acquire a violet color when approaching a neutral pH, and decrease the intensity of the color as the pH increases. Under very high

pH conditions, anthocyanins are irreversibly destroyed. Further, during the making, conservation, and aging of wine, the formation of new compounds and their polymerization modify the red wine color and determine its stability [23]. These molecules are partially degraded due to hydrolysis or oxidation reactions [24,25], while other molecules participate in cyclo-addition reactions with metabolites produced by yeasts [26]. Other anthocyanins are condensed with catechins [27,28]. A significant fraction of the anthocyanins extracted from grape skins will be adsorbed by yeasts and will precipitate in the lees [29], whereas there is also a fixation of these compounds in the solid parts of the grapes [21].

In the last few decades, several alternative techniques of maceration have been proposed that allow a differentiated extraction of the phenolic and aromatic compounds of the grape to the wine to improve quality and aging potential [11,13,30]. Most of these techniques have had a substantial impact on the color of red wines [13,31]. More recently, some research groups have evaluated different winemaking techniques to regulate the ethanol content and pH of wines in response to the effect of global warming on the composition of grapes [32–34]. The results obtained with the application of these procedures have allowed the reduction of the ethanol content and pH of the wines, but the effects on the sensory characteristics, particularly on the color, have not been conclusive [32,33,35].

In Uruguay, Tannat is the most relevant red cultivar due to its adaptation to the country's eco-physiological conditions. The polyphenolic and anthocyanin richness of Tannat wines is related to the enological potential of their grapes. The grapes have a low extraction capacity of anthocyanins and lower proportions of malvidin and acetylated glycosides compared with other red cultivars, such as Cabernet Sauvignon and Merlot [30]. Consequently, the color stability of Tannat wines is lower than wines of other varieties [3,8], although they maintain the characteristic anthocyanin profile of the grape of origin for a specified period. Additionally, high interannual climate variability has been recorded during the ripening period, which strongly affects the composition of the grape. In particular, high temperatures during the ripening period cause a high accumulation of sugars and degradation of acidity [36] due to the consumption of malic acid [37] and alter the synthesis of polyphenols [38,39]. Thermal stress during the maturation period causes the degradation and inhibition of the accumulation of

anthocyanins, compounds responsible for the color of grapes and red wines [38]. Currently, there is a growing concern of winemakers regarding having tools that allow regulation of the contents of ethanol and pH and the concentrations of phenolic compounds without causing detriment to the color of Tannat red wines. The intensity and hue of the color of Tannat red wines determine the target market and commercial value.

This research aims to study the impact of must replacement and hot pre-fermentative maceration in the color of Uruguayan Tannat red wines produced in three consecutive vintages. Both techniques have been proposed to obtain red wines with lower alcohol content and pH and higher phenolic compound concentration [35]. Hot pre-fermentative maceration consists of the degradation of cellular structures, mainly of the grape skins, through the heating of the must before alcoholic fermentation at a temperature and a period variable [40]. These techniques increase the extraction of phenolic compounds. Moreover, must replacement consists of the substitution of a percentage of grape juice of very ripe grapes with the grape juice of unripe grapes before alcoholic fermentation to reduce the alcohol content and the pH of the wines [35].

5.3. MATERIALS Y METHODS

5.3.1. Chemicals and equipment

Methanol, acetonitrile, formic acid, and acetic acid were of HPLC grade (>99%) and purchased from Panreac (Barcelona, Spain). Acetaldehyde (>99.5%), ascorbic acid (>99%), and sodium acetate (>99%) were purchased from Sigma-Aldrich (Madrid, Spain). Absolute ethanol and hydrochloric acid (37%) were purchased from Panreac. Malvidin-3-O-glucoside chloride (≥95%), was purchased from Extrasynthese (Genay, France). A Winescan TM Autosampler 79,000 infrared analyzer (Foss, USA) and Foss Integrator software version 154 (Foss, Denmark) were used to determine the alcohol content, total acidity, and pH of the wines. The HPLC analyses were performed using an Agilent 1200 series liquid chromatograph equipped with a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1316A column oven, and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). All the spectrophotometric measurements were performed using a

Helios Alpha UV–Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA, USA).

5.3.2. Grapes and wines

This research was carried out with grapes of Tannat *Vitis vinifera* L., Vitis International Variety Catalogue (VIVC) number 12,257 [41], in 2016, 2017, and 2018 vintages. The grapes were manually harvested from a commercial vineyard located in Canelones in the south of Uruguay.

At the beginning of veraison, 100 kg of Tannat grapes were harvested to obtain a must with high acidity and low sugar concentration. The grapes were crushed (Alfa 60 R crusher, Italcom, Piazzola Sul Brenta, Italy) and lightly pressed in a manual press to obtain 50 L of an unripe grape must. The grape must was immediately sulphited with 100 mg/L of $K_2S_2O_2$, settled overnight, packaged in a 50-L recipient, and conserved at 4 °C until use. When the grapes reached technological maturity, 120 kg of grapes were collected and randomly distributed into 12 lots of 10 kg. The grapes were destemmed and crushed (Alfa 60 R crusher, Italcom, Piazzola Sul Brenta, Italy), and the must was sulphited with 100 mg/L of $K_2S_2O_2$ and distributed in 12 polyethylene containers (each of 10-L capacity). The must containers were randomly divided into two groups of six containers each. Six containers were considered to be controls (original must—OM), whereas in the other six containers (must replacement—MR), 3 L of the original grape must were replaced with 3 L of unripe grapes must with the aim of decreasing sugar content and pH.

Next, three containers of each experimental group (OM and MR) were traditionally macerated (TM), whereas the other three were subjected to hot pre-fermentative maceration (HM) for 1 h at a temperature between 60 and 70 °C. The heating was carried out by transferring the pomace to 11-L stainless-steel tanks that were submerged in a hot water bath (at 80–90 °C). During warming, the pomace was homogenized manually. At the end of the heat treatment, the stainless-steel tanks were submerged in a cold water bath in order to refrigerate them to ambient temperature (around 26 °C). After that, the must was transferred to the original 10-L polyethylene containers. Thus, four experimental groups for each cultivar were

obtained: control wine with traditional maceration (OM-TM), must replacement with reduced alcohol and pH in the wine obtained by traditional maceration (MR-TM), control wine with hot pre-fermentative maceration (OM-HM), and must replacement and hot pre-fermentative maceration (MR-HM) (Figure 1).

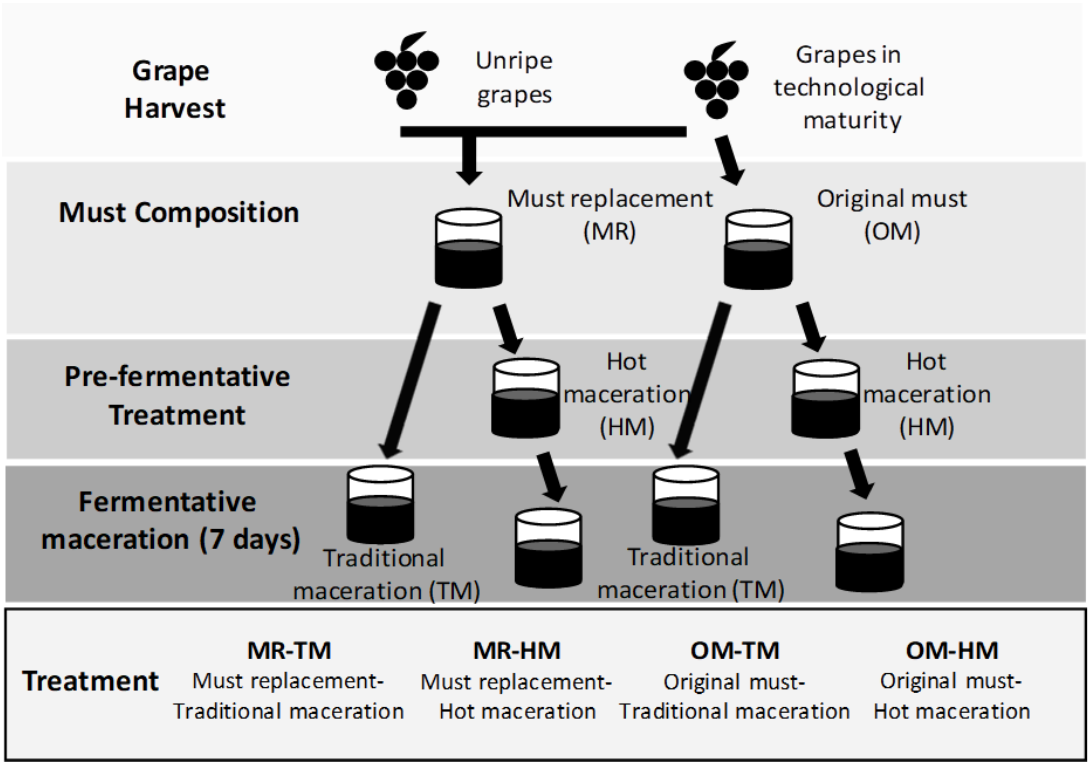

Figure 1. Diagram of the experimental design.

All the containers were inoculated with 200 mg/L of active dry yeast (*Saccharomyces cerevisiae* ex *bayanus* Natuferm 804; Oenobiotech, Paris, France) and were fermented in contact with the skins and seeds. During maceration, all the containers were manually pumped over once daily, followed by a manual punching down of the cap to favor polyphenol extraction. The fermentation temperature ranged between 26 and 29 °C in the 2016 vintage, between 22 and 27 °C in the 2017 vintage, and between 25 and 29 °C in the 2018 vintage. After 7 days of maceration, the free-run wine was extracted by gravity, and the resting pomace was lightly pressed in a manual press. The free-run wine and the lightly pressed wine of each

tank were blended and maintained in 5-L vessels at room temperature (18 ± 2 °C). The alcoholic fermentation was completed when the daily measurements of the must density were less than 998 g/L for three consecutive days. The wines were preserved in polyethylene containers of 5 L of capacity at laboratory room temperature (18 ± 2 ˚C), and once spontaneous malolactic fermentation was finished (around 35 days later), all the wines were stabilized with 100 mg/L of $K_2S_2O_2$ and 300 mg/L of lysozyme (Delvo®Zyme, Delft, the Netherlands). Finally, the wines were bottled and stored in a dark cellar at laboratory ambient temperature until analysis. The analyses started 2 months after bottling and ended 3 weeks later.

5.3.3. Standard grape juice and wine analysis

Analytical methods recommended by the International Organization of Vine and Wine [42] were used to determine the sugar concentration, pH, and titratable acidity of the grape juices. During the fermentation, the temperature and density of the must were monitored daily. The ethanol content, titratable acidity, pH, residual sugars, and volatile acidity of the wines were analyzed using an infrared analyzer Winescan TM Autosampler 79,000 (Foss, USA) and Foss Integrator software version 154 (Foss, Denmark).

5.3.4. Color parameters

The color parameters were determined directly on wine samples placed in a 1-mm pathlength cuvette. Color intensity (CI) was estimated using the method proposed by Glories [43]. The CIELAB coordinates, lightness (L*), chroma (C*), hue (h*), red-greenness (a*), and yellow-blueness (b*), were determined according to the method described by Ayala et al. [44]. Thus, data processing was performed with MSCV software [45].

5.3.5. Spectrophotometric analysis of anthocyanins and related parameters

The total anthocyanin content of the grapes, their extractability, and their total phenolic index were determined, according to the procedure outlined by González-Neves et al. [46].

The polyphenolic composition was evaluated using classical spectrophotometric indices. The total polyphenols were determined using the Folin–Ciocalteu method, according to Singleton and Rossi [47], and their contents in the wines are expressed in mg of gallic acid per liter. The total pigment and anthocyanin content were analyzed using the technique described by Ribéreau-Gayon and Stonestreet [48], and they are expressed as mg of malvidin-3-glucoside equivalent (EMG) per liter. Catechins were quantified using the method proposed by Swain and Hillis [49], and their concentrations are expressed in mg of D-catechin per liter. Proanthocyanidins were determined according to Ribéreau-Gayon and Stonestreet [50], and their contents are expressed in mg of cyanidin chloride per liter of wine. The ionization index (which indicates the proportion of red-colored anthocyanins at wine pH) and the PVPP index (which indicates the proportion of anthocyanins combined with proanthocyanidins) were determined in line with the method described by Glories [43]. The copigmentation index was measured in accordance with the procedure outlined by Boulton [4].

5.3.6. HPLC anthocyanidin analysis

Reversed-phase HPLC analyses of the anthocyanidins were carried out by injecting 40 μL of wine into an Agilent 1200 series liquid chromatographer (HPLC-DAD) and using an Agilent Zorbax Eclipse XDBC18, 4.6 × 250 mm, 5-μm column (Agilent Technologies). The solvents used were 10% aqueous formic acid (solvent A) and a mixture of 45% methanol, 45% water, and 10% formic acid (solvent B), following the method described by Valls [51]. Chromatograms were recorded at 530 nm, and anthocyanin standard curves were made using malvidin-3-O-glucoside chloride. Compounds were identified considering the relative retention times between the compounds and by recording their UV spectra with a diode array detector and comparing these with the UV spectra reported by Valls [51]. The five anthocyanidin-3-monoglucosides of wine (delphinidin, cyanidin, peonidin, petunidin, and malvidin) and their respective acetylated and p-coumarylated anthocyanins were quantified.

5.3.7. Statistical analysis

All the data are expressed as the arithmetic average ± standard deviation of three replicates. Multifactorial analysis of variance (MANOVA) was carried out with INFOSTAT [52] (version 2017, Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina), and multiple comparisons between samples were performed by using the Hotelling test.

5.4. RESULTS AND DISCUSSION

5.4.1. Fermentation kinetics

Figure 2 shows the fermentation kinetics of the treatments evaluated according to the year of vintage. In the treatments with must replacement (MR-TM and MR-HM), the density was lower due to lower concentrations of sugars. Therefore, these musts finished alcoholic fermentation before the must without substitution and traditional maceration (OM-TM). These results were expected, because the sugar concentrations of the musts were low, and the level of alcohol generated did not affect the development of the yeasts, achieving a complete fermentation of the musts. Moreover, the musts produced by hot pre-fermentative maceration finished alcoholic fermentation before the traditional maceration musts. When a must is subjected to temperatures above 40 °C, the populations of lactic and acetic bacteria, as well as yeasts, disappear [53]. Additionally, the extraction of growth factors during warming favors the subsequent development of inoculated yeasts [54], which explains the results obtained for this treatment. These results are more clearly observed for the wines produced from the 2016 and 2018 vintages, as the climatic conditions allowed the grape to reach a higher degree of maturity. On the contrary, in the vintage of 2017, the ripening stopped, so the harvested grapes were immature.

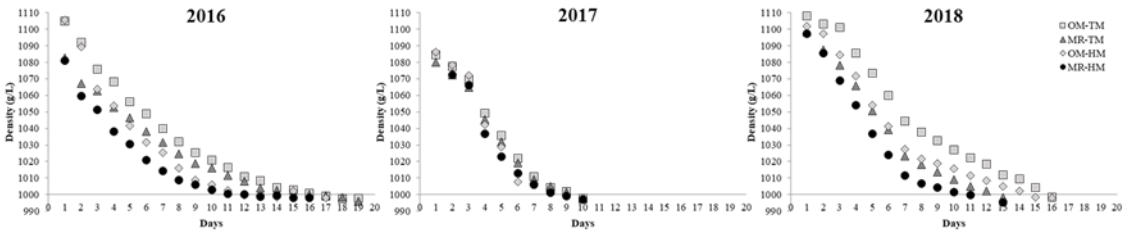

Figure 2. Fermentation kinetics of the treatments by the year of vintage. Average of three wines. OM-TM: original must and traditional maceration; MR-TM: must replacement and traditional maceration; OM-HM: original must and hot pre-fermentative maceration; MR-HM: must replacement and hot pre-fermentative maceration.

5.4.2. General composition of wines

Table 1 shows the effects of the year of vintage, must composition, and winemaking technique factors on the contents of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of wines.

The vintage factor expresses the average content of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of all the wines produced in the same vintage, regardless of the must composition and winemaking procedure. Wines produced from the 2018 vintage had the highest ethanol content, and those of the 2017 vintage had the lowest. The highest values of titratable acidity and pH were recorded in the wines produced in 2016 and the lowest in 2017. During the ripening of the grapes, the sugar concentration and the pH increased, whereas titratable acidity decreased. However, climatic conditions during the ripening determine the composition of the grape [14,41]. The ripeness conditions were different between vintages. The grapes harvested in 2016 and 2018 had better maturation conditions, with high concentrations of sugar and an optimum pH. In contrast, in 2017, grape maturation halted, resulting in lower concentrations of sugars and pH. The wines produced from the 2016 and 2017 vintages presented residual sugar concentrations lower than 2 g/L [53], whereas the 2018 wines presented a slightly higher value. These results may be related to a higher concentration of non-fermentable sugars in the 2018 vintage, because the grapes showed a high concentration of sugars. Another

possibility may be that the high levels of alcohol generated during alcoholic fermentation affected the development of yeasts in the final stages of alcoholic fermentation [53]. The volatile acidity of the wines elaborated in the different vintages were expected according to the winemaking system used.

Table 1. General composition of wines

Factor analyzed		Ethanol (% v/v)	Titratable acidity (gH_2SO_4/L)	pH	Residual sugars (g/L)	Volatile acidity (gH_2SO_4/L)
Year of vintage (*)	2016	14.0 ± 0.1 b	4.30 ± 0.27 a	3.92 ± 0.16 a	1.47 ± 0.41 c	0.36 ± 0.07 b
	2017	11.2 ± 0.2 c	2.93 ± 0.05 c	3.86 ± 0.04 c	1.85 ± 0.21 b	0.43 ± 0.09 a
	2018	15.4 ± 0.2 a	3.85 ± 0.03 b	3.89 ± 0.09 b	2.44 ± 0.44 a	0.44 ± 0.07 a
Must composition (**)	OM	14.0 ± 0.1 a	3.51 ± 0.17 b	3.95 ± 0.09 a	2.07 ± 0.59 a	0.43 ± 0.09 a
	MR	13.0 ± 0.1 b	3.88 ± 0.06 a	3.83 ± 0.09 b	1.83 ± 0.39 a	0.39 ± 0.08 b
Maceration technique (***)	TM	13.3 ± 0.2 b	3.74 ± 0.19 a	3.87 ± 0.09 a	2.01 ± 0.55 a	0.47 ± 0.06 a
	HM	13.7 ± 0.1 a	3.64 ± 0.04 a	3.92 ± 0.09 a	1.89 ± 0.46 a	0.35 ± 0.05 b
Must composition - Maceration techinque (****)	OM-TM	14.0 ± 0.2 a	3.61 ± 0.30 b	3.92 ± 0.09 b	2.30 ± 0.56 a	0.50 ± 0.06 a
	MR-TM	12.6 ± 0.2 c	3.87 ± 0.09 a	3.81 ± 0.12 d	1.72 ± 0.37 c	0.45 ± 0.06 b
	OM-HM	14.0 ± 0.1 a	3.40 ± 0.03 c	3.98 ± 0.08 a	1.84 ± 0.53 bc	0.36 ± 0.05 c
	MR-HM	13.4 ± 0.1 b	3.88 ± 0.04 a	3.85 ± 0.09 c	1.95 ± 0.39 b	0.33 ± 0.05 c

(*) Average of 12 wines ± standard deviation regardless of the grape juice composition and the winemaking technique. (**) Average of the 18 wines ± standard deviation regardless of the year of vintage and the winemaking technique. (***) Average of 18 wines ± standard deviation regardless of the year of vintage and the grape juice composition. (****) Average of 9 wines ± standard deviation regardless of the year of vintage. Different letters indicate statistical differences ($p < 0,05$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot pre-fermentative maceration.

The must composition factor expresses the average contents of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of all the wines produced with original must (OM) or must replacement (MR), independent of the vintage or the maceration technique. The MR wines had lower ethanol content and pH and higher titratable acidity than the OM wines. These results were expected, because the must replacement of the well-ripened grapes with the must of unripe grapes implicated a decrease in sugar content and pH and an increase of titratable acidity. These data agree with those obtained by Kontoudakis et al. [32] and Role et al. [33], who proposed a similar but different procedure. Kontoudakis et al. [39] proposed the simultaneous reduction of the ethanol content and the pH of the wine by mixing wines, one of them obtained with green grapes and the other with ripe grapes [32].

Moreover, Role et al. [33] proposed three alternative procedures to achieve alcohol reduction: (i) pre-fermentation addition of liquid derived from grape must (reverse osmosis byproduct); (ii) mixed fermentations with strains of Starmerella bacillaris and *Saccharomyces cerevisiae*; and (iii) dealcoholization of wine post-fermentation with a polypropylene membrane. In our research, the partial replacement of grape juice had a low impact on the chemical composition of the wines. The concentration of residual sugars in the wine was not affected by the must replacement, whereas the volatile acidity was slightly lower.

The maceration technique factor expresses the average contents of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of all the wines produced by traditional maceration or hot pre-fermentative maceration, without considering the initial must composition and the vintage. The HM wines presented higher ethanol content than the TM wines, without significant differences in the total acidity or pH. The highest levels of ethanol were observed in the HM wines. These results agree with those obtained by other authors [54,55] and could be explained by two factors, the first of which is due to how the hot pre-fermentation maceration was carried out. Weak evaporation of water could have occurred during the pre-fermentative stage, which may have contributed to the small concentration of all the compounds of the must, particularly the sugars. Second, a higher level of amino acids has been reported in thermovinified musts [54]. This increase in amino acids could contribute to improving ethanol yields [56]. However, the residual sugar concentrations of the wines were not affected by the winemaking technique, whereas the volatile acidity was slightly lower.

The must composition x maceration technique factor expresses the average contents of ethanol, titratable acidity, pH, residual sugars, and volatile acidity of all the wines produced with the original must and traditional maceration (OM-TM), must replacement and traditional maceration (MR-TM), original must and pre-fermentative hot pre-fermentative maceration (OM-HM), or must replacement and hot pre-fermentative maceration (MR-HM), regardless of the vintage. The ethanol content of the OM-TM and OM-HM wines was significantly higher than that of the MR-TM and MR-HM wines, which evidenced significant differences due to the

maceration techniques used. In contrast, the ethanol content of the MR-HM wine was significantly higher than that of the MR-TM wine, probably because of the maceration technique described previously. As expected, the MR-TM and MR-HM wines presented the highest values of titratable acidity and the lowest pH values in comparison with the OM-TM and OM-HM wines. When analyzing the combination of both winemaking techniques, changes in pH were observed, associated with the initial composition of the must and the maceration technique. In this sense, it has been reported that wines developed via hot pre-fermentative maceration have shown higher pH values, because, during the pre-fermentative heating, the extraction of cations increases, which results in a rise in the pH mainly given by the salification of tartaric acid [57]. Additionally, the wines produced with must replacement and/or hot pre-fermentative maceration showed the lowest concentrations of residual sugars and lower values of volatile acidity.

5.4.3. Spectrophotometrical phenolic composition and related parameters

The phenolic composition of the wines was different according to the vintage (Table 2). Wines produced in 2016 were characterized by the highest concentrations of total polyphenols, anthocyanins, and proanthocyanidins, whereas the wines produced in 2017 presented the lowest values. The concentrations of catechins in the wines produced in 2018 were significantly higher than those in the wines produced in other vintages. The concentration of anthocyanins did not significantly differ from the wines produced in 2016, whereas the concentrations of total polyphenols and proanthocyanidins were intermediate (Table 2). These results indicate that the ripening stage of the grapes strongly determined the wine composition. Fourment et al. [58] reported that for the conditions of Uruguay, the interannual climate variability strongly modifies the composition of the grape, especially in the concentration of secondary metabolites.

Total polyphenols, anthocyanins, catechins, and proanthocyanidins of the MR wines did not differ significantly from those of the OM wines. The techniques proposed by Role et al. [33] to reduce the alcohol content of the wines reduced the concentration of highly polymerized flavonols without substantially modifying the

concentration of anthocyanins. According to these authors, the lower ethanol concentration could be the extraction of high polymerized flavanols from the grapes during fermentation. Moreover, they suggest that although lower concentration of anthocyanins would be expected, because a portion of must was eliminated, this does not necessarily imply anthocyanin losses, because the replacement was done before maceration. With ripe berries, however, these red pigments are more easily extracted from the skins during the crushing process and the short time of skin contact, and therefore, the fraction removed could contain a considerable amount of anthocyanin [59]. This was not observed in our results. Meanwhile, Kontoudakis et al. [32] found that anthocyanins remained almost unchanged when the ethanol concentration was reduced by 3.0% v/v by replacing a part of the total volume of the grape juice with the same volume of a low-ethanol wine. These authors reported that proanthocyanidin was less abundant in the reduced alcohol wines than in the control wines.

Table 2. Polyphenolic composition of wines.

Factor analyzed		Total polyphenol (mg/L)	Anthocyanins (mg/L)	Catechins (mg/L)	Proanthocyanidins (mg/L)
Year of vintage (*)	2016	2479 ± 252 a	1052 ± 156 a	1769 ± 455 b	4172 ± 714 a
	2017	1624 ± 68 c	614 ± 68 b	1420 ± 58 c	2690 ± 60 c
	2018	2140 ± 43 b	1165 ± 43 a	1883 ± 86 a	3260 ± 80 b
Must composition (**)	OM	2045 ± 140 a	960 ± 67 a	1667 ± 239 a	3397 ± 372 a
	MR	2117 ± 102 a	994 ± 73 a	1714 ± 160 a	3352 ± 197 a
Maceration technique (***)	TM	1784 ± 112 b	838 ± 69 b	1281 ± 215 b	2764 ± 261 b
	HM	2379 ± 129 a	1117 ± 71 a	2100 ± 184 a	3985 ± 308 a
Must composition - Maceration techinque (****)	OM-TM	1821 ± 131 c	832 ± 69 c	1273 ± 268 b	2792 ± 352 b
	MR-TM	1747 ± 94 d	843 ± 69 c	1289 ± 161 b	2735 ± 170 b
	OM-HM	2345 ± 149 b	1088 ± 66 b	2061 ± 209 a	4001 ± 390 a
	MR-HM	2413 ± 109 a	1146 ± 77 a	2141 ± 159 a	3968 ± 225 a

(*) Average of 12 wines ± standard deviation regardless of the grape juice composition and the winemaking technique. (**) Average of the 18 wines ± standard deviation regardless of the year of vintage and the winemaking technique. (***) Average of 18 wines ± standard deviation regardless of the year of vintage and the grape juice composition. (****) Average of 9 wines ± standard deviation regardless of the year of vintage. Different letters indicate statistical differences ($p < 0,05$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot maceration.

In contrast, total polyphenols, anthocyanins, catechins, and proanthocyanidins of the HM wines were significantly higher than those of the TM wines (Table 2).

These results agree with previous studies [30,40,57,60] and confirm that this technique is useful to improve polyphenol extraction, because pre-fermentative heating contributes to degrading the tissues of the skins, releasing these compounds into the must.

When we analyzed the joint effect of the grape juice composition and the maceration technique, it was observed that the wines produced by hot pre-fermentative maceration presented the highest concentrations of the different phenolic families evaluated. In particular, the HM-OM wines presented lower contents of total polyphenols and anthocyanins than the HM-MR wines, whereas no significant differences were observed in the concentrations of catechins and proanthocyanidins given by the initial composition of the must. Similar results were observed between the OM and MR wines made by traditional maceration. These results indicate that the combination of must replacement and hot pre-fermentative maceration increased the concentration of anthocyanins in wines, whereas the concentration of catechins and proanthocyanidins was affected only by this winemaking technique, as was discussed previously.

Table 3 shows the effects of the vintage, must composition, maceration technique, and the combination of must composition–maceration technique on the ionization, copigmentation, and PVPP indices. The ionization index represents the percentage of anthocyanins colored given the standard pH and free SO_2 concentration of the wine [4], the copigmentation index represents the percentage of color due to the copigmentation process [4], and the PVPP index measures the percentage of anthocyanins combined with proanthocyanidins [49]. These indices were different according to the vintage. These results could be explained by the effects of ripening conditions on the concentration and the relationship between the phenolic compounds that subsequently interact in the wine. Thus, the highest indices of ionization and PVPP were recorded in the 2016 vintage together with the highest concentrations of total polyphenols, anthocyanins, and proanthocyanidins, whereas the lowest values of these indices were recorded in the 2017 harvest. In the 2018 harvest, the highest value of the copigmentation index was probably associated with

a higher concentration of catechins, whereas in the 2016 harvest, it was the lowest value.

Table 3. Color fractions of wines

Factor analyzed		Ionization index (%)	Copigmentation index (%)	PVPP index (%)
Year of vintage (*)	2016	33.9 ± 2.3 a	16.5 ± 3.7 c	45.2 ± 0.8 a
	2017	15.7 ± 2.4 c	17.8 ± 4.2 b	35.9 ± 0.8 c
	2018	17.7 ± 0.6 b	31.7 ± 3.1 a	40.0 ± 1.2 b
Must composition (**)	OM	20.1 ± 1.8 b	20.9 ± 3.2 b	38.2 ± 0.9 b
	MR	24.8 ± 1.7 a	23.1 ± 4.0 a	42.4 ± 0.9 a
Maceration technique (***)	TM	18.0 ± 2.1 b	18.4 ± 3.4 b	35.9 ± 0.9 b
	HM	26.9 ± 1.4 a	26.6 ± 3.9 a	44.7 ± 0.9 a
Must composition - Maceration techinque (****)	OM-TM	16.0 ± 2.6 d	15.7 ± 3.0 c	35.2 ± 0.9 c
	MR-TM	20.0 ± 1.7 c	21.0 ± 3.9 b	36.6 ± 1.0 c
	OM-HM	24.2 ± 1.1 b	26.0 ± 3.4 a	41.3 ± 0.9 b
	MR-HM	29.6 ± 1.7 a	25.3 ± 4.3 a	48.2 ± 0.9 a

(*) Average of 12 wines ± standard deviation regardless of the grape juice composition and the winemaking technique. (**) Average of the 18 wines ± standard deviation regardless of the year of vintage and the winemaking technique. (***) Average of 18 wines ± standard deviation regardless of the year of vintage and the grape juice composition. (****) Average of 9 wines ± standard deviation regardless of the year of vintage. Different letters indicate statistical differences ($p < 0,05$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot maceration.

Nevertheless, an effect of the must replacement treatments on the different indices was found. The MR wines presented higher ionization, copigmentation, and PVPP indices. The color of red wine is the result of the concentration of ionized free anthocyanins and the interactions between these and other components of the wine that produce new pigments [22]. During the winemaking, the new pigment produced when anthocyanins combine with tannins is much less sensitive to bleaching by pH and SO_2, so the percentage of coloring increases [12,27].

This effect and the result obtained in the pH (Table 1) of the wines could explain the differences registered in both indices. Further, the HM wines presented higher values of all these indices than the TM wines (Table 4). This effect could be determined by the increase in the concentrations of anthocyanins, catechins, and proanthocyanidins registered in the wines produced with hot pre-fermentative

maceration, which could promote their interaction in the wine by increasing copigmentation and condensation between anthocyanins and tannins [24].

The wines presented significant differences in the evaluated indices given by the initial composition of the must and the winemaking technique with which they were developed. The OM-HM and RM-HM wines presented higher ionization, copigmentation, and PVPP indices than the OM-TM and RM-TM wines, but the highest values recorded were in the wines where pre-fermentative treatment was carried out on the must replacement. The anthocyanin, catechins, and proanthocyanidin contents of the HM wines were higher than those of the TM wines (Table 3). These results suggest that hot pre-fermentation maceration favors the reactions between anthocyanins and tannins, which suggests greater color stability over time, according to [61]. Moreover, when hot pre-fermentative maceration was carried out on the replaced grape juice, the values registered in the indices were substantially higher, suggesting that the combination of both techniques improves the stability of the wine color.

5.4.4. Wine anthocyanin composition

Figure 3 a,b shows the average of the levels and profiles of the anthocyanin composition of the wines elaborated in the 2016, 2017, and 2018 vintages, according to treatment. As observed, total anthocyanin concentrations determined by HPLC-DAD were lower than the total anthocyanin concentrations measured by spectrophotometry. It should be considered that spectrophotometric analysis includes contributions from other pigments in the measurement and therefore overestimates the total anthocyanin concentration, whereas the HPLC-DAD analysis only detects free anthocyanins. In general, Tannat wines had a high non-acylated glucosides, delphinidin, and petunidin proportions and low acylated anthocyanin (acetylated and coumarylated) proportions, as has been previously reported [1,8].

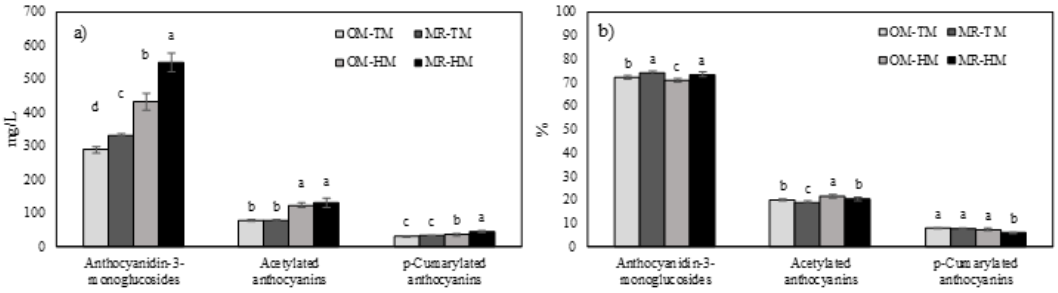

Figure 3. Concentration (a) and proportion (b) of anthocyanidin-3-monoglucosides, acetylated anthocyanins, and p-coumarylated anthocyanins. Average of nine wines ± standard deviation. Different letters indicate statistical differences ($p < 0.05$). OM-TM: original must and traditional maceration; MR-TM: must replacement and traditional maceration; OM-HM: original must and hot pre-fermentative maceration; MR-HM: must replacement and hot pre-fermentative maceration.

Figure 3a shows the effect of the treatments evaluated on the concentration of monoglucosylated, acetylated, and coumarylated anthocyanins. Both must replacement and the hot pre-fermentative maceration contributed to increase the concentrations of monoglucosylated and p-coumarylated anthocyanins compared with those of the wine produced by original must followed by a traditional maceration. Instead, the concentration of acetylated anthocyanins was differentiated between wines only by the maceration technique used. These results confirm those obtained through spectrophotometric analysis. The must replacement seemed to increase the concentration of monoglucosides, probably because these wines had a lower pH, whereas the hot pre-fermentation maceration seemed to generate an increase in the monoglucosylated, acetylated, and p-coumarylated anthocyanins concentration. However, when analyzing the proportion of different anthocyanins, we observed that the differences between treatments were attenuated (Figure 3b).

In general, it was observed that in the wines produced from must replacement the percentage of monoglucocylated anthocyanins was lower, and the percentage of acetylated anthocyanins was higher compared with the wines produced from the original grape must. In this sense, it could be said that there was a modification in the proportion of the different anthocyanin forms that was more affected by the must

replacement than by the hot pre-fermentative maceration. In a previous investigation where must replacement and hot pre-fermentative maceration were evaluated on the composition of Pinot Noir and Tannat wines produced from the 2016 vintage, a differential behavior was observed according to the cultivar [35]. The monoglucosylated anthocyanin concentration of Pinot Noir wines with must replacement was significantly lower in relation to that of the control wines, especially when they were subjected to hot pre-fermentation maceration. This behavior was explained because the lower pH caused by the substitution of must could favor the formation of other pigments at high temperatures. However, in the Tannat wines, the changes in monoglucosylated, acetylated, and p-coumarylated anthocyanin concentrations caused by the must replacement and the hot pre-fermentation maceration were different. In general, no significant effect of the must substitution was observed on the concentration of these anthocyanins, but its concentration was increased when hot pre-fermentative maceration was carried out. The results obtained in this research help to clarify the effect of both winemaking techniques, where must replacement and hot pre-fermentation maceration increase the concentrations of monoglucosylated, acetylated, and p-coumarylated anthocyanins in Tannat wines without modifying their proportions.

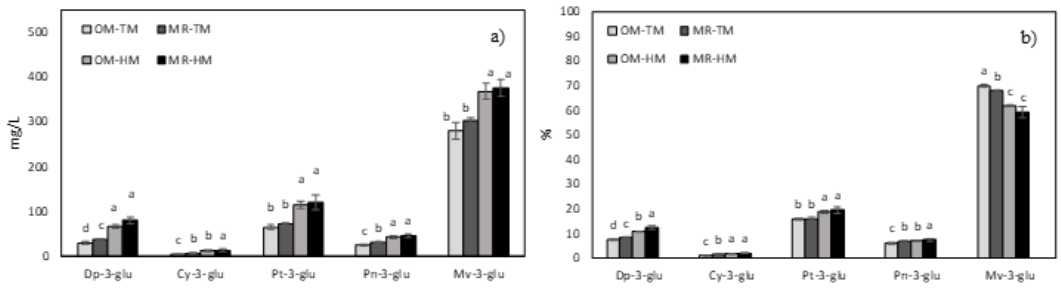

Figure 4. Concentration (a) and proportion (b) of different anthocyanidin forms. Average of nine wines ± standard deviation. Different letters indicate statistical differences ($p < 0,05$). OM-TM: original must and traditional maceration; MR-TM: must replacement and traditional maceration; OM-HM: original must and hot pre-fermentative maceration; MR-HM: must replacement and hot pre-fermentative maceration.

The average concentration of the different anthocyanin forms and the anthocyanin profile of wines produced in the 2016, 2017, and 2018 vintages are shown in Figure 4 a,b, respectively. As can be observed, the concentrations of the different anthocyanin forms of the wines were increased by the must replacement and the hot pre-fermentative maceration with the sole exception of petunidin-3-glucoside, whose concentration in the MR-TM wines did not differ from that in the OM-TM wines.

Wines produced by the combination of both techniques presented the highest concentrations of all anthocyanin forms independent of the composition of the must. It is known that pH and the ethanol content of the medium are factors that contribute to the extraction of the phenolic compounds during the fermentative maceration [24]. As seen in Figure 4b, the anthocyanin profile of the wines was modified by the winemaking techniques used. In general, must replacement and hot pre-fermentative maceration increased the percentages of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, and peonidin-3-glucoside. In particular, the winemaking in which hot pre-fermentation maceration was carried out presented the highest values. In contrast, the percentage of malvidin-3-glucoside was lower in the OM-HM and MR-HM wines than in the MR-TM and OM-TM wines. As previously discussed, hot pre-fermentative maceration allows greater extraction of the anthocyanins by degrading the cellular structures of the skins [34]. The effect of hot pre-fermentation maceration was also observed in the anthocyanin profile of the wines where, in the three vintages, the HM wines had higher percentages of delphinidin, petunidin, and peonidin and a significantly lower percentage of malvidin than the TM wines. At this point, the results obtained in our research are contradictory, because it was shown that wines produced by hot pre-fermentation maceration had a higher percentage of less stable anthocyanidins and a lower percentage of the more stable anthocyanidins. As is known, malvidin is more resistant to thermal degradation than other anthocyanin forms [40], so the idea that hot pre-fermentation maceration affects malvidin more than the other anthocyanidins does not seem to be the correct explanation. On the other hand, it has been shown that pre-fermentative heating above 60 °C degrades polyphenoloxidases enzymes,

which are responsible for the oxidation of phenolic compounds in the early stages of winemaking [24,60]. Because the adjacent hydroxyl groups of o-diphenols are sensitive to oxidation, the malvidin-3-O-glucoside and peonidin-3-O-glucoside that do not possess ortho-positioned hydroxyl groups are comparatively more resistant to oxidation than cyanidin-3-O-glucoside [22]. Therefore, it could be thought that the increase in the proportions of petunidin, delphinidin, and cyanidin occurred, because these forms were preserved from enzymatic oxidation during winemaking by hot pre-fermentation maceration.

5.4.5. Wine color

Table 4 shows the chromatic parameters of the wines produced. The wines produced from the 2016 vintage were characterized by having the highest coloring intensity and the greatest hue, whereas those produced from the 2017 harvest presented the highest lightness and the lowest speed of coloring intensity, chroma, and hue. The wines produced during the 2018 vintage presented the highest chroma value with intermediate values of coloring intensity and hue. In general, the MR wines had a deeper red color, because the color intensity, chroma, and hue were significant higher and the lightness was significant lower than that of the OM wines, while the HM wines also had a deeper color than the TM wines due to the fact that the color intensity and the chroma were significantly higher and the lightness was significantly lower in the HM wines. No significant differences were observed due to the hot pre-fermentative maceration.

When analyzing the effect of the combination of the initial must composition and the maceration technique, it was observed that the MR-HM wines presented the highest intensity of color and chroma and the lowest lightness, whereas the OM-TM wines presented the lowest values. Meanwhile, the OM-HM wines presented a lower value of hue, which suggests that these wines are more bluish. For the other chromatic parameters, the RM-HM and OM-TM wines presented intermediate values.

Table 4. Color of wines

Factor analyzed		Color intensity	Lightness (L*)	Chroma (C*)	Hue (h_{ab})
Year of vintage (*)	2016	32.5 ± 1.4 a	31.5 ± 1.2 b	45.0 ± 1.0 b	348.1 ± 1.6 a
	2017	16.0 ± 0.5 c	60.5 ± 1.5 a	28.1 ± 1.5 c	10.6 ± 1.3 c
	2018	24.2 ± 0.5 b	25.5 ± 0.9 c	53.1 ± 0.8 a	11.8 ± 0.5 b
Must composition (**)	OM	23.2 ± 0.9 b	40.2 ± 1.3 a	41.0 ± 1.2 b	3.27 ± 1.0 a
	MR	25.1 ± 0.8 a	37.9 ± 1.2 b	43.1 ± 1.4 a	3.74 ± 1.2 a
Maceration technique (***)	TM	20.4 ± 0.7 b	44.8 ± 1.2 a	41.4 ± 1.3 b	4.66 ± 0.8 a
	HM	27.9 ± 0.9 a	33.3 ± 1.3 b	42.7 ± 1.3 a	2.35 ± 1.4 a
Must composition - Maceration techinque (****)	OM-TM	19.6 ± 1.0 d	45.9 ± 1.4 a	40.4 ± 1.0 c	5.11 ± 0.6 a
	MR-TM	21.2 ± 0.4 c	43.8 ± 0.9 b	42.6 ± 1.7 b	4.21 ± 0.9 a
	OM-HM	26.8 ± 0.8 b	34.6 ± 1.2 c	41.6 ± 1.5 bc	1.43 ± 1.4 c
	MR-HM	29.0 ± 1.1 a	32.0 ± 1.4 d	43.7 ± 1.0 a	3.27 ± 1.5 b

(*) Average of 12 wines ± standard deviation regardless of the grape juice composition and the winemaking technique. (**) Average of the 18 wines ± standard deviation regardless of the year of vintage and the winemaking technique. (***) Average of 18 wines ± standard deviation regardless of the year of vintage and the grape juice composition. (****) Average of nine wines ± standard deviation regardless of the year of vintage. Different letters indicate statistical differences ($p < 0,05$). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot maceration.

The differences in the chromatic parameters of the wines were associated with the differences in the concentrations of phenolic compounds found, in particular, those of the anthocyanins; the pH of the wine and the percentage of ionized, copigmented, and polymerized anthocyanins were also different among the wines produced in different vintages and from different treatments, as was previously discussed. Furthermore, hot pre-fermentative maceration increasing the extraction of anthocyanins explains the differences in the color parameters. Other authors have previously described similar results [31]. Moreover, in this sense, the increase in the extraction of anthocyanins from the first stages of the maceration and the increase in the extraction of tannins allowed a greater association of these molecules, which has been reported as a determining factor to improve the color stabilization [12]. The results obtained in this investigation in the ionization, copigmentation, and PVPP indices support this theory.

While it is true that in a sensory evaluation, the chromatic characteristics of these wines can be challenging to differentiate, even for a panel of experts, it must be considered that the wines were evaluated two months after bottling. As is known, the color of the wine evolves during conservation, decreasing its coloring intensity and

increasing its angle. The results obtained in this research suggest that wines made by both winemaking techniques could have a more stable color over time and, consequently, a greater potential for aging.

5.4.6. Multifactorial analysis of variance

Multifactorial analysis of the variance shows the effect of each factor and its interaction on the different components of the wines (Table 5). In general, it was verified that the year of vintage (Y), the composition of the grape must (M), the maceration techniques (V), and their interactions (YxM, YxV, MxV, YxMxV) influenced differently the color and the concentration of the phenolic composition of the wine.

The results obtained in the ethanol content, pH, and titratable acidity of the wines seem logical, because the initial composition of the grape must (concentration of sugars, pH, and titratable acidity) at harvest was very different in the vintages due to the climatic conditions of maturation. In this sense, in the treatments where a must replacement for immature grape must was produced, the initial composition of the must, and therefore the wine, was also affected. Moreover, the maceration technique strongly influenced the ethanol content and the pH of the wines. The results obtained regarding the concentration of residual sugars and the volatile acid content of the wines corresponded to the initial composition of the grape and the conditions in which the alcoholic fermentation took place. The vintage and the maceration technique strongly influenced all the phenolic compounds and the ionization, copigmentation, and PVPP indices. Several authors have shown that the phenolic composition of a grape and a wine is determined by the maturation conditions of each year in particular [15]. Moreover, hot pre-fermentative maceration strongly degrades the cellular structures of the skins, extracting their content toward the grape juice and favoring the interaction between them, as mentioned above. The composition of the grape must influences significantly the concentrations of total polyphenols and anthocyanins and the ionization, copigmentation, and PVPP indices.

Table 5. Multifactorial analysis of variance.

	Year of vintage (Y)	Must composition (M)	Vinification technique (V)	Y x M	Y x V	M x V	Y x M x V
Ethanol	5152.9 ***	939.6 ***	137.5 ***	61.8 ***	52.5 ***	131.1 ***	120.2 ***
Titratable acidity	185.5 ***	38.8 ***	2.93 *	25.1 ***	6.9 ***	3.3 *	3.9 **
pH	10.6 ***	101.0 ***	18.3 ***	41.9 ***	10.8 ***	0.4	21.9 ***
Reducing sugars	80.9 ***	9.1 **	2.2	8.0 **	4.6 **	43.4 ***	9.4 ***
Volatile acidity	21.5 ***	11.7 ***	193.1 ***	7.2 **	10.1 ***	0.2	17.8 ***
Total polyphenols	574.8 ***	11.7 ***	824.7 ***	11.9 ***	25.8 ***	0.1	2.9
Anthocyanins	1232.6 ***	10.8 ***	728.2 ***	14.4 ***	89.5 ***	5.11 **	10.1 ***
Catechins	92.4 ***	2.7	800.3 ***	9.5 ***	12.0 ***	1.2	3.0 *
Proanthocyanidins	193.6 ***	0.5	387.0 ***	2.2	0.2	0.1	0.4
Ionization index	248.7 ***	41.6 ***	149.9 ***	4.1 **	28.2 ***	0.9	3.6 **
Copigmentation index	690.4 ***	36.8 ***	385.1 ***	3.8 **	12.9 ***	66.1 ***	3.6 **
PVPP index	15.4 ***	9.33 ***	41.4 ***	1.6	28.8 ***	4.0 *	6.6 ***
Color intensity	1526.4 ***	60.9 ***	966.5 ***	7.6 *	29.5 ***	2.6	2.7
Lightness (L*)	5272.7 ***	65.0 ***	1519.0 ***	10.8 ***	9.6 ***	0.8	5.5 ***
Chroma (C*)	1180.2 ***	25.3 ***	8.0 ***	6.4 ***	94.7 ***	0.1	5.8 ***
Hue (h_{ab})	2160.5 ***	2.0	48.3 ***	5.8 ***	37.0 ***	17.0 ***	7.4 ***
Anthocyanidin-3-monoglucosides	566.1 ***	25.3 ***	364.3 ***	15.5 ***	53.6 ***	1.4	4.6 **
Acetylated anthocyanins	138.1 ***	1.2	231.5 ***	1.2	25.1 ***	1.3	0.1
p-Coumarylated anthocyanins	439.0 ***	16.3 ***	91.2 ***	22.7 ***	1.7	41.9 ***	3.8 **
Delphinidin-3-glucoside	219.3 ***	31.1 ***	531.8 ***	4.9 *	85.2 ***	3.6 *	11.3 ***
Cyanidin-3-glucoside	58.1 ***	2.4	71.2 ***	1.5	20.0 ***	3.7 *	2.6 *
Petunidin-3-glucoside	117.1 ***	3.3 *	158.0 ***	4.9 **	16.6 ***	0.1	0.6
Peonidin-3-glucoside	208.5 ***	12.9 ***	209.2 ***	0.5	12.3 ***	3.2 *	1.6
Malvidin-3-glucoside	623.8 ***	1.6	207.5 ***	8.4 ***	33.6 ***	7.7 ***	1.6

F values and its statistical significance (p <0.001 = ***; p <0.01 = **; p <0.1 = *). OM: original must; MR: must replacement; TM: traditional maceration; HM: hot maceration.

As discussed above, the ethanol content and pH are factors that contribute to the extraction during fermentative maceration, but this effect was only observed in the concentrations of total polyphenols and anthocyanins. A strong interaction between YxV was detected for the phenolic compounds and the indices analyzed, except for the concentration of proanthocyanidins, which was not significant. The YxM interaction was not significant for the concentration of proanthocyanidins or for the PVPP index, whereas the MxV interaction was highly significant only for the anthocyanin concentration and the copigmentation index. The year of harvest and the technique of maceration strongly influenced the concentrations of the different anthocyanin forms, while the initial composition of the grape must only affected the concentrations of monoglucosylated anthocyanins, p-coumarylated, delphinidin-3-glucoside, and petunidin-3-glucoside. Again, a strong interaction was detected in the anthocyanin composition of the wines between the harvest year and the maceration technique (YxV), while the other interactions were significant in the concentrations of some anthocyanin forms.

As discussed earlier, the color of red wine results from the concentration of anthocyanins, their interactions with other phenolic compounds or metabolites of alcoholic fermentation, and the physical–chemical conditions of the medium in which these pigments are found. Therefore, any modification of these factors determines a change in the wine color. The year of vintage, the composition of the grape must, and the maceration technique had a strong impact on all the color parameters, with the only exception being the effect of the composition of the grape must on the hue (hab), which showed a lower significance. All the interactions were significant with respect to the chromatic parameters, except for the MxV interaction, which was only significant for the hue of the wine.

5.5. CONCLUSIONS

The must replacement of mature grape juice for immature grape juice and hot pre-fermentative maceration are technological alternatives to improve the color of Tannat red wines.

The effect of MR on the color and the general composition of wines is highly dependent on the composition of the grape. In contrast, HM improved the intensity and quality of the wine color by increasing the extraction of phenolic compounds and promoting condensation between anthocyanins and tannins, suggesting greater color stability. The results obtained in our research are relevant, because this winemaking technique allows us to mitigate the limitations in the extractability of anthocyanins presented by the Tannat cultivar. Moreover, this winemaking technique modified the anthocyanin profile of the wines in which a relative increase of the most oxidizable forms was obtained. Further studies should be focused on determining the effect of pre-fermentation heating on the degradation of oxidation enzymes and how that influences the phenolic profile of wines.

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6. INFLUENCE OF THE USE OF ACIDIFIED WATER AND LOW-SUGAR WHITE GRAPE JUICE PREVIOUSLY TREATED WITH CATION EXCHANGE RESINS AS STRATEGY FOR REDUCING SIMULTANEOUSLY ETHANOL CONTENT AND PH OF OVER-RIPE RED GRAPES *

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6.1. SUMMARY

This study researches treatments for reducing the ethanol content and pH of wine, by either adding or replacing a portion of overripe red grape juice with acidified water or with a white grape juice of low potential ethanol content previously treated with cationic exchange. All treatments resulted in wines with lower ethanol content; however, the treatments did not always correct wine acidity effectively and sometimes the wine composition was negatively affected because the other wine components were diluted. Specifically, both adding and substituting with acidified water caused an increase in wine pH and a general dilution of the other wine components, particularly when the water was added. In contrast, adding acidified must, unlike acidified water, significantly reduced wine pH and the dilution effect was lower, especially when a portion of the original must was replaced by a low sugar content white must treated by cationic exchange. Moreover, this practice is not unauthorized and seems not to affect, but rather improve, the sensory quality of the wine.

Keywords: Climate change; Ethanol reduction; pH reduction, Red winemaking

6.2. INTRODUCTION

In recent years the alcohol content and pH of most wines have gradually increased (GODDEN AND MUHLACK 2010) probably because winemakers are looking for grapes with high phenolic and/or aromatic maturity (KONTOUDAKIS et al. 2010; KONTOUDAKIS et al. 2011a), and also because climate change is increasing this tendency (JONES et al. 2005; MIRA DE ORDUÑA 2010). If the temperature during ripening is higher, the grape pulp matures faster, and the pH and sugar concentration become too high. The period between veraison and industrial maturity is therefore shorter, which leads to an earlier harvest date. This makes it more difficult to determine the appropriate aromatic and phenolic maturity with precision, and frequently leads to obtain unbalanced wines (ZAMORA 2014).

The Australian Wine Research Institute (AWRI) reported an increase in the mean alcohol level from 12.4% to 14.4% for red wines and from 12.2% to 13.2% for white wines between 1984 and 2008 (GODDEN AND MUHLACK 2010). In another example, the alcohol level of Alsace wines increased from 9% to 12% between 1970 and 2005 (DUCHÊNE AND SCHNEIDER 2005). This trend has also been observed in many other wine-producing countries (SCHULTZ AND JONES 2010; VAN LEEUWEN AND DARRIET 2016).

An excess of alcohol may cause several drawbacks that are associated with slowdowns of alcoholic (BISSON 1999) and malolactic (LONVAUD-FUNEL et al. 1988) fermentations, increases in volatile acidity (ZAMORA 2009), and alterations in the wine's sensory qualities (FISCHER AND NOBLE 2004; LE BERRE et al. 2007). Moreover, excessive alcohol consumption has negative effects on human health (GRØNBÆK 2009) and therefore a high ethanol content on the label of a wine bottle can discourage potential consumers who prefer to be responsible and drink a light wine (SALIBA et al. 2013). Evidently, the wine industry is very concerned with these issues and is therefore interested in producing wines with a moderate alcohol level.

High pH values in wines can also cause certain problems (PATTERSON 2009). A correct pH is needed for a good sensory balance and correct conservation of the wines. When pH is higher than usual, wines lack freshness (NAGEL et al. 1982)

and usually age faster than desired (SIMS AND MORRIS 1984; KONTOUDAKIS et al. 2011c:). Moreover, the higher the pH the lower the antimicrobial effect of sulfur dioxide (USSEGLIO-TOMASSET 1992), which is probably why the problems caused by volatile phenols and biogenic amines have become more common in recent years (LANDETE et al. 2005; ROMANO et al. 2007). Moreover, the color of red wine is drastically affected by pH since the percentage of the red form of anthocyanins, the flavylium cation, decreases greatly when pH increases (KONTOUDAKIS et al. 2011c).

In light of this problem, winemakers can either harvest their grapes when the potential alcohol value and pH are appropriate, or they can harvest them when complete phenolic and aromatic maturity has been reached. In the first case, the grapes would not have reached complete maturity. In the second case, the ethanol content and pH of the grapes would probably be excessive. Neither of these options is conducive to obtain high quality wines and winemakers are therefore concerned about it.

Some ways have been proposed for reducing the increased ethanol content and pH and thus counteracting the impact of climate change on wine production. These include introducing new cultivars and modifying culture techniques (SCHULTZ 2000), harvesting the grapes at an early ripening stage (SCHMIDTKE et al. 2012), adding water and mineral acids to the grape juice before fermentation begins (HARBERTSON et al. 2009), using yeasts (*Saccharomyces* and non-*Saccharomyces*) with a low yield in the sugar-ethanol transformation ratio (CIANI AND FERRARO 1996; TILLOY et al. 2014) and even using glucose oxidase (EC 1.1.3.4) (PICKERING et al. 1998).

Despite the different possibilities, currently the most commonly used methods for reducing alcohol content and pH in wines are physical methods (SCHMIDTKE et al. 2012). More specifically, to reduce ethanol content, the spinning cone column (BELISARIO-SÁNCHEZ et al., 2009) and reverse osmosis (Gil et al., 2013) are used, and to reduce pH, the cationic exchange (LASANTA et al. 2013) or electrodialysis (WALKER et al., 2004) are used.

Using unripe grapes harvested during cluster thinning (KONTOUDAKIS, et al. 2011b) has also been proposed to overcome the problems inherent to overripe red grapes. Briefly, the grape juice of these unripe grapes is fermented, and the resulting wine is treated with charcoal and bentonite to eliminate aromas and phenolic compounds. This green wine, which has a very low ethanol content and pH, is used to substitute some of the grape juice of the overripe Cabernet Sauvignon, Merlot and Bobal grapes just after destemming and crushing. This procedure has been shown to be very effective for simultaneously reducing ethanol content and pH, it is easy to apply and does not require any additional equipment. Moreover, this procedure improves the color intensity of red wines because it decreases pH very effectively and consequently the proportion of the red form of the anthocyanins, the flavylium cation, increases. Recently PICCARDO et al. (2019) evaluated the substitution of immature grape must for grape must overripe prior to alcoholic fermentation in Tannat and Pinot noir overripe grapes, reaching similar results.

Also recently, SCHELEZKI et al. (2018a and 2018b) compared the procedure described by KONTOUDAKIS et al. (2011) and a procedure that substitutes a portion of the grape juice with water. Both treatments effectively reduced the ethanol content; however, surprisingly, this article concluded that substitution with water is more suitable than substitution with green wine because the changes in the volatile composition and sensory qualities of the final wine are less pronounced. However, the OIV and most wine producing countries have not authorized adding water, and it can be analytically detected (THOMAS et al. 2013).

Given the interest of these kinds of treatments in the current context of climate change, this work aimed to study the effect of using acidified water or a low-sugar white grape juice, previously treated with cation exchange resins, as strategies for reducing the ethanol content and pH of overripe red grapes. The acidified water and the treated grape juice were either added directly or they replaced a portion of red grape juice.

6.3. MATERIAL AND METHODS

6.3.1. Chemicals

Methanol, acetonitrile, formic acid, and acetic acid were of HPLC grade (>99%) and purchased from Panreac (Barcelona, Spain). Acetaldehyde (>99.5%), phloroglucinol (>99%), ascorbic acid (>99%), sodium acetate (>99%), and ammonium formate (>99%) were purchased from Sigma-Aldrich (Madrid, Spain). Absolute ethanol and hydrochloric acid (37%) were purchased from Panreac. Malvidin-3-O-glucoside chloride (≥95%), proanthocyanidin dimer B2 (≥90%), (+)-catechin (≥99%), (−)-epicatechin (≥99%), (−)-epigallocatechin (≥98%), and (−)-epicatechin- 3-O-gallate (≥97.5%) were purchased from Extrasynthese (Genay, France). A pullulan calibration kit Shodex P-82 (P-5, MW = 5.9 kDa; P-10, MW = 11.8 kDa; P-20, MW = 22.8 kDa; P-50, MW = 47.5 kDa; P-100, MW = 112 kDa; P-200, MW = 212 kDa; P-400, MW = 404 kDa; P-800, MW = 788 kDa) was obtained from Waters (Barcelona, Spain), while a pullulan 1.3 kDa and four dextrans BioChemika (12, 25, 50, and 80 kDa) were obtained from Fluka (St. Louis, MO, USA). A Winescan TM Autosampler 79000 infrared analyzer (Foss, USA) and the Foss Integrator software version 154 (Foss, Denmark) were used to determine the alcohol content, total acidity and pH of the wines. The polysaccharides used as external standards for quantification were pectins from citrus fruit (≥90%) and dextrans synthesized by Leuconostoc mesenteroides (≥99.9%) purchased from Sigma-Aldrich (St. Louis, MO, USA).

6.3.2. Equipment

The HPLC analyses were performed using an Agilent 1200 series liquid chromatograph equipped with a G1362A refractive index detector (RID), a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1316A column oven, and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). All the spectrophotometric measurements were performed using a Helios Alpha UV−vis spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA, USA).

6.3.3. Grapes and wines

The study was carried out using grapes from Tempranillo Tinto (Vitis International Variety Catalogue number VIVC 12350) and Merlot Noir (Vitis International Variety Catalogue number VIVC 7657) cultivars (*Vitis vinifera* L.) from the 2017 vintage. The grapes of both cultivars were manually picked in a commercial vineyard located in Els Guiamets [AOC Montsant; 41º 06′ 20.92″ (N) and 0º 45′ 42.59″ (E)] and were harvested at two different ripening stages. The first harvest was carried out when the potential degree of alcohol was between 13.0 and 14.0%. The second harvest was carried out when the grapes reached optimum phenolic maturity. Specifically, the grapes of the first harvest were picked at 22.8 oBrix (Merlot) and 23.3 oBrix (Tempranillo), whereas the grapes of the second harvest were picked at 25.1 oBrix (Merlot) and 24.9 oBrix (Tempranillo).

6.3.4. Cationic exchange treatment of the white grape juice

Just after settling, a white grape juice from the Macabeo cultivar (Vitis International Variety Catalogue number VIVC 13127) was treated with an industrial cationic exchange column (FreeK+, Agrovin, Alcazar de San Juan, Spain) to reduce its pH as much as possible. The initial characteristics of this grape juice were 16.6 ^{o}Brix, a titratable acidity of 5.7 g/L (expressed as tartaric acid), and a pH of 3.21. After the treatment no changes were observed in the Brix degree, but the titratable acidity increased to 8.5 g/L and the pH decreased to 2.40.

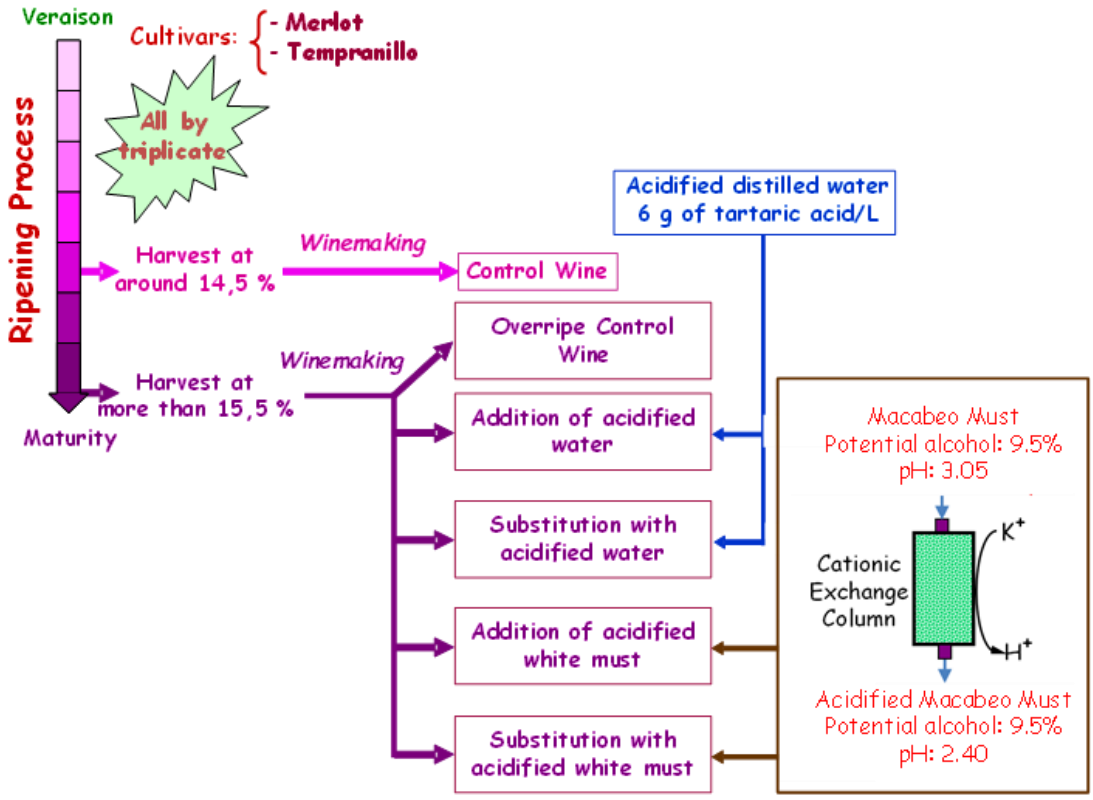
6.3.5. Winemaking experimental conditions

Figure 1 illustrates the outline of the experimental conditions. Merlot and Tempranillo grapes were carefully destemmed (Delta, Bucher-Vaslin, Chalonnes-sur-Loire, France) and the intact grapes were randomly distributed in batches of 6 kg to avoid differences due to the heterogeneity of the grapes as much as possible. The grapes of each batch were then crushed with a manual crusher, sulphited (100 mg of $K_2S_2O_5$/kg) and placed in 8-L and 6-L tanks equipped with a submerged cap system (SAMPAIO et al. 2007). The grapes of the first harvest (normal control wine) and a quantity of grapes from the second harvest (overripe control wine) were vinified

without any additional treatment. The rest of the grapes from the second harvest were used to study the four different treatments for mitigating the effects of overripening. The treatments applied to these grapes were: addition of acidified water (6 g of tartaric acid/L), substitution with acidified water (6 g of tartaric acid/L), addition of acidified must, and substitution with acidified must. The proportion of addition/substitution of acidified water or acidified must was calculated to reduce the ethanol content of the wines by around 1.0 degree. Specifically, 333 mL of acidified water was added to the Merlot grapes, and 335 mL was added to the Tempranillo grapes; 312 mL of acidified water was used to substitute the original Merlot grape must and 314 mL of acidified water was used to substitute the Tempranillo grape must; 980 mL of acidified grape must was added to the Merlot grapes and 1000 mL to the Tempranillo grapes. Finally, 814 mL of Merlot grape must and 828 mL of Tempranillo grape must were substituted with acidified grape must. We made these calculations with the aim of decreasing the alcohol content by 1.0 degree taking into account the potential ethanol content of both the original grapes and the acidified grape must and also that 80 % of the grapes' weight is liquid.

All tanks were immediately inoculated with 200 mg/kg yeast (EC1118; Lallemand Inc., Montreal, Canada) and maintained at a room temperature of 25 ± 1ºC until racking. Density and temperature were measured daily to monitor the alcoholic fermentation and two manual punch-downs of the cap were made at around 1060 and 1020 density units to improve color and phenolic extraction. After 14 days of maceration, the wines were racked. Once alcoholic fermentation had completely finished, wines were sulphited (100 mg of $K_2S_2O_2$/L) and kept at 4 °C for three months to allow the tartaric salts to stabilize. Therefore, malolactic fermentation was inhibited to avoid any variations resulting from it. The wines were then bottled and stored in a dark cellar at 15 °C until analysis. All these microvinifications were performed in triplicate.

Figure 1. Experimental designe



6.3.6. Standard grape juice and wine analysis

We used the analytical methods recommended by OIV (2012) to determine the sugar concentration, pH and titratable acidity of the grape juices as well as the ethanol content, titratable acidity and pH of the wines. The total anthocyanin content of the wines was estimated with the spectrophotometric method proposed by NIKETIC-ALEKSIC & HRAZDRINA (1972). The total phenolic index (TPI) was estimated by measuring the absorbance at 280 nm (RIBÉREAU-GAYON et al. 2006).

6.3.7. HPLC anthocyanin analysis

Reversed-phase HPLC analyses of the anthocyanins were carried out by injecting 40 µL of wine into an Agilent 1200 series liquid chromatograph (HPLC-

DAD) and using an Agilent Zorbax Eclipse XDBC18, 4.6 × 250 mm, 5 μm column (Agilent Technologies). The solvents used were 10% aqueous formic acid (solvent A) and a mixture of 45% methanol, 45% water, and 10% formic acid (solvent B) in accordance with the method described by GIL et al. (2004). Chromatograms were recorded at 530 nm, and anthocyanin standard curves were made using malvidin-3-O-glucoside chloride. Compounds were identified considering the relative retention times between the compounds and by recording their UV spectra with a diode array detector and comparing these with the UV spectra. We quantified the five anthocyanidin-3-monoglucosides of wine (delphinidin, cyanidin, peonidin, petunidin, and malvidin) and their respective acetylated and p-coumaroylated anthocyanins.

6.3.8. HPLC proanthocyanidin analysis

The proanthocyanidins of the wines were extracted and analyzed by acid depolymerization in the presence of an excess of phloroglucinol (PASTOR DEL RIO AND KENNEDY, 2006); the products of the reaction were separated by RP-HPLC-DAD (KENNEDY AND JONES 2001). Proanthocyanidins were analyzed with an Agilent 1200 Series HPLC equipped with a G1362A refractive index detector (RID), a G1315D DAD, a G1311A quaternary pump, a G1316A column oven and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). The chromatographic system was managed by an Agilent Chem Station (version B.01.03) data processing station.

6.3.9. HPLC polysaccharides analysis

Samples were processed using the methodology described by AYESTARÁN et al. (2004). Briefly, 10mL of sample was concentrated to a final volume of 2 mL using a vacuum evaporator (Univap 148 100ECH; Progen Scientific, London, UK). The total soluble polysaccharides were precipitated by adding 10 mL cold acidified ethanol (hydrochloric acid 0.3mol L−1 in absolute ethanol) and kept for 24 h at 4 °C. The samples were then centrifuged (10 000 × g for 15min) and the supernatants discarded. Finally, the precipitates were dissolved in 1mL ultra-pure water, frozen to −20 °C and freeze-dried. The polysaccharides were analyzed as described in

ESTERUELAS et al. (2015) by high-resolution size-exclusion chromatography (HRSEC) using a refraction index detector (RID) and an HPLC Agilent 1200 Series system (Agilent Technologies Inc., Santa Clara, CA, USA).

6.3.10. Sensory analysis

All sensory analyses were performed in the tasting room of the Faculty of Enology in Tarragona (University Rovira i Virgili), which was designed in accordance with UNE87004.197 (AENOR 2010). Official ISO tasting glasses (ISO-3591 1997) were used for the tasting. Each sample consisted of 30 mL of wine at room temperature (20 °C) covered with a clear plastic petri dish to minimize the escape of volatile components. They were randomly coded with three-digit numbers.

A panel of ten trained wine tasters tasted all the samples. The panel was made up of 4 females and 6 males aged between 26 and 58. Two tasting sessions were held, one for Merlot wines and the other for Tempranillo wines. For each sample, tasters were required to evaluate the intensity of seven sensory attributes on a scale of 1 to 10 (1 = 'slight intensity', 10 = 'maximum intensity'): fruit, vegetal, spicy, acidity, astringency, bitterness and structure. The intensity level of each descriptor was then expressed as the mean value of all the tasters. A sensory training session was held beforehand so that the panelists could agree on the criteria for each of the different sensory attributes. Samples were served randomly to avoid the influence of the tasting order. Tasters were also required to classify the different wines in order of preference (from 1, the one they considered the best, to 6 the one they considered the worst).

6.3.11. Statistics

All analytical data are expressed as the arithmetic mean ± standard deviation of the samples from three replicates. The multifactor analysis of variance (ANOVA) was carried out using XLSTAT software in order to compare the different samples. All sensory data are expressed as the arithmetic mean of the scores of the 10 panelists.

6.4. RESULTS AND DISCUSSION

Table 1 shows the general parameters of the different wines. As expected, the sugar content of the must and the ethanol content and pH of the overripe control wines were significantly higher and the titratable acidity significantly lower than in normal control wines. This confirms that the grapes of the two cultivars really ripened between the two harvest dates. The total phenolic indexes of the overripe control Merlot and Tempranillo wines were also significantly higher than those of their corresponding normal controls, which suggest that the total phenolic content and its extractability increased between the two harvest dates. Similar results have been previously reported (GIL et al., 2012; PASCUAL et al. 2016). No significant changes were detected in the total anthocyanin concentration measured spectrophotometrically between the two maturation stages; however, a tendency to decrease was observed in both cultivars. This trend is probably because the grapes have already reached the maximal anthocyanin concentration at normal maturity. The HPLC analysis of the anthocyanins (Table 2) confirms these data because the total anthocyanin concentration tended to decrease in the overripe control wines, although these differences were only significant in the Tempranillo wine. In general, the proportion of the different anthocyanins was very similar between both control wines (normal grapes and over-ripe grapes) although small but significant differences were observed in two anthocyanins. Specifically, paeonidin-3-O-glucoside was significant higher and acetylated malvidin-3-O-glucoside significant lower in the over-ripe control wine. Regarding the different treatments, no large differences were found with respect to the control. It was only observed that acetylated delphinidin-3-O-glucoside decreased significantly in the treatments performed with must and that coumarylated malvidin-3-O-glucoside increased significantly in all the treatments. In any case, these differences can be considered as not very important in relation to the global composition in anthocyanins of these wines. TPI and anthocyanins have been previously reported to behave similarly throughout the grape ripening process (PÉREZ-MAGARINO AND GONZALEZ-SAN JOSE 2004; PASCUAL et al. 2016).

Table 3 shows the total proanthocyanidin concentration of the different wines as well as their mean degree of polymerization (mDP) and the percentages of prodelphinidins and galloylation. The results indicate that the total proanthocyanidin concentration was significantly lower in the overripe control wines of the two cultivars than in the corresponding normal control wines. This decrease in the total proanthocyanidin concentration contrasts with TPI, which followed the opposite trend. A possible explanation for these results could be that acid catalysis with phloroglucinol is not completely efficient (KONTOUDAKIS et al. 2011c) and thus the proanthocyanidin concentration of overripe grape wines may have been underestimated. No significant differences were found in the mDP or the percentage of galloylation in the proanthocyanidins of the overripe control wine and the normal control wine. In contrast a significant increase in the percentage of prodelphinidins was detected in the overripe control Merlot wine. This indicates that skin proanthocyanidins make a higher contribution when the grapes are riper because prodelphinidins are only present in skin tannins (SOUQUET et al. 1996; GIL et al., 2012). This increase was not detected in the Tempranillo wine.

The color intensity of the overripe control wine was significantly higher than in the corresponding normal control wine for Merlot grapes but it was significantly less intense for Tempranillo grapes. This different behavior can be because color not only depends on the wine anthocyanin composition. Other factors such pH and the presence of copigments can exert a very important effect on wine color intensity and hue (KONTOUDAKIS et al., 2011c). In both cultivars, pH was significant higher in the wines obtained from over-ripe grapes. This data can explain why the color intensity decrease in the wine obtained from over-ripe grapes in the case of Tempranillo but do not explain why in the case of Merlot happens the opposite. In this case it can be hypothesized that the over-ripening of the Merlot grapes could have favored the release of more copigments. Finally, the total polysaccharide concentration (Table 4) tended to increase in the overripe control wines of both cultivars but these differences were not significant. These data agree with previously published results (GIL et al. 2012).

Table 1. General parameters

Parameter	Cultivar	Normal grapes	Over-ripe grapes			
		Control wine	Control wine	Addition of acidified water	Substitution of acidified water	Addition of acidified must	Susbtitution of acidified must
Sugar content of the must (g/L)	M	241 ± 3.4 A	263 ± 2.0 B	243 ± 2.9 A	248 ± 5.1 A	242 ± 3.4 A	247 ± 2.9 A
	T	245 ± 4.3 A	257 ± 1.7 B	243 ± 5.1 A	251 ± 3.4 A	241 ± 5.1 A	244 ± 3.4 A
Ethanol content (%)	M	14.2 ± 0.2 A	15.4 ± 0.1 B	14.4 ± 0.2 A	14.7 ± 0.3 A	14.3 ± 0.2 A	14.5 ± 0.2 A
	T	14.4 ± 0.5 A	15.3 ± 0.1 B	14.2 ± 0.3 A	14.7 ± 0.2 A	14.3 ± 0.3 A	14.5 ± 0.2 A
Titratable acidity (g/L)	M	4.77 ± 0.06 E	4.30 ± 0.10 D	2.73 ± 0.12 A	2.77 ± 0.06 A	3.13 ± 0.06 B	3.32 ± 0.03 C
	T	3.90 ± 0.10 E	3.67 ± 0.09 D	2.87 ± 0.15 A	2.77 ± 0.06 A	3.10 ± 0.10 B	3.32 ± 0.03 C
pH	M	3.45 ± 0.02 B	3.56 ± 0.02 C	3.83 ± 0.06 D	3.87 ± 0.12 D	3.30 ± 0.01 A	3.32 ± 0.01 A
	T	3.76 ± 0.03 A	3.94 ± 0.01 C	3.98 ± 0.02 D	3.93 ± 0.05 CD	3.81 ± 0.01 B	3.81 ± 0.03 B
TPI	M	67.6 ± 1.4 B	75.4 ± 1.8 C	63.3 ± 1.1 A	66.1 ± 2.1 AB	67.2 ± 2.8 A	78.6 ± 1.5 B
	T	67.3 ± 0.5 A	75.0 ± 1.9 C	70.1 ± 0.8 B	73.9 ± 1.0 CD	70.3 ± 1.5 B	74.1 ± 2.0 C
Anthocyanins (mg/L)	M	1096 ± 60 BC	1040 ± 14 B	949 ± 57 A	1029 ± 32 AB	1042 ± 67 B	1152 ± 27 C
	T	897 ± 5 C	845 ± 6 B	825 ± 13 A	840 ± 15 AB	815 ± 13 A	842 ± 10 B
Color Intensity	M	15.9 ± 0.4 B	17.0 ± 0.1 C	15.2 ± 0.1 A	17.4 ± 0.1 C	16.9 ± 0.4 C	20.3 ± 0.2 D
	T	8.6 ± 0.1 B	8.1 ± 0.2 A	8.1 ± 0.1 A	8.3 ± 0.1 A	8.2 ± 0.2 A	8.7 ± 0.2 B

All data are expressed as the average values of 3 replicates ± standard deviation. M: Merlot; T: Tempranillo. Different letters indicate the existence of statistical differences ($p < 0.05$). TPI corresponds to the wine total phenolic index.

In general, all the treatments reduced the sugar content of the must and the ethanol content of the wine very effectively with an average decrease of 0.9 degrees, thus making it possible to obtain an ethanol content similar to that of the corresponding normal control wine. The transformation ratio of sugar in ethanol was very similar in all the experimental groups with a minimal value of 16.80 g/L for obtaining 1 % of ethanol and a maximal value of 17.10 with an average value of 16.95. These values are close to that established by OIV (16.83). However, the different treatments did not always correct wine acidity effectively and sometimes they affected the wine composition negatively because other wine components were probably diluted.

Table 2. Anthocyanins

Anthocyanins (mg/l)		Cultivar	Normal grapes		Over-ripe grapes									
			Control wine		Control wine		Addition of acidified water		Substitution of acidified water		Addition of acidified must		Susbtitution of acidified must	
Total Anthocyanins		M	846 ± 55	A	800 ± 23	A	770 ± 42	A	801 ± 36	A	811 ± 14	A	994 ± 14	B
		T	646 ± 65	B	503 ± 27	A	505 ± 36	A	488 ± 8	A	485 ± 14	A	522 ± 59	AB
Non-acylated anthocyanins (%)	Dp-3-*O*-G	M	10.3 ± 0.3	A	10.7 ± 0.2	A	10.4 ± 0.2	A	10.6 ± 0.3	A	10.9 ± 0.3	A	10.9 ± 0.3	A
		T	14.6 ± 0.6	A	13.6 ± 0.7	A	13.4 ± 0.7	A	13.5 ± 0.7	A	14.6 ± 0.3	A	13.6 ± 0.8	A
	Cy-3-*O*-G	M	1.6 ± 0.1	A	1.5 ± 0.1	A	1.5 ± 0.1	A	1.5 ± 0.1	A	1.5 ± 0.1	A	1.6 ± 0.1	A
		T	1.0 ± 0.1	A	1.0 ± 0.0	A	1.0 ± 0.1	A	1.1 ± 0.0	A	1.1 ± 0.1	A	1.0 ± 0.1	A
	Pt-3-*O*-G	M	10.5 ± 0.1	A	10.2 ± 0.1	A	10.0 ± 0.2	A	9.9 ± 0.3	A	10.3 ± 0.2	A	10.4 ± 0.3	A
		T	14.5 ± 0.3	A	13.7 ± 0.7	A	13.5 ± 0.4	A	14.1 ± 0.3	A	13.7 ± 0.3	A	13.7 ± 0.5	A
	Pn-3-*O*-G	M	6.8 ± 0.4	A	8.7 ± 0.2	B	8.9 ± 0.1	B	8.5 ± 0.1	B	8.3 ± 0.1	B	8.2 ± 0.2	B
		T	4.5 ± 0.2	A	4.9 ± 0.4	A	4.7 ± 0.1	A	4.9 ± 0.2	A	4.8 ± 0.3	A	4.7 ± 0.3	A
	Mv-3-*O*-G	M	46.2 ± 2.0	A	45.7 ± 1.8	A	46.0 ± 2.3	A	44.6 ± 0.8	A	42.8 ± 3.6	A	41.9 ± 3.5	A
		T	53.2 ± 2.1	A	56.4 ± 1.2	A	55.3 ± 1.3	A	56.3 ± 0.6	A	54.7 ± 2.1	A	54.6 ± 1.6	A
	Total non-acylated anthocyanins	M	75.5 ± 2.7	A	76.8 ± 1.4	A	76.6 ± 1.5	A	75.1 ± 1.6	A	73.9 ± 2.8	A	73.1 ± 3.2	A
		T	87.8 ± 3.2	A	89.7 ± 3.0	A	87.9 ± 2.2	A	90.0 ± 1.8	A	88.8 ± 1.9	A	87.7 ± 3.2	A
Acetylated anthocyanins (%)	Dp-3-*O*-G-Ac	M	3.7 ± 0.6	B	3.8 ± 0.7	B	2.8 ± 0.6	AB	2.8 ± 0.5	AB	1.6 ± 0.4	A	1.8 ± 0.4	A
		T	2.2 ± 1.5	A	0.7 ± 0.5	A	1.1 ± 0.5	A	0.7 ± 0.3	A	0.7 ± 0.1	A	1.0 ± 0.7	A
	Cy-3-*O*-G-Ac	M	2.0 ± 1.3	A	2.7 ± 1.1	A	1.5 ± 0.3	A	2.0 ± 0.3	A	1.3 ± 0.5	A	1.6 ± 0.3	A
		T	1.6 ± 0.8	A	0.6 ± 0.2	A	0.7 ± 0.2	A	0.4 ± 0.4	A	0.6 ± 0.2	A	0.7 ± 0.3	A
	Pt-3-*O*-G-Ac	M	4.0 ± 2.9	A	2.3 ± 0.8	A	2.1 ± 0.1	A	2.6 ± 0.3	A	2.2 ± 0.4	A	2.4 ± 0.2	A
		T	0.8 ± 0.1	A	0.8 ± 0.3	A	0.9 ± 0.3	A	0.6 ± 0.1	A	1.0 ± 0.1	A	1.5 ± 0.9	A
	Pn-3-*O*-G-Ac	M	2.2 ± 1.1	A	2.1 ± 0.5	A	1.6 ± 0.3	A	1.9 ± 0.4	A	2.0 ± 0.3	A	2.1 ± 0.1	A
		T	0.5 ± 0.2	A	0.5 ± 0.1	A	0.8 ± 0.4	A	0.5 ± 0.1	A	0.5 ± 0.1	A	0.6 ± 0.1	A
	Mv-3-*O*-G-Ac	M	10.3 ± 0.2	B	8.7 ± 0.3	A	8.3 ± 0.1	A	8.5 ± 0.1	A	8.4 ± 0.1	A	8.5 ± 0.1	A
		T	4.1 ± 0.3	A	4.5 ± 0.2	A	5.7 ± 1.7	A	4.5 ± 0.2	A	4.8 ± 0.2	A	4.6 ± 0.1	A
	Total acetylated anthocyanins	M	22.1 ± 5.8	A	19.6 ± 3.3	A	16.5 ± 1.6	A	17.8 ± 1.3	A	15.6 ± 1.7	A	16.4 ± 1.4	A
		T	9.1 ± 3.8	A	7.0 ± 1.1	A	9.2 ± 3.1	A	6.7 ± 1.1	A	7.6 ± 1.3	A	8.4 ± 2.0	A
Coumarylated anthocyanins (%)	Dp-3-*O*-G-Cou	M	0.4 ± 0.3	A	0.3 ± 0.1	A	0.3 ± 0.1	A	0.4 ± 0.1	A	0.5 ± 0.1	A	0.4 ± 0.1	A
		T	0.3 ± 0.1	A	0.4 ± 0.1	A	0.6 ± 0.2	A	0.4 ± 0.1	A	0.4 ± 0.1	A	0.5 ± 0.1	A
	Cy-3-*O*-G-Cou	M	0.1 ± 0.1	A	0.3 ± 0.3	A	0.4 ± 0.1	A	0.5 ± 0.2	A	0.5 ± 0.2	A	0.6 ± 0.3	A
		T	0.1 ± 0.1	A	0.1 ± 0.2	A	0.1 ± 0.1	A	0.1 ± 0.1	A	0.1 ± 0.1	A	0.5 ± 0.3	A
	Pt-3-*O*-G-Cou	M	0.4 ± 0.1	A	0.5 ± 0.2	A	0.7 ± 0.4	A	0.5 ± 0.2	A	1.0 ± 0.4	A	0.6 ± 0.2	A
		T	0.4 ± 0.1	A	0.3 ± 0.3	A	0.3 ± 0.3	A	0.5 ± 0.1	A	0.5 ± 0.1	A	0.5 ± 0.1	A
	Pn-3-*O*-G-Cou	M	0.3 ± 0.3	A	0.9 ± 0.4	A	1.3 ± 0.3	A	1.1 ± 0.0	A	0.7 ± 0.6	A	1.4 ± 0.1	A
		T	0.5 ± 0.1	A	0.6 ± 0.1	A	0.2 ± 0.3	A	0.6 ± 0.1	A	0.6 ± 0.1	A	0.6 ± 0.1	A
	Mv-3-*O*-G-Cou	M	1.3 ± 0.4	A	1.6 ± 1.3	A	4.1 ± 1.7	B	4.6 ± 0.4	B	7.8 ± 0.3	C	7.5 ± 0.1	C
		T	1.7 ± 0.1	A	1.8 ± 0.3	A	1.8 ± 0.1	A	1.8 ± 0.1	A	1.9 ± 0-3	A	1.8 ± 0.2	A
	Total coumarylated anthocyanins	M	2.4 ± 0.6	A	3.6 ± 2.1	A	6.9 ± 1.6	B	7.1 ± 0.6	B	10.5 ± 1.3	C	10.5 ± 0.3	C
		T	3.1 ± 0.4	A	3.3 ± 0.7	A	2.9 ± 0.9	A	3.3 ± 0.1	A	3.6 ± 0.3	A	3.9 ± 0.3	A

All data are expressed as the average values of 3 replicates ± standard deviation. M: Merlot; T: Tempranillo. Dp: delphinidin; Cy: cyanidin; Pt: petunidin; Pn: paeonidin; Mv: malvidin; 3-*O*-G: 3-ortho-monoglucoside; Ac: acetylated; Cou: Coumarylated. Different letters indicate the existence of statistical differences ($p < 0.05$).

Specifically, adding acidified water caused a significant increase in pH and significant decrease in titratable acidity in both cultivars. In addition, adding water also significantly decreased TPI and the total anthocyanin concentration measured by spectrophotometry; however, no significant differences were detected in the different types of anthocyanins measured by HPLC (Table 2). The color intensity also decreased significantly in Merlot wines although no differences were found in Tempranillo wines. However, adding acidified water did not affect the proanthocyanidin composition (Table 3) and only significantly decreased the total polysaccharide concentration of Tempranillo wines (Table4). These results show that adding acidified water causes a general decrease in the concentration of most wine components.

Table 3. Proanthocyanidins

Parameter	Cultivar	Normal grapes	Over-ripe grapes				
		Control wine	Control wine	Addition of acidified water	Substitution of acidified water	Addition of acidified must	Susbtitution of acidified must
Proanthocyanidins	M	1027 ± 74 D	773 ± 70 B	757 ± 29 B	619 ± 22 A	865 ± 18 C	1142 ± 41 D
	T	1248 ± 43 C	1086 ± 43 B	1055 ± 70 AB	945 ± 38 A	1324 ± 84 C	1253 ± 40 C
Mean degree of polymerization (mDP)	M	4.5 ± 0.3 A	4.7 ± 0.3 A	4.7 ± 0.3 A	4.3 ± 0.3 A	4.7 ± 0.2 A	4.9 ± 0.3 A
	T	7.5 ± 0.5 A	6.9 ± 0.4 A	6.5 ± 0.5 A	6.8 ± 0.7 A	6.7 ± 0.8 A	6.6 ± 0.3 A
% Prodelphinidins	M	19.2 ± 0.4 A	21.3 ± 0.4 CB	21.9 ± 0.9 CB	19.5 ± 0.1 A	20.3 ± 0.6 B	22.0 ± 0.7 C
	T	19.2 ± 0.4 A	18.5 ± 0.4 A	19.4 ± 1.3 AB	18.4 ± 0.6 A	20.8 ± 0.9 BC	21.7 ± 0.6 C
% Galloylation	M	10.3 ± 0.6 A	9.6 ± 0.5 A	9.8 ± 0.5 A	10.3 ± 0.6 A	9.2 ± 0.4 A	9.3 ± 0.6 A
	T	5.7 ± 0.4 A	6.1 ± 0.2 A	6.5 ± 0.4 A	6.3 ± 0.6 A	5.8 ± 0.4 A	6.1 ± 0.3 A

All data are expressed as the average values of 3 replicates ± standard deviation. M: Merlot; T: Tempranillo. Different letters indicate the existence of statistical differences ($p < 0.05$).

Table 4. Polysaccharides

Parameter	Cultivar	Normal grapes	Overripe grapes				
		Control wine	Control wine	Addition of acidified water	Substitution of acidified water	Addition of acidified must	Susbtitution of acidified must
Total Polysaccharides (mg/L)	M	525 ± 75 A	602 ± 77 A	633 ± 34 A	611 ± 35 A	563 ± 40 A	590 ± 18 A
	T	1072 ± 61 B	1165 ± 50 B	883 ± 79 A	908 ± 58 A	783 ± 60 A	879 ± 61 A

All data are expressed as the average values of 3 replicates ± standard deviation. M: Merlot; T: Tempranillo. Different letters indicate the existence of statistical differences ($p < 0.05$).

Substituting a portion of the original grape must with acidified water generated very similar results to those obtained by simply adding acidified water. Similar reductions in ethanol content and titratable acidity and increases in wine pH in both cultivars were obtained. A general decrease in other wine components was also observed although these differences were in general less intense than when acidified water was added. For example, the decreases in the TPI and the total anthocyanins were somewhat lower, and the color intensity of the Merlot wine was not affected by this treatment.

Adding acidified must effectively reduce the wine ethanol content and also significantly reduced wine pH, unlike the two acidified water treatments. This treatment also caused a significant decrease in the TPI in both cultivars, but it seems to affect other parameters to a lesser extent, such as total anthocyanin concentration

and color intensity in the case of Merlot. Moreover, the total proanthocyanidin concentration was even significantly higher than in the overripe control wine, which indicates that substituting a portion of the original must with the acidified must favors proanthocyanidin extraction during winemaking. This could be due to the decrease in pH. In contrast, the total polysaccharide concentration was significantly lower than in the overripe control Tempranillo wine at a similar level as that in the acidified water treatments.

Finally, substituting a portion of the original must with acidified must was probably the more interesting treatment since it reduced the wine ethanol content and pH to a similar extent as adding acidified must, but the dilution of the other wine components was quite low or even inexistent. The TPI and the total anthocyanin concentration were not affected in Tempranillo wines and were even significantly increased in Merlot wines. Similar results were observed in the anthocyanins analyzed by HPLC. In addition, the color intensity and the total proanthocyanidin concentration were significantly higher in both cultivars. It must be highlighted that the percentage of prodelphinidins was significantly higher in Tempranillo wines, which indicates a higher extraction of skin tannins (SOUQUET et al. 1996; GIL et al., 2012). In contrast, the polysaccharide concentration of Tempranillo wines was significantly lower than in the non-treated wine similarly to all the other treatments.

Figure 2 shows the results of the descriptive sensory analysis of the different Merlot (Figure 2.A) and Tempranillo (Figure 2.B) wines. Spider web graphics are used to compare first the normal control wine with the overripe control wine, second the overripe control wine with wines with added or substituted acidified water, and third the overripe control wine with wines with added or substituted acidified must.

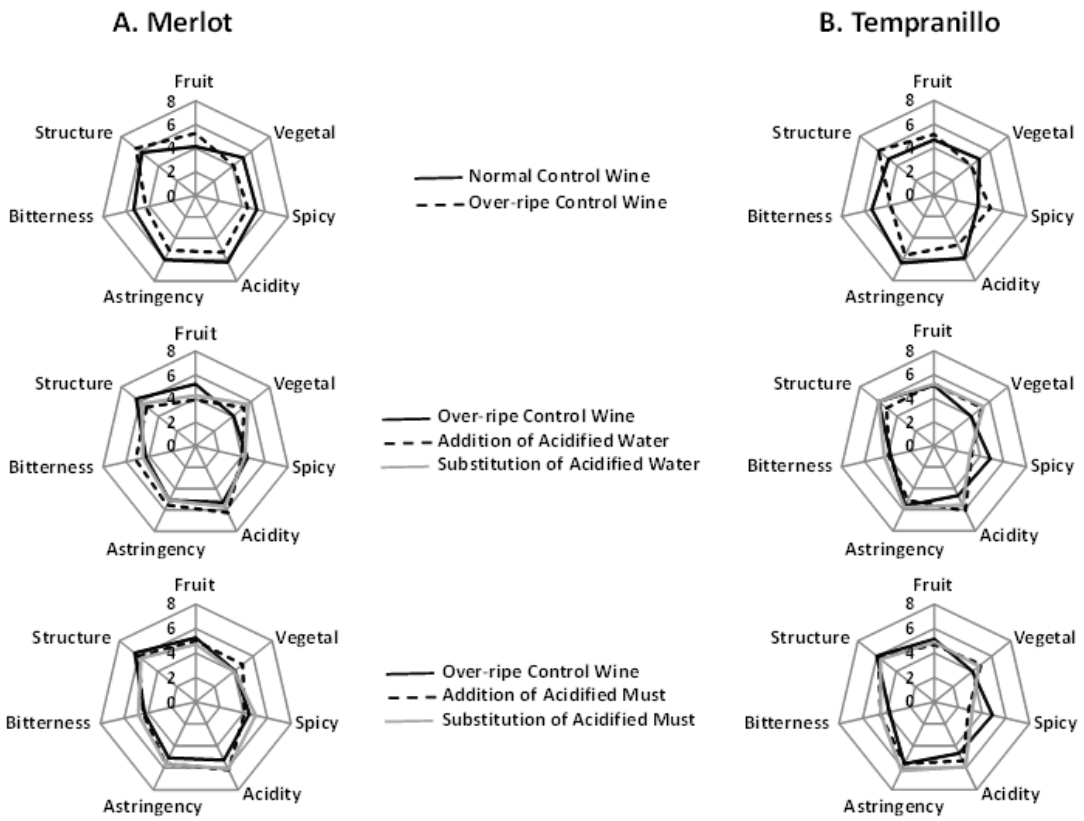


Figure 2. Descriptive sensory analysis

The comparison between overripe control wines and normal control wines showed the expected results. In general, the panelists considered that the overripe control wines for both cultivars were less vegetal, acidic, astringent and bitter than the normal control wines. They also found that overripe control Merlot wine was more fruity and less spicy, and the Tempranillo wine was more spicy and more structured. In general, these differences can be associated with the different maturity stages of the grapes and similar results have been previously reported (GIL et al. 2012; CASASSA et al. 2013).

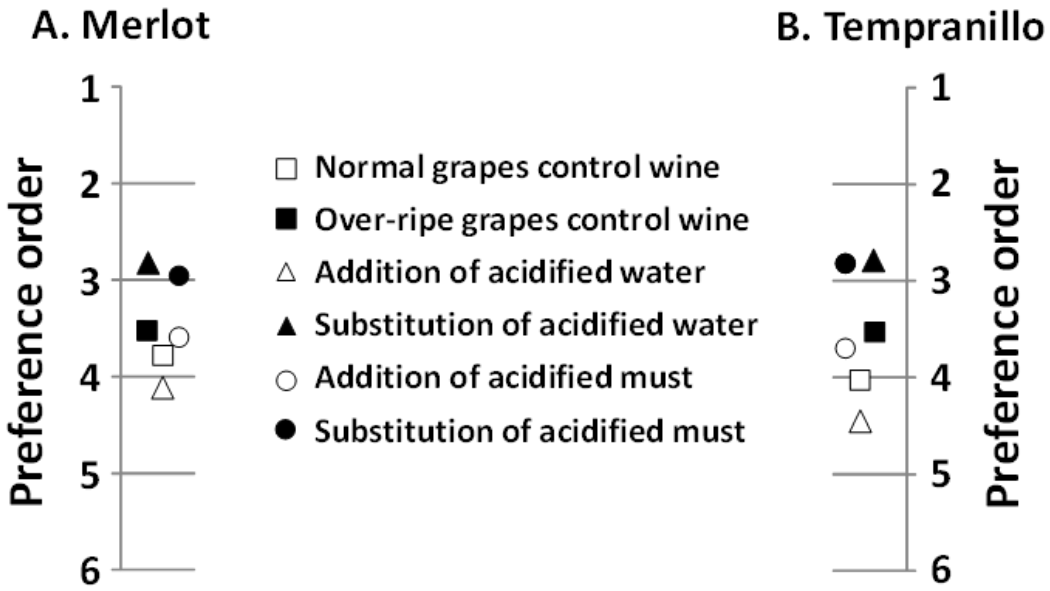


Figure 3. Preference sensory analysis

In general, the panelists considered the wines obtained by adding or substituting acidified water to be more vegetal and acidic in both cultivars. Moreover, the Tempranillo wine was considered less spicy and the Merlot wine was considered less fruity and structured. In addition, the wines obtained by adding water were considered less structured for Tempranillo grapes and more bitter for Merlot grapes.

Finally, the wines obtained by adding or substituting acidified must seem to be less affected at a sensory level than wines obtained by the other treatments. Specifically, wines obtained by adding acidified must were considered more vegetal and acidic than overripe control wines but to a lesser extent than the equivalent wines treated with acidified water. The Tempranillo wines obtained with this treatment were considered noticeably less spicy. In contrast, wines obtained by substituting a portion of the original must with acidified must were more similar to the overripe control wine, although they were considered more acidic and in Tempranillo wines less spicy.

The panelists were also required to classify the wines by order of preference and the results are shown in Figure 3. The classification by order of preference was very similar for both cultivars. The preferred wines were those obtained by substituting a portion of the original must with acidified water or acidified must, followed by the overripe control wine and then the wine obtained by adding acidified must. Finally, the normal control wine and especially the wine obtained by adding acidified water were classed as the least preferred wines.

6.5. CONCLUSIONS

It can be concluded that all the studied treatments are useful for reducing the ethanol content of wines elaborated with overripe grapes. However, adding or substituting with acidified water has the considerable drawback of increasing pH and decreasing titratable acidity and other wine components, which affects the wine sensory appreciation, especially when water is added. Moreover, adding or substituting with water is not authorized by the OIV or most wine producing countries, and can be analytically detected. In addition, adding water implies an increase in wine production, which is also a problem in the context of the global overproduction of wine and it is not acceptable for most wine consumers who seek authenticity. In contrast, adding or substituting with low ethanol white must acidified by cationic exchange reduces ethanol content and pH, does not dilute the wine as much, does not increase the wine volume produced, is not an unauthorized practice and would probably be well accepted by consumers. Furthermore, using acidified must, especially for substitutions, does not affect, and in fact can even improve, the sensory quality of the wine.

6.6. ACKNOWLEDGEMENTS

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7. CONCLUSIONES Y PERSPECTIVAS

7.1. CONCLUSIONES

1- ***Las investigaciones realizadas en este proyecto contribuyen al conocimiento sobre técnicas alternativas de vinificación que permiten elaborar vinos tintos diferenciados.***

Las investigaciones realizadas abordaron diferentes técnicas de vinificación que permitieron elaborar vinos tintos diferenciados ya sea por una reducción en el contenido de alcohol y pH como por un incremento en su color, composición fenólica y en polisacáridos (Capítulos 2, 3, 4, 5 y 6).

Las técnicas evaluadas para reducir el contenido de alcohol y el pH del vino fueron: la sustitución y la adición de agua acidificada; la sustitución y la adición de mosto de uva inmadura blanca y acidificado; y la sustitución de mosto de uvas del mismo cultivar con diferente grado de maduración. En general, estas técnicas fueron efectivas para reducir el contenido de alcohol de los vinos, mientras que su efecto sobre el pH, el color, la composición fenólica y en polisacáridos fue variable.

El agregado y la sustitución con agua acidificada generó un aumento del pH en los vinos Tempranillo y Merlot y una disminución en la acidez titulable y en las concentraciones de los demás componentes de calidad afectando su apreciación sensorial, especialmente cuando se agregó agua. Además, se debe considerar que la OIV y la mayoría de los países productores de vino, entre los que se encuentra Uruguay, no autorizan agregar o sustituir con agua, ya que se considera una adulteración, que es controlada y detectada analíticamente. Además, el agregado de agua implica un aumento en la producción de vino, que también es un problema en el contexto de la sobreproducción mundial de vino y no es aceptable para la mayoría de los consumidores que buscan genuinidad.

El agregado o la sustitución de mosto de uva madura por mosto de uva blanca inmadura y acidificado redujo el contenido de etanol y también el pH en los vinos Tempranillo y Merlot, sin diluir los demás componentes del vino. Además, el uso de

mosto acidificado, especialmente cuando se sustituyó, no afectó, e incluso podría mejorar, la calidad sensorial del vino.

La sustitución de mosto de uva madura por mosto de uva inmadura del mismo cultivar previo a la fermentación alcohólica permitió elaborar vinos Tannat y Pinot noir con menor pH y mayor acidez total. No se detectaron cambios relevantes en otros componentes del vino tales como polifenoles totales, catequinas o antocianos. Sin embargo, el efecto sobre la concentración de proantocianidinas y el color fue variable de acuerdo con el cultivar y el año de vendimia.

En general, la sustitución de mosto de uvas maduras por mosto de uvas inmaduras ya sea del mismo cultivar como de un cultivar blanco, no aumenta el volumen de vino producido, no está prohibida y probablemente sería bien aceptada por consumidores.

La maceración pre-fermentativa en caliente fue efectiva para incrementar el color en vinos Tannat y Pinot noir. Esto se debió principalmente a una mayor extracción de compuestos fenólicos durante la etapa pre-fermentativa debido a la degradación de las partes solidas de la uva por efecto de la temperatura. Los vinos presentaron mayor concentración de polifenoles totales, antocianos, proantocianidinas y polisacáridos en relación con vinos elaborados por maceración tradicional. Las diferencias entre estos vinos se mantuvieron durante su conservación. En consecuencia, estos vinos podrían soportar mejor el proceso de envejecimiento y crianza.

2- ***El efecto de las técnicas de vinificación evaluadas depende del potencial enológico de cada cultivar.***

Las investigaciones realizadas en este trabajo se han evaluado sobre cuatro cultivares de *Vitis vinífera.* La sustitución de mosto de uva madura por mosto de uva inmadura del mismo cultivar y la maceración pre-fermentativa en caliente se evaluaron durante tres vendimias sobre los cultivares Tannat y Pinot noir (Capítulos 2 y 3). La adición o sustitución de mosto por agua acidificada o por mosto de uva

blanca inmadura y acidificado se evaluaron sobre los cultivares Tempranillo y Merlot (Capitulo 6).

En general, los vinos Tannat elaborados por sustitución de mosto y maceración pre-fermentativa en caliente presentaron mejores características cromáticas, en tanto que en los vinos Pinot noir el efecto sobre el color no fue claro. También se observó que el impacto de estas técnicas de vinificación sobre la concentración de proantocianidinas fue diferente dependiendo del cultivar. Se debe considerar que estas variedades tienen un perfil fenólico distinto determinando la composición y características sensoriales del vino. En particular, Tannat se caracteriza por presentar contenidos muy elevados de pigmentos (antocianos) y taninos mientras que Pinot noir presenta una gran riqueza polifenólica (sobre todo en semillas), constituida casi exclusivamente por taninos y con muy baja concentración de antocianos.

El efecto de los tratamientos de adición y sustitución con agua acidificada y adición y sustitución con mosto de uva blanca inmaduro y acidificado también fue dependiente del cultivar. La adición y sustitución de agua acidificada afectó en mayor medida a la composición de los vinos tintos Merlot. En tanto que la adición y sustitución de mosto de uva blanca inmaduro y acidificado no modifico la composición de los vinos Tempranillo, pero provocó un incremento en la concentración de algunos compuestos fenólicos en los vinos Merlot. También se detectó un aumento significativo en el porcentaje de prodelfinidinas en los vinos Merlot elaborados a partir de uvas sobremaduras, lo que indica que las proantocianidinas de la piel contribuyen más cuando las uvas son más maduras porque las prodelfinidinas solo están presentes en los hollejos. Este aumento no se detectó en el vino Tempranillo. La intensidad del color del vino elaborado a partir de uvas sobremaduras Merlot fue significativamente mayor que en el vino control, pero fue significativamente menos intenso con las uvas Tempranillo. Nuevamente, se debe considerar que ambos cultivares presentan diferencias en su riqueza y aporte de compuestos fenólicos. Las uvas del cultivar Tempranillo presentaron un tamaño medio, con un importante contenido de antocianos, pero relativamente pobre en taninos tanto de hollejos como de semillas. Mientras que las uvas del cultivar Merlot

tuvieron mayor tamaño con semillas grandes, con una importante concentración de antocianos y una concentración media de taninos de hollejo y de semillas.

3- ***Las vinificaciones evaluadas pueden utilizarse como herramientas para mitigar los efectos de la variabilidad climática interanual sobre la composición del vino en las condiciones de Uruguay.***

Esta conclusión se desprende del análisis del efecto de la sustitución de mosto y la maceración pre-fermentativa en caliente sobre la composición de los vinos tintos Tannat y Pinot noir uruguayos, elaborados en tres vendimias consecutivas.

Los resultados presentados en los Capítulos 2, 3, 4 y 5 demuestran que sustitución de mosto de uva madura por mosto de uva inmadura del mismo cultivar, la maceración pre-fermentativa en caliente e incluso la combinación de las técnicas de vinificación, son herramientas interesantes para mitigar simultáneamente los problemas que causa el cambio climático con relación a la madurez de la uva e incrementar el color y los contenidos de compuestos fenólicos y polisacáridos del vino.

Por un lado, se observó que el impacto de las técnicas de vinificación evaluadas sobre el contenido de etanol, el pH y la acidez titulable de los vinos fue mayor en los años en que las condiciones climáticas permitieron la maduración completa de las uvas. Por otro lado, los resultados muestran que el impacto sobre la composición fenólica del vino también está determinado por las condiciones de maduración de cada año en particular.

En general, a través de las técnicas de vinificación evaluadas es posible intervenir a fin de mejorar el aprovechamiento del potencial enológico de la uva y regular la extracción de compuestos fenólicos durante la maceración. Es así como en los años en donde las condiciones climáticas permiten una buena maduración de la uva, la sustitución de mosto podría ser una técnica para reducir el contenido de alcohol sin afectar los demás componentes del vino. Mientras que en los años en donde las condiciones climáticas limitan la maduración de la uva, la maceración pre-

fermentativa en caliente podría ser una alternativa para maximizar la extracción de los compuestos localizados en las partes solidas a fin de mejorar la calidad del vino.

4- Las técnicas de vinificación evaluadas permiten mejorar el color de los vinos tintos Tannat nacionales.

Los vinos tintos Tannat presentan elevados contenidos de polifenoles totales, antocianos, catequinas y proantocianidinas, una intensidad colorante superior y mayores tonalidades rojas respecto a los vinos elaborados a partir de Cabernet Sauvignon, Merlot, Marselan y Syrah (Gonzáles-Neves et al., 2005 y 2016). Además, la riqueza polifenólica y antociánica de los vinos Tannat está relacionada con el potencial enológico de sus uvas (González-Neves, 1999, González-Neves et al., 2015 a y b, 2006, 2005, 2004 a y b, 2003 y 1998, González-Neves y Ferrer, 2008 y 2000, González-Neves y Gatto, 2001, Boido et al., 2006 y 2011). Sin embargo, los vinos Tannat presentan un perfil antociánico característico, con menores proporciones de malvidina y glucósidos acetilados, respecto a los vinos Cabernet-Sauvignon y Merlot, sugiriendo un color menos estable en el tiempo (González-Neves et al., 2005). Se ha constatado que los vinos tintos Tannat elaborados mediante maceraciones tradicionales, la estabilidad del color es menor a la de los vinos de otras variedades (González-Neves et al., 2007 y 2005).

Las investigaciones realizadas permitieron determinar que la sustitución de mosto de uva madura por mosto de uva inmadura y la maceración pre-fermentativa en caliente son alternativas tecnológicas que permiten mejorar el color de los vinos tintos Tannat (Capítulos 2, 3, 4 y 5).

El efecto de la sustitución de mosto sobre el color y la composición general de los vinos Tannat está determinado fundamentalmente por las modificaciones en el pH y depende en gran medida de la composición de la uva en cada vendimia.

Por su parte, en los vinos elaborados por maceración pre-fermentativa en caliente se observó un incremento en la intensidad y la calidad del color al aumentar la extracción de compuestos fenólicos, particularmente antocianos, y promover la

condensación entre antocianos y taninos, lo que sugiere una mayor estabilidad del color.

Los resultados obtenidos en estas investigaciones son relevantes para el sector vitivinícola nacional ya que estas técnicas de vinificación permiten levantar las limitantes que presenta este cultivar durante la vinificación.

7.2. PERSPECTIVAS

A partir de los resultados y las conclusiones obtenidas en esta tesis doctoral surgen las siguientes perspectivas:

1. ***Estudiar el efecto de la sustitución de mosto y la maceración pre-fermentativa en caliente en otros cultivares de Vitis vinifiera relevantes para el país.***

El efecto de las técnicas de vinificación evaluadas depende de las características varietales y de su potencial enológico en nuestras condiciones de cultivo. Si bien Tannat es el cultivar emblematico de Uruguay, la demanda de los mercados internacionales por una mayor diversificación de los vinos hace necesario evaluar y ajustar las técnicas de vinificación a otros cultivares de *Vitis vinífera* que puedan contribuir al desarrollo del sector vitivinícola nacional.

2. ***Estudiar el efecto de estas técnicas de vinificación con uvas provenientes de diferentes regiones vitivinícolas del país.***

Las investigaciones fueron realizadas sobre uvas Tannat y Pinot noir cosechadas de viñedos comerciales localizados en la región sur del país. El clima tiene un comportamiento local, por lo que las condiciones de maduración y el potencial enológico de la uva son diferentes dependiendo de su región de origen. Sería interesante poder evaluar y ajustar las diferentes alternativas de vinificación al potencial enológico de la uva en cada región de origen, tratando de mantener la tipicidad de sus vinos.

3. ***Incorporar otras técnicas de vinificación que puedan contribuir a minimizar el efecto de la variabilidad climática interanual y mejorar el aprovechamiento del potencial fenólico de la uva.***

La adición y sustitución de mosto de uva blanca con bajo contenido de etanol y acidificada fue evaluada sobre uvas Tempranillo y Merlot cosechadas de viñedos comerciales localizados en Els Guiamets, AOC Montsant, (Tarragona, España). Dado los resultados obtenidos con estas técnicas de reducción del contenido de etanol, sería interesante implementarles sobre cultivares locales a fin de evaluar su efecto.

Adicionalmente, en las investigaciones realizadas, la maceración pre-fermentativa en caliente fue seguida por una maceración fermentativa. Los resultados obtenidos, al igual que los reportados por otros investigadores, sugieren que la mayor extracción de compuestos fenólicos de los hollejos ocurre en la etapa pre-fermentativa, mientras que la extracción de taninos de semilla requiere de una maceración fermentativa. Dadas las características del cultivar Tannat, sería interesante evaluar el efecto de esta técnica de vinificación seguida de una fermentación en ausencia de partes sólidas. Asimismo, es necesario evaluar el efecto de esta técnica de vinificación sobre las actividades de las enzimas responsables de la oxidación de compuestos fenólicos, ya sean propias de la uva o provenientes del desarrollo de microorganismos causales de podredumbres de racimos.

Finalmente, el grupo de investigación de Enología de la Facultad de Agronomía ha evaluado el efecto de varias técnicas alternativas de vinificación sobre la composición del vino de variedades como Tannat, Merlot, Cabernet Sauvignon, Cabernet Franc y Maselan. Sería interesante continuar con el ajuste de estas técnicas de vinificación de acuerdo con el potencial enológico de la uva en el contexto del cambio climático.

4. ***Continuar generando conocimiento sobre tecnologías innovadoras para la elaboración de vinos de calidad que contribuyan al desarrollo sustentable del sector vitivinícola nacional.***

Actualmente, la competitividad de las bodegas uruguayas está condicionada por la eficiencia en el aprovechamiento de los recursos y las tecnologías disponibles para la elaboración de vinos de calidad. En este marco, generar conocimiento sobre técnicas que permitan elaborar vinos diferenciados, haciendo un uso eficiente de la materia prima y que puedan ser ajustadas a las diferentes realidades productivas, son fundamentales para el desarrollo sustentable del sector vitivinícola uruguayo.

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9. ANEXOS

9.1. VALORIZACIÓN DE LA TESIS

9.1.1. Publicaciones

9.1.1.1. Artículos publicados en revistas arbitradas

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & Gonzalez G. 2019. Influence of the use of unripe grapes to reduce ethanol content and pH on the color, polyphenol and polysaccharide composition of conventional and hot macerated Pinot Noir and Tannat wines. European Food Research and Technology, 245 (6): 1321-1335.
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Piccardo D, Gonzalez-Neves G, Favre G, Pascual O, Canals JM, Zamora F. 2019. Impact of must replacement and hot pre-fermentative maceration on the color of Uruguayan Tannat red wines. Fermentation, 5 (80): 1-17.
DOI: https://doi.org/10.3390 / fermentación 5030080

Piccardo D, Gombau J, Pascual O, Vignault A, Pons P, Canals JM, González-Neves G, Zamora F. 2019. Influence of two prefermentative treatments to reduce the ethanol content and pH of red wines obtained from overripe grapes. Vitis, 58: 59-67.
DOI: 10.5073/vitis.2019.58.special-issue.59-67

9.1.1.2. Artículos publicados en revistas con comité de lectura

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & Gonzalez G. 2019. Reducción del contenido de alcohol y pH de vinos tintos Pinot noir y Tannat empleando uvas con diferentes niveles de maduración. BIO Web Conferences, 12: 1-6.
DOI: https://doi.org/10.1051/bioconf/20191202023

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & Gonzalez G. 2019. Evaluación de la composición y calidad del color de vinos tintos Tannat elaborados por maceración pre-fermentativa en caliente. BIO Web Conferences, 12: 1-7.
DOI: https://doi.org/10.1051/bioconf/20191202006

9.1.1.3. Trabajos completos publicados en actas de congreso

Piccardo D, González-Neves G, Favre G, Pascual O, Zamora F. XVI Congreso Latinoamericano de Viticultura y Enología Trabajo (26-29 de noviembre de 2019). Estudio del color de vinos tintos Tannat uruguayos elaborados a distintas escalas de producción y con diferentes tecnologías de vinificación. ICA/ Peru (Trabajo completo enviado).

Piccardo D, Pascual O, Favre G, Zamora F, González-Neves G. XVI Congreso Latinoamericano de Viticultura y Enología Trabajo (26-29 de noviembre de 2019). Estudio del remplazo de mosto y la maceración pre-fermentativa en caliente sobre la composición de vinos tintos Pinot noir uruguayos. ICA/ Peru (Trabajo completo enviado).

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & Gonzalez G. 41° Congreso Mundial de la Viña y el Vino (Conferencista, 19/11/2018). Evaluation of the composition and color quality of Tannat red wines produced by pre-fermentative hot maceration. Punta del Este/Uruguay (Trabajo Completo)

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & Gonzalez G. 41° Congreso Mundial de la Viña y el Vino (Conferencista, 19/11/2018). Reduction of the alcohol content and pH of Pinot noir and Tannat red wines using grapes with different maturation level. Punta del Este/Uruguay (Trabajo Completo)

9.1.1.4. Resúmenes publicados en actas del congreso

Piccardo D, González-Neves G, Favre G, Pascual O, Zamora F. XVI Congreso Latinoamericano de Viticultura y Enología Trabajo (26-29 de noviembre de 2019). Estudio del color de vinos tintos Tannat uruguayos elaborados a distintas escalas de producción y con diferentes tecnologías de vinificación. ICA/ Peru (Resumen aceptado para su publicación).

Piccardo D, Pascual O, Favre G, Zamora F, González-Neves G. XVI Congreso Latinoamericano de Viticultura y Enología Trabajo (26-29 de noviembre de 2019). Estudio del remplazo de mosto y la maceración pre-fermentativa en caliente sobre la composición de vinos tintos Pinot noir uruguayos. ICA/ Peru (Resumen aceptado para su publicación).

Piccardo D, Favre G, Pascual O, Cannals JM, Zamora F & Gonzalez G. Macrowine 2018 (Presenta trabajo, 28/05/2018). Influence of unripe grapes to reduce ethanol content and pH on the color, polyphenols and polysaccharides composition of Pinot Noir and Tannat wines. Zaragoza/España (Resumen).

Piccardo D, Gambau J, Pascual O, Vignault A, Pons P, Canals JM & Zamora F. International Congress on Grapevine and Wine Sciences (Presenta trabajo, 07/11/2018). Influence of replacing a proportion of the grape juice of very ripe red grapes by acidified water or grape juice previously treated with cationic exchange as strategy for reducing ethanol content and pH. Logroño/España (Resumen).

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & González G. 41° Congreso Mundial de la Viña y el Vino (Conferencista, 19/11/2018). Evaluation of the composition and color quality of Tannat red wines produced by pre-fermentative hot maceration. Punta del Este/Uruguay (Resumen).

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & González G. 41° Congreso Mundial de la Viña y el Vino (Conferencista, 19/11/2018). Reduction of the alcohol content and pH of Pinot noir and Tannat red wines using grapes with different maturation level. Punta del Este/Uruguay (Resumen).

9.1.1.5. Resúmenes publicados en jornadas y seminarios

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & González G. Jornadas de Investigación FAGRO 2018 (Presenta trabajo, 08/11/2018). Elaboración de vinos tintos con menores contenidos de alcohol y enriquecidos en compuestos fenólicos. Montevideo/Uruguay (Resumen)

9.1.2. Presentaciones

9.1.2.1. Presentaciones orales

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & González G. Seminarios de vinos orgánicos y biodiámicos de la región, y la realidad en Uruguay. (Conferencista, 25/07/2019). Reducción del contenido de alcohol y pH de vinos tintos como herramientas para mitigar la variabilidad climática interanual. Montevideo/Uruguay.

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & González G. 41° Congreso Mundial de la Viña y el Vino (Conferencista, 19/11/2018). Evaluation of the composition and color quality of Tannat red wines produced by pre-fermentative hot maceration. Punta del Este/Uruguay.

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & Gonzalez G. 41° Congreso Mundial de la Viña y el Vino (Conferencista, 19/11/2018). Reduction of the alcohol

content and pH of Pinot noir and Tannat red wines using grapes with different maturation level. Punta del Este/Uruguay

Piccardo D., Gambau J., Pascual O., Vignault A., Pons P., Canals J. & Zamora F. International Congress on Grapevine and Wine Sciences (Presenta trabajo, 07/11/2018). Influence of replacing a proportion of the grape juice of very ripe red grapes by acidified water or grape juice previously treated with cationic exchange as strategy for reducing ethanol content and pH. Logroño/España (Resumen)

9.1.2.2. Poster

Piccardo D, Favre G, Pascual O, Cannals JM, Zamora F & Gonzalez G. Macrowine 2018. (Presenta trabajo, 28/05/2018). Influence of unripe grapes to reduce ethanol content and pH on the color, polyphenols and polysaccharides composition of Pinot Noir and Tannat wines. Zaragoza/España (Resumen).

Piccardo D, Favre G, Pascual O, Canals JM, Zamora F & Gonzalez G. Jornadas de Investigación FAGRO 2018 (Presenta trabajo, 08/11/2018). Elaboración de vinos tintos con menores contenidos de alcohol y enriquecidos en compuestos fenólicos. Montevideo/Uruguay (Resumen). **Mención mejor poster de Producción Vegetal.**

9.1.3. <u>Proyectos de investigación asociados</u>

Piccardo D, Gonzalez-Neves G, Favre G. 2018. Evaluación de la estabilidad del color de vinos tintos Tannat elaborados a escala experimental e industrial utilizando diferentes tecnologías de vinificación. CSIC–Iniciación a la investigación. Modalidad 2.