High-impact wine aroma production by *Saccharomyces* yeasts and generation of yeast hybrids for industrial application

PhD thesis

María Dolores Pérez Pérez

Doctorado en Ciencias de la Alimentación

Supervisors:

Dra. Amparo Querol and Dr. José Manuel Guillamón

iata
Instituto de Agroquímica y Tecnología de Alimentos

CSIC

LALLEMAND

Valencia, March 2022

This thesis has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement number 764364, Aromagenesis, and from Generalitat Valenciana grant PROMETEO/2020/014.

La **Dra. Amparo Querol Simón** y el **Dr. José Manuel Guillamón Navarro**, Profesores de investigación del Consejo Superior de Investigaciones Científicas (CSIC) en el Instituto de Agroquímica y Tecnología de los Alimentos (IATA):

CERTIFICAN

Que Doña **María Dolores Pérez Pérez**, ha realizado bajo su dirección el trabajo titulado: "High-impact wine aroma production by *Saccharomyces* yeasts and generation of yeast hybrids for industrial application", que presenta para optar al grado de Doctor en el programa de Ciencias de la Alimentación de la Universitat de València. Asimismo, certifican haber dirigido y supervisado tanto los distintos aspectos del trabajo como su redacción.

Y para que así conste a los efectos oportunos, firman el presente certificado en

Valencia, 10 de marzo de 2022

Fdo. Amparo Querol Simón

Fdo. José Manuel Guillamón Navarro

Agradecimientos

Se acerca el cierre de esta etapa de mi vida que tantos cambios positivos me ha dado. Un sinfín de oportunidades para crecer personal y profesionalmente que nunca olvidaré. Por eso necesito agradecer a todos y cada uno de los que han estado en este camino, ayudándome siempre a lo largo del mismo.

En primer lugar, quiero agradecer a mis directores, Amparo y José Manuel, por darme la oportunidad de formar parte de un proyecto tan interesante y por confiarme las herramientas para llevar a cabo esta tesis. Pepe, gracias por ocuparte siempre de mi bienestar durante todo este tiempo, siempre apreciaré todo lo que has hecho por mí. Gracias por la gran confianza y fe que depositaste en mi trabajo en Logroño. Vicente, gracias por recibirme en el LAAE de Zaragoza, la inmensidad de conocimientos sobre aromas que surgen de ese laboratorio es increíble.

En segundo lugar, nada de esto hubiera sido posible si mis compañeros del INTA, Ceci, Mariana, Santi y Esteban no me hubieran animado a solicitar esta beca. Gracias Santi por estar siempre ahí pase lo que pase, fuiste el primero que creyó en mi trabajo y en mis capacidades en la bodega, y gracias a eso tuve esta gran oportunidad. Siempre has sido mi apoyo en mi trabajo, dándome fuerzas y animándome a progresar. Esteban, siempre con la bodega en perfecto estado, gracias a vos aprendí mucho sobre el manejo de la planta piloto. Cómo extraño los matecitos antes de comenzar la jornada. Ceci, mi mamá valenciana, gracias por enseñarme Valencia y por estar siempre ahí charlando, personalmente o a través de audios eternos ¡jajaja! Mariana, mi fugaz compañera en Logroño, siempre tan alegre, gracias por darme una mano para contactar con Amparo.

A los pocos días de llegar al laboratorio, llegó mi compañero de beca, el chileno más singular que he conocido, Seba, con su fuerte acento que tuve que traducir durante unos meses (jejeje). Gracias Seba por ser mi principal compañero en todo esto, eres un excelente "cabro" como persona y profesionalmente. ¿Quién sería Seba sin su francesito, súper mejor amigo, Romain? Gracias Romain por ser tan gentil y ayudarme; siempre te pregunto de todo y me respondes muy bien y bajito (jejeje); me escuchas y me aconsejas sobre los gatetes. Gracias por presentarme a Laura, siempre tan alegre, deportista y entusiasta. Gracias a los tres por compartir conmigo momentos divertidos en Jávea y Bejis, y todos los encuentros que hicimos y seguiremos haciendo.

Además, cuando llegué al laboratorio me encontré con este enorme grupo, "La Secta", de estudiantes de doctorado y doctores recientes que

siempre se lo pasan muy bien, ¡un grupo muy divertido! Gracias, Anto, Adri, Alba, María, Laura, Sara, Rick, Walter, Peris, Lucía, Miguel y Javi, por incluirme al instante en las salidas, las cañas y el baloncesto.

Unos meses después, en agosto, cuando empiezo a darme cuenta del intenso calor que hace en Valencia, me encuentro con Ying de camino a clases de inglés donde ella iba a perfeccionar su español, aunque no lo necesitaba. Gracias Ying por ser siempre tan sensible y amable, ¡todos te echamos de menos!

Luego, ese año y el siguiente, tuve la suerte de conocer a un grupo espectacular de personas con las que comparto algunas costumbres y orígenes: Dani, Thelma, Sofi, Cande, Lainy y Flor. ¡Qué bien lo pasamos! Gracias por acompañarme. Lainy, ¡una verdadera trabajadora y amiga! Flor, gracias por ser tan Sat Nam, escucharme y preocuparte por mí.

También se unieron a nosotros dos personas con costumbres diferentes: Inés y Marie. Inés, gracias por ayudarme en ese trabajo gigante y hacer las cosas tan bien, casi robóticamente, jeje. Marí-Sofí-Julí, mi mejor amiga AROMAGENESIS, cuántas risas, caminatas, paseos en bicicleta, qué delicadita tan divertida, ¡qué suerte tuve de conocerte!

Thank you AROMAGENESIS ESR, such a diverse and spectacular group of people, wonderful to meet you, always enjoying amazing cities with you, after the stressful meetings we went through. Madina, always telling me the stories of your country so interesting and immense. How hot it was trying to go around Milan! We are still waiting for a trip to Argentina. Thanks for everything.

Volviendo al laboratorio de Valencia, no sería lo que es sin la presencia de dos pilares esenciales: Laura y Anacris. Laura, ¿sabes que tengo tus papelitos explicando cada cosa que me has enseñado? ¡Gracias por ayudarme siempre! Anacris, gracias por atender con tanta paciencia y amabilidad cada cosa que se me ocurría preguntar o hacer. Roberto, iniciando tu etapa como investigador después de tanto esfuerzo cuanto me alegro!, gracias por tus consejos. Eladio, introduciéndome en la historia de Valencia y también sobre Argentina, ¡gracias!

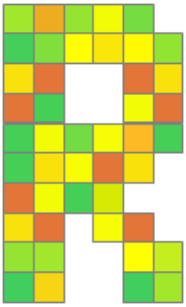
Por último, los nuevos doctorandos llegan a la tercera planta, con una pandemia de por medio: Albas, Sonia, Tania, Andrés, Noemi, Raquel, Helena y David. ¡Qué juventud en ese grupo, cuánta energía y cuánto deporte mezclado con cerveza! ¡Gracias por compartir momentos tan divertidos!

¡Alba y Sonia gracias por el enorme apoyo durante los últimos pasos de esta tesis!

Dejando Valencia y yendo a Argentina, no puedo dejar atrás a mi familia, a mi madre y a mis abuelos que me dieron todo lo necesario para desarrollarme profesionalmente, ellos son mis ejemplos de vida. Mis abuelos que emigraron a esa tierra llena de oportunidades y ahora yo hago el camino inverso, gracias por enseñarme a valorar lo esencial de la vida. Mamá, eres el mejor ejemplo de trabajo y esfuerzo que he tenido. Volviendo a España, Mario y Charo, mis "tíos de Barcelona", que nos ayudaron en todo desde que llegamos a España, gracias por formar parte de mi familia. Por último, mi moni a mi lado, empezando este viaje juntos, confiando en mí y yo en él, gracias por su paciencia y tranquilidad en todo.

Contents

Resumen Amplio



El vino se diferencia de la mayoría de las bebidas fermentadas, como por ejemplo de la cerveza, por su gran complejidad y diversidad aromática. En su aroma se distinguen aquellos compuestos que constituyen la nota vinosa, base similar en todos los vinos, y los aromas de alto impacto, compuestos menos abundantes, pero de potentes notas florales y frutales. Así, el aroma del vino se produce a lo largo de la vinificación, en su mayor parte durante la fermentación alcohólica y en él influyen principalmente la variedad de uva y la acción de la levadura.

Las levaduras han sido explotadas durante muchos años por el ser humano para la producción de vino, y en el caso de la fermentación las cepas vínicas de *Saccharomyces cerevisiae* son las más utilizadas, caracterizadas por la gran capacidad de producir alcohol y adaptarse a las condiciones iniciales del mosto de uva y del proceso de vinificación. A partir de los años 70 en el siglo pasado, cepas de esta especie se extendieron por todas las zonas vitivinícolas del mundo en forma de levaduras secas activas, las cuales aseguraban una fermentación sin problemas, altos rendimientos de etanol, evitando defectos aromáticos y reproduciendo perfiles de vino entre añadas.

Sin embargo, el vino, como cualquier producto comercial, se rige ante todo por las tendencias del consumidor, lo que marca los cambios a nivel industrial para responder a estas demandas. Actualmente los consumidores buscan una mayor diversificación de esta bebida; incluyendo vinos con perfiles aromáticos afrutados y frescos y con un contenido alcohólico equilibrado.

Por otra parte, la vid está sufriendo las adversidades producidas por el calentamiento global, el cual genera un desequilibrio en la madurez óptima de las uvas. El calentamiento global ha generado un aumento de la temperatura media anual, una mayor frecuencia de olas de calor e irregularidad en las precipitaciones. Las temperaturas extremas (>30ºC) y las frecuentes sequías

concentran los azúcares, mientras que los compuestos relacionados con la calidad del aroma, el sabor y el color aún están en proceso de desarrollo. Por lo tanto, esto lleva a la decisión de retrasar la cosecha para conseguir la madurez fenólica y aromática, arriesgando una deshidratación severa de la baya. Esta deshidratación repentina puede interrumpir la biosíntesis de compuestos polifenólicos (taninos y antocianos) y aromáticos, mientras que se produce una concentración de azúcares, aumento del pH y disminución del contenido en nitrógeno fácilmente asimilable por las levaduras. En el plano sensorial en vinos de uvas marchitas o con avanzado estado de madurez, los compuestos aromáticos positivos se ven afectados en gran medida y aparecen notas a "vino de oporto" o "alcohólicas". Además, el alto contenido alcohólico influye negativamente disminuyendo la percepción de notas florales y frutales.

Dado que los vinos que usualmente se obtienen con las levaduras vínicas *S. cerevisiae* no consiguen resolver los problemas relacionados con el cambio climático como, reducción del grado alcohólico, aumento de la acidez y de los aromas; actualmente existe un gran interés en la búsqueda y estudio de levaduras novedosas, no convencionales, con propiedades enológicas capaces de satisfacer las expectativas de los consumidores y de mitigar el efecto del cambio climático en las uvas. Por este motivo, en los últimos años se han incrementado los estudios de cepas de otras especies del género *Saccharomyces* o de géneros diferentes a *Saccharomyces* aisladas de fermentaciones espontáneas, de otros procesos fermentativos más tradicionales o encontradas en la naturaleza. Estas levaduras son usadas como co-cultivos con cepas vínicas de *S. cerevisiae* por contribuir positivamente en la complejidad aromática, favoreciendo la liberación de aromas varietales y fermentativos, como también organolépticamente, disminuyendo en este sentido el rendimiento en etanol y aumentando el contenido de ácidos orgánicos del vino (málico, succínico o cítrico). Estas propiedades también

contribuyen a disminuir el efecto del cambio climático sobre la vid, el cual provoca exactamente lo opuesto: incremento del alcohol potencial, disminución de la acidez y degradación de precursores aromáticos.

En este contexto, cabe destacar que las especies criotolerantes *S. eubayanus*, *S. kudriavzevii* y *S. uvarum* e incluso las cepas no vínicas de *S. cerevisiae* además de ser capaces de crecer y fermentar a bajas temperaturas, tienen la capacidad de producir menor contenido de etanol y una mayor producción de productos secundarios como: eritritol, glicerol, 2,3-butanediol, ácido succínico, etc. Estos compuestos son mayormente producidos por estas levaduras como alternativas diferentes a la producción de etanol buscando compensar de esta manera el exceso de cofactores reducidos derivados de la glicólisis. Asimismo, en la mayoría de los estudios llevados a cabo sobre estas levaduras, se ha encontrado una notable producción de compuestos volátiles cuya síntesis también estaría relacionada con estrategias de equilibrio redox y con el metabolismo nitrogenado, concretamente nos referimos a alcoholes superiores, sus acetatos, ácidos grasos de cadena media y sus ésteres etílicos. Sin embargo, estos compuestos tienen una relevancia aromática limitada, ya que la mayoría de ellos sólo participan en la nota vinosa, apenas superan sus umbrales de percepción y se degradan fácilmente durante el envejecimiento en botella. Por otra parte, pocos estudios se han centrado en la producción y liberación de aromas de mayor importancia aromática por parte de estas levaduras, es decir, aromas procedentes de precursores varietales o del proceso de fermentación cuyos umbrales de percepción son, en cambio, realmente bajos: terpenos, C_{13}-norisoprenoides, mercaptanos polifuncionales, ésteres etílicos de ácidos grasos de cadena ramificada, etc.

Estas especies/cepas poseen ciertas debilidades y baja competitividad frente a las levaduras vínicas de *S. cerevisiae*, ya que estas últimas, al ser usadas durante muchos años en fermentaciones, se han adaptado

perfectamente a las condiciones de fermentaciones y han sufrido lo que se conoce como domesticación. Hay varias posibilidades de usar estas cepas, o bien como inoculaciones secuenciales o co-inoculaciones, las cuales son muy utilizadas en la industria con las levaduras no convencionales pertenecientes a otros géneros. Debido a que estas técnicas pueden resultar muy laboriosas en bodega, otra alternativa es la generación de levaduras híbridas para obtener una cepa que herede las competencias y propiedades organolépticas de ambos parentales.

La hibridación ha sido un mecanismo de adaptación de las levaduras a condiciones ambientales variables, incluyendo también una forma de adaptación a condiciones estresantes como las que se presentan en ambientes vínicos. Varios trabajos han descrito la presencia de híbridos naturales entre *S. cerevisiae* y especies criotolerantes como *Saccharomyces uvarum* o *Saccharomyces kudriavzevii* que exhiben propiedades fisiológicas y enológicas de ambas especies. Estos híbridos han adquirido de su parental criotolerante una mayor capacidad fermentativa a bajas temperaturas, mientras que de su parental vínico una mayor tolerancia a altas concentraciones de azúcares y al etanol. Esto ha llevado a la generación de híbridos como técnica de mejora en levaduras. Entre las diferentes técnicas que se pueden usar para obtener híbridos destaca la denominada *rare-mating*, la cual es muy sencilla de llevar a cabo y no implica la generación de microorganismos genéticamente modificados (GMO), por lo tanto, éstos resultan aptos para la aplicación en la industria del vino.

En este contexto, esta tesis doctoral forma parte de un proyecto del programa Marie Skłodowska-Curie Innovative Training Network (ITN), concretamente del proyecto Aromagénesis (nº 764364), financiado por la Comisión Europea, Horizonte 2020. Este proyecto supuso la creación de una red de trabajo entre 10 grupos beneficiarios, incluyendo tanto empresas como

instituciones de investigación pertenecientes a 7 países de la Unión Europea. Este proyecto se centra en apoyar científicamente a dos de las industrias más importantes de Europa a nivel económico, el vino y la cerveza. El objetivo general del proyecto es el estudio de la producción de compuestos aromáticos por parte de las levaduras tanto cerveceras como vínicas para mejorar el perfil aromático de estas bebidas. Para alcanzar este objetivo se analizó la variabilidad de levaduras aisladas de diferentes orígenes en la síntesis de los aromas tanto mayoritarios como minoritarios y se desarrollaron nuevos métodos analíticos para determinar estos compuestos. A nivel molecular se analizó la variabilidad genética de todos los genes que participan en las rutas de síntesis de los aromas y la regulación de dichas rutas. Como aplicación a la industria se propuso generar nuevas cepas de levaduras que adquieran perfiles organolépticos mejorados, incluyendo nuevas selecciones, el uso de la co-inoculación o mejoras de las levaduras comerciales. Otro aspecto relevante es el desarrollo de nuevos nutrientes de levaduras que puedan contribuir a mejorar los perfiles aromáticos y organolépticos en el vino.

Esta tesis se centró en estudiar la capacidad para producir y liberar una amplia gama de compuestos volátiles de importancia aromática por parte de cepas no convencionales pertenecientes a especies criotolerantes del género *Saccharomyces* (*S. eubayanus*, *S. kudriavzevii* y *S. uvarum*) y cepas no vínicas de *S. cerevisiae*. Dado que algunos de estos compuestos están relacionados con el metabolismo de ciertos aminoácidos (valina, leucina, isoleucina y fenilalanina) y que adquieren relevancia durante el envejecimiento en botella, en esta tesis también se tuvo en cuenta estos factores. Por último, se generaron cepas híbridas entre las mejores levaduras no convencionales y una levadura vínica comercial de la empresa Lallemand Bio S.L., empresa líder en el desarrollo, producción y comercialización de levaduras, y que forma parte del consorcio Aromagenesis.

En el capítulo 1 se analizó una colección de 33 levaduras pertenecientes a las especies criotolerantes de ambientes naturales (*S. eubayanus, S. kudriavzevii, S. uvarum*) y *S. cerevisiae* no vínicas aisladas de cerveza, cachaça, masato, agave, cacao fermentado, insectos y roble. Estas cepas fermentaron en mostos con diferentes fuentes de nitrógeno, lo que nos permitió estudiar tanto la diversidad de levaduras, así como si con estas fuentes de nitrógeno se podían obtener atributos enológicos diferentes, como compuestos relacionados con la fermentación (etanol, glicerol, ácidos orgánicos, u otros productos secundarios de la fermentación incluyendo aromas). Al mismo tiempo, se pudo aportar información sobre aspectos relacionados con el metabolismo del nitrógeno en estas especies/cepas poco estudiadas. Las fermentaciones se llevaron a cabo en fermentadores a pequeña escala (fermentadores en 10 mL) en mosto sintético de composición y contenido de nitrógeno fácilmente asimilable (NFA) similar al mosto de uva (240 g/L de azúcares y 140 mg/L de NFA) y en mostos que contenían este mismo nivel de NFA pero aportado solamente por sal de amonio o por un amino ácido (valina, leucina, isoleucina o fenilalanina). A partir de los 6 mostos y las 33 levaduras se obtuvo un experimento de diseño factorial, que nos permitió discriminar entre los efectos individuales de los factores nitrógeno y levadura y su interacción sobre el rendimiento de la fermentación y la composición final del vino.

Se estudió la capacidad fermentativa de cada levadura y el efecto de las fuentes de nitrógeno sobre este parámetro mediante la medida de la pérdida de peso diario. Por otra parte, se determinaron mediante cromatografía gaseosa (GC-FID), los compuestos aromáticos mayoritarios y de origen fermentativo. Por último, mediante cromatografía líquida de alto rendimiento (HPLC), se analizaron otros compuestos fermentativos importantes, como alcoholes, azúcares y ácidos orgánicos producidos por parte de las levaduras e influenciados por las diferentes fuentes nitrogenadas.

En cuanto a los resultados sobre la capacidad fermentativa de las cepas, cabe destacar que las cepas pertenecientes a la especie *S. uvarum* se agruparon por una mayor dificultad fermentativa con fenilalanina, mientras que esta dificultad en la fermentación también se observó principalmente con leucina en las cepas de *S. kudriavzevii* aisladas en España, y al fermentar con isoleucina en las de *S. eubayanus*. Por el contrario, estas cepas no-*cerevisiae* mostraron una mejora en su velocidad fermentativa en mosto con valina, especialmente en el caso de cepas de *S. eubayanus*. Por otra parte, la mayoría de las levaduras *S. uvarum* y *S. eubayanus* se caracterizaron por alcanzar los mayores niveles de β-feniletanol y su éster de acetato, mientras que las cepas de *S. kudriavzevii* se relacionaron positivamente con la alta producción de glicerol y las de *S. cerevisiae* con la producción de etanol.

Las fuentes de nitrógeno, especialmente los aminoácidos, influyeron sobre la producción de ciertos compuestos volátiles y no volátiles. Además de observar la mayor producción de compuestos relacionados directamente con su catabolismo, es decir, alcoholes superiores y acetatos relacionados con la ruta de Ehrlich, así como otros metabolitos como eritritol, etanol, 2,3-butanediol, entre otros. En este sentido, la fenilalanina aumentó el contenido de eritritol y de los ésteres etílicos hexanoato y octanoato de etilo. Por otra parte, la leucina produjo cierta disminución del grado alcohólico, principalmente en las cepas de *S. cerevisiae* mientras que la valina aumentó el 2,3-butanediol.

Cabe destacar, que las cepas *S. uvarum* mostraban mayor producción de β-feniletanol y su acetato en todos los medios, tal y como ya estaba descrito por otros autores, en cambio, al fermentar en presencia de fenilalanina era mayor el aumento de eritritol que de estos compuestos aromáticos. Probablemente, estas levaduras producen estos compuestos volátiles a partir de la ruta de los azúcares, en concreto a través de la ruta del ácido shikímico,

y en presencia de fenilalanina esta ruta resultaría inhibida en su punto inicial, lo que lleva a derivarse hacia la mayor formación de eritritol.

A partir de estos datos se realizó una primera selección de cepas de cada especie en base a la capacidad fermentativa y los mayores niveles producidos de ésteres etílicos afrutados, ácido succínico y glicerol para ser incluidas en los estudios realizados en los capítulos 2 y 3. En el capítulo 2 se estudió cómo las cepas no vínicas pueden contribuir a la síntesis de aromas en mostos semi-sintéticos de la variedad Tempranillo (*Vitis vinifera* L) y en el capítulo 3 en la variedad Albariño (*Vitis vinifera* L). Para ello, las levaduras fermentaron en dos tipos de mostos sintéticos que contenían fracciones fenólicas y aromáticas naturales (PAF) de cada una de estas variedades de uva. Además, al mosto de Albariño se le adicionó una solución sintética de precursores glutatiónicos y cisteínicos de mercaptanos polifuncionales: 3-mercaptohexanol y 4-metil-mercaptopentanona. Tanto las soluciones de PAF como de mercaptanos fueron provistos por el laboratorio de análisis de aroma y enología (LAAE) de la Universidad de Zaragoza. Además, gran parte de los análisis aromáticos detallados a continuación fueron realizados durante la estancia desarrollada en este mismo laboratorio de la Universidad de Zaragoza en 2019 bajo la supervisión del Profesor Vicente Ferreira.

Las fermentaciones se llevaron a cabo en fermentadores de 100 ml de capacidad sellados y con válvulas de escape de gases y conteniendo 50 mL de mosto en agitación. El proceso fermentativo se realizó a 20ºC en mosto de Tempranillo y se utilizaron 14 cepas, mientras que en Albariño las fermentaciones fueron a 16ºC utilizando 8 cepas. Además, se incluyeron como controles fermentadores conteniendo mostos sin inocular. El seguimiento de la fermentación y análisis de alcoholes, azúcares y ácidos orgánicos se realizó como en el capítulo 1.

Una parte del volumen de los vinos resultantes y los mostos se sometieron a un envejecimiento anóxico acelerado con el cual se consigue simular el envejecimiento o guarda en botella durante un año. Para conseguir este proceso acelerado, dentro de una cámara desprovista de oxígeno las muestras de vino se introdujeron en viales sin espacio de cabeza y se colocaron en bolsas de plástico cerradas herméticamente, eliminando de esta manera la presencia o entrada de oxígeno en los vinos. Posteriormente, estas bolsas con los viales se mantuvieron en estufa a 50ºC durante 5 semanas, y a continuación se analizaron los aromas.

El análisis aromático realizado en estos dos capítulos fue más exhaustivo utilizando diferentes técnicas cromatográficas según la abundancia del aroma (aromas mayoritarios, minoritarios o trazas) y momento en el que se realizó el análisis (aromas desprendidos durante la fermentación, en el vino joven y en el vino añejado). En el ensayo de Tempranillo se colocaron en la salida de gases de los fermentadores cartuchos de resinas acondicionadas que adsorbieron los compuestos volátiles liberados durante el proceso fermentativo, posteriormente el contenido de los cartuchos de cada fermentador fue eludido utilizando disolventes orgánicos y los extractos obtenidos de cada uno se mezclaron en una única solución representando los aromas liberados por todas estas cepas no convencionales durante la fermentación. Esta solución mezcla se analizó a través de cromatografía de gases-Olfatometría utilizando detector de ionización de llama (FID) y puerto olfatométrico por el cual los aromas que fueron percibidos y evaluados por un panel de catadores, determinando el tiempo e intensidad del olor percibido. Luego estos compuestos fueron identificados molecularmente por comparación de los índices cromatográficos de retención en diferentes columnas y utilizando un detector de masas (GC-MS). En esta solución se identificaron once compuestos aromáticos, en su mayoría de origen fermentativo y cuyo orden de relevancia fue notablemente diferente al

encontrado en otro trabajo que utilizó el mismo mosto pero con cepas comerciales. Además, una característica muy notable fue la identificacion de un aroma varietal, la 4-mercapto-4-metilpentan-2-ona (4MMP), la cual es un tiol volátil responsable del aroma a "boj" que caracteriza a los vinos de Sauvignon Blanc.

En los vinos jóvenes de Albariño y Tempranillo se realizó un análisis de los compuestos aromáticos mayoritarios, es decir aquellos que se encuentran en concentraciones superiores a 1 mg/L, los cuales fueron obtenidos a partir de las muestras mediante micro-extracciones entre fases líquido/líquido y fueron analizados por cromatografía de gases acoplada a detector de ionización de llama (GC-FID).

Por otra parte, los aromas minoritarios, (0.1-200 μg/L) se analizaron tanto en vinos jóvenes como envejecidos de ambas variedades y en este caso los extractos se analizaron por cromatografía de gases acoplada de detector de masas (GC-MS).

Por último, los mercaptanos polifuncionales, también conocidos como tioles cuyas concentraciones rondan los ng/L fueron analizados por una técnica recientemente puesta a punto en el laboratorio LAAE. Las muestras fueron derivatizadas y extraídas por fase sólida junto con analitos deuterados incluidos como estándares internos. En este caso estos aromas fueron determinados por cromatografía de gases de doble columna acoplada a detector de masas (GC-GC-MS).

Como resultados generales de estos dos capítulos, se encontró una cepa no fermentativa de *S. uvarum* (CECT12600), que además de la acidez proporcionada por su mayor producción de ácido succínico y la mayor producción de aceto de feniletilo en ambos varietales, produjo vinos jóvenes y envejecidos de Tempranillo con gran concentración de aromas de notas

florales y frutales como monoterpenos, β-ionona, isovalerato de etilo e isobutirato de etilo. Esta misma cepa también desarrolló vinos de Albariño de gran interés enológico, sintetizando la mayor concentración de geraniol, ésteres lineales y ramificados afrutados y, además, mostró una importante capacidad para liberar mercaptanos polifuncionales varietales, principalmente 3-mercaptohexanol y su acetato.

En cuanto a estos últimos compuestos aromáticos, dos cepas de *S. kudriavzevii,* aisladas de ambientes naturales (nombre común CR89D1 y CA111F1), revelaron una notable capacidad para liberar grandes cantidades de mercaptanos polifuncionales, principalmente 4-metil-4-mercaptopentan-2-ona (4MMP) y 3-mercaptohexanol (3MH) en los vinos de Albariño. Por otra parte, una cepa *S. cerevisiae* aislada de cerveza de sorgo mostró un perfil aromático particular debido a la mayor producción de 4-metilvalerato de etilo (notas lácticas y afrutadas), γ-octalactona (coco) y, furfuriltiol (café tostado). Este último compuesto es un mercaptano de origen fermentativo cuya presencia en el vino se asocia al contacto del vino con las duelas de madera, formándose de esta manera durante la fermentación o añejamiento en barrica. Sin embargo, su detección en vinos sin madera ha generado dudas sobre su origen. Una de las posibles rutas de síntesis propuestas en la bibliografía es a partir de la ribosa y la cisteína. Considerando esto último y evidenciando en esta una misma levadura una gran producción tanto de furfuriltiol como eritritol, el cual deriva de la ruta de las pentosas fosfatos (PPP), propusimos que el furfuriltiol pudiera sintetizarse en *S. cerevisiae* a partir de la ribosa-5-fosfato, intermediaria en esta misma ruta (PPP).

Por otra parte, una de estas cepas de *S. kudriavzevii* (CA111F1) se destacó por la mayor producción de los 3 ésteres etílicos ramificados analizados isovalerato de etilo, isobutirato de etilo, y 2-metilbutirato de etilo en las fermentaciones realizadas con la variedad Tempranillo. En cuanto a las

cepas de *S. cerevisiae* que fermentaron mosto con la variedad Tempranillo destacó la cepa vínica T73 por su sorprende expresión aromática, siendo muy similar a las cepas *S. uvarum* y *S. eubayanus*, mientras que una cepa de *S. cerevisiae* asilada de cachaça (nombre común CSC1) desarrolló niveles significativos de C_{13}-norisoprenoides y derivados vanillínicos.

En general, el envejecimiento acelerado produjo un gran efecto aumentando los ésteres etílicos de cadena ramificada afrutados, mientras que los acetatos y algunos terpenos disminuyeron. Interesantemente, los vinos de las cepas criotolerantes tras ser sometidos al envejecimiento, seguían mostrando diferencias significativas en la composición volátil, manteniendo en varios casos sus perfiles diferenciales. Los vinos de cepas de *S. uvarum* produjeron un importante aumento de estos ésteres principalmente isobutirato de etilo, un atributo interesante tras el envejecimiento. Asimismo, la cepa de *S. cerevisiae* de agave desarrolló los mayores contenidos de un aroma esencial propio del envejecimiento, leucato de etilo, cuyas notas aromáticas recuerdan a las moras frescas.

El tercer objetivo de este estudio fue generar híbridos a partir de las levaduras no vínicas seleccionadas en función de los datos obtenidos en los capítulos anteriores con una levadura vínica, con el objetivo de potenciar o mejorar su potenciales aromáticos u otras propiedades como la capacidad de fermentar a bajas temperaturas, manteniendo sus propiedades enológicas de la cepa vínica comercial. Las levaduras seleccionadas fueron la cepa CECT12600 perteneciente a *S. uvarum*, dos cepas de *S. kudriavzevii* (CR89D1 y CA111F1) y la cepa asilada de cachaça (CSC1) de *S. cerevisiae*. Mediante la técnica de *rare-mating* se obtuvieron híbridos, y tal y como se ha indicado previamente, estos híbridos no se consideran GMO y por tanto no tienen limitación legal para ser usados en la industria. Esta técnica inicialmente requiere generar la obtención de mutantes auxotróficos espontáneos en las

cepas a cruzar para poder así aislar la cepa híbrida resultante con las auxotrofías compensadas. Para esto, se sembraron alícuotas de cada cepa en los siguientes medios selectivos: ácido α-aminoadípico (α-AA), ácido 5-fluoroorótico (5-FOA) y ácido 5-fluoroantranílico (5-FAA) con el fin de seleccionar colonias mutantes naturales para los genes LYS2, URA3 y TRP1 respectivamente. Una vez obtenidos los mutantes naturales de las cepas se cruzaron auxótrofos complementarios y tras la de estabilización genética de estos híbridos, se obtuvieron 7 levaduras híbridas de cruces intra e interespecíficos. Los 7 híbridos obtenidos corresponden a 2 híbridos de cada cepa de *S. kudriavzevii*, 2 híbridos de la S. *uvarum* y un híbrido con la *S. cerevisiae* de cachaça y la levadura vínica de *S. cerevisiae*.

Teniendo en cuenta las propiedades enológicas de los híbridos obtenidos se seleccionaros los 4 híbridos de *Sc* × *Sk* y los dos de *Su* × *Sc* para fermentar mosto Albariño a 16ºC y el híbrido *Sc* × *Sc* y uno de *Sc* × *Sk* para fermentar mosto Tempranillo a 25ºC. Las composiciones de estos mostos, las condiciones de fermentación y los análisis de compuestos aromáticos fueron similares a lo descripto en los capítulos 2 y 3, aunque solamente los vinos de Tempranillo fueron sometidos a envejecimiento acelerado anóxico.

Los resultados de este capítulo revelaron que las levaduras híbridas que heredaron la mitocondria de la levadura vínica mostraron mejores capacidades fermentativas, produciendo vinos con valores similares a ésta en cuanto a la producción de etanol y glicerol. Por otra parte, los híbridos con mitocondrias diferentes a la vínica produjeron vinos con niveles de etanol menor y mayor producción de glicerol, 2,3-butanediol y ácidos orgánicos.

En cuanto a los perfiles aromáticos, todos los híbridos produjeron vinos con mayores contenidos en varios compuestos aromáticos afrutados y florales en comparación con la levadura vínica. Cabe destacar una mayor producción de acetato de feniletilo, acetato de isobutilo, γ-octalactona,

cinamato de etilo en ambos vinos varietales. Los cruces *Sc* × *Sk* produjeron vinos Albariño con un mayor contenido de 3-6 veces mayor en mercaptanos polifuncionales, 4-mercapto-4-metilpentan-2-ona (4MMP), y 3-mercaptohexanol (3MH) en comparación con la cepa vínica parental.

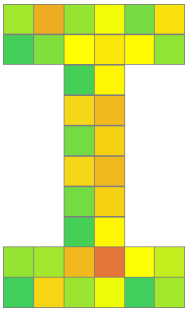
Resulta interesante destacar, que teniendo en cuenta las diferencias en ciertos compuestos aromáticos entre las diferentes cepas/especies, hemos propuesto rutas de síntesis de aromas poco definidas en la actualidad, especialmente en levaduras relacionadas con el vino. En este sentido, propusimos el furfuriltiol como derivado de la ruta de las pentosas fosfatos; la γ-butirolactona y el succinato de dietilo como aromas derivados de la ruta del GABA y atribuimos la mayor liberación de mercaptanos polifuncionales en cepas *S. kudriavzevii* a una reacción de desintoxicación de metilglioxal derivado de la gran producción de glicerol.

También como resumen de los resultados más relevantes, cabe destacar que las cepas silvestres no vínicas de *S. cerevisiae* y las especies criotolerantes aumentan la complejidad del aroma de los vinos. Al mismo tiempo, producen mayores cantidades de glicerol y ácidos orgánicos y un menor rendimiento de etanol, lo que contrarresta el efecto del cambio climático en las uvas. Por último, obtuvimos híbridos interespecíficos a partir de estas cepas no-*cerevisiae* que mostraron mejores características enológicas y aromáticas que la cepa vínica parental.

Por otra parte, los datos obtenidos en concreto los del capítulo 1, pueden ser la base para el desarrollo de suplementos nutricionales que permitan modular el grado alcohólico, el rendimiento en glicerol y ácidos orgánicos, así como de ciertos aromas. Siendo la valina la fuente que favorece fermentación en cepas no-*cerevisiae* (especialmente en *S. eubayanus*), mientras que en *S. cerevisiae* la fenilalanina potenció la producción de esteres

de aromas afrutados y el rendimiento de etanol disminuyó en mostos conteniendo leucina.

Introduction



I- Global warming affecting wine quality

Today, viticulture and enology face major challenges to reduce the effects of climate change on grape vines and wine's aromatic and organoleptic quality. Indeed, global warming has caused major changes in viticulture, especially in regions with a Mediterranean climate characterized by hot, dry summers. With the increase in average annual temperature, more frequent heatwaves, and irregular rainfall, there has been an advance of 2 to 3 weeks in the phenological stages of the grapevine (Holland and Smit, 2010; Mira de Orduña, 2010; Ramos and Martínez-Casasnovas, 2010).

Grape harvest time is usually determined by technical or oenological maturity, i.e., the ratio between the level of sugars and acids reached in the grape pulp, which will also establish the potential alcoholic level of the wine (Ribéreau-Gayon et al., 2006a). Extreme temperatures (>30ºC) and frequent droughts concentrate sugars at the same time that compounds related to aroma, flavor, and color quality are in the process of development (Figure 1) (Mori et al., 2007; Pons et al., 2017). Therefore, phenolic and aromatic maturity do not coincide with technical maturity, which leads to the decision to delay harvesting, risking severe dehydration of the berry (Bonada et al., 2015). If this dehydration occurs, not only does have an impact on plant yield, but the berry composition is also altered. The biosynthesis of polyphenolic and aromatic compounds is interrupted; there is an increase in potential alcohol, and significant malic acid degradation, resulting in an overall deterioration in the organoleptic wine quality (Gambetta et al., 2020; Mira de Orduña, 2010; Pons et al., 2017).

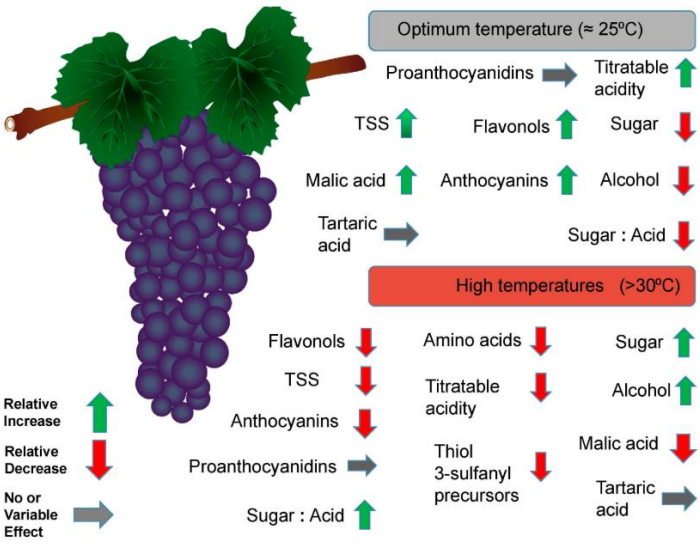


Figure 1. Effect of high temperatures on grape metabolites. TSS: Total soluble solids. (Venios et al., 2020).

ii. Biotechnological solutions: yeast for the future

Wine aroma is affected chemically and sensorially. Free varietal aromas and aroma precursors are degraded by the effect of high temperatures during the grape development and ripening phases (Park and Morrison, 1991; Peyrot des Gachons et al., 2000; Pons et al., 2017). The resulting wine from these grapes is distinguished by unfavorable notes such as Port wine, cooked fruits, or kerosene, while the high ethanol content (>14.5% v/v) also suppresses the fruity notes (Escudero et al., 2007). Furthermore, high alcoholic beverages are taxed in many countries and are now less demanded due to growing consumer concern about the harmful effects of alcohol consumption (Schelezki et al., 2020, 2018). In addition to the low ethanol content, new consumer trends are looking for fresh, fruity, and floral wines

(Fleet, 2008; Querol et al., 2018), as opposed to what is achieved shriveling grapes (Figure 2).

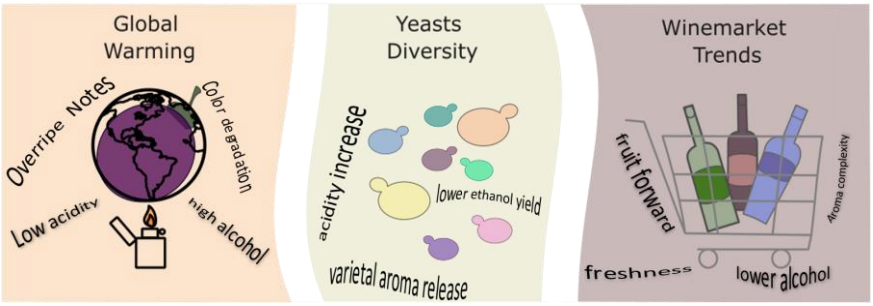


Figure 2. Schematic representation of the relationship between the effects of climate change, actual consumer demands, and phenotypic characteristics of yeasts on wine.

Several viticultural and oenological practices have been developed to reduce ethanol content while maintaining aroma quality (Varela et al., 2015). Nevertheless, some of them are economically expensive or affect the wine aromas by reducing the fruity and floral aromas or keeping the typical aromas of over-ripe grapes. (Gardner et al., 2021; Schelezki et al., 2018).

The use of non-conventional yeasts from the *Saccharomyces* genus or different genera has become a potential solution to these problems as they can reduce alcohol content, increase acidity, and favor the varietal and fermentative aroma profile (Mérillon et al., 2017) (Figure 2). However, non-wine strains possess lower tolerance to oenological conditions and low competitiveness against *Saccharomyces cerevisiae* wine yeasts. Due to this, strategies such as sequential fermentations, co-inoculations, or generation of hybrid yeasts are emerging to solve these strains' weaknesses (Querol et al., 2018; Varela et al., 2016).

II- Yeasts as wine modulating organisms

> *"It is possible to agree with me when, first, it is accepted that fermentations proper require as an absolute prerequisite the presence of microorganisms…"* Pasteur, 1878 (Friedmann, 1997).

Yeasts are unicellular eukaryote microorganisms belonging to the kingdom Fungi, widely present in natural habitats: flowers, fruits, tree bark, insects, soil. In addition, yeasts are involved in several biotechnological processes to produce food, beverages, medicines, and even biofuels.

Although man first recognized and identified yeasts as a fermenting microorganism in the 19th century, the involvement of yeasts in the production of bread, beer, and wine date back many centuries before, with the latest findings referring to 3150 BC (Barnett, 2000; Cavalieri et al., 2003). Since then, yeasts have been the model organism for many studies, mainly since the complete sequencing of *Saccharomyces cerevisiae* genome just two decades ago (Goffeau et al., 1996). In addition, many studies have successfully engineered the yeast (*S. cerevisiae*) systems towards the formation of aromas (Zebec et al., 2016). Further research has continued beyond *S. cerevisiae*, digging into its ecology, evolution, origins, and expansion throughout the world, discovering a huge phenotypic diversity within and out of this species. Thus, yeasts have always been close to the man providing food and beverages, and consequently, the phenomenon of domestication occurred; yeast metabolism, particularly that of *S. cerevisiae*, gradually adapted to the raw materials and fermentation conditions encountered (Camarasa et al., 2011; Gallone et al., 2016).

Among the grape and vineyard microbiota, non-*Saccharomyces* species are the predominant yeasts in percentages higher than 50% (*Rhodotorula, Pichia, Candida, Metschnikowia, and Hansenula)*, while *Saccharomyces* fermentative yeasts (*S. cerevisiae* and *S. uvarum*) are found in low proportions. These *Saccharomyces* yeasts are mainly found inside the winery (equipment, tanks, surfaces), whether autochthonous or commercial strains. As non-*Saccharomyces* yeasts have a low tolerance to ethanol or SO_2, they are unable to consume all the sugars during alcoholic grape-must fermentation, so *Saccharomyces* yeasts gradually prevail over the others and eventually completes the process on their own (Fleet et al., 1984; Mercado et al., 2007; Torija, 2002).

i. *Saccharomyces genus*, the wine leaders

As mentioned above, the *Saccharomyces* genus is the most closely linked to wine production. Yeasts belonging to the genus *Saccharomyces* are morphologically and phenotypically similar; their cells are ovoid or ellipsoid and can ferment sugars except for pentoses under aerobic or anaerobic conditions (Boynton and Greig, 2014; Vontrobová et al., 2019).

The taxonomic classification of the *Saccharomyces* genus recognizes eight species: S. *cerevisiae, S. paradoxus, S. mikatae, S. kudriavzevii, S. arboricola, S. jurei, S. uvarum* and, *S. eubayanus*, where *S. eubayanus* is the most recently identified species (Figure 3). In addition, two well-studied hybrid yeasts arise from some of these species, namely are *S. bayanus* (*S. uvarum* × *S. eubayanus*) and *S. pastorianus*. Where strains belonging to *S. pastorianus* have been reported as *S. cerevisiae* × *S. eubayanus* hybrids and *S. cerevisiae* × *S. uvarum* × *S. eubayanus* hybrids (Almeida et al., 2014; Peŕez-Través et al., 2014).

Among the members of the *Saccharomyces* genus, *S. cerevisiae* is the most domesticated and studied species, followed by *S. paradoxus* in the latter

respect. In this way, while some species have only been found in nature (*S. mikatae, S. kudriavezii, S. arboricola, S. eubayanus*), others have been isolated in both natural and human-associated environments (*S. cerevisiae* and *S uvarum*). Likewise, they are found geographically distributed according to optimal growth temperature, where *S. kudriavzevii, S. uvarum,* and *S. eubayanus* are considered cryotolerant species as their optimal growth and fermentation temperatures are usually lower than those of *S. cerevisiae* while they are not able to grow at 37ºC (Belloch et al., 2008; Boynton and Greig, 2014; Salvadó et al., 2011; Tronchoni et al., 2009). In this study, we will focus on *S. cerevisiae* and the cryotolerant species *S. uvarum, S. kudriavzevii* and *S. eubayanus* due to recent oenological interest.

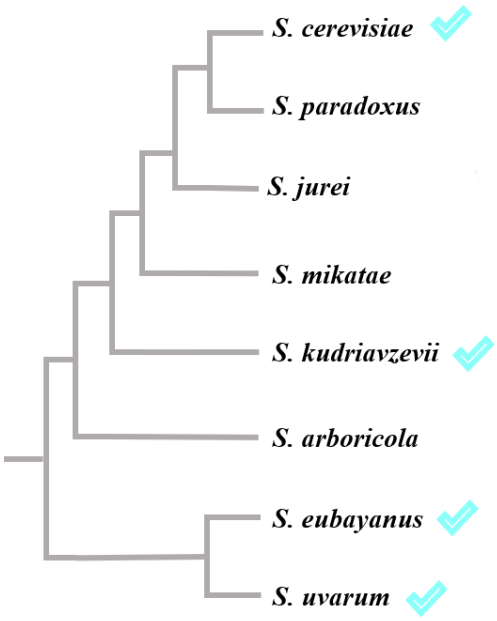


Figure 3. Phylogenetic relationships of the *Saccharomyces* genus (Boynton and Greig, 2014; Vontrobová et al., 2019, modified).

a. Saccharomyces cerevisiae

As explained above, *Saccharomyces cerevisiae* is the most widespread, studied, and domesticated species found in nature and related

human environments. Its popularity in scientific fields is partly due to the similarity of its biological cycle and cellular system with higher eukaryotes and its easy propagation and simple genetic system (Replansky et al., 2008). Most importantly for our study, *S. cerevisiae* is the main microorganism responsible for transforming grape must into wine.

Several studies have determined the extensive participation of *S. cerevisiae* in the spontaneous fermentation of cereals, fruits, or plants to obtain traditional beverages (e.g., chicha, cachaça, sorghum beer, sake) from different geographical locations (e.g., South America, West Africa, Asia). Many of these works have determined that all *S. cerevisiae* ended up genetically related to European wine *S. cerevisiae* yeasts (Badotti et al., 2014; Olivera et al., 2016; Rodríguez et al., 2016; Van Khle et al., 2001). However, by adapting to different ecological niches, these strains developed great phenotypic and physiological diversity within the same species (Camarasa et al., 2011; Rodríguez et al., 2016; Van Khle et al., 2001). Consequently, when faced with winemaking conditions, these non-wine *S. cerevisiae* strains have been shown to differ in the production of wine metabolites, such as ethanol, glycerol, higher alcohols, esters, and acetates (Brice et al., 2018; Furdíková et al., 2017; Molina et al., 2009).

b. *Saccharomyces uvarum*

Many earlier studies have reported it under *Saccharomyces bayanus* var. *uvarum*, now formally defined as *Saccharomyces uvarum*. Like many other species, it has been isolated in nature, such as hardwood bark, soil, or *Araucaria araucana* seeds, mostly from geographic locations with cold climates. After *S. cerevisiae, S. uvarum* is the most frequently found in fermentation processes, mainly at low temperatures such as Amarone wine, beer, cider and, some American beverages such as chicha and cachaça (Almeida et al., 2014; Naumov et al., 2011; Rodríguez et al., 2014;

Vontrobová et al., 2019). Therefore, under the grape must condition, *Saccharomyces uvarum* exhibits tolerances similar to those of *S. cerevisiae*, such as resistance to high sugar concentrations up to 27% sugar, as in the case of isolates in must for Amarone wine (Torriani et al., 1999). However, these two species differ in some tolerances, such as the greater cryotolerant capacity of *S. uvarum* and the greater ethanol resistance of *S. cerevisiae* (Belloch et al., 2008; Origone et al., 2017).

Many wine metabolites have been associated with *S. uvarum* species such as glycerol, malic acid, isobutyl alcohol, ethyl lactate, and others (Gangl et al., 2009), yet currently, the most widely described properties of their wines are the higher yields of succinic acid, erythritol, β-phenylethanol and its acetate ester, and the lower yields of ethanol and acetic acid (López-Malo et al., 2013; Minebois et al., 2020; Stribny et al., 2015). Also, the ability to release thiols and *de novo* production of varietal aromas are an interesting features in recent works since the contribution to desired wine flavors (Gamero et al., 2011; Masneuf et al., 2002; Ugliano et al., 2006).

c. *Saccharomyces kudriavzevii*

The most striking aspect of this cryophilic species is that it has not been found as a pure culture involved in industrial fermentations so far. Its first isolation source was in fallen leaves in Japan (Naumov et al., 2011), and subsequently, it was reported in Portugal and Spain in similar natural environments (Lopes et al., 2010; Sampaio and Gonçalves, 2008). When it is inoculated in natural must or under industrial wine conditions, *S. kudriavzevii* as pure species is usually displaced by the presence of other more competitive microorganisms, such as *S. cerevisiae* (Alonso-del-Real et al., 2017; Arroyo-López et al., 2009). However, under sterile and low-temperature conditions, *S. kudriavzevii* manages to carry out a complete fermentation, giving rise to wines mainly characterized by a higher content of glycerol and

lower ethanol yields (Gangl et al., 2009; González et al., 2007; Peris et al., 2016; Querol et al., 2018; Tronchoni et al., 2009). Wines fermented by *S. kudriavzevii* yeasts have also been characterized for increased higher alcohol levels, like isobutanol and β-phenylethanol (Stribny et al., 2015), but more recently, they have been associated with the presence of metabolites derivatives of acetoin pathway, such as 2,3-butanediol (Minebois et al., 2020).

d. *Saccharomyces eubayanus*

Within the *Saccharomyces* genus, *S. eubayanus* was the last recognized and identified as a pure *Saccharomyces* species. This event occurred after the first isolation in Patagonian Nothofagus trees by Libkind et al. (2011). To date, its discovery has spread to other geographical areas: Tibetan plateau and in Fagus and Acer trees in Wisconsin, USA (Peris et al., 2014). All the geographic areas where this species has been found are characterized by cold climates, which gives them their cryotolerant capacity.

So far, like *S. kudriavzevii*, there are no records of *S. eubayanus* presence in fermentation environments. However, it has been suggested that it may once have been involved in the fermentation of traditional Patagonian beverages but was then displaced by the advent of European baker's and wine yeasts. In addition to this, *S. eubayanus* exhibits certain weaknesses in carrying out the process (Olivera et al., 2016; Rodríguez et al., 2014).

Although it seems a species with little value for fermented beverages, since its discovery as a *Saccharomyces* species, it was revealed as one of the progenitors among two important interspecific hybrids: *S. pastorianus* and *S. bayanus*, so from then, the vision of this species has become more relevant.

ii. Hybridization, yeast natural choice for improvement

According to today's requirements, most of the early described, *S. uvarum*, *S. kudriavzevii*, and *S. eubayanus*, have interesting oenological potential. However, they show a poor competitive ability in different fermentation environments (Alonso-del-Real et al., 2017; Arroyo-López et al., 2010; Origone et al., 2017; Su et al., 2019b). Therefore, industrial application as pure culture starters is restricted to a certain extent (Deroite et al., 2018). At the same time, the bibliography indicates that most of them have been found as natural interspecific hybrids having greater tolerance to beer or wine fermentation conditions (Belloch et al., 2008; Gangl et al., 2009; Ortiz-Tovar et al., 2019; Silva et al., 2015).

The natural hybrid yeast between *S. uvarum* and *S. cerevisiae* was one of the first to be studied and found in wine environments. While being able to ferment at low temperatures, these hybrids showed a great capacity to release higher concentrations of pleasant thiols and β-phenylethyl acetate (Masneuf et al., 2002; Murat et al., 2001).

S. kudriavzevii species has only been associated to a diverse fermentative process under its interspecific hybrid form *S. cerevisiae* × *S. kudriavzevii and* the triple hybrid *S. cerevisiae* × *S. kudriavzevii* × *S. uvarum* (González et al., 2007, 2006; Peris et al., 2012). Many hybrids have been oenologically characterized, showing several improved traits over their parental species in terms of metabolite production and fermentative capacity. For instance, a group of *S. cerevisiae* x *S. kudriavzevii* hybrids, although initially mistaken as a pure *S. cerevisiae* species, became popular in the wine industry due to their high ability to release pleasant polyfunctional mercaptans and by the great fermentability at low temperatures (Dubourdieu et al., 2006; Erny et al., 2012; Murat et al., 2001).

It has been determined with certainty that *S. eubayanus*, as parental of the hybrid *S. pastorianus*, is responsible for conferring the cryotolerant nature to this brewer's yeast allowing fermentation temperatures between 10-15°C, necessary for the production of lager-style beers (Gibson and Liti, 2014). This interspecific hybrid was the first yeast used as a starter culture for beer fermentations. This breakthrough occurred in 1883 at the Carlsberg Laboratory in Copenhagen, so it was initially named *Saccharomyces carlsbergensis*. Now, it is recognized as *S. pastorianus*, having a huge economic impact as it is the most lager-yeast used in the beer industry (Hebly et al., 2015).

All these studies showed that natural interspecific hybrids in fermentative environments are very frequent, thus confirming that hybridization is a very common evolutionary mechanism in the *Saccharomyces* genus (Barrio et al., 2006). This process occurs due to a natural adaptation to the prevailing environmental conditions, originating organisms with inherited physiological properties from both parents (Peris, 2018).

Therefore, induced hybridization is one of the most suitable techniques for yeast physiological improvement, overcoming deficiencies of a particular yeast strain or combining the best of each parent in one yeast (Pérez-Través et al., 2012; Schillberg et al., 1991). The hybridization process can be tailored to cover many oenological traits in yeasts, such as increased ethanol tolerance, high glycerol synthesis, higher capacity to release volatile thiols, improved growth temperature tolerance, and decreased nutritional requirements (Lairón-Peris et al., 2020; Masneuf et al., 2002; Pérez-Través et al., 2015; Su et al., 2019a). In addition, the use of artificial hybrid yeasts results more practical for the wine industry than the mixed culture strategies.

iii. Laboratory hybridization strategies

Different methods have been used to obtain hybrids, such as spore conjugation, rare-mating or protoplast fusion.

a. Protoplast fusion

Briefly, this is the main hybridization method used in laboratories, in which the cell wall of both parents is removed enzymatically, and then the protoplasts are mixed and fused in the presence of polyethylene glycol and Ca^{2+} ions (Spencer and Spencer 1996). The main advantage is that interspecies, intraspecies, and intergeneric crosses can be made (Curran and Bugeja 1996). However, the main problem is that as the type of crossing is forced, i.e., it would not occur in nature; it is considered a method of producing genetically modified organisms.

b. Spore conjugation

This hybridization process involves the union of two haploid cells of different sexual types, which occurs after germination to form a diploid individual. Mass-mating is a hybridization technique also based on strain sporulation and spore union, yet as its name indicates, in a massive manner. However, industrial yeasts sporulate poorly depending on the cell's ploidy, and also, its spore viability varies greatly (between 0 and 98%). Therefore, it is not effective when crosses between this type of yeast are required.

c. Rare-mating

This strategy is an alternative to the spore crossing technique when the strains have low sporulation frequency or viability. The crossing is based on the occasional change of mating-type in yeasts, which could be diploid, of higher ploidy, or may display aneuploidy as well. Eventually, one of these individuals must become homozygous and thus cross with a cell of the opposite sex, either haploid or diploid (Barre et al., 1993). Hybridization

occurs once this change is achieved, which happens with a very low frequency. This would be the major disadvantage of the technique (Spencer and Spencer 1996). The isolation of the resulting hybrid requires selection techniques in which is usually chosen the use of parents that have some auxotrophy that will be complemented in the resulting hybrid (Pretorius, 2000).

One of its advantages is that intra- and inter-species crosses can be achieved within the *Saccharomyces* genus (de Barros Lopes et al., 2002). In addition, its main advantage over sporulation technique is that rare-mating avoids the loss of large genetic material, which can result in the loss of interesting features of the parental yeasts. Likewise, this technique does not require the time-consuming aspect of handling spores and the loss of viability when generating them (Pérez-Través et al., 2012). On the other hand, being the result of a natural process, it is not considered a GMO technique as the protoplast fusion, therefore its hybrids are allowed in the food industry.

III- Wine aroma

Wine aroma is one of the parameters most judged by consumers and oenologists when assessing the quality of this beverage. However, this parameter is difficult to define, analyze and control, as factors of different origins influence its composition and final perception (Ferreira et al., 2021).

From a qualitative point of view, grapes of *Vitis vinifera* L. cultivar are the initial and most important factor that will define the flavor profile of the wine, while in quantitative terms, most of the aromas are generated during the winemaking process. The synthesis of this large group of compounds begins from the reception of grapes when pre-fermentative treatments are applied. Then the microorganisms play the most important role during the alcoholic and malolactic fermentation stages. During these processes, the initial composition of the must, the yeast or bacteria strain, routine oenological practices, the use of additives, the fermentation temperature, oxygenation, aging, and storage conditions, among others, will influence the final aromatic content (Lambrechts and Pretorius, 2000; Molina et al., 2009, 2007; Swiegers et al., 2006).

Around 1,000 compounds responsible for wine aroma are currently known, in different concentration ranges, from ng/L to g/L (Pons et al., 2017). Their origin results from a series of biological, biochemical, and technological reactions during the berry's development up to wine conservation. Following the classification proposed by Oliveira, (2019), the aromas of wine according to their genesis are grouped as follows:

- Aroma compounds derived from specific grape precursors
- Aroma compounds derived from unspecific grape precursors

i. Aroma compounds derived from specific grape precursors

This group consists of volatile and non-volatile compounds present in grapes and wine from ng to µg/L, contributing to varietal differentiation (Bayonove et al., 2003; Günata et al., 1999; Rapp, 1995). These compounds are formed through metabolic grapevine pathways and accumulate in the pulp and the berry skin. The volatile and odorant compounds are called free varietal aroma or free fraction, while the non-volatile and non-odorant substances are called varietal aroma precursors or bound fractions. The latter fraction consists of non-volatile molecule, (e.g., sugars, cysteine, glutathione) covalently bound to an aroma molecule which, will retain its original chemical structure upon release (Marais, 1983). Moreover, in the case of C_{13}-norisoprenoids, the aglycones may not be sugars, but rather intermediary compounds that, after reactions during vinification and aging, release volatile substances.

The volatile fraction is generally found in smaller proportion than the bound one, constituting between them the aroma potential of the grapes (Bayonove et al., 2003). Thus, some varieties, such as Albariño, Muscat, Gewürztraminer, are called aromatic or floral due to the greater presence of these free aromas perceptible in the berry itself. On the other hand, the vast majority of the grape varieties are considered neutral or non-aromatic, as these odorant molecules are absent or in concentrations below their odor thresholds (López et al., 2004; Ugliano et al., 2006). In any case, in both neutral and aromatic varieties, varietal aromas will be released during vinification or aging, constituting the character or typicity of each varietal (Roland et al., 2011).

a. Cysteinylated and glutathionylated precursors

They are the precursors of the pleasant polyfunctional mercaptans (PFMs) and consist of a cysteine or glutathione molecule bound to the aroma. They are found in most grape varieties, mainly in Sauvignon Blanc (*Vitis vinifera* L), giving its varietal character to the wines (Tominaga et al., 1995; Tominaga et al., 1998a; Roland et al. 2011). The S-Cys-conjugated precursors of 3-mercaptohexanol (3MH, or 3-sulfanyl hexanol) and 4-methyl-4-mercaptopentan-2-one (4MMP, or 4-methyl-4sulfanylpentan-2-one) were first identified in the form: Cys-3MH and Cys-4MMP (Tominaga et al., 1998b). Subsequent studies determined the presence of 3MH and 4MMP bound to glutathione, forming the precursors Glt-3MH and Glt-4MMP (Fedrizzi et al., 2009; Peyrot des Gachons et al., 2002). In addition, other possible precursors have been proposed, such as 2-*E*-hexanal, mesityl oxide, the cysteine-glycine, and glutamyl-cysteine conjugates (Bonnaffoux et al., 2017; Capone et al., 2011; Schneider et al., 2006).

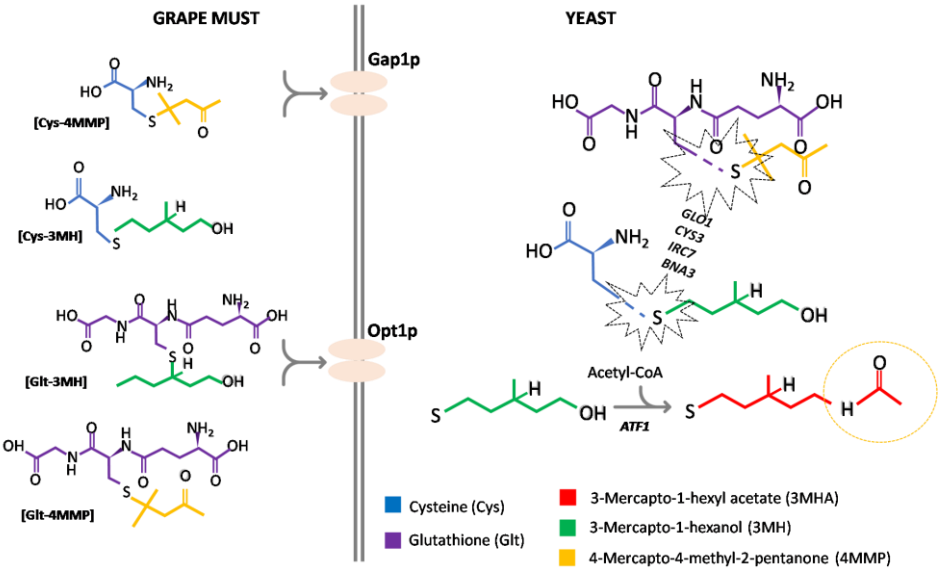


Figure 4. Schematic representation of cysteinylated and glutathiolated (non-volatile) precursors in grape must, the uptake into yeast cell by amino acid permeases and the

release of the volatilized fractions, 3MH and 4MMP, and the formation of 3MHA by yeast enzymes, as well as the genes involved.

In grapes, 4MMP and 3MH are always bound to a non-volatile molecule, cysteine or glutathione, which is why they do not impart aromas to the berries (Roland et al., 2011). Their release occurs exclusively during fermentation under the action of yeasts, although to some extent, they can also be released during bottle aging by acid hydrolysis (Alegre et al., 2020; López et al., 2004). The mechanism studied involves releasing the aromatic fraction, 4MMP or 3MH, by enzymatic cleavage of the carbon-sulfur (C-S) bond that links it to the non-volatile fraction cysteine or glutathione (Tominaga et al., 1995) (Figure 4). These β-lyase enzymes have so far been found to be encoded by the following genes, *BNA3, GLO1*, *CYS3,* and *IRC7*, some of which are controlled by the Nitrogen catabolic repression (NCR) and whose functions are not entirely specific (Howell et al., 2005; Thibon et al., 2008). On the other hand, 3MH is acetylated to 3MHA by the action of yeast's acyltransferase enzymes (Swiegers et al., 2006).

For yeast to carry out these release reactions, precursors must enter the cell (Figure 4). Recent studies have determined that precursors enter the yeast cell via the general amino acid permeases, Gap1p and Opt1p, and other specific amino acid transporters recently reported (Pinu, 2018; Ruiz et al., 2019; Thibon et al., 2008). These transporters could be controlled by NCR or Ssy-Ptr3-Ssy5 (SPS), whose activation depends on the yeast's preference for nitrogen sources available in the medium (Pinu, 2018; Subileau et al., 2008). Thus, yeasts' genetic and physiological diversity is key in these compounds. Indeed, several studies have found yeasts of different species and interspecies hybrids within the genus *Saccharomyces* as well as non-*Saccharomyces* strains having a high capacity to release polyfunctional

mercaptans (Belda et al., 2015; Deroite et al., 2018; Masneuf et al., 2002; Zott et al., 2011).

The most distinctive varietal free forms of PFMs or thiols are 3MH and 4MMP, and 3-mercaptohexanol acetate (3MHA, or 3-sulfanyl hexanoate acetate). Although this acetate is not directly derived from a conjugated precursor, it is formed from free 3MH during alcoholic fermentation by an alcohol acetyltransferase enzyme encoded by the *ATF1* gene (Swiegers et al., 2006).

They are key sulfur compounds essential for wine aroma quality. Although they are found in concentrations of the order of ng/L, their perception thresholds are often highly exceeded in young wines (Mateo-Vivaracho et al., 2010; Roland et al., 2011; Ruiz et al., 2019). Thus, their aromas contribute positively to the varietal aroma quality in wines and are easily recognized by their tropical fruity notes. At normal concentrations, they provide desired notes, such as box tree, blackcurrant (4MMP, 3MHA), grapefruit, passion fruit (3MH, 3MHA; Rolland et al., 2011). The first identified free-form compound in Sauvignon Blanc wines was 4MMP (Darriet et al., 1995; Du Plessis and Augustyn, 1981). Later, 3MH and its acetate were identified (Swiegers et al., 2006), which along with 4MMP were found in other varieties besides Sauvignon Blanc, such as Verdejo, Albariño, Chardonnay, Riesling, Pinot Gris, Merlot, Cabernet Sauvignon, among others (Bouchilloux et al., 1998; Capone et al., 2011; Mateo-Vivaracho et al., 2010; Murat et al., 2001; Schüttler et al., 2015).

Furfurylthiol or 2-furanmethanethiol (FFT) is a fermentative PFM of great interest within this chemical family. FFT has a roasted-coffee aroma, and its known synthesis route is during fermentation or aging by the contact of furfural, released from roasted wood barrels, with SO_2. However, this origin is not entirely accurate since furfurylthiol has been detected in wines without

wood contact, suggesting alternative synthesis routes during alcoholic fermentation (Blanchard et al., 2001; Mateo-Vivaracho et al., 2010; Tominaga et al., 2000).

b. Glycosidic precursors

Although non-volatile, glycosidic precursors are part of the aromatic reserve of the grape and hence their importance in wine aroma. They are constituted by a sugar linked to the odorant molecule. The non-volatile fraction is called glycone and can be formed by a single glucose molecule or by a disaccharide: apiose, arabinose, rhamnose, or xylose linked to a glucose molecule. In any case, the glucose molecule is the one that retains by a glycosidic bond the aromatic substance called aglycone or volatile fraction. The release of the aglycone can be triggered by acid-catalyzed or enzymatic hydrolysis. Enzymes can be grape or yeast-derived.

b.1. Release mechanisms

b.1.1. By mild acid hydrolysis

Acid hydrolysis depends on the media acidity level, with the optimum pH range between 3 and 3.5. The reactions occur spontaneously and slowly during all stages of winemaking, especially during the wine storage or aging in the bottle. This process allows the formation of volatile compounds such as C_{13}-norisoprenoids and terpenes, from their non-aromatic varietal precursors. In the case of C_{13}-norisoprenoids, is not a direct release, instead an intermediate compound is released by this hydrolysis, which then gives rise to these volatiles (Waterhouse et al., 2016).

The aglycone liberated via acid-catalyzed hydrolysis can be submitted to chemical rearrangements on these free molecules, as in the case of monoterpenes (Figure 5), generating volatile compounds with different chemical structures or decreasing their concentration through degradations

(López et al., 2004). For instance, once released during winemaking, grape-derived monoterpenes are susceptible to changes in their structure due to the acidic environment or by the action of yeasts. In the case of geraniol, after hydrolysis, several monoterpenes can be formed by different chemical rearrangements (Figure 5), while after the hydrolysis of linalool, a cyclization mechanism could lead to the generation of 1,8-cineole (Waterhouse et al., 2016).

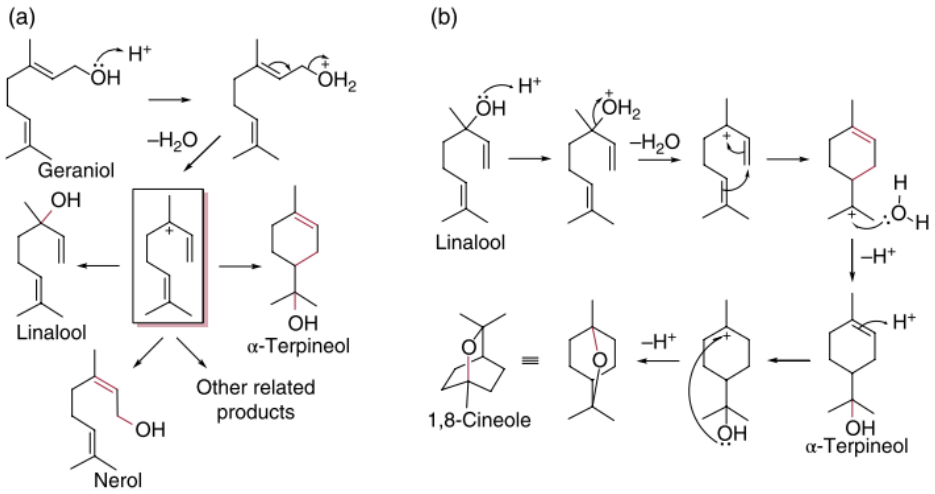


Figure 5. Acid-catalyzed hydrolysis followed by chemical rearrangements of grape-derived geraniol (a) and linalool (b) terpene compounds, under winemaking conditions (Waterhouse et al., 2016).

b.1.2. By enzymatic β-D-glucosidase hydrolysis

When the glycone is a disaccharide, the enzymatic hydrolysis process occurs in two steps (Figure 6), whereas in the case of glucose it occurs in one step only. First specific enzymes such as α-L-arabinofuranosidase, β-D-apiosidase, β-D-xylosidase, α-L-rhamnosidase break the glycosidic bonds linking the monosaccharide (arabinose, apiose, xylose, or rhamnose) to β-D-glucose. Then the enzyme β-D-glucosidase releases the aglycone, from the glycosidic bond linking it to glucose (Günata et al., 1988; Liu et al., 2017).

Unlike acid hydrolysis, enzymatic hydrolysis does not generate a subsequent alteration in the chemical structure of the released molecule.

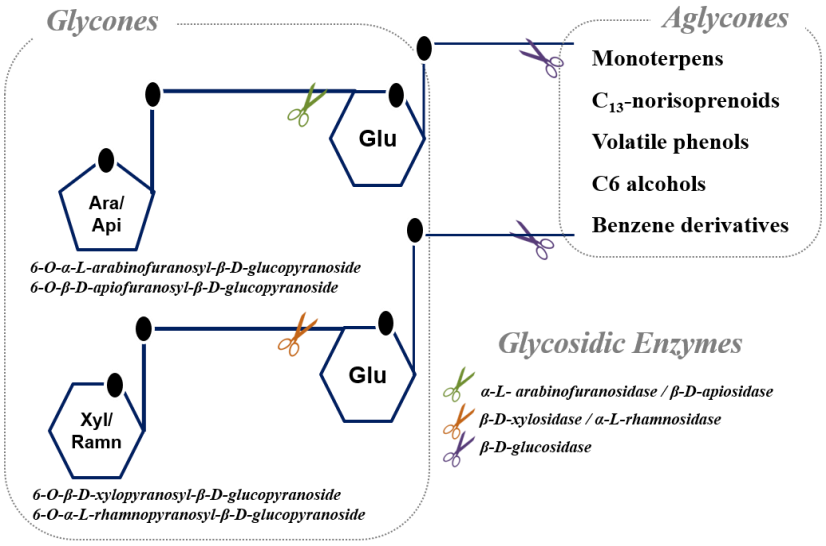


Figure 6. Schematic representation of the enzymatic hydrolysis of glycosidic precursors by cleavage of glycosidic bonds and release of aglycones.

Only a few of these enzymes are found in grapes and *S. cerevisiae* yeasts, which most of them are not particularly efficient under winemaking conditions: high levels of sugars and ethanol, low pH, and presence of polyphenols (Fernández-González and Di Stefano, 2004; Ugliano et al., 2006). On this basis, commercial preparations of exogenous enzymes from filamentous fungi such as *Aspergillus niger* have been developed. Their corresponding β-D-glucosidase enzymes have a high activity at low pH, and although they are sensitive to high sugar concentrations, they remain active towards the end of fermentation (Baumes, 2009). However, these preparations possess other enzymes whose action on other wine compounds can impair wine quality, e.g., by releasing large amounts of C6 alcohols and polyphenols (Armada et al., 2010). On the other hand, it has been determined that non-*Saccharomyces* yeasts, despite their low fermentative capacity, possess highly

active and specific enzymes for the release of varietal aromas (Belda et al., 2015; Fernández-González et al., 2003).

b.2. Free forms of the glycosidic precursors, or aglycones

Glycosidic precursors containing monoterpenes as aglycone were the first to be discovered and eventually characterized the aromatic Muscat grape varieties (Park and Morrison, 1991; Sánchez-Palomo et al., 2006). Next, in terms of aromatic importance and abundance in grapes, C_{13}-norisoprenoids are the second most abundant aglycones. Likewise, benzyl derivatives (benzene alcohol, benzaldehyde), 2-phenylethanol, C6-alcohols, and volatile phenols are also aglycones (Baumes, 2009; Fenoll et al., 2009; Fernández-González and Di Stefano, 2004; Ugliano and Moio, 2008).

b.2.1. C_{13}-norisoprenoids

They are compounds of great aromatic importance found and characterizing several fruits (Winterhalter and Schreier, 1994). In grapes, they are found mostly in the skin and originate from veraison to berry ripening, from the oxidative breakdown of carotenoid pigments, mainly from β-carotene, neoxanthin, and lutein. Then through a series of enzymatic reactions, the corresponding glycosidic conjugates are obtained (Keller, 2010; Winterhalter and Ebeler, 2013). Consequently, they are mostly found in grapes in this non-volatile form (Baumes, 2009; Winterhalter and Schreier, 1994). The release and transformation of their precursors occurs by acid hydrolysis during fermentation and mainly bottle aging (Ugliano and Moio, 2008). These free and volatile compounds have low perception thresholds and contribute significantly to wine aroma.

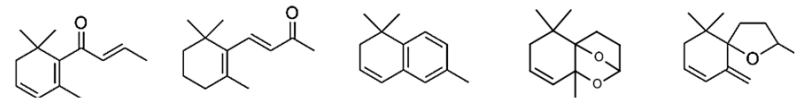


Figure 7. Main C_{13}-norisoprenoids in wine, in free form (aglycone).

TDN (Figure 7) is considered a typical constituent of bottle-aged wines, mainly of the *Vitis vinifera* L. Riesling cultivar (Oliveira, 2019). Its glycosidic precursors are estimated to be 3,6-dihydroxy-7,8-hydro-α-ionone, in the form of hemiacetal and dihydroxyketone. The aromatic impact of TDN in wine is of great importance due to its low perception threshold and its kerosene or hydrocarbon note, which at high concentrations is considered unpleasant. Its content is higher in wines from grapes grown in warm climates or exposed to high solar radiation, so it is considered one of the adverse effects of climate change on grapevines (Winterhalter and Gök, 2013).

On the other hand, it has been hypothesized that Riesling acetal and Vitispirane A and B are also generated during bottle aging from TDN precursors and chemical rearrangements on the TDN molecule (Oliveira, 2019; Waldmann and Winterhalter, 1992).

β-damascenone and β-ionone (Figure 7) are essential in the aromatic base of wine, contributing positively, favoring the perception of fruity notes in young wines and raisin-dry plum notes in aged wines (Escudero et al., 2007; Pineau et al., 2007; San-Juan et al., 2011). The β-damascenone, whose specific notes are cooked apple, honey, and flowers, is a key compound in the aromatic quality of red (Ferreira et al., 2016) and white (Li et al., 2008) wines. As an impact compound in red wines, without the need to exceed its perception threshold, it intensifies fruity notes of certain volatiles while suppressing herbaceous notes (Pineau et al., 2007; Escudero et al., 2007). However, recent studies have determined that the positive impact of this compound depends mainly on the wine matrix, i.e., the type of wine. The opposite is the case with the violet-scented β-ionone, which favors floral and red and black fruit notes regardless of the type of red wine (Ferreira et al., 2016; Tomasino and Bolman, 2021).

b.2.2. Terpenes

These compounds are found in all grapevine varieties, but characterizing mainly the aromatic Muscat family (Marais, 1983), such as Muscat of Alexandria (Park and Morrison, 1991), Moscatel "à petits grains" (Castro Vázquez et al., 2002), Gewürztraminer (Rusjan et al., 2009), Albariño (Armada et al., 2010), Riesling (Schüttler et al., 2015) Torrontés Riojano (Pérez et al., 2018).

In grapes, they are mostly present in the skin in their non-volatile glycosidic form and, to a lesser extent, are found in the free state as the volatile and aromatic fraction (Park and Morrison, 1991). Their concentration increases from veraison to grape maturity. The glycone attached to the monoterpene (aglycone) is generally a disaccharide and, to a lesser extent, can be a glucose or a trisaccharide (Hjelmeland and Ebeler, 2015). The most important and abundant are geraniol, linalool, nerol, β-citronellol, α-terpineol, hotrienol, limonene, rose, and linalool oxide (Marais, 1983; Mateo and Jimenez, 2000), as they have low perception thresholds providing floral and fruity notes.

Besides yeasts releasing monoterpenes from glycosidic precursors, several works have determined the ability of yeasts belonging to different species to incorporate and transform free-monoterpenes, as well as *de novo* synthesize these compounds from their metabolism (Figure 8) (Fernández-González et al., 2003; Gamero et al., 2011; Pardo et al., 2015).

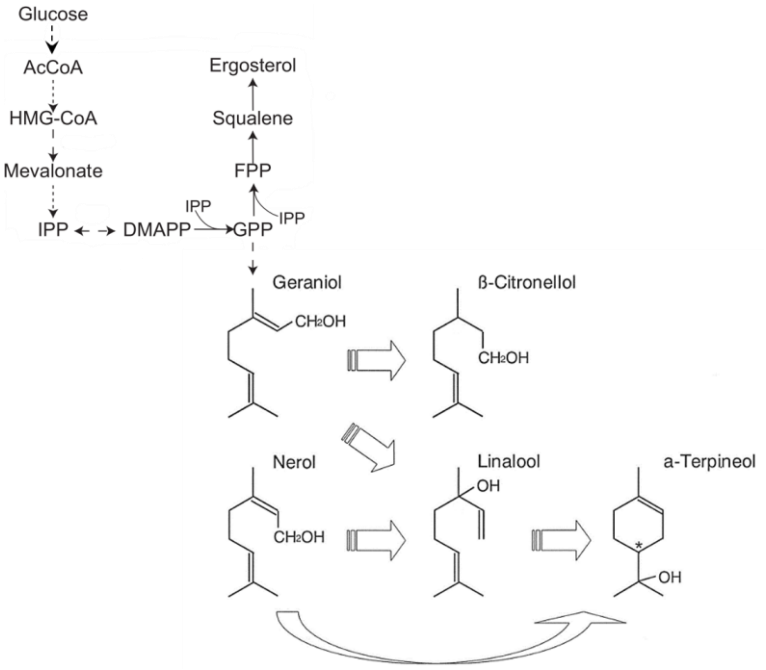


Figure 8. *De novo* synthesis and biotransformation pathways of monoterpenes by yeasts (Takoi et al., 2014; Liu et al., 2014, modified)

Monoterpenes can be synthesized by all living organisms (plants, animals, yeasts, and bacteria), through two possible synthesis routes, MVA (mevalonate pathway) and MEP (methylerythritol 4-phosphate pathway). Plants, such as the grapevine *Vitis vinifera*, are by far the most important producers of these aroma compounds as they have both routes active, whereas in our case, yeast can only synthesize monoterpenes from the MVA route (Zebec et al., 2016). In this way, yeasts can produce these compounds during the ergosterol synthesis (MVA pathway) from the intermediate, geranyl pyrophosphate (GPP, Figure 8). However, they reach only trace concentrations of monoterpenes due to the lack of monoterpene synthases (Pardo et al., 2015). On the other hand, yeasts can incorporate extracellular monoterpenes, like geraniol, to use them to produce membrane sterols. Overall, it has been suggested that yeasts *S. cerevisiae* consumed geraniol during the growth phase and transformed it into linalool and α-terpineol while

the cryotolerant species *S. uvarum* and *S. kudriavzevii* stored geraniol in their cells to be able to generate membrane sterols faster under low temperatures or oxygen deficiency conditions (Carrau et al., 2005; Gamero et al., 2011; Takoi et al., 2014; Vaudano et al., 2004).

On the other hand, there is strong evidence of yeast playing an important role in β-citronellol synthesis, since it has been determined that its presence in wine comes directly from the reduction of geraniol (Figure 8), whether intra- or extracellular, carried out entirely by yeast metabolism (Fernández-González et al., 2003; Steyer et al., 2013; Vaudano et al., 2004). Nerol has also been suggested as a precursor of other terpenes either by yeast action or acid-catalyzed reactions (King and Dickinson, 2000; Takoi et al., 2014).

b.2.3. Volatile phenols

Major volatile phenols in wines can be classified into vinylphenols and ethyl phenols, the former mostly present in white wines, and the latter are known as off-flavors mainly produced by the spoilage *Brettanomyces* spp. yeasts.

Vinylphenols can be found in glycosylated form in grapes, being released by acid or enzymatic hydrolysis, or they can also come from the grape cinnamic acids (mainly p-coumaric and ferulic acid) released by the action of yeasts during alcoholic fermentation (Chatonnet et al., 1993). Cinnamic acids, together with benzoic acids, anthocyanins, flavonoids, and tannins, are part of the polyphenolic compounds of grapes, responsible for color, bitterness, and astringency, which are found in greater proportion in red grapes than in white wines.

In addition to these compounds, other pleasant volatile phenols, such as vanillin, cresols, guaiacol and eugenol, can be found scarcely in grapes in

glycosylated form, although a large part generally originates during the wine's contact with wood, giving smoky and clove notes.

ii. **Aroma compounds derived from unspecific grape precursors**

According to their chemical structure, the following compounds of fermentative origin with non-specific precursors are found in wine: volatile fatty acids, higher alcohols, acetate of higher alcohols, ethyl esters of fatty acids, and carbonyls. Their concentration depends mainly on the basic non-volatile composition of the must: sugar content, nitrogen, and lipid composition (Cordente et al., 2019; Torrea et al., 2011; Varela et al., 2012). Since all these compounds are closely related to yeast metabolism, yeast is a key factor in their formation.

Most of these molecules constitute the buffer aroma or the vinous note of the wine. This mixture consists of 27 aroma molecules, plus ethanol, that are the foundation of all wines, from which no particular aroma can be distinguished. The term "buffer" refers to the fact that any modification of its composition does not generate major perceptible alterations at the sensory level. In other words, the omission of some compound or group of compounds is "compensated", as well as the ability to suppress the aromas of some aromatic compounds different from their composition (Ferreira et al., 2021).

Within this set of aromas, those that generate a greater impact by increasing their concentration are ethanol, acetic acid, and the higher alcohols, isobutanol, and isoamyl alcohol, which suppress fruity and woody notes, in addition to decreasing consumer preference (De-la-Fuente-Blanco et al., 2017; San-Juan et al., 2011). Conversely, the increased presence of fatty acids, straight or branched, increases fruity notes and decreases or masks notes related to volatile phenols.

Certain compounds or groups of compounds of similar chemical structures can break the aroma buffer. These compounds are called impact

aromas, and they share global aromatic notes called aroma vectors (Ferreira et al., 2016).

a. Medium-chain fatty acids (MCFAs)

Within this group of compounds found in wine are butyric, hexanoic, octanoic, and decanoic acids, which are medium and straight-chain fatty acids that also contribute to the aroma of wines (Ferreira et al., 2021). Many studies have related their presence in wine to stuck fermentation, attributing a toxic effect on yeasts intensified by parameters affecting membrane permeability, low pH, low temperatures, and high ethanol content (Robinson et al., 2014; Torija et al., 2003a, 2003b)

However, it has also been suggested that they could be found in wine because of stuck fermentation, rather than being the cause of it. In this regard, these volatile fatty acids are intermediates in synthesizing essential components of the plasma membrane, long-chain fatty acids (e.g., palmitic, stearic, oleic and, palmitoleic acids), and of more complex molecules, such as phospholipids. The latter is synthesized by the repeated condensation of acetyl-CoA, going on to form malonyl-CoA and catalyzed by the fatty acid synthase complex (FAS) (Figure 9), where elongations continue to form higher and unsaturated fatty acids (16 to 20 carbons). Under oxygen starvation, unsaturated fatty acids cannot be synthesized, so the resulting saturated fatty acids inhibit the key enzyme action (acetyl-CoA carboxylase) and disrupt the FAS complex, releasing the medium-chain fatty acid (C4, C6, C8, C10, and C12) as intermediates into the environment (Saerens et al., 2010; Waterhouse et al., 2016). Along with this, greater availability of Acetyl-CoA is left for the esterification of these acids.

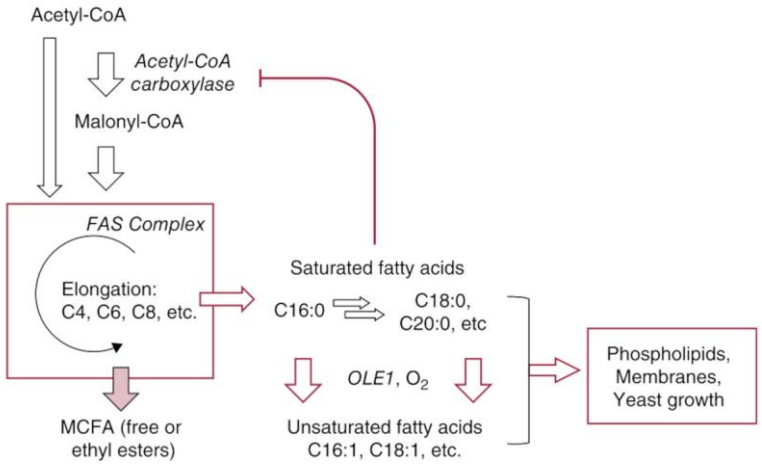


Figure 9. Biosynthesis of medium chain fatty acids adapted from Waterhouse et al., 2016.

Given the relationship of MCFAs with the biosynthesis of UFAs and phospholipids, their concentrations are increased by factors that decrease membrane fluidity or affect its composition, i.e., factors related to sluggish fermentations or stuck fermentations, such as anaerobic conditions, cold temperatures, depletion of UFAs and sterols, an increase of long-chain saturated fatty acids, the presence of ethanol (Bardi et al., 1999; Molina et al., 2007; Torija et al., 2003b; Varela et al., 2012). On the other hand, the grape variety, maturity, and pre-fermentation treatments influence these compounds since the grapevine contributes to its MCFAs to the must (Peinado et al., 2004; Sánchez-Palomo et al., 2007).

Despite having unpleasant aromas such as sweat, cheese, and rancid (Culleré et al., 2019), the ethyl esters formed from them confer desired aromatic properties. Although, recent works determined the positive participation of these acids in wine aroma (Lukić et al., 2008; San-Juan et al., 2011).

b. Higher alcohols and fatty acids from carbon and nitrogen metabolism

Higher alcohols (more than two carbons), also known as fusel alcohols (fusel refer to "bad liquor"), quantitatively are the most important secondary fermentative volatiles produced during alcoholic fermentation. Together with branched and straight acids, these alcohols are formed by yeasts to incorporate nitrogen into the metabolism to support cell growth (Fairbairn et al., 2017; Fleet, 1998; Lambrechts and Pretorius, 2000), either by the catabolism or anabolism of amino acids. Briefly, its formation consists in transamination of the amino acid releasing the carbon skeleton, an α-ketoacid, which is decarboxylated to aldehyde and subsequently reduced to generate the higher alcohol (**Figure 10**).

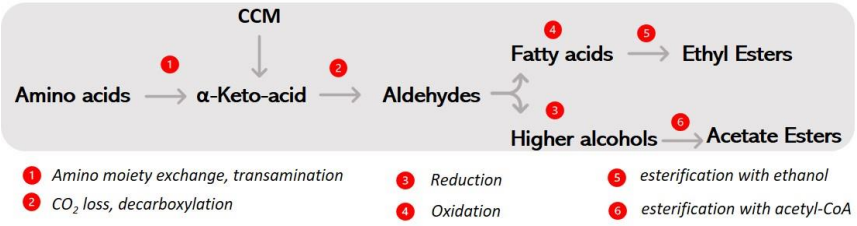


Figure 10. Synthesis of volatile compounds from α-keto-acids through the anabolism (central carbon metabolic pathway, CMM) and catabolism (Ehrlich pathway) of amino acids.

In the last step of this reaction, the synthesis of either higher alcohols or fatty acids (fusel acids) depends on the REDOX balance required to restore NAD+/NADH levels in the yeast metabolism. Although, in general, under fermentative conditions, alcohols formations prevail over acids (<1%) (Fariña et al., 2012; Hazelwood et al., 2008). Therefore, the higher alcohols are usually found in wine at concentrations above 100 mg/L, while the related acids are found under 1mg/L (Culleré et al., 2019).

Recent studies have established that in *S. cerevisiae* and non-*Saccharomyces* strains, only a small proportion of higher alcohols and their

acetate esters, such as isoamyl acetate and isobutanol, are derived from the catabolism of extracellular amino acids. Instead, almost 75 % of these compounds are synthesized from the central carbon metabolism (CCM) (Crépin et al., 2017; Rollero et al., 2017; Su et al., 2020).

Several factors related to nutrition affect its production: the available nitrogen sources, the management and timing of addition and fermentation environmental parameters such as temperature and oxygen, where if conditions favor cell growth, but the nitrogen source is limited, an increase in higher alcohols is observed (Bell and Henschke, 2005; Carrau et al., 2008; Hernández-Orte et al., 2006a, 2006b; Keller, 2010; Torija, 2002; Valero et al., 2003). Therefore, if the yeast requires nitrogen (amino groups) to carry out transamination and they are not available or are poorly assimilable nitrogen sources, the greater the availability of higher alcohols will be in the medium (Beltrán et al., 2005; Carrau et al., 2008; Hazelwood et al., 2008). On the other hand, the greater the presence of organic sources, i.e., amino acids, the greater the release of their corresponding higher alcohols, since this is their only possible nitrogen supply source (Wang et al., 2016). Therefore, yeast is the most important factor in the formation of these compounds, as the diverse variability between nitrogen and sugar metabolic networks, which determine the needs, preferences, and, therefore, the uptake of nitrogen sources (Fairbairn et al., 2017; Hernández-Orte et al., 2005; Rollero et al., 2018; Seguinot et al., 2020).

A great diversity of amino acids is metabolized by yeast, releasing their respective acids and higher alcohols. Among these, the most important in yeast metabolism are aromatic (phenylalanine, tyrosine, and tryptophan), branched (Valine, Leucine, Isoleucine), and sulfur amino acids (Cysteine and Methionine) (Figure 11).

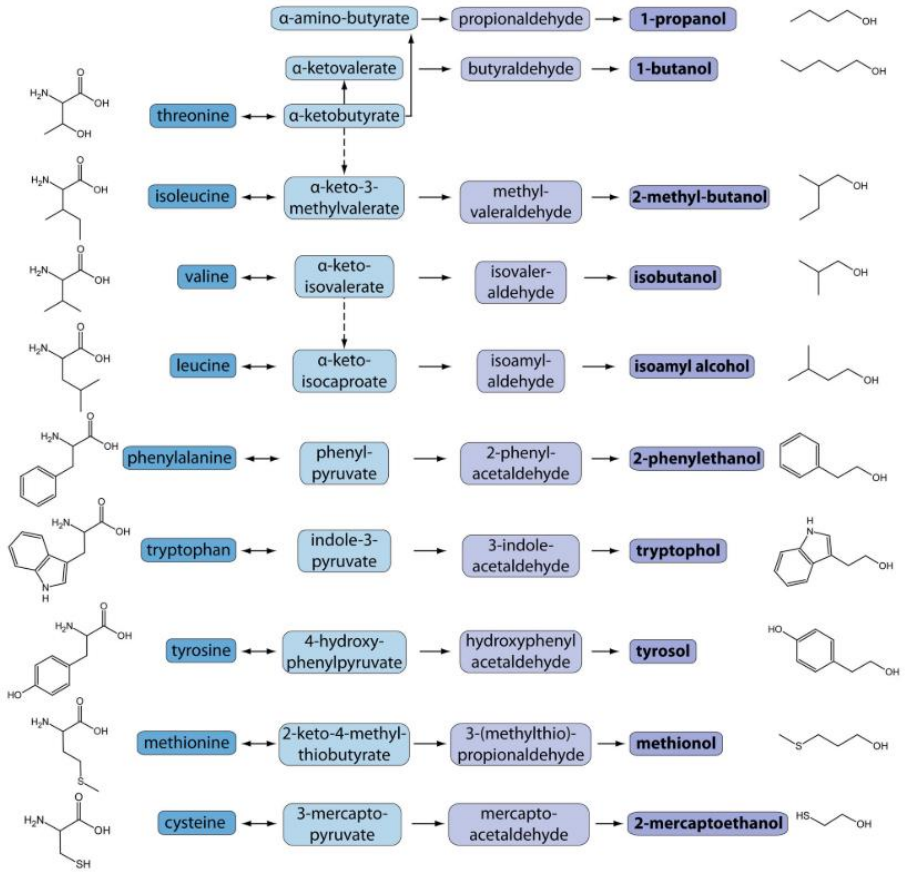


Figure 11. α-ketoacids and higher alcohols released from amino acids metabolized by yeast (Dzialo et al., 2017).

Sensorially, the presence of higher alcohols in wine also lies in their activity as precursors of acetate esters related to some desired aromas in wines, such as fruity and floral. Higher alcohols, such as isoamyl alcohol and isobutanol are often associated with a decrease in the aromatic quality of wines (Lukić et al., 2008; Peinado et al., 2004; San-Juan et al., 2011), while β-phenylethanol and methionol contribute only minorly to wine aroma (De-La-Fuente-Blanco et al., 2016). Furthermore, special attention should be paid to the branched-chain fatty acids derived from this metabolism, such as isobutyric, isovaleric, 2-methyl butyric acids, not because of their aromatic impact, i.e., they have unpleasant aromas and high perception thresholds, but

because they are precursors of esters with a high aromatic impact, especially in aged wines.

c. Esters

In contrast to the fatty acids and higher alcohols, esters compounds are usually found in wines at concentrations above their perception thresholds, producing a great aromatic impact (Lukić et al., 2008; San-Juan et al., 2011). In general, all are considered desirable for wine aroma, producing floral and fruity notes (Liu et al., 2014; Verstrepen et al., 2003), except ethyl acetate, which despite being the most produced during fermentation, its solvent-like aroma is considered an off-flavor, although only when exceeding a certain concentration (Lambrechts and Pretorius, 2000).

Two kinds of esterification can be distinguished. In brief, acetates formation results from the condensation between higher alcohol (or ethanol) with an acyl-CoA while ethyl esters derived from ethanol with an activated fatty acid. Of the latter, ethyl esters can be derived from fatty acids coming from different routes: from lipid synthesis (MCFAs: hexanoic, octanoic, decanoic, etc.) or nitrogen metabolism (branched or short fatty acids from α-keto acids, those along with the higher alcohols).

In wines, the acetates are predominant compared to ethyl esters and also diffuse faster through the membrane, while the others diffuse slower as their carbon chain is longer. These volatile compounds also differ because the formation of acetate esters is primarily influenced by the enzyme activity rather than substrate concentration. The opposite occurs for the synthesis of ethyl esters, where enzyme activity is not a limiting factor. However, the substrate concentration is (Saerens et al., 2010).

Other origins are derived from phenylpropanoid compounds, such as ethyl cinnamate or ethyl dihydrocinnamate, whose initial precursors are derived from the shikimate pathway of grapevine, but the esterification

process occurs during alcoholic fermentation or aging (Schüttler, 2013). These esters have low perception thresholds, aromas reminiscent of strawberry and in combination with other esters or impact aromas have been shown to enhance the aromatic quality of the wine (Ferreira et al., 1999; Puertas et al., 2018). In addition, there are esters formed from succinic and lactic acids, such as diethyl succinate and ethyl lactate, and are formed by the action of yeast or lactic acid bacteria during alcoholic or malolactic fermentation. Contrary to the previous ones, their perception thresholds are high and have not been determined to greatly influence the wine aroma (Robinson et al., 2014).

c.1. MCFA ethyl esters

Esterification of fatty acids occurs by condensation of ethanol and mid-chain fatty acid-CoA catalyzed by Eht1p and Eeb1p ethanol O-acyltransferase enzymes, so far described (Figure 12, Saerens et al., 2010). We can find ethyl hexanoate, ethyl octanoate, and ethyl decanoate within this group. They are characterized by producing fruity and fresh aromas typical of young wines; however, during bottle storage, they hydrolyze and disappear progressively (Díaz-Maroto et al., 2005; Ribéreau-Gayon et al., 2006b).

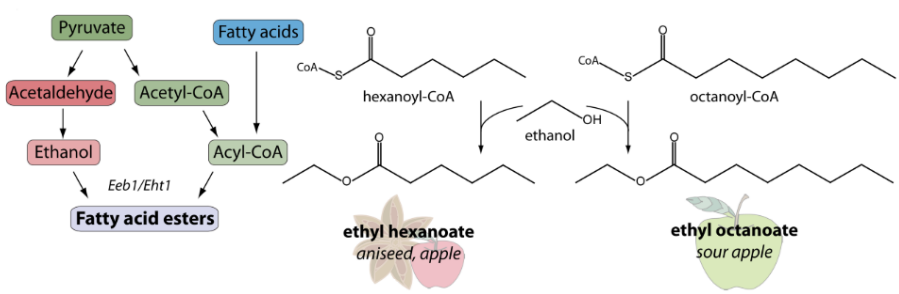


Figure 12. Synthesis of the main MCFA-ethyl esters (Dzialo et al., 2017).

c.2. Acetate esters of higher alcohols

Acetates are synthesized from the reaction between acetyl-CoA and higher alcohols, catalyzed by the action of alcohol acetyltransferase enzymes (Aft1p and Atf2p) encoded by *ATF1* and *ATF2* genes. Although there are still

other ester synthases to be developed, ATF1 plays a major role in forming these esters, yet its overexpression implies the undesired increase of ethyl acetate (Saerens et al., 2010). The most important acetate esters are active amyl/isoamyl and β-phenylethyl acetates, which have a banana and rose-honey nuances, respectively (Culleré et al., 2019). While the latter, together with isobutyl acetate, would provide "stone fruit" notes to white wines (Puertas et al., 2018).

The synthesis of acetates is greater at the end of alcoholic fermentation because lipid synthesis at this stage decreases (unsaturated fatty acids and sterols), with greater availability of acetyl-CoA. Hydrolysis generates the disappearance of these acetate esters during storage at elevated temperatures (Robinson et al., 2014).

c.3. Branched-chain fatty acid ethyl esters

As mentioned above, esters from short or branched fatty acids are gaining importance in wine aroma studies. The importance lies in three main aspects, the appreciated red- and black-berry fruity nuances, their lower perception thresholds, and above all, their significant increase by slow esterification during bottle aging, being key impact-aromas of aged wines. In addition, its greater presence in high-quality wines determines its relationship with wine quality (San-Juan et al., 2012). As is shown in Figure 13, the ethyl esters derived from branched-chain amino acids, such as ethyl 2-methylbutyrate, ethyl isobutyrate, and ethyl isovalerate, are usually present in wine at a lower concentration than their corresponding fatty acids precursors and their related higher alcohols. However, they had a significant fruity aroma impact due to the lowest perception odor.

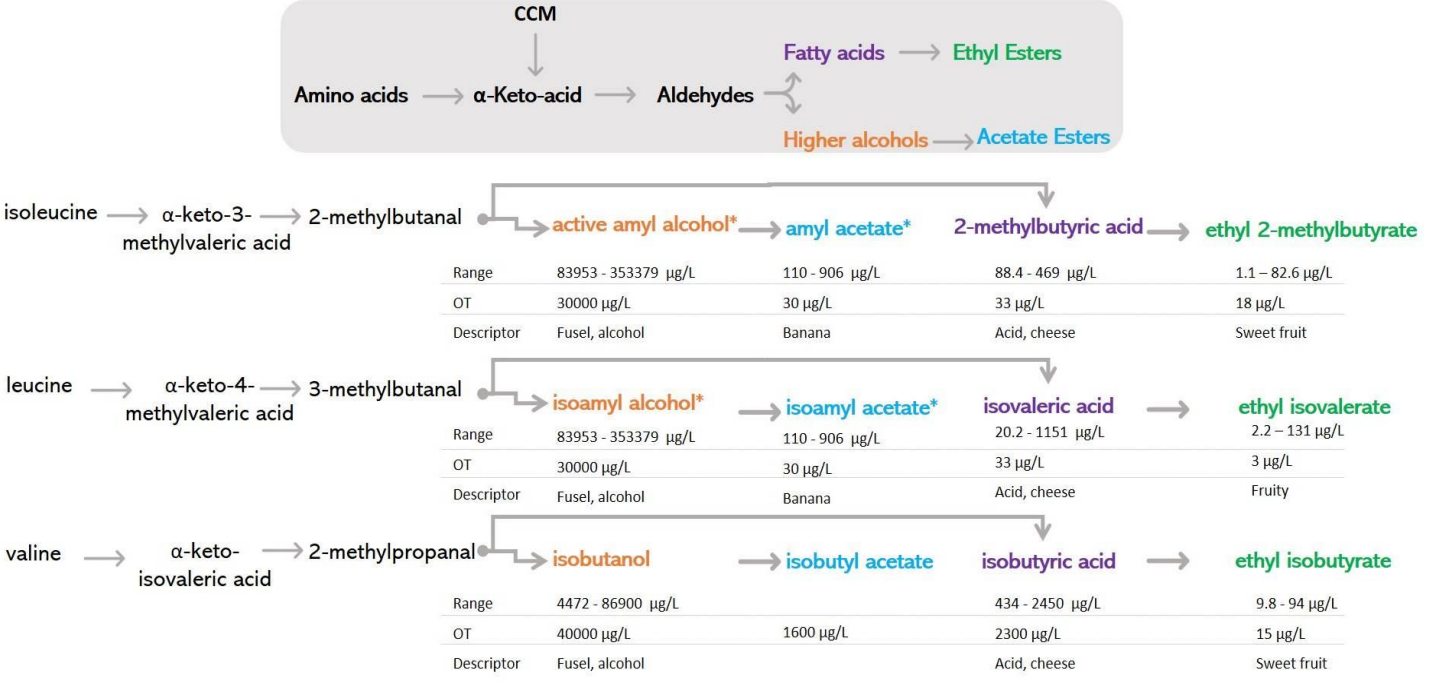


Figure 13. Acids, alcohols and esters synthesized from three of the most important branched amino acids, normal concentrations in wines, perception thresholds and aromatic descriptors according to Culleré et al., 2019.

Esters involved in the overall perception of red and black fruit nuances impacting the aroma of red wines have recently gained attention in wine aroma studies. On the one hand, strawberry-smelling esters, ethyl cyclohexanoate, ethyl 2-hydroxy-3-methylbutyrate, and ethyl 2-hydroxy-4-methylpentanoate were found for the first time in aged and spirit wines, and it was suggested that they increase by slow esterification during aging (Campo et al., 2006). Then, Falcao et al. (2012) identified a compound involved in the fresh blackberry aroma of Bordeaux wines, 2-hydroxy-4-methylpentanoate, also known as ethyl leucate.

On the other hand, several works have studied their role in wine aroma, performing omission or addition tests between them and assessing their sensory impact (De-la-Fuente-Blanco et al., 2020; Lytra et al., 2012). For instance, Pineau et al. (2009) determined that these esters are related to the specific red wine aroma grouped, imparting similar aromas. In this way, one group was related to the general aroma of redberry fruits (ethyl propanoate, ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate) while another group, including MCFA-ethyl esters, was related to blackberry fruits (ethyl hexanoate, ethyl octanoate, and ethyl 3-hydroxybutanoate).

d. Carbonyls compounds

Another group of aroma compounds derived from the sugar metabolism are carbonyls, such as diacetyl, acetoin, and lactones.

Diacetyl is a ketone (2,3-butanedione) which, together with acetoin (3-hydroxybutanone), are mainly produced by lactic acid bacteria as by-products of malolactic fermentation. Recently, however, diacetyl synthesis by certain yeasts has gained importance because it participates in the positive buttery flavor of wines without undergoing malolactic fermentation (Ferreira et al., 2021; Morata et al., 2021). Starting from pyruvate (Figure 14), acetoin formation can occur by three related pathways. The main route involves the

condensation of a free acetaldehyde with an active acetaldehyde-thiamine pyrophosphate molecule, catalyzed by the pyruvate decarboxylase (PDC) directly forming acetoin. The other two routes produce acetoin from diacetyl reduction, which can be derived from the non-enzymatic and spontaneous decarboxylation of α-acetolactate (intermediate in valine/leucine pathway metabolism) or from the condensation of acetaldehyde with acetyl-CoA, catalyzed by diacetyl synthetase (DS). Once formed, by the action of NADPH-dependent Bdh1 enzyme, acetoin is reduced to 2,3-butanediol, a secondary polyol with no sensory influence on the wine (Dzialo et al., 2017; Ehsani et al., 2009).

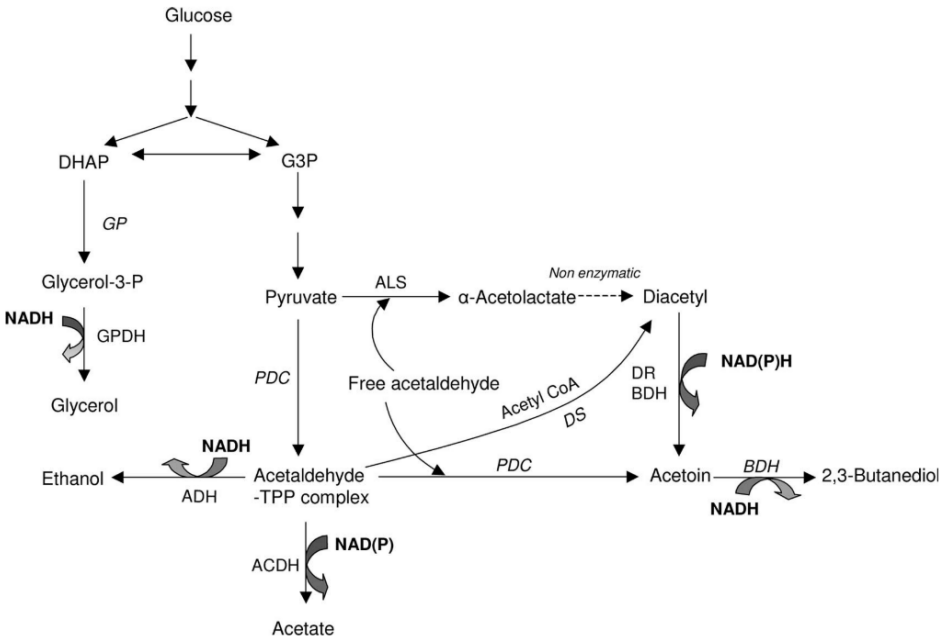


Figure 14. Yeast pathways for the formation of acetoin, diacetyl and 2,3-butanediol (Ehsani et al., 2009). GP, glycerol phosphatase; GPDH, glycerol phosphate dehydrogenase; PDC, pyruvate decarboxylase; ACDH, acetaldehyde dehydrogenase, ADH, alcohol dehydrogenase, ALS, acetolactate synthase, DS, diacetyl synthetase; DR, diacetyl reductase; G3P, glycerol-3-phosphatase; DHAP, dihydroxyacetone phosphate; acetyl CoA, acetyl coenzyme A; TPP, thiamine PPi.

On the other hand, lactones are another chemical group of carbonyls. Within this group, the main wine lactone is γ-butyrolactone, followed by γ-

octa, γ-nona and γ-decalactone. In flavor and fragrance chemical industries these volatiles are highly appreciated by their aromas such as tangerine, peach and coconut (Romero-Guido et al., 2011). However, their odor thresholds are hardly surpassed at the normal concentrations in wines, and their synthesis routes, although assumed to be of a fermentative origin (Culleré et al., 2019), remain to be elucidated in *S. cerevisiae*.

Another important lactone in wine is “oak lactone” or “whiskey lactone” which is more related to wines stored or aged in oak barrels, having coconut, walnut, and sweet wood notes (Wamhoff and Gribble, 2012).

IV- Wine polyols and carboxylic acids

Ethanol is the most important by-product of the fermentation process and consequently, the main ingredient of alcoholic beverages, although its reputation has changed over time. In the past, the presence of this compound in beverages was appreciated as it prolonged the preservation time by avoiding possible pathogenic contaminants in the ingredients (e.g., water) or contaminations that were produced during storage. In addition, not so long ago, winemakers were looking for concentrated wines, with a lot of color and therefore a lot of ethanol and aging time, which shed light on water stress practice on grapevine. Now, with the increased awareness of the harmful effects of ethanol on health and the previously detailed climate effect on grapes, there is an increasing tendency towards the consumption and production of beverages with lower alcoholic content.

Yeasts will ferment sugars to ethanol under glucose levels higher than 0.1% and even under aerobic conditions instead of going for the more energy-efficient respiratory mechanism. This phenomenon is known as the Crabtree effect (Fugelsang and Edwards, 2007). Briefly, during fermentative metabolism, sugars are transformed into pyruvic acid via glycolysis and then two molecules of pyruvate are reduced to ethanol. Although fermentation appears to be a process that fully compensates for the redox balance, instead, in the initial stage of this process, an excess of NADH co-factors is produced due to the energetic need for cell growth. Therefore, the reduced environment implies choosing alternative pathways to compensate for this redox imbalance. So, from this point, yeasts can redirect this NADH to alternative routes other than the one producing ethanol, such as through the glycolytic pathway, the tricarboxylic acid cycle (TCA), and the pentose phosphate pathway (or PPP). All these pathways lead to the excretion of secondary

compounds; among the most important and which we will explain are succinic acid, glycerol, erythritol, and 2,3-butanediol (Waterhouse et al., 2016).

The production of polyols and organic acids by strains has an important influence on the mouth feel of wine whereas its main function during fermentation is to compensate the redox balance (Schulze et al., 1996).

Succinic acid is an important organic acid in wine since its increase contributes to the drop of the pH and attenuates the loss of total acidity from potassium bitartrate precipitation (Coulter et al., 2004). This carboxylic acid during alcoholic fermentation can be produced by yeast from the reductive or oxidative branches of the tricarboxylic acid cycle (Camarasa et al., 2003). In addition, it has recently been determined the great involvement of the GABA shunt in the production of this acid, which is a pathway interconnected with TCA through a-ketoglutarate (Bach et al., 2009).

Glycerol is the most important secondary metabolite of alcoholic fermentation. It is produced by a diversion in the sugar flux to ethanol, to maintain NADH as oxidative form with a subsequent decrease in ethanol content (Cadière et al., 2011). While erythritol is another polyol, derived from the pentose phosphate pathway which has recently been reported in *Saccharomyces* yeasts under wine conditions (Minebois et al., 2020). Despite being an important contributor of sweetness in foods and beverages (Moon et al, 2010), at the concentrations found in wines its role in wine is only indicative of an alternative route for redox balance by yeasts. Lastly, 2,3-butanediol would come from the reduction of acetoin as explained above.

References

Alegre, Y., Sáenz-Navajas, M.P., Hernández-Orte, P., Ferreira, V., 2020. Sensory, olfactometric and chemical characterization of the aroma potential of Garnacha and Tempranillo winemaking grapes. Food Chem. 331. https://doi.org/10.1016/j.foodchem.2020.127207

Almeida, P., Gonçalves, C., Teixeira, S., Libkind, D., Bontrager, M., Masneuf-Pomarède, I., Albertin, W., Durrens, P., Sherman, D.J., Marullo, P., Hittinger, C.T., Gonçalves, P., Sampaio, J.P., 2014. A Gondwanan imprint on global diversity and domestication of wine and cider yeast *Saccharomyces uvarum*. Nat. Commun 5, 1-12. https://doi.org/10.1038/ncomms5044

Alonso-del-Real, J., Contreras-Ruiz, A., Castiglioni, G.L., Barrio, E., Querol, A., 2017. The use of mixed populations of *Saccharomyces cerevisiae* and *S. kudriavzevii* to reduce ethanol content in wine: Limited aeration, inoculum proportions, and sequential inoculation. Front. Microbiol. 8. https://doi.org/10.3389/fmicb.2017.02087

Armada, L., Fernández, E., Falqué, E., 2010. Influence of several enzymatic treatments on aromatic composition of white wines. LWT - Food Sci. Technol. 43, 1517–1525. https://doi.org/10.1016/j.lwt.2010.06.009

Arroyo-López, F.N., Orlić, S., Querol, A., Barrio, E., 2009. Effects of temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae*, *S. kudriavzevii* and their interspecific hybrid. Int. J. Food Microbiol. 131, 120–127.

Arroyo-López, F.N., Salvadó, Z., Tronchoni, J., Guillamón, J.M., Barrio, E., Querol, A., 2010. Susceptibility and resistance to ethanol in *Saccharomyces* strains isolated from wild and fermentative environments. Yeast 191–198. https://doi.org/10.1002/yea

Bach, B., Meudec, E., Lepoutre, J.P., Rossignol, T., Blondin, B., Dequin, S., Camarasa, C., 2009. New insights into γ-aminobutyric acid catabolism: Evidence for γ-hydroxybutyric acid and polyhydroxybutyrate synthesis in *Saccharomyces cerevisiae.* Appl. Environ. Microbiol. 75, 4231–4239. https://doi.org/10.1128/AEM.00051-09

Badotti, F., Vilaça, S.T., Arias, A., Rosa, C.A., Barrio, E., 2014. Two interbreeding populations of *Saccharomyces cerevisiae* strains coexist in cachaça fermentations from Brazil. FEMS Yeast Res. 14, 289–301. https://doi.org/10.1111/1567-1364.12108

Bardi, L., Cocito, C., Marzona, M., 1999. *Saccharomyces cerevisiae* cell fatty acid composition and release during fermentation without aeration and in absence of exogenous lipids. Int. J. Food Microbiol. 47, 133–140. https://doi.org/10.1016/S0168-1605(98)00203-7

Barre P, Vézinhet F, Dequin S, Blondin B., 1993. Genetic improvement of wine yeast. In: Fleet GH (ed) Wine Microbiology and Biotechnology, 1 Harwood Academic Publishers, Chur, Switzerland, 265-287

Barnett, J.A., 2000. A history of research on yeasts 2: Louis Pasteur and his contemporaries, 1850-1880. Yeast 16, 755–771. https://doi.org/10.1002/1097-0061(20000615)16:8<755::AID-YEA587>3.0.CO;2-4

Barrio, E., González, S.S., Arias, A., Belloch, C., Querol, A., 2006. molecular mechanisms involved in the adaptive evolution of industrial yeasts, in: The Yeast Handbook: Yeasts in Food and Beverages. Springer, Berlin, Heidelberg, pp. 153–174.

Baumes, R., 2009. Wine aroma precursors, in: Moreno-Arribas, M. V., Polo, M.C. (Eds.), Wine Chemistry and Biochemistry. Springer Science Business Media, New York, pp. 251–274.

Bayonove, C., Baumes, R., Crouzet, J., Günata, Z., 2003. Aromas, in: A. Madrid Vicente (Ed.), Enología : Fundamentos Científicos y Tecnológicos / Coord. Por Claude Flanzy. Mundiprensa, Madrid, pp. 137–176.

Belda, I., Benito, S., Ruiz, J., Conchillo, L.B., Alonso, A., Marquina, D., Calderón, F., Navascués, E., Santos, A., 2015. Directed metabolomic approaches for the characterization and development of new yeast strains. BIO Web Conf. 5, 02003. https://doi.org/10.1051/bioconf/20150502003

Bell, S.J., Henschke, P.A., 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11, 242–295.

Belloch, C., Orlic, S., Barrio, E., Querol, A., 2008. Fermentative stress adaptation of hybrids within the *Saccharomyces* sensu stricto complex. Int. J. Food Microbiol. 122, 188–195. https://doi.org/10.1016/j.ijfoodmicro.2007.11.083

Beltrán, G., Esteve-Zarzoso, B., Rozès, N., Mas, A., Guillamón, J.M., 2005. Influence of the timing of nitrogen additions during synthetic grape must fermentations on fermentation kinetics and nitrogen consumption. J. Agric. Food Chem. 53, 996–1002.

Blanchard, L., Tominaga, T., Dubourdieu, D., 2001. Formation of furfurylthiol exhibiting a strong coffee aroma during oak barrel fermentation from furfural released by toasted staves. J. Agric. Food Chem. 49, 4833–4835. https://doi.org/10.1021/jf010539w

Bonada, M., Jeffery, D.W., Petrie, P.R., Moran, M.A., Sadras, V.O., 2015. Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. Aust. J. Grape Wine Res. 21, 240–253. https://doi.org/10.1111/ajgw.12142

Bonnaffoux, H., Roland, A., Rémond, E., Delpech, S., Schneider, R., Cavelier, F., 2017. First identification and quantification of S-3-(hexan-1-ol)-γ-glutamyl-cysteine in grape must as a potential thiol precursor, using UPLC-MS/MS analysis and stable isotope dilution assay. Food Chem. 237, 877–886. https://doi.org/10.1016/j.foodchem.2017.05.116

Bouchilloux, P., Darriet, P., Henry, R., Lavigne-Cruège, V., Dubourdieu, D., 1998. Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. J. Agric. Food Chem. 46, 3095–3099.

https://doi.org/10.1021/jf971027d

Boynton, P.J., Greig, D., 2014. The ecology and evolution of non-domesticated *Saccharomyces* species. Yeast 31, 449–462. https://doi.org/10.1002/yea.3040

Brice, C., Cubillos, F.A., Dequin, S., Camarasa, C., Martínez, C., 2018. Adaptability of the *Saccharomyces cerevisiae* yeasts to wine fermentation conditions relies on their strong ability to consume nitrogen. PLoS One 13, 1–20. https://doi.org/10.1371/journal.pone.0192383

Cadière, A., Ortiz-Julien, A., Camarasa, C., & Dequin, S. (2011). Evolutionary engineered *Saccharomyces cerevisiae* wine yeast strains with increased in vivo flux through the pentose phosphate pathway. Metabolic Engineering, 13(3), 263–271. https://doi.org/10.1016/j.ymben.2011.01.008

Camarasa, C., Grivet, J. P., & Dequin, S. (2003). Investigation by 13C-NMR and tricarboxylic acid (TCA) deletion mutant analysis of pathways of succinate formation in *Saccharomyces cerevisiae* during anaerobic fermentation. Microbiology, 149(9), 2669–2678. https://doi.org/10.1099/mic.0.26007-0

Camarasa, C., Sanchez, I., Brial, P., Bigey, F., Dequin, S., 2011. Phenotypic landscape of *Saccharomyces cerevisiae* during wine fermentation: Evidence for origin-dependent metabolic traits. PLoS One 6, 1-12. https://doi.org/10.1371/journal.pone.0025147

Campo, E., Cacho, J., Ferreira, V., 2006. Multidimensional chromatographic approach applied to the identification of novel aroma compounds in wine. Identification of ethyl cyclohexanoate, ethyl 2-hydroxy-3-methylbutyrate and ethyl 2-hydroxy-4-methylpentanoate. J. Chromatogr. A 1137, 223–230. https://doi.org/10.1016/j.chroma.2006.10.020

Capone, D.L., Sefton, M.A., Jeffery, D.W., 2011. Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. J. Agric. Food Chem. 59, 4649–4658. https://doi.org/10.1021/jf200116q

Carrau, F., Medina, K., Boido, E., Farina, L., Gaggero, C., Dellacassa, E., Versini, G., Henschke, P.A., 2005. De novo synthesis of monoterpenes by *Saccharomyces cerevisiae* wine yeasts. FEMS Microbiol. Lett. 243, 107–115.

Carrau, F.M., Medina, K., Farina, L., Boido, E., Henschke, P.A., Dellacassa, E., 2008. Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: Effects of yeast assimilable nitrogen on two model strains. FEMS Yeast Res. 8, 1196–1207.

Castro Vázquez, L., Pérez-Coello, M.S., Cabezudo, M.D., 2002. Effects of enzyme treatment and skin extraction on varietal volatiles in Spanish wines made from Chardonnay, Muscat, Airén, and Macabeo grapes. Anal. Chim. Acta 458, 39–44.

Cavalieri, D., McGovern, P.E., Hartl, D.L., Mortimer, R., Polsinelli, M., 2003. Evidence for *S. cerevisiae* Fermentation in Ancient Wine. J. Mol. Evol. 57, 226–232. https://doi.org/10.1007/s00239-003-0031-2

Chatonnet, P., Dubourdieu, D., Boidron, J. N., & Lavigne, V., 1993. Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. J. Sci. Food Agric., 62, 191–202.

Cordente, A.G., Schmidt, S., Beltran, G., Torija, M.J., Curtin, C.D., 2019. Harnessing yeast metabolism of aromatic amino acids for fermented beverage bioflavouring and bioproduction. Appl. Microbiol. Biotechnol. 103, 4325–4336. https://doi.org/10.1007/s00253-019-09840-w

Crépin, L., Truong, N.M., Bloem, A., Sanchez, I., Dequin, S., Camarasa, C., 2017. Management of multiple nitrogen sources during wine fermentation by *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 83, 1–21. https://doi.org/10.1128/AEM.02617-16

Culleré, L., López, R., Ferreira, V., 2019. The Instrumental analysis of aroma-active compounds for explaining the flavor of red wines, in: Red Wine Technology. pp. 283–307. https://doi.org/10.1016/b978-0-12-814399-5.00020-7

Coulter, A., Godden, P. W., & Pretorius, I. S. (2004). Succinic Acid, How is it formed, what is its effect on titratable acidity, and what factors influence its concentration in wine? Wine Industry Journal, 19(6), 16–25. https://doi.org/10.1002/9780470995327.ch163

Curran, B. P. G., Bugeja, V. C., 1996. Protoplast fusion in *Saccharomyces cerevisiae*. In: Evans, I. (Ed.), Methods in Molecular Biology:Yeast protocols. Humana Press Inc., Totowa, New Jersey, 45-49.

Darriet, P., Tominaga, T., Lavigne, V., Boidron, J.-N., Dubourdieu, D., 1995. Identification of a powerful aromatic component of *Vitis vinifera* L. var. sauvignon wines: 4-mercapto-4-methylpentan-2-one. Flavour Fragr. J 10, 385–392.

de Barros Lopes M., Bellon J.R., Shirley N.J., Ganter P.F (2002) Evidence for multiple interspecific hybridization in *Saccharomyces* sensu stricto species. FEMS Yeast Res 1:323-331

De-la-Fuente-Blanco, A., Sáenz-Navajas, M.P., Ferreira, V., 2017. Levels of higher alcohols inducing aroma changes and modulating experts' preferences in wine model solutions. Aust. J. Grape Wine Res. 23, 162–169. https://doi.org/10.1111/ajgw.12273

De-La-Fuente-Blanco, A., Sáenz-Navajas, M.P., Ferreira, V., 2016. On the effects of higher alcohols on red wine aroma. Food Chem. 210, 107–114. https://doi.org/10.1016/j.foodchem.2016.04.021

De-la-Fuente-Blanco, A., Sáenz-Navajas, M.P., Valentin, D., Ferreira, V., 2020. Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration. Food Chem. 307, 125553. https://doi.org/10.1016/j.foodchem.2019.125553

Deroite, A., Legras, J.L., Rigou, P., Ortiz-Julien, A., Dequin, S., 2018. Lipids modulate acetic acid and thiol final concentrations in wine during fermentation by *Saccharomyces cerevisiae* × *Saccharomyces kudriavzevii* hybrids. AMB

Express 8. https://doi.org/10.1186/s13568-018-0657-5

Díaz-Maroto, M.C., Schneider, R., Baumes, R., 2005. Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. J. Agric. Food Chem. 53, 3503–3509. https://doi.org/10.1021/jf048157o

Du Plessis, C.S., Augustyn, O.P.H., 1981. Initial Study on the Guava Aroma of Chenin Blanc and Colombar Wines. South African J. Enol. Vitic. 2, 101–103. https://doi.org/10.21548/2-2-2401

Dubourdieu, D., Tominaga, T., Masneuf, I., Des Gachons, C.P., Murat, M.-L., 2006. The role of yeasts in grape flavor development during fermentation: The example of Sauvignon blanc. Am. J. Enol. Vitic. 57, 81–88.

Dzialo, M.C., Park, R., Steensels, J., Lievens, B., Verstrepen, K.J., 2017. Physiology, ecology and industrial applications of aroma formation in yeast. FEMS Microbiol. Rev. 41, S95–S128. https://doi.org/10.1093/femsre/fux031

Ehsani, M., Fernández, M.R., Biosca, J.A., Julien, A., Dequin, S., 2009. Engineering of 2,3-butanediol dehydrogenase to reduce acetoin formation by glycerol-overproducing, low-alcohol *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 75, 3196–3205. https://doi.org/10.1128/AEM.02157-08

Erny, C., Raoult, P., Alais, A., Butterlin, G., Delobel, P., Matei-Radoi, F., Casaregola, S., Legras, J.L., 2012. ecological success of a group of *Saccharomyces cerevisiae/Saccharomyces kudriavzevii* hybrids in the Northern European wine-making environment. Appl. Environ. Microbiol. 78, 3256–3265. https://doi.org/10.1128/AEM.06752-11

Escudero, A., Campo, E., Fariña, L., Cacho, J., Ferreira, V., 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. J. Agric. Food Chem. 55, 4501–4510. https://doi.org/10.1021/jf0636418

Fairbairn, S., McKinnon, A., Musarurwa, H.T., Ferreira, A.C., Bauer, F.F., 2017. The impact of single amino acids on growth and volatile aroma production by *Saccharomyces cerevisiae* strains. Front. Microbiol. 8, 1–12. https://doi.org/10.3389/fmicb.2017.02554

Falcao, L.D., Lytra, G., Darriet, P., Barbe, J.C., 2012. Identification of ethyl 2-hydroxy-4-methylpentanoate in red wines, a compound involved in blackberry aroma. Food Chem. 132, 230–236. https://doi.org/10.1016/j.foodchem.2011.10.061

Fariña, L., Medina, K., Urruty, M., Boido, E., Dellacassa, E., Carrau, F., 2012. Redox effect on volatile compound formation in wine during fermentation by *Saccharomyces cerevisiae*. Food Chem. 134, 933–939. https://doi.org/10.1016/j.foodchem.2012.02.209

Fedrizzi, B., Pardon, K.H., Sefton, M.A., Elsey, G.M., Jeffery, D.W., 2009. First identification of 4-S-glutathionyl-4-methylpentan-2-one, a potential precursor of 4-mercapto-4-methylpentan-2-one, in Sauvignon Blanc juice. J. Agric. Food Chem. 57, 991–995. https://doi.org/10.1021/jf802799w

Fenoll, J., Manso, A., Hellín, P., Ruiz, L., Flores, P., 2009. Changes in the aromatic composition of the *Vitis vinifera* grape Muscat Hamburg during ripening. Food Chem. 114, 420–428. https://doi.org/10.1016/j.foodchem.2008.09.060

Fernández-González, M., Di Stefano, R., 2004. Fractionation of glycoside aroma precursors in neutral grapes. Hydrolysis and conversion by *Saccharomyces cerevisiae*. LWT - Food Sci. Technol. 37, 467–473. https://doi.org/10.1016/j.lwt.2003.11.003

Fernández-González, M., Di Stefano, R., Briones, A., 2003. Hydrolysis and transformation of terpene glycosides from muscat must by different yeast species. Food Microbiol. 20, 35–41. https://doi.org/https://doi.org/10.1016/S0740-0020(02)00105-3

Ferreira, V., De-la-Fuente-Blanco, A., Sáenz-Navajas, M.P., 2021. A New classification of perceptual interactions between odorants to interpret complex aroma systems. Application to model wine aroma. Foods 10, 1–19. https://doi.org/https://doi.org/10.3390/foods10071627

Ferreira, V., López, R., Hernández, P., Cacho, J.F., 1999. Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study. Journal of the Science of Food and Agriculture, 79, 1461, 1999. J. Sci. Food Agric. 1467, 1461–1467.

Ferreira, V., Sáenz-Navajas, M.P., Campo, E., Herrero, P., de la Fuente, A., Fernández-Zurbano, P., 2016. Sensory interactions between six common aroma vectors explain four main red wine aroma nuances. Food Chem. 199, 447–456. https://doi.org/10.1016/j.foodchem.2015.12.048

Fleet, G.H., 2008. Wine yeasts for the future. FEMS Yeast Res. 8, 979–995. https://doi.org/10.1111/j.1567-1364.2008.00427.x

Fleet, G.H., 1998. The microbiology of alcoholic beverages, in: B. J. B. Wood (Ed.), Microbiology of Fermented Foods. pp. 217–262. https://doi.org/10.1007/978-1-4613-0309-1_9

Fleet, G.H., Lafon-Lafourcade, S., Ribereau-Gayon, P., 1984. Evolution of yeasts and lactic acid bacteria during fermentation and storage of Bordeaux wines. Appl. Environ. Microbiol. 48, 1034–1038. https://doi.org/10.1128/aem.48.5.1034-1038.1984

Friedmann, H.C., 1997. From Friedrich Wöhler'S Urine To Eduard Buchner'S Alcohol, in: Cornish-Bowden, A. (Ed.), New Beer in an Old Bottle. Universitat de València, Spain, pp. 67–122.

Fugelsang, K. C., & Edwards, C. G., 2007. Wine Microbiology: Practical Applications and Procedures (2nd Ed.; Springer, ed.). New York: Springer Science + Business Media.

Furdíková, K., MakyŠová, K., Špánik, I., 2017. Effect of indigenous *S. cerevisiae* strains on higher alcohols, volatile acids, and esters in wine. Czech J. Food Sci. 35, 131–142. https://doi.org/10.17221/79/2016-CJFS

Gallone, B., Steensels, J., Prahl, T., Soriaga, L., Saels, V., Herrera-Malaver, B.,

Merlevede, A., Roncoroni, M., Voordeckers, K., Miraglia, L., Teiling, C., Steffy, B., Taylor, M., Schwartz, A., Richardson, T., White, C., Baele, G., Maere, S., Verstrepen, K.J., 2016. Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. Cell 166, 1397-1410.e16. https://doi.org/10.1016/j.cell.2016.08.020

Gambetta, G.A., Herrera, J.C., Dayer, S., Feng, Q., Hochberg, U., Castellarin, S.D., 2020. The physiology of drought stress in grapevine: Towards an integrative definition of drought tolerance. J. Exp. Bot. 71, 4658–4676. https://doi.org/10.1093/jxb/eraa245

Gamero, A., Manzanares, P., Querol, A., Belloch, C., 2011. Monoterpene alcohols release and bioconversion by *Saccharomyces* species and hybrids. Int. J. Food Microbiol. 145, 92–97. https://doi.org/10.1016/j.ijfoodmicro.2010.11.034

Gangl, H., Batusic, M., Tscheik, G., Tiefenbrunner, W., Hack, C., Lopandic, K., 2009. Exceptional fermentation characteristics of natural hybrids from *Saccharomyces cerevisiae* and *S. kudriavzevii*. N. Biotechnol. 25, 244–251. https://doi.org/10.1016/j.nbt.2008.10.001

Gardner, J.M., Walker, M.E., Boss, P.K., Jiranek, V., 2021. The effect of grape juice dilution and complex nutrient addition on oenological fermentation and wine chemical composition. J. Food Compos. Anal. 105, 104241. https://doi.org/10.1016/j.jfca.2021.104241

Gibson, B., Liti, G., 2014. *Saccharomyces pastorianus*: genomic insights inspiring innovation for industry. Yeast 32, 17–27. https://doi.org/10.1002/yea.3033

Goffeau, A., Barrell, G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J.D., Jacq, C., Johnston, M., Louis, E.J., Mewes, H.W., Murakami, Y., Philippsen, P., Tettelin, H., Oliver, S.G., 1996. Life with 6000 genes. Science. 274, 546–567. https://doi.org/10.1126/science.274.5287.546

González, S.S., Barrio, E., Gafner, J., Querol, A., 2006. Natural hybrids from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces kudriavzevii* in wine fermentations. FEMS Yeast Res. 6, 1221–1234. https://doi.org/10.1111/j.1567-1364.2006.00126.x

González, S.S., Gallo, L., Climent, M.D., Barrio, E., Querol, A., 2007. Enological characterization of natural hybrids from *Saccharomyces cerevisiae* and *S. kudriavzevii*. Int. J. Food Microbiol. 116, 11–18. https://doi.org/10.1016/j.ijfoodmicro.2006.10.047

Günata, Z., Bitteur, S., Brillouet, J.-M., Bayonove, C., Cordonnier, R., 1988. Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. Carbohydr. Res. 184, 139–149.

Günata, Z., Razungles, A., Baumes, R., 1999. Aroma compounds in Muscat grapes. J. Int. des Sci. la Vigne du Vin 33, 129–135.

Hazelwood, L.A., Daran, J.M., Van Maris, A.J.A., Pronk, J.T., Dickinson, J.R., 2008. The Ehrlich pathway for fusel alcohol production: A century of research on *Saccharomyces cerevisiae* metabolism. Appl. Environ. Microbiol. 74, 3920.

https://doi.org/10.1128/AEM.00934-08

Hebly, M., Brickwedde, A., Bolat, I., Driessen, M.R.M., Hulster, E.A.F. De, Van, M., 2015. *Saccharomyces cerevisiae* x *Saccharomyces eubayanus* interspecific hybrid , the best of both worlds and beyond 1–46.

Hernández-Orte, P., Bely, M., Cacho, J., Ferreira, V., 2006a. Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different *Saccharomyces cerevisiae* strains during fermentation in controlled synthetic media. Aust. J. Grape Wine Res. 12, 150–160.

Hernández-Orte, P., Ibarz, M.J., Cacho, J., Ferreira, V., 2006b. Addition of amino acids to grape juice of the Merlot variety: Effect on amino acid uptake and aroma generation during alcoholic fermentation. Food Chem. 98, 300–310. https://doi.org/10.1016/j.foodchem.2005.05.073

Hernández-Orte, P., Ibarz, M.J., Cacho, J., Ferreira, V., 2005. Effect of the addition of ammonium and amino acids to musts of Airen variety on aromatic composition and sensory properties of the obtained wine. Food Chem. 89, 163–174.

Hjelmeland, A.K., Ebeler, S.E., 2015. Glycosidically bound volatile aroma compounds in grapes and wine: A review. Am. J. Enol. Vitic. 66, 1–11. https://doi.org/10.5344/ajev.2014.14104

Holland, T., Smit, B., 2010. Climate change and the wine industry: Current research themes and new directions. J. Wine Res. 21, 125–136. https://doi.org/10.1080/09571264.2010.530095

Howell, K.S., Klein, M., Swiegers, J.H., Hayasaka, Y., Elsey, G.M., Fleet, G.H., Høj, P.B., Pretorius, I.S., De Barros Lopes, M.A., 2005. Genetic determinants of volatile-thiol release by *Saccharomyces cerevisiae* during wine fermentation. Appl. Environ. Microbiol. 71, 5420–5426. https://doi.org/10.1128/AEM.71.9.5420-5426.2005

Keller, M., 2010. Developmental Physiology, 1st ed, The Science of Grapevines: Anatomy and Physiology. Elsevier Inc. https://doi.org/10.1016/b978-0-12-374881-2.00006-4

King, A., Dickinson, J.R., 2000. Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Kluyveromyces lactis*. Yeast 16, 499–506.

Lairón-Peris, M., Pérez-Través, L., Muñiz-Calvo, S., Guillamón, J.M., Heras, J.M., Barrio, E., Querol, A., 2020. Differential contribution of the parental genomes to a *S. cerevisiae* × *S. uvarum* hybrid, inferred by phenomic, genomic, and transcriptomic analyses, at different industrial stress conditions. Front. Bioeng. Biotechnol. 8. https://doi.org/10.3389/fbioe.2020.00129

Lambrechts, M.G., Pretorius, I.S., 2000. Yeast and its importance to wine aroma - A Review. South African J. Enol. Vitic. 21, 97–129.

Li, H., Tao, Y.S., Wang, H., Zhang, L., 2008. Impact odorants of Chardonnay dry white wine from Changli County (China). Eur. Food Res. Technol. 227, 287–

292. https://doi.org/10.1007/s00217-007-0722-9

Libkind, D., Hittinger, C.T., Valério, E., Gonçalves, C., Dover, J., Johnston, M., Gonçalves, P., Sampaio, J.P., 2011. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc. Natl. Acad. Sci. U. S. A. 108, 14539–14544. https://doi.org/10.1073/pnas.1105430108

Liu, J., Toldam-Andersen, T.B., Petersen, M.A., Zhang, S., Arneborg, N., Bredie, W.L.P., 2014. Instrumental and sensory characterisation of Solaris white wines in Denmark. Food Chem. 166, 133–142.

Liu, J., Zhu, X.L., Ullah, N., Tao, Y.S., 2017. Aroma glycosides in grapes and wine. J. Food Sci. 82, 248–259. https://doi.org/10.1111/1750-3841.13598

Lopes, C.A., Barrio, E., Querol, A., 2010. Natural hybrids of *S. cerevisiae* × *S. kudriavzevii* share alleles with European wild populations of *Saccharomyces kudriavzevii*. FEMS Yeast Res. 10, 412–421. https://doi.org/10.1111/j.1567-1364.2010.00614.x

López-Malo, M., Querol, A., Guillamon, J.M., 2013. Metabolomic comparison of *Saccharomyces cerevisiae* and the cryotolerant species *S. bayanus* var. *uvarum* and *S. kudriavzevii* during wine fermentation at low temperature. PLoS One 8. https://doi.org/10.1371/journal.pone.0060135

López, R., Ezpeleta, E., Sánchez, I., Cacho, J., Ferreira, V., 2004. Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry. Food Chem. 88, 95–103. https://doi.org/10.1016/j.foodchem.2004.01.025

Lukić, I., Plavša, T., Sladonja, B., Radeka, S., Peršurić, D., 2008. Aroma compounds as markers of wine quality in the case of Malvazija Istarska Young wine. J. Food Qual. 31, 717–735. https://doi.org/10.1111/j.1745-4557.2008.00230.x

Lytra, G., Tempere, S., Revel, G. De, & Barbe, J. (2012). Impact of perceptive interactions on red wine fruity aroma. Journal of Agricultural and Food Chemistry, 60, 12260–12269. https://doi.org/dx.doi.org/10.1021/jf302918q

Marais, J., 1983. Terpenes in the aroma of grapes and wines: a Review. South African J. Enol. Vitic. 4, 49–58.

Masneuf, I., Murat, M.L., Naumov, G.I., Tominaga, T., Dubourdieu, D., 2002. Hybrids *Saccharomyces cerevisiae* x *Saccharomyces bayanus* var. *uvarum* having a high liberating ability of some sulfur varietal aromas of *Vitis vinifera* Sauvignon blanc wines. J. Int. des Sci. la Vigne du Vin 36, 205–212. https://doi.org/10.20870/oeno-one.2002.36.4.965

Mateo-Vivaracho, L., Zapata, J., Cacho, J., Ferreira, V., 2010. Analysis, occurrence, and potential sensory significance of five polyfunctional mercaptans in white wines. J. Agric. Food Chem. 58, 10184–10194. https://doi.org/10.1021/jf101095a

Mateo, J.J., Jimenez, M., 2000. Monoterpenes in grape juice and wines, Review. J. Chromatogr. 881, 557–567.

Mercado, L., Dalcero, A., Masuelli, R., Combina, M., 2007. Diversity of *Saccharomyces* strains on grapes and winery surfaces: Analysis of their contribution to fermentative flora of Malbec wine from Mendoza (Argentina) during two consecutive years. Food Microbiol. 24, 403–412. https://doi.org/10.1016/j.fm.2006.06.005

Mérillon, J. M., & Ramawat, K. G. (2017). Fungal Metabolites (J. M. Mérillon & K. G. Ramawat, eds.). https://doi.org/10.1007/978-3-319-25001-4

Minebois, R., Pérez-Torrado, R., Querol, A., 2020. A time course metabolism comparison among *Saccharomyces cerevisiae*, *S. uvarum* and *S. kudriavzevii* species in wine fermentation. Food Microbiol. 90, 1–14. https://doi.org/10.1016/j.fm.2020.103484

Mira de Orduña, R., 2010. Climate change associated effects on grape and wine quality and production. Food Res. Int. 43, 1844–1855. https://doi.org/10.1016/j.foodres.2010.05.001

Moon, H. J., Jeya, M., Kim, I. W., & Lee, J. K. (2010). Biotechnological production of erythritol and its applications. Applied Microbiology and Biotechnology, 86(4), 1017–1025. https://doi.org/10.1007/s00253-010-2496-4

Molina, A.M., Guadalupe, V., Varela, C., Swiegers, J.H., Pretorius, I.S., Agosin, E., 2009. Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. Food Chem. 117, 189–195. https://doi.org/10.1016/j.foodchem.2009.03.116

Molina, A.M., Swiegers, J.H., Varela, C., Pretorius, I.S., Agosin, E., 2007. Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. Appl. Microbiol. Biotechnol. 77, 675–687. https://doi.org/10.1007/s00253-007-1194-3

Morata, A., Escott, C., Loira, I., Del Fresno, J.M., Vaquero, C., Bañuelos, M.A., Palomero, F., López, C., González, C., 2021. pH control and aroma improvement using the Non-*Saccharomyces Lachancea thermotolerans* and *Hanseniaspora* spp. Yeasts to improve wine freshness in warm areas, in: Grapes and Wine. IntechOpen, pp. 1–14. https://doi.org/http://dx.doi.org/10.5772/intechopen.100538

Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K., 2007. Loss of anthocyanins in red-wine grape under high temperature. J. Exp. Bot. 58, 1935–1945. https://doi.org/10.1093/jxb/erm055

Murat, M.L., Tominaga, T., Dubourdieu, D., 2001. Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. J. Agric. Food Chem. 49, 5412–5417. https://doi.org/10.1021/jf0103119

Naumov, G.I., Naumova, E.S., Martynenko, N.N., Masneuf-Pomaréde, I., 2011. Taxonomy, ecology, and genetics of the yeast *Saccharomyces bayanus*: A new object for science and practice. Microbiology 80, 735–742.

https://doi.org/10.1134/S0026261711060154

Oliveira, I., 2019. Contribution of wine microorganisms to the aroma composition of wine and its sensory impact.

Olivera, N.L., Libkind, D., Donati, E., 2016. Biology and biotechnology of patagonian microorganisms, Biology and Biotechnology of Patagonian Microorganisms. https://doi.org/10.1007/978-3-319-42801-7

Origone, A.C., del Mónaco, S.M., Ávila, J.R., González Flores, M., Rodríguez, M.E., Lopes, C.A., 2017. Tolerance to winemaking stress conditions of Patagonian strains of *Saccharomyces eubayanus* and *Saccharomyces uvarum.* J. Appl. Microbiol. 123, 450–463. https://doi.org/10.1111/jam.13495

Ortiz-Tovar, G., Minebois, R., Barrio, E., Querol, A., Pérez-Torrado, R., 2019. Aroma production and fermentation performance of *S. cerevisiae* × *S. kudriavzevii* natural hybrids under cold oenological conditions. Int. J. Food Microbiol. 297, 51–59. https://doi.org/10.1016/j.ijfoodmicro.2019.03.005

Pardo, E., Rico, J., Gil, J.V., Orejas, M., 2015. *De novo* production of six key grape aroma monoterpenes by a geraniol synthase - engineered *S . cerevisiae* wine strain. Microb. Cell Fact. 1–8. https://doi.org/10.1186/s12934-015-0306-5

Park, S., Morrison, J., 1991. Distribution of free and glycosidically bound monoterpenes in the skin and mesocarp of Muscat of Alexandria grapes during development. J. Agric. Food Chem. 39, 514–518.

Peinado, R.A., Moreno, J., Bueno, J.E., Moreno, J.A., Mauricio, J.C., 2004. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. Food Chem. 84, 585–590. https://doi.org/10.1016/S0308-8146(03)00282-6

Pérez-Través, L., Lopes, C.A., Barrio, E., Querol, A., 2012. Evaluation of different genetic procedures for the generation of artificial hybrids in *Saccharomyces* genus for winemaking. Int. J. Food Microbiol. 156, 102–111. https://doi.org/10.1016/j.ijfoodmicro.2012.03.008

Pérez-Través, L., Lopes, C.A., González, R., Barrio, E., Querol, A., 2015. Physiological and genomic characterisation of *Saccharomyces cerevisiae* hybrids with improved fermentation performance and mannoprotein release capacity. Int. J. Food Microbiol. 205, 30–40. https://doi.org/10.1016/j.ijfoodmicro.2015.04.004

Peŕez-Través, L., Lopes, C.A., Querol, A., Barrio, E., 2014. On the complexity of the *Saccharomyces bayanus* taxon: Hybridization and potential hybrid speciation. PLoS One 9. https://doi.org/10.1371/journal.pone.0093729

Pérez, D., Assof, M., Bolcato, E., Sari, S., Fanzone, M., 2018. Combined effect of temperature and ammonium addition on fermentation profile and volatile aroma composition of Torrontés Riojano wines. LWT - Food Sci. Technol. 87, 488–497. https://doi.org/10.1016/j.lwt.2017.09.020

Peris, D., Pérez-Torrado, R., Hittinger, C. T., Barrio, E., & Querol, A. (2018). On the origins and industrial applications of *Saccharomyces cerevisiae* x

Saccharomyces kudriavzevii hybrids. Yeast, 35(1), 51–69. https://doi.org/10.1002/yea.3283

Peris, D., Lopes, C. A., Belloch, C., Querol, A., & Barrio, E. (2012). Comparative genomics among *Saccharomyces cerevisiae* × *Saccharomyces kudriavzevii* natural hybrid strains isolated from wine and beer reveals different origins. BMC Genomics, 13(407), 1–14. https://doi.org/10.1186/1471-2164-13-407

Peris, D., Pérez-Través, L., Belloch, C., Querol, A., 2016. Enological characterization of Spanish *Saccharomyces kudriavzevii* strains, one of the closest relatives to parental strains of winemaking and brewing *S. cerevisiae* × *S. kudriavzevii* hybrids. Food Microbiol. 53, 31–40. https://doi.org/10.1016/j.fm.2015.07.010

Peris, D., Sylvester, K., Libkind, D., Gonçalves, P., Sampaio, J.P., Alexander, W.G., Hittinger, C.T., 2014. Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. Mol. Ecol. 23, 2031–2045. https://doi.org/10.1111/mec.12702

Peyrot des Gachons, C., Tominaga, T., Dubourdieu, D., 2002. Sulfur aroma precursor present in S-glutathione conjugate form: Identification of S-3-(Hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon blanc. J. Agric. Food Chem. 50, 4076–4079. https://doi.org/10.1021/jf020002y

Peyrot des Gachons, C., Tominaga, T., Dubourdieu, D., 2000. Measuring the aromatic potential of *Vitis vinifera L*. cv. Sauvignon Blanc grapes by assaying S-Cysteine conjugates, precursors of the volatile thiols responsible for their varietal aroma 3387–3391.

Pineau, B., Barbe, J.C., Leeuwen, C. Van, Dubourdieu, D., 2009. Examples of perceptive interactions involved in specific "Red-" and "Black-berry" aromas in red wines. J. Agric. Food Chem. 57, 3702–3708. https://doi.org/10.1021/jf803325v

Pineau, B., Barbe, J.C., Van Leeuwen, C., Dubourdieu, D., 2007. Which impact for β-damascenone on red wines aroma? J. Agric. Food Chem. 55, 4103–4108. https://doi.org/10.1021/jf070120r

Pinu, F.R., 2018. Grape and wine metabolomics to develop new insights using untargeted and targeted approaches. Fermentation 4. https://doi.org/10.3390/fermentation4040092

Pons, A., Allamy, L., Schüttler, A., Rauhut, D., Thibon, C., Darriet, P., 2017. What is the expected impact of climate change on wine aroma compounds and their precursors in grape? Oeno One 51, 141–146. https://doi.org/10.20870/oeno-one.2016.0.0.1868

Puertas, B., Jimenez-Hierro, M.J., Cantos-Villar, E., Marrufo-Curtido, A., Carbú, M., Cuevas, F.J., Moreno-Rojas, J.M., González-Rodríguez, V.E., Cantoral, J.M., Ruiz-Moreno, M.J., 2018. The influence of yeast on chemical composition and sensory properties of dry white wines. Food Chem. 253, 227–235. https://doi.org/10.1016/j.foodchem.2018.01.039

Querol, A., Pérez-Torrado, R., Alonso-del-Real, J., Minebois, R., Stribny, J., Oliveira,

B.M., Barrio, E., 2018. New trends in the uses of yeasts in oenology, in: Advances in Food and Nutrition Research. Elsevier Inc., Amsterdem, pp. 177–210. https://doi.org/10.1016/bs.afnr.2018.03.002

Ramos, M.C., Martínez-Casasnovas, J.A., 2010. Effects of precipitation patterns and temperature trends on soil water available for vineyards in a Mediterranean climate area. Agric. Water Manag. 97, 1495–1505. https://doi.org/10.1016/j.agwat.2010.05.003

Rapp, A., 1995. Possibilities of characterizing wine varieties by means of volatile flavor compounds. Dev. Food Sci. 37, 1703–1722. https://doi.org/https://doi.org/10.1016/S0167-4501(06)80259-1.

Replansky, T., Koufopanou, V., Greig, D., Bell, G., 2008. *Saccharomyces* sensu stricto as a model system for evolution and ecology. Trends Ecol. Evol. 23, 494–501. https://doi.org/10.1016/j.tree.2008.05.005

Ribéreau-Gayon, Pascal, Dubourdieu, D., Doneche, B., Lonvaud, A., 2006a. Handbook of Enology, Vol. 1:The Microbiology of Wine and Vinifications., 2nd Ed. ed, The Microbiology of Wine and Vinifications. John Wiley and Sons Ltd, Chichester, UK.

Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., 2006b. Handbook of Enology, Vol. 2: The chemistry of wine stabilization and treatments., 2nd Ed. ed, The Chemistry of Wine Stabilization and Treatments. John Wiley and Sons Ltd, Chichester, UK.

Robinson, A.L., Boss, P.K., Solomon, P.S., Trengove, R.D., Heymann, H., Ebeler, S.E., 2014. Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. Am. J. Enol. Vitic. 65, 1–24.

Rodríguez, M.E., Origone, A.C., González Flores, M., Lopes, C.A., 2016. *Saccharomyces* in traditional and industrial fermentations from Patagonia, in: Olivera, N.L., Libkind, D., Donati, E. (Eds.), Biology and Biotechnology of Patagonian Microorganisms. Switzerland, pp. 1–360. https://doi.org/10.1007/978-3-319-42801-7

Rodríguez, M.E., Pérez-Través, L., Sangorrín, M.P., Barrio, E., Lopes, C.A., 2014. *Saccharomyces eubayanus* and *Saccharomyces uvarum* associated with the fermentation of *Araucaria araucana* seeds in Patagonia. FEMS Yeast Res. 14, 948–965. https://doi.org/10.1111/1567-1364.12183

Roland, A., Schneider, R., Razungles, A., Cavelier, F., 2011. Varietal thiols in wine: Discovery, analysis and applications. Chem. Rev. 111, 7355–7376. https://doi.org/10.1021/cr100205b

Rollero, S., Bloem, A., Ortiz-Julien, A., Camarasa, C., Divol, B., 2018. Fermentation performances and aroma production of non-conventional wine yeasts are influenced by nitrogen preferences. FEMS Yeast Res. 18, 1–11. https://doi.org/10.1093/femsyr/foy055

Rollero, S., Mouret, J.R., Bloem, A., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.M., Dequin, S., Camarasa, C., 2017. Quantitative 13C-isotope labelling-based

analysis to elucidate the influence of environmental parameters on the production of fermentative aromas during wine fermentation. Microb. Biotechnol. 10, 1649–1662. https://doi.org/10.1111/1751-7915.12749

Romero-Guido, C., Belo, I., Ta, T.M.N., Cao-Hoang, L., Alchihab, M., Gomes, N., Thonart, P., Teixeira, J.A., Destain, J., Waché, Y., 2011. Biochemistry of lactone formation in yeast and fungi and its utilisation for the production of flavour and fragrance compounds. Appl. Microbiol. Biotechnol. 89, 535–547. https://doi.org/10.1007/s00253-010-2945-0

Ruiz, J., Kiene, F., Belda, I., Fracassetti, D., Marquina, D., Navascués, E., Calderón, F., Benito, A., Rauhut, D., Santos, A., Benito, S., 2019. Effects on varietal aromas during wine making: a review of the impact of varietal aromas on the flavor of wine. Appl. Microbiol. Biotechnol. 103, 7425–7450. https://doi.org/10.1007/s00253-019-10008-9

Rusjan, D., Strli, M., Komerl, T., Prosen, H., 2009. The response of monoterpenes to different enzyme preparations in Gewürztraminer (*Vitis vinifera* L.) Wines. S. Afr. J. Enol. Vitic. 30, 56–64.

Saerens, S.M.G., Delvaux, F.R., Verstrepen, K.J., Thevelein, J.M., 2010. Production and biological function of volatile esters in *Saccharomyces cerevisiae*. Microb. Biotechnol. 3, 165–177. https://doi.org 10.1111/j.1751-7915.2009.00106.x

Salvadó, Z., Arroyo-López, F.N., Guillamón, J.M., Salazar, G., Querol, A., Barrio, E., 2011. Temperature adaptation markedly determines evolution within the genus *Saccharomyces*. Appl. Environ. Microbiol. 77, 2292–2302. https:// doi.org/10.1128/AEM.01861-10

Sampaio, J.P., Gonçalves, P., 2008. Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with Oak bark and are sympatric with *S. cerevisiae* and *S. paradoxus*. Appl. Environ. Microbiol. 74, 2144–2152. https://doi.org/10.1128/AEM.02396-07

San-Juan, F., Cacho, J., Ferreira, V., Escudero, A., 2012. Aroma chemical composition of red wines from different price categories and its relationship to quality. J. Agric. Food Chem. 60, 5045–5056. https://doi.org/10.1021/jf2050685

San-Juan, F., Ferreira, V., Cacho, J., Escudero, A., 2011. Quality and aromatic sensory descriptors (mainly fresh and dry fruit character) of spanish red wines can be predicted from their aroma-active chemical composition. J. Agric. Food Chem. 59, 7916–7924. https://doi.org/10.1021/jf1048657

Sánchez-Palomo, E., Díaz-Maroto, M.C., Viñas, M.A.G., Soriano-Pérez, A., Pérez-Coello, M.S., 2007. Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. Food Control 18, 398–403. https://doi.org/10.1016/j.foodcont.2005.11.006

Sánchez-Palomo, E., Pérez-Coello, M.S., Díaz-Maroto, M.C., González Viñas, M.A., Cabezudo, M.D., 2006. Contribution of free and glycosidically-bound volatile compounds to the aroma of muscat "a petit grains" wines and effect of skin contact. Food Chem. 95, 279–289.

Schelezki, O.J., Antalick, G., Šuklje, K., Jeffery, D.W., 2020. Pre-fermentation approaches to producing lower alcohol wines from Cabernet Sauvignon and Shiraz: Implications for wine quality based on chemical and sensory analysis. Food Chem. 309. https://doi.org/10.1016/j.foodchem.2019.125698

Schelezki, O.J., Šuklje, K., Boss, P.K., Jeffery, D.W., 2018. Comparison of consecutive harvests versus blending treatments to produce lower alcohol wines from Cabernet Sauvignon grapes: Impact on wine volatile composition and sensory properties. Food Chem. 259, 196–206. https://doi.org/10.1016/j.foodchem.2018.03.118

Schillberg, S., Zimmermann, M., Emeis, C.C., 1991. Analysis of hybrids obtained by rare-mating of *Saccharomyces* strains. Appl. Microbiol. Biotechnol. 35, 242–246. https://doi.org/10.1007/BF00184695

Schneider, R., Charrier, F., Razungles, A., Baumes, R., 2006. Evidence for an alternative biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one in wines. Anal. Chim. Acta 563, 58–64. https://doi.org/10.1016/j.aca.2006.01.057

Schüttler, A., 2013. Influencing factors on aromatic typicality of wines from *Vitis vinifera* L. cv. Riesling.

Schüttler, A., Friedel, M., Jung, R., Rauhut, D., Darriet, P., 2015. Characterizing aromatic typicality of Riesling wines: merging volatile compositional and sensory aspects. Food Res. Int. 69, 26–37.

Schulze, U., Lidén, G., Nielsen, J., & Villadsen, J. (1996). Physiological effects of nitrogen starvation in an anaerobic batch culture of *Saccharomyces cerevisiae*. Microbiology, 142(8), 2299–2310. https://doi.org/10.1099/13500872-142-8-2299

Seguinot, P., Bloem, A., Brial, P., Meudec, E., Ortiz-Julien, A., Camarasa, C., 2020. Analysing the impact of the nature of the nitrogen source on the formation of volatile compounds to unravel the aroma metabolism of two non-*Saccharomyces* strains. Int. J. Food Microbiol. 316, 1–12. https://doi.org/10.1016/j.ijfoodmicro.2019.108441

Silva, T., Albertin, W., Dillmann, C., Bely, M., Vienne, D. De, Marullo, P., 2015. Hybridization within *Saccharomyces* genus results in homoeostasis and phenotypic novelty in winemaking conditions. PLoS ONE. 6, 1–24. https://doi.org/10.1371/journal.pone.0123834

Spencer J.F.T., Spencer D.M., 1996. Rare-Mating and Cytoduction in *Saccharomyces cerevisiae*. In: Evans I (ed) Methods in Molecular Biology:Yeast protocols, 53 Humana Press Inc., Totowa, New Jersey, 39-44

Steyer, D., Erny, C., Claudel, P., Riveill, G., Karst, F., Legras, J.L., 2013. Genetic analysis of geraniol metabolism during fermentation. Food Microbiol. 33, 228–234. https://doi.org/10.1016/j.fm.2012.09.021

Stribny, J., Gamero, A., Pérez-Torrado, R., Querol, A., 2015. *Saccharomyces kudriavzevii* and *Saccharomyces uvarum* differ from *Saccharomyces cerevisiae*

during the production of aroma-active higher alcohols and acetate esters using their amino acidic precursors. Int. J. Food Microbiol. 205, 41–46. https://doi.org/10.1016/j.ijfoodmicro.2015.04.003

Su, Y., Gamero, A., Rodríguez, M.E., Lopes, C.A., Querol, A., Guillamón, J.M., 2019a. Interspecific hybridisation among diverse *Saccharomyces* species: A combined biotechnological solution for low-temperature and nitrogen-limited wine fermentations. Int. J. Food Microbiol. 310, 108331. https://doi.org/10.1016/j.ijfoodmicro.2019.108331

Su, Y., Origone, A.C., Rodríguez, M.E., Querol, A., Guillamón, J.M., Lopes, C.A., 2019b. Fermentative behaviour and competition capacity of cryotolerant *Saccharomyces* species in different nitrogen conditions. Int. J. Food Microbiol. 291, 111–120. https://doi.org/10.1016/j.ijfoodmicro.2018.11.020

Su, Y., Seguinot, P., Bloem, A., Ortiz-Julien, A., Heras, J.M., Guillamón, J.M., Camarasa, C., 2020. Isotopic tracers unveil distinct fates for nitrogen sources during wine fermentation with two non-*Saccharomyces* strains. Microorganisms 8, 1–19. https://doi.org/10.3390/microorganisms8060904

Subileau, M., Schneider, R., Salmon, J.M., Degryse, E., 2008. Nitrogen catabolite repression modulates the production of aromatic thiols characteristic of Sauvignon Blanc at the level of precursor transport. FEMS Yeast Res. 8, 771–780. https://doi.org/10.1111/j.1567-1364.2008.00400.x

Swiegers, J.H., Willmott, R., Hill-Ling, A., Capone, D.L., Pardon, K.H., Elsey, G.M., Howell, K.S., de Barros Lopes, M.A., Sefton, M.A., Lilly, M., Pretorius, I.S., 2006. Modulation of volatile thiol and ester aromas by modified wine yeast. Dev. Food Sci. 43, 113–116. https://doi.org/10.1016/S0167-4501(06)80027-0

Takoi, K., Itoga, Y., Takayanagi, J., Kosugi, T., Shioi, T., Nakamura, T., Watari, J., 2014. Screening of geraniol-rich flavor hop and interesting behavior of β-citronellol during fermentation under various hop-addition timings. J. Am. Soc. Brew. Chem. 72, 22–29. https://doi.org/10.1094/ASBCJ-2014-0116-01

Thibon, C., Marullo, P., Claisse, O., Cullin, C., Dubourdieu, D., Tominaga, T., 2008. Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation. FEMS Yeast Res. 8, 1076–1086. https://doi.org/10.1111/j.1567-1364.2008.00381.x

Tomasino, E., Bolman, S., 2021. The potential effect of β-ionone and β-damascenone on sensory perception of Pinot noir wine aroma. Molecules 26. https://doi.org/10.3390/molecules26051288

Tominaga, T., Blanchard, L., Darriet, P., Dubourdieu, D., 2000. A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. J. Agric. Food Chem. 48, 1799–1802. https://doi.org/10.1021/jf990660r

Tominaga, T., Furrer, A., Henry, R., & Dubourdieu, D. (1998a). Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. Flavour and Fragrance Journal, 13(3), 159–162. https://doi.org/10.1002/(SICI)1099-1026(199805/06)13:3<159::AID-

FFJ709>3.0.CO;2-7

Tominaga, T., Peyrot des Gachons, C., & Dubourdieu, D. (1998b). A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon Blanc: S-cysteine conjugates. Journal of Agricultural and Food Chemistry, 46(12), 5215–5219.

Tominaga, T., Masneuf, I., Dubourdieu, D., 1995. Mise en évidence d'un s-conjugué de la cystéine, précurseur d'arôme du Sauvignon. J. Int. des Sci. la Vigne du Vin 29, 227–232.

Torija, M.J., 2002. Ecología de levaduras: selección y adaptación a fermentaciones vínicas. Universidad Rovira i Virgili, Tarragona, España.

Torija, M.J., Beltrán, G., Novo, M., Poblet, M., Guillamón, J.M., Mas, A., Rozès, N., 2003a. Effects of fermentation temperature and *Saccharomyces* species on the cell fatty acid composition and presence of volatile compounds in wine. Int. J. Food Microbiol. 85, 127–136.

Torija, M.J., Beltrán, G., Novo, M., Poblet, M., Rozès, N., Guillamón, J.M., Mas, A., 2003b. Effect of the nitrogen source on the fatty acid composition of *Saccharomyces cerevisiae*. Food Microbiol. 20, 255–258.

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Leigh Francis, I., Henschke, P.A., 2011. Comparison of inorganic and organic nitrogen supplementation of grape juice - Effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae* yeast. Food Chem. 127, 1072–1083. https://doi.org/10.1016/j.foodchem.2011.01.092

Torriani, S., Zapparoli, G., Suzzi, G., 1999. Genetic and phenotypic diversity of *Saccharomyces* sensu stricto strains isolated from Amarone wine Diversity of *Saccharomyces* strains from Amarone wine. Antonie Van Leeuwenhoek 75, 207–215.

Tronchoni, J., Gamero, A., Arroyo-López, F.N., Barrio, E., Querol, A., 2009. Differences in the glucose and fructose consumption profiles in diverse *Saccharomyces* wine species and their hybrids during grape juice fermentation. Int. J. Food Microbiol. 134, 237–243. https://doi.org/10.1016/j.ijfoodmicro.2009.07.004

Ugliano, M., Bartowsky, E.J., McCarthy, J., Moio, L., Henschke, P.A., 2006. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. J. Agric. Food Chem. 54, 6322–6331. https://doi.org/10.1021/jf0607718

Ugliano, M., Moio, L., 2008. Free and hydrolytically released volatile compounds of *Vitis vinifera* L. cv. Fiano grapes as odour-active constituents of Fiano wine. Anal. Chim. Acta 621, 79–85. https://doi.org/10.1016/j.aca.2008.03.002

Valero, E., Millán, C., Ortega, J.M., Mauricio, J.C., 2003. Concentration of amino acids in wine after the end of fermentation by *Saccharomyces cerevisiae* strains. J. Sci. Food Agric. 83, 830–835.

Van Khle, A.D.A., Jesperen, L., Glover, R.L.K., Diawara, B., Jakobsen, M., 2001. Identification and characterization of *Saccharomyces cerevisiae* strains isolated

from West African sorghum beer. Yeast 18, 1069–1079. https://doi.org/10.1002/yea.756

Varela, C., Kutyna, D.R., Francis, I.L., Curtin, C., 2015. Strategies for reducing alcohol concentration in wine. https://doi.org/10.1111/ajgw.12187

Varela, C., Sengler, F., Solomon, M., Curtin, C., 2016. Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. Food Chem. 209, 57–64. https://doi.org/10.1016/j.foodchem.2016.04.024

Varela, C., Torrea, D., Schmidt, S.A., Ancin-Azpilicueta, C., Henschke, P.A., 2012. Effect of oxygen and lipid supplementation on the volatile composition of chemically defined medium and Chardonnay wine fermented with *Saccharomyces cerevisiae*. Food Chem. 135, 2863–2871.

Vaudano, E., Moruno, E.G., Di Stefano, R., 2004. Modulation of geraniol metabolism during alcohol fermentation. J. Inst. Brew. 110, 213–219. https://doi.org/10.1002/j.2050-0416.2004.tb00205.x

Venios, X., Korkas, E., Nisiotou, A., & Banilas, G. (2020). Grapevine responses to heat stress and global warming. Plants, 9(12), 1–15. https://doi.org/10.3390/plants9121754

Verstrepen, K.J., Derdelinckx, G., Dufour, J.P., Winderickx, J., Thevelein, J.M., Pretorius, I.S., Delvaux, F.R., 2003. Flavor-active esters: Adding fruitiness to beer. J. Biosci. Bioeng. 96, 110–118.

Vontrobová, E., Kubizniaková, P., & Fiala, J. (2019). Autochthonous yeasts as one of the tools to produce wines by original technologies. Kvasny Prumysl, 65, 38–45. https://doi.org/10.18832/kp2019.65.38

Waldmann, D., Winterhalter, P., 1992. Identification of a novel vitispirane precursor in Riesling wine. Vitis 31, 169–174.

Wamhoff, H., Gribble, G.W., 2012. Wine and Heterocycles, Advances in Heterocyclic Chemistry. https://doi.org/10.1016/B978-0-12-396531-8.00003-1

Wang, Y.Q., Ye, D.Q., Liu, P.T., Duan, L.L., Duan, C.Q., Yan, G.L., 2016. Synergistic effects of branched-chain amino acids and phenylalanine addition on major volatile compounds in wine during alcoholic fermentation. South African J. Enol. Vitic. 37, 169–175. https://doi.org/10.21548/37-2-683

Waterhouse, A.L., Sacks, G.L., Jeffery, D.W., 2016. Understanding Wine Chemistry. John Wiley & Sons. https://doi.org/10.1002/9781118730720

Winterhalter, P., Ebeler, S.E., 2013. Carotenoid cleavage products: An introduction, in: Society, A.C. (Ed.), Carotenoid Cleavage Products. Washington, DC, pp. 3–9. https://doi.org/10.1021/bk-2013-1134.ch001

Winterhalter, P., Gök, R., 2013. TDN and β-Damascenone: two important carotenoid metabolites in wine, in: Society, A.C. (Ed.), Carotenoid Cleavage Products. Washington, DC, pp. 125–137. https://doi.org/10.1021/bk-2013-1134.ch011

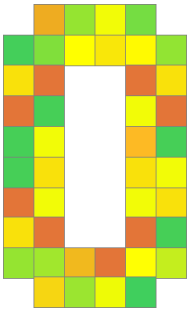
Winterhalter, P., Schreier, P., 1994. C_{13}-Norisoprenoid glycosides in plant tissues: An

overview on their occurrence, composition and role as flavour precursors. Flavour Fragr. J. 9, 281–287.

Zebec, Z., Wilkes, J., Jervis, A.J., Scrutton, N.S., Takano, E., Breitling, R., 2016. Towards synthesis of monoterpenes and derivatives using synthetic biology. Curr. Opin. Chem. Biol. 34, 37–43. https://doi.org/10.1016/j.cbpa.2016.06.002

Zott, K., Thibon, C., Bely, M., Lonvaud-Funel, A., Dubourdieu, D., Masneuf-Pomarede, I., 2011. The grape must non-*Saccharomyces* microbial community: Impact on volatile thiol release. Int. J. Food Microbiol. 151, 210–215. https://doi.org/10.1016/j.ijfoodmicro.2011.08.026

Objectives



This doctoral thesis is part of the Marie Skłodowska-Curie Innovative Training Network program, namely Aromagenesis project (nº 764364), funded by the European Commission, Horizon 2020. This grand project generated a network between 10 beneficiary groups composed of companies and research institutions that exchanged technological experiences and academic knowledge on two of the most important industries in Europe, wine, and beer, which have yeast as a common worker. The focus of this project was on the production of aroma compounds by yeasts, so the main objectives were: to examine the biochemistry and genetics of aroma compound production in yeasts used in wine and beer fermentation, generate new yeast strains with improved or more varied flavor profiles, and to develop novel approaches to extend flavor profiles by co-fermentation of different yeasts.

Especially, this thesis focused on the screening of yeasts not previously used in the wine industry that show interesting flavor profiles to generate hybrid strain having improved flavor profiles for wine industry applications. These objectives were assigned to the company Lallemand Bio S.L., the leader in developing, producing, and marketing yeasts, bacteria, and products for the wine, among other industries. The network for this Ph.D. included the Institute of Agrochemistry and Food Technology (IATA- CSIC) in Valencia and Zaragoza University. More precisely, the work was performed within the research group headed by Prof. Amparo Querol, experts in yeast biotechnology, with great knowledge on non-conventional yeasts (especially on *S. uvarum*, *S. kudriavzevii*, and *S. eubayanus* species) as well as hybrid yeast generation. Regarding the determination and analysis of aromas, through a secondment, we have the great contribution of the LAE laboratory headed by Prof. Vicente Ferreira, in Zaragoza.

Several studies have determined that yeasts from different genera and species can provide interesting sensory and organoleptic characteristics to the wines. Within the group of *Saccharomyces*, the so-called non-conventional

yeasts included the wild *S. cerevisiae*, i.e., not isolated from wine fermentation, and the cryotolerant species. However, two important aspects must consider: these yeasts are poorly studied and adapted to the winemaking environment compared to the wine *S. cerevisiae* yeast.

On the first aspect, numerous studies have contributed to the knowledge of these yeasts, allowing them to be characterized phenologically and metabolically under winemaking conditions. Their nutritional needs, production of volatile and non-volatile compounds, preferred metabolic pathways, competitive capacities, and tolerances have been investigated. However, there is great biodiversity among them, and aspects remain to be studied, such as performance towards flavor-inducing amino acids, high impact aromas production, and bottle aging influence on their wines.

Therefore, this thesis started from a large collection of yeasts by evaluating their capacities to ferment and produce metabolites using musts with aroma-inducing amino acids. Then, with a more selected group of yeasts, we evaluated the ability to produce or release impact aromas from grape extracts of one aromatic (Albariño) and one non-aromatic (Tempranillo) grape variety, including a solution of thiol precursors.

Regarding the second aspect, several biotechnological strategies have been developed to solve this yeast's intolerance or poor competitiveness, which is the case of induced hybrid yeast generation. At this point is where the other fundamental component of this thesis appears, generating hybrid yeasts that achieve the aromatic profiles of non-conventional yeasts and being adapted for industrial winemaking application, i.e., non-GMO yeasts with a background of wine yeast.

Therefore, the objectives to be achieved in this thesis in more detail are as follows:

1- Identification and selection of non-conventional yeast strains for their fermentation capacity and volatile and non-volatile metabolites under different nitrogen sources. Objective addressed in **Chapter 1**.

   a. Evaluate the fermentation capability of a set of non-wine *Saccharomyces* strains through the fermentation on complete grape nitrogen must and in 5 musts with a single nitrogen source: Valine, Leucine, Isoleucine, Phenylalanine, and Ammonium.

   b. Evaluate the volatiles (fermentative aromas) and non-volatiles (ethanol, polyols, and organic acids) compounds produced by non-wine *Saccharomyces* strains under these different nitrogen sources.

   c. Determine the individual effect of the branched and aromatic nitrogen sources on wine aroma and metabolites production for future development of yeast nitrogen supplements.

   d. Select the best strains of each species for the next objective.

2- Study of aroma and chemical compounds produced in young and bottle-aged wines fermented by non-wine yeasts on semi-synthetic must containing Albariño and Tempranillo extracts. Objective addressed in **Chapters 2 and 3**.

   a. Olfactometric identification and detection of impact aromas released during fermentation in Tempranillo semi-synthetic musts by non-wine yeasts

b. Analysis of major and minor impact aromas produced from Albariño and Tempranillo varietal precursors by non-wine yeasts.

c. Evaluation of the aromas released and degraded by bottle aging and the interaction with non-wine yeasts.

d. Characterize cryotolerant species according to the production of metabolites and flavors of interest from Albariño and Tempranillo varietal precursors.

e. Tentatively determine aroma synthesis pathways.

f. Select strains with the best flavor profiles of each species for the next objective.

3- Generate hybrid yeasts using a non-GMO strategy having enhanced flavor profiles. Objective addressed in **Chapter 4.**

a. Generate intra- and interspecific hybrid yeast between a wine strain and the best non-wine yeasts.

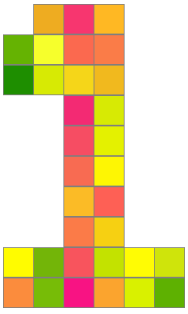
b. Evaluate the fermentation capability of the hybrid yeast generated and compare with their parental wine strain using Tempranillo and Albariño semi-synthetic musts.

c. Compare the flavor profiles of the *Saccharomyces* hybrid yeasts with those of their parental wine strain in Tempranillo and Albariño semi-synthetic wines.

d. Tentatively determine aroma synthesis pathways.

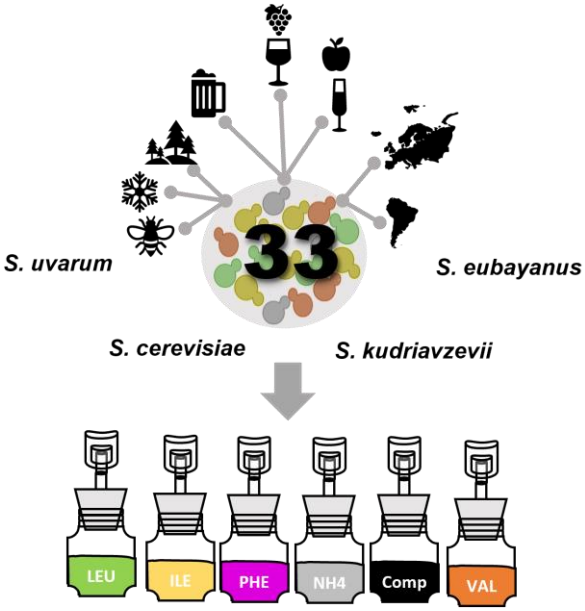
e. Choose hybrid yeast for pilot-scale fermentations.

Chapter 1



Screening of *Saccharomyces* strains for the capacity to produce desirable fermentative compounds under the influence of different nitrogen sources in synthetic wine fermentations

Dolores Pérez; Inés Jaehde; José Manuel Guillamón, José María Heras and Amparo Querol



Published in Food Micribiology (2021)

DOI10.1016/j.fm.2021.103763

Abstract

A collection of 33 *Saccharomyces* yeasts were used for wine fermentation with a sole nitrogen source: ammonium and four individual aroma-inducing amino acids. The fermentation performance and chemical wine composition were evaluated. The most valuable nitrogen sources were valine as a fermentation promoter on non-*cerevisiae* strains, phenylalanine as fruity aromas enhancer whereas the ethanol yield was lessened by leucine and isoleucine. *S. cerevisiae* SC03 and *S. kudriavzevii* SK02 strains showed to be the greatest producers of fruity ethyl esters while *S. kudriavzevii* strains SK06 and SK07 by shortening the fermentation duration. *S. uvarum* strains produced the greatest succinic acid amounts and, together with *S. eubayanus*, they reached the highest production of 2-phenylethanol and its acetate ester, whereas *S. kudriavzevii* strains were found to be positively related to high glycerol production.

1. Introduction

Nowadays, wine consumers are looking for new sensory profiles, and market trends are guided by higher aromatic intensity, fruit forward, freshness, and lower alcohol content (Querol et al., 2018). The higher alcohol level is concerning for wine drinkers through its negative impact on health (Lindberg & Amsterdam, 2008). On the other hand, associated with climate change events, the grapes have higher levels of sugar, fewer organic acids, and alterations in aroma and phenolic synthesis (Drappier et al., 2019). Therefore, winemakers have to come up with enological strategies that mitigate the high potential alcoholic grade or other factors affecting wine quality.

During winemaking, alcoholic fermentation is a complex process where the principal reaction is the transformation of grape hexoses into ethanol and carbon dioxide by yeasts (Fleet, 1998). *Saccharomyces cerevisiae* is the most frequently used species in wine industry; it is well adapted to must and fermentative conditions like high sugar concentration, high ethanol content, and low levels of pH, besides they produce rapid and predictable fermentations (Rodríguez et al., 2016; Querol et al., 2018). However, other species of the genus *Saccharomyces* such as *S. kudriavzevii*, *S. uvarum,* and *S. eubayanus* are also able to ferment grape must. These yeast species are particularly known for being cryotolerant, causing high glycerol and low ethanol concentrations, and having low nitrogen requirements (Tronchoni et al., 2012; Su et al., 2019; Minebois et al., 2020). Moreover, they can produce higher amounts of fermentative aromatic compounds like higher alcohols and acetate esters (Gamero et al., 2014; Stribny et al., 2015). These characteristics can influence the composition of the final wine contributing to the development of new styles that satisfy the consumer preferences, such as a high intensity of fruity aromas, lower alcohol content, and improvement of the mouthfeel.

An essential step during winemaking is the management of yeast nutrition with nitrogen supplements. Yeasts, under anaerobic conditions, consume nitrogen in the forms of ammonium cations, α-amino acids, and some small peptides which all are described by the term YAN, yeast assimilable nitrogen (Ugliano et al.,2007; Waterhouse et al., 2016). The amount and proportion of YAN in must depends on many factors such as cultivar, the sanitary state and maturity of grapes, as well as the cultural practices carried out in the vineyard (Nicolini et al., 2004; Bell & Henschke, 2005; Swiegers et al., 2005; Carrau et al., 2008). Yeasts use the nitrogen sources for multiple purposes, mainly for biomass production, fermentation activity, and also, there is evidence to influence the production of volatile and non-volatile wine compounds (Fleet, 1998; Fairbairn et al., 2017). Some of the volatile compounds produced are higher alcohols and their acetate esters that can provide fruity and floral notes to wine (Lambrechts& Pretorius, 2000). Recent studies have established that in some *S. cerevisiae* and non-*Saccharomyces* strains only a small proportion of higher alcohols and their acetate esters, such as isoamyl acetate and isobutanol, are derived from the catabolism of amino acids, like from leucine and valine (Su et al., 2020; Crépin et al., 2017, Rollero et at., 2017).

Besides, each yeast strain, within species and genus, can have specific nutritional requirements, either on the amount needed as on the preferred nitrogen source used (Fairbairn et al., 2017; Suet al., 2019). These differences in the nitrogen requirements can have an impact on the aromatic profile of the resulting wine (Rollero et al., 2018; Seguinot et al., 2020). Some studies revealed that *Saccharomyces cerevisiae* strains produced different wine flavour profiles, which differ in their capacity to produce esters, higher alcohols, and volatile fatty acids from aromatic and branched-chain amino acids (Cordente et al., 2019).

Since non-conventional yeasts have been described as potential wine strains that can fulfil the current requirements on the wine industry (Querol et

al., 2018), it was of great interest to continue expanding the actual knowledge in a greater variety of strains about their enological traits and their nutritional metabolism. For this reason, the objective of this research is to study several wild yeast strains of the species *S. cerevisiae*, *S. uvarum*, *S. kudriavzevii*, and *S. eubayanus* using synthetic musts containing a single nitrogen source and analysing their fermentative behaviour and the resulting volatile and non-volatile metabolic end-products.

2. Materials and Methods

2.1 Yeast strains and inocula making

The yeast strains used for conducting the alcoholic fermentations are listed in **Table 1**. They belong to the species *S. cerevisiae, S. uvarum, S. kudriavzevii,* and *S. eubayanus,* and were isolated from natural habitats and spontaneous fermentations. As reference wine yeast, we used the commercial *S. cerevisiae* Lalvin EC1118®strain (Lallemand Inc, Montreal, Canada).

Each strain was grown on complete GPY (2% glucose, 0.5% yeast extract, and 0.5% peptone) agar plates at 30 ºC for 48 h and stored at 4 ºC as reference stocks. A day before starting the fermentation, an amount of yeast cells from the stocks was transferred and incubated at 25 ºC on 5 mL of GPY medium overnight. The pre-cultures were centrifuged and suspended in 3.5 mL of sterile water. Immediately thereafter, each must was inoculated with an initial population of $2x10^6$ cells/mL (OD_{600nm}approx. 0.2).

Table 1. *Saccharomyces cerevisiae*, *Saccharomyces eubayanus*, *Saccharomyces kudriavzevii* and *Saccharomyces uvarum* strains used in this study

Species	Code used	Strain name	Source of isolation	Geographic origin
S. cerevisiae	**EC1118**	EC1118	Wine, Commercial (Lallemand)	France
	SC01	B2-1	Wasp	Peru
	SC02	CBS 1591	Fermenting cacao	Indonesia
	SC03	CBS 8857	Sorghum beer	Burkina Faso
	SC04	Chr. 96.2	Oak (*Q. Faginea*)	Spain
	SC05	CSC 1	Cachaça	Brazil
	SC06	G1	Beer	Belgium
	SC07	PE 15 M	Agave	Peru
	SC08	PE 93 M	Masato	Peru
	SC09	Temohaya-MI 26	Agave	Mexico
	SC10	UFMG CAY 1007	Cachaça	Brazil
S. eubayanus	**SE01**	LGMUSC210	Oak (*N. pumili)*	Chile
	SE02	LGMUSC215	Oak (*N. pumili)*	Chile
	SE03	NPCC1282	Tree seeds (*A. araucana*)	Argentina
	SE04	NPCC1283	Tree seeds (*A. araucana*)	Argentina
	SE05	NPCC1286	Tree seeds (*A. araucana*)	Argentina
	SE06	NPCC1287	Tree seeds (*A. araucana*)	Argentina
	SE07	NPCC1292	Tree bark (*A. araucana*)	Argentina
	SE08	NPCC1296	Tree bark (*A. araucana*)	Argentina
	SE09	NPCC1301	Tree bark (*A. araucana*)	Argentina
S. kudriavzevii	**SK01**	CA111F1	Monosporic derivative from oak (*Q. Faginea)* isolate	Spain
	SK02	CBS12751	Soil	Taiwan
	SK03	CBS12752	Soil	Taiwan
	SK04	CR85	Oak (*Q. Faginea*)	Spain
	SK05	CR89	Oak (*Q. Faginea*)	Spain
	SK06	CR89D1	Monosporic derivative from oak (*Q. Faginea)* isolate	Spain
	SK07	CR90F4	Monosporic derivative from oak (*Q. Faginea)* isolate	Spain
S. uvarum	**BMV58**	BMV58	Wine, Commercial (Lallemand)	Spain
	SU02	CBS7001	Insect	Spain
	SU03	CECT12600	Sweet wine	Spain
	SU04	NPCC1290	Tree seeds (*A. araucana*)	Argentina
	SU06	NPCC1314	Apple Chicha	Chile
	SU07	S10	Cider	Ireland

NPCC, North Patagonian Culture Collection, Neuquén, Argentina. LGMUSC, Molecular Genetic Laboratory of Santiago de Chile University.

2.2 Synthetic grape must composition

The fermentations were performed with synthetic musts based on the recipe of Rossignol et al., (2003), with slight modifications: 220 g/L of reducing sugars (110 g/L glucose +110 g/L fructose); 3 g/L L-tartaric acid; 0.5 g/L citric acid; 5 g/L L-malic acid; 0.75 g/L K_2HPO_4; 0.25 g/L $MgSO_4.7H_2O$; 4 mg/L $MnSO_4$; 4 mg/L $ZnSO_4.7H_2O$; 1 mg/L $CuSO_4.5H_2O$; 1 mg/L KI; 0.4 mg/L $CoCl_2.6H_2O$; 1 mg/L H_3BO_3; 0.5 g/L K_2SO_4; 0.155 g/L $CaCl_2$. $2H_2O$; 0.2 g/L NaCl; 1 mg/L $Na_2MoO_4.2H_2O$; 20mg/L myo-inositol; 0.003 mg/L biotin; 0.25 mg/L thiamin hydrochloride; 25 mg/L pyridoxine hydrochloride; 2 mg/L nicotinic acid; 1.5 mg/L calcium pantothenate; 1 mg/L *p*-aminobenzoic acid; 15 mg/L ergosterol; 0.05% Tween 80 in ethanol (v/v). Specific nitrogen sources were added individually at a concentration of 140 mg N/L acquiring five synthetic musts with the following single nitrogen sources: NH_4Cl 0.535 g/L (NH_4), L-Isoleucine 1.31 g/L (Ile), L-Leucine 1.31 g/L (Leu), L- Phenylalanine 1.65 g/L (Phe), L- Valine 1.17 g/L (Val). As a control, a synthetic must that mimics an average nitrogen grape must (Comp) was also prepared at the same nitrogen concentration (140 mg N/L): NH_4Cl 0.2147 g/L, and a mix of the follow amino acids: Tyrosine 9.15 mg/L; Tryptophan 81.74 mg/L; Isoleucine 15.25 mg/L; Aspartic acid 20.74 mg/L; Glutamic acid 56.12 mg/L; Arginine 172.63 mg/L; Leucine 22.57 mg/L; Threonine 35.39 mg/L; Glycine 8.54 mg/L; Glutamine 234.24 mg/L; Alanine 68.32 mg/L; Valine 20.74 mg/L; Methionine 14.64 mg/L; Phenylalanine 17.69 mg/L; Serine 36.6 mg/L; Histidine 15.86 mg/L; Lysine 7.93 mg/L; Cysteine 9.15 mg/L and Proline 281.21 mg/L. The pH was adjusted to 3.3 with NaOH (1M), and then must solution was filtered for sterilisation (0.2 µm).

2.3. Microfermentations conditions

Following the standardized fermentation method proposed by Peltier et al., (2018), fermentations were carried out in 10-mL screw-top vials

(Thermo Scientific, Langerwehe, Germany) containing 7.5 mL of must. They were closed with 18mm magnetic screw caps and seals out of silicone/PTFE septum (Thermo scientific, Langerwehe Germany). The septum was pierced by a needle (27G x ½"-0.4x13mm; BD Microlance 3; Becton, Dickinson and Company Limited, Louth, Ireland), which allowed CO_2 release. Each combination of yeast and nitrogen source was performed in triplicate.

The fermentations were maintained at 20°C and shaken at 170 rpm by an orbital shaker (Shaker DOS-20L, ELMI, Calabasas, California, USA). The progress of the fermentation was monitored by their daily weight loss through CO_2 release. The process was considered as finished, when the daily weight loss was less than 0.005 g or when 700 hours of fermentation had passed.

The fermentation curves were adjusted with the mathematical model of a non-linear regression by Gompertz, like it was proposed by Zwietering et al., (1990) for bacterial growth curves, with the following expression:

$$y = A*\exp\{-\exp[((\mu_{max}*e/A)*(\lambda - t)) + 1]\}$$

The parameters λ, $\mu_{max,}$ and A of the resulting growth equation can be interpreted biologically as the lag phase (λ), the maximum specific growth rate (μ_m) and the maximal asymptotic y-value (A), at which the specific growth rate reaches zero. In this study, biological parameters were reassigned to kinetic parameters of fermentation curves being λ, the time when fermentation starts vigorously; Vmax, the maximum specific fermentation rate, and A; the maximal lost weight. Also, times at which there was 10% and 75% weight loss of the maximal lost weight (T10% and T75%) were calculated to have a better description of the fermentation.

2.4. Determinations of organic acids, sugars, and alcohols by HPLC

The amounts of sugars, acids, and alcohols of the final wines were determined by HPLC (Thermo Fisher Scientific, Waltham, MA, USA) with a

HyperREZTM XP Carbohydrate H+ 8µm column (Thermo Fisher Scientific) equipped with a HyperRETZM XP Carbohydrate Guard (Thermo Fisher Scientific). The sugars and alcohols were detected by a refraction index detector and the acids by a UV detector. The samples were filtered through a 0.22 µm nylon filter and diluted according to their estimated residual sugar amount. The analysis conditions were: 1.5 mM of H_2SO_4. 0.6 mL/min flux, a pressure of 35 bars, and an oven temperature of 50°C. The concentrations of these compounds, in g/L, were determined by using the calibration curves of the corresponding standard compounds.

2.5. Determination of volatile and fermentative compounds by HS-SPME-GC/FID

The higher alcohols, acetate and ethyl esters produced during the fermentation were extracted and analysed in the final wines by the HS-SPME-GC/FID (headspace solid-phase microextraction gas chromatography) method adapted from Stribny et al., (2015). A Thermo Science TRACE GC Ultra gas chromatograph equipped with SPME autosampler (Thermo Fisher Scientific, Waltham, MA, USA) with a flame ionization detector (FID) was used and the separation of the compounds was carried out on a 30m x 0.25mm x 0.25 µm HP-INNOWax capillary column, which is coated with a layer of cross-linked polyethylene glycol (Agilent, Santa Clara, USA).

The samples were prepared by centrifugation (3150 *x g*,10 minutes) to remove the yeast cells and 1.5 mL of the supernatant was put into a 20 mL screw-top vial containing 0.35 g NaCl, 750 µl of MiliQ water, 20 µl of the internal standard (2-Heptanone 0.005% w/v). It was closed by a magnetic screw cap with an 18 mm silicone/PTFE septum (Supelco, Bellefonte, PA, USA). The sample was shaken and heated at 35 ºC by a stirrer for 30 minutes, so that there could be created a balance between the volatile compounds in the liquid and the gas phase. Then the septum was pierced by a needle and a 100-µm poly-dimethylsiloxane (PDMS) fused silica fiber (Supelco, Sigma-Aldrich,

Spain) was introduced through the septum and exposed to the headspace for 15 minutes with further shaking. The fiber was then injected into the GC inlet port, which held a temperature of 220 °C, with a helium flow of 1 mL/min and stayed exposed for four minutes (splitless mode). The temperature program for the run was 5 min at 35 °C, 2 °C/min to 150 °C, 20 °C/min to 250 °C and 2 min at 250 °C and the detector temperature was 300°C.

Calibration curves were obtained by using pure standards solutions at three different concentrations to define the retention times and to calculate the amount (mg/L) of the volatile compounds. The high polarity of the column used did not allow a separation of active amyl alcohol (2-methyl-1-butanol) and isoamyl alcohol (3-methyl-1-butanol) compounds, so both were calibrated from the isoamyl alcohol areas and named "active amyl/isoamyl alcohol".

The instrument reproducibility and the method repeatability were tested by injecting a reference wine five times in three different working days. The values obtained were on average below 20% for all the compounds.

2.6. Statistical Analysis

The results of every parameter and analyte were expressed as the arithmetic means of three repetitions with their corresponding standard deviation. The results of HPLC and GC were standardized to the yield (g of compound produced/100g of sugar consumed), as not all fermentations finished. A factorial design experiment was used to analyse the influence of nitrogen sources (“N”; six nitrogen sources) and yeast strain used (“Y”; 33 yeast strains) on every analyte, aroma compound, and oenological parameter.Therefore, an application of two-way ANOVA with Tukey test for the individual and interaction factor effects was applied anda non-parametric Kruskal-Wallis test were used for multiple comparisons between treatment for each parameter considering significance when *p*-value was below 0.05. Genesis software 1.7.7 (Graz University of Technology, Austria) was used to

acquire the heat map, the hierarchical clustering using Euclidean distance and the average linkage as agglomeration rule. Principal component analysis (PCA), as a multivariate methodology, was applied to obtain a visualisation in a reduced dimension of data and to determine which variable contributes the most to the differences observed. All statistical analyses and plots were obtained by the use of Infostat software, version 2011 (Grupo Infostat, Córdoba, Argentina), and GraphPad Prism version 8.0 (Graph-Pad Software, Inc., La Jolla, CA).

3. Results

Microfermentations were performed using six different musts, each containing a specific nitrogen source (Phe, Ile, Leu, Val, NH_4, Comp), and 33 different yeast strains of four *Saccharomyces* species (Table 1) to explore their influence on the fermentation kinetics, organic acid and alcohol production, as well as aroma production.

3.1. Fermentation performances

The alcoholic fermentation curves were assessed by the daily measurement of weight loss due to the release of CO_2 during the fermentation and adjusted by the mathematical non-linear regression of Gompertz model. The lag phase was represented by the estimation of the time needed to reach 10% of the weight loss. The results of every fermentation parameter were expressed as the arithmetic mean of the repetitions (n=3) with its corresponding standard deviation (Table S1). In the next sections, we will describe the effect of the different nitrogen sources used, as well as the species/strains effects on the fermentative parameters: the lag phase, the time to reach 75% of the fermentation duration (T75%), and the maximum fermentation rate (Vmax).

The heat map (Figure 1A) represented the normalized values of the lag phase, Vmax, and T75% by the mean of the values of all strains. The clustering applied in this graph shows the distribution of the yeast strains in groups regarding the nutritional individual effect on their fermentation performance. In most of the strains, shorter lag phases were obtained using NH_4 and complete medium (dark green), and maximum values were obtained using isoleucine (dark pink) with few exceptions. Contrary to the expected, Comp and NH_4 mediums did not improve the fermentation performance, instead, a great part of the strains had their highest velocity and the shortest T75% in valine musts. Isoleucine and phenylalanine musts led to slower fermentation rates and longer times to finish the process, with the exception of *S. kudriavzevii* strains and SC06, where using leucine must the lag phase was longer than in must containing only isoleucine.

The strains were separated into five main clusters by the application of the hierarchical clustering (Figure 1A). Two fermentative strains SC07 and SC10 were separated (Group I) from the rest due to the slow fermentation rates (Lag phase and T75%) with valine. Group II included the majority of *S. eubayanus* strains, two *S. kudriavzevii*, and two *S. cerevisiae* strains. This cluster was characterized by strains that had greater maximum fermentation rates with valine and the longest times with isoleucine. Most of the strains of this cluster were isolated from trees and soil, with the exceptions of SC02 from fermentative cocoa, and SC06 from beer. The third group included the wine strain EC1118, five strains from fermentative environments (four *S. cerevisiae* and one *S. uvarum*), and two from non-fermentative sources. They reached the highest fermentation rates in complete must and the shortest time to finish the fermentation (T75%) in this condition. Finally, the last two groups, showed different fermentation profiles, including most of the *S. uvarum* and *S. kudriavzevii* strains. Both groups presented a high fermentation rate with valine followed by isoleucine and the longer T75% times with phenylalanine.

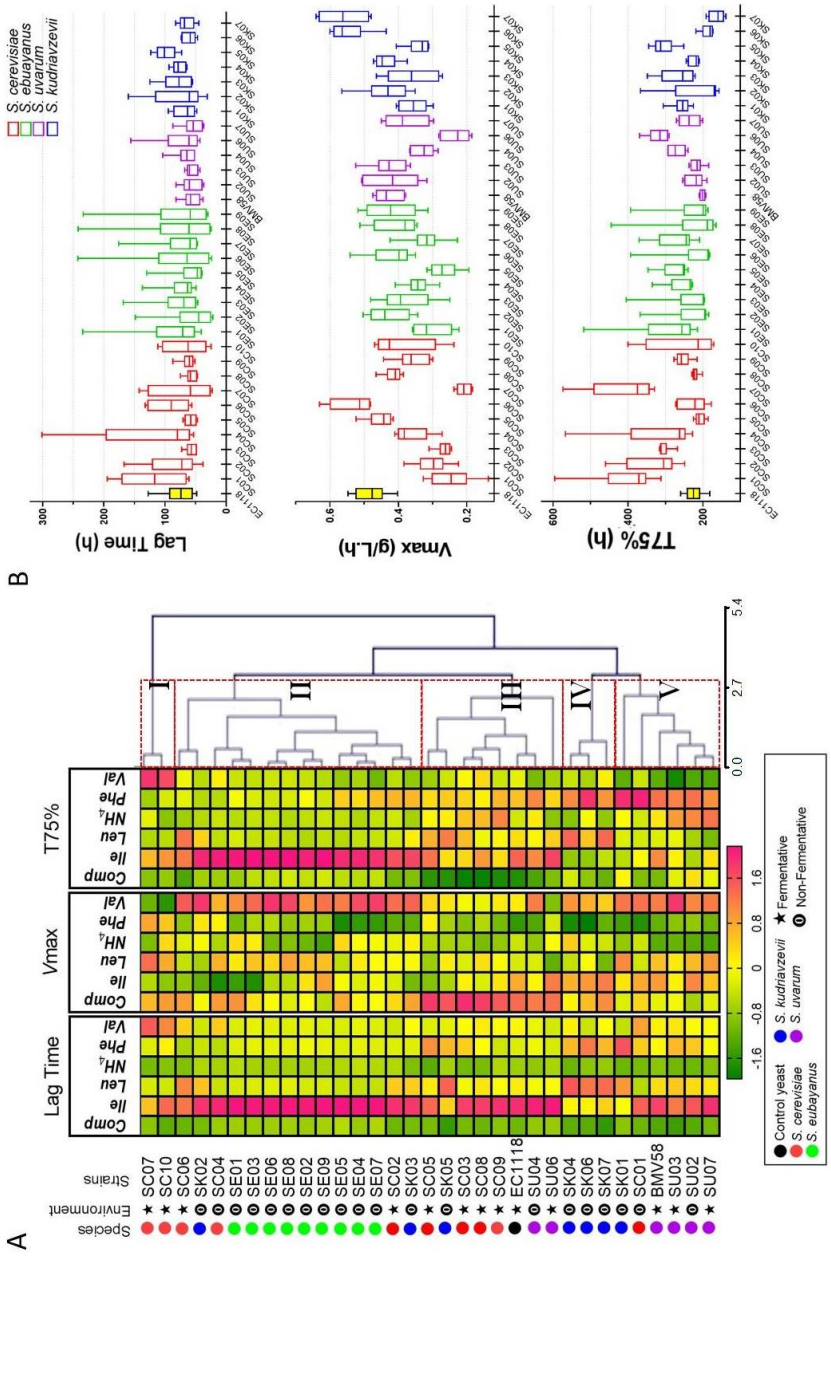


Figure 1. (A) Heat map of fermentation parameters: lag phase (time to reach the 10% of fermentation), Vmax (maximal fermentation rate) and T75% (time to reach the 75% of the fermentation) in which each row indicates the different nitrogen musts: Comp (complete); Ile (isoleucine); Leu (leucine); NH_4 (ammonium); Phe (phenylalanine) and Val (valine). The color key bar indicated the normalized value of each parameter by the average value of all yeast strains: low values, green and high values, pink. The hierarchical clustering of the yeast (on the right) was calculated based on Euclidean distance and the average linkage: the yeast was separated in five main clusters represented by red squares. **(B)** Box plots indicate for each fermentation parameter the distribution of all the nitrogen sources values for each strain, showing median, upper and lower quartile, vertical bars showing the minimum and maximum value respectively.

The differences between group IV and V were that the three *S. kudriavzevii* strains from group IV produced longer lag phases and T75% when they fermented with leucine. Figure 1B represents the results obtained when yeast strain was the analyzed factor and each box represents one strain. In terms of maximum fermentation rates, EC1118 together with two *S. kudriavzevii* strains isolated from nature (SK07 and SK06), and two *S. cerevisiae* strains (SC06 and SC05), isolated from beer and cachaça fermentation, showed remarkably higher speed than the rest of the strains in all the musts. It is also notable that no yeast strain displayed a shorter lag phase than the reference yeast. Moreover, four *S. cerevisiae* strains SC03, SC05, SC08, and SC09 showed similar lag phases with all the nitrogen sources. On the other hand, *S. eubayanus* presented a long lag phase and T75%, as an effect of isoleucine, as was mentioned above (Figure 1A, Table S1).

The wine strain EC1118 showed the best fermentation parameters in all the conditions, followed by SK06, SK07 and SC06 strain, reaching the highest Vmax values and the shortest fermentation times. The strain that showed the poorest fermentative traits was the SC07 mainly by the lower maximum fermentation rate, and longer time to finish the process.

We can conclude that the lag phase is affected more by the nitrogen source than by the yeast used, as this effect was more pronounced in *S. eubayanus* (Figure S1). However, the Vmax and fermentation time (measure by T75%) were more strain-dependent except in the case of *S. eubayanus*, which was more nitrogen source dependent.

3.2. Major fermentation metabolites

Concentrations, expressed as g/100 g of consumed sugars, of ethanol, erythritol, glycerol, 2,3-butanediol, acetic acid, and succinic acid generated by yeast during sugar metabolism are shown in Supplementary Material (Tables S2 to S7). The application of two-way ANOVA on these metabolite

concentrations (Figure 2A) revealed a significant effect of each factor, yeast (Y, 33 yeast strains) and nitrogen media (N, six musts with different nitrogen composition), as well as the interactions between them (Y x N). We observed a high influence of yeast factor (Y) and a very low effect of the nitrogen media for the synthesis of these compounds. The production of 2,3-butanediol (81%) and glycerol (79%) were most related to the yeast factor.

To visualize the individual effect of the factors, the values were normalized by the average of the total concentration yielded by the strains (Figure 2B) and by the nitrogen media (Figure 2C). The significant variations ($p< 0.05$) between each strain and the wine strain EC1118 (black bar), were indicated by a red asterisk in Figure 2B. EC1118 strain showed the highest ethanol yield and the lowest amounts of polyols and succinic acid whereas half of the tested strains produced significantly higher concentrations (Figure 2B). All the *S. kudriavzevii* and *S. eubayanus* species (blue and green bars) reached the highest values of glycerol and 2,3-butanediol and were statistically different from the industrial yeast (black bar). Interestingly, the strain SK02 showed the highest concentration of glycerol (6.36 ± 1.01 g/100g) and the lowest concentration of erythritol (0.07±0.05 g/100g) and ethanol (39.92 ± 3.55 g/100g). *S. uvarum* strains, mainly the wine yeast BMV58, produced the highest amount of succinic acid (4.26±2.34 g/100g), 26-fold higher than the control wine yeast (0.16±0.04 g/100g).

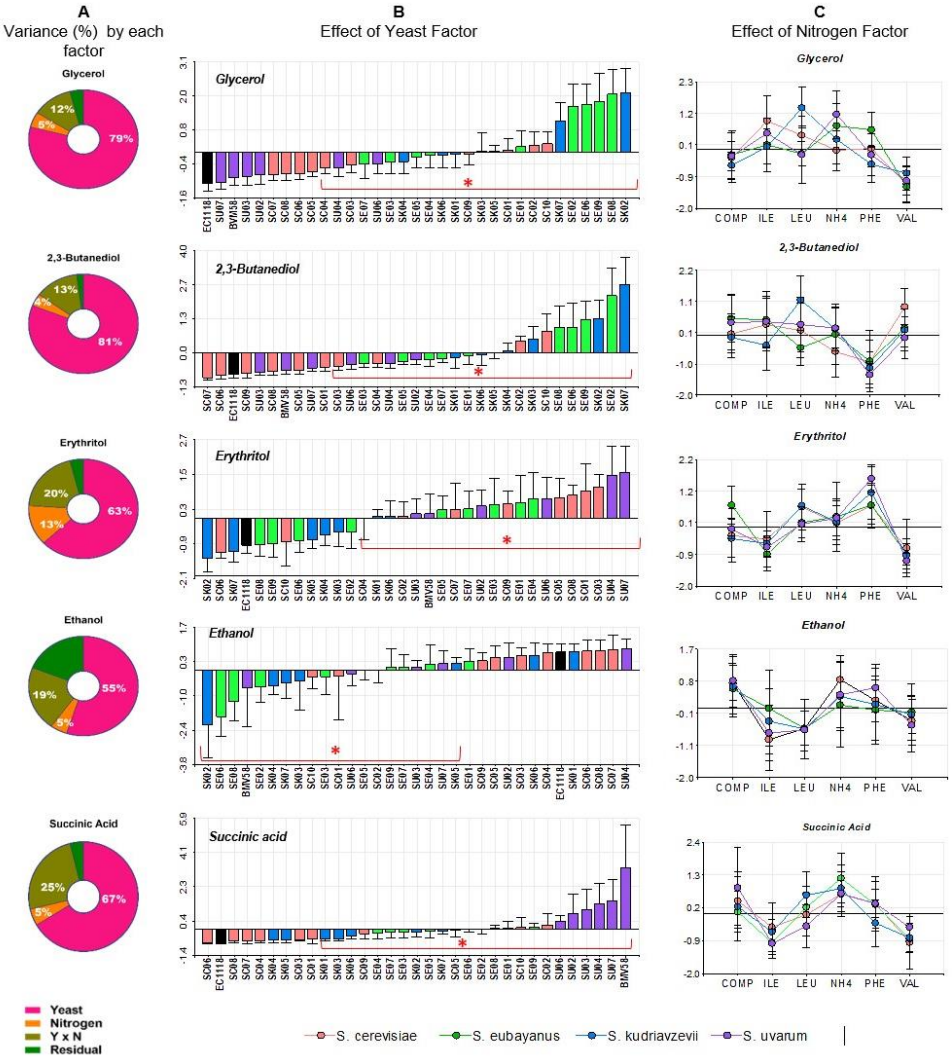


Figure 2. (A) Two-way ANOVA for the oenological traits: glycerol, 2,3-butanediol, erythritol, ethanol, and succinic acid was applied, and data are presented as the percent of variance explained by each factor: Yeast strains (Y), Nitrogen musts (N), the interaction effects (Y x N) and the residuals. **(B)** Bars indicate normalized values by the average concentration produced by strain for each metabolite (bar; n = 18; error bars = SD). Kruskal-Wallis test and pair-wise comparison at a significance level of 95% were performed respect to the control yeast strain Lalvin EC1118® (Black bar), Groups indexed with a red asterisk (*) indicate that the yeast strains differed significantly from the control ($p<0.05$). **(C)** The concentration of the different metabolites was normalised by the average concentration measured in each must per species. Each dots represent the mean and the error lines, SD for each species in each nitrogen media connecting by lines: *S. cerevisiae*, n=33; *S. uvarum*, n=18; *S. eubayanus*, n=27; *S. kudriavzevii* n=21.

Nitrogen sources' effect on metabolites is presented as the normalized values (mean ± SD) by the average concentration reached for all strains, then grouped by species (Figure 2C). The minimum values for glycerol, erythritol, and succinic acid were obtained with valine and for 2,3-butanediol with phenylalanine. However, phenylalanine maximized the erythritol synthesis in all the species but was more pronounced in *S. uvarum* (Table S5). Isoleucine also decreased the synthesis of erythritol and succinic acid concentration in all the species but was more significant in *S. uvarum* strains (Table S6). Ethanol yield, in all species, seemed to be reduced with isoleucine and leucine and was most remarkable in *S. cerevisiae* (decrease of 3 - 4 % respect the complete media). The use of the complete medium or ammonium resulted in higher ethanol levels in most of the synthetic wines.

In summary, the wine strain EC1118 used as a control, produced low levels of polyols and organic acid comparing with the other species and even comparing with the rest of *S. cerevisiae* strains analyzed. *S. kudriavzevii* and *S. eubayanus* were the species with the highest glycerol and 2,3-butanediol levels and the lower yield of ethanol. Most of the *S. uvarum* strains showed a huge production of succinic acid and surprisingly the lowest concentrations in glycerol. *S. cerevisiae* strains were characterized by the low production of 2,3-butanediol and the high levels of ethanol. The lowest percentages of variance were mainly attributable to individual nitrogen factor (less than 13%) due to the low influence in the modulation of these alcohols and acids; nevertheless, valine led to a decrease in all the parameters (except for 2,3-butanediol), isoleucine and leucine reduced the ethanol concentration, and phenylalanine favored the erythritol production. Acetic acid values have not been analyzed due to the values were high in all the fermentations (**Table S7**), which could be attributed to the small fermentation volume and the use of synthetic media (Beltrán et al., 2008).

3.3. Fermentative aromatic compounds

The fermentative aromas on wine are mainly higher alcohols, their acetate esters, and medium-chain fatty acids (MCFA) as well as their corresponding ethyl esters. Three MCFA ethyl esters, three higher alcohols, and one acetate ester were identified and quantified in this work (Table S8-S11) by the GC-FID methodology. These compounds are synthesized by yeast from sugar and amino acid metabolism, and it is expected that their variability could be linked to the yeast strain used or to the nitrogen composition of the must. The variance attributed to the yeast factor (33 yeast strains), or nitrogen (six nitrogen media) are shown in Table S12.The concentrations of ethyl esters were more affected by the yeast factor while higher alcohols and 2-phenylethyl acetate production were extremely dependent on the nitrogen source. The individual effects of the factors showed to be statistically significant (p-value<0.05), however, as the p-value associated with the interaction was highly significant too, the factors studied are not entirely independently.

3.3.1. MCFA ethyl esters compounds

The capability of each strain to produce these three fruity aroma compounds is shown in Figure 3A, which represents the values obtained for each strain normalized by the mean concentration reached for all the strains. Figure 3B represents the normalized concentration in each nitrogen sources by the total mean value produced.

The normalized values showed that EC1118 and SE01 reached average concentrations of these compounds (Figure 3A). SC03 and SK02 strains produced the highest amounts of MCFA ethyl esters with respect EC1118 (p<0.05), while the lowest producers were SC05, SE02, and SU07. SK07 strain produced the highest ethyl decanoate concentration and the lowest concentrations of the other ethyl esters. The production of these compounds

was not species-dependent; for example, in the cases of *S. uvarum*, SU06 was the only one of its species that was particularly noted by the high synthesis of these compounds whereas SU03 and SU07 reached the lowest concentrations.

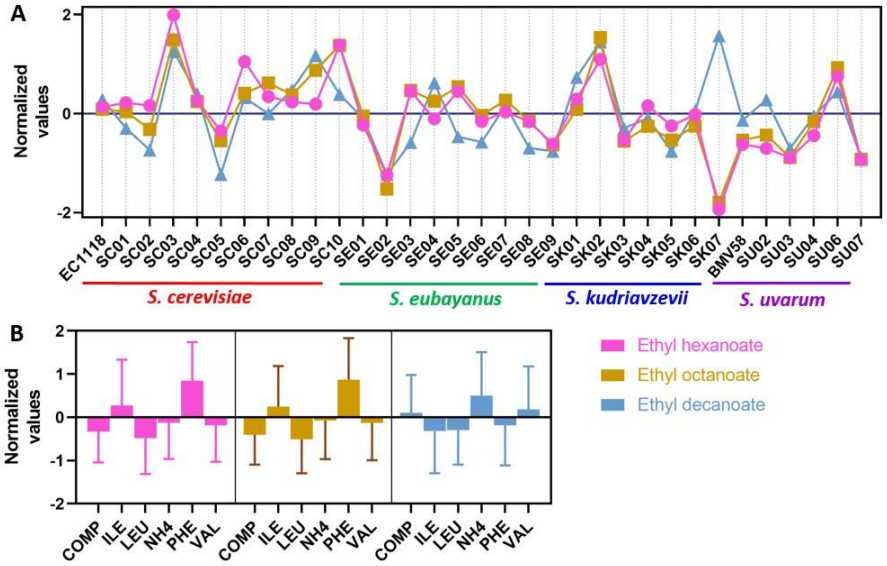


Figure 3. A. Individual capacity of the strains to produce ethyl hexanoate, ethyl octanoate, and ethyl decanoate presented as the mean (n= 18) of the normalized concentration reached by strain regardless of nitrogen musts. **B.** Ethyl hexanoate, ethyl octanoate and ethyl decanoate normalized concentration measured in each nitrogen must (n=99, error bars= SD) regardless the yeast strain.

Among the nitrogen effects, Leu decreased the levels of all ethyl esters compounds (Figure 3B). On the contrary, Phe and Ile tended to stimulate the synthesis of ethyl hexanoate and ethyl octanoate. In the case of ethyl decanoate, NH_4 favored its synthesis, followed by Val and complete media. Except for Leu, there could be observed a similar pattern for the production of ethyl hexanoate and ethyl octanoate: the nitrogen media that favor the production of these two aromas, decrease the concentration of ethyl decanoate and vice versa.

3.3.2. Fusel alcohols and acetate esters

Higher alcohols and their acetates are produced either by central carbon metabolism or from their amino acid precursors. The amino acids used in this study are directly related to the production of fusel alcohols and their acetate esters (Table S11). Therefore, in this case, we focused on the strains/species effect in the production of these compounds.

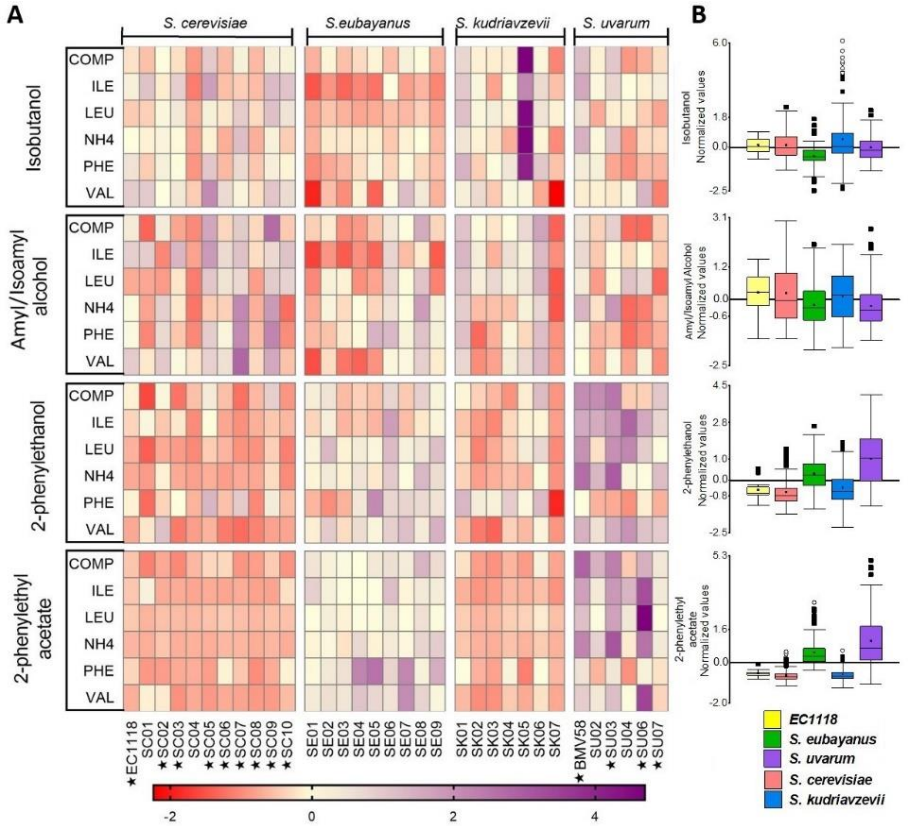


Figure 4. A Heat map of the fermentative aromas: isobutanol, active amyl/isoamyl alcohol, 2-phenylethanol and 2-phenylethyl acetate in which each row indicates the nitrogen must: Comp (complete); Ile (Isoleucine); Leu (Leucine); NH_4 (Ammonium); Phe (Phenylalanine) and Val (Valine). The color key bar indicated the normalized value of each compound by the average value of each nitrogen musts: low values, red and high values, violet. The stars in the name of the strains indicate a fermentative environment isolated. **B** Box plots indicate for each aroma compound the concentration normalized by each species, showing median, upper and lower quartile, vertical bars showing the minimum and maximum value respectively.

The production of aroma concentrations using different nitrogen sources and strains were integrated into a heat map (Figure 4A). For each compound, columns indicate the strains and rows of the nitrogen media, the maximum (violet) and the minimum (red) represent the normalized values by the average concentration produced by all the strains in the different nitrogen musts. To visualize the differences between species, Figure 4B represents the normalized values by the total average of these volatile compounds organized by species and compared to the wine strain EC1118 (yellow).

In the case of isobutanol, there was a remarkably great production of this compound by the SK05 yeast strain, except with valine as nitrogen source. Conversely, the strains SK07, SC04, SU07, and all the *S. eubayanus* strains showed the lowest values of isobutanol contents (Figure 4A and 4B). However, isobutanol production was not species-dependent. Moreover, Ile seemed to encourage isobutanol synthesis in most of the strains while ammonium and complete media decreased it (Table S11).

As expected, isoleucine and leucine amino acids enhanced the amyl/isoamyl alcohols production, although isoleucine seems to produce larger quantities than leucine in the majority of strains (Table S11). This was especially evident for *S. cerevisiae*, which increased the synthesis with isoleucine and valine. The strains that produced a higher concentration of these alcohols in the majority of nitrogen sources were SC05, SC07, SC09, SE08, SK01 and SK06 (Figure 4A).

S. *uvarum* strains, as many studies have described, is characterized by the high production of 2-phenylethanol and its corresponding acetate ester (Figure 4B). However, most of *S. eubayanus* and *S. kudriavzevii* synthesized higher concentrations than *S. uvarum* with phenylalanine (Figure 4A; Table S11). SE05 was the highest producer with phenylalanine whereas BMV58, SU03 and SU06 produced the highest concentrations with the rest of the

mediums, mainly with $NH_4$and LEU. Ammonium led toa little increased production of this alcohol in some *S. eubayanus* and *S. uvarum* strains, whereas in the rest of nitrogen sources lower concentrations were found (Figure 4A).

Regarding 2-phenylethyl acetate, the differences between the species were more evident (Figure 4B). All *S. cerevisiae* and *S. kudriavzevii* strains were the lowest producers whereas *S. uvarum* and *S. eubayanus* were the highest producers of this acetate ester in synthetic must.

To summarize all the aroma results, a PCA for yeast strains and nitrogen media on the seven fermentative aroma compounds quantified was applied. The first two principal components (PC1 and PC2) determined a variance of 64.5% for yeasts and 82.6% for the individual effect of nitrogen musts (Figure 5 A and B). A clear separation between species was detected, *S. cerevisiae* fermentations were characterized by a high content in MCFA ethyl esters and the fermentations conducted by the other three species showed higher 2-phenylethanol and 2-phenylethyl acetate synthesis. Furthermore, SK07 strain differs extremely from the rest of the strains, producing the lowest amounts of these aroma compounds, although, as previously described, it reached the highest values on ethyl decanoate.

Figure 5B showed the effect of the nitrogen source on the aroma synthesis. As mentioned above, there can be seen an influence of the amino acid precursor on the synthesis of the fusel alcohols and the respective acetate ester. Besides the effect on their corresponding aroma compounds, the PCA showed the important effect of phenylalanine and valine on the production of ethyl octanoate, hexanoate, and decanoate, respectively.

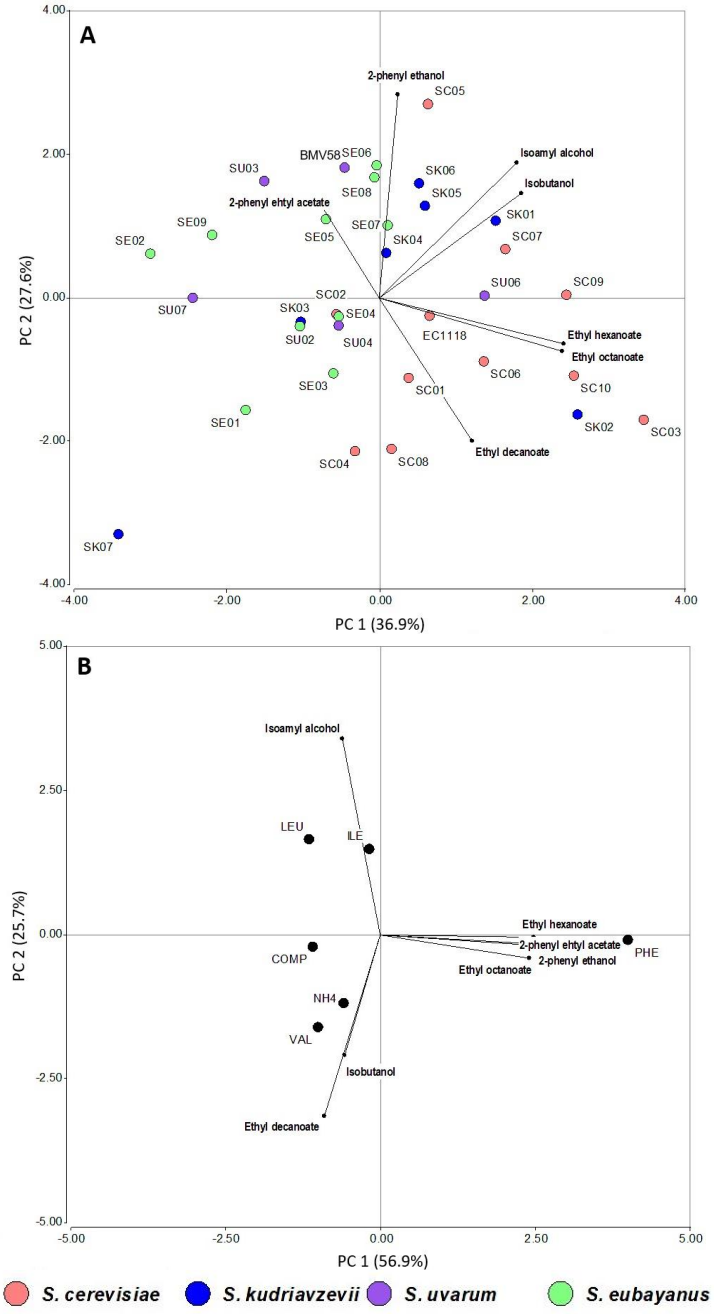


Figure 5. Principal component analysis (PCA) of fermentative volatile compounds produced by 33 strains in 6 six different nitrogen musts. **A** Distinction among yeast strains: each point represents one of the 33 yeast strains (n= 18) and the relation between the aroma variables **B** Distinction among nitrogen musts: each point represents one of the six nitrogen sources (n= 99) and the relation between the aroma variables.

The importance of these results lies in the fact that it was possible to observe how certain yeasts and species were characterized by the production capacity of these compounds without the need to have the amino acid that stimulates their production in the medium. At the same time, it was possible to find other nitrogen sources that could enhance these aromas.

4. Discussion

Oenology is facing new challenges on account of new consumer demands and global warming. As a potential solution, the use of novel yeasts and the management of yeast nutrition appear to be the keys to tackle them.

Non-*cerevisiae* strains have attracted attention in the past decade as they have shown the ability to positively modify the chemical and sensory composition of wine (Querol et al., 2018). Furthermore, there are plenty of wild *Saccharomyces cerevisiae* strains found in natural environments and fermentation processes that are of special interest for wine application (Camarasa et al., 2011). Additionally, very little is known about their nutritional aspects under winemaking conditions (Brice et al., 2018; Su et al., 2019). Therefore, this work aimed is to discover novel natural strains and nitrogen supplements that attenuate the global warming effect and satisfy the sensory demands of wine consumers.

For this purpose, the effects on the aromatic, oenological and fermentative properties of 33 different yeasts of the species *S. cerevisiae, S. uvarum, S. kudriavzevii*, and *S. eubayanus*, were evaluated under the influence of six different nitrogen sources.

Fermentation performance: the main effects of yeast and nitrogen sources

According to the literature, *S. cerevisiae* has been considered the main species responsible for carrying out the alcoholic fermentation as strains were

adapted to this specific process, known as domestication (Querol et al., 2003). During this process, yeasts have been adapted to different environmental habitats, such as specific nitrogen composition or osmotic stress conditions, which resulted in different yeast phenotypes (Camarasa et al., 2011; Legras et al., 2018). In the present study, *S. cerevisiae* strains from wine, cachaça, and beer showed great fermentation performances, while the rest of *S. cerevisiae* strains, from natural and other not industrial fermentation environments, did not show a good fermentation capacity. Probably, the most different process is agave fermentation where the fructose is the main sugar (50-150 g/L of a 96% fructose content) and high fermentation temperature (30-35 ºC) (Arrizon & Gschaedler, 2002), this may be the reason why strain SC07 (Peruvian agave fermentation) is the most different within the *S. cerevisiae* group. Therefore, strains/species showed different capacities to adapt to the specific stresses (temperature, nitrogen, and sugar conditions), which could be related to the original environmental conditions (Camarasa et al., 2011; Brice et al., 2018).

The species *S. eubayanus, S. kudriavzevii,* and *S. uvarum* have been characterized as cryotolerant species as they show a better fermentation activity at low temperatures than *S. cerevisiae* strains (Su et al., 2019). However, in our work, this fact was not appreciated, since fermentations conducted at 20 ºC did not provide clear differentiation between these species and *S. cerevisiae*. Furthermore, natural non-*cerevisiae* strains have been described as poor fermentative yeast compared to *S. cerevisiae* wine strains (Querol et al., 2018; Minebois et al., 2020), however, we found that the monosporic derivatives of the natural *S. kudriavzevii* strains SK06 and SK07 exhibited the best fermentation capacity, even quite similar to EC1118 performance.

It has been well established that nitrogen sources have a direct impact on yeast growth and fermentation capacity (Bely et al., 1990), and not all the nitrogen compounds are equally preferred by yeasts (Brice et al., 2018;

Gutiérrez et al., 2016). Consequently, some authors have classified the nitrogen sources according to the yeast-preferred level, in which valine was positioned by Godard et al., (2007) as an "intermediate" source to sustain growth and fermentation among *S. cerevisiae* species. In our study, *S. cerevisiae* strains did not perform the best fermentation in must containing only this compound. Valine was demonstrated to be the most preferred source for most of the non-*cerevisiae* yeasts, mainly for *S. eubayanus* strains, as they showed their best fermentation activity in valine-musts. This greater preference for this source could be related to the isolation source (oak, tree bark, and seeds, soil) in which they may find this amino acid more available (Camarasa et al., 2011; Brice et al., 2018). Furthermore, our results showed that valine, in almost all strains, led to lower production of the secondary end-products erythritol and glycerol in agreement with Fairbairn et al., (2017), who observed that valine as a sole nitrogen source, increases biomass production.

Production of desirable extracellular metabolites

The production of polyols and organic acids by strains has an important influence on the mouth feel of wine whereas its main function during fermentation is to compensate the redox balance (Schulze et al., 1996).

Glycerol is the most important secondary metabolite of alcoholic fermentation. It is produced by a diversion in the sugar flux to ethanol, to maintain NADH as oxidative form with a subsequent decrease in ethanol content (Cadière et al., 2011). Our results showed that most of *S. eubayanus* and *S. kudriavzevii* strains produced the highest glycerol and the lowest ethanol concentrations. This feature has been attributed to these species, which are found in cold environments, producing glycerol to protect the cells from low temperatures (Oliveira et al., 2014). Additionally, it has been proposed that glycerol synthesis decreases along with biomass formation and increases as a response to extracellular succinic acid release (Schulze et al., 1996;

Minebois et al., 2020). Regarding this fact, in our case, fermentations conducted in isoleucine and leucine musts showed a decrease of ethanol and succinic acid content along with an increase in glycerol production, suggesting a poor yeast preference for these nitrogen sources to produce biomass.

Succinic acid is an important organic acid in wine since its increase contributes to the drop of the pH and attenuates the loss of total acidity from potassium bitartrate precipitation (Coulter et al., 2004). This carboxylic acid during alcoholic fermentation can be produced by yeast from the reductive or oxidative branches of the tricarboxylic acid cycle (Camarasa et al., 2003). The high production of succinic acid by *S. uvarum* observed in our results was consistent with several studies that attribute this trait to *S. uvarum* being a cryotolerant strain (Coulter et al., 2004; Varela, et al., 2016; Minebois et al., 2020). Additionally, previous studies (Schulze et al., 1996; Coulter et al., 2004) found a positive correlation between organic acids secreted, especially succinic acid, and glycerol production for the redox balance maintenance. However, in our study this correlation was only found in *S. kudriavzevii* but not in *S. uvarum* (data not shown). *S. uvarum* can compensate their redox balance by producing other alcohols than glycerol, such as 2-phenylethanol by the chorismate pathway and erythritol by the pentose pathway as Minebois et al., (2020) proposed for the BMV58 strain.

Regarding ethanol content, our results showed that with a few exceptions, the lowest ethanol yields were characteristic for most of the *S. eubayanus* and *S. kudriavzevii* strains comparing with *S. cerevisiae* suggesting a major ethanol resistance of *S. cerevisiae* strains acquired from fermentative environments (Arroyo-López et al., 2010; Origone et al., 2017). Furthermore, as mentioned above an important decrease effect on ethanol content was found using the organic nitrogen sources instead of ammonium, mainly by isoleucine and leucine.

Production of desirable aromatic metabolites

MCFA ethyl esters contribute significantly to the fruity aroma of wine, and their precursor fatty acids (4-12 carbons fatty acids) are secondary products of the yeast lipid metabolism. According to our data, the nitrogen source did not appear to be a determining factor in the concentration of these aromatic compounds. However, a consistent effect of certain nitrogen sources was observed, phenylalanine was found to improve ethyl octanoate and hexanoate yields whereas leucine led to a decrease of the three MCFA ethyl esters in most of the strains. This variability in the production of these compounds in relation to nitrogen sources has been reported as result of an imbalance in the intracellular NADP/NADHP (Seguinot et al., 2020; Bloem et al., 2016).

Higher alcohols and mainly their acetate esters have been described to significantly contribute to the desirable fermentation flavor of wines (Lambrechts & Pretorius, 2000). Several studies have established how the production of these aromatic compounds can derive from two main routes, in which their precursors can be glucose or branched-chain or aromatic amino acids (Bell & Henschke, 2005). Since the effect of these amino acids on their related aromas is widely known, we focused particularly on the strain/species capability of producing them as well as understanding the interaction between these nitrogen sources and the strains. Firstly, a distinction at species-level was observed. As in previous reports, we also found that *S. uvarum* species produced the highest concentration of 2-phenylethanol and its acetate ester (Minebois et al., 2020; Varela et al., 2016) followed by *S. eubayanus*, whereas *S. kudriavzevii* and *S. cerevisiae* produced the highest amounts of active amyl/isoamyl alcohols and isobutanol. On the other hand, many studies have been established that the aromatic amino acid phenylalanine enhances the synthesis of 2-phenylethanol and its acetate (Stribny, et al., 2015; Fairbairn et al., 2017; Cordente et al., 2019). Unexpectedly, when the fermentations were

carried out in musts containing only phenylalanine, *S. uvarum* produced lower concentrations of 2-phenylethanol and its acetate ester than the rest of the species. Probably, in *S. uvarum* the presence of phenylalanine as a sole nitrogen source is repressed by the *novo*-synthesis of these volatile aroma compounds, as proposed Etschmann et al., (2002). Furthermore, SK05 considered in this study as the highest isobutanol producer, presented the same effect under the presence of valine as a sole nitrogen source, reducing the synthesis of isobutanol in this condition. Furthermore, it has been reported by Mendes-Ferreira et al., (2011) that the presence of branched or aromatic amino acids produced a feedback or enzymatic repression in favor of Ehrlich pathway over the biosynthetic pathway.

5. Conclusion

The results presented above shed light on the actual industry demands regarding wine quality improvement, specifically providing a better approach to the interactions between the non-conventional strains with certain nitrogen sources.

Regarding nitrogen effects, most positive aspects were observed in phenylalanine and valine and, to a lesser extent, in leucine and isoleucine musts compared with complete and ammonium mediums. As expected, we observed overproduction of their related catabolic compounds; however, valine also promoted the fermentation process in non-*cerevisiae* strains. On the other hand, phenylalanine increased erythritol, ethyl hexanoate and ethyl octanoate production while leucine and isoleucine showed the advantage of leading to low ethanol production. Nevertheless, the impact of the nitrogen source on the production of fermentation metabolites, volatile and non-volatile, was not entirely independent as a high level of interaction was observed between the factors, suggesting a complex relationship between the nitrogen source and the yeast metabolism.

The screening on the non-conventional strains allowed us to deeply differentiate and characterize species. Among them, the *S. cerevisiae* SC03 and the *S. kudriavzevii* SK02 strains were found to be great producers of fruity aromas, while BMV58 follow by the rest of *S. uvarum* strains were especially characterized by a great production of succinic acid. Other strains of interest were the *S. kudriavzevii* strains SK06 and SK07, which showed a significantly higher fermentation velocity in comparison to all the non-conventional researched strains. As remarkable features within the species, most of *S. eubayanus* and *S. uvarum* strains were characterized by the highest production of fermentative aromas which give a pleasant rose-like aroma to wine, whereas *S. kudriavzevii* strains were found to be positively related to high glycerol production.

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement number 764364, Aromagenesis, and from Generalitat Valenciana grant PROMETEO/2020/014. We are also grateful to Christian A. Lopes of the Universidad Nacional del Comahue, Argentina, and Francisco Cubillos of the Universidad de Santiago de Chile for kindly providing the use of yeast strains from their collection (NPCC and LGMUSC).

References

Arrizon, J., Gschaedler, A., 2002. Increasing fermentation efficiency at high sugar concentrations by supplementing an additional source of nitrogen during the exponential phase of the tequila fermentation process. Can. J. Microbiol. 48, 965–970. https://doi.org/10.1139/w02-093

Arroyo-López, F.N., Salvadó, Z., Tronchoni, J., Guillamón, J.M., Barrio, E., Querol, A., 2010. Susceptibility and resistance to ethanol in *Saccharomyces* strains isolated from wild and fermentative environments. Yeast 191–198. https://doi.org/10.1002/yea

Bell, S.J., Henschke, P.A., 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11, 242–295.

Beltrán, G., Novo, M., Guillamón, J.M., Mas, A., Rozès, N., 2008. Effect of fermentation temperature and culture media on the yeast lipid composition and wine volatile compounds. Int. J. Food Microbiol. 121, 169–177.

Bloem, A., Sanchez, I., Dequin, S., Camarasa, C., 2016. Metabolic impact of redox cofactor perturbations on the formation of aroma compounds in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 82, 174–183. https://doi.org/10.1128/AEM.02429-15

Bely, M., Sablayrolles, J.M., Barre, P., 1990. Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in oenological conditions. J. Ferment. Bioeng. 70, 246–252. https://doi.org/10.1016/0922-338X(90)90057-4

Brice, C., Cubillos, F.A., Dequin, S., Camarasa, C., Martínez, C., 2018. Adaptability of the *Saccharomyces cerevisiae* yeasts to wine fermentation conditions relies on their strong ability to consume nitrogen. PLoSOne 13, 1–20. https://doi.org/10.1371/journal.pone.0192383

Cadière, A., Ortiz-Julien, A., Camarasa, C., Dequin, S., 2011. Evolutionary engineered *Saccharomyces cerevisiae* wine yeast strains with increased in vivo flux through the pentose phosphate pathway. Metab. Eng. 13, 263–271. https://doi.org/10.1016/j.ymben.2011.01.008

Camarasa, C., Grivet, J.P., Dequin, S., 2003. Investigation by ^{13}C-NMR and tricarboxylic acid (TCA) deletion mutant analysis of pathways of succinate formation in *Saccharomyces cerevisiae* during anaerobic fermentation. Microbiology 149, 2669–2678. https://doi.org/10.1099/mic.0.26007-0

Camarasa, C., Sanchez, I., Brial, P., Bigey, F., Dequin, S., 2011. Phenotypic landscape of *Saccharomyces cerevisiae* during wine fermentation: Evidence for origin-dependent metabolic traits. PLoSOne 6. https://doi.org/10.1371/journal.pone.0025147

Carrau, F.M., Medina, K., Farina, L., Boido, E., Henschke, P.A., Dellacassa, E., 2008. Production of fermentation aroma compounds by *Saccharomyces cerevisiae* wine yeasts: Effects of yeast assimilable nitrogen on two model strains. FEMS Yeast Res. 8, 1196–1207.

Cordente, A.G., Schmidt, S., Beltrán, G., Torija, M.J., Curtin, C.D., 2019. Harnessing yeast metabolism of aromatic amino acids for fermented beverage bioflavouring and bioproduction. Appl. Microbiol. Biotechnol. 103, 4325–4336. https://doi.org/10.1007/s00253-019-09840-w

Coulter, A., Godden, P.W., Pretorius, I.S., 2004. Succinic Acid, How is it formed, what is its effect on titratable acidity, and what factors influence its concentration in wine? Wine Ind. J. 19, 16–25. https://doi.org/10.1002/9780470995327.ch163

Crépin, L., Truong, N.M., Bloem, A., Sanchez, I., Dequin, S., Camarasa, C., 2017. Management of multiple nitrogen sources during wine fermentation by *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 83, 1–21. https://doi.org/10.1128/AEM.02617-16

Drappier, J., Thibon, C., Rabot, A., Geny-Denis, L., 2019. Relationship between wine composition and temperature: Impact on Bordeaux wine typicity in the context of global warming—Review. Crit. Rev. Food Sci. Nutr. 59, 14–30. https://doi.org/10.1080/10408398.2017.1355776

Etschmann, M., Bluemke, W., Sell, D., Schrader, J., 2002. Biotechnological production of 2-phenylethanol. Appl. Microbiol. Biotechnol. 59, 1–8. https://doi.org/10.1007/s00253-002-0992-x

Fairbairn, S., McKinnon, A., Musarurwa, H.T., Ferreira, A.C., Bauer, F.F., 2017. The impact of single amino acids on growth and volatile aroma production by *Saccharomyces cerevisiae* strains. Front. Microbiol. 8, 1–12. https://doi.org/10.3389/fmicb.2017.02554

Fleet, G.H., 1998. The microbiology of alcoholic beverages, in: B. J. B. Wood (Ed.), Microbiology of Fermented Foods. pp. 217–262. https://doi.org/10.1007/978-1-4613-0309-1_9

Gamero, A., Belloch, C., Ibáñez, C., Querol, A., 2014. Molecular analysis of the genes involved in aroma synthesis in the species *S. cerevisiae*, *S. kudriavzevii* and *S. bayanus* var. *uvarum* in winemaking conditions. PLoS One 9, 1–10. https://doi.org/10.1371/journal.pone.0097626

Godard, P., Urrestarazu, A., Vissers, S., Kontos, K., Bontempi, G., van Helden, J., André, B., 2007. Effect of 21 different nitrogen sources on global gene expression in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. 27, 3065–3086. https://doi.org/10.1128/mcb.01084-06

Gutiérrez, A., Sancho, M., Beltrán, G., Guillamon, J.M., Warringer, J., 2016. Replenishment and mobilization of intracellular nitrogen pools decouples wine yeast nitrogen uptake from growth. https://doi.org/10.1007/s00253-015-7273-y

Lambrechts, M.G., Pretorius, I.S., 2000. Yeast and its importance to wine aroma - A Review. South African J. Enol. Vitic. 21, 97–129.

Legras, J., Galeote, V., Camarasa, C., Marsit, S., Nidelet, T., Sanchez, I., Couloux, A., Guy, J., Franco-duarte, R., Marcet-houben, M., Gabaldon, T., Schuller, D., Sampaio, P., Dequin, S., 2018. Adaptation of *S. cerevisiae* to fermented food environments reveals remarkable genome plasticity and the footprints of

domestication. Mol. Biol. Evol. 35, 1712–1727. https://doi.org/10.1093/molbev/msy066

Lindberg, M.L., Amsterdam, E.A., 2008. Alcohol, wine, and cardiovascular health. Clin. Cardiol. 31, 347–351. https://doi.org/10.1002/clc.20263

Mendes-Ferreira, A., Barbosa, C., Lage, P., Mendes-Faia, A., 2011. The impact of nitrogen on yeast fermentation and wine quality. Cienc. e Técnica Vitivinícola 26, 17–32.

Minebois, R., Pérez-Torrado, R., Querol, A., 2020. A time course metabolism comparison among *Saccharomyces cerevisiae*, *S. uvarum* and *S. kudriavzevii* species in wine fermentation. Food Microbiol. 90, 1–14. https://doi.org/10.1016/j.fm.2020.103484

Nicolini, G., Larcher, R., Versini, G., 2004. Status of yeast assimilable nitrogen in Italian grape musts and effects of variety, ripening and vintage. Vitis - J. Grapevine Res. 43, 89–96.

Oliveira, B.M., Barrio, E., Querol, A., Pérez-Torrado, R., 2014. Enhanced enzymatic activity of glycerol-3-phosphate dehydrogenase from the cryophilic *Saccharomyces kudriavzevii*. PLoSOne 9. https://doi.org/10.1371/journal.pone.0087290

Origone, A.C., del Mónaco, S.M., Ávila, J.R., González Flores, M., Rodríguez, M.E., Lopes, C.A., 2017. Tolerance to winemaking stress conditions of Patagonian strains of *Saccharomyces eubayanus* and *Saccharomyces uvarum*. J. Appl. Microbiol. 123, 450–463. https://doi.org/10.1111/jam.13495

Peltier, E., Bernard, M., Trujillo, M., Barbe, C., Gibon, Y., Marullo, P., 2018. Wine yeast phenomics: A standardized fermentation method for assessing quantitative traits of *Saccharomyces cerevisiae* strains in enological conditions. PLoSOne 1–23. https://doi.org/10.1371/journal.pone.0190094

Querol, A., Fernández-Espinar, M.T., Del Olmo, M., Barrio, E., 2003. Adaptive evolution of wine yeast. Int. J. Food Microbiol. 86, 3–10. https://doi.org/10.1016/S0168-1605(03)00244-7

Querol, A., Pérez-Torrado, R., Alonso-del-Real, J., Minebois, R., Stribny, J., Oliveira, B.M., Barrio, E., 2018. New trends in the uses of yeasts in oenology, in: Advances in Food and Nutrition Research. Elsevier Inc., Amsterdem, pp. 177–210. https://doi.org/10.1016/bs.afnr.2018.03.002

Rodríguez, M.E., Origone, A.C., González Flores, M., Lopes, C.A., 2016. *Saccharomyces* in traditional and industrial Fermentations from Patagonia, in: Olivera, N.L., Libkind, D., Donati, E. (Eds.), Biology and Biotechnology of Patagonian Microorganisms. Switzerland, pp. 1–360. https://doi.org/10.1007/978-3-319-42801-7

Rollero, S., Bloem, A., Ortiz-Julien, A., Camarasa, C., Divol, B., 2018. Fermentation performances and aroma production of non-conventional wine yeasts are influenced by nitrogen preferences. FEMS Yeast Res. 18, 1–11. https://doi.org/10.1093/femsyr/foy055

Rollero, S., Mouret, J.R., Bloem, A., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.M., Dequin, S., Camarasa, C., 2017. Quantitative 13C-isotope labelling-based

analysis to elucidate the influence of environmental parameters on the production of fermentative aromas during wine fermentation. Microb. Biotechnol. 10, 1649–1662. https://doi.org/10.1111/1751-7915.12749

Rossignol, T., Dulau, L., Julien, A., Blondin, B., 2003. Genome-wide monitoring of wine yeast gene expression during alcoholic fermentation. Yeast 20, 1369–1385. https://doi.org/10.1002/yea.1046

Schulze, U., Lidén, G., Nielsen, J., Villadsen, J., 1996. Physiological effects of nitrogen starvation in an anaerobic batch culture of *Saccharomyces cerevisiae*. Microbiology 142, 2299–2310. https://doi.org/10.1099/13500872-142-8-2299

Seguinot, P., Bloem, A., Brial, P., Meudec, E., Ortiz-Julien, A., Camarasa, C., 2020. Analysing the impact of the nature of the nitrogen source on the formation of volatile compounds to unravel the aroma metabolism of two non-*Saccharomyces* strains. Int. J. Food Microbiol. 316, 1–12. https://doi.org/10.1016/j.ijfoodmicro.2019.108441

Stribny, J., Gamero, A., Pérez-Torrado, R., Querol, A., 2015. *Saccharomyces kudriavzevii* and *Saccharomyces uvarum* differ from *Saccharomyces cerevisiae* during the production of aroma-active higher alcohols and acetate esters using their amino acidic precursors. Int. J. Food Microbiol. 205, 41–46. https://doi.org/10.1016/j.ijfoodmicro.2015.04.003

Su, Y., Origone, A.C., Rodríguez, M.E., Querol, A., Guillamón, J.M., Lopes, C.A., 2019. Fermentative behaviour and competition capacity of cryotolerant *Saccharomyces* species in different nitrogen conditions. Int. J. Food Microbiol. 291, 111–120. https://doi.org/10.1016/j.ijfoodmicro.2018.11.020

Su, Y., Seguinot, P., Bloem, A., Ortiz-Julien, A., Heras, J.M., Guillamón, J.M., Camarasa, C., 2020. Isotopic tracers unveil distinct fates for nitrogen sources during wine fermentation with two non-*Saccharomyces* strains. Microorganisms 8, 1–19. https://doi.org/10.3390/microorganisms8060904

Swiegers, J.H., Bartowsky, E.J., Henschke, P.A., Pretorius, I.S., 2005. Yeast and bacterial modulation of wine aroma and flavour. Aust. J. Grape Wine Res. 11, 139–173. https://doi.org/10.1111/j.1755-0238.2005.tb00285.x

Tronchoni, J., Rozès, N., Querol, A., Guillamón, J.M., 2012. Lipid composition of wine strains of *Saccharomyces kudriavzevii* and *Saccharomyces cerevisiae* grown at low temperature. Int. J. Food Microbiol. 155, 191–198. https://doi.org/10.1016/j.ijfoodmicro.2012.02.004

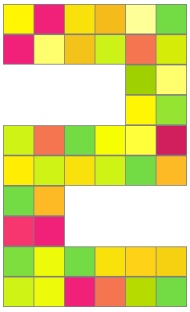
Ugliano, M., Henschke, P.A., Herderich, M.J., Pretorius, I.S., 2007. Nitrogen management is critical for wine flavour and style. Aust. New Zeal. Wine Ind. J. 22, 24–30.

Varela, C., Sengler, F., Solomon, M., Curtin, C., 2016. Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. Food Chem. 209, 57–64. https://doi.org/10.1016/j.foodchem.2016.04.024

Waterhouse, A.L., Sacks, G.L., Jeffery, D.W., 2016. Understanding Wine Chemistry. John Wiley & Sons.

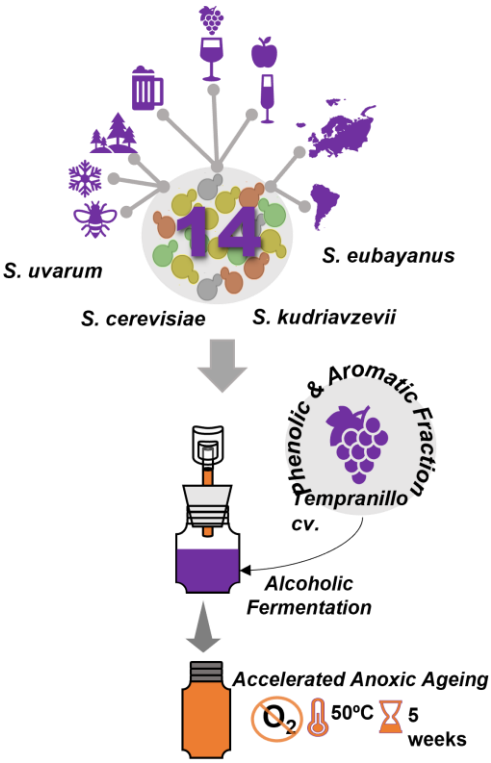
Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K., 1990. Modeling of the bacterial growth curve. Appl. Environ. Microbiol. 56, 1875–1881. https://doi.org/0099-2240/90/061875-07$02.00/0

Chapter 2



Effect of non-wine *Saccharomyces* yeasts and bottle aging on the release and generation of aromas in semi-synthetic Tempranillo wines

Dolores Pérez, Marie Denat, José María Heras, José Manuel Guillamón, and Amparo Querol



Published in International Journal of Food Microbiology (2022)

DOI: 10.1016/j.ijfoodmicro.2022.1095

Abstract

Interest in the use of non-conventional yeasts in wine fermentation has been increased in the last years in the wine sector. The main objective of this manuscript was to explore the aromatic diversity produced by wild and non-wine strains of *S. cerevisiae*, *S. eubayanus*, *S. kudriavzevii*, and *S. uvarum* species in young and bottle-aged Tempranillo wines as well as evaluate their fermentation capacity and the yield on ethanol, glycerol, and organic acids, that can contribute to diminishing the effects of climate change on wines.

S. uvarum strain U1 showed the highest ability to release, or *de novo* produce monoterpenes, such as geraniol and citronellol, whose values were 1.5 and 3.5-fold higher than those of the wine *S. cerevisiae* strain. We found that compared to the normal values for red wines, β-phenylethyl acetate was highly synthesized by U1 and E1 strains, achieving 1 mg/L. Additionally, after aging, wines of *S. eubayanus* strains contained the highest levels of this acetate. Malic acid was highly degraded by *S. kudriavzevii* yeasts, resulting in the highest yields of lactic acid (>5-fold) and ethyl lactate (>2.8-fold) in their wines. In aged wines, we observed that the modulating effects of yeast strain were very high in β-ionone. *S. uvarum* strains U1 and BMV58 produced an important aging attribute, ethyl isobutyrate, which was highly enhanced during the aging. Also, the agave *S. cerevisiae* strain develops an essential aroma after aging, reaching the highest ethyl leucate contents.

According to the results obtained, the use of wild non-wine strains of *S. cerevisiae* and strains of the cryotolerant species *S. eubayanus*, *S. kudriavzevii*, and *S. uvarum* in Tempranillo wine fermentation increase the aroma complexity. In addition, wines from *S. kudriavzevii* strains had twice additional glycerol, those from *S. uvarum* 4-fold more succinic acid, while wines from wild strains yielded 1% v/v less ethanol which may solve wine problems associated with Climate Change.

1. Introduction

Wine aroma is widely known to be one of the most relevant determinants of the overall wine quality (Charters and Pettigrew, 2007; San-Juan et al., 2011). Its composition consists of several volatile molecules at concentrations ranging from ng/L to mg/L. According to their origin, they can be divided into three main categories, the aroma of varietal, fermentative, and aging origin. The most abundant volatile compounds are from fermentative origin, such as ethyl esters, acetate esters, higher alcohols, and volatile fatty acids, mainly derived from nitrogen and carbon yeast metabolisms (Rollero et al., 2017). However, the varietal aroma is the most influential group in terms of the substantial aroma contribution of the odorants to wine. Among others, this group includes polyfunctional mercaptans, norisoprenoids, terpenoids, volatile phenols, and vanillin derivatives (Ferreira and López, 2019). Initially, most of these molecules do not contribute directly to wine aroma as they are presented at bound forms, such as a glycosylated, whereas only a small fraction is found as free aroma molecules (Ferreira and López, 2019; Hjelmeland and Ebeler, 2015; Liu et al., 2017). In general, aglycones are released from their non-volatile precursors by a slow process of acid hydrolysis that mainly occurs during the aging process (Alegre et al., 2020a; Ferreira and López, 2019). On the other hand, yeasts can release the aromatic aglycone from sugar moieties through endogenous enzymatic reactions (Ugliano, 2009). They can even generate some varietal aromas within their cells through the *denovo*-synthesis process (Gamero et al., 2011a, 2011 b).

Recent studies have determined the importance of yeast and bottle aging in the generation or release of this type of compounds from the grapes non-volatile aroma precursors, such as β-damascenone, linalool, rose oxide, α-terpineol, guaiacol, eugenol, methoxyeugenol, 2,6-dimehtoxyphenol, and vanillin derivates (Alegre et al., 2020a; Denat et al., 2021). These insights

have established new aromas origin sub-categories and have emphasized the importance of yeast strain and aging time on the development of varietal and fermentative aromas.

Currently, wine researchs have shown that the most widely used and adapted strains for wine fermentation have been set aside in favor of what are known as indigenous or wild yeasts. These wild *S. cerevisiae* or *S.* non-*cerevisiae* strains have been isolated from spontaneous fermentations or found in nature. The implementation of non-conventional yeasts in winemaking could lead to the discovery of new aromatic profiles as well as some improvements in wine quality (Querol et al., 2018). For instance, cryotolerant species, *S. kudriavzevii*, *S. uvarum,* and *S. eubayanus* have been characterized to produce wines with low alcohol levels, high glycerol content, and high concentrations of certain higher alcohols and their acetate esters, thus decreasing the effect of climate change on grapes (Minebois et al., 2020a; Stribny et al., 2015). Additionally, some studies revealed that some *S. cerevisiae* strains could differ in the final wine flavor profile due to their capacity to produce ethyl esters, higher alcohols, and volatile fatty acids from their nitrogen metabolism (Cordente et al., 2019). In general, when isolated from natural or non-wine environments, these yeasts usually show poor fermentative abilities due to some must and fermentation conditions: high fermentation temperature, pH, high sugar content, increased ethanol and SO_2 levels (Arroyo-López et al., 2010; Origone et al., 2017). However, through screening studies, some non-*cerevisiae* and non-wine *S. cerevisiae* strains have been selected for their optimal wine fermentation capacities (Pérez et al., 2021).

Tempranillo cv. (*Vitis vinifera* L) is one of the most important red grape varieties in Spain, especially in the region of La Rioja. Despite being a neutral grape variety, its wines have a distinctive aroma profile originated after yeast

and bottle-aging action on varietal-specific odorless precursors (Hernández-Orte et al., 2008; López et al., 2004, Alegre et al., 2020b). In this context, given the impact of yeasts and bottle aging on aroma modulation, in this work we aim to explore in-depth the variability and contribution of yeast strains, not used previously in wine fermentations, on the aroma of young and aged Tempranillo wines. We also evaluate these strains in terms of their fermentation capacity and their ability to provide oenological qualities that diminish the effects of climate change on wines.

2. Materials and Methods

2.1 Semi-synthetic must composition

Must composition was prepared according to Hernández-Orte et al. (2006) with minor modifications: 210 g/L reducing sugars (105 g/L glucose + 105 g/L fructose); 4 g/L L-tartaric acid; 0.3 g/L citric acid; 3 g/L L-malic acid; 2 mg/L KH_2PO_4; 0.2 g/L $MgSO_4.7H_2O$; 4.7 mg/L $MnCl_2.4H_2O$; 1.35 mg/L $CuSO_4.5H_2O$; 1.29 mg/L KIO_3; 0.22 mg/L $CoCl_2$; 1 mg/L H_3BO_3; 2mg/L $ZnCl_2$; 0.155 g/L $CaCl_2.2H_2O$; 0.19 mg/L $NaMoO_4.2H_2O$; 0.3 g/L myo-inositol; 0.04 mg/L biotin; 1 mg/L thiamin hydrochloride; 1 mg/L pyridoxine hydrochloride; 1 mg/L nicotinic acid; 1 mg/L calcium pantothenate;1 mg/L *p*-aminobenzoic acid; 15 mg/L ergosterol; 0.05% Tween 80 in ethanol (v/v); 0.2 mg/L folic acid and 0.2 mg/L riboflavin.

The nitrogen compounds were defined by two fractions with a final nitrogen concentration (YAN) of 355 mg/L. The basal nitrogen content was composed of 0.22 g/L $(NH_4)_2HPO_4$; 14.34 mg/L tyrosine; 44.37 mg/L γ-aminobutyric acid (GABA); 14.43 mg/L isoleucine; 13.42 mg/L leucine; 58.51 mg/L alanine and 17.73 mg/L valine. The second was a specific Tempranillo amino acids fraction, composed by 86.52 mg/L aspartic acid; 85.24 mg/L glutamic acid; 60.08 mg/L serine; 6.47 mg/L glycine; 137.4 mg/L

histidine; 72.27 mg/L threonine; 673.1 mg/L arginine; 302.3 mg/L proline, 25.2 mg/L methionine; 7.53 mg/L phenylalanine;13.69 mg/L lysine and 177.3 mg/L glutamine.

Before the must was filtered by sterilization (0.2 μm), the pH was adjusted to 3.5, and 10 mL/L of phenolic and of aromatic fraction of Tempranillo grapes (PAF) was aseptically added.

The phenolic and aromatic non-volatile extract from Tempranillo grapes (PAF) was prepared at Zaragoza University (Spain) following the protocol described by Alegre et al. (2020a) and was conserved in ethanol. Before being introduced into the synthetic must, the alcohol content was entirely evaporated to dryness under vacuum using a rotary evaporator. Finally, the extract was reconstituted in the same volume with sterile water and added to the must immediately before the inoculation to avoid possible oxidation.

2.2 Yeast strains and fermentation conditions

Yeast strains belonging to the species *S. cerevisiae, S. uvarum, S. kudriavzevii*, and *S. eubayanus*, isolated from natural habitats and spontaneous fermentation, were used to conduct the alcoholic fermentation (Table 1).

A day before starting the fermentation, each strain was grown at 25ºC in 5 mL of GPY (2 % glucose, 0.5 % yeast extract, and 0.5% peptone, PanReac AppliChem, Spain), then each must was inoculated with these pre-cultures at an initial population of $1x10^6$ cells/mL.

As fermenters, 100 mL sterile glass flasks with a stirrer magnet were used. Standard cartridges filled with previously conditioned LiChrolut-EN resin (Denat et al., 2021) were inserted at the airlock output. The silicone caps

were pierced with fixed syringes to collect samples without introducing oxygen or losing aroma.

For each strain, three fermentations were carried out in 50 mL of must containing PAF and as controls, non-inoculated musts containing PAF, were included. Fermentations were carried out at 20 ºC with agitation (100 rpm) while daily weight loss was monitored. Once the fermenter's weight was constant and reducing sugars contents were less than 2 g/L, the fermentation process was considered finished. By the mathematical model of non-linear regression, the fermentation curves of weight loss were adjusted using the reparametrized Gompertz equation proposed by Zwietering et al., (1990) for bacterial growth:

$$y = A*\exp\{-\exp[((\mu_{max}*e/A)*(\lambda - t)) + 1]\}$$

For the adjustment to the fermentation process, these kinetic parameters were adapted, being λ the time to the vigorous starts of fermentation, Vmax the maximum specific fermentation rate, and A, as the maximum weight lost reached. In addition, from these parameters, the time needed to reach 75% of fermentation was calculated (T75%).

2.3 Analysis of organic acids, sugars, and alcohols

After fermentation, samples were analyzed by HPLC (High-Performance Liquid Chromatography, Thermo Fisher Scientific, Waltham, MA, USA). The concentrations of the following metabolites were determined: glucose, fructose, ethanol, glycerol, succinic, and malic acid. The instrument was composed of a Hyper REZTM XP Carbohydrate H+ 8 μm column equipped with a Hyper RETZM XP Carbohydrate Guard and a refraction index detector for sugars and alcohols, and a UV detector for organic acids (Thermo Fisher Scientific). The analysis conditions were: 1.5 mM of H_2SO_4. 0.6 mL/min flux, a pressure of 30 bars, and oven temperature were held at

50ºC. Samples were previously filtered by a nylon filter (0.22 μm), and the concentrations of these compounds (g/L and % v/v for ethanol) were determined using standard calibration curves.

Table 1. *Saccharomyces cerevisiae* , *Saccharomyces eubayanus* , *Saccharomyces kudriavzevii,* and *Saccharomyces uvarum* strains were used in this study.

Species	Code used	AQ code[a]	Source of isolation	Geographic origin
S. cerevisiae	T73	AQ0029	Wine, Commercial (Lallemand), T73 ™	Spain
	C1	AQ2543	Cachaça fermentation	Brazil
	C2	AQ2493	Agave fermentation	Mexico
	C3	AQ2458	Wasp	Peru
S. eubayanus	E1	AQ2875	Oak (*N. pumili)*[b]	Chile
	E2	AQ2596	Tree seeds (*A. araucana*)[c]	Argentina
	E3	AQ2600	Tree bark (*A. araucana*)[c]	Argentina
S. kudriavzevii	K1	AQ2641	Monosporic derivative from oak (*Q. Faginea)* isolate	Spain
	K2	AQ2148	Oak (*Q. Faginea*)	Spain
	K3	AQ2619	Monosporic derivative from oak (*Q. Faginea)* isolate	Spain
S. uvarum	BMV58	AQ1580	Wine, Commercial (Lallemand), Velluto BMV58™	Spain
	U1	AQ1124	Non fermented liquor (Mistela)	Spain
	U2	AQ1179	Cider fermentation	Ireland
S. cer x S. uv	VellEvol	AQ2868	Wine, Commercial (Lallemand);VellutoEvolution ™	Spain

[a] Dr. Querol yeast collection at IATA (CSIC). [b]Molecular Genetic Laboratory of Santiago de Chile University. [c]North Patagonian Culture Collection, Neuquén, Argentina.

2.4 Determination of volatile aromatic compounds

2.4.1 Analysis of aromas volatilized during fermentation

Once fermentations were finished, each cartridge was removed from the airlock and immediately dried under vacuum. Then, the retained volatile compounds were extracted and collected by elution with 1.6 mL of

dichloromethane solution containing 5% v/v of methanol. Next, 50 μL of each sample extract was mixed in one solution to concentrate under nitrogen flow.

This extract was analyzed by Gas-Chromatography-Olfactometry (GC-O) using a gas chromatograph (Thermo 8000 series) equipped with a Flame Ionization Detector (FID) and a sniffing port ODO-1 (SGE, Ringwood, Australia), which was attached to the column output by a flow splitter. The methodology used for GC-O was as described in San-Juan et al. (2010).

The sensory evaluations in the GC-O were realized by a panel of six experienced judges, two men and four women. The aroma evaluation consisted of a 40 minutes chromatographic run, so judges were asked to do it in separate 20 minutes sessions to avoid fatigue and twice on different days. They were asked to describe the odor perceived, the intensity on a scale of 0 to 3 (being 0 not detected; 1 weak, hardly recognizable odor; 2 clear but no intense odor; 3 intense), and write down the detection time.

The data collected from the panel were analyzed using the model proposed by Dravnieks (1985), obtaining scores that correspond to the detection frequency of aromatic attributes and the average intensity of their maximum intensity. Then the compounds were identified by comparing the descriptors, the chromatographic retention index obtained in DB-WAX and DB-5 columns, and the MS spectra with those of pure reference compounds. The GC-MS conditions were as Denat et al. (2021) described.

2.4.2 Analysis of major aromas compounds in young wines samples

Immediately after fermentation, higher alcohols, volatile fatty acids, and major esters were extracted by liquid-liquid microextraction and quantified by GC-FID analysis using the protocol described by Ortega et al. (2001). The quantification of each compound (mg/L) was done in reference to

the relative response factor obtained from the areas and concentrations of each standard compound dosed to a wine.

2.4.3 Analysis of trace aromas compounds in young and aged wines samples

Part of each finished wine was introduced in 18 mL vials with a non-metallic screw cap and treated in an anoxic chamber (Jacomex, Dagneux, France). Samples were introduced in vacuum plastic bags containing oxygen scavengers AnaeroGen (Thermo Scientific, USA). These bags containing 18 mL-vials with the free-O_2 wine samples were incubated at 50ºC for 5 weeks to simulate bottle aging. Those compounds found at concentrations ranging from 0.1 to 200 ug/L (minor or traces compounds) were analyzed in young and aged wines following the protocol described by López et al. (2002). The identification and quantification of the minor compounds were carried out by a gas chromatograph coupled to a mass spectrometer detector, GC-MS (Shimadzu QP2010, Quioto, Japan), and the concentrations were obtained in reference to the relative response factor obtained from the areas and concentrations of each standard compound dosed to a wine.

2.5 Statistical Analysis

Every fermentation parameter and compound was expressed as the arithmetic means of three repetitions with their corresponding standard deviation. One-way ANOVA with Tukey test was applied considering significant differences at p-value < 0.05. Genesis software 1.7.7 (Graz University of Technology, Austria) was used to acquire the heat map, the hierarchical clustering using Euclidean distance, and the average linkage as aglomeration rule. Principal component analysis (PCA), as a multivariate methodology, was applied to obtain visualization in a reduced dimension of data and to determine which variable contributes the most and how the

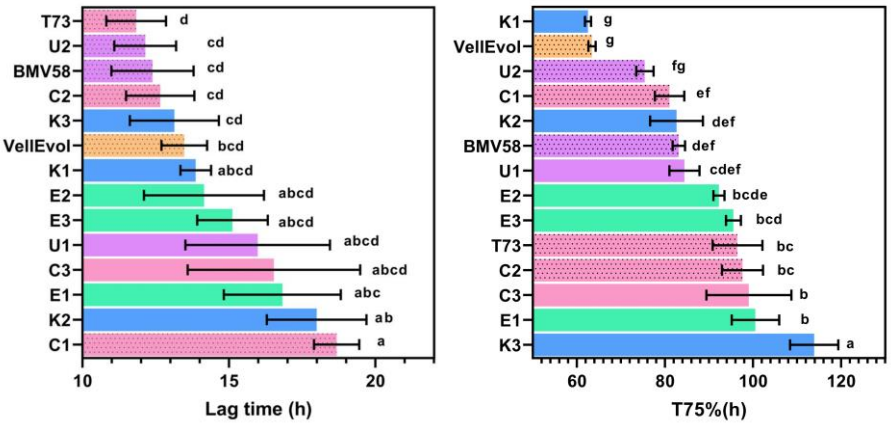
samples are grouped around them. All statistical analyses and plots were obtained by the use of Infostat software, version 2011 (Grupo Infostat, Córdoba, Argentina), and GraphPad Prism version 8.0 (Graph-Pad Software, Inc., La Jolla, CA).

3. Results and discussion

Microfermentations were carried out by 14 different yeast strains of four *Saccharomyces* species (Table 1) in synthetic musts containing PAF (phenolic and aromatic fraction) extracted from Tempranillo grapes.

3.1. Fermentation performances

Lag phase, Vmax, and T75 resulting from the fermentation of each strain are plotted in Figure 1. Most strains with fermentative background (dot-filled bars) showed a better adaptation to the must conditions at the beginning of the fermentation, resulting in shorter lag times. In contrast, most of the wild strains presented the longest lag times.



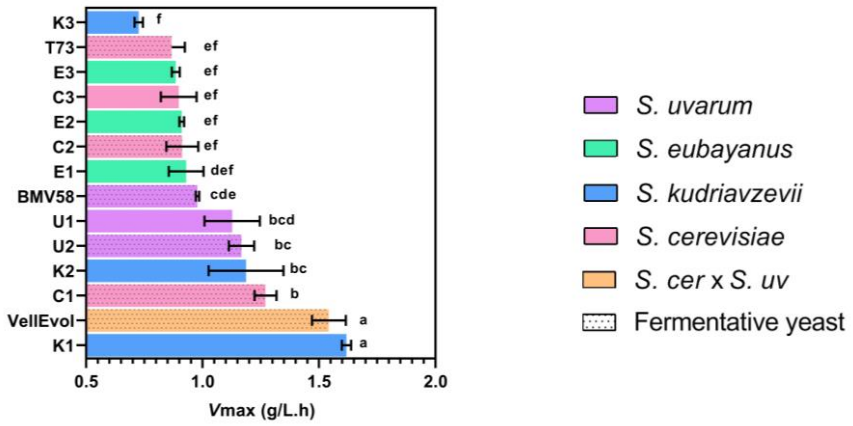


Figure 1. Kinetic parameters(mean±SD) of the alcoholic fermentation curves: lag phase, Vmax (maximal fermentation rate), and T75% (time to reach 75% of the fermentation) of the different strains represented in each bar, differentiating the species by color and the dotted filing indicates that they are of fermentative origin. ANOVA results are presented in the supplementary material (Table S1).

On the other hand, as the fermentation progressed, the natural *S. kudriavzevii* strains K1 and K2 as well as VellEvol and C1 displayed a better performance, achieving the 75% of the fermentation earlier and with the fastest Vmax. On the contrary, the other *S. kudriavzevii* strain, K3, had the opposite tendency; it started fermenting quite fast, but its T75% was the longest, and its Vmax was the slowest. Such different fermentation behaviors are probably due to different tolerances among yeast strains to factors encountered during fermentation, such as pH, temperature, hexose concentrations, and ethanol increments (Origone et al., 2017). However, the most relevant finding in this work was that the natural *S. kudriavzevii* K1 strain showed a fermentation capacity equal or even better than the wine strains VellEvol (*S. cerevisiae* x *S. uvarum* hybrid), T73 (*S. cerevisiae*), and BMV58 (*S. uvarum*).

3.2. Major fermentation metabolites

Relevant differences in organic acids and glycerol production have been observed (Figure 2; Table S2). Malic acid contents ranged from 2.53 to 4.23 g/L, with the highest values for C2 and U2 strains (4.23 and 4.18 g/L, respectively) and lowest for the *S. kudriavzevii* strains (2.53 to 2.74 g/L), resulting in wines with 1.5 g/L below C2 and U1 contents. Considering that the original must contain 3 g/L malic acid, strain C2, U1, E2, T73, E1, BMV58, and C1 seem to have produced this acid through the reductive branch of Krebs cycle (TCA), whereas *S. kudriavzevii* yeasts appear to have degraded the must's malic acid, as a different way to recycle redox cofactors (Redzepovic et al., 2003; Waterhouse et al., 2016). In line with this last statement, the degradation of malic acid by *S. kudriavzevii* strains could be related to the highest lactic acid synthesized by them, having statistically different values and up to five-fold higher than the rest of the yeasts (Figure 2). In the case of succinic acid, *S. uvarum* BMV58 and the commercial hybrid (*S. cerevisiae* x *S. uvarum*) produced the highest levels (4.24 and 3.54 g/L, respectively). In contrast, *S. cerevisiae* strains produced the lowest levels of this acid, up to 3 g/L below those maxima values. In the case of glycerol, differences as high as 2-3.5 g/L were observed between strains from different sources: strains from fermentation environments reached the lowest values, whereas strains from natural environments had the highest values of this alcohol.

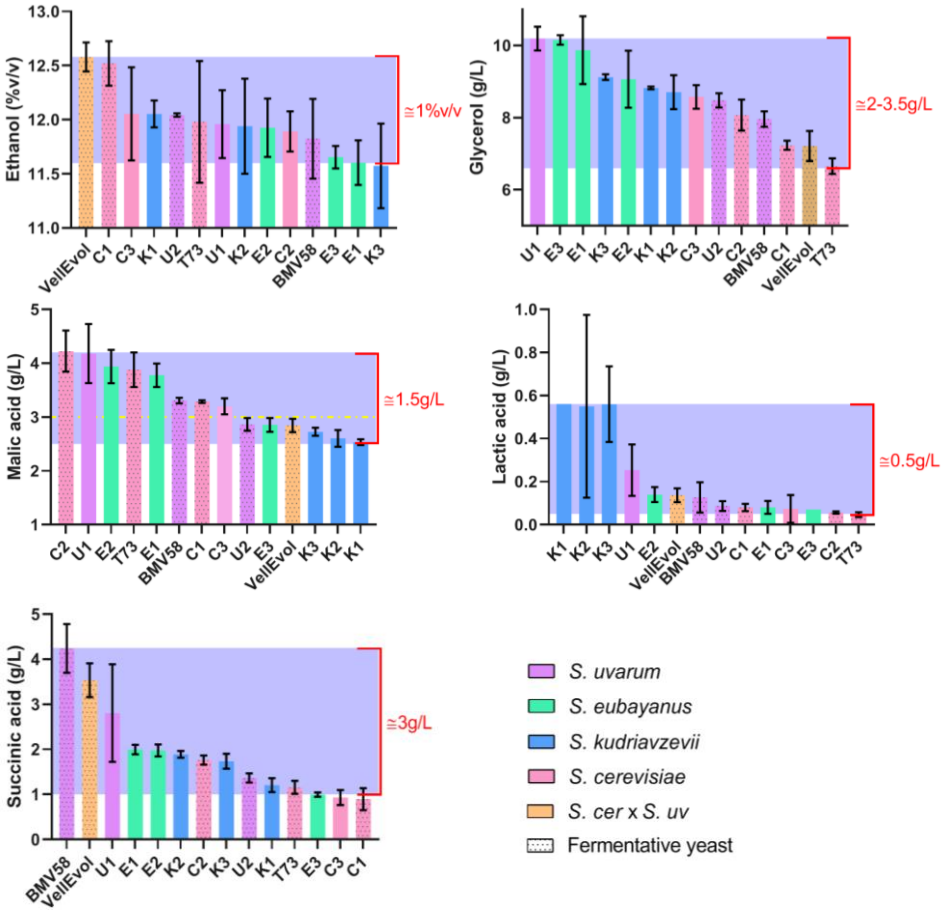


Figure 2. Main metabolites (mean±SD) determined in young wines fermented by the different strains: ethanol (%v/v), glycerol (g/L), malic acid (g/L), succinic acid (g/L) and lactic acid (g/L). The color of each bar indicates the species and dot-filling strains with fermentative origin. The differences between wines with maximum and minimum average values are indicated in red. The dashed yellow line indicates the original malic acid value in must. ANOVA results are presented in the supplementary material (Table S2).

According to these results, we confirmed the ability of *S. uvarum* and *S. kudriavzevii* strains as well as the wild/natural *S. cerevisiae* strains to produce higher amounts of glycerol and organic acids as well as to reduce the ethanol yield (Minebois et al., 2020a, 2020b, 2021; Pérez et al., 2021; Querol et al., 2018). Differences among glycerol and organic acids synthesis (mainly succinic acid) have been related to different metabolic strategies used by the cryophilic non-*cerevisiae*, and wild *S. cerevisiae* strains to maintain the

NAD^+/NADH balance during alcoholic fermentation (Minebois et al., 2020a, 2020b,2021; Oliveira et al., 2014). So, we confirm the applications of non-conventional yeasts to solve the current winemaking demands.

3.3. Aroma analysis

3.3.1. Volatiles lost during fermentation

The aromas volatilized during fermentation were retained into aroma trap systems containing LiChrolut-EN resins installed in the airlocks. The trapped aroma was further eluted with solvent. For olfactometric screening, aliquots from all fermentation extracts were mixed, concentrated by evaporation, and then analyzed by GC-Olfactometry using a sensory panel with six trained judges. This extract representing the "average aroma effluent" from all fermentations was composed of 11 identified odorants given in **Table 2** and by some other weaker odorants with a MF<30% not given in the table.

Odorants in Table 2 are ranked attending to their olfactometric score so that those in the first position are the most intense. Two central observations can be deduced from these data. First, all compounds in the table except o-cresol and 4-mercapto-4-methylpentan-2-one (4MMP) have a fermentative origin. Second, the order of relevance is quite different from that found in a similar experiment with the same semi-synthetic must using commercial strains (Denat et al., 2021). A most remarkable difference is the presence in this extract of 4MMP, a volatile thiol responsible for the "box tree" aroma that characterized Sauvignon Blanc wines (Tominaga et al., 1995). Polyfunctional mercaptans can be released from their non-odorous form by yeast action (Roland et al., 2011; Swiegers and Pretorius, 2007). However, using wine strains in the previous experiment, PFMs were not detected in volatile fraction released during fermentation, which strongly suggests that some of the non-conventional yeasts used in this experiment release it from its specific non-

aromatic precursor since the early stages of fermentation. Also most remarkable is the absence in the list of the Strecker aldehyde isovaleraldehyde, which was the second most intense odorant emitted by commercial *Saccharomyces* yeasts, and together with isobutyraldehyde, a major component of the effluent of those fermentations (Denat et al., 2021).

Table 2. Odorants identified in a mix of volatiles released during all fermentations that were trapped into the LiChrolut-EN cartridges placed in each airlock. Determination of the retentions indexes (RI) in DB-WAX and DB-5 columns, olfatometrics scores (MF%), and odor descriptors.

RI $_{DB-WAX}$	RI $_{DB-5}$	Compound	Odor description*	MF (%)
1224	**<900**	isoamyl alcohol	*Glue, Cheese*	73
1932	**1115**	β-phenylethanol	*Roses, Flowery*	68
953	**<900**	ethyl isobutyrate	*Strawberry Cream*	65
1244	**988**	ethyl hexanoate	*Fruity, Flowery*	61
1037	**<900**	ethyl butyrate	*Strawberry*	59
2030		o-cresol	*Metalic*	58
1388		4-mercapto-4-methylpentan-2-one	*Tropical Fruit, Sweat*	50
1515	**1193**	ethyl octanoate	*Plastic, Woody, Green*	50
974	**<900**	isopropyl acetate	*Strawberry, Fruity*	41
1129	**<900**	isoamyl acetate	*Banana, nail polish*	37
1832	**1263**	β-phenylethyl acetate	*Roses, Flowery*	30

*Odor descriptors correspond to those identified in the mix-sample by the panel that performed the GC-O test.

3.3.2 Aroma composition in young wines

Among the major volatile compounds (22 volatile compounds found at concentrations >0.2 mg/L), 1-hexanol was the only compound that did not present differences between young wines. However, PCA analysis (**Figure 3**) shows that differences between strains were not very large, explaining 38.2% of the total variance. Although all *S. eubayanus* strains were in the up-left part of the plot because of decanoic acid and butanol highest levels, this should not represent any sensory mismatch. Nevertheless, some differences and observations should be mentioned; first, the significant levels of decanoic acid

reached by the three *S. eubayanus* strains, particularly E3 producing 3 mg/L. Secondly, decanoic acid, also known as capric acid, is a known toxic compound able to inhibit the growth of cells by altering their membranes (Lafon-Lafourcade et al., 1984; Viegas et al., 1989).

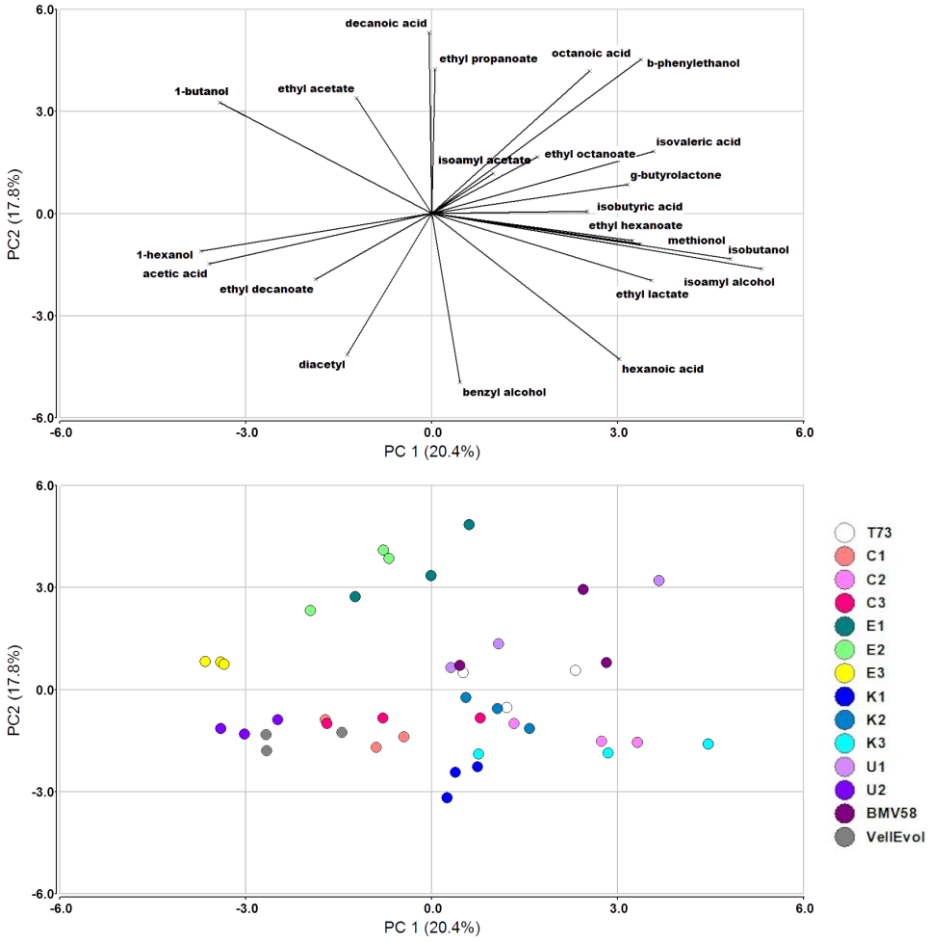
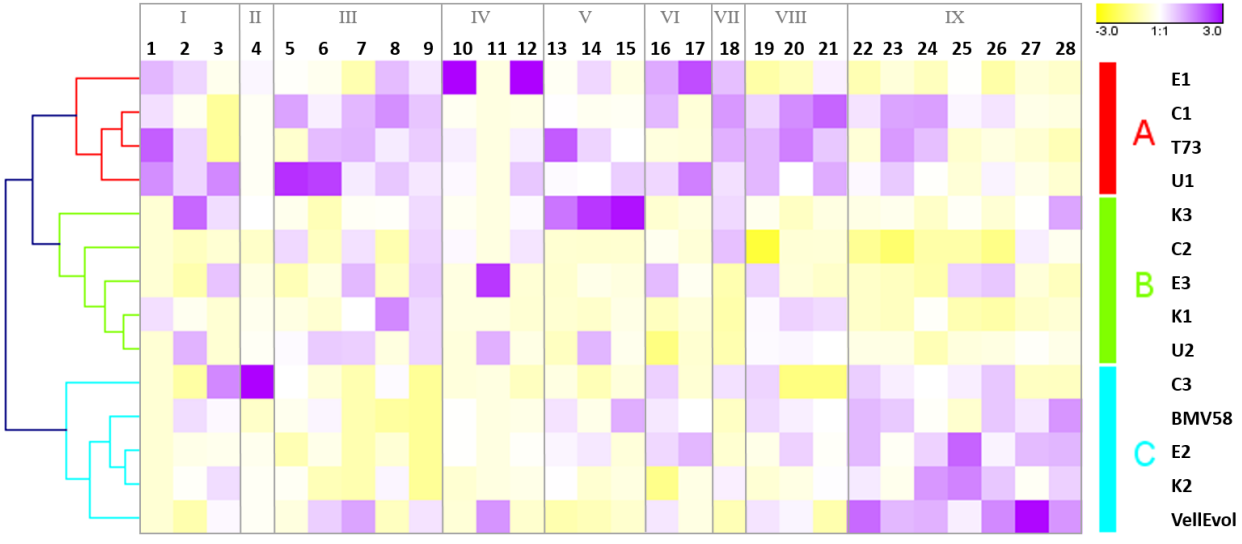


Figure 3. Principal component analysis (PCA) applied on the major aroma compounds (> 0.2 mg/L) determined in young wines fermented by the different strains where each replicate is represented.ANOVA results are presented in the supplementary material (Table S3).

S. kudriavzevii strains contained the highest levels of ethyl lactate, which corresponds with the highest lactic acid production observed. As it is well documented in other works (Minebois et al., 2020a, 2020b; Pérez et al., 2021; Stribny et al., 2015), the species *S. uvarum* (particularly, U1 and BMV58 strains), followed by the *S. eubayanus* (particularly, E1 and E2 strains), were characterized by a high production of β-phenylethanol.

Since the esters are not particularly abundant in red wines, and they are very easily vaporized when fermentation is carried out in little volumes, it should be noted that some positive traits were found among the fruity ethyl esters. Namely, ethyl octanoate was only detected in BMV58 strain, the *S. cerevisiae* C1 produced ethyl decanoate highest levels, and ethyl propanoate was 8 and 4-fold higher in E1 and U1 (0.22 mg/L and 0.12 mg/L, respectively) than in the rest of the strains (0.03 mg/L). The latter result is relevant because ethyl propanoate, not exceeding its perception threshold, can act together with other esters as a "mature fruit" aroma enhancer (Puertas et al., 2018). Finally, K1 strain synthesized diacetyl at higher levels. This compound may give a butter or lactic character to young wine (Ugliano and Henschke, 2009), but it is very reactive towards polyphenols so that it will not survive aging.

The aromatic diversity of trace compounds in young wines introduced by the yeast is summarized in the heatmap shown in Figure 4. Strains can be classified into three main groups. Cluster A contained the yeasts E1, U1, C1, and T73 and is characterized by major levels of C_{13}-norisoprenoids, monoterpenes, vanillin derivatives, acetates, and ethyl leucate. Strains in cluster C were C3, BMV58, E2, K2, and VellEvol had higher levels of varietal volatile phenols, while strains in cluster B seem to be more neutral.



I **C13-norisoprenoides**
1 α-Ionone
2 β-Ionone
3 TDN
II **Benzenic esters**
4 Ethyl dihydrocinnamate
III **Monoterpenes**
5 β-Citronellol
6 Geraniol
7 Linalool
8 Nerol
9 α-Terpineol
IV **Lactones**
10 γ-Nonalactone
11 γ-Decalactone
12 Whiskylactone
V **Ethyl esters**
13 Ethyl isovalerate
14 Ethyl 2-methylbutyrate
15 Ethyl isobutyrate
VI **Acetates**
16 Isobutyl acetate
17 Phenylethyl acetate
VII **Hidroxy esters**
18 Ethvl Leucate
VIII **Vanillin derivatives**
19 Acetovanillone
20 Vanillin
21 Syringaldehyde
IX **Volatile phenols**
22 trans-Isoeugenol
23 Eugenol
24 Guaiacol
25 Syringol
26 Methoxyeugenol
27 4-Vinylguaicol
28 4-Vinylphenol

Figure 4. For each aroma compound determined in the young wines fermented by 14 yeast strains, data were normalized by the average value of all the strains and were represented by a color scale being in yellow the lowest values and violet the highest values of each compound. Using these aroma data, hierarchical grouping was applied to obtain a dendrogram representing the separation of the yeasts according to their similarities in the aroma profiles The values of each concentration and the ANOVA results are presented in Supplementary Material Table S4.

Regarding this group of aroma compounds, measured levels of all of them also fell within the normal range of these compounds in red wines; however, the most significant group of yeasts was cluster A. Within this cluster, the single compound found at sensory-relevant levels and slightly above usual red wine contents (Ferreira et al., 2000) was phenylethyl acetate, found in E1 and U1 close to 1 mg/L (Table S4).Besides the monoterpenes were found at concentrations under their OTs (odor thresholds), it is interesting that *S. uvarum* U1 achieved the maximal levels of β-citronellol and geraniol. This characteristic is attributed to the ability of *S. uvarum* strains to release geraniol from the odorless precursors (Gamero et al., 2011a, 2011b) whereas, some studies have determined that β-citronellol is completely synthesized from geraniol by yeasts (Fernández-González and Di Stefano, 2004; King and Dickinson, 2000; Takoi et al., 2014).

Since Tempranillo is a neutral variety, yeast's role in releasing its aromas in young wines is considered of great significance (Ferreira and López, 2019). Based on the most relevant major and minor aroma compounds found in young wines, we observed that the wild strains of *Saccharomyces* species could improve the aroma profile of Tempranillo. According to the obtained, E1 strain was characterized by ethyl propanoate, lactones, and acetates; C1 strain by vanillin derivatives and ethyl decanoate; K3 strain by fruity branched ethyl esters and β-ionone and U1 by monoterpens.

Table 3. Concentration (μg/L) and odor threshold of trace aroma compounds detected in wines and must contain PAF after aging.

	Must	T73	C1	C2	E1	E2	K1	K2	U1	BMV58	VellEv	OT (μg/L)
C_{13}-norisoprenoids												
α-ionone	0.15±0.03^{ab}	0.13±0.01^{b}	0.13±0.04^{b}	0±0^{c}	0.15±0.01^{ab}	0.18±0.02^{a}	0±0^{c}	0.14±0.01^{ab}	0±0^{c}	0±0^{c}	0±0^{c}	2.6^{III}
β-ionone	**0.33±0.01^{bcd}**	**0.86±0.07^{a}**	**0.43±0.21^{bc}**	**0.18±0.05^{cd}**	**0.86±0.11^{a}**	**0.33±0.02^{bcd}**	**0.34±0.04^{bcd}**	**0.31±0.01^{bcd}**	**0.86±0.08^{a}**	**0.59±0.2^{ab}**	**0.13±0.03^{d}**	0.09^{IV}
TDN	**18.29±4.67^{b}**	**34.1±7.17^{a}**	**25.37±3.75^{ab}**	**35.96±5.62^{a}**	**29.21±4.05^{ab}**	**22.49±2.33^{ab}**	**28.11±5.26^{ab}**	**23.72±1.14^{ab}**	**33.28±5.92^{a}**	**26.41±5.35^{ab}**	**23.49±2.68^{ab}**	2^{VII}
β-damascenone	**11.2±1.36^{b}**	**14.46±0.99^{ab}**	**13.88±0.71^{ab}**	**13.69±1.43^{ab}**	**14.98±0.91^{ab}**	**13.12±0.93^{ab}**	**14.33±0.25^{ab}**	**15.64±0.69^{a}**	**11.96±2.1^{ab}**	**13.47±3.06^{ab}**	**13.36±1.31^{ab}**	0.05^{VI}
vitispirane A and B*	0.4±0.05^{abc}	0.49±0.04^{ab}	0.41±0.04^{abc}	0.51±0.06^{a}	0.46±0.05^{abc}	0.38±0.03^{bc}	0.4±0.04^{abc}	0.37±0.02^{c}	0.46±0.05^{abc}	0.43±0.03^{abc}	0.39±0.04^{bc}	
riesling acetal*	0.23±0.01^{a}	0.23±0.02^{a}	0.22±0.01^{a}	0.22±0.02^{a}	0.23±0.01^{a}	0.2±0.0049^{a}	0.22±0.01^{a}	0.21±0.01^{a}	0.22±0.02^{a}	0.21±0.01^{a}	0.22±0.02^{a}	
Monoterpenes												
β-citronellol	0.17±0.02^{d}	1.01±0.07^{abc}	1.34±0.06^{ab}	0.44±0.09^{cd}	0.93±0.37^{abcd}	0.23±0.06^{cd}	0.79±0.18^{abcd}	0.69±0.11^{bcd}	1.54±0.67^{a}	0.88±0.4^{abcd}	0.54±0.11^{cd}	40^{IX}
geraniol	0±0^{d}	1.28±0.38^{a}	1.05±0.19^{abc}	0.52±0.12^{bcd}	0.76±0.06^{abc}	0.44±0.24^{cd}	0.8±0.06^{abc}	0.7±0.09^{abc}	1.13±0.43^{ab}	0.75±0.08^{abc}	0.71±0.17^{abc}	30^{VI}
linalool	0±0^{b}	2.06±0.12^{a}	0±0^{b}	0±0^{b}	1.83±0.22^{a}	0±0^{b}	1.61±0.42^{a}	0±0^{b}	1.87±0.38^{a}	1.69±0.14^{a}	0±0^{b}	25.2^{IV}
linalool oxide	3.81±0.39^{ab}	4.14±0.4^{a}	3.49±0.52^{ab}	4.14±0.48^{a}	3.72±0.41^{ab}	2.79±0.22^{b}	3.14±0.43^{ab}	2.68±0.24^{b}	3.86±0.42^{ab}	3.45±0.56^{ab}	3.12±0.4^{ab}	4000(*c*)/4000(*t*)^{X}
α-terpineol	4.83±0.74^{d}	11.2±1.04^{ab}	10.55±0.88^{abc}	8.76±2.25^{bcd}	10.36±1.04^{abc}	7.69±0.3^{bcd}	8.95±0.31^{abcd}	6.68±0.33^{cd}	13.15±3.38^{a}	9.14±0.76^{abcd}	10.39±2^{abc}	250^{IV}
Lactones												
γ-nonalactone	0.88±0.44^{b}	2.7±0.3^{a}	2.37±0.11^{a}	2.42±0.41^{a}	2.46±0.15^{a}	2.23±0.05^{a}	2.14±0.09^{a}	2.38±0.25^{a}	2.53±0.24^{a}	2.49±0.26^{a}	2.07±0.09^{a}	30^{XI}
γ-decalactone	0±0^{e}	0.74±0.03^{d}	0.97±0.11^{bcd}	1.03±0.07^{abcd}	1.68±0.55^{a}	1.07±0.1^{abcd}	1.5±0.16^{abc}	1.25±0.26^{abcd}	1.64±0.39^{ab}	1.08±0.12^{abcd}	0.83±0.1^{cd}	88^{II}
whiskylactone	0±0^{f}	5.7±0.69^{bcd}	3.9±0.27^{cde}	5.74±0.77^{bc}	12.6±1.88^{a}	4.07±0.11^{cde}	3.3±0.14^{de}	3.82±0.08^{cde}	7.13±1.47^{b}	3.85±0.74^{cde}	2.9±0.27^{e}	790 (*t*)/67 (*c*)^{XII}
massoia lactone	**1.69±0.04^{b}**	**1.51±0.25^{bc}**	**1.3±0.17^{bc}**	**1.69±0.22^{b}**	**2.37±0.28^{a}**	**1.4±0.13^{bc}**	**1.17±0.11^{bc}**	**1.28±0.16^{bc}**	**1.42±0.33^{bc}**	**1.29±0.19^{bc}**	1.07±0.08^{c}	11^{XIV}
Ethyl esters												
ethyl isovalerate	0±0^{d}	**72.96±9.41^{a}**	**32.19±8.14^{bc}**	**22.46±6.39^{bcd}**	**25.65±8.47^{bcd}**	**28.31±6.31^{bcd}**	**26.82±3.36^{bcd}**	**35.9±1.79^{bc}**	**44.8±23.45^{ab}**	**42.12±13.74^{b}**	**8.74±2.09^{cd}**	3^{IV}
ethyl 2-methylbutyrate	0±0^{d}	**75.42±13.53^{a}**	**34.28±6.66^{bc}**	**27.83±6.48^{bc}**	**48.65±14.07^{ab}**	**50.88±3.98^{ab}**	**27.69±3.14^{bc}**	**31.05±1.77^{bc}**	**50.77±17.3^{ab}**	**42.56±12.41^{b}**	10.52±2.49^{cd}	18^{IV}
ethyl isobutyrate	0±0^{c}	**283.34±39.19^{b}**	**202.76±50.23^{bc}**	**130.01±45.35^{bc}**	**146.69±34.38^{bc}**	**140.72±3.48^{bc}**	**254.33±29.12^{b}**	**205.59±38.65^{bc}**	**556.57±153.05^{a}**	**659.67±201.42^{a}**	**96.05±28.87^{bc}**	15^{IV}
ethyl D/L-leucate	0±0^{c}	**90.71±40.88^{ab}**	**61.4±11.43^{bc}**	**160.09±65.1^{a}**	45.46±6.55^{bc}	40.53±4.06^{bc}	34.88±4.87^{bc}	**73.97±9.89^{bc}**	**69.37±26.42^{bc}**	53.21±18.15^{bc}	34.46±8.15^{bc}	900 (D)/300 (L)^{XIII}

Acetates												
β-phenylethyl acetate	0±0^{c}	**28.95±7.67^{bc}**	**27.38±4.21^{bc}**	24.44±0.46^{bc}	**162.05±21.83^{a}**	**167.11±55.35^{a}**	**33.71±3.46^{bc}**	**42.29±1.73^{bc}**	**78.22±8.85^{b}**	**75.12±4.59^{b}**	**41.42±7.57^{bc}**	250^{VI}
isobutyl acetate	0±0^{f}	23.22±1.2^{abc}	27.18±6.08^{ab}	20.08±7.72^{abcd}	8.77±1.5^{def}	16.37±2.06^{bcde}	20.22±4.65^{abcd}	4.95±0.52^{ef}	10.6±1.19^{cdef}	32.77±10.82^{a}	18.54±0.69^{bcde}	1605^{V}
Vanillin derivatives												
acetovanillone	2.98±1.04^{c}	24.5±2.09^{ab}	26.54±2.21^{a}	20.18±1.46^{b}	23.71±1.44^{ab}	25.75±0.43^{a}	24.97±0.27^{ab}	25.21±3.97^{ab}	25.73±1.55^{a}	24.77±0.96^{ab}	22.04±0.61^{ab}	1000^{I}
vanillin	5.87±0.55^{d}	12.62±1.36^{ab}	14.11±3.32^{a}	8.24±0.69^{bcd}	8.6±1.21^{bcd}	11.02±0.01^{abc}	9.03±1.35^{bcd}	7.53±2.28^{cd}	10.88±1.59^{abc}	8.85±0.68^{bcd}	10.93±0.28^{abc}	200^{VI}
syringaldehyde	145.57±11.09^{ab}	94.93±29.19^{b}	110.53±4.45^{b}	205.07±51.43^{a}	60.21±12.71^{b}	103.67±8.87^{b}	113.15±1.97^{ab}	140.13±58.5^{ab}	139.25±3.42^{ab}	124.97±27.59^{ab}	119.93±53.4^{ab}	50000^{I}
Volatile phenols												
trans-isoeugenol	0±0^{b}	0.41±0.08^{a}	0.5±0.09^{a}	0.34±0.04^{a}	0.34±0.09^{a}	0.39±0.07^{a}	0.42±0.04^{a}	0.36±0.07^{a}	0.43±0.09^{a}	0.45±0.03^{a}	0.4±0.06^{a}	6^{I}
eugenol	0±0^{d}	0±0^{d}	0.51±0.02^{a}	0±0^{d}	0±0^{d}	0±0^{d}	0.33±0.03^{c}	0±0^{d}	0.42±0.04^{b}	0±0^{d}	0.4±0.04^{bc}	6^{IV}
guaiacol	**5.04±0.6^{a}**	**3.47±0.36^{a}**	**5.15±0.87^{a}**	**3.26±0.81^{a}**	**3.86±1.41^{a}**	**3.04±0.58^{a}**	**4.2±0.43^{a}**	**3.4±0.75^{a}**	**5.08±1.12^{a}**	**3.06±0.25^{a}**	**4.82±0.9^{a}**	9.5^{IV}
syringol	**91.86±9.85^{a}**	43.53±9.41^{b}	55.35±4.28^{b}	44.57±7.01^{b}	44.77±21.71^{b}	53.79±12.5^{b}	39.1±4.26^{b}	33.04±8.25^{b}	**57.85±15.8^{b}**	52.57±4.54^{b}	43.77±5.24^{b}	570^{VIII}
methoxyeugenol	1.78±0.23^{d}	3.79±0.64^{ab}	3.88±0.14^{a}	2.86±0.37^{bc}	2.97±0.12^{abc}	2.78±0.28^{c}	2.91±0.33^{abc}	2.46±0.23^{cd}	3.36±0.16^{abc}	3.23±0.51^{abc}	2.77±0.31^{c}	1200^{I}
4-Vinylguaicol	22.18±5.55^{c}	24.13±2.34^{c}	28.46±4.87^{bc}	19.07±4.06^{c}	19.8±2.37^{c}	23.3±0.1^{c}	49.89±2.77^{a}	40.06±11.71^{ab}	23.84±1.66^{c}	26.33±6.2^{bc}	23.38±0.62^{c}	1100^{IV}
4-Vinylphenol	**119.56±23.94^{ab}**	**120.66±25.79^{ab}**	**124.16±17.78^{a}$_{b}$**	**98.22±14.74^{ab}**	**87.75±9.59^{b}**	**114.59±9.94^{ab}**	**151.35±7.88^{a}**	**117.36±41.92^{ab}**	**106.31±6.21^{ab}**	**110.56±17.39^{ab}**	**108.67±6.15^{ab}**	180^{III}

Data are expressed as mean value ± SD (n=3). Different letters in the same row indicate significant differences according to HSD Tukey test (p<0.05).*data expressed as relative area. I, Escudero et al., 2007; II, Etievant, 1991; III, Boidron et al, 1988; IV, Ferreira et al., 2000; V, Ferreira et al., 2002; VI, Guth, 1997; VII, Sacks et al, 2012; VIII, López et al, 2002; IX, Ohloff, 1978; X, Ribéreau-Gayon et al, 1975; XI, Nakamura et al, 1988; XII, Otsuka et al, 1973; XIII, Falcao et al, 2012; XIV, Pons et al, 2017; XV, Simpson, 1978 . Each compound whose concentration exceeded its threshold of perception about OAV>0.1 , marked in bold type.

3.3.3 Trace aroma compounds in aging wines

Accelerated anoxic aging (5 weeks at 50ºC) was applied only with the ten strains that presented a better fermentation capability and to the unfermented control must. As expected, aging involved an intense aroma formation, and many aroma compounds reached sensory-relevant concentrations, particularly true for two norisoprenoids, and the ethyl esters of branched acids.

The two C_{13}-norisoprenoids β-ionone and β-damascenone reach maxima levels close to 1 ppb and 16 ppb, respectively (Table 3). These two aroma compounds play the most relevant role in red wine aroma. β-ionone is a powerful aroma usually found in red wines providing floral, red, or dark berry aroma notes (Tomasino and Bolman, 2021), and β-damascenone is a potent aroma enhancer and modulator (Pineau et al., 2007). At low concentrations, this compound contributes to fresh fruity notes, and at higher levels, it can increase the ripen character of the fruit (San-Juan et al., 2011). Its effects are strongly dependent on the sensory context (Tomasino and Bolman, 2021). The levels of both compounds found in the present work are exceptionally high (San-Juan et al., 2012). The effects of the yeast strain were very high in the case of β-ionone, whose levels ranged from 0.13 in VellEv to 0.86 in T73, E1, and U1, and moderate in the case of β-damascenone (Table 3). Levels between yeasts were not significantly different, as all yeasts produced levels of this compound above those found in the unfermented control. This result confirms that yeast cannot produce this compound but can accelerate its release, as recently reported (Denat et al., 2021).

Levels of ethyl esters of branched acids are also relatively high compared to commercial *S. cerevisiae* strains, such as the levels of ethyl leucate (Denat et al., 2021). In any case, levels of these families of esters increase with aging because they are slowly formed by esterification with

ethanol of their corresponding acids (Díaz-Maroto et al., 2005). Differences between maxima and minima levels reached were close to 9, 7.5, 7.0, and 5-fold in ethyl isovalerate, 2-methylbutyrate, isobutyrate, and leucate, respectively, depending on the strain in all cases (Table 3). Ethyl isovalerate and ethyl 2-methylbutyrate were maximal in T73, followed by U1 and BMV58, with the highest ethyl isobutyrate concentrations. In contrast, the maxima levels of ethyl leucate were found in C2. Some authors have suggested, generally working with elementary models, that some of the individual ethyl esters could play a role in the specific aroma nuances of wines (Lytra et al., 2012). However, it has been recently demonstrated that 14 different fruity ethyl esters (including the ethyl esters of linear fatty acids) qualitatively integrate within a unique fruity descriptor (De-la-Fuente-Blanco et al, 2020), and the intensity is the addition of the individual aromas (Ferreira et al., 2021). Therefore, the aroma of these compounds is the nuclear part of the fruity perception in red wines, and the results presented here reveal the most notable differences between yeast strains.

To summarize, when aging was applied to young wines, a synergic effect with some non-conventional yeasts was observed, favoring the release and generation of compounds of relevant aroma impact and importance for the aged wines. Some of the red-fruit smelling compounds were ethyl leucate and ethyl isobutyrate, which were found at high levels in C2 and U1 strain, respectively. While another important compound such as β-ionone, with floral-fruity notes, was highly increased by U1 and E1 yeasts.

4. Conclusion

In this study, *S. cerevisiae* and *S.* non-*cerevisiae* strains isolated from wild and fermentative sources were used to ferment synthetic must with the addition of odorless aroma precursors and phenolic compounds extracted from Tempranillo grapes. Then, accelerated anoxic aging was applied to the young

wines. The main objective was to determine the aromatic diversity introduced by using non-wine strains in young and aged Tempranillo wines.

Considering the aromas volatilized during fermentation, the presence of 4MMP showed that the non-conventional yeasts used could have a greater capacity to release these compounds than the commercial strains.

The young wines obtained with non-wine strains as U1 (*S. uvarum*), E1 (*S. eubayanus*), C3, C1 (*S. cerevisiae*), and K3 (*S. kudriavzevii*) were characterized by the highest levels of fruity ethyl esters, acetates, monoterpenes, and C_{13}-norisoprenoids. *S. uvarum* strains were also characterized by the ability to release or novo-produced geraniol and citronellol, as well as by the highest production of β-phenylethyl acetate, as has been previously described by other authors. In addition, *S. eubayanus* strains can release high levels of this acetate in young and aged wines. Due to their non-fermentative origin, these yeasts showed lower fermentative capacity compared to the wine *S. cerevisiae* strain. However, they were able to complete the fermentation process and the resulting wines reached enological parameters of interest in the wine industry (lower ethanol level, higher organic acid, and glycerol content).

Several compounds were highly enhanced during aging by the yeast action. T73, U1, and BMV58 strains exhibited important aging compounds: ethyl isobutyrate, ethyl 2-methylbutyrate, and ethyl isovalerate. E1, T73, and U1 strains were shown to accelerate the release of β-ionone, reaching the highest levels. In addition, aging allowed some yeast that had not been prominent in the young wines to develop relevant aromas after aging, as the case of the highest production of ethyl leucate determined in C2 aged wine.

Therefore, according to the results obtained in this study and considering the current consumer preferences for more complex aromatic

profiles, wild non-wine and cryotolerant strains would be recommended for the wine industry.

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement number 764364, Aromagenesis, and from Generalitat Valenciana grant PROMETEO/2020/014. We are also grateful to Christian A. Lopes of the Universidad Nacional del Comahue, Argentina, and Francisco Cubillos of the Universidad de Santiago de Chile for kindly providing the use of yeast strains from their collection (NPCC and LGMUSC).

References

Alegre, Y., Arias-Pérez, I., Hernández-Orte, P., Ferreira, V. (2020a). Development of a new strategy for studying the aroma potential of winemaking grapes through the accelerated hydrolysis of phenolic and aromatic fractions (PAFs). *Food Research International*, *127*(September 2019), 108728. https://doi.org/10.1016/j.foodres.2019.108728

Alegre, Y., Sáenz-Navajas, M. P., Hernández-Orte, P., Ferreira, V. (2020b). Sensory, olfactometric and chemical characterization of the aroma potential of Garnacha and Tempranillo winemaking grapes. *Food Chemistry*, *331*(May). https://doi.org/10.1016/j.foodchem.2020.127207

Arroyo-López, F. N., Salvadó, Z., Tronchoni, J., Guillamón, J. M., Barrio, E., & Querol, A. (2010). Susceptibility and resistance to ethanol in *Saccharomyces* strain isolated from wild and fermentative environments. *Yeast*, (September), 191–198. https://doi.org/10.1002/yea

Charters, S., Pettigrew, S. (2007). The dimensions of wine quality. *Food Quality and Preference*, *18*(7), 997–1007. https://doi.org/10.1016/j.foodqual.2007.04.003

Cordente, A. G., Schmidt, S., Beltran, G., Torija, M. J., Curtin, C. D. (2019). Harnessing yeast metabolism of aromatic amino acids for fermented beverage bioflavouring and bioproduction. *Applied Microbiology and Biotechnology*, *103*(11), 4325–4336. https://doi.org/10.1007/s00253-019-09840-w

Dravnieks, A. (1985). Atlas of odor character profiles. Philadelphia: ASTM.

De-la-Fuente-Blanco, A., Sáenz-Navajas, M. P., Valentin, D., Ferreira, V. (2020). Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration. *Food Chemistry*, *307*(April 2019), 125553. https://doi.org/10.1016/j.foodchem.2019.125553

Denat, M., Pérez, D., Heras, J. M., Querol, A., Ferreira, V. (2021). The effects of *Saccharomyces cerevisiae* strains carrying alcoholic fermentation on the fermentative and varietal aroma profiles of young and aged Tempranillo wines. *Food Chemistry*, *9*(December 2020). https://doi.org/10.1016/j.fochx.2021.100116

Díaz-Maroto, M. C., Schneider, R., Baumes, R. (2005). Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. *Journal of Agricultural and Food Chemistry*, *53*(9), 3503–3509. https://doi.org/10.1021/jf048157o

Falcao, L. D., Lytra, G., Darriet, P., Barbe, J. C. (2012). Identification of ethyl 2-hydroxy-4-methylpentanoate in red wines, a compound involved in blackberry aroma. *Food Chemistry*, *132*(1), 230–236. https://doi.org/10.1016/j.foodchem.2011.10.061

Fernández-González, M., Di Stefano, R. (2004). Fractionation of glycoside aroma precursors in neutral grapes. Hydrolysis and conversion by *Saccharomyces*

cerevisiae. *LWT - Food Science and Technology*, *37*(4), 467–473. https://doi.org/10.1016/j.lwt.2003.11.003

Ferreira, V., López, R., Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. *Journal of the Science of Food and Agriculture*, *80*(12), 1659–1667.

Ferreira, V., Ortín, N., Escudero, A., López, R., & Cacho, J. (2002). Chemical characterization of the aroma of Grenache rosé wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *Journal of Agricultural and Food Chemistry*, *50*(14), 4048–4054. https://doi.org/10.1021/jf0115645

Ferreira, V., López, R. (2019). The actual and potential aroma of winemaking grapes. *Biomolecules*, *9*(12). https://doi.org/10.3390/biom9120818

Ferreira, V., de-la-Fuente-Blanco, A., Sáenz-Navajas, M. P. (2021). Odorants to interpret complex aroma systems. Application to model wine aroma. *Foods*, *10*(1627), 1–19. https://doi.org/https://doi.org/10.3390/foods10071627

Gamero, A., Hernández-Orte, P., Querol, A., Ferreira, V. (2011a). Effect of aromatic precursor addition to wine fermentations carried out with different *Saccharomyces* species and their hybrids. *International Journal of Food Microbiology*, *147*(1), 33–44. https://doi.org/10.1016/j.ijfoodmicro.2011.02.035

Gamero, A., Manzanares, P., Querol, A., Belloch, C. (2011b). Monoterpene alcohols release and bioconversion by *Saccharomyces* species and hybrids. *International Journal of Food Microbiology*, *145*(1), 92–97.https://doi:10.1016/j.ijfoodmicro.2010.11.034

Hernández-Orte, P., Bely, M., Cacho, J., Ferreira, V. (2006). Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different *Saccharomyces cerevisiae* strains during fermentation in controlled synthetic media. *Australian Journal of Grape and Wine Research*, *12*(2), 150–160.

Hernández-Orte, P., Cersosimo, M., Loscos, N., Cacho, J., García-Moruno, E., Ferreira, V. (2008). The development of varietal aroma from non-floral grapes by yeasts of different genera. *Food Chemistry*, *107*(3), 1064–1077. https://doi:10.1016/j.foodchem.2007.09.032

Hjelmeland, A. K., Ebeler, S. E. (2015). Glycosidically bound volatile aroma compounds in grapes and wine: A review. *American Journal of Enology and Viticulture*, *66*(1), 1–11. https://doi.org/10.5344/ajev.2014.14104

King, A., Dickinson, J. R. (2000). Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Kluyveromyces lactis*. *Yeast*, *16*(6), 499–506. https://doi.org/10.1002/(SICI)1097-0061(200004)16:6<499::AID-YEA548>3.0.CO;2-E

Lafon-Lafourcade, S., Geneix, C., Ribereau-Gayon, P. (1984). Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their

elimination by yeast ghosts. *Applied and Environmental Microbiology*, *47*(6), 1246–1249. https://doi.org/10.1128/aem.47.6.1246-1249.1984

Liu, J., Zhu, X. L., Ullah, N., Tao, Y. S. (2017). Aroma glycosides in grapes and wine. *Journal of Food Science*, *82*(2), 248–259. https://doi.org/10.1111/1750-3841.13598

López, R., Aznar, M., Cacho, J., Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A*, *966*(1–2), 167–177. https://doi.org/10.1016/S0021-9673(02)00696-9

López, R., Ezpeleta, E., Sánchez, I., Cacho, J., Ferreira, V. (2004). Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry. *Food Chemistry*, *88*(1), 95–103. https://doi.org/10.1016/j.foodchem.2004.01.025

Lytra, G., Tempere, S., De Revel, G., Barbe, J. C. (2012). Distribution and organoleptic impact of ethyl 2-hydroxy-4-methylpentanoate enantiomers in wine. *Journal of Agricultural and Food Chemistry*, *60*(6), 1503–1509. https://doi.org/10.1021/jf204378u

Minebois, R., Pérez-Torrado, R., Querol, A. (2020a). A time course metabolism comparison among *Saccharomyces cerevisiae*, *S. uvarum* and *S. kudriavzevii* species in wine fermentation. *Food Microbiology*, *90*, 1–14. https://doi.org/10.1016/j.fm.2020.103484

Minebois, R., Pérez-Torrado, R. Querol, A. (2020b). Metabolome segregation of four strains of *Saccharomyces cerevisiae*, *Saccharomyces uvarum* and *Saccharomyces kudriavzevii* conducted under low temperature oenological conditions. *Environmental Microbiology*, *22*(9), 3700–3721. https://doi.org/10.1111/1462-2920.15135

Minebois, R., Lairón-Peris, M., Barrio, E., Pérez-Torrado, R., Querol, A. (2021). Metabolic differences between a wild and a wine strain of *Saccharomyces cerevisiae* during fermentation unveiled by multi-omic analysis. *Environmental Microbiology*, *23*, 3059–3076. https://doi.org/10.1111/1462-2920.15523

Oliveira, B. M., Barrio, E., Querol, A., Pérez-Torrado, R. (2014). Enhanced enzymatic activity of glycerol-3-phosphate dehydrogenase from the cryophilic *Saccharomyces kudriavzevii*. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0087290

Origone, A. C., del Mónaco, S. M., Ávila, J. R., González Flores, M., Rodríguez, M. E., Lopes, C. A. (2017). Tolerance to winemaking stress conditions of Patagonian strains of *Saccharomyces eubayanus* and *Saccharomyces uvarum*. *Journal of Applied Microbiology*, *123*(2), 450–463. https://doi.org/10.1111/jam.13495

Ortega, C., López, R., Cacho, J., Ferreira, V. (2001). Fast analysis of important wine volatile compounds - Development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane

microextracts. *Journal of Chromatography A*, *923*(1–2), 205–214. https://doi.org/10.1016/S0021-9673(01)00972-4

Pérez, D., Jaehde, I., Guillamón, J. M., Heras, J. M., Querol, A. (2021). Screening of *Saccharomyces* strains for the capacity to produce desirable fermentative compounds under the influence of different nitrogen sources in synthetic wine fermentations. *Food Microbiology*, *97*(September 2020), 103763. https://doi.org/10.1016/j.fm.2021.103763

Pineau, B., Barbe, J. C., Van Leeuwen, C., Dubourdieu, D. (2007). Which impact for β-damascenone on red wines aroma? *Journal of Agricultural and Food Chemistry*, *55*(10), 4103–4108. https://doi.org/10.1021/jf070120r

Pons, A., Allamy, L., Lavigne, V., Dubourdieu, D., Darriet, P. (2017). Study of the contribution of massoia lactone to the aroma of Merlot and Cabernet Sauvignon musts and wines. *Food Chemistry*, *232*, 229–236. https://doi.org/10.1016/j.foodchem.2017.03.151

Puertas, B., Jimenez-Hierro, M. J., Cantos-Villar, E., Marrufo-Curtido, A., Carbú, M., Cuevas, F. J., … Ruiz-Moreno, M. J. (2018). The influence of yeast on chemical composition and sensory properties of dry white wines. *Food Chemistry*, *253* (June 2017), 227–235. https://doi.org/10.1016/j.foodchem.2018.01.039

Querol, A., Pérez-Torrado, R., Alonso-del-Real, J., Minebois, R., Stribny, J., Oliveira, B. M., Barrio, E. (2018). New trends in the uses of yeasts in oenology. In *Advances in Food and Nutrition Research* (1st ed., Vol. 85, pp. 177–210). https://doi.org/10.1016/bs.afnr.2018.03.002

Redzepovic, S., Orlic, S., Majdak, A., Kozina, B., Volschenk, H., & Viljoen-Bloom, M. (2003). Differential malic acid degradation by selected strains of *Saccharomyces* during alcoholic fermentation. *International Journal of Food Microbiology*, *83*(1), 49–61. https://doi.org/10.1016/S0168-1605(02)00320-3

Roland, A., Schneider, R., Razungles, A., Cavelier, F. (2011). Varietal thiols in wine: Discovery, analysis and applications. *Chemical Reviews*, *111*(11), 7355–7376. https://doi.org/10.1021/cr100205b

Rollero, S., Mouret, J. R., Bloem, A., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J. M., … Camarasa, C. (2017). Quantitative 13C-isotope labelling-based analysis to elucidate the influence of environmental parameters on the production of fermentative aromas during wine fermentation. *Microbial Biotechnology*, *10*(6), 1649–1662. https://doi.org/10.1111/1751-7915.12749

Sacks, G. L., Gates, M. J., Ferry, F. X., Lavin, E. H., Kurtz, A. J., Acree, T. E. (2012). Sensory threshold of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and concentrations in young Riesling and non-Riesling wines. *Journal of Agricultural and Food Chemistry*, *60* (12), 2998–3004. https://doi.org/10.1021/jf205203b

San-Juan, F., Pet'ka, J., Cacho, J., Ferreira, V., Escudero, A. (2010). Producing headspace extracts for the gas chromatography–olfactometric evaluation of wine aroma. *Food Chemistry*, 123(1), 188–195. https://doi.org/10.1016/j.foodchem.2010.03.129.

San-Juan, F., Ferreira, V., Cacho, J., Escudero, A. (2011). Quality and aromatic sensory descriptors (mainly fresh and dry fruit character) of Spanish red wines can be predicted from their aroma-active chemical composition. *Journal of Agricultural and Food Chemistry*, *59*(14), 7916–7924. https://doi.org/10.1021/jf1048657

San-Juan, F., Cacho, J., Ferreira, V., Escudero, A. (2012). Aroma chemical composition of red wines from different price categories and its relationship to quality. *Journal of Agricultural and Food Chemistry*, *60*(20), 5045–5056. https://doi.org/10.1021/jf2050685

Stribny, J., Gamero, A., Pérez-Torrado, R., Querol, A. (2015). *Saccharomyces kudriavzevii* and *Saccharomyces uvarum* differ from *Saccharomyces cerevisiae* during the production of aroma-active higher alcohols and acetate esters using their amino acidic precursors. *International Journal of Food Microbiology*, *205*, 41–46. https://doi.org/10.1016/j.ijfoodmicro.2015.04.003

Swiegers, J. H., Pretorius, I. S. (2007). Modulation of volatile sulfur compounds by wine yeast. *Applied Microbiology and Biotechnology*, *74*(5), 954–960. https://doi.org/10.1007/s00253-006-0828-1

Takoi, K., Itoga, Y., Takayanagi, J., Kosugi, T., Shioi, T., Nakamura, T., Watari, J. (2014). Screening of geraniol-rich flavor hop and interesting behavior of β-citronellol during fermentation under various hop-addition timings. *Journal of the American Society of Brewing Chemists*, *72*(1), 22–29. https://doi.org/10.1094/ASBCJ-2014-0116-01

Tomasino, E., Bolman, S. (2021). The potential effect of β-ionone and β-damascenone on sensory perception of pinot noir wine aroma. *Molecules*, *26*(5). https://doi.org/10.3390/molecules26051288

Tominaga, T., Masneuf, I., Dubourdieu, D. (1995). Mise en évidence d'un s-conjugué de la cystéine, précurseur d'arôme du Sauvignon. *Journal International Des Sciences de La Vigne et Du Vin*, *29*(4), 227–232.

Ugliano, M., (2009) Enzymes in winemaking. In: *Wine Chemistry and Biochemistry*. Springer, New York, pp. 103–126.

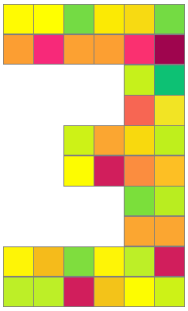
Ugliano, M., Henschke, P. A. (2009). Yeast and wine flavour. In *Wine Chemistry and Biochemistry* In: *Wine Chemistry and Biochemistry*. Springer, New York, pp. 313–392.

Viegas, C. A., Rosa, M. F., Correia, I. S., Novais, J. M. (1989). Inhibition of yeast growth by octanoic and decanoic acids produced during ethanolic fermentation. *Applied and Environmental Microbiology*, *55*(1), 21–28. https://doi.org/10.1128/aem.55.1.21-28.1989

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016). *Understanding Wine Chemistry*. John Wiley & Sons.

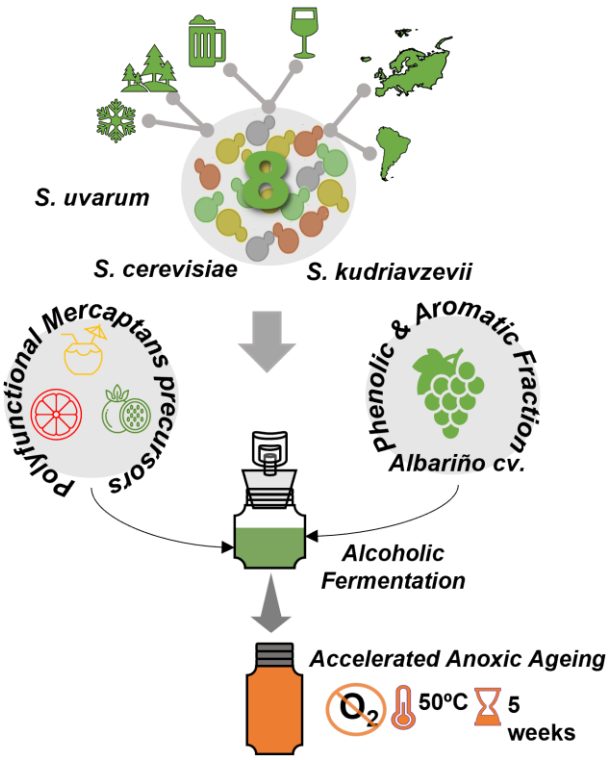
Zwietering, M. H., Jongenburger, I., Rombouts, F. M., Van't Riet, K. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology*, *56*(6), 1875–1881. https://doi.org/0099-2240/90/061875-07$02.00/0

Chapter 3



Modulation of aroma and chemical composition of Albariño semi-synthetic wines by non-wine *Saccharomyces* yeasts and bottle aging

Pérez, Dolores; Denat Marie; Minebois, Romain; Heras José María; Guillamón José Manuel; Ferreira, Vicente; Querol Amparo



Published in Food Microbiology (2022)
DOI: 10.1016/j.ijfoodmicro.2022.109554

Abstract

Saccharomyces yeasts from different origins and species fermented in a semi-synthetic must containing aroma precursor of cv. Albariño and polyfunctional mercaptans precursors. The resulting wines were subjected to accelerate anoxic aging. Afterward, aroma profiles were analyzed by distinct gas chromatography methodologies.

Cryotolerant strains showed better fermentation performances with significant differences in volatile and non-volatile fermentation products than *Saccharomyces cerevisiae* (*S. cerevisiae*). We suggested that the highest levels γ-butyrolactone and diethyl succinate in *Saccharomyces uvarum* (*S. uvarum*) strains, together with their substantial succinic acid yields, could be related to greater flux through the GABA shunt. These strains also had the highest production of β-phenylethyl acetate, geraniol, and branched-chain ethyl esters. The latter compounds were highly increased by aging, while acetates and some terpenes decreased. *S. kudriavzevii* strains showed a remarkable ability to release polyfunctional mercaptans, with SK1 strain yielding up to 47-fold and 8-fold more 4-methyl-4-mercaptopentan-2-one (4MMP) than *S. cerevisiae* and *S. uvarum* strains, respectively. The wild *S. cerevisiae* beer isolate showed a particular aroma profile due to the highest production of ethyl 4-methylvalerate (lactic and fruity notes), γ-octalactone (coconut), and furfurylthiol (roasted coffee). The latter compound is possibly produced from the pentose phosphate pathway (PPP). Since erythritol, another PPP intermediate was largely produced by this strain.

1. Introduction

The aroma of white wines is an essential factor defining their quality and varietal character. It results from the sensory contribution of aromatic metabolites proceeding from grapes (varietal aromas) and fermentation, including those produced during alcoholic fermentation and bottle aging. Among the most important varietal aroma compounds family, we found terpenes, C_{13}-norisoprenoids, and polyfunctional mercaptans (also known as thiols) (Parker et al., 2017). In grape musts, these varietal aromas can be found in a free (i.e., volatile) state or a non-volatile state when linked to a so-called non-volatile varietal precursor, except for polyfunctional mercaptans, which are only found in non-volatile form (Roland et al., 2011). This distinction allows discriminating between neutral (or non-aromatic) grapevines whose varietal aromas composition is mainly made of linked aromatic compounds and aromatic grape varieties containing a substantial fraction of volatile varietal aromas (Ferreira and López, 2019).

During winemaking, two mechanisms can participate in releasing the odorous compounds linked to varietal precursors. On the one hand, acid-catalyzed hydrolysis, resulting from the acidic nature of grape must, occurs throughout the winemaking process and participates in the release of bound-aromas (López et al., 2004; Liu et al., 2017). On the other hand, enzymatic hydrolysis of bound aroma compounds can be carried out by enzymes provided by grapes or, to a lesser extent, by *Saccharomyces cerevisiae*, the main species used in winemaking (Fernández-González et al., 2003, Ugliano, 2009; Sieiro et al., 2014). Further reactions differentially affect aromas during bottle aging, such as esterification, chemical rearrangements, and hydrolysis. These chemical reactions mainly occur in the absence of oxygen, at low pH, and are time-dependent (Ferreira and López, 2019). Besides, it has recently been determined that the yeast strain used during alcoholic fermentation also

affects the way aromas are modulated during bottle aging, like the level of massoia lactone, guaiacol, or TDN produced (Oliveira and Ferreira, 2019; Denat et al., 2021). Similarly, esters produced by yeast during alcoholic fermentation are further differentially affected by aging. For instance, while straight-chain esters are rapidly hydrolyzed, branched ethyl esters increase by esterification with their free fatty acids (Díaz-Maroto et al., 2005).

In this regard, yeast strains other than *Saccharomyces cerevisiae* have gained attention in wine research not only for their ability to produce higher levels of fermentative aromas (e.g., fusel alcohols and esters) (Querol et al. 2018). In addition, they could contribute to liberating a higher quantity of bounded varietal aromas, thus providing wines with more complex flavor profiles (Swiegers et al., 2005; Oliveira and Ferreira, 2019; Borren and Tian, 2021; Feng et al., 2021). For example, cryotolerant species *S. uvarum* can release terpenes due to its enhanced hydrolytic activity (Ugliano et al., 2006) and biosynthesize some of them from sugar metabolism (Fernández-González and Di Stefano, 2004; Gamero et al., 2011). Likewise, *S. uvarum* and *S. kudriavzevii* species in their interspecific hybrid forms with *S. cerevisiae* exhibit a strong capacity to release polyfunctional mercaptans from their odorless grape precursors during wine fermentation (Murat et al., 2001; Dubourdieu et al., 2006). Finally, *S. uvarum* and *S. kudriavzevii* species, along with some wild strains of *S. cerevisiae,* have also been characterized as high-producers of relevant fermentative by-products such as glycerol, erythritol, succinic acid, 2,3-butanediol, ethyl, and acetate esters that could contribute to improving the aromatic and organoleptic quality of the wine (Oliveira et al., 2014; Stribny et al., 2015; Minebois et al., 2020).

In this context, the present work attempts to study how non-wine strains of different *Saccharomyces* species and bottle aging modulate the production of a high range of volatile compounds in *Vitis vinifera* L. Albariño wines. This cultivar was selected for this study because it exhibits a high content of

monoterpenes, C_{13}-norisoprenoids, and polyfunctional mercaptans. These aromas constitute an important varietal aroma reserve that can be significantly affected by the aforementioned factors (Vilanova and Vilariño, 2006; Mateo-Vivaracho et al., 2010; Carrascosa et al., 2012). Additionally, by combining the aroma data with that of the rest of the fermentative by-products quantified, we propose several hypotheses of metabolic pathways involved in producing some varietal flavors still little described in the literature.

2. Materials and Methods

2.1 Fermenters setup

2.1.1 Semi-synthetic must composition

The synthetic grape must was prepared as reported Hernández-Orte et al. (2006) with some modifications. Reducing sugars, 100 g/L glucose + 100 g/L fructose; 2.5 g/L L-tartaric acid; 0.4 g/L citric acid; 3 g/L L-malic acid. The nitrogen composition was prepared as described Hernández-Orte et al. (2002), simulating the nitrogen content profile of Spanish varieties. Synthetic glutathionylated (Glu) and cysteinylated (Cys) precursors of volatile mercaptans compounds were added in the following composition and proportions: 0.1 mg/L of Cys-MH, 0.05 mg/L Cys-MMP, 1 mg/L Glu-MH, 0.05 mg/L Glu-MMP. This precursor solution was added as they are estimated to be removed together with the amino acid fraction during the extraction of the Albariño aromatic and phenolic fraction (PAF). Then, the pH of the must was adjusted to 3.3 and filtered by sterilization (0.2 μm).

Phenolic and Aroma extract from Albariño grapes (PAF) was provided by Zaragoza University (Spain) in ethanolic solution (45% v/v of ethanol) prepared as described by Alegre et al. (2020). Once the volume of ethanol was removed by vacuum, it was replaced by the same amount of sterile water and was added to the must (100 mL/L) just before inoculation. This 10%

PAF addition represents the initial quantity of grapes from which the concentrated alcoholic solution was obtained.

2.1.2 Yeast strains and fermentation conditions

The yeast strains used in this study belonged to the species *S. cerevisiae, S. uvarum,* and *S. kudriavzevii*. Lalvin MSB™, Lalvin T73™, and BMV58™ are wine strains provided by Lallemand Bio SL, Spain. The commercial *Saccharomyces cerevisiae* strains T73 and MSB were included as controls. T73 is a reference wine and commercial strain that we usually employ in our studies; MSB is a wine and commercial strain isolated in New Zealand as a great thiol producer. The rest of the strains were isolated from natural habitats and spontaneous fermentations (**Table 1**).

Table 1. Natural and commercial *Saccharomyces cerevisiae*, *Saccharomyces kudriavzevii,* and *Saccharomyces uvarum* strains used in this study.

Species	Code used	Source of isolation	Geographic origin
S. cerevisiae	T73	Wine, Commercial (Lallemand)	Spain
	MSB	Wine, Commercial (Lallemand)	New Zealand
	SC1	Cachaça fermentation	Brazil
	SC2	Sorghum beer	Burkina Faso
S. kudriavzevii	SK1	Monosporic derivative of CR89, oak (*Q. Faginea)*	Spain
	SK3	Monosporic derivative of CA111, oak (*Q. Faginea)*	Spain
S. uvarum	BMV58	Wine, Commercial (Lallemand)	Spain
	U1	Non fermented liquor (Mistela)	Spain

Fermentations were carried out in triplicates at 16ºC in 100 mL-glass flasks containing 70 mL of must, a stirrer magnet (100 rpm), and closed with an airlock valve. Each fermenter was inoculated with the pre-cultures at an initial 1×10^6 cells/mL population. In addition, the same non-inoculated grape must was used as a control during fermentation and aging processes and is referred to as "young must" and "aged must" in the following sections.

Fermentation progress was monitored by measuring daily weight loss and sugar consumption, and the total yeast population was monitored by flow cytometry. The obtained curves were fitted to the non-linear regression Gompertz model (Zwietering et al., 1990). The process was considered finished at residual sugars < 1 g/L.

Inside a free-O_2 chamber (Jacomex, Dagneux, France), 18 ml-vials containing each wine sample were placed in plastic bags with oxygen scavengers, and then, bags were vacuum-sealed. Once the samples were free-O_2 conditioned, they were incubated at 50ºC for 5 weeks for the accelerated bottle aging (Vela et al., 2017; Oliveira and Ferreira, 2019).

2.2 Analysis of main metabolites

Concentrations of ethanol (% v/v), glycerol (g/L), erythritol (g/L), succinic, citric, and malic acid (g/L), glucose (g/L), fructose (g/L) were analyzed in the finished wines and during fermentation by HPLC (High-Performance Liquid Chromatography, Thermo Fisher Scientific, Waltham, MA, USA) using the same methodology, standard calibration curves and conditions previously described in Pérez et al. 2021. Additionally, pH levels were determined in the final wines.

2.3 Determination of volatile aromatic compounds in young and aged wines samples

Only in young samples (immediately after fermentation) higher alcohols, volatile fatty acids, and major esters, which are usually present in concentrations above 0.2 mg/L, were acquired by liquid-liquid microextraction and analyzed by GC-FID (Gas Chromatography with flame ionization detector), following the methodology of Ortega et al. 2001.

Polyfunctional mercaptans were analyzed as described in Mateo-Vivaracho et al. (2010) with some modifications. Briefly, the sample extract

was obtained by derivatization, in solid-phase extraction (SPE) in which deuterated analytes as internal standards were added: 3MH-d5 at 700 ppt, 3MHA-d5 at 200 ppt, 4MMP-d10 at 100 ppt (EptesSarl, Switzerland). After washing with brine and drying with anhydrous sodium sulfate, extracts were injected into the GC-GC-MS (NCI) system. Concentrations were obtained using a response factor calculated by analyzing table wines spiked with known amounts of the analytes.

Minor aromatic compounds (0.1-200 μg/L) in aged and young wines were acquired by SPE following the protocol described by López et al. 2002. Their identification and quantification were carried out using a GC coupled to a mass detector (Shimadzu QP2010, Quioto, Japan).

2.4 Statistical Analysis

Each metabolite was expressed as the arithmetic means of biological triplicates with its corresponding standard deviation. One-way ANOVA followed by Tukey's HSD test was applied, considering statistical significance when the *p*-value was below 0.05. A hierarchical clustering (Euclidean distance and average linkage) was applied to determine the distribution and grouping of treatments according to the multiple variables. All statistical analyses and plots were obtained using Infostat software, version 2011 (Grupo Infostat, Córdoba, Argentina) and GraphPad Prism version 8.0 (Graph-Pad Software, Inc., La Jolla, CA).

3 Results

3.1 Fermentation activity and main metabolites produced

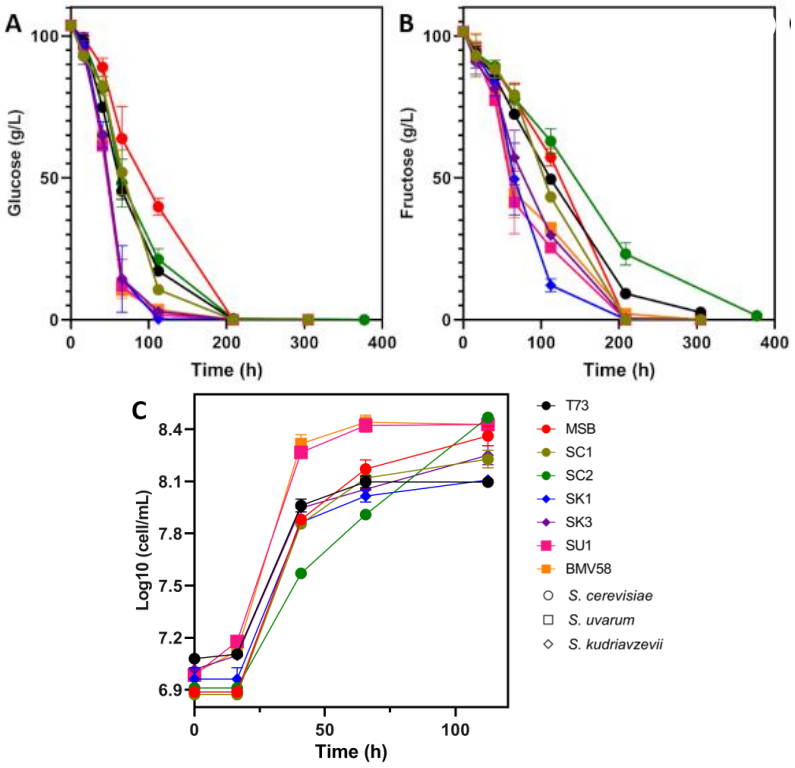


Figure 1. Sugar consumption and cell growth curves by yeasts during fermentation in semi-synthetic Albariño musts.

All fermentations, carried out at 16ºC, successfully reached trace sugars in less than 14 days. As shown in Fig. 1A and 1B, yeast strains of cryotolerant species (squares and diamonds) broadly consumed hexose sugars faster than *S. cerevisiae* strains (circles). For instance, besides glucose, *S. kudriavzevii* strain SK1 was also the quickest in consuming fructose. Considering cell growth, the highest biomass producers were the two *S. uvarum* strains (Fig. 1C). On the contrary, from the early stages of

fermentation, the *S. cerevisiae* strain SC2 mainly showed sluggish fructose consumption, resulting in a low fermentation and a slow biomass production (Fig. 1B and C).

Regarding fermentation by-products, an influence of species and isolation origin was observed (Fig. 2). Wine strains (T73, MSB, and BMV58) were associated with slightly higher malic acid content in the final wines compared to the initial content in the grape must, suggesting production of this compound across fermentation. On the other hand, wines fermented with *S. uvarum* strains presented the highest succinic acid content, with concentrations of 4.7 and 7.3 g/L reported for SU1 and BMV58, respectively. For the rest of the yeast strains, the succinic acid content ranged between 0.40 and 1.3 g/L. This difference observed in the succinic acid yielded by *S. uvarum* strains could be due to a great production of this acid through the GABA shunt, which aligns with the differential production by *S. uvarum* strains of certain aromas related to this pathway, as detailed below.

Interestingly, the wine produced with SC2 strain was characterized by the lowest malic and citric acid content, which correlated with the highest pH level (Table S2). Wines of *S. cerevisiae* strains, T73 and MSB, were also characterized by their highest ethanol and lowest glycerol and erythritol amounts. On the contrary, natural *S. kudriavzevii* strains SK1 and SK3 produced the highest levels of glycerol (Fig. 2).

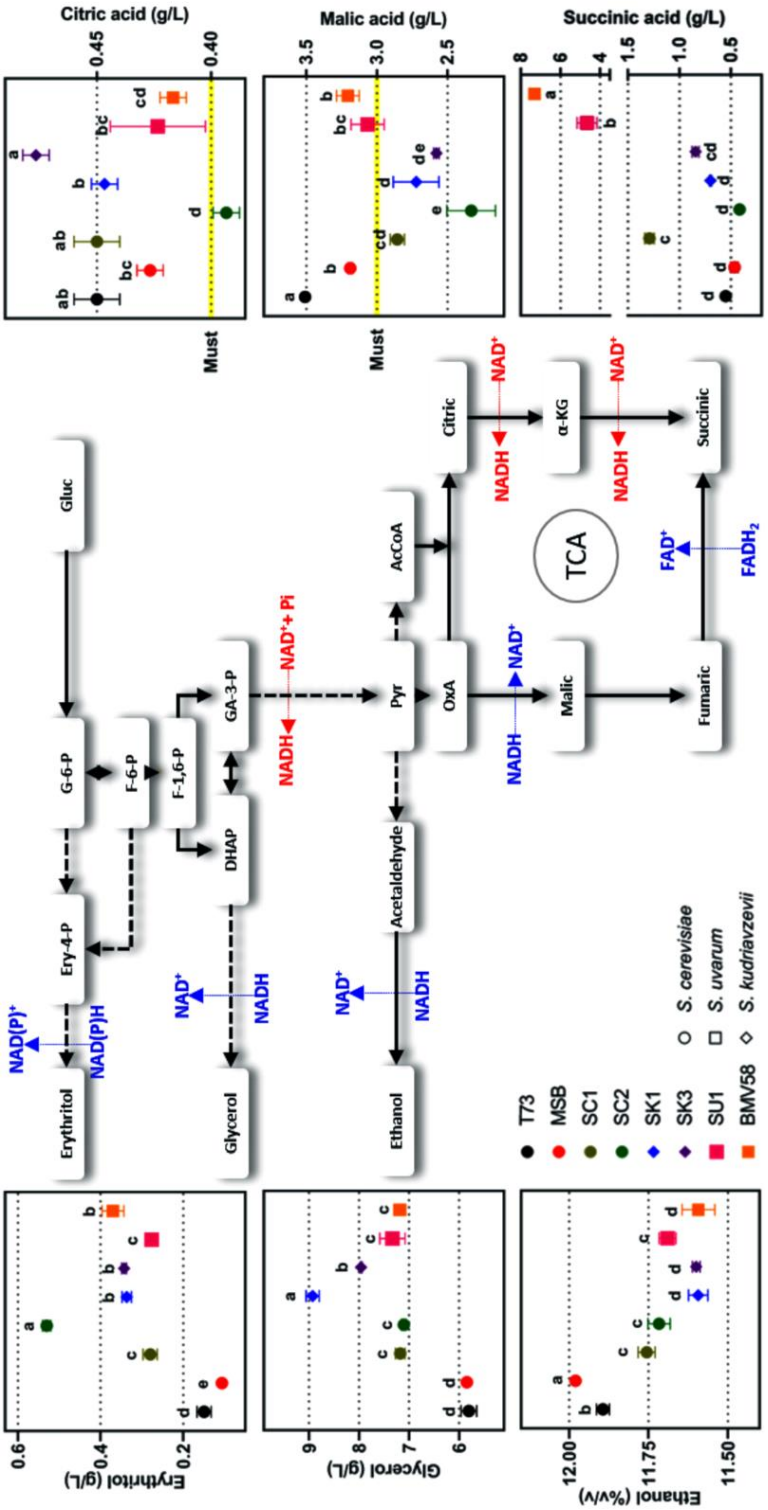


Figure 2. Primary metabolites (mean ± SD) determined in young wines fermented by the different strains and a simplified representation of their yeast's synthesis routes. Gluc: glucose; G-6-P: glucose-6-phosphate; F-6-P: fructose-6-phosphate; Ery-4-P: erythrose-4-phosphate; DHAP: dihydoxyacetone phosphate;GA-3-P: glyceraldehyde-3-phosphate; Pyr: pyruvate; AcCoA: acetyl coenzyme A; OxA: oxaloacetate; α-KG: α-ketoglutarate; TCA: tricarboxylic acid cycle.

3.2 General effect of yeast strain and aging on Albariño wine and must aromas

Sixty volatile compounds classified into ten categories (volatile acids, higher alcohols, acetate esters, ethyl esters, miscellaneous and carbonyls compounds, phenols, lactones, C_{13}-norisoprenoids, monoterpenes, and polyfunctional mercaptans) were determined in young wines and musts (Fig. 3A). After accelerated anoxic aging, thirty-eight minor volatile compounds (0.1-200 μg/L) were also determined in these samples (Fig. 3B). The values displayed in Fig. 3A and 3B represent the relative concentration of each aroma compound compared to the mean calculated from the concentrations of all strains and the non-inoculated must.

The following compounds were detected in young control grape must: volatile phenols, lactones, terpenes except for citronellol, polyfunctional mercaptans, and β-damascenone (Fig. 3A and 3B). We assumed that they represented the initial Albariño volatile fraction or could also have been released by acid hydrolysis during the time of fermentation. The aromas only found in the young wines represented fermentative aromas, which greatly differed according to the strain used. Similarly, we could discern the aroma fraction that proceeded entirely from the aging process, aromas that were only found in the aged must and the aged wines. So, we identified massoia lactone, vitispirane A and B, TDN, riesling acetal, vanillin, and 1,8-cineole as aromas formed or liberated during the aging process. The data also suggested a combined effect between yeast and aging on vanillin derivatives, ethyl cinnamate, and ethyl dihydrocinnamate. During fermentation, their precursors were modified by yeast, and subsequent aging led to the formation of volatiles.

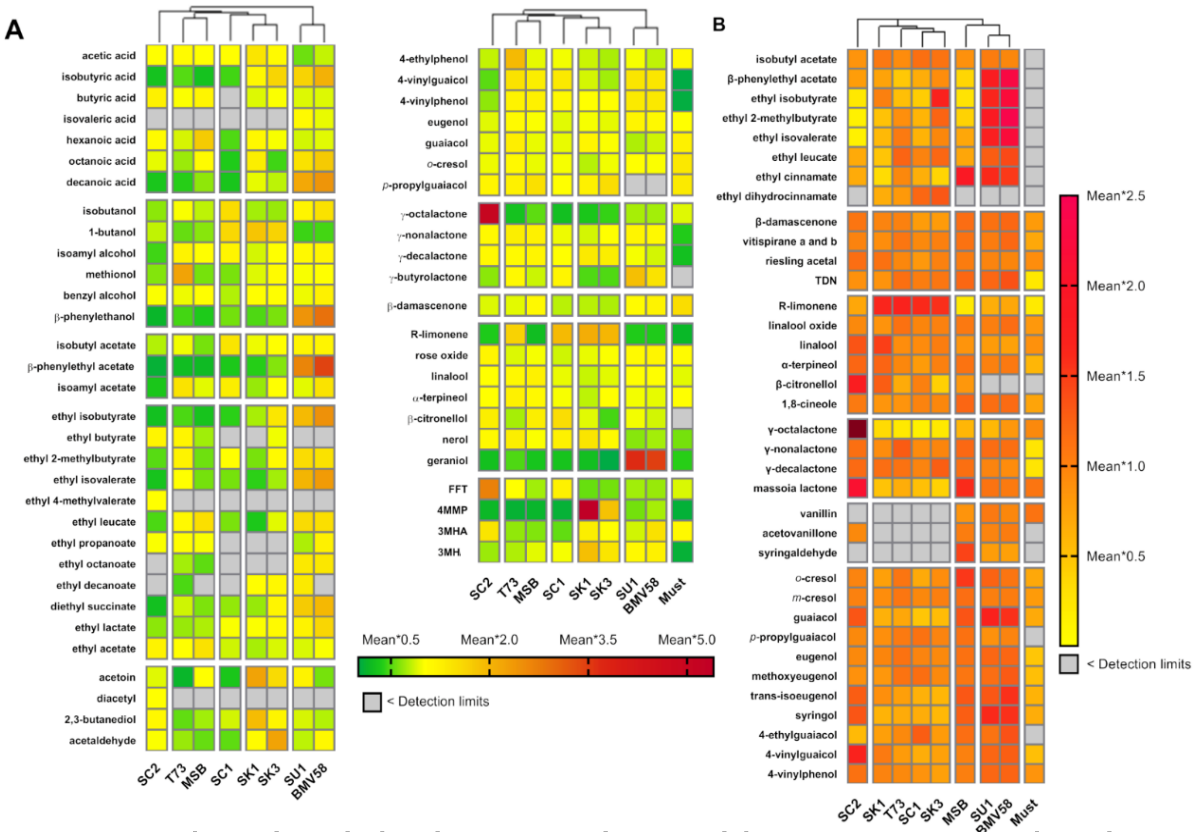


Figure 3. Heat map represents by a color scale the relative aroma values around the average concentration obtained in young (A) and aged (B) wines resulting from fermentation with different yeasts strains. Dataset of each condition was submitted to hierarchical clustering analysis resulting in a dendrogram representing the separation of the yeasts according to their similarities in the aroma profiles. Gaps between volatile indicate the different aromatic groups, starting from the top in A: acids, higher alcohols; acetate esters; ethyl esters; compounds related to 2,3-butanediol synthesis, volatile phenols, lactones, C_{13}-norisoprenoids, terpenes, polyfunctional mercaptans; in B, acetate esters and ethyl esters; C_{13}-norisoprenoids, terpenes, lactones, vanillin derivatives, volatile phenols. The average concentration of aromas in the aged wines was subtracted and divided by the average value of the young wines and expressed as a percentage: %VAR, thus obtaining the variability caused by the aging. Aromas not found in either of the two conditions: %VAR= n.a, not applicable

The percentage variability between the average concentration of each compound in young and aged wines determined that acetates, linalool, β-citronellol, *p*-propylguaiacol, 4-vinylguaiacol, and 4-vinylphenol decreased due to the aging process. These compounds underwent a decrease of between 40 to 90 %, while the rest of the compounds increased by between 20 to 7000 %. While lactones increased slightly, the highest increases were found for linalool oxide (4525 %), ethyl isovalerate (6694 %), ethyl 2-methylbutryate (4457 %), ethyl isobutyrate (3886 %), and ethyl leucate (1420 %). On the contrary, among the compounds that were no longer detected after aging, we found nerol, geraniol, rose oxide and, 4-ethylphenol. These compounds may have undergone isomerization reactions leading to the formation of other compounds or degradation. On the other hand, β-citronellol was the only monoterpene not found in the unfermented must.

3.2.1 Aromatic diversity in young Albariño wines according to yeast strains

The hierarchical cluster analysis applied on the aroma's concentration data sets of young wines (Fig. 3A) grouped yeasts into 3 groups. The SC2 strain stood alone on the left side of the plot and opposite the two *S. uvarum* strains were clustered together. In the middle, we found strains T73, MSB, SK1, SK3, and SC1. Yeasts were sub-grouped according to species within this last group, *S. cerevisiae* wine strains (T73 and MSB), and the environmental *S. kudriavzevii* strains (SK1 and SK3). The lowest levels of 2,3-butanediol characterized T73 and MSB strains. SC1 was in between these subgroups, sharing some traits, such as the high R-limonene content found equally in T73, SC1, SK1, and SK3 and was 13-fold higher than the one detected in the young must (Fig. 3A).

SK1 and SK3 strains were distinguished from T73, MSB, and SC1 by yielding the highest amounts of compounds related to 2,3-butanediol (i.e.,

acetaldehyde, acetoin, and 2,3-butanediol). Besides, their most distinctive trait was their great release of polyfunctional mercaptans (PFMs), particularly 4-methyl-4-mercaptopentan-2-one (4MMP), derived from the added precursors. For instance, strain SK1 reached a concentration of 4MMP 47-fold and 8-fold higher than the concentration yielded by *S. cerevisiae* and *S. uvarum* strains, respectively.

Regarding the *S. uvarum* cluster, their young wines were characterized by the highest yields of linear and branched-chain fatty acids, higher alcohols, ethyl, and acetates esters (Fig. 3A). The most notable aromas were β-phenylethanol and its acetate, with concentrations for both compounds almost 3-fold higher than the mean value. Additionally, octanoic and decanoic medium-chain fatty acids were highly present in these wines, as well as γ-butyrolactone and diethyl succinate, whose concentrations determined in these young wines doubled the average value (Fig. 3A). Curiously, SC2 was the only strain that produced detectable amounts of ethyl 4-methylvalerate (strawberry notes), while it yielded the lowest concentrations for the rest of the ethyl and acetate esters. Besides, this strain was also the one that produced the highest level (5-fold higher) of γ-octalactone in young wines.

Finally, geraniol amounts in young wines of *S. uvarum* strains were above the odor threshold (OT) and 3.5-fold higher than the average value. However, for nerol, the levels detected in SU1 and BMV58 wines were statistically equal to unfermented young musts and two orders of magnitude lower than in the other strains (Fig. 3A).

3.2.2 Aromatic diversity of aged Albariño wines according to yeast strains

Like young wines, the hierarchical cluster analysis applied on aromas of aged wines grouped yeasts into 3 groups (Fig. 3B). *S. uvarum* strains BMV58 and SU1 still clustered together, mainly because of their highest

concentrations of most esters, except ethyl cinnamate, which was not detected (Fig. 3B). Interestingly, ethyl isobutyrate significantly increased by aging in these strains, exceeding its OTs (19.23 to 647.3 μg/L in SU1 and 24.7 to 848.5 μg/L in BMV58; Table S4 and S5).

Again, SC2 strain stood alone at the opposite end of the dendrogram. It was characterized by having the lowest amounts of most aroma compounds but was still the strain with the highest γ-octalactone levels after aging (Fig. 3B). SC2 aged wines were also characterized by 2.5-fold higher massoia lactone and 2-fold higher 4-vinylguaiacol concentrations.

After aging, wines fermented with *S. kudriavzevii* strains SK1 and SK3 developed an aromatic profile more similar to those fermented with T73 and SC1 strains and were characterized by the highest levels of R-limonene and the lowest amount of γ-octalactone. On the contrary, the wine fermented with MSB strain evolved after aging towards an aromatic profile similar to*S. uvarum* strains, coinciding in the detection of vanillin compounds which were only found with these strains.

4 Discussion

Albariño grapevine is a cultivar with an important reservoir of varietal aromas, and few studies have been carried out to determine the influence of different yeast strains and bottle aging on the release of their volatile form in the resulting wine. The existing bibliography generally focused on specific aroma groups, including C_{13}-norisoprenoids, monoterpenes, and fermentative aromas, often using *S. cerevisiae* strains (Lema et al., 1996; Vilanova and Sieiro, 2006; Oliveira et al., 2008; Carrascosa et al., 2012). For this reason, in this study, we aimed to determine how wild *S. cerevisiae*, cryotolerant *S. uvarum*, and *S. kudriavzevii* yeasts and aroma maturation during bottle aging modulate a variety of Albariño aromas. Ultimately some hypotheses on the metabolic origin of these aroma compounds within the yeast metabolic

network are presented and summarized in Figure 4 that will be used in the discussion section.

Regarding the effect caused by aging, we observed several compounds highly modified in Albariño aged wines. As several studies have determined, the anoxic aging favored the esterification of branched ethyl esters, which was visualized in this study in the high increase of ethyl isovalerate and ethyl 2-methylbutyrate. We also corroborated the effect of aging on the degradation of isobutyl and β-phenylethyl acetates by hydrolysis (Díaz-Maroto et al., 2005). Regarding monoterpenes, geraniol, the principal precursor of monoterpenes, was degraded via acid-catalyzed hydrolysis during aging resulting in α-terpineol and linalool. Likewise, linalool initially present in grape must also undergo a chemical evolution resulting in 1,8-cineole and α-terpineol (Waterhouse et al., 2016).

Besides the general effect caused by aging, yeast also played a major role in modulating these aromas. A good example of this is the case of R-limonene observed in wines fermented with SK1, SK3, T73, and SC1 strains. The high concentration could be attributed to the synthesis of R-limonene from GPP through the mevalonate pathway (Fig. 4C). However, the biosynthetic pathway of R-limonene is not fully known on yeast (Duetz et al., 2003). After aging, its concentration still increases, which could be linked to the further release of this compound from yeast cells during the aging process.

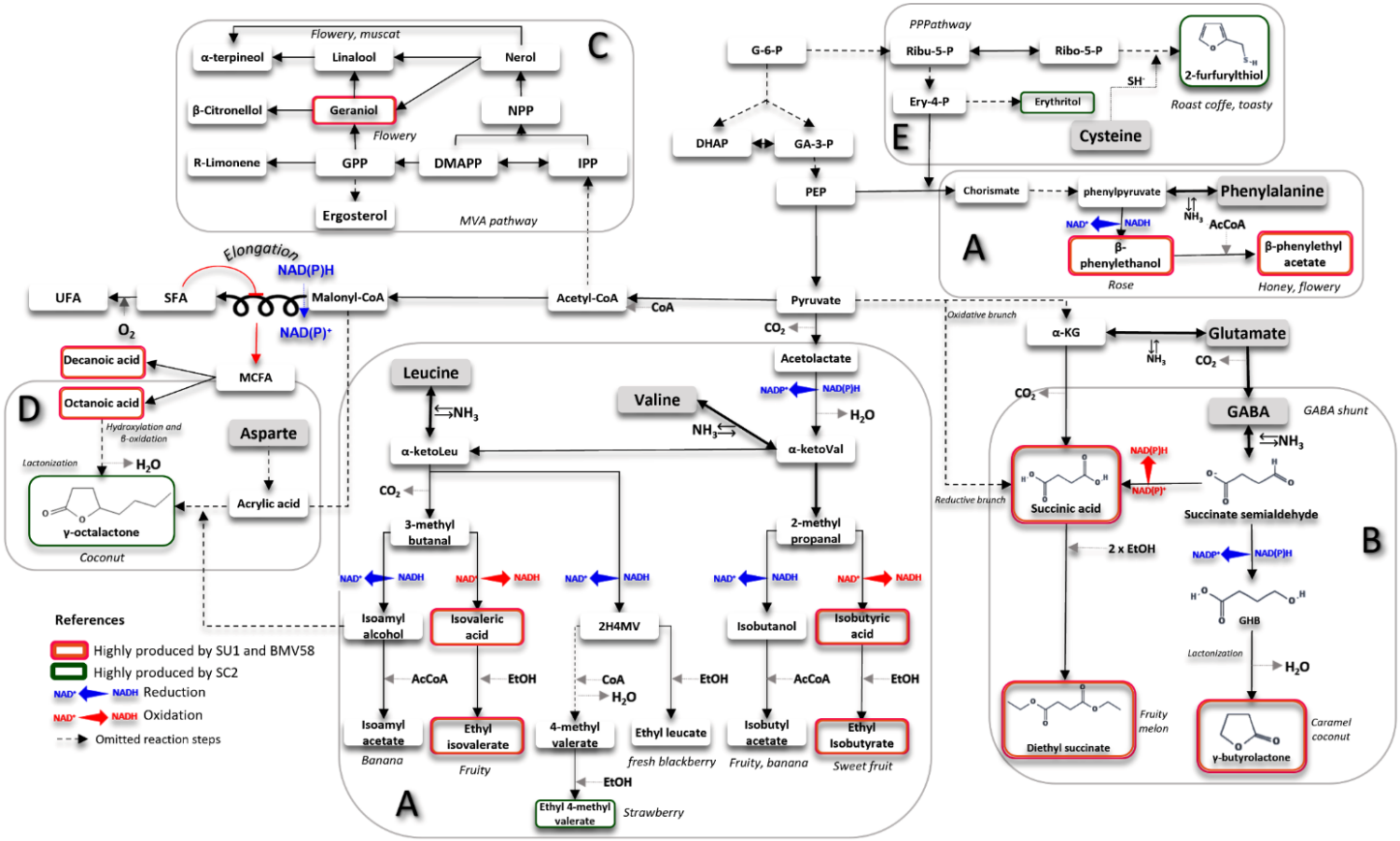


Figure 4. Summary of yeast metabolic pathways involved in the synthesis of relevant aroma compounds determined in this study. UFA: unsaturated fatty acids; SFA: saturated fatty acids; MCFA: medium-chain fatty acids; 2H4MP: 2-hydroxy 4-methylpentanoic acid; GPP: geranyl diphosphate; DMAPP: dimethylallyl diphosphate; IPP: isopentenyl diphosphate. Odor descriptors were taken from Culleré et al. (2019).

4.1 Aroma modulation by *S. uvarum* strains

The most striking result obtained with the *S. uvarum* strains was their great capacity to release γ-butyrolactone and diethyl succinate. Although the sensory effect was not relevant according to their perception thresholds, their biosynthetic origin can be related to the catabolic pathway of glutamate, most commonly known as the GABA shunt (Fig. 4B). In this pathway, glutamate is decarboxylated to γ-aminobutyric acid (GABA), transaminated to succinate semi-aldehyde (SSA), and oxidized to succinate. When SSA is not oxidized to succinate, it can be reduced to γ-hydroxybutyric acid (GHB) (Bach et al., 2009), which is the substrate of lactonization to γ-butyrolactone (Ribéreau-Gayon et al., 2006). On the other hand, the end product of the GABA shunt is succinate, which after double esterification with ethanol, can lead to diethyl succinate (Sieiro-Sampedro et al., 2019). Therefore, the overproduction of γ-butyrolactone and diethyl succinate by *S. uvarum* strains is likely the result of a greater flux through the GABA shunt in this species. This is concordant with the higher succinate yields found for SU1 and BMV58 strains in our study. The recent work of Henriques et al. (2021) highlights the role of this pathway in succinic acid synthesis and balancing redox homeostasis in *S. uvarum* strains.

In young and aged wines fermented with *S. uvarum* strains, we also observed a significant amount of higher alcohols, ethyl esters, and acetates related to the catabolism of branched-chain and aromatic amino acids. As shown in Figure 4A, all these compounds can be formed from their exogenous and endogenous (i.e., *de novo* synthesized from sugars metabolism) amino acids precursors, valine, leucine, or phenylalanine, via the Ehrlich pathway. Briefly, the Ehrlich pathway consists of the catabolism of branched-chain and aromatic amino acids, or their related keto-acids, in a three-step reaction. The last reaction can be a reduction (leading to a fusel alcohol) or oxidation

(leading to a fusel acid). As presented in Figure 4A, the higher amounts of isobutyric and isovaleric acids and their related ethyl esters (i.e., ethyl isobutyrate and ethyl isovalerate) in *S. uvarum* strains suggested that they mainly directed the catabolism of leucine and valine through the oxidative branch of the Ehrlich pathway. In addition, we also observed that *S. uvarum* strains were the fastest in growing while SC2 strain was the slowest, which could be the reason for an initial higher demand for reductive equivalents (NADPH) by *S. uvarum* (Bakker et al., 2001).

Regarding the highest levels of geraniol detected in the young wines of these strains, this phenotype is probably related to the blockage of ergosterol synthesis at the level of squalene in the absence of oxygen during fermentation (Vaudano et al., 2004). The accumulation of geranyl diphosphate (GPP), an intermediate of ergosterol synthesis and situated above squalene, might have contributed to geraniol synthesis in *S. uvarum* strains (Fig. 4C). From this step, the bioconversion of this terpene into other terpenes could have been generated in several of the strains, giving rise, among others, to citronellol (Gamero et al., 2011).

Moreover, under the conditions of our study, we also found *S. uvarum* strains with a high capacity to release various PFMs. This aptitude of *S. uvarum* has also been reported on Sauvignon Blanc fermentation (Masneuf et al., 2002). However, in our work, differences in 4MMP and 3MH production between commercial strains of *S. cerevisiae* and *S. uvarum* strains were much greater.

According to the literature, ethyl isobutyrate results from the slow esterification of isobutyric acid during aging (Díaz-Maroto et al., 2005). Therefore, the highest amounts found in young wines and the major increase observed after aging are directly related to the high levels of the branched

acids found, mainly in BMV58. The most significant increase of this compound by the action of aging was reported in our *S. uvarum* strains.

4.2 SC2 strain a particular strain

Curiously, SC2 had the lowest amount of most ethyl esters, but it was the only strain that produced a detectable amount of ethyl 4-methylvalerate. This ester was first identified in wines by Campo et al. (2006) as an isomer of ethyl hexanoate but with a much lower odor threshold, contributing to the strawberry aroma. Its synthesis has not been entirely determined but is suggested to result from the esterification of 4-methylvaleric acid with ethanol (Gracia-Moreno et al., 2015). Here we hypothesize that the latter acid could derive from 2H4MV (2-hydroxy 4-methylpentanoic acid), the hydroxy acid precursor of ethyl leucate (Shimizu et al., 2016) (Fig. 4A).

Lactones in wine have a relevant flavor role, but their synthesis by yeasts remains to be fully discovered. From the limited literature and observations in this strain, we traced two metabolic pathways that could result in γ-octalactone (Fig. 4D). On the one hand, γ-octalactone could derive from yeast lipid metabolism. In this scenario, it would be produced from octanoic acid after being hydroxylated, β-oxidized, and finally lactonized (Romero-Guido et al., 2011). The second hypothetical pathway involved acrylic acid, a compound little studied in yeasts and wine but could proceed from aspartate or malonyl-CoA. Acrylic acid could be bound with isoamyl alcohol and, after losing a water molecule by lactonization, γ-octalactone would be formed (Berger and Zorn, 2004).

High content in furfurylthiol also characterized SC2 wines. This compound is another key volatile thiol with a strong roast coffee aroma. The presence of FFT in wine is generally associated with contact with toasted staves either during fermentation or aging (Tominaga et al., 2000; Blanchard et al., 2001). However, its detection in oak-free wines and the variability

between cultivars or vintages has raised concerns about its origin (Tominaga et al., 2000). According to Hofmann and Schieberle (1998), FFT is formed from the reaction between ribose and cysteine under dry heat during food processing. In this aspect, we notably found that SC2 produced high levels of erythritol, a pentose phosphate pathway derivative, suggesting that this pathway might be more active in this strain. Consequently, the higher availability of other pentose phosphate intermediates, such as ribose-5-phosphate, might have contributed to the higher synthesis of FFT in SC2.

4.3 The great capacity of *S. kudriavzevii* strains in releasing polyfunctional mercaptans

The most important related trait to *S. kudriavzevii* strains was a large concentration of polyfunctional mercaptans (4MMP and 3MH) found in their young wines. These compounds are considered very potent aroma molecules that impart important tropical notes, even at very low concentrations in wines (Roland et al., 2011). 4MMP has the lowest perception threshold, and its aroma is related to the box tree or cat's pee notes, typical of Sauvignon Blanc wines (Tominaga et al., 1998). The release of 4MMP from its nonvolatile S-cysteinylated precursor, *S*-3-(4-mercapto-4-methylpentan-2-one)-cysteine (Cys4MMP), has been reported to be carried out by different carbon-sulfurylase enzymes whose coding genes have only been identified in laboratory *S. cerevisiae* strain so far (Howell et al., 2005). Among them, protein Irc7p is the main responsible for the release of 4MMP, and its activity is controlled by genes of the nitrogen catabolic repression (NCR) system (Thibon et al., 2008). Therefore, greater activity of carbon-sulfurylase enzymes in SK1 and SK3 or a distinct regulation of their coding genes by the NCR complex could explain the higher levels of 4MMP. In line with this, previous works have already pointed out the higher volatile-thiol release capacity of an interspecific *S. kudriavzevii* × *S. cerevisiae* hybrid widely used

in white vinifications (Murat et al.,2001; Erny et al., 2012). Additionally, the first step in the release of 4MMP and 3MH consists of the incorporation by yeast of Cys4MMP and Cys3MH (S-3-hexan-1-ol-cysteine) precursors. This can be accomplished via the general amino-acid permease Gap1p (Subileau et al., 2008) or cysteine-specific permeases (Thibon et al., 2008). In another essay (data not shown), we notably found that *S. kudriavzevii* strains grown better on cysteine as the sole nitrogen source than other strains. This could be a reason for the higher incorporation of S-Cys-conjugates into their cells, cleaving and releasing into the free form of 4MMP and 3MH.

5 Conclusion

To recapitulate, the most relevant findings in this work regarding the impact of yeast strain and aging on the modulation of Albariño aroma profile were found in young and aged wines obtained with *S. uvarum*, SC2, and *S. kudriavzevii* strains.

First, we give several evidence elements that, besides its contribution to the higher succinic acid production by the *S. uvarum* strains, the GABA shunt probably provides the precursors (SSA and succinate) required for γ-butyrolactone and diethyl succinate synthesis. Secondly, besides the fact that *S. kudriavzevii* strains had the best fermentative performances in Albariño fermentations, they also stood out for their great capacity to release PFMs. We related this with more efficient incorporation of their precursor inside their cells and a consequent better ability to be cleaved and released by these yeasts. At this point, we found that these two groups of yeasts demonstrated the ability to enhance the floral character of this grape variety, providing floral profiles by *S. uvarum* and tropical fruits profiles by *S. kudriavzevii*.

On the other hand, the wild *S. cerevisiae* SC2 strain, isolated from sorghum beer, was noted for its high production of the PFMs furfurylthiol,

whose synthesis route we related to the pentose phosphate pathway since this strain also produced the highest concentrations of erythritol. In addition, this strain was noted for the high production of γ-octalactone, and ethyl 4-methylvalerate, although at concentrations of low sensory significance.

Finally, aging favored the increase of several important aromatic compounds, among which the aged wines of *S. uvarum* stood out for their higher content of branched esters with fruity notes.

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement number 764364, Aromagenesis, and from Generalitat Valenciana grant PROMETEO/2020/014.

References

Alegre, Y., Arias-Pérez, I., Hernández-Orte, P., Ferreira, V., 2020. Development of a new strategy for studying the aroma potential of winemaking grapes through the accelerated hydrolysis of phenolic and aromatic fractions (PAFs). Food Res. Int. 127, 108728. https://doi.org/10.1016/j.foodres.2019.108728

Bach, B., Meudec, E., Lepoutre, J.P., Rossignol, T., Blondin, B., Dequin, S., Camarasa, C., 2009. New insights into γ-aminobutyric acid catabolism: Evidence for γ-hydroxybutyric acid and polyhydroxybutyrate synthesis in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 75, 4231–4239. https://doi.org/10.1128/AEM.00051-09

Bakker, B.M., Overkamp, K.M., Van Maris, A.J.A., Kötter, P., Luttik, M.A.H., Van Dijken, J.P., Pronk, J.T., 2001. Stoichiometry and compartmentation of NADH metabolism in *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. 25, 15–37. https://doi.org/10.1016/S0168-6445(00)00039-5

Berger, R.G., and Zorn, H., 2004. Flavors and Fragrances. Adv. Fungal Biotechnol. Ind. Agric. Med. 341–358. https://doi.org/10.1007/978-1-4419-8859-1_13

Blanchard, L., Tominaga, T., Dubourdieu, D., 2001. Formation of furfurylthiol exhibiting a strong coffee aroma during oak barrel fermentation from furfural released by toasted staves. J. Agric. Food Chem. 49, 4833–4835. https://doi.org/10.1021/jf010539w

Borren, E., and Tian, B., 2021. The important contribution of non-*Saccharomyces* yeasts to the aroma complexity of wine: A review. *Foods*, *10*(1). https://doi.org/10.3390/foods10010013

Campo, E., Cacho, J., Ferreira, V., 2006. Multidimensional chromatographic approach applied to the identification of novel aroma compounds in wine. Identification of ethyl cyclohexanoate, ethyl 2-hydroxy-3-methylbutyrate and ethyl 2-hydroxy-4-methylpentanoate. J. Chromatogr. A 1137, 223–230. https://doi.org/10.1016/j.chroma.2006.10.020

Carrascosa, a. V., Bartolome, B., Robredo, S., Leon, A., Cebollero, E., Juega, M., Nuñez, Y.P., Martinez, M.C., Martinez-Rodriguez, a. J., 2012. Influence of locally-selected yeast on the chemical and sensorial properties of Albariño white wines. LWT - Food Sci. Technol. 46, 319–325. doi:10.1016/j.lwt.2011.09.011

Culleré, L., López, R., & Ferreira, V., 2019. The Instrumental Analysis of Aroma-Active Compounds for Explaining the Flavor of Red Wines. In *Red Wine*

Technology (pp. 283–307). https://doi.org/10.1016/b978-0-12-814399-5.00020-7

Denat, M., Pérez, D., Heras, J.M., Querol, A., Ferreira, V., 2021. The effects of *Saccharomyces cerevisiae* strains carrying alcoholic fermentation on the fermentative and varietal aroma profiles of young and aged Tempranillo wines. FoodChem. 9. https://doi.org/10.1016/j.fochx.2021.100116

Díaz-Maroto, M.C., Schneider, R., Baumes, R., 2005. Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. J. Agric. Food Chem. 53, 3503–3509. https://doi.org/10.1021/jf048157o

Dubourdieu, D., Tominaga, T., Masneuf, I., Des Gachons, C. P., & Murat, M.-L., 2006. The Role of Yeasts in Grape Flavor Development during Fermentation: The Example of Sauvignon blanc. *American Journal of Enology and Viticulture*, *57*(1), 81–88.

Duetz, W.A., Bouwmeester, H., Van Beilen, J.B., Witholt, B., 2003. Biotransformation of limonene by bacteria, fungi, yeasts, and plants. Appl. Microbiol. Biotechnol. 61, 269–277. https://doi.org/10.1007/s00253-003-1221-y

Erny, C., Raoult, P., Alais, A., Butterlin, G., Delobel, P., Matei-Radoi, F., Casaregola, S., Legras, J.L., 2012. Ecological Success of a Group of *Saccharomyces cerevisiae*/*Saccharomyces kudriavzevii* hybrids in the Northern European wine-making environment. Appl. Environ. Microbiol. 78, 3256–3265. https://doi.org/10.1128/AEM.06752-11

Feng, C. T., Du, X., Wee, J., 2021. Microbial and chemical analysis of non-*Saccharomyces* yeasts from Chambourcin hybrid grapes for potential use in winemaking. *Fermentation*, *7*(1). https://doi.org/10.3390/fermentation7010015

Fernández-González, M., Di Stefano, R., 2004. Fractionation of glycoside aroma precursors in neutral grapes. Hydrolysis and conversion by *Saccharomyces cerevisiae*. LWT - Food Sci. Technol. 37, 467–473. https://doi.org/10.1016/j.lwt.2003.11.003

Fernández-González, M., Di Stefano, R., Briones, A., 2003. Hydrolysis and transformation of terpene glycosides from muscat must by different yeast species. Food Microbiol. 20, 35–41. https://doi.org/10.1016/S0740-0020(02)00105-3

Ferreira, V., and López, R., 2019. The actual and potential aroma of winemaking grapes. *Biomolecules*, *9*(12), 1–35. https://doi.org/10.3390/biom9120818

Gamero, A., Manzanares, P., Querol, A., & Belloch, C., 2011. Monoterpene alcohols release and bioconversion by *Saccharomyces* species and hybrids. *International Journal of Food Microbiology*, *145*(1), 92–97. https://doi.org/10.1016/j.ijfoodmicro.2010.11.034

Gracia-Moreno, E., López, R., Ferreira, V., 2015. Quantitative determination of five hydroxy acids, precursors of relevant wine aroma compounds in wine and other alcoholic beverages. Anal. Bioanal. Chem. 407, 7925–7934. https://doi.org/10.1007/s00216-015-8959-9

Hernández-Orte, P., Bely, M., Cacho, J., Ferreira, V., 2006. Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different *Saccharomyces cerevisiae* strains during fermentation in controlled synthetic media. Aust. J. Grape Wine Res. 12, 150–160.

Hernández-Orte, P., Cacho, J.F., Ferreira, V., 2002. Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric study. J. Agric. Food Chem. 50, 2891–2899. https://doi.org/10.1021/jf011395o

Hofmann, T., Schieberle, P., 1998. Quantitative model studies on the effectiveness of different precursor systems in the formation of the intense food odorants 2-furfurylthiol and 2-methyl-3-furanthiol. J. Agric. Food Chem. 46, 235–241. https://doi.org/10.1021/jf9705983

Howell, K.S., Klein, M., Swiegers, J.H., Hayasaka, Y., Elsey, G.M., Fleet, G.H., Høj, P.B., Pretorius, I.S., De Barros Lopes, M.A., 2005. Genetic determinants of volatile-thiol release by *Saccharomyces cerevisiae* during wine fermentation. Appl. Environ. Microbiol. 71, 5420–5426. https://doi.org/10.1128/AEM.71.9.5420-5426.2005

Lema, C., Garcia-Jares, C., Orriols, I., Angulo, L., 1996. Contribution of *Saccharomyces* and non-*Saccharomyces* populations to the production of some components of Albariño wine aroma. Am. J. Enol. Vitic. 47, 206–216.

Liu, J., Zhu, X. L., Ullah, N., Tao, Y. S., 2017. Aroma Glycosides in Grapes and Wine. Journal of Food Science, 82(2), 248–259. https://doi.org/10.1111/1750-3841.13598

López, R., Ezpeleta, E., Sánchez, I., Cacho, J., Ferreira, V., 2004. Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from Tempranillo and Grenache grapes using gas chromatography-olfactometry. Food Chemistry, 88(1), 95–103. https://doi.org/10.1016/j.foodchem.2004.01.025

López, R., Aznar, M., Cacho, J., Ferreira, V., 2002. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. J. Chromatogr. A 966, 167–177. https://doi.org/10.1016/S0021-9673(02)00696-9

Masneuf, I., Murat, M.L., Naumov, G.I., Tominaga, T., Dubourdieu, D., 2002. Hybrids *Saccharomyces cerevisiae* x *Saccharomyces bayanus* var. *uvarum* having a high liberating ability of some sulfur varietal aromas of *Vitis vinifera* Sauvignon Blanc wines. J. Int. Des Sci. la Vigne du Vin 36, 205–212. https://doi.org/10.20870/oeno-one.2002.36.4.965

Mateo-Vivaracho, L., Zapata, J., Cacho, J., Ferreira, V., 2010. Analysis, occurrence, and potential sensory significance of five polyfunctional mercaptans in white wines. J. Agric. Food Chem. 58, 10184–10194. https://doi.org/10.1021/jf101095a

Minebois, R., Pérez-Torrado, R., Querol, A., 2020. A time course metabolism comparison among *Saccharomyces cerevisiae*, *S. uvarum* and *S. kudriavzevii* species in wine fermentation. Food Microbiol. 90, 1–14. https://doi.org/10.1016/j.fm.2020.103484

Murat, M.L., Tominaga, T., Dubourdieu, D., 2001. Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. J. Agric. Food Chem. 49, 5412–5417. https://doi.org/10.1021/jf0103119

Oliveira, B.M., Barrio, E., Querol, A., Pérez-Torrado, R., 2014. Enhanced enzymatic activity of glycerol-3-phosphate dehydrogenase from the cryophilic *Saccharomyces kudriavzevii*. PLoSOne 9. https://doi.org/10.1371/journal.pone.0087290

Oliveira, I., and Ferreira, V., 2019. Modulating Fermentative, Varietal and Aging Aromas of Wine Using non- *Saccharomyces* Yeasts in a Sequential Inoculation Approach. *Microorganisms*, *7*(6), 1–23. https://doi.org/doi:10.3390/microorganisms7060164

Oliveira, J.M., Oliveira, P., Baumes, R.L., Maia, O., 2008. Changes in aromatic characteristics of Loureiro and Alvarinho wines during maturation. J. Food Compos. Anal. 21, 695–707. https://doi.org/10.1016/j.jfca.2008.08.002

Ortega, C., López, R., Cacho, J., Ferreira, V., 2001. Fast analysis of important wine volatile compounds - Development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. J. Chromatogr. A 923, 205–214. https://doi.org/10.1016/S0021-9673(01)00972-4

Parker, M., Capone, D.L., Francis, I.L., Herderich, M.J., 2017. Review aroma precursors in grapes and wine: flavor release during wine production and consumption. J. Agric. Food Chem. https://doi.org/10.1021/acs.jafc.6b05255

Pérez, D., Jaehde, I., Guillamón, J.M., Heras, J.M., Querol, A., 2021. Screening of *Saccharomyces* strains for the capacity to produce desirable fermentative compounds under the influence of different nitrogen sources in synthetic wine fermentations. Food Microbiol. 97, 103763. https://doi.org/10.1016/j.fm.2021.103763

Querol, A., Pérez-Torrado, R., Alonso-del-Real, J., Minebois, R., Stribny, J., Oliveira, B.M., Barrio, E., 2018. New Trends in the Uses of Yeasts in Oenology, in: Advances in Food and Nutrition Research. Elsevier Inc., Amsterdam, pp. 177–210. https://doi.org/10.1016/bs.afnr.2018.03.002

Ribéreau-Gayon, P., Dubourdieu, D., Doneche, B., Lonvaud, A., 2006. Handbook of Enology, Vol. 1: The Microbiology of Wine and Vinifications., 2nd Ed. John Wiley and Sons Ltd, Chichester, UK.

Roland, A., Schneider, R., Razungles, A., Cavelier, F., 2011. Varietal thiols in wine: Discovery, analysis and applications. Chem. Rev. 111, 7355–7376. https://doi.org/10.1021/cr100205b

Romero-Guido, C., Belo, I., Ta, T.M.N., Cao-Hoang, L., Alchihab, M., Gomes, N., Thonart, P., Teixeira, J.A., Destain, J., Waché, Y., 2011. Biochemistry of lactone formation in yeast and fungi and its utilization for the production offlavour and fragrance compounds. Appl. Microbiol. Biotechnol. 89, 535–547. https://doi.org/10.1007/s00253-010-2945-0

Shimizu, M., Yamamoto, T., Okabe, N., Sakai, K., Koide, E., Miyachi, Y., Kurimoto, M., Mochizuki, M., Yoshino-Yasuda, S., Mitsui, S., Ito, A., Murano, H., Takaya, N., Kato, M., 2016. Novel 4-methyl-2-oxopentanoate reductase involved in synthesis of the Japanese sake flavor, ethyl leucate. Appl. Microbiol. Biotechnol. 100, 3137–3145. https://doi.org/10.1007/s00253-015-7182-0

Sieiro, C., Villa, T.G., Da Silva, A.F., García-Fraga, B., Vilanova, M., 2014. Albariño wine aroma enhancement through the use of a recombinant polygalacturonase from *Kluyveromyces marxianus*. Food Chem. 145, 179–185. https://doi.org/10.1016/j.foodchem.2013.08.050

Sieiro-Sampedro, T., Figueiredo-González, M., González-Barreiro, C., Simal-Gandara, J., Cancho-Grande, B., Rial-Otero, R., 2019. Impact of mepanipyrim and tetraconazole in Mencía wines on the biosynthesis of volatile compounds during the winemaking process. Food Chem. 300, 125223. https://doi.org/10.1016/j.foodchem.2019.125223

Stribny, J., Gamero, A., Pérez-Torrado, R., Querol, A., 2015. *Saccharomyces kudriavzevii* and *Saccharomyces uvarum* differ from *Saccharomyces cerevisiae* during the production of aroma-active higher alcohols and acetate esters using their amino acidic precursors. Int. J. Food Microbiol. 205, 41–46. https://doi.org/10.1016/j.ijfoodmicro.2015.04.003

Subileau, M., Schneider, R., Salmon, J.M., Degryse, E., 2008. New insights on 3-mercaptohexanol (3MH) biogenesis in Sauvignon Blanc wines: Cys-3MH and (E)-hexen-2-al are not the major precursors. J. Agric. Food Chem. 56, 9230–9235. https://doi.org/10.1021/jf801626f

Swiegers, J.H., Bartowsky, E.J., Henschke, P.A., Pretorius, I.S., 2005. Yeast and bacterial modulation of wine aroma and flavour. Aust. J. Grape Wine Res. 11, 139–173. https://doi.org/10.1111/j.1755-0238.2005.tb00285.x

Thibon, C., Marullo, P., Claisse, O., Cullin, C., Dubourdieu, D., Tominaga, T., 2008. Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation. FEMS Yeast Res. 8, 1076–1086. https://doi.org/10.1111/j.1567-1364.2008.00381.x

Tominaga, T., Blanchard, L., Darriet, P., Dubourdieu, D., 2000. A powerful aromatic volatile thiol, 2-furanmethanethiol, exhibiting roast coffee aroma in wines made from several *Vitis vinifera* grape varieties. J. Agric. Food Chem. 48, 1799–1802. https://doi.org/10.1021/jf990660r

Tominaga, T., des Gachons, P. C., Dubourdieu, D., 1998. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon Blanc: S-cysteine conjugates. J. Agric. Food Chem. 46, 5215–5219.

Ugliano, M., 2009. Enzymes in winemaking. In M. V. Moreno-Arribas& M. C. Polo (Eds.), *Wine Chemistry and Biochemistry* (pp. 103–126). https://doi.org/10.1007/978-0-387-74118-5_6

Ugliano, M., Bartowsky, E.J., McCarthy, J., Moio, L., Henschke, P.A., 2006. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. J. Agric. Food Chem. 54, 6322–6331. https://doi.org/10.1021/jf0607718

Vaudano, E., Moruno, E.G., Di Stefano, R., 2004. Modulation of geraniol metabolism during alcohol fermentation. J. Inst. Brew. 110, 213–219. https://doi.org/10.1002/j.2050-0416.2004.tb00205.x

Vela, E., Hernández-Orte, P., Franco-Luesma, E., Ferreira, V., 2017. The effects of copper fining on the wine content in sulfur off-odors and on their evolution during accelerated anoxic storage. Food Chem. 231, 212–221. https://doi.org/10.1016/j.foodchem.2017.03.125

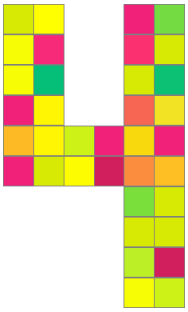
Vilanova, M., and Vilariño, F., 2006. Influence of geographic origin on aromatic descriptors of Spanish Albariño wine. Flavour and Fragrance Journal, 21(2), 373–378. https://doi.org/10.1002/ffj.1677

Vilanova, M., and Sieiro, C., 2006. Contribution by *Saccharomyces cerevisiae* yeast to fermentative flavour compounds in wines from cv. Albariño. J. Ind. Microbiol. Biotechnol. 33, 929–933. https://doi.org/10.1007/s10295-006-0162-8

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W., 2016. *Understanding Wine Chemistry* (J. W. & Sons, ed.). https://doi.org/10.1002/9781118730720

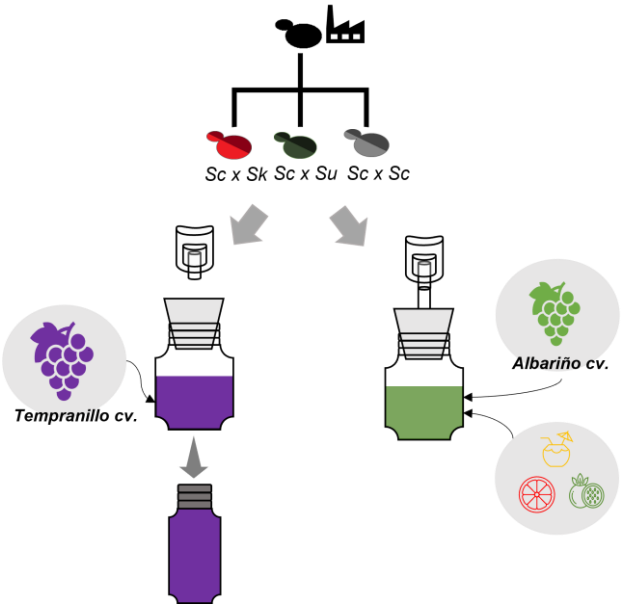
Zwietering, M. H., Jongenburger, I., Rombouts, F. M., Van't Riet, K., 1990. Modeling of the Bacterial Growth Curve. *Applied and Environmental Microbiology*, *56*(6), 1875–1881. https://doi.org/0099-2240/90/061875-07$02.00/0

Chapter 4



Generation of intra- and interspecific *Saccharomyces* hybrids with improved oenological and aromatic properties

Pérez, Dolores; Denat, Marie; Pérez-Través, Laura; Heras, José María; Guillamón, José Manuel; Ferreira, Vicente; Querol, Amparo



Submitted in Microbial Biotechnology

Abstract

Non-wine yeasts could enhance the aroma and organoleptic profile of wines. However, compared to wine strains, they have specific intolerances to winemaking conditions. To solve this problem, we generated intra- and interspecific hybrids using a non-GMO technique (rare-mating) in which non-wine strains of *S. uvarum*, *S. kudriavzevii*, and *S. cerevisiae species* were crossed with a wine *S. cerevisiae* yeast. The hybrid that inherited the wine yeast mitochondrial showed better fermentation capacities, whereas hybrids carrying the non-wine strain mitotype reduced ethanol levels and increased glycerol, 2,3-butanediol, and organic acid production.

Moreover, all the hybrids produced several fruity and floral aromas compared to the wine yeast: β-phenylethyl acetate, isobutyl acetate, γ-octalactone, ethyl cinnamate in both varietal wines. *Sc* × *Sk* crosses produced 3-6 fold higher polyfunctional mercaptans, 4-mercapto-4-methylpentan-2-one (4MMP), and 3-mercaptohexanol (3MH). We proposed that the exceptional 3MH release observed in an *S. cerevisiae* × *S. kudriavzevii* hybrid was due to the cleavage of the non-volatile glutathione precursor (Glt-3MH) to detoxify the cell from the presence of methylglyoxal, a compound related to the high glycerol yield reached by this hybrid.

In conclusion, hybrid generation allows us to obtain aromatically improved yeasts concerning their wine parent. In addition, they reduced ethanol and increased organic acids yields, which counteracts climate change effect on grapes.

1. Introduction

Today, the role of yeasts in winemaking is no longer only focused on producing ethanol but on specific objectives such as providing aroma complexity, reducing ethanol, increasing acidity, and others. As a result, the research and selection of yeasts have been extended to other species and genera different from the wine yeasts *Saccharomyces cerevisiae*. Many interesting yeasts of non-*Saccharomyces* genera have been selected, fulfilling the objectives mentioned above (Gobbi et al., 2013; Varela et al., 2016; Ravasio et al., 2018; Oliveira et al., 2019). However, due to their low competitiveness and poor winemaking tolerance, co-inoculation or sequential fermentation with *S. cerevisiae* wine yeasts are crucial to complete the fermentation.

Within *Saccharomyces* genus, yeasts of cryophilic species and non-wine *S. cerevisiae* yeasts also have the ability to decrease alcohol yield, increase acidity and enhance fruity fermentative and varietal aromas in wines (Gamero et al., 2011a; Oliveira et al., 2014; Stribny et al., 2015). In addition, cryotolerant species such as *S. kudriavzevii* and *S. uvarum* can grow and ferment at low temperatures, thus favoring wine aroma retention. Moreover, these attributes can mitigate the effects of climate change on grapes and fulfill new consumer demands (Querol et al., 2018).

These wild *Saccharomyces* strains also show poor competitive ability under different fermentation environments (Arroyo-López et al., 2010; Alonso-del-Real et al., 2017; Origone et al., 2017; Su et al., 2019a). Consequently, as for non-*Saccharomyces* yeasts, their application as pure culture starters at the industrial level is somehow restricted (Deroite et al., 2018). However, despite these limitations, many studies have found natural hybrid yeasts derived from these wild strains having enhanced winemaking tolerance (Belloch et al., 2008; Gangl et al., 2009; Gamero et al., 2011b; Silva

et al., 2015; Ortiz-Tovar et al., 2019). The presence of natural hybrids in fermentative and wild environments is relatively frequent and results from adapting to the prevailing environmental conditions (González et al., 2006; Peris et al., 2018). Thus, this process originates organisms (intra- and interspecific) with inherited physiological properties from both parents, resulting in improved oenological characteristics (González et al., 2007).

Therefore, the use of hybrid yeasts that overcome some wild strain limitations is more practical for the wine industry than the mixed culture strategies required with non-*Saccharomyces* yeasts. In addition, the resulting hybrids can cover a wide range of oenological objectives. For example, *S. cerevisiae* × *S. uvarum* hybrids have been generated with increased ethanol tolerance, high glycerol synthesis, and increased capacity to release volatile thiols (Masneuf et al., 2002; Lairón-Peris et al., 2020). Likewise, *S. cerevisiae* × *S. uvarum* and *S. cerevisiae* × *S. eubayanus* hybrids with improved fermentative capacity at low temperatures and under low nitrogen conditions have been achieved (Su et al., 2019b). Commercial wine yeasts have also been improved by generating *S. cerevisiae* × *S. cerevisiae* hybrids (Pérez-Través et al., 2015).

Hybrid yeasts can be generated by different techniques, including the so-called rare-mating. Besides being a non-GMO (Genetic Modified Organisms) and easy-to-use method, rare-mating results in the formation of organisms with sufficient genetic information from both parental yeasts (Schillberg et al., 1991; Pérez-Través et al., 2012).

In our previous studies, wild yeasts having attractive oenological traits for Tempranillo and Albariño wines have been characterized. In this regard, a wild *S. uvarum* strain producing a significant number of high-impact aromas in Tempranillo wines compared to a wine *S. cerevisiae* strain was found. This *S. uvarum* strain also developed Albariño wines of great oenological interest,

releasing typical varietal aromas. Besides the acidity provided by its highest succinic acid production, it synthesized the highest concentration of fruity linear and branched ethyl esters and showed a significant ability to release varietal polyfunctional mercaptans (Pérez et al., 2022a).

Regarding the latter aroma compounds, two *S. kudriavzevii* strains, isolated from natural niches, revealed a remarkable capacity to release high amounts of polyfunctional mercaptans (PMFs), mainly 4-methyl-4-mercaptopentan-2-one (4MMP) and 3-mercaptohexanol (3MH) in Albariño wines (Pérez et al. 2022a). This ability and their cryophilic aptitude make them suitable for white varieties fermentation, such as Sauvignon Blanc. Additionally, among young Tempranillo wines, one *S. kudriavzevii* strain showed the highest yields of fruity ethyl esters, while a cachaça *S. cerevisiae* strain developed significant C_{13}-norisoprenoids and vanillin derivatives levels (Pérez et al., 2022b).

In this context, these strains described above are ideal for achieving wines according to the current wine sector demands. However, for their application in the winemaking industry as pure culture, they need to acquire certain aptitudes specific to wine strains. Therefore, this work aims to generate non-GMO hybrid yeasts with intermediate or superior oenological characteristics to those desired from their parental strains. More precisely, hybrid yeasts with optimal fermentation activity inherited from the wine yeast and producing wines with high content of desired aromas and better oenological characteristics (i. e., low ethanol levels, high acidity, and low acetic acid production) inherited from the wild yeasts.

2. Materials and Methods

2.1. Parental yeast strains used for hybridization.

Hybrid yeasts used in this work were generated from 4 strains of *S. kudriavzevii* (SKR and SKA), *S. uvarum* (UE), and *S. cerevisiae* (SC1) isolated from natural environments. These four strains were previously selected for their significant capacity to produce high-impact aromas of Albariño and Tempranillo wines (Table 1). Each of these selected strains was crossed with a wine strain (*S. cerevisiae*, coded as LALL) provided by Lallemand Bio S.L.

2.2. Hybrids generation by rare-mating methodology

The hybridization process was carried out following the steps described in Pérez-Través et al. (2012) with some modifications. The first step consisted in the isolation of auxotrophic organisms from each candidate parental strain. Therefore, strains were grown overnight in 15 ml of GPY liquid medium (2% glucose, 0.5% peptone, 0.5% yeast extract) at 28ºC in an agitated incubator. Then, the recovered cellular content was spread on multiple agar plates containing selective media, named α-aminoadipic acid (α-AA), 5-fluorotic acid (5-FOA), and 5-fluoroantranilic acid (5-FAA) (Zarett and Sherman, 1985; Boeke et al., 1987; Toyn et al., 2000). Such mediums enable the growth of natural lys-, ura- and trp- mutants, respectively. Once mutants were obtained, their auxotrophic trait was corroborated, as explained in Lairón-Peris *et al*. 2020.

Secondly, these auxotrophic yeasts were grown overnight in 50 ml of GPY. The recovered cells were mixed in pairs according to the hybrids to be obtained (Table 2) and having opposed auxotrophies. Next, 100 µL of each yeast pair was incubated at 28°C without shaking in 15-mL slant tubes containing 2 mL of GPY. Every 1, 3, 5 days, 500 µL of each cross was seeded in MM plates (0.17% Yeast Nitrogen Base without amino acids, 2% glucose, and 2% agar) and incubated for a maximum of 5 days at 25ºC (*S. cerevisiae* × *S.* non-*cerevisiae*) or 28ºC (*S. cerevisiae* × *S. cerevisiae*). As colonies

appeared, they were individually purified for further molecular analysis to confirm the hybrid profiles. Accordingly, to Pérez-Través et al. (2012), for intraspecific hybrids, PCR-microsatellite analysis was applied. In contrast, interspecific hybrids were verified by PCR-RFLP analysis of nuclear genes (Pérez-Través et al., 2012). In this case, MAG2 and GSY1 were selected for hybrid confirmation. DNA content was then measured in hybrids and their parent strains by flow cytometry (Lopes et al., 2010). At the same time, their molecular profiles were determined by mtDNA-RFLP analysis (Querol et al., 1992) and inter-δ sequence analysis (Legras and Karst, 2003).

Five rounds of successive seven-day laboratory fermentations were carried out to achieve genetic stabilization of the obtained hybrid yeasts (Pérez-Través et al., 2014). From rounds 4 and 5, the molecular patterns of ten colonies were compared with each original hybrid. If colonies with different molecular profiles were observed, they were selected for greater diversity and included in the yeast stabilization program. Once the stabilization of the different selected hybrids has been achieved, the fermentative capacity of these stable hybrids was compared with the parental strains. The fermentations were carried out in shaken flasks with 50 ml synthetic must (220 g/L of reducing sugars) at 16°C and 25°C. Finally, the best fermenting stable hybrids were selected for the present work.

2.3. Must composition and fermentation conditions

The semi-synthetic musts of the two varieties, Albariño and Tempranillo, were prepared as we described in previous works (Pérez et al., 2022a, 2022b) with some modifications. Solutions of phenolic and aromatic varietal fractions (PAFs) and polyfunctional mercaptans (PFMs) precursors were provided by Zaragoza University. Due to the higher contribution of PMFs in white wines, the solution of their cysteine and glutathione precursors was added only to Albariño must at the following concentrations: 0.05 mg/L of Glut-3MH, 0.1 mg/L of Glut-4MMP, 0.05 mg/L of Cys-3MH, 1 mg/L of Cys-4MMP. The

sugar must content was 210 g/L (105 g/L glucose and 105 g/L fructose) for Albariño must and 230 g/L (115 g/L glucose and 115 g/L fructose) for Tempranillo must. Must pH was adjusted to 3.3 and then filtered for sterilization (0.22 μm).

Fermentations were carried out in a 100 mL sterile flask containing 80 mL of each varietal must, a stirrer magnet (100 rpm), and closed by a rubber stopper equipped airlock valve (n=3). Temperature fermentation was set at 16 ºC for Albariño and 25 ºC for Tempranillo fermentations. Yeast strains were inoculated at 1 x 10^6 cells/mL from an overnight culture grown in GPY. Fermentations were monitored by daily weight loss, and once the reducing sugar content was less than 1 g/L, the fermentation process was considered finished. Then, 18 mL of Tempranillo wines were subjected to accelerated anoxic aging (50ºC, 5 weeks), simulating bottle aging and following the methodology proposed by Oliveira and Ferreira (2019).

2.4. Determination of kinetic parameters and main fermentation by-products

Weight loss curves were fitted by the non-linear regression Gompertz model proposed by Zwietering et al. (1990), adapted for maximum fermentation rate (Vmax), as explained in other works (Pérez et al., 2021). The daily weight loss in each must volume (80 mL in Albariño and 50 mL in Tempranillo) was expressed in grams lost per liter.

In the final wines and during the last days of fermentation, the content, in g/L, of glucose, fructose, erythritol, glycerol, tartaric, citric, malic and succinic acids, and ethanol (%v/v) were analyzed by HPLC (High-Performance Liquid Chromatography, Thermo Fisher Scientific, Waltham, MA, USA) under the same equipment conditions and following the protocol described in Pérez *et al.*, 2021.

2.5. Volatile compound analysis on the final wines

Major (> 0.2 mg/L) and minor (0.1-200 μg/L) volatile compounds were analyzed in young wines of both varieties. Additionally, in Albariño wines, PFMs were analyzed, and in aged Tempranillo samples, minor volatile compounds were quantified.

Major aromas were liquid-liquid micro-extracted, identified, and quantified by a gas chromatograph with flame ionization detector as has been described by Ortega et al. (2001). Minor volatile compounds were solid-phase extracted (SPE) and quantified using a gas chromatograph coupled to a mass spectrometer detector (GC-MS, Shimadzu QP2010, Quioto, Japan) based on the methodology developed by López et al. (2002). PFMs were extracted by solid-phase, followed by derivatization, and analyzed by thermal desorption-GC-GC-MS, following the methods of Mateo-Vivaracho et al. (2010) with some modifications as described elsewhere (Pérez et al., 2022a).

2.6. Statistical data analysis

All parameters (Vmax, primary metabolites, and aroma compounds) were treated by one-way ANOVA followed by Tukey's HSD test, considering a significance level of $p < 0.05$. The results were expressed as the average value ± standard deviation from three replicates. The aroma data set was treated by hierarchical clustering using Euclidean distance and, principal component analysis (PCA) was applied to visualize the treatments to these measured multi-variables. For the statistical treatment, Infostat software (2011 version, Grupo Infostat, Córdoba, Argentina) was used and, plots were generated using GraphPad Prism version 8.0 software (Graph-Pad Software, Inc., La Jolla, CA).

3. Results

3.1. Hybrid obtention and molecular characterization of the stable hybrids

Four crosses were made using the wine yeast LALL as a common parental strain and four wild strains (Table 1), resulting in 5 different original hybrids (Table 2), one for each cross and two for the cross with KR strain.

According to the parental ploidy (Table 1) and DNA content of the resulting hybrids (Table 2), the rare-mating crosses were indeed made between two spores (n × n crosses) or between a spore and a diploid cell (n × 2n crosses), indicating the ability of all the parental strains to sporulate in these conditions.

Initial hybrids from KR (*S. kudriavzevii, Sk*) and CS (*S. cerevisiae, Sc*) parental yeasts inherited LALL mitochondrial DNA (mtDNA), whereas crosses with KA (*S. kudriavzevii*) and UE (*S. uvarum, Su*) parental yeasts presented a mixture of mtDNA profiles. Each original HKA and HUE hybrid derived in two different stable profiles that differed in their mtDNA (HKA4, HKA5, HUE2, and HUE5), which were again subjected to stabilization. Contrary, HKRI, HKRII, and HCS original hybrids maintained the same stable profile after the stabilization process naming them HKR1, HKR8, and HCS3. For this reason, 7 stable hybrids were finally recovered from the 4 crosses made.

Table 1. Physiological characteristics of parental strains.

Parental strain	Species	Designation	Source	DNA content*	Aromas in Tempranillo[#]	Aromas in Albariño[#]	Oenologicalproperties[#]
CR89D1	*S. kudriavzevii*	**KR**	oak (*Q. Faginea*)	2.20±0.002	neutral	3MHA, 4MMP,3MH, R-limonene	Low ethanol, high glycerol
CA111F1	*S. kudriavzevii*	**KA**	oak (*Q. Faginea*)	2.10±0.006	branched ethyl esters	3MHA, 4MMP,3MH, R-limonene	Low ethanol, high glycerol
CECT12600	*S. uvarum*	**UE**	Non fermented liquor	2.01±0.053	geraniol, β-citronellol, α-ionone, vanillin derivatives, PEA	3MHA, 4MMP,3MH, geraniol, PEA, branched ethyl esters	low ethanol, high glycerol, high succinic acid, low acetic acid
CSC1	*S. cerevisiae*	**CS**	Cachaça fermentation	0.99±0.021	vanillin derivatives, ethyl leucate, monoterpenes, isobutyl acetate	neutral	neutral
LALL	*S. cerevisiae*	**LALL**	Wine commercial	1.98±0.01	-	-	-

*DNA content values measured from two replicates (mean ± SD). # Data obtained in previous studies (Pérez et al., 2022a; Pérez et al., 2022b). 3MHA, 3-mercaptohexyl acetate; 4MMP, 4-mercapto-4-methylpentan- 2-one; 3MH, 3-mercaptohexanol, PEA, β-phenylethyl acetate.

Table 2. Molecular characterization of original and stable hybrids showing differential molecular profiles.

Crosses	Original hybrid	*DNA content (crosstype)	mtDNA	δ-PCR	Stable hybrid	*DNA content	mtDNA	δ-PCR
LALL x KR (*Sc* × *Sk*)	**HKRI**	2.13±0.022[a] (nxn)	LALL	1	**HKR1**	2.01±0.002[b]	LALL	1
	HKRII	3.08±0.026[a] (nx2n)	LALL	2	**HKR8**	2.95±0.012[b]	LALL	2
LALL x KA (*Sc* × *Sk*)	**HKA**	2.03±0.038 (nxn)	mixture	1	**HKA4**	1.81±0.105	r1	1
					HKA5	2.07±0.011	LALL	1
LALL x UE (*Sc* × *Su*)	**HUE**	2.86±0.014(nx2n)	mixture	1	**HUE2**	2.87±0.025	r1	1
					HUE5	2.83±0.011	UE	1
LALL x CS (*Sc* × *Sc*)	**HCS**	3.02±0.021[a] (nx2n)	LALL	1	**HCS3**	2.48±0.008[b]	LALL	1

Sc, Saccharomyces cerevisiae; Sk, Saccharomyces kudriavzevii; Su, Saccharomyces uvarum, r1, recombinant mitochondrial genome. *DNA content values measured from two replicates (mean ± SD). According to ANOVA ($p < 0.05$) and Tuckey HSD test, letters superscripts denote DNA content statistically different from the original hybrid. Molecular profiles were determined by mtDNA-RFLP analysis (Querol *et al*., 1992) and inter-δ sequence analysis (Legras and Karst, 2003).

More precisely, mtDNA analysis revealed that *Sc*× *Sk* and *Sc*× *Sc* crosses mainly retained *Sc* LALL mtDNA except for the recombinant mitochondrial profile (r1) found in the HKA4 stable hybrid. While *Sc*× *Su* crosses had the UE (*Su*) mtDNA and a recombinant mtDNA in each stable yeast. In addition, the delta pattern (δ-PCR) remained unchanged compared with their original hybrids, whereas a poorly DNA loss was observed. Indeed, a statistically significant reduction ($p < 0.05$) was determined concerning ploidy after stabilization in HCS3 (*Sc*× *Sc*), by 0.5 points, and to a lesser extent in the hybrids HKR1 and HKR8 (Table 2).

3.2. Characterization of fermentation capacity and metabolite production of hybrids

Albariño fermentations were carried out at 16 ºC by the two *Sc*× *Su* hybrids and the four *Sc*× *Sk* hybrids. Tempranillo fermentations were performed at 25ºC with three hybrids: HSC3 (*Sc* × *Sc),* HUE5 (*Sc* × *Su),* and HKA5 (*Sc*× *Sk*). Additionally, the shared parental strain among all hybrids (wine *Sc* strain, LALL) was included as a control in both fermentation sets (Figure 1). Fermentations were completed in 14 days (< 1 g/L sugar).

In Albariño fermentations, hybrids HUE2 and HUE5 (*Sc*× *Su*) and HKR8 (*Sc*× *Sk*) showed shorter lag phases than LALL. Despite starting fermentations earlier, their specific maximum rates (Vmax) were lower to LALL and HKR1. The latter hybrid, HKR1, showed the best performance, equal to LALL, while HKA4 was the slowest, with a more extended lag phase and the lowest maximum rate. However, HKA4 finished fermentation simultaneously as LLAL, while it took longer for HUE2 and HUE5 strains.

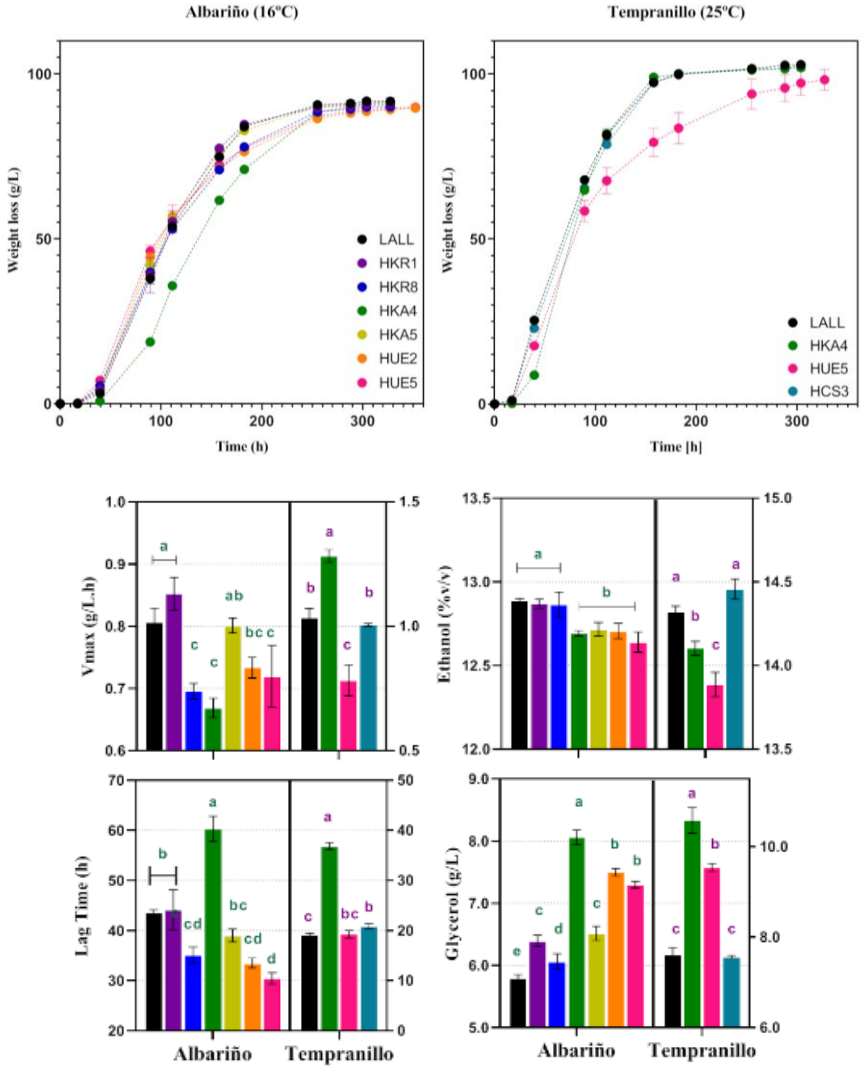


Figure 1. Weight loss curves, kinetic fermentation parameters, and primary metabolites (mean ± SD) produced by hybrid yeasts and parental wine strain LALL during fermentation in semi-synthetic Albariño and Tempranillo musts at 16ºC and 25ºC, respectively.

The Vmax was considerably higher in Tempranillo fermentations than Albariño, probably because of the higher fermentation temperature, resulting in a greater volume of CO_2 released in less time (Figure 1). HCS3 and HKA4 hybrids displayed the best fermentation performances in these conditions. This latter strain also started the fermentation delayed, but then it reached the

highest maximum rate, even higher than LALL, finishing the process without difficulty. HUE5 strain showed the slowest fermentation rates as in Albariño fermentations.

In Albariño wines, an ethanol difference of 0.2 %v/v was observed in HKA4, HKA5, HUE2, and HUE5 hybrids, differing statistically from LALL, HKR1, and HKR8 strains (Figure 1). For Tempranillo wines, the differences were more significant between the strains. HKA4 and HUE5 hybrids produced wines with 0.3 %v/v and 0.48 % v/v less ethanol than LALL and HCS3.

In the case of glycerol, HKA4 produced the highest amounts, reaching 8 g/L in Albariño and 10.5 g/L in Tempranillo (Figure 1). *Sc* × *Su* hybrids also produced large amounts of glycerol (7.5 g/L in Albariño and 9.5 g/L in Tempranillo). HCS3, HKR1, HKR8, HKA5, and the parental *Sc* LALL strain reached the lowest glycerol values in both musts.

Regarding wine's organic acid content (Table S1), HKA4 strain exhibited the highest citric acid contents, though differing slightly. The two *Sc* × *Su* hybrids (HUE2 and HUE5) showed the highest malic acid values in Albariño and the highest succinic acid values in both varietal wines. On the other hand, it was also interesting to note the high amount of 2,3-butanediol determined in both young wines of the HKA4 hybrid.

Interestingly, the hybrids HKR1, HKR8, HCS3, and HKA5 having *Sc* LALL mtDNA, were the most similar to the wine yeast in terms of fermentation performance and by-products production. The rest of the hybrids with *Su* mtDNA (HUE5) or recombinant mtDNA (HKA4 and HUE2), reduced ethanol and increased organic acid yields, highlighting HKA4 to be the highest glycerol producer in both varietal wines.

3.3. Production of volatile compounds by hybrid strains

3.3.1. Aroma composition of Albariño wines

Of the 58 aroma compounds determined above detection limits, 37 showed statistical differences between strains ($p < 0.05$; Table S2) in Albariño wines. To estimate the potential sensorial impact of yeasts on Albariño wines, concentrations of volatiles considered active aroma vectors (Ferreira et al., 2021) were normalized by their odor threshold (OT) and grouped according to a shared sensory descriptor (Table S3).

Therefore, a principal component analysis was applied to the significant compounds and their sensory descriptors associated according to the literature (Figures 2a and 2b). In addition, the assembly of a dendrogram allowed a more general view of the differences between hybrids (Figure 2c). The resulting PCA plot describes 64.3% of the total variance, separating strains into three main groups. PC1 (44.5%) showed the most significant separation between wines, clustering the *Sc* × *Su* hybrids, HUE2 and HUE5, on PCA's right quadrant. Indeed, HUE2 and HUE5 were also separated from the rest by the highest dissimilarity in the dendrogram plot (Figure 2c). Their wines were composed of most fermentative compounds, including branched acids and ethyl esters, β-phenylethanol and acetate, and two lactones, γ-butyrolactone and γ-octalactone. In contrast, the lowest values of 1-butanol and 4-methyl-4-mercaptopentan-2-one (4MMP) were found in their wines. Given the estimated OAVs, these wines would be sensorially characterized by the floral, spicy/woody and lactic/acidic descriptors (Figure 2a).

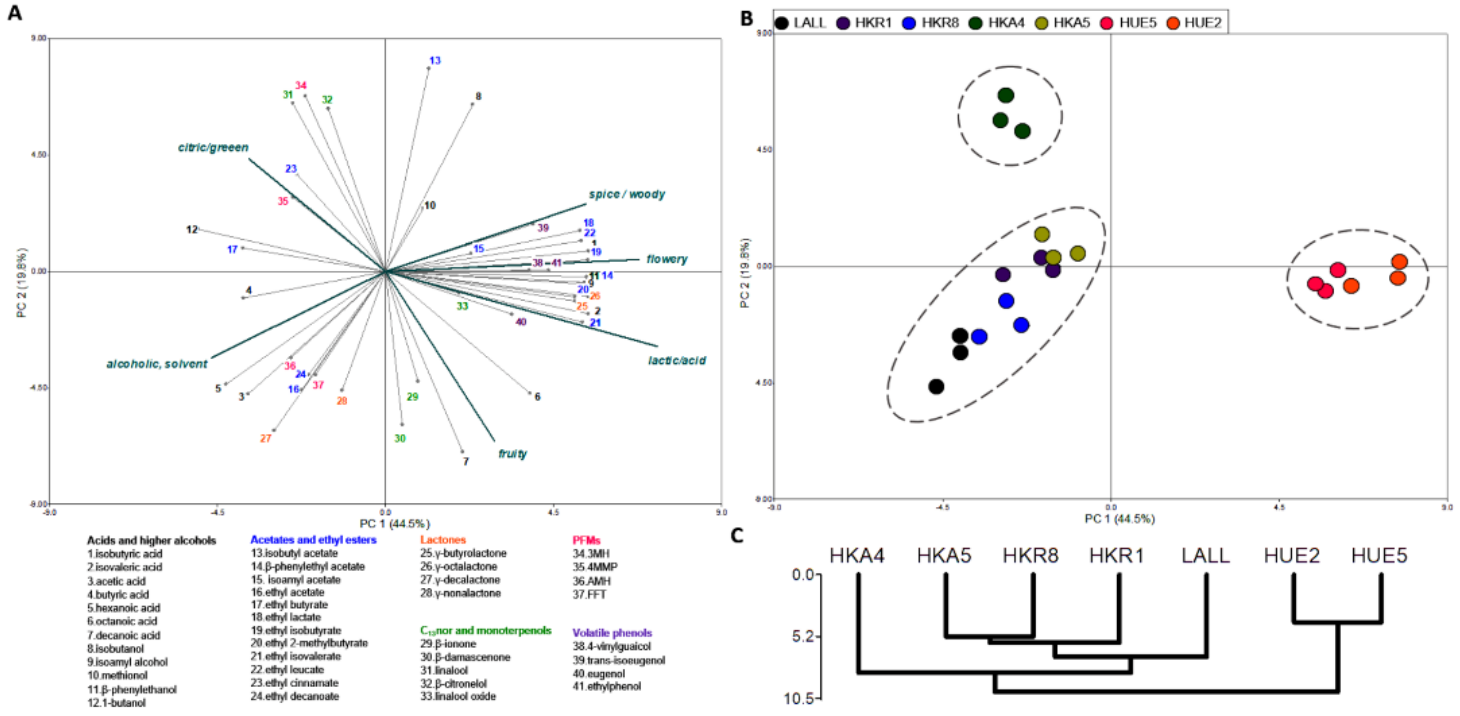


Figure 2. Principal component analysis (A and B) and hierarchical clustering analysis (C) of the major and minor aroma compounds produced by yeast hybrids and parental wine strain LALL in Albariño wines. 3MH, 3-mercaptohexanol; 4MMP, 4-mercapto-4-methylpentan-2-one; 3MHA, 3-mercpatohexyl acetate; FFT, 2-furfurylthiol.

The rest of the strains were mainly opposed to HUE2 and HUE5 due to the superior contents of PFMs. HKA4 wines were located in the upper-left PCA's quadrant characterized by 3-mercaptohexanol (3MH) and the floral compounds ethyl cinnamate, linalool, and β-citronellol. Due to the highest content in 3MH, their wines would be mainly characterized by the citric/green notes. LALL, HKR1, and HKR8 strains showed the highest acetic acid values, and their wines were identified by the most increased ethanol and ethyl acetate contents, involved into the alcoholic/solvent aroma vector. However, HKA5, positioned within this group but within the upper quadrant, showing shared aromatic characters with HKA4.

Finally, the parental yeast, LALL, had the highest values of hexanoic acid while the lowest acetates concentrations, and it was the only strain with detectable TDN and furfurylthiol amounts in the wines recently fermented.

In summary, in Albariño wines, *Sc*× *Su* hybrids were noted for their typical *S. uvarum* aromas, fruity and floral ethyl, and acetate esters, while the *Sc*× *Sk* hybrids were noted for their higher ability to release PFMs. However, HKR1 and HKR8 were the *Sc*× *Sk* hybrids most similar to LALL by the higher acetic acid content involved into the alcoholic/solvent aroma vector. It is worth to note that HKA4 (*Sc*× *Sk*) stood out for its high content in ethyl cinnamate and most varietal compounds, such as monoterpenes and PFMs.

3.3.2. Aroma composition of young and aged Tempranillo wines

In young Tempranillo wines, 42 out of 59 aroma compounds were found to have statistically different concentrations between strains. In contrast, in aged wines, only 16 of the 40 were statistically different (Table S4 and S5). As in previous studies, the aging application to wines (A) produced a significant aromatic differentiation from the young wines (Y) as can be seen in Figure 3a. Nevertheless, each strain's wines were still very different in each of the fermentation conditions, highlighting the great modulating effect of the

yeasts. Regarding this effect, the HKA4 strain appeared to be the most different among young wines (Y:HKA4), while HUE5 yeast was for aged wines (A:HUE5). On the other hand, LALL and HCS3 yeasts remained together in both conditions, indicating that they share similar aroma profiles.

The 44 most relevant aromas determined in young Tempranillo wines showed 70.6% of the total variance among yeasts, as shown in the PCA (Figure 3b), where PC1 (38.3%) separated the parental strain *S. cerevisiae* (LALL) and the *Sc* × *Sc* hybrid (HCS3) from the *S. cerevisiae* × non-*cerevisiae* hybrids HUE5 and HKA4. As in Albariño, isobutyl acetate was highly produced by the HKA4 strain, while HUE5 achieved the highest levels of isobutyric and isovaleric acids, the latter strain associated once more with the lactic/acidic vector. Furthermore, the highest γ-octalactone and β-phenylethyl acetate characterized these hybrids' young wines. This last compound, β-phenylethyl acetate, would be responsible for linking the flowery vector to the young wines of these hybrids.

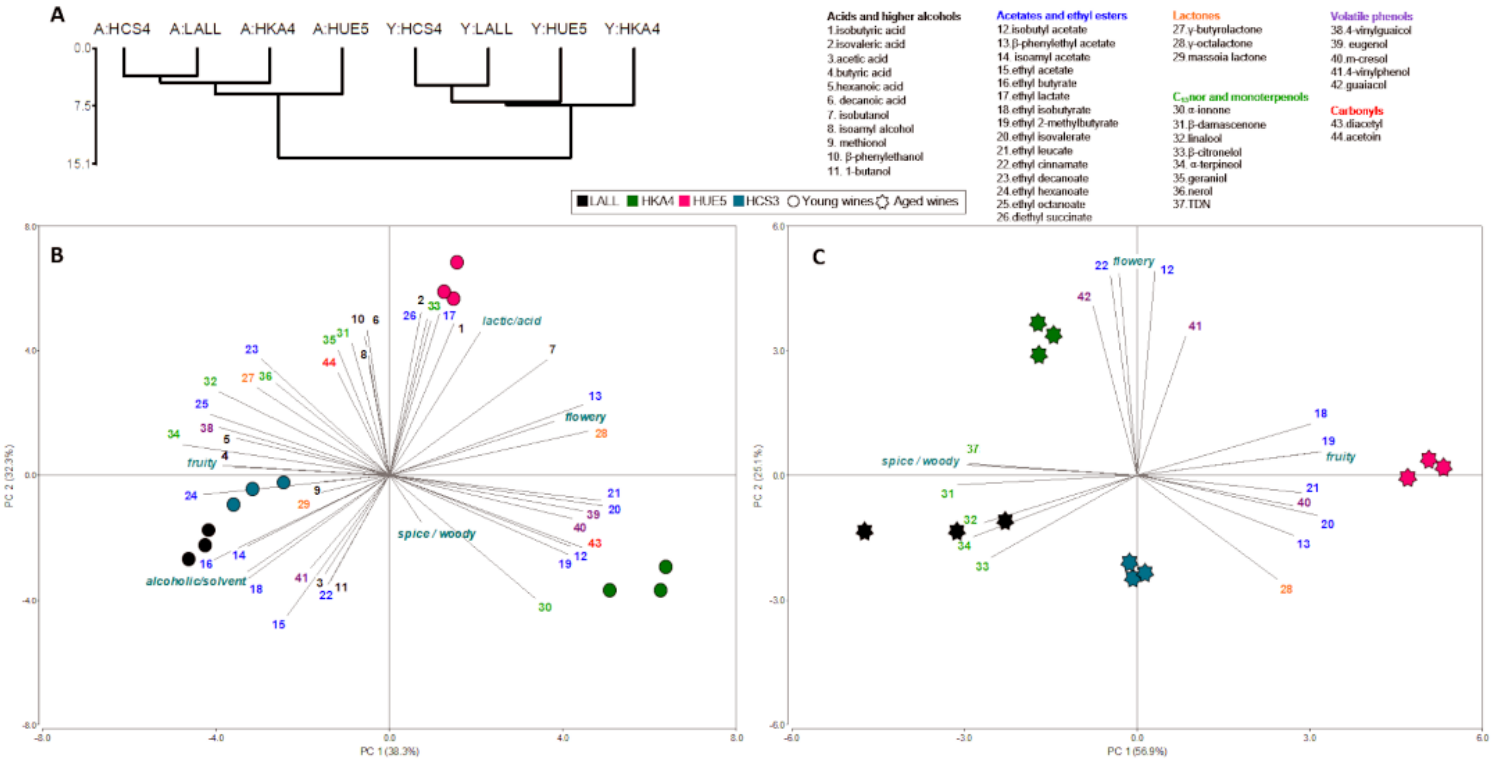


Figure 3. Hierarchical clustering analysis (A) and principal component analysis (B and C) of major and minor aroma compounds produced by yeast hybrids and parental wine strain LALL in Tempranillo young wines (“ Y”) and in Tempranillo aged wines (“ A”).

On the other hand, LALL and HCS3 (only *Sc* genome background) were characterized by fruity compounds, such as ethyl octanoate, hexanoate, butyrate, isobutyrate, and isoamyl acetate. However, the alcoholic/solvent aroma vector would be characterizing their wines due to the highest yields in ethanol, ethyl acetate in LALL, and 1-butanol and methionol in HCS3.

After accelerated aging application, yeasts' sensory profiles changed (Figure 3c). PC1 (56.9%) distinguished HUE5 from the rest of the yeasts; its wines were characterized by fruity volatiles. This hybrid, which in its young wines had a high content of branched fatty acids, now, due to their esterification during accelerated aging process, the corresponding fruity ethyl esters reached the maximal concentrations. In this regard, concentrations of ethyl isobutyrate, ethyl isovalerate, and ethyl 2-methylbutyrate increase 1267, 165, and 220-fold compared to its young wines (Tables S4 and S5). Likewise, ethyl cinnamate concentrations also increase after aging, around 25-fold compared to young wines. This floral ethyl ester, highly produced by LALL in young wines, was now detected in aged HKA4 wines at the highest levels, 3.5-fold higher than the rest of the aged wines. In contrast, isobutyl and β-phenylethyl acetates decreased due to aging.

The aged LALL wines were characterized by a higher content of some terpenes and C_{13}-norisoprenoids. Here, the most significant differences were found in β-damascenone and TDN, the latter conferring spice/woody notes was detected in higher amounts in aged LLAL wines. Finally, HCS3 and HUE5 aged wines had the highest γ-octalactone and ethyl leucate concentrations, where ethyl leucate values were close to 250 μg/L and 1.5-fold higher than LALL and HKA4 yeasts.

As a summary, in young Tempranillo wines, the *S. cerevisiae*× *S. no-cerevisiae* hybrids showed similar characteristics to Albariño wines, such as the highest contents of acetate esters, γ-octalactone, and branched-chain fatty

acids. However, when aging was applied, these wines showed much more interesting profiles, with the highest yields of fruity esters in HUE5 and ethyl cinnamate and ethyl leucate in HKA4, having fruity and floral as general descriptors.

3.3.3. Aroma differences with the wine strain LALL

Wines fermented by the hybrids achieved the production of desirable compounds, usually associated with attractive aromatic attributes, at concentrations significantly higher than LALL wine parental yeast ($p < 0.05$). Figure 4 represents the ratio between aroma concentrations in hybrids versus LALL.

Most hybrid yeasts differed from LALL wine strain by the highest production of fruity aromas: γ-octalactone, isobutyl acetate, branched ethyl esters, and PFMs (Figure 4). The most significant differences were found in γ-octalactone, 8 to 12-fold higher in hybrids than LALL, highlighting the hybrids HKA4, HCS3 in Tempranillo wines and *S. uvarum* hybrids in Albariño. As for isobutyl acetate, all hybrids in Albariño wines highly synthesized this pear-like compound, more than doubling LALL concentration. HKA4 was the higher producer of this aroma, 4.5 to 6 times higher than the reference yeast (LALL) in all the wines analyzed. Hybrids (except HKR1 and HKR8) also produced fruity esters at concentrations, over twice that of LALL, where HUE2 and HUE5 strains had the highest values, reaching 5-fold and 8-fold higher contents in Albariño and aged Tempranillo, respectively.

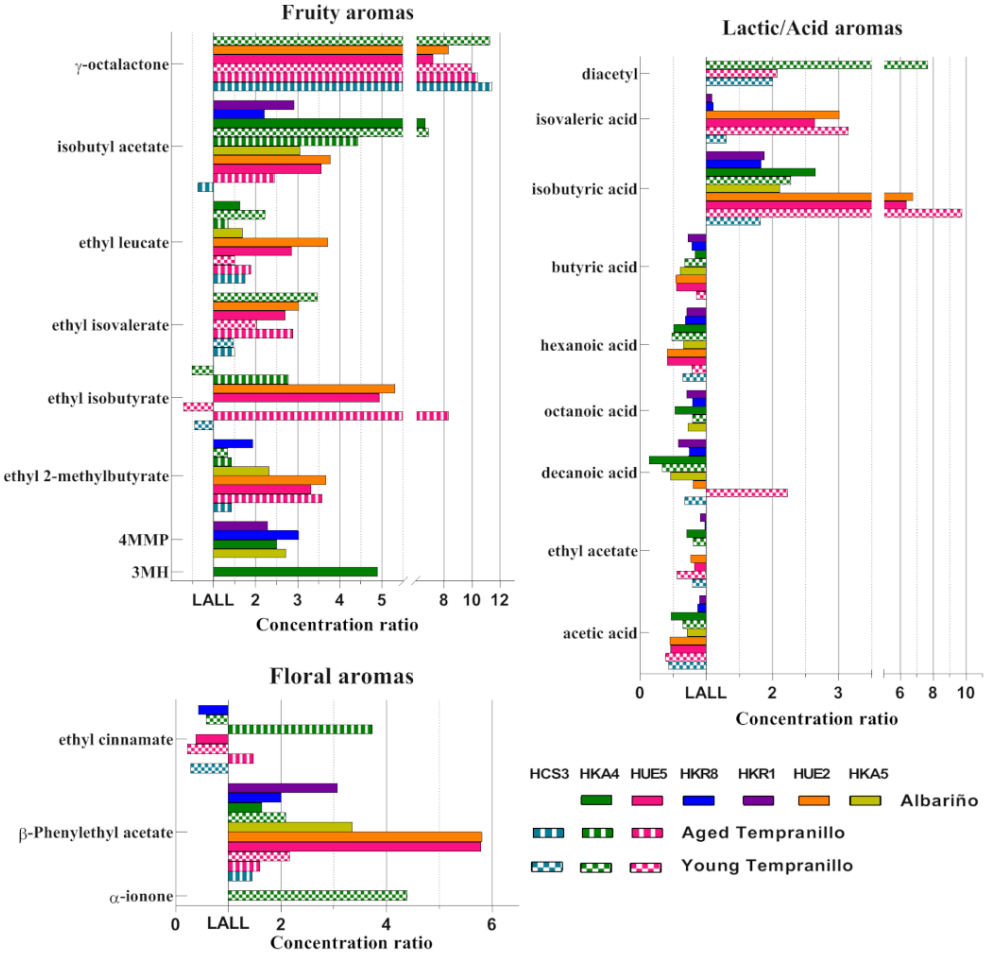


Figure 4. Average aroma concentration of the hybrids relative to the concentrations determined in LALL wine strain, in Albariño (fulfill), young Tempranillo (square fill), and aged Tempranillo (line fill). 3MH, 3-mercaptohexanol; 4MMP, 4-mercapto-4-methylpentan-2-one.

Concerning polyfunctional mercaptans (PFMs) determined in Albariño wines, all *S. kudriavzevii* hybrids were the major 4MMP releasers, reaching concentrations about 2 to 3-fold higher than those of LALL parental strain. Interestingly, the HKA4 strain released the highest concentrations of 3MH, another important PFM, 5-fold above all yeasts, including LALL.

Regarding compounds with floral aromas, hybrids involving *S. uvarum* species produced β-phenylethyl acetate at almost 6-fold higher concentrations than LALL in Albariño, whereas HKA4 produced a 4.5-fold higher concentration of α-ionone in young Tempranillo; additionally, these concentrations exceeded their odor thresholds by around 1.5 OAV (Table S3) in both aroma compounds. The HKA4 wines quadrupled the LLAL wines in ethyl cinnamate content, a compound prevalent in the other LALL wines.

Volatiles imparting lactic/acid notes (major compounds) were only determined in Albariño and Tempranillo young wines. Within this group, diacetyl was only detected in Tempranillo young wines, showing a great production by the hybrids, where HKA4 produced concentrations almost 8-fold higher than LALL (Figure 4). On the other hand, both isobutyric and isovaleric acids were overproduced by all hybrids, between 3 to 10-fold higher than LALL. On the contrary, HKR1, and HKR8 did not differ much from LALL, having the lowest amounts of these two acids and consequently the lowest values of their fruity branched esters in Albariño. In addition, all hybrids were characterized by having lower amounts than the LALL strain in the rest of fatty acids such as butyric, hexanoic, octanoic, and decanoic. HUE2 and HUE5 were notable for reducing the content of these compounds, whereas HKA4 produced almost 8-fold less decanoic acid in Albariño and Tempranillo young wines.

Among the undesirable flavors in the wines, all hybrids, whether Albariño or Tempranillo, produced less ethyl acetate and up to 50% less acetic acid than LALL.

4. Discussion

Wild and non-wine yeasts have been extensively characterized for producing fermentative compounds that can favor the organoleptic quality of

wine and counteract the effects of climate change on grapes. Despite the recent interest in non-*Saccharomyces* yeasts to reduce the ethanol content of wines and to improve their aromas, most of these species are easily replaced by *S. cerevisiae* during wine fermentations. A similar situation has been also described for other *Saccharomyces* species (*S. uvarum* and *S. kudriavzevii*), they can be replaced by *S. cerevisiae* in wine fermentation conditions (Arroyo-López et al., 2010; Alonso-del-Real et al., 2017). Since several studies have found natural hybrid yeasts possessing enhanced fermentative properties acquired from wine and cryotolerant wild strains (González *et al*., 2007; Gangl et al., 2009; Ortiz-Tovar et al., 2019), hybrid-induced generation would be a solution. In previous studies (Pérez et al., 2022a, 2022b) we selected non-wine yeasts of the species *S. uvarum*, *S. kudriavzevii*, and *S. cerevisiae* with attractive oenological attributes, and we generated hybrids with a wine *S. cerevisiae* strain by rare-mating.

Hybrid strains can inherit the nuclear genome of both parentals. This statement suggests that they may acquire different capabilities, such as tolerance to low temperatures from their cryophilic parental or resistance to high ethanol concentrations or high fermentation temperatures from their wine parental *S. cerevisiae* strain (Masneuf et al., 2002; González et al., 2006; Belloch et al., 2008; Arroyo-López et al. 2010; Lairón-Peris et al., 2020). In our study, we observed that hybrids HKR1, HKR8, HKA5, and HCS3 that inherited the mitochondrial DNA of the wine strain, were the most similar to LALL in terms of fermentation pattern, ethanol and glycerol production in both, Albariño (16ºC) and Tempranillo (25ºC) fermentations. However, hybrids having recombinant or *S. uvarum* mtDNA such as HKA4, HUE2, and HUE5 strains, showed a higher lag phase affecting the fermentation beginning, lower maximum fermentation rates (Vmax), and longer fermentations indicating a low tolerance to high-temperature fermentation, and low tolerance to ethanol presence resembling those characteristics of the

cryotolerant *S. uvarum* and *S. kudriavzevii* parental species (Alonso-del-Real et al., 2017; Lairón-Peris et al., 2020).

But the most interesting is that these hybrids showed differences in the ethanol, glycerol, and organic acids yields depending on the mtDNA inherited. The hybrids with *S. cerevisiae* mitochondria have more evident similarities with *S. cerevisiae* parental strain (LALL) showing lower glycerol production and high ethanol yields. On the contrary, HKA4 (*Sc* × *Sk*) produced the highest glycerol and 2,3-butanediol yields, while *Sc* × *Su* hybrids having the most significant amounts of succinic acid and the lowest of acetic acid reinforces the fact that they mainly inherited typical traits wildly studied in their cryotolerant parental species (Querol et al., 2018). It has been suggested that hybrid yeast carrying the mitochondrial genome of one parental yeast confer the optimal growth temperature of the donor parent (Baker et al., 2019), however, according to our results, the mitotype inhered could be contributing to fermentation capability as well as with the main fermentative by-products involved in redox homeostasis, namely glycerol, succinic acid, 2,3-butanediol.

Aroma profiles inherited

We also observed a good correlation between the mitochondria and the aroma in Albariño wines, where yeast sharing the same mitochondrial DNA of *S. cerevisiae* (LALL) aromatically resembled the wine strain by the low fruity ethyl ester content. At the same time, the ability to produce certain aroma compounds typical of non-*cerevisiae* strains seems to have been inherited, such as the high production of PFMs in Albariño wines and acetates esters in both varietal wines. However, they shared with LALL the highest levels of ethanol, belonging to the alcoholic/solvent aroma vector and that tend to decrease the fruity flavors (Ferreira et al., 2021). Therefore HKA4, HUE2, and HUE5 hybrids possessing mitochondria of non-*cerevisiae* species were the most different on the aroma production level, noted by the floral and fruity

descriptors. In future works, we need to confirm if this is only associated with inherited mitochondria, or there may also be a larger fraction of genomic DNA from *S. cerevisiae* or non-*cerevisiae* parentals.

The differences observed in HUE2 and HUE5 compared to LALL were mainly on the production of fermentative aromas typically found in *S. uvarum* species, such as fruity ethyl esters (Pérez et al., 2022a, 2022b), phenylethyl acetate, and its alcohol (Stribny et al., 2015). Minebois et al. (2020) have recently reported that this species highly produced these fermentative compounds to compensate for the fermentation redox balance, and in addition, they are high acetyl-CoA producers by consuming acetate during the first stages of fermentation. For this reason, the two *Sc*× *Su* hybrids liberated the lowest values of acetic acid. As observed previously in their parental *S. uvarum* strain (Pérez et al., 2022a, 2022b, Table 1), these hybrids produced large amounts of fruity ethyl esters, corresponding to the large production of isobutyric and isovaleric acid. During accelerated aging, these acids continued to be esterified, which resulted in the significantly highest concentrations of ethyl isobutyrate and ethyl isovalerate observed in HUE5 aged Tempranillo wines. In addition, during the aging process, acetates, such as the rose-like aroma β-phenylethyl acetate, suffered a decrease by hydrolysis (Díaz-Maroto et al., 2005). As a result of this chemical process, we could expect an evolution from a floral profile in young wines to a fruity profile in aged wines. At the same time, the opposite happened with the HKA4 wines, where the floral-like ethyl cinnamate compound increased considerably during aging, evolving from fruity to floral aged wines.

Polyfunctional mercaptans are desired high-impact aromas that, despite being at very low concentrations in wines, are generally above their perception thresholds (Mateo-Vivaracho et al., 2010), providing tropical-citric notes to white wines such as Sauvignon Blanc (Tominaga et al., 1998; Roland et al.,

2011). These aromas, mainly 4MMP, were previously determined to be highly released by the parental strains KR (CR89D1), followed by KA (CA111F1) (Pérez et al., 2022a). Fortunately, one interesting finding was that all the *Sc*× *Sk* hybrids generated using these latter wild yeasts showed a solid ability to free 4MMP (2-3 fold higher than LALL) and 3MH. Natural yeast hybrids of *Sc* × *Sk* cross have already been reported to release PFMs in high amounts, but they are also shown to produce high acetic acid yields under certain winemaking conditions (Murat et al., 2001; Gangl et al., 2009; Deroite et al., 2018). Among this interspecific cross, we found that the HKA4 hybrid produced the lowest acetic acid levels than the wine strain LALL and the other *Sc*× *Sk* hybrids while yielding the maximal 3MH levels.

This latter compound (3MH) is one of the most appreciated thiols in Sauvignon blanc wines (Tominaga et al., 1998), providing tropical notes such as grapefruit (Swiegers and Pretorius, 2007). Recently, several studies have determined that Gluthatione-3MH is the 3MH major precursor (Subileau et al., 2008; Alegre et al., 2017). PFMs are released from their precursors by the action of a β-lyase enzyme. Protein encoded by gene *GLO1* is known to participate in the condensation reaction between methylglyoxal and glutathione as a detoxification mechanism (Inoue and Kimura, 1996), but also was reported to have C-S lyase activity on thiol precursors (Howell et al., 2005). Since methylglyoxal and glycerol shares the same precursor, namely dihydroxyacetone phosphate, and given the very high level of glycerol produced by HKA4 strain, we inferred that this strain could take advantage Glut-3MH to utilize glutathione for the methylglyoxal detoxification, ultimately releasing 3MH.

Finally, since these precursors enter through specific amino acid transporters whose efficiency depends on several regulatory mechanisms, this implies differences in the uptake of the two precursors depending on the yeast strain (Thibon et al., 2008; Winter et al., 2011; Pinu, 2018). According to this,

overexpression of genes related to amino acids transporters or β-lyases enzymes could explain the substantial 4MMP release by *S. cerevisiae*× *S. kudriavzevii* strains from Cys-4MMP precursor. In contrast, overexpression of the gene *GLO1* involved in the methylglyoxal detoxification mechanism could explain the 3MH massive release from Glut-3MH by HKA4 yeast.

On the other hand, HUE2 and HUE5 hybrids, whose parental yeast UE was described as good PFMs-producer (Pérez et al., 2022a), produced the lowest amounts and did not differ from LALL among these compounds, which also contrasts with previous works that have characterized *Sc*× *Su* hybrids as high PFMs releasing (Masneuf et al., 2002; Dubourdieu et al., 2006).

Another interesting finding was that in both varietal wines, γ-octalactone and isobutyl acetate seem to be distinct aroma components from all hybrids generated, where the acetate was another compound that described the HKA4 aroma profile. Despite having a very high perception threshold, γ–octalactone, coconut-like lactone, could be a key compound in non-wine yeasts since all these non-wine yeast-derived hybrids highly produced it. In addition, its presence in wild strains has been reported in our previous work (Pérez et al., 2022a). However, to explore this regard, there is still little information on its synthesis pathway in *Saccharomyces* spp. Theoretically, γ–octalactone could be derived from the octanoic acid lactonization or isoamyl alcohol and acrylic acid conjugation, an acid not yet identified in wine (Berger and Zorn, 2004; Romero-Guido et al., 2011).

5. Conclusion

In summary, in this work, we generated non-GMO hybrid yeasts that were shown to have inherited the best aromatic and chemical properties of their wild parents in Albariño and Tempranillo wines. The wine *S. cerevisiae* parent gave to some hybrids a better fermentation capacity, which we related to

having inherited its mitochondria. The hybrids that inherited non-*cerevisiae* mitochondria reduced ethanol levels by around 0.3-0.45 %. In addition, hybrids showed typical traits inhered from its cryotolerant parental species, such as in HKA4 hybrid, which showed the highest levels of citric acid, 2,3-butanediol and glycerol, or the *Sc*× *Su* hybrids, which produced the highest succinic acid and the lowest acetic acid contents.

Regarding the aromatic profiles, several appreciated aroma compounds were significantly produced by all the hybrids compared to *S. cerevisiae* strain, such as isobutyl acetate, β-phenylethyl acetate, and γ-octalactone. The *Sc*× *Su* crosses were characterized by higher fruity esters in both wines, mainly in the aged Tempranillo, where a significant increase was observed. Whereas the *Sc*× *Sk* crosses were mainly characterized by higher production of PFMs in Albariño, where the highest levels of 3MH noted HKA4 hybrid. We also postulated that HKA4 releases 3MH from glutathione nonvolatile precursor to detoxify the cell from the presence of methylglyoxal, a compound related to its highest glycerol synthesis.

Acknowledgments

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement number 764364, Aromagenesis, and from Generalitat Valenciana grant PROMETEO/2020/014.

Conflict of interest

The authors declare no competing interests.

References

Alonso-del-Real, J., Lairón-Peris, M., Barrio, E., and Querol, A. (2017). Effect of temperature on the prevalence of *Saccharomyces* non *cerevisiae* species against a *S. cerevisiae* wine strain in wine fermentation: Competition, physiological fitness, and influence in final wine composition. *Frontiers in Microbiology*, **8**(150). https://doi.org/10.3389/fmicb.2017.00150

Arroyo-López, F.N., Salvadó, Z., Tronchoni, J., Guillamón, J.M., Barrio, E., and Querol, A. (2010). Susceptibility and resistance to ethanol in *Saccharomyces* strains isolated from wild and fermentative environments. *Yeast* 191–198. https://doi.org/10.1002/yea

Baker, E. P., Peris, D., Moriarty, R. V., Li, X. C., Fay, J. C., and Hittinger, C. T. (2019). Mitochondrial DNA and temperature tolerance in lager yeasts. *Science Advances*, 5(1), 1–8. https://doi.org/10.1126/sciadv.aav1869

Belloch, C., Orlic, S., Barrio, E., and Querol, A. (2008). Fermentative stress adaptation of hybrids within the *Saccharomyces* sensu stricto complex. Int. J. Food Microbiol. 122, 188–195. https://doi.org/10.1016/j.ijfoodmicro.2007.11.083

Berger, R.G., and Zorn, H. (2004). Flavors and Fragrances. Adv. Fungal Biotechnol. Ind. Agric. Med. 341–358. https://doi.org/10.1007/978-1-4419-8859-1_13

Boeke, J.D., Trueheart, J., Natsoulis, G., and Fink, G.R. (1987). 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. 154, 164–175. https://doi.org/10.1016/0076-6879(87)54076-9

Deroite, A., Legras, J.L., Rigou, P., Ortiz-Julien, A., and Dequin, S. (2018). Lipids modulate acetic acid and thiol final concentrations in wine during fermentation by *Saccharomyces cerevisiae* × *Saccharomyces kudriavzevii* hybrids. AMB Express 8:1-14. https://doi.org/10.1186/s13568-018-0657-5

Díaz-Maroto, M.C., Schneider, R., and Baumes, R. (2005). Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. J. Agric. Food Chem. 53: 3503–3509. https://doi.org/10.1021/jf048157o

Dubourdieu, D., Tominaga, T., Masneuf, I., Peyrot, C., and Murat, M. L. (2006). The role of yeasts in grape flavor development during fermentation: The example of Sauvignon blanc. Am. J. Enol. Vitic. 57: 81–88.

Ferreira, V., de-la-Fuente-Blanco, A., and Sáenz-Navajas, M.P. (2021). Odorants to Interpret Complex Aroma Systems. Application to Model Wine Aroma. Foods 10: 1–19. https://doi.org/https://doi.org/10.3390/foods10071627

Gamero, A., Manzanares, P., Querol, A., and Belloch, C. (2011a). Monoterpene alcohols release and bioconversion by *Saccharomyces* species and hybrids. Int.

J. Food Microbiol, 145 (1): 92–97. https://doi.org/10.1016/j.ijfoodmicro.2010.11.034

Gamero, A., Hernández-Orte, P., Querol, A., and Ferreira, V. (2011b). Effect of aromatic precursor addition to wine fermentations carried out with different *Saccharomyces* species and their hybrids. Int. J. Food Microbiol. 147: 33–44. https://doi.org/10.1016/j.ijfoodmicro.2011.02.035

Gangl, H., Batusic, M., Tscheik, G., Tiefenbrunner, W., Hack, C., and Lopandic, K. (2009). Exceptional fermentation characteristics of natural hybrids from *Saccharomyces cerevisiae* and S. *kudriavzevii*. N. Biotechnol. 25: 244–251. https://doi.org/10.1016/j.nbt.2008.10.001

Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., and Ciani, M. (2013). *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and sequential co-fermentation: A strategy to enhance acidity and improve the overall quality of wine. *Food Microbiology*, 33(2): 271–281. https://doi.org/10.1016/j.fm.2012.10.004

González, S.S., Barrio, E., Gafner, J., and Querol, A. (2006). Natural hybrids from *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces kudriavzevii* in wine fermentations. FEMS Yeast Res. 6: 1221–1234. https://doi.org/10.1111/j.1567-1364.2006.00126.x

González, S.S., Gallo, L., Climent, M.D., Barrio, E., and Querol, A. (2007). Enological characterization of natural hybrids from *Saccharomyces cerevisiae* and S. *kudriavzevii*. Int. J. Food Microbiol. 116: 11–18. https://doi.org/10.1016/j.ijfoodmicro.2006.10.047

Howell, K.S., Klein, M., Swiegers, J.H., Hayasaka, Y., Elsey, G.M., Fleet, G.H. *et al.* (2005). Genetic determinants of volatile-thiol release by *Saccharomyces cerevisiae* during wine fermentation. Appl. Environ. Microbiol. 71: 5420–5426. https://doi.org/10.1128/AEM.71.9.5420-5426.2005

Inoue, Y., and Kimura, A. (1996). Identification of the structural gene for glyoxalase I from *Saccharomyces cerevisiae*. J. Biol. Chem. 271: 25958–25965. https://doi.org/10.1074/jbc.271.42.25958

Lairón-Peris, M., Pérez-Través, L., Muñiz-Calvo, S., Guillamón, J.M., Heras, J.M., Barrio, E., and Querol, A. (2020). Differential contribution of the parental genomes to a *S. cerevisiae* × *S. uvarum* Hybrid, inferred by phenomic, genomic, and transcriptomic analyses, at different industrial stress conditions. Front. Bioeng. Biotechnol. 8. https://doi.org/10.3389/fbioe.2020.00129

Legras, J.L., and Karst, F. (2003). Optimisation of interdelta analysis for *Saccharomyces cerevisiae* strain characterisation. FEMS Microbiol. Lett. 221: 249–255. https://doi.org/10.1016/S0378-1097(03)00205-2

Lopes, C.A., Barrio, E., and Querol, A. (2010). Natural hybrids of *S. cerevisiae* × *S. kudriavzevii* share alleles with European wild populations of *Saccharomyces kudriavzevii*: FEMS Yeast Res. 10: 412–421. https://doi.org/10.1111/j.1567-1364.2010.00614.x

López, R., Aznar, M., Cacho, J., and Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. J. Chromatogr. A 966, 167–177. https://doi.org/10.1016/S0021-9673(02)00696-9

Masneuf, I., Murat, M.L., Naumov, G.I., Tominaga, T., and Dubourdieu, D. (2002). Hybrids *Saccharomyces cerevisiae* × *Saccharomyces bayanus* var. *uvarum* having a high liberating ability of some sulfur varietal aromas of *Vitis vinifera* Sauvignon blanc wines. J. Int. desSci. la Vigne du Vin 36: 205–212. https://doi.org/10.20870/oeno-one.2002.36.4.965

Mateo-Vivaracho, L., Zapata, J., Cacho, J., and Ferreira, V. (2010). Analysis, occurrence, and potential sensory significance of five polyfunctional mercaptans in white wines. J. Agric. Food Chem. 58: 10184–10194. https://doi.org/10.1021/jf101095a

Murat, M.L., Tominaga, T., and Dubourdieu, D. (2001). Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. J. Agric. Food Chem. 49: 5412–5417. https://doi.org/10.1021/jf0103119

Oliveira, B.M., Barrio, E., Querol, A., and Pérez-Torrado, R. (2014). Enhanced enzymatic activity of glycerol-3-phosphate dehydrogenase from the cryophilic *Saccharomyces kudriavzevii*. PLoS One 9. https://doi.org/10.1371/journal.pone.0087290

Oliveira, I., and Ferreira, V. (2019). Modulating fermentative, varietal and aging aromas of wine using non- *Saccharomyces* yeasts in a sequential inoculation approach. *Microorganisms*, 7(6): 1–23. https://doi.org/doi:10.3390/microorganisms7060164

Origone, A.C., del Mónaco, S.M., Ávila, J.R., González Flores, M., Rodríguez, M.E., and Lopes, C.A. (2017). Tolerance to winemaking stress conditions of Patagonian strains of *Saccharomyces eubayanus* and *Saccharomyces uvarum*. J. Appl. Microbiol. 123, 450–463. https://doi.org/10.1111/jam.13495

Ortega, C., López, R., Cacho, J., and Ferreira, V. (2001). Fast analysis of important wine volatile compounds - Development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. J. Chromatogr. A 923, 205–214. https://doi.org/10.1016/S0021-9673(01)00972-4

Ortiz-Tovar, G., Minebois, R., Barrio, E., Querol, A., and Pérez-Torrado, R. (2019). Aroma production and fermentation performance of *S. cerevisiae* × *S.*

kudriavzevii natural hybrids under cold oenological conditions. Int. J. Food Microbiol. 297, 51–59. https://doi.org/10.1016/j.ijfoodmicro.2019.03.005

Pérez, D., Jaehde, I., Guillamón, J.M., Heras, J.M., and Querol, A. (2021). Screening of *Saccharomyces* strains for the capacity to produce desirable fermentative compounds under the influence of different nitrogen sources in synthetic wine fermentations. Food Microbiol. 97: 103763. https://doi.org/10.1016/j.fm.2021.103763

Pérez, D., Denat, M., Minebois, R., Heras, J. M., Guillamón, josé M., Ferreira, V., & Querol, A. (2022a). Modulation of aroma and chemical composition of Albariño semi-synthetic wines by non-wine *Saccharomyces* yeasts and bottle aging. *Food Microbiology*, *104*(November 2021), 103981. https://doi.org/10.1016/j.fm.2022.103981

Pérez, D., Denat, M., Heras, J. M., Guillamón, J. M., Ferreira, V., & Querol, A. (2022b). Effect of non-wine *Saccharomyces* yeasts and bottle aging on the release and generation of aromas in semi-synthetic Tempranillo wines. *International Journal of Food Microbiology*, *365*. https://doi.org/10.1016/j.ijfoodmicro.2022.109554

Pérez-Torrado, R., Carrasco, P., Aranda, A., Gimeno-Alcañiz, J., Pérez-Ortín, J.E., Matallana, E., and Del Olmo, M. (2002). Study of the first hours of microvinificationby the use of osmotic stress-response genes as probes. Syst. Appl. Microbiol. 25, 153–161. https://doi.org/10.1078/0723-2020-00087

Pérez-Través, L., Lopes, C. A., Barrio, E., and Querol, A. (2012). Evaluation of different genetic procedures for the generation of artificial hybrids in *Saccharomyces* genus for winemaking. Int. J. Food Microbiol. 156, 102–111. https://doi.org/10.1016/j.ijfoodmicro.2012.03.008

Pérez-Través, L., Lopes, C. A., Barrio, E., and Querol, A. (2014). Stabilization process in *Saccharomyces* intra- and interspecific hybrids in fermentative conditions. Int. Microbiol. 17: 213–224. https://doi.org/10.2436/20.1501.01.224

Pérez-Través, L., Lopes, C.A., González, R., Barrio, E., and Querol, A. (2015). Physiological and genomic characterisation of *Saccharomyces cerevisiae* hybrids with improved fermentation performance and mannoprotein release capacity. Int. J. Food Microbiol. 205, 30–40. https://doi.org/10.1016/j.ijfoodmicro.2015.04.004

Peris, D., Pérez-Torrado, R., Hittinger, C. T., Barrio, E., and Querol, A. (2018). On the origins and industrial applications of *Saccharomyces cerevisiae* × *Saccharomyces kudriavzevii* hybrids. *Yeast (Chichester, England)*, *35*(1), 51–69. https://doi.org/10.1002/yea.3283

Pinu, F. R., (2018). Grape and wine metabolomics to develop new insights using untargeted and targeted approaches. *Fermentation*, 4(4). https://doi.org/10.3390/fermentation4040092

Querol, A., Barrio, E., Huerta, T., and Ramón, D., (1992). Molecular monitoring of wine fermentations conducted by active dry yeast strains. Appl. Environ. Microbiol. 58, 2948–2953.

Querol, A., Pérez-Torrado, R., Alonso-del-Real, J., Minebois, R., Stribny, J., Oliveira, B.M., and Barrio, E. (2018). New Trends in the Uses of Yeasts in Oenology, in: Advances in Food and Nutrition Research. Elsevier Inc., Amsterdem, pp. 177–210. https://doi.org/10.1016/bs.afnr.2018.03.002

Ravasio, D., Carlin, S., Boekhout, T., Groenewald, M., Vrhovsek, U., Walther, A., and Wendland, J. (2018). Adding flavor to beverages with non-conventional yeasts. *Fermentation*, 4(1): 1-16. https://doi.org/10.3390/fermentation4010015

Roland, A., Schneider, R., Razungles, A., and Cavelier, F. (2011). Varietal thiols in wine: Discovery, analysis and applications. Chem. Rev. 111: 7355–7376. https://doi.org/10.1021/cr100205b

Romero-Guido, C., Belo, I., Ta, T.M.N., Cao-Hoang, L., Alchihab, M., Gomes, N., *et al.* (2011). Biochemistry of lactone formation in yeast and fungi and its utilization for the production of flavour and fragrance compounds. Appl. Microbiol. Biotechnol. 89: 535–547. https://doi.org/10.1007/s00253-010-2945-0

Schillberg, S., Zimmermann, M., and Emeis, C.C. (1991). Analysis of hybrids obtained by rare-mating of *Saccharomyces* strains. Appl. Microbiol. Biotechnol. 35: 242–246. https://doi.org/10.1007/BF00184695

Stribny, J., Gamero, A., Pérez-Torrado, R., and Querol, A. (2015). *Saccharomyces kudriavzevii* and *Saccharomyces uvarum* differ from *Saccharomyces cerevisiae* during the production of aroma-active higher alcohols and acetate esters using their amino acidic precursors. Int. J. Food Microbiol. 205: 41–46. https://doi.org/10.1016/j.ijfoodmicro.2015.04.003

Su, Y., Origone, A.C., Rodríguez, M.E., Querol, A., Guillamón, J.M., and Lopes, C.A. (2019a). Fermentative behaviour and competition capacity of cryotolerant *Saccharomyces* species in different nitrogen conditions. Int. J. Food Microbiol. 291: 111–120. https://doi.org/10.1016/j.ijfoodmicro.2018.11.020

Su, Y., Gamero, A., Rodríguez, M.E., Lopes, C.A., Querol, A., and Guillamón, J.M. (2019b). Interspecific hybridisation among diverse *Saccharomyces* species: A combined biotechnological solution for low-temperature and nitrogen-limited wine fermentations. Int. J. Food Microbiol. 310, 108331. https://doi.org/10.1016/j.ijfoodmicro.2019.108331

Subileau, M., Schneider, R., Salmon, J.M., and Degryse, E. (2008). New insights on 3-mercaptohexanol (3MH) biogenesis in Sauvignon Blanc wines: Cys-3MH and (E)-hexen-2-al are not the major precursors. J. Agric. Food Chem. 56: 9230–9235. https://doi.org/10.1021/jf801626f

Swiegers, J.H., and Pretorius, I.S. (2007). Modulation of volatile sulfur compounds by wine yeast. Appl. Microbiol. Biotechnol. 74, 954–960. https://doi.org/10.1007/s00253-006-0828-1

Thibon, C., Marullo, P., Claisse, O., Cullin, C., Dubourdieu, D., and Tominaga, T. (2008). Nitrogen catabolic repression controls the release of volatile thiols by *Saccharomyces cerevisiae* during wine fermentation. FEMS Yeast Res. 8: 1076–1086. https://doi.org/10.1111/j.1567-1364.2008.00381.x

Tominaga, T., Furrer, A., Henry, R., and Dubourdieu, D. (1998). Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. Flavour Fragr. J. 13, 159–162. https://doi.org/10.1002/(SICI)1099-1026(199805/06)13:3<159::AID-FFJ709>3.0.CO;2-7

Toyn, J.H., Gunyuzlu, P., White, W.H., Thompson, L.A., and Hollis, G.F. (2000). A counterselection for the tryptophan pathway in yeast: 5-Fluoroanthranilic acid resistance. Yeast 16: 553–560. https://doi.org/10.1002/(SICI)1097-0061(200004)16:6<553::AID-YEA554>3.0.CO;2-7

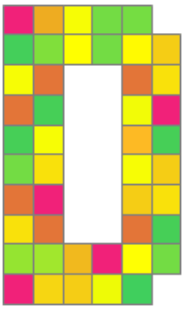
Varela, C., Sengler, F., Solomon, M., and Curtin, C. (2016). Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. Food Chemistry, 209: 57–64. https://doi.org/10.1016/j.foodchem.2016.04.024

Winter, G., Van der Westhuizen, T., Higgins, V. J., Curtin, C., and Ugliano, M. (2011). Contribution of cysteine and glutathione conjugates to the formation of the volatile thiols 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) during fermentation by *Saccharomyces cerevisiae*. The Australian Wine Research Institute, 285–290. https://doi.org/10.1111/j.1755-0238.2011.00127.x

Zarett, K.S., and Sherman, F. (1985). α-Aminoadipate as a primary nitrogen source for *Saccharomyces cerevisiae* mutants. J. Bacteriol. 162, 579–583.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., and Van't Riet, K., (1990). Modeling of the Bacterial Growth Curve. Appl. Environ. Microbiol. 56: 1875–1881. https://doi.org/0099-2240/90/061875-07$02.00/0

General Discussion



Many attractive oenological characteristics are found in the literature about cryotolerant species and *S. cerevisiae* strains from fermentative or natural environments other than wine. These characteristics refer mainly to the lower production of ethanol and consequently higher production of secondary products (e.g., erythritol, glycerol, 2,3-butanediol, succinic acid), produced as different alternatives to compensate the excess of reduced co-factors derived from glycolysis. Likewise, most of these studies have found a remarkable volatiles production whose synthesis would also be related to redox balance strategies by these yeasts, namely higher alcohols, acetate esters, medium-chain fatty acids, and their ethyl esters. However, these compounds have limited aromatic relevance, as most of them only take part in the vinous note, hardly pass their perception thresholds, and are easily degraded during bottle aging. Thus, few studies have focused on producing and releasing aromas of major aromatic importance by these alternative *Saccharomyces* yeasts, i.e., those coming from varietal precursors or fermentation and aging process (e.g., terpenes, C_{13}-norisoprenoids, branched-chain fatty acid ethyl esters).

Therefore, this thesis focused on the ability of these non-conventional strains belonging to cryotolerant species (*S. eubayanus*, *S. uvarum*, *S. kudriavzevii*) and non-wine strains of *S. cerevisiae* species to produce and release a wide range of volatile compounds of greater aromatic importance. Since these compounds are related to the metabolism of certain amino acids (valine, leucine, isoleucine, and phenylalanine), and some of them become relevant during bottle aging, this thesis also considered these factors.

In chapter 1, starting from a large collection of 33 yeasts, we studied the fermentative capacity and metabolite production (major volatiles and non-volatiles) and characterized in depth these yeasts (at the species and strain level) as well as their relationship with nitrogen sources. In chapters 2 and 3,

we reduced the number of studied yeasts to the best candidate strains and continued adding phenotypic knowledge about these strains/species, including the ability to produce impact aromas from varietal precursors and the relationship with bottle aging. This information provided further guidelines for selecting the best candidate strains to generate industry-applicable hybrids yeasts. Finally, this leads us to chapter 4, where we generated hybrids yeasts by rare-mating (a non-GMO procedure) and corroborated the inheritance aptitudes of their rigorously selected progenitors. Therefore, under the same conditions of chapters 2 and 3, the hybrid yeasts were evaluated on aroma and secondary by-products production and fermentation capacity, including the common wine parental strain as control yeast.

With the information gathered from each chapter, this section discusses the relevant results that best define the characteristics of these yeasts and their resulting hybrid yeasts.

Saccharomyces uvarum

In chapter 1, we started the evaluation of this species using 6 strains from non-fermentative environments and fermentative environments, including as a control one wine *S. uvarum* strain, BMV58. They all differed from the wine *S. cerevisiae* strain (EC1118) in their high capacity to produce succinic acid, erythritol, phenylethanol, its acetate ester, and reduced ethanol and acetic acid yields. Interestingly, in the presence of phenylalanine as the only nitrogen source, we observed that the expected increase of phenylethanol and its acetate ester was not as large as in other species; instead, a more significant increase in erythritol yield was observed. Furthermore, the time to finish fermentation was longer in the presence of this amino acid, indicating a low preference as a nitrogen source by *S. uvarum* yeasts. These data gave us evidence that the large production of these aroma compounds by this species

comes mainly from the central carbon metabolism (i.e., the shikimate pathway) rather than from extracellular phenylalanine catabolism, as studies using labeled amino acids have suggested (Crépin et al., 2017; Su et al., 2020). In addition, this extracellular amino acid could be producing an inhibition in the first step of the shikimate pathway where enzymes are encoded by ARO3 and ARO4 genes sensitive to this amino acid (Etschmann et al., 2002). Therefore, erythrose-4-phosphate, one of the initial precursors in this pathway, would now be directed exclusively to erythritol formation, thus yielding higher levels of this alcohol.

From the results of chapter 1, two *S. uvarum* strains were selected for Tempranillo fermentations, Chapter 2: the strain isolated from unfermented liquor (*Mistela*) that yielded the highest β-phenylethanol and its acetate levels, and the strain isolated from cider reaching high erythritol yields (coded in chapter 2 as U1, and U2 respectively). In addition, we included the wine strain BMV58. Then, in chapter 3, using synthetic musts with Albariño and PFMs (polyfunctional mercaptans) varietal precursors, we only evaluated the wine BMV58 strain and the *Mistela* strain (code in this chapter as SU1) since the cider strain was showed a weaker aroma profile in Tempranillo wines (chapter 2).

Results from chapters 2 and 3 showed that these two strains continued displaying *S. uvarum* phenotypic profile regarding the by-products and fermentative aromas production mentioned in chapter 1. All these traits have been widely attributed to metabolic differences towards a redox balance compensation and a higher activity of this species towards the shikimate pathway (López-Malo et al., 2013; Minebois et al., 2020).

More importantly, in these two chapters, we found other relevant characteristics of this species by analyzing aroma compounds deeply. On the one hand, in both Albariño and Tempranillo semi-synthetic musts, we

determined a high production of branched-chain fatty acids by this species. Some of these compounds are related to the valine and leucine catabolism/anabolism, such as isobutyric and isovaleric acid. These acids are derived from the oxidation of the keto-acids corresponding to the amino acids above, which commonly are reduced to higher alcohols by yeasts (e.g., isobutanol and isoamyl alcohol). Although, these acids do not provide desired aromas, e.g., lactic or cheese notes, their importance lies in the fact that they are precursors of fruity ethyl esters, ethyl isobutyrate and isovalerate, produced by time-dependent esterification, mainly during bottle storage or aging. Therefore, maximum yields of these fruity ethyl esters were found after bottle aging application on these yeast's aged wines. These aromas, highly appreciated in red wines and never observed in these species, revealed the great aromatic potential of these species for red wines.

Finally, since we found higher amounts of geraniol and citronellol in their wines than in the unfermented must, we confirmed the high capacity to *de novo* produce monoterpenes by this species, as previously reported Gamero et al. (2011). In this way, we reported that geraniol could result from the blockage of ergosterol synthesis maybe by the absence of oxygen (Vaudano et al., 2004), while citronellol could be originated from the reduction of geraniol, as others have reported (Fernández-González and Di Stefano, 2004; Takoi et al., 2014).

So far, the common component in the synthesis pathways of all the above aroma compounds is acetyl-CoA. Recent studies indicated that *S. uvarum* strains initially produce acetate but then consume it to generate acetyl-CoA, leading to the high availability of this compound (Minebois et al., 2020). Therefore, we hypothesized that all reactions involving acetyl-CoA in *S. uvarum* would be producing more of these derivatives.

On the other hand, we found a great production by these yeasts of diethyl succinate and γ-butyrolactone, whose synthesis we associate with their high succinic acid production. In this regard, it has recently been suggested that this acid in *S. uvarum* is produced mainly through the alternative GABA pathway instead of being produced through the TCA to compensate for the REDOX balance (Henriques et al., 2021). Since GHB (γ-hydroxybutyric acid) is an intermediate of this pathway (Bach et al., 2009), and reported as a precursor of γ-butyrolactone (Ribéreau-Gayon et al., 2006), we suggest γ-butyrolactone formation via the GABA shunt pathway. On the other hand, we also suggest the further formation of diethyl succinate from the esterification of succinic acid from this route with ethanol.

In addition, in chapter 3, when additionally analyzing the PFMs released from the added precursors, we found a high capacity in these strains to release these compounds, being the second species, after *S. kudriavzevii*, in releasing the highest amount of these compounds, mainly 3MH and 3MHA. This trait has been little documented in this species, yet it has been specially found under its interspecific hybrids with *S. cerevisiae* (Masneuf et al., 2002).

Finally, considering the properties evaluated and found in these two strains, the non-wine strain (formally named CECT12600) was the parental candidate to generate hybrids in Chapter 4. Therefore, from this strain (code in this chapter as UE), we obtained 2 stable hybrids, namely HUE2 and HUE5, by crossing UE with a wine strain. They were mainly differentiated molecularly by their mitochondrial type, one, HUE2, having a recombinant mitochondrial, and the other, HUE5, having inherited the mitochondrial from the parental UE *S. uvarum* strain.

Both hybrids fermented Albariño semi-synthetic musts while only HUE5 fermented in Tempranillo. Although both parents have good

fermentation capacity (UE or CECT12600 exhibited this property throughout chapters 1, 2, and 3), the resulting hybrids were not very efficient fermenting, especially at 25ºC, which could be due to some genetic loss in this respect during the stabilization process. Despite this, they did finish the fermentation, and the resulting wines yielded lower ethanol and higher glycerol than the control yeast (parental wine strain *S. cerevisiae*). Most importantly, they retained the ability of their parental *S. uvarum* strain to produce branched fatty acids and their fruit ethyl esters in young wines, which corresponded to a significant increase of these esters in the resulting aged Tempranillo wines. On the contrary, although the parental *S. uvarum* showed a high capacity to release PFMs in chapter 3, its hybrids did not produce large amounts, i.e., they were like the wine parental in this aspect. These two traits confirm the suitability of these hybrid strains for red wines production, although their low fermentation capacity, especially at high temperatures, would be inconvenient for this type of wine. Therefore, the search for hybrids *Su* × *Sc* with better fermentation capabilities should be continued.

Saccharomyces kudriavzevii

From the initial screening (chapter 1), this species displayed some of its specific features, being distinguished from the wine strain by the higher glycerol and 2,3-butanediol production. All isolated from natural non-fermentative environments, 5 oak-related strains from Spain were distinguished by their longer lag phase in leucine and phenylalanine than in isoleucine, as the other species displayed. Among these 5 strains, two monosporic derivatives, CA111F1, CR89D1, and the CR85 strain were selected for the next study in chapter 2, coded as K1, K3, and K2, respectively. Then, in chapter 3, only CR89D1 and CA111F1 were used (code as SK1 and SK3, respectively).

Therefore, these *S. kudriavzevii* strains fermenting semi-synthetic musts of Tempranillo and Albariño (chapters 2 and 3) revealed other characters above these species in terms of by-product production. Here we gathered further evidence on one of its pathways involved in cofactor recycling, which refers to malic acid degradation. According to the literature, the yeasts can degrade malic acid and convert it either into ethanol (via malic enzyme) or fumaric and succinic acids (via fumarase) (Redzepovic et al., 2003). However, we did not observe an increase of any of these compounds, ethanol, or succinic acid in wines fermented with *S. kudriavzevii* yeasts. Instead, we did observe an increase in pyruvic acid and acetaldehyde derivatives towards the acetoin route, thus finding high levels of 2,3-butanediol and diacetyl in both chapters, while acetoin in Albariño and lactic acid and ethyl lactate in Tempranillo. This implies that *S. kudriavzevii* could oxidize extracellular malic acid to pyruvic (via the malic enzyme), which instead of ethanol produces acetolactate, which leads to the formation of the compounds mentioned above.

Regarding the analysis of impact aroma in Tempranillo, the most remarkable strain was CA111F1 (K3), which in young wine developed the highest concentration of fruit ethyl esters linked to the branched amino acids isoleucine, valine, and leucine. Unfortunately, this young wine was not one of those chosen for the aging application, so in this case, we could not observe the evolution of these esters during bottle maturation.

Apart from this strain, no major aromatic contributions from this species were observed in the Tempranillo wines. However, in chapter 3, when we analyzed the polyfunctional mercaptans in Albariño, without doubt, this species gained great aromatic importance. Thus, we discovered the potent ability of *S. kudriavzevii* species, mainly in the CR89D1 strain (code in chapter 3 as SK1), releasing the highest 4MMP and 3MH values, followed by CA111F1 (SK3). Now, if we go back to Tempranillo's results in chapter 2,

we could state that the 4MMP tentatively identified by the panel of tasters by GC-Olfactometry would be due to the action of one of these strains.

Although previous studies had already reported high thiol release capacity in yeast hybridized with *S. kudriavzevii* species (Erny et al., 2012; Murat et al., 2001), in this study, this capacity was reported for the first time in strains of this pure species and under wine conditions.

The release of these 3MH and 4MMP compounds from their cysteine or glutathione precursors involves the activity of carbon-sulfurylase enzymes which are so far encoded by 4 genes: *BNA3, GLO1, CYS3,* and *IRC7* (Howell et al., 2005). In addition to the cleavage, the precursors must first enter the yeast cell through general or amino acid-specific permeases. Both permeases and enzyme genes are regulated by repressive complexes: NRC (Nitrogen catabolic repression) and SSP (Ssy-Ptr3-Ssy5) (Subileau et al., 2008; Thibon et al., 2008). As we observed in chapter 1, there is a marked difference in this group of *S. kudriavzevii* strains (isolated from Spain) when fermenting in the presence of one amino acid as the sole nitrogen source, for example, in leucine. Therefore, this suggests different management of nitrogen sources by this species, so further investigation on genes related to nitrogen regulation is required to elucidate the great PFMs release displays by *S. kudriavzevii* and the relationship with nitrogen metabolism.

Overall, CR89D1 and CA111F1 strains were used for hybrid generation resulting in 2 hybrid strains from each one: HKR1 and HKR8 from CR89D1 and HKA3 and HKA4 from CA111F1. Fortunately, these strains maintained their high capacity to release PFMs, with HKA4 standing out for the highest release of 3MH. This 3MH production by this strain gave us evidence of a relationship with its particularly high glycerol production. In this sense, we theorized that the high glycerol production leads to a higher accumulation of methylglyoxal, a toxic compound that yeast cell eliminates

using glutathione, a reaction encoded by the *GLO1* gene. Since this gene is the same that Howell et al. (2005) correlated with the release of polyfunctional mercaptans, it is likely to hypothesize that yeasts use the glutathione bound to 3MH (Glt-3MH) to detoxify its cell, and consequently, high free 3MH is detected in wines.

On the other hand, we determined that the hybrids with mitochondria inherited from the wine parental (HKR1 and HKR8) showed fermentation and metabolite production profiles, such as ethanol and glycerol, similar to the wine parental.

Non-wine *S. cerevisiae*

Initially, in chapter 1, the group of *S. cerevisiae*, mostly from fermentative, but non-wine environments, showed certain similarities. Mainly in terms of aroma production, this group of yeasts produced low levels of phenylethanol and its acetate and, conversely, high levels of isoamyl alcohol and MCFA ethyl esters. They also showed similarities with the wine control strain (EC1118) in terms of lower production of secondary metabolites and higher ethanol production, with leucine favoring its reduction. All these characteristics could be due to the greater influence of the domestication process on this species, which has been gradually adapting to fermentative conditions such as temperatures, nitrogen composition, and sugar levels.

Four yeasts of this species were used in Tempranillo fermentations (Chapter 2), where the most outstanding was the yeast isolated from cachaça (coded in Chapter 2 as C1) due to its high content of lactones, vanillin derivatives, and positive volatile phenols. When fermented in Albariño (Chapter 3, coded as SC1), this strain showed a different profile, without the presence of vanillin derivatives or lactones, where it only highlighted for the higher production of limonene. In addition to this strain, a strain isolated from

agave (coded as C2) curiously evolved from a poor aroma profile in young wine, towards the highest synthesis in ethyl leucate after aging, which is a very valuable compound in red wines, whose synthesis may be related to the leucine or valine pathways.

Finally, the cachaça yeast was chosen to cross with the wine yeast. As a result, we obtained a hybrid (HCS3), which, having inherited the mitochondria of the parental wine yeast, did not differ aromatically or in terms of metabolite production from this parent.

On the other hand, a strain isolated from sorghum beer in Chapter 1 (coded as SC03) was noted for its highest production of fruity ethyl esters and higher erythritol content on any must of different nitrogen composition. Therefore, due to the importance of these medium-chain fatty acid ethyl esters in white wines (fruity notes), this strain was selected for Albariño fermentations (chapter 3). Surprisingly, under these conditions, ethyl esters of MCFA were not the main characteristic of this strain (code as SC2), yet it exhibited a different profile to any strain studied in Albariño and certainly to any other *S. cerevisiae*. In this regard, despite its low production of ethyl esters, it was the only strain producing ethyl 4-methylvalerate (strawberry aroma), a compound that could derive from the esterification of 4-methylvaleric acid with ethanol (Campo et al., 2006; Gracia-Moreno et al., 2015).

Furthermore, the erythritol yield by the SC2 strain was the highest of all, conversely to the lower succinic and malic acid content. This difference provided evidence that this strain derives a low carbon flux to TCA and higher toward the pentose phosphate pathway (PPP). In addition, we found a high production of furfurylthiol by this strain, a polyfunctional mercaptan whose synthesis in oak-free wines is not well understood. Considering that this

compound may have ribose and cysteine as precursors (Hofmann and Schieberle, 1998), a plausible explanation for its presence in wood-free wines would be its formation from ribose-5-phosphate, an intermediate of the same PPP route, like erythritol.

The γ-octalactone yield was another impacting difference in this strain, which we suggested could derive from yeast lipid metabolism, from octanoic acid after being hydroxylated, β-oxidized, and finally lactonized (Romero-Guido et al., 2011).

Despite of all this, this beer yeast did not show a greater aromatic contribution, so it was not selected to generate hybrids.

Saccharomyces eubayanus

Finishing with the least studied species at present, the most interesting aspect found in chapter 1 was that all the strains exhibited a similar fermentative performance among musts of different nitrogen compositions. While the fermentation rate was highly increased in the presence of valine, with isoleucine, it was drastically decreased. Thus, the similar fermentation performance observed in the 7 *S. eubayanus* strains could indicate regulatory analogies in their nitrogen metabolism that could be species-specific or due to the similar isolation sources from which they originate: trees from cold regions of Argentina and Chile.

Being cryotolerant strains, they also showed the typical character of higher glycerol yields. That, together with the higher production of 2,3-butanediol and lower ethanol, evidenced the higher carbon flow to other routes than ethanol, similar to *S. kudriavzevii* strains. In addition, this species was also noted for a higher production of β-phenylethyl acetate and erythritol,

suggesting a greater activity by the shikimate route and greater disposition of acetyl Co-A, such as the case of *S. uvarum* strains.

Then, only 3 of the 7 strains went on to the second experiment fermenting in semi-synthetic must with the addition of aromatic precursors extracted from Tempranillo grapes (chapter 2). Without the influence of the nitrogen sources studied above, these strains continued to be characterized by a high glycerol production similar to that of *S. kudriavzevii* and a low ethanol yield. In addition, one of them (coded as E1) showed a particular aroma profile in young wines, having high levels of acetates and two lactones. Despite this, screening of this species was not continued due to its low interest as a hybrid for wine and its greater relationship with beer.

In summary, first, the different strains and species showed preferences for fermentation in the presence of different nitrogen sources. The most preferred source for non-*cerevisiae* strains was valine, whereas the least preferred for *S. uvarum* was phenylalanine, leucine for *S. kudriavzevii*, and isoleucine for *S. eubayanus*. Secondly, non-wine *S. cerevisiae* strains and cryotolerant species increase the aroma complexity of wines. Where *S. uvarum* produced higher fruity ethyl esters, highly increased during bottle aging, and S. *kudriavzevii* strains increased the release of polyfunctional mercaptans up to 10-fold more than wine yeast. On the other hand, the *S. eubayanus* strains shared certain characteristics between the species mentioned above.

Moreover, the non-*cerevisiae* species produce higher amounts of glycerol and organic acids and a lower ethanol yield, which counteracts the effect of climate change on grapes. Among the *S. cerevisiae*, one strain stood out for its high furfurylthiol production, possibly related to its high erythritol yields. Finally, we obtained interspecific hybrids from these non-

cerevisiae strains that showed better oenological and aromatic characteristics than the parental wine strain.

References

Bach, B., Meudec, E., Lepoutre, J.P., Rossignol, T., Blondin, B., Dequin, S., Camarasa, C., 2009. New insights into γ-aminobutyric acid catabolism: Evidence for γ-hydroxybutyric acid and polyhydroxybutyrate synthesis in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 75, 4231–4239. https://doi.org/10.1128/AEM.00051-09

Campo, E., Cacho, J., Ferreira, V., 2006. Multidimensional chromatographic approach applied to the identification of novel aroma compounds in wine. Identification of ethyl cyclohexanoate, ethyl 2-hydroxy-3-methylbutyrate and ethyl 2-hydroxy-4-methylpentanoate. J. Chromatogr. A 1137, 223–230. https://doi.org/10.1016/j.chroma.2006.10.020

Crépin, L., Truong, N.M., Bloem, A., Sanchez, I., Dequin, S., Camarasa, C., 2017. Management of multiple nitrogen sources during wine fermentation by *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 83, 1–21. https://doi.org/10.1128/AEM.02617-16

Erny, C., Raoult, P., Alais, A., Butterlin, G., Delobel, P., Matei-Radoi, F., Casaregola, S., Legras, J.L., 2012. Ecological success of a group of *Saccharomyces cerevisiae/Saccharomyces kudriavzevii* hybrids in the Northern European wine-making environment. Appl. Environ. Microbiol. 78, 3256–3265. https://doi.org/10.1128/AEM.06752-11

Etschmann, M., Bluemke, W., Sell, D., Schrader, J., 2002. Biotechnological production of 2-phenylethanol. Appl. Microbiol. Biotechnol. 59, 1–8. https://doi.org/10.1007/s00253-002-0992-x

Fernández-González, M., Di Stefano, R., 2004. Fractionation of glycoside aroma precursors in neutral grapes. Hydrolysis and conversion by *Saccharomyces cerevisiae*. LWT - Food Sci. Technol. 37, 467–473. https://doi.org/10.1016/j.lwt.2003.11.003

Gamero, A., Manzanares, P., Querol, A., Belloch, C., 2011b. Monoterpene alcohols release and bioconversion by *Saccharomyces* species and hybrids. Int. J. Food Microbiol. 145, 92–97. https://doi.org/10.1016/j.ijfoodmicro.2010.11.034

Gracia-Moreno, E., López, R., Ferreira, V., 2015. Quantitative determination of five hydroxy acids, precursors of relevant wine aroma compounds in wine and other alcoholic beverages. Anal. Bioanal. Chem. 407, 7925–7934. https://doi.org/10.1007/s00216-015-8959-9

Hofmann, T., Schieberle, P., 1998. Quantitative model studies on the effectiveness of different precursor systems in the formation of the intense food odorants 2-Furfurylthiol and 2-methyl-3-furanthiol. J. Agric. Food Chem. 46, 235–241. https://doi.org/10.1021/jf9705983

Howell, K.S., Klein, M., Swiegers, J.H., Hayasaka, Y., Elsey, G.M., Fleet, G.H., Høj, P.B., Pretorius, I.S., De Barros Lopes, M.A., 2005. Genetic determinants of volatile-thiol release by *Saccharomyces cerevisiae* during wine fermentation. Appl. Environ. Microbiol. 71, 5420–5426. https://doi.org/10.1128/AEM.71.9.5420-5426.2005

López-Malo, M., Querol, A., Guillamon, J.M., 2013. Metabolomic comparison of *Saccharomyces cerevisiae* and the cryotolerant species *S. bayanus* var. *uvarum* and *S. kudriavzevii* during wine fermentation at low temperature. PLoS One 8. https://doi.org/10.1371/journal.pone.0060135

Masneuf, I., Murat, M.L., Naumov, G.I., Tominaga, T., Dubourdieu, D., 2002. Hybrids *Saccharomyces cerevisiae* x *Saccharomyces bayanus* var. *uvarum* having a high liberating ability of some sulfur varietal aromas of *Vitis vinifera* Sauvignon blanc wines. J. Int. des Sci. la Vigne du Vin 36, 205–212. https://doi.org/10.20870/oeno-one.2002.36.4.965

Minebois, R., Pérez-Torrado, R., Querol, A., 2020. A time course metabolism comparison among *Saccharomyces cerevisiae*, *S. uvarum* and *S. kudriavzevii* species in wine fermentation. Food Microbiol. 90, 1–14. https://doi.org/10.1016/j.fm.2020.103484

Murat, M.L., Tominaga, T., Dubourdieu, D., 2001. Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. J. Agric. Food Chem. 49, 5412–5417. https://doi.org/10.1021/jf0103119

Redzepovic, S., Orlic, S., Majdak, A., Kozina, B., Volschenk, H., Viljoen-Bloom, M., 2003. Differential malic acid degradation by selected strains of *Saccharomyces* during alcoholic fermentation. Int. J. Food Microbiol. 83, 49–61. https://doi.org/10.1016/S0168-1605(02)00320-3

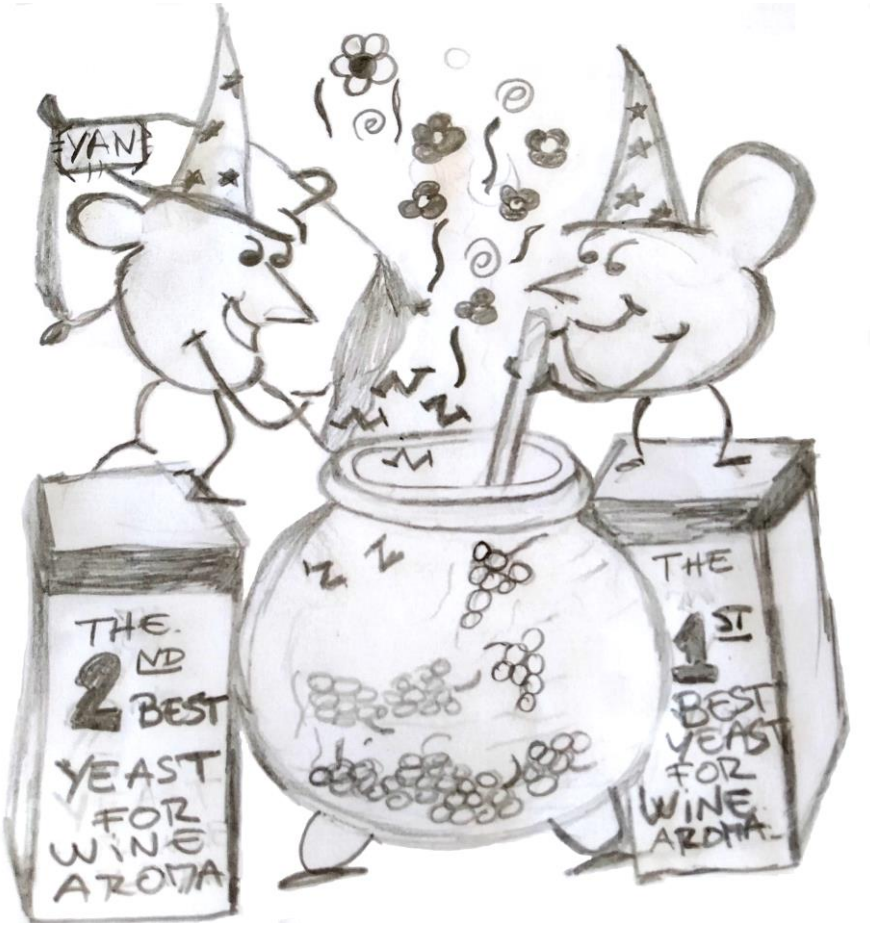
Ribéreau-Gayon, Pascal, Dubourdieu, D., Doneche, B., Lonvaud, A., 2006. Handbook of Enology, Vol. 1:The Microbiology of Wine and Vinifications., 2nd Ed. ed, The Microbiology of Wine and Vinifications. John Wiley and Sons Ltd, Chichester, UK.

Romero-Guido, C., Belo, I., Ta, T.M.N., Cao-Hoang, L., Alchihab, M., Gomes, N., Thonart, P., Teixeira, J.A., Destain, J., Waché, Y., 2011. Biochemistry of lactone formation in yeast and fungi and its utilisation for the production of flavour and fragrance compounds. Appl. Microbiol. Biotechnol. 89, 535–547. https://doi.org/10.1007/s00253-010-2945-0

Su, Y., Seguinot, P., Bloem, A., Ortiz-Julien, A., Heras, J.M., Guillamón, J.M., Camarasa, C., 2020. Isotopic tracers unveil distinct fates for nitrogen sources during wine fermentation with two non-*Saccharomyces* strains. Microorganisms 8, 1–19. https://doi.org/10.3390/microorganisms8060904

Takoi, K., Itoga, Y., Takayanagi, J., Kosugi, T., Shioi, T., Nakamura, T., Watari, J., 2014. Screening of geraniol-rich flavor hop and interesting behavior of β-citronellol during fermentation under various hop-addition timings. J. Am. Soc. Brew. Chem. 72, 22–29. https://doi.org/10.1094/ASBCJ-2014-0116-01

Conclusions



1. Valine as the sole nitrogen source increased the fermentation rate in non-*cerevisiae* yeasts.

2. Isoleucine was the least nitrogen source suitable for fermenting, mainly for *S. eubayanus* strains but not for the Spanish *S. kudriavzevii* strains. In this respect, these *S. kudriavzevii* strains showed a considerable delay in initiating fermentation on leucine, and along with the *S. uvarum* strains, they showed similar difficulty to complete fermentation in the presence of phenylalanine as a sole nitrogen source.

3. Leucine mainly reduced the ethanol yield in *S. cerevisiae* while phenylalanine increased ethyl esters.

4. The presence of phenylalanine inhibited the production of β-phenylethanol and its acetate ester from the shikimate pathway in *S. uvarum*, so erythrose-4-phosphate was directed towards a higher formation of erythritol.

5. *S. uvarum* yeasts produce a large amount of branched chain fatty acids ethyl ester which are highly valuable for red wines after bottle aging.

6. In addition to the contribution of GABA shunt in the production of succinic acid in *S. uvarum*, this pathway would be involved in the synthesis of γ-butyrolactone and diethyl succinate.

7. S. *kudriavzevii* are characterized by low ethanol levels and increased production of the secondary products, glycerol and the related to the acetoin pathway (2,3-butanediol, diacetyl, acetoin, lactic acid and ethyl lactate). The latter can be

derived from glucose or the degradation of extracellular malic acid.

8. An overproduction of glycerol by yeasts could trigger a methylglyoxal intoxication from which the yeasts would use glutathione to eliminate it through a reaction encoded by the *GLO1* gene, a gene previously associated with the release of polyfunctional mercaptans. Thus, the use of the glutathione bound to 3MH (Glt-3MH) for cell detoxification may be related to the high liberation of 3MH.

9. *S. kudriavzevii* has a great capacity to release polyfunctional mercaptans, which may be related to their differences in the management of nitrogen sources.

10. Hybrid yeasts that inherit mitochondrial belonging to wine yeasts acquire the same fermentative properties with respect to ethanol and glycerol yields.

11. The synthesis of furfurylthiol in wood-free wines could come from the pentose phosphate pathway, i.e., from ribose-5-phosphate and cysteine.

Annex I

Supplementary Material

Chapter 1

TableS1. Fermentation parameters obtained by the yeast strains during fermentation in the six nitrogen musts.

	Lag phase (h)					
	Comp	**Ile**	**Leu**	**NH_4**	**Phe**	**Val**
EC1118	48.1±0.9^{a}	127.8±8.5^{d}	81.7±1.8^{cd}	57.1±0.8^{ab}	69.6±0.6^{abc}	78.4±2.3^{bcd}
SC01	60.9±1.4^{a}	194.5±3.6^{d}	90.1±1.8^{abc}	66.0±2.2^{ab}	143.9±5.6^{bcd}	163.7±1.7^{cd}
SC02	37.63±4.43^{a}	167.12±4.99^{d}	105.86±13.4^{cd}	60.13±6.83^{ab}	75.49±2.38^{bcd}	69.78±1.6^{abc}
SC03	48.1±2.2^{a}	72.9±1.7^{b}	56.6±0.5^{ab}	48±1.5^{a}	61.3±0.3^{b}	57.7±2.2^{ab}
SC04	53.4±1.3^{a}	301.0±9.0^{d}	84.8±1.0^{bcd}	61.3±0.4^{ab}	74.8±3.6^{abc}	162.2±5^{cd}
SC05	49.0±0.9^{ab}	70.6±1.9^{d}	59.8±0.8^{bcd}	46.9±0.2^{a}	67.3±0.4^{cd}	56.0±0.5^{abc}
SC06	56.3±0.1^{a}	132.5±0.9^{c}	127.6±7.7^{c}	62.2±0.2^{ab}	72.8±1.8^{abc}	106.6±2.2^{bc}
SC07	26.2±0.1^{a}	123.4±9.5^{b}	58.0±2.3^{ab}	22.7±0.7^{a}	58.6±0.4^{ab}	142.2±11.7^{b}
SC08	46.6±1.0^{a}	74.9±2.5^{c}	60.0±1.2^{bc}	47.6±0.5^{ab}	57.6±2.0^{abc}	59.3±1.8^{bc}
SC09	50.9±3.2^{a}	87.0±1.5^{c}	62.6±13.6^{abc}	54.7±1.4^{ab}	57.7±0.6^{abc}	62.8±1.8^{bc}
SC10	24.5±1.6^{a}	111.8±7.8^{d}	54.2±2.7^{abc}	34.8±0.8^{ab}	70.0±6.0^{bcd}	102.7±4.1^{cd}
SE01	40.8±0.9^{a}	234.8±3.6^{d}	69.8±0.7^{abc}	54.9±3.3^{ab}	74.2±1.1^{cd}	72.4±1.4^{bcd}
SE02	21.9±6.5^{a}	148.5±6.4^{d}	42.5±2.0^{abc}	25.5±4.5^{ab}	52.1±5.9^{cd}	47.7±2.6^{bcd}
SE03	46.4±1.5^{a}	168.2±8.0^{c}	67.3±2.4^{abc}	49.9±1.5^{ab}	71.7±3.1^{bc}	71.0±2.3^{bc}
SE04	49.3±3.0^{a}	137.2±3.5^{c}	59.3±1.3^{ab}	58.4±2.3^{ab}	67.1±2.3^{bc}	68.2±1.1^{bc}
SE05	40.1±0.5^{ab}	130.0±2.9^{c}	44.7±0.8^{ab}	39.2±0.6^{a}	49.8±1.7^{bc}	49.9±0.3^{bc}
SE06	23.8±1.8^{a}	242.8±9.4^{c}	62.7±3.8^{abc}	28.8±1.9^{ab}	66.9±1.4^{bc}	64.8±0.8^{abc}
SE07	46.7±1.2^{a}	175.9±3.0^{d}	54.6±1.2^{abc}	47.7±1.3^{ab}	64.3±1.7^{cd}	63.7±0.5^{bcd}
SE08	24.5±1.2^{a}	241.9±9.9^{d}	62.0±1.3^{bcd}	26.1±0.4^{ab}	63.6±0.8^{cd}	59.9±1.3^{abc}
SE09	29.9±5.7^{a}	233.7±2.5^{c}	57.6±1.6^{ab}	32.6±1.8^{a}	65.7±0.4^{bc}	60.0±0.1^{abc}
SK01	52.0±0.5^{ab}	67.0±0.5^{bcd}	83.6±0.8^{cd}	47.9±2.1^{a}	94.7±0.5^{d}	59.3±0.9^{abc}
SK02	30.9±0.8^{a}	160.2±6.9^{b}	101.3±11.1^{b}	50.0±7.3^{a}	59.3±1.5^{ab}	60.9±0.7^{ab}
SK03	54.9±1.8^{a}	124.8±5.3^{c}	91.1±0.7^{bc}	56.2±0.9^{a}	74.4±1.9^{ab}	80.0±1.2^{abc}
SK04	64.3±0.9^{a}	78.4±1.3^{ab}	93.4±0.5^{b}	65.6±1.3^{a}	83.9±1.2^{b}	78.5±0.4^{ab}
SK05	72.9±2.4^{a}	106.5±4.5^{bc}	123.4±3.4^{c}	87.1±1.2^{ab}	109.7±0.5^{bc}	95.7±3.7^{ab}
SK06	46.9±0.3^{a}	61.4±0.6^{bc}	73.5±1.1^{c}	50.9±1.3^{ab}	73.0±0.9^{c}	55.8±0.3^{abc}
SK07	44.7±1.8^{a}	70.8±1.2^{bcd}	82.6±1.2^{d}	54.6±4.1^{ab}	73.3±1.4^{cd}	65.4±1.8^{abc}
BMV58	37.7±0.6^{a}	81.8±1.8^{b}	61.3±0.8^{b}	43.9±0.7^{a}	57.6±0.4^{ab}	58.6±2.2^{ab}
SU02	36.7±1.5^{a}	82.0±1.4^{d}	62.3±1.4^{bcd}	39.2±2.4^{ab}	65.2±0.9^{cd}	57.6±0.8^{abc}
SU03	42.7±0.7^{a}	68±1.0^{d}	61.2±0.6^{bcd}	46.4±1.4^{ab}	62.4±0.9^{cd}	58.5±0.8^{abc}
SU04	51.2±1.4^{ab}	103.7±3.5^{c}	65.1±1.7^{bc}	51.2±1.4^{a}	64.6±1.3^{abc}	63.9±0.5^{abc}
SU06	42.7±1.0^{a}	156.1±0.9^{d}	75.2±3.1^{cd}	47.7±2.1^{ab}	61.9±1.5^{bcd}	60.1±0.4^{abc}
SU07	36.6±0.1^{a}	87.2±1.4^{b}	58.2±0.5^{b}	38.8±0.8^{a}	53.9±1.2^{ab}	54.1±0.5^{ab}

TableS1.Continued

	Maximum fermentation rate (g/L.h)					
	Comp	**Ile**	**Leu**	**NH_4**	**Phe**	**Val**
EC1118	0.55±0.01^{c}	0.52±0.02^{bc}	0.46±0.03^{ab}	0.46±0.02^{ab}	0.4±0^{a}	0.49±0.01^{abc}
SC01	0.27±0.01^{bc}	0.3±0^{bc}	0.22±0.01^{ab}	0.22±0.01^{ab}	0.14±0.01^{a}	0.33±0.0^{c}
SC02	0.32±0.07^{b}	0.28±0.01^{ab}	0.3±0.04^{b}	0.29±0.07^{ab}	0.22±0.01^{a}	0.38±0.03^{b}
SC03	0.31±0.01^{c}	0.27±0.01^{bc}	0.26±0^{ab}	0.25±0.01^{ab}	0.24±0.01^{a}	0.26±0^{bc}
SC04	0.41±0.03^{c}	0.27±0.01^{a}	0.4±0.02^{c}	0.33±0.01^{ab}	0.37±0.01^{abc}	0.40±0.01^{bc}
SC05	0.52±0.01^{c}	0.42±0.03^{a}	0.42±0.01^{a}	0.43±0^{ab}	0.46±0.01^{abc}	0.47±0.0^{bc}
SC06	0.59±0^{bc}	0.48±0.01^{a}	0.51±0.05^{ab}	0.52±0.01^{abc}	0.48±0.02^{a}	0.63±0.05^{c}
SC07	0.22±0^{abc}	0.20±0.0^{ab}	0.24±0.0^{c}	0.19±0.01^{a}	0.23±0.01^{bc}	0.18±0.01^{a}
SC08	0.47±0.03^{c}	0.41±0.01^{ab}	0.42±0.01^{bc}	0.39±0.02^{a}	0.4±0.01^{ab}	0.41±0.01^{abc}
SC09	0.44±0.07^{c}	0.37±0.01^{bc}	0.36±0.05^{abc}	0.31±0.01^{ab}	0.3±0.0^{a}	0.37±0^{bc}
SC10	0.47±0.02^{c}	0.31±0.01^{ab}	0.46±0.04^{c}	0.42±0.04^{abc}	0.43±0.05^{bc}	0.24±0.01^{a}
SE01	0.36±0.01^{c}	0.22±0.01^{a}	0.31±0.01^{abc}	0.32±0.01^{bc}	0.25±0^{ab}	0.36±0.01^{c}
SE02	0.43±0.14	0.45±0.05	0.47±0.01	0.34±0.01	0.37±0.03	0.5±0
SE03	0.4±0.01^{ab}	0.25±0.01^{a}	0.42±0^{b}	0.39±0.01^{ab}	0.33±0^{a}	0.48±0^{b}
SE04	0.34±0.02^{abc}	0.33±0.02^{ab}	0.35±0.02^{bc}	0.34±0.01^{abc}	0.28±0.01^{a}	0.41±0.01^{c}
SE05	0.30±0.02^{c}	0.26±0.01^{abc}	0.25±0^{ab}	0.28±0.01^{bc}	0.19±0^{a}	0.32±0^{c}
SE06	0.42±0.03^{bc}	0.38±0.02^{ab}	0.44±0.02^{bc}	0.35±0.02^{a}	0.38±0^{ab}	0.54±0.01^{c}
SE07	0.32±0.01^{ab}	0.31±0.01^{ab}	0.32±0^{ab}	0.31±0.01^{a}	0.23±0^{a}	0.42±0.01^{b}
SE08	0.39±0.01^{abc}	0.37±0.02^{ab}	0.46±0.02^{bc}	0.35±0.01^{a}	0.35±0^{a}	0.51±0.01^{c}
SE09	0.38±0.02^{ab}	0.49±0.01^{bc}	0.46±0.02^{bc}	0.31±0.01^{a}	0.36±0.01^{ab}	0.52±0^{c}
SK01	0.35±0.0^{abc}	0.37±0.0^{bcd}	0.40±0.0^{cd}	0.32±0.01^{ab}	0.3±0.01^{a}	0.41±0.0^{d}
SK02	0.43±0.02^{abc}	0.35±0.01^{a}	0.39±0.02^{ab}	0.43±0.04^{bc}	0.45±0.02^{bc}	0.57±0.01^{c}
SK03	0.42±0.02^{b}	0.28±0.03^{a}	0.36±0.02^{ab}	0.37±0.0^{ab}	0.27±0.02^{a}	0.46±0.01^{b}
SK04	0.44±0.01^{abc}	0.46±0.01^{bc}	0.42±0.02^{ab}	0.45±0.01^{bc}	0.37±0.02^{a}	0.47±0.01^{c}
SK05	0.41±0.03	0.31±0.01	0.35±0.01	0.31±0.01	0.31±0.01	0.35±0.06
SK06	0.58±0^{bcd}	0.60±0.01^{d}	0.55±0^{abc}	0.53±0.01^{ab}	0.44±0.01^{a}	0.59±0^{cd}
SK07	0.63±0.02^{b}	0.64±0.01^{b}	0.49±0.04^{a}	0.53±0.01^{ab}	0.48±0.01^{a}	0.59±0.02^{ab}
BMV58	0.42±0.01^{ab}	0.46±0.01^{bc}	0.45±0.01^{abc}	0.38±0.0^{a}	0.38±0.0^{a}	0.47±0.0^{c}
SU02	0.36±0^{abc}	0.51±0.01^{c}	0.47±0.02^{bc}	0.32±0.0^{a}	0.35±0.01^{ab}	0.51±0.0^{c}
SU03	0.42±0.01^{abc}	0.43±0.01^{bc}	0.44±0.01^{bc}	0.36±0^{a}	0.38±0.01^{ab}	0.52±0.01^{c}
SU04	0.37±0^{b}	0.33±0.01^{ab}	0.32±0.01^{ab}	0.3±0^{a}	0.28±0^{a}	0.37±0.01^{b}
SU06	0.28±0.01^{c}	0.28±0.01^{c}	0.19±0^{a}	0.23±0^{bc}	0.22±0^{abc}	0.19±0^{ab}
SU07	0.37±0^{abc}	0.41±0.01^{bcd}	0.43±0^{cd}	0.3±0^{a}	0.31±0^{ab}	0.45±0.01^{d}

TableS1.Continued

	T75% (h)					
	Comp	**Ile**	**Leu**	**NH_4**	**Phe**	**Val**
EC1118	181.8±1^{a}	259.2±3.6^{c}	231.2±9.2^{bc}	215.3±1.4^{ab}	238.1±1.5^{bc}	219.3±1.8^{ab}
SC01	312±8.1^{a}	406.5±1.4^{bc}	371.8±20.2^{ab}	370.2±11.4^{abc}	595.5±50.8^{c}	364.1±5^{ab}
SC02	248.43±39.86^{a}	460.18±16.79^{c}	305.24±35.43^{ab}	304.43±79.4^{ab}	386.21±13.45^{bc}	291.87±15.67^{ab}
SC03	268±12.3	311±3.4	315.5±10.7	314.3±17.4	311.7±2.1	305.3±4.1
SC04	227.9±16.2^{a}	567.2±20.7^{c}	257.2±5.5^{ab}	267.3±4.6^{bc}	252.2±3.5^{ab}	335.1±4.7^{bc}
SC05	186.1±4.1^{a}	225.2±5^{d}	217.9±2.3^{cd}	209.2±0.8^{abc}	212.4±2.1^{bcd}	198.3±2.1^{ab}
SC06	177.7±1.2^{a}	270±3.4^{b}	272.7±14^{b}	200.8±2^{a}	219.4±2.8^{ab}	222.5±5.6^{ab}
SC07	329±5.7^{a}	465.4±16.5^{bc}	357.2±4.5^{ab}	392±14.3^{abc}	346.7±19.3^{a}	573.8±14.5^{c}
SC08	200.9±6.4^{a}	229.5±0.3^{c}	219±0.3^{ab}	226.5±5.4^{bc}	221.4±3.1^{abc}	223.7±1.2^{bc}
SC09	215.8±17.7^{a}	260.2±2.9^{abc}	254.6±9.8^{ab}	277.4±2.6^{c}	267.7±3.6^{bc}	245±3^{ab}
SC10	177.7±4.6^{a}	338±27.8^{b}	201.7±10^{ab}	170.9±9.8^{a}	223.6±35.3^{ab}	400.1±6.4^{b}
SE01	214.3±3.3^{a}	518±17.2^{b}	262±14.6^{ab}	238.3±3.8^{a}	289.8±5.3^{b}	250.1±7.2^{ab}
SE02	194.3±45.5^{ab}	367.4±27.8^{c}	184.4±6.6^{a}	193.3±15^{ab}	224±19.3^{bc}	196±4.8^{ab}
SE03	195.3±5.5^{a}	404.9±11.7^{c}	198.6±4.5^{ab}	198.4±3.9^{ab}	212.2±4^{bc}	196.4±2.2^{ab}
SE04	232.9±5.4^{ab}	335.7±17.6^{c}	236.2±6.8^{abc}	229.6±10.9^{a}	267.4±11.3^{bc}	227.9±9^{a}
SE05	251.1±13.1^{ab}	346.4±7^{c}	249.1±0.7^{ab}	252.5±4.8^{abc}	288.5±8.7^{bc}	239.7±0.2^{a}
SE06	181.4±18	392.8±19.1	183.6±6.2	182.7±8.1	188.9±7.8	189.2±9.3
SE07	243.9±3.4^{ab}	370.4±8.6^{c}	244.7±4.2^{ab}	243.5±0.9^{ab}	299.8±3.9^{bc}	209±5.3^{a}
SE08	189.8±10.1^{abc}	444.7±10.9^{c}	173.8±4.8^{ab}	165±11.5^{a}	192.7±7.2^{bc}	187.4±8.6^{abc}
SE09	204.3±15.4^{abc}	393.1±2.8^{c}	192±3.1^{ab}	197.4±3.5^{abc}	200.6±4.5^{bc}	186.6±4.4^{a}
SK01	258.6±7.2^{bc}	242.8±0.9^{ab}	250±2.4^{ab}	257.3±4.9^{bc}	305.9±3.2^{c}	225.1±0.7^{a}
SK02	157.2±9.9^{a}	366.6±14.1^{c}	243.5±7.1^{bc}	167.8±9.3^{ab}	167.5±2.9^{ab}	171.2±6.5^{abc}
SK03	220.4±4.4^{a}	348.4±24.1^{d}	273.9±7.4^{bcd}	233.3±3.8^{abc}	298±18.4^{cd}	226.5±0.9^{ab}
SK04	218.4±3.5^{ab}	210.7±2.9^{a}	244.3±7.1^{b}	209.8±4.7^{a}	239.5±12.4^{b}	216.8±3.9^{ab}
SK05	250.9±15.5^{a}	315.4±10.2^{abc}	344.9±4.1^{c}	310.7±6.3^{ab}	322±1.8^{bc}	293±33.8^{ab}
SK06	172.7±2^{a}	173.1±2.2^{a}	196.1±1.7^{bc}	186.2±3.4^{abc}	218.8±1.8^{c}	175.9±1^{ab}
SK07	138.6±9^{a}	153.1±7.6^{ab}	191.7±7.8^{c}	145.3±10.4^{a}	182.8±4.5^{bc}	166±7.4^{abc}
BMV58	193.5±1.9^{a}	208.6±1^{bc}	199.1±0.9^{ab}	202.6±1.9^{abc}	210.4±0.9^{c}	192.1±1.5^{a}
SU02	228.7±3.7^{bc}	206.5±1.8^{ab}	205.1±5.3^{ab}	248.6±5.5^{bc}	253.7±4^{c}	189.1±0.6^{a}
SU03	212.5±5.6^{ab}	218.3±3.7^{abc}	213.4±1.4^{a}	232.3±2.3^{bc}	236.7±5^{c}	184.3±4.5^{a}
SU04	239.5±3.4^{a}	295±7.1^{b}	272.8±9.2^{ab}	275.1±3.6^{ab}	295.1±9.9^{b}	247.4±3.5^{a}
SU06	290±6.5^{a}	369.3±9.8^{c}	332.3±1.8^{bc}	293.4±9.2^{a}	330.6±2.3^{abc}	298.9±3.4^{ab}
SU07	231.6±2.1^{abc}	242.8±5.8^{bcd}	208.2±1.6^{ab}	270.4±3.5^{d}	263±0.5^{cd}	200.2±4.2^{a}

Data is expressed as the mean value ± SD (n=3). Different superscript letters in a same row and a same fermentation parameter indicate significant differences ($p< 0.05$) by Kruskal-Wallis test. Lag phase: time to reach the 10% of the maximum weight lost on the fermentation; Vmax: maximum fermentation rate; T75%: time to reach the 75% of the maximum weight lost on the fermentation. Comp (complete); Ile (Isoleucine); Leu (Leucine); NH4 (Ammonium); Phe (Phenylalanine) and Val (Valine).

Table S2. Glycerol mean concentration expressed in g produced per 100g sugar consumed by the 33 yeast strains in the six nitrogen media.

	Comp*	Ile	Leu	NH4	Phe	Val	Yeast**
EC1118	2.45±0.1^{ab}	3.18±0.08^{c}	2.66±0.09^{bc}	2.59±0.02^{abc}	2.65±0.02^{bc}	2.28±0.03^{a}	2.64±0.29
SC01	3.94±0.07^{ab}	4.18±0.16^{b}	4.44±0.73^{b}	3.67±0.06^{ab}	4.41±0.35^{b}	3.31±0.04^{a}	**3.99±0.52**
SC02	4.7±0.1^{b}	4.11±0.13^{ab}	4.55±0.16^{b}	4.48±0.56^{b}	4.11±0.13^{ab}	3.28±0.29^{a}	**4.21±0.54**
SC03	3.06±0.03^{a}	3.88±0.05^{d}	3.43±0.06^{bcd}	3.17±0.01^{ab}	3.49±0.06^{cd}	3.25±0.03^{abc}	**3.38±0.28**
SC04	3.13±0.07^{abc}	3.45±0.06^{c}	3.52±0.3^{bc}	3.46±0.03^{c}	3.02±0.01^{a}	3.06±0.03^{ab}	**3.27±0.24**
SC05	3.14±0.01^{ab}	3.27±0.04^{b}	3.27±0.04^{b}	3.14±0.08^{ab}	3.01±0.03^{a}	2.8±0.03^{a}	3.11±0.17
SC06	3.11±0.04^{ab}	3.23±0.01^{b}	3.27±0.03^{b}	2.97±0.02^{a}	3.11±0.02^{ab}	2.61±0.02^{a}	3.05±0.23
SC07	2.82±0.05^{ab}	3.19±0.03^{c}	3.01±0.02^{abc}	3.05±0.15^{bc}	3.21±0.06^{c}	2.67±0.01^{a}	2.99±0.21
SC08	2.81±0.07^{a}	3.52±0.06^{c}	3.11±0.03^{bc}	2.94±0.05^{ab}	2.97±0.01^{abc}	2.91±0.04^{ab}	3.04±0.24
SC09	3.22±0.02^{a}	4.55±0.07^{d}	4.02±0.08^{cd}	3.53±0.05^{ab}	3.87±0.05^{bcd}	3.78±0.02^{abc}	**3.83±0.42**
SC10	4.82±0.08^{c}	4.13±0.54^{ab}	4.18±0.1^{abc}	4.67±0.12^{bc}	4.18±0.27^{abc}	3.6±0.12^{a}	**4.26±0.46**
S. cerevisiae	3.38±0.75^{ab}	3.7±0.49^{d}	3.59±0.64^{cd}	3.43±0.64^{bc}	3.46±0.58^{bc}	3.05±0.44^{a}	
SE01	3.73±0.04^{ab}	5.42±0.11^{c}	3.8±0.08^{ab}	4.09±0.08^{bc}	4.35±0.05^{bc}	3.49±0.16^{a}	**4.15±0.66**
SE02	6.35±0.76^{bc}	5.05±0.16^{ab}	5.66±0.22^{abc}	6.98±0.47^{c}	5.93±0.34^{bc}	4.7±0.22^{a}	**5.78±0.86**
SE03	3.3±0.19^{ab}	3.91±0.08^{cd}	3.39±0.07^{abc}	3.61±0.09^{bcd}	3.94±0.14^{d}	2.78±0.18^{a}	**3.49±0.42**
SE04	3.89±0.39^{abc}	3.8±0.12^{bc}	3.78±0.08^{abc}	4.41±0.5^{c}	3.7±0.08^{ab}	3.04±0.08^{a}	**3.77±0.47**
SE05	3.42±0.04^{a}	3.71±0.03^{ab}	3.99±0.04^{b}	3.71±0.04^{ab}	4.34±0.01^{b}	3.07±0.07^{a}	**3.71±0.42**
SE06	5.84±0.68	6.31±0.12	5.76±0.41	6.6±0.75	6.17±0.13	4.44±0.15	**5.83±0.79**
SE07	2.49±0.02^{a}	3.51±0.2^{abc}	3.19±0.06^{ab}	3.66±0.15^{bc}	4.22±0.17^{c}	3.43±0.11^{abc}	**3.42±0.55**
SE08	6.69±0.07^{bc}	5.66±0.29^{ab}	6.03±0.35^{ab}	7.75±0.47^{c}	6.69±0.12^{bc}	4.75±0.09^{a}	**6.26±0.99**
SE09	6.43±0.56^{bc}	4.9±0.08^{ab}	5.63±0.67^{abc}	7.44±0.63^{c}	6.81±0.24^{c}	4.61±0.3^{a}	**6.03±1.12**
S. eubayanus	4.68±1.6^{b}	4.7±0.97^{b}	4.58±1.14^{b}	5.36±1.75^{b}	5.13±1.21^{b}	3.81±0.78^{a}	
SK01	3.35±0.1^{ab}	3.7±0.03^{abc}	4.7±0.04^{d}	3.85±0.03^{bcd}	3.96±0.06^{cd}	3.27±0.02^{a}	**3.8±0.48**
SK02	6.24±0.49^{abc}	6.74±0.82^{bc}	7.38±0.49^{c}	6.98±0.93^{c}	5.29±0.6^{ab}	5.11±0.04^{a}	**6.36±1.01**
SK03	3.25±0.05^{a}	4.52±0.06^{bc}	5.09±0.07^{c}	3.56±0.37^{ab}	3.27±0.24^{a}	4.15±0.04^{abc}	**3.97±0.72**
SK04	3.18±0^{ab}	3.61±0.01^{bc}	4.46±0.11^{c}	3.27±0.07^{ab}	3.52±0.13^{bc}	3.1±0.01^{a}	**3.53±0.47**
SK05	3.83±0.05^{ab}	4.1±0.09^{bc}	4.04±0.04^{abc}	4.32±0.08^{c}	3.75±0.06^{a}	3.82±0.21^{ab}	**3.96±0.22**
SK06	3.54±0.02^{abc}	3.45±0.02^{ab}	4.68±0.04^{d}	4.05±0.06^{cd}	3.71±0.02^{bcd}	3.28±0.04^{a}	**3.79±0.48**
SK07	5.23±0.15^{abc}	4.91±0.3^{ab}	6.13±0.86^{c}	5.69±0.55^{bc}	4.65±0.39^{a}	4.45±0.26^{a}	**5.18±0.72**
S. kudriavzevii	4.09±1.13^{a}	4.43±1.12^{a}	5.21±1.14^{b}	4.53±1.31^{a}	4.02±0.71^{a}	3.88±0.71^{a}	
BMV58	2.88±0.09^{ab}	2.89±0.13^{ab}	2.57±0.08^{a}	3.3±0.28^{b}	3.11±0.26^{b}	2.58±0.07^{a}	2.89±0.32
SU02	2.93±0.02^{abc}	3.17±0.07^{bc}	2.89±0.38^{ab}	3.53±0.1^{c}	2.88±0.09^{ab}	2.51±0.09^{a}	2.98±0.35
SU03	2.79±0.07^{ab}	3.01±0.1^{bc}	2.96±0.06^{bc}	3.63±0.02^{c}	2.7±0.04^{ab}	2.5±0.06^{a}	2.93±0.37
SU04	3.44±0.07^{bc}	3.62±0.05^{c}	3.34±0.22^{abc}	3.47±0.01^{abc}	3.15±0.03^{ab}	2.73±0.09^{a}	**3.28±0.32**
SU06	3.03±0.03^{a}	3.89±0.08^{b}	3.88±0.08^{b}	3.39±0.18^{ab}	3.04±0.04^{a}	3.28±0.03^{ab}	**3.42±0.37**
SU07	2.63±0.03^{abc}	2.74±0.03^{bcd}	2.34±0.03^{a}	3.15±0.12^{d}	2.84±0.05^{cd}	2.47±0.03^{ab}	2.7±0.27
S. uvarum	2.95±0.27^{b}	3.22±0.42^{bc}	3±0.54^{b}	3.41±0.21^{c}	2.95±0.19^{b}	2.68±0.3^{a}	

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different ($p< 0.05$) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S3. 2,3-butanediol mean concentration expressed in g of 2,3-butanediol produced per 100g of sugars consumed by the 33 yeast strains in the six nitrogen media.

	Comp*	Ile	Leu	NH4	Phe	Val	Yeast**
EC1118	0.21±0.04^{ab}	0.31±0.02^{c}	0.27±0.01^{bc}	0.2±0.01^{ab}	0.18±0.01^{a}	0.27±0.01^{bc}	0.24±0.05
SC01	0.34±0.01^{ab}	0.35±0.02^{ab}	0.33±0.08^{ab}	0.27±0.01^{a}	0.3±0.05^{a}	0.4±0.01^{b}	0.33±0.05
SC02	0.72±0.05	0.59±0.02	0.77±0.02	0.71±0.1	0.7±0.05	0.7±0.03	**0.7±0.07**
SC03	0.39±0.01^{c}	0.38±0.01^{bc}	0.29±0.02^{a}	0.3±0.01^{ab}	0.34±0.01^{abc}	0.4±0^{c}	**0.35±0.05**
SC04	0.36±0.01^{ab}	0.38±0.01^{abc}	0.45±0.03^{c}	0.4±0.02^{bc}	0.31±0^{a}	0.38±0.01^{abc}	**0.38±0.05**
SC05	0.3±0.01^{abc}	0.25±0.01^{ab}	0.32±0^{bc}	0.33±0.01^{c}	0.2±0.01^{a}	0.33±0.01^{c}	0.29±0.05
SC06	0.25±0.01^{c}	0.23±0.01^{abc}	0.24±0.01^{bc}	0.2±0^{ab}	0.15±0.01^{a}	0.25±0.01^{c}	0.22±0.04
SC07	0.18±0.03^{abc}	0.2±0.01^{bc}	0.18±0^{ab}	0.15±0.01^{a}	0.19±0^{abc}	0.22±0.01^{c}	0.19±0.02
SC08	0.25±0.01^{ab}	0.31±0.01^{b}	0.26±0^{ab}	0.23±0.01^{a}	0.23±0.01^{a}	0.34±0.01^{b}	0.27±0.04
SC09	0.22±0.01^{ab}	0.31±0.01^{b}	0.23±0.01^{ab}	0.19±0^{a}	0.21±0.01^{a}	0.31±0.04^{b}	0.24±0.05
SC10	0.87±0.05^{abc}	1.03±0.21^{bc}	0.69±0.16^{ab}	0.67±0.09^{a}	0.57±0.1^{a}	1.18±0.06^{c}	**0.84±0.24**
S. cerevisiae	0.37±0.21^{abc}	0.39±0.23^{bc}	0.37±0.19^{bc}	0.33±0.19^{ab}	0.31±0.17^{a}	0.44±0.27^{c}	
SE01	0.43±0.01^{a}	0.73±0.02^{b}	0.39±0.04^{a}	0.52±0.1^{ab}	0.42±0.01^{a}	0.52±0.07^{ab}	**0.5±0.13**
SE02	1.42±0.44	1.34±0.15	1.47±0.14	1.33±0.5	0.75±0.09	1.67±0.18	**1.33±0.38**
SE03	0.39±0.03^{bc}	0.46±0.02^{c}	0.34±0.01^{ab}	0.38±0.02^{abc}	0.34±0.02^{a}	0.37±0.03^{abc}	**0.38±0.05**
SE04	0.52±0.09^{c}	0.44±0.01^{bc}	0.4±0.02^{ab}	0.53±0.09^{bc}	0.32±0.01^{a}	0.42±0.05^{abc}	**0.44±0.09**
SE05	0.42±0.01^{bc}	0.39±0^{ab}	0.42±0.01^{bc}	0.37±0^{a}	0.46±0.01^{c}	0.39±0.01^{ab}	**0.41±0.03**
SE06	1.12±0.3^{bc}	0.83±0.12^{abc}	0.67±0.06^{ab}	1.19±0.23^{c}	0.41±0.01^{a}	1.14±0.23^{c}	**0.91±0.33**
SE07	0.51±0.01^{b}	0.51±0.02^{b}	0.4±0.01^{a}	0.41±0.07^{a}	0.43±0.05^{a}	0.45±0.01^{ab}	**0.45±0.05**
SE08	1.26±0.09^{c}	0.77±0.07^{ab}	0.69±0.1^{ab}	1.09±0.02^{bc}	0.48±0.04^{a}	1.07±0.21^{bc}	**0.89±0.29**
SE09	1.11±0.18^{bc}	1.28±0.05^{c}	0.95±0.19^{abc}	0.9±0.05^{ab}	0.53±0.03^{a}	1.16±0.07^{bc}	**0.97±0.26**
S. eubayanus	0.8±0.43^{bc}	0.75±0.35^{c}	0.64±0.37^{ab}	0.75±0.4^{bc}	0.46±0.13^{a}	0.8±0.46^{bc}	
SK01	0.39±0.02^{a}	0.38±0.01^{a}	0.78±0.02^{c}	0.4±0.01^{ab}	0.44±0.01^{bc}	0.41±0.01^{abc}	**0.47±0.15**
SK02	0.86±0.08^{ab}	1.32±0.12^{c}	1.16±0.08^{bc}	0.97±0.15^{abc}	0.61±0.07^{a}	1.15±0^{bc}	**1±0.26**
SK03	0.63±0.03^{ab}	0.8±0.01^{bc}	0.87±0.01^{bc}	0.63±0.06^{ab}	0.44±0.06^{a}	0.96±0.03^{c}	**0.72±0.18**
SK04	0.6±0.01^{b}	0.47±0.02^{a}	0.77±0.04^{b}	0.55±0.01^{ab}	0.47±0.03^{a}	0.55±0.01^{ab}	**0.57±0.11**
SK05	0.62±0.04^{b}	0.49±0.02^{a}	0.53±0.02^{ab}	0.6±0.03^{b}	0.45±0^{a}	0.51±0.07^{ab}	**0.53±0.07**
SK06	0.45±0.01^{abc}	0.37±0.01^{a}	0.76±0^{d}	0.56±0.02^{cd}	0.42±0.01^{ab}	0.47±0.01^{bcd}	**0.5±0.13**
SK07	1.48±0.1^{abc}	1.14±0.09^{ab}	1.75±0.09^{c}	1.97±0.09^{c}	0.89±0.01^{a}	1.63±0.12^{bc}	**1.48±0.38**
S. kudriavzevii	0.72±0.35^{b}	0.71±0.37^{ab}	0.95±0.38^{c}	0.81±0.52^{bc}	0.53±0.17^{a}	0.81±0.43^{bc}	
BMV58	0.33±0.01^{c}	0.29±0.01^{abc}	0.24±0.01^{a}	0.29±0.03^{abc}	0.25±0.02^{ab}	0.32±0.06^{bc}	0.29±0.04
SU02	0.42±0.03^{ab}	0.42±0.02^{ab}	0.49±0.04^{b}	0.49±0.02^{b}	0.35±0.01^{a}	0.43±0.02^{ab}	**0.43±0.05**
SU03	0.24±0.02^{ab}	0.32±0.01^{c}	0.29±0^{bc}	0.26±0^{abc}	0.22±0.01^{a}	0.25±0^{ab}	0.26±0.03
SU04	0.51±0.04^{c}	0.35±0.04^{ab}	0.37±0.02^{abc}	0.44±0.01^{bc}	0.33±0^{a}	0.35±0.02^{ab}	**0.39±0.07**
SU06	0.42±0.01^{cd}	0.45±0.03^{d}	0.39±0.01^{bcd}	0.34±0.01^{ab}	0.26±0^{a}	0.36±0^{abc}	**0.37±0.06**
SU07	0.31±0^{ab}	0.34±0^{bc}	0.37±0.01^{c}	0.33±0.03^{bc}	0.26±0^{a}	0.31±0^{ab}	0.32±0.04
S. uvarum	0.37±0.09^{b}	0.36±0.06^{b}	0.36±0.08^{b}	0.35±0.08^{b}	0.28±0.05^{a}	0.34±0.06^{b}	

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different ($p< 0.05$) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S4. Erythritol mean concentration expressed in g per 100g of sugars consumed by the 33 yeast strains in the six nitrogen musts.

	Comp*	Ile	Leu	NH4	Phe	Val	Yeast**
EC1118	0.11±0.02^{ab}	0.08±0.01^{a}	0.15±0.01^{b}	0.11±0^{ab}	0.15±0^{b}	0.09±0.01^{a}	0.12±0.03
SC01	0.46±0.03^{c}	0.24±0.01^{ab}	0.3±0.07^{abc}	0.38±0.03^{bc}	0.29±0.01^{abc}	0.19±0.01^{a}	**0.31±0.1**
SC02	0.18±0.01	0.17±0.05	0.25±0.02	0.25±0.06	0.24±0.02	0.23±0.07	**0.22±0.05**
SC03	0.33±0.03^{abc}	0.3±0.02^{ab}	0.36±0.01^{bc}	0.32±0.03^{abc}	0.38±0.02^{c}	0.26±0.01^{a}	**0.32±0.04**
SC04	0.19±0.01^{abc}	0.11±0.01^{a}	0.34±0.04^{d}	0.23±0.01^{bcd}	0.25±0^{cd}	0.16±0.01^{ab}	**0.21±0.08**
SC05	0.19±0.01^{a}	0.38±0.02^{c}	0.36±0.01^{c}	0.24±0.02^{ab}	0.28±0.01^{bc}	0.27±0^{abc}	**0.29±0.07**
SC06	0.08±0.01^{ab}	0.09±0^{abc}	0.07±0.01^{a}	0.1±0.01^{bc}	0.12±0.01^{c}	0.07±0^{a}	0.09±0.02
SC07	0.28±0^{ab}	0.15±0.01^{a}	0.28±0.01^{ab}	0.32±0.02^{b}	0.35±0.01^{b}	0.1±0.01^{a}	**0.25±0.09**
SC08	0.25±0.01^{a}	0.31±0.01^{bc}	0.33±0.01^{c}	0.29±0.01^{abc}	0.34±0.01^{c}	0.26±0.01^{ab}	**0.3±0.03**
SC09	0.21±0.02^{a}	0.29±0.02^{bc}	0.33±0.01^{c}	0.25±0.01^{ab}	0.3±0.03^{bc}	0.21±0.02^{a}	**0.26±0.05**
SC10	0.14±0	0.03±0.06	0.15±0.02	0.13±0.01	0.1±0.09	0.22±0.15	0.13±0.09
S. cerevisiae	0.22±0.1^{abc}	0.2±0.11^{ab}	0.27±0.1^{d}	0.24±0.09^{bcd}	0.26±0.09^{cd}	0.19±0.08^{a}	
SE01	0.39±0.03^{b}	0.12±0.01^{a}	0.27±0.03^{ab}	0.35±0.04^{b}	0.29±0.01^{ab}	0.19±0.03^{a}	**0.27±0.1**
SE02	0.15±0.03^{ab}	0.1±0.01^{a}	0.23±0.01^{b}	0.14±0.02^{ab}	0.24±0.02^{b}	0.12±0.03^{a}	0.16±0.06
SE03	0.39±0.04^{b}	0.16±0.01^{a}	0.24±0.01^{ab}	0.36±0.01^{b}	0.27±0.02^{ab}	0.16±0^{a}	**0.26±0.09**
SE04	0.34±0.05^{bc}	0.14±0.01^{a}	0.32±0.02^{abc}	0.32±0.08^{bc}	0.39±0.01^{c}	0.19±0.01^{ab}	**0.28±0.09**
SE05	0.29±0.01^{bc}	0.2±0^{a}	0.24±0^{abc}	0.32±0.01^{c}	0.22±0.01^{ab}	0.19±0.01^{a}	**0.24±0.05**
SE06	0.16±0.03^{c}	0.1±0.02^{ab}	0.13±0.01^{abc}	0.16±0.05^{bc}	0.18±0.01^{c}	0.08±0.01^{a}	0.13±0.04
SE07	0.29±0.01^{b}	0.17±0.01^{a}	0.26±0.01^{ab}	0.31±0.04^{b}	0.3±0.05^{b}	0.17±0.01^{a}	**0.25±0.06**
SE08	0.14±0.01^{ab}	0.12±0.01^{ab}	0.1±0.03^{a}	0.08±0.07^{a}	0.17±0.01^{b}	0.1±0.02^{a}	0.12±0.04
SE09	0.16±0.05^{bc}	0.11±0^{abc}	0.14±0.03^{bc}	0.07±0.06^{ab}	0.16±0.01^{c}	0.08±0.01^{a}	0.12±0.05
S. eubayanus	0.26±0.11^{b}	0.14±0.03^{a}	0.21±0.07^{b}	0.23±0.12^{b}	0.25±0.07^{b}	0.14±0.05^{a}	
SK01	0.2±0.01^{ab}	0.21±0^{abc}	0.24±0.01^{c}	0.25±0^{c}	0.23±0.01^{bc}	0.18±0.01^{a}	**0.22±0.02**
SK02	0.04±0.07	0.05±0.02	0.08±0.01	0.11±0.02	0.09±0.08	0.04±0.02	0.07±0.05
SK03	0.17±0.03^{abc}	0.11±0.01^{ab}	0.22±0.01^{c}	0.19±0.01^{bc}	0.18±0.01^{abc}	0.09±0.01^{a}	0.16±0.05
SK04	0.13±0.01^{ab}	0.15±0.01^{abc}	0.17±0.02^{bc}	0.15±0.01^{bc}	0.2±0.03^{c}	0.11±0^{a}	0.15±0.03
SK05	0.13±0.02	0.11±0.01	0.13±0.01	0.13±0.02	0.19±0	0.12±0.02	0.13±0.03
SK06	0.2±0^{ab}	0.18±0.01^{a}	0.28±0.01^{b}	0.2±0.01^{ab}	0.3±0^{b}	0.16±0.01^{a}	**0.22±0.05**
SK07	0.07±0^{ab}	0.08±0.03^{ab}	0.12±0.02^{bc}	0.06±0.01^{a}	0.15±0.02^{c}	0.08±0.02^{abc}	0.09±0.04
S. kudriavzevii	0.14±0.06^{abc}	0.13±0.06^{ab}	0.18±0.07^{cd}	0.16±0.06^{bcd}	0.19±0.07^{d}	0.11±0.05^{a}	
BMV58	0.26±0.02^{bc}	0.17±0.01^{a}	0.24±0.01^{abc}	0.2±0.03^{ab}	0.35±0.05^{c}	0.16±0.01^{a}	**0.23±0.07**
SU02	0.26±0.02^{abc}	0.24±0.01^{ab}	0.23±0.03^{ab}	0.33±0.01^{c}	0.31±0.01^{bc}	0.17±0.01^{a}	**0.26±0.06**
SU03	0.22±0.01^{abc}	0.18±0.01^{ab}	0.29±0^{c}	0.24±0.01^{bc}	0.3±0.01^{c}	0.15±0.01^{a}	**0.23±0.05**
SU04	0.31±0.01^{ab}	0.35±0.01^{abc}	0.38±0.04^{bc}	0.36±0.01^{abc}	0.56±0.01^{c}	0.25±0.02^{a}	**0.37±0.1**
SU06	0.29±0^{bc}	0.2±0.01^{a}	0.27±0.01^{ab}	0.29±0^{bc}	0.43±0.01^{c}	0.21±0.01^{ab}	**0.28±0.08**
SU07	0.36±0.01^{ab}	0.29±0.01^{a}	0.35±0.01^{ab}	0.42±0.03^{b}	0.55±0.01^{b}	0.29±0.01^{a}	**0.38±0.09**
S. uvarum	0.28±0.05^{bc}	0.24±0.07^{ab}	0.29±0.06^{c}	0.3±0.08^{c}	0.42±0.11^{d}	0.21±0.05^{a}	

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different ($p< 0.05$) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S5. Ethanol mean concentration expressed in g per 100 g of sugars consumed by the 33 yeast strains in the six nitrogen musts.

	Comp*	Ile	Leu	NH4	Phe	Val	Yeast**
EC1118	47.89±0.32^{ab}	46.39±0.55^{a}	47.26±0.63^{a}	48.44±0.08^{b}	47.89±0.32^{ab}	46.79±0.95^{a}	47.42±0.87
SC01	49.00±0.87^{c}	47.02±2.05^{bc}	39.77±6.86^{ab}	44.97±0.24^{abc}	40.95±3.55^{a}	47.58±0.87^{c}	**44.58±4.50**
SC02	45.68±0.47	45.76±0.24	43.71±0.95	47.26±1.97	45.29±0.63	45.60±0.47	**45.53±1.34**
SC03	48.13±0.39^{d}	45.92±0.16^{a}	46.71±0.08^{abc}	47.97±0.32^{cd}	47.42±0.16^{bcd}	46.55±0.32^{ab}	47.10±0.87
SC04	47.73±0.24^{bc}	46.08±0.08^{a}	47.81±1.34^{bc}	47.66±0.32^{abc}	48.13±0.16^{c}	46.95±0.32^{ab}	47.42±0.87
SC05	48.05±0.16^{c}	45.05±0.08^{a}	45.68±0.24^{ab}	48.05±0.32^{c}	46.87±0.32^{abc}	47.42±0.08^{bc}	46.87±1.18
SC06	48.60±0.16^{b}	46.16±0.24^{a}	46.47±0.24^{a}	49.08±0.63^{b}	47.89±0.47^{ab}	47.26±0.55^{ab}	47.58±1.10
SC07	48.60±0.55^{bc}	46.79±0.16^{abc}	46.24±0.16^{ab}	49.23±1.50^{c}	49.39±0.71^{c}	45.92±0.24^{a}	47.66±1.58
SC08	49.00±0.87^{c}	46.00±0.16^{a}	46.63±0.08^{ab}	48.60±1.03^{bc}	47.66±0.16^{abc}	47.81±0.39^{bc}	47.58±1.18
SC09	47.58±0.39^{c}	45.13±0.16^{a}	46.55±0.24^{abc}	47.02±0.24^{bc}	46.95±0.47^{bc}	46.16±0.08^{ab}	46.55±0.79
SC10	45.05±0.32	44.89±1.97	44.97±0.79	45.92±1.03	45.05±0.32	43.08±0.16	**44.82±1.18**
S. cerevisiae	47.73±1.34^{e}	45.92±1.03^{a}	45.60±2.84^{ab}	47.66±1.50^{de}	46.63±2.37^{cd}	46.47±1.34^{bc}	
SE01	47.97±0.16^{c}	43.95±0.24^{a}	46.08±0.32^{ab}	47.58±0.55^{bc}	46.24±0.24^{abc}	47.10±0.63^{bc}	46.47±1.42
SE02	44.11±0.63^{abc}	45.37±0.16^{c}	42.76±1.18^{ab}	42.37±0.71^{a}	45.21±1.66^{bc}	42.53±0.24^{a}	**43.71±1.50**
SE03	47.10±1.74	45.29±1.18	43.79±0.24	45.37±0.87	44.66±2.05	42.84±1.74	**44.82±1.81**
SE04	46.87±0.39	45.05±0.95	45.68±0.55	48.21±4.73	45.45±0.47	45.84±0.24	**46.16±1.97**
SE05	46.95±0.32^{c}	45.21±0.16^{ab}	44.42±0.16^{a}	46.24±0.32^{bc}	44.50±0.32^{a}	45.60±0.16^{abc}	**45.53±0.95**
SE06	40.00±1.18	41.34±1.03	40.16±1.89	39.37±3.79	40.32±0.95	42.61±1.74	**40.48±2.13**
SE07	46.63±0.24^{c}	44.18±0.08^{a}	45.21±0.08^{ab}	46.55±0.24^{c}	46.95±2.52^{bc}	45.76±0.08^{abc}	**45.84±1.34**
SE08	42.76±1.10	44.82±0.24	40.24±0.95	41.97±1.03	40.87±1.42	42.53±2.68	**42.21±1.89**
SE09	46.39±2.13	47.66±1.26	44.89±0.87	44.42±3.47	46.63±1.34	44.89±0.87	**45.76±2.05**
S. eubayanus	45.37±2.68	44.74±1.74	43.71±2.29	44.66±3.39	44.50±2.60	44.42±2.05	
SK01	47.73±1.18^{b}	47.26±0.63^{ab}	46.31±0.08^{a}	48.29±0.16^{b}	47.89±0.24^{b}	47.58±0.08^{ab}	47.50±0.79
SK02	41.58±2.45	40.00±4.73	38.50±2.60	41.97±4.50	39.69±4.50	37.00±1.26	**39.92±3.55**
SK03	46.47±0.32	46.00±0.87	45.05±0.71	43.71±3.47	39.13±2.21	45.84±0.24	**44.34±3.00**
SK04	45.13±0.08^{c}	43.47±0.08^{ab}	42.61±0.08^{a}	44.66±0.16^{bc}	43.63±0.39^{ab}	43.55±0.08^{abc}	**43.87±0.87**
SK05	46.95±0.16^{b}	45.45±0.16^{a}	45.84±0.63^{a}	46.47±0.16^{ab}	47.10±0.16^{b}	45.68±0.24^{a}	**46.31±0.71**
SK06	48.37±0.47^{c}	44.82±0.39^{a}	46.08±0.24^{ab}	48.13±0.47^{c}	48.05±0.08^{bc}	47.26±0.08^{abc}	47.10±1.34
SK07	43.40±1.81	44.89±0.47	44.74±1.66	42.76±2.45	44.97±0.39	44.11±1.26	**44.18±1.58**
S. kudriavzevii	45.68±2.52	44.58±2.68	44.18±2.84	45.13±3.08	44.34±3.87	44.42±3.47	
SU01	43.87±3.87	39.84±6.31	44.03±0.47	40.87±1.74	45.92±4.18	47.18±0.39	**43.63±4.10**
SU02	46.87±0.79	47.42±0.71	45.76±1.74	46.95±0.63	48.44±2.05	45.76±1.66	46.87±1.50
SU03	46.95±0.16^{c}	45.21±0.24^{a}	45.45±0.39^{ab}	46.00±0.16^{abc}	46.55±0.16^{bc}	45.29±0.16^{a}	**45.92±0.71**
SU04	49.31±0.24^{c}	46.79±0.39^{a}	47.26±0.55^{ab}	48.52±0.24^{bc}	48.21±0.08^{bc}	47.34±0.16^{ab}	47.89±0.95
SU06	46.55±0.32^{c}	44.42±0.16^{abc}	43.63±0.32^{a}	46.47±0.24^{c}	46.16±0.16^{bc}	43.79±0.08^{ab}	**45.21±1.26**
SU07	47.10±0.39^{b}	45.05±0.39^{a}	45.45±0.16^{a}	48.05±2.21^{b}	46.24±0.16^{ab}	45.53±0.32^{a}	**46.24±1.34**
S. uvarum	46.79±2.13^{c}	44.82±3.31^{a}	45.29±1.34^{a}	46.00±2.76^{abc}	46.95±1.89^{bc}	45.84±1.42^{ab}	

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different ($p< 0.05$) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S6. Succinic acid mean concentration expressed in g per100 g of sugars consumed by the 33 yeast strains in the six nitrogen musts.

	Comp*	Ile	Leu	NH_4	Phe	Val	Yeast**
EC1118	0.19±0.06^{bc}	0.14±0.04^{abc}	0.13±0.02^{ab}	0.17±0.02^{bc}	0.21±0.01^{c}	0.11±0.01^{a}	0.16±0.04
SC01	0.21±0.01^{a}	0.56±0.11^{b}	0.21±0.04^{a}	0.27±0.01^{ab}	0.2±0.02^{a}	0.63±0.04^{b}	0.35±0.19
SC02	0.89±0.1^{ab}	0.74±0.66^{ab}	1.8±0.2^{c}	1.43±0.4^{bc}	1.69±0.21^{bc}	0.43±0.43^{a}	**1.16±0.61**
SC03	0.34±0.02	0.33±0.03	0.35±0.06	0.37±0.03	0.34±0.14	0.24±0.01	0.33±0.07
SC04	0.32±0.05^{bc}	0.2±0.02^{ab}	0.24±0.03^{ab}	0.41±0.01^{c}	0.32±0.05^{bc}	0.15±0.01^{a}	0.27±0.09
SC05	1.52±0.04^{c}	0.63±0.03^{ab}	0.5±0.01^{a}	0.76±0.01^{bc}	1±0.06^{bc}	0.64±0.06^{ab}	**0.84±0.35**
SC06	0.15±0.01^{bc}	0.13±0.01^{ab}	0.14±0.01^{abc}	0.19±0.01^{c}	0.14±0.02^{abc}	0.09±0.01^{a}	0.14±0.03
SC07	0.29±0.05^{ab}	0.14±0.01^{a}	0.36±0.01^{b}	0.36±0.03^{b}	0.3±0.02^{ab}	0.1±0.01^{a}	0.26±0.11
SC08	0.3±0.02^{bc}	0.17±0.01^{a}	0.23±0.02^{abc}	0.33±0.02^{c}	0.19±0.01^{ab}	0.18±0.02^{a}	0.23±0.06
SC09	0.92±0.01^{cd}	0.38±0.01^{ab}	0.54±0.01^{abc}	0.67±0.01^{bcd}	1.08±0.13^{d}	0.31±0.03^{a}	**0.65±0.29**
SC10	1.12±0.19	0.83±0.8	1.28±0.05	1.49±0.4	1.12±0.52	0.18±0.32	**1±0.57**
S. cerevisiae	0.57±0.45^{bc}	0.39±0.36^{ab}	0.52±0.52^{bc}	0.59±0.47^{c}	0.6±0.53^{bc}	0.28±0.24^{a}	
SE01	1.19±0.16^{bc}	0.42±0.02^{a}	1.45±0.12^{c}	1.08±0.21^{bc}	1.06±0.03^{abc}	0.76±0.07^{ab}	**0.99±0.35**
SE02	0.99±0.17^{ab}	0.62±0.1^{a}	0.89±0.07^{ab}	1.21±0.17^{b}	1.07±0.04^{b}	0.67±0.07^{a}	**0.91±0.24**
SE03	0.8±0.07^{bc}	0.47±0.02^{a}	0.73±0.02^{bc}	1.25±0.04^{c}	0.53±0.08^{ab}	0.58±0.04^{ab}	**0.73±0.27**
SE04	0.49±0.21^{ab}	0.48±0.1^{a}	0.77±0.03^{bc}	0.62±0.03^{abc}	1.15±0.03^{c}	0.53±0.07^{ab}	**0.67±0.26**
SE05	0.7±0.05^{bc}	0.59±0.02^{a}	0.62±0.02^{ab}	1.46±0.07^{c}	0.61±0.02^{ab}	0.7±0.03^{bc}	**0.78±0.32**
SE06	0.97±0.1^{b}	0.82±0.02^{ab}	0.84±0.07^{ab}	1.05±0.14^{b}	0.98±0.03^{b}	0.64±0.09^{a}	**0.89±0.16**
SE07	0.46±0.02^{a}	0.53±0.02^{ab}	1±0.08^{c}	1.05±0.06^{c}	0.65±0.13^{bc}	0.53±0.03^{ab}	**0.7±0.25**
SE08	1.07±0.03^{cd}	0.71±0.02^{ab}	0.87±0.1^{abc}	1.26±0.08^{d}	1.06±0.07^{bcd}	0.71±0.08^{a}	**0.95±0.22**
SE09	1.08±0.05^{ab}	0.75±0.02^{a}	1.09±0.16^{ab}	1.32±0.11^{b}	1.1±0.07^{ab}	0.8±0.07^{a}	**1.02±0.21**
S. eubayanus	0.86±0.27^{b}	0.6±0.14^{a}	0.92±0.25^{b}	1.15±0.25^{c}	0.91±0.24^{b}	0.66±0.11^{a}	
SK01	0.33±0.03^{ab}	0.3±0.01^{a}	0.42±0.02^{bc}	0.52±0.02^{c}	0.33±0.03^{abc}	0.3±0.0^{a}	**0.37±0.08**
SK02	1.02±0.09^{b}	0.52±0.05^{a}	0.78±0.07^{ab}	1±0.15^{b}	0.74±0.1^{ab}	0.42±0.01^{a}	**0.75±0.24**
SK03	0.45±0.07^{bc}	0.3±0.01^{ab}	0.55±0.04^{c}	0.4±0.06^{bc}	0.28±0.01^{ab}	0.24±0.02^{a}	**0.37±0.12**
SK04	0.18±0.01^{a}	0.28±0.01^{bc}	0.36±0.04^{c}	0.41±0.09^{c}	0.2±0.01^{ab}	0.26±0.01^{abc}	0.28±0.09
SK05	0.41±0.18	0.28±0.03	0.29±0.07	0.32±0.02	0.2±0.01	0.28±0.07	0.3±0.09
SK06	0.44±0.01^{abc}	0.38±0.02^{a}	0.58±0.03^{bc}	0.69±0.02^{c}	0.71±0.04^{c}	0.41±0.02^{ab}	**0.54±0.14**
SK07	0.9±0.08^{c}	0.73±0.02^{ab}	0.89±0.16^{bc}	0.81±0.06^{bc}	0.75±0.04^{abc}	0.69±0.02^{a}	**0.79±0.11**
S. kudriavzevii	0.53±0.3^{bcd}	0.4±0.16^{ab}	0.55±0.22^{cd}	0.59±0.24^{d}	0.46±0.25^{abc}	0.37±0.15^{a}	
BMV58	8.21±0.32^{b}	1.61±0.69^{a}	4.64±2.53^{ab}	4.32±0.36^{ab}	4.31±0.35^{ab}	2.44±0.48^{a}	**4.26±2.34**
SU02	3.35±0.19^{c}	1.02±0.36^{a}	0.85±0.17^{a}	2.83±0.33^{bc}	1.7±0.1^{abc}	0.96±0.01^{ab}	**1.78±1.02**
SU03	4.03±0.19^{d}	0.84±0.03^{a}	1.22±0.07^{ab}	2.44±0.22^{cd}	2±0.03^{bcd}	1.35±0.05^{abc}	**1.98±1.09**
SU04	0.08±0.03^{a}	2.06±0.45^{ab}	2.86±0.43^{bc}	3.18±0.11^{bc}	3.3±0.23^{c}	2.48±0.35^{abc}	**2.27±1.16**
SU06	1.59±0.13^{bc}	0.94±0.08^{a}	1.19±0.09^{abc}	1.63±0.16^{c}	1.66±0.04^{c}	1.11±0.06^{ab}	**1.35±0.3**
SU07	3.89±0.08^{d}	0.75±0.02^{a}	1.36±0.06^{ab}	3.46±0.06^{cd}	2.75±0.12^{bcd}	2.55±0.08^{abc}	**2.46±1.13**
S. uvarum	3.52±2.59^{c}	1.21±0.58^{a}	2.02±1.63^{ab}	2.96±0.91^{c}	2.62±1^{c}	1.81±0.74^{b}	

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different ($p< 0.05$) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S6. Succinic acid mean concentration expressed in g per100 g of sugars consumed by the 33 yeast strains in the six nitrogen musts.

	Comp*	Ile	Leu	NH_4	Phe	Val	Yeast**
EC1118	0.19±0.06^{bc}	0.14±0.04^{abc}	0.13±0.02^{ab}	0.17±0.02^{bc}	0.21±0.01^{c}	0.11±0.01^{a}	0.16±0.04
SC01	0.21±0.01^{a}	0.56±0.11^{b}	0.21±0.04^{a}	0.27±0.01^{ab}	0.2±0.02^{a}	0.63±0.04^{b}	0.35±0.19
SC02	0.89±0.1^{ab}	0.74±0.66^{ab}	1.8±0.2^{c}	1.43±0.4^{bc}	1.69±0.21^{bc}	0.43±0.43^{a}	**1.16±0.61**
SC03	0.34±0.02	0.33±0.03	0.35±0.06	0.37±0.03	0.34±0.14	0.24±0.01	0.33±0.07
SC04	0.32±0.05^{bc}	0.2±0.02^{ab}	0.24±0.03^{ab}	0.41±0.01^{c}	0.32±0.05^{bc}	0.15±0.01^{a}	0.27±0.09
SC05	1.52±0.04^{c}	0.63±0.03^{ab}	0.5±0.01^{a}	0.76±0.01^{bc}	1±0.06^{bc}	0.64±0.06^{ab}	**0.84±0.35**
SC06	0.15±0.01^{bc}	0.13±0.01^{ab}	0.14±0.01^{abc}	0.19±0.01^{c}	0.14±0.02^{abc}	0.09±0.01^{a}	0.14±0.03
SC07	0.29±0.05^{ab}	0.14±0.01^{a}	0.36±0.01^{b}	0.36±0.03^{b}	0.3±0.02^{ab}	0.1±0.01^{a}	0.26±0.11
SC08	0.3±0.02^{bc}	0.17±0.01^{a}	0.23±0.02^{abc}	0.33±0.02^{c}	0.19±0.01^{ab}	0.18±0.02^{a}	0.23±0.06
SC09	0.92±0.01^{cd}	0.38±0.01^{ab}	0.54±0.01^{abc}	0.67±0.01^{bcd}	1.08±0.13^{d}	0.31±0.03^{a}	**0.65±0.29**
SC10	1.12±0.19	0.83±0.8	1.28±0.05	1.49±0.4	1.12±0.52	0.18±0.32	**1±0.57**
S. cerevisiae	0.57±0.45^{bc}	0.39±0.36^{ab}	0.52±0.52^{bc}	0.59±0.47^{c}	0.6±0.53^{bc}	0.28±0.24^{a}	
SE01	1.19±0.16^{bc}	0.42±0.02^{a}	1.45±0.12^{c}	1.08±0.21^{bc}	1.06±0.03^{abc}	0.76±0.07^{ab}	**0.99±0.35**
SE02	0.99±0.17^{ab}	0.62±0.1^{a}	0.89±0.07^{ab}	1.21±0.17^{b}	1.07±0.04^{b}	0.67±0.07^{a}	**0.91±0.24**
SE03	0.8±0.07^{bc}	0.47±0.02^{a}	0.73±0.02^{bc}	1.25±0.04^{c}	0.53±0.08^{ab}	0.58±0.04^{ab}	**0.73±0.27**
SE04	0.49±0.21^{ab}	0.48±0.1^{a}	0.77±0.03^{bc}	0.62±0.03^{abc}	1.15±0.03^{c}	0.53±0.07^{ab}	**0.67±0.26**
SE05	0.7±0.05^{bc}	0.59±0.02^{a}	0.62±0.02^{ab}	1.46±0.07^{c}	0.61±0.02^{ab}	0.7±0.03^{bc}	**0.78±0.32**
SE06	0.97±0.1^{b}	0.82±0.02^{ab}	0.84±0.07^{ab}	1.05±0.14^{b}	0.98±0.03^{b}	0.64±0.09^{a}	**0.89±0.16**
SE07	0.46±0.02^{a}	0.53±0.02^{ab}	1±0.08^{c}	1.05±0.06^{c}	0.65±0.13^{bc}	0.53±0.03^{ab}	**0.7±0.25**
SE08	1.07±0.03^{cd}	0.71±0.02^{ab}	0.87±0.1^{abc}	1.26±0.08^{d}	1.06±0.07^{bcd}	0.71±0.08^{a}	**0.95±0.22**
SE09	1.08±0.05^{ab}	0.75±0.02^{a}	1.09±0.16^{ab}	1.32±0.11^{b}	1.1±0.07^{ab}	0.8±0.07^{a}	**1.02±0.21**
S. eubayanus	0.86±0.27^{b}	0.6±0.14^{a}	0.92±0.25^{b}	1.15±0.25^{c}	0.91±0.24^{b}	0.66±0.11^{a}	
SK01	0.33±0.03^{ab}	0.3±0.01^{a}	0.42±0.02^{bc}	0.52±0.02^{c}	0.33±0.03^{abc}	0.3±0.0^{a}	**0.37±0.08**
SK02	1.02±0.09^{b}	0.52±0.05^{a}	0.78±0.07^{ab}	1±0.15^{b}	0.74±0.1^{ab}	0.42±0.01^{a}	**0.75±0.24**
SK03	0.45±0.07^{bc}	0.3±0.01^{ab}	0.55±0.04^{c}	0.4±0.06^{bc}	0.28±0.01^{ab}	0.24±0.02^{a}	**0.37±0.12**
SK04	0.18±0.01^{a}	0.28±0.01^{bc}	0.36±0.04^{c}	0.41±0.09^{c}	0.2±0.01^{ab}	0.26±0.01^{abc}	0.28±0.09
SK05	0.41±0.18	0.28±0.03	0.29±0.07	0.32±0.02	0.2±0.01	0.28±0.07	0.3±0.09
SK06	0.44±0.01^{abc}	0.38±0.02^{a}	0.58±0.03^{bc}	0.69±0.02^{c}	0.71±0.04^{c}	0.41±0.02^{ab}	**0.54±0.14**
SK07	0.9±0.08^{c}	0.73±0.02^{ab}	0.89±0.16^{bc}	0.81±0.06^{bc}	0.75±0.04^{abc}	0.69±0.02^{a}	**0.79±0.11**
S. kudriavzevii	0.53±0.3^{bcd}	0.4±0.16^{ab}	0.55±0.22^{cd}	0.59±0.24^{d}	0.46±0.25^{abc}	0.37±0.15^{a}	
BMV58	8.21±0.32^{b}	1.61±0.69^{a}	4.64±2.53^{ab}	4.32±0.36^{ab}	4.31±0.35^{ab}	2.44±0.48^{a}	**4.26±2.34**
SU02	3.35±0.19^{c}	1.02±0.36^{a}	0.85±0.17^{a}	2.83±0.33^{bc}	1.7±0.1^{abc}	0.96±0.01^{ab}	**1.78±1.02**
SU03	4.03±0.19^{d}	0.84±0.03^{a}	1.22±0.07^{ab}	2.44±0.22^{cd}	2±0.03^{bcd}	1.35±0.05^{abc}	**1.98±1.09**
SU04	0.08±0.03^{a}	2.06±0.45^{ab}	2.86±0.43^{bc}	3.18±0.11^{bc}	3.3±0.23^{c}	2.48±0.35^{abc}	**2.27±1.16**
SU06	1.59±0.13^{bc}	0.94±0.08^{a}	1.19±0.09^{abc}	1.63±0.16^{c}	1.66±0.04^{c}	1.11±0.06^{ab}	**1.35±0.3**
SU07	3.89±0.08^{d}	0.75±0.02^{a}	1.36±0.06^{ab}	3.46±0.06^{cd}	2.75±0.12^{bcd}	2.55±0.08^{abc}	**2.46±1.13**
S. uvarum	3.52±2.59^{c}	1.21±0.58^{a}	2.02±1.63^{ab}	2.96±0.91^{c}	2.62±1^{c}	1.81±0.74^{b}	

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different ($p< 0.05$) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S7. Acetic acid mean concentration expressed in g per 100 g of sugars consumed by the 33 yeast strains in the six nitrogen musts.

	Comp*	Ile	Leu	NH4	Phe	Val	Yeast**
EC1118	0.3±0.01^{b}	0.27±0.03^{b}	0.18±0.02^{a}	0.23±0.02^{ab}	0.22±0.01^{ab}	0.18±0^{a}	0.23±0.05
SC01	0.66±0.01^{c}	0.36±0.01^{ab}	0.62±0.12^{c}	0.41±0.01^{abc}	0.58±0.1^{bc}	0.3±0.01^{a}	**0.49±0.15**
SC02	1.26±0.04	1±0.18	1.17±0.15	1.23±0.1	1.09±0.11	1.34±0.3	**1.18±0.18**
SC03	0.31±0.02^{c}	0.22±0.01^{bc}	0.16±0.01^{a}	0.19±0.01^{ab}	0.26±0.02^{bc}	0.2±0.01^{ab}	0.22±0.05
SC04	0.49±0.01^{c}	0.34±0.01^{abc}	0.28±0.02^{a}	0.42±0.01^{bc}	0.27±0.02^{a}	0.29±0.01^{ab}	**0.35±0.09**
SC05	0.34±0.01^{c}	0.14±0.02^{a}	0.2±0.01^{abc}	0.3±0.01^{bc}	0.2±0^{ab}	0.2±0.01^{ab}	0.23±0.07
SC06	0.58±0.02^{c}	0.34±0.02^{ab}	0.41±0.01^{bc}	0.37±0.01^{abc}	0.3±0.02^{a}	0.33±0.01^{ab}	**0.39±0.09**
SC07	0.47±0.01^{c}	0.39±0.01^{bc}	0.25±0.01^{a}	0.33±0.01^{ab}	0.36±0.01^{abc}	0.37±0.02^{abc}	**0.36±0.07**
SC08	0.37±0.01^{c}	0.28±0.01^{bc}	0.21±0.01^{a}	0.27±0.01^{abc}	0.22±0^{ab}	0.29±0.01^{c}	0.27±0.06
SC09	0.34±0.01^{ab}	0.46±0.02^{b}	0.27±0.02^{a}	0.26±0.01^{a}	0.32±0.01^{ab}	0.45±0.01^{b}	**0.35±0.08**
SC10	0.47±0.05^{bc}	1.08±1.13^{bc}	0.26±0.03^{a}	0.39±0.02^{abc}	0.34±0.04^{ab}	1.74±1.11^{c}	**0.71±0.77**
S. cerevisiae	0.51±0.27^{c}	0.44±0.42^{b}	0.36±0.29^{a}	0.4±0.28^{ab}	0.38±0.25^{ab}	0.52±0.58^{ab}	
SE01	0.45±0.03^{c}	0.39±0.01^{bc}	0.22±0.01^{a}	0.35±0.04^{bc}	0.26±0.01^{ab}	0.24±0^{ab}	**0.32±0.09**
SE02	0.54±0.08^{c}	0.22±0.04^{a}	0.25±0.02^{abc}	0.37±0.02^{bc}	0.22±0.04^{a}	0.24±0.03^{ab}	0.31±0.13
SE03	0.39±0.01^{d}	0.32±0.01^{cd}	0.21±0.01^{ab}	0.23±0^{abc}	0.28±0.02^{bcd}	0.2±0.01^{a}	0.27±0.07
SE04	0.68±0.04^{c}	0.32±0.01^{ab}	0.3±0.02^{a}	0.51±0.09^{bc}	0.3±0.02^{a}	0.34±0.02^{abc}	**0.41±0.15**
SE05	0.46±0.01^{b}	0.33±0.01^{b}	0.25±0^{a}	0.31±0.01^{ab}	0.31±0.01^{ab}	0.23±0.01^{a}	**0.31±0.08**
SE06	0.47±0.15^{c}	0.16±0.03^{bc}	0.09±0.02^{a}	0.15±0.04^{abc}	0.13±0.04^{ab}	0.13±0.03^{ab}	0.19±0.14
SE07	0.53±0.01^{c}	0.39±0^{bc}	0.28±0.01^{ab}	0.39±0.01^{abc}	0.38±0.02^{abc}	0.28±0.01^{a}	**0.37±0.09**
SE08	0.5±0.16	0.16±0.03	0.12±0.06	0.14±0.08	0.14±0.01	0.14±0.03	0.2±0.15
SE09	1.11±0.16^{c}	0.27±0.02^{ab}	0.29±0.12^{ab}	0.6±0.13^{bc}	0.44±0.05^{abc}	0.22±0.05^{a}	**0.49±0.32**
S. eubayanus	0.57±0.22^{c}	0.28±0.09^{b}	0.22±0.08^{a}	0.34±0.16^{b}	0.27±0.1^{ab}	0.22±0.06^{a}	
SK01	0.55±0.02^{b}	0.49±0.02^{ab}	0.52±0.02^{b}	0.45±0.01^{a}	0.5±0.02^{ab}	0.44±0.01^{a}	**0.49±0.04**
SK02	0.12±0.04^{ab}	0.18±0.03^{bc}	0.27±0.02^{c}	0.06±0.02^{a}	0.08±0.02^{a}	0.14±0.02^{abc}	0.14±0.08
SK03	0.39±0.01^{ab}	0.44±0.03^{b}	0.45±0.02^{b}	0.35±0.02^{a}	0.35±0.04^{a}	0.38±0.02^{ab}	**0.39±0.04**
SK04	0.47±0.01^{d}	0.35±0.01^{abc}	0.43±0.02^{cd}	0.29±0.03^{ab}	0.42±0.01^{bcd}	0.3±0.01^{a}	**0.38±0.07**
SK05	0.42±0.02^{c}	0.24±0.01^{ab}	0.2±0.06^{a}	0.32±0.03^{bc}	0.32±0.06^{bc}	0.27±0.04^{ab}	0.3±0.08
SK06	0.41±0.01^{c}	0.2±0.02^{a}	0.37±0.01^{bc}	0.27±0.02^{ab}	0.35±0.03^{bc}	0.22±0^{ab}	**0.3±0.08**
SK07	0.24±0.07	0.21±0.04	0.28±0.02	0.21±0.04	0.14±0.01	0.19±0.02	0.21±0.06
S. kudriavzevii	0.37±0.14	0.3±0.12	0.36±0.11	0.28±0.12	0.31±0.14	0.28±0.1	
BMV58	0.38±0.02^{c}	0.09±0.01^{a}	0.12±0.06^{a}	0.18±0.03^{abc}	0.3±0.04^{bc}	0.18±0.1^{ab}	0.21±0.11
SU02	0.26±0.01^{c}	0.16±0.01^{ab}	0.16±0.02^{ab}	0.18±0.01^{abc}	0.2±0.01^{bc}	0.15±0.01^{a}	0.18±0.04
SU03	0.1±0^{c}	0.07±0.01^{bc}	0.05±0.01^{ab}	0.04±0^{a}	0.08±0.01^{bc}	0.04±0.01^{ab}	**0.06±0.02**
SU04	0.1±0^{a}	0.22±0.03^{abc}	0.24±0.01^{bc}	0.31±0^{c}	0.28±0.03^{c}	0.2±0.01^{ab}	0.2±0.1
SU06	0.39±0.03^{c}	0.25±0.02^{bc}	0.23±0.01^{ab}	0.26±0.01^{bc}	0.23±0.01^{ab}	0.18±0.01^{a}	0.26±0.07
SU07	0.23±0^{c}	0.12±0.01^{ab}	0.11±0^{a}	0.16±0.02^{abc}	0.17±0.02^{bc}	0.11±0.01^{a}	0.15±0.05
S. uvarum	0.23±0.14^{b}	0.15±0.07^{a}	0.15±0.07^{a}	0.18±0.09^{ab}	0.21±0.08^{b}	0.14±0.06^{a}	

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different ($p< 0.05$) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S8. Ethyl hexanoate mean concentration expressed in µg/L produced per 100 g of sugars consumed by the 33 yeast strains in the six nitrogen musts.

	Comp*	Ile	Leu	NH4	Phe	Val	Yeast**
EC1118	238.58±12.86	242.13±26.73	224.34±25.16	225.09±27.1	232.63±19.07	242.7±31.78	234.75±22.03
SC01	221.59±8.23^{a}	268.54±21.87^{b}	218.45±11.52^{a}	229.95±1.16^{ab}	270.35±10.06^{b}	229.84±22.95^{a}	230.55±25.26
SC02	251.88±13.99	224.4±12.22	248.14±16.92	240.53±24.39	221.73±10.01	218.96±9.32	231.88±18.66
SC03	250.32±5.29	266.49±30.5	293.83±48.47	310.68±61.56	382.35±86.96	275.23±21.88	**275.79±61.1**
SC04	239.01±4.29	245.76±25.67	233.68±31.29	233.62±22.6	250±25.81	232.75±24.91	244.6±21.28
SC05	207.09±2.33^{ab}	230.92±4.91^{c}	215.5±7.5^{abc}	203.18±2.86^{a}	226.24±0.6^{bc}	239.61±20.08^{c}	220.4±15.4
SC06	256.91±28.43^{ab}	257.19±2.15^{a}	238.39±7.02^{a}	268.52±6.21^{ab}	292.97±7.4^{b}	268.14±1.46^{ab}	**266.83±20**
SC07	236.4±6.07^{ab}	251.12±3.9^{bc}	214.14±6.81^{a}	248.17±1.85^{bc}	268.32±7.53^{c}	236.88±8.18^{ab}	246.44±17.81
SC08	226.05±22.16	260.48±33.45	234.86±30.5	220.06±18.63	237.97±18.41	249.36±27.22	241.77±25.74
SC09	222.49±2.67	223.06±1.2	233.69±41.58	244.88±49.99	261.17±41	241.49±35.36	224.02±32.15
SC10	231.5±3.24	338.84±60	286.93±45.1	262.66±19.33	265.49±68.77	263.77±11.94	**261.12±49.24**
SE01	220.33±3.39^{ab}	243.02±5.23^{b}	213.03±1.78^{a}	223.54±1.34^{ab}	242.13±1.18^{b}	208.47±0.88^{a}	222.77±13.86
SE02	201.59±9.92	164.46±0.98	193.61±14.34	201.45±17.85	201.37±14.13	193.71±7.65	**193.87±16.9**
SE03	245.65±3.02^{bc}	243.19±5.42^{abc}	233.03±6.81^{ab}	242.59±3.07^{abc}	286.83±2.94^{c}	226.05±7.64^{a}	241.76±20.41
SE04	225.43±4.96^{bcd}	213.52±0.62^{abc}	213.56±3.04^{ab}	262.35±26.12^{d}	247.8±1.16^{cd}	209.59±1.07^{a}	219.6±22.28
SE05	235.53±10.18^{ab}	239.16±2.55^{abc}	254.68±10.78^{bc}	232.91±1.01^{ab}	290.56±4.3^{c}	226.32±8.15^{a}	238.49±22.94
SE06	202.18±13.14^{a}	251.58±17.37^{bc}	217.89±11.53^{abc}	209.18±16.05^{ab}	257.32±19.59^{c}	232.11±27.96^{abc}	224.16±26.26
SE07	236.69±6.37^{bc}	223.96±3.86^{ab}	214.77±4^{a}	238.6±6.07^{bc}	246.41±7.3^{c}	231.68±6.01^{abc}	231.69±11.63
SE08	220.41±16.12	201.61±10.81	227.19±14.25	238.14±16.96	260.3±21.67	216.26±9.88	223.77±22.98
SE09	211.27±5.45^{abc}	181.47±3.79^{a}	194.35±10.95^{ab}	221.24±19.2^{bc}	254.48±6.87^{c}	217.35±7.83^{bc}	**210±25.13**
SK01	241.67±11.97	243.65±12.52	229.37±17.74	236.17±9.39	253.56±5.38	235.7±14.4	238.78±13.1
SK02	289.06±4.01	241.04±12.42	246.55±11.5	271.41±31.21	287.78±17.65	245.24±7.56	**257.1±24.95**
SK03	212.23±1.16^{abc}	220.74±1.42^{cd}	204.63±5.7^{ab}	220.35±4.44^{bcd}	227.52±4.69^{d}	205.73±1.81^{a}	**214.88±9.14**
SK04	239.83±13.54	235.13±14.53	220.39±15.95	238.37±13.41	253.28±6.39	228.86±3.15	235.53±14.55
SK05	221.79±1.9^{ab}	232.93±9.37^{b}	215.69±6.94^{a}	214.18±3.96^{a}	233.95±8.08^{b}	223.53±11.7^{ab}	221.99±10.18
SK06	220.42±6.29^{a}	254.52±17.7^{b}	226.78±6.79^{ab}	216.95±2.92^{a}	245.94±4.63^{b}	224.47±8.31^{ab}	227.98±16.14
SK07	162.72±13.94	194.03±16.91	164.51±7.06	165.63±7.2	185.71±35.58	168.14±9.98	**171.32±19.51**
BMV58	206.35±1.2^{ab}	219.34±1.31^{c}	203.77±1.47^{a}	214.69±0.62^{bc}	222±2.84^{c}	209.38±1.52^{abc}	**212.61±6.98**
SU02	202.17±0.64^{a}	217.88±2.31^{b}	207.1±5.92^{ab}	204.41±2.31^{a}	209.38±0.74^{ab}	217.29±4.61^{b}	**209.08±6.79**
SU03	198.36±0.92^{a}	212.82±0.2^{c}	199.5±1.65^{a}	202.09±3.83^{ab}	208.62±0.34^{bc}	203.53±0.15^{abc}	**203.47±5.43**
SU04	219.36±2.27	228.35±8.78	214.74±4.18	211.49±9.45	219.11±5.46	210.72±7.01	**217.72±8.31**
SU06	217.75±3.79^{a}	240.32±8.93^{ab}	279.41±15.11^{bc}	246.5±4.23^{abc}	240.37±13.38^{ab}	296.06±10.18^{c}	247.6±28.4
SU07	193.81±0.25^{a}	213.17±0.37^{d}	201.07±2.25^{abc}	197.43±0.67^{ab}	209.31±0.35^{cd}	203.69±0.74^{bcd}	**203.23±6.87**

For all the data Kruskal-Wallis test and pair-wise comparison at asignificance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different ($p< 0.05$) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S9. Ethyl octanoate mean concentration expressed in µg/L produced per 100 g of sugars consumed by the 33 yeast strains in the six nitrogen musts.

	Comp*	Ile	Leu	NH4	Phe	Val	Yeast**
EC1118	471.15±24.48	474.71±25.97	450.89±38.42	457.14±44.62	489.32±51.07	496.9±57.69	471.83±39.19
SC01	427.79±7.42^{a}	521.51±20.89^{bc}	440.22±14.26^{ab}	438.5±6.82^{a}	533.58±20.1^{c}	474.68±32.2^{abc}	448.53±45.61
SC02	499.51±14.06	440.24±15.17	488.99±28.04	445.23±70.81	415.16±15.65	402.04±28.94	447.12±46.9
SC03	509.05±28.07	545.43±31.77	517.58±30.47	572.17±30.96	631.82±31.67	531.9±48.93	**549.07±51.4**
SC04	481.27±8.18	477.11±24.38	464.88±48.28	489.8±38.21	504.69±39.49	474.63±43.58	485.05±33.14
SC05	417.8±2^{ab}	458.5±5.55^{c}	430.58±1.01^{abc}	412.77±1.81^{a}	454.41±0.94^{c}	463.7±35.32^{bc}	**434.17±24.1**
SC06	484.24±29.92	481.92±5.6	480.71±26.04	491.64±12.8	523.05±8.11	485.1±3.81	489.2±21.12
SC07	500.23±10.47^{ab}	505.37±4.67^{abc}	444.63±14.94^{a}	526.8±7.39^{bc}	557.39±13.27^{c}	485.29±12.73^{ab}	502.8±37
SC08	481.48±40.12	524.14±36.22	473.05±48.77	458.24±24.98	475.67±16.53	516.95±33.06	484.47±38.29
SC09	483.23±8.51	490.88±3.64	506.58±81.41	537.27±81.41	558.97±85.41	525.52±63.76	**491.2±60.36**
SC10	472.36±5.78	642.22±72.87	554.23±29.03	523.74±25.86	509±98.78	558.56±21.54	**541.7±70.43**
SE01	457.83±7.55^{ab}	502.02±10.63^{b}	441.76±3.58^{a}	465.53±4.02^{ab}	501.9±4.19^{b}	431.34±2.01^{a}	463.21±28.45
SE02	398.79±19.71	335.39±1.58	386.09±20.75	411.95±40.12	398.12±29.25	390.34±17.71	**387.42±32.43**
SE03	488.23±7.7^{abc}	489.32±4.65^{abc}	466.88±5.57^{ab}	498.37±7.37^{bc}	572.53±5.08^{c}	455.22±4.51^{a}	489.25±38.99
SE04	471.72±11.79^{abc}	441.59±2.05^{ab}	442.78±7.63^{a}	592.79±96.08^{c}	519.93±6.14^{bc}	437.43±2.63^{a}	457.71±66.77
SE05	469.03±9.04^{a}	486.21±5.97^{ab}	509.33±7.58^{b}	485.75±4.51^{ab}	580.53±4.27^{b}	465.22±14.38^{a}	487.91±40.75
SE06	407.34±20.83^{a}	525.31±37.76^{b}	445.91±23.23^{ab}	434.05±31.97^{a}	523.99±43.76^{b}	476.78±58.21^{ab}	462.79±55.78
SE07	500.3±10.01^{bcd}	459.7±7.37^{ab}	449.38±11.25^{a}	514.81±17.46^{d}	508.59±15.33^{cd}	466.13±23.24^{abc}	490.17±29.2
SE08	444.75±27.66	416.2±21.7	465.26±27.58	485.46±43.54	517.61±41.34	442.42±21.56	457.83±42.99
SE09	431.17±9.24^{abc}	376.58±7.59^{a}	395.22±22.54^{ab}	459.91±40.4^{bc}	509.65±16.09^{c}	442.88±17.37^{bc}	**426.58±48.19**
SK01	468.76±7.46	477.41±6.79	459.69±25.33	463.75±7.76	508.89±0.17	463.19±19.94	471.03±20.93
SK02	589.64±4.8	529.29±15.66	506.81±25.59	569.45±64.91	581.93±38.25	525.97±33.34	**540.27±44.02**
SK03	426.78±4.15^{ab}	459.23±1.58^{cd}	414.79±4.05^{a}	442.63±13.24^{bcd}	465.12±7.59^{d}	428.42±2.67^{abc}	**431.97±19.41**
SK04	444.41±6.46^{ab}	458.4±4.38^{bc}	430.45±2.52^{a}	457.02±11.78^{bc}	496.38±2.28^{c}	449.04±12.97^{ab}	451.22±21.93
SK05	429.26±5.86^{ab}	448.69±2.53^{bc}	420.43±3.38^{a}	422.28±3.74^{a}	483.8±3.57^{c}	439.81±8.01^{abc}	**434.72±22.62**
SK06	416.65±2.5^{a}	493.28±14.14^{c}	450.15±2.51^{abc}	429.72±7.65^{ab}	485.69±4.5^{c}	461.39±2.36^{bc}	456.24±29.02
SK07	337.95±28.62	420.87±38.79	338.39±16.63	372.98±18.61	426.02±102.58	349.22±24.86	**361.92±55.54**
BMV58	430.55±3.86^{ab}	454.62±2.03^{cd}	418.17±1.61^{a}	445.61±2.1^{bcd}	457.82±5.22^{d}	435.27±4.21^{abc}	**441.64±14.54**
SU02	439.53±12.45	456.97±0.44	442.85±14.79	432.28±10.48	433.77±3.05	463±10.81	**441.57±14.53**
SU03	411.67±0.83^{a}	439.79±0.36^{c}	410.3±3.54^{a}	415.6±5.21^{ab}	430.72±0.09^{bc}	420.06±0.22^{abc}	**419.93±11.16**
SU04	461.18±9.6	483.68±22.26	458.41±1.76	444.21±23.95	457.13±14.25	451.52±30.3	459±20.64
SU06	447.13±4.84^{a}	478.69±3.68^{ab}	573.47±14.45^{bc}	508.81±8.5^{abc}	496.81±27.26^{ab}	608.47±8.19^{c}	**507.83±58.07**
SU07	400.98±0.67^{a}	440.07±0.6^{d}	413.03±1.14^{abc}	408.16±1.08^{ab}	433.4±0.47^{cd}	421.64±1.22^{bcd}	**417.27±14.17**

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different (p< 0.05) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S10. Ethyl decanoate mean concentration expressed in µg/L produced per 100 g sugar consumed by the 33 yeast strains in the six nitrogen musts.

	Comp*	Ile	Leu	NH4	Phe	Val	Yeast**
EC1118	838.67±116.39	759.4±53.42	796.19±94.42	882.22±154.61	1003.17±152.76	1007.33±131.24	894.78±142.15
SC01	490.5±9.93^{a}	1057.77±105.11^{c}	564.82±36.74^{abc}	513.33±13.2^{ab}	774.48±34.91^{bc}	1012.54±166.63^{c}	**665.7±247.96**
SC02	825.28±58.83^{c}	525±17.69^{ab}	656.41±55.53^{bc}	737.27±184.98^{bc}	512.22±35.48^{ab}	496.39±47.52^{a}	**565.96±147.14**
SC03	1061.37±82.36	1146.32±105.94	1009.69±228.01	1423.69±119.39	1191.41±19.15	969.45±259	**1151.36±204.21**
SC04	912.84±36.19^{ab}	771.05±104.18^{a}	870.97±60.18^{a}	1195.21±182.04^{b}	841.44±37.17^{a}	962.09±112.74^{ab}	884.87±162.78
SC05	458.75±2.44^{ab}	504.25±3.01^{bc}	496.27±32.05^{abc}	452.67±3.75^{a}	574.96±12.64^{c}	515.87±66.78^{bc}	**484.43±49.04**
SC06	927.87±71.25	713.44±3.74	934.4±210.7	1026.01±43.15	911.53±43.22	830.68±31.9	891.21±128.25
SC07	879±63.17^{abc}	663.29±5.91^{ab}	691.56±78.43^{ab}	936.96±15.71^{bc}	1014.26±47.5^{c}	656.35±21.82^{a}	799.34±151.74
SC08	943.87±220.84	994.2±154.23	871.43±58.8	952.09±162.13	746.93±67.89	1100.48±235.54	893.83±177.7
SC09	879.72±22.01^{ab}	835.29±8.87^{a}	1171.91±342.72^{bc}	1278±313.34^{c}	1311.02±387.83^{c}	1158.23±305.49^{bc}	1030.23±299.94
SC10	800.36±9.04	901.17±102.01	1028.57±96.3	965.43±88.96	810.68±166.5	932.82±57.32	916.32±117.82
SE01	977±102.01^{bc}	644.48±20.76^{a}	690.56±113.75^{ab}	1038.07±104.84^{c}	746.52±93^{abc}	669.2±60.54^{ab}	719.28±175.9
SE02	566.48±26.85^{c}	401.14±2.62^{a}	532.79±57.82^{bc}	578.64±63.55^{c}	454.57±36.47^{ab}	508.66±27.04^{abc}	**510.23±72.85**
SE03	670.38±8.37^{ab}	579.77±25.05^{a}	649.83±72.39^{ab}	730.05±25.92^{b}	706.52±49.41^{b}	657.22±53.6^{ab}	**675.17±61.58**
SE04	1065.55±150.07^{bc}	556.29±28.04^{a}	731.45±115.34^{ab}	2179.55±131.85^{c}	785.41±30.93^{ab}	914.53±23.91^{bc}	869.27±555.08
SE05	680.89±26.93^{ab}	585.43±54.2^{a}	688.56±52.04^{abc}	823.96±13.49^{c}	743.67±38.49^{bc}	664.94±38.03^{ab}	**696.43±82.34**
SE06	669.51±111.52	670.72±70.68	612.13±30.68	633.37±30.76	692.18±96.28	716.24±93.41	**638.62±75.25**
SE07	1096.56±78.4^{bc}	557.74±18.68^{a}	761.49±76.27^{ab}	1260.23±112.83^{c}	744.04±6.48^{ab}	804.06±208.84^{abc}	754±258.58
SE08	707.73±54.37	576.08±32.32	627.37±41.65	699.98±50.95	609.44±73.36	609.54±41.24	**636.38±65.82**
SE09	723.47±116.06^{bc}	478.63±14.42^{a}	535.04±34.74^{ab}	722.01±93.07^{c}	643.79±55.43^{bc}	648.53±53.36^{bc}	**621.37±110.49**
SK01	1161.33±293.19	898.86±77.94	998.05±230.97	1032.38±27.72	838.7±53.68	1003.27±239.51	977.91±187.95
SK02	1207.94±177.12^{abc}	1207.41±94.49^{bc}	858.35±80.01^{a}	1285.2±254.7^{bc}	970.91±137.44^{ab}	1620.16±306.05^{c}	**1130.04±297.67**
SK03	657.03±52.92^{ab}	1043.76±75.36^{b}	519.53±10.6^{a}	526.83±57.72^{a}	600.36±34.63^{ab}	1085.75±41.58^{b}	**626.53±245.72**
SK04	898.82±72.65	791.65±237.02	718.79±166.8	893.87±214.45	695.48±71.4	745.16±14.55	752.44±152.84
SK05	588.83±51.65	546.41±27.22	571.32±49.86	518.45±20.48	790.67±116.49	656.03±139.72	**560.47±115.33**
SK06	560.08±21.49^{a}	1020.66±68.69^{bc}	909.42±132.69^{bc}	698.87±60.85^{ab}	685.24±43.4^{ab}	1067.49±34.6^{c}	773.43±201.26
SK07	1127.44±143.61	1312.67±169.13	904.43±176.64	1760.23±203.74	1197.24±215.8	1099.78±187.09	**1194.53±313.83**
BMV58	858.35±73.1^{b}	874.93±77.58^{b}	569.21±57.09^{a}	866.71±111.36^{b}	645.7±41.99^{ab}	913.38±215.09^{b}	780.95±164.09
SU02	1066.61±142.19^{c}	769.93±253.25^{ab}	910.57±95.79^{abc}	811.67±43.41^{abc}	628.76±89.79^{a}	1068.13±150.78^{bc}	865.18±203.69
SU03	778.53±49.88^{b}	585.6±26.19^{ab}	577.65±24.23^{a}	748.91±54.72^{b}	559.46±28.18^{a}	585.74±5.34^{ab}	**588.5±96.14**
SU04	742.87±43.12^{ab}	928.39±151.51^{b}	893.27±145.7^{b}	735.42±2.65^{ab}	599.93±21.86^{a}	871.49±148.73^{b}	756.53±147.56
SU06	783.08±54.51	855.98±265.03	1028.38±171.17	877.92±46.27	752.41±126.13	1234.98±378.37	870.62±244.75
SU07	559.68±14.14	662.1±67.81	553.26±30.33	607.27±37.28	564.99±35.77	581.99±69.5	**575.29±55.17**

For all the data Kruskal-Wallis test and pair-wise comparison at a significance level of 95% was applied. *mean ± SD (n=3) superscript letters in the same row indicate significant differences respectively. **Yeast effect: Mean ± SD (n=18), data in bold are significantly different (p< 0.05) to Lalvin EC1118® control yeast by Kruskal-Wallis test.

Table S11. Higher alcohols and 2-phenylethyl acetate mean concentration produced by the 33 yeast strains in the six nitrogen musts.

Isobutanol(mg/L)

	Comp	Ile	Leu	NH4	Phe	Val
EC1118	7.17±0.03^{a}	29.92±1.83^{bc}	9.87±2.03^{ab}	11.11±0.01^{ab}	22.83±0.84^{bc}	273.22±0.7^{c}
SC01	5.46±0.32^{a}	39.81±2.55^{bc}	10.26±2.06^{ab}	10.17±0.81^{ab}	12.76±0.59^{bc}	276.1±22.44^{c}
SC02	8.64±1.33^{a}	25.31±1.13^{cd}	15.89±0.26^{abc}	12.58±2.09^{ab}	20.52±0.08^{bcd}	226.81±2.44^{d}
SC03	7.04±0.45^{a}	37.07±2.01^{b}	18.41±4.7^{ab}	10.72±1.11^{a}	18.11±5^{ab}	247.24±65.27^{b}
SC04	3.51±0.14^{a}	14.18±1.54^{bc}	7.52±0.07^{abc}	5.33±0.23^{ab}	7.47±2.21^{abc}	204.29±4.09^{c}
SC05	13.9±1.16^{ab}	46.76±1.2^{bc}	16.97±2.44^{ab}	10.19±0.82^{a}	28.65±0.37^{bc}	335.09±8.58^{c}
SC06	5.15±0.92^{a}	20.82±0.66^{b}	12.47±1.34^{ab}	5.85±0.08^{a}	10.93±0.92^{ab}	260.62±14.06^{b}
SC07	9.87±0.63^{a}	22.15±0.98^{abc}	23.74±2.24^{bc}	19.75±1.22^{ab}	24.59±1.66^{bc}	237.24±8.85^{c}
SC08	5.13±0.11^{a}	20.48±1.05^{b}	11.36±0.86^{ab}	6.01±0.12^{a}	12.26±2.92^{ab}	212.84±6.63^{b}
SC09	10.18±0.23^{a}	41.14±0.15^{cd}	25.79±2.65^{bcd}	12.77±1.25^{ab}	20.63±1.56^{abc}	279.94±26.96
SC10	9.74±0.4^{ab}	39.12±9.43^{cd}	19.53±1.18^{bcd}	7.96±0.61^{a}	16.54±2.02^{abc}	259.91±12.17^{d}
SE01	4.72±0.14^{a}	10.18±0.54^{bc}	6.75±1.77^{abc}	6.19±0.21^{ab}	6.5±0.19^{abc}	129.22±3.32^{c}
SE02	8.09±0.64^{a}	16.46±1.04^{bc}	9.29±0.66^{ab}	9.84±1.7^{abc}	9.46±0.72^{ab}	197.92±7.13^{c}
SE03	5.46±0.28^{a}	11.85±3.22^{bc}	7.67±2.11^{ab}	9.69±0.33^{abc}	10.8±0.49^{bc}	176.07±40.92^{c}
SE04	6.79±0.53^{ab}	15.09±0.53^{cd}	6.61±0.19^{a}	9.78±0.42^{abc}	14.46±1.23^{bcd}	220.52±4.14^{d}
SE05	8±0.46^{a}	13.29±2.35^{abc}	7.15±1.03^{a}	10.09±0.58^{ab}	19.23±1.95^{bc}	157.04±49.19^{c}
SE06	5.33±0.94^{a}	26.89±1.88^{cd}	8.73±0.47^{abc}	7.64±0.8^{ab}	13.61±2.35^{bcd}	233.54±30.34^{d}
SE07	9.89±0.66^{ab}	17.52±0.92^{bc}	8.19±0.35^{a}	10.2±0.63^{ab}	18.33±7.89^{bc}	289.31±21.5^{c}
SE08	8.45±0.94^{a}	19.8±2.66^{bc}	10.44±1.44^{ab}	10.98±0.89^{ab}	13.85±2.84^{abc}	247.73±24.21^{c}
SE09	4.94±0.74^{a}	14.92±0.83^{bc}	6.48±0.93^{ab}	6.7±0.81^{ab}	11.59±0.9^{bc}	193.53±9.59^{c}
SK01	12.66±0.73^{a}	38.41±1.83^{cd}	26.21±2.22^{abc}	14.52±1.65^{ab}	32.75±0.52^{bcd}	241.77±41.96^{d}
SK02	7.89±1.55^{a}	28.89±1.39^{cd}	15.95±0.49^{bcd}	10.32±1.45^{ab}	12.99±0.26^{abc}	260.16±13.36^{d}
SK03	7.72±1.23^{a}	17.92±1.55^{abc}	17.45±4.07^{bc}	9.31±0.72^{ab}	18.09±4.2^{bc}	203.04±9.69^{c}
SK04	7.73±0.13^{ab}	27.75±1.51^{cd}	13.14±0.53^{abc}	5.85±0.23^{a}	22.23±0.88^{bcd}	234.3±5.13^{d}
SK05	35.12±1.97^{a}	51.59±14.2^{ab}	59.98±6.16^{bc}	45.06±5.76^{ab}	61.68±21.41^{abc}	234.95±70.53^{c}
SK06	9.33±0.7^{a}	34.56±2.54^{cd}	19.64±2.68^{abc}	10.44±0.4^{ab}	28.44±0.62^{bcd}	188.87±11.54^{d}
SK07	2.54±0.33^{a}	20.89±3.17^{b}	13.35±1.58^{ab}	3.7±0.04^{a}	11.74±2.78^{ab}	113.43±9.47^{b}
BMV58	15.34±1.61^{a}	44.97±6.32^{bc}	19.37±3.88^{ab}	21.42±0.93^{abc}	21.16±1.14^{ab}	219.59±9.63^{c}
SU02	7.84±1.05^{a}	28.45±0.76^{bc}	7.09±1.51^{a}	11.93±0.89^{ab}	20.27±0.15^{abc}	220.92±17.94^{c}
SU03	14.39±1.15^{abc}	35.77±1.62^{cd}	12.94±0.32^{ab}	18.58±1.05^{bcd}	9.2±0.2^{a}	217.31±2.02^{d}
SU04	3.46±0.21^{a}	27.2±6.74^{cd}	13.19±1.94^{bcd}	4.49±0.74^{ab}	7.03±0.61^{abc}	235.06±5.03^{d}
SU06	4.65±0.66^{a}	25.74±5.15^{b}	10.13±1.11^{ab}	8.85±0.53^{a}	10.95±1.23^{ab}	295.76±22.46^{b}
SU07	6.94±0.17^{a}	30.46±1.18^{bc}	6.73±1.58^{a}	8.81±0.19^{ab}	10.46±0.34^{abc}	169.99±9.62^{c}

Table S11. Continued.

	Amyl/Isoamyl Alcohol (mg/L)					
	Comp	**Ile**	**Leu**	**NH4**	**Phe**	**Val**
EC1118	73.31±10.72^{a}	622.64±30.38^{c}	394.01±68.27^{bc}	84.72±10.4^{ab}	80±17.04^{ab}	98.01±20.17^{abc}
SC01	31.63±0.87^{a}	627.11±50.9^{d}	389.45±66.55^{cd}	57.41±5.87^{abc}	47.34±1.28^{ab}	74.07±5.87^{bcd}
SC02	72.94±17^{a}	373.87±19.86^{bc}	379.01±9.86^{c}	108.81±8.74^{abc}	96.47±6.21^{ab}	99.22±9.96^{ab}
SC03	45.45±4.41^{a}	680.05±63.03^{c}	445.31±123.98^{bc}	76.2±10.29^{abc}	63.49±14.3^{ab}	80.17±20.44^{abc}
SC04	39.52±0.96^{a}	404.39±25.87^{c}	351.8±0.93^{bc}	45.26±6.57^{a}	48.01±9.07^{ab}	73.53±3.71^{abc}
SC05	103.92±10.16^{abc}	749.53±23.52^{c}	569.94±78.55^{bc}	68.08±5.01^{a}	103.07±1.4^{abc}	96.18±3.17^{ab}
SC06	56.19±10.07^{a}	593.95±11.58^{b}	518.26±38.71^{b}	69.62±0.44^{a}	81.99±7.08^{ab}	90.01±6.26^{ab}
SC07	87.49±4.93^{a}	656.91±33.26^{c}	536.41±45.69^{bc}	148.3±5.54^{bc}	137±7.95^{ab}	137.11±3.72^{ab}
SC08	56.46±2.83^{a}	474.1±15.62^{c}	382.2±11.94^{bc}	60.56±1.32^{ab}	60.55±5.4^{ab}	71.64±1.95^{abc}
SC09	130.47±5.72^{ab}	596.48±2.88^{c}	563.5±61.12^{bc}	137.62±14.89^{abc}	141.39±17.93^{abc}	111.8±10.77^{a}
SC10	53.66±3.4^{abc}	655.18±108.33^{d}	551.16±75.2^{cd}	42.97±1.84^{a}	48.82±7.84^{ab}	67.08±1.9^{bcd}
SE01	54.45±4.65^{a}	296.08±23.37^{b}	362.41±51.6^{b}	59.77±7.36^{ab}	60.04±4.01^{ab}	45.66±7.01^{a}
SE02	60.44±1.77^{a}	390.86±16.72^{bc}	492.1±71.51^{c}	88.08±5.56^{abc}	62.13±4.15^{a}	72.07±3.18^{ab}
SE03	40.45±2.53^{a}	325.43±53.75^{b}	456.75±35.57^{b}	72.33±3.7^{ab}	71.87±16.36^{ab}	52.86±3.62^{a}
SE04	42.83±4.16^{a}	382.95±5.15^{b}	368.19±29.25^{b}	82.96±9.59^{ab}	75.96±6.1^{ab}	49.63±1.1^{a}
SE05	52.09±2.91^{a}	336.77±47.99^{bc}	387.55±10.6^{c}	71.08±4.51^{ab}	116.86±8.69^{abc}	58.5±9.19^{a}
SE06	74.22±10.88^{a}	609.47±40.63^{c}	548.29±37.58^{bc}	97.62±13.88^{ab}	114.41±19.93^{abc}	81.37±12.78^{a}
SE07	65.58±2.7^{a}	435.73±5.98^{b}	465.15±50.52^{b}	81.08±7.6^{ab}	83.43±9.14^{ab}	80.91±7.7^{ab}
SE08	102.4±8.02^{a}	486.79±64.92^{b}	579.13±72.31^{b}	136.31±6.79^{ab}	108.96±19.83^{a}	96.35±6.91^{a}
SE09	56.07±6.42^{a}	324.99±12.09^{b}	356.28±40.11^{b}	89.57±12.24^{ab}	84.72±8.19^{ab}	75.11±5.62^{a}
SK01	91.54±5.39^{a}	683.18±35.27^{b}	577.36±30.83^{b}	109.19±10.02^{ab}	109.49±2.38^{ab}	93.69±17.77^{a}
SK02	66.08±14.68^{abc}	549.13±30.66^{c}	497.28±40.45^{bc}	64.41±4.11^{abc}	40.24±1.82^{a}	57.33±1.83^{ab}
SK03	59.23±8.07^{a}	422.81±33.52^{b}	541.33±120.4^{b}	65.36±5.56^{ab}	57.62±17.08^{a}	61.58±5.37^{a}
SK04	65.05±2.75^{a}	582.96±41.22^{b}	495.34±2.14^{b}	65.66±3^{a}	82.48±4.58^{ab}	85.05±3.09^{ab}
SK05	81.15±2.86^{a}	539.64±68.23^{b}	514.56±133.33^{b}	88.95±7.7^{ab}	67.81±13.11^{a}	92.58±16.33^{ab}
SK06	105.44±9.28^{ab}	671.39±58.62^{c}	612.74±60.41^{bc}	104.97±2.07^{a}	110.94±0.87^{abc}	105.45±6.88^{a}
SK07	31.07±3.43^{a}	382.25±67.64^{c}	317.38±30.5^{bc}	36.68±2.87^{a}	49.94±10.7^{ab}	59.42±4.68^{abc}
BMV58	76.03±4.76^{a}	599.73±85.06^{c}	500.92±99.04^{bc}	123.06±8.4^{abc}	80.82±4.42^{ab}	78.19±6.45^{a}
SU02	55.15±4.19^{a}	447.92±26.67^{c}	380.72±18.24^{bc}	74.39±6.75^{abc}	58.86±0.46^{a}	64±8.41^{ab}
SU03	85.39±6.59^{ab}	517.01±20.47^{c}	463.63±12.06^{bc}	139.58±5.72^{bc}	62.25±2.53^{a}	80.11±4.3^{ab}
SU04	33.19±3.08^{a}	469.05±46.64^{bc}	580.99±145.64^{c}	42.22±1.73^{ab}	41.68±4.82^{ab}	62.51±18.02^{bc}
SU06	32.34±3.7^{a}	509.61±83.32^{cd}	542.58±59^{d}	47.39±3.09^{ab}	49.51±6.47^{abc}	82.73±2.36^{bcd}
SU07	54.14±1.54^{a}	448.67±17.36^{c}	336.85±23.47^{bc}	65.36±0.94^{abc}	57.58±1.77^{ab}	57.76±3.4^{ab}

Table S11. Continued.

	2-Phenylethanol (mg/L)					
	Comp	**Ile**	**Leu**	**NH4**	**Phe**	**Val**
EC1118	40.31±9.61^{bc}	21.4±3.53^{ab}	20.5±2.41^{ab}	17.63±3.58^{a}	718.87±161.18^{c}	20.2±2.96^{ab}
SC01	6.68±0.7^{ab}	30.34±2.1^{cd}	5.63±1.42^{a}	13.58±1.13^{abc}	515.45±24.26^{d}	29.59±3.99^{bcd}
SC02	45.58±5.81^{abc}	19.25±1.51^{a}	18.82±0.73^{a}	37.73±6.91^{ab}	672.06±19.72^{c}	52.56±4.42^{bc}
SC03	15.26±1^{a}	24.45±3.57^{bc}	16.35±2.31^{ab}	18.07±2.25^{abc}	737.18±190.09^{c}	13.25±3.2^{a}
SC04	30.08±1.78^{cd}	9.94±0.68^{a}	13.16±0.52^{ab}	15.34±0.89^{abc}	622.86±58.28^{d}	18.6±0.78^{bcd}
SC05	50.61±3.58^{bc}	28.04±2.98^{abc}	25.08±6.37^{ab}	18.73±2.21^{a}	969.17±20.78^{c}	21.97±3.56^{ab}
SC06	30.14±4.6^{bc}	14.15±0.3^{ab}	20.83±6.02^{abc}	14.24±0.65^{ab}	643.43±56.89^{c}	13.56±0.93^{a}
SC07	14.54±1.3^{ab}	10.53±0.14^{a}	14.57±1.05^{ab}	17.78±0.09^{b}	899.84±66.7^{b}	11.42±0.58^{a}
SC08	28.72±3.25^{bc}	15.64±0.8^{ab}	14.93±1.46^{a}	17.6±0.19^{abc}	557.65±20.38^{c}	14.62±0.52^{a}
SC09	62.49±5.05^{bc}	19.41±0.67^{a}	25.64±2.55^{ab}	31.53±5.98^{abc}	794±160.01^{c}	19.5±3.67^{a}
SC10	22.02±1.02^{cd}	17±1.49^{abc}	11.62±0.7^{ab}	9.32±0.69^{a}	640.66±127.58^{d}	19.72±1.55^{bcd}
SE01	49.36±4.9^{b}	21.4±0.69^{a}	37.88±2.44^{ab}	38.83±2.96^{ab}	627.51±46.33^{b}	30.98±4.41^{a}
SE02	54.2±2.29^{abc}	32.85±2.66^{a}	59.66±6.27^{bc}	54.01±5.16^{abc}	563.9±62.72^{c}	49.19±1.96^{ab}
SE03	29.68±5.87^{ab}	16.36±0.54^{a}	31.57±2.8^{abc}	37.88±2.69^{bc}	619.58±9.18^{c}	28.1±0.95^{ab}
SE04	33.58±6.27^{a}	19.67±1.59^{a}	40.13±0.89^{ab}	58.5±10.1^{b}	810.25±26.85^{b}	43.73±5.8^{ab}
SE05	30.53±5.83^{ab}	16.4±2.76^{a}	36.58±2.4^{bc}	35.87±4.77^{abc}	1101.17±73.85^{c}	35.95±1.65^{abc}
SE06	59.47±7.79^{ab}	59.18±6.81^{ab}	58.51±8.34^{ab}	73.41±8.89^{bc}	863.64±160.56^{c}	47.7±12.45^{a}
SE07	32.11±0.78^{a}	17.56±0.11^{a}	39.26±5.72^{ab}	43.34±4.69^{ab}	871.53±13.14^{b}	52.75±6.97^{b}
SE08	62.16±3.54^{bc}	24.36±1.45^{a}	43.03±1.94^{ab}	83.75±3.66^{bc}	856.9±199.42^{c}	39.25±3.53^{ab}
SE09	47.08±5.09^{ab}	30.89±0.9^{a}	56.76±5.41^{bc}	62.43±9.92^{bc}	821.89±43.18^{c}	57.16±5.45^{abc}
SK01	40.76±4.74^{bc}	19.02±1.81^{a}	27.85±1.67^{ab}	29.38±2.44^{abc}	961.71±50.44^{c}	23.52±6.13^{ab}
SK02	33.61±6.55^{bc}	12.16±0.5^{a}	12.08±1.21^{a}	14.93±1.07^{abc}	795.37±45.47^{c}	13.3±1.48^{ab}
SK03	28.68±6.9^{b}	9.34±0.61^{a}	19.05±4.27^{ab}	19.18±1.2^{ab}	775.18±176^{b}	10.12±0.95^{a}
SK04	21.42±0.81^{a}	22.14±2.44^{ab}	25.61±5.58^{abc}	24.22±2.12^{abc}	895.26±133.03^{c}	26.42±1.53^{bc}
SK05	39.38±12^{bc}	25.83±1.92^{ab}	39.93±4.28^{bc}	21.54±1.29^{a}	758.56±115.7^{c}	22.87±2.03^{ab}
SK06	70.39±6.63^{b}	26.28±2.77^{a}	48.47±9.21^{ab}	43.71±2.12^{ab}	953.92±19.52^{b}	35.83±2.98^{a}
SK07	21.69±1.16^{bc}	13±1.34^{ab}	13.29±0.87^{ab}	11.25±0.94^{a}	408.23±51.98^{c}	17.5±1.69^{bc}
BMV58	90.11±9.55^{abc}	55.36±7.18^{a}	73.75±13.26^{ab}	128.02±6.35^{bc}	734.12±91.85^{c}	57.37±3.69^{a}
SU02	89.33±2.21^{cd}	54.77±10.52^{ab}	28.44±4.25^{a}	84.47±2.52^{bcd}	621.43±4.59^{d}	42.43±7.73^{ab}
SU03	98.67±7.04^{bcd}	60.49±1.77^{ab}	74.46±8.56^{abc}	139.23±10.47^{cd}	609.56±7.36^{d}	59.15±0.13^{a}
SU04	32.69±7.19^{a}	77.47±19.36^{bc}	77.82±6.76^{bc}	46.67±0.02^{ab}	568.78±49.97^{c}	66.45±11.38^{abc}
SU06	33.61±6.93^{a}	55.33±9.12^{bc}	51.8±1.76^{abc}	41.3±1.86^{ab}	710.98±146.73^{c}	52.21±0.47^{bc}
SU07	65.26±1^{abc}	41.47±5.92^{a}	34.84±4.44^{a}	80.9±19.41^{bc}	618.75±16.71^{c}	49.38±2.35^{ab}

Table S11. Continued.

2-phenylethyl acetate (µg/L)

	Comp	Ile	Leu	NH4	Phe	Val
EC1118	83.21±2.01^{bc}	57.12±12.34^{abc}	46.9±6.82^{ab}	41.57±5.64^{a}	2921.13±612.6^{c}	44.54±6.98^{ab}
SC01	19.72±1.41^{a}	122.73±14.51^{bc}	18.92±2.46^{a}	26±1.29^{ab}	1825.5±94.24^{c}	90.2±14.4^{abc}
SC02	56.1±15.54^{ab}	36.46±1.19^{a}	35.05±5.13^{a}	64.92±21.02^{ab}	1910.54±238.62^{b}	86.44±18.21^{b}
SC03	26.65±1.1	36.44±11.4	28.9±6.44	27.75±4.09	2169.36±633.17	23.71±3.65
SC04	71.08±0.95^{bc}	31.84±2.03^{ab}	27.32±1.78^{a}	32.4±1.07^{ab}	2219.44±296.07^{c}	41.48±2.17^{bc}
SC05	100.93±15.33^{b}	36.33±2.88^{ab}	36.92±3.33^{ab}	26.26±2.94^{a}	1914.33±79.81^{b}	29.5±1.58^{a}
SC06	102.02±14.51^{bc}	32.38±1.08^{a}	49.37±9.98^{abc}	42.32±1.12^{ab}	3426.48±317.85^{c}	42.43±2.09^{ab}
SC07	39.15±4.03^{abc}	37.03±1.88^{ab}	32.38±2.98^{a}	40.84±3.07^{bc}	2644.4±171.33^{c}	37.4±2.31^{ab}
SC08	47.39±6.13^{bc}	28.06±2.25^{ab}	24.55±0.65^{a}	27.1±0.61^{ab}	1642.58±116.37^{c}	29.86±1.17^{abc}
SC09	88.83±13.49^{bc}	30.22±1.29^{a}	36.42±9.05^{ab}	41.06±11.05^{abc}	2196.88±591.82^{c}	31.94±4.8^{a}
SC10	36.6±0.22^{ab}	77.46±8.2^{bc}	36.68±3.07^{ab}	26.8±1.44^{a}	3409.39±1150.11^{c}	54.1±3.74^{bc}
SE01	188.01±15.86^{bc}	177.01±19.06^{abc}	116.33±22.95^{a}	138.85±17.46^{ab}	3853.69±980.43^{c}	129.06±44.28^{ab}
SE02	163.18±44.78^{abc}	123±25.4^{a}	157.95±3.01^{ab}	178.35±20.27^{bc}	4446.12±668.72^{c}	145.21±20.9^{ab}
SE03	146.22±17.56^{cd}	105.65±6.14^{ab}	99.99±7.21^{a}	112.78±1.33^{abc}	4727.66±57.44^{d}	135.49±17.38^{bcd}
SE04	138.12±19.36^{ab}	102.59±18.72^{a}	124.19±18.77^{ab}	284.56±67.9^{bc}	8127.19±608.81^{c}	185.78±13.68^{bc}
SE05	180.63±20.63^{cd}	99.56±20.27^{a}	126.63±7.85^{ab}	135.5±9.82^{abc}	8841.32±844.3^{d}	157.43±6.48^{bcd}
SE06	199.23±67.23	205.77±5.04	173.87±42.49	220.21±37.6	5175.53±1535.69	140.66±19.56
SE07	173.71±4.46^{ab}	93.17±5.61^{a}	118.34±14.31^{a}	178.41±17.38^{ab}	8084.19±287.07^{b}	267.1±45.84^{b}
SE08	284.15±58.99^{bc}	134.32±20.8^{a}	196.09±50.64^{ab}	296.84±21.35^{bc}	4101.31±1096.3^{c}	162.18±12.74^{ab}
SE09	205.11±29.03^{abc}	128.53±13.26^{ab}	125.87±34.16^{a}	320.44±85.21^{bc}	6631.95±2095.43^{c}	110.86±24.96^{a}
SK01	110.99±19.66^{bc}	40.32±4^{a}	51.77±6^{ab}	64.88±8.61^{abc}	3694.22±280^{c}	52.41±8.7^{ab}
SK02	46.35±13.2^{bc}	18.73±3.25^{a}	22.55±2^{ab}	34.14±5.2^{abc}	2283.97±150.81^{c}	21.13±0.91^{a}
SK03	35.47±3.12^{bc}	25.53±0.22^{ab}	27.31±4.42^{ab}	28.4±1.72^{abc}	2002.39±397.98^{c}	23.67±0.7^{a}
SK04	69.86±4.64^{bc}	47.08±0.65^{a}	53.65±6.6^{ab}	58.77±4.94^{ab}	4029.42±887.71^{c}	63.22±7.73^{abc}
SK05	45.3±7.51^{abc}	41.39±4.88^{ab}	59.07±17.33^{bc}	35.67±1.57^{a}	1371.53±271.97^{c}	41.53±2.09^{ab}
SK06	163.98±2.71^{cd}	52.6±4.92^{a}	90.59±9.44^{bcd}	72.53±0.22^{ab}	3717.34±221.9^{d}	79.43±3.82^{abc}
SK07	53.16±2.71^{bc}	16.5±2.44^{a}	34.46±10.78^{ab}	32.44±8.15^{ab}	2394.29±774.28^{c}	39.99±7.98^{abc}
BMV58	442.07±37.27^{bc}	254.7±33^{ab}	286.4±46.42^{ab}	562.82±63.24^{bc}	4666.37±487.56^{c}	171.03±16.61^{a}
SU02	290.4±23.03^{abc}	134.78±24.39^{ab}	123.07±24.04^{a}	304.62±5.79^{bc}	2335.63±280.23^{c}	131.51±25.19^{ab}
SU03	386.81±52.54^{ab}	266.53±36.18^{a}	366.57±34.26^{ab}	723.62±46.02^{b}	4553.35±248.75^{b}	194.29±8.41^{a}
SU04	87.67±14.6^{a}	219.25±48.44^{bc}	178.74±8.87^{bc}	125.48±4.55^{ab}	1696.37±185.47^{c}	104.11±22.14^{ab}
SU06	292.53±53.1^{a}	388.06±125.42^{ab}	803.25±51.95^{bc}	623.99±134.21^{abc}	4095.3±1031.83^{c}	370.36±30.17^{ab}
SU07	183.18±5.17^{bc}	135.37±18.81^{ab}	119.45±23.72^{ab}	226.03±21.36^{bc}	3148.95±133.62^{c}	71.44±11.85^{a}

Data is expressed as the mean ± SD (n=3). Superscript letters in the same row indicate significant differences respectively (p<0.05) by the application of Kruskal-Wallis test.

Table S12. Percentage of variance on volatile fermentative compounds from each factor obtained by a two-way ANOVA

	Yeast Strain (Y)	Nitrogen Must (N)	Y x N	Residual
Ethyl hexanoate	52	6	22	20
Ethyl octanoate	53	7	24	16
Ethyl decanoate	48	5	34	13
2-phenylethanol	1.18	92.85	4.58	1.39
2-phenylethyl acetate	5.60	71.75	20.31	2.34
Isobutanol	1.97	92.63	4.30	1.10
Amyl/Isoamyl alcohol	3.86	88.65	5.79	1.71

All factor effects are expressed in % of variance resulted significant with p-values<0.001 by the two-way ANOVA.

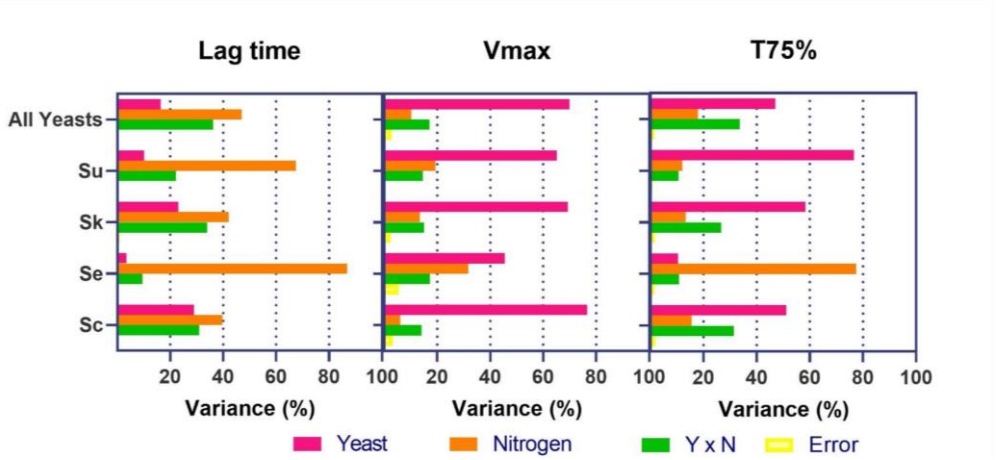


Figure 1S. Variance percentage of all the strains, and separated by species, of the fermentation parameters obtained by a multi-factorial ANOVA. Lag time (time to reach 10% of fermentation), Vmax (maximal fermentation rate), and T75% (time to reach 75% of the fermentation).

Chapter 2

Table S1. Fermentation curve parameters obtained by the different yeast strains in must containing PAF.

	Fermentation performance		
	Lag time[1]	**T75%[2]**	**Vmax[3]**
T73	11.84±1.02^{d}	96.46±5.64^{bc}	0.87±0.06^{ef}
C1	18.68±0.77^{a}	81.05±3.33^{ef}	1.27±0.05^{b}
C2	12.65±1.16^{cd}	97.6±4.67^{bc}	0.91±0.07^{ef}
C3	16.53±2.94^{abcd}	99.04±9.64^{b}	0.9±0.08e^{f}
E1	16.82±1.99^{abc}	100.53±5.37^{b}	0.93±0.07^{def}
E2	14.15±2.05^{abcd}	92.25±1.24^{bcde}	0.91±0.01^{ef}
E3	15.12±1.2^{abcd}	95.55±1.68^{bcd}	0.88±0.02^{ef}
K1	13.87±0.52^{abcd}	62.52±0.7^{g}	1.62±0.02^{a}
K2	18±1.7^{ab}	82.63±6^{def}	1.19±0.16^{bc}
K3	13.14±1.52^{cd}	113.88±5.47^{a}	0.73±0.02^{f}
U1	15.98±2.46^{abcd}	84.42±3.42^{cdef}	1.13±0.12^{bcd}
U2	12.14±1.05^{cd}	75.44±1.97^{fg}	1.17±0.05^{bc}
BMV58	12.39±1.4^{cd}	83.14±1.41^{def}	0.98±0.01^{cde}
VellEvol	13.48±0.78^{bcd}	63.43±0.81^{g}	1.54±0.07^{a}

Data is expressed as the mean value ± SD (n=3). Different superscript letters in the same column indicate significant differences ($p < 0.05$) in samples with PAF using HSD Tukey test. [1] h; [2] time (h) to reach the 75% of the maximum weight loss of the fermentation; [3]maximun fermentation rate (g/L.h).

Table S2. Main metabolites produced during fermentation by the different yeast strains in must containing PAF.

	Ethanol[1]	**Glycerol[2]**	**Malic acid[2]**	**Succinic acid[2]**	**Lactic acid[2]**	**pH**
T73	11.98±0.56^{abc}	6.66±0.22^{f}	3.88±0.32^{abc}	1.16±0.14^{de}	0.05±0.01^{b}	3.62±0.03
C1	12.52±0.21^{ab}	7.23±0.13^{def}	3.29±0.03^{bcd}	0.9±0.24^{e}	0.08±0.02^{b}	3.51±0.13
C2	11.89±0.19^{abc}	8.07±0.43^{cde}	4.23±0.38^{a}	1.76±0.1^{cde}	0.06±0.01^{b}	3.63±0.03
C3	12.05±0.43^{abc}	8.58±0.33^{c}	3.2±0.15^{cde}	0.93±0.17^{de}	0.07±0.06^{b}	3.56±0.03
E1	11.6±0.21^{c}	9.87±0.94^{ab}	3.78±0.22^{abc}	2±0.11^{cd}	0.08±0.03^{b}	3.56±0.09
E2	11.93±0.27^{abc}	9.07±0.79^{abc}	3.94±0.31^{ab}	1.98±0.13^{cd}	0.14±0.03^{b}	3.6±0.05
E3	11.65±0.1^{bc}	10.15±0.13^{a}	2.85±0.13^{de}	1±0.05^{de}	0.07±0^{b}	3.57±0.01
K1	12.05±0.12^{abc}	8.82±0.04^{bc}	2.53±0.06^{e}	1.21±0.16^{de}	0.56±0^{a}	3.58±0.04
K2	11.94±0.44^{abc}	8.71±0.47^{bc}	2.6±0.16^{de}	1.89±0.08^{cde}	0.55±0.42^{a}	3.62±0.03
K3	11.57±0.39^{c}	9.12±0.08^{abc}	2.73±0.08^{de}	1.74±0.17^{cde}	0.56±0.18^{a}	3.62±0.05
U1	11.96±0.31^{abc}	10.19±0.33^{a}	4.18±0.55^{a}	2.81±1.08^{bc}	0.25±0.12^{ab}	3.52±0.08
U2	12.04±0.02^{abc}	8.48±0.2^{cd}	2.86±0.12^{de}	1.37±0.1^{de}	0.09±0.02^{b}	3.63±0.03
BMV58	11.82±0.37^{abc}	7.96±0.22^{cde}	3.31±0.05^{bcd}	4.24±0.54^{a}	0.13±0.07^{b}	3.55±0.09
VellEvol	12.58±0.13^{a}	7.21±0.42^{ef}	2.84±0.13^{de}	3.54±0.38^{ab}	0.14±0.03^{b}	3.52±0.1

Data is expressed as the mean value ± SD (n=3). Different superscript letters in the same column indicate significant differences ($p < 0.05$) in samples with PAF using HSD Tukey test. [1] %v/v; [2] g/L. pH no significant differences, *p*-value 0.2490

Table S3. Concentration in mg/L of major compounds and their corresponding odor threshold (OT) found in wines recently fermented by 14 yeasts in a synthetic must supplemented with PAF.

	Acids						*Alcohols*						
Yeasts	acetic acid	decanoic acid	hexanoic acid	isobutyric acid	isovaleric acid	octanoic acid	1-butanol	1-hexanol	benzyl alcohol	isoamyl alcohol	isobutanol	methionol	β-phenylethanol
T73	300.32±81.31^{bcd}	0.28±0.16^{b}	0.3±0.06^{cd}	4.6±0.69^{cd}	0.97±0.16^{ab}	0.13±0.04^{c}	0.36±0.04^{c}	0.01±0.0038^{a}	0.08±0.01^{ab}	292.67±53.17^{bc}	33.5±2.81^{cdefg}	8.3±3.01^{ab}	35.38±14.48^{ab}
C1	234.92±36.39^{de}	0.19±0.04^{b}	0.23±0.02^{cdefg}	3.62±0.99^{d}	0.6±0.09^{bcd}	0.11±0.02^{c}	1.13±0.29^{bc}	0.02±0.0002^{a}	0.08±0.01^{ab}	257.63±45.29^{bcd}	55.89±10.85^{abcd}	1.68±0.46^{e}	27.1±4.83^{b}
C2	269.49±69.43^{cd}	0.3±0.06^{b}	0.53±0.02^{a}	3.67±0.37^{d}	0.35±0.1^{cd}	0.17±0.02^{abc}	0.98±0.1^{bc}	0.01±0.0018^{a}	0.08±0.01^{ab}	453.84±90.88^{a}	57.38±9.74^{abc}	10.98±3^{a}	62.61±15.94^{ab}
C3	520±105.21^{a}	0.25±0.09^{b}	0.3±0.04^{cde}	6.12±0.24^{bcd}	0.29±0.1^{cd}	0.15±0.03^{bc}	0.9±0.04^{bc}	0.01±0.0028^{a}	0.07±0.0047^{ab}	284.28±77.27^{bc}	50.11±16.14^{abcde}	5.9±1.06^{bcd}	29.3±10.86^{ab}
E1	93.49±16.8^{efg}	1.32±0.9^{b}	0.19±0.08^{defg}	7.1±1.57^{bcd}	0.77±0.18^{abc}	0.22±0.08^{abc}	2.61±0.56^{a}	0.02±0.005^{a}	0.06±0.01^{b}	218.98±20.77^{bcd}	28.53±9.86^{efg}	6.25±0.97^{bcd}	87.12±30.11^{ab}
E2	447.57±84.02^{ab}	3.18±0.88^{a}	0.12±0.02^{g}	6.32±0.45^{bcd}	0.74±0.06^{abc}	0.23±0.1^{abc}	2.99±0.74^{a}	0.01±0.0011^{a}	0.07±0.01^{ab}	209.77±10.57^{bcd}	20.75±1.25^{fg}	5.07±0.47^{bcde}	95.89±21.21^{ab}
E3	509±35.37^{a}	1.03±0.14^{b}	0.14±0.03^{fg}	6.98±0.33^{bcd}	0.43±0.03^{cd}	0.11±0.04^{c}	2.39±0.37^{a}	0.02±0.0018^{a}	0.07±0.0048^{ab}	151.96±21.7^{cd}	19.07±2.25^{fg}	3.06±0.24^{de}	36.73±3.74^{ab}
K1	312.58±73.51^{bcd}	0.47±0.07^{b}	0.44±0.04^{ab}	7.32±1.25^{bcd}	0.51±0.03^{bcd}	0.17±0.0022^{abc}	0.81±0.04^{bc}	0.02±0.0029^{a}	0.08±0.0049^{ab}	284.29±1.34^{bc}	43.25±6.84^{bcdef}	5.78±0.37^{bcd}	51.01±7.13^{ab}
K2	45.95±6.89^{g}	0.72±0.09^{b}	0.34±0.06^{bc}	6.14±0.51^{bcd}	0.72±0.08^{abc}	0.12±0.02^{c}	1.04±0.34^{bc}	0.01±0.0022^{a}	0.08±0.01^{ab}	301.8±8.91^{b}	31.26±4.65^{defg}	7.26±0.21^{abc}	79.98±9.97^{ab}
K3	328.4±28.83^{bcd}	0.51±0.28^{b}	0.29±0.04^{cde}	16.07±5.07^{a}	**1.13±0.44^{a}**	0.1±0.02^{c}	0.81±0.13^{bc}	0.01±0.0034^{a}	0.08±0.01^{a}	325.45±94.81^{ab}	65.8±5.28^{ab}	5.83±0.5^{bcd}	50.13±13.98^{ab}
U1	59.58±7.65^{fg}	0.98±0.62^{b}	0.33±0.01^{bc}	8.54±0.55^{bc}	0.68±0.24^{abcd}	0.34±0.14^{a}	0.34±0.02^{c}	0.02±0.0029^{a}	0.07±0.0038^{ab}	247.46±63.53^{bcd}	53.44±11.92^{abcde}	2.58±0.6^{de}	90.47±69.52^{ab}
U2	411.1±18.61^{abc}	0.25±0.12^{b}	0.24±0.03^{cdef}	7.34±0.51^{bcd}	0.41±0.12^{cd}	0.13±0.01^{c}	1.42±0.18^{b}	0.02±0.0026^{a}	0.08±0.0018^{a}	114.64±14.3^{d}	16.76±2.05^{g}	2.94±0.25^{de}	24.62±4.41^{b}
BMV58	211.62±29.85^{def}	0.88±0.43^{b}	0.18±0.02^{efg}	9.91±0.97^{b}	0.59±0.12^{bcd}	0.31±0.08^{ab}	0.26±0.05^{c}	0.02±0.0013^{a}	0.07±0.01^{ab}	239.03±20.96^{bcd}	72.13±13.35^{a}	3.86±0.92^{cde}	107.73±51.18^{a}
VellEv	423.98±39.72^{abc}	0.47±0.12^{b}	0.34±0.03^{bc}	4.5±0.27^{cd}	0.19±0.04^{d}	0.14±0.01^{bc}	0.94±0.07^{bc}	0.02±0.0024^{a}	0.08±0.004^{ab}	153.04±28.39^{cd}	23.8±2.4^{fg}	4.49±0.78^{bcde}	38.03±13.7^{ab}
p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0001**	**<0.0001**	0.111	**0.0171**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0029**
OT (mg/L)	300^{V}	1^{IV}	0.42^{IV}	2.3^{IV}	0.033^{IV}	0.5^{IV}	150^{III}	8^{VI}	200^{II}	30^{VI}	40^{VI}	1^{IV}	14^{IV}

Table S3. Continue

	Esters						*Miscellaneous and lactone*		
Yeasts	ethyl acetate	ethyl decanoate	ethyl hexanoate	ethyl octanoate	ethyl propanoate	isoamyl acetate	ethyl lactate	γ-butyrolactone	diacetyl
T73	30.87±0.59^{a}	0.18±0.06^{bc}	0.05±0.02^{ab}	0±0^{b}	0.06±0.01^{bc}	0.1±0.03^{a}	0.84±0.25^{de}	0.61±0.1^{a}	0.1±0.01^{f}
C1	18.52±1.34^{cde}	0.41±0.09^{a}	0.05±0.01^{ab}	0±0^{b}	0±0^{e}	0.05±0.01^{abcd}	0.59±0.12^{e}	0.26±0.05^{bc}	0.64±0.15^{def}
C2	20.03±0.69^{bc}	0.21±0.03^{bc}	0.04±0.01^{ab}	0±0^{b}	0.04±0.01^{c}	0.08±0.01^{abc}	0.9±0.21^{cde}	0.39±0.06^{b}	0.29±0.03^{ef}
C3	32.47±3.13^{a}	0.29±0.03^{abc}	0.06±0.01^{ab}	0±0^{b}	0.05±0.02^{c}	0.06±0.02^{abcd}	0.66±0.11^{de}	0.3±0.05^{bc}	1.23±0.22^{bc}
E1	29.56±5.28^{a}	0.33±0.08^{ab}	0.04±0.01^{ab}	0±0^{b}	0.22±0.07^{a}	0.08±0.01^{abc}	0.73±0.07^{de}	0.32±0.01^{bc}	0.11±0.02^{f}
E2	26.62±3.52^{abc}	0.16±0.04^{bc}	0.03±0.0036^{b}	0±0^{b}	0.04±0.01^{c}	0.07±0.02^{abc}	0.92±0.08^{bcde}	0.24±0.03^{bc}	0.47±0.01^{ef}
E3	24.32±1.84^{abc}	0.3±0.03^{abc}	0±0^{c}	0±0^{b}	0.05±0.02^{bc}	0.04±0.0041^{cd}	0.53±0.16^{e}	0.17±0.01^{c}	0.38±0.09^{ef}
K1	10.98±0.76^{c}	0.28±0.02^{abc}	0.06±0.01^{ab}	0±0^{b}	0±0^{e}	0.05±0.01^{bcd}	1.51±0.22^{b}	0.29±0.04^{bc}	2.22±0.55^{a}
K2	11.49±2.49^{de}	0.26±0.06^{abc}	0.03±0.01^{ab}	0±0^{b}	0.04±0.0045^{c}	0.04±0.01^{cd}	1.48±0.13^{bc}	0.25±0.04^{bc}	0.51±0.07^{ef}
K3	18.75±4.71^{bcde}	0.29±0.08^{abc}	0.05±0.02^{ab}	0±0^{b}	0±0^{e}	0.04±0.01^{bcd}	3.43±0.36^{a}	0.31±0.11^{bc}	0.77±0.16^{cde}
U1	19.28±1.87^{bcd}	0.23±0.05^{bc}	0±0^{c}	0±0^{b}	0.12±0.03^{b}	0.04±0.01^{cd}	1.22±0.34^{bcd}	0.66±0.07^{a}	0.38±0.07^{ef}
U2	20.01±1.64^{bc}	0.22±0.04^{bc}	0±0^{c}	0±0^{b}	0.05±0.0019^{c}	0.03±0.01^{d}	0.92±0.14^{bcde}	0.24±0.03^{bc}	1.09±0.23^{bcd}
BMV58	26.83±3.23^{ab}	0.14±0.02^{c}	0.06±0.01^{a}	0.06±0.02^{a}	0±0^{e}	0.04±0.01^{cd}	0.83±0.21^{de}	0.31±0.08^{bc}	0.65±0.1^{def}
VellEv	21.04±1.72^{bc}	0.28±0.08^{abc}	0±0^{c}	0±0^{b}	0±0^{e}	0.09±0.03^{ab}	0.72±0.15^{de}	0.26±0.03^{bc}	1.62±0.07^{b}
p-value	**<0.0001**	**0.0003**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
OT (mg/L)	12.3^{I}	0.2^{IV}	0.062^{VII}	0.58^{III}	5.5^{VII}	0.03^{VI}	15.4^{III}	3.5^{II}	0.1^{VI}

Data is expressed as the mean value ± SD (n=3). Different superscript letters in a same row and *p*-values in bold indicate significant differences ($p< 0.05$) according to HSD Tukey test. I, Escudero et al., 2004; II, Escudero et al., 2007; III, Etievant, 1991; IV, Ferreira, Lopez, & Cacho, 2000; V, Ferreira et al., 2002; VI, Guth, 1997.

Table S4. Concentrations (µg/L) of trace aroma compounds detected in young wines and must containing PAF.

	C13-norisoprenoides				Monoterpenes						Lactones			
	α-ionone	β-ionone	TDN	**Total**	β-citronellol	geraniol	linalool	nerol	α-terpineol	**Total**	γ-nonalactone	γ-decalactone	Whisky lactone	**Total**
Must	0±0^{c}	0±0^{f}	0.07±0.03^{bc}	0.07±0.03^{g}	0±0^{g}	0±0^{f}	0±0^{a}	0±0^{f}	0±0^{c}	0±0^{d}	0±0^{b}	0±0^{d}	0±0^{g}	0±0^{e}
T73	0.05±0.01^{a}	0.21±0.01^{bc}	0.07±0.01^{c}	0.32±0.02^{bcd}	1.41±0.14^{ef}	2.95±0.62^{b}	2.09±0.11^{a}	0.54±0.03^{abcde}	3.68±0.29^{a}	7.71±0.43^{ab}	0.41±0.06^{ab}	0±0^{d}	3.16±0.35^{bcd}	3.57±0.34^{bc}
C1	0.02±0.01^{b}	0.14±0.04^{cde}	0.07±0.01^{c}	0.23±0.04^{def}	3.71±0.39^{b}	2.04±0.29^{bcd}	2.07±0.47^{a}	0.71±0.07^{ab}	3.84±0.31^{a}	10.32±1.14^{a}	0.35±0.13^{ab}	0±0^{d}	2.22±0.41^{cdef}	2.57±0.4^{cd}
C2	0±0^{c}	0.08±0.01^{def}	0.09±0.02^{bc}	0.17±0.03^{fg}	2.86±0.21^{c}	0.59±0.05^{ef}	1.5±0.15^{a}	0.35±0.04^{de}	3.51±0.26^{ab}	8.22±0.17^{ab}	0.39±0.18^{ab}	0±0^{d}	3.32±0.5^{bc}	3.71±0.65^{bc}
C3	0±0^{c}	0.05±0.0012^{ef}	0.15±0.02^{a}	0.21±0.02^{ef}	2.23±0.11^{cd}	1.02±0.16^{def}	0±0^{a}	0.51±0.03^{abcde}	0±0^{c}	2.73±0.13^{c}	0.31±0.08^{ab}	0±0^{d}	1.42±0.33^{f}	1.74±0.41^{d}
E1	0.03±0.0036^{ab}	0.21±0.02^{bc}	0.1±0.01^{abc}	0.35±0.02^{abc}	2.14±0.27^{cde}	1.46±0.58^{cde}	0±0^{a}	0.63±0.15^{abc}	3.07±0.05^{b}	5.84±0.4^{b}	0.79±0.78^{a}	0±0^{d}	7.04±0.88^{a}	7.83±1.37^{a}
E2	0±0^{c}	0.13±0.01^{cde}	0.1±0.03^{abc}	0.23±0.03^{def}	0.99±0.16^{f}	1.34±0.12^{de}	0±0^{a}	0.42±0.07^{cde}	0±0^{c}	1.42±0.21^{cd}	0.37±0.26^{ab}	0±0^{d}	2.63±0.53^{cdef}	3±0.78^{bcd}
E3	0±0^{c}	0.06±0.01^{ef}	0.13±0.01^{ab}	0.19±0.02^{f}	1.03±0.21^{f}	1.21±0.17^{def}	2.03±0.58^{a}	0.39±0.12^{cde}	3.7±0.04^{a}	7.15±0.87^{b}	0.32±0.02^{ab}	0.75±0.03^{a}	1.87±0.53^{def}	2.94±0.52^{bcd}
K1	0.02±0.01^{b}	0.14±0.07^{cde}	0.09±0.02^{abc}	0.26±0.09^{cdef}	1.76±0.1^{def}	0.9±0.29^{def}	1.08±0.96^{a}	0.72±0.04^{a}	3.44±0.08^{ab}	6.99±1.06^{b}	0.32±0.12^{ab}	0±0^{d}	1.82±0.2^{ef}	2.14±0.28^{cd}
K2	0±0^{c}	0.15±0.01^{cd}	0.12±0.02^{abc}	0.27±0.02^{cdef}	2.04±0.2^{de}	0.45±0.03^{ef}	0±0^{a}	0.52±0.02^{abcde}	0±0^{c}	2.56±0.22^{cd}	0.29±0.02^{ab}	0±0^{d}	2.28±0.1^{cdef}	2.57±0.09^{cd}
K3	0±0^{c}	0.33±0.05^{a}	0.12±0.02^{abc}	0.45±0.04^{a}	1.91±0.13^{de}	0.42±0.05^{ef}	0.96±0.83^{a}	0.49±0.07^{abcde}	3.35±0.39^{ab}	6.72±0.48^{b}	0.35±0.01^{ab}	0±0^{d}	2.91±0.25^{bcde}	3.26±0.23^{bcd}
U1	0.04±0.01^{a}	0.21±0.01^{bc}	0.15±0.04^{a}	0.4±0.03^{ab}	5.17±0.38^{a}	4.81±1.11^{a}	1.36±1.18^{a}	0.61±0.23^{abcd}	3.05±0.03^{b}	10.19±1.49^{a}	0.39±0.07^{ab}	0±0^{d}	3.96±0.68^{b}	4.35±0.67^{b}
U2	0±0^{c}	0.25±0.02^{ab}	0.09±0.01^{abc}	0.35±0.01^{abc}	2.33±0.43^{cd}	2.69±0.42^{b}	1.74±0.36^{a}	0.44±0.09^{bcde}	3.46±0.23^{ab}	7.96±0.51^{ab}	0.31±0.06^{ab}	0.4±0.03^{c}	2.2±0.25^{cdef}	2.91±0.26^{bcd}
BMV58	0±0^{c}	0.2±0.06^{bc}	0.11±0.01^{abc}	0.31±0.06^{bcde}	1.93±0.39^{de}	1.95±0.04^{bcd}	0±0^{a}	0.32±0.04^{e}	0±0^{c}	2.25±0.43^{cd}	0.37±0.17^{ab}	0±0^{d}	2.22±0.49^{cdef}	2.6±0.56^{cd}
VellEvol	0±0^{c}	0.06±0.01^{def}	0.11±0.004^{abc}	0.17±0.01^{fg}	1.68±0.27^{def}	2.62±0.36^{bc}	2.29±2.51^{a}	0.38±0.03^{cde}	3.13±0.02^{b}	7.47±2.23^{b}	0.3±0.05^{ab}	0.49±0.02^{b}	1.71±0.23^{ef}	2.5±0.3^{cd}

Table S4. Continue

	Acetates			Volatile phenols							
	isobutyl acetate	β-phenylethyl acetate	**Total**	*trans*-Isoeugenol	eugenol	guaiacol	syringol	methoxyeugenol	4-vinylguaiacol	4-vinylphenol	**Total**
Must	0±0^{d}	0±0^{c}	0±0^{c}	0.46±0.18^{c}	0±0^{h}	2.12±0.51^{abc}	3.23±1.22^{a}	0.25±0.19^{b}	5.14±1.39^{bc}	107.68±27.94^{a}	118.88±29.97^{a}
T73	6.91±1.05^{abc}	130.18±54.69^{c}	137.09±55.26^{c}	0.57±0.06^{abc}	0.52±0.04^{a}	2.46±0.12^{abc}	2.46±0.3^{a}	0.36±0.04^{ab}	3.44±0.54^{c}	22.87±7.11^{c}	32.69±7.17^{d}
C1	11.22±1.2^{a}	114.62±25.52^{c}	125.84±25.52^{c}	0.67±0.04^{abc}	0.51±0.01^{ab}	2.65±0.62^{ab}	2.76±0.34^{a}	0.4±0.03^{ab}	4.35±0.57^{bc}	38.78±12.33^{bc}	50.12±13.77^{abcd}
C2	7.64±0.61^{abc}	109.84±25.07^{c}	117.48±25.09^{c}	0.48±0.04^{bc}	0.26±0.03^{g}	1.51±0.27^{c}	2.27±0.14^{a}	0.3±0.03^{ab}	5.97±0.9^{bc}	44.47±15.03^{abc}	55.25±16.06^{abcd}
C3	10.3±2.77^{a}	96.88±31.12^{c}	107.18±32.69^{c}	0.7±0.11^{abc}	0.43±0.0027^{bcdef}	2.05±0.23^{abc}	2.79±0.31^{a}	0.42±0.06^{ab}	2.81±0.3^{c}	26.61±3.68^{bc}	35.8±4.52^{cd}
E1	11.79±0.94^{a}	1186.66±497.6^{a}	1198.45±496.73^{a}	0.52±0.0029^{bc}	0.37±0.02^{f}	1.64±0.44^{abc}	2.69±0.18^{a}	0.32±0.02^{ab}	3.74±0.56^{c}	30.5±12.41^{bc}	39.77±12.37^{cd}
E2	9.95±2.89^{ab}	715.91±260.73^{ab}	725.85±263.14^{ab}	0.73±0.01^{ab}	0.4±0.04^{cdef}	2.34±0.16^{abc}	3.43±0.14^{a}	0.39±0.0041^{ab}	7.95±2.21^{b}	78.38±20.92^{abc}	93.63±23.14^{abcd}
E3	11.09±4.19^{a}	252.23±58.8^{bc}	263.33±62.97^{bc}	0.55±0.08^{bc}	0.34±0.02^{fg}	1.53±0.09^{c}	2.93±0.26^{a}	0.42±0.07^{ab}	3.69±0.16^{c}	25.29±1.54^{bc}	34.76±2.16^{cd}
K1	5.65±1.58^{abcd}	124.44±29.39^{c}	130.09±30.97^{c}	0.55±0.1^{bc}	0.34±0.02^{fg}	2.02±0.09^{abc}	2.32±0.19^{a}	0.32±0.03^{ab}	3.1±0.23^{c}	34.24±2.78^{bc}	42.9±3.27^{bcd}
K2	3.69±0.96^{bcd}	187.69±29.5^{bc}	191.38±29.97^{bc}	0.66±0.12^{abc}	0.39±0.04^{def}	2.69±0.69^{a}	3.3±0.65^{a}	0.42±0.09^{ab}	4.85±1.46^{bc}	68.89±24.24^{abc}	81.21±27.01^{abcd}
K3	6.37±1.57^{abcd}	154.55±34.72^{bc}	160.92±35.94^{bc}	0.59±0.07^{abc}	0.39±0.02^{def}	1.7±0.16^{abc}	2.66±0.43^{a}	0.35±0.01^{ab}	5.15±1.73^{bc}	84.1±63.8^{abc}	94.93±64.86^{abcd}
U1	10±3.55^{ab}	975.01±460.85^{a}	985.01±463.24^{a}	0.64±0.1^{abc}	0.47±0.02^{abcde}	2.03±0.14^{abc}	2.5±0.37^{a}	0.39±0.06^{ab}	4.31±0.88^{bc}	32.71±13.23^{bc}	43.07±13.69^{bcd}
U2	3.4±0.63^{cd}	78.63±15.52^{c}	82.03±16.14^{c}	0.59±0.06^{abc}	0.38±0.01^{ef}	1.59±0.11^{bc}	2.53±0.15^{a}	0.36±0.01^{ab}	5.03±0.87^{bc}	41.66±4.02^{bc}	52.14±5.07^{abcd}
BMV58	9.24±2.34^{abc}	331.54±22.76^{bc}	340.79±23.89^{bc}	0.73±0.11^{ab}	0.47±0.06^{abcd}	2.02±0.49^{abc}	2.46±0.51^{a}	0.42±0.05^{ab}	6.26±2.07^{bc}	89.54±19.61^{ab}	101.9±20.2^{abc}
VellEvol	9.36±3.01^{abc}	179.08±41.08^{bc}	188.44±41.13^{bc}	0.83±0.09^{a}	0.49±0.03^{abc}	2.54±0.37^{abc}	2.79±0.33^{a}	0.46±0.05^{a}	13.13±1.62^{a}	89.67±12.54^{ab}	109.91±15^{ab}

Table S4. Continue

	Ethyl esters						Vanillin derivatives			
	ethyl isovalerate	ethyl 2-methylbutyrate	ethyl isobutyrate	ethyl dihydrocinnamate	ethyl D/L-leucate	**Total**	acetovanillone	vanillin	syringaldehyde	**Total**
Must	0±0^{c}	0±0^{g}	0±0^{c}	0±0^{d}	0±0^{e}	0±0^{g}	4.11±2.41^{c}	8.06±8.95^{a}	403.68±64.38^{a}	415.85±72.45^{a}
T73	0.8±0.09^{a}	0.87±0.16^{bc}	5.17±0.83^{cd}	0.05±0.01^{bc}	18.5±9.98^{ab}	25.4±9.53^{abc}	34.05±0.79^{a}	4.58±0.56^{a}	44.36±7.3^{b}	82.99±6.89^{b}
C1	0.36±0.08^{bc}	0.58±0.06^{bcdef}	4.45±0.47^{cd}	0.05±0.01^{bc}	20.31±1.82^{a}	25.75±2.32^{ab}	33.33±0.88^{a}	4.48±0.63^{a}	60.65±8.3^{b}	98.45±6.8^{b}
C2	0.22±0.06^{bcde}	0.41±0.1^{defg}	2.4±0.91^{de}	0±0^{d}	17.23±4.31^{abc}	20.26±5.09^{bcdef}	28.07±0.44^{b}	2.99±0.54^{a}	25.96±11.77^{b}	57.02±12.63^{b}
C3	0.27±0.05^{bcd}	0.29±0.04^{fg}	3.05±0.37^{de}	0.31±0.01^{a}	14.58±2.61^{abcd}	18.49±2.16^{bcdef}	33.34±1.71^{a}	2.22±0.12^{a}	10.94±5.62^{b}	46.5±5.35^{b}
E1	0.32±0.04^{bcd}	0.86±0.31^{bcd}	3.48±0.22^{de}	0.07±0.01^{b}	17.31±3.82^{abc}	22.04±4.31^{abcde}	30.17±1.17^{ab}	2.75±0.45^{a}	36.75±15.73^{b}	69.67±17.32^{b}
E2	0.39±0.07^{bc}	0.77±0.05^{bcde}	2.93±0.34^{de}	0.04±0.01^{bc}	7.91±2.5^{bcde}	12.04±2.76^{cdefg}	31.72±1.58^{ab}	3.86±0.04^{a}	32.59±11.69^{b}	68.17±13.16^{b}
E3	0.21±0.05^{bcde}	0.54±0.02^{cdef}	3.26±0.32^{de}	0.03±0.004^{cd}	5.36±0.21^{de}	9.4±0.51^{efg}	33.31±1.12^{a}	3.01±0.47^{a}	23.59±6.96^{b}	59.91±6.29^{b}
K1	0.22±0.03^{bcde}	0.39±0.04^{efg}	3.63±0.39^{de}	0.04±0.003^{bc}	4.86±0.57^{de}	9.13±0.61^{efg}	32.45±1.08^{ab}	3.86±0.55^{a}	40.61±8.03^{b}	76.91±8.89^{b}
K2	0.36±0.02^{bc}	0.42±0.04^{cdefg}	3.34±0.46^{de}	0.05±0.01^{bc}	13.21±2.05^{abcd}	17.37±2.35^{bcdef}	31.22±1.84^{ab}	3.13±0.32^{a}	34.29±9.85^{b}	68.64±9.74^{b}
K3	0.74±0.16^{a}	1.53±0.41^{a}	16.74±4^{a}	0.06±0.01^{bc}	15.18±6.35^{abcd}	34.25±9.75^{a}	31.83±1.3^{ab}	2.81±0.22^{a}	28.22±4.63^{b}	62.86±3.89^{b}
U1	0.37±0.16^{bc}	0.65±0.11^{bcdef}	8.14±1.59^{bc}	0.05±0.03^{bc}	14.62±3.18^{abcd}	23.82±5.04^{abcd}	34.03±3.23^{a}	3.39±0.29^{a}	49.22±11.15^{b}	86.64±14.15^{b}
U2	0.17±0.02^{cde}	1.02±0.05^{b}	4.03±0.45^{d}	0.05±0.01^{bc}	5.34±1.32^{de}	10.61±1.77^{defg}	32.4±1.38^{ab}	3.48±0.23^{a}	33.91±3^{b}	69.8±3.98^{b}
BMV58	0.44±0.1^{b}	0.54±0.14^{cdef}	9.92±2.12^{b}	0±0^{d}	6.93±2.18^{cde}	17.83±4.34^{bcdef}	33.2±2.21^{a}	3.56±0.79^{a}	33.52±8.28^{b}	70.28±10^{b}
VellEvol	0.12±0.02^{de}	0.3±0.04^{fg}	2.21±0.39^{de}	0.05±0.01^{bc}	5.77±1.37^{de}	8.44±1.8^{fg}	32.95±1.04^{ab}	3.5±0.28^{a}	18.61±4.26^{b}	55.06±5.13^{b}

Data is expressed as mean value ± SD. Different letters in the same row indicate significant differences according to HDS Tukey test (p<0.05).

Chapter 3

Table S1. Fermentation parameters and main metabolites analyzed in Albariño wines fermented by 8 different yeast strains

	μ_{max} (cells/mL.h)	erythritol (g/L)	glycerol (g/L)	ethanol (%v/v)	citric acid (g/L)	malic acid (g/L)	succinic acid (g/L)	pH	acetic acid (g/L)
T73	0.12±0.01^{bc}	0.15±0.02^{d}	5.81±0.16^{d}	11.89±0.02^{b}	0.45±0.01^{ab}	3.51±0.04^{a}	0.56±0.01^{d}	3.22±0.03^{d}	0.62±0.03^{ab}
MSB	0.11±0.001^{bc}	0.11±0.01^{e}	5.85±0.04^{d}	11.98±0.01^{a}	0.43±0.01^{bc}	3.19±0.02^{b}	0.47±0.04^{b}	3.23±0.01^{d}	0.46±0.02^{cd}
SC1	0.12±0.01^{bc}	0.28±0.02^{c}	7.18±0.1^{c}	11.76±0.03^{c}	0.45±0.01^{ab}	2.86±0.05^{cd}	1.29±0.05^{c}	3.25±0.02^{bcd}	0.51±0.03^{bc}
SC2	0.05±0.0005^{d}	0.53±0.01^{a}	7.1±0.05^{c}	11.72±0.04^{c}	0.39±0.01^{d}	2.33±0.17^{e}	0.42±0.01^{cd}	3.38±0.03^{a}	0.48±0.03^{c}
SK1	0.12±0.02^{bc}	0.34±0.01^{b}	8.93±0.13^{a}	11.59±0.03^{d}	0.45±0.01^{b}	2.72±0.16^{d}	0.7±0.01^{d}	3.31±0.02^{b}	0.64±0.06^{a}
SK3	0.1±0.0043^{c}	0.34±0.01^{b}	7.96±0.02^{b}	11.6±0.01^{d}	0.48±0.01^{a}	2.58±0.02^{de}	0.84±0.03^{d}	3.29±0.02^{bc}	0.52±0.03^{abc}
SU1	0.14±0.0046^{ab}	0.28±0.01^{c}	7.33±0.25^{c}	11.69±0.03^{c}	0.42±0.02^{bc}	3.07±0.12^{bc}	4.68±0.51^{d}	3.24±0.02^{cd}	0.28±0.09^{e}
BMV58	0.17±0.02^{a}	0.37±0.03^{b}	7.19±0.12^{c}	11.59±0.05^{d}	0.42±0.01^{cd}	3.21±0.08^{b}	7.31±0.03^{a}	3.24±0.01^{d}	0.35±0.02^{de}

Values are expressed as mean value ± SD (n=3). Different letters in the same column denote significant differences between yeast strains at $p< 0.05$ according to Tuckey test HSD.

Table S2. Concentrations and odour thresholds of volatile compounds detected in Albariño synthetic wines immediately after fermentation and in musts.

	Unit	*p*-value	T73	MSB	SC1	SC2	SK1	SK3	SU1	BMV58	MUST	OT*
I-acetic acid	mg/L	0.0005	**448.11±26.79^{ab}**	**418.15±22.15^{abc}**	**442.01±144.52^{ab}**	**425.65±33.99^{ab}**	**599.67±62.14^{a}**	**453.84±31.32^{ab}**	235.57±53.23^{c}	**341.37±60.28^{bc}**	n.d.	300
I-isobutyric acid	mg/L	<0.0001	1.39±0.12^{d}	0.72±0.02^{d}	1.26±0.4^{d}	0.7±0.03^{d}	**3.26±0.42^{c}**	**4.72±0.49^{b}**	**4.75±0.17^{ab}**	**5.96±0.93^{a}**	n.d.	2.3
I-butyric acid	mg/L	<0.0001	0.03±0.01^{bc}	0.04±0^{ab}	n.d.	0.04±0.01^{a}	0.03±0^{c}	0.03±0.01^{bc}	0.03±0^{c}	0.03±0^{c}	n.d.	0.173
I-isovaleric acid	mg/L	<0.0001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.48±0.04^{a}**	**0.4±0.04^{b}**	n.d.	0.033
I-hexanoic acid	mg/L	<0.0001	**0.65±0.06^{c}**	**1.33±0.15^{a}**	0.36±0.09^{d}	**0.81±0.04^{bc}**	**0.86±0.04^{b}**	**0.73±0.03^{bc}**	**0.64±0.01^{c}**	**0.67±0.08^{bc}**	n.d.	0.42
I-octanoic acid	mg/L	<0.0001	**0.44±0.06^{def}**	**0.68±0.12^{bcd}**	0.21±0.05^{f}	**0.57±0.09^{cde}**	**0.82±0.04^{bc}**	**0.28±0.06^{ef}**	**0.92±0.03^{ab}**	**1.13±0.25^{a}**	n.d.	0.5
I-decanoic acid	mg/L	<0.0001	0.32±0.02^{bc}	0.57±0.08^{bc}	0.25±0.06^{c}	0.26±0.01^{c}	0.78±0.12^{b}	0.68±0.04^{bc}	**1.9±0.15^{a}**	**2.11±0.41^{a}**	n.d.	1
II-isobutanol	mg/L	<0.0001	26.06±1.83^{bc}	21.35±1.87^{bc}	**41.58±8.85^{a}**	17.57±0.61^{c}	19.44±2.56^{c}	18.49±1.36^{c}	31.46±1.46^{ab}	**39.59±3.27^{a}**	n.d.	40
II-1-butanol	mg/L	<0.0001	0.25±0.01^{bc}	0.3±0.01^{bc}	0.76±0.13^{a}	0.38±0.02^{b}	0.86±0.1^{a}	0.79±0.04^{a}	0.2±0.01^{c}	0.21±0.02^{bc}	n.d.	150
II-isoamyl alcohol	mg/L	<0.0001	**237.03±5.42^{a}**	**202.16±6.1^{ab}**	**222.92±44.73^{a}**	**84.84±5.56^{c}**	**163.17±12.07^{b}**	**191.65±10.45^{ab}**	**213.29±8.8^{ab}**	**225.46±17.08^{a}**	n.d.	30
II-methionol	mg/L	<0.0001	**11.4±0.72^{a}**	**2.92±0.05^{d}**	**2.95±0.36^{d}**	**3.11±0.09^{cd}**	**4.4±0.61^{bc}**	**5.26±0.46^{b}**	**4.82±0.37^{b}**	**4.99±0.68^{b}**	n.d.	1
II-benzyl alcohol	mg/L	0.001	0.05±0.01^{bc}	0.06±0^{ab}	0.04±0.01^{c}	0.06±0.01^{bc}	0.06±0^{ab}	0.06±0^{ab}	0.05±0.01^{bc}	0.07±0.01^{a}	n.d.	200
II-β-phenylethanol	mg/L	<0.0001	**25±0.16^{cd}**	**18.17±0.35^{cd}**	**32.57±7.68^{c}**	**7.22±0.49^{d}**	**26.61±1.84^{cd}**	**33.6±2.57^{c}**	**140.34±11.89^{b}**	**166.4±15.15^{a}**	n.d.	14
II-1-hexanol	mg/L	0.5989	0.04±0	0.05±0	0.05±0.01	0.04±0.01	0.05±0.01	0.04±0	0.05±0.02	0.04±0.01	n.d.	8
III- isobutyl acetate	µg/L	<0.0001	40.97±1.71^{cd}	25.45±2.25^{e}	62.1±5.25^{a}	33.21±5de	40.44±2.55^{cd}	43.16±0.86^{bcd}	50.13±4.28^{bc}	51.46±4.36^{b}	n.d.	1605
III- β-phenylethyl acetate	µg/L	<0.0001	90.56±8.97^{cd}	77.16±9.19^{cd}	138.43±17.61^{cd}	32.81±6.42^{d}	164.05±7.73^{cd}	**277.62±18.14^{c}**	**1189.97±128.03^{b}**	**1563.96±172.63^{a}**	n.d.	250
III- isoamyl acetate	mg/L	<0.0001	**0.11±0.01^{a}**	**0.07±0.02^{ab}**	**0.1±0.03^{a}**	0.02±0.01^{c}	**0.05±0.01^{bc}**	**0.08±0.02^{ab}**	**0.07±0.01^{ab}**	**0.11±0.02^{a}**	n.d.	0.03

Table S2. Continue

	Unit	*p*-value	T73	MSB	SC1	SC2	SK1	SK3	SU1	BMV58	MUST	OT*
IV- ethyl butyrate	mg/L	0.752	0.02±0.01	0.01±0.00	n.d.	0.02±0.01	n.d.	0.01±0.01	n.d.	n.d.	n.d.	0.125
IV- ethyl isobutyrate	µg/L	<0.0001	4.55±0.22^{de}	2.7±0.27^{c}	3.87±0.52^{de}	2.28±0.61^{c}	7.17±0.24^{d}	**13.76±1.23^{c}**	**19.23±3.28^{b}**	**24.7±0.94^{a}**	n.d.	15
IV- ethyl 2-methylbutyrate	µg/L	<0.0001	1.83±0.24^{ab}	0.85±0.09^{cd}	1.51±0.23^{bcd}	0.74±0.2^{d}	0.88±0.09^{cd}	1.62±0.64^{abc}	2.35±0.11^{a}	2.22±0.17^{ab}	n.d.	18
IV- ethylisovalerate	µg/L	<0.0001	0.95±0.05^{c}	0.54±0.02^{de}	0.54±0.09^{de}	0.25±0.08^{f}	0.37±0.02^{ef}	0.67±0.05^{d}	1.89±0.14^{b}	2.22±0.07^{a}	n.d.	3
IV- ethyl 4-methylvalerate	µg/L		n.d.	n.d.	n.d.	0.21±0.03	n.d.	n.d.	n.d.	n.d.	n.d.	10
IV- ethyl leucate	µg/L	<0.0001	2.94±0.5^{bc}	4.1±0.31^{a}	1.59±0.25^{de}	1.24±0.47^{e}	0.84±0.05^{e}	2.39±0.14^{cd}	4.21±0.61^{a}	4.06±0.53^{ab}	n.d.	900/300
IV- ethyl propanoate	mg/L	**0.1955**	0.05±0.01	0.04±0.02	n.d.	0.04±0.01	n.d.	n.d.	0.03±0.01	0.06±0.01	n.d.	5.5
IV- ethyl hexanoate	mg/L	**0.1656**	**0.11±0.01**	**0.1±0.02**	**0.08±0.01**	**0.13±0.01**	**0.12±0.02**	**0.12±0.01**	**0.08±0.04**	**0.12±0.05**	n.d.	0.062
IV- ethyl octanoate	mg/L	**0.0838**	0.06±0.03	0.04±0.02	n.d.	n.d.	n.d.	n.d.	0.11±0.01	0.12±0.06	n.d.	0.58
IV- ethyl decanoate	mg/L	**0.107**	0.03±0.01	n.d.	n.d.	n.d.	0.07±0.01	0.07±0.04	0.09±0.03	n.d.	n.d.	0.2
IV- diethyl succinate	mg/L	<0.0001	1.64±0.15^{c}	1.18±0.18^{c}	1.31±0.31^{c}	0.46±0.02^{d}	1.34±0.08^{c}	2.33±0.18^{b}	3.46±0.31^{a}	3.94±0.4^{a}	n.d.	200
IV- ethyllactate	mg/L	<0.0001	0.8±0.06^{c}	0.87±0.02^{c}	1.13±0.22^{bc}	0.76±0.04^{c}	1.14±0.14^{bc}	1.26±0.05^{bc}	1.44±0.18^{ab}	1.91±0.41^{a}	n.d.	154
IV- ethy lacetate	mg/L	<0.0001	**21.75±0.92^{bc}**	**31.12±2.42^{a}**	**17.79±3.03^{cd}**	**26.09±1.29^{b}**	**15.41±0.56^{d}**	**18.34±0.44^{cd}**	**18.34±0.18^{cd}**	**20.57±1.61^{c}**	n.d.	12.3
V- acetoin	mg/L	0.037	0.1±0.09^{b}	0.8±1.07^{ab}	0.24±0.07^{ab}	0.7±0.4^{ab}	1.81±0.34^{a}	1.26±0.28^{ab}	0.96±1^{ab}	0.46±0.27^{ab}	n.d.	150
V- 2,3-butanediol**	mg/L	<0.0001	403.3±46.2^{d}	550±52^{d}	650±26.5^{c}	**873.3±20.8^{b}**	**1496.7±55.1^{a}**	**923.3±23.1^{b}**	**660±70^{c}**	590±20^{c}	n.d.	669
V- diacetyl	mg/L	-	n.d.	n.d.	n.d.	0.02±0.01	n.d.	n.d.	n.d.	n.d.	n.d.	0.1
V- acetaldehyde	mg/L	0.0007	**2.03±0.59^{b}**	**1.58±0.16^{b}**	**1.54±0.43^{b}**	**2.88±1.48^{b}**	**3.15±0.65^{b}**	**6.72±1.83^{a}**	**2.32±0.08^{b}**	**3.32±1.8^{b}**	n.d.	0.5
V- dihydromyrcenol	µg/L	**0.1747**	0.83±0.3	0.65±0.26	0.97±0.18	1.03±0.06	0.99±0.03	1.04±0.34	1.01±0.22	0.9±0.12	0.67±0.07	

Table S2. Continue

	Unit	*p*-value	T73	MSB	SC1	SC2	SK1	SK3	SU1	BMV58	MUST	OT*
VI- 4-ethylphenol	µg/L	0.011	0.24±0.11^{a}	0.12±0.01^{b}	0.12±0.04^{b}	0.1±0.02^{b}	0.1±0.03^{b}	0.09±0.01^{b}	0.12±0.02^{b}	0.1±0.01^{b}	0.14±0.01^{ab}	440
VI- 4-vinylguaicol	µg/L	<0.0001	**2922.59±102.67^{a}**	**2639.93±192.55^{a}**	**2689.71±197.14^{a}**	**1017.82±545.16^{c}**	**1708.05±66.05^{b}**	**1433.22±118.38^{bc}**	**2832.07±45.42^{a}**	**2806.95±202.53^{a}**	9.12±6.17^{d}	1100
VI- 4-vinylphenol	µg/L	<0.0001	**697.59±57.21^{ab}**	**722.79±65.96^{ab}**	**639.12±47.92^{ab}**	**376.83±152.66^{c}**	**582.55±5.47^{b}**	**567.27±3.99^{b}**	**762.35±35.75^{a}**	**779.89±30.02^{a}**	18.86±3.18^{d}	180
VI- eugenol	µg/L	0.001	2.07±0.12^{ab}	1.87±0.04^{ab}	1.7±0.1^{b}	1.7±0.07^{b}	1.84±0.08^{ab}	1.73±0.12^{b}	2.25±0.22^{a}	2.09±0.06^{ab}	1.95±0.28^{ab}	6
VI- guaiacol	µg/L	0.004	0.47±0.04^{a}	0.34±0.02^{ab}	0.34±0.1^{ab}	0.34±0.03^{ab}	0.43±0.05^{ab}	0.36±0.05^{ab}	0.28±0.04^{b}	0.31±0.05^{ab}	0.46±0.09^{a}	9.5
VI- *trans*-isoeugenol	µg/L	<0.0001	0.47±0.06^{a}	0.28±0.02^{ab}	0.47±0.15^{a}	0.31±0.04^{ab}	0.39±0.03^{a}	0.33±0.09^{a}	0.42±0.05^{a}	0.45±0.06^{a}	0.12±0.03^{b}	6
VI- methoxyeugenol	µg/L	0.0135	0.24±0.02^{ab}	0.17±0.02^{ab}	0.28±0.1^{a}	0.23±0.02^{ab}	0.22±0.01^{ab}	0.21±0.02^{ab}	0.25±0.02^{ab}	0.27±0^{a}	0.15±0.05^{b}	1200
VI- o-cresol	µg/L	0.004	1.76±0.18^{ab}	1.46±0.05^{b}	1.51±0.23^{b}	1.46±0.06^{b}	1.23±0.06^{b}	1.49±0.22^{b}	1.62±0.13^{ab}	1.55±0.16^{ab}	2.16±0.48^{a}	120
VI- *m*-cresol	µg/L	<0.0001	0.23±0.01^{b}	0.23±0.01^{b}	0.21±0.01^{b}	0.22±0.01^{b}	0.21±0.01^{b}	0.22±0.01^{b}	0.21±0.01^{b}	0.21±0.01^{b}	0.28±0.02^{a}	200
VI- *p*-propylguaiacol	µg/L	<0.0001	0.1±0.01^{abc}	0.11±0.01^{ab}	0.07±0.01^{d}	0.09±0.01^{cd}	0.09±0.01^{bcd}	0.12±0.01^{a}	n.d.	n.d.	0.1±0.01^{abc}	10
VII- γ-nonalactone	µg/L	<0.0001	3.92±0.37^{a}	3.86±0.07^{ab}	2.89±0.24^{de}	3.38±0.06^{bcd}	2.62±0.05^{e}	3.11±0.09^{cde}	3.53±0.11^{abc}	3.37±0.13^{bcd}	1.09±0.23^{f}	30
VII- γ -butyrolactone	mg/L	<0.0001	0.45±0.01^{c}	0.33±0.02^{d}	0.46±0.1^{bc}	0.26±0.02^{de}	0.19±0.02^{e}	0.19±0.02^{e}	0.75±0.03^{a}	0.58±0.02^{b}	n.d.	88
VIII- β-damascenone	µg/L	<0.0001	**1.64±0.06^{cd}**	**2.03±0.1^{bc}**	**1.42±0.22^{d}**	**1.59±0.08^{cd}**	**1.42±0.1^{d}**	**1.36±0.1^{d}**	**1.92±0.07^{bc}**	**2.23±0.11^{b}**	**2.76±0.37^{a}**	0.05
VIII- β-ionone	µg/L	**0.1058**	0.06±0.02	0.04±0.01	0.05±0.02	0.06±0.01	0.06±0.01	0.06±0.01	0.05±0.01	0.06±0	0.04±0.01	0.09

Table S2. Continue

	Unit	*p*-value	T73	MSB	SC1	SC2	SK1	SK3	SU1	BMV58	MUST	OT*
IX- R-limonene	µg/L	<0.0001	5.19±1.39^{a}	0.64±0.1^{b}	6.06±0.94^{a}	1.02±0.14^{b}	6.57±1.27^{a}	6.2±0.62^{a}	1.06±0.13^{b}	1.05±0.05^{b}	0.47±0.07^{b}	34
IX- rose oxide	µg/L	0.004	0.25±0.02^{b}	0.27±0.02^{ab}	0.24±0.02^{b}	0.32±0.03^{a}	0.26±0.04^{ab}	0.28±0.01^{ab}	0.3±0.04^{ab}	0.32±0.03^{a}	0.3±0.01^{ab}	50 (cis)/80 (trans)
IX- linalool	µg/L	0.002	5.07±0.33^{ab}	5.64±0.74^{a}	4.14±0.57^{bc}	4.58±0.34^{abc}	3.67±0.04^{c}	4±0.65^{bc}	4.5±0.08^{abc}	4.69±0.49^{abc}	4.03±0.42^{bc}	25.2
IX- β-citronellol	µg/L	<0.0001	3.04±0.35^{cd}	5.44±0.78^{ab}	6.36±0.84^{a}	5.46±0.64^{ab}	3.73±0.31^{bc}	1.89±0.17^{d}	3.96±1.1^{bc}	3.54±0.35^{cd}	n.d.	40
IX- nerol	µg/L	<0.0001	0.78±0.08^{ab}	0.59±0.06^{bc}	0.75±0.17^{ab}	0.67±0.09^{ab}	0.86±0.01^{a}	0.66±0.02^{ab}	0.39±0.07^{cd}	0.44±0.04^{cd}	0.36±0^{d}	300
IX-linalool oxide	µg/L	**0.2365**	0.48±0.02	0.44±0.04	0.49±0.08	0.47±0.02	0.43±0.04	0.46±0.03	0.47±0.02	0.5±0.03	0.51±0.04	4000 (cis)/4000 (trans)
IX- geraniol	µg/L	<0.0001	4.73±0.12^{b}	2.81±0.47^{bcd}	2.64±0.15^{bcd}	2.45±0.1^{bcd}	2.25±0.13^{cd}	0.88±1.43^{d}	**36.87±1.5^{a}**	**34.92±0.98^{a}**	3.83±0.65^{bc}	30
X- 2-furfurythiol	ng/L	<0.0001	**4.53±0.63^{b}**	**3.2±0.38^{b}**	**5.51±2.37^{b}**	**11.93±2.06^{a}**	**2.43±0.13^{b}**	**2.58±0.07^{b}**	**3.19±0.19^{b}**	**3.07±0.18^{b}**	**3.88±0.19^{b}**	0.4
X- 4-methyl-4-mercatopentan-2-one	ng/L	<0.0001	**21.12±0.43^{d}**	**29.25±0.71^{d}**	**18.86±0.3^{d}**	**35.35±2.09^{d}**	**1236.07±48.74^{a}**	**427.32±33.22^{b}**	**133.8±25.2^{c}**	**167.93±8.42^{c}**	**15.89±2.76^{d}**	0.8
X- 3-mercaptohexyl acetate	ng/L	<0.0001	**9.64±0.74^{cd}**	**8.95±0.41^{cd}**	**7.63±1.02^{d}**	**20.47±3.12^{a}**	**18.92±2.43^{ab}**	**15.2±0.88^{abc}**	**12.25±4.44^{bcd}**	**21.35±3.6^{a}**	**16.03±2.31^{abc}**	4
X- 3-mercapto-1-hexanol	ng/L	<0.0001	**591.26±73.32^{ef}**	**702.68±69.82^{def}**	**751.86±38.34^{de}**	**540.21±9.84^{f}**	**1463.85±21.77^{a}**	**1105.37±11.63^{b}**	**866.42±95.49^{cd}**	**963.65±101.66^{bc}**	**59.47±21.23^{g}**	60

Values are expressed as mean value ± SD (n=3). Different letters in the same row denote significant differences between yeast strains at $p< 0.05$ according to Tuckey test HSD*p*-value in bold indicated no diffferences.*Odour Thresholdexpressed in the same units that each aroma concentration. n.d. indicates that the compound was not detected or below detection limits. I, acids; II, higher alcohols; III, acetate esters; VI, ethyl esters; V, miscellneus and carbonyls; VI, phenols; VII, lactones; VIII, C13-norisoprenoids; IX, terpenes, X, polyfunctional mercaptans. Odor threshold values estimated by Averbeck et al, 2009; Boidron et al, 1988; Cooke et al, 2009; Escudero et al, 2004, 2007; Étievant, 1991; Falc; Takeoka et al, 1995ao et al., 2012; Ferreira et al., 2002, 2000; Guth, 1997; López et al, 2002; Moreno et al., 2005; Nakamura et al, 1988; Ohloff, 1978; Otsuka et al, 1973; Poitou et al, 2017; Pons et al, 2017; Ribéreau-Gayon et al, 1975; Sacks et al, 2012; San Juan et al, 2012; Simpson, 1978; Takeoka et al, 1995; Tominaga et al, 1998; Yamamoto, 2002.** This compound was detected by HPLC together with ethanol, glycerol, etc., but it was more convenient to include it in this table for a better interpretation of the results.

Table S3. Concentrations (µg/L) ofvolatilecompoundsdetected in Albariño syntheticwines and must after aging.

	T73	MSB	SC1	SC2	SK1	SK3	SU1	BMV58	Must	Odour Threshold*
I-isobutyl acetate	6.33±0.39^{ab}	6.02±0.7^{ab}	7.86±0.28^{a}	5.91±0.46^{b}	7.48±0.48^{ab}	7.7±0.85^{ab}	7.23±1.02^{ab}	6.66±0.74^{ab}	n.d.	1605
I-β-phenylethyl acetate	98.54±10.18^{de}	79.61±1.61^{e}	141.91±13.05^{cd}	168.41±35.76^{c}	159.26±8.33^{cd}	196.26±12.55^{c}	**382.93±6.99^{b}**	**489.25±43.16^{a}**	n.d.	250
II-ethyl isobutyrate	**207.22±1.25^{d}**	**101.98±7.06^{d}**	**169.14±9.84^{d}**	**83.72±7.75^{d}**	**398.08±52^{c}**	**664.15±60.54^{b}**	**647.3±112.25^{b}**	**848.53±17.79^{a}**	n.d.	15
II-ethyl 2-methylbutyrate	**61.24±3.18^{d}**	**27.27±1.13^{ef}**	**41.11±7.53^{e}**	9.74±1.51^{f}	**34.84±2.21^{e}**	**83.86±3.94^{c}**	**126.76±13.61^{b}**	**162.56±9.61^{a}**	n.d.	18
II-ethyl isovalerate	**67.1±2.33^{c}**	**44.92±1de**	**38.87±7.77^{e}**	**9.03±1^{f}**	**35.72±1.94^{e}**	**60.45±3^{cd}**	**111.27±11.76 ^{b}**	**137.64±5.95^{a}**	n.d.	3
II-ethyl leucate	49.84±3.98^{ab}	29.01±0.5^{c}	40.24±7.15^{b}	27.92±2.4^{c}	19.52±0.93^{c}	49.01±2.25^{ab}	51.97±4.21^{a}	57.49±1.13^{a}	n.d.	900/300
II-ethylcinnamate	**2.67±0.74^{b}**	**5.3±0.28^{a}**	**1.83±0.54^{bc}**	**1.91±0.33^{bc}**	0.91±0.19^{c}	**1.07±0.25^{c}**	**4.56±0.46^{a}**	**4.3±0.72^{a}**	n.d.	1.1
II-ethyl dihydrocinnmate	0.02±0.02^{a}	n.d.	0.03±0.01^{a}	n.d.	0.01±0.01^{a}	0.03±0.00^{a}	n.d.	n.d.	n.d.	1.6
III-β-damascenone	**10.81±0.27^{b}**	**12.55±0.28^{a}**	**8.58±0.81^{c}**	**11.89±0.19^{ab}**	**10.68±0.09^{b}**	**8.7±0.44^{c}**	**12.25±0.61^{a}**	**13.06±0.39^{a}**	**8.7±0.77^{c}**	0.05
III-riesling acetal*	0.1±0.01^{cde}	0.11±0.01^{abc}	0.09±0^{e}	0.13±0.01^{a}	0.12±0.01^{ab}	0.11±0^{bcd}	0.11±0.01^{cd}	0.1±0.01^{cde}	0.1±0.01^{de}	
III-vitispirane a and b*	1.1±0.04^{ab}	1.12±0.05^{ab}	0.95±0.1^{c}	1±0.02^{bc}	0.91±0.01^{c}	1.03±0.02^{bc}	1.05±0.05^{bc}	1.2±0.05^{a}	0.7±0.06^{d}	
III-TDN	**66.45±4.18^{bc}**	**70.2±4.58^{b}**	**60.5±4.95^{c}**	**50.92±3.16^{d}**	**50.3±1.81^{d}**	**63.2±0.4^{bc}**	**70.59±2.34^{ab}**	**79.44±2.85^{a}**	**12.47±1.82^{e}**	2
IV-R-limonene	**38.13±2.64^{a}**	4.69±0.32^{c}	**36.48±1.53^{a}**	16.05±1.21^{b}	**37.44±1.21^{a}**	**35.71±2.47^{a}**	14.77±1.9^{b}	15.94±1.29^{b}	4.46±0.44^{c}	34
IV-linalool oxide	24.89±0.92^{a}	23.5±0.88^{ab}	21.19±1.87^{bcd}	20.71±0.7^{bcd}	18.94±0.92^{cd}	21.89±0.29^{abc}	22.34±0.96^{abc}	25.38±1.03^{a}	18.05±2.29^{d}	4000 (cis)/4000 (trans)
IV-linalool	0.87±0.06^{bc}	0.82±0.09^{bc}	0.89±0.04^{bc}	1.28±0.15^{a}	1.43±0.07^{a}	0.99±0.07^{b}	0.77±0.09^{bc}	0.72±0.09^{c}	0.84±0.03^{bc}	25.2
IV-α-terpineol	15.83±0.99^{bc}	18.27±0.76^{ab}	13.18±1.21^{c}	20.05±1.04^{a}	20.11±1.25^{a}	16.69±0.63^{b}	16.59±1.75^{b}	17.58±0.87^{ab}	9.63±0.3^{d}	250
IV-β-citronellol	0.12±0.04^{bc}	0.15±0.02^{bc}	0.18±0.05^{abc}	0.32±0.09^{a}	0.23±0.05^{ab}	0.07±0.06^{c}	n.d.	n.d.	n.d.	40
IV-1,8-cineole	0.35±0.04^{abc}	0.49±0.06^{a}	0.4±0.09^{abc}	0.43±0.03^{abc}	0.34±0.04^{bc}	0.39±0.09^{abc}	0.46±0.02^{ab}	0.47±0.02^{ab}	0.29±0.02^{c}	1.1
V-γ-octalactone	0.25±0.05^{bc}	0.71±0.09^{bc}	0.18±0.01^{c}	6.19±0.42^{a}	0.4±0.05^{bc}	0.25±0.09^{bc}	0.7±0.28^{bc}	1±0.13^{bc}	1.13±0.79^{b}	238
V-γ-nonalactone	4.58±0.26^{a}	4.26±0.14^{ab}	3.29±0.26^{c}	4.05±0.2^{b}	3.27±0.07^{c}	3.46±0.28^{c}	4.12±0.1^{ab}	4.07±0.07^{ab}	0.88±0.12^{d}	30
V-γ-decalactone	2.39±0.17^{b}	2.45±0.21^{ab}	2.11±0.01^{c}	2.54±0.08^{ab}	2.48±0.07^{ab}	2.72±0.02^{a}	2.1±0.03^{c}	1.96±0.06^{c}	0.53±0.02^{d}	88
V-massoia lactone	0.54±0.06^{de}	1.3±0.17^{b}	0.42±0.06^{e}	1.69±0.18^{a}	0.41±0.01^{e}	0.32±0.1^{e}	0.9±0.15^{c}	0.85±0.14^{cd}	0.89±0.05^{c}	11

Table S3. Continue

VI-vanillin	n.d.	9.57±0.33^{a}	n.d.	n.d.	n.d.	n.d.	11.9±0.8^{a}	10.67±1.54^{a}	12.47±3.76^{a}	200
VI-acetovanillone	n.d.	33.13±1.55^{a}	n.d.	29.85±0.94^{b}	n.d.	n.d.	31.17±0.96^{ab}	33.26±0.3^{a}	n.d.	1000
VI-syringadlehyde	n.d.	38.49±6.98^{a}	n.d.	n.d.	n.d.	n.d.	20.47±3.56^{b}	19.86±4.6^{b}	n.d.	50000
VII-o-cresol	4.97±0.29^{bc}	6.91±0.05^{a}	3.08±0.38^{f}	4.4±0.4^{cd}	3.43±0.21^{ef}	4.08±0.23^{de}	5.56±0.33^{b}	4.93±0.33^{bc}	3.12±0.19^{f}	120
VII-m-cresol	0.34±0.03^{ab}	0.36±0.01^{a}	0.3±0.0026^{bcd}	0.31±0.02^{abc}	0.28±0.01^{cd}	0.32±0.03^{abc}	0.32±0.01^{abc}	0.33±0.0049^{abc}	0.25±0.02^{d}	200
VII-guaiacol	0.56±0.04^{c}	1.06±0.08^{b}	0.37±0.02^{c}	1.05±0.1^{b}	0.5±0.07^{c}	0.43±0.02^{c}	1.35±0.09^{a}	1.24±0.12^{ab}	0.54±0.04^{c}	9.5
VII-*p*-propylguaiacol	0.05±0.00071^{ab}	0.05±0.0013^{a}	0.05±0.0011^{a}	0.04±0.0047^{ab}	0.04±0.0032^{b}	0.04±0.0045^{ab}	0.04±0.01^{b}	0.04±0.0028^{ab}	n.d.	10
VII-eugenol	2.6±0.09^{abc}	2.63±0.07^{ab}	2.3±0.2^{bcd}	2.18±0.13^{d}	2.26±0.1^{cd}	2.2±0.17^{d}	2.82±0.0024^{a}	2.84±0.05^{a}	1.1±0.17^{e}	6
VII-methoxyeugenol	1.51±0.07^{abc}	1.47±0.01^{bc}	1.56±0.13^{ab}	1.19±0.11^{d}	1.13±0.07^{d}	1.28±0.11^{cd}	1.56±0.06^{ab}	1.77±0.1^{a}	0.71±0.1^{e}	1200
VII-*trans*-isoeugenol	0.76±0.04^{c}	1.3±0.11^{a}	0.62±0.05^{c}	1.26±0.28^{ab}	0.89±0.13^{bc}	0.58±0.02^{c}	1.3±0.12^{a}	1.55±0.19^{a}	0.6±0.05^{c}	6
VII-syringol	0.62±0.0035^{c}	1.16±0.07^{b}	0.61±0.11^{c}	1.22±0.14^{ab}	0.56±0.03^{c}	0.53±0.05^{c}	1.47±0.13^{a}	1.43±0.1^{a}	0.61±0.05^{c}	570
VII-4-ethylphenol	1.54±1.69^{a}	0.24±0.02^{a}	0.28±0.08^{a}	0.22±0.02^{a}	0.23±0.09^{a}	0.53±0.57^{a}	0.27±0.02^{a}	0.24±0.01^{a}	0.19±0.02^{a}	440
VII-4-ethylguaiacol	0.07±0.01^{bcd}	0.09±0.01^{abc}	0.1±0.01^{ab}	0.04±0.01^{e}	0.06±0.01^{de}	0.06±0.01^{cde}	0.09±0.02^{abcd}	0.11±0.01^{a}	n.d.	33
VII-4-vinylguaicol	199.28±9.34^{cde}	251.12±31.18^{bcd}	167.09±4.69^{de}	421.99±68.63^{a}	263.16±20.7^{bcd}	186.07±22.45^{de}	303.39±77.1^{bc}	315.28±29.88^{ab}	130.97±3.66^{e}	1100
VII-4-vinylphenol	60.01±2.21^{bcd}	65.32±4.68^{abcd}	55.23±1.82^{cd}	71.9±9.09^{abc}	63.13±1.9^{abcd}	48.3±3.73^{d}	76.94±14.05^{ab}	78.42±5.86^{a}	54.44±2.75^{cd}	180
VIII-dihydromyrcenol	0.75±0.3^{ab}	0.96±0.24^{ab}	0.85±0.08^{ab}	1.17±0.07^{a}	0.86±0.01^{ab}	0.85±0.25^{ab}	1.02±0.15^{ab}	1.05±0.12^{ab}	0.6±0.1^{b}	

Values are expressed as mean value ± SD (n=3). Different letters in the same row denote significant differences between yeasts trains at $p< 0.05$ acording to Tuckey test HSD.*Is expressed as relative areas. n.d., indicates that the compound was no tdetected or below detection limits. I, acetate esters; II, ethyl esters; III, C13-norisoprenoids; IV, terpenes; V, lactones; VI, vanillin derivatives; VII, phenols; VIII, miscellneus.

Chapter 4

Table S1. Maximum fermentation rate (Vmax) and main metabolites analyzed in Albariño wines and young Tempranillo wines fermented by the different hybrids yeast strains and the commercial parental strain (LALL).

	Albariño wines							*Young Tempranillo wines*			
	LALL	**HKR1**	**HKR8**	**HKA4**	**HKA5**	**HUE2**	**HUE5**	**LALL**	**HCS3**	**HKA4**	**HUE5**
V max (g/L.h)	0.81±0.02^{a}	0.85±0.03^{a}	0.7±0.01^{c}	0.67±0.02^{c}	0.8±0.01^{ab}	0.73±0.02^{bc}	0.72±0.05^{c}	1.03±0.04^{b}	1.01±0.01^{b}	1.28±0.03^{a}	0.78±0.06^{c}
ethanol (%v/v)	12.89±0.01^{a}	12.87±0.03^{a}	12.86±0.08^{a}	12.69±0.02^{b}	12.72±0.04^{b}	12.71±0.05^{b}	12.64±0.06^{b}	14.32±0.03^{a}	14.46±0.06^{a}	14.1±0.04^{b}	13.89±0.07^{c}
Glycerol (g/L)	5.8±0.06^{e}	6.4±0.09^{c}	6.07±0.12^{d}	8.06±0.12^{a}	6.51±0.12^{c}	7.5±0.06^{b}	7.3±0.06^{b}	7.61±0.15^{c}	7.57±0.03^{c}	10.58±0.28^{a}	9.54±0.09^{b}
citric acid (g/L)	0.55±0.01^{b}	0.56±0.01^{b}	0.56±0.01^{b}	0.62±0.02^{a}	0.56±0.02^{b}	0.57±0.02^{b}	0.57±0.01^{b}	0.57±0.01^{c}	0.61±0.01^{b}	0.71±0.02^{a}	0.59±0.02^{bc}
malic acid (g/L)	3.31±0.03^{bc}	3.14±0.04^{d}	3.39±0.03^{b}	3.32±0.07^{bc}	3.24±0.03^{cd}	3.77±0.05^{a}	3.7±0.02^{a}	3.27±0.01^{a}	3.6±0.02^{a}	3.66±0.14^{a}	3.5±0.25^{a}
2,3-butanediol (g/L)	0.53±0.04^{ab}	0.47±0.11^{bc}	0.32±0.01^{d}	0.67±0.01^{a}	0.36±0.02^{cd}	0.47±0.06^{bc}	0.44±0.02^{bcd}	1.04±0.09^{b}	0.5±0.02^{c}	1.44±0.07^{a}	1.1±0.04^{b}
erythritol (g/L)	0.11±0.01^{e}	0.21±0.01^{c}	0.21±0.01^{cd}	0.19±0^{cd}	0.18±0.01^{d}	0.3±0.01^{b}	0.33±0.02^{a}	0.12±0.01^{b}	0.2±0.01^{b}	0.2±0.06^{b}	0.33±0.03^{a}
succinic acid (g/L)	0.47±0.02^{c}	0.52±0.07^{c}	0.51±0.02^{c}	0.78±0.01^{a}	0.62±0.05^{b}	0.87±0.01^{a}	0.84±0.01^{a}	0.61±0.03^{d}	0.82±0.01^{c}	0.95±0.08^{b}	1.13±0.04^{a}

Values are expressed as mean value ± SD (n=3). Different letters in the same row denote significant differences between yeast strains at $p < 0.05$ according to TuckeyHSD test

Table S2. Concentrations (μg/L) of volatile compounds determined in Albariño wines fermented by the different hybrids yeast strains and the commercial parental strain (LALL).

	***p*-value**	**LALL**	**HKR1**	**HKR8**	**HKA4**	**HKA5**	**HUE2**	**HUE5**
acetic acid	**0.00063**	438502.72±30018.21^{a}	395881.46±61727.21^{a}	381052.19±96893.61^{a}	206154.67±53711.18^{b}	313304.57±94726.26^{ab}	196638.22±34753.6^{b}	201912.21±17032.14^{b}
isobutyric acid	**0**	772.61±69.87^{c}	1451.24±305.14^{bc}	1415.85±262.73^{bc}	2049.73±163.56^{b}	1636.46±507.77^{bc}	5223.57±446.25^{a}	4916.91±351.03^{a}
isovaleric acid	**0**	1708.05±88.18^{b}	1859.43±445.59^{b}	1898.18±402.78^{b}	1220.28±95.27^{b}	2125.1±601.73^{b}	5153.64±359.84^{a}	4512±326.75^{a}
butyric acid	**0.00364**	1224.9±60.31^{a}	886.5±151.32^{ab}	956.25±190.7^{ab}	1015.86±205.04^{ab}	743.53±213.94^{b}	661.25±9.08^{b}	669.07±81.88^{b}
hexanoic acid	**0**	1347.11±43.39^{a}	952.74±102.45^{b}	923.67±151.91^{b}	689.72±70.94^{cd}	883.71±20.76^{bc}	552.26±36.81^{d}	554.37±47.41^{d}
octanoic acid	**0.00002**	448.81±37.03^{ab}	315.63±54.44^{bc}	356.37±49.66^{bc}	237.7±39.07^{c}	323.69±75.07^{bc}	509.89±62.01^{a}	573.65±36.51^{a}
decanoic acid	**0**	277.12±14.61^{a}	158.69±37.04^{bc}	204.53±42^{abc}	38.7±13.46^{d}	128.51±24.45^{cd}	221.44±53.81^{ab}	284.88±18.59^{a}
1-butanol	**0**	409.03±10.49^{ab}	308.63±23.51^{bc}	349.64±17.43^{abc}	432.3±55.15^{a}	304.56±71.51^{c}	158.38±12.32^{d}	159.57±2.82^{d}
isobutanol	**0**	20926.41±1424.9^{c}	32097.05±2879.36^{c}	32026.69±2857.17^{c}	119720.23±7566.21^{a}	28552.26±6859.92^{c}	80517.06±4623.35^{b}	76668.51±3391.16^{b}
isoamyl alcohol	**0.00001**	238711±5770.82^{c}	314238.07±39766.57^{b}	307515.67±32673.36^{bc}	267927.5±7022.71^{bc}	297937±40960.3^{bc}	425562.07±6818.57^{a}	389667.37±21669.67^{a}
β-phenylethanol	**0**	32228.26±550.89^{d}	60214.07±10540.23^{b}	54335.25±2126.38^{bc}	41376.18±945.73^{cd}	60015.85±6080.42^{b}	94641.86±3716.87^{a}	88706.88±5445.18^{a}
methionol	**0.00894**	4991.92±196.49^{b}	9339.53±1601.32^{a}	7320.59±1068.03^{ab}	7351.92±708.89^{ab}	8899.34±2093.53^{a}	7734.39±89.03^{ab}	7369.85±565.99^{ab}
benzyl alcohol	0.38169	93.64±9.15^{a}	94.25±8.21^{a}	81.92±18.2^{a}	80.42±6.63^{a}	79.66±10.19^{a}	86.08±4.93^{a}	82.26±5.52^{a}
isoamyl acetate	**0.04189**	48.75±4.88^{a}	83.48±24.89^{a}	51.99±9^{a}	58.13±17.49^{a}	83.83±14.4^{a}	76.58±13.19^{a}	79.95±15.66^{a}
isobutyl acetate	**0**	0.87±0.09^{e}	2.53±0.11^{cd}	1.92±0.14^{d}	5.77±0.28^{a}	2.65±0.33^{bc}	3.27±0.32^{b}	3.09±0.37^{bc}
β-phenylethyl acetate	**0**	62.82±3.31^{d}	193.13±13.1^{b}	126.15±6.25^{c}	103.06±2.88^{c}	210.47±6.88^{b}	364.78±19.54^{a}	363.83±17.11^{a}
ethyl acetate	**0.02104**	23613.91±1077.83^{a}	21415.46±926.63^{a}	22991.34±1118.24^{a}	16524.26±3116.02^{a}	23550.23±2068.87^{a}	18103.37±5154.82^{a}	19369.87±1470.28^{a}
ethyl butyrate	**0.0448**	24.8±3.17^{a}	22.71±0.94^{a}	27.18±6.56^{a}	26.66±6.5^{a}	26.89±2.04^{a}	17.9±2.43^{a}	18.05±3.25^{a}
trans-ethyl cinnamate	**0.00015**	0.13±0.04^{ab}	0.14±0.02^{ab}	0.060.01^{c}	0.17±0.03^{a}	0.08±0.02^{bc}	0.1±0.02^{bc}	0.05±0.02^{c}
ethyl decanoate		n.d.	n.d.	50.22±8.32	n.d.	n.d.	n.d.	n.d.
ethyl hexanoate	0.08751	88.71±8.54^{a}	87.73±27.16^{a}	105.72±15.37^{a}	70.14±20.69^{a}	99.97±18.82^{a}	74.88±9.02^{a}	67.32±6.1^{a}
ethyl isobutyrate	**0**	3.07±0.27^{b}	5.34±0.61^{b}	5.32±0.39^{b}	6.26±0.27^{b}	5.87±1.07^{b}	16.27±1.39^{a}	15.15±2.49^{a}
ethyl isovalerate	**0**	0.73±0.05^{bc}	0.73±0.12^{bc}	0.97±0.19^{b}	0.5±0.07^{c}	0.93±0.16^{bc}	2.2±0.17^{a}	1.97±0.27^{a}
ethyl lactate	**0**	1176.03±30.42^{c}	1290.96±135.91^{c}	1417.93±205.45^{c}	1971.9±109.93^{b}	1356.55±328.41^{c}	3084.15±84.27^{a}	2882.48±76.74^{a}
ethyl D/L-leucate	**0**	6.91±1.33^{b}	7.52±2.48^{b}	6.71±1.41^{b}	11.26±2.21^{b}	11.7±0.78^{b}	25.66±4.51^{a}	19.7±2.98^{a}
ethyl 2-methylbutyrate	**0**	0.39±0.1^{d}	0.46±0.11^{cd}	0.76±0.19^{bc}	0.45±0.05^{cd}	0.91±0.07^{b}	1.44±0.17^{a}	1.3±0.14^{a}
diethylsuccinate	0.21424	93.57±42.96^{a}	84.12±40.41^{a}	32.79±5.27^{a}	87.44±19.59^{a}	57.08±20.62^{a}	109.49±61.07^{a}	78.67±16.16^{a}
γ-butyrolactone	**0**	994.41±80.9^{b}	936.47±60.87^{b}	778.38±129.93^{b}	851.43±51.61^{b}	923.35±251.55^{b}	1633.77±147.84^{a}	1560.08±21.14^{a}
γ-decalactone	**0**	2.38±0.11^{a}	2.12±0.19^{a}	2.31±0.25^{a}	1.4±0.15^{b}	1.65±0.01^{b}	1.62±0.16^{b}	1.53±0.04^{b}
γ-nonalactone	**0.04227**	2.05±0.27^{a}	1.85±0.1^{ab}	1.99±0.33^{ab}	1.68±0.11^{ab}	1.53±0.03^{b}	1.83±0.11^{ab}	1.71±0.11^{ab}
γ-octalactone	**0**	0.37±0.17^{b}	0.75±0.33^{b}	0.61±0.24^{b}	0.32±0.02^{b}	0.81±0.15^{b}	3.05±0.71^{a}	2.65±0.21^{a}
massoia lactone	0.70185	2.2±0.72^{a}	1.95±0.6^{a}	1.84±0.51^{a}	1.78±0.17^{a}	1.6±0.35^{a}	1.88±0.3^{a}	1.61±0.16^{a}
β-ionone	**0.00196**	0.05±0.01^{bc}	0.05±0.01^{bc}	0.07±0.01^{a}	0.04±0.01^{c}	0.06±0.01^{abc}	0.06±0.01^{ab}	0.05±0.01^{bc}
β-damascenone	**0.00472**	0.68±0.04^{a}	0.67±0.05^{a}	0.63±0.02^{ab}	0.54±0.02^{b}	0.57±0.06^{ab}	0.61±0.05^{ab}	0.68±0.04^{a}

Table S2. Continue

	p-value	LALL	HKR1	HKR8	HKA4	HKA5	HUE2	HUE5
α-terpineol	0.11536	0.68±0.1^{a}	0.61±0.02^{a}	0.6±0.06^{a}	0.71±0.08^{a}	0.58±0.02^{a}	0.63±0.03^{a}	0.63±0.02^{a}
β-citronellol	**0.00196**	4.14±0.31^{b}	4.72±0.41^{ab}	4.14±0.19^{b}	5.64±0.72^{a}	4.19±0.24^{b}	4.38±0.36^{b}	3.92±0.33^{b}
geraniol	0.25656	5.23±0.73^{a}	7.59±1.98^{a}	6.93±0.19^{a}	8.41±1.05^{a}	8.16±0.1^{a}	4.7±2.69^{a}	6.71±3.94^{a}
nerol	0.52296	0.82±0.1^{a}	0.85±0.11^{a}	0.82±0.04^{a}	0.88±0.1^{a}	0.74±0.08^{a}	0.78±0.07^{a}	0.79±0.08^{a}
linalool	**0.00015**	4.33±0.17^{b}	4.56±0.14^{b}	4.19±0.14^{b}	5.69±0.73^{a}	4.68±0.09^{b}	3.94±0.19^{b}	4.2±0.04^{b}
R-limonene	0.16736	6.13±0.65^{a}	7±1.21^{a}	6.31±0.43^{a}	6.46±0.16^{a}	5.74±0.32^{a}	6.04±0.24^{a}	5.72±0.22^{a}
linalool oxide	**0.01627**	1.13±0.04^{ab}	1.25±0.07^{a}	1.08±0.05^{ab}	1.11±0.04^{ab}	1.05±0.04^{b}	1.17±0.07^{ab}	1.2±0.1^{ab}
(+)-rose oxide	0.17627	0.13±0.03^{a}	0.13±0.02^{a}	0.11±0.01^{a}	0.12±0.01^{a}	0.11±0.01^{a}	0.12±0.02^{a}	0.11±0.01^{a}
1,8-cineole	0.39876	0.2±0.02^{a}	0.19±0.05^{a}	0.24±0.05^{a}	0.19±0.03^{a}	0.23±0.07^{a}	0.22±0.03^{a}	0.26±0.02^{a}
3MH	**0**	107.97±26.6^{bc}	87.99±26.43^{bc}	160±31.35^{b}	527.6±56.29^{a}	154.75±5.47^{b}	84.14±35.06^{bc}	55.36±7.7^{c}
4MMP	**0**	24.45±2.05^{c}	55.91±8.32^{b}	73.95±1.55^{a}	61.25±6.64^{ab}	66.55±4.87^{ab}	25.76±8.37^{c}	39.8±0.8^{c}
3MHA	**0.00138**	46.06±10.2^{ab}	28.69±1.28^{c}	48.21±2.1^{a}	36.21±4.98^{abc}	44.38±2.44^{ab}	34.76±4.98^{abc}	32.54±1.93^{bc}
FFT		0.17±0.15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
dihydromyrcenol	0.17245	1.13±0.24^{a}	1.21±0.22^{a}	1.08±0.17^{a}	0.99±0.25^{a}	0.98±0.34^{a}	0.89±0.23^{a}	1.45±0.21^{a}
eugenol	**0.03704**	0.75±0.11^{ab}	0.69±0.18^{ab}	0.61±0.09^{ab}	0.64±0.08^{ab}	0.6±0.04^{b}	0.91±0.14^{a}	0.79±0.07^{ab}
guaiacol	0.06126	1.36±0.11^{a}	1.2±0.21^{a}	1.23±0.06^{a}	2.03±0.99^{a}	1.45±0.13^{a}	3.13±1.55^{a}	1.92±0.51^{a}
m-cresol	0.07908	0.75±0.17^{a}	0.79±0.15^{a}	0.69±0.12^{a}	0.8±0.23^{a}	0.93±0.07^{a}	1.06±0.09^{a}	0.74±0.12^{a}
o-cresol	0.22345	2.52±0.21^{a}	2.48±0.23^{a}	2.74±0.27^{a}	2.38±0.27^{a}	2.7±0.17^{a}	2.88±0.36^{a}	2.75±0.13^{a}
p-propylguaiacol	0.3574	0.16±0.05^{a}	0.16±0.06^{a}	0.12±0.03^{a}	0.12±0.02^{a}	0.11±0.01^{a}	0.11±0.02^{a}	0.11±0.02^{a}
trans-isoeugenol	**0.00057**	10.2±1.35^{b}	9.15±0.37^{b}	9.35±1.15^{b}	11.83±1.53^{b}	10.42±0.59^{b}	16.22±2.74^{a}	11.56±1.09^{b}
ethylphenol	0.60809	0.92±0.42^{a}	0.77±0.12^{a}	0.76±0.26^{a}	0.64±0.05^{a}	0.62±0.02^{a}	0.68±0.02^{a}	0.7±0.11^{a}
4-vinylguaiacol	**0.01671**	361.77±24.44^{b}	331.27±41.3^{b}	376.9±43.05^{ab}	378.53±64.54^{ab}	345.83±16.99^{b}	534.93±125.65^{a}	414.37±27.31^{ab}
4-vinylphenol	**0.02448**	47.41±2.88^{a}	44.72±5.69^{a}	51.32±3.17^{a}	51.73±7.57^{a}	57.4±6.16^{a}	76.92±24.95^{a}	78.65±19.51^{a}

Values are expressed as mean value ± SD (n=3). Different letters in the same row denote significant differences between yeast strains at $p < 0.05$ (marked in bold letters) according to Tuckey HSD test. 3MH, 3 mercaptohexanol; 4MMP, 4-mercapto-4-methylpentan-2-one; 3MHA, 3-mercpatohexyl acetate; FFT, 2-furfurylthiol. n.d. indicates that the compound was not detected or was below detection limits.

Table S3: Volatiles defined as active aroma vectors and their general aroma descriptor according to Ferreira et al., 2021. Mean (n=3) of the odor activity values (OAV) calculated from the ratio between the concentrations of each yeast in Albariño wines and their corresponding odor thresholds (OT) for each aroma compound.

General descriptor	Active Aroma Vector	Compounds	OT (µg/L)	OAVs Albariño						
				LALL	HKR1	HKR8	HKA4	HKA5	HUE2	HUE5
alcoholic / solvent	ethanol	ethanol	**11**	4083.33	4076.67	4076.67	4043.33	4030	4026.67	4006.67
	ethyl acetate	ethyl acetate	**12300**	1.92	1.74	1.87	1.34	1.91	1.47	1.57
	higher alcohols	β-phenylethanol	**14000**	2.3	4.3	3.88	2.96	4.29	6.76	6.34
		isoamyl alcohol	**30000**	7.96	10.47	10.25	8.93	9.93	14.19	12.99
		isobutanol	**40000**	0.52	0.8	0.8	2.99	0.71	2.01	1.92
		benzyl alcohol	**200**	0.47	0.47	0.41	0.4	0.4	0.43	0.41
		1-butanol	**150**	2.73	2.06	2.33	2.88	2.03	1.06	1.06
		methionol	**1000**	4.99	9.34	7.32	7.35	8.9	7.73	7.37
	Alcoholic / solvent total OAVs			*4104.22*	*4105.85*	*4103.53*	*4070.18*	*4058.17*	*4060.32*	*4038.33*
flowery	cinnamates	*trans* -ethyl cinnamate	**1**	0.12	0.13	0.05	0.16	0.07	0.09	0.05
	ionones	β-ionone	**0.09**	0.55	0.51	0.83	0.51	0.61	0.67	0.61
	β-phenylethyl acetate	β-phenylethyl acetate	**250**	0.25	0.77	0.5	0.41	0.84	1.46	1.46
	terpenes	(+)-*cis*/*trans* -rose oxide	**80**	0.0016	0.0017	0.0014	0.0015	0.0013	0.0015	0.0013
		β-citronellol	**40**	0.1	0.12	0.1	0.14	0.1	0.11	0.1
		geraniol	**30**	0.17	0.25	0.23	0.28	0.27	0.16	0.22
		linalool	**25.2**	0.17	0.18	0.17	0.23	0.19	0.16	0.17
		nerol	**2**	0.003	0.003	0.003	0.003	0.003	0.003	0.003
		1,8-cineole	**1.1**	0.19	0.17	0.22	0.17	0.21	0.2	0.23
		R-limonene	**34**	0.18	0.21	0.19	0.19	0.17	0.18	0.17
		α-terpineol	**250**	0.003	0.002	0.002	0.003	0.002	0.003	0.003
	Flowery total OAVs			*1.737*	*2.3469*	*2.2965*	*2.0972*	*2.4661*	*3.0366*	*3.0164*
citric / green	polyfunctional mercaptans	3-mercaptohexyl acetate	**4**	11.51	7.17	12.05	9.05	11.09	8.69	8.14
		3-mercaptohexanol	**60**	1.8	1.47	2.67	8.79	2.58	1.4	0.92
		4-methyl-4-mercaptopentan-2-one	**0.8**	30.56	69.89	92.44	76.56	83.19	32.2	49.74
	Citric/green total OAVs			*43.87*	*78.53*	*107.16*	*94.4*	*96.86*	*42.29*	*58.8*
fruity	acetates	isoamyl acetate	**30**	1.62	2.78	1.73	1.94	2.79	2.55	2.67
		isobutyl acetate	**1605**	0.0005	0.0016	0.0012	0.0036	0.0016	0.002	0.0019
	β-damascenone	β-damascenone	**0.05**	13.64	13.4	12.55	10.79	11.46	12.21	13.47
	ethyl esters	ethyl 2-methylbutyrate	**18**	0.02	0.03	0.04	0.02	0.05	0.08	0.07
		ethyl butyrate	**125**	0.21	0.22	0.18	0.22	0.14	0.14	0.2
		ethyl hexanoate	**62**	1.43	1.41	1.71	1.13	1.61	1.21	1.09
		ethyl isobutyrate	**15**	0.2	0.36	0.35	0.42	0.39	1.08	1.01
		ethyl isovalerate	**3**	0.24	0.24	0.32	0.17	0.31	0.73	0.66
		ethyl D/L-leucate	**900**	0.01	0.01	0.01	0.01	0.01	0.03	0.02
		diethyl succinate	**200000**	0.00047	0.00042	0.00016	0.00044	0.00029	0.00055	0.00039
		ethyl decanoate	**200**	0.08	0	0.25	0	0	0	0
	γ-lactones	γ-nonalactone	**30**	0.07	0.06	0.07	0.06	0.05	0.06	0.06
		γ-decalactone	**88**	0.03	0.02	0.03	0.02	0.02	0.02	0.02
		γ-octalactone	**238**	0	0	0	0	0	0.01	0.01
	Fruity total OAVs			*17.55251*	*18.53512*	*17.24396*	*14.78534*	*16.83529*	*18.12255*	*19.28229*
lactic / acid	branched acids	isobutyric acid	**2300**	0.34	0.63	0.62	0.89	0.71	2.27	2.14
		isovaleric acid	**33**	51.76	56.35	57.52	36.98	64.4	156.17	136.73
	linear fatty acids	decanoic acid	**1000**	0.28	0.16	0.2	0.04	0.13	0.22	0.28
		hexanoic acid	**420**	3.21	2.27	2.2	1.64	2.1	1.31	1.32
		octanoic acid	**500**	0.9	0.63	0.71	0.48	0.65	1.02	1.15
		butyric acid	**173**	7.08	5.12	5.53	5.87	4.3	3.82	3.87
	Lactic/ acid total OAVs			*63.57*	*65.16*	*66.78*	*45.9*	*72.29*	*164.81*	*145.49*
spice / woody	volatile phenols	guaiacol	**30**	0.14	0.13	0.13	0.21	0.15	0.33	0.2
		trans -isoeugenol	**6**	1.7	1.53	1.56	1.97	1.74	2.7	1.93
		eugenol	**6**	0.13	0.12	0.1	0.11	0.1	0.15	0.13
		p -propylguaiacol	**200**	0.02	0.02	0.01	0.01	0.01	0.01	0.01
	Spice / woody total OAVs			*1.99*	*1.8*	*1.8*	*2.3*	*2*	*3.19*	*2.27*

Table S4. Concentrations (μg/L) of volatile compounds determined in young Tempranillo wines fermented by the different hybrids yeast and the commercial parental strain (LALL).

	***p*-value**	**LALL**	**HCS3**	**HKA4**	**HUE5**
acetic acid	**0.000194**	704216.05±118862.25^{a}	304335.02±13950.94^{bc}	454935.78±27106.31^{b}	270911.25±58164.91^{c}
butyric acid	**0.010476**	780.61±113.72^{ab}	973.99±86.17^{a}	528.04±53.99^{b}	663.16±184.76^{ab}
isobutyric acid	**0.000118**	737.4±119.92^{b}	1339±82.15^{b}	1682.63±277.85^{b}	7203.74±1900.71^{a}
isovaleric acid	**0.002955**	1025.02±82.1^{b}	1332.31±407.82^{b}	1071.82±196.05^{b}	3222.12±974^{a}
hexanoic acid	**0.000644**	766.9±38.7^{a}	491.95±43.15^{bc}	364.73±15.65^{c}	597.22±125.21^{ab}
octanoic acid	0.078657	280.79±66.23^{a}	335.75±77.43^{a}	221.09±14.05^{a}	355.63±52.05^{a}
decanoic acid	**0.001237**	171.2±44.25^{b}	115.95±39.76^{b}	57.78±7.69^{b}	381.77±111.84^{a}
1-butanol	**0.000003**	702.8±93.56^{b}	1182.26±71.33^{a}	813.67±30.51^{b}	355.67±52.05^{c}
1-hexanol	0.543335	21.45±4.14^{a}	24.42±3.27^{a}	23.39±0.84^{a}	24.76±2.49^{a}
isobutanol	**0.000005**	19971.24±2441.25^{c}	30515.18±1571.94^{c}	67800.38±6798.47^{b}	98234.03±13251.79^{a}
methionol	**0.000025**	3105.51±478.94^{b}	8268.49±599.16^{a}	3857.07±235.17^{b}	3738.95±943.19^{b}
isoamyl alcohol	**0.000007**	192424.68±9286.85^{b}	363892.77±19700.49^{a}	224756.23±3520.3^{b}	338279.55±29883.64^{a}
benzyl alcohol	0.328257	115.55±12.91^{a}	104.72±24.48^{a}	93.56±2.07^{a}	116.07±15.68^{a}
β-phenylethanol	**0.000006**	34740.24±360.25^{b}	103216.89±10005.67^{a}	38568.29±1658.88^{b}	116762.58±15399.78^{a}
isobutyl acetate	**0.000535**	0.29±0.04^{b}	0.53±0.1^{b}	2±0.58^{a}	0.54±0.2^{b}
isoamyl acetate	**0.002163**	133.72±33.58^{ab}	190.22±36.11^{a}	92.97±15.62^{b}	68.85±4.9^{b}
β-phenylethyl acetate	**0.000002**	157.67±8.05^{b}	197.57±16.13^{b}	330.16±12.79^{a}	340.34±26.25^{a}
ethyl acetate	**0.000009**	31450.31±467.02^{a}	24803.37±618.76^{b}	25209.63±2126.71^{b}	17526.45±1281.13^{c}
ethyl butyrate	**0.001744**	25.58±5.06^{a}	22.84±2.36^{a}	13.45±3.03^{b}	11.03±2.1^{b}
ethyl isobutyrate	**0.000005**	2.62±0.19^{a}	1.43±0.19^{b}	1.25±0.17^{b}	0.74±0.09^{c}
ethyl isovalerate	**0**	0.29±0.03^{d}	0.43±0.01^{c}	1±0.02^{a}	0.59±0.01^{b}
ethyl 2-methylbutyrate	**0.000294**	0.56±0.04^{b}	0.55±0.02^{b}	0.75±0.05^{a}	0.57±0.01^{b}
ethyl D/L-leucate	**0.000006**	13.06±0.19^{c}	13.33±2.47^{c}	28.96±1.55^{a}	19.62±1.37^{b}
ethyl hexanoate	**0.0016**	73.53±4.77^{a}	59.98±5.5^{ab}	38.98±7.97^{c}	48.18±8.87^{bc}
ethyl octanoate	**0.002839**	19.56±4.43^{a}	21.65±7.24^{a}	n.d.	17.81±5.6^{a}
ethyl decanoate	**0.000063**	54.86±8.45^{ab}	34.85±5.97^{b}	n.d.	65.54±13.48^{a}
trans-ethyl cinnamate	**0**	0.63±0.05^{a}	0.18±0.02^{c}	0.37±0.03^{b}	0.14±0.02^{c}
ethyl lactate	**0.000051**	2101.43±308.82^{b}	3129.32±205.43^{b}	2629.65±84.84^{b}	5373.12±730.42^{a}
diethyl succinate	**0.000139**	175.12±7.5^{b}	191.16±2.62^{b}	167.72±9.87^{b}	394.31±70.2^{a}
γ-butyrolactone	**0.005079**	2079.77±276.66^{ab}	2660.95±226.37^{a}	1494.99±29.67^{b}	2376.68±429^{a}
γ-octalactone	**0.000021**	0.12±0.01^{b}	0.19±0.04^{b}	1.35±0.3^{a}	1.18±0.13^{a}
γ-nonalactone	0.104924	1.53±0.03^{a}	1.69±0.12^{a}	1.57±0.01^{a}	1.61±0.07^{a}
γ-decalactone	0.531126	1.04±0.13^{a}	1.14±0.16^{a}	1.00±0.1^{a}	1.04±0.06^{a}
massoia lactone	**0.012496**	2.05±0.15^{b}	2.91±0.45^{a}	2.13±0.15^{b}	2.05±0.22^{b}
α-ionone	**0.000005**	0.04±0.0035^{b}	0.05±0.0047^{b}	0.17±0.03^{a}	n.d.
β-ionone	0.476866	0.06±0.01^{a}	0.07±0.01^{a}	0.07±0.01^{a}	0.08±0.01^{a}
β-damascenone	**0.026516**	0.48±0.04^{ab}	0.53±0.05^{ab}	0.46±0.03^{b}	0.56±0.01^{a}
TDN	0.4184	0.16±0.01^{a}	0.18±0.06^{a}	0.15±0.01^{a}	0.14±0.01^{a}
α-terpineol	**0.000039**	0.55±0.02^{a}	0.49±0.03^{ab}	0.32±0.03^{c}	0.46±0.02^{b}
β-citronellol	**0.000003**	2.7±0.08^{b}	2.35±0.26^{b}	2.52±0.11^{b}	4.47±0.25^{a}
geraniol	**0.026454**	4.59±0.36^{ab}	5.9±1.58^{ab}	3.94±0.31^{b}	6.37±0.38^{a}
nerol	**0.036665**	0.67±0.05^{ab}	0.6±0.05^{ab}	0.48±0.08^{b}	0.71±0.11^{a}
R-limonene	0.897387	8.82±0.53^{a}	9.42±1.87^{a}	8.88±0.1^{a}	8.96±0.88^{a}
linalool	**0.00001**	3.13±0.19^{a}	2.52±0.19^{b}	1.47±0.08^{c}	2.92±0.2^{ab}
linalool oxide	0.645273	0.66±0.08^{a}	0.73±0.17^{a}	0.75±0.11^{a}	0.65±0.07^{a}
(+)-rose oxide	0.189088	0.08±0.01^{a}	0.09±0.01^{a}	0.08±0.0015^{a}	0.08±0.0018^{a}
eugenol	**0.001921**	0.37±0.02^{b}	0.4±0.02^{b}	0.51±0.01^{a}	0.42±0.05^{b}
guaiacol	0.142742	58.3±6.45^{a}	94.23±27.45^{a}	76.7±26.38^{a}	57.99±2.29^{a}
o-cresol	0.243959	2.09±0.09^{a}	1.81±0.24^{a}	1.87±0.28^{a}	1.74±0.14^{a}
m-cresol	**0.006835**	0.99±0.11^{b}	1.05±0.27^{b}	2.09±0.21^{a}	1.27±0.48^{b}
methoxyeugenol	0.066864	5.09±0.36^{a}	4.97±0.2^{a}	6.46±1.24^{a}	5.03±0.21^{a}
p-propylguaiacol	0.174227	0.16±0.02^{a}	0.15±0.02^{a}	0.14±0.02^{a}	0.13±0.01^{a}
syringol	0.222811	183.66±10.88^{a}	167.51±28.48^{a}	237.57±80.04^{a}	164.02±17.98^{a}
trans-isoeugenol	0.202715	21.85±0.73^{a}	23.53±1.65^{a}	25.8±3^{a}	22.74±2.35^{a}
4-vinylguaicol	**0.012674**	114.04±8.04^{a}	107.32±7.25^{a}	86.91±1.92^{b}	105.58±10.55^{ab}
4-vinylphenol	**0.017824**	3505.16±135.79^{a}	2914.79±140.56^{b}	3068.18±333.99^{ab}	2770.62±216.58^{b}
ethylphenol	0.061944	0.58±0.06^{a}	0.66±0.05^{a}	0.67±0.05^{a}	0.56±0.03^{a}
acetoin	**0.002338**	1352.55±401.96^{a}	452.51±110.15^{b}	463.37±30.13^{b}	1541.08±388.63^{a}
diacetyl	**0.000001**	292.13±39.31^{b}	585.19±74.76^{b}	2247.2±123.56^{a}	605.43±265.53^{b}
dihydromyrcenol	0.409187	1.89±0.11^{a}	2.15±0.49^{a}	1.75±0.22^{a}	1.9±0.11^{a}

Values are expressed as mean value ± SD (n=3). Different letters in the same row denote significant differences between yeast strains at $p < 0.05$ (marked in bold letters) according to Tuckey HSD test, n.d. indicates that the compound was not detected or was below detection limits.

Table S5. Concentrations (µg/L) of volatile compounds determined in aged Tempranillo wines fermented by the different hybrids yeast strains and the commercial parental strain (LALL).

	***p*-value**	**LALL**	**HCS3**	**HKA4**	**HUE5**
isobutyl acetate	**0**	3.09±0.48^{c}	1.93±0.22^{d}	13.72±0.43^{a}	7.57±0.39^{b}
β-phenylethyl acetate	**0.003**	38.65±1.03^{c}	56.15±7.06^{ab}	40.83±2^{bc}	61.95±9.15^{a}
ethyl isobutyrate	**0**	112.32±24.62^{c}	170.78±16.62^{bc}	311.89±24.04^{b}	936.24±123.34^{a}
ethyl isovalerate	**0**	44.74±3.16^{c}	67.61±2.9^{b}	37.14±2.83^{c}	129.24±11.43^{a}
ethyl 2-methylbutyrate	**0**	26.01±1.74^{c}	37.3±1.45^{b}	37.26±1.51^{b}	93±6.72^{a}
ethyl 4-methylvalerate	0.14511	0.23±0.05^{a}	0.2±0.02^{a}	0.14±0.05^{a}	0.17±0.05^{a}
ethyl D/L-leucate	**0.00002**	130.24±3.54^{c}	227.55±7.11^{a}	177.69±16.05^{b}	246.74±18.77^{a}
trans-ethyl cinnamate	**0**	2.53±0.36^{c}	1.78±0.2^{c}	9.42±0.71^{a}	3.73±0.4^{b}
γ-octalactone	**0**	0.15±0.0044^{b}	1.77±0.12^{a}	0.14±0.01^{b}	1.61±0.33^{a}
γ-nonalactone	0.21236	2.49±0.07^{a}	2.32±0.08^{a}	2.42±0.02^{a}	2.16±0.35^{a}
γ-decalactone	0.0757	1.69±0.15^{a}	1.59±0.07^{a}	1.41±0.07^{a}	1.29±0.29^{a}
massoia lactone	0.91035	1.1±0.15^{a}	1.19±0.04^{a}	1.12±0.13^{a}	1.13±0.25^{a}
β-ionone	0.34164	0.04±0.004^{a}	0.04±0.0025^{a}	0.05±0.0017^{a}	0.04±0.01^{a}
β-damascenone	**0.00013**	0.95±0.03^{a}	0.81±0.02^{b}	0.86±0.01^{ab}	0.62±0.08^{c}
TDN	**0.017**	76.32±6.92^{a}	69.04±3.66^{ab}	73.08±1.89^{ab}	62.57±1.53^{b}
riesling acetal	0.31151	0.1±0.01^{a}	0.09±0.0013^{a}	0.1±0.00078^{a}	0.09±0.01^{a}
vitispirane a and b	0.07605	0.49±0.0046^{a}	0.46±0.01^{a}	0.48±0.0037^{a}	0.46±0.03^{a}
α-terpineol	**0.00006**	0.65±0.07^{a}	0.66±0.03^{a}	0.58±0.02^{a}	0.37±0.02^{b}
β-citronellol	**0.03993**	0.42±0.1^{a}	0.38±0.03^{ab}	0.33±0.05^{ab}	0.26±0.03^{b}
geraniol	0.10162	0.85±0.23^{a}	0.67±0.02^{a}	0.77±0.08^{a}	0.56±0.05^{a}
R-limonene	0.68975	5.27±0.7^{a}	4.96±0.44^{a}	5.41±0.29^{a}	5.05±0.47^{a}
linalool	**0.02352**	0.14±0.02^{a}	0.11±0.02^{ab}	0.11±0.02^{ab}	0.08±0.0033^{b}
linalool oxide	0.05018	5.09±0.62^{a}	3.98±0.37^{b}	4.42±0.2^{ab}	4.65±0.27^{ab}
(+)-rose oxide	0.82711	0.13±0.02^{a}	0.12±0.01^{a}	0.13±0.01^{a}	0.13±0.02^{a}
1,8-cineole	0.69135	0.19±0.05^{a}	0.14±0.03^{a}	0.16±0.03^{a}	0.17±0.08^{a}
eugenol	0.19317	0.4±0.11^{a}	0.3±0.0044^{a}	0.32±0.04^{a}	0.41±0.05^{a}
guaiacol	**0.01688**	3.46±0.19^{ab}	2.98±0.08^{b}	3.96±0.4^{a}	3.39±0.33^{ab}
o-cresol	0.25812	2.22±0.26^{a}	2.3±0.14^{a}	2.68±0.24^{a}	2.42±0.39^{a}
m-cresol	**0.00302**	1.27±0.21^{b}	1.48±0.5^{b}	1.14±0.13^{b}	2.41±0.2^{a}
methoxyeugenol	0.46382	9±1.63^{a}	10.08±1.82^{a}	8.75±1.78^{a}	10.94±1.97^{a}
p-propylguaiacol	0.28168	0.14±0.03^{a}	0.12±0.0036^{a}	0.14±0.02^{a}	0.12±0.01^{a}
trans-isoeugenol	0.49584	8.31±2.62^{a}	6.57±0.46^{a}	6.8±1.05^{a}	7.11±0.3^{a}
syringol	0.55514	20.52±4.97^{a}	19.51±1.92^{a}	23.65±3.31^{a}	21.12±3.28^{a}
4-vinylguaicol	0.51173	4.75±0.58^{a}	4.76±0.54^{a}	5.38±0.89^{a}	5.26±0.37^{a}
4-vinylphenol	**0.00743**	21.36±0.57^{b}	30.12±4.68^{ab}	37.47±6.26^{a}	30.82±0.96^{ab}
ethylphenol	0.81779	0.3±0.17^{a}	0.23±0.03^{a}	0.29±0.14^{a}	0.23±0.04^{a}
vanillin	0.93379	13.76±2.64^{a}	14.2±0.78^{a}	14.59±2.03^{a}	15.08±3.93^{a}
acetovanillone	0.52539	17.58±1.4^{a}	16.25±0.39^{a}	15.67±1.74^{a}	17.97±3.53^{a}
syringaldehyde	0.12172	446.37±112.91^{a}	629.69±59.78^{a}	508.69±82.33^{a}	536.86±58.23^{a}
dihydromyrcenol	0.09154	1.41±0.38^{a}	1.3±0.14^{a}	1.14±0.12^{a}	0.91±0.07^{a}

Values are expressed as mean value ± SD (n=3). Different letters in the same row denote significant differences between yeast strains at $p< 0.05$ (marked in bold letters) according to Tuckey HSD test. n.d. indicates that the compound was not detected or was below detection limits.

Table S6. Volatiles defined as active aroma vectors and their general aroma descriptor according to Ferreira et al., 2021. Mean (n=3) of the odor activity values (OAV) calculated from the ratio between the concentrations of each yeast in Tempranillo young wines and their corresponding odor thresholds (OT) for each aroma compound.

General descriptor	Active Aroma Vector	compounds	OT (µg/L)	OAVs Tempranillo young wines			
				LALL	HCS3	HKA4	HUE5
alcoholic / solvent	ethanol	ethanol	**11**	4537.543	4580.847	4468.89	4400.127
	ethyl acetate	ethyl acetate	**12300**	2.557	2.017	2.047	1.423
	higher alcohols	β-phenylethanol	**14000**	2.48	7.373	2.753	8.34
		isoamyl alcohol	**30000**	6.417	12.13	7.493	11.273
		isobutanol	**40000**	0.5	0.76	1.697	2.457
		benzyl alcohol	**200**	0.577	0.523	0.47	0.58
		1-butanol	**150**	4.687	7.883	5.427	2.37
		methionol	**1000**	3.103	8.27	3.86	3.74
	Alcoholic / solvent total OAVs			*4557.864*	*4619.803*	*4492.637*	*4430.31*
flowery	cinnamates	*trans*-ethyl cinnamate	**1**	0.573	0.163	0.337	0.13
	ionones	α-ionone	**2.6**	0.017	0.02	0.063	0
	β-phenylethyl acetate	β-ionone	**0.09**	0.71	0.83	0.78	0.85
	terpenes	β-phenylethyl acetate	**250**	0.633	0.79	1.323	1.363
		(+)-*cis/trans*-rose oxide	**80**	0.0009758	0.001169	0.0010585	0.0009782
		β-citronellol	**40**	0.07	0.057	0.063	0.113
		geraniol	**30**	0.153	0.2	0.133	0.213
		linalool	**25.2**	0.123	0.097	0.06	0.113
		nerol	**2**	0.0022333	0.002	0.0016	0.0023667
		1,8-cineole	**1.1**				
		R-limonene	**34**	0.26	0.277	0.26	0.263
		α-terpineol	**250**	0.00219	0.0019433	0.00129	0.0018267
	Flowery total OAVs			*2.5443991*	*2.439113*	*3.0229485*	*3.0501716*
fruity	acetates	isoamyl acetate	**30**	4.46	6.34	3.1	2.297
		isobutyl acetate	**1605**	0	0	0	0
	β-damascenone	β-damascenone	**0.05**	9.563	10.563	9.18	11.29
	ethyl esters	ethyl 2-methylbutyrate	**18**	0.03	0.03	0.04	0.03
		ethyl butyrate	**125**	0.207	0.183	0.11	0.087
		ethyl hexanoate	**62**	1.187	0.967	0.63	0.777
		ethyl octanoate	**580**	0.033	0.04	0	0.03
		ethyl isobutyrate	**15**	0.173	0.097	0.087	0.047
		ethyl isovalerate	**3**	0.1	0.14	0.337	0.193
		ethyl D/L-leucate	**900**	0.01	0.013	0.03	0.02
		diethyl succinate	**200000**	0.0008756	0.0009558	0.0008386	0.0019716
		ethyl decanoate	**200**	0.273	0.173	0	0.327
	γ-lactones	γ-nonalactone	**30**	0.05	0.053	0.05	0.053
		γ-decalactone	**88**	0.01	0.013	0.01	0.01
		γ-octalactone	**238**	0	0	0.007	0.003
	Fruity total OAVs			*16.0968756*	*18.6129558*	*13.5818386*	*15.1659716*
lactic / acid	diacetyl	diacetyl	**100**	2.921	5.852	22.472	6.054
	branched acids	isobutyric acid	**2300**	0.321	0.582	0.732	3.132
		isovaleric acid	**33**	31.061	40.373	32.479	97.64
	linear fatty acids	decanoic acid	**1000**	0.171	0.116	0.058	0.382
		hexanoic acid	**420**	1.826	1.171	0.868	1.422
		octanoic acid	**500**	0.562	0.671	0.442	0.711
		butyric acid	**173**	4.512	5.63	3.052	3.833
	Lactic/ acid total OAVs			*38.453*	*48.543*	*37.631*	*107.12*
spice / woody	volatile phenols	guaiacol	**30**	6.137	9.919	8.073	6.104
		trans-isoeugenol	**6**	3.641	3.922	4.3	3.79
		eugenol	**6**	0.062	0.067	0.086	0.07
		methoxyeugenol	**1200.00**	0.004	0.004	0.005	0.004
		p-propylguaiacol	**200**	0.016	0.015	0.014	0.013
	Spice / woody total OAVs			*9.86*	*13.927*	*12.478*	*9.981*

Table S7. Volatiles defined as active aroma vectors and their general aroma descriptor according to Ferreira et al., 2021. Mean (n=3) of the odor activity values (OAV) calculated from the ratio between the concentrations of each yeast in Tempranillo aged wines and their corresponding odor thresholds (OT) for each aroma compound.

General descriptor	Active Aroma Vector	compounds	OT (µg/L)	OAVs Tempranillo agedwines			
				LALL	HCS3	HKA4	HUE5
flowery	cinnamates	*trans*-ethylcinnamate	**1**	2.30	1.62	8.56	3.39
	ionones	β-ionone	**0.09**	0.50	0.48	0.54	0.46
	β-phenylethylacetate	β-phenylethyl acetate	**250**	0.16	0.22	0.16	0.25
	terpenes	(+)-*cis/trans*-rose oxide	**80**	0.0017	0.0016	0.0016	0.0016
		β-citronellol	**40**	0.010	0.010	0.008	0.006
		geraniol	**30**	0.030	0.020	0.027	0.020
		linalool	**25.2**	0.0054	0.0043	0.0042	0.0033
		1,8-cineole	**1.1**	0.17	0.13	0.15	0.15
		R-limonene	**34**	0.16	0.14	0.16	0.15
		α-terpineol	**250**	0.0026	0.0027	0.0023	0.0015
		Flowery total OAVs		*3.32*	*2.64*	*9.62*	*4.43*
fruity	acetates	isobutylacetate	**1605**	0.0019	0.0012	0.0086	0.0047
	β-damascenone	β-damascenone	**0.05**	18.9	16.3	17.2	12.4
	ethyl esters	ethyl 2-methylbutyrate	**18**	1.45	2.07	2.07	5.16
		ethyl 4-methylvalerate	**10**	0.023	0.020	0.013	0.017
		ethyl isobutyrate	**15**	7.49	11.38	20.79	62.42
		ethylisovalerate	**3**	14.92	22.54	12.38	43.08
		ethyl D/L-leucate	**900**	0.15	0.25	0.20	0.27
	ɣ-lactones	ɣ-nonalactone	**30**	0.08	0.08	0.08	0.07
		ɣ-decalactone	**88**	0.020	0.020	0.020	0.013
		ɣ-octalactone	**238**	0.0007	0.0074	0.0006	0.0068
		Fruity total OAVs		*43.03*	*52.65*	*52.72*	*123.46*
spice / woody	volatile phenols	guaiacol	**30**	0.36	0.31	0.42	0.36
		trans-isoeugenol	**6**	1.39	1.10	1.13	1.18
		eugenol	**6**	0.07	0.05	0.05	0.07
		methoxyeugenol	**1200**	0.01	0.01	0.01	0.01
		p-propylguaiacol	**200**	0.013	0.010	0.013	0.010
	vanillin derivatives	vanillin	**200**	0.067	0.070	0.073	0.077
		acetovanillone	**1000**	0.020	0.020	0.017	0.020
	TDN	TDN	**2**	38.16	34.52	36.54	31.29
		Spice / woody total OAVs		*40.1*	*40.08*	*36.09*	*38.26*

Annex II

Publications

Food Microbiology 97 (2021) 103763

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Screening of *Saccharomyces* strains for the capacity to produce desirable fermentative compounds under the inf uence of different nitrogen sources in synthetic wine fermentations

Dolores Pérez[a,b,c], Inés Jaehde[c,d], José Manuel Guillamón[c], José María Heras[a], Amparo Querol[c,*]

[a] Lallemand Bio S.L., 08028, Barcelona, Spain
[b] Estación Experimental Agropecuaria Mendoza (EEA), Instituto Nacional de Tecnología Agropecuaria (INTA), 5507, Luján de Cuyo, Mendoza, Argentina
[c] Departamento de Biotecnología de Los Alimentos, Grupo de Biología de Sistemas en Levaduras de Interés Biotecnológico, Instituto de Agroquímica y Tecnología de Los Alimentos (IATA)-CSIC, 46980, Valencia, Spain
[d] University of Bonn, Regina-Pacis-Weg 3, 53113, Bonn, Germany

ARTICLE INFO

Keywords:
S. uvarum
S. kudriavzevii
S. eubayanus
Fermentative aroma compound
Nitrogen source
Branched-chain amino acids
Non-volatile metabolic end-products

ABSTRACT

A collection of 33 *Saccharomyces* yeasts were used for wine fermentation with a sole nitrogen source and four individual aroma-inducing amino acids. The fermentation performance and chemical wine were evaluated. The most valuable nitrogen sources were valine as a fermentation promoter on n strains, phenylalanine as fruity aromas enhancer whereas the ethanol yield was lessened by isoleucine. *S. cerevisiae* SC03 and *S. kudriavzevii* SK02 strains showed to be the greatest producers of esters while *S. kudriavzevii* strains SK06 and SK07 by shortening the fermentation duration. *S. uv* produced the greatest succinic acid amounts and, together with *S. eubayanus*, they reached the duction of 2-phenylethanol and its acetate ester; whereas *S. kudriavzevii* strains were found to b related to high glycerol production.

1. Introduction

Nowadays, wine consumers look for new sensory prof les, and market trends are guided by higher aromatic intensity, fruit forward, freshness, and lower alcohol content (Querol et al., 2018). The higher alcohol level is concerning for wine drinkers because of its negative impact on health (Lindberg and Amsterdam, 2008). On the other hand, associated with climate change events, the grapes have higher levels of sugar, fewer organic acids, and alterations in aroma and phenolic synthesis (Drappier et al., 2019). Therefore, winemakers have to come up with enological strategies that mitigate the high potential alcoholic grade or other factors affecting wine quality.

During winemaking, alcoholic fermentation is a complex process where the principal reaction is the transformation of grape hexoses into ethanol and carbon dioxide by yeasts (Fleet, 1998). *Saccharomyces cerevisiae* is the most frequently used species in the wine industry; it is well adapted to must and fermentative conditions like high sugar concentration, high ethanol content, and low levels of pH. Besides they produce rapid and predictable fermentations (Rodríguez et al., 20 et al., 2018). However, other species of the genus *Saccharom* *S. kudriavzevii*, *S. uvarum*, and *S. eubayanus* are also able to fer must. These yeast species are particularly known for being cr causing high glycerol and low ethanol concentrations, and nitrogen requirements (Tronchoni et al., 2012; Su et al., 201 et al., 2020). Moreover, they can produce higher amounts o tive aromatic compounds like higher alcohols and ace (Gamero et al., 2014; Stribny et al., 2015). These charact inf uence the composition of the f nal wine contributing to opment of new styles that satisfy the consumer preferences high intensity of fruity aromas, lower alcohol content, and im of the mouthfeel.

An essential step during winemaking is the manageme nutrition with nitrogen supplements. Yeasts, under anaer tions, consume nitrogen in the forms of ammonium cation acids, and some small peptides which all are described by the yeast assimilable nitrogen (Ugliano et al., 2007; Waterho

* Corresponding author.
E-mail address: aquerol@iata.csic.es (A. Querol).

https://doi.org/10.1016/j.fm.2021.103763
Received 19 September 2020; Received in revised form 3 February 2021; Accepted 7 February 2021

International Journal of Food Microbiology 365 (2022) 109554

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Effect of non-wine *Saccharomyces* yeasts and bottle aging on the release and generation of aromas in semi-synthetic Tempranillo wines

Dolores Pérez [a,b], Marie Denat [c], José María Heras [a], José Manuel Guillamón [d], Vicente Ferreira [c], Amparo Querol [d,*]

[a] Lallemand Bio S.L., 08028 Barcelona, Spain
[b] Estación Experimental Agropecuaria Mendoza (EEA), Instituto Nacional de Tecnología Agropecuaria (INTA), 5507 Luján de Cuyo, Mendoza, Argentina
[c] Laboratory for Aroma Analysis and Enology (LAAE), Department of Analytical Chemistry, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), Universidad de Zaragoza, c/Pedro Cerbuna 12, 50009 Zaragoza, Spain
[d] Departamento de Biotecnología de Los Alimentos, Grupo de Biología de Sistemas en Levaduras de Interés Biotecnológico, Instituto de Agroquímica y Tecnología de Los Alimentos (IATA)-CSIC, 46980, Valencia, Spain

ARTICLE INFO

Keywords:
Saccharomyces eubayanus
Saccharomyces uvarum
Saccharomyces kudriavzevii
Wine longevity
Fruity branched ethyl esters
Ethyl leucate
β-Ionone

ABSTRACT

Interest in the use of non-conventional yeasts in wine fermentation has been increased in the last wine sector. The main objective of this manuscript was to explore the aromatic diversity produced non-wine strains of *S. cerevisiae*, *S. eubayanus*, *S. kudriavzevii*, and *S. uvarum* species in young and Tempranillo wines as well as evaluate their fermentation capacity and the yield on ethanol, g organic acids, that can contribute to diminishing the effects of climate change on wines.

S. uvarum strain U1 showed the highest ability to release or *de novo* produce monoterpenes, such and citronellol, whose values were 1.5 and 3.5-fold higher than those of the wine *S. cerevisiae* strai that compared to the normal values for red wines, β-phenylethyl acetate was highly synthesized by strains, achieving 1 mg/L. Additionally, after aging, wines of *S. eubayanus* strains contained the high this acetate. Malic acid was highly degraded by *S. kudriavzevii* yeasts, resulting in the highest yields (>5-fold) and ethyl lactate (>2.8-fold) in their wines. In aged wines, we observed that the modulati yeast strain were very high in β-ionone. *S. uvarum* strains U1 and BMV58 produced an important agi ethyl isobutyrate, which was highly enhanced during the aging. Also, the agave *S. cerevisiae* strain essential aroma after aging, reaching the highest ethyl leucate contents.

According to the results obtained, the use of wild non-wine strains of *S. cerevisiae* and strains otolerant species *S. eubayanus*, *S. kudriavzevii*, and *S. uvarum* in Tempranillo wine fermentation aroma complexity. In addition, wines from *S. kudriavzevii* strains had twice additional glycerol, *S. uvarum* 4-fold more succinic acid, while wines from wild strains yielded 1% v/v less ethanol whic wine problems associated with climate change.

1. Introduction

Wine aroma is widely known to be one of the most relevant determinants of the overall wine quality (Charters and Pettigrew, 2007; San-Juan et al., 2011). Its composition consists of several volatile molecules at concentrations ranging from ng/L to mg/L. According to their origin, they can be divided into three main categories, the aroma of varietal, fermentative, and aging origin. The most abundant volatile compounds are from fermentative origin, such as ethyl esters, acetate esters, higher alcohols, and volatile fatty acids, mainly derived from nitrogen and carbon yeast metabolisms (Rollero et al., 2017) the varietal aroma is the most inf uential group in terms stantial aroma contribution of the odorants to wine. Among group includes polyfunctional mercaptans, norisoprenoids, volatile phenols, and vanillin derivatives (Ferreira and Ló Initially, most of these molecules do not contribute direct aroma as they are presented at bound forms, such as a gl whereas only a small fraction is found as free aroma molecul and López, 2019; Hjelmeland and Ebeler, 2015; Liu et al. general, aglycones are released from their non-volatile prec

* Corresponding author.
E-mail address: aquerol@iata.csic.es (A. Querol).

https://doi.org/10.1016/j.ijfoodmicro.2022.109554
Received 17 October 2021; Received in revised form 6 January 2022; Accepted 20 January 2022

Food Microbiology 104 (2022) 103981

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Modulation of aroma and chemical composition of Albariño semi-synthetic wines by non-wine *Saccharomyces* yeasts and bottle aging

Dolores Pérez[a,b,d], Marie Denat[c], Romain Minebois[d], José María Heras[a], José Manuel Guillamón[d], Vicente Ferreira[c], Amparo Querol[d,*]

[a] Lallemand Bio S.L., 08028, Barcelona, Spain
[b] Estación Experimental Agropecuaria Mendoza (EEA), Instituto Nacional de Tecnología Agropecuaria (INTA), 5507, Luján de Cuyo, Mendoza, Argentina
[c] Laboratory for Aroma Analysis and Enology (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), C/Pedro Cerbuna 12, 50009, Zaragoza, Spain
[d] Departamento de Biotecnología de Los Alimentos, Grupo de Biología de Sistemas en Levaduras de Interés Biotecnológico, Instituto de Agroquímica y Tecnología de Los Alimentos (IATA)-CSIC, 46980, Valencia, Spain

ARTICLE INFO

Keywords:
Saccharomyces uvarum
Saccharomyces kudriavzevii
Polyfunctional mercaptans
2-furfurylthiol
4MMP
GABA shunt
Wine longevity

ABSTRACT

Saccharomyces yeasts from different origins and species fermented in a semi-synthetic must containing aroma precursor of cv. Albariño and polyfunctional mercaptans precursors. The resulting wines were subjected to accelerate anoxic aging. Afterward, aroma profiles were analyzed by distinct gas chromatography methodologies.

Cryotolerant strains showed better fermentation performances with significant differences in volatile and non-volatile fermentation products than *Saccharomyces cerevisiae* (*S. cerevisiae*). We suggested that the highest levels γ-butyrolactone and diethyl succinate in *Saccharomyces uvarum* (*S. uvarum*) strains, together with their substantial succinic acid yields, could be related to greater flux through the GABA shunt. These strains also had the highest production of β-phenylethyl acetate, geraniol, and branched-chain ethyl esters. The latter compounds were highly increased by aging, while acetates and some terpenes decreased. *S. kudriavzevii* strains showed a remarkable ability to release polyfunctional mercaptans, with SK1 strain yielding up to 47-fold and 8-fold more 4-methyl-4-mercaptopentan-2-one (4MMP) than *S. cerevisiae* and *S. uvarum* strains, respectively. The wild *S. cerevisiae* beer isolate showed a particular aroma profile due to the highest production of ethyl 4-methylvalerate (lactic and fruity notes), γ-octalactone (coconut), and furfurylthiol (roasted coffee). The latter compound is possibly produced from the pentose phosphate pathway (PPP). Since erythritol, another PPP intermediate was largely produced by this strain.

1. Introduction

The aroma of white wines is an essential factor defining their quality and varietal character. It results from the sensory contribution of aromatic metabolites proceeding from grapes (varietal aromas) and fermentation, including those produced during alcoholic fermentation and bottle aging. Among the most important varietal aroma compounds family, we found terpenes, C_{13}-norisoprenoids, and polyfunctional mercaptans (also known as thiols) (Parker et al., 2017). In grape musts, these varietal aromas can be found in a free (i.e., volatile) state or a non-volatile state when linked to a so-called non-volatile varietal precursor, except for polyfunctional mercaptans, which are only found in non-volatile form (Roland et al., 2011). This distinction allows discriminating between neutral (or non-aromatic) grapevines whose varietal aromas composition is mainly made of linked aromatic compounds and aromatic grape varieties containing a substantial fraction of volatile varietal aromas (Ferreira and López, 2019).

During winemaking, two mechanisms can participate in the release of the odorous compounds linked to varietal precursors. On the one hand, acid-catalyzed hydrolysis, resulting from the acidic nature of grape must, occurs throughout the winemaking process and participates in the release of bound-aromas (López et al., 2004; Liu et al., 2017). On the other hand, enzymatic hydrolysis of bound aroma compounds can be carried out by enzymes provided by grapes or, to a lesser extent, by

* Corresponding author.
E-mail address: aquerol@iata.csic.es (A. Querol).

https://doi.org/10.1016/j.fm.2022.103981
Received 13 October 2021; Received in revised form 30 November 2021; Accepted 12 January 2022
Available online 15 January 2022