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PROGRAMA DE DOCTORADO EN RECURSOS Y TECNOLOGÍAS AGRARIAS, AGROAMBIENTALES Y ALIMENTARIAS

Caracterización fisicoquímica, aromática y sensorial de Fondillón, el vino más representativo de la Denominación de Origen Protegida Alicante

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La presente Tesis Doctoral, titulada "**Caracterización fisicoquímica, aromática y sensorial de Fondillón, el vino más representativo de la Denominación de Origen Protegida Alicante**", se presenta bajo la modalidad de **tesis por compendio** de las siguientes **publicaciones:**

- Issa-Issa, H.; Noguera-Artiaga, L.; Sendra, E.; Pérez-López, A. J.; Burló, F.; Carbonell-Barrachina, Á. A.; & López-Lluch, D. (2019). Volatile composition, sensory profile, and consumers' acceptance of *Fondillón*. Journal of Food Quality, 1-10 5981762. https://doi.org/10.1155/2019/5981762.

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- Issa-Issa, H.; Hernández, F.; Lipan, L.; López-Lluch, D.; Carbonell-Barrachina, Á.A. (2021). Quality, Nutritional, Volatile and Sensory Profiles and Consumer Acceptance of *Fondillón*, a Sustainable European Protected Wine. Agronomy, 11(9). https://doi.org/10.3390/agronomy11091701.

- Issa-Issa, H.; Noguera-Artiaga, L.; Mora, M.; Carbonell-Barrachina, Á.A.; López-Lluch, D. (2021). Consumer profile and drivers influencing consumer behavior towards *Fondillón*, a European Protected naturally sweet red wine. Foods, 10(11). https://doi.org/10.3390/foods10112651.

- Issa-Issa, H.; Hernández, F.; López-Lluch, D.; Reyhan Selin Uysal; Carbonell-Barrachina, Á.A. (2023). *Fondillón* wine adulteration by addition of other *Monastrell* wines. Beverages, 9(1). https://doi.org/10.3390/beverages9010028.

PUBLICACIÓN 1

Issa-Issa, H.; Noguera-Artiaga, L.; Sendra, E.; Pérez-López, A.J.; Burló, F.; Carbonell-Barrachina, Á.A.; López-Lluch, D. 2019. Volatile composition, sensory profile, and consumers' acceptance of *Fondillón. Journal of Food Quality.* Article ID 5981762, 10 pages.

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JCR®	Cuartil	Rank	Factor de impacto (2019)	Factor de impacto (5 años)
Food Science & Technology	Q3	83/139	1,763	1,781

PUBLICACIÓN 2

Issa-Issa, H.; Guclu, G.; Noguera-Artiaga, L.; López-Lluch, D.; Poveda, R.; Kelebek, H.; Selli, S.; Carbonell-Barrachina, Á.A. 2020. Aroma-active compounds, sensory profile, and phenolic composition of *Fondillón*. *Food Chemistry.* Vol. 316, 126353.

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JCR®	Cuartil	Rank	Factor de impacto (2020)	Factor de impacto (5 años)
Food Science & Technology	Q1	7/143	7,514	7,516

PUBLICACIÓN 3

Issa-Issa, H.; Hernández, F.; Lipan, L.; López-Lluch, D.; Carbonell-Barrachina, Á.A. 2021. Quality, nutritional, volatile and sensory profiles and consumer acceptance of *Fondillón*, a sustainable European Protect wine. *Agronomy.* Vol: 11(9), 1701.

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Agronomy	Q1	18/124	3,949	4,117

PUBLICACIÓN 4

Issa-Issa, H.; Noguera-Artiaga, L.; Mora, M.; Carbonell-Barrachina, Á.A.; López-Lluch, D. 2021. Consumer profile and drivers influencing consumer behavior towards *Fondillón*, a European protected naturally sweet red wine. *Foods.* Vol: 10(11), 2651.

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JCR®	Cuartil	Rank	Factor de impacto (2021)	Factor de impacto (5 años)
Food Science & Technology	Q1	37/165	5,561	5,940

PUBLICACIÓN 5

Issa-Issa, H.; Hernández, F.; López-Lluch, D.; Reyhan Selin Uysal; Carbonell-Barrachina, Á.A. (2023). *Fondillón* wine adulteration by addition of other *Monastrell* wines. *Beverage*s, 9(1).

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Food Science & Technology	Q3	91/169	3,68

El Dr. Ángel Antonio Carbonell Barrachina, director y el Dr. David Bernardo López Lluch, codirector de la tesis doctoral titulada “**Caracterización fisicoquímica, aromática y sensorial de Fondillón, el vino más representativo de la Denominación de Origen Protegida Alicante**”

INFORMAN:

Que Dña. Hanán Issa Issa ha realizado bajo nuestra supervisión el trabajo titulado “**Caracterización fisicoquímica, aromática y sensorial de Fondillón, el vino más representativo de la Denominación de Origen Protegida Alicante**” conforme a los términos y condiciones definidos en su plan de investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

Lo que firmamos para los efectos oportunos, en Orihuela a 27 de Junio de 2023.

Director de la tesis	Codirector de la tesis
Dr. Ángel Antonio Carbonell Barrachina	Dr. David Bernardo López Lluch

La Dra. Juana Fernández López, Coordinadora del Programa de Doctorado en Recursos y Tecnologías Agrarias, Agroambientales y Alimentarias,

INFORMA:

Que Dña. Hanán Issa Issa ha realizado bajo la supervisión de nuestro Programa de Doctorado el trabajo titulado "**Caracterización fisicoquímica, aromática y sensorial de Fondillón, el vino más representativo de la Denominación de Origen Protegida Alicante**" conforme a los términos y condiciones definidos en su plan de investigación y de acuerdo al Código de Buenas Prácticas de la Universidad Miguel Hernández de Elche, cumpliendo los objetivos previstos de forma satisfactoria para su defensa pública como tesis doctoral.

Lo que firmamos para los efectos oportunos, en Orihuela a 27 de Junio de 2023.

Profa. Dra. Juana Fernández López

Coordinadora del Programa de Doctorado en Recursos y
Tecnologías Agrarias, Agroambientales y Alimentarias

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A Esther, Paqui y Paco por su ayuda en los momentos en los que los he necesitado.

A Arturo Torrecilla por su amistad y cariño.

Las imágenes que aparecen en la tesis que no se derivan del trabajo experimental han sido tomadas de:

- La bevanda di Vivina. 2023. Uva Mourvedre. https://www.labevandadiviviana.com/cepas-rojas/mourvedre [Visitada 22 de junio de 2023]

- Croatian wines emerge from the dark. 2023. https://suruchimohan.com/2016/06/24/croatian-wines/ [Visitada 22 de junio de 2023]

TABLA DE CONTENIDO

TABLA DE CONTENIDOS

	Página
1. ESTRUCTURA DE LA TESIS DOCTORAL ……	1
2. ABREVIATURAS ……	5
3. RESUMEN / ABSTRACT ……	9
4. INTRODUCCIÓN ……	19
5. OBJETIVOS ……	43
6. MATERIALES Y MÉTODOS ……	47
7. PUBLICACIONES ……	65
8. RESULTADOS Y DISCUSIÓN ……	153
9. CONCLUSIONES ……	179
10. FUTURAS INVESTIGACIONES ……	183
11. REFERENCIAS ……	187

1. ESTRUCTURA DE LA TESIS DOCTORAL

1. ESTRUCTURA DE LA TESIS DOCTORAL

Esta Tesis Doctoral se ha escrito siguiendo el reglamento interno vigente de la Universidad Miguel Hernández de Elche para la presentación de la Tesis Doctoral bajo la modalidad de tesis por compendio de publicaciones. Esta memoria se ha estructurado de la siguiente forma:

- **Resumen y Abstract.** Están detallados los principales objetivos y los resultados más relevantes.
- **Introducción.** Contextualización del estado del arte del vino, la importancia del Fondillón y el hilo conductor de esta Tesis Doctoral.
- **Objetivos.** Se indica cual es el **o**bjetivo principal y los objetivos específicos de la investigación.
- **Materiales y Métodos.** Se resumen y referencian las muestras de vino utilizadas, y los métodos analíticos y sensoriales y de tratamiento de datos utilizados para la realización de esta Tesis Doctoral.
- **Publicaciones científicas.** Se presenta la transcripción literal de las publicaciones científicas incluidas en esta Tesis:

1. «*Volatile composition, sensory profile, and consumers' acceptance of Fondillón*». Publicado en la revista *Journal of Food Quality.*
2. «*Aroma-active compounds, sensory profile, and phenolic composition of Fondillón*». Publicado en la revista *Food Chemistry.*
3. «*Quality, nutritional, volatile and sensory profiles and consumer acceptance of Fondillón, a sustainable European protected wine*». Publicado en la revista *Agronomy.*
4. «*Consumer profile and drivers influencing consumer behavior towards Fondillón, a European protected naturally sweet red wine*». Publicado en la revista *Foods.*
5. «*Fondillón wine adulteration by addition of other Monastrell wines*». Publicado en la revista *Beverages.*

- **Resultados y discusión**. En esta sección se hace un breve resumen de los principales resultados obtenidos.
- **Conclusiones.** Se enumeran las principales conclusiones de la Tesis Doctoral.
- **Referencias.** En esta sección se indican las referencias utilizadas para la redacción y justificación de esta Tesis Doctoral.

2. ABREVIATURAS

AA	Actividad antioxidante
$ABTS^{+\bullet}$	2,2′-azino-bis-(ácido 3-etilbenzotiazolina-6-sulfónico)
AEDA	"*Aroma extract dilution analysis*", Análisis de dilución del extracto aromático
ANOVA	Análisis de varianza
CA	Análisis de correspondencia
CATA	*Check all that apply*
CD	Densidad de color
CI	Intensidad de color
DOP	Denominación de Origen Protegida
DOCa	Denominación de Origen Calificada
$DPPH^{\bullet}$	2,2-difenil-1-picrilhidracilo
ENAC	Entidad Nacional de Acreditación
FID	Detector de ionización de llama
FRAP	Poder de reducción antioxidante del ion férrico
GC	Cromatografía de gases
HPLC	Cromatografía líquida de alta resolución
HS-SPME	Microextracción en fase sólida del espacio de cabeza
JAR	*Just about right*
MS	Espectrometría de masas
MSD	Detector selectivo de masas
OIV	Organización Internacional de la Viña y el Vino
ODP-2	Dispositivo de olfatometría
PCA	Análisis de componentes principales
PLS	Regresión mínima cuadrática parcial
SAFE	"*Solvent assisted flavor evaporation*", Extracción de compuestos volátiles por evaporación de aromas
T	Tonalidad
TAC	Contenido total de antocianinas
TDN	1,1,6-trimetil-1,2-dihidronaftaleno
TPI	Índice de polifenoles totales
TTC	Contenido total de taninos condensados

3. RESUMEN Y ABSTRACT

RESUMEN

El Fondillón es un tipo de vino de Alicante único en el mundo; está reconocido por la Unión Europea en su base de datos E-bachus y es el núcleo de la Denominación de Origen Alicante (DOP Alicante). Está elaborado con uvas de la variedad Monastrell sobremaduradas en la cepa; por tanto, su contenido en alcohol (≥16 % v/v) procede exclusivamente de la uva, y necesita de un envejecimiento mínimo de 10 años.

Al comienzo de esta tesis doctoral (octubre de 2018), sólo existía una publicación científica recogida en la base de datos SCOPUS sobre el Fondillón. Por tanto, no existía un conocimiento científico sobre qué hace especial a este vino; aunque cualquiera que haya probado este producto sabe que es único y diferente al resto de vinos.

A partir de este momento, nos propusimos estudiar (*i*) el Fondillón y (*ii*) sus consumidores, para poder entender esta relación y así poder ver sus debilidades y fortalezas. Para ello establecimos unos objetivos específicos que incluían evaluar (*i*) las principales características fisicoquímicas del Fondillón, (*ii*) su perfil volátil, incluyendo los compuestos aromáticos activos (*odor-active compounds*), (*iii*) su perfil polifenólico, (*iv*) su perfil sensorial (definido por un panel entrenado y por un panel de consumidores), (*v*) el perfil tipo del consumidor actual, y (*vi*) marcadores claves para identificar posibles adulteraciones o fraudes en la comercialización de este vino.

Con estos objetivos en mente, se comenzó a trabajar con el apoyo del Consejo Regulador de la DOP Alicante, y de dos bodegas BOCOPA y Monóvar (MGWines); sin embargo, hay que reseñar que para la realización de esta tesis doctoral no se ha contado con financiación de ningún tipo y que los recursos económicos han venido únicamente del grupo de investigación Calidad y Seguridad Alimentaria (CSA) de la Universidad Miguel Hernández de Elche. Se plantearon diversos experimentos encaminados al conocimiento progresivo del producto y de sus consumidores. Finalmente se publicaron 5 artículos en revistas internacionales en las que se estudiaron los parámetros que se detallan a continuación:

- <u>Publicación 1</u> (*Journal of Food Quality*): características fisicoquímicas y primera aproximación al perfil volátil y sensorial de los tipos de Fondillón comercializados al comienzo de esta tesis doctoral. Este artículo es importante porque en él se desarrollaron el léxico y la ficha de cata para la evaluación sensorial descriptiva del Fondillón.

- ✦ Publicación 2 (*Food Chemistry*): compuestos aromáticos activos y perfil polifenólico. Esta publicación se realizó en colaboración con investigadores de la Çukurova University (Adana, Turquía), y es el núcleo de esta tesis doctoral.
- ✦ Publicación 3 (*Agronomy*): actividad antioxidante, características cromáticas, contenidos totales de taninos y antocianinas, y se comenzó a profundizar en el conocimiento del consumidor habitual de este vino.
- ✦ Publicación 4 (*Foods*): grado de satisfacción de los consumidores al respecto de los tipos de Fondillón comercializados y sobre sus principales atributos organolépticos. Además, se estudió el perfil tipo del consumidor actual de Fondillón.
- ✦ Publicación 5 (*Beverages*): desarrollo de un método para la identificación de muestras potencialmente adulteradas y que de ser comercializadas puedan suponer un fraude. Se estableció una huella digital inicial basada en la presencia de distintos parámetros a concentraciones específicas, para que por comparación se determine si las muestras bajo estudio son sospechosas de adulteración o no.

Fruto de estos estudios y de las correspondientes publicaciones, el conocimiento sobre el Fondillón y sobre quién lo consume, cuándo lo consume y por qué lo consume ha mejorado; si bien, todavía son diversas las preguntas que tenemos y que orientarán nuestra línea de trabajo sobre el vino de la DOP Alicante.

Algunas de las conclusiones principales que se han derivado de esta tesis doctoral son:

- ✦ Se ha desarrollado un léxico y una ficha de cata específicos para la descripción objetiva del Fondillón.
- ✦ Se tienen definiciones bastante coincidentes de Fondillón por parte de un panel entrenado y de un panel de consumidores:
  - o Según los expertos "*vino dulce, equilibrado (aunque con un marcado contenido alcohólico), con olor y aroma intensos en los que predominan los frutos maduros, los frutos secos, las notas tostadas de café, con madera bien integrada y con una persistencia muy larga*"
  - o Según los consumidores "*vino con buen equilibrio entre alcohol, dulzor y amargor, con notas florales, afrutadas y tostadas intensas, y con un prolongado y agradable postgusto*".

- Se han identificado **marcadores de envejecimiento**: TDN, vitispirano, furfural, y benzaldehído.
- Se han identificado **marcadores de autenticidad**: fructosa, ácido acético, furfural, benzaldehído, vitispirano, TDN, etil octanoato, etil decanoato, entre otros.
- La totalidad de muestras evaluadas en esta tesis doctoral, cumplen con los requisitos para el Fondillón, según el pliego de condiciones de la DOP Alicante. Pero, para poder establecer un control sobre la **autenticidad** de las muestras en comercialización y detectar **posibles fraudes**, se ha desarrollado un modelo inicial (que necesita mejora en la cantidad de datos en que se basa, y en la automatización en la toma de decisiones), basado en los marcadores de autenticidad, anteriormente enumerados.
- Se ha identificado el **perfil tipo del consumidor actual** de Fondillón "*hombre de entre 42 y 52 años, con un elevado nivel cultural/educativo, y con unos ingresos anuales entre 25.000 y 50.000 euros, y que bebe este vino principalmente en casa y en ocasiones especiales*". Además, se han identificado las principales **razones para no consumir Fondillón** que son: (*i*) el enorme desconocimiento del producto por parte del consumidor, (*ii*) su elevado precio, y (*iii*) que no es fácil de encontrar (ni en la cadena habitual de compra ni en los restaurantes).

Toda la información generada en esta tesis doctoral nos permite tener un conocimiento inicial sobre el Fondillón y sobre quién lo consume. Sin embargo, todavía son muchas las incógnitas que envuelven a este tipo de vino de la DOP Alicante y que tenemos que seguir tratando de desvelar en un futuro cercano. Las principales líneas de trabajo a seguir son:

- Evaluar un mayor número de muestras de Fondillón de distintas bodegas, de distintas soleras, de distintas añadas, para poder confirmar el perfil volátil (marcadores de envejecimiento y de autenticidad). Pero, para ello es imprescindible la colaboración de todas y cada una de las bodegas que elaboran y comercializan Fondillón.
- Automatizar la toma de decisiones sobre la posible adulteración de muestras de Fondillón empleando técnicas de metabolómica (dirigida y no-dirigida) y métodos estadísticos más robustos.
- Ampliar el estudio sobre la aceptación de Fondillón y el perfil de consumidor a mercados internacionales.

Y sin duda, la línea principal de investigación y trabajo es colaborar con las bodegas y la DOP Alicante en el desarrollo de estrategias conjuntas y basadas en la ciencia para la promoción del Fondillón, garantizando la calidad y especificidad del vino comercializado.

ABSTRACT

Fondillón is a unique type of Alicante wine in the world; it is recognized by the European Union in its E-bachus database and is the core of the Alicante Denomination of Origin (PDO Alicante). It is made with grapes of the Monastrell variety that are overripe on the vine; thus, its alcohol content (≥16% v/v) comes exclusively from the grape, and it needs a minimum aging period of 10 years.

At the beginning of this doctoral thesis (October 2018), there was only one scientific publication on *Fondillón* gathered in the SCOPUS database. Therefore, there was no scientific knowledge about what makes this wine special; although anyone who has tried this product knows that it is unique and different from other wines.

From this moment on, we set out to study (*i*) *Fondillón* and (*ii*) its consumers, to understand this relationship and thus be able to see its strengths and weaknesses. For this, we established specific objectives that included evaluating (*i*) the main physicochemical characteristics of *Fondillón*, (*ii*) its volatile profile, including the odor-active compounds, (*iii*) its polyphenolic profile, (*iv*) its sensory profile (defined by a trained panel and by a panel of consumers), (*v*) the typical profile of the current consumer, and (*vi*) key markers to identify possible adulterations or fraud in the marketing of this wine.

With these objectives in mind, work began with the support of the Regulatory Council of the DOP Alicante, and two wineries BOCOPA and Monóvar (MGWines); however, it should be noted that no funding of any kind has been received for the completion of this doctoral thesis and that the economic resources have come solely from the Food Quality and Safety (CSA) research group of the Miguel Hernández University of Elche. Various experiments aimed at the progressive knowledge of the product and its consumers were proposed. Finally, 5 articles were published in international journals in which the parameters detailed below were studied:

- ✦ Publication 1 (*Journal of Food Quality*): physicochemical characteristics and the first approach to the volatile and sensory profile of the types of *Fondillón* commercialized at the beginning of this doctoral thesis. This article is important because in it the lexicon and tasting sheet/questionnaire for the descriptive sensory evaluation of *Fondillón* were developed.

- ✦ Publication 2 (*Food Chemistry*): odor-active compounds and polyphenolic profile. This publication was carried out in collaboration with researchers from Çukurova University (Adana, Turkey), and is the core of this doctoral thesis.

- Publication 3 (*Agronomy*): antioxidant activity, chromatic characteristics, total tannin and anthocyanin contents, and began to deepen the knowledge of the regular consumer of this wine.
- Publication 4 (*Foods*): degree of consumer satisfaction regarding the types of *Fondillón* marketed and its main organoleptic attributes. In addition, the profile of the current consumer of *Fondillón* was studied.
- Publication 5 (*Beverages*): development of a method for the identification of potentially adulterated samples that, if sold, could imply fraud. An initial fingerprint was established based on the presence of different parameters at specific concentrations, so that by comparison it can be determined whether the samples under study are suspicious of adulteration or not.

As a result of these studies and the corresponding publications, knowledge about *Fondillón* and who consumes it, when it is consumed and why it is consumed has improved; Although, there are still various questions that we have and that will guide our line of work on PDO Alicante wine.

Some of the main conclusions that have been derived from this doctoral thesis are:

- A specific lexicon and tasting sheet have been developed for an objective description of *Fondillón*.
- There are fairly consistent definitions of *Fondillón* by a trained panel and a panel of consumers:
  - According to the experts, "*sweet, balanced wine (although with a marked alcoholic content), with an intense smell and aroma in which ripe fruits, dried fruits, and toasted notes of coffee predominate, with well-integrated wood and a very long aftertaste*"
  - According to consumers, "*a wine with a good balance between alcohol, sweetness and bitterness, with intense floral, fruity and toasted notes, and a long and pleasant aftertaste*".
- **Aging markers** have been identified: TDN, vitispirane, furfural, and benzaldehyde.
- **Authenticity markers** have been identified: fructose, acetic acid, furfural, benzaldehyde, vitispirane, TDN, ethyl octanoate, ethyl decanoate, among others.
- All the samples evaluated in this doctoral thesis meet the requirements for *Fondillón*, according to the specifications of the DOP Alicante. But, in order to establish control

over the **authenticity** of the samples being sold and detect possible **fraud**, an initial model has been developed (which needs improvement in the amount of data on which it is based, and in decision-making automation), based on the authenticity markers, listed above.

- The **typical profile of the current consumer** of *Fondillón* has been identified as "a man between 42 and 52 years of age, with a high cultural/educational level, and with an annual income between 25,000 and 50,000 euros, and who drinks this wine mainly at home and in special occasions". In addition, the main **reasons for not consuming *Fondillón*** have been identified, which are: (*i*) the enormous lack of knowledge of the product on the part of the consumer, (*ii*) its high price, and (*iii*) that it is not easy to find it (neither in shops nor in restaurants).

All the information generated in this doctoral thesis allows us to have an initial knowledge about *Fondillón* and who consumes it. However, there are still many unknowns that surround this type of PDO Alicante wine and that we want to continue trying to reveal in the near future. The main lines of work to be followed are:

- Evaluate a greater number of *Fondillón* samples from different wineries, from different soleras, and from different vintages, to confirm the volatile profile (aging and authenticity markers). But, for this, the collaboration of all wineries producing and marketing *Fondillón* is essential.
- Automate decision-making on the possible adulteration of *Fondillón* samples using metabolomics techniques (directed and non-directed) and more robust statistical methods.
- Expand the study on the acceptance of *Fondillón* and the consumer profile to international markets.
- And without a doubt, the main line of research and work is to collaborate with the wineries and the DOP Alicante in the development of joint strategies based on science for the promotion of *Fondillón*, guaranteeing the quality and specificity of the wine marketed.

4. INTRODUCCIÓN

4.1 El vino en Alicante

El vino es una de las bebidas más antiguas que se conocen. El cultivo de la (hermafrodita) *Vitis vinifera* a partir de su antepasada salvaje (dioica) *Vitis vinifera sylvestris*, se origina en el Neolítico en una región que incluye lo que hoy es el noroeste de Irán, Armenia, Azerbaiyán y la Georgia rusa (Celestino-Pérez y Blánquez-Pérez, 2013). La prueba química y arqueológica más antigua de la vinificación hasta la fecha proviene de Hajji Firuz Tepe, un sitio neolítico en el noroeste de Irán que data del 5000 al 5400 a.C. La forma de las pepitas de uva encontradas en el sitio demuestra que se utilizaron vides hermafroditas (cultivadas). Las evidencias de vinificación más antiguas conocida se encuentran en el sur de Armenia en un sitio arqueológico llamado Areni-1, no muy lejos al norte de Hajji Firuz Tepe y está fechado en el 4100 a. C. La elaboración del vino consistía en triturar racimos de uvas, probablemente pisándolas, y el mosto fluiría a tinajas de barro enterradas para su fermentación. Aquí nuevamente, la presencia de pepitas proporciona evidencias del cultivo de la vid.

El vino se fue extendiendo a lo largo de los tiempos y, en el 3000 a.C. llega a Egipto y a Fenicia, aunque es en el antiguo Egipto donde se le atribuye un papel más importante, ya que se utilizaba esta bebida durante ritos religiosos y celebraciones paganas (Robinson & Johnson, 2003). De hecho, en Egipto se producía el vino en grandes vasijas de barro, incluso a los antiguos faraones se les enterraba con vasijas llenas de vino, ya que era un indicador de estatus social.

Desde esta parte del Mediterráneo la cultura de la vid y del vino se expandió hacia Occidente a través de las costas del Mediterráneo y saltando de isla en isla, merced a la actividad comercial de fenicios y griegos. Fueron ellos los que lo trajeron hasta la Península Ibérica hacia el siglo VII a.C. Así, en la Comunidad Valenciana destacan los yacimientos de la Solana de las Pilillas (Requena) y de *L'Alt de Benimaquía* (Denia) datados en el siglo VI a.C. El material encontrado en *L'Alt de Benimaquia*, entre otros, fueron 7000 pepitas de uva (*Vitis vinifera*) (Gómez-Bellard *et al.*, 1995). Su estudio, por su tamaño, nos permite identificarla como vides cultivadas. Además, nos da a conocer unas estructuras para pisar las uvas "lagares" (siglos VII-VI a.C.), y una industria de vasijas fenicias, ánforas, como introducción a la viticultura en el medio indígena.

Ahora bien, debemos a los romanos el desarrollo de la tradición vitícola y, sobre todo, el profundo sentido religioso, cultural y social con todo el simbolismo y ritual que esto supone. Además, debe ser enfatizado el carácter más o menos uniforme de estos rituales y simbolismo especialmente en las provincias occidentales de su Imperio (de la misma forma que posteriormente se heredó la religión cristina, el latín y todo el derecho civil romano). Este hecho es fundamental ya que el cristianismo, latín y derecho romano están estrechamente vinculadas a la cultura del vino durante la Edad Media. L (Márquez-Villora, 1999) as evidencias de este vínculo, de cómo las técnicas romanas de viticultura y vinificación eran prácticamente iguales tanto en Hispania, Italia y la Galia como en los territorios del norte de África e incluso en la Galia Belga, que alcanzaba hasta las orillas del Rhin, se pueden encontrar en los restos que han llegado hasta nuestros días. En ese sentido, las representaciones en mosaicos, esculturas, enterramientos, así como los elementos para la vinificación (prensas, ánforas, etc.) y herramientas para el cultivo de la vid son muy similares tanto en Italia como en España, Túnez, Francia o Alemania. Mención especial merecen las evidencias (encontradas en innumerables mosaicos y esculturas) de la popularidad del culto a Baco (Liber Pater) y que se proyecta en las imágenes cristianas en la Edad Media.

Fue en la época Imperial (siglos I y II) cuando las provincias occidentales (Tarraconense entre otras) fueron las encargadas de proveer de vino a Roma. Los vinos más citados son los de Saguntum y Tarraco. La romanización de nuestras tierras a partir del siglo II a.C. consolidó el cultivo de la vid y la elaboración de vinos para su exportación, como se desprende de los talleres de ánforas vinarias descubiertos en la playa de la Almadraba (Dénia) y de El Campello. A partir del siglo III el cultivo de la vid y la elaboración de vino se había extendido a la totalidad de la Península Ibérica.

El vino y su cultura contaron con un aliado fundamental a finales del siglo IV, la proclamación del cristianismo como religión oficial en el Imperio Romano por parte del emperador Teodosio. Esto implicó la sacralización del vino en la liturgia. No hay que olvidar que el vino ya era parte fundamental de los ritos religiosos romanos paganos (Márquez-Villora, 1999).

Tras la desaparición del Imperio Romano de Occidente, fueron los monasterios los responsables del desarrollo y difusión del cultivo de la vid y la elaboración de vino, especialmente en Europa Central. Esto no fue así en el Mediterráneo, donde esta tradición vitícola romana se mantenía viva entre la población autóctona. Esta cultura se mantuvo viva incluso en la época de dominación islámica. En la Edad Media, el vino era la bebida común de todas las clases sociales del sur de Europa, donde se cultivaba la uva. Sin embargo, en el norte y el este de Europa, donde se cultivaban pocas o ninguna uva, la cerveza era la bebida habitual.

Si bien el vino se exportaba a las regiones del norte, pero debido a su costo relativamente alto rara vez era consumido por las clases más bajas.

El vino en la Edad Media era algo más que una bebida placentera objeto de controversias. La sociedad de aquella época era una gran consumidora de vino, ya que no conocían otra bebida aparte del agua, y esta no solía ser de buena calidad. En una época salpicada de epidemias de peste, las aguas de pozos y fuentes eran precisamente el principal conducto difusor de la plaga.

La edad mínima para empezar a beber vino solía ser a partir de los seis años, aunque fuera rebajado con agua o mezclado con pan (el sopanvino) (Bendicho, 1991). Así, en España y en concreto en Alicante, ni siquiera la dominación islámica restringió el cultivo de la vid, y posiblemente incluso se toleró la producción de vino a pequeña escala.

La ocupación musulmana y el proceso de islamización de la población autóctona no acabó como algunos piensan con el cultivo de la vid ni con la producción y consumo de vino, aunque fuera más importante la elaboración de pasas y arrope. Los propios geógrafos árabes, como alIdrisi (siglo XII) nos hablan de los extensos viñedos que cubrían la llanura de Denia.

Después de la conquista cristiana, los moriscos de La Marina y del valle del Vinalopó mantuvieron la elaboración de pasas para abastecer a los comerciantes que se encargaban de exportarlas a Francia, Italia, Flandes e Inglaterra, comercio que constituía a finales del siglo XV la principal fuente de ingresos en los puertos de Denia y Alacant. Tampoco olvidaron la elaboración y consumo de vino, manteniendo tabernas separadas de las de los cristianos, como ocurría en Biar, Crevillent y el rabal de Sant Joan (Elx).

La pervivencia de conventos y templos cristianos permitía a los musulmanes comprar lo que denominaban "vino de monasterio", al ser adquirido en estos inmuebles, y luego consumido de manera privada en jardines y en momentos de regocijo, como se refiere el poeta cordobés Ibs Shuhayd. Poetas musulmanes hispanos refieren la existencia de tabernas y taberneras (*al-Gazal*), aunque la ingesta de esta bebida se asocia con jardines, lugares deleitosos, la presencia de la noche o la alborada, tertulias y buena compañía (*Ibn Jatima*) (VitiEno, 2016).

De hecho, en la primera "Exposición del Vino" que tuvo lugar en París en 1212, el vino de Alicante alcanzó el cuarto lugar, indicando la calidad del vino de nuestra región y lo más importante el hecho de que se estaba produciendo esta bebida alcohólica. En ese momento, Alicante estaba bajo dominio islámico, ligada al reino islámico de Murcia, pero bajo la influencia de Castilla, según el Tratado de Cazorla (1179) (Piqueras, 2014).

Alicante fue reconquistada en diciembre de 1248. Fueron los cristianos, siguiendo prácticas enológicas griegas y romanas transmitidas por los musulmanes, los que durante el siglo XV crearon un vino con nombre propio, el Alacant, que habría de alcanzar renombre y proyección universal. El consumo del vino de Alacant en las ricas ciudades del Norte de Europa debió iniciarse a comienzos del XV, como deduce Amerigo Melis de la correspondencia entre el representante de la casa Orlandini en Brujas y el de la casa Datini en Barcelona, que le había enviado una partida de vino «*della Chantera*» (así transcribía el «d'Alacant» valenciano).

Por su parte, el historiador francés Roger Dion afirma que el vino de Alacant empezó a cobrar fama en Francia, Flandes e Inglaterra a finales del siglo XV y cita en su argumento los Libros de Privilegios de Amberes (Flandes) en los que en 1482 figuran los nombres de los vinos licorosos que por entonces estaban entrando en aquella ciudad, y entre los que destacaban solamente uno de España: el Alacant (Desde 1510, la historia más bonita jamás servida, 2022).

Por su parte, Michel Mollat ha destacado que en el comercio marítimo normando de finales del siglo XV, algunos mercaderes de Rouen, Dieppe y Honfleur compraban grandes cantidades de vino dulce de Alacant que, en parte, era reexpedido a Inglaterra, en donde entraba por los puertos de Chichester, Londres, Lynn y Boston. El transporte se hacía tanto en barcos normandos como vizcaínos, estos últimos a un ritmo de dos o tres por año, y en el viaje de ida llevaban, además de comestibles y vino, hierro de Bilbao.

Desde la Reconquista hasta el Renacimiento, Alicante fue famosa como puerto comercial y el vino se cargaba en barcos para abastecer a toda Europa. A tal fin, los comerciantes nórdicos, británicos y estadounidenses mantuvieron agentes comerciales en Alicante. De hecho, los reyes de España concedieron privilegios de comercialización preferente a los vinos de Alicante frente a otros vinos de nuestro país (Montojo-Montojo, 2007).

Ninguna descripción mejor que la que Ieronimus Münzer, médico de Nüremberg, hizo de Alacant y su puerto a mediados del mes de octubre de 1494, cuando aquel puerto estaba en plena campaña de exportación vinícola: "*Se crían también en las regiones marítimas hacia oriente [se refiere sin duda a la Huerta de Alacant] una gran cantidad de vino blanco; pero más todavía del tinto llamado de Alacant, que se envía a Inglaterra, Escocia, Flandes y otros lugares de Europa. Son vinos muy espesos y dan mucho color. En Flandes, con él tiñen el vino del Rin y lo fortifican, pues se produce en tanta abundancia que causa admiración. Aquel día había allí veintiséis naves de Vizcaya, de Flandes, etc., que allí se cargan de vino y de otras cosas*" Münzer, 1991).

Fernando el Católico en 1510 prohibió la distribución en Alicante de vinos procedentes de otras tierras. Felipe II en 1596 confirma el privilegio anterior: "*La collita de vi sia la mes principal de la qual se sustenta molta gent així principal com plebeyos*". Todos estos privilegios vienen dados para proteger el vino autóctono ya que su fama había traspasado nuestras fronteras.

Lo que ocurrió con el volumen de las exportaciones del vino de Alacant durante los siglos XVI y casi todo el XVII es muy difícil de saber, puesto que al ser un producto de tan alto valor, su control debió ser apartado de los *Llibres de Mercaderíes* y entregado a algún organismo especial, probablemente a una Junta Local Municipal, como la que a partir de 1679 empezó a recopilar la valiosa información sobre cosechas, cosecheros y comercio en los Libros del Manifiesto del Vino, base documental fundamental para conocer la evolución desde finales del XVII hasta finales del XVIII (Giménez-López, 1981).

Sabemos que el negocio del vino siguió siendo de capital importancia, como relatan Martín de Viciana en 1564 y el cronista local V. Bendicho en 1640 (Bendicho, 1991). Ciertos documentos confirman que en 1607 salieron al menos tres barcos con casi 100 botas de 40 cántaros de vino cada uno con destino a Inglaterra y que muchos barcos gallegos que venían a traer bacalao y sardina cargaban de retorno entre 10 y 20 botas de vino cada uno. No cabe ninguna duda que el vino de Alacant figuró entre los vinos más cotizados del mercado internacional del siglo XVII. Así lo demuestran varias investigaciones de Tim Unwin, en *Wine and the Vine: An Historical Geography of Viticulture and the Wine Trade* (1991), donde se hace una valiosa recopilación al tratar sobre los impuestos y los precios de los vinos en la Inglaterra de mediados del XVII. En 1632 el impuesto a la introducción de vinos fue establecido en 32 libras por tonel para los vinos de calidad y grado superior, como eran el Canary, el Muscadel y el Alacant; 26 libras deberían pagar el sack (Jerez) y el Málaga, 18 los vinos de Gasconia (Burdeos), 15 los de La Rochelle y sólo 12 el resto de los vinos menores procedentes de Francia.

El vino siguió siendo la verdadera insignia de la exportación alicantina por todo el siglo XVIII, como muy bien demostró Giménez-López, (1981) en su libro Alacant en el siglo XVIII. A lo largo del siglo XVIII la exportación de vino por el puerto de Alacant creció de 85.000 cántaros en 1712 a 107.000 en 1723 y a una media superior a los 230.000 a finales del mismo siglo, como se deduce de los «Estados de frutos y manufacturas» que por aquellas fechas elaboraban las autoridades de Alacant, en un momento en que la cosecha media oscilaba en torno a los 26.5000 cántaros, lo que equivaldría a suponer que se exportaba casi el 90 % de la producción local, si bien sospechamos que por estas fechas ya había fisuras en la Junta de Inhibición del Vino Forastero y se exportaban también vinos cosechados en pueblos del interior, como Novelda, Aspe y Monòver.

Aunque los vecinos del término general de Alacant procuraron mediante leyes y privilegios mantener la exclusiva de exportación de los vinos de su jurisdicción, ello no pudo impedir que en las comarcas del interior, sobre todo en la cuenca del Vinalopó, se desarrollara la viticultura comercial. La expansión del viñedo en estas tierras interiores se hizo sobre la misma variedad Monastrell que había dado fama internacional a los vinos de Alacant, aunque las diferentes coyunturas del mercado hicieron que primero en el siglo XVIII y luego en los siglos XIX y XX, la mayor parte de la producción se orientara a la producción de vinos de mezcla (Giménez-López, 1981).

El proceso de expansión del viñedo se hizo primero por la comarca conocida como *Vinalopó Mitjà*, la más cercana al puerto de Alacant, y luego se propagó hacia el Alto Vinalopó, la *Foia de Castalla* y *les Valls d'Alcoi*, mientras que en la Marina seguían una expansión paralela pero orientada hacia la elaboración de pasas.

El primer paso hacia la expansión del viñedo de masa tuvo lugar en la segunda mitad del siglo XVIII y estuvo motivado sobre todo por la demanda internacional de aguardiente, como confirmaba Cavanilles, (1797) al escribir sobre algunos municipios cercanos a Alacant y otros del valle del Vinalopó. Así, la producción de Monforte se había multiplicado por cuatro gracias, por una parte, a que sus vinos estaban acogidos al mismo privilegio que los de la Huerta de Alacant para poder ser exportados y, por otra, a la existencia en el propio Monforte de media docena de fábricas que destilaban 16.000 arrobas de aguardiente, para lo que precisaban más de 7.000 hl de vino.

Algo similar sucedía en Elda y Petrer, pueblos que dedicaron al cultivo de la vid los nuevos regadíos conseguidos mediante la traída de agua desde Villena, combinando la producción de uva fresca y pasas con la de vino y aguardientes.

Pero en ningún lugar esta industria alcanzaba el relieve que en la comercial Novelda, cuyas cuatro fábricas producían cada año del orden de 13.000 hl de vino. Algo más al sur, en Aspe, la vid había invadido la huerta del río Tarafa y se extendía hacia el suroeste del término por cerros y eriales que habían sido roturados por este motivo, alcanzándose una cosecha de 45.000 hl, cifra espectacular para aquella época (Giménez-López, 1981).

En *Monòver*, cuyo extenso término comprendía también los actuales del *Pinós* y *L'Alguenya*, la expansión había sido todavía mayor ya que, a comienzos del XVIII, la cosecha local no bastaba para surtir a la taberna del pueblo y era preciso traer vino de Elda, mientras que hacia 1790 se cosechaban nada menos que 60.000 hl, que hacían de *Monòver* el mayor productor de vino de todo el antiguo reino de Valencia. De acuerdo con la producción de vino estimada por el citado Cavanilles, podemos calcular que el viñedo de lo que ahora es la provincia de Alacant ocupaba a finales del siglo XVIII unas 22.000 hectáreas y estaba muy repartido por todos los municipios, dado que el vino era la bebida habitual en la llamada dieta mediterránea y había un consumo doméstico muy generalizado (Giménez-López, 1981).

El segundo gran impulso del viñedo se inició a mediados del siglo XIX, cuando el mercado exterior (Francia, Alemania, Suiza, etc.) empezó a demandar vino en grandes cantidades debido a dos causas principales:

- el fuerte incremento de la población urbana provocado por la revolución industrial, que significada mayor consumo de vino; y
- la crisis de producción que, sobre todo Francia, padeció a partir de 1.845 por culpa de las tres grandes plagas del viñedo venidas de América: el oídium (1.845-1.860), el mildiu y la filoxera.

Esta última fue la más terrible pues supuso la muerte de más de dos millones de hectáreas de viñas en Francia y de otro millón en España, entre 1870 y 1905, año hasta el cual las viñas alicantinas se habían visto libres de la misma, mientras que habían sido poco importantes los daños causados por el oídium y el mildiu, dos plagas criptogámicas que sólo prosperan en ambientes húmedos, algo que en Alacant sólo se da en la Marina (Dion Roger, 2010).

La gran demanda exterior vino a coincidir con una revolución de los medios de transporte que facilitaban la exportación en grandes cantidades, y que en nuestro caso se concretaron en el ferrocarril Madrid-Alacant, inaugurado en 1.858, y las mejoras en los muelles del puerto para poder albergar a los nuevos buques de vapor que fueron sustituyendo a los viejos veleros. Las estaciones de Villena y *Monòver* se convirtieron en grandes puntos de expedición y en sus alrededores se agolpaban las grandes bodegas comerciales.

La construcción en 1884 de otro ferrocarril, esta vez de vía estrecha, con la intención de extraer los vinos de Jumilla y Yecla, de una parte, y los de Alcoi y Cocentaina, de otra, siempre a través de Villena, atrajo a esta ciudad a gran número de comerciantes bodegueros y sociedades de exportación tanto de origen nacional como extranjero. Al mismo tiempo, algunos de los grandes cosecheros locales se convirtieron en comerciantes por cuenta propia o comisionistas de firmas francesas y catalanas.

No se debe perder de vista la vertiente granelística exportadora de Alicante. Así, los vinos de Burdeos, en el siglo XIX iban bastante cortos de alcohol. Esto explica una técnica que hoy nos puede parecer descabellada. Según señala André Julien en *Topographie de tous les vignobles connus, contenant : leur position géographique, l'indication du genre et de la qualité des produits de chaque cru, les lieux où se font les chargements et le principal commerce de vin, le nom et la capacité des* *tonneaux et des mesures en usage, les moyens de transport ordinairement employés, suivie d'une classification générale des vins* en 1816, hasta mediados del siglo XIX, los vinos destinados a Inglaterra (los mejores de Burdeos) se sometían al "travail à l' anglaise" (Dion Roger, 2010) *(Travail à l'anglaise: Pratique oenologique consistant à remettre en fermentation une partie des vins avec un ajout de 12 à 18 % de vin provenant de la vallée du Rhône, du Roussillon (cépage Benicarlo) ou d'Espagne avc un moût partiellement fermenté et de l'esprit de vin (alcool éthylique). Le but recherché étant de « renforcer » les vins en obtenant un vin plus alcoolisé et aromatique*).

La receta requería el uso de 30 L de vino español (Alicante o Benicarló), 2 L de mosto blanco sin fermentar y una botella de aguardiente por cada barrica de clarete. En el verano posterior a la cosecha, el vino se ponía a fermentar de nuevo con estos aditivos, después se trataba como los demás vinos y se conservaba varios años en barricas de roble antes de su embarque. El resultado era un vino fuerte y de buen sabor, aunque "cabezón y no apto para todos los estómagos" (Piqueras-Haba, 1981).

Hacia 1890 operaban en los alrededores de la estación de Villena más de veinte exportadores, de ellos varios franceses (*Moullé et Jeune*, *Blondeau*, *Reverchon*, etc.) y la mayoría de origen comarcal, tales como García Poveda, Salvador Amorós, Pedro Conesa, Galvis e Hijos, Francisco Hernández, etc., alguno de los cuales ha contado con sucesores hasta nuestros días.

Por su parte, la estación de *Monòver* atrajo a bodegueros de toda la comarca, como los Quiles, los Amorós, los Pérez Verdú, los Amat, etc. y a algunas firmas del resto de España, como Bodegas Bilbaínas.

Mayor fue la aglomeración de bodegas exportadoras en las inmediaciones del puerto de Alacant. A finales del XIX había allí comerciantes autóctonos como los Maluenda, Cerdá, Amorós, y otros forasteros, sobre franceses, como *Moullé et Jeune*, socios de Penalva, *Laguillon*, *Isaujou*, *Carrière*, *Meziat*, *Ribeil Frères*, etc. a los que ya entrado el siglo XX se les unirían Federico Madrid, José de Barrio, Luis Brotons, León Dupuy y, ya en los años treinta, la firma suiza Bodegas Schenk, que con el tiempo habría de convertirse en la primera firma exportadora de vinos de Alacant a granel.

El volumen de exportación por el puerto de Alacant y la playa de Santa Pola creció de forma espectacular alcanzando los 130.000 hL en 1861, los 550.000 hL en 1880 y los 2.500.000 hL en 1891, momento en que también salían por aquí vinos de Murcia y La Mancha, pues la cosecha provincial no bastaba para tanta demanda (Piqueras-Haba, 1981).

Luego decayó notablemente, debido a la recuperación de las cosechas en Francia, y al comenzar el siglo XX, justo cuando nuestras viñas empezaban a ser destruidas por la filoxera, la exportación apenas alcanzaba los 300.000 hectolitros anuales y a precios muy bajos.

Pero la buena coyuntura vivida durante medio siglo significó una auténtica "edad de oro" para la exportación de vinos de mezcla, para remontar el color y el grado de los vinos franceses, alemanes y suizos, y además provocó una fiebre de plantaciones de viñas, cuya superficie creció de las 22.000 ha estimadas a fines del XVIII a 32.000 ha en 1857, 62.000 ha en 1886 y un máximo de 104.000 ha en 1islam904 (Giménez-López, 1981).

El afán por obtener uvas que dieran un mayor tono de color propició la aclimatación de la variedad híbrida Alicante-Bouchet o garnacha tintorera, descubierta por el ampelógrafo francés Bouchet en 1855 e introducida en España por Antonio Sánchez Almodóvar en su finca del término de Aspe, desde donde se propagó valle del Vinalopó arriba hasta afincarse con notable intensidad en tierras de Villena, donde su presencia es todavía significativa.

La filoxera llego a la Comunidad Valenciana 61 años después de haberlo hecho en la Provenza francesa. Los viticultores de nuestra tierra lograron mantener fuera esta amenaza, mientras iba llegando y destruyendo los viñedos de las demás regiones españolas. Este es uno de los logros más valorados en la historia de la gente de esta tierra, pues mientras evitaban esa pandemia incluso llegaron a aumentar la superficie vitícola de la región pasando de 129.000 a 259.000 ha (Martín J.C., 2011).

La plaga filoxérica llegó a tierras alicantinas con mucho retraso con respecto a otras regiones de España. El primer foco fue detectado en 1900 en Dolores, por invasión natural procedente de Murcia, y desde allí se propagó hacia el Camp d'Alacant (1905) y el valle del Vinalopó, llegando en 1906 a *Monòver* y en 1909 a Villena.

Mientras tanto, la introducción fraudulenta de plantas americanas provocó nuevos focos: Gata de Gorgos (1904) desde donde se extendió rápidamente por toda La Marina, y en la comarca valenciana de la *Vall d'Albaida* (1906), de donde saltó al año siguiente a los viñedos de *La Foia d'Alcoi*. La difusión de la plaga, excepto los áridos glacis calizos del Vinalopó Occidental donde el terrible insecto no encontró un medio apropiado para su desarrollo, provocaba el arranque masivo de todas las vides europeas y la replantación con planta americana. Pero dicha replantación, que se prolongó durante varias décadas, sólo se llevaría a término en *La Foia de Castalla* y en el valle del Vinalopó (Fundación MARQ, 2014).

En ambos casos la superficie del viñedo en 1977 volvía a ser prácticamente la misma que a finales del XIX, antes de la plaga. Por el contrario, en la comarca de la Marina y en *Les Valls* *d'Alcoi* se replantaron muy pocas viñas y en 1977 el viñedo ocupaba diez veces menos de tierra que antes de la filoxera, desapareciendo totalmente en muchos de los municipios.

Las causas de aquel abandono fueron la mala coyuntura del sector (tanto del vino como de las pasas) en los mismos años en que había que replantar, y los mayores gastos de cultivo que exigía la planta americana, que sólo resultaba rentable en las tierras llanas. Es por eso por lo que en los bancales escalonados de la Marina y *Vall d'Alcoi*, la vid fue sustituida por otros cultivos más rústicos y adaptables a las fuertes pendientes, como el olivo y el almendro (Fundación MARQ, 2014).

El balance total del viñedo provincial fue una reducción a la mitad, desde las más de 86.000 ha de 1889 a las casi 43.000 ha de 1945 y las 41.500 ha de 1997.

La recuperación definitiva del sector vitivinícola se basó en la creación de la Denominación de Origen Alicante (DOP Alicante) en 1932. En 1931, la II República había publicado "el Estatuto de la Viña y el Vino", que dio lugar al año siguiente a la creación de las primeras diecisiete DOs españolas, entre las cuales había cuatro de la región valenciana: Alicante, Valencia, Utiel-Requena y Cheste. El 13 de septiembre de 1932 se publicó en la Gaceta de Madrid (Boletín Oficial) la creación de las DOs con una estructura que recordaba a la de la *Junta d'Inhibició del Vi Foraster d'Alacant*; este documento definía para cada DO su ámbito geográfico, sus órganos de gestión y control, su organismo regulador, sus normas, su zona de crianza, su reglamento y su misión (Fundación MARQ, 2014).

La crisis provocada por la filoxera, los mayores costos de cultivo de la planta americana (más abonos, tratamientos contra el oidium y el mildiu, etc.) y la necesidad de liberarse de la "tiranía" comercial que significaba para los pequeños cosecheros tener que vender sus uvas a los bodegueros comisionistas, eran un terreno propicio para el desarrollo del movimiento cooperativista, que se inició con la fundación de sindicatos agrícolas promovidos casi siempre por los párrocos ya a comienzos del siglo XX.

En los primeros momentos, aquellos sindicatos tenían como principal y casi único objetivo la creación de cajas de ahorros o montepíos para ayudar a los pequeños agricultores y obreros. Imitando el modelo alemán, en las zonas rurales el colectivismo empezó a aplicarse también a la elaboración en común de ciertos productos que, como el aceite y el vino, requerían unas instalaciones (almazaras y bodegas) que muy pocos cosecheros podían establecer por su cuenta, viéndose obligados a tener que llevar la aceituna y la uva a establecimientos privados, que se la compraban al precio que querían o bien las trabajan a maquila.

Pero las bodegas cooperativas tardarían todavía algunos años en cristalizar y ello gracias al empeño de algunos de los llamados «apóstoles» del cooperativismo (párrocos, médicos, abogados, ingenieros, etc.) entre los que destacó de forma singular un alicantino de Sax, el ingeniero agrónomo Pascual Carrión, quien tras el Congreso Nacional de Viticultores celebrado en Valencia en 1926, logró convencer a otros grandes propietarios (él también lo era) de Sax, *Monòver* y el *Pinós*, para poner al servicio de los pequeños cosecheros sus bodegas familiares y fundar así las tres primeras bodegas cooperativas de la provincia: las de Sax (1928), *Monòver* (1929) y el *Pinós* (1932). En la primera figuraba el propio Carrión como mayor propietario al frente de casi medio centenar de pequeños cosecheros.

En *Monòver* fueron los hermanos Eduardo y Ricardo Pérez Gutiérrez, quienes lideraron a los 32 socios fundadores, que en el primer año de cosecha (1930) lograron reunir 1.386.000 kg de uva, de los que más de 400.000 kg eran de la familia Pérez, que había puesto, como Carrión en Sax, su propia bodega al servicio de los cooperativistas.

Pero aquellas primeras iniciativas tardaron mucho tiempo en tener seguidores y se tuvo que esperar a los años cuarenta, ya bajo la tutela y promoción del régimen franquista, cuando se fundó la de Castalla (1947), a la que siguieron las de *Beneixama* (1952), Villena (1961), el *Manyà* (1962), la Romana (1964) y, por último, *L'Alguenya* (1976), por citar sólo las más grandes.

Sin embargo, en el resto de la provincia el cooperativismo tuvo poco éxito. En *Les Valls d'Alcoi* sólo se crearon dos bodegas muy modestas en *Gaianes* y *Beniarrés*, mientras que en la Marina, tras varios intentos por crear cooperativas paseras, sólo lograron prosperar dos bodegas: la de *Xalò* (1962) y la de Teulada, ambas todavía en plena actividad.

Aunque nunca faltaron bodegueros particulares que lucharon por mantener una línea de vinos de marca embotellados, la verdad es que la coyuntura del mercado internacional surgida de aquellos años de gran demanda de finales del siglo XIX, orientó la mayor parte de la producción de vinos alicantinos (casi todos de la variedad Monastrell) hacia los vinos de pasto, ideales por su color y alta graduación, para ser mezclados de forma anónima con otros, no sólo de Francia, Suiza y Alemania, sino también de otras regiones de España, sin olvidar la vecina Jumilla, donde algunas grandes firmas todavía siguen elaborando uvas y vinos del Vinalopó.

Fue ya a partir de 1970, coincidiendo cronológicamente con la revitalización de la DO Alicante, cuando se inició la nueva etapa que llega a nuestros días y que está caracterizada por la apuesta cada vez más generalizada de los vinos de marca embotellados. Entre los innovadores pioneros hay que destacar por orden cronológico en primer lugar a Salvador Poveda Luz, quien en 1968 inició el embotellado a gran escala, con marcas propias como «Viña

Vermeta» que pronto habría de convertirse en símbolo de la nueva enología alicantina. El ejemplo tardó un tiempo en tener seguidores, y aunque hubo varios intentos de escasa importancia en cuanto al volumen se refiere, hubo que esperar hasta 1989 para la aparición de otra gran firma, la de Bodegas Mendoza (*L'Alfàs del Pí*) para que los vinos alicantinos dieran un segundo paso hacia el reconocimiento de calidad internacional. Casi al mismo tiempo, año 1987, varias bodegas cooperativas se unían para fundar otra de segundo grado con el nombre de BOCOPA, destinada a embotellar y comercializar vinos de calidad, que tras la construcción en el año 2000 de una gran bodega en el término de Petrer y una campaña de publicidad radiofónica de gran alcance, se ha convertido en el tercer gran pilar, de la nueva imagen de los vinos alicantinos.

El vino de Alicante ha mantenido una inquebrantable identidad histórica, por ejemplo, más antigua que la de Burdeos, más permanente que la de Oporto, y más directa que la de Jerez. El vino de Alicante se caracterizó a lo largo de la historia por su estabilidad enológica, por su intenso color, por su rico sabor y por su capacidad evolutiva. Estas virtudes y sus características provienen de la asociación de la variedad (Monastrell) y el clima, dando como resultado un vino que ha mantenido su personalidad y sus bondades desde el comienzo de su elaboración hasta la actualidad (Fundación MARQ, 2014).

4.2 La Denominación de Origen Protegida Alicante

La Denominación de Origen Protegida (DOP) Alicante es una figura de protección agroalimentaria reconocida por la Unión Europea y acreditada como certificadora de producto por ENAC. Es una de las más antiguas de España, se originó en 1932 y se regulo en 1957, con el objetivo de proteger las zonas históricas y de mejor producción y los vinos más particulares de este entorno geográfico. Esta figura de calidad es garantía del origen de los productos vínicos elaborados y etiquetados de acuerdo con el Pliego de Condiciones de la DOP Alicante (Resolución 4/8/2021 de la Conselleria de Agricultura, Pesca, Alimentación y Agua de la Generalitat Valenciana) y tiene como principal objetivo el impulso de la calidad de los vinos, garantizar la autenticidad de los mismos y la defensa de todos los productores de vino dentro de la DOP Alicante (Conselleria de Agricultura, Pesca, Alimentación y Agua de la Generalitat Valenciana, 2021). La zona de producción amparada por la DOP Alicante está dividida en subzonas que coinciden con las comarcas naturales de la provincia de Alicante, e incluyen áreas con climas diferentes, donde varía la humedad y la naturaleza de los suelos (**Figura 1**).

Figura 1. Principales regiones vitivinícolas de la DOP Alicante (Vinos alicante DOP, 2021).

En la actualidad, la DOP Alicante tiene registrados unos 2.100 viticultores y hay 38 bodegas certificadas (Vinos alicante DOP, 2021). El Consejo Regulador de la DOP Alicante trabaja de acuerdo con la norma internacional de certificación de productos alimentarios, UNE 17065, y está acreditado por la Entidad Nacional de Acreditación ENAC para esta labor; este es un hecho histórico que respalda el trabajo realizado desde hace años, pero que, sobre todo, revela una adaptación a los nuevos tiempos y a las nuevas normas que el consumidor del día de hoy demanda. Con esta garantía, además, se han abierto mercados internacionales gracias a la reputación de este sello.

4.2.1 Variedades de la Denominación de Origen Protegida Alicante

Los suelos de la DOP Alicante son areno-francos y arenosos, por lo tanto, tienen una granulometría y textura especialmente aptos para el cultivo de la vid. La climatología de Alicante también es un factor importante, ya que, gracias a esta, se puede encontrar una amplia diversidad de variedades en la zona. Las variedades actuales amparadas por la Denominación de Origen son:

- Blancas: Moscatel de Alejandría, Airén, Subirat Parent (o Malvasia), Chardonnay, Macabeo, Merseguera, Planta fina de Pedralba, Sauvignon blanc, Verdil.
- Tintas: Monastrell, Garnacha Tintorera (o Alicante Bouschet), Garnacha tinta (o Gironet), Bobal, Cabernet Sauvignon, Merlot, Pinot Noir, Petit Verdot, Syrah, Tempranillo.

Pero de entre todas las anteriormente enumeradas, las de mayor implantación y mejor adaptación son: (*i*) Monastrell, (*ii*) Moscatel de Alejandría, y (*iii*) Garnacha Tintorera (o Alicante Bouschet). A continuación, se presentan detalles de la variedad Monastrell al ser esta con la que se elabora el Fondillón, base de esta tesis doctoral.

4.2.1.1 Variedad de uva Monastrell

Dentro de la DOP Alicante esta variedad representa aproximadamente el 75 % de su cultivo y es la variedad que mejor representa la adaptación al terreno. Es de origen español y se sitúa en la Comunidad Valenciana y en la región de Cataluña, si bien después del siglo XVI, se empezó a cultivar en Francia (Vinos alicante DOP, 2021).

El tamaño de las vides es mediano pero muy compacto y fuerte, lo que permite aguantar las horas de sol e incluso sobremadurar en la cepa (**Figura 2**). Las condiciones de sequía de la región la han hecho fuerte y resistente, con un gran poder aromático y versatilidad para realizar vinos monovarietales, vinos con mezclas varietales (*coupages*), vinos de licor y, por supuesto, Fondillón, ya que la maduración es tardía lo que implica que tiene el potencial de madurar y alcanzar altos niveles de azúcar para luego ser transformados en alcohol durante el proceso de fermentación.

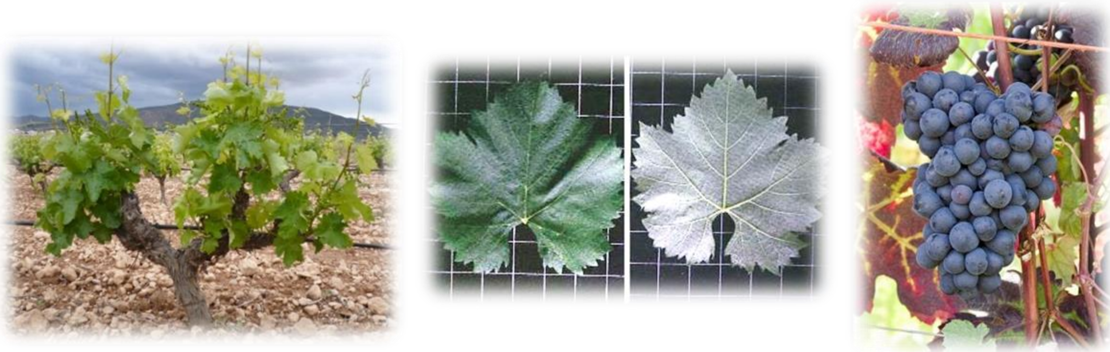

Figura 2. Cepa, hoja y racimo de la variedad Monastrell (Ministerio de agricultura, pesca y alimentación, 2023).

Respecto a las hojas (**Figura 2**) adultas, presentan un tamaño y longitud medianos, tienen forma pentagonal-orbicular, con tres lóbulos, haz verde oscuro y envés algodonoso. En cuanto a los racimos, son de tamaño pequeño a mediano, compacto y longitud de pedúnculo muy corto (**Figura 2**). El tamaño de la baya es de pequeña a mediana y uniforme, la epidermis presenta un color azul-negra, con forma esférica, grosor de la piel media, la pulpa no presenta pigmentación, cuya consistencia es blanda a ligeramente dura y jugosa, desabor neutro y con presencia de pepitas.

A modo de resumen, podemos indicar que la variedad utilizada para elaborar Fondillón es la Monastrell, una variedad española, adaptada al clima mediterraneo de Alicante. Es una variedad que florece y madura muy tarde, produciendo vinos ricos en alcohol y taninos, baja acidez, muy afrutados y con gran estructura.

4.2.2 Tipos de vino

La clasificación de los vinos de la DOP Alicante está recogida en el Diario Oficial de la Comunidad Valenciana RESOLUCIÓN de 4 de agosto de 2021, de la consellera de Agricultura, Desarrollo Rural, Emergencia Climática y Transición Ecológica, por la que se adopta y hace pública la decisión favorable a la modificación del pliego de condiciones de la Denominación de Origen Protegida Alicante.), donde se establecen los atributos o características organolépticas que deben contener los principales vinos protegidos bajo esta figura de calidad:

- Vino blanco: Los principales en la zona de Alicante son los elaborados con la variedad Moscatel. Estos vinos presentan tonos muy pálidos y limpios, con aromas a flores blancas como el azahar, el jazmín. En boca son ligeros, persistentes y con buena acidez.
- Vino rosado: El vino rosado que procede de Monastrell, por su carga fenólica, suele ser intenso, con tonalidad rosado frambuesa o gominola, con ligeros toque aromáticos a fresas y con una muy buena estructura.
- Vino tinto: Hay dos tipos de vino tinto:
  - *Vinos jóvenes*, los cuales no han pasado por barrica o lo han hecho por un periodo de tiempo inferior a 6 meses. Estos vinos tienen un color que van desde rubí a tonos violetas; en nariz destaca la fruta fresca roja. Destacan por su buen equilibrio y su buena estructura.
  - *Vinos con envejecimiento*, los cuales han pasado, como mínimo, 6 meses de envejecimiento en barrica. Se trata de vinos de capa media o alta, con aromas a fruta madura (ciruelas, cerezas) e intensos en nariz y en boca.
- Fondillón: El principal y más característico de los vinos de la DOP Alicante. Tiene una gran intensidad aromática, un postgusto largo y buen equilibrio. Tiene aroma a fruta madura, frutos secos y toques balsámicos.

4.2.3 El Fondillón

El nombre Fondillón proviene del término latino "fondus", o "fondo", que se refiere a la forma en la que el vino se envejece utilizando un sistema de solera, como usan en Jerez, donde el vino se extrae de una barrica y se mezcla con otros más envejecidos de otras barricas diferentes (5 barricas amantes del vino, 2023).

El Fondillón, a pesar de ser un vino desconocido de la provincia de Alicante, reinó durante varias épocas en las cortes europeas y llegó a ser el vino más caro del mundo y uno de los de mayor exportación. Según el catálogo de vinos de Maisonnave de finales del siglo XIX, el Fondillón alcanzaba el precio de 800 francos/hectolitro, en comparación con otros vinos apreciados de esa época como el Jerez o el Oporto, que alcanzaban los 204 y los 153 francos, respectivamente.

Hasta finales del siglo XVIII era el único vino español de renombre en Alemania, donde junto al Malvasier y al Peter Simens, era uno de los tres vinos dulces más cotizados del mundo. Gozaba de tanta estima que había que beberlo en copas de plata, no de cristal, como los vinos de Rhin.

El origen del Fondillón tiene que ver con las leyes de distribución y cesión de tierras después de la reconquista en la Huerta de Alicante, una superficie de aproximadamente 3.800 ha, ubicada al norte de la ciudad. La enfiteusis, una legislación proveniente del Derecho Romano, se dio como solución para repoblar las tierras en el antiguo Reino de Valencia tras la expulsión definitiva de los moriscos en el año 1609. El instrumento para repoblar estos terrenos fueron las cartas pueblas que incluían la entrega de tierras y viviendas a los arrendatarios a censo enfitéutico. Esto significaba que, mientras al arrendatario le quedasen las viñas en producción, le pertenecían los derechos de explotación de las mismas. Por tanto, los agricultores no arrancaban las cepas más viejas, para no perder sus derechos. La recogida de estas cosechas se hacía después de la vendimia, en plan familiar por los propios arrendatarios. Después estos arrendatarios, las estrujaban en el lagar y ponían a fermentar el mosto en los toneles más viejos de la bodega. La fermentación era muy lenta y la transformación del mosto en vino se retrasaba hasta la primavera. El resultado era un vino con alta graduación alcohólica.

En el año 1453, los turcos conquistaron Constantinopla, y tanto los venecianos como los genoveses empezaron a perder sus colonias comerciales y sus influencias en el Oriente del Mediterráneo. Entre los siglos XVI y XVII, se refuerza la presencia de italianos en Alicante, sobre todo de genoveses (Ansaldo, Bonfante, Canicia, Forneli, Imperial, Patucio, Pavia, Rivanegra, Scoria) y milaneses (Paravecino). En esta época, son ellos quienes dominaban el comercio del vino tinto de Alicante.

Por otra parte, también está confirmada la presencia de comerciantes ingleses en Alicante, que compraban el vino tinto para mejorar los vinos de Burdeos que comercializaban. A mediados del siglo XVII el vino de Alicante era el más caro de Inglaterra.

La primera referencia escrita sobre la palabra española Fondillón o su homóloga valenciana *Fondellol*, está vinculada a un milagro atribuido al Beato Andrés Hibernón (1534-1602) que llenó y vació una tina de Fondillón para dar de beber a un enfermo que luego sanó; esta referencia aparece en su causa general de beatificación (Cavanilles, 1797).

En el diccionario Covarrubias en la edición de 1611, apareció la primera definición de Fondillón: "*El asiento y la madre de la cuba cuando la mediada se vuelve a volver a utilizar y suele conservarse por muchos años, y el vino de Fondillón de ordinario es fuerte y generoso*". Sin embargo, esta no es una definición de vino sino una definición de proceso de envejecimiento. La primera definición de "Fondillón" como vino aparecerá en el Diccionario de la Real Academia Española en 1791 como "vino rancio de Alicante". Desde este momento, las referencias a su origen son inequívocas (Carmona-Escribano, 2015).

La primera descripción detallada de la elaboración aparece en 1808, gracias al trabajo de Don Francisco de Paula Valcárcel y Pio de Saboya, Conde de Lumiares. Es interesante que esta descripción se refiera a la uva Morastel (sinónimo de Graciano) y no a la Monastrell.

En el Siglo XVIII, el Fondillón, fue uno de los vinos más prestigiosos del mundo, se decía que era el favorito de Luis XIV de Francia e Isabel I de Inglaterra. Igualmente aparece mencionado en la obra de Alejandro Dumas "El Conde de Montecristo", y también lo nombran Dostoievski y Shakespeare, con el término "Alicante", ya que era así como se conocía (Mark O´Neill, 2018).

Los problemas del Fondillón aparecen con la filoxera, y no, como sería normal, por la destrucción del viñedo, sino más bien por la enorme demanda mundial de vino, especialmente de Francia. Ya se ha visto que el parásito no llegó a España hasta la primera década del siglo XX, cuarenta años después de arruinar los viñedos franceses. Durante esos cuarenta años de destrucción del viñedo europeo, Alicante multiplicó sus viñedos hasta alcanzar el máximo histórico de 93.000 ha en 1895.

El afán de producir vino en cantidad hizo olvidar el cuidado de las viñas viejas y la tradición de dejar algunas viñas sin cosechar hasta bastante después de la vendimia principal quedó únicamente en algunas casas solariegas. Tras la crisis de sobreproducción global y la destrucción del viñedo de la huerta de Alicante por la filoxera, sólo quedaron viñas de Monastrell en el valle del Vinalopó para elaborar Fondillón. La presión urbanística terminó de condenar a muerte el viñedo de la huerta de Alicante. De este modo, el Fondillón desapareció progresivamente del comercio y sólo algunos productores locales lo conservaron. Los saqueos de la Guerra Civil terminaron con muchos toneles viejos, muy pocos han sobrevivido y están todavía en uso.

A comienzos del siglo XX, con la filoxera, los cambios en los tipos de vinos que se elaboran y en los gustos de los consumidores, hay graves problemas con la elaboración y comercialización del Fondillón, llegando a una completa desaparición de este vino; sin embargo, posteriormente fue recuperándose poco a poco hasta lograr nuevamente el gran prestigio enológico del que goza en la actualidad. El resurgimiento del vino Fondillón se dio en los años 50 cuando empezó a elaborarse de nuevo en las tierras del interior de la provincia de Alicante, especialmente en el Vinalopó (Campello-Quereda *et al.*, 2009). El trabajo de las familias Quiles y Poveda, ambas de Monóvar, ha mantenido y hecho renacer la producción de Fondillón.

El Fondillón vio reconocida su singularidad enológica e histórica al incorporarse en el reglamento de la DOP Alicante en 1986 y desde 2011 está reconocido por la Unión Europea en su base de datos E-bachus.

4.2.3.1 Proceso de elaboración

La vinificación del Fondillón comienza con la fermentación de las bayas (sobremaduradas en la cepa) estrujadas y despalilladas. Esta fermentación alcohólica va ocurriendo lentamente por causa de los altos niveles de azúcares y por el contenido en flora microbiana desarrollada durante el proceso de sobremaduración. Posteriormenre, se produce una parada en la fermentación de forma natural cuando el grado alcohólico llega a los 16°-18°, quedando el vino con un nivel medio de azúcar residual oscilando entre los 10 y los 40 g/L.

El envejecimiento o crianza del Fondillón, es la etapa más importante, ya que este proceso implica varios años, como minímo 10 años. Los toneles empleados para el envejecimiento/crianza son los tradicionales monoveros o alicantinos con capacidad de hasta 1.200 L y se realiza mediante dos técnicas:

(i) <u>Sistema de criaderas y solera</u> (**Figura 3**) el cual consiste en la mezcla de las crideras más antiguas con un porcentaje controlado de otras más nuevas; es decir, cada año se vacía un tercio del volumen de la solera en donde se encuentra el vino más "viejo" llamado madre. Este es el vino que se embotella para su venta. El hueco dejado se

rellena con un tercio del volumen de cada criadera anterior y este se rellena con el de la anterior, así sucesivamente hasta llegar a la criadera más moderna. El relleno máximo en cada saca será del 10 % anual más la merma (máximo 5%) (DOP Alicante, 2019; DOP Alicante, 2022). El vino que se encuentra en las criaderas, debe tener como minimo 10 años para que pueda ser usado para rellenar la solera. El hueco de los toneles de esta última criadera, se rellena con vino del año llamado tambie sobretabla, siendo su graduación alcohólica entre 15 y 18 % vol/vol. (Cernuda-Juan *et al.,* 1975).

(ii) <u>Sistema de añadas</u>, es decir, con la mezcla de las uvas procedentes de una única campaña y empleando un tonel nuevo para su envejecimiento. Se necesita 10 años, como minimo, para que el vino pueda ser declarado como Fondillón.

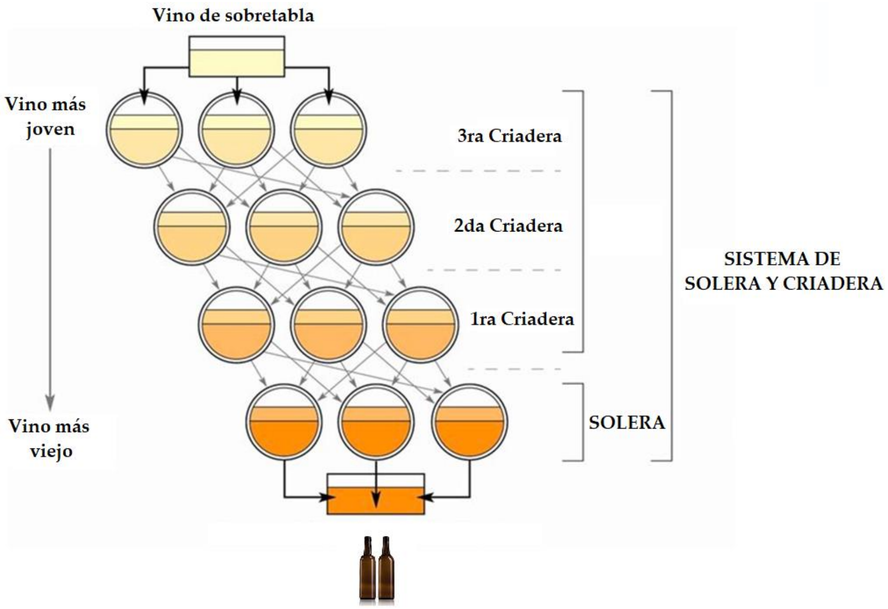

Figura 3. Sistema de solera y criaderas (Wine and other stories, 2023)

Su peculiar proceso de envejecimiento/crianza da vinos con unas características únicas que lo diferencian del resto de vinos; el Fondillón tiene un tono ámbar, es ligeramente dulce, con aromas de pasa y madera y con un aroma distintivo debido a su proceso de oxidación lento y prolongado en el tiempo (Cernuda-Juan *et al.,* 1975).

4.2.3.2 *Bodegas y producción*

Tal y como se ha descrito para la elaboración del Fondillón se necesita mucho tiempo de envejecimiento, por lo cual son muy pocas las bodegas que lo elaboran. Adicionalmente, las bodegas tienen que solicitar una certificación específica del cumplimiento de los requisitos establecidos en el pliego de condiciones. La producción de Fondillón certificado por la DOP Alicante en el 2022 se muestra en la **Tabla 1** (Datos proporcionados por el Consejo Regulador de la DOP Alicante).

Tabla 1. Registro del Fondillón certificado y embotellado por bodegas privadas y por cooperativas durante 2022.

Bodega/Marca comercial	Volumen certificado	Volumen embotellado	Total (L)
Bodegas Monóvar -Bodegas Monóvar	139.654	1.258	140.912
BOCOPA Coop. Val. -BOCOPA	2.100	0	2.100
Primitivo Quiles S.L. -Primitivo Quiles	20.650	0	20.650
Bodega Coop. N. Sra. de las Virtudes -Bod. Las Virtudes	11.940	274	12.214
Brotons, Vinos y Aceites S.L. -Brotons	54.166	375	54.541
Bodegas Alejandro S.L. -Bodegas Alejandro	15.300	0	15.300
Bod. Coop. Santa Catalina -Santa Catalina del Mañán	12.650	0	12.650
Hacienda la Serrata S.L. -Francisco Gómez	76.656	0	76.656
Bod. Cooperativa de Algueña -Vinos de Algueña	44.100	0	44.100
Naoual Bouizgaren - Bodegas El Pinaret	7.800	910	8.710
TOTAL	***385.016***	***2.817***	***387.833***
Total Bodegas privadas	314.226	**82 %**	
Total Cooperativas	70.790	**18 %**	
Total Solera		**80 %**	
Total Añada		**20 %**	

En la **Tabla 1** se puede ver, que el 82 % de la producción de Fondillón depende de las bodegas privadas, mientras que el 18 % de las cooperativas y el 80 % de este vino está producido por el sistema de solera. Como puede verse fácilmente, el porcentaje de Fondillón que se embotella (0,73 %) representa una cifra minúscula en comparación con la totalidad de vino certificado bajo esta categoría. Este hecho indica claramente que hay un potencial de desarrollo enorme para este vino que como hemos reseñado es el núcleo de la DOP Alicante.

4.2.3.3 *Características fisicoquímicas y sensoriales*

Las características fisicoquímicas (**Tabla 2**) que indica el Pliego de Condiciones de la DOP Alicante para el Fondillón se muestran a continuación.

Tabla 2. Parámetros fisicoquímicos del Fondillón.

Características analíticas	
Grado alcohólico total mínimo (% Vol.)	16
Grado alcohólico adquirido mínimo (% Vol.)	16
Azúcares reductores (g/L)	< 45
Acidez total mínima (g/L en ácido tartárico)	3,5
Acidez volátil máxima (g/L en ácido acético)	2,2
Dióxido de azufre total máximo (mg/L en SO_2)	200

Todo el alcohol proviene exclusivamente de la propia fermentación y está totalmente prohibido añadir, encabezar, remontar o fortificar. Con el paso de los años, los toneles pueden sufrir evaporación de agua y de etanol por causa de la sequedad ambiental; esto tiene como consecuencia el aumento del grado alcohólico de forma natural. Por consiguiente, es posible que Fondillones muy viejos presenten contenidos de alcohol de 22-23 % sin que haya añadidos en ningún momento (DOP Alicante, 2019; DOP Alicante, 2022).

Respecto al azúcar que contiene el Fondillón, proviene exclusivamente del mosto; estos azucares son residuales, es decir, son los que no han sido transformados en alcohol por la incapacidad de las levaduras de seguir activas a un nivel elevado de alcohol. Por lo tanto, está totalmente prohibido adicionar azucares, mostos concentrados o cualquier otro edulcorante al Fondillón.

El perfil sensorial del Fondillón es:

- ✓ color caoba, ámbar y tonalidades cobrizas;
- ✓ aromas frutales, entre ellos fruta madura y frutos secos, notas a tostado, madera y café;
- ✓ en boca también destacan la fruta madura, los frutos secos, notas a especias, tostado, madera, ácido, amargo y una larga persistencia.

4.2.3.4 Estado actual de la investigación sobre Fondillón

Una revisión bibliográfica en la base de datos SCOPUS a fecha 8 de junio de 2023 empleando como palabra clave "Fondillón" arroja como resultado 7 artículos: los 5 incluidos en esta tesis doctoral, 1 publicado por nuestro grupo en 2023 y posterior al cierre de esta tesis (https://doi-org.publicaciones.umh.es/10.1007/s00217-023-04256-3) y una única publicación previa al comienzo de esta tesis doctoral:

- ✓ Arrieta M.P., Prats-Moya M.S. 2012. Free amino acids and biogenic amines in Alicante Monastrell wines. *Food Chem.* 135(3): 1511-1519.

Como puede observarse la necesidad de investigación vinculada directamente al Fondillón es evidente e incuestionable y el crecimiento en la comercialización de este vino tan especial irá ligado, sin duda alguna, al mayor conocimiento científico de este vino. Además, este mayor conocimiento del producto constituirá una base incuestionable para mejorar el conocimiento que los consumidores españoles e internacionales tienen sobre el Fondillón y así aumentar su demanda por este vino de la DOP Alicante.

5. OBJETIVOS

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El objetivo principal de esta tesis doctoral fue realizar una caracterización fisicoquímica y sensorial del Fondillón de diferentes bodegas y soleras/cosechas, para evaluar su aceptación por parte de los consumidores nacionales y determinar los principales factores (*drivers*) que determinan su acto de compra y su satisfacción.

Para alcanzar el objetivo principal, los objetivos específicos fueron:

1. Realizar una caracterización fisicoquímica del Fondillón e identificar su perfil volátil.
2. Identificar los compuestos aromáticos activos del olor del Fondillón y su perfil polifenólico.
3. Determinar el perfil sensorial descriptivo de Fondillón.
4. Establecer el perfil general del consumidor típico de Fondillón.
5. Estudiar el efecto del envejecimiento y potencial adulteración sobre los principales parámetros fisicoquímicos, composición volátil y perfil sensorial del Fondillón.

6. MATERIALES Y MÉTODOS

Los materiales y métodos utilizados en esta tesis doctoral se detallarán en función de los artículos publicados.

4.1 PUBLICACIÓN 1

4.1.1 Muestras de vino

En este estudio se analizaron siete muestras de Fondillón (F1-F7), por triplicado (de diferentes lotes), para obtener las principales características de este tipo de vino (parámetros de calidad, perfil sensorial descriptivo y composición volátil). Las muestras fueron tomadas de las siete bodegas alicantinas que elaboran este producto en 2015 y fueron amablemente donadas por el Consejo Regulador de la DOP Alicante. Las muestras consistieron en 3 botellas comerciales (3 lotes diferentes) de cada una de las 7 bodegas, con al menos 10 años de envejecimiento, aunque algunas de las muestras tenían hasta 25 años.

4.1.2 Parámetros de calidad

Los principales parámetros fisicoquímicos de calidad (grado alcohólico total, acidez volátil y total, pH, densidad relativa, extracto seco total, SO_2 total, y azúcares reductores) se analizaron de acuerdo con los métodos oficiales de la Organización Internacional de la Viña y el Vino (OIV), de acuerdo con los métodos publicados en el artículo 120 g del primer párrafo del Reglamento (CE) Nº 1234/2007 del Consejo (publicado de conformidad con el artículo 15 del Reglamento (CE) Nº 606/2009 de la Comisión, de 10 de julio de 2009, y pueden consultarse en la página web de la OIV) (OIV, 2021).

4.1.3 Composición de compuestos volátiles

El método seleccionado para la identificación y cuantificación del perfil volátil de las muestras de Fondillón fue la microextracción en fase sólida del espacio de cabeza (HS-SPME). Para este análisis, se colocaron 5 mL de vino, 1,5 g de cloruro de sodio (NaCl) y 10 mL de agua ultrapura en un vial de 50 mL con una tapa de polipropileno y un tabique de PTFE/silicona. Las muestras se equilibraron durante 15 min a 40 °C en los viales, y una fibra DVB/CAR/PDMS (50/30 μm) se expuso al espacio de cabeza de la muestra a 40 °C durante 50 min. Las condiciones de extracción (HS-SPME) se optimizaron para obtener un perfil volátil correlacionado positivamente con las características sensoriales del olor (Alonso *et al.*, 2009).

Para la cuantificación de la composición volátil se utilizó un cromatógrafo de gases, Shimadzu GC-2010, con detector de ionización de llama (FID). Los experimentos de extracción y los estudios volátiles se realizaron por triplicado. El patrón interno utilizado fue el acetato de bencilo con una concentración 1,0 g/L).

4.1.4 Análisis sensorial descriptivo

Quince panelistas (5 mujeres y 10 hombres) de entre 24 y 61 años (edad media 38 años) participaron en este estudio que tuvo lugar en las instalaciones del Consejo Regulador de la Denominación de Origen Protegida de Alicante, DOP Alicante, en Alicante (España). Estos panelistas fueron:

- *(i)* seleccionados (3 sesiones de 1,5 h), de acuerdo, a los resultados obtenidos en pruebas previas de discriminación sensorial, ranking y reconocimiento;
- *(ii)* formados (12 sesiones de 1,5 h, durante 4 meses), fueron entrenados íntegramente en sensorial descriptiva de vinos de la DOP Alicante; y,
- *(iii)* validados (2 sesiones de 1,5 h).

No fue necesaria ninguna sesión de orientación porque los panelistas de la DOP Alicante están acostumbrados a evaluar este tipo de vino. Durante la capacitación del panel, los panelistas fueron instruidos sobre el protocolo de cata, la estructura del cuestionario y el orden de los atributos a evaluar.

Para la evaluación de las muestras, se sirvieron muestras de vino de ~35 mL en la copa negra oficial de cata de vinos (ISO, 1977) para la evaluación de las fases olfativas y gustativa de las muestras, incluyendo atributos positivos y defectos. Más tarde, se sirvieron ~20 mL en la copa transparente oficial de cata de vinos (ISO, 1977). Las muestras se evaluaron a temperatura ambiente (20 ± 1 °C) y bajo luz blanca y se sirvieron codificadas aleatoriamente con tres dígitos junto con el cuestionario apropiado, uno a la vez, y esperando 5 min entre muestras.

Para la limpieza del paladar, se proporcionó agua y galletas sin sal a los panelistas. En cada cuestionario, se pidió a los panelistas que evaluaran la intensidad de los siguientes atributos: sabor (alcohol, afrutado, floral, vegetal, especiado, animal y tostado), sabores básicos (dulce, ácido y amargo), sensación astringente, atributos globales (desequilibrio y persistencia), apariencia (limpidez, color e intensidad del color) y defectos (vegetal, manzana podrida, vinagre, pegamento, jabón, azufre, huevo podrido, cebolla, coliflor, caballo, terroso y corcho). Se puntuó la intensidad del defecto más relevante, pero los descriptores sensoriales de "todos" los defectos encontrados fueron marcados en el cuestionario de cata. Los panelistas utilizaron una escala de 11 puntos para la evaluación, en la que 10 fue intensidad extremadamente alta y 0 fue intensidad extremadamente baja o no perceptible. Se prepararon materiales de referencia para cada atributo. Las evaluaciones se llevaron a cabo en tres sesiones de 1 h para tener 3 repeticiones.

4.1.5 Análisis sensorial afectivo

Para este estudio, participaron 60 consumidores en la Universidad Miguel Hernández de Elche, UMH (España), y consistió en 25 hombres y 35 mujeres con edades comprendidas entre 22 y 67 años. Los consumidores vivían en el este de España (Comunidad Valenciana, Región de Murcia, Andalucía y Comunidad de Castilla La Mancha). El principal requisito para poder participar era que consumieran bebidas alcohólicas, principalmente vino "envejecido", al menos una vez al mes. El estudio de consumidores se realizó en la UMH durante 4 sesiones (15 consumidores por sesión). En cada sesión, los consumidores probaron las 7 muestras de Fondillón. Se sirvieron 20 mL de la muestra (junto con el cuestionario apropiado) a temperatura ambiente (20 ± 1 ° C), codificadas con números de 3 dígitos, uno a la vez, y con un intervalo de 5 minutos entre las muestras. Para la limpieza del paladar, se proporcionó agua y galletas sin sal a los panelistas.

En cada cuestionario, se preguntó a los consumidores sobre su grado de satisfacción para las muestras de Fondillón, utilizando una escala hedónica de 9 puntos (9 = me gusta extremadamente, 5 = ni me gusta ni me disgusta, y 1 = no me gusta extremadamente). Además, también se pidió a los consumidores que clasificaran las muestras según sus preferencias, la muestra que más les gustaba y la que menos les gustaba.

4.1.6 Análisis estadístico

Todos los datos incluidos en este estudio son la media de, al menos, 3 repeticiones para los parámetros fisicoquímicos, 15 para los datos sensoriales descriptivos y 60 para los datos afectivos. Todos los datos se sometieron primero a análisis de varianza (ANOVA) y posteriormente a una prueba de rango múltiple (prueba de Tukey), utilizando el software StatGraphics Plus 5.0 (Manugistics, Inc., Rockville, MD). Las diferencias se consideraron estadísticamente significativas para $p < 0.05$.

4.2 PUBLICACIÓN 2

4.2.1 Muestras de vino

Para este estudio, se analizaron 3 muestras de vino de Fondillón de diferente solera que fueron amablemente donadas por Bodegas Monóvar-MGWines (Monóvar, Alicante, España) y fueron recogidas por triplicado (3 muestras de ~1 L por cada solera: 1944, 1987 y 1996) fueron tomadas e inmediatamente transportadas a las instalaciones de la Universidad Miguel Hernández de Elche (Orihuela, Alicante, España) para su análisis. Posteriormente, aproximadamente 500 mL fueron llevados a Adana para el análisis de los compuestos aromáticos activos y la composición fenólica.

4.2.2 Extracción de compuestos volátiles por evaporación de aromas SAFE (Solvent-Assisted Flavor Evaporation)

Los compuestos volátiles de los vinos Fondillón se aislaron utilizando el equipo SAFE (Glasblaserei Bahr, Manching, Alemania) al vacío (10−3 Raya Vacuubrand DCP 3000, Wertheim, Alemania). La extracción de los compuestos aromáticos se realizó de acuerdo con el procedimiento descrito por *Selli et al.,* (2004) con ligeras modificaciones. Se colocaron 100 mL de vino y 100 mL de diclorometano en un matraz de 500 mL. El contenido se agitó a 4 °C durante 60 min bajo un N_2 atmósfera y, a continuación, centrifugado a 4 °C, 5500 rpm durante 15 min. La fase orgánica (disolvente) se introdujo lentamente en el embudo de caída del cabezal de transferencia. La separación de la mezcla se llevó a cabo en el recipiente de destilación (10 mL/min) que se sumergió parcialmente en un baño de agua caliente a 38 °C. Para garantizar una temperatura continua durante toda la destilación y evitar la condensación de los compuestos volátiles, el sistema se termostatizó completamente con agua a 4 °C. Los compuestos volátiles separados pasaron a través del cabezal de separación a un recipiente receptor/colector donde se condensaron y congelaron por la caída repentina de la temperatura debida al N_2 líquido en la trampa de enfriamiento. Una vez que se completó la separación, se retiró el recipiente receptor y se dejó descongelar a temperatura ambiente durante 30 min. Después de la deshidratación con sulfato de sodio anhidro, el extracto orgánico resultante se concentró en 200 μL (Sonmezdag, 2018).

4.2.3 Determinación del aroma y de los compuestos aromáticos activos

En los análisis de aroma y compuestos aromáticos se utilizó un cromatógrafo de gases (GC) (Agilent 6890) equipado con un detector selectivo de masas (MSD) (5973-Network 159

MSD, Agilent Technologies, Wilmington, Delaware, USA), un detector de ionización de llama (FID) y un dispositivo de olfatometría Gerstel ODP-2 (Baltimore, Maryland, USA) con aire humidificado a 40 °C y se utilizó una columna capilar de sílice fundida desactivada (30 cm × 0,3 mm). Este sistema coordinado proporcionó un conocimiento profundo sobre los perfiles aromáticos de las muestras de Fondillón mediante la combinación de MS, FID y aparatos de olfatometría dividiendo el efluente en 3 flujos iguales (1:1:1) para identificar y cuantificar el aroma y los compuestos aroma-activos. Los compuestos aromáticos se diferenciaron en una columna DB-Wax (30 m de longitud × 0,25 mm i.d. × 0,5 μm de espesor, J&W Scientific, Folsom, California, USA). La temperatura del horno de la columna se incrementó gradualmente de la siguiente manera: (*i*) inicialmente se mantuvo a 40 °C durante 10 min, luego (*ii*) aumentó de 40 a 160 °C a una velocidad de 3 °C/min, (*iii*) posteriormente aumentado a 240 °C a 6 °C/min, (*iv*) y finalmente se mantuvo a 240 °C durante 25 min. La presión de la columna se estableció en un valor constante de 20 psi, y se inyectaron 3 μL de extracto en modo sin división pulsada. Los detectores de inyector y FID se ajustaron a 270 °C y 280 °C, respectivamente. Las condiciones MS (ionización electrónica por impacto) fueron energía de ionización de 70 eV, rango de masa m/z de 33 a 300 amu a 2.0 scan/s.

Las concentraciones de los compuestos aromáticos se determinaron mediante la aplicación del método patrón interno y se expresaron como μg/L resultante del área pico relativa al estándar interno (equivalentes de 4-nonanol con una concentración de 40 μg/L) (de Saint Laumer *et al.*, 2010). Todos los compuestos se determinaron comparando sus índices de retención y sus espectros de masas en la columna DB-Wax con los de una base de datos de espectros comerciales (Wiley 6, Flavor 2, NBS 75 k) y de la biblioteca interna del instrumento creada en estudios anteriores. Los índices de retención lineal se calcularon mediante la inyección de la serie comercial de n-alcanos lineales (C6-C32).

4.2.4 Análisis de dilución del extracto aromático AEDA (Aroma Extract Dilution Analysis)

Los compuestos odoríferos activos del Fondillón fueron identificados por GC-MS-O con la aplicación del análisis de dilución de extracto aromático (AEDA). Un extracto aromático concentrado (200 μg) de las muestras de Fondillón se diluyó gradualmente con Cl_2CH_2 comenzando desde 1:1, continuando con 1:2, 1:4, y concluyendo con una relación de dilución de 1:1024, en la que surgió el último compuesto odorífero detectable. Cada muestra diluida (3 μL) se inyectó en el equipo GC-MS-O y todos los compuestos aromáticos clave fueron olfateados por panelistas experimentados. El aumento del número de factores FD representó el nivel de actividad de los compuestos aromáticos en las muestras bajo análisis (Selli *et al.*, 2014).

4.2.5 *Análisis sensorial descriptivo*

Diez panelistas (6 hombres y 4 mujeres), de 30 a 62 años, evaluaron las 3 muestras de Fondillón en las instalaciones del grupo de investigación de Calidad y Seguridad Alimentaria (CSA) de la Universidad Miguel Hernández de Elche (UMH). El léxico y el cuestionario utilizado aquí fueron los desarrollados por el Consejo Regulador de la Denominación de Origen Protegida Alicante, DOP Alicante y el método de análisis fue el descrito recientemente en la sección *5.1.4*.

4.2.6 *Composición fenólica (LC-MS-MS)*

Las muestras de Fondillón se filtraron a través de un filtro de membrana de 0,45 μm de tamaño de poro antes de la inyección. Se utilizó un sistema HPLC Agilent 1100 (Agilent Technologies, Palo Alto CA-USA) operado por el software ChemStation. El equipo HPLC se utilizó con un detector de matriz de diodos (DAD). El sistema consistía en una bomba binaria, desgasificador y automuestreador. La columna utilizada fue una Beckman Ultrasphere ODS (Roissy CDG, Francia): 4,6 mm × 250 mm, 5 μm equipada con una precolumna de 4,6 mm × 10 mm (misma granulometría). La fase móvil consistió en dos disolventes: disolvente *A* [agua/ácido fórmico (95:5; v/v)] y disolvente *B* [acetonitrilo/disolvente *A* (60:40; v/v)]. Los compuestos fenólicos se eluyeron bajo las siguientes condiciones: 1 mL/min caudal y 25 °C, condiciones isocráticas de 0 a 10 min con 0 % *B*, condiciones de gradiente de 0 a 5 % *B* en 30 min, de 5 a 15 % *B* en 18 min, de 15 a 25 % *B* en 14 min, de 25 a 50 % *B* en 31 min, de 50 a 100 % *B* en 3 min, seguido del lavado y reacondicionamiento de la columna. Los espectros ultravioleta-visibles (escaneo de 200 nm a 600 nm) se registraron para todos los picos. Se realizaron los análisis por triplicados para cada muestra. La identificación de compuestos fenólicos se obtuvo utilizando estándares auténticos y comparando los tiempos de retención y los espectros ultravioleta-visibles con los encontrados en la literatura, mientras que la cuantificación se realizó mediante calibración externa con estándares también confirmados por el análisis LC-MS/MS según Kelebek, 2016.

4.2.7 Análisis estadístico

El tratamiento estadístico para todos los datos de este estudio, están descritos en la sección *5.1.6*.

4.3 PUBLICACIÓN 3

4.3.1 Muestras de vino

El factor principal en este estudio fue la "solera" (tiempo de envejecimiento). Se utilizaron 9 muestras de diferentes tipos de soleras: 1930, 1944, 1950, 1960, 1969, 1975, 1980, 1987 y 1996. Estas muestras (nueve botellas de 500 mL por solera) fueron suministradas amablemente por Bodegas Monóvar-MGWines (Monóvar, Alicante, España). Las muestras fueron transportadas y almacenadas en condiciones óptimas en las instalaciones de la Universidad Miguel Hernández (Orihuela, Alicante, España) para posteriormente realizar los análisis correspondientes. Todos los análisis se realizaron, al menos, por triplicado, utilizando diferentes botellas para cada repetición.

4.3.2 Parámetros enológicos

Los parámetros enológicos básicos [pH, densidad relativa, grado alcohólico total (% Vol.), acidez total (g ácido tartárico/L), y acidez volátil (g ácido acético/L)] se determinaron, por triplicado, según los métodos de análisis de la Organización Internacional del Compendio de la Viña y el Vino (OIV, 2020).

4.3.3 Características cromáticas

Las características cromáticas: (*i*) intensidad de color, (*ii*) tonalidad y (*iii*) densidad de color en las muestras de vino se determinaron según los métodos OIV-MA-AS2-07B (OIV, 2020).y Glories (OIV, 2020) utilizando un espectrofotómetro (ThermoSpectronic Heγios γ, Cambridge, Inglaterra). Todos los análisis se realizaron por triplicado.

La intensidad del color (CI) se calculó como la suma de la absorbancia a 420, 520 y 620 nm (IC = A420 + A520 + A620). La tonalidad (T) se calculó como la relación entre absorbancia a 420 nm y absorbancia a 520 nm (T = A420/Un520). La densidad de color (CD) se calculó como la suma de la absorbancia a 420 y 520 nm (CD = A420 + A520). Además, los porcentajes del índice colorimétrico Glories (Glories, 1984) de los colores amarillo, rojo y azul se obtuvieron utilizando los valores de absorbancia a 420, 520 y 620 nm de la siguiente manera:

- Amarillo, Y (%) = (A420/CI) × 100
- Rojo, R (%) = (A520/CI) × 100
- Azul, B (%) = (A620/CI) × 100

4.3.4 *Índice de polifenoles totales (TPI)*

El índice de polifenoles totales se evaluó midiendo la absorbancia a 280 nm en un espectrofotómetro UV-visible (modelo Helios Gamma, UVG 1002E) (Luna *et al.*, 2010). El TPI se determinó después de diluir la muestra de vino 100 veces. Los análisis se realizaron por triplicado.

4.3.5 *Contenido de antocianinas totales (TAC)*

El contenido de antocianinas totales (TAC) se determinó de acuerdo con Rocco *et al.*, (1989). Brevemente, la absorbancia de las muestras se midió a 540 nm utilizando un espectrofotómetro UV-visible (modelo Helios Gamma, UVG 1002E) y el contenido total de antocianinas se calculó utilizando la fórmula de Rocco *et al.*, (1989). El análisis se realizó por triplicado y los resultados se expresaron como equivalentes de malvidina 3-O-glucósido (mg/L).

4.3.6 *Actividad Antioxidante (AA)*

Para la determinación de la AA se utilizaron 3 métodos: (*i*) FRAP para evaluar el poder reductor/antioxidante férrico, (*ii*) DPPH$^{\bullet}$ (2,2-difenil-1-picrilhidrazilo) y (*iii*) ABTS$^{\bullet+}$ [2, 2-azinobis-(ácido 3-etilbenzotiazolina -6-sulfónico), estos métodos se basan en la medición de la respuesta inhibitoria, que generan los compuestos antioxidantes presentes en una muestra, ante la liberación de radicales libres. Para la evaluación de FRAP se siguió el método descrito por Benzie *et al.*, (1996) con algunas modificaciones, midiendo la absorbancia a 593 nm. El método para la determinación de DPPH$^{\bullet}$ fue el indicado por Katalinic *et al.*, (2006), registrándose la absorbancia a 517 nm. Finalmente, se determinó el ABTS$^{\bullet+}$ siguiendo el método propuesto por Re *et al.*, (1999), utilizando una absorbancia a 734 nm. Se utilizó un espectrofotómetro UV-vis UVG1002E (Helios, Cambridge, Reino Unido).

Para la preparación de la muestra se tomaron 5 mL de vino, adicionalmente se agregaron 5 mL de extractante [metanol/agua (80:20, v/v) + 1% HCl] y se trató con ultrasonidos durante 15 min; luego, la mezcla se dejó reposar durante 12 h a 4 °C. Luego, las muestras se tratan nuevamente con ultrasonidos durante 15 min. Una vez terminada la extracción, las muestras se centrifugaron durante 10 min a 10 000 rpm (12,880 g). El sobrenadante se recogió y se colocó en viales de color ámbar para la medición en los ensayos antes mencionados. Todos los análisis se realizaron por triplicado y los resultados se expresaron como mmol Trolox/L.

4.3.7 Taninos condensados totales (TTC)

Los taninos condensados totales (TTC) se determinaron según Ribéreau-Gayon y Stonestreet, (1965). Brevemente, las muestras se prepararon en dos tubos de ensayo, uno para el control (tubo 2) y el otro para la hidrólisis (tubo 1); en este tubo, se agregaron 4 mL de vino diluido en agua destilada 1:50, 2 mL de agua destilada y 6 mL de HCl 12 N sucesivamente. Posteriormente, el tubo cerrado de hidrólisis se colocó en un baño de agua a 100 °C durante 30 min, y luego se enfrió en un baño de agua helada. Luego, a los dos tubos se le agregó 1 mL de etanol al 95 % para solubilizar el color rojo que apareció y finalmente se midió la absorbancia (Abs) de los dos tubos a 550 nm bajo un recorrido óptico de 1 cm, utilizando agua como referencia. Los taninos se determinaron mediante la siguiente ecuación:

$$\text{Taninos (g/L)} = 19{,}33 \times (\text{Abs del tubo 1} - \text{Abs del tubo 2})$$

donde, el coeficiente de 19,33 corresponde al coeficiente de extinción molar de la cianidina obtenido por la hidrólisis ácida de los taninos condensados, corregido para dar directamente el resultado en g/L.

4.3.8 Composición de compuestos volátiles

La extracción de compuestos volátiles se realizó mediante la técnica HS-SPME con fibra DVB/CAR/PDMS de 1 cm (Supelco, Bellegonte, PA, USA). Brevemente, se colocaron 10 mL de muestra en un vial de espacio de cabeza de 20 mL junto con 5 µL de acetato de bencilo (1000 mg/L) como patrón interno y 1 g de NaCl. Posteriormente, la muestra se colocó en un muestreador automático (AOC-6000 Plus, Shimadzu) a 250 rpm y 40 °C, durante 40 min. La identificación y cuantificación de los compuestos volátiles se llevó a cabo utilizando un cromatógrafo de gases Shimadzu GC2030 y un espectrómetro de masas de triple cuadrupolo TQ8040 NX como detector (Shimadzu Scientific Instruments, Inc., Columbia, MD, EUA) equipado con un muestreador automático AOC-6000 Plus. Los datos se adquirieron utilizando el software de solución GCMS versión 4.4 (Shimadzu). La columna utilizada fue una X5MS (polímero de silfenileno; Teknokroma, Barcelona, España) con dimensiones de 30 m (longitud), 0,25 mm (diámetro interno) y 0,25 µm (espesor de película). El programa de temperatura GC fue, de la siguiente manera: 50 °C mantener durante 2 min, luego se aumentó usando una rampa de 3 °C/min hasta 170 °C y un incremento de 20 °C/min hasta 230 °C. La presión de la cabeza de helio fue de 18 kPa (modo de velocidad lineal constante 30 cm/s). El inyector, la fuente de iones y la interfaz estaban a 230, 230 y 280 °C, respectivamente. Se utilizó helio como gas portador con un flujo de 0,6 mL/min con una relación de división de 1:10.

Los compuestos volátiles se identificaron mediante la comparación de: (*i*) espectros de masas obtenidos experimentalmente con los disponibles en NIST 17 Mass Spectral, y (*ii*) índices de retención lineal calculados utilizando la mezcla de *n*-alcanos C6-C20 (sigma-Aldrich, Steinheim, Alemania). Sólo los compuestos con similitud de espectros >90 % se consideraron como aciertos correctos; además, el umbral de similitud de retención lineal se estableció en ± 10 unidades. Los análisis se realizaron por triplicado y los resultados se expresaron como mg/L.

4.3.9 Análisis sensorial afectivo

Se utilizó un panel entrenado, compuesto por ocho panelistas (cuatro mujeres y cuatro hombres), con edades comprendidas entre 35 y 60 años, para realizar el análisis sensorial descriptivo de las muestras de Fondillón en estudio. Este panel contaba con más de 300 h de experiencia, pertenece al Consejo Regulador de los Vinos de Alicante Denominación de Origen Protegida (DOP Alicante), se formó según ISO 8586:2012 (ISO, 2012a) y está certificado por la Agencia Nacional de Acreditación (ENAC) bajo la norma ISO 17065 (ISO, 2012b). El método de análisis utilizado fue el descrito recientemente en la sección *5.1.4*.

4.3.10 Análisis estadístico

Los resultados se sometieron primero a un análisis unidireccional de varianza (ANOVA) utilizando la edad solera como factor, y más tarde la prueba de rango múltiple de Tukey o LSD (datos sensoriales descriptivos y afectivos). Las diferencias fueron consideradas estadísticamente significativas a $p < 0,05$. Para realizar estos análisis estadísticos, se utilizaron los programas XLSTAT Premium 2016 (Addinsoft, Nueva York, NY, USA) y Statgraphics Plus (versión 3.1, Statistical Graphics Corp., Rockville, MA, USA).

4.4 PUBLICACIÓN 4

4.4.1 Muestras de vino

En este estudio se analizaron 5 muestras de Fondillón de 5 bodegas diferentes. Las muestras (3 botellas de 750 mL por cada muestra) fueron amablemente facilitadas por el Consejo Regulador de la DOP Alicante. Las muestras analizadas fueron:

- F1, Gran Reserva 1987 (dulce, moscatel, pasas y color ámbar).
- F2, Solera 1948 (dulce, amargo, leñoso, cartón y caramelo).
- F3, Reserva Especial (color amargo, ácido, alcohol y caoba).
- F4, Gran Reserva 1964 (dulce, amargo, tostado, tofe y postgusto largo).
- F5, Solera 1996 (dulce, tostado, caramelo, vainilla y color ámbar).

4.4.2 Análisis sensorial descriptivo

Para la evaluación sensorial de las muestras de Fondillón se utilizó el panel de cata del Consejo Regulador de la DOP Alicante. Este panel estuvo formado por 12 panelistas (6 mujeres y 6 hombres), con edades comprendidas entre los 30 y los 60 años, con más de 400 h de experiencia en la descripción de vinos alicantinos. El método de análisis para la evaluación de las muestras fue el descrito en la sección *5.1.4*.

4.4.3 Análisis sensorial afectivo

Este estudio se llevó a cabo para establecer el grado de satisfacción general del consumidor, junto con el grado de satisfacción de los atributos sensoriales clave: color, alcohol (olor y sabor, *o* y *f*), tostado / caramelo / café (*o*, *f*), químico (*o*, *f*), dulzura, acidez y postgusto. También se realizó una prueba de preferencia. El estudio afectivo se realizó con 100 consumidores españoles, reclutados en el campus de Orihuela de la UMH, y que bebían vino dulce u oxidado (Oporto, Madeira, Jerez, Fondillón, etc.) al menos dos veces al mes. El perfil del consumidor fue el siguiente: 44% mujeres y 56% hombres, de los cuales el 20% tenían entre 18 y 24 años, el 40% entre 25-35, el 20% entre 36-44 y el 20% mayores de 45 años. El método de análisis y preparación de las muestras fue el descrito en la sección *5.1.5*.

4.4.4 Encuesta online

Se diseñó y realizó un estudio online (con 294 consumidores, siendo un 62% hombres) para determinar el nicho de consumidores del Fondillón y entender las principales razones del consumo o no consumo de este vino. Los participantes procedían de dos regiones vecinas

(Valencia y Murcia, que comprenden 4 provincias: Castellón, Valencia, Alicante y Murcia). Las condiciones que debían de tener los consumidores para poder participar en el estudio eran: beber (*i*) vino DOP Alicante al menos dos veces por semana, o (*ii*) vino dulce u oxidado (Oporto, Madeira, Jerez, Fondillón, etc.) al menos dos veces al mes. El cuestionario contenía preguntas relacionadas con el consumo de Fondillón, y preguntas demográficas para clasificarlos según sexo, edad, ingresos y educación.

4.4.5 Análisis estadístico

Los resultados se sometieron a un análisis unidireccional de varianza (ANOVA) y luego, se realizó una prueba de rango múltiple de LSD sobre los atributos analizados con el panel entrenado. Las diferencias fueron consideradas estadísticamente significativas para $p < 0,05$. La regresión mínima cuadrática parcial (PLS) se realizó utilizando descriptores sensoriales y el gusto general del consumidor. Los datos de JAR se analizaron mediante un análisis de penalización, que se llevó a cabo para obtener información sobre aquellos puntos débiles donde se puede mejorar el perfil sensorial de cada una de las muestras. Los resultados de CATA se analizaron utilizando un gráfico de Análisis de Correspondencias (CA), que se creó a través de una tabla de contingencia con 20 y 300 columnas y filas, respectivamente. Para realizar todos estos análisis estadísticos, se utilizó el software XLSTAT Premium 2016 (Addinsoft, Nueva York, NY, USA) y Statgraphics Plus (versión 3.1, Statistical Graphics Corp., Rockville, MA, USA).

4.5 PUBLICACIÓN 5

4.5.1 *Muestras de vino*

Las tres muestras de vino (joven, crianza/crianza y Fondillón) utilizadas para este estudio fueron cedidas amablemente por Bodegas BOCOPA (Petrer, Alicante, España); se suministraron por triplicado a partir de tres lotes diferentes y se trasladaron a las instalaciones de la Universidad Miguel Hernández de Elche (Orihuela, Alicante, España).

4.5.2 *Ácidos Orgánicos y Azúcares*

Para la identificación y cuantificación de los ácidos orgánicos y azúcares, se utilizó un cromatógrafo líquido de alto rendimiento (HPLC) serie HP 1100 (Wilmington, DE, USA). Para la preparación de las muestras se extrajeron 5 mL de los vinos y se centrifugaron a 15.000 rpm y 4 °C durante 10 min. Posteriormente, el sobrenadante se filtró a través de filtros Millipore de 0,45 µm y se almacenó en viales ámbar, y luego se inyectaron 10 µL en el cromatógrafo utilizando un tampón de elución (ácido fosfórico al 0,1 % con un caudal de 0,5 mL/min). Los ácidos orgánicos se aislaron utilizando una columna Supelcogel TM C-610H (30 cm × 7,8 mm) y una precolumna Supelguard (5 cm × 4,6 mm; Supelco, Bellefonte, PA, USA). La absorbancia se midió a 210 nm con un detector de matriz de diodos (DAD). Los azúcares se detectaron usando un detector de índice de refracción (RID), usando las mismas condiciones cromatográficas.

Se desarrollaron curvas de calibración de azúcares (glucosa y fructosa) y ácidos orgánicos (ácido cítrico, tartárico, láctico, acético y málico) de acuerdo con los estándares adecuados, con un rango de concentración entre 1 y 10 g/L. Los análisis se realizaron por triplicado y los resultados se expresaron como g/L.

4.5.3 *Actividad antioxidante, Índice de polifenoles totales y Características cromáticas*

El método de análisis para la evaluación de las características cromáticas, índice de polifenoles totales y actividad antioxidante fueron los descritos en las secciones *5.3.3*, *5.3.4* y 5.3.6, respectivamente.

4.5.4 *Minerales*

Las concentraciones de las macro- y micro-elementos se determinaron utilizando un espectrómetro de masas de plasma acoplado inductivamente (ICP-MS): Shimadzu ICPMS-2030 (*Shimadzu Scientific Instruments*, Inc., Columbia, MD, USA). Para la preparación de las muestras se diluyó 1 mL de vino a 10 mL con HNO_3 1 % (dilución 1:10); luego, esta porción se pasó a través de filtros Millipore de 0.45 μm y se almacenó en tubos Falcon de 15 mL.

Se crearon curvas de calibración con los estándares minerales, para los macroelementos (Ca, Mg, Na y K), la concentración oscila entre 1 y 20 mg/L, mientras que para los microelementos (Fe, Cu, Mn y Zn) la concentración osciló entre 0,01 y 0,2 mg/L. Los análisis se realizaron por triplicado y los resultados se expresaron como mg/L para los macroelementos y como μg/L para los microelementos.

4.5.5 Compuestos volátiles

El método de análisis para la evaluación de los compuestos volátiles bajo estudio fue el descrito en la sección *5.3.8*.

4.5.6 Análisis estadístico

El procesamiento de los resultados se realizó inicialmente con un análisis de varianza de una vía (ANOVA) seguido de la prueba de rango múltiple de Tukey. Las diferencias se consideraron estadísticamente significativas para $p < 0,05$. El software utilizado para realizar los análisis estadísticos fue XLSTAT Premium 2016 (Addinsoft, Nueva York, NY, USA).

Posteriormente, el análisis de datos de las mediciones de GC-MS se realizó mediante el análisis de componentes principales (PCA) con el software de quimiometría independiente (versión Solo 9.1. para Windows, Eigenvector Research Inc., Wenatchee, WA, USA).

7. PUBLICACIONES

PUBLICACIÓN 1

Volatile Composition, Sensory Profile, and Consumers' Acceptance of *Fondillón*

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Research Article

Volatile Composition, Sensory Profile, and Consumers' Acceptance of *Fondillón*

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No scientific information exists on quality attributes of *Fondillón*, a special naturally sweet wine produced from overripe Monastrell grapes and one of the only six wines that can use its own name according to European Union Regulations. The aim of this study was to analyze the composition (physicochemical and volatile profile) and sensory quality of this special wine. A specific lexicon to describe wines under the Alicante PDO was developed, using 28 attributes (11 flavor notes, 3 visuals, 2 global, and 12 defects). Forty volatile compounds were isolated, and esters were the main chemical family of volatile compounds of *Fondillón* (~70 %), followed by alcohols (~20 %). Furthermore, two volatile compounds (TDN and vitispirane) were positively correlated with the age of the *Fondillón* samples, under the specific working conditions used in this study. According to a sensory study, this wine was appreciated by Spanish consumers as having intense fruity notes, high alcohol content, and some bitter and balsamic notes; however, further research is needed to identify the proper profile of *Fondillón* consumers and their buying and acceptance drivers.

1. Introduction

Wine fermentation turns grapes into wine. In this process, yeasts take natural sugars from mature grapes and convert them into alcohol and CO_2. Hence, most wines are dry or almost dry (they have no sweetness or residual sugar). However, we can find several wines produced through different processes that have some different amount of sugar. This sort of wines is called sweet or dessert wines. Along history, the amount of sugar in the finished wines was a key factor for conservation. Sweet wines were highly valued in ancient Rome and in the Middle Ages and were promoted and marketed within the Dutch and British wine trade of the early 18th century. However, nowadays, they represent a very small percentage of the global wine business. Nevertheless, there is a growing interest in high-quality sweet wines [1].

Dehydrating grapes can be reached in two ways, on-vine or off-vine [2]. Grapes can become overripe through several techniques, such as by exposure to sunlight (Passito, Pedro Ximenez, Málaga); by dehydration in closed rooms of hot or fresh air (Recioto, Vin Santo, Vin de Paille); by grape colonization by fungus *Botrytis cinerea*, causing noble rot (Alsace, Loire, Montbazillac, Sauternes, Tokaji, Trockenbeerenauslese); by leaving grapes to shrivel in the plot, where they may also be occasionally affected by noble rot (*Fondillón*, Spatlese, Tokaji Late harvest, Vendage Tardive); or by waiting until winter, causing grape dehydration by ice (Eiswein, Vi de Gel, ice wine) [3]. These on-vine overripe grape lead to naturally concentrated must, rich in sugars and volatile compounds. Natural sweet wines are mainly featured by their high sugar level, and their quality mainly depends on their aroma compounds [1]. However, still, little is known about the biochemistry behind this special sort of wine Wines produced according to this method are for example, Alsatia, Fiano, *Fondillón*, Jurançon, Pacherenc du VicBilh, Picolit, Priorat rancid sweet, and Malvasia from La Palma and Lanzarote [4].

It is possible to classify sweet wines according to the winemaking process: fortified musts, fortified wines, and naturally sweet wines. Fortified musts (Muscat, mistelle) and fortified wines (Port wine, Sherry) are those in which fer- mentation is stopped by adding alcohol to the must or wine, respectively. On the other hand, naturally sweet wines, including *Fondillón*, come from overripe grapes and are nonfortified wines. These wines with a total alcoholic strength of not less than 15 % by volume (abv), and an actual alcoholic strength of not less than 12 % abv are produced without enrichment [5]. Owing to the high grape original sugar content, yeast metabolism implies high levels of alcohol (naturally above 15 % abv); this high alcohol content is the most usual cause of cessation of fermentation in non- fortified dessert wines.

Fondillón is a naturally sweet wine (included by the European Union in its E-Bacchus database) produced in the Alicante Protected Designation of Origin, Alicante PDO [6]. *Fondillón* is a red wine produced in an oxidized (*rancio*) style from overripe Monastrell grapes; it is typically bottled and sold after a long aging period in oak barrel (minimum 10 years). *Fondillón* production was almost lost during the end of the 19th century, but fortunately, it was recovered around 1950; but, since then, no scientific approach has been done to fully characterize this wine and to promote its distinctive characteristics.

Consequently, the aim of this study was to develop a sensory protocol (mainly the lexicon) to properly describe the quality of the *Fondillón* wines being marketed in Spain. This is essential to guarantee that only those wines fulfilling the requirements of the PDO Alicante get the proper seal. To back up sensory data with instrumental and physicochemical data, the basic quality parameters and the volatile profile were also analyzed.

2. Materials and Methods

2.1. Wine Samples

Seven *Fondillón* samples (F1–F7) under the Alicante PDO were analyzed in this study, in triplicate from different batches), to get the main characteristics of this type of wine (quality parameters, typical descriptive sensory profile, and volatile composition). During the first stage of the experiment, *Fondillón* samples were taken from the seven wineries in Alicante producing this product in 2015 and were kindly donated by the Regulatory Council of the Alicante PDO. Samples consisted of 3 commercial bottles (3 different batches) from each of the 7 wineries, with at least 10 years of aging, but some of the samples had up to 25 years.

At a second stage (validation of the panel and the lexicon), 5 of the previous samples were used to validate the sensory lexicon developed in this study and were randomly labeled as F8–F12. Three samples were used as taken from the wineries, while, to simulate common wide defects, another two of them (randomly selected) were spiked with concentrations of SO_2 (sensory descriptor sulfur) and ethyl acetate (sensory descriptor glue) above their detection thresholds. The concentrations used were 250 mg SO_2 L^{-1} (sample F10) (which maximum legal value is 200 mg·L^{-1} [7]) and 20 mg·L^{-1} of ethyl acetate (sample F12) (which detection threshold is 12.27 mg·L^{-1} [8]) and it is reported to range between 8.64 and 17.24 mg·L^{-1} in alcoholic beverages [9].

Finally, these two samples (F10 and F12) were left for 1 week in a hot room (reaching temperatures up to 35–40 °C) to induce slight deterioration of the wines, by simulating real conditions of the wines in hot regions, such as Spain, with no proper control of the storage temperature.

2.2. Quality Parameters

The main physicochemical quality parameters (total alcohol content, volatile and total acidity, pH, relative density, total dry extract, total SO2, and reducing sugars) were analyzed according to the official methods of the International Organisation of Vine and Wine (OIV), in accordance with the methods published in the first paragraph of Article 120 g of Council Regulation (EC) No. 1234/2007 (published in accordance with Article 15 of Commission Regulation (EC) No. 606/2009 of 10 July 2009, and can be located at the OIV web page) [10].

2.3. Volatile Composition.

The method selected to determine the composition and quantify the volatile profile of Fondillón samples was headspace solid phase microextraction (HS- SPME). For this analysis, 5 mL of wine, 1.5 g of NaCl, and 10 mL of ultrapure water were placed in a 50 mL vial with a polypropylene cap and a PTFE/silicone septum. The samples were equilibrated for 15 min at 40 °C on the vials, and a DVB/ CAR/PDMS fiber (50/30 μm) was exposed to the sample headspace at 40 °C for 50 min. The extraction conditions (HS-SPME) were optimized to obtain a volatile profile positively correlated with sensory odor characteristics [11]. Similar extraction procedures have been successfully used in fruit liquors [12, 13] and pomegranate wine [14].

The isolation and identification of the volatile compounds were performed using the GC-MS conditions previously described [13]. A gas chromatograph, Shimadzu GC- 2010, with a flame ionization detector (FID) was used for the quantification of the volatile composition of samples. The column and chromatographic conditions were the same as those reported previously by Gironés-Vilaplana et al. [13]. The extraction experiments and volatile studies were run in triplicate.

The proposed internal standard, benzyl acetate, was checked for its suitability for our GC analyses. It was found to be absent in the volatile profiles of Fondillón samples, it did not react with water, it possessed similar FID and MS response factors to most of the wine volatiles, and its chromatographic peak did not overlap with any of those of the wine volatiles. Therefore, this compound (50 μL) was used as internal standard (concentration 1.0 $g \cdot L^{-1}$).

Calibration curves were performed with the following compounds (Sigma-Aldrich, Madrid, Spain) as representative of each chemical family, and with intermediate molecular weights: octanoic acid (organic acids), 1-hexanol (alcohols), nonanal (aldehydes), ethyl hexanoate (esters), limonene (monoterpenes), and γ-nonalactone (lactones), and specific calibration curves were prepared for the two key compounds under study, TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) and vitispirane.

These calibration curves were done using synthetic wine as matrix; this wine was prepared by diluting 3.5 g of tartaric acid and 160 mL of ethanol with Milli-Q quality water until 1 L and, then, pH was adjusted to pH 3.5 [15, 16]. The correlation coefficients (R^2) for all compounds were above 0.995, and results were expressed as $\mu g \cdot L^{-1}$.

2.4. Descriptive Sensory Analysis with Trained Panel

Fifteen panelists (5 women and 10 men) aged 24–61 years (mean age 38 years) participated in this study which took place at the facilities of the Regulatory Board of the Alicante Protected Designation of Origin, Alicante PDO, in Alicante (Spain). They were (i) selected (3 sessions of 1.5 h), according to their results in previous sensory discrimination, ranking, and recognition tests, (ii) trained (12 sessions of 1.5 h, during 4 months) (they were fully trained in descriptive sensory of wines from the Alicante PDO), and (iii) validated (2 sessions of 1.5 h), and are included in the control tools of the Regulatory Board to control the quality of their wines; this tool is included among those certified by the ISO/IEC 17065 : 2012 [17], with the reference number 118/C-PR198. These panelists are paid for their involvement in the current study and any other evaluations they perform.

No orientation session was needed because the panelists of the Alicante PDO are used to evaluate this type of wine. During the panel training, the panelists were instructed about the tasting protocol, the questionnaire structure and the order of the attributes to be evaluated, the lexicon (**Table 1**), and the scale to be used.

2.4.1. Wine Evaluation

Initially, wine samples of ~35 mL were served in the official "black" wine tasting cup [18] for the evaluation of the flavor of the samples, including positive and defect attributes. Later, ~20 mL was served in the official "transparent" wine-tasting cup [18]. It was decided that the visual stage of the evaluation should be conducted at the end of the tasting to avoid any influence on the objective description of the wines [19–21]. Samples were evaluated at room temperature (20 ± 1 °C) and under white light and were served coded randomly with three digits together with the appropriate questionnaire, one at a time, and waiting 5 min between samples. Between samples and for palate cleansing, water and unsalted crackers were provided to panelists. In each questionnaire, panelists were asked, to evaluate the intensity of the following attributes: flavor (alcohol, fruity, floral, vegetable, spicy, animal, and toasted), basic tastes (sweet, sour, and bitter), chemical feelings (astringent), global attributes (imbalance and persistence), appearance (limpidity, color, and color intensity), and defects (vegetal, rotten apple, vinegar, glue, soap, sulfur, rotten egg, onion, cauliflower, horse, earthy, and cork). The intensity of the most relevant defect was scored, but the sensory descriptors of "all" found defects were marked in the tasting questionnaire. Panelists used an 11-point scale for the evaluation, in which 10 was extremely high intensity and 0 was extremely low or nonperceptible intensity. Reference materials for each attribute were prepared and were available for all panelists.

Evaluations were carried out in three 1 h sessions to have 3 replications. In each session, the 7 *Fondillón* samples under evaluation were monadically presented according to a William's Latin Square design balanced for order and carryover effects.

The panel was validated by analyzing five *Fondillón* samples, two of which were spiked with chemicals (SO_2 and ethyl acetate) leading to odor/aroma and flavor defects, as previously described. Besides, in each session, reproducibility (1 wine from a previous session is evaluated again) and repeatability (1 wine is evaluated twice in each session) are checked, and 1 wine with a significant defect is also introduced. These are the general rules for the working protocol of this accredited panel.

2.5. Affective Sensory Analysis with Consumers' Panel.

A sample group of 60 consumers was recruited at Miguel Hernández University of Elche, UMH (Spain), and consisted of 25 men and 35 women aged between 22 and 67 years. Consumers lived in the East of Spain (Valencian Com- munity, Murcia Region, Andalucía, and Castilla La Mancha Community). The main requirement for their recruitment was that they consumed alcoholic beverages, mainly "aged" wine, at least once a month. The consumer study was conducted at UMH during 4 sessions (15 consumers *per* session). In each session, consumers tested the 7 *Fondillón* samples; the 7 samples (F1–F7) under evaluation were monadically presented according to a William's Latin Square design balanced for order and carry-over effects. Twenty millilitre samples (along with the appropriate questionnaire) were served at room temperature (20 ± 1 °C), coded with 3 digit numbers, one at a time, and with a 5 min gap between samples. Between samples and for palate cleansing, water and unsalted crackers were provided to panelists.

In each questionnaire, consumers were asked about their satisfaction degree for the *Fondillón* samples, using 9-point hedonic scale (9 like extremely, 5 = neither like or dislike, and 1 = dislike extremely). Besides, consumers were also asked to rank samples according to their preference, from the least preferred sample to the most preferred one.

4.3 Statistical Analysis

All data included in this study are the mean of, at least, 3 replicates for the physicochemical parameters, 15 for descriptive sensory data, and 60 for affective data. All the data were first subjected to analysis of variance (ANOVA) and later to a multiple range test (Tukey's test), using StatGraphics Plus 5.0 software (Manugistics Inc., Rockville, MD). Differences were considered statistically significant at $p < 0.05$.

Table 1: Main physicochemical parameters defining the quality of the *Fondillón* wine.

Parameters	ANOVA	F1	F2	F3	F4	F5	F6	F7
Total alcohol content (% v/v)	**	21.13 a‡	18.63 ab	20.17 a	16.97 b	16.60 b	16.16 b	18.50 ab
Volatile acidity (g acetic acid L−1)	*	0.71 b	0.62 b	1.05 b	1.03 b	0.75 b	0.99 b	1.50 a
Total acidity (g tartaric acid L−1)	*	5.30 b	5.46 b	5.50 b	7.80 a	6.50 a	7.27 a	6.60 a
pH	*	3.56 b	3.25 b	3.36 b	3.54 b	3.26 b	3.45 b	3.80 a
Relative density (20°C)	NS	1.0183	0.9986	1.0041	0.9935	1.0052	1.0077	0.9978
Total dry extract (g·L−1)	*	100 a	56.2 b	68.5 b	71.4 b	65.7 b	65.2 b	54.7 b
Total SO2 (mg·L−1)	**	76.0 b	35.0 c	48.0 c	53.0 c	124 a	142 a	78.0 b
Reducing sugars (g·L−1)	**	39.70 a	29.30 b	39.20 a	23.00 c	34.50 ab	37.98 a	22.00 c

*NS = not significant at $p > 0.05$; * and ** significant at $p < 0.05$, and 0.01, respectively. ‡Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different, $p > 0.05$, according to Tukey's least significant difference test.*

5 Results and Discussion

3.1 Quality Parameters

This is a type of wine with a high alcohol content; the minimum legal threshold is 16 % (v/v) [7], and the experimental values ranged from 16.2 upto 21.2 % (v/v) (**Table 1**).

The *Fondillón* wine is prepared with overripe Monastrell grapes. Then, the reducing sugars should be above a content of 15 g·L^{-1}; although there is no legislation for this minimum value, there is an official maximum threshold for the content of sugars, 40 g·L^{-1} [7]. As can be seen in Table 1, the experimental values found for the total reducing sugars ranged from 22.0 up to 39.7 g·L^{-1} (mean of 32.24 g·L^{-1}), proving that all samples used overripe grapes, with a very high content of initial sugars. It is interesting to mention that, in some cases, it is believed that there is an inverse relationship between the alcohol and the reducing sugar contents; however, this was not the case of *Fondillón* samples, and these two parameters showed no significant relationship ($R^2 = 0.1044$).

The total acidity of *Fondillón* should be above 3.5 g tartaric acid L^{-1} and had a mean of 6.35 g·L^{-1} (range between 5.30 and 7.80 g·L^{-1}) in the studied samples. The legislation also establishes maximum values for the volatile acidity and total SO2 at 1.50 g acetic acid L^{-1} and 200 mg·L^{-1}, respectively, and the experimental ranges for these two parameters were 0.62–1.50 g·L^{-1} and 35–142 mg·L^{-1}, respectively (**Table 1**).

As a summary of this section, it can be stated that the 7 wine samples analyzed fulfilled all legal requirements and, then, they can be legally classified and sold as *Fondillón* and, then, have Alicante PDO label.

3.2. Volatile Profile and Composition

Forty volatile compounds were isolated, identified, and quantified in the headspace of the seven *Fondillón* samples analyzed using HS-SPME (**Table 2**). The volatile aroma compounds found in this specific type of Spanish wine can be grouped in 8 chemical groups: (a) esters (17 compounds): e.g., ethyl acetate, ethyl propionate, and ethyl 2-methylpropanoate; (b) alcohols (7 compounds): e.g., isoamyl alcohol, 2,3- butanediol, and 1-hexanol; (c) aldehydes (5 compounds): e.g., benzaldehyde, nonanal, and decanal; (d) terpenes (4 compounds): e.g., α-thujene, α-pinene, and limonene; (e) organic acids (3 compounds): e.g., acetic acid, octanoic acid, and decanoic acid; (f) ketones (1 compound): β-methyl- γ-octalactone; (g) sulfur compounds (1 compound): sulfur dioxide; and (h) others (2 compounds): vitispirane and TDN (1,1,6-trimethyl-1,2-dihydronaphthalene). The mean relative abundance of these 8 chemical families in the *Fondillón* samples under study was

$$\text{Lactones (0.13 \%)} < \text{terpenes (0.85 \%)} < \text{others (0.94 \%)} < \text{sulfur compounds (1.41 \%)} < \text{organic acids (2.24 \%)} < \text{aldehydes (2.49 \%)} < \text{alcohols (20.6 \%)} < \text{esters (71.4 \%)} \quad (1)$$

The overripe character of the Monastrell grapes used for the preparation of the *Fondillón* wine together with a long aging determined the volatile composition of the final wine, which was dominated by the ester family (71.4 ± 2.9 %), followed by alcohols (20.6 ± 2.2 %), as a result of the high alcoholic content of this type of wine. However, the most abundant group, esters, was not the key chemical group in determining the drivers for consumers' satisfaction degree, which was basically linked to the content of alcohols, according to a preliminary consumer study. There was a statistically significant negative correlation between the percentages of esters and alcohols ($R^2 = 0.9134$); that is, the higher the esters, the lower the alcohols.

Table 2: Identification and contents ($\mu g \cdot L^{-1}$) of volatile compounds in *Fondillón* wine.

Volatile Compounds	Code	Descriptor	RT	RI		ANOVA	F1	F2	F3	F4	F5	F6	F7
			(min)	Exp.	Lit.[‡]		Concentration ($\mu g \cdot L^{-1}$)						
Sulfur dioxide	V1		4.48	na	na	**	139 b[ϒ]	16 d	14 d	163 b	292 a	61 c	38 cd
Acetic acid	V2	Vinegar	4.83	na	na	***	70 b	49 b	73 b	56 b	632 a	49 b	64 b
Ethyl acetate	V3	Anise, ethereal	5.14	na	na	***	737 c	746 c	959 c	631 c	3655 a	339 d	1733 b
Ethyl propanoate	V4	Pineapple, wine	5.58	712	714	***	9 d	19 d	28 d	20 d	119 c	353 b	1126 a
Isoamyl alcohol	V5	Whiskey	6.03	735	732	***	836 b	1262 a	1057 a	474 c	1100 a	106 d	27 d
Ethyl 2-methylpropanoate	V6		6.49	759	747	NS	20	18	23	12	172	8	125
(*Z*,*Z*)-2,3-Butanediol[¥]	V7		6.84	776	782	NS	21	23	24	50	38	9	88
(*E*,*E*)-2,3-Butanediol[¥]	V8		7.24	797	803	*	14 b	41 b	136 a	14 b	19 b	6 b	152 a
Ethyl lactate	V9	Butter, fruity	7.55	812	815	NS	184	155	123	231	97	35	230
Ethyl 2-methylbutyrate	V10	Apple, green, plum	8.19	845	847	NS	23	54	69	61	33	28	62
Ethyl 3-methylbutyrate	V11	Apple, green, plum	8.26	848	853	NS	31	54	51	53	51	19	56
1-Hexanol	V12	Green, herb	8.61	866	864	NS	33	54	40	74	38	29	48
Isoamyl acetate	V13	Banana, pear	8.76	874	875	*	76 b	72 b	66 b	103 a	89 ab	27 c	122 a
α-Thujene	V14		10.42	938	930	**	2 c	33 b	10 b	1 c	85 a	2 c	2 c
α-Pinene	V15	Woody	10.55	943	940	***	0 c	0 c	22 b	0 c	126 a	0 c	1 c
Benzaldehyde	V16	Almond, cherry	11.68	981	980	***	2c	141 a	132 a	65 b	18 c	65 b	104 ab
Ethyl hexanoate	V17	Fruity, wine	12.32	1002	1000	NS	202	271	216	389	253	158	284
Limonene	V18	Citrus	13.78	1040	1033	NS	21	31	18	21	29	17	21
cis-*β*-Ocimene	V19		14.78	1066	1059	NS	5	8	5	5	8	5	4
Ethyl heptanoate	V20	Berry, fruity	16.16	1101	1100	NS	20	9	13	19	11	8	15
Ethyl sorbate	V21	Fruity, ethereal	16.40	1106	1111	***	557 a	14 b	20 b	2 b	2 b	4 b	42 b
Nonanal	V22	Fruity, nutty, citrus	16.77	1115	1112	NS	41	40	18	72	29	38	38
Phenethyl alcohol	V23	Honey, rose	17.48	1131	1127	*	416 c	589 b	423 c	617 a	706 a	270 c	550 bc
Octanoic acid	V24	Oily	19.48	1176	1180	NS	31	47	36	36	97	11	35
Benzyl acetate	IS		19.53	1177	1168								
1-Nonanol	V25	Citrus, rose	19.66	1180	1173	NS	13	37	26	25	37	15	14
Diethyl succinate	V26	Grape, fruity, wine	19.98	1187	1191	**	1051 b	1842 a	1643 ab	1949 a	1970 a	729 b	2053 a
Ethyl octanoate	V27	Apricot, floral	20.78	1205	1204	***	637 b	709 b	757 b	1959 a	1701 a	727 b	968 b
Decanal	V28	Floral, citrus	21.42	1219	1212	NS	28	27	17	39	19	29	22
Ethyl-2-phenyl acetate	V29		23.34	1260	1255	NS	11	16	21	29	24	10	22
Phenethyl acetate	V30	Fruity, grape, wine	23.92	1273	1265	NS	14	14	11	23	31	4	20
Vitispirane	V31	Camphor, eucalyptus	25.34	1303	1286	***	26 c	20 cd	83 a	63 b	8 d	19 cd	46 b
Ethyl nonanoate	V32	Fruity, nutty	25.48	1306	1297	**	1 b	0 b	2 b	22 a	10 b	1 b	0 b
γ-Nonalactone	V33	Whiskey	27.23	1344	1344	*	1 b	3 b	6 b	10 b	44 a	6 b	7 b
Decanoic acid	V34	Fatty, citrus	28.60	1374	1373	NS	1	9	6	4	15	0	4
TDN	V35	Petroleum	29.06	1384	1367	***	1 d	1 d	53 a	39 b	16 c	12 c	48 ab
Ethyl decanoate	V36	Grape, oily	30.19	1409	1405	***	24 d	21 d	40 d	237 b	548 a	81 c	48 cd
Dodecanal	V37	Herb, floral	31.05	1428	1420	**	3 b	2 b	4 b	10 b	81 a	3 b	2 b
(*Z*)-4-Dodecenol[¥]	V38		31.78	1445	1457	*	21 c	54 b	70 ab	76 ab	119 a	20 c	49 b
Ethyl dodecanoate[¥]	V39	Green, fruity, floral	39.12	1615	1598	NS	1	0	0	2	23	1	1
Tetradecanal[¥]	V40		40.13	1640	1625	NS	2	1	1	3	25	1	1
Total ($mg \cdot L^{-1}$)						***	5.33 c	6.50 b	6.32 b	7.66 b	12.4 a	3.30 c	8.27 b

[¥]*Tentatively identified; RT: retention time; RI: retention indexes; Exp.: experimental; Lit.: literature.* [‡]*Reference [22]. NS = not significant at $p > 0.05$; *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively.* [ϒ]*Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test.*

that is, the higher the esters, the lower the alcohols.

The main volatile aroma compounds found in the *Fondillón* samples, their relative abundance, and their sensory descriptors were as follows:

(*i*) Diethyl succinate (mean for all 7 samples of 22.7 %; descriptors: grape, fruity, wine)

(*ii*) Ethyl octanoate (15.0 %; descriptors: apricot, floral) and ethyl acetate (17.7 %; descriptors: anise, ethereal)

(*iii*) Isoamyl alcohol (9.8 %; descriptors: whiskey) and phenethyl alcohol (7.2 %; descriptors: honey, rose)

The most important esters in wines (e.g., ethyl acetate and ethyl octanoate) are considered to be the fatty acid ethyl esters, while branched-chain higher alcohols, including isoamyl alcohol, are synthesized from branched-chain amino acids [23]. Thus, there is nothing unusual in the main aroma compounds found in *Fondillón* wines. Similar to findings by Bailly et al. [24] in Sauternes wines, *Fondillón* samples, after a minimum aging period of 10 years, still contained odorants found in young Monastrell wines, such as varietal aroma (α-pinene, limonene), fermentation alcohols (phenethyl alcohol), and esters (ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate), but also contained maturation-related compounds (γ-nonalactone, vitispirane, TDN). Vitispirane and TDN are norisoprenoids that could come from the degradation of carotenoid molecules during wine aging [25].

Factors such as oxygen, temperature, and pH are key parameters, influencing the oxidative changes of *Fondillón* during its prolonged aging in oak vats, which are permeable to the entrance of oxygen. The specific volatile compounds that develop during its aging are what control the commercial value of the *Fondillón* wines. Five compounds were key for the aroma quality of Port wine, and their concentrations were markedly different between young and aged samples [26]. These compounds were β-damascenone (sensory descriptor: rose and citrus), β-ionone (floral, violet and rose), 2,2,6-trimethylcyclohexanone, TCH (rose), 1,1,6-trimethyl-1,2-dihydronaphthalene, TDN (petroleum), and vitispirane (camphor and eucalyptus). Some of these norisoprenoid molecules were responsible for floral and violet notes at low concentrations; however, some others (TDN and vitispirane) have nonpleasant aroma notes (e.g., petroleum or camphor), especially at high concentrations, but have been correlated positively correlated with the age of Port wine [27]. There were 15, 5, and 3 times higher levels of TDN, vitispirane, and TCH in 40-year-old than in young ports [26]. In the *Fondillón* samples, and under the working conditions assayed (HS-SPME and DVB/CAR/PDMS fiber), only 2 of these compounds, vitispirane and TDN, were found using HS-SPME. Future studies will be conducted using other extraction techniques and SPME fibers to check whether all these 5 compounds can also be found in *Fondillón*. The levels of these two compounds (vitispirane and TDN) were positively correlated (R^2 = 0.8410 and 0.7797, respectively) with the age of the solera and can be initially considered a good indicator of the age of the *Fondillón* samples. Besides, there is a need for further research to determine the key odorants in this special Alicante wine, by using gas chromatography and olfactometry [28, 29].

3.3 *Descriptive Sensory Analysis with Trained Panel*

Legal sensory definition of the *Fondillón* wine [7] is as follows:

Color: mahogany and amber and with copper tone.

Nose: aromatically intense, ripe fruit nuts, well-integrated wood, high roasted.

Taste: balanced, good structure, big volume, persistent, and slight sweet.

This definition is certainly not wide enough to fully express the whole personality of this type of wine. Besides, there is a pressing need to have methods certified by official accreditation bodies to score the sensory quality of foods [30], and wine is not an exemption. In 2015, the Alicante PDO selected, trained, and validated their sensory panel to evaluate the wines protected by this organization. During the training, the panel developed, together with the UMH researchers who were responsible for this training, the lexicon compiled in **Table 3.** This lexicon was prepared according to experience of the panelists included in the panel, who were oenologists, sommeliers, researchers, etc., and to previous studies developing similar lexicons for other Spanish wines, such as Rioja Alavesa [19, 31] and txakoli [32]. The lexicon was divided into 4 phases or steps: (*i*) flavor (including odor (perception of volatile compounds with the wine in the cup) and aroma (perception of the volatile compounds with the wine in the mouth), (*ii*) global, (*iii*) visual, and (*iv*) defects for each one of the previous three phases. The visual evaluation was conducted in a black cup to avoid any subjective color bias.

The Alicante PDO has prepared "typical profiles" for each one of the wines under their protection, including *Fondillón*, and the wines under evaluation should be as close to the Alicante profiles as possible, with a tolerance level established by the Regulatory Body. The sensory profile of the first 7 *Fondillón* samples (F1–F7) fully agreed with the scores of the typical *Fondillón* profile of the Alicante PDO, shown in **Table 4,** in the column "PDO profile."

Table 3: Lexicon used for the descriptive analysis of *Fondillón*.

Attributes	Definition	References and intensities
Flavor		
Alcohol	A flavor reminiscent of alcoholics compounds	Ethanol solution 7 % = 2.0; ethanol solution 11 % = 5.0; ethanol solution 18 % = 9.5
Fruity	A flavor blend that is sweet and reminiscent of a variety of fruits	Citral 16 $\mu g \cdot L^{-1}$ = 6.0; isoamyl acetate 30 $\mu g \cdot L^{-1}$ = 6.0; benzaldehyde 100 $\mu g \cdot L^{-1}$ = 6.0
Floral	A sweet, heavy aromatic blend of a combination of flowers	Geraniol 10 $\mu g \cdot L^{-1}$ = 6.0; β-ionone 0.10 $\mu g \cdot L^{-1}$ = 6.0
Vegetable	Flavor reminiscent of a variety of different vegetables	2-Isobutyl-3-methoxypyrazine 0.02 $\mu g \cdot L^{-1}$ = 6.0; cis-3-hexen-1-ol 70 $\mu g \cdot L^{-1}$ = 6.0; 1-octen-3-ol 1 $\mu g \cdot L^{-1}$ = 6.0
Spicy	Flavor reminiscent of different species, which are directly related to the passage of wine barrels	Eugenol 15 $\mu g \cdot L^{-1}$ = 6.0; anethole 70 $\mu g \cdot L^{-1}$ = 6.0
Animal	Flavor reminiscent of animals or products derivatives thereof	Albona butter flavor 6 $\mu g \cdot L^{-1}$ = 6.0; "le nez du vin" flavor no. 45 = 9.0
Toasted	Aromas reminiscent of roasted products and generally coming from the toasting of the barrels	Vainillin 20 $\mu g \cdot L^{-1}$ = 6.0; 2-acetylthiazole 5 $\mu g \cdot L^{-1}$ = 6.0
Sweet	The fundamental taste factor associated with a sucrose solution	Sucrose solution 4 % = 2.5; sucrose solution 8 % = 5.0; sucrose solution 16 % = 9.5
Sour	The taste stimulated by acids, such as citric and malic	Tartaric acid solution 0.05 % = 2.5; tartaric acid solution 0.08 % = 4.0; tartaric acid solution 0.20 % = 9.5
Bitter	The taste stimulated by substances such as quinine or caffeine	Caffeine solution 0.05 % = 2.5; caffeine solution 0.08 % = 4.0; caffeine solution 0.20 % = 9.5
Astringent	The complex of drying, puckering, and shrinking sensations in the oral cavity	Alum solution 0.05 % = 1.5; alum solution 0.10 % = 3.0; alum solution 0.20 % = 6.0
Global		
Imbalance	Wine attribute or attributes that prevail over the rest, breaking the balance	Sour: tartaric acid 2 $g \cdot L^{-1}$ = 6; astringent: tannin 4 $g \cdot L^{-1}$ = 6; bitter: quinine sulphate 0.03 $g \cdot L^{-1}$ = 6; alcohol: ethanol 60 $mL \cdot L^{-1}$ = 6
Persistence	Time it remains in the mouth, the characteristic flavor of the fruit after swallowing the sample	5–8 s = 5.0; 15–18 s = 10
Visual		
Limpidity	Without particles or coloidal elements in suspension	Isolated elements = 5; without particles = 10
Color	Visual evaluation of the color intensity of the sample	Pantone 1675C = 2.0; Pantone 201C = 4.0; Pantone 200C 0 6.0
Color int.	Depth of color when you put a text under the glass	If you can read the text = 1.0; if you can see the text but you can't read it = 5.0; if you can't see the text = 10
Defects		
Vegetal	Defect caused by immature grapes or insufficient cleaning of bunches	"Le nez du vin, faults" no. 1 = 8
Rotten apple	Wine oxidation by Candida mycoderma, with formation of acetaldehyde	"Le nez du vin, faults" no. 2 = 8
Vinegar	Formation of acetic acid by Gluconobacter and Acetobacter	"Le nez du vin, faults" no. 3 = 8
Glue	Formation of ethyl acetate by reaction of acetic acid with ethanol	"Le nez du vin, faults" no. 4 = 8
Soap	Soapy notes caused by the salts of certain fatty acids, mainly decanoic acid	"Le nez du vin, faults" no. 5 = 8
Sulfur	Sulfurous notes from too much sulfite	"Le nez du vin, faults" no. 6 = 8
Rotten Egg	Formation of hydrogen sulfide by reduction of sulfiting by yeasts	"Le nez du vin, faults" no. 7 = 8
Onion	Ethanethiol formation by reaction of H2S with ethanol	"Le nez du vin, faults" no. 8 = 8
Cauliflower	Note characteristic aromatic wines made from poorly debourbaged musts	"Le nez du vin, faults" no. 9 = 8
Horse	Unpleasant animal note (mainly phenolic) that resembles the horse stable smells, is defect may occur due to presence of the Brettanomyces	"Le nez du vin, faults" no. 10 = 8
Earthy	Notes that smells like wet earth	"Le nez du vin, faults" no. 11 = 8
Cork	Aromatic note caused by the poor quality of cork Employed, is complex defect includes simple notes like solvents and moisture	"Le nez du vin, faults" no. 12 = 8

Table 4: Descriptive sensory analysis of commercial samples of Fondillón used to validate the sensory lexicon.

Attribute	ANOVA †	PDO profile	F8	F9	F10	F11	F12
			Sensory intensity (scale 0-10)				
Odor (o)							
Alcohol	NS	7.0	7.0	7.0	7.0	6.5	7.0
Fruity	*	6.0	6.0 a ‡	6.0 a	5.5 b	6.0 a	4.0 c
Floral	NS	2.0	2.0	2.0	3.0	2.0	2.0
Vegetal	***	2.5	3.0 b	3.5 ab	4.0 a	2.0 c	2.5 b
Spicy	NS	3.5	2.5	4.0	4.0	3.0	3.5
Animal	**	3.0	3.0 b	3.0 b	4.0 a	2.8 b	3.0 b
Toasted	NS	6.0	5.5	7.0	5.0	6.3	6.0
Defects	***	0	0 b	0 b	2.8 a	0 b	2.5 a
Flavor (f)							
Alcohol	NS	7.0	7.0	7.0	7.0	7.0	7.0
Fruity	***	6.0	6.0 a ‡	6.5 a	4.0 b	6.0 a	3.0 b
Floral	NS	2.0	2.0	1.0	2.0	2.0	2.0
Vegetal	NS	2.0	2.0	2.0	2.0	2.0	3.0
Spicy	NS	4.0	3.0	4.0	4.0	2.8	4.0
Animal	NS	3.0	3.0	2.0	3.0	2.0	3.0
Toasted	NS	6.0	6.0	6.8	5.0	6.3	6.0
Sweet	***	3.0	2.0 b	5.0 a	3.0 b	4.0 ab	3.0 b
Sour	**	4.0	4.0 ab	3.0 b	5.0 a	4.0 ab	5.0 a
Bitter	NS	2.0	2.0	2.0	2.0	1.3	3.0
Astringent	NS	2.0	2.0	2.0	3.0	1.0	2.5
Defects	**	0	0 c	0 c	2.5 a	0 c	1.0 b
Global							
Imbalances	***	0	1.0 b	0 c	3.0 a	0 c	2.0 ab
Aftertaste	*	7.0	7.0 b	8.0 a	6.5 b	7.0 ab	6.0 b
Appearance (a)							
Limpidity	NS	9.0	9.0	9.0	8.5	9.0	8.0
Color	**	5.0	5.0 b	5.0 b	6.0 a	5.0 b	3.0 c
Color intensity	**	3.0	3.5 b	3.0 b	5.0 a	4.8 a	3.0 b
Defects	NS	0	0	0	0	0	0.5
Qualification			OK	OK	NOT OK	OK	NOT OK
Liking¶	***		6.0 b	8.0 a		6.7 b	
Ranking¶	***		b	a		b	

*†NS = not significant at $p > 0.05$; *, **, and *** significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡Values (mean of 15 trained panelists) followed by the same letter, within the same row, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test. ¶Mean satisfaction degree of 30 consumers is denoted by liking, and statistical results of Friedman's test are denoted by ranking.*

However, the trained panel of the Alicante PDO, using the sensory lexicon specific for *Fondillón* (Table 3), identified two samples (F10 and F12) during the validation step, which were considered as having significant problems, which should preclude their labeling with the Alicante PDO seal (Table 4). The problems in these two samples were mainly due to (i) defects in the olfactory phase, with defects having scores of 2.8 and 2.5 and (ii) imbalances (sour, astringent, and bitter) in the global phase, with scores being 3.0 and 2.0, for samples F10 and F12, respectively. Eleven out of 15 panelists described the defect found in the sample F10 (SO_2) as sulfur, while all panelists properly described the excessive occurrence of ethyl acetate as "glue".

Besides, two parameters were used to validate the quality of the sensory lexicon and the performance of the sensory panel: (*i*) repeatability in attribute identification and scores: ability to identify the same attributes (including defects) and give similar scores when the same wine is evaluated in two replications in the same session and (*ii*) reproducibility in attribute identification and scores: ability to identify the same attributes (including defects) and give similar scores when the same wine is evaluated in replicate in different sessions [19]. The values of these two parameters for the panel sessions were acceptable for the requirements of the PDO Alicante; standard deviation of the same wine sample should be ≤ 1.3 for all the identified attributes.

Thus, the conclusion of this section was that the sensory lexicon and questionnaire developed especially for *Fondillón* samples under the Alicante PDO have been validated by detecting the two spiked and spoiled samples.

3.4 Affective Sensory Analysis with Consumers' Panel.

A preliminary consumer study (with only 60 consumers) seemed to indicate that the highest satisfaction degree (8.0 in sample F2) was linked to the fruity notes, the alcoholic content, the aftertaste, and the presence of key volatile compounds, such as vitispirane (which sensory descriptor is eucalyptus) and benzaldehyde, with a bitter almond note. The satisfaction degree ranged between 4.4 and 8.0. However, more complex affective tests (regular Fondillón consumers, a consumer number > 100, and 4-5 locations in different regions of Spain) must be conducted to prove the hypothesis raised in this preliminary affective study.

4. Conclusions

The combined use of instrumental (HS-SPME-GC-MS/FID) and sensory (descriptive sensory analysis and consumer studies) tools has allowed proper classification of the *Fondillón* samples. To have a full description of this wine, a specific lexicon to describe wines under the Alicante PDO label was developed. This wine (Fondillón), historically known as Alicante wine, was highly appreciated by today's Spanish consumers when having intense fruity notes, but at the same time, high alcoholic content and some bitter and balsamic notes, such as those coming from benzaldehyde (bitter almond) and vitispirane (eucalyptus). However, further affective studies are needed using a higher number of consumers and including more locations within different regions of Spain and also in the European Union as the initial potential market for this wine. The age of the Fondillón samples has been successfully linked with the contents of two key compounds TDN and vitispirane, but other extraction and analysis techniques must be assayed to fully prove this statement.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References

[1] P. Reboredo-Rodríguez, C. González-Barreiro, R. Rial-Otero, B. Cancho-Grande, and J. Simal-Gándara, "Effects of sugar concentration processes in grapes and wine aging on aroma compounds of sweet wines-a review," *Critical Reviews in Food Science and Nutrition*, vol. 55, no. 8, pp. 1053–1073, 2015.

[2] L. Rolle, F. Torchio, S. Giacosa, and V. Gerbi, "Modifications of mechanical characteristics and phenolic composition in berry skins and seeds of mondeuse winegrapes throughout the on-vine drying process," *Journal of the Science of Food and Agriculture*, vol. 89, no. 11, pp. 1973–1980, 2009.

[3] A. Valero, S. Marín, A. J. Ramos, and V. Sanchis, "Survey: ochratoxin a in European special wines," *Food Chemistry*, vol. 108, no. 2, pp. 593–599, 2008.

[4] M. Figuereido-González, B. Cancho-Grande, and J. Simal- Gándara, "Effects of colour and phenolic composition of sugar concentration processes in dried-on-or dried-off-vine grapes and their aged or not natural sweet wines," *Trends in Food Science and Technology*, vol. 31, no. 1, pp. 36–54, 2013.

[5] EC, *Council Regulation (EC) No 479/2008 of April 2008 On the Common Organisation of the Market in Wine, in 479/2008*, European Union, Brussels, Belgium, 2008.

[6] D. Alicante, Fondillón de Alicante, time is a luxury, 2016, http://vinosalicantedop.org/fondillon/en/.

[7] G. Valenciana, *Orden 5/2011, de 16 de Noviembre, de la Conselleria de Agricultura, Pesca, Alimentación y Agua, Por la que se Aprueba el Texto del Reglamento y Pliego de Condiciones de la Denominación de Origen Protegida Alicante y su Consejo Regulador*, Diario Oficial de la Comunitat Valenciana, Valencia, Spain, 2011.

[8] C. Lorenzo, F. Pardo, A. Zalacain, G. L. Alonso, and M. R. Salinas, "Differentiation of co-winemaking wines by their aroma composition," *European Food Research and Technology*, vol. 227, no. 3, pp. 777–787, 2008.

[9] G. A. Burdock, *Fenaroli's Handbook of Flavor Ingredients*, CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, 6th edition, 2010.

[10] OIV, *Compendium of International Methods of Analysis of Wines and Musts*, vol. 2, OIV, Paris, France, 2016, http://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis/compendium-of-international-methods-of-analysis-of-wines-and-musts-2-vol.

[11] A. Alonso, L. Vázquez-Araújo, S. García-Martínez, J. J. Ruiz, and Á. A. Carbonell-Barrachina, "Volatile compounds of traditional and virus-resistant breeding lines of Muchamiel tomatoes," *European Food Research and Technology*, vol. 230, no. 2, pp. 315–323, 2009.

[12] Á. A. Carbonell-Barrachina, P. J. Szychowski, M. V. Vásquez, F. Hernández, and A. Wojdyło, "Technological aspects as the main impact on quality of quince liquors," *Food Chemistry*, vol. 167, pp. 387–395, 2015.

[13] A. Gironés-Vilaplana, Á. Calín-Sánchez, D. A. Moreno, Á. A. Carbonell-Barrachina, and C. García-Viguera, "Novel maqui liquor using traditional pacharán processing," *Food Chemistry*, vol. 173, pp. 1228–1235, 2015.

[14] A. J. Andreu-Sevilla, P. Mena, N. Martí, C. García Viguera, and ´ A. A. Carbonell-Barrachina, "Volatile composition and descriptive sensory analysis of pomegranate juice and wine," Food Research International, vol. 54, no. 1, pp. 246–254, 2013.

[15] L. Vera, M. Mestres, R. Boqué, O. Busto, and J. Guasch, "Use of synthetic wine for models transfer in wine analysis by HS- MS e-nose," *Sensors and Actuators B: Chemical*, vol. 143, no. 2, pp. 689–695, 2010.

[16] A. de-la-Fuente-Blanco, M.-P. Sáenz-Navajas, and V. Ferreira, "Levels of higher alcohols inducing aroma changes and modulating experts' preferences in wine model solutions," *Australian Journal of Grape and Wine Research*, vol. 23, no. 2, pp. 162–169, 2017.

[17] ISO 17065:2012, *Conformity Assessment. Requirements for Bodies Certifying Products, Processes and Services in 17065: 2012*, ISO/IEC, New York, NY, USA, 2012.

[18] ISO 3591:1977, *Sensory Analysis. Apparatus. Wine Tasting Glass*, ISO, Geneva, Switzerland, 1977.

[19] I. Etaio, M. Albisu, M. Ojeda, P. F. Gil, J. Salmerón, and F. J. Pérez Elortondo, "Sensory quality control for food certification: a case study on wine. Panel training and qualification, method validation and monitoring," *Food Control*, vol. 21, no. 4, pp. 542–548, 2010.

[20] G. Morrot, F. Brochet, and D. Dubourdieu, "Toe color of odors," *Brain and Language*, vol. 79, no. 2, pp. 309–320, 2001.

[21] W. V. Parr, K. G. White, and D. A. Heatherbell, "Toe nose knows: influence of colour on perception of wine aroma," *Journal of Wine Research*, vol. 14, no. 2-3, pp. 79–101, 2003.

[22] NIST. National Institute of Standards and Technology, 2016, http://webbook.nist.gov/chemistry/name-ser.html.

[23] A. L. Robinson, P. K. Boss, P. S. Solomon, R. D. Trengove, H. Heymann, and S. E. Ebeler, "Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts," *American Journal of Enology and Viticulture*, vol. 65, no. 1, pp. 1–24, 2014.

[24] S. Bailly, V. Jerkovic, A. Meurée, A. Timmermans, and S. Collin, "Fate of key odorants in sauternes wines through aging," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 18, pp. 8557–8563, 2009.

[25] A. Silvaferreira and P. Guedes de Pinho, "Nor-isoprenoids profile during port wine ageing—influence of some technological parameters,"

Analytica Chimica Acta, vol. 513, no. 1, pp. 169–176, 2004.

[26] N. Moreira and P. Guedes de Pintho, "Port wine," in *Advances in Food and Nutrition Research*, R. S. Jackson, Ed., pp. 119– 146, Elsevier, Amsterdam, Netherlands, 2011.

[27] C. F. Ross, A. C. Zwink, L. Castro, and R. Harrison, "Odour detection threshold and consumer rejection of 1,1,6- trimethyl-1,2-dihydronaphthalene in 1-year-old riesling wines," *Australian Journal of Grape and Wine Research*, vol. 20, no. 3, pp. 335–339, 2014.

[28] R. M. Callejon, M. L. Morales, A. M. Troncoso, and A. C. Silva Ferreira, "Targeting key aromatic substances on the typical aroma of sherry vinegar," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 15, pp. 6631–6639, 2008.

[29] L. Culleré, A. Escudero, J. Cacho, and V. Ferreira, "Gas chromatography−olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 6, pp. 1653–1660, 2004.

[30] F. J. P. Elortondo, M. Ojeda, M. Albisu, J. Salmerón, I. Etayo, and M. Molina, "Food quality certification: an approach for the development of accredited sensory evaluation methods," *Food Quality and Preference*, vol. 18, no. 2, pp. 425–439, 2007.

[31] I. Etaio, M. Albisu, M. Ojeda, P. F. Gil, J. Salmerón, and F. J. P. Elortondo, "Sensory quality control for food certification: a case study on wine. Method development," Food Control, vol. 21, no. 4, pp. 533–541, 2010.

[32] I. Etaio, P. F. Gil, M. Ojeda, M. Albisu, J. Salmerón, and F. J. Pérez Elortondo, "Improvement of sensory quality control in PDO products: an example with txakoli white wine from Bizkaia," Food Quality and Preference, vol. 23, no. 2, pp. 138–147, 2012

PUBLICACIÓN 2

Aroma-active compounds, sensory profile, and phenolic composition of *Fondillón*

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Aroma-active compounds, sensory profile, and phenolic composition of *Fondillón*

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ABSTRACT

The *Fondillón* is a wine made from overripe grapes of the Monastrell variety, which is characterized by a high alcohol content and a minimum barrel ageing of 10 years. The objective of this study was to analyze the *Fondillón* volatile composition, key aroma-active compounds, sensory profile and phenolic composition. Fifty-four volatile compounds were identified, quantified and classified as alcohols, esters, acids, aldehydes, lactones, phenols, hydrocarbons, and ketone. From these compounds, 22 aroma-active compounds were identified, with phenylethyl alcohol, diethyl succinate and ethyl lactate having the highest flavor dilution factor. The *Fondillón* wines were characterized by having high intensity of alcohol, fruity and toasted odor and flavor notes, and long aftertaste. Besides, 25 phenolic compounds were also identified and quantified; the phenolic acids (gallic, protocatechuic and syringic acids) were the predominant phenolic compounds.

1. Introduction

Wineries face two main producing strategies (*i*) bulk wines or (*ii*) differentiated wines. Some of the world most exclusive differentiated wines are the naturally sweet ones. The main characteristic of these wines is that they come from grapes with higher than normal sugar concentration and that are non-fortified with alcohol. Sweeter grapes can be produced both on- and off-vine, through different ways (Valero, Marín, Ramos, & Sanchis, 2008), including:

(i) Exposure of grapes to sunlight after harvest. Examples of this procedure are Passito (Italy) and Pedro Ximenez and Málaga (Spain).

(ii) Grape dehydration in closed rooms with fresh or hot ventilation. This procedure is used for Vin de Paille (France) and Vin Santo and Recioto (Italy).

(iii) Noble rot grape colonization by *Botrytis cinerea.* This strategy is used in Trockenbeerenauslese (Germany), Tokaji (Hungary) and Loire, Montbazillac, Sauternes and Alsace (France).

(iv) Using overripe grapes after a late harvest. Examples can be found in *Fondillón* (Spain), Vendage Tardive (France), Tokaji Late harvest (Hungary) and Spatlese (Germany). Noble rot can also be present in the last three wines.

(*v*) Grape dehydration by ice after delaying harvest in cold climates. This is the case of ice wine (Canada), Vi de Gel (Spain) and Eiswein (Germany).

These wines must have an alcohol by volume content (abv) above 15 %; however, they are produced without alcohol enrichment (EC, 2008). The quality of these wines is strongly determined by their high sugar level and their aroma compounds, which precursors are mainly developed during the on-vine dehydration (Reboredo-Rodríguez, González-Barreiro, Rial-Otero, Cancho-Grande, & Simal-Gándara, 2015). In this process, not only sugars are accumulated, but also phenolic and aroma precursors and/or compounds are either concentrated or produced (Bellincontro, De Santis, Botondi, Villa, & Mencarelli, 2004; Costantini, Bellincontro, De Santis, Botondi, & Mencarelli, 2006). *Fondillón* is a naturally sweet red wine produced in an oxidized (rancio) style from overripe *Monastrell* grapes. It can only be produced within the Alicante Protected Designation of Origin, Alicante PDO (Alicante, 2016). It is included by the European Union in its E-Bacchus database. In the case of *Fondillón*, dehydration is done on-vine; thus, it can be considered as a late-harvest wine. These on-vine overripe grapes produce a naturally concentrated must, rich in sugars and volatile compounds. This wine is bottled and sold after a minimum ageing period of 10 years in oak barrels. It can either be vintage-dated or come from a solera. It was recently reported that: (*i*) esters (e.g. diethyl succinate) and alcohols (e.g. isoamyl alcohol) are the predominant chemical families, and (*ii*) Spanish consumers like *Fondillón* when it has intense fruity notes, high alcohol content, and some bitter and balsamic notes (Issa-Issa et al., 2019).

Fondillón production almost stopped at the end of XIX century due to the increased demand of "low-quality and low-price" wine from France because of *Phyloxera* crisis. It was recovered around 1950, when the demand of "high-quality" wines increased again. Until the last couple of years, Alicante wineries were not fully aware of the full potential of this wine and paid not enough attention to its production.

Considering all the above information, the aims of this study were
(*i*) to define which are the aroma-active compounds controlling the sensory quality of this wine, and (*ii*) to determine which are the main phenolic compounds found in this aged Alicante wine. The knowledge generated in this study will allow enologists to understand the *Fondillón* winemaking process (mainly ageing) and will provide unique information for wineries to develop marketing campaigns to promote the consumption of this highly differentiated wine. In fact, the most important aim of the PDO Alicante is to develop a full marketing campaign to make *Fondillón* the core wine of this region based on key sensory attributes, including aroma-active compounds.

2. Materials and Methods

2.1 Wine samples

Fondillón samples were kindly donated by Bodegas Monóvar- MGWines (Monóvar, Alicante, Spain) and were collected in triplicate (3 samples of ~1 L per each solera: 1944, 1987, and 1996) were taken and immediately transported to the facilities of the Universidad Miguel Hernández de Elche (Orihuela, Alicante, Spain) for analyses. Later, approximately 0.5 L were taken personally by plane to Adana for the analysis of the aroma-active compounds and the phenolic composition.

2.2 Extraction of volatile compounds by solvent-assisted flavor evaporation (SAFE)

The volatile compounds of *Fondillón* wines were isolated using SAFE (Glasblaserei Bahr, Manching, Germany) apparatus under vacuum (10^{-3} Pa; Vacuubrand DCP 3000, Wertheim, Germany). The extraction of the aroma compounds was performed according to the procedure described by Selli et al. (2004) with slight modifications. A 100 mL of wine and 100 mL of dichloromethane were placed into a 500-mL flask. The content was stirred at 4 °C for 60 min under a N_2 atmosphere, and, then, centrifuged at 4 °C, 5500 rpm for 15 min. The organic phase (solvent) was slowly fed into the dropping funnel of the transfer head. Separation of the mixture was carried out in the distillation vessel (10 mL min^{-1}) that was partially submerged in a warm water bath of 38 °C. To ensure a continuous temperature throughout distillation and to avoid condensation of the volatile compounds, the system was completely thermostated with water at 4 °C. The separated volatile compounds passed through the separation head into a receiving vessel where they condensed and froze because of the sudden drop in temperature under the liquid N_2 in the cooling trap. Once the separation was complete, the receiving vessel was removed and allowed to thaw out at room temperature for 30 min. After dehydration by anhydrous sodium sulfate, the resulting organic extract was concentrated to 200 μL (Engel, Bahr, & Schieberle, 1999; Sonmezdag, 2018).

2.3 Determination of aroma and aroma-active compounds

A gas chromatography (GC) instrument (Agilent 6890) equipped with a mass selective detector (MSD) (5973-Network 159 MSD, Agilent Technologies, Wilmington, Delaware, USA), flame ionization detector (FID), and a Gerstel ODP-2

(Baltimore, Maryland, USA) olfactometry sniffing device coupled with humidified air at 40 °C using deactivated fused silica capillary column (30 cm × 0.3 mm) were used in the analyses of aroma and aroma-active compounds. This coordinated system provided deep knowledge about aroma profiles of *Fondillón* samples by combining MS, FID, and sniffing apparatus dividing the effluent into 3 equal flows (1:1:1) to identify and quantify aroma and aroma-active compounds. Aroma compounds were differentiated in a DB-Wax column (30 m length × 0.25 mm i.d. × 0.5 μm thickness, J&W Scientific, Folsom, California, USA). The oven temperature of the column was gradually increased as follows: (1) initially held at 40 °C for 10 min, then (2) increased from 40 to 160 °C at a rate of 3 °C min^{-1}, (3) subsequently increased to 240 °C at 6 °C min^{-1}, (4) and finally held at 240 °C for 25 min. The column pressure was set up at a constant value of 20.0 psi, and 3 μL of extract were injected in pulsed splitless mode. Injector and FID detectors were set at 270 °C and 280 °C, respectively. The MS (electronic impact ionization) conditions were ionization energy of 70 eV, mass range *m/z* of 33 to 300 amu at 2.0 scan s^{-1}. Concentrations of aroma compounds were determined by the application of internal standard method and expressed as μg L^{-1} resulting from the relative peak area to internal standard (4-nonanol equivalents with a concentration of 40 μg L^{-1}) (de Saint Laumer, Cicchetti, Merle, Egger, & Chaintreau, 2010). All compounds were determined by comparing their retention indexes and their mass spectra on the DB-Wax column with those of a commercial spectra database (Wiley 6, Flavor 2, NBS 75 k) and of the internal library of the instrument created in earlier studies. Linear retention indexes were calculated by the injection of commercial linear *n*-alkane (C6-C32) series.

2.4 Aroma extract dilution analysis (AEDA)

Key odorants of *Fondillón* wines were identified by GC-MS-O with the application of aroma extract dilution analysis (AEDA). The method was applied by experienced panelists determining the impact of in- dividual volatile compounds on overall aroma. A concentrated aromatic extract (200 μg) of *Fondillón* samples were gradually diluted with Cl_2CH_2 starting from 1:1, continuing with 1:2, 1:4, and concluding with 1:1024 dilution ratio, at which the last detectable odor emerged. Each diluted sample (3 μL) was injected into the GC-MS-O equipment and all key aroma compounds was sniffed by experienced panelists. Increasing the number of FD factors represented the activity level of aroma compounds in the samples under analysis (Selli, Kelebek, Turan Ayseli, & Tokbas, 2014).

2.5 Descriptive sensory analysis with trained panel

Ten panelists (6 males and 4 females), aged 30–62 years, evaluated 3 *Fondillón* samples at the facilities of the Food Quality and Safety (CSA) research group of the Universidad Miguel Hernández de Elche (UMH). The lexicon and questionnaire used here were those developed by the Regulatory Board of the Alicante Protected Designation of Origin, Alicante PDO and recently described by (Issa-Issa et al., 2019). For the analysis of the odor (perception of volatile compounds with the wine outside the mouth), flavor [combination of odor, aroma (perception of volatile compounds with the wine inside the mouth), basic tastes and chemical feeling factors] and global attributes, 35 mL was served in a black cup and for the appearance 25 mL were used but in a transparent cup. Samples were evaluated at room temperature (16–18 °C), which is the official temperature range for this specific wine and under white light. Samples were randomly served coded with 3-digit numbers, together with the appropriate questionnaire, one at a time, and waiting 5 min between samples. Between samples and for palate cleansing, water and unsalted crackers were provided to panelists. The attributes under evaluation were: odor (alcohol, fruity, floral, vegetable, spicy, animal and toasted), flavor (alcohol, fruity, floral, vegetable, spicy, animal and toasted), basic tastes (sweet, acid and bitter), chemical sensations (astringent), global attributes (imbalance and aftertaste), appearance (cleanliness, color and color intensity), and defects (rotten apple, vinegar, glue, soap, sulfur, rotten egg, onion, cauliflower, horse, earthy and cork). Panelists used a scale of 0 to 10 points for the evaluation, where 10 was extremely high intensity and 0 was extremely low intensity or not noticeable.

2.6 Phenolic composition (LC-MS-MS)

Fondillón wine samples were filtered through a 0.45-μm pore size membrane filter before injection. An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto CA-USA) operated by Windows NT based ChemStation software was used. The HPLC equipment was used with a diode array detector (DAD). System consisted of a binary pump, degasser and autosampler. The column used was a Beckman Ultrasphere ODS (Roissy CDG, France): 4.6 mm × 250 mm, 5 μm equipped with a precolumn 4.6 mm × 10 mm (same granulometry). The mobile phase consisted of two solvents: Solvent A, water/formic acid (95:5; v/v) and Solvent B, acetonitrile/solvent A (60:40; v/v). Phenolic compounds were eluted under the following conditions: 1 mL min^{-1} flow rate and 25 °C, isocratic conditions from 0 to 10 min with 0 % B, gradient conditions from 0 to 5 % B in 30 min, from 5 to 15 %

B in 18 min, from 15 to 25 % B in 14 min, from 25 to 50 % B in 31 min, from 50 to 100 % B in 3 min, followed by washing and reconditioning the column. The ultra-violet-visible spectra (scanning from 200 nm to 600 nm) were recorded for all peaks. Triplicate analyses were per- formed for each sample. The identification of phenolic compounds was obtained out by using authentic standards and by comparing the retention times and ultra-violet-visible spectra with those found in the literature, while quantification was performed by external calibration with standards also confirmed by LC–MS/MS analysis according to Kelebek (2016).

2.7 Statistical analysis

All data included in this study consisted of the mean of, at least, 3 replicates for the volatile and phenolic compounds and 10 (number of panelists) for descriptive sensory data. All the data were first subjected to analysis of variance (ANOVA) and later to a multiple range test (Tukey's test), using StatGraphics Plus 5.0 software (Manugistics, Inc., Rockville, MD). Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1 Aroma compounds of aged Fondillón wines

Aromatic extracts of *Fondillón* wines from 3 soleras (1944, 1987 and 1996) were obtained by the application of direct solvent extraction followed by SAFE technique. According to GC–MS results, a total of 54 volatile compounds were identified and quantified and are displayed in Table 1. These compounds can be classified in 8 chemical groups, in- cluding alcohols, esters, acids, aldehydes, lactones, phenols, hydro- carbons, and ketones, with esters predominating qualitatively (n = 18) and alcohols quantitatively (Fig. 1). The total content of volatile com- pounds ranged between 24.96 and 41.84 mg L^{-1}.

In the aromatic profile of *Fondillón* wines, esters embodied the highest number of volatiles with 18 compounds in total. These compounds are stated to be produced by yeast during the fermentation and play a substantial role in the fruity odor, aroma and flavor of wines (Flamini & Traldi, 2010). There was not a clear relationship between the total ester content and the age of the *Fondillón* wines; the contents were 6995, 4720, and 8814 μg L^{-1} in F-1944, F-1987 and F-1996 samples, respectively (Fig. 1). In general, esters are formed from the reactions of existing alcohols and acids throughout wine ageing (Fan & Qian, 2005). In previous studies, it was reported that diethyl esters of succinic and glutaric acids arose during wine ageing (Wang, Gambetta, & Jeffery, 2016). It is important to remember that this wine has gone to at least 10 years of ageing; thus, it is possible that esters are formed and later reorganization reactions occurred decreasing their content but it is also possible that the differences observed are due to different raw materials; the *Fondillón* samples under analysis come from vintage-dated wines/oak barrels and it is possible that the overripe grapes used for their preparation were different at harvest; each season is different due to specific weather conditions, among other factors. Thus, further research is needed to "fully" clarify the simultaneous effects of age and raw materials on Fondillón quality.

Ethyl lactate was detected as the most abundant ester (4057 μg L^{-1}) in all samples followed by diethyl succinate (1337 μg L^{-1}). This trend (high contents of ethyl lactate and diethyl succinate) agreed with previous results in other type of wines. For instance, the high content of ethanol favored esterification and ethyl lactate accumulation in Sherry wines (Pozo-Bayón & Moreno-Arribas, 2011), while diethyl succinate predominated in the aroma profile of Madeiras (Perestrelo, Silva, Pereira, & Câmara, 2016). The other determined compounds in this group were mainly ethyl and acetate esters, including for example ethyl propanoate, ethyl 2-methylpropanoate, etc.

Another important chemical group in *Fondillón* wines was alcohols. Fourteen alcohols were found with their total content ranging between 17.06 and 28.43 mg L^{-1}, representing approximately 68 % of the total volatile content respectively (Fig. 1). Isoamyl alcohol was found to be the most abundant alcohol (13831 μg L^{-1}) in all 3 *Fondillón* samples, followed by phenylethyl alcohol (5373 μg L^{-1}). Total alcohol con- centration displayed a fluctuation between samples, with the oldest sample (F-1944) showing an intermediate value (21.04 mg L^{-1}) respectively (Fig. 1). Alcohols are reported to be produced by sugar catabolism and/or amino acid degradation in wine matrix (Ebeler, 2001). Isoamyl alcohol was found in the aroma profiles of fino Sherries (Perestrelo et al., 2016), while phenethyl alcohol was found in Amontillado wines (Pozo-Bayón & Moreno-Arribas, 2011).

After the two most complex chemical groups (esters and alcohols), 7 organic acids were found in *Fondillón* wine samples, with acetic acid being the most abundant one. The volatile profile of fino Sherries also appears to be characterized by the presence of acetic acid (Perestrelo et al., 2016). Acids with the remaining groups of lactones, phenols, aldehydes and ketones represented about 10 % of the total volatile content of *Fondillón*, with the highest content of these groups being found in the youngest sample (F-1996).

3.2 Key odorants of Fondillón wine determined with GC–MS–O

In an attempt to entirely understand the aroma profile of aged *Fondillón* wines, aroma extract dilution analysis (AEDA) was conducted to determine the key aroma-active compounds. A total of 24 aroma- active compounds were identified by sniffing the effluent of GC–MS–O and the results are summarized in **Table 2.** Similar as previously described for the volatile profile, esters dominated the aroma of aged *Fondillón* with 8 compounds with FD (flavor dilution) factor ranging between 4 and 512. Apart from esters (8), alcohols (4), aldehydes (3) and lactones (3), acids (2), ketones (1) and phenolic compounds (1) contributed to the potent aroma of the *Fondillón* samples. Additionally, two unknown compounds [LRI (linear retention index): 1733 and LRI:1748] were perceived by panelists and were described as having cooked vegetable and onion odor/aroma notes, but could not be identified by GC–MS (FD factors of 8–16).

Table 1. Volatile compounds identified in *Fondillón* wines (µg L^{-1}).

Chemical Family	Compound	Code	RT†	LRI‡	ANOVA¶	F-1944	F-1987	F-1996
Esters	Ethyl propanoate	V1	6.4	915	***	56.7 b	ND c	97.9 a
	Ethyl 2-methylpropanoate	V2	6.5	975	**	14.5 b	ND c	25.1 a
	Ethyl butyrate	V4	9.6	1045	*	ND¥ b	55.0 a	ND b
	Ethyl 3-methylbutyrate	V6	10.4	1067	*	ND b	25.3 a	ND b
	Butyl acetate	V7	11.1	1091	*	ND b	33.4 a	ND b
	Isoamyl acetate	V9	12.1	1126	*	10.4 c	32.4 b	46.9 a
	Ethyl hexanoate	V13	17.8	1223	NS	19.4	18.0	16.1
	Ethyl lactate	V15	21.4	1316	***	3942 b	2766 c	5463 a
	Ethyl 3-hydroxybutyrate	V24	28.8	1522	*	6.7 b	29.4 a	10.5 b
	Diethyl succinate	V30	35.5	1661	**	1521 b	747 c	1743 a
	Ethyl phenylacetate	V34	38.4	1763	*	ND b	ND b	12.1 a
	Diethyl glutarate	V35	39.4	1768	**	69.4 a	67.4 a	ND b
	Ethyl-4-hydroxybutanoate	V36	40.0	1790	*	91.1 b	108 b	138 a
	Phenylethyl acetate	V38	40.6	1825	NS	242	292	240
	Diethyl DL malate	V47	46.9	2039	***	320 a	86.5 c	230 b
	Diethyl-2-hydroxy-pentadioate	V49	49.7	2140	NS	125	120	124
	Monoethyl succinate	V52	54.2	2350	**	571 b	340 c	655 a
	Ethyl vanillate	V54	59.3	2676	*	5.9 b	ND c	12.0 a
Alcohols	1-Propanol	V5	9.9	1052	*	103 c	377 a	338 b
	Isobutyl alcohol	V8	11.4	1122	***	1019 c	1161 b	3195 a
	1-Butanol	V10	12.4	1148	**	33.9 b	71.9 a	69.2 a
	3-Penten-2-ol	V11	14.0	1163	*	ND b	21.6 a	ND b
	Isoamyl alcohol	V12	15.0	1197	***	13917 b	10492 c	17083 a
	1-Hexanol	V16	23.1	1339	*	287 b	278 b	320 a
	(E)-3-Hexen-1-ol	V17	23.3	1361	*	11.1 a	ND b	ND b
	3-Ethoxy-1-propanol	V18	23.7	1370	*	6.3 ab	13.0 a	ND b
	(Z)-3-Hexen-1-ol	V19	23.9	1382	*	4.9 a	ND b	ND b
	2,3-Butanediol	V26	29.5	1539	NS	286	289	324
	Furfuryl alcohol	V28	33.6	1614	*	6.9 c	30.8 b	54.0 a
	Methionol	V31	36.6	1715	**	75.5 b	100 a	ND c
	Benzyl alcohol	V41	42.0	1846	NS	159	141	146
	Phenylethyl alcohol	V42	43.1	1858	***	5131 b	4085 a	6902 b
Aldehydes	Furfural	V21	26.2	1425	*	373 b	349 c	468 a
	Benzaldehyde	V23	28.5	1501	*	84.1 a	68.9 b	94.4 a
	Vanillin	V53	57.8	2560	NS	89.4	94.8	91.5
Lactones	γ-Butyrolactone	V27	32.3	1595	*	861 c	1067 b	1356 a
	Whiskey lactone	V43	44.8	1898	*	68.4 b	74.8 a	76.3 a
	Pantolactone	V45	46.2	1998	NS	224	214	218
Acids	Acetic acid	V20	24.0	1403	**	359 c	459 b	658 a
	Propanoic acid	V25	28.8	1526	*	41.6 a	27.5 b	34.0 b
	Isovaleric acid	V29	34.2	1627	*	154 b	134 c	193 a
	(E)-2-Butenoic acid	V33	38.3	1740	NS	6.0	5.7	10.0
	Hexanoic acid	V39	41.2	1831	*	32.5 a	17.9 b	35.1 a
	Octanoic acid	V48	47.3	2050	*	32.8 a	9.0 c	20.4 b
	Decanoic acid	V51	52.1	2279	NS	5.3	8.6	4.7
Phenolic compounds	Guaiacol	V40	41.4	1841	**	ND b	ND b	37.2 a
	Phenol	V44	45.6	1992	*	17.3 b	24.6 a	16.2 b
	Ethyl guaiacol	V46	46.5	2011	NS	13.8	15.4	14.4
	4-Ethylphenol	V50	49.9	2164	NS	18.6	19.4	20.5
Ketones	Acetoin	V14	18.6	1255	**	509 a	338 c	441 b
	2(5H)-Furanone	V32	37.4	1732	NS	56.2	56.9	63.1
Others	2-Methyl-1,3-dioxane	V3	9.1	1044	**	28.1 b	ND c	37.8 a
	(Z)-2-Methyl-1,3-dioxane-5-ol	V22	27.7	1494	*	277 b	131 c	511 a
	(E)-2-Methyl-1,3-dioxane-5-ol	V37	40.4	1798	*	107 b	47.3 c	174 a
Total					*	31402 b	24957 c	41841 a

†*RT: Retention times on DB-Wax column.* ‡*LRI: linear retention index on DB-Wax column.* ¶*NS: not significant at $p > 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ($p > 0.05$), Tukey's least significant difference test.* ¥*ND: Not detected.*

Esters represented the largest number of aroma-active compounds in all *Fondillón* samples and will provide fruity odor and aroma notes to wines. Esters have a substantial contribution on the aroma of a broad array of wines and were generally considered as the potent aroma compound (Welke, Manfroi, Zanus, Lazarotto, & Alcaraz Zini, 2012; Zhao, Qian, He, Li, & Qian, 2017). The compounds giving out fruity notes were ethyl propanoate, diethyl succinate, ethyl-4-hydroxybutyrate, phenylethyl acetate and diethyl-2-hydroxy-pentanedioate. Most of the esters identified as potential odorants in *Fondillón* have been previously reported in other wine types (Kopka, Walther, William Allwood, & Goodacre, 2011; Reboredo-Rodríguez et al., 2015; Zhao et al., 2017). Within the ester family, ethyl lactate (described as popcorn and creamy) and diethyl succinate (fermented and fruity) were perceived as the most potent aroma-active compounds having FD values of 512, 256 and 512 for both compounds, from the oldest to the youngest *Fondillón* sample, respectively. Ethyl lactate was also reported to be one of the most important odorants of Riesling, Müller-Thurgau and sweet wines (Kopka et al., 2011; Reboredo-Rodríguez et al., 2015). Similarly, diethyl succinate was also found in Cabernet Sauvignon wines as aroma-active compounds using AEDA (Wang et al., 2016). These two compounds were derived from the chemical esterification of acids in the presence of alcohols (Bartowsky & Pretorius, 2009). Moreover, the previous reports stated that ethyl lactate and diethyl succinate can be also formed as a result of malolactic fermentation and they accumulate in stored wines (Celik, Cabaroglu, & Krieger-Weber, 2019; Izquierdo-Cañas, Mena-Morales, & García-Romero, 2016). Furthermore, Reboredo-Rodríguez et al. (2015) observed an increase of these two compounds during oxidative ageing.

Alcohols were the second important potential aroma compounds of aged *Fondillón* wines and included phenylethyl alcohol, isoamyl alcohol, 2,3-butanediol (being common in all samples), and methionol. Among alcohols, especially phenylethyl alcohol stood out with the highest FD values as 1024, 512 and 1024 in F-1944, F-1987 and F-1996, respectively; this compound is responsible for floral and rosy odor notes. Many studies reported phenylethyl alcohol as having rose odor and showing the highest FD factor in Pinot noir wine, Dornfelder red wine, Chardonnay-Semillon wines and aged red wine (Fan & Qian, 2005; Frank, Wollmann, Schieberle, & Hofmann, 2011; Mayr et al., 2014; Rutan, Herbst-Johnstone, Pineau, & Kilmartin, 2014).

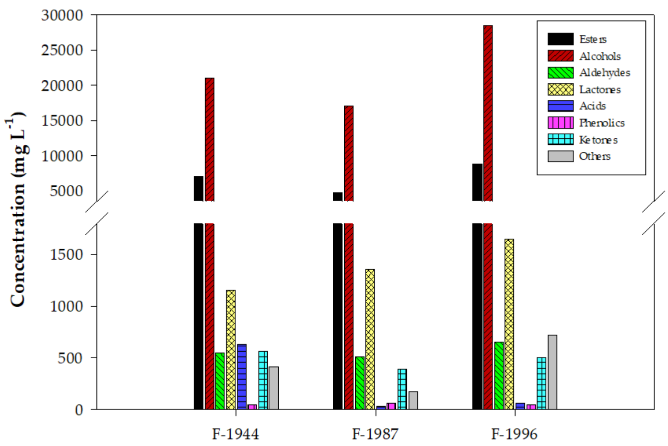

Fig. 1. Chemical families found in the *Fondillón* samples under evaluation.

In literature, this compound was reported as increasing during ageing (Tóth- Markus, Magyar, Kardos, Bánszky, & Maráz, 2002); thus, it was expected that the contribution of phenylethyl alcohol to whole *Fondillón* aroma was higher in aged samples; however, this was not fully demonstrated. Apart from this compound, isoamyl alcohol (fermented, alcohol), methionol (cooked, earthy) and 2,3-butanediol (fatty, sweet) were also perceived by the sniffing-port assessors but had minor effects.

After alcohols, 3 aldehydes and 3 lactones were identified as potent aroma compounds of *Fondillón*. Vanillin (vanilla) and furfural (burnt, bread) were notable among aldehydes with the same FD factor (16) in all three samples. Regarding lactones, γ-butyrolactone with a creamy note was found prominently in all samples, with its FD factor reaching its highest value (64) in the sample F-1996. However, pantolactone (floral) showed the opposite trend, and had its highest value (32) in the oldest sample, F-1944.

The remaining odorants included 2 acids, 1 ketone and 1 phenolic compound; they had FD values in the range 4 to 32. Among these compounds, acetic acid (which main aroma descriptor is vinegar) and acetoin (creamy) were perceived with the highest intensity in the samples F-1996 (FD 32) and F-1944 (FD 16), respectively.

With all the data obtained from this study, it can be stated that an initial characterization of the odor properties of aged *Fondillón* wines was done. The odor characteristics of these wines can be summarized as having floral, rosy, fruity and creamy notes preponderantly resulting from phenylethyl alcohol, ethyl lactate and diethyl succinate, in general. As the information about aged *Fondillón* wines was still very scarce, further researchers should be conducted in this special

Table 2. Aroma-active compounds of *Fondillón* wines identified by using the AEDA method (FD ≥ 4).

Chemical Family	Code	Compound	Aroma descriptor†	LRI‡	FD Factor¶		
					F-1944	F-1987	F-1996
Esters	V1	Ethyl propanoate	Sweet, tropical fruity	915	64	ND	64
	V15	Ethyl lactate	Popcorn, creamy	1316	512	256	512
	V36	Ethyl 4-hydroxybutyrate	Caramel, fruity	1790	16	16	32
	V30	Diethyl succinate	Fermented, fruity	1661	512	256	512
	V35	Diethyl glutarate	Caramel, burnt	1768	4	4	ND
	V38	Phenylethyl acetate	Fruity	1825	4	4	4
	V47	Diethyl DL malate	Caramel, burnt	2039	128	32	128
	V49	Diethyl-2-hydroxy-pentadioate	Dried fruit	2140	32	32	32
Alcohols	V12	Isoamyl alcohol	Fermented, alcohol	1197	16	4	32
	V26	2,3-Butanediol	Fatty, sweet	1539	8	8	16
	V31	Methionol	Cooked, earthy	1715	4	4	ND
	V42	Phenylethyl alcohol	Floral, rosy	1858	1024	512	1024
Aldehydes	V21	Furfural	Burnt, bread	1425	16	16	16
	V23	Benzaldehyde	Roasted nuts	1501	8	4	8
	V53	Vanillin	Vanilla	2560	16	16	16
Lactones	V27	γ-Butyrolactone	Creamy	1595	32	32	64
	V43	Whiskey lactone	Fruity, sweet	1898	16	16	16
	V45	Pantolactone	Floral	1998	32	16	16
Acids	V20	Acetic acid	Vinegary	1403	16	16	32
	V29	Isovaleric acid	Chemical, rotten	1627	4	4	8
Phenolic compounds	V40	Guaiacol	Smoke	1841	ND	ND	8
Ketone	V14	Acetoin	Creamy	1255	16	8	8
Unknown		Unknown	Cooked	1733	ND	16	8
		Unknown	Cooked vegetable, onion	1748	16	16	16

†Aroma descriptor: aroma perceived by panelists during olfactometry. ‡LRI: linear retention index calculated on DB-Wax capillary column. ¶FD factor is the highest dilution of the extract at which an odorant is determined by aroma extract dilution analysis; ND: Not detected.

Alicante wine.

3.3 Sensory analysis of Fondillón wines

Fondillón is legally defined as having mahogany, amber color with copper tones, in the nose it is aromatically intense, with ripe fruit nuts, well integrated wood and roasted notes, and regarding flavor, it is balanced, with good structure, big volume, persistent and slightly sweet (Valenciana, 2011). However, some of the attributes included in this definition are not easy to be scientifically quantified (e.g. volume) and a specific and certified lexicon and tasting protocol was recently developed (Issa-Issa et al., 2019) and have been used here to describe the *Fondillón* samples under analysis.

Only 10 of the 26 studied sensory attributes were affected by the ageing of the wine (**Table 3**). Results showed that, in general, samples from 1944 and 1996 had higher intensity than that of 1987; this was the case of fruity odor, spicy flavor, toasted flavor, sweetness, sourness, aftertaste, and color-hue (**Fig. 1S**, supplementary material). This trend agreed well with the data on the volatile compounds and the aroma- active compounds.

Pearson correlations were conducted to study the correlation between the aroma-active volatile compounds and the key sensory attributes (**Table 4**). In this way, esters (e.g. ethyl lactate, diethyl succinate and diethyl malate), alcohols (e.g. phenylethyl alcohol) and aldehydes (e.g. furfural and vanillin) affected positively fruity and vegetal flavor notes (but not the odor ones, which were less sensitive) as well as the bitterness, aftertaste, and even color of *Fondillón* samples. While, organic acids (acetic and isovaleric acids) affected more the floral and animal notes but at both odor and flavor levels. Finally, lactones (e.g. γ- butyrolactone and pantolactone) seemed not to have a significant influence on the sensory profiles of this wine; however, the only phenolic compound found, guaiacol, had a significant effect on floral and animal (e.g. butter) odor and flavor notes. These results again proved that esters, alcohols and aldehydes are the key chemical families controlling the sensory quality of *Fondillón*.

Table 3. Descriptive sensory analysis of *Fondillón* samples.

Attribute	ANOVA	SAMPLE		
		1944	1987	1996
Odor				
Alcohol	NS	5.9 a	4.8 a	5.3 a
Fruity	*	6.0 a	5.0 b	5.4 ab
Floral	NS	1.4 a	1.1 a	2.0 a
Vegetable	*	2.1 a	1.2 b	1.4 ab
Spicy	NS	3.8 a	2.9 a	3.4 a
Animal	NS	2.0 a	2.0 a	1.6 a
Toasted	***	5.6 a	4.0 b	5.5 a
Defects	NS	0.0 a	0.2 a	0.0 a
Flavor				
Alcohol	***	6.9 a	5.2 b	5.6 b
Fruity	NS	5.6 a	4.5 a	5.7 a
Floral	NS	1.6 a	1.5 a	1.9 a
Vegetable	NS	1.5 a	1.3 a	1.5 a
Spicy	***	4.2 a	2.9 b	4.3 a
Animal	NS	1.8 a	1.9 a	1.5 a
Toasted	*	5.4 a	3.9 a	5.5 a
Sweet	**	4.0 a	2.3 b	3.8 a
Sour	**	3.6 a	2.4 b	3.1 ab
Bitter	NS	1.4 a	1.3 a	1.2 a
Astringent	NS	1.4 a	1.1 a	1.2 a
Defects	NS	0.0 a	0.0 a	0.0 a
Global				
Imbalance	NS	0.0 a	0.0 a	0.0 a
Aftertaste	***	6.8 a	4.9 b	6.8 a
Appearance				
Limpidity	NS	9.3 a	8.7 a	9.3 a
Color (hue)	*	4.8 a	5.6 a	4.8 a
Color intensity	NS	2.2 a	2.0 a	2.2 a
Defects	NS	0.0 a	0.0 a	0.0 a

*†NS: not significant at p > 0.05; *, **, and ***, significant at p < 0.05, 0.01, and 0.001, respectively. Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different (p > 0.05), Tukey's least significant difference test.*

3.4 Phenolic compounds of Fondillón wines

The phenolic composition of the *Fondillón* wines are listed in Table 5 and model chromatograms are provided as supplementary material (**Fig. 2S**). A total of 25 phenolic compounds were characterized in wine samples, including phenolic acids (cis and trans isomers) and derivatives, flavanols, stilbenes, flavones and one unknown compound. Phenolic acids were the most abundant compounds in all three wine samples.

Sixteen phenolic acids were identified in the studied *Fondillón* samples and included gallic acid, protocatechuic acid, protocatechuic acid-*O*-hexoside, syringic acid, etc. Hydroxybenzoic acids (gallic, protocatechuic, syringic acids) were the most important group of phenols in all three *Fondillón* samples. In *oloroso-Sherries*, benzoic and cinnamic acids remain at constant values through oxidative ageing. However, other phenolic acids, such as gallic, syringic, and caffeic acids, experienced important changes during ageing. In addition, the content of esterified derivatives was lower in *oloroso-* than in *fino*-Sherries (Pozo-Bayón & Moreno-Arribas, 2011).

With respect to the phenolic acids, the protocatechuic acid was the predominant compound, with contents ranging from 39.15 to 52.47 mg L^{-1} in the samples F-1944 and F-1996, respectively. While, the most abundant phenolic acid was gallic acid in the F-1987 wine, with 48.58 mg L^{-1}. It has been mentioned in the literature that gallic acid content depends on the origin of the wine and on the enological techniques to which it has been subjected. According to the literature, the content in this phenolic compound increases with the ageing in wood, because of the hydrolysis of gallic tannins during ageing (Natera, Castro, De Valme García- Moreno, Hernández, & García-Barroso, 2003).

Table 4. Pearson correlation between the chemical families of the aroma-active compounds and the descriptive sensory attributes of Fondillón samples. The values shown between parenthesis are the *p*-values for each "significant" correlation.

	Esters	Alcohols	Organic acids	Aldehydes	Lactones	Phenols	Ketones	Unknown
Odor								
Alcohol	NS†	NS	NS	NS	NS	NS	NS	*(0.0334)
Fruity	NS	NS	NS	NS	NS	NS	NS	***(0.0001)
Floral	NS	NS	***(0.0001)	NS	NS	***(0.0001)	NS	NS
Vegetal	NS	NS	NS	NS	NS	NS	***(0.0001)	NS
Spicy	NS	NS	NS	NS	NS	NS	NS	NS
Animal	NS	NS	***(0.0001)	NS	NS	***(0.0001)	NS	NS
Toasted	NS	NS	NS	NS	NS	NS	NS	NS
Flavor								
Alcohol	NS	NS	NS	NS	NS	NS	NS	NS
Fruity	** (0.0098)	*(0.0206)	NS	***(0.0001)	NS	NS	NS	NS
Floral	NS	NS	***(0.0001)	NS	NS	***(0.0001)	NS	NS
Vegetal	**(0.0098)	*(0.0206)	NS	***(0.0001)	NS	NS	NS	NS
Spicy	NS	NS	NS	NS	NS	NS	NS	NS
Animal	NS	NS	***(0.0001)	NS	NS	***(0.0001)	NS	NS
Toasted	NS	NS	NS	NS	NS	NS	NS	NS
Sweet	NS	NS	NS	NS	NS	NS	NS	NS
Sour	NS	NS	NS	NS	NS	NS	NS	***(0.0001)
Bitter	**(0.0098)	*(0.0206)	NS	***(0.0001)	NS	NS	NS	NS
Astringent	NS	NS	NS	NS	NS	NS	***(0.0001)	NS
Aftertaste	**(0.0098)	*(0.0206)	NS	***(0.0001)	NS	NS	NS	NS
Color	**(0.0098	*(0.0206)	NS	***(0.0001)	NS	NS	NS	NS

*†NS: not significant at $p > 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ($p > 0.05$), Tukey's least significant difference test.*

Moreover, it was also mentioned that the ageing span of such wines is as effective as the prefermentative treatments, in particular maceration length, to increase the phenolic content of the wines (Bautista-Ortín, Fernández-Fernández, López-Roca, & Gómez-Plaza, 2007; Tetik, Sevindik, Kelebek, & Selli, 2018) examined *Monastrell* wines and re- ported that the hydroxybenzoic acids, particularly gallic acid, was the most abundant phenolic compound and its concentration increased with ageing from 21.6 to 27.9 mg L^{-1}. As reported in **Table 5**, F-1987 sample wine had twice the content of gallic acid as compared to the F-1944 sample, but its content decreased in the youngest sample, F-1996. Among hydroxybenzoic acids, although, protocatechuic, gallic, and syringic acids were found at higher concentrations, 2-*S*-glutathionyl-caffeoyltartaric acid, *trans*-caftaric acid, *cis*-coutaric acid, *trans*-coutaric acid, *trans*-caffeic acid, *cis*-fertaric acid, *trans*-fertaric acid, *p*-coumaric acid, ferulic acid caftaric acid *o*-quinones were only present in small contents.

In all three *Fondillón* samples, catechin ($[M-H]^-$, *m/z* 289) was the only flavan-3-ol detected and quantified. The highest catechin con- centration was detected in the sample F-1996 (4.96 mg L^{-1}) and the lowest in the F-1987 one (3.62 mg L^{-1}). The crucial role of catechin in red wines astringency and bitterness has been widely described in the literature (Kelebek, Canbas, Jourdes, & Teissedre, 2010). According to previous studies, the catechin content in red young and aged *Monastrell* wines is higher than in highly-aged *Fondillón* wines. This could be explained with polimerisation, precipitation and oxidation phenomena, as the *Fondillón* wines undergoes oxidative ageing (Cerezo et al., 2008). In an earlier study, (Gómez-Plaza, Gil-Muñoz, López-Roca, & Martínez, 2000) studied freshly bottled *Monastrell* wines and found *trans*-caftaric and *trans*-coutaric acid levels ranging between 171 and 187 and 117–124 mg L^{-1}, respectively. Besides, several researchers have pro- posed the ratio *trans*-coutaric acid to *trans*-caftaric acid as being a good indicator of the varietal origin of the wine (Gómez-Alonso, García- Romero, & Hermosín-Gutiérrez, 2007).

Quercetin, quercetin-3-*O*-glucuronide, dihydrokaempferol 3-*O*-*ß*-D-glucoside, and isorhamnetin-*O*-hexoside were the compounds identified and quantified within the flavonols group.

Table 5. Retention time, mass spectral characteristics, and concentration of phenolic compounds (mg L^{-1}) present in Fondillón wine samples.

Peak	Compounds	RT (min)	UV λ_{max} (nm)	$[M–H]^-$ (*m*/*z*)	MS/MS (*m*/*z*)	ANOVA	F-1944	F-1987	F-1996
Phenolic acids and derivatives									
2	Gallic acid	14.18	276	169	125	**	23.46 c	48.58 a	27.51 b
3	Protocatechuic acid-*O*-hexoside	17.45	296	315	153, 109	*	3.59 b	7.52 a	3.09 b
4	2-S-glutathionyl-caffeoyltartaric acid	18.53	330	616	484, 440, 272	*	0.33 a	0.19 b	0.17 b
5	Protocatechuic acid	21.04	294	153	109	**	39.15 b	42.30 b	52.47 a
6	Hydroxy-caffeic acid dimer isomer 1	22.61	315	373	305, 193	NS	0.08 b	0.22 a	0.23 a
7	(*E*)-Caftaric acid	25.24	328	311	179, 149	**	5.05 a	3.13 b	2.42 c
8	Hydroxy-caffeic acid dimer isomer 2	25.66	320	373	327, 305, 281	NS	0.04	0.09	0.06
9	Hydroxy-caffeic acid dimer isomer 3	27.66	320	373	355, 327, 305, 175	*	1.22 b	2.32 a	2.08 a
12	(*Z*)-Coutaric acid	32.46	310	295	163, 149	**	1.06 a	0.42 c	0.60 b
13	(*E*)-Coutaric acid	33.72	314	295	163	NS	5.16	5.05	4.92
14	(*Z*)-Fertaric acid	36.06	322	325	193, 149	NS	0.09	0.08	0.05
15	(*E*)-Caffeic acid	36.75	323	179	135	*	1.09 a	1.13 a	0.35 b
16	(*E*)-Fertaric acid	37.09	328	325	193, 149	*	1.89 a	1.85 a	1.14 b
17	Ferulic acid	37.09	323	193	178, 149, 134	NS	1.66	1.59	1.41
20	Syringic acid	45.41	272	197	182, 167, 153	**	15.11 b	22.79 a	14.78 b
21	*p*-Coumaric acid	46.93	310	163	119	*	3.22 a	2.76 b	2.86 b
22	Ellagic acid	48.43	275	301	284, 257, 229, 185	*	0.27 b	0.47 a	0.42 a
Flavan-ols									
11	Catechin	31.01	280	289	245, 175	*	4.56 b	3.62 c	4.96 a
Stilbenes									
1	(*Z*)-Piceid	13.45	282	389	227	***	0.17 b	1.33 a	0.07 b
10	Tyrosol	28.39	275	137	93	*	0.54 b	0.50 b	0.95 a
Flavones									
18	Dihydrokaempferol 3-*O*-*ß*-d-glucoside	39.93	290	449	287	**	1.97 b	1.88 b	2.89 a
19	Quercetin-3-*O*-glucuronide	45.37	355	477	301	*	0.32 a	0.41 a	0.10 b
23	Isorhamnetin-*O*-hexoside	49.26	356	477	315, 301, 300, 299	*	0.34 a	0.45 a	0.10 b
25	Quercetin	64.33	355	301	151	***	0.20 b	1.13 a	0.01 c
Others									
24	Unkown	54.32	280	713	600, 389, 335, 133	***	0.01 b	134 a	0.01 b
	TOTAL					***	**111 c**	**284 a**	**124 b**

†NS: not significant at $p > 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively. ‡Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different ($p > 0.05$), Tukey's least significant difference test.

Two peaks were identified as quercetin derivatives according to their UV spectra and MS fragmentation leading to the quercetin aglycone at *m*/*z* 301 in negative mode. The flavonols identified by this method were quercetin-3-*O*-glucuronide (peak 19, $[M-H]^-$ detected at *m*/*z* 477), and quercetin (peak 25, $[M-H]^-$ detected at *m*/*z* 301). One peak was identified as isorhamnetin derivative according to their UV spectra and MS fragmentation leading to the isorhamnetin aglycone at *m*/*z* 315 in negative mode. The flavonol identified by this method was isorhamnetin-3-*O*-hexoside (peak 23, $[M-H]^-$ detected at *m*/*z* 477).

Quercetin is one of an important flavonol in several wines produced with *Cabernet Sauvignon*, *Syrah*, *Merlot*, and *Tempranillo* grapes (Makris, Kallithraka, & Kefalas, 2006). Among these flavonols, dihydrokaempferol 3-*O*-*ß*-*D*- glucoside was the most abundant one and its concentration ranged between 1.88 and 2.89 mg L^{-1}.

In addition to these phenolic compounds, *cis*-piceid was also found in all 3 samples. The spectra of *cis*-piceid (*m*/*z* 389) gave a fragment ion at *m*/*z* 227 due to the loss of a glucose moiety (**Table 5**).

4. Conclusions

Fifty-four volatile compounds were identified and quantified in Fondillón wines. However only 22 of those were identified as aroma- active compounds, with phenylethyl alcohol (floral), diethyl succinate (fruity), and ethyl lactate (popcorn) having a predominant role. Ageing slightly affected the sensory profile of Fondillón, mainly by affecting alcohol, fruity, vegetable, and toasted notes, together with sweetness, sourness and aftertaste. Finally, 25 phenolic compounds were identified and quantified, with phenolic acids predominating, especially protocatechuic and gallic acids. The generated information is essential to start new research projects checking the effects of viticulture practices and winemaking unit operations on the volatile composition, sensory profile and phenolic composition of Fondillón wines

CRediT authorship contribution statement

Hanán Issa-Issa: Formal analysis, Methodology, Writing - original draft. **Gamze Guclu:** Investigation, Methodology, Formal analysis. **Luis Noguera-Artiaga:** Formal analysis, Methodology, Writing - original draft. **David López-Lluch:** Conceptualization, Supervision, Writing - review & editing. **Rafael Poveda:** Funding acquisition, Investigation. Hasim Kelebek: Investigation, Methodology, Formal analysis. Serkan Selli: Investigation, Methodology, Formal analysis. **Ángel A. Carbonell-Barrachina:** Conceptualization, Supervision, Writing - review & editing. All authors contributed to the writing of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

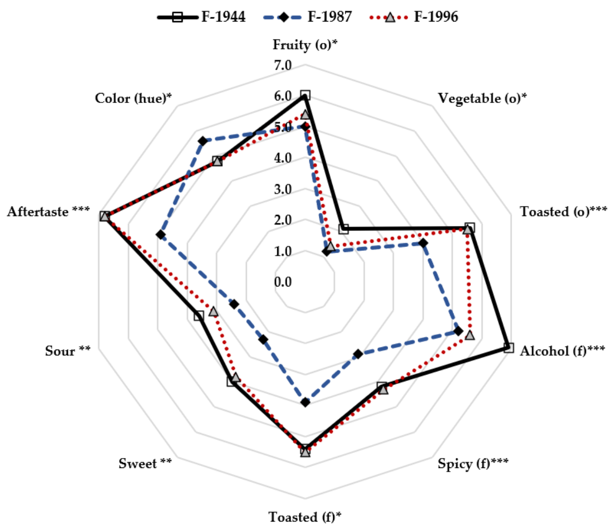

Fig. 1S. Descriptive sensory profile of the Fondillón samples under evaluation (statistics: *, **, and ***, significant at $p < 0.05$, 0.01, and 0.001, respectively).

References

Alicante, DOP. (2016). Fondillón de Alicante, time is a luxury. http://vinosalicantedop.org/fondillón/en/.

Bartowsky, E. J., & Pretorius, I. S. (2009). Microbial formation and modification of flavor and off-flavor compounds in wine. In H. König, G. Unden, & J. Fröhlich (Eds.). *Biology of microorganisms on grapes, in must and in wine* (pp. 209–231). Berling (Germany): Springer-Verlag.

Bautista-Ortín, A. B., Fernández-Fernández, J. I., López-Roca, J. M., & Gómez-Plaza, E. (2007). The effects of enological practices in anthocyanins, phenolic compounds and wine colour and their dependence on grape characteristics. *Journal of Food Composition and Analysis, 20*(7), 546–552.

Bellincontro, A., De Santis, D., Botondi, R., Villa, I., & Mencarelli, F. (2004). Different postharvest dehydration rates affect quality characteristics and volatile compounds of Malvasia, Trebbiano and Sangiovese grapes for wine production. *Journal of the Science of Food and Agriculture, 84*(13), 1791–1800.

Celik, Z. D., Cabaroglu, T., & Krieger-Weber, S. (2019). Impact of malolactic fermentation on the volatile composition of Turkish Kalecik karası red wines. *Journal of the Institute of Brewing, 125*(1), 92–99.

Cerezo, A. B., Tesfaye, W., Torija, M. J., Mateo, E., García-Parrilla, M. C., & Troncoso, A. M. (2008). The phenolic composition of red wine vinegar produced in barrels made from different woods. *Food Chemistry, 109*(3), 606–615.

Costantini, V., Bellincontro, A., De Santis, D., Botondi, R., & Mencarelli, F. (2006). Metabolic changes of Malvasia grapes for wine production during postharvest drying. *Journal of Agricultural and Food Chemistry, 54*(9), 3334–3340.

de Saint Laumer, J.-Y., Cicchetti, E., Merle, P., Egger, J., & Chaintreau, A. (2010). Quantification in gas chromatography: Prediction of flame ionization detector response factors from combustion enthalpies and molecular structures. *Analytical Chemistry, 82*(15), 6457–6462.

Ebeler, S. E. (2001). Analytical chemistry: Unlocking the secrets of wine flavor. *Food Reviews International, 17*(1), 45–64.

EC, C. R. (2008). (EC) No 479/2008 of April 2008 On the Common Organisation of the Market in Wine, in 479/2008. Brussels, Belgium: European Union2008.

Engel, W., Bahr, W., & Schieberle, P. (1999). Solvent assisted flavour evaporation - A new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. European Food Research and Technology, 209(3–4), 237–241.

Fan, W., & Qian, M. (2005). Headspace solid phase microextraction and gas chromato-graphy−olfactometry dilution analysis of young and aged Chinese "Yanghe Daqu" liquors. Journal of Agricultural and Food Chemistry, 53, 7931–7938.

Flamini, R., & Traldi, P. (2010). Mass spectrometry in grape and wine chemistry. Chichester (UK): John Wiley & Sons Ltd.

Frank, S., Wollmann, N., Schieberle, P., & Hofmann, T. (2011). Reconstitution of the flavor signature of Dornfelder red wine on the basis of the natural concentrations of its key aroma and taste compounds. Journal of Agricultural and Food Chemistry, 59(16), 8866–8874.

Generalitat Valenciana. (2011). Orden 5/2011, de 16 de noviembre, de la Conselleria de Agricultura, Pesca, Alimentación y Agua, por la que se aprueba el texto del reglamento y pliego de condiciones de la Denominación de Origen Protegida Alicante y su consejo regulador. 2011, Diario Oficial de la Comunitat Valenciana: Valencia, Spain.

Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. Journal of Food Composition and Analysis, 20(7), 618–626.

Gómez-Plaza, E., Gil-Muñoz, R., López-Roca, J. M., & Martínez, A. (2000). Color and phenolic compounds of a young red wine. Influence of wine-making techniques, storage temperature, and length of storage time. Journal of Agricultural and Food Chemistry, 48(3), 736–741.

Issa-Issa, H., Noguera-Artiaga, L., Sendra, E., Pérez-López, A. J., Burló, F., Carbonell- Barrachina, Á. A., & López-Lluch, D. (2019). Volatile composition, sensory profile, and consumers' acceptance of Fondillón. Journal of Food Quality, 2019, 1–10. https://doi.org/10.1155/2019/5981762.

Izquierdo-Cañas, P. M., Mena-Morales, A., & García-Romero, E. (2016). Malolactic fer-mentation before or during wine aging in barrels. LWT - Food Science and Technology, 66, 468–474.

Kelebek, H. (2016). LC-DAD–ESI-MS/MS characterization of phenolic constituents in Turkish black tea: Effect of infusion time and temperature. Food Chemistry, 204, 227–238.

Kelebek, H., Canbas, A., Jourdes, M., & Teissedre, P. L. (2010). Characterization of colored and colorless phenolic compounds in Öküzgözü wines from Denizli and Elazig regions using HPLC-DAD-MS. Industrial Crops and Products, 31(3), 499–508.

Kopka, J., Walther, D., William Allwood, J., & Goodacre, R. (2011). Progress in chemo- metrics and biostatistics for plant applications, or: A good red wine is a bad white wine. In R. D. Hall (Ed.). Annual Plant Reviews Volume 43: Biology of Plant Metabolomics (pp. 317–342). Chichester (UK): Blackwell Publishing Ltd.

Makris, D. P., Kallithraka, S., & Kefalas, P. (2006). Flavonols in grapes, grape products and wines: Burden, profile and influential parameters. Journal of Food Composition and Analysis, 19(5), 396–404.

Mayr, C. M., Geue, J. P., Holt, H. E., Pearson, W. P., Jeffery, D. W., & Francis, I. L. (2014). Characterization of the key aroma compounds in shiraz wine by quantitation, aroma reconstitution, and omission studies. Journal of

Agricultural and Food Chemistry, 62(20), 4528–4536.

Natera, R., Castro, R., De Valme García-Moreno, M., Hernández, M. J., & García-Barroso, C. (2003). Chemometric studies of vinegars from different raw materials and processes of production. Journal of Agricultural and Food Chemistry, 51(11), 3345–3351.

Perestrelo, R., Silva, C., Pereira, J., & Câmara, J. (2016). Wines: Madeira, Port and Sherry fortified wines – The sui generis and notable peculiarities. Major differences and chemical patterns. Encyclopedia of Food and Health, 5, 534–555.

Pozo-Bayón, M. A., & Moreno-Arribas, M. A. (2011). Sherry wines. Advances in Food and Nutrition Research, 63, 17–40.

Reboredo-Rodríguez, P., González-Barreiro, C., Rial-Otero, R., Cancho-Grande, B., & Simal-Gándara, J. (2015). Effects of sugar concentration processes in grapes and wine aging on aroma compounds of sweet wines—A review. Critical Reviews in Food Science and Nutrition, 55(8), 1051–1071.

Rutan, T., Herbst-Johnstone, M., Pineau, B., & Kilmartin, P. A. (2014). Characterization of the aroma of central otago pinot noir wines using sensory reconstitution studies. American Journal of Enology and Viticulture, 65(4), 424–434.

Selli, S., Cabaroglu, T., Canbas, A., Erten, H., Nurgel, C., Lepoutre, J. P., & Gunata, Z. (2004). Volatile composition of red wine from cv. Kalecik Karası grown in central Anatolia. Food Chemistry, 85(2), 207–213.

Selli, S., Kelebek, H., Turan Ayseli, M., & Tokbas, H. (2014). Characterization of the most aroma-active compounds in cherry tomato by application of the aroma extract dilution analysis. Food Chemistry, 165, 540–546.

Sonmezdag, A. S. (2018). Characterization of aroma and aroma-active composition of Gaziantep cheese by solvent-assisted flavor evaporation (SAFE) and aroma extract dilution analysis (AEDA). Journal of Food Processing and Preservation, 43(6), e13840.

Tetik, M. A., Sevindik, O., Kelebek, H., & Selli, S. (2018). Screening of key odorants and anthocyanin compounds of cv. Okuzgozu (Vitis vinifera L.) red wines with a free run and pressed pomace using GC-MS-Olfactometry and LC-MS-MS. Journal of Mass Spectrometry, 53(5), 444–454.

Tóth-Markus, M., Magyar, I., Kardos, K., Bánszky, L., & Maráz, A. (2002). Study of Tokaji Aszú wine flavour by solid phase microextraction method. Acta Alimentaria, 31(4), 343–354.

Valero, A., Marín, S., Ramos, A. J., & Sanchis, V. (2008). Survey: Ochratoxin A in European special wines. Food Chemistry, 108(2), 593–599.

Wang, J., Gambetta, J. M., & Jeffery, D. W. (2016). Comprehensive study of volatile compounds in two Australian rose wines: Aroma extract dilution analysis (AEDA) of extracts prepared using solvent-assisted flavor evaporation (SAFE) or headspace solid-phase extraction (HS-SPE). Journal of Agricultural and Food Chemistry, 64(19), 3838–3848.

Welke, J. E., Manfroi, V., Zanus, M., Lazarotto, M., & Alcaraz Zini, C. (2012). Characterization of the volatile profile of Brazilian Merlot wines through comprehensive two dimensional gas chromatography time-of-flight mass spectrometric detection. Journal of Chromatography A, 1226, 124–139.

Zhao, P., Qian, Y., He, F., Li, H., & Qian, M. (2017). Comparative characterization of aroma compounds in Merlot wine by LiChrolut-EN-based aroma extract dilution analysis and odor activity value. Chemosensory Perception, 10(4), 149–160.

PUBLICACIÓN 3

Quality, Nutritional, Volatile and Sensory Profiles and Consumer Acceptance of *Fondillón*, a Sustainable European Protected Wine

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Article

Quality, Nutritional, Volatile and Sensory Profiles and Consumer Acceptance of *Fondillón*, a Sustainable European Protected Wine

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Abstract: Sustainable irrigation strategies in Southeast Spain (one of the most arid regions in Europe) are essential to fight against desertification and climate change mitigation. In this way, *Fondillón* production is based on rain-based vineyards, over-ripe *Monastrell* grapes, and non-alcohol fortification. Thus, *Fondillón* is a naturally sweet red wine, protected within the Alicante Denomination of Origin, recognized by the European Union in its E-bachus database. The study aim was to evaluate the effect of the aging (*solera* factor) on *Fondillón:* (*i*) basic enological parameters (e.g., total, and volatile acidity), (*ii*) chromatic characteristics, (*iii*) antioxidant activity ($ABTS^{\bullet+}$, FRAP and $DPPH^{\bullet}$), (*iv*) total contents of condensed tannins and anthocyanins, (*v*) volatile composition, (*vi*) sensory profile, and (*vii*) overall liking. Experimental data proved that the wine (1960 *solera*) with the highest total contents of condensed tannins and anthocyanins and total antioxidant activity was the most liked by Spanish consumers. Experimental results clearly established a positive relationship among *Fondillón* chemical composition, its antioxidant activity, and overall consumer liking. Exceptional harvest with grapes having extremely high antioxidant power (e.g., 1960 *solera*) will result, even more than 50 years later, in high quality wines with high consumer acceptance and a high monetary worth.

Keywords: affective sensory analysis; antioxidant activity; descriptive sensory analysis; $DPPH^{\bullet}$; phenolic content; rain-based farming; wine color.

1. Introduction

Sustainability regarding environmental issues related to agriculture must be considered [1]. The coastal strip of the Southeast of Spain is one of the most arid regions of Europe, with an average rainfall of less than 400 mm [2]. There is still a need to promote public awareness, to truly reduce water consumption in the most demanding sectors, re- duce uptakes form water bodies and, thus, the pressure made over them [3]. Thus, agriculture with low water requirement should be encouraged in arid and semi-arid regions rather than irrigation agriculture. In this sense, *Fondillón* vineyards located in Alicante (Valencian Community) are rain-fed and are fully hydroSOStainable, as defined by Sánchez-Bravo et al. [4]. The effect on preventing desertification through this crop is clear because vines are one of the few agricultural options due to the edaphic and climatic conditions in many areas. Moreover, encouraging the cultivation and production of this grape cultivar in the mentioned conditions, could be a way of increasing *Fondillón* production and profitability as well as creating jobs and value in rural areas. As explained by other authors, this can be a successful tool to mitigate climate change in arid and semiarid regions considering adequate farm and cultivation practices and systems [5].

Fondillón is a strong *rancio* wine from Alicante made from *Monastrell* grape and matured like an Oloroso sherry (oxidized for a long aging period in oak barrels; minimum of 10 years). The main difference with Oloroso sherry is that *Fondillón* is not fortified. Thus, *Fondillón* is a naturally sweet wine (included by the European Union in its E-Bacchus database) produced in the Alicante Protected Designation of Origin, Alicante PDO [6].

Naturally sweet wines come from overripe and or dehydrated grapes, are non-fortified wines, and have a total alcoholic strength of not less than 15% by volume (abv) [7].

Rancio is an imprecise tasting term used in many languages for a distinctive style of wine achieved by deliberately maderizing the wine by exposing it (*i*) to oxygen and/or (*ii*) heat. Key flavor compounds identified in aged *vin doux naturels* arise by Maillard reaction of sugars with amino acids and by oxidation [8]. Maderization implies mild oxidation over a long period of time and sometimes heat. It should be properly applied only to wines with (*i*) a high enough alcoholic strength to inhibit the action of *Agrobacter*, which would otherwise transform the wine into vinegar, and (*ii*) a high antioxidant capacity, which is needed to preserve the maximum and guarantee that the transformation of aroma precursors into the proper volatile compounds is achieved without generation of any type of off- flavors.

Very few maderized wines are made today by simply ageing the wine at cellar temperature; the oxidation process is instead hastened by heating or "baking" the wine as on the Madeira Island. Oxidation reactions, like most organic chemical reactions, can be roughly doubled in speed by a temperature rise of 10 °C [9,10]. A high level of oxidation is a key part of the style of fortified wines (such as Oloroso Sherry or Madeira); the oxidation is evident from the brown color of the wine and a lack of fresh fruit on the palate, which is instead replaced by nuts and dried fruits. In this case, the wine is stored for years in barrels that are not filled to the top, allowing oxygen within the headspace of the barrel (ullage). More commonly, for un-fortified red wines, the exposure to oxygen is limited and therefore has a much slower, subtler effect.

Aging and oxidation will affect wine composition [11]. However, changes with time of chromatic characteristics, antioxidant activity, and total polyphenolic composition un- der oxidation conditions in oak barrels for a long period of time without fortifying and/or heating the wine are not well known. Consequently, the aim of this study was to evaluate in *Fondillón* wine the effect of the aging (*solera* factor) on (*i*) basic enological parameters (e.g., total and volatile acidity), (*ii*) chromatic characteristics, (*iii*) antioxidant activity ($ABTS^{\bullet+}$, FRAP, and $DPPH^{\bullet}$), (*iv*) total contents of condensed tannins and anthocyanins, (*v*) volatile composition, (*vi*) sensory profile, and (*vii*) overall liking of *Fondillón* consumers.

2. Materials and Methods

2.1. *Wine Samples and Experimental Design*

The factor under study was "*solera*" (aging time) and included nine levels: 1930, 1944, 1950, 1960, 1969, 1975, 1980, 1987, and 1996. The samples used (nine bottles of 500 mL per *solera*) were kindly supplied by Bodegas Monóvar-MGWines (Monóvar, Alicante, Spain). The samples were transported and stored under optimal conditions at the facilities of the Miguel Hernández University (Orihuela, Alicante, Spain) to later carry out the corresponding analyses. All analyses were run, at least, in triplicate, using different bottles for each replication; this is, samples from three bottles were taken and analyzed.

2.2. *Enological Parameters*

The basic enological parameters [pH, relative density, total alcohol content (% vol.), total acidity (g tartaric acid L^{-1}), and volatile acidity (g acetic acid L^{-1})] were determined, in triplicate, according to the International Organization of Vine and Wine Compendium methods of analysis [12].

2.3. *Chromatic Characteristics, Total Polyphenol Index (TPI), Total Anthocyanin Content (TA), Antioxidant Activity (AA) and Total Condensed Tannins (TCT)*

The chromatic characteristics: (i) color intensity, (ii) tonality, and (iii) color density in the wine samples were determined according to methods OIV-MA-AS2-07B [13] and Glories [13] using a spectrophotometer (ThermoSpectronic Heγios γ, Cambridge, England). All analyses were carried out in triplicate.

The color intensity (CI) was calculated as the sum of the absorbance at 420, 520, and 620 nm ($CI = A_{420} + A_{520} + A_{620}$). The tonality (T) was calculated as the ratio of absorbance at 420 nm to absorbance at 520 nm ($T = A_{420}/A_{520}$).

The color density (CD) was calculated as the sum of the absorbance at 420 and 520 nm ($CD = A_{420} + A_{520}$). Also, Glories colorimetric index [14] percentages of the colors yellow, red, and blue were obtained using the absorbance values at 420, 520, and 620 nm as follows:

- Yellow, $Y (\%) = (A_{420}/CI) \times 100$
- Red, $R (\%) = (A_{520}/CI) \times 100$
- Blue, $B (\%) = (A_{620}/CI) \times 100$

Total polyphenol index (TPI) was evaluated by measuring the absorbance at 280 nm on a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E) [15]. The TPI was determined after diluting the wine sample 100 times. Analyses were run in triplicate.

Total anthocyanin content (TA) was determined according to Di Stefano et al. [16]. Briefly, the absorbance of the samples was measured at 540 nm using a UV–visible spectrophotometer (Helios Gamma model, UVG 1002E) and the total anthocyanin content was calculated using the formula by Di Stefano et al. [16]. Analysis was run in triplicate, and results were expressed as equivalents of malvidin 3-*O*-glucoside (mg L^{-1}).

Antioxidant activity (AA) was evaluated using three methods:

(i) The free radical scavenging capacity using the $ABTS^{\bullet+}$ [2,2′-azino-bis (3-ethylbenzo- thiazoline-6-sulphonic acid)] method described by Re et al. [17], with absorbance being measured at 734 nm (UV-visible spectrophotometer, Helios Gamma model, UVG 1002E). Calibration curve (3.5–5.0 mmol Trolox L^{-1}) with good linearity ($R^2 \geq 0.999$) was used for the quantification. Analyses were run in triplicate and results were ex- pressed as mmol Trolox L^{-1}.

(ii) The free radical scavenging capacity was also measured using the $DPPH^{\bullet}$ method as proposed by Katalinic et al. [18], with absorbance being measured at 517 nm. The inhibition percentage of the $DPPH^{\bullet}$ radical was determined according to the following Equation (1):

$$\left[\frac{AC\ (0) - AA\ (t)}{AC\ (0)}\right] \times 100 \tag{1}$$

where *AC* (0) is the absorbance of the sample at $t = 0$ min, and *AA* (*t*) is the absorbance of sample at $t = 16$ min. Analyses were carried out in triplicate.

(iii) The ferric reducing/antioxidant power (FRAP) was performed on a modified version of the method by Benzie et al. [19]. It is based on the reducing power of antioxidants, which will reduce the Fe^{3+} to Fe^{2+} in the form of a blue complex (Fe^{2+}/TPTZ). Absorbance was measured at 593 m. Analyses were run in triplicate and results were ex- pressed as mmol Trolox L^{-1}.

Total condensed tannins (TCT) were determined according to Ribéreau-Gayon and Stonestreet [20]. Briefly, the samples were prepared in two test tubes, one for the control (tube 2) and the other for the hydrolysis (tube 1); in this tube, 4 mL of wine diluted in 1:50 distilled water, 2 mL of distilled water, and 6 mL of HCl 12 N were added successively. Subsequently, the closed hydrolysis tube was placed in a water bath at 100 °C for 30 min, and then cooled in an ice water bath. Then, 1 mL of 95 % ethanol was added to the two tubes to solubilize the red color that appeared and finally the absorbance (Abs) of the two tubes was measured at 550 nm under an optical path of 1 cm, using water as a reference. Tannins were determined by the following equation: Tannins (g L^{-1}) = 19.33 × (Abs tube 1–Abs tube 2). The coefficient of 19.33 corresponds to the molar extinction coefficient of the cyanidin obtained by the acid hydrolysis of the condensed tannins, corrected to directly give the result in g L^{-1}.

2.4 Volatile Compounds

The extraction of volatile compounds was done by HS-SPME technique with 1 cm DVB/CAR/PDMS fiber (Supelco, Bellegonte, PA, USA). Briefly, 10 mL of sample were placed into 20 mL headspace vial together with 5 µL of benzyl acetate (1000 mg L^{-1}) as internal standard and 1 g of NaCl. Thereafter, the sample was placed on autosampler (AOC-6000 Plus, Shimadzu) at 250 rpm and 40 °C, for 40 min. The identification and quantification of the volatile compounds was carried out using a Shimadzu GC2030 gas chromatograph and a TQ8040 NX triple quadrupole mass spectrometer as detector (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) equipped with an AOC-6000 Plus autosampler. Only the single quadrupole acquisition mode was exploited on the TQ8040 NX (Q3 Scan). Data were acquired by using the GCMS solution software ver. 4.4 (Shimadzu). The column used was an X5MS (silphenylene polymer; Teknokroma, Barcelona, Spain) with dimensions of 30 m (length), 0.25 mm (internal diameter), and 0.25 µm (film thickness). The GC temperature program was, as follows: 50 °C hold for 2 min, then +3 °C min^{-1} ramp up to 170 °C, and an increment of 20 °C min^{-1} up to 230°C. Helium head pressure was 18 kPa (constant linear velocity mode 30 cm s^{-1}). Injector, ion source, and interface were at 230, 230, and 280 °C, respectively. Helium with flow 0.6 mL min^{-1} was used as carrier gas with a split ratio of 1:10. Volatile compounds were identified by comparison of: (i) experimentally obtained mass spectra with those available in NIST 17 Mass Spectral, and (ii) linear retention indices calculated using the C6-C20 n-alkane mix (sigma-Aldrich, Steinheim, Germany). Only compounds with spectra similarity > 90 % were considered as correct hits; besides, the linear retention similarity threshold was set up at ±10 units. Analyses were conducted in triplicate and results were expressed as mg L^{-1}.

2.5 Descriptive Sensory Analysis with Trained Panel

A trained panel, consisting of eight panelists (four women and four men), aged between 35 and 60 years, was used to conduct the descriptive sensory analysis of the *Fondillón* samples under study. This panel had more than 300 h of experience, it belongs to the Regulatory Council of the Wines of Alicante Protected Designation of Origin (DOP Alicante), was trained according to ISO 8586:2012 [21] and is certified by the National Accreditation Agency (ENAC) under the ISO 17065 [22].

The attributes and vocabulary used by the panelists to evaluate the *Fondillón* samples were those found in the official lexicon of the DOP Alicante [6]. The attributes under evaluation were: (*i*) appearance: color; (*ii*) odor (alcohol, fruity, floral, Mediterranean forest, spicy, animal, toasted, and chemical); (*iii*) basic tastes: sweetness, sourness, and bitterness; (*iv*) flavor: alcohol, fruity, floral, Mediterranean forest, spicy, animal, toasted, chemical, and aftertaste); and (*v*) chemical sensations (astringent). The panelists used a structure 10- point scale (0 was extremely low or no intensity, and 10 was extremely high intensity), with increments of 0.5 units, to evaluate the intensity of each of the attributes.

The nine samples under analysis were run in triplicate in four sessions of ~2.0 h. In each of the sessions, two quality control parameters were included to evaluate the panel performance: (*i*) reproducibility (same sample evaluated in two different sessions) and (*ii*) repeatability (same sample evaluated twice in the same session); to validate the panel performance, the deviation of these two parameters must be below 20 % for all sensory descriptors. A maximum of 10 samples can be evaluated in a single session of 2.0 h, including three quality control samples (reproducibility, repeatability, and sample with a defect). Samples were randomly served coded with a three-digit numbers, together with the ap- propriate questionnaire, one at a time, and waiting 10 min between samples. Between samples and for palate cleansing, water and unsalted crackers were provided to panelists. For the descriptive analysis, each panelist was initially provided with a black cup with 35 mL of the sample for the analysis of the odor, basis tastes, flavor and chemical sensations, and later to a transparent cup with 20 mL for the color analysis, at a temperature of 16–18 °C, breadsticks and water were also provided to clean the palate between samples. This evaluation was carried out in normalized sensory booths with white light at a temperature of 22 ± 2 °C.

2.6 *Affective Sensory Analysis*

The affective sensory analysis was carried out with 123 Spanish consumers, which were recruited from the Province of Alicante. The key recruitment requirement was that consumers should be regular *Fondillón* or oxidized wines consumers. The study was developed according to a balanced incomplete block design (split-plot), with participants testing five of the nine *Fondillón* samples. The samples were served to each consumer un- der the same tasting conditions (wine temperature, wine volume, and palate cleansers) previously mentioned for the descriptive study, in a random order, and coded with three- digit codes. Overall liking together with the satisfaction degree of the sample sweetness and aftertaste were evaluated, using a 9-point hedonic scale: 1 = dislike extremely; 5 = neither like or dislike; and 9 = like extremely. Finally, questions were asked about wine preference and purchase intention for the samples under study (**Table S1**).

2.7 *Statistical Analysis*

The results were first subjected to a one-way analysis of variance (ANOVA) using the *solera* age as the factor, and later to the Tukey or LSD (descriptive and affective sensory data) multiple range test. For the sensory data, a requirement of the ENAC-certified sensory panel is that the factor "judge" must be preliminary analyzed to ensure that it has no significant effect on the results of the panel. If any of the panelists produce significantly different results, its data must be removed, and the statistical analysis must be redone. Then, the factor or factors under study (e.g., *solera*) is considered. Differences were considered statistically significant at $p < 0.05$. To perform these statistical analyses, the soft- ware XLSTAT Premium 2016 (Addinsoft, New York, NY, USA) and Statgraphics Plus (version 3.1, Statistical Graphics Corp., Rockville, MA, USA) were used.

3. Results and Discussion

3.1 *Enological Parameters*

The legislation of the DOP Alicante establishes several thresholds for the physicochemical parameters in *Fondillón* samples. In this way, the total acidity must be above 3.50 g of tartaric acid L^{-1}, the volatile acidity must be below 1.50 g of acetic acid L^{-1}, and the total alcohol content must be above 16 % *v*/*v* [23]. Regarding the present results, the factor "*solera*" did not significantly affect either the pH or the relative density; however, there were significant effects on the rest of parameters, although without a clear trend with the "*solera*" age (**Table 1**).

Table 1. Basic enological parameters of *Fondillón* wines as affected by the "solera" factor.

Solera	pH	Relative density (20 °C) (g mL^{-1})	Total alcohol content (% v/v)	Total acidity (g tartaric acid L^{-1})	Volatile acidity (g acetic acid L^{-1})
	ANOVA[†]				
	NS	NS	**	**	***
	Tukey Multiple Range Test[‡]				
1930	3.55	0.9967	21.17 a	8.63 ab	1.16 cd
1944	3.67	1.0098	20.46 ab	8.40 ab	1.46 ab
1950	3.59	0.9972	19.30 ab	8.63 ab	1.13 d
1960	3.44	0.9985	19.60 ab	10.01 a	1.31 bc
1969	3.68	0.9978	18.53 ab	9.00 ab	1.37 ab
1975	3.82	1.0000	18.93 ab	7.80 b	1.01 d
1980	3.35	0.9959	18.90 ab	8.33 ab	1.50 a
1987	3.51	0.9984	18.44 ab	7.65 b	1.13 cd
1996	3.43	0.9970	18.30 b	7.28 b	1.16 cd
Minimum	3.35	0.9959	18.30	7.28	1.01
Maximum	3.82	1.0098	21.17	10.01	1.50
Legal threshold[¥]			> 16	> 3.5	< 1.5

[†] *NS = not significant at $p > 0.05$; ** and *** significant at $p < 0.01$, and 0.001, respectively.* [‡] *Values (mean of three replications) followed by the same letter, within the same column, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test* [¥] *[20].*

The pH plays an important role in the stability and sensory quality of the wine as it is linked to longevity, aroma, and color [24]. The pH of red wines must range between 3.30 and 3.60, because above these values the wines could have a flat flavor and on the contrary, below this threshold the wines would be harsh and could be unpleasant for consumption [21]. The pH of the *Fondillón* samples under study ranged between 3.35 (*solera* 1980) and 3.82 (*solera* 1975) and presented a mean value of 3.56 (**Table 1**). Thus, these values are within the previously mentioned parameters to meet the normality range, except for three values, which were slightly above the legal thresholds, perhaps implying a low negative effect of the aging process. Nogueira et al. [25] studied the effect of (*i*) type and (*ii*) age of on the main physico-chemical parameters of Madeira wines and concluded that their pH was unaffected by these two factors and ranged between 3.26 and 3.42.

Acidity is one of the most important characteristics to be controlled when making a wine. The total acidity is the result of the sum of all the organic acids present in the wine and has a direct and positive influence on the conservation of the wine, because it inhibits the development of microorganisms. Volatile acidity is another important parameter in wines, because above a threshold it shows deterioration of the wine due to production of acetic acid during yeast fermentation or also during aging in oak barrels by the chemical hydrolysis of hemicellulose [26]. The experimental ranges for these two parameters were: (i) 7.28 (*solera* 1996) and 10.01 g tartaric acid L^{-1} (*solera* 1960), and (ii) 1.01 (*solera* 1975) to 1.50 g acetic acid L^{-1} (*solera* 1980), respectively (Table 1).

Consequently, all experimental values of the total and volatile acidities were above and below the legal thresholds for *Fondillón* wines, respectively, and can be declared as appropriate; the sample *solera* 1980 was just in the legal threshold for the volatile acidity.

Nogueira et al. [25] also reported that total and volatile acidity seem to increase with aging for all types of Madeira wines, for samples up to 10 years old, and then, the contents were stable and below the maximum concentration admissible, for example 1.2 g L^{-1} for the volatile acidity. It is important to consider that the *Fondillón* samples under analysis in the current study were around 25 years old, and the reported increase in these two parameters in the Madeira wines could have happened at the beginning of the aging period also in the *Fondillón*, but the values reported in the current study are representative of the final steady state. This is a possible explanation for the fact that the "*solera*" factor did not affect the values of these enological parameters.

Regarding the alcohol content, it is reported that it must have at least 16% and it is of the outmost importance to remark that "no fortification can be done" [23]. The alcoholic content of the analyzed wines ranged between 18.30 (*solera* 1996) and 21.17 % *v/v* (*solera* 1930), with a mean of 19.28 % (**Table 1**). A general trend was found, with alcohol content increasing with the wine *solera* ($R^2 = 0.848$; $p < 0.01$). A study carried out by

Gómes-Cebrián [27], on the aromatic characterization of the *Fondillón* samples being marketed on 2013 (10 brands), showed that the mean alcohol content was 16.65 % *v/v*, with values ranging between 15.7 and 18.10 %. The values found in the current study are higher than those reported in the older study, and this might occur due to the age of the studied wines. Nogueira et al. [25] found that the average alcoholic content for young Madeira wines was up to 18 %, while a slight increase above 19 % was reported for 10-year-old wines, although no statistical support was provided. It is important to remember that Madeira wines are "fortified", reaching alcoholic contents in the range 15–22 % [28], while the alcoholic con- tent of *Fondillón* is only due to the fermentation of the natural sugars of Monastrell grapes by the yeast.

As a conclusion for this section, it can be stated that all *Fondillón* samples under analysis were within the legal limits established by the regulation of the DOP Alicante, and the physico-chemical profile of *Fondillón* seems not to be affected by the "*solera*" factor due to the long aging time of the wines, above 25 years.

3.2 Chromatic Characteristics, Total Polyphenol Index (TPI), Total Anthocyanin Content (TA), Antioxidant Activity (AA) and Total Condensed Tannins (TCT)

Color is one of the most important organoleptic aspects of a wine, as it can be an indicator of quality and can influence consumer acceptance and its satisfaction degree. This attribute depends mainly on (*i*) the grape type and enological parameters at harvest, (*ii*) vinification processes, and (iii) aging type and time [29].

The hue and intensity of the color are parameters mainly linked to the content of anthocyanins, which are extracted from the grape during maceration. Anthocyanins have a high antioxidant capacity, which are very beneficial for people's health [30]. During *Fondillón* oxidative aging, anthocyanins undergo different oxidation reactions such as condensation and polymerization, producing changes in the initial bluish tones transforming them into orange [31].

The parameter "tonality" (*Y*/*R*) measures the relationship between the intensity of the yellow component, *Y* (420 nm), and red one, *R* (520 nm). In the studied *Fondillón* samples, significant positive and negative correlations were observed among the tonality and (*i*) the *Y* component ($R^2 = 0.987$; $p < 0.001$) and (*ii*) *R* component ($R^2 = 0.982$; $p < 0.001$), respectively (**Figure 1a**). These relationships meant that during aging there was an increase in the *Y* component and a decrease in the *R* component, leading to an increase in the wine tonality. These results agreed with those reported by Del Fresno et al. [32] in their study of the changes of phenolic fraction of red wines (cultivar Tempranillo) aged in oak barrels.

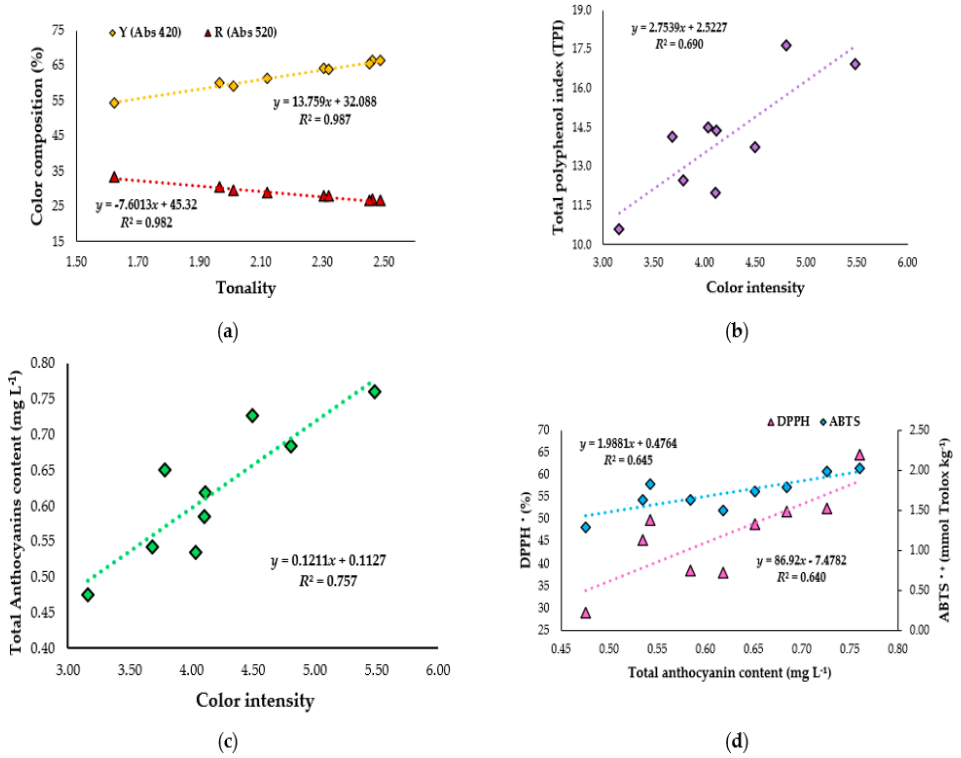


Figure 1. Correlation between color composition (*Y*, *R*) and tonality (**a**); correlation between color intensity and phenolics index (**b**); correlation between color intensity and total anthocyanins (**c**); and correlation between total anthocyanins content and antioxidant activity (ABTS•+, DPPH•) (**d**).

Regarding the parameter "color intensity", a positively correlation was observed with TPI (R^2 = 0.690; p < 0.01) and the TA (R^2 = 0.757; p < 0.01) (**Figure 1b, c**). These correlations meant that a high color intensity was linked to a high content of polyphenols and anthocyanins. In this way, the *Fondillón* sample with the highest values of color intensity (5.49), color density (4.80), R component (33.3), B component (12.5), and TA (0.76 mg L^{-1}) was that of the 1960 *solera*, which at the same time had the lowest values of the tonality (1.62) and Y component (54.1) (**Table 2**); all these values made *Fondillón solera* 1960 the most special one within the studied wine collection.

The presence of phenolic compounds in wines has a direct effect on their antioxidant activity. The most important phenolics in wine are flavonoids (anthocyanins, flavanols and flavanols), which play a key role in the wine antioxidant activity and sensory profile, by influencing color, astringency, and bitterness [33]. In this way, a significant positive correlation was found between TPI and TCT (R^2 = 0.615; p < 0.05); however, no other significant correlations were found among TPI, TCT, and TA (Table S2).

For the analysis of the antioxidant activity, three methods were used: $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP, because each one of them has different mechanisms of action. Table 2 shows that the *Fondillón* that presented the highest value for the three methods again was that of the *solera* 1960: $ABTS^{\bullet+}$ (2.02 mmol Trolox kg^{-1}), FRAP (4.97 mmol Trolox kg^{-1}), and $DPPH^{\bullet}$ (64.4 %), with the *solera* 1996 (the youngest *Fondillón* under study) being on the opposite side (lowest AA values): $ABTS^{\bullet+}$, (1.28 mmol Trolox kg^{-1}), FRAP (4.28 mmol Trolox kg^{-1}), and finally $DPPH^{\bullet}$ (29.9 %).

It was also observed that there were significant positive correlations among the TA and $ABTS^{\bullet+}$ (R^2 = 0.645; p < 0.01) and $DPPH^{\bullet}$ (R^2 = 0.640; p < 0.01) (Figure 1d); no significant effect was observed on FRAP. These results agreed with those presented by Luna et al. [15], when they studied the phenolic composition and antioxidant activity of minor grape varieties (Sabater, Gorgollassa, Escursac, Giró Ros, and Quigat) from the Balearic Islands (Spain) and concluded that the higher the total phenolic content, the higher the antioxidant activity.

The current experimental data showed a general trend of an increase in the $DPPH^{\bullet}$ with the aging time (R^2 = 0.224; p < 0.05). No clear trend was found for the $ABTS^{\bullet+}$ or FRAP data. The $DPPH^{\bullet}$ trend found in *Fondillón* agreed with the results published by Larrauri et al. [34], who reported higher values of $DPPH^{\bullet}$ for older Spanish wines prepared using Tempranillo grapes from fours Appellation of Origin (Madrid, Rioja, Ribera de Duero, and Valdepeñas). Later, contradictory results regarding the antioxidant activity were reported by Rivero-Pérez et al. [35], who studied 162 samples of red wine from Castilla y León. Their FRAP data showed an increase with the aging time, perhaps due to the trans- fer of ellagitannins from the wood to the wine in the first stages of aging; however, the opposite trend was found for $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ data, with values decreasing with the aging time. This behavior could be justified because these two last methods have the same antioxidant mechanism, that is, single-electron transfer mechanism [35]. Another possible explication for the increase in antioxidant activity with the aging time found in different wine types, including some *Fondillón soleras*, could be the formation of polar products due to Maillard reactions happening during wine storage [36,37]. In this way, Moreno et al. [36] studied the antioxidant activity of Pedro Ximenez musts and reported an increase in the antioxidant values linked to Maillard reactions.

Considering all the above, it can be concluded that the $DPPH^{\bullet}$ method seemed the most suitable one to study the antioxidant activity of *Fondillón*, and that the antioxidant activity in this type of wine was clearly linked to the total anthocyanins content.

Another important conclusion of this section was that the *Fondillón solera* 1960 was the most special sample because it had the highest contents of phenolic compounds, condensed tannins, and anthocyanins, which led to (*i*) the highest values of antioxidant activity (all three methods under study, $ABTS^{\bullet+}$, FRAP, and $DPPH^{\bullet}$), and (*ii*) the highest values of color density and intensity, as defined by intense red and blue colors.

Table 2. Chromatic Characteristics, antioxidant activity, total phenolic index, and total contents of condensed tannins and anthocyanins of *Fondillón* wines as affected by the "solera" factor.

Solera	Color intensity	Tonality	Color density	Y¶	R¶	B¶	ABTS•+	FRAP	DPPH•	Total phenolic index	Total condensed tannins content	Total anthocyanins content
					(%)		(mmol Trolox kg^{-1})		(%)	(TPI)	(g L^{-1})	(mg L^{-1})
	ANOVA†											
	***	***	***	***	***	***	***	**	***	***	***	***
	Tukey Multiple Range Test‡											
1930	4.03 cd	2.30 c	3.72 bcd	64.2 b	27.9 d	7.88 f	1.63 c	4.61 ab	45.3 e	14.5 b	0.60 bc	0.53 ef
1944	3.69 d	2.46 ab	3.43 cd	66.2 a	26.9 e	6.96 g	1.82 b	4.48 ab	49.7 cd	14.2 bc	1.24 a	0.54 ef
1950	4.81 ab	2.01 de	4.27 ab	59.3 d	29.5 c	11.3 b	1.78 b	4.54 ab	51.6 bc	17.7 a	1.07 a	0.69 abc
1960	5.49 a	1.62 f	4.80 a	54.1 e	33.3 a	12.5 a	2.02 a	4.97 a	64.4 a	17.0 a	0.98 ab	0.76 a
1969	3.73 d	2.37 bc	3.42 d	64.5 b	27.2 de	8.29 e	1.49 d	4.37 ab	37.9 f	14.4 b	0.38 cd	0.62 cde
1975	3.79 cd	2.49 a	3.52 cd	66.2 a	26.6 e	7.14 g	1.73 bc	4.15 b	48.8 d	12.5 bcd	0.45 cd	0.65 bcd
1980	4.50 bc	1.97 e	4.07 bc	60.0 d	30.5 b	9.46 d	1.98 a	4.91 ab	52.3 b	13.8 bc	0.61 bc	0.73 ab
1987	4.10 bcd	2.12 d	3.70 bcd	61.2 c	28.9 c	9.93 c	1.63 c	4.47 ab	38.5 f	12.0 cd	0.18 d	0.59 de
1996	3.16 d	2.45 abc	2.90 d	65.2 ab	26.6 e	8.14 ef	1.28 e	4.28 ab	29.0 g	10.6 d	0.10 d	0.48 f

*† ** and *** significant at $p < 0.01$, and 0.001, respectively. ‡Values (mean of three replications) followed by the same letter, within the same row, were not significantly different ($p < 0.05$), according to Tukey's least significant difference test. ¶ Y mean yellow color, R mean red color, and B mean blue color.*

3.3 Volatile Compounds

The HS-SPME-GC-MS analysis identified a total of 56 volatile compounds in all the analyzed wines, among which 34 showed significant differences ($p < 0.05$). The chemical profile consisted of 10 chemical families: 27 esters, 11 alcohols, 4 aldehydes, 4 alkanes, 3 ketones, 3 norisoprenoids, 1 organic acid, 1 terpene, 1 dioxolane, and 1 acetal, with esters being the predominant chemical group contributing to *Fondillón* odor and aroma (**Table S3**). No clear trends were found for the effect of the "*solera*" factor on the content of volatile compounds; thus, general comments will be presented in this section.

Esters are aromatic compounds providing and enhancing fruit aromas in wines; in general, the most important ones are ethyl fatty acid esters and acetates [38]. Diethyl butanedioate, ethyl octanoate, and ethyl acetate were the most abundant esters in the nine *Fondillón* samples under study and contributed to their red berries fragrance (**Table 3**).

Table 3. Volatile compounds content (mg L^{-1}) of the *Fondillón* wines as affected by the "solera" factor.

Code	Volatile compounds	ANOVA†	1930	1944	1950	1960	1969	1975	1980	1987	1996
			mg L^{-1}								
			Tukey Multiple Range Test ‡								
1	Acetaldehyde	NS	0.03	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.02
2	Ethanol	**	12.3 a	10.9 ab	10.9 ab	11.4 a	6.98 ab	8.82 ab	5.66 b	9.09 ab	8.10 ab
3	Isopropyl alcohol	NS	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
4	Acetic acid	NS	0.33	0.34	0.30	0.37	0.26	0.23	0.28	0.32	0.27
5	Hexane	***	0.15 a	0.02 b	0.01 b	0.01 b	0.01 b	0.01 b	0.00 b	0.01 b	0.01 b
6	Ethyl acetate	**	6.38 a	6.03 ab	5.16 b	6.09 ab	3.84 bc	3.66 c	3.66 c	4.51 bc	3.69 bc
7	Isobutyl alcohol	**	0.58 a	0.44 abc	0.44 abc	0.49 ab	0.33 abc	0.34 abc	0.23 c	0.39 abc	0.33 bc
8	3-Methyl butanal	NS	0.04	0.03	0.03	0.03	0.02	0.02	0.01	0.03	0.02
9	Ethyl propionate	***	0.18 a	0.11 bc	0.12 abc	0.14 ab	0.10 bc	0.10 bc	0.06 c	0.09 bc	0.07 bc
10	2,4,5-trimethyl-1,3-dioxolane	***	1.62 a	1.38 ab	1.48 ab	1.59 a	0.82 bc	1.03 abc	0.57 c	0.98 abc	0.78 bc
11	Isoamyl alcohol	**	7.44 a	6.19 ab	5.38 abc	6.00 ab	3.98 bc	4.46 bc	3.04 c	5.17 abc	4.57 abc
12	2-Methyl-1-butanol	**	2.93 a	2.33 ab	2.00 abc	2.22 abc	1.56 bc	1.68 bc	1.15 c	2.05 abc	1.72 bc
13	Ethyl isobutyrate	**	0.15 a	0.10 abc	0.11 ab	0.12 ab	0.07 bc	0.08 bc	0.04 c	0.10 abc	0.08 bc
14	Isobutyl acetate	NS	0.03	0.03	0.02	0.03	0.02	0.01	0.02	0.02	0.02
15	2,3-Butanediol	NS	0.22	0.19	0.23	0.24	0.14	0.20	0.14	0.15	0.16
16	2-Hexanol	NS	0.04	0.02	0.04	0.04	0.03	0.03	0.02	0.02	0.02
17	Ethyl butanoate	***	0.18 ab	0.13 b	0.12 b	0.26 a	0.10 b	0.09 b	0.07 b	0.17 ab	0.12 b
18	Ethyl lactate	***	0.98 ab	0.80 abc	0.84 abc	1.09 a	0.52 bc	0.60 bc	0.40 c	0.54 bc	0.48 c
19	Furfural	**	0.27 abc	0.25 abc	0.28 ab	0.35 a	0.17 bc	0.22 abc	0.11 c	0.19 bc	0.17 bc
20	Ethyl 2-methylbutyrate	**	0.12 a	0.09 ab	0.10 ab	0.09 abc	0.06 bc	0.07 abc	0.04 c	0.09 abc	0.07 bc
21	Ethyl isovalerate	**	0.25 a	0.21 abc	0.21 abc	0.23 ab	0.12 bc	0.16 abc	0.10 c	0.22 ab	0.16 abc
22	1-Hexanol	***	0.30 a	0.22 ab	0.17 bc	0.20 abc	0.16 bc	0.16 bc	0.12 c	0.23 ab	0.21 abc
23	Isoamyl acetate	**	0.62 a	0.51 ab	0.37 b	0.52 ab	0.37 ab	0.29 b	0.36 b	0.43 ab	0.34 b
24	Ethyl pentanoate	NS	0.03	0.02	0.02	0.03	0.01	0.02	0.01	0.02	0.02
25	Butyrolactone	NS	0.03	0.03	0.02	0.03	0.02	0.02	0.01	0.02	0.02
26	1,1-Diethoxy-3-methylbutane	NS	0.07	0.04	0.05	0.05	0.03	0.03	0.01	0.03	0.02
27	Benzaldehyde	***	0.50 a	0.35 ab	0.36 ab	0.36 ab	0.29 bc	0.29 bc	0.14 c	0.28 bc	0.31 bc
28	Ethyl hexanoate	**	1.35 ab	1.08 abc	1.05 abc	1.44 a	0.66 c	0.82 abc	0.52 c	0.89 abc	0.74 bc
29	Hexyl acetate	NS	0.03	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.01
30	Limonene	NS	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02	0.00
31	Benzyl alcohol	NS	0.02	0.02	0.02	0.01	0.01	0.02	0.01	0.01	0.02
32	Ethyl E-2-hexenoate	NS	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
33	Ethyl 2-furoate	NS	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
34	Isoamyl butyrate	NS	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01
35	2-Nonanol	NS	0.02	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01
36	3-Nonanone	NS	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
37	2-Nonanone	NS	0.03	0.03	0.02	0.03	0.02	0.02	0.01	0.03	0.02
38	Ethyl heptanoate	NS	0.06	0.06	0.05	0.05	0.03	0.04	0.02	0.04	0.03
39	Phenethyl alcohol	**	4.24 a	3.84 ab	3.09 abc	3.46 abc	2.35 bc	2.65 abc	1.78 c	3.15 abc	2.90 abc
40	Ethyl benzoate	**	0.09 ab	0.09 ab	0.05 bc	0.10 a	0.06 abc	0.07 abc	0.04 c	0.06 abc	0.06 abc
41	Diethyl butanedioate	**	8.19 a	7.64 a	7.34 a	8.36 a	4.55 ab	5.68 ab	3.14 b	5.30 ab	4.64 ab
42	Ethyl octanoate	***	7.45 a	5.55 abc	6.67 ab	5.23 abc	3.40 c	4.77 abc	2.72 c	4.88 abc	4.25 bc
43	Ethylphenyl acetate	***	0.10 ab	0.08 abc	0.05 bc	0.12 a	0.07 abc	0.05 bc	0.03 c	0.12 a	0.10 ab
44	Phenethyl acetate	**	0.08 a	0.07 ab	0.05 bc	0.08 a	0.06 abc	0.04 c	0.05 bc	0.06 abc	0.05 abc

45	Ethyl salicylate	***	0.05 bcd	0.06 abc	0.10 a	0.07 ab	0.03 cd	0.07 ab	0.03 cd	0.04 bcd	0.02 d
46	Ethyl glutarate	NS	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.01
47	Vitispirane	***	0.20 a	0.15 ab	0.12 bc	0.10 bc	0.08 c	0.11 bc	0.10 bc	0.16 ab	0.11 bc
48	Ethyl nonanoate	**	0.07 a	0.05 abc	0.07 ab	0.06 abc	0.03 bc	0.05 abc	0.03 c	0.05 abc	0.04 bc
49	Tridecane	NS	0.03	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01
50	TDN	***	0.19 a	0.12 b	0.13 b	0.09 bc	0.06 c	0.09 bc	0.07 c	0.13 ab	0.08 bc
51	Ethyl decanoate	***	2.12 ab	1.82 abc	2.83 a	1.82 abc	1.17 bc	1.70 abc	0.83 c	1.52 bc	1.46 bc
52	Tetradecane	**	0.08 a	0.01 b	0.01 b	0.01 b	0.00 b	0.01 b	0.00 b	0.01 b	0.01 b
53	Ethyl 3-methylbutyl butanedioate	**	0.11 a	0.09 ab	0.08 ab	0.10 ab	0.06 b	0.07 ab	0.04 c	0.06 b	0.06 b
54	Isoamyl octanoate	NS	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.01
55	Pentadecane	***	0.11 a	0.02 b	0.01 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
56	2,3,5-Trimethylnaphthalene	NS	0.03	0.03	0.04	0.03	0.02	0.02	0.02	0.03	0.03

*† NS = not significant at p > 0.05; **, ***, significant at p < 0.01, and 0.001, respectively. ‡ Values (mean of three replications) followed by the same letter, within the same row, were not significantly different (p > 0.05), according to Tukey's least significant difference test.*

In the same way, alcohols played an important role in the wines under analysis; they can come from the grapes themselves or from the yeast metabolism [38]. In *Fondillón*, the predominant ones were isoamyl alcohol, 2-methyl-1-butanol, and phenethyl alcohol and were responsible for fruity, floral, and honey notes.

The norisoprenoids are key compounds in the wine aroma profile and belong to the carotenoid metabolism. During wine aging, glycosidically-bound aroma compounds coming from the carotenoid metabolism can be subjected to slow acid hydrolysis rendering norisoprenoids such as 1,1,6-trimethyl-l,2-dihydronaphthalene (TDN) and vitispirane isomers [38,39]. TDN has been reported as the compound responsible for the kerosene bottle-aged character of Riesling wines [38,40], while vitispirane contributes to nut flavor notes [41]. There was no significant effect of the "*solera*" factor on the contents of (i) TDN (range 0.06 and 0.19 mg L^{-1}, with a mean of 0.13 mg L^{-1}) and (ii) vitispirane (range 0.08 and 0.20 mg L^{-1}, with a mean of 0.14 mg L^{-1}).

Regarding the *Fondillón solera* 1960 (the sample identified as the one having the highest antioxidant activity and highest contents of bioactive compounds, such as phenolic compounds), it contained high contents of key aroma compounds (ethyl butanoate, ethyl lactate, furfural, ethyl hexanoate, ethyl benzoate, etc.) (Table 3), which contributed to in- tense fruity, nutty, caramel, sweet, and floral notes.

3.4 Descriptive Sensory Analysis with Trained Panel

According to the legislation, *Fondillón* is defined as a wine being sweet, balanced, with good structure, and having intense aroma with predominance of ripe fruit, nuts, well-integrated wood and toasted coffee, and long aftertaste [23]. The descriptive profiles of the nine *Fondillón* samples (66 years of difference in the aging time) under analysis only showed statistically significant differences for 11 of the 22 attributes, which were used to evaluate the quality of these wines; these attributes were: alcohol (*o, f*), fruity (*o, f*), spicy (*o, f*), sweetness, acidity, astringency, and aftertaste (**Table 4**).

The attribute "aftertaste" is defined as a pleasant sensation, and it is considered by most of the experts and consumers as one of the most relevant; besides, it was significantly affected by the "*solera*" factor (**Table 4**). It was found that the sample with the longest aftertaste was that of the *solera* 1960. While the samples of *soleras* 1950 and 1987 were on the opposite side (shortest aftertaste, indicating perhaps a less complex organoleptic pro-file. Positive and significant correlations were found among aftertaste and (i) sweetness (R^2 = 0.462; $p < 0.05$) and (ii) fruity flavor (R^2 = 0.467; $p < 0.05$).

Table 4. Descriptive sensory analysis of wines aged in *Fondillón* wines as affected by the "*solera*" factor.

Attribute	ANOVA†	*Fondillón solera* 1930	1944	1950	1960	1969	1975	1980	1987	1996
		Tukey Multiple Range Test‡								
Appearance										
Color	NS	5.2	5.2	5.2	5.0	5.2	5.7	5.8	5.3	5.7
Odor										
Alcohol	*	6.0 a	5.0 ab	3.7 b	4.5 ab	4.2 ab	4.5 ab	5.0 ab	4.0 ab	3.7 b
Fruity	**	7.0 a	5.2 ab	3.3 b	5.0 ab	4.7 ab	4.8 ab	5.2 ab	4.5 b	3.7 b
Floral	NS	1.3	1.5	1.5	1.7	1.7	1.7	1.8	1.7	1.5
Mediterranean forest	NS	2.0	1.8	2.0	1.8	1.5	1.8	1.7	1.7	1.7
Spicy	*	4.2 a	3.5 ab	2.0 b	3.7 ab	3.3 ab	3.3 ab	3.8 a	3.5 ab	3.0 ab
Animal	NS	1.5	2.2	1.0	1.7	1.7	2.2	2.0	1.7	1.3
Toasted	NS	6.7	5.7	4.0	4.2	5.0	4.7	5.3	4.7	4.2
Chemical	NS	0.8	2.5	2.5	2.7	2.3	2.7	3.5	2.0	1.3
Basic taste										
Sweetness	*	3.0 ab	5.0 a	2.7 b	3.8 ab	3.7 ab	3.7 ab	3.2 ab	3.0 ab	2.7 b
Sourness	**	4.5 a	3.5 abc	2.5 c	3.5 abc	3.0 bc	3.8 abc	4.2 ab	3.3 abc	2.8 bc
Bitterness	NS	1.5	1.3	2.2	2.0	2.5	2.5	1.8	1.8	1.5
Flavor										
Alcohol	*	5.7 a	5.0 ab	4.0 b	5.2 ab	4.5 ab	4.7 ab	4.7 ab	4.5 ab	4.5 ab
Fruity	*	4.8 a	4.7 a	3.3 b	4.7 a	4.8 a	4.5 a	4.0 ab	4.0 ab	3.5 ab
Floral	NS	0.8	1.5	1.2	2.0	2.0	1.8	1.5	1.5	1.3
Mediterranean forest	NS	1.7	1.7	0.8	1.7	2.0	2.0	1.7	1.7	1.5
Spicy	*	3.8 a	3.0 ab	2.8 b	3.3 ab	3.0 ab	3.0 ab	3.2 ab	3.0 ab	2.8 b
Animal	NS	1.0	1.5	1.0	1.7	1.7	1.8	1.3	1.7	1.2
Toasted	NS	5.5	4.8	3.3	4.7	5.3	5.0	4.8	4.2	3.8
Chemical	NS	0.8	1.5	1.8	2.0	1.3	2.3	2.2	1.7	1.7
Astringency	**	1.0 b	0.8 b	0.8 b	0.8 b	1.2 b	2.0 a	1.2 b	1.2 b	1.0 b
Aftertaste	***	6.3 bc	6.8 ab	3.7 d	7.2 a	6.3 bc	6.3 bc	5.8 c	4.2 d	6.3 bc

*† NS = not significant at p > 0.05; *, **, *** significant at p < 0.05, 0.01, and 0.001, respectively. ‡ Values (mean of 8 trained panelists) followed by the same letter, within the same row, were not significantly different (p > 0.05), according to LSD least significant difference test.*

3.5 Affective Sensory Analysis

The consumer profile was: 63 % men (37 % women), of which 5 %, 18 %, 45 %, and 32 % belonged to the age ranges 18–24, 25–39, 40–59, and 60–74 %, respectively.

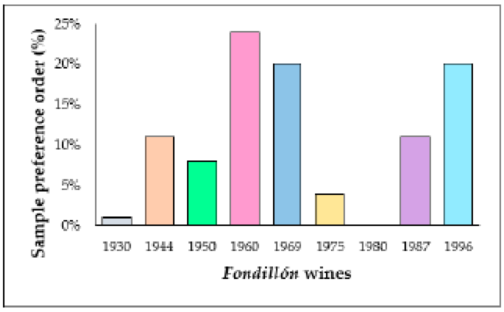
Samples of *soleras* 1944 (6.3), 1960 (6.2), and 1996 (6.1) obtained the highest overall liking values, followed by 1930 (5.8) and 1987 (5.8) samples (**Table 5**); these values led to an overall mean of ~6.0, which means that consumers liked the wines "slightly". This value can be taken as a high degree of satisfaction because consumers tend not to use the extreme values of the scale, concentrating their opinions in the middle part. On the other hand, *Fondillón* samples of *soleras* 1980, 1975, 1969, and 1950 showed the lowest liking values, with a mean of 5.1, which means that the wines were neither like nor dislike. Similar trends were found for the other two affective parameters under study, sweetness and aftertaste, with *Fondillón solera* 1960 having the highest liking scores.

When the effects of the gender and age factors were studied, it was surprisingly seen that young women, below 35 years old, were the ones with the highest liking score. This observation did not agree with the preconceived idea that the typical *Fondillón* consumer was a man of 40–50 years old, with high education and income. This surprising experimental observation opened a whole new perspective of research and revealed the important need to prepare a detailed study to determine which is the current profile of *Fondillón* and which are the options to promote *Fondillón* consumption among new consumers.

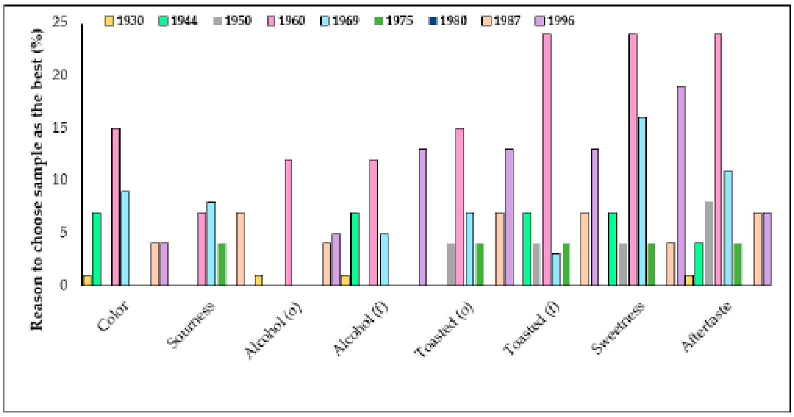
Table 5. Affective sensory analysis of *Fondillón* wines as affected by the "*solera*" factor.

Solera	Overall	Sweetness	Aftertaste
	ANOVA†		
	***	***	***
	Tukey Multiple Range Test‡		
1930	5.8 b	5.1 bc	5.9 ab
1944	6.3 a	5.8 ab	6.3 a
1950	5.4 bc	5.1 bc	6.1 a
1960	6.2 a	6.1 a	6.1 a
1969	5.5 bc	5.1 bc	5.7 ab
1975	5.2 bc	5.1 bc	5.6 ab
1980	4.3 c	4.8 c	4.9 b
1987	5.8 b	5.7 b	5.9 ab
1996	6.1 ab	6.3 a	6.2 a

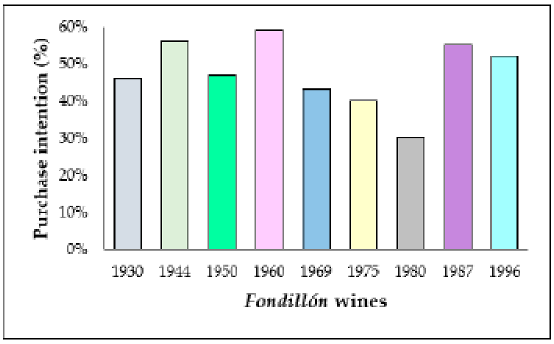
*† *** significant at $p < 0.001$. ‡Values (mean of 123 consumers) followed by the same letter, within the same column, were not significantly different ($p > 0.05$), according to LSD least significant difference test.*



(a)



(b)



(c)

Figure 2. Consumer preference (**a**); main reasons to choose 1960 *Fondillón* as the favorite (**b**); and purchase intention (**c**).

Figure 2a shows the preference percentage of the Spanish consumers for the *Fondillón* samples under study, and Figure 2b the reasons why they chose their preferred sample. The *Fondillón solera* 1960 was chosen as the most liked one (24 %), mainly because of its color, its toasted (woody) odor and flavor, its sweetness, its toffee and coffee notes, and its long aftertaste. Besides, the wine obtaining the highest purchase intention (59 %) was that of the *solera* 1960 (Figure 2c), with sample of *solera* 1980 being on the opposite side (30 %).

4. Conclusions

The effect of the *solera* age on nine *Fondillón* samples was studied, by analyzing several quality, functional, and sensory parameters together with their consumer liking. In most of the cases, it seems that no clear trend was found for the "*solera*" factor perhaps due to the long aging time of the wines, above 25 years. Independently of the *solera* effect, the *Fondillón solera* 1960 was the most special one due to its high contents of phenolic com- pounds, condensed tannins, anthocyanins, high antioxidant activity, intense red and blue notes, and due to its high contents of key volatile compounds (e.g., ethyl butanoate and furfural), which provides the wine with nutty, caramel, and sweet notes. There was a positive correlation among overall liking with a long aftertaste and intense sweetness. The most important satisfaction and buying drivers for *Fondillón* consumers were color, toasted odor and flavor, sweetness, toffee and coffee notes, and long aftertaste. Additional conclusions were: (*i*) the DPPH• method showed the biggest differences among samples and can be considered as the most suitable one to study the antioxidant activity of *Fondillón*; and (*ii*) the *Fondillón* antioxidant activity showed a positive and significant correlation with the total anthocyanins content. The final recommendation based on the findings and conclusions of this study could be that *Fondillón* quality and consumer acceptance depend strongly on high contents of key compounds (e.g., anthocyanins) and high antioxidant capacity. Thus, *Fondillón* should be only prepared when the Monastrell grapes have high antioxidant potential besides proper total sugar content.

Supplementary Materials:

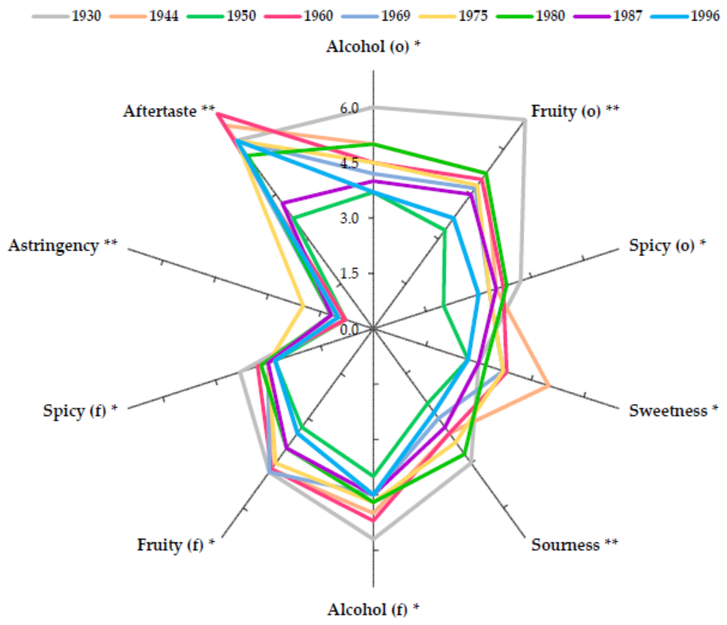


Figure S1. Descriptive sensory profile of the *Fondillón* wines as affected by the "solera" factor (*, **, and *** mean $p < 0.05$, 0.01, and 0.001, respectively).

Table S1. Affective questionnaire used in this study.

Question #	Text			
Satisfaction degree/Liking and preference				
Q1	How much do you like this sample from a **global** point of view?			
Q2	How much do you like the **sweetness** of this sample?			
Q3	How much do you like the **aftertaste** of this sample?			
Q4	Check all those **aroma families** that you have perceived in this sample, if any?			
	Fruity	*Vanilla*	*Coffee*	*Floral*
	Caramel	*Nutty*	*Vegetal*	*Raisins*
	Toffee	*Defect, if any (please, specify): ______________*		
Q5	Will you buy this sample?			
	Yes		*No*	
Q6	After tasting all nine samples, which was the sample that **you liked the most**?			
Q7	Regarding your preferred sample, please indicate the reasons why you have chosen it?			
	Color	*Alcohol odor*	*Toasted/woody odor*	*Sweetness*
	Sourness	*Alcohol flavor*	*Toasted/woody flavor*	*Aftertaste*
	Others (please, specify): ___________________________________			
Demographics and food habits				
Q8	Which is your gender?			
	Male	*Female*	*Other (please, specify):*	*I prefer not to answer*
Q9	Which is your age?			
	18-24	*25-35*	*36-45*	*Above 45*
Q10	How often do you consume this type of product?			
	Daily	*Twice a week*	*On weekends*	
	Twice a month	*On special occasions*		
Q11	Which are the main two reasons driving your selection of wine?			
	Price	*Year/vintage*	*Brand*	*The one on sale*
	The trendy one	*Other (please, specify): ____________________*		

Table S2. Volatile compounds, descriptors, and retention index of the *Fondillón* wines as affected by the "*solera*" factor.

Code	Volatile compounds†	Chemical Family	Odor Descriptor†	Retention Time ‡ (min)	Retention Index Exp.	Retention Index Lit.
1	Acetaldehyde	Aldehyde	Ethereal, coffee	2.097	493	427
2	Ethanol	Alcohol	Alcohol	2.186	508	493
3	Isopropyl alcohol	Alcohol	Butter	2.525	563	530
4	Acetic acid	Acid	Vinegar	2.615	577	580
5	tetradecane	Alkane	Gasoline	2.772	603	600
6	Ethyl acetate	Ester	Anise, pineapple	2.851	616	616
7	Isobutyl alcohol	Alcohol	Fruity	2.975	636	635
8	3-Methyl butanal	Aldehyde	Chocolate, peach, fatty	3.270	684	686
9	Ethyl propionate	Ester	Fruity, pineapple, sweet	3.965	723	725
10	2,4,5-trimethyl-1,3-dioxolane	Dioxolane	Honey, floral, green	4.267	735	735
11	Isoamyl alcohol	Alcohol	Oily, whiskey	4.408	741	742
12	2-Methyl-1-butanol	Alcohol	Roasted, fruity, fusel	4.482	744	743
13	Ethyl isobutyrate	Ester	Citrus, strawberry	4.883	759	759
14	Isobutyl acetate	Ester	Apple, banana, pear, sweet	5.232	773	771
15	2,3-Butanediol	Alcohol	Fruity, creamy, buttery	5.428	781	782
16	2-Hexanol	Alcohol	Chemical, cauliflower, fruity	5.710	792	793
17	Ethyl butanoate	Ester	Fruity, pineapple, fruity	5.925	800	800
18	Ethyl lactate	Ester	Butter, fruity	6.236	809	806
19	Furfural	Aldehyde	Almond, woody, sweet	6.870	827	825
20	Ethyl 2-methylbutyrate	Ester	Apple, sweet, green, fruity	7.456	844	845
21	Ethyl isovalerate	Ester	Apple	7.603	848	848
22	1-Hexanol	Alcohol	Green, herbaceous, woody, sweet	8.161	865	872
23	Isoamyl acetate	Ester	Banana, pear	8.433	872	872
24	Ethyl pentanoate	Ester	Apple	9.369	900	900
25	Butyrolactone	Ketone	Creamy, oily, fatty	9.748	908	908
26	1,1-Diethoxy-3-methylbutane	Acetal	Honey, floral, green	11.559	949	952
27	Benzaldehyde	Aldehyde	Almond, cherry, sweet	12.005	959	959
28	Ethyl hexanoate	Ester	Apple, banana, pineapple	13.726	997	997
29	Hexyl acetate	Ester	Apple, cherry, floral, pear sweet	14.377	1011	1011
30	Limonene	Terpene	Lemon, herbaceous, citrus, sweet	15.238	1029	1029
31	Benzyl alcohol	Alcohol	Berry, cherry, grapefruit, walnut	15.378	1031	1031
32	Ethyl (E)-2-hexenoate	Ester	Sweet, green, vegetable, fruity	15.910	1042	1040
33	Ethyl 2-furoate	Ester	Floral, plum	16.192	1048	1047
34	Isoamyl butyrate	Ester	Apricot, banana, pineapple	16.497	1054	1056
35	2-Nonanol	Alcohol	Melon, fatty, green	17.069	1066	1076
36	3-Nonanone	Ketone	Cheese, herbaceous, fruity, green	17.941	1084	1085
37	2-Nonanone	Ketone	Cheese, coconut, oily, fatty, floral	18.208	1089	1088
38	Ethyl heptanoate	Ester	Berry, melon, peach, pineapple	18.525	1096	1097
39	Phenethyl alcohol	Alcohol	Honey, rose	19.196	1110	1110
40	Ethyl benzoate	Ester	Anise, vanilla, floral, fruity	22.050	1168	1168
41	Diethyl butanedioate	Ester	Cooked, apple, fruity	22.469	1177	1176
42	Ethyl octanoate	Ester	Apricot, floral, pear, pineapple	23.365	1195	1195
43	Ethylphenyl acetate	Ester	Sweet, floral, honey, rose, balsam	25.474	1240	1243
44	Phenethyl acetate	Ester	Apple, apricot, caramel, honey	26.040	1252	1250
45	Ethyl salicylate	Ester	Floral, fruity, minty, sweet	26.757	1267	1265
46	Ethyl glutarate	Ester		27.220	1277	1279
47	Vitispirane	Norisoprenoid	Floral, fruity, nuts	27.368	1280	1280
48	Ethyl nonanoate	Ester	Oily, fruity, nutty	28.033	1294	1294
49	Tridecane	Alkane	Floral	28.336	1300	1300
50	1,1,6-trimethyl-l,2-dihydronaphthalene (TDN)	Norisoprenoid	Petrol	30.689	1353	1354
51	Ethyl decanoate	Ester	Grape, oily, pear	32.493	1393	1392
52	Tetradecane	Alkane	Gasoline	32.829	1401	1400
53	Ethyl 3-methylbutyl butanedioate	Ester		33.862	1425	1400
54	Isoamyl octanoate	Ester	Apple, coconut, green, fruity	34.724	1445	1445
55	Pentadecane	Alkane	Gasoline	37.077	1501	1500
56	2,3,5-Trimethylnaphthalene	Norisoprenoid	Earthy	39.569	1559	1558

†National Institute of Standards and Technology, NIST [42]; SAFC [43]. ‡RT = retention time; Exp = experimental; Lit = literature.[42,43]

Table S3. Pearson's correlation coefficients (R) among basic enological parameters, chromatic characteristics, antioxidant activity ($ABTS^{•+}$, FRAP, $DPPH^{•}$), total contents of condensed tannins (TCT) and anthocyanins (T) and total polyphenol index (TPI) of the *Fondillón* wines as affected by the "solera" factor.

Parameters	pH	Total alcohol content (% v/v)	Total acidity (g tartaric acid L^{-1})	Volatile acidity (g acetic acid L^{-1})	Color intensity	Tonality	Color density	Y	R	B	$ABTS^{•+}$	FRAP	$DPPH^{•}$	TPI	TCT	TA
								(%)			(mmol Trolox kg^{-1})		(%)			
pH	**1**															
Total alcohol content (% v/v)	0.124	**1**														
Total acidity (g tartaric acid L^{-1})	-0.079	0.392	**1**													
Volatile acidity (g acetic acid L^{-1})	-0.351	0.139	0.401	**1**												
Color intensity	-0.289	0.133	**0.818****	0.196	**1**											
Tonality	0.590	0.018	**-0.669***	-0.235	**-0.932*****	**1**										
Color density	-0.249	0.171	**0.833****	0.218	**0.996*****	**-0.910*****	**1**									
Y(%)	0.568	0.058	-0.658	-0.171	**-0.925*****	**0.993*****	**-0.896****	**1**								
R (%)	-0.582	0.031	**0.705***	0.287	**0.927*****	**-0.991*****	**0.909*****	**-0.978*****	**1**							
B (%)	-0.519	-0.160	0.566	0.027	**0.871****	**-0.941*****	**0.831****	**-0.970*****	**0.898****	**1**						
$ABTS^{•+}$ (mmol Trolox kg^{-1})	-0.144	0.344	0.585	0.401	**0.750***	-0.663	**0.775***	-0.613	**0.709***	0.465	**1**					
FRAP (mmol Trolox kg^{-1})	**-0.699***	0.302	0.662	0.562	**0.773***	**-0.857****	**0.772***	**-0.806****	**0.886****	**0.669***	**0.731***	**1**				
$DPPH^{•}$ (%)	-0.051	0.435	**0.730***	0.264	**0.813****	**-0.667***	**0.833****	-0.650	**0.728***	0.522	**0.945*****	**0.688***	**1**			
TPI	0.002	0.428	**0.838****	0.208	**0.830****	-0.639	**0.841****	-0.642	0.623	0.629	0.620	0.567	**0.750***	**1**		
TCT (g L^{-1})	0.120	0.626	0.600	0.388	0.508	-0.329	0.529	-0.319	0.354	0.260	**0.701***	0.450	**0.764***	**0.784***	**1**	
TA (mg L^{-1})	-0.148	-0.117	0.623	0.221	**0.870****	**-0.786***	**0.880****	**-0.765***	**0.797***	**0.684***	**0.803****	0.616	**0.800****	0.635	0.392	**1**

*, **, ***, significant at $p < 0.05$, 0.01, and 0.001, respectively.

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References

1. Post, W.M.; Kwon, K.C. Soil carbon sequestration and land-use change: Processes and potential. *Glob. Chang Biol.* **2000**, *6*, 317– 327, doi:10.1046/j.1365-2486.2000.00308.x.
2. Agencia Estatal de Meteorología. Accumulated precipitation (mm). Available online: https://www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos (accessed on 30 July 2021).
3. Escribano Francés, G.; Quevauviller, P.; San Martín González, E.; Vargas Amelin, E. Climate change policy and water resources in the EU and Spain. A closer look into the Water Framework Directive. *Environ. Sci. Policy* **2017**, *69*, 1–12, doi:10.1016/j.envsci.2016.12.006.
4. Sánchez-Bravo, P.; Edgar Chambers, V.; Noguera-Artiaga, L.; Sendra, E.; Edgar Chambers, I.V.; Carbonell-Barrachina, A.A. How consumers perceivewater sustainability (hydrosostainable) in food products and how to identify it by a logo. *Agronomy* **2020**, *10*, 1495, doi:10.3390/agronomy10101495.
5. Andreu-Coll, L.; Cano-Lamadrid, M.; Noguera-Artiaga, L.; Lipan, L.; Carbonell-Barrachina, Á.A.; Rocamora-Montiel, B.; Legua, P.; Hernández, F.; López-Lluch, D. Economic estimation of cactus pear production and its feasibility in Spain. *Trends Food Sci. Technol.* **2020**, *103*, 379–385, doi:10.1016/j.tifs.2020.07.003.
6. Issa-Issa, H.; Noguera-Artiaga, L.; Sendra, E.; Pérez-López, A.J.; Burló, F.; Carbonell-Barrachina, A.A.; López-Lluch, D. Volatile Composition, Sensory Profile, and Consumers' Acceptance of *Fondillón*. *J. Food Qual.* **2019**, *2019*, doi:10.1155/2019/5981762.
7. Council Regulation (EC) No 479/2008, 29 April 2008. *Common Organisation of the Market in Wine*. Official Journal of the European Union, No L 148/1-84.
8. Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J. Agric. Food Chem.* **1999**, *47*, 2837–2846, doi:10.1021/jf981224s.
9. Oliveira, C.M.; Ferreira, A.C.S.; De Freitas, V.; Silva, A.M.S. Oxidation mechanisms occurring in wines. *Food Res. Int.* **2011**, *44*, 1115–1126, doi:10.1016/j.foodres.2011.03.050.
10. Pereira, V.; Albuquerque, F.; Cacho, J.; Marques, J.C. Polyphenols, antioxidant potential and color of fortified wines during accelerated ageing: The madeira wine case study. *Molecules* **2013**, *18*, 2997–3017, doi:10.3390/molecules18032997.
11. Gambuti, A.; Rinaldi, A.; Ugliano, M.; Moio, L. Evolution of phenolic compounds and astringency during aging of red wine: Effect of oxygen exposure before and after bottling. *J. Agric. Food Chem.* **2013**, *61*, 1618–1627, doi:10.1021/jf302822b.

12. OIV (International Organization of Vine and Wine). *Compendium of International Methods of Wine and Must Analysis, Vol 1;* OIV: Paris, France, 2020.
13. OIV (International Organization of Vine and Wine). *Compendium of International Methods of Wine and Must Analysis, Vol 2*; OIV: Paris, France, 2021.
14. Glories, Y. The color of red wines. *Connaissance Vignevini* **1984**, *18*, 253–271.
15. Luna, J.M., Garau, M.C., Negre, A., March, J., Martorell, A. Composición Fenólica y Actividad Antioxidante de Variedades Minoritarias de Vid de las Islas Baleares. In Proceedings of the VII Foro Mundial del Vino, Logroño, La Rioja, Spain, 12–14 May 2010; Sáenz-Navajas, M.P., Fernández-Zurbano, P., Valentín, D., Ferreira-González V (Eds.); Consejería de Agricultura, Ganadería y Desarrollo Rural: Logroño, La Rioja, Spain, 2010; p. 62.
16. Di Stefano, R.C., M.C.; Gentilini, N. Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico* **1989**, 83–89.
17. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237, doi:10.1016/S0891-5849(98)00315-3.
18. Katalinic, V.; Milos, M.; Kulisic, T.; Jukic, M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem.* **2006**, *94*, 550–557, doi:10.1016/j.foodchem.2004.12.004.
19. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability 7, of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76, doi:10.1006/abio.1996.0292.
20. Ribéreau-Gayon, P.; Stonestreet, E. Determination of anthocyanins in red wine. *Bulletin de la Societe Chimique de France* **1965**, *9*, 2649–2652.
21. International Organization of Standardization. ISO 8586:2012. Sensory Analysis—General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors. Available online: https://www.iso.org/standard/45352.html (accessed on 8 June 2021).
22. International Organization of Standardization. ISO/IEC 17065:2012. Conformity Assessment—Requirements for Bodies Certifying Products, Processes and Services. Available online: https://www.iso.org/standard/46568.html (accessed on 8 June 2021).
23. Orden 5/2011, 16 November 2011. *Reglamento y Pliego de Condiciones de la Denominación de Origen Protegida Alicante y su Consejo Regulador*. Diario Oficial de la Comunitat Valenciana, DOCV, No 6661/39267-39289, 2011.
24. Forino, M.; Picariello, L.; Rinaldi, A.; Moio, L.; Gambuti, A. How must pH affects the level of red wine phenols. *LWT* **2020**, 129. 109546, doi:10.1016/j.lwt.2020.109546.
25. Nogueira, J.M.F.; Nascimento, A.M.D. Analytical characterization of Madeira wine. *J. Agric. Food Chem.* **1999**, *47*, 566–575, doi:10.1021/jf9804490.
26. Miranda, A.; Pereira, V.; Pontes, M.; Albuquerque, F.; Marques, J.C. Acetic acid and ethyl acetate in Madeira wines: Evolution with ageing and assessment of the odour rejection threshold. *Ciencia e Tecnica Vitivinicola* **2017**, *32*, 1–11, doi:10.1051/ctv/20173201001.
27. Gómez-Cebrián, R. *Characterization of Fondillón Wines from the Denomination of Origin Alicante (Master Thesis, In Spanish)*. Universidad Miguel Hernández de Elche: Elche, Alicante, Spain, 2015.
28. Council Regulation (EEC) No 4252/88, 21 December 1988. *Preparation and Marketing of Liquer Wines Produced in the Community*. Official Journal of the European Communities, No L 373/59-65, 1988.
29. González-Neves, g., Balado, J., Barreiro, L., Bochicchio, R., Gatto, G., Gil, G., Tessore, A., Ferrer, M. Efecto de Algunas Prácticas de Manejo del Viñedo y de la Vinificación en la Composición Fenólica y el Color de los Vinos Tintos. In Proceedings of the X Congresso Brasileiro de Viticultura e Enologia, Anais, Bento Gonçalves, RS, Brazil, 3–5 December 2003.
30. Reyes-Cortés, C.A. *Red Wine Quality usign Analysis of the Color. Intensity and Anthocyanins Content (Degree Thesis, in Spanish)*; Universidad de Chile: Santiago de Chile, Chile, 2020.
31. Gómez-Cordovés, C.; González-SanJosé, M.L. Interpretation of Color Variables during the Aging of Red Wines: Relationship with Families of Phenolic Compounds. *J. Agric. Food Chem.* **1995**, *43*, 557–561, doi:10.1021/jf00051a001.
32. Del Fresno, J.M.; Morata, A.; Loira, I.; Escott, C.; Suárez Lepe, J.A. Evolution of the Phenolic Fraction and Aromatic Profile of Red Wines Aged in Oak Barrels. *ACS Omega* **2020**, *5*, 7235–7243,

doi:10.1021/acsomega.9b03854.

33. Rivero-Pérez, M.D.; González-Sanjosé, M.L.; Muñiz, P.; Pérez-Magariño, S. Antioxidant profile of red-single variety wines microoxygenated before malolactic fermentation. *Food Chem.* **2008**, *111*, 1004–1011, doi:10.1016/j.foodchem.2008.04..

34. Larrauri, J.A.; Sánchez-Moreno, C.; Rupérez, P.; Saura-Calixto, F. Free radical scavenging capacity in the aging of selected red spanish wines. *J. Agric. Food Chem.* **1999**, *47*, 1603–1606, doi:10.1021/jf980607n.

35. Rivero-Pérez, M.D.; González-Sanjosé, M.L.; Ortega-Herás, M.; Muñiz, P. Antioxidant potential of single-variety red wines aged in the barrel and in the bottle. *Food Chem.* **2008**, *111*, 957–964, doi:10.1016/j.foodchem.2008.05.013.

36. Moreno, J.; Peinado, J.; Peinado, R.A. Antioxidant activity of musts from Pedro Ximénez grapes subjected to off-vine drying process. *Food Chem.* **2007**, *104*, 224–228, doi:10.1016/j.foodchem.2006.11.028.

37. López de Lerma, N.; Peinado, J.; Moreno, J.; Peinado, R.A. Antioxidant activity, browning and volatile Maillard compounds in Pedro Ximénez sweet wines under accelerated oxidative aging. *LWT−Food Sci. Technol.* **2010**, *43*, 1557–1563, doi:10.1016/j.lwt.2010.06.004.

38. Robinson, A.L.; Boss, P.K.; Solomon, P.S.; Trengove, R.D.; Heymann, H.; Ebeler, S.E. Origins of grape and wine aroma. Part 1. Chemical components and viticultural impacts. *Am. J. Enol. Vitic.* **2014**, *65*, 1–24, doi:10.5344/ajev.2013.12070.

39. Full, G.; Winterhalter, P. Application of on-line coupled mass spectrometric techniques for the study of isomeric vitispiranes and their precursors of grapevine cv. Riesling. *Vitis* **1994**, *33*, 241–244.

40. Winterhalter, P., Sefton, M.A., Williams, P.J. Volatile C13-Norisoprenoid Compounds in Riesling Wine Are Generated From Multiple Precursors. *Am. J. Enol. Vitic.* **1990**, *41*, 277–283.

41. Ángeles Pozo-Bayón, M.; Victoria Moreno-Arribas, M. Sherry wines. *Adv. Food Nutr. Res.* **2011**, *63*, 17–40, doi:10.1016/B978-0- 12-384927-4.00002-6.

42. National Institute of Standards and Technology (NIST), NIST Chemistry Webbook. Available online: https://webbook.nist.gov/chemistry (accessed on 8 July 2021).

43. Sigma-Aldrich. *Flavors & Fragrances*; Sigma-Aldrich: Saint Louis, MO, USA, 2012.

PUBLICACIÓN 4

Consumer Profile and Drivers Influencing Consumer Behavior towards *Fondillón*, a European Protected Naturally Sweet Red Wine

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Consumer Profile and Drivers Influencing Consumer Behavior towards Fondillón, a European Protected Naturally Sweet Red Wine

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Abstract: Sustainable irrigation strategies in Southeast Spain (one of the most arid regions in Europe) are essential to fight against desertification and climate change mitigation. In this way, *Fondillón* production is based on rain-based vineyards, over-ripe *Monastrell* grapes, and non-alcohol fortification. Thus, *Fondillón* is a naturally sweet red wine, protected within the Alicante Denomination of Origin, recognized by the European Union in its E-bachus database. The study aim was to evaluate the effect of the aging (*solera* factor) on *Fondillón:* (*i*) basic enological parameters (e.g., total, and volatile acidity), (*ii*) chromatic characteristics, (*iii*) antioxidant activity ($ABTS^{•+}$, FRAP and $DPPH^{•}$), (*iv*) total contents of condensed tannins and anthocyanins, (*v*) volatile composition, (*vi*) sensory profile, and (*vii*) overall liking. Experimental data proved that the wine (1960 *solera*) with the highest total contents of condensed tannins and anthocyanins and total antioxidant activity was the most liked by Spanish consumers. Experimental results clearly established a positive relationship among *Fondillón* chemical composition, its antioxidant activity, and overall consumer liking. Exceptional harvest with grapes having extremely high antioxidant power (e.g., 1960 *solera*) will result, even more than 50 years later, in high quality wines with high consumer acceptance and a high monetary worth.

Keywords: Alicante wine; consumer acceptance; descriptive sensory analysis; Monastrell; oxidized wine

1. Introduction

Wine is an alcoholic beverage that is consumed worldwide. Wine quality is determined by several factors such as cultivar, environmental conditions, quality of raw materials at harvest, and winemaking process, especially storage conditions and hygiene. All these factors transform each wine into a unique product with aromatic and gustatory complexity and typicity [1].

A special group of wines are those with residual sugar; they include a large range of styles. Some inexpensive wines contain low to moderate levels of residual sugar, making them appealing to a larger consumer segment. There are different ways of producing wines with residual sugar (*i*) by concentrating the sugar in the grape must (overripening of the grape); (*ii*) stopping the fermentation before dryness (adding alcohol or by freezing), or (*iii*) blending in a sweetening component (e.g., adding must) to the wine. The method selection will be determined by the vineyard climate, the local wine legislation, the wine style and quality, and the final market price that the winemaker wants for their products. In any case, the global objective is to produce a wine with balanced residual sugar and acidity, and with a flavor profile appropriate for the final sugar content [2].

Concentrating the sugar in the grape must is the method used to produce many sweet and luscious wines. The concentration of sugars in the grape must be sufficient to stop the fermentation before reaching dryness; yeasts struggle in very sugary environments, especially when alcohol is also present, and naturally stop fermentation even at relatively low levels of alcohol. These wines are known as *naturally sweet wines*. This process not only concentrates sugar, but also other grape components such as organic acids and flavor precursors.

The increase in sour and flavor components helps in keeping the balance of these wines by avoiding that sweetness becomes too dominant; this is the key reason why many of these wines have an outstanding organoleptic quality. In general, these wines can have more complexity than those simply made by stopping the fermentation by chilling or adding a sweetening component [3]. However, these grapes are difficult to process, because (*i*) the volume of juice obtained from the grapes is low, and (*ii*) the very sugary pulp is also often hard to extract during pressing. These factors must be added to the normal production cost, making them only appropriate to be sold for premium and super premium wines with a "high selling price". In fact, there is a growing interest in high-quality naturally sweet wines [4], but this is not supported by a deep research on consumer preferences and drivers controlling sweet wine consumption [5], especially if this research is compared with the number of papers on dry wine consumption. This lack of research is hampering a full development of suitable marketing strategies by wineries producing sweet wines, including those producing *Fondillón*.

Fondillón is a naturally sweet wine made only in the province of Alicante and having a set of specific characteristics that makes it unique. Its main features are: (*i*) it comes from the overripening of *Monastrell* (also known as Mourvèdre) grapes, with very high amounts of sugars, aroma precursors, and color components; (*ii*) its alcohol content is entirely produced by fermentation of the grape sugars, that is, no fortification takes place at any stage; (*iii*) the minimum alcohol allowed is 16° abv (alcohol by volume); and (*iv*) it must be aged in oak barrels for, at least, 10 years [6]. *Fondillón* production was almost lost at the end of the 19th century due to, among other reasons, a high demand of cheap wine from Europe because of *Phylloxera*. Production was recovered around 1950; however, there has not been an appropriate approach to understand the special chemistry behind this wine and to promote it among non-highly-specialized wine consumers [7].

The perception of distinction is not an objective process but a social one taking place in a field of cultural production [8]. Consumers look for and are willing to pay high prices for wine if its symbolic position allows for their social distinction [9]. In this way, the initial working hypothesis of this study was that consumers do not know the peculiarities and singular characteristics of *Fondillón* mainly because (*i*) consumers with high purchasing power were not interested on this specific wine from a non-trendy wine PDO (Alicante) due to a lack of scientific information on its peculiarities, and (*ii*) consequently, winemakers lost their interest in producing and selling this wine, especially due to the long inversion in time needed for its production. However, this situation is changing and the few manuscripts characterizing *Fondillón* [10] and comparing it to other high-quality sweet wines, such as *Tokaj* and *Sauternes*, are getting winemakers and consumer attention and making this wine more and more popular again. As a result, in the last two-three years the *Fondillón* selling price in wine shops has increased by about 30–40 %, showing that consumers are starting to be aware of its uniqueness and willing to buy more and more *Fondillón* bottles and this higher demand has made winemakers concentrate on this wine and has even made them quick to renew the *Fondillón* packaging to communicate to consumers its exclusivity. In this way, 11,050 bottles of *Fondillón* were labeled under the Alicante PDO in the season 2019–2020 as compared to only 5720 in the season 2014–2015, showing an increase of ~93% (data kindly provided by the Regulatory Council of the Alicante PDO). In 2020, a *Fondillón* (*Brotons Gran Fondillón Reserva* 1964) was included among the best Spanish wines and this prize (Best Wine 2020, Spanish Ministry of Agriculture, Fisheries and Food) has also positioned this Alicante wine in the focus of the specialized media.

Consequently, the aims of this study were: (*i*) to evaluate the degree of consumer acceptance and satisfaction towards *Fondillón*, (*ii*) to establish key drivers controlling consumer satisfaction, and (*iii*) to establish a general profile of the typical *Fondillón* consumer, including the main reasons why *Fondillón* is not as popular as other naturally sweet wines, such as *Sherry*, *Porto*, or *Madeira*. This information will be essential for Alicante winemakers to understand consumer behavior towards *Fondillón* (buying and satisfaction drivers) and adjust their marketing/selling strategies to help *Fondillón* recover its symbolic position of social distinction and make consumers order this wine in modern restaurants, particularly in those specialized in Mediterranean cuisine.

2. Materials and Methods

2.1 *Wine Samples*

In 2019, only 9 wineries in Alicante produced *Fondillón* under the PDO Alicante. To reduce the number of samples to be evaluated, a preliminary grouping of the commercial *Fondillón* samples was conducted using a napping test with 20 trained panelists. They classified wines into 5 clusters (the main characteristics/sensory descriptors of each cluster are described below); one sample from each of these clusters was selected and fully evaluated in the current study. Samples (3 bottles of 750 mL for each sample) were kindly provided by the Regulatory Council of the Alicante PDO.

The samples analyzed were:

(i) **F1**, *Gran Reserva* 1987 (sweet, Muscat, raisins, and amber color).
(ii) **F2**, *Solera* 1948 (sweet, bitter, woody, paperboard, and toffee).
(iii) **F3**, *Reserva Especial* (bitter, sour, alcohol, and mahogany color).
(iv) **F4**, *Gran Reserva* 1964 (sweet, bitter, roasted, toffee, and long aftertaste).
(v) **F5**, *Solera* 1996 (sweet, roasted, caramel, vanilla, and amber color).

2.2 Descriptive Sensory Analysis

For the sensory evaluation of *Fondillón* samples, the tasting panel of the Regulatory Council of the Alicante PDO was used. This panel consisted of 12 panelists (6 women and 6 men), aged between 30 and 60 years old, with more than 400 h of experience in describing Alicante wines. These panelists were (*i*) selected (3 sessions of 1.5 h), according to results in discrimination, ranking, and recognition tests, (*ii*) trained (12 sessions of 1.5 h sessions, for 4 months), and (*iii*) validated (2 sessions of 1.5 h). The panel is included in the control tools of the Regulatory Board to monitor the quality of their wines and is accredited by ENAC (National Entity of Accreditation), following the norm ISO/IEC 17065:2012 [11]. Panelists were paid for their involvement in the current study and any other evaluation they performed. A maximum of 10 wines can be tasted in a single session, including 1 repeatability (same sample evaluated twice in the same session), 1 reproducibility (same sample evaluated in two different sessions), and 1 defect sample.

The vocabulary used to describe the *Fondillón* samples was that of the official lexicon of the PDO Alicante and the attributions, definitions, and reference materials are described in Issa-Issa et al. [7]; these attributes were selected by the panel during the lexicon development. The attribute "Mediterranean forest" replaced "vegetable" because during the last 3 years of experience of the panel, it was demonstrated that the term vegetable was too general and did not properly describe the notes found in *Fondillón* samples; a new reference material was prepared and consisted of rosemary dried at 40 °C for 24 h and its intensity was 8.0. The main attributes used for the evaluation of *Fondillón* samples were classified into 4 complex properties: appearance, odor, basic tastes, and flavor. In the appearance, only color was evaluated. Regarding odor and flavor (olfactory and gustative phases), the analyzed attributes were alcohol, fruity/nutty, floral, Mediterranean forest, spicy, animal, toasted/caramel, and chemical, together with the basic tastes: sweetness, sourness, and bitterness. Finally, astringency and aftertaste or persistence (time that the characteristic *Fondillón* wine flavor remains in the mouth after swallowing the sample) were also studied.

The 5 samples under analysis were run in triplicate in 3 sessions of ~2.0 h. The deviation of quality control samples must be below 20 % for all sensory descriptors. A maximum of 10 samples can be evaluated in a single session of 2.0 h, including 3 quality control samples (reproducibility, repeatability, and sample with a defect). Samples were randomly served coded with 3-digit numbers, together with the appropriate questionnaire, one at a time, and with waiting 10 min between samples. Initially, ~35 mL of each sample were served in black cups at 16–18 °C for the evaluation of the flavor and later ~20 mL were served in transparent cups in normalized booths at a controlled temperature of 22 ± 2 °C. To avoid carry over effects, mineral water and breadsticks were provided as palate cleansers.

To quantify the intensity of each of the attributes studied, a scale from 0 to 10 was used, with increments of 0.5 units; where, 0 indicates extremely low or no intensity, and 10 indicates extremely high intensity.

2.3 Affective Sensory Analysis

This study was carried out to establish the degree of overall consumer satisfaction, together with the satisfaction degree for key and easy-to-understand-by-consumers sensory attributes: color, alcohol (odor and flavor, *o* and *f*), toasted/caramel/coffee (*o*, *f*), chemical (*o*, *f*), sweetness, sourness, and aftertaste. A preference test was also conducted. The affective study was carried out with 100 Spanish consumers, recruited at the Orihuela campus of the UMH, and who drank sweet or oxidized wine (*Porto*, *Madeira*, *Sherry*, *Fondillón*, etc.) at least twice a month. The consumer profile was as follows: 44 % women and 56 % men, of which 20 % were between 18 and 24 years old, 40 % between 25–35, 20 % between 36–44, and 20 % above 45 years old.

All samples were coded using 3-digit numbers and served to each of the consumers one at a time in a random order. Wine service conditions (cups, wine temperature, and palate cleansers) were the same as those previously described for the descriptive study. A questionnaire was developed, where a liking for each of the studied attributes was evaluated, using a 9-point hedonic scale (9 = like it extremely, 5 = neither like it nor dislike it, and 1 = dislike it extremely). The intensity of the main *Fondillón* attributes were evaluated using a 9-point JAR (Just About Right) scale (1 = much too weak, 5 = just about right, and 9 = much too strong). Additionally, to identify the main sensory attributes driving overall liking, a Check-All-That-Apply (CATA) ballot was used; a list of 18 notes, including 9 odor and 9 flavor notes (fruity, vanilla, coffee, floral, caramel, nutty, Mediterranean forest, raisins, and toffee) was built for the intuitive description of the *Fondillón* samples by consumers; these 20 attributes were obtained from the preliminary napping test carried out to group wine samples and, thus, reduce the number of samples to be used in descriptive and affective studies. Finally, consumers were asked about (*i*) their preference (after tasting these 5 *Fondillón* samples, which sample did you like the most or prefer? This question was located at the end of the affective questionnaire and after the consumers tasted the 5 *Fondillón* samples); and, (*ii*) purchase intention (are you willing to buy this wine? This question was located at the end of the affective test for each one of the samples).

2.4 Online Survey

An online study (with 294 consumers, consisting of 62 % men) was designed and conducted to determine the consumers niche of *Fondillón* and to understand the main reasons for the consumption or no-consumption of this wine. Participants were from two neighboring regions (Valencia and Murcia, comprising 4 provinces: Castellón, Valencia, Alicante, and Murcia) and should drink (*i*) Alicante PDO wine at least twice a week, or (*ii*) sweet or oxidized wine (*Porto*, *Madeira*, *Sherry*, *Fondillón*, etc.) at least twice a month. In this way, consumers were expected to have a basic knowledge or information on *Fondillón* due to their geographical proximity to its producing area; in further studies, consumers from all Spanish regions will be included, but initially only consumers from these two regions were targeted. The questionnaire had questions related to the consumption of *Fondillón*, and demographic questions to classify them according to gender, age, income, and education (**Table S1**).

2.5 Statistical Analysis

Results were subjected to a one-way analysis of variance (ANOVA) and then, a LSD multiple-range test was performed on the attributes analyzed with the trained panel and on liking. Differences were considered statistically significant at $p < 0.05$. Partial least square regression (PLS) was performed using sensory descriptors and consumer overall liking. JAR data were analyzed by a penalty analysis, which was carried out to obtain information on those weak points where the sensory profile of each of the samples can be improved. The normal distribution of the hedonic data was checked using the Jarque-Bera test for each sample. CATA results were analyzed using a Correspondence Analyses (CA) plot, which was created through a contingency table with 20 and 300 columns and rows, respectively. To perform all these statistical analyses, the software XLSTAT Premium 2016 (Addinsoft, Nueva York, NY, USA) and Statgraphics Plus (version 3.1, Statistical Graphics Corp., Rockville, MA, USA) were used.

3. Results and Discussion

3.1 Descriptive Sensory Analysis

A trained panel was used to develop the descriptive sensory profiles of the *Fondillón* samples under analysis; these profiles will be later used to determine which of their attributes are key drivers of consumer acceptance. In this way, Issa-Issa et al. [7], in a preliminary affective study on *Fondillón*, concluded that Spanish consumers appreciated samples with a high intensity of fruity notes, high alcohol content, and some bitter and Mediterranean forest notes.

Twenty-two sensory attributes were analyzed, with 16 of them showing significant differences (**Table 1**). It is important to highlight that sample F4 was the one that had the highest intensity (> 5.0) of most of the attributes evaluated, including sweetness, alcohol (*o, f*) and fruity/nutty, and aftertaste, while sample F1 was characterized by a high intensity of color and toasted/caramel notes.

Vitispirane (having as sensory descriptors eucalyptus and camphor) and TDN (1,1,6- trimethyl-1,2-dihydronaphthalene, which has sensory descriptors of petroleum or smoke) were identified as potential markers of the *Fondillón* age [7]. These two compounds can be linked with the descriptors (*i*) Mediterranean forest, which includes balsamic (vitispirane), and (*ii*) chemical (TDN) notes. Perhaps concerning the aging intensity, related attributes (Mediterranean forest and chemical) could be one of the factors affecting consumer acceptance and satisfaction. In this way, sample F4 showed the highest intensity of the attribute "chemical" (odor and flavor: *o, f*), which includes the petroleum note (TDN); on the other hand, there were not significant differences for the attribute "Mediterranean forest" (vitispirane).

3.2 Affective Sensory Analysis

Table 2 shows the overall satisfaction degree of Spanish consumers, and their liking regarding key sensory attributes of these wines, including color, alcohol (*o, f*), toasted (*o, f*), sweetness, sourness, and aftertaste.

The main parameter to be studied was the overall liking; that is, the "global" opinion of the consumer when tasting the wine samples F4 (6.9) and F1 (6.7) were the most liked ones, with scores close to 7.0 (like moderately); this value can be taken as a relatively high acceptance because consumers tend not to use the extreme values of the scale. Comparing the overall satisfaction degree for the 100 consumers participating in this study, it was shown that only 5 % of the consumers who liked the F4 sample also liked the F1 sample.

Table 1. Descriptive sensory profile of the *Fondillón* samples under analysis.

Attribute	ANOVA†	F1	F2	F3	F4	F5
		LSD Multiple Range Test ‡				
Appearance						
Color	***	6.0 a	4.0 ab	3.0 c	4.0 ab	5.0 b
Odor						
Alcohol	***	5.3 b	6.1 ab	6.6 a	6.5 a	5.7 ab
Fruity	**	4.8 b	5.2 ab	5.3 ab	5.4 ab	5.7 a
Floral	*	2.0 a	1.6 ab	0.9 b	1.0 b	2.0 a
Mediterranean forest	NS	1.9	0.9	0.8	0.8	1.7
Spicy	NS	3.3	3.6	3.4	3.7	3.0
Animal	NS	1.3	1.4	1.4	0.9	1.3
Toasted	**	5.8 a	4.9 ab	5.1 ab	4.2 b	4.2 b
Chemical	**	2.2 c	2.7 b	2.0 c	3.2 a	2.7 b
Basic taste						
Sweetness	***	5.4 a	4.0 b	3.2 c	5.3 a	3.7 bc
Sourness	***	3.5 b	3.4 b	4.3 ab	3.4 b	3.8 ab
Bitterness	***	1.6 bc	2.1 b	3.3 a	1.1 c	1.5 bc
Flavor						
Alcohol	**	6.1 b	6.7 a	6.7 a	6.7 a	6.1 b
Fruity	**	5.7 a	4.9 ab	5.5 a	5.0 ab	4.5 b
Floral	*	1.4 b	0.9 b	2.1 a	1.1 b	2.3 a
Mediterranean forest	NS	1.3	0.9	1.2	0.9	1.5
Spicy	NS	3.6	4.0	3.5	3.4	3.8
Animal	NS	0.9	1.1	1.6	0.8	1.2
Toasted	***	7.2 a	6.5 b	5.7 c	5.8 c	4.8 d
Chemical	**	2.5 b	3.0 ab	2.7 b	3.7 a	3.0 ab
Astringency	***	0.8 b	1.6 a	1.2 b	0.8 b	1.0 b
Aftertaste	**	5.7 b	5.5 b	6.0 ab	6.7 a	6.0 ab

*† NS = not significant at p > 0.05; *, **, ***, significant at p < 0.05, 0.01, and 0.001, respectively. ‡ Values (mean of 12 trained panelists) followed by the same letter, within the same row, were not significantly different (p > 0.05), according to LSD least significant difference test.*

Table 2. Liking scores of Spanish consumers regarding the Fondillón samples under analysis.

Samples	Overall	Color	Alcohol (o)¥	Toasted (o)¥	Chemical (o)¥	Sweetness	Sourness	Alcohol (f)¥	Toasted (f)¥	Chemical (f)¥	Aftertaste
	ANOVA Test†										
	***	***	***	***	**	***	***	***	**	**	***
	Tukey Multiple Range Test‡										
F1	6.7 a	6.8 ab	6.8 a	6.8 a	5.4 ab	6.2 a	6.6 a	6.5 a	6.8 a	6.0 ab	6.6 ab
F2	5.8 b	6.5 ab	5.6 b	5.9 b	4.6 b	5.2 b	5.6 b	5.5 b	5.5 b	5.0 b	5.6 c
F3	5.9 b	6.9 a	6.4 ab	6.4 ab	4.1 b	5.3 b	5.5 b	5.5 b	6.0 ab	4.5 b	5.4 c
F4	6.9 a	7.0 a	6.0 ab	6.3 ab	6.3 a	6.5 a	6.1 ab	5.9 ab	6.7 a	7.0 a	7.0 a
F5	6.0 b	6.1 b	5.6 b	6.0 b	5.7 ab	5.9 ab	5.9 ab	5.4 b	6.2 ab	6.3 ab	6.2 bc

*†, **, ***, significant at p < 0.01, and 0.001, respectively. ‡ Values (mean of 100 consumers) followed by the same letter, within the same column, were not significantly different (p < 0.05), according to LSD least significant difference test. ¥ (o) = odor; (f) = flavor*

Moving to the liking of each of the 10 main sensory attributes selected for this study, samples F1 and F4 were those showing the highest satisfaction scores. For instance, the acceptance of sample F1 was based on high liking scores (> 6.2), for all studied attributes except by chemical (*o*). This sample (F1) was defined by consumers in their comments as a balanced wine, easy to drink, fresh, and clean. This description could be linked to the high score observed for the satisfaction degree on sourness; besides, the high score (6.6) on the aftertaste liking indicated that despite the long ageing—above 10 years—no off-flavor notes were perceived by consumers. The high overall liking scores of the F4 sample were based on high scores for color, sweetness, toasted (*f*), and aftertaste; in this case, aftertaste can play a key role because it is very long, pleasant, and it is linked with the complexity of this special Alicante wine.

Figure 1 shows the first two components of the correspondence analyses (CA) plot, which explained 95.74 % of the CATA data variability; three groups can be recognized based on the position of the samples in the CA. The first group included sample F4, which was linked with nutty (*o*) and coffee (*f*) notes. A second group consisted of samples F1, F2, and F3, which had more relevant descriptors such as caramel (*o*, *f*) and Mediterranean forest (*f*). Finally, sample F5 was characterized by its floral (*o*) note.

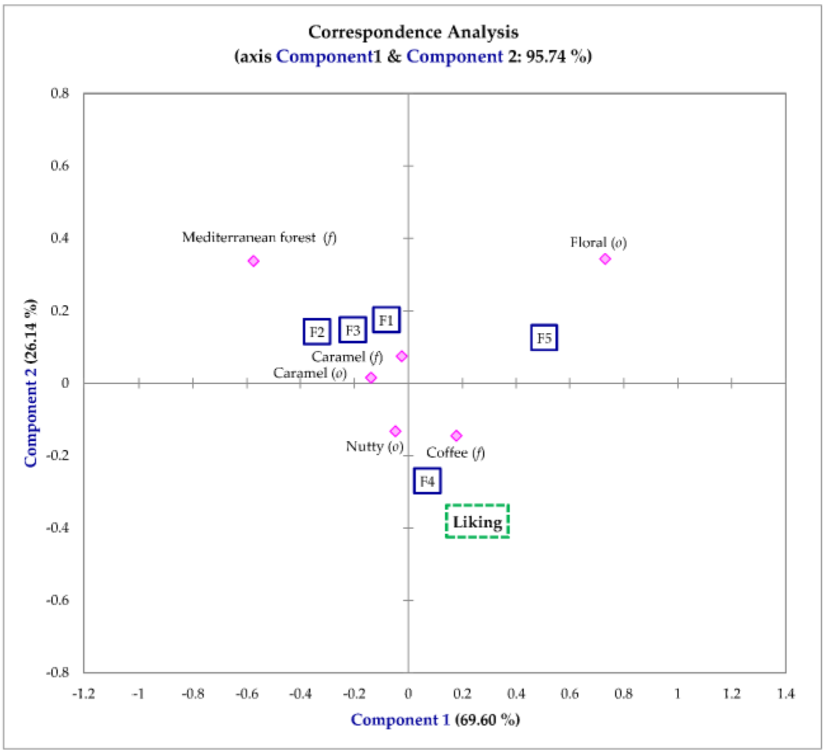

Figure 1. Differences among the samples according to the CATA descriptors used to study the *Fondillón* samples under study (Correspondence Analysis). Legend: □ wine samples; ◇ sensory attributes. --- consumers 'overall liking.

Results from the preference test showed that the two most preferred samples were F4 and F1 (30 and 28 %, respectively); while on the other hand, only 10, 13, and 18 % of consumers preferred samples F2, F3, and F5, respectively. Finally, and regarding the purchase intention, 100 and 8 3% of the consumers were willing to buy samples F4 and F1, respectively, while ~60% were willing to buy the other three samples (60, 60, and 53 % for F3, F5, and F2, respectively).

3.3 Partial Least Square Regression (PLS) and Correspondence Analysis

A PLS analysis was performed to analyze whether descriptive data was able to properly explain consumer overall liking (**Figure 2**). For this analysis, only those descriptive attributes being statistically affected ($p < 0.05$) by the type of *Fondillón* were included. It is important to mention that the low number of samples used in the study, five, limits the ability to assess relationships, but the number of samples was limited by the complexity and long aftertaste of the *Fondillón* samples analyzed. In this way, 70 % of the variation of the descriptive data (X−axis) explained 47 % of the variation of the consumer overall liking (Y−axis). Figure 2 shows that ~50 % of consumers liked sweet *Fondillón* samples having floral (*o*) notes (F1 and F5). On the other hand, ~40 % of consumers liked *Fondillón* samples being sweet, with fruity (*o*, *f*) notes, having characteristic chemical notes (*o*, *f*), and having a long aftertaste (F4).

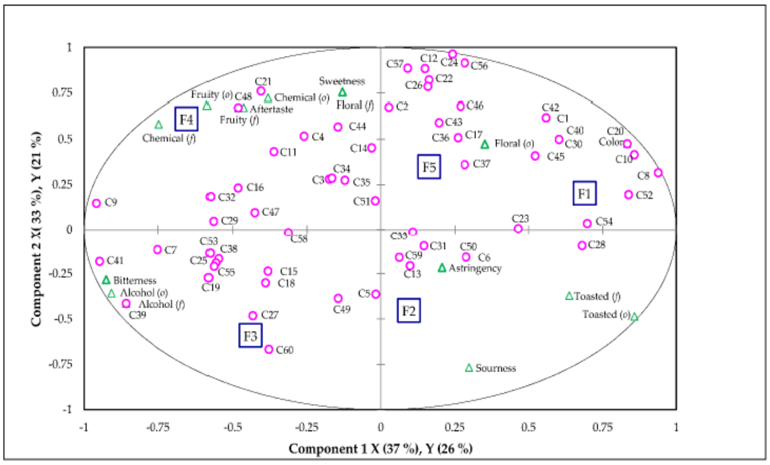

Figure 2. Partial Least Squares (PLS) regression of descriptive sensory attributes (X-axis) and consumers overall liking (Y-axis) of *Fondillón* samples under analysis. Legend: C consumers; □ Sample; △ sensory attributes, ○ consumer's overall liking.

In general, the PLS analysis demonstrated that *Fondillón* consumers do not like samples with high intensity of bitterness, sourness, astringency, and/or alcohol or toasted/caramel notes (*o*, *f*) (F2 and F3). This experimental statement agreed with results from penalty analysis, indicating that the key drivers controlling consumer satisfaction towards *Fondillón* [12] were long aftertaste, high sweetness, and low alcohol and sourness (**Figure S1**). These consumer opinions/demands (limiting excessive sourness and alcohol notes) can be linked to some of the *Fondillón* samples slightly starting to be too oxidized, with sugars starting to be converted into organic acids, such as acetic acid [13], providing an slightly higher sourness, decreasing their characteristic sweetness and complexity, and starting to lose their balance.

Correspondence Analysis (CA) was used to evaluate the CATA results and showed the key role of the following descriptors: coffee, floral, caramel, nutty, and Mediterranean forest/rosemary (**Table 3**). Coffee and caramel notes (CATA) can be linked to the descriptive attribute "toasted/caramel" considering their definitions; in a similar way, the nutty note can be linked to the descriptive attribute "fruity" (reference material benzaldehyde, which has a sensory descriptor of bitter almond) [7]. This linkage among affective CATA notes and descriptive sensory attributes were initially established to understand which descriptive attributes were more easily perceived by consumers. Future studies with a higher number of consumers will be conducted to confirm these relationships, including volatile compounds generating the sensory attributes/descriptors.

Table 3. Results of the Cochran's Q test for each note used in the CATA questionnaire.

Note	*p*-values†	F1	F2	F3	F4	F5
			ODOR			
Fruity	NS	0.23	0.18	0.28	0.23	0.10
Vanilla	NS	0.38 a	0.32	0.40	0.35	0.30
Coffee	NS	0.42	0.35	0.32	0.35	0.55
Floral	***	0.12 ab	0.05 a	0.15 ab	0.05 a	0.30 b
Caramel	***	0.77 b	0.75 b	0.73 b	0.67 ab	0.40 a
Nutty	*	0.40 a	0.52 a	0.53 a	0.63 a	0.35 a
Mediterranean forest	NS	0.08	0.10	0.18	0.08	0.10
Raising	NS	0.45	0.52	0.58	0.62	0.60
Toffee	NS	0.57	0.38	0.43	0.42	0.35
				FLAVOR		
Fruity	NS	0.22	0.10	0.15	0.15	0.05
Vanilla	NS	0.25	0.17	0.28	0.27	0.30
Coffee	*	0.50 ab	0.33 a	0.57 ab	0.72 b	0.55 ab
Floral	NS	0.13	0.05	0.10	0.10	0.10
Caramel	*	0.80 ab	0.67 ab	0.83 b	0.62 ab	0.55 a
Nutty	*	0.58 b	0.58 b	0.53 b	0.70 a	0.45 b
Mediterranean forest	*	0.13 ab	0.18 b	0.17 b	0.03 ab	0.00 a
Raising	NS	0.53	0.57	0.52	0.53	0.55
Toffee	NS	0.48	0.33	0.40	0.45	0.40

*† NS = not significant at $p > 0.05$; *, ***, significant at $p < 0.05$ and 0.001, respectively. Values followed by the same letter, within the same row, were not significantly different ($p > 0.05$).*

3.4 Consumers Profile

In this online study, results showed that 50 % of the respondents were *Fondillón* consumers, although all participants drank Alicante wine, at least twice a week, and oxidized wine, at least twice a month. This first result is important because it highlights the fact that *Fondillón* is not so popular, even for people consuming other types of Alicante wines and living in regions close to its production zone (Alicante PDO). The profile of the *Fondillón* consumers (not from all respondents but only those consuming *Fondillón* regularly) was as follows: 68 % men (**Figure 3A**), of which 29 % were between 24 and 41 years old, 41 % between 42 and 52, and 27 % between 53 and 73 years old (**Figure 3B**). Regarding the education and socioeconomic levels of consumers, 51% and 33% of the *Fondillón* consumers have bachelor or higher degree of education and an income between € 25,000 and € 50,000 (**Figure 3C,D**). The results also showed that only 3 % of the participants drank *Fondillón* 2–3 times a week, 8 % once a week, 16 % 2–3 times a month, 14% 1 time a month, 23 % 2–3 times a year, and 37 % consumed it only on special occasions (Figure 3E). The reasons why they consume this type of wine were that (*i*) they like its characteristic flavor (78 %), (*ii*) it is a local and traditional product (40 %), (*iii*) it is an exclusive wine from the province of Alicante (2 9 %), and (*iv*) it is a luxury wine (24 %) (Figure 3F). The main sensory attributes driving consumers to drink this wine were sweetness, toasted/caramel aroma, raisin aroma, nutty aroma, long aftertaste, and its balance. In the specific case of consumption, 77 % of the people surveyed answered that they only consumed *Fondillón* at home, while only 16 % consumed it in restaurants. A potential explanation for this experimental finding can be the high price of *Fondillón* at restaurants or the fact that most of the restaurants do not have this type of wine on their wine list.

The reasons why wine consumers do not consume *Fondillón* are: (*i*) it is very expensive (9 %), (*ii*) it is difficult to find (14 %), (*iii*) it is not in the stores where they usually buy alcoholic beverages, including wine (27 %), or (iv) simply because they do not know it (18 %).

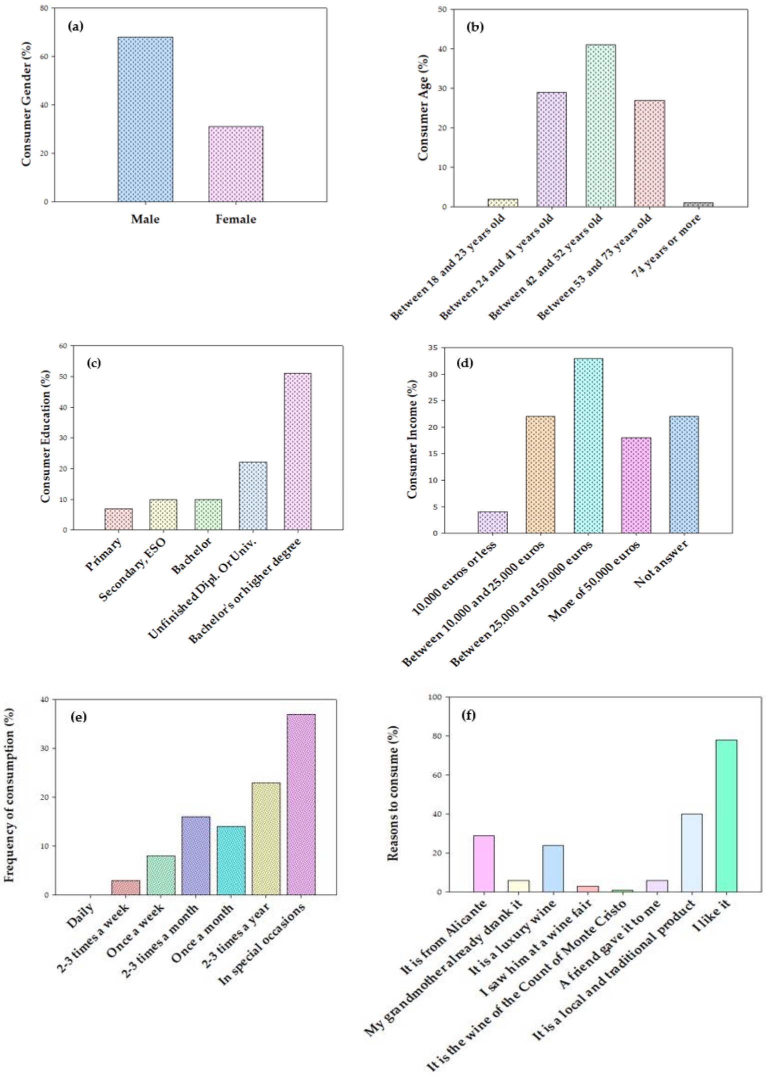

Figure 3. *Fondillón* consumer profile as affected by (**a**) gender, (**b**) age, (**c**) educational level, and (**d**) annual income, together with key consumption habits: (**e**) consumption frequency and (**f**) main reasons to buy and consume this wine.

4. Conclusions

The sensory profile of the most liked *Fondillón* samples was: (*i*) good balance between alcohol, sweetness, and bitterness, (*ii*) intense floral and/or fruity notes, and (*iii*) a long and pleasant aftertaste. When wine consumers try *Fondillón*, they like it and are willing to keep drinking it, as happened with samples *F4* and *F1*. The main problem is that a wide percentage of consumers still do not know or have never tried *Fondillón*. The typical *Fondillón* consumer is a man between 42 and 52 years old, with bachelor or higher degree, and earning between 25,000 and 50,000 euros/year. They drink *Fondillón* mainly during special occasions at home and the two main reasons why they drink it are: (*i*) it is a traditional Alicante wine and (*ii*) because of its characteristic and distinctive flavor. The reasons for not ordering *Fondillón* at restaurants are: (*i*) its high price, and (*ii*) it is not generally included on the restaurant wine list. Producers should develop their marketing strategies regarding *Fondillón,* focusing on communication and distribution and keeping in mind that the two major constraints limiting its consumption are consumer value perception (consumers are not fully aware of the real value and high quality of *Fondillón* and then consider it is too expensive) and the lack of availability in both on and off trade. Further studies at the Alicante PDO can be conducted to study the profile of tourists visiting these wineries and their opinion on *Fondillón* after their first tasting, as has been done with similar wines, such as *Sherry* [14] and Porto [15] wines. Additionally, in future studies, the number of consumers participating in the affective test will be increased and consumers from all Spanish regions will be included, and then, the approach used to link descriptive and affective data will be preference mapping.

Supplementary Materials:

Table S1. Questionnaire used in this study to establish the consumer profile.

Code	Question	
Q1	Do you know *Fondillón* wine?	
	Yes	No
Q2	Are you a *Fondillón* consumer?	
	Yes	No
Q3	If your previous answer was NO, why you do not consume it?	
	It is very expensive	It is not in the stores where I usually buy wine
	I do not like its taste	It is a wine only for special occasions
	It is hard to find	It has an excessive alcohol content
	I do not like its color	Others (please, specify):
Q4	If your previous answer was YES, how often do you consume *Fondillón*?	
	Daily	2-3 times a month
	2-3 times a week	Once a month
	Once a week	2-3 times a year
	Only in special occasions	
Q5	I drink *Fondillón* because (choose all the answers you consider appropriate):	
	It is from Alicante	It is the wine of the Count of Monte Cristo
	My grandmother already drank it	A friend recommended it to me
	It is a luxury wine	It is a local and traditional product
	I discover it while visiting a wine fair	I tried once, and I like it
Q6	What *Fondillón* brand do you know?	
	Fondillón Laudum	*Fondillón MGW*
	Fondillón Primitivo Quiles	*Fondillón Algueña*
	Fondillón Tesoro de Villena	*Fondillón Mañan*
	Fondillón Culebrón	Others (please, specify):
Q7	Which or which of them have you consumed or consume?	
	Fondillón Laudum	*Fondillón MGW*
	Fondillón Primitivo Quiles	*Fondillón Algueña*
	Fondillón Tesoro de Villena	*Fondillón Mañan*
	Fondillón Culebrón	Others (please, specify):
Q8	Where do you usually buy *Fondillón*?	
	Gourmet shop	Specialized wine shop
	Hypermarket	Straight from the winery
	Supermarket	Neighborhood store
Q9	Where do you usually consume *Fondillón*?	
	Home	Restaurant
Q10	Demographics	
Q10.1	Which is your gender?	
Q10.2	Which is your age?	
Q10.3	Which is the highest education level you have completed?	
Q10.4	How many adults live in your household including yourself?	
Q10.5	How much is your approximate annual income?	

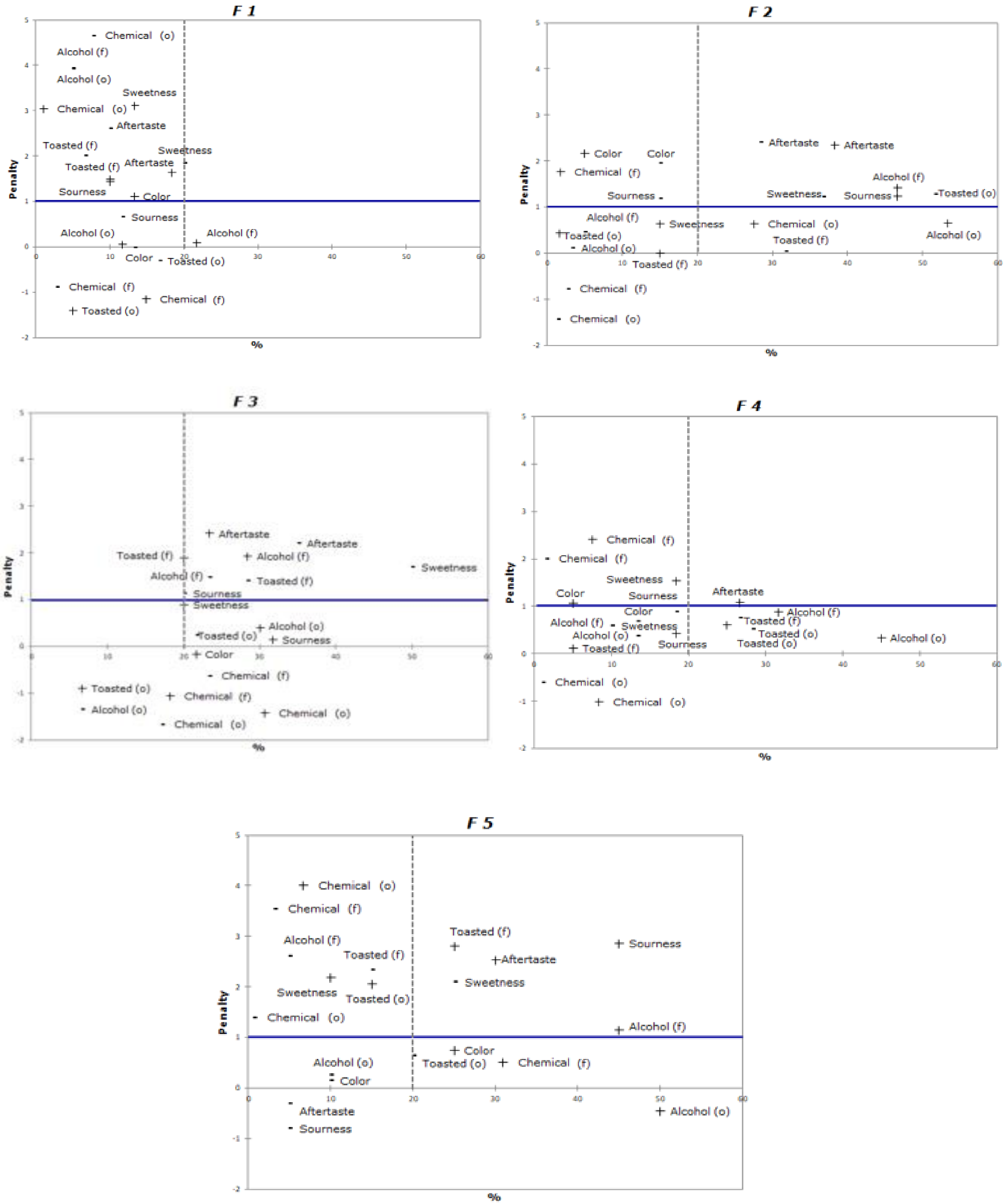

Figure S1. Penalty analysis of intensities of attributes different *Fondillón* wines by Spanish consumers. (Sample code effect indicated on the graphic title of each figure; "too low intensity" is indicated by the symbol "-" and "too high intensity" by the symbol "+".

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References

1. Souza Gonzaga, L.; Capone, D.L.; Bastian, S.E.P.; Danner, L.; Jeffery, D.W. Sensory typicity of regional Australian Cabernet Sauvignon wines according to expert evaluations and descriptive analysis. *Food Res. Int.* **2020**, *138*, 109760. [CrossRef] [PubMed]
2. Figueiredo-González, M.; Cancho-Grande, B.; Simal-Gándara, J. Effects on colour and phenolic composition of sugar concentration processes in dried-on- or dried-off-vine grapes and their aged or not natural sweet wines. *Trends Food Sci. Technol.* **2013**, *31*, 36–54. [CrossRef]
3. Rolle, L.; Torchio, F.; Giacosa, S.; Gerbi, V. Modifications of mechanical characteristics and phenolic composition in berry skins and seeds of Mondeuse winegrapes throughout the on-vine drying process. *J. Sci. Food Agric.* **2009**, *89*, 1973–1980. [CrossRef]
4. Reboredo-Rodríguez, P.; González-Barreiro, C.; Rial-Otero, R.; Cancho-Grande, B.; Simal-Gándara, J. Effects of sugar concentration processes in grapes and wine aging on aroma compounds of sweet wines—A review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1053–1073. [CrossRef] [PubMed]
5. Blackman, J.; Saliba, A.; Schmidtke, L. Sweetness acceptance of novices, experienced consumers and winemakers in Hunter Valley Semillon wines. *Food Qual. Prefer.* **2010**, *21*, 679–683. [CrossRef]
6. Alicante PDO. Fondillón de Alicante, Time Is a Luxury. Available online: http://vinosalicantedop.org/fondillon/en/ (accessed on 12 June 2021).
7. Issa-Issa, H.; Noguera-Artiaga, L.; Sendra, E.; Pérez-López, A.J.; Burló, F.; Carbonell-Barrachina, A.A.; López-Lluch, D. Volatile composition, sensory profile, and consumers' acceptance of *Fondillón*. *J. Food Qual.* **2019**, 5981762. [CrossRef]
8. Bourdieu, P. Distinction: A social critique of the judgement of taste. *Int. J. Cult. Policy* **1984**, *16*, 63–65.
9. Beckert, J.; Rössel, J.; Schenk, P. Wine as a cultural product: Symbolic capital and price formation in the wine field. *Sociol. Perspect.* **2017**, *60*, 206–222. [CrossRef]
10. Issa-Issa, H.; Guclu, G.; Noguera-Artiaga, L.; López-Lluch, D.; Poveda, R.; Kelebek, H.; Selli, S.; Carbonell-Barrachina, Á.A. Aroma-active compounds, sensory profile, and phenolic composition of *Fondillón*. *Food Chem.* **2020**, *316*, 126353. [CrossRef]
11. ISO/IEC 17065:2012. *Conformity Assessment—Requirements for Bodies Certifying Products, Processes and Services*; ISO: Geneva, Switzerland, 2012; p. 30.
12. Lipan, L.; Cano-Lamadrid, M.; Corell, M.; Sendra, E.; Hernández, F.; Stan, L.; Vodnar, D.C.; Vázquez-Araújo, L.; Carbonell- Barrachina, Á.A. Sensory profile and acceptability of hydrosostainable almonds. *Foods* **2019**, *8*, 64. [CrossRef]
13. Mas, A.; Torija, M.J.; García-Parrilla, M.D.C.; Troncoso, A.M. Acetic acid bacteria and the production and quality of wine vinegar. *Sci. World J.* **2014**, 394671. [CrossRef] [PubMed]
14. López-Guzmán, T.; Vieira-Rodríguez, A.; Rodríguez-García, J. Profile and motivations of European tourists on the Sherry wine route of Spain. *Tour. Manag. Perspect.* **2014**, *11*, 63–68. [CrossRef]
15. Santos, V.; Ramos, P.; Almeida, N.; Marôco, J.; Santos-Pavón, E. Wine tourist profiling in the Porto wine cellars: Segmentation based on wine product involvement. *Anatolia* **2020**, *31*, 577–590. [CrossRef]

PUBLICACIÓN 5

Fondillón Wine Adulteration by Addition of Other *Monastrell* Wines

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Article

Fondillón Wine Adulteration by Addition of Other *Monastrell* Wines

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Abstract: Authenticity and traceability are two issues of great importance to quality and food safety in the food industry. For wine producers and authorities, it is essential to know how to detect adulterations because wine is one of the alcoholic beverages most prone to adulteration, as indicated by the European Commission. *Fondillón* is one of the most important naturally sweet Spanish wines and is certainly the core of the Alicante PDO. *Fondillón* is a wine that is prone to be adulteration due to its limited production and high price. The aim of this study was to identify potential markers of *Fondillón* adulteration by mixing it with other *Monastrell* wines. The experimental results showed that *Fondillón* is characterized by high concentrations of acetic acid, furfural, benzaldehyde, vitispirane, and TDN and low concentrations of citric, tartaric, and malic acids; a low total phenolic content; and low values of antioxidant activity.

Keywords: acetic acid; authentication; fraud; fructose; minerals; TDN; vitispirane; volatile composition

1. Introduction

Traceability and authenticity are two key aspects for both the food and beverages industries because they are associated with the quality and safety of the produced items and the protection of consumers [1]. Traceability is a measure concerning the full control over marketed products from their origin to the production process, storage, and, finally, acquisition by consumers. Traceability increases food safety and is a tool that both producers and consumers can use to obtain all the necessary information to be able to manage any problem more easily and quickly by accessing a product's history [2]. Authenticity is the key to guaranteeing the quality of food and beverages; it is a component of food safety as it helps producers adequately comply with national legislation [3].

Regarding wine, the study of authenticity is a central part of production because wine is one of the most easily imitated food products [4]. Wine is produced following a series of long and complex processes involving physical, chemical, and biological reactions, where every step has a strong influence on the desired quality of the final product [5]. However, it is a very easy product to adulterate due to its chemical features (low pH and high alcoholic content) and its availability throughout the world. In this sense, wine is one of the products with the most complete and complex legislation [6]. In addition, it is also one of the products with the most extensive analytical procedures [7]. Both facts are directly linked to the fight against the massive quantities of adulterations available in the international wine market.

In particular, when wines are scarce and appreciated, the number of imaginative schemes with which to take advantage of their prestige and price increases. This can result in a lack of consumer confidence, and thus the system must develop new control methods [8]. Wine adulteration is a worldwide problem. In 2021, almost 1,800,000 L of wine and alcoholic beverages was seized in a specific raid led by the European Anti-Fraud Office

(OLAF) [9]. Wine fraud costs the EU wine sector an estimated EUR 1.3 billion each year, amounting to around 3 % of the total value of sales. These adulterations refer to different types of fraud, such as the (*i*) addition of alcohol, (*ii*) dilution with water, (*iii*) the addition of flavorings, (*iv*) substitution or mixture with lower quality wine, and (*v*) mislabeling (fraud with respect to the origin and the cultivar) [1,10,11].

The authenticity and commercial value of wines are linked to their geographical origin, the grape variety employed, and the year of the harvest. Therefore, the methods currently used to counterfeit wine include (*i*) fraud regarding the geographical region of origin, (*ii*) fraud regarding the grape variety used, and/or (iii) fraud regarding the vintage year [12]. This is the case of wines within a Protected Designation of Origin (PDO). These wines are produced using specific grape varieties cultivated in specific geographical regions under controlled growing and production conditions. In this sense, one of the main purposes of PDO Regulatory Councils is the prevention of fraud by guaranteeing the origin and quality of wines [7].

Until recently, tasting by experts was the most widely used method with which to evaluate and certify wine's authenticity. However, this technique is time consuming and too expensive because it requires a highly trained panel to be able to detect some of these types of fraud; thus, a new strategy has been developed that combines the latest analytical technology and advanced mathematical tools. Studies have developed a series of methods used to assess the authenticity of wines, which include the determination of polyphenols, the identification and quantification of odor-active compounds, the determination of minerals, and determination of organic acids and sugars [13,14].

Fondillón, an Alicante PDO wine with a production process constituting at least 10 years of aging and an alcoholic strength of over 16 %, which is obtained from overripe on-vine *Monastrell* grapes and is produced without the addition of alcohol, is a perfect candidate for adulteration, which can occur through different methods, but one of the most difficult to identify is its mixing with cheaper *Monastrell* wines, even with those coming from the Alicante PDO. Depending on the season, approximately 2000 to 5000 bottles are sold annually, and this wine has a high price, namely, ~EUR 75 per 700 mL bottle; however, there is no validated methodology to prevent the production and circulation of fraudulent versions of this wine.

Consequently, the aim of this study was to find chemical markers to identify potential instances of the adulteration of *Fondillón* by mixing it with other *Monastrell* wines, which are the closest ones to the sensory profile of this wine. The following parameters have been considered as potential markers and have been evaluated accordingly: sugars, organic acids, minerals, antioxidant activity, and volatile compounds. Another goal of this study was to analyze the authenticity of pure *Fondillón* using gas chromatography and mass spectrometry (GC–MS) combined with chemometrics. For this purpose, two other *Monastrell* wine samples were used to prepare adulterated *Fondillón* samples; measurements of key parameters of these samples were conducted using GC–MS, and the data were evaluated using principal component analysis (PCA).

2. Materials and Methods

2.1 Reagents and Chemicals

The standards for identification of volatile compounds shown in Table S1 were all purchased from Sigma Aldrich (Steinheim, Germany). The compounds 2,2-diphenyl-1-picrylhydrazyl and 2,2-azinobis-(3- ethyl benzothiazoline acid-6-sulfonic used for the analysis of the antioxidant activity were supplied by Fisher Scientific (Hampton, NH, USA). The standards for the minerals were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2 Wine Samples

The three wine samples (young, aged/crianza, and *Fondillón*) used for this study were kindly provided by Bodegas BOCOPA (Petrer, Alicante, Spain); they were supplied in triplicate from three different batches and taken to the facilities of the Miguel Hernández University of Elche (Orihuela, Alicante, Spain).

The main physicochemical quality parameters (total and volatile acidity; total alcoholic content and reducing sugars) were analyzed following the official methods of the International Organization of Vine and Wine (OIV) (published in accordance with article 15 Commission Regulation (EC) No. 606/2009 of 10 July 2009, which can be found on the OIV website) [15]. The main characteristics of the wines used were as follows: (*i*) young wine—prepared using exclusively *Monastrell* grapes from the 2019 season, with 15 % alcohol, total acidity equal to 3.9 g L^{-1} tartaric acid, and volatile acidity equal to 0.4 g L^{-1} acetic acid; (*ii*) aged/crianza wine—prepared using exclusively *Monastrell* grapes from the 2017 season, aged for 24 months in total, including 6 months in oak barrels, with 15 % alcohol, a content of reducing sugars of 48 g L^{-1}, a total acidity of 4.2 g L^{-1} tartaric acid, and a volatile acidity of 0.7 g L^{-1} acetic acid; and (*iii*) *Fondillón*—prepared exclusively from overripe *Monastrell* grapes from the 1988 season, aged (using the vintage system) for 32 years in old oak barrels and a subsequent 2 years in a bottle, with 21 % alcohol, reducing sugars of 40 g L^{-1}, total acidity of 5.3 g L^{-1} tartaric acid, and volatile acidity of 0.7 g L^{-1} acetic acid.

Pure aged/crianza *Monastrell* and young *Monastrell* wine samples were used separately to prepare wine mixtures (*Fondillón*-aged wine and *Fondillón*-young wine) to simulate *Fondillón* adulteration. Both "*Fondillón*–aged" and "*Fondillón*–young" mixed samples were prepared with different *Fondillón* replacement percentages of 0, 30, 50, 70, and 100 %.

2.3 Sugars and Organic Acids

For the identification and quantification of sugars and organic acids, an HP 1100 series high-performance liquid chromatography (HPLC) machine (Wilmington, DE, USA) was used, as previously described [16], with slight modifications. For the preparation of the samples, 5 mL of the wines was extracted and centrifuged at 15,000 rpm (28.980 g) and 4 °C for 10 min. Subsequently, the supernatant was filtered through 0.45 μm Millipore filters and stored in amber vials, and 10 μL was then injected into the chromatograph using an elution buffer (0.1 % phosphoric acid with a flow rate of 0.5 mL min^{-1}). Organic acids were isolated using a column (Supelcogel TM C-610H 30 cm × 7.8 mm) and a pre-column (Supelguard 5 cm × 4.6 mm; Supelco, Bellefonte, PA, USA). Absorbance was measured at 210 nm with a diode array detector (DAD). Sugars were detected for analysis using a refractive index detector (RID), using the same HPLC conditions (elution buffer, flow rate, and column).

Calibration curves of sugars (glucose and fructose) and organic acids (citric, tartaric, lactic, acetic, and malic acid) were developed in accordance with the proper standards, with a concentration range between 1 and 10 g L^{-1}. The analyses were performed in triplicate and the results were expressed as g L^{-1}.

2.4 Antioxidant Activity (AA), Total Polyphenol Index (TPI), and Chromatic Characteristics

2.4.1 Antioxidant Activity (AA)

For the determination of AA, 3 methods were used: (*i*) FRAP to evaluate the ferric reducing/antioxidant power and (*ii*) DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) and (*iii*) ABTS$^{\bullet+}$ [2,2-azinobis-(3-ethylbenzothiazoline acid-6-sulfonic) to assess the ability to scavenge free radicals. For the evaluation of FRAP, the method described by Iris and Benzie et al. [17], with slight modifications, was followed, wherein absorbance was measured at 593 nm. The method for the determination of DPPH$^{\bullet}$ was that reported by Katalinic et al. [18], wherein absorbance was recorded at 517 nm. Finally, the ABTS$^{\bullet+}$ was determined following the method proposed by Re et al. [19], employing a measurement of absorbance at 734 nm.

A UVG1002E UV–vis spectrophotometer (Helios, Cambridge, UK) was used. For the preparation of the sample, 5 mL of wine was extracted; additionally, 5 mL of extractant was added [methanol/water (80:20, v/v) + 1 % HCl] and sonicated for 15 min. Then, the mixture was left to stand overnight at 4 °C. The samples were sonicated again for 15 min and centrifuged for 10 min at 10,000 rpm (12.880 g). The supernatant was collected and placed in amber vials. All analyses were performed in triplicate and results were expressed as mmol Trolox L^{-1}.

2.4.2 Total Polyphenol Index (TPI)

The extract used for the analysis of the antioxidant activity was also used for the quantification of the TPI. Absorbance was measured at a wavelength of 280 nm in a UV–visible spectrophotometer (Helios Gamma, UVG 1002E, ThermoSpectronic Helios, Cambridge, UK) [20]. The TPI was determined after carrying out a 100-fold dilution of the sample. Analyzes were performed in triplicate.

2.4.3 Chromatic Characteristics

The chromatic characteristics, namely, (*i*) tonality, (*ii*) color intensity, and (*iii*) color density, of the wine samples were analyzed according to the method described in [21]. For this analysis, a spectrophotometer ThermoSpectronic Helios (Cambridge, UK) was used. The Glories color index percentages [22] of red, yellow, and blue were evaluated by measuring absorbances at 420, 520, and 620 nm, respectively.

2.5 Minerals

The concentrations of macro- and micro-elements were determination using an inductively coupled plasma mass spectrometer (ICP-MS): Shimadzu ICPMS-2030 (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). For the preparation of the samples, 1 mL of wine was diluted to 10 mL with HNO_3 1 % (1:10 dilution); then, this portion was passed through 0.45 μm Millipore filters and stored in 15 mL Falcon tubes.

Calibration curves were created with the mineral standards, where for the macroelements (Ca, Mg, Na, and K) the concentration ranges between 1 and 20 mg L^{-1}, while for the micro-elements (Fe, Cu, Mn, and Zn) the concentration ranges between 0.01 and 0.2 mg L^{-1}. The analyses were performed in triplicate and the results were expressed as mg L^{-1}.

2.6 Volatile Compounds

The volatile profile was determined according to Issa-Issa et al. [23]. The identification and semi quantification of volatile compounds was carried out using a Shimadzu GC2030 gas chromatograph and a TQ8040 NX triple quadrupole mass spectrometer as detector, as well as GC–MS (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) with an AOC-6000Plus. For the preparation of the samples, 10 mL of wine was extracted and placed in a 20 mL vial with 10 μL of benzyl acetate used as internal standard (100 mg L^{-1}); finally, 1.5 g of NaCl was added. Volatile compounds were extracted using the HS-SPME technique and a DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA). The sample was then placed in an autosampler at 500 rpm and 40 C for 20 min. The GC program temperature scheme was as follows: holding at 40 °C for 2 min, followed by a +3 °C min^{-1} increase to 250 °C. The helium pressure was 50.4 kPa, and the flow control mode was linear velocity (at 36.3 cm s^{-1}). The injection temperature was 260 °C, the ion source temperature was 200 ° C, and the interface temperature was 250 °C. Helium was used as carrier gas, with a total flow rate of 1.01 mL min^{-1}. Most of the volatile compounds were identified by 3 different methods: (*i*) retention indices of the analyzed compounds, calculated using the C7 to C16 n-alkane mix (Sigma-Aldrich, Steinheim, Germany); (*ii*) retention indices of standards; and (*iii*) comparison of the mass spectra obtained with those of the standards and those of the NIST 14 and Wiley 229 spectrum libraries. All analyses were performed in triplicate and the results were expressed as mg L^{-1}.

2.7 Statistical Analysis

The processing of the results was initially carried out with one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. Differences were considered statistically significant at $p < 0.05$. The software used to perform the statistical analyses was XLSTAT Premium 2016 (Addinsoft, New York, NY, USA). Subsequently, data analysis of GC–MS measurements was performed using principal component analysis (PCA) with Stand-alone Chemometrics Software (Version Solo 9.1. for Windows, Eigenvector Research Inc., Wenatchee, WA, USA). The data obtained from GS-MS measurements of pure (*Fondillón* (n = 3), aged (n = 3), and young (n = 3)) wines and *Fondillón* samples adulterated with aged (n = 9) or young (n = 9) *Monastrell* wines at different ratios (30 %, 50 %, and 70 %, v/v) were used to develop a PCA model. Autoscale pre-processing step was applied to the data while developing the model. Three separate PCA models were constructed. The first PCA model consisted of the three pure wine sample groups (*Fondillón*, aged and young *Monastrell* wines). The second PCA model was created using pure *Fondillón*, aged sample groups, and adulterated *Fondillón* sample groups by addition of aged wine at different ratios 30 % (n = 3), 50 % (n = 3), and 70 % (n = 3). Finally, the last PCA model also incorporated the pure *Fondillón*, young sample groups, and adulterated *Fondillón* sample groups doctored by the addition of young wine at different ratios (30 % (n = 3), 50 % (n = 3), and 70 % (n = 3)). The number of principal components (PCs) for the PCA models was selected by leave-one-out cross validation, where all calibration samples were validated by one. The success of the models was evaluated based on root mean squared error calibration (RMSEC), root mean squared error cross-validation (RMSECV) values, percent per variance, and percent cumulative variance of the PC.

3. Results and Discussion

3.1 Sugars and Organic Acids

It is important to remember that the organic acids in wine limit oxidation and, together with ethanol, are responsible for the physicochemical and microbial stability of wine. They also influence its flavor and improve its color, rendering it more stable. The organic acids contained in wine are produced by the oxidation of sugars (e.g., citric, tartaric, and malic acid) or by alcoholic fermentation (e.g., lactic, acetic, and succinic acid) [24]. *Fondillón* contained the lowest concentrations of all organic acids, except acetic acid. All three samples had a similar amount of lactic acid, which is logical as this compound is produced during the lactic fermentation of the *Monastrell* must and remains constant during aging. The decrease in the concentrations of *Fondillón* citric, tartaric, and malic acid reached values of 66.4, 51.1, and 72.2 %, respectively, compared to the young wine (**Table 1**). A similar trend was recently reported by Valcárcel-Muñoz et al. [25], who studied the characterization of Fino and Amontillado Sherries during aging in the criaderas and solera system. These authors reported that the concentration of malic acid (originating from the grapes) decreased significantly because it was consumed by flor yeasts and lactic bacteria; citric acid (which is a component of yeast metabolism during the formation of the flor yeast veil) also showed a decreasing trend during the aging of the oxidative *Monastrell* wines. Tartaric acid, which originated from the (*i*) grapes and (*ii*) was supplemented during the vinification process because *Monastrell* grapes grown under the dried conditions of the Mediterranean vineyards require tartaric acid to be added during the operation of the grape-crushing unit and before the start of fermentation because their pH would otherwise be too high, was precipitated as calcium tartrate and potassium bitartrate during the aging of the wine; thus, the *Fondillón* samples had the lowest concentrations of the studied *Monastrell* wines.

Table 1. Concentration (g L^{-1}) of organic acids and sugars identified in *Monastrell* young, aged and *Fondillón* wines.

Wine type	Organic acids					Sugars	
	Citric	Tartaric	Malic	Lactic	Acetic	Glucose	Fructose
	(g L^{-1})						
	ANOVA†						
	***	***	***	*	***	***	***
	Tukey Multiple Range Test‡						
Young	2.50 b	5.15 b	6.95 b	4.73 ab	0.87 b	5.82 b	4.10 b
Aged	3.72 a	6.95 a	9.78 a	5.44 a	0.63 c	6.89 b	3.88 b
Fondillón	0.84 c	2.52 c	1.93 c	4.42 b	1.27 a	7.12 a	6.01 a

** and *** significant at $p < 0.05$ and 0.001, respectively. ‡ Values (mean of three replications) followed by the same letter, within the same column, were not significantly different ($p > 0.05$), according to Tukey's least significant difference test.*

Regarding acetic acid, the opposite trend occurred, with its content increasing after the long aging of *Fondillón*, reaching a value of 1.27 g L^{-1}. This concentration is below the detection threshold for this volatile compound of 3.185 g L^{-1} reported in ice wine, which is also a naturally sweet wine; however, it is above the detection threshold reported for table wine 0.7 g L^{-1} [26]. Acetic acid is formed by the oxidation of ethanol via acetaldehyde and acetyl groups that are present in wood xylans. As reported by Caldeira et al. [27], the same trend occurred in his study on the kinetics of odorant compounds in a wine brandy aged in two aging systems (in barrels and in steel tanks with staves inside); the acetic acid presented a significant increase over time in the studied aging systems. It is important to highlight that the presence of low concentrations of acetic acid is not considered an off-flavor but rather a characteristic attribute of this type of aged-wines [27].

Regarding sugars, these compounds are responsible for the formation of ethanol and other secondary products. The main sugars used by yeasts during alcoholic fermentation are glucose and fructose, which are used to determine the optimal maturity of the grape [28,29]; however, in the current study, the highest sugar concentrations were found in *Fondillón* (**Table 1**). The increase in sugars may be influenced by the hydrolysis of wood hemicellulose during oxidative aging [30]. In the study carried out by Valcárcel-Muñoz et al. [25], the sugar concentration increased over time due to the transfer of certain compounds such as pentoses, hexoses, and hemicellulose polysaccharides from the wooden barrels to the wine.

3.2 Antioxidant Activity (AA), Total Polyphenol Index (TPI), and Chromatic Characteristics

Previous studies showed a non-consistent behavior of the antioxidant potential of wine as affected by the aging time. Authors such as Echeverry et al. [31] reported that the aging of wine increased its antioxidant potential; however, other authors, e.g., Roginsky et al. [32], found that young wines presented higher values of ABTS+. In the current study, the experimental results showed a global trend, with the antioxidant activity, measured by three methods ($ABTS^{\bullet+}$, FRAP, and $DPPH^{\bullet}$), increasing at the beginning of the aging period but significantly decreasing after the minimum of 10 years of aging required for the preparation of *Fondillón* (**Table 2**). These results agree quite well with those published by Rivero-Pérez et al. [33], who studied the total antioxidant capacity ($ABTS^{\bullet+}$, $DPPH^{\bullet}$, DMPD, ORAC, and FRAP), the scavenging activity, and biomarkers of oxidative stress of wines and concluded that young wines presented higher values of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$; this behavior occurs in these two specific methods of measuring AA because they have the same antioxidant mechanism, which is a single electron transfer mechanism. In general, during the wine-aging process, the concentrations of phenolic compounds normally show a slow decrease due to adsorption, precipitation, and/or reduction in the degree of polymerization and astringency [34]. The experimental results demonstrate that the *Fondillón* samples had the lowest content of TPC (**Table 2**); this trend agreed with that previously reported by Chira et al. [35].

Table 2. Antioxidant activity, total phenolic index, and chromatic characteristics identified in *Monastrell* young, aged and *Fondillón* wines.

Wine Type	ABTS•+	FRAP	DPPH•	Total Phenolic Index	Color Intensity	Tonality	Color Density	Y ¶	R ¶	B ¶
	(mmol Trolox L^{-1})			(mg AG/100 mL Wine)				(%)		
	ANOVA †									
	***	***	***	***	***	***	***	***	**	**
	Tukey Multiple Range Test ‡									
Young	2.30 a	7.42 b	2.18 b	285 a	9.13 a	0.60 b	7.88 a	32.4 b	53.9 a	13.7 a
Aged	2.35 a	13.9 a	2.43 a	289 a	8.70 a	0.60 b	7.48 a	32.4 b	53.5 a	14.1 a
Fondillón	1.28 b	3.52 c	1.49 c	221 b	3.92 b	1.87 a	3.62 b	60.1 a	32.2 b	7.73 b

*** and *** indicate significance at $p < 0.01$ and 0.001, respectively. ‡ Values (mean of three replications) followed by the same letter within the same row are not significantly different ($p > 0.05$) according to Tukey's least significant difference test. ¶ Y mean yellow color, R mean red color, and B mean blue color.*

Regarding tonality (**Table 2**), the *Fondillón* samples were characterized by the highest value of the yellow component and the lowest ones of the red and blue components. During aging, there is an increase in the *Y* component and a decrease in the *R* component, which leads to an increase in the wine's tonality, rendering it more brownish. These results agreed with those reported by Del Fresno et al. [36] in their study on the changes in the phenolic fraction of red wines aged in oak barrels, where they indicated that all the wines studied, after several days of aging, exhibited an increase in absorbance at 420 nm and a decrease in absorbance at 520 nm that corresponded to the *Y* and *R* components, respectively, leading to an increase in tonality.

3.3 Minerals

There are three main sources of metals in wines [37]:

1. Natural source: This is related to the grape variety, its maturity at harvest, and the type of soil and weather conditions during the growth process.
2. Anthropogenic source: This refers to the (external) impurities of the environment that the wine acquires while the grape is growing or during the different winemaking processes (e.g., bottling, aging, etc.).
3. Oenological source: This refers to the different steps of wine production.

The concentrations of some elements can play a very important role in the vinification process; an example is Zn, which, at low concentrations, is important for the proper development of alcoholic fermentation, while other elements such as Fe, Mn, and Cu at higher concentrations can influence the organoleptic profile of the wine [38]. Very high Fe content can cause stabilization problems, causing wine to suffer from oxidation.

In **Table 3**, Na, Cu, Mn, and Zn from *Fondillón* presented higher concentrations compared to the young and aged *Monastrell* wines. Cu and Mn are involved in changes in the stability of aged wines. During wine storage and maturation, these elements form stable complexes with polyphenols, meloids, and amino acids, leading to the characteristic color, flavor, and final aroma of aged wines [39].

Table 3. Concentration (mg L^{-1}) of micro- and macro-elements quantified in *Monastrell* young, aged and *Fondillón* wines.

Wine Type	Ca	Mg	Na	K	Fe	Cu	Mn	Zn
	(mg L^{-1})				(µg L^{-1})			
	ANOVA †							
	**	***	***	**	***	**	***	***
	Tukey Multiple Range Test ‡							
Young	8.88 a	14.2 c	2.87 c	148 a	30 c	Traces b	60 c	10 c
Aged	8.36 b	16.1 a	3.79 b	125 b	170 a	Traces b	60 b	20 b
Fondillón	7.93 c	15.3 b	4.94 a	144 a	140 b	10 a	110 a	40 a

*** and *** indicate significance at $p < 0.01$ and 0.001, respectively. ‡ Values (mean of three replications) followed by the same letter within the same column are not significantly different ($p > 0.05$) according to Tukey's least significant difference test.*

All the *Monastrell* wines under analysis contained relatively high concentrations of K and Mg, which are elements related to the grape's maturity and, consequently, its sweetness [40]. The Ca concentrations were low compared to those reported in international wines, including Spanish ones, for which Ca ranged from 12 to 241 mg L^{-1} [39]. A similar phenomenon was found for K, which ranged from 338 to 2032 mg L^{-1} in Spanish wines [39].

3.4 Volatile Compounds

Wine is made up of many volatile compounds; some of them come from the grape, some from the yeast and the alcoholic fermentation, and others from the aging of the wine [41]. In fact, during the aging period, some compounds from the oak barrels can be transferred into the wine while others are produced by the reaction of the aroma-precursors, leading to a complex final aromatic profile [42].

To detect a possible *Fondillón* fraud, it is very important to establish possible aging markers that are not present in other younger *Monastrell* wines; these compounds can be considered as indicators and can be used to guarantee and ensure the authenticity of this Alicante wine [43].

A total of 93 volatile compounds of the wines under study (young and aged *Monastrell* wines and *Fondillón*) were identified and quantified (**Table S1**), with 18 compounds playing a key role in identifying possible adulterations of *Fondillón* (**Table 4**). Some of the volatile compounds identified in *Fondillón*, such as furfural (0.60 mg L^{-1}), guaiacol (0.01 mg L^{-1}), and eugenol (0.01 mg L^{-1}), were previously reported by Perestrelo et al. [44] as wood markers. The intensity of the release of these volatile compounds can be controlled by several factors, including the type of barrel, the toasting of the barrel, the time the wine remained in contact with the barrel, etc. As reported by Perestrelo et al. [43], the concentration of furfural shows a tendency to increase during the aging process and is considered a good age marker. This compound can be formed by (*i*) the dehydration of sugars due to the Maillard reaction, (*ii*) the pyrolysis of carbohydrates, and(*iii*) caramelization.

Another key volatile compound was benzaldehyde, which was present in *Fondillón* in a relatively high concentration (0.28 mg L^{-1}) compared to the trace concentrations found in the young and aged/crianza *Monastrell* wines. This trend agreed with the one reported by Castro-Vázquez et al. [45], who reported low benzaldehyde concentrations in young "Tempranillo" wines. In general, benzaldehyde can be considered as a typical aroma of the volatile profile of aged red wines.

Table 4. Concentrations (mg L^{-1}) of the key volatile compounds in *Monastrell* (young and aged) and *Fondillón* wines.

Code	Volatile Compounds	Young	Aged	*Fondillón*
			(mg L^{-1})	
V1	1,1-diethoxyethane	nd	nd	0.13
V6	Furfural	nd	0.09	0.60
V13	1,1-diethoxy-2-methylpropane	nd	nd	0.02
V14	Benzaldehyde	nd	0.01	0.28
V15	1-(1-ethoxyethoxy)-pentane	nd	nd	0.03
V28	Isoamyl butyrate	nd	nd	0.04
V30	Guaiacol	nd	nd	0.01
V31	Ethyl sorbate	nd	nd	0.13
V33	Nonanal	nd	nd	0.11
V39	Diethyl butanedioate	1.86	1.90	2.80
V53	4-Ethylguaiacol	nd	nd	0.02
V55	Vitispirane	0.12	0.22	0.37
V57	trans-Whiskey lactone	0.02	0.05	0.06
V60	cis-Whiskey lactone	0.11	0.09	0.17
V64	Eugenol	nd	0.01	0.01
V65	TDN	0.00	0.04	0.35

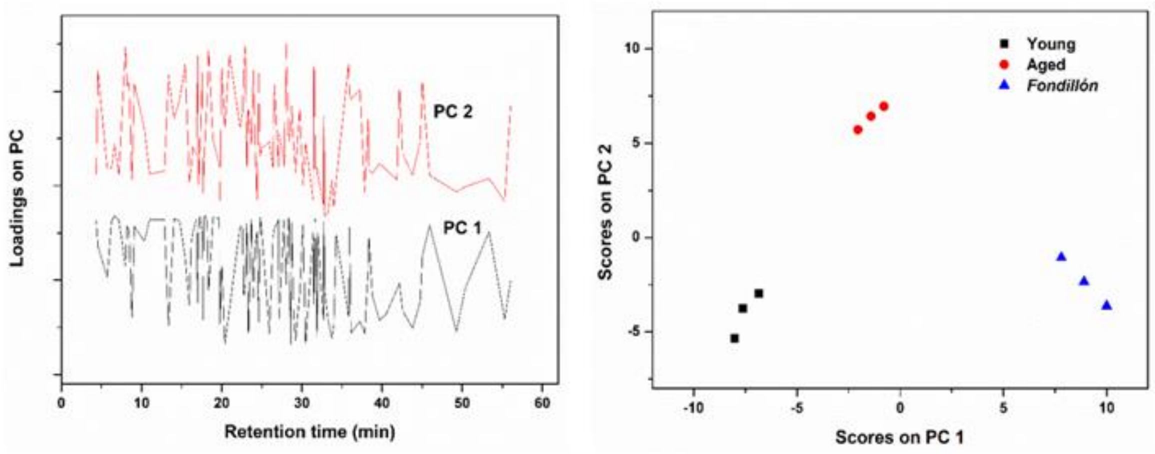
nd: not detected.

In general, the concentrations of norisoprenoids, such as vitispirane and TDN, increase during the aging of wine (**Table 4**) and have previously been identified as the odor-active compounds of *Fondillón* [46]. The concentration of TDN in wine can be influenced by four main factors: (*i*) the exposure of the grapes to the sun, (*ii*) the pH, (*iii*) the storage temperature, and (*iv*) the wine's age [47]. This same trend was reported by Marais et al. [48], who concluded that TDN is a key compound because it is responsible for the kerosene-like flavor that develops during the aging process of Weisser Riesling wines. The same trend was reported for vitispirane by Khairallah et al. [49]. Both compounds (vitispirane and TDN) are generated from the decomposition of carotenoid precursors and are released through acid hydrolysis or enzymatic reactions.

Although not in very high concentrations, other compounds, such as 1,1-diethoxyethane;1,1-diethoxy-2-methyl-propane; and 1-(1-ethoxyethoxy)-pentane, are only present in *Fondillón*. These compounds are formed during fermentation and the concentrations increase during aging, possibly as a result of oxidation, as reported by Versari et al. [44].

3.5 Principal Component Analysis (PCA)

The classification of pure wines and adulterated sample groups was performed using the measurements obtained by the GC–MS chromatogram. The GC–MS chromatograms of the pure wine samples (*Fondillón* + aged/crianza + young) and adulterated *Fondillón* sample groups via the addition of aged *Monastrell* and young *Monastrell* wines are shown in **Figure S1a–c.** As can be seen in **Figure S1a**, the chromatograms of pure wines are quite different from each other due to the change in the concentration of volatile compounds. The difference in the chromatogram between *Fondillón* and other wines is most evident for the furfural, benzaldehyde, ethyl hexanoate, phenyl ethyl alcohol, diethyl butanediate, vitispirane, TDN, and ethyl decanoate components. Chromatographic data analyses of the GC–MS measurements of the pure and adulterated sample (*Fondillón* + young *Monastrell* wine and *Fondillón* + aged/crianza *Monastrell* wine) groups were performed separately via PCA. To classify the three pure wine sample groups (young, aged, and *Fondillón*), a PCA model was created using the first two PCs, which explained 50.45 % (PC1) and 23.56 %(PC2) of the per cumulative data variance. The PCA loading and score plot of the three wine samples are presented in **Figure 1a, b**. The PCA plot results showed that a good classification was achieved for all the wine sample groups. The explained per variance, cumulative variance, and error values (RMSEC and RMSECV) of the PCA models are given in **Table 5**; as can be seen in this table, low error values for RMSEC (0.48) and RMSECV (3.64) were obtained from this PCA model.



(a) (b)

Figure 1. Principal component analysis (PCA) loading (**a**) and score plot (**b**) of GC–MS measurements of pure *Monastrell Fondillón,* aged, and young wines.

Table 5. The per cumulative variance of the principal components (PCs), root mean square error calibration (RMSEC), and root mean square error cross-validation (RMSECV) values of the principal component analysis (PCA) models formed using the *Monastrell* (young and aged) and *Fondillón* wines.

Wine Type	PCs	Variance (%)		RMSEC	RMSECV
		This PC	Cumulative		
Young/Aged—*Fondillón*	1	50.45	50.45	0.48	3.64
	2	23.60	74.01		
Fondillón—Aged	1	33.12	33.12	0.62	2.16
	2	26.78	59.90		
Fondillón—Young	1	40.29	40.29	0.65	1.84
	2	16.10	56.39		

The second PCA model was developed for the adulterated *Fondillón* sample groups prepared by the addition of *Monastrell* aged/crianza wine. Using the first two PCs, the model was constructed for the separation of five sample groups (pure *Fondillón*, 70 %*Fondillón*, 50 % *Fondillón*, 30 % *Fondillón*, and pure aged/crianza *Monastrell* wine). The PCA loading and score plot of the pure and adulterated sample groups is presented in **Figure 2a, b**.

The explained per PC variance, total variance, RMSEC and RMSECV values of the PCA model are listed in Table 5. The explained per PC variance values for PCs 1 and 2were 33.12 and 26.78, respectively, and low error values (0.62 and 2.16) were also obtained for RMSEC and RMSECV, respectively. As can be seen from the figure, the classification of the each of the sample groups (pure and adulterated) has been achieved. The loading plots (**Figure 2a**) of PC1 and PC2 show that the main differences among the chromatograms were found in the region of the retention times between 5–10 min (furfural and isoamyl acetate),10–20 min (benzaldehyde, ethyl hexanoate, and limonene) and 20–30 min (phenyl ethyl alcohol, diethyl butanedioate, and vitispirane), and 30–40 min (TDN, ethyl decanoate, and isoamyl octanoate).

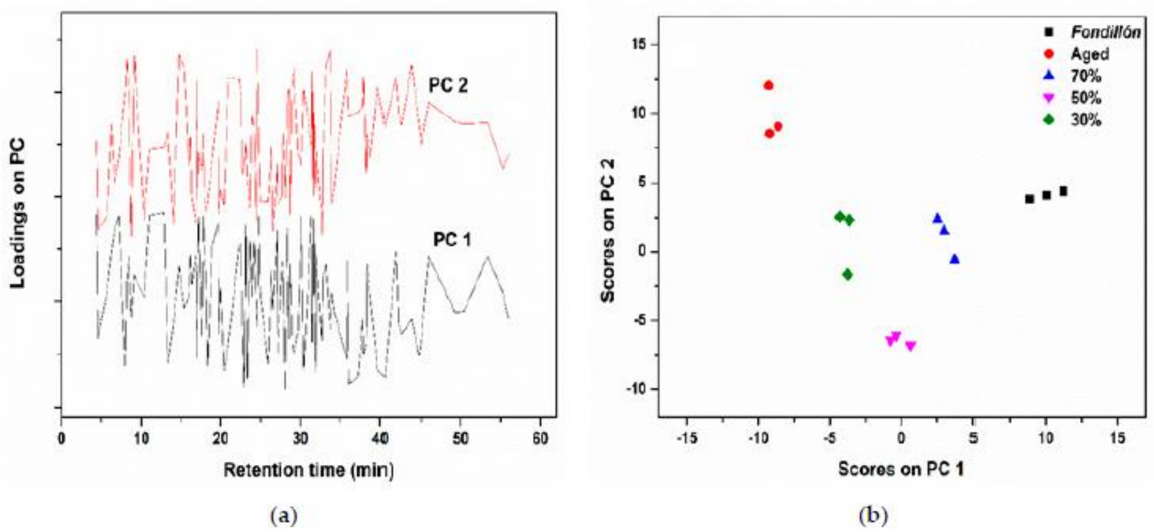


Figure 2. Principal component analysis (PCA) loading (a) and score plot (b) of GC–MS measurements of adulterated *Fondillón* samples via the addition of *Monastrell* aged wine.

Finally, another PCA model with the first two PCs was created for the classification of the adulterated *Fondillón* sample groups by the addition of pure, young *Monastrell* wine (pure 100% *Fondillón*, 70 % *Fondillón*, 50 % *Fondillón*, 30 % *Fondillón*, and pure young wine). **Figure 3a, b** demonstrate the PCA loadings and score plots of the all the pure and adulterated sample groups. As can be seen in Table 5, low RMSEC (0.65) and RMSECV (1.84) values were obtained. The explained per PC variance values for PC1 and PC2 were deter-mined to be 40.16 and 16.10, respectively. The main chromatographic contributions observed in the loadings of PC1 and PC2 (**Figure 3a**) were in the regions of the retention times between 4–10 min (isoamyl alcohol, furfural, and 1-Hexanol), 10–20 min (benzaldehyde, ethyl hexanoate, and limonene), 20–30 min (diethyl butanedioate, vitispirane, and ethyl nonanoate), 30–40 min (TDN and ethyl dodecanoate), and 40–50 min (ethyl tetradecanoate).

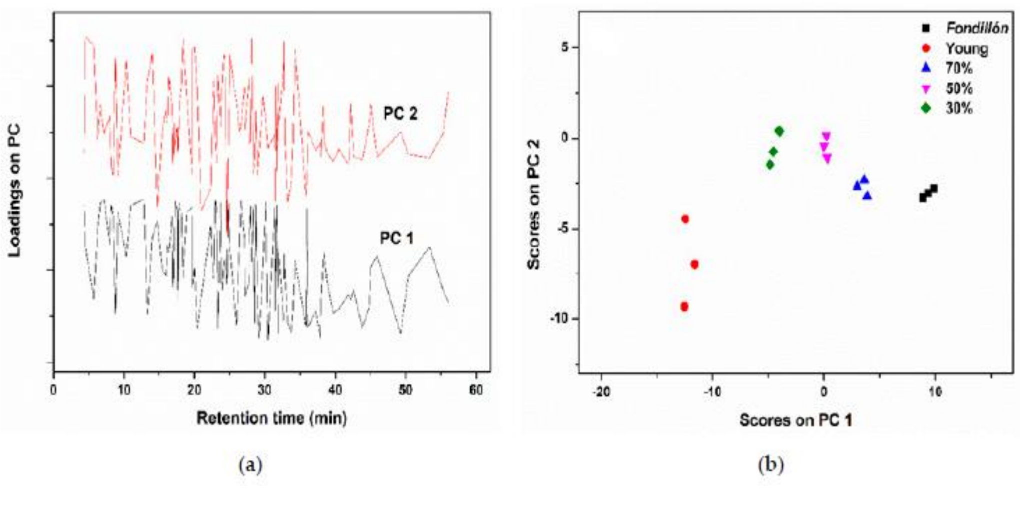


Figure 3. Principal component analysis (PCA) loadings (a) and score plot (b) of GC–MS measurements of *Fondillón* samples adulterated by the addition of *Monastrell* young wine.

As a result, firstly, based on the difference in the concentration of volatile components, the pure *Fondillón* wine was successfully differentiated from the other pure wine (aged and young) samples. Then, adulterated *Fondillón* sample groups could be distinguished from each other in the different regions of the PCA score plot. This study demonstrated that measurements of volatile compounds can be used to determine the adulteration of *Fondillón* by the addition of cheaper types of wine. To the best of our knowledge, regarding wine studies, *Fondillón* adulteration and volatile compound analysis of *Fondillón* have not been carried out using the method applied herein.

4. Conclusions

To summarize the results and identify the main markers of potential *Fondillón* adulteration using *Monastrell* wines, it can be stated that *Fondillón* is characterized by

- ✓ Relatively high concentrations of Cu (~10 μg L^{-1}), Mn (~110 μg L^{-1}), Zn (~40 μg L^{-1}), and Na (~4.5 g L^{-1});
- ✓ Relatively high concentrations of fructose (~6.0 g L^{-1}) and acetic acid (>1.0 g L^{-1});
- ✓ Relatively high concentrations of the following volatile compounds: 1,1-diethoxyethane (~10 mg L^{-1}), furfural (~0.6 mg L^{-1}), benzaldehyde (~0.3 mg L^{-1}), vitispirane (~0.3 mg L^{-1}), and TDN (~0.3 mg L^{-1});
- ✓ Relatively low concentrations of the following volatile compounds: ethyl octanoate (≤ 2.5 mg L^{-1}) and ethyl decanoate (≤ 2.0 mg L^{-1}).

Thus, if a sample marketed under the name *Fondillón* does not fulfill all the above listed criteria it is likely an adulterated sample and deserves further study by a trained sensory panel to finally certify its authenticity or adulteration. One drawback of this study is that it was conducted using wine samples from only one winery, but these preliminary statements will be further studied in *Fondillón* samples from as many Alicante wineries as possible (n = 5–8) to build a robust mathematical model that clearly classifies whether a sample being commercialized as *Fondillón* is a real *Fondillón* or a fake *Fondillón*.

Supplementary Materials:

Table S1. Concentrations (mg L-1) of volatile compounds in *Monastrell* young, aged and *Fondillón* wines.

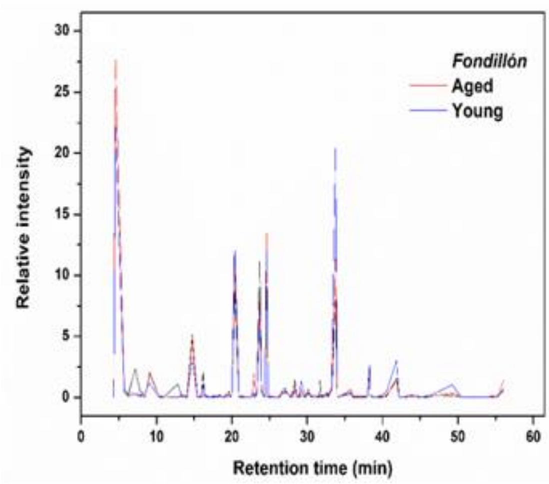
Code	Volatile compound	Retention Time (min)	Chemical Family	Odor Threshold† (µg L⁻¹) †	Odor Descriptor ‡	ANOVA¥	Young	Aged	*Fondillón*
							(mg L⁻¹)		
							Tukey Multiple Range Test £		
V1	1,1-diethoxyethane	4.333	Acetals	-	Honey, floral, green	***	0.00 b	0.00 b	0.13 a
V2	Isoamyl alcohol	4.541	Alcohols	30000	Cheese	NS	6.26	8.19	7.1
V3	2,3-Butanediol	5.689	Alcohols	150000	Buttery	NS	0.18	0.16	0.17
V4	Ethyl butanoate	6.240	Esters	20	Fruity	NS	0.02	0.02	0.04
V5	Ethyl Lactate	6.591	Esters	-	Butter, fruity	**	0.07 b	0.15 b	0.27 a
V6	Furfural	7.222	Aldehydes	14100	Almond, woody, sweet	***	0.00 b	0.09 b	0.60 a
V7	Ethyl 2-methylbutanoate	7.978	Esters	0.1	Apple, green, fruity	*	0.02 b	0.05 a	0.03 ab
V8	Ethyl 3-methylbutanoate	8.158	Esters	3	Fruity, sweet apple, cherry	**	0.05 b	0.13 a	0.13 a
V9	Ethyl Isovalerate	8.426	Esters	3	Apple	NS	0.00	0.01	0.01
V10	1-Hexanol	8.788	Alcohols	8000	Herbaceous, woody, sweet	NS	0.29	0.24	0.22
V11	Isoamyl acetate	9.111	Esters	30	Banana, pear	NS	0.35	0.56	0.45
V12	Butyrolactone	10.339	Lactones	-	Caramel	NS	0.02	0.03	0.03
V13	1,1-diethoxy-2-methylpropane	10.898	Acetals	-	-	**	0.00 b	0.00 b	0.02 a
V14	Benzaldehyde	12.773	Aldehydes	2000	Almond, anise, vanilla	***	0.00 b	0.01 b	0.28 a
V15	1-(1-ethoxyethoxy)-pentane	13.388	Acetals	-	-	**	0.00 b	0.00 b	0.03 a
V16	Hexanoic acid	14.056	Acids	420	Cheese, fatty, sour	NS	0.01	0.02	0.02
V17	Ethyl hexanoate	14.744	Esters	14	Fruity, floral, tropical fruit	NS	0.94	1.32	1.32
V18	Hexyl acetate	15.414	Esters	400	Fruity, sweet floral	NS	0.01	0.02	0.02
V19	*p*-Cymene	15.941	Terpenes	-	Citrus	NS	0.02	0.01	0.01
V20	Limonene	16.206	Terpenes	200	Herbaceous, citrus, sweet	NS	0.17	0.15	0.4
V21	Benzyl alcohol	16.352	Alcohols	200000	Berry, cherry, citrus	NS	0.04	0.04	0.05
V22	Phenyl acetaldehyde	16.817	Aldehydes	-	-	*	0.01 b	0.01 ab	0.02 a
V23	Ethyl 2-hexenoate	16.966	Esters	-	-	NS	0.01	0.02	0.01
V24	Ethyl 2-furoate	17.239	Esters	-	Floral, plum	***	0.00 b	0.01 b	0.02 a

V25	γ-Terpinene	17.643	Terpenes		Herbaceous, citrus	NS	0.02	0.00	0.00
V26	1-(5-Methyl-2-furyl)-2-propanone§	17.755	Furans	-	-	***	0.00 b	0.00 b	0.03 a
V27	Acetophenone	17.873	Ketones	0.24 - 590	Almond, sweet, floral	***	0.00 b	0.01 b	0.01 a
V28	Isoamyl butyrate	18.126	Esters	-	Apricot, banana, pineapple	**	0.00 b	0.00 b	0.04 a
V29	1-Octanol	18.343	Alcohols	120	Citrus, fatty, woody	NS	0.04	0.07	0.04
V30	Guaiacol	18.897	Phenols	9.5	Medicinal, smoky	**	0.00 b	0.00 b	0.01 a
V31	Ethyl sorbate	19.651	Esters	-	Fruity	***	0.00 b	0.00 b	0.13 a
V32	Linalool	19.753	Terpenes	25.2	Lemon, orange, sweet	**	0.01 a	0.01 b	0.01 b
V33	Nonanal	20.005	Aldehydes	1	Apple, coconut, grape	***	0.00 b	0.00 b	0.11 a
V34	Phenylethyl Alcohol	20.349	Alcohols	14000	Honey, rose	NS	3.28	3.17	2.23
V35	Methyl octanoate	20.949	Esters	200	Fruity, green, citrus	**	0.02 b	0.04 a	0.01 b
V36	3-Ethylphenol	23.052	Phenols	-	-	***	0.01 b	0.59 a	0.06 b
V37	Ethyl benzoate	23.122	Esters	575	Anise, banana, grape, floral	***	0.02 b	0.02 b	0.05 a
V38	1-Nonanol	23.339	Alcohols	58	Citrus, rose	*	0.12 a	0.12 a	0.05 b
V39	Diethyl butanedioate	23.768	Esters	-	Fruity	**	1.86 b	1.90 b	2.80 a
V40	Octanoic acid	23.899	Acids	500	Rancid, cheese, oily	*	0.16 ab	0.24 a	0.13 b
V41	Methyl salicylate	24.179	Esters	-	Spicy, minty, sweet	NS	0.04	0.04	0.04
V42	α -Terpineol	24.426	Terpenes	250	Lilac	NS	0.01	0.01	0.02
V43	Ethyl octanoate	24.589	Esters	5	Apricot, floral, pear	NS	3.12	3.65	2.31
V44	Diethyl methylsuccinate	24.875	Esters	-	-	***	0.00 b	0.00 b	0.01 a
V45	Decanal	24.968	Aldehydes	1000	Floral, citrus, sweet	NS	0.02	0.02	0.04
V46	Citronellol	26.000	Terpenes	-	Geranium, rose	*	0.02 a	0.01 ab	0.00 b
V47	Neral	26.393	Terpenes	-	Sweet, citral, lemon, peel	NS	0.01	0.01	0.03
V48	Ethyl phenyl acetate	26.573	Esters	-	Anise, apple, honey	NS	0.07	0.13	0.12
V49	Linalyl acetate	27.082	Esters	-	Floral, fruity, sweer	NS	0.12	0.13	0.19
V50	Phenethyl acetate	27.128	Esters	250	Caramel, honey, fruity	NS	0.14	0.14	0.12
V51	Ethyl salicylate	27.796	Esters	-	Floral, fruity, minty	*	0.01 b	0.01 ab	0.02 a
V52	Geranial	27.803	Terpenes	-	Lemon, orange, sweet	NS	0.00	0.01	0.03
V53	4-Ethylguaiacol	28.022	Phenols	33	Smoky, meaty	**	0.00 b	0.00 b	0.02 a

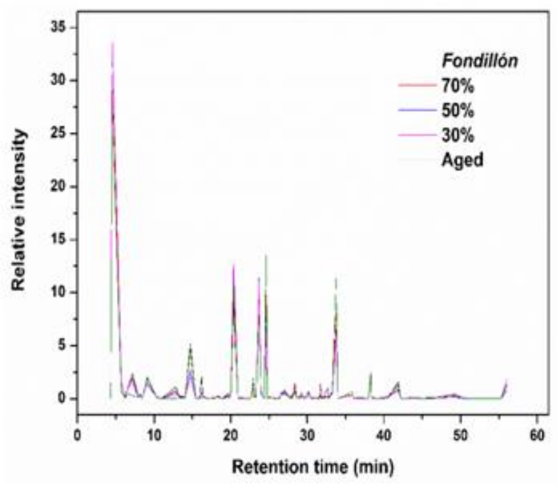
V54	1-Decanol	28.104	Alcohols	400	Fatty, fruity, rose	NS	0.06	0.07	0.06
V55	Vitispirane	28.372	Norisoprenoid	-	Woody, spicy	***	0.12 c	0.22 b	0.37 a
V56	Geraniol	28.596	Terpenes	20 - 30	Apple, berry, sweet, floral	***	0.04 a	0.03 a	0.00 b
V57	trans-Whiskey lactone	28.701	Lactones	67	Coconut, green	*	0.02 b	0.05 a	0.06 a
V58	Ethyl nonanoate	29.184	Esters	-	Oily, fruity. Nutty	***	0.35 a	0.15 b	0.05 b
V59	Undecanal	29.719	Aldehydes	-	Orange, fatty, rose	NS	0.00	0.00	0.01
V60	*cis*-Whiskey lactone	30.139	Lactones	67	Coconut, green	**	0.11 b	0.09 b	0.17 a
V61	Methyl decanoate	30.448	Esters	-	Oily, wine-like, fruity	**	0.05 a	0.03 a	0.01 b
V62	Ethyl 3-phenyl propionate	31.403	Esters	-	-	**	0.01 b	0.01 b	0.02 a
V63	Isobutyl caprylate	31.511	Esters	-	-	***	0.01 b	0.02 a	0.00 b
V64	Eugenol	31.603	Phenols	5 - 6	Cinnamon, clove, spicy	*	0.00 b	0.01 a	0.01 a
V65	TDN	31.677	Norisoprenoid	2 - 50	Kerosene	***	0.00 b	0.04 b	0.35 a
V66	2(3H)-Furanone, dihydro-5-pentyl §	31.888	Lactones	-	-	***	0.03 a	0.02 b	0.01 c
V67	Geranyl acetate	31.978	Esters	-	Fruity, floral, rose, sweet	NS	0.00	0.00	0.023
V68	Decanoic acid	32.423	Acids	1000	Fatty, citrus	***	0.16 a	0.04 b	0.05 b
V69	Damascenone	32.833	Ketones	0.05	Apple, woody, nutty	NS	0.06	0.03	0.03
V70	Ethyl 9-decenoate	33.249	Esters	-	Fruity	NS	0.25	0.17	0.17
V71	Ethyl decanoate	33.673	Esters	200	Grape, oily, pear	**	5.17 a	2.96 b	2.00 b
V72	Tetradecane	33.951	Alkanes	-	-	**	0.03 a	0.01 b	0.01 b
V73	Dodecanal	34.242	Aldehydes	-	Herbaceous, floral, sweet	NS	0.02	0.02	0.04
V74	Isoamyl octanoate	35.824	Esters	125	Coconut, fruity, sweet	*	0.12 ab	0.17 a	0.08 b
V75	Isoamyl caprylate	35.934	Terpenes	-	Woody, spicy	NS	0.03	0.07	0.06
V76	*trans*-Caryophyllene	36.104	Terpenes	-	Herbal	*	0.02 a	0.02 a	0.00 b
V77	α - Curcumene	37.231	Esters	-	Coconut	NS	0.02	0.02	0.02
V78	Ethyl undecanoate	37.843	Terpenes	-	-	**	0.03 a	0.01 b	0.01 b
V79	α – Muurolene §	37.972	Phenols	-	-	NS	0.22	0.03	0.01
V80	2,4-Di-tert-butylphenol §	38.269	Terpenes	-	Balsamic, woody	NS	0.50	0.63	0.48
V81	β – Bisabolene §	38.375	Terpenes	-	Woody	NS	0.02	0.02	0.05
V82	β- Cadinene §	38.792	Terpenes	-	-	NS	0.01	0.01	0.01

V83	α-Calacorene §	39.652	Norisoprenoid	-	Earthy	NS	0.05	0.02	0.01
V84	2,3,5-Trimethylnaphthalene	40.461	Norisoprenoid	-	Earthy	NS	0.30	0.05	0.02
V85	Ethyl dodecanoate	41.845	Esters	1500	Coconut, creamy, soapy	NS	0.59 a	0.30 b	0.37 b
V86	Hexadecane	42.171	Alkanes	-	-	NS	0.01	0.08	0.01
V87	Tetradecanal	42.572	Aldehydes	-	Fatty, waxy, creamy	NS	0.02	0.01	0.02
V88	Isoamyl decanoate	43.799	Esters	-	Fruity banana green	NS	0.04	0.03	0.02
V89	1-Tetradecanol	45.026	Alcohols	-	-	NS	0.00	0.01	0.01 a
V90	Ethyl tetradecanoate	49.266	Esters	-	Waxy, soapy	***	0.33 a	0.06 b	0.04 b
V91	Isopropyl myristate	50.370	Esters	-	Cheese, cherry, cinnamon	NS	0.02	0.01	0.01
V92	Ethyl 9-hexadecenoate	55.284	Esters	-	Fruity	**	0.05 a	0.01 b	0.01 b
V93	Ethyl hexadecanoate	56.033	Esters	-	Fruity	NS	0.19	0.28	0.15

*† References: Ferreira, V.; López, R.; Cacho, J.F. Quantitative determination of the odorants of young red wines from different grape varieties. J. Sci. Food Agric. 2000, 80, 1659–1667. Gómez-Míguez, M.J.; Cacho, J.F.; Ferreira, V.; Vicario, I.M.; Heredia, F.J. Volatile components of Zalema white wines. Food Chem. 2007, 100, 1464–1473. Jiang, B.; Xi, Z.; Luo, M.; Zhang, Z. Comparison on aroma compounds in Cabernet Sauvignon and Merlot wines from four wine grape-growing regions in China. Food Res. Int. 2013, 51, 482–489. ‡ Reference: Sigma-Aldrich. Flavors & Fragrances; Sigma-Aldrich: Saint Louis, MO, USA, 2012. ¥ NS = not significant at p > 0.05; *, **, ***, significant at p < 0.05, 0.01, and 0.001, respectively. £ Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different (p > 0.05), according to Tukey's least significant difference test. §Tentatively identified (no standard was available).*



(a)



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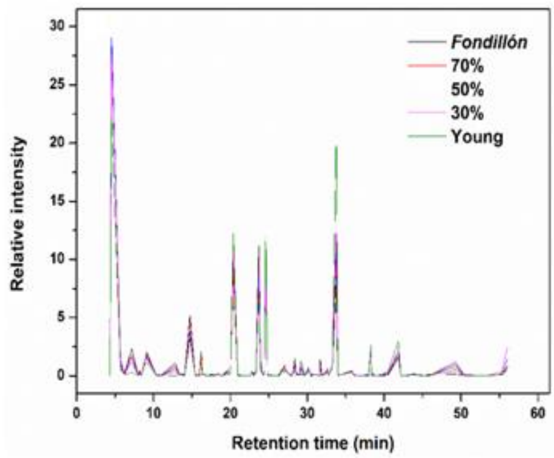

(c)

Figure S1. Plot of GC-MS chromatogram of (a) pure *Monastrell Fondillón*, aged and young wines, and adulterated *Fondillón* by the addition of (b) aged *Monastrell* wine, and (c) young *Monastrell* wine.

Author Contributions: H.I.-I.: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Original draft, and Visualization. R.S.U.: Data curation, Writing—review and editing. D.L.-L.: Conceptualization, Validation, Writing—review and editing, Funding acquisition, Supervision. F.H.: Resources, Methodology. Á.A.C.-B.: Conceptualization, Methodology, Validation, Writing—review and editing, Visualization, Funding acquisition, Supervision. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kamiloglu, S. Authenticity and traceability in beverages. *Food Chem.* **2019**, 277, 12–24. [CrossRef] [PubMed]
2. Pasvanka, K.; Tzachristas, A.; Proestos, C. Quality tools in wine traceability and authenticity. In *Quality Control in the Beverage Industry: Volume 17: The Science of Beverages*; Woodhead Publishing: Duxford, UK, 2019; pp. 289–334.
3. Preti, R. Progress in beverages authentication by the application of analytical techniques and chemometrics. In *Quality Control in the Beverage Industry: Volume 17: The Science of Beverages;* Woodhead Publishing: Duxford, UK, 2019; pp. 85–121.
4. Sáenz-Navajas, M.P.; Jeffery, D.W. Perspectives on Wines of Provenance: Sensory Typicality, Quality, and Authenticity. *ACS Food Sci. Technol*. **2021**, 1, 986–992. [CrossRef]
5. Chandra, S.; Chapman, J.; Power, A.; Roberts, J.; Cozzolino, D. Origin and Regionality of Wines—The Role of Molecular Spectroscopy. *Food Anal. Methods* **2017**, 10, 3947–3955. [CrossRef]
6. Swinnen, G.M.a.J. The Political Economy of EuropeanWine Regulations. *J. Wine Econ*. **2013**, 8, 244–284. [CrossRef]
7. Medina, B. Wine authenticity. In *Food Authentication*; Ashurst, P.R., Dennis, M.J., Eds.; Chapman & Hall: London, UK, 1996; pp. 60–107.
8. Gonzálvez, A.; Llorens, A.; Cervera, M.L.; Armenta, S.; de la Guardia, M. Elemental fingerprint of wines from the protected designation of origin Valencia. *Food Chem*. **2009**, 112, 26–34. [CrossRef]
9. Office, E.A.-F. Over 1 Million Litres of Wine and Alcoholic Beverages Seized under OLAF-led Operation. Available online: https://anti-fraud.ec.europa.eu/media-corner/news/over-1-million-litres-wine-and-alcoholic-beverages-seized-underolaf-led-operation-2021-07-22_en (accessed on 28 November 2022).
10. Canizo, B.V.; Escudero, L.B.; Pellerano, R.G.; Wuilloud, R.G. Quality monitoring and authenticity assessment of wines: Analytical and chemometric methods. In *Quality Control in the Beverage Industry: Volume 17: The Science of Beverages;* Woodhead Publishing: Duxford, UK, 2019; pp. 335–384.
11. Astray, G.; Martinez-Castillo, C.; Mejuto, J.C.; Simal-Gandara, J. Metal and metalloid profile as a fingerprint for traceability of wines under any Galician protected designation of origin. *J. Food Compost. Anal.* **2021**, 102, 104043. [CrossRef]
12. Sun, X.; Zhang, F.; Gutiérrez-Gamboa, G.; Ge, Q.; Xu, P.; Zhang, Q.; Fang, Y.; Ma, T. Real wine or not? Protecting wine with traceability and authenticity for consumers: Chemical and technical basis, technique applications, challenge, and perspectives. *Crit. Rev. Food Sci. Nutr*. **2022**, 62, 6783–6808. [CrossRef]
13. Popîrda, A.; Luchian, C.E.; Cotea, V.V.; Colibaba, L.C.; Scutaras, u, E.C.; Toader, A.M. A review of representative methods used in wine authentication. *Agriculture* **2021**, 11, 225. [CrossRef]
14. Nuncio-Jáuregui, N.; Munera-Picazo, S.; Calín-Sánchez, Á.;Wojdyło, A.; Hernández, F.; Carbonell-Barrachina, Á.A. Bioactive compound composition of pomegranate fruits removed during thinning. *J. Food Compost. Anal.* **2015**, 37, 11–19. [CrossRef]
15. OIV. Compendium of International Methods of Analysis of Wines and Musts. OIV: Paris, France; vol. 2. Available online: http://www.oiv.int/en/technical-standards-and-documents/methods-ofanalysis/compendium-of-international-methodsof-analysisof-wines-and-musts-2-vol (accessed on 4 March 2023).
16. 16. Carbonell-Barrachina, A.A.; Calín-Sánchez, A.; Bagatar, B.; Hernández, F.; Legua, P.; Martínez-Font, R.; Melgarejo, P. Potential of Spanish sour-sweet pomegranates (cultivar C25) for the juice industry. *Food Sci. Technol. Int.* **2012**, 18, 129–138. [CrossRef]
17. Iris, F.F.; Benzie, a.J.J.S. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Anal. Biochem*. **1996**, 239, 70–76.
18. Katalinic, V.; Milos, M.; Kulisic, T.; Jukic, M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem.* **2006**, 94, 550–557. [CrossRef]
19. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med*. **1999**, 26, 1231–1237. [CrossRef] [PubMed]

20. Luna, J.M.; Garau, M.C.; Negre, A.; March, J.; Martorell, A. Composición Fenólica y Actividad Antioxidante de Variedades Minoritarias de Vid de las Islas Baleares. In Proceedings of the VII Foro Mundial del Vino, Logroño, La Rioja, Spain, 12–14 May 2010; pp. 12–14.
21. OIV. *Compendium of International Methods of Wine and Must Analysis*, 2022nd ed.; OIV: Paris, France, 2022; Volume 1.
22. Glories, Y. The color of red wines. *Connaiss. Vignevini* **1984**, 18, 195–217.
23. Issa-Issa, H.; Hernández, F.; Lipan, L.; López-Lluch, D.; Carbonell-Barrachina, Á.A. Quality, nutritional, volatile and sensory profiles and consumer acceptance of *Fondillón*, a sustainable european protected wine. *Agronomy* **2021**, 11, 1701. [CrossRef]
24. Geana, E.I.; Popescu, R.; Costinel, D.; Dinca, O.R.; Ionete, R.E.; Stefanescu, I.; Artem, V.; Bala, C. Classification of red wines using suitable markers coupled with multivariate statistic analysis. *Food Chem.* **2016**, 192, 1015–1024. [CrossRef] [PubMed]
25. Valcárcel-Muñoz, M.J.; Guerrero-Chanivet, M.; Rodríguez-Dodero, M.C.; García-Moreno, M.V.; Guillén-Sánchez, D.A. Analytical and Chemometric Characterization of Fino and Amontillado Sherries during Aging in Criaderas y Solera System. *Molecules* **2022**, 27, 365. [CrossRef]
26. Cliff, M.; Pickering, G. Determination of odour detection thresholds for acetic acid and ethyl acetate in ice wine. *J. Wine Res.* **2006**, 17, 45–52. [CrossRef]
27. Caldeira, I.; Santos, R.; Ricardo-Da-Silva, J.M.; Anjos, O.; Mira, H.; Belchior, A.P.; Canas, S. Kinetics of odorant compounds in wine brandies aged in different systems. *Food Chem.* **2016**, 211, 937–946. [CrossRef]
28. de Villiers, A.; Alberts, P.; Tredoux, A.G.J.; Nieuwoudt, H.H. Analytical techniques for wine analysis: An African perspective; a review. *Anal. Chim.* Acta **2012**, 730, 2–23. [CrossRef]
29. Coelho, E.M.; da Silva Padilha, C.V.; Miskinis, G.A.; de Sá, A.G.B.; Pereira, G.E.; de Azevêdo, L.C.; dos Santos Lima, M. Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. *J. Food Compost.* Anal. **2018**, 66, 160–167. [CrossRef]
30. Del Alamo, M.; Bernal, J.L.; Del Nozal, M.J.; Gómez-Cordovés, C. Red wine aging in oak barrels: Evolution of the monosaccharides content. *Food Chem.* **2000**, 71, 189–193. [CrossRef]
31. Echeverry, C.; Ferreira, M.; Reyes-Parada, M.; Abin-Carriquiry, J.A.; Blasina, F.; González-Neves, G.; Dajas, F. Changes in antioxidant capacity of Tannat red wines during early maturation. *J. Food. Eng.* **2005**, 69, 147–154. [CrossRef]
32. Roginsky, V.; De Beer, D.; Harbertson, J.F.; Kilmartin, P.A.; Barsukova, T.; Adams, D.O. The antioxidant activity of Californian red wines does not correlate with wine age. *J. Sci. Food Agric*. **2006**, 86, 834–840. [CrossRef]
33. Rivero-Pérez, M.D.; González-Sanjosé, M.L.; Ortega-Herás, M.; Muñiz, P. Antioxidant potential of single-variety red wines aged in the barrel and in the bottle. *Food Chem*. **2008**, 111, 957–964. [CrossRef]
34. Pfahl, L.; Catarino, S.; Fontes, N.; Graça, A.; Ricardo-Da-silva, J. Effect of barrel-to-barrel variation on color and phenolic composition of a red wine. *Foods* **2021**, 10, 1669. [CrossRef]
35. Chira, K.; Jourdes, M.; Teissedre, P.L. Cabernet sauvignon red wine astringency quality control by tannin characterization and polymerization during storage. *Eur. Food Res. Technol*. **2012**, 234, 253–261. [CrossRef]
36. Del Fresno, J.M.; Morata, A.; Loira, I.; Escott, C.; Suárez Lepe, J.A. Evolution of the Phenolic Fraction and Aromatic Profile of Red Wines Aged in Oak Barrels. *ACS Omega* **2020**, 5, 7235–7243. [CrossRef]
37. Kment, P.; Mihaljeviˇc, M.; Ettler, V.; Šebek, O.; Strnad, L.; Rohlová, L. Differentiation of Czech wines using multielement composition—A comparison with vineyard soil. *Food Chem*. **2005**, 91, 157–165. [CrossRef]
38. Cozzolino, D. Elemental composition in grapes and wine: Role, analytical methods and their use. In *Handbook of Mineral Elements in Food*; Wiley Blckwell: Chichester, UK, 2015; pp. 473–487.
39. Pohl, P. What do metals tell us about wine? *TrAC Trends Analyt Chem*. **2007**, 26, 941–949. [CrossRef]
40. Frías, S.; Conde, J.E.; Rodríguez-Bencomo, J.J.; García-Montelongo, F.; Pérez-Trujillo, J.P. Classification of commercial wines from the Canary Islands (Spain) by chemometric techniques using metallic contents. *Talanta* **2003**, 59, 335–344. [CrossRef] [PubMed]

41. Cordente, A.G.; Curtin, C.D.; Varela, C.; Pretorius, I.S. Flavour-active wine yeasts. *Appl. Microbiol. Biotechnol.* **2012**, 96, 601–618. [CrossRef] [PubMed]
42. Jarauta, I.; Cacho, J.; Ferreira, V. Concurrent phenomena contributing to the formation of the aroma of wine during aging in oak wood: An analytical study. *J. Agric. Food Chem*. **2005**, 53, 4166–4177. [CrossRef] [PubMed]
43. Perestrelo, R.; Barros, A.S.; Câmara, J.S.; Rocha, S.M. In-depth search focused on furans, lactones, volatile phenols, and acetals as potential age markers of Madeira wines by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry combined with solid phase microextraction. *J. Agric. Food Chem.* **2011**, 59, 3186–3204. [CrossRef] [PubMed]
44. Versari, A.; Laurie, V.F.; Ricci, A.; Laghi, L.; Parpinello, G.P. Progress in authentication, typification and traceability of grapes and wines by chemometric approaches. *Food Res. Int*. **2014**, 60, 2–18. [CrossRef]
45. Castro-Vázquez, L.; Alañón, M.E.; Calvo, E.; Cejudo, M.J.; Díaz-Maroto, M.C.; Pérez-Coello, M.S. Volatile compounds as markers of ageing in Tempranillo red wines from La Mancha D.O. stored in oak wood barrels*. J. Chromatogr*. **2011**, 1218, 4910–4917. [CrossRef]
46. Issa-Issa, H.; Guclu, G.; Noguera-Artiaga, L.; López-Lluch, D.; Poveda, R.; Kelebek, H.; Selli, S.; Carbonell-Barrachina, Á.A. Aroma-active compounds, sensory profile, and phenolic composition of *Fondillón*. *Food Chem*. **2020**, 316, 126353. [CrossRef]
47. Winterhalter, P.; Gök, R. TDN and β-damascenone: Two important carotenoid metabolites in wine. *ACS Symp. Ser*. **2013**, 1134, 125–137. [CrossRef]
48. Marais, J.; van Wyk, C.J.; Rapp, A. Effect of Sunlight and Shade on N orisoprenoid Levels in Maturing Weisser Riesling and Chenin blanc Grapes and Weisser Riesling Wines. *Outh African J. Enol. Vitic*. **1992**, 13, 23–32. [CrossRef]
49. Khairallah, R.; Reynolds, A.G.; Bowen, A.J. Harvest date effects on aroma compounds in aged Riesling icewines. *J. Sci. Food Agric*. **2016**, 96, 4398–4409. [CrossRef]
50. Ferreira, V.; López, R.; Cacho, J.F. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric*. **2000**, 80, 1659–1667. [CrossRef]
51. Gómez-Míguez, M.J.; Cacho, J.F.; Ferreira, V.; Vicario, I.M.; Heredia, F.J. Volatile components of Zalema white wines. *Food Chem*. **2007**, 100, 1464–1473. [CrossRef]
52. Jiang, B.; Xi, Z.; Luo, M.; Zhang, Z. Comparison on aroma compounds in Cabernet Sauvignon and Merlot wines from four wine grape-growing regions in China. *Food Res. Int*. **2013**, 51, 482–489. [CrossRef]
53. Sigma-Aldrich. *Flavors & Fragrances*; Sigma-Aldrich: Saint Louis, MO, USA, 2012.

8. RESULTADOS Y DISCUSIÓN

Esta sección contiene los principales **Resultados** obtenidos en las 5 publicaciones incluidas en esta tesis doctoral, junto con su **Discusión,** agrupados de acuerdo con los 5 objetivos generales planteados al principio del trabajo. Los detalles de los resultados expuestos en esta sección pueden consultarse en cada una de las publicaciones anteriormente presentadas.

Objetivo 1.
Realizar una caracterización fisicoquímica del Fondillón e identificar su perfil volátil.

Los resultados relativos a este objetivo se encuentran recogidos en las publicaciones 1 y 3 e incluyen los siguientes puntos: (*i*) parámetros de calidad, (*ii*) características cromáticas, (*iii*) contenidos totales de polifenoles (TPI) y de antocianinas (TAC), (*iv*) actividad antioxidante (AA), y (*v*) composición volátil.

En la publicación 1 se trabajó con las 7 muestras comerciales de Fondillón que estaban disponibles en 2015; mientras que, en la publicación 3 se trabajó con muestras de Fondillón proporcionadas por Bodegas Monóvar-MGWines de 9 soleras: 1930, 1944, 1950, 1960, 1969, 1975, 1980, 1987 y 1996.

Parámetros de calidad

Este vino tiene un alto *contenido alcohólico*, siendo el mínimo legal 16 % (v/v) (Conselleria de Agricultura, Pesca, Alimentación y Agua, 2011), los valores experimentales encontrados para las muestras de Fondillón evaluados oscilaron entre 16,2 y 21,2 %.

La *acidez total* tiene que ser superior a 3,5 g ácido tartárico/L (Conselleria de Agricultura, Pesca, Alimentación y Agua, 2011) y los valores oscilaron entre 5,30 y 7,80 g/L en las muestras comerciales (publicación 1) y entre 7,28 y 10,01 g/L en las muestras de MGWines (publicación 3).

Por otro lado, el valor máximo legal de la *acidez volátil* es de 1,50 g ácido acético/L (Conselleria de Agricultura, Pesca, Alimentación y Agua, 2011). Los valores experimentales fueron superiores a 0,62 g/L y tuvieron un valor medio de 1,13 ± 0,06 (datos de las publicaciones 1 y 3) y solo en una ocasión se obtuvo un valor igual al máximo legal.

En definitiva, se puede concluir que todas las muestras de Fondillón estudiadas en esta tesis doctoral cumplían con los requisitos fisicoquímicos establecidos en el Pliego de Condiciones de la DOP Alicante.

<u>Características colorimétricas y contenidos totales de polifenoles (TPI) y de antocianinas (TA)</u>

Según el Pliego de Condiciones de la DOP Alicante el color del Fondillón es *caoba, ámbar y tonalidades cobrizas*. Durante el envejecimiento oxidativo del Fondillón, las antocianinas sufren distintas reacciones de oxidación, tales como condensación y polimerización, que causan una transformación de las tonalidades azuladas iniciales en tonalidades más anaranjadas. En general, no se encontró un efecto claro de la solera sobre los parámetros de color (intensidad de color, tonalidad y densidad de color). Parece razonable que los cambios en el color se den durante los primeros años de envejecimiento (y que las diferencias con respecto al color inicial del vino a envejecer sean significativas), pero que las características cromáticas de los Fondillones (ya tras los 10 años mínimos de envejecimiento) sean similares entre sí y que, por tanto, el factor "año de la solera" pase a jugar un papel secundario. Sin embargo, el parámetro **tonalidad** (Y/R) mostró una correlación positiva con la componente amarilla y negativa con la roja (**Figura 4a**).

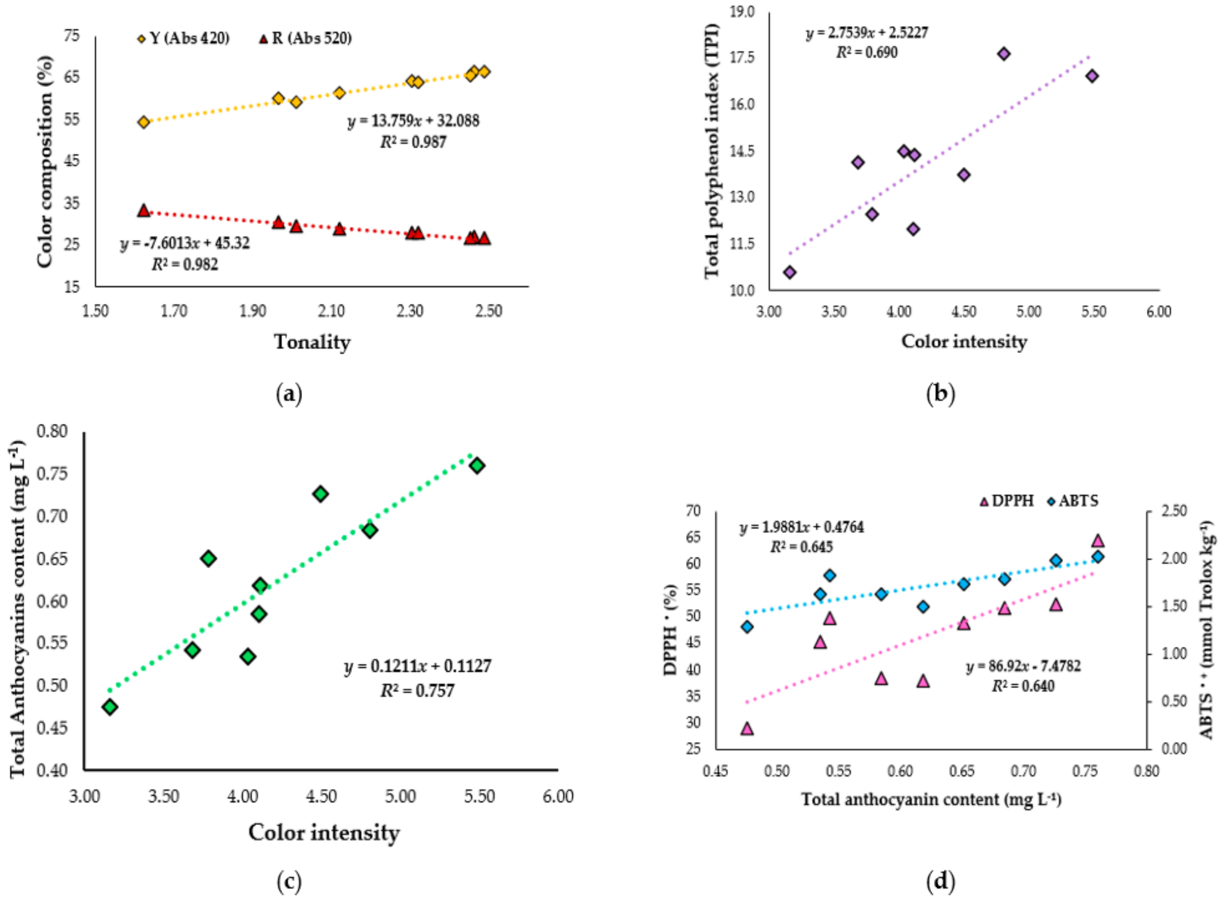


Figura 4. Correlación entre composición de color (Y, R) y tonalidad (a); correlación entre la intensidad del color y el índice de fenoles (b); correlación entre la intensidad del color y las antocianinas totales (c); y correlación entre el contenido total de antocianinas y la actividad antioxidante ($ABTS^{•+}$, $DPPH^{•}$) (d).

También se encontraron correlaciones positivas y estadísticamente significativas entre la intensidad de color y el TPI (polifenoles) y TA (antocianinas) (**Figura 4b, c**).

Actividad antioxidante

Se evaluó la actividad antioxidante mediante tres métodos complementarios ($ABTS^{\bullet+}$, FRAP y $DPPH^{\bullet}$) y se concluyó que el método que mejores resultados dio fue $DPPH^{\bullet}$ ya que mostró una correlación positiva con el contenido total de antocianinas (TAC).

En general, se observó como la muestra de la solera de 1960 (Bodegas Monóvar-MGWines) fue la más especial ya que tenía los mayores contenidos totales de polifenoles (TPI), de taninos condensados (TTC), de antocianinas (TAC) y los valores más elevados de actividad antioxidante, por los tres métodos estudiados, lo que le permitió mantener unos valores elevados de densidad e intensidad de color.

Perfil volátil

El perfil volátil del Fondillón (espacio de cabeza analizado usando fibras de SPME) está constituido por hasta 10 familias químicas, predominando los ésteres, alcoholes y aldehídos. Dentro de los *ésteres*, los compuestos predominantes fueron aquellos derivados de los ácidos grasos etílicos y del ácido acético, por ejemplo, el dietil butanodioato, el etil octanoato y el etil acetato. Los ésteres proporcionan aromas afrutados a los vinos, especialmente a frutos rojos y frutos del bosque. Los *alcoholes* provienen directamente de la uva y/o del metabolismo de las levaduras, predominando el alcohol isoamílico, el 2-metil-1-butanol y el alcohol fenetílico, que tienen descriptores afrutados, florales y a miel (Robinson *et al.*, 2014).

Sin embargo, los *norisoprenoides* son los compuestos clave en el perfil aromático de este tipo de vino. Durante el envejecimiento del vino, estos compuestos procedentes del metabolismo de los carotenoides pueden ser sometidos a una hidrólisis ácida lenta que generan norisoprenoides tales como el 1,1,6-trimetil-1,2-dihidronaftaleno (TDN) y el vitispirano. El TDN ha sido referenciado como el responsable del olor a queroseno característico de los vinos Riesling envejecidos en botella (Winterhalter *et al.*, 1990), y el vitispirano contribuye con notas a fruto seco (Pozo-Bayón y Moreno-Arribas, 2011).

La *abundancia relativa* (%) de las principales familias químicas que intervienen en el perfil volátil del Fondillón se resume en la ordenación siguiente:

Ésteres (~70 %) >>> alcoholes (~20 %) >>> aldehídos (2 %) ≥ ácidos orgánicos (2 %) ≥ compuestos azufrados (~1 %) ≥ terpenos (~1 %) > lactonas (0,1 %)

Conclusiones del objetivo 1

Las muestras de Fondillón empleadas en las publicaciones de esta tesis doctoral siempre cumplieron con los requisitos establecidos por el Pliego de Condiciones de la DOP Alicante [grado alcohólico >16 %, acidez volátil < 1,50 g ácido acético/L, contenido total de SO_2 < 200 mg/L, etc.] y los valores medios experimentales fueron grado alcohólico 19,86 ± 0,37 %, acidez total 7,51 ± 0,34 g ácido tartárico/L, acidez volátil 1,12 ± 0,07 g ácido acético/L, contenido total de SO_2 < 200 mg/L, etc.

El color del vino tiene una correlación positiva con el contenido total de compuestos fenólicos (TPI) y antocianinas (TA); por tanto, el control de las coordenadas colorimétricas puede proporcionarnos información interesante sobre el contenido de estos compuestos bioactivos.

El método de evaluación de la actividad antioxidante que mejor resultados parece proporcionar es el DPPH$^{\bullet}$ ya que se vincula al contenido total de antocianinas (TA).

El perfil aromático del Fondillón está dominado (en lo que respecta a la abundancia) por ésteres (dietil butanodioato, etil octanoato y etil acetato) y alcoholes (alcohol isoamílico, 2-metil-1-butanol, alcohol fenetílico), representando entre ambos casi un 90 % del contenido total de compuestos volátiles. Sin embargo, compuestos con una abundancia menor, tales como los norisoprenoides (TDN y vitispirano), pueden jugar un papel importante en la determinación de la edad y autenticidad de este tipo de vino.

Objetivo 2.
Identificar los compuestos aromáticos activos del olor del Fondillón y su perfil polifenólico.

Los resultados relativos a este objetivo se encuentran recogidos en las publicaciones 1, 2 y 3, si bien la información más relevante es la generada en la publicación 2, donde se incluyen los siguientes puntos: (*i*) extracción de compuestos volátiles mediante la técnica (SAFE, *solvent-assisted flavor evaporation*), (*ii*) análisis de dilución del extracto aromático (AEDA, *aroma extract dilution analysis*), y (*iii*) composición fenólica.

En la publicación 2 se trabajó con 3 muestras de Fondillón (Bodegas Monóvar, MGWines), concretamente de soleras de 1944, 1987 y 1996.

Extracción de compuestos volátiles mediante la técnica SAFE (*solvent-assisted flavor evaporation*)

Para poder identificar los compuestos aromáticos activos del Fondillón lo primero es obtener un extracto que sea representativo del aroma de la muestra bajo estudio. Para ello se utilizó la técnica SAFE, en la que ~100 mL de Fondillón se extrajeron con diclorometano, Cl_2CH_2 (100 mL) para su posterior destilación a vacío y temperatura controlada (4 °C), y así obtener un extracto final de ~200 µL.

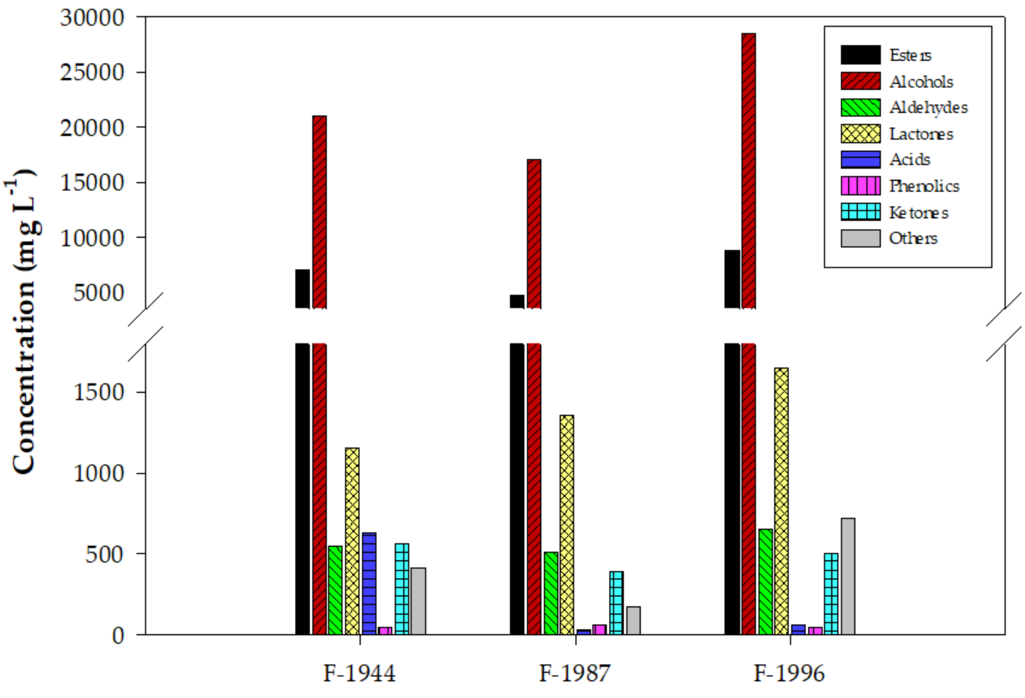


Figura 5. Familias químicas encontradas en las muestras de Fondillón evaluadas.

Posteriormente y mediante el empleo de la cromatografía de gases con detector de espectrometría de masas (GC-MS) se identificaron 54 compuestos volátiles, que se agruparon en 8 familias químicas (alcoholes, ésteres, ácidos, aldehídos, lactonas, fenoles, hidrocarburos y cetonas). El contenido total de compuestos volátiles osciló entre los 25,0 y los 41,8 mg/L. El grupo químico predominante fue el de los ésteres con un total de 18 compuestos, aunque la concentración total de los alcoholes fue superior (**Figura 5**). Los compuestos predominantes fueron isoamil alcohol (13831 μg/L) y etil lactato (4057 μg/L). Es importante indicar que, tras los ésteres y alcoholes, los ácidos orgánicos fueron el siguiente grupo químico por abundancia, incluyendo contenidos relativamente elevados de ácido acético.

<u>Análisis de dilución del extracto aromático, AEDA</u> (*aroma extract dilution analysis*)

Los compuestos aromáticos activos se identificaron mediante GC-MS-olfatometría (cromatografía de gases con detector de espectrometría de masas y puerto de olfatometría) con la aplicación de la técnica AEDA. El extracto anterior (200 μL) fue gradualmente diluido con diclorometano (Cl_2CH_2) comenzando por una proporción 1:1, continuando con 1:2, 1:4, y concluyendo con una proporción de 1:1024, a la cual emergió el último compuesto/olor detectable.

Usando la combinación GC-MS-olfatometría y AEDA (**Tabla 3**) se detectaron 24 compuestos aromáticos activos (con factores de dilución, FD, entre 4 y 1024); incluyendo 8 ésteres, 4 alcoholes, 3 aldehídos, 3 lactonas, 2 ácidos, 1 cetona y 1 compuestos fenólico, si bien 2 de estos 24 compuestos no pudieron ser identificados mediante GC-MS.

Los cuatro compuestos aromáticos activos con una mayor aportación al aroma del Fondillón fueron:

1. *Alcohol feniletílico* (cuyos descriptores sensoriales son floral, rosa), cuyo factor de dilución, FD, osciló entre 512 y 1024.
2. *Etil lactato* (palomitas, crema), con FDs entre 256 y 512.
3. *Dietil succinato* (afrutado, fermentado), con FDs entre 256 y 512.
4. *Dietil malato* (caramelo, quemado), con FDs entre 32 y 128.

Por tanto, el perfil sensorial del Fondillón tiene aportaciones significativas de compuestos químicos (principalmente ésteres, alcoholes y aldehídos) cuyas notas aromáticas principales pueden describirse como florales (rosa), afrutadas y tostadas (palomitas y caramelo).

Finalmente, y mediante correlaciones de Pearson entre los compuestos aromáticos activos y los principales atributos sensoriales se estableció que tanto los ésteres como los alcoholes y aldehídos están positivamente correlacionados con la intensidad de las notas afrutadas y vegetales del sabor del Fondillón. Mientras que los ácidos orgánicos y el guaiacol (compuesto fenólico) afectaron las notas florales y animales (mantequilla).

Tabla 3. Compuestos aromáticos activos del Fondillón identificados mediante el método AEDA (FD≥4).

Chemical Family	Code	Compound	Aroma descriptor†	LRI‡	FD Factor¶		
					F-1944	F-1987	F-1996
Esters	V1	Ethyl propanoate	Sweet, tropical fruity	915	64	ND	64
	V15	Ethyl lactate	Popcorn, creamy	1316	512	256	512
	V36	Ethyl 4-hydroxybutyrate	Caramel, fruity	1790	16	16	32
	V30	Diethyl succinate	Fermented, fruity	1661	512	256	512
	V35	Diethyl glutarate	Caramel, burnt	1768	4	4	ND
	V38	Phenylethyl acetate	Fruity	1825	4	4	4
	V47	Diethyl DL malate	Caramel, burnt	2039	128	32	128
	V49	Diethyl-2-hydroxy-pentadioate	Dried fruit	2140	32	32	32
Alcohols	V12	Isoamyl alcohol	Fermented, alcohol	1197	16	4	32
	V26	2,3-Butanediol	Fatty, sweet	1539	8	8	16
	V31	Methionol	Cooked, earthy	1715	4	4	ND
	V42	Phenylethyl alcohol	Floral, rosy	1858	1024	512	1024
Aldehydes	V21	Furfural	Burnt, bread	1425	16	16	16
	V23	Benzaldehyde	Roasted nuts	1501	8	4	8
	V53	Vanillin	Vanilla	2560	16	16	16
Lactones	V27	γ-Butyrolactone	Creamy	1595	32	32	64
	V43	Whiskey lactone	Fruity, sweet	1898	16	16	16
	V45	Pantolactone	Floral	1998	32	16	16
Acids	V20	Acetic acid	Vinegary	1403	16	16	32
	V29	Isovaleric acid	Chemical, rotten	1627	4	4	8
Phenolic compounds	V40	Guaiacol	Smoke	1841	ND	ND	8
Ketones	V14	Acetoin	Creamy	1255	16	8	8
Unknown		Unknown	Cooked	1733	ND	16	8
		Unknown	Cooked vegetable, onion	1748	16	16	16

†Aroma descriptor: aroma perceived by panelists during olfactometry. ‡LRI: linear retention index calculated on DB-Wax capillary column. ¶FD factor is the highest dilution of the extract at which an odorant is determined by aroma extract dilution analysis; ND: Not detected.

<u>Composición fenólica</u>

Los compuestos fenólicos presentes en el Fondillón se identificaron mediante HPLC-DAD y se cuantificaron mediante LC-MS/MS. Por primera vez se ha estudiado el perfil polifenólico del Fondillón y se identificaron 25 compuestos, incluyendo 17 ácidos fenólicos y derivados, 1 flavanol, 2 estilbenos, 4 flavonas y un compuesto no identificado (**Tabla 4**). Por tanto, los ácidos fenólicos son la familia química predominante en este tipo de vinos.

El contenido total de compuestos fenólicos osciló entre 11 y los 284 mg/L. Los ácidos hidroxibenzoicos (gálico, protocatecuico y siríngico) fueron los predominantes en Fondillón, destacando el contenido del ácido protocatecuico que llegó a un contenido superior de los 50 mg/L y tuvo un contenido medio de 44,6 mg/L, seguido del ácido gálico con un contenido medio de 33 mg/L y del ácido siríngico con 17,6 mg/L.

Tabla 4. Tiempo de retención, características espectrales de masas y concentración de compuestos fenólicos (mg/L) presentes en muestras de Fondillón.

Peak	Compounds	RT (min)	UV λ_{max} (nm)	$[M–H]^-$ (*m/z*)	MS/MS (*m/z*)	ANOVA	F-1944	F-1987	F-1996
Phenolic acids and derivatives									
2	Gallic acid	14.18	276	169	125	**	23.46 c	48.58 a	27.51 b
3	Protocatechuic acid-*O*-hexoside	17.45	296	315	153, 109	*	3.59 b	7.52 a	3.09 b
4	2-S-glutathionyl-caffeoyltartaric acid	18.53	330	616	484, 440, 272	*	0.33 a	0.19 b	0.17 b
5	Protocatechuic acid	21.04	294	153	109	**	39.15 b	42.30 b	52.47 a
6	Hydroxy-caffeic acid dimer isomer 1	22.61	315	373	305, 193	NS	0.08 b	0.22 a	0.23 a
7	(*E*)-Caftaric acid	25.24	328	311	179, 149	**	5.05 a	3.13 b	2.42 c
8	Hydroxy-caffeic acid dimer isomer 2	25.66	320	373	327, 305, 281	NS	0.04	0.09	0.06
9	Hydroxy-caffeic acid dimer isomer 3	27.66	320	373	355, 327, 305, 175	*	1.22 b	2.32 a	2.08 a
12	(*Z*)-Coutaric acid	32.46	310	295	163, 149	**	1.06 a	0.42 c	0.60 b
13	(*E*)-Coutaric acid	33.72	314	295	163	NS	5.16	5.05	4.92
14	(*Z*)-Fertaric acid	36.06	322	325	193, 149	NS	0.09	0.08	0.05
15	(*E*)-Caffeic acid	36.75	323	179	135	*	1.09 a	1.13 a	0.35 b
16	(*E*)-Fertaric acid	37.09	328	325	193, 149	*	1.89 a	1.85 a	1.14 b
17	Ferulic acid	37.09	323	193	178, 149, 134	NS	1.66	1.59	1.41
20	Syringic acid	45.41	272	197	182, 167, 153	**	15.11 b	22.79 a	14.78 b
21	*p*-Coumaric acid	46.93	310	163	119	*	3.22 a	2.76 b	2.86 b
22	Ellagic acid	48.43	275	301	284, 257, 229, 185	*	0.27 b	0.47 a	0.42 a
Flavan-ols									
11	Catechin	31.01	280	289	245, 175	*	4.56 b	3.62 c	4.96 a
Stilbenes									
1	(*Z*)-Piceid	13.45	282	389	227	***	0.17 b	1.33 a	0.07 b
10	Tyrosol	28.39	275	137	93	*	0.54 b	0.50 b	0.95 a
Flavones									
18	Dihydrokaempferol 3-*O*-*ß*-d-glucoside	39.93	290	449	287	**	1.97 b	1.88 b	2.89 a
19	Quercetin-3-*O*-glucuronide	45.37	355	477	301	*	0.32 a	0.41 a	0.10 b
23	Isorhamnetin-*O*-hexoside	49.26	356	477	315, 301, 300, 299	*	0.34 a	0.45 a	0.10 b
25	Quercetin	64.33	355	301	151	***	0.20 b	1.13 a	0.01 c
Others									
24	Unkown	54.32	280	713	600, 389, 335, 133	***	0.01 b	134 a	0.01 b
	TOTAL					***	**111 c**	**284 a**	**124 b**

*†NS: not significant at p > 0.05; *, **, and ***, significant at p < 0.05, 0.01, and 0.001, respectively. ‡Values (mean of 3 replications) followed by the same letter, within the same row, were not significantly different (p > 0.05), Tukey's least significant difference test.*

Conclusiones del objetivo 2

Se demostró que la combinación de cromatografía de gases (GC-MS) con las técnicas de SAFE y AEDA y la olfatometría proporciona una información esencial para la caracterización del perfil aromático del Fondillón. Se identificaron 54 compuestos volátiles en el perfil volátil del Fondillón, de los cuales solo **22 juegan un papel activo en su perfil aromático**. Y hay que destacar el papel de cuatro compuestos: **alcohol feniletílico**, **etil lactato**, **dietil succinato** y **dietil malato** que aportan notas florales (rosa), afrutadas y tostadas (palomitas y caramelo).

Además, y por primera vez, se ha estudiado el perfil polifenólico del Fondillón y se han identificado 25 compuestos, predominando significativamente los ácidos fenólicos, especialmente los **ácidos protocatecuico y gálico**.

Objetivo 3.
Determinar el perfil sensorial descriptivo de Fondillón.

Los resultados relativos a este objetivo se encuentran recogidos en las publicaciones 1, 2 y 3 e incluyen los siguientes puntos: (*i*) desarrollo de un léxico para la descripción sensorial del Fondillón, y (*ii*) desarrollo de perfiles sensoriales.

Léxico sensorial

Se desarrolló un léxico específico para la descripción sensorial del Fondillón (publicación **1**), de acuerdo con la definición de este vino en el Pliego de Condiciones de la DOP Alicante. El léxico consta de las siguientes propiedades complejas y atributos:

A evaluar usando **copa negra** normalizada:

- ✓ **Olor y aroma** (n=7 × 2): alcohol, afrutado, floral, vegetal, especiado, animal y tostado.
- ✓ **Gustos básicos** (n=3): dulce, ácido y salado.
- ✓ **Sensaciones somatosensoriales** (n=1): astringencia.
- ✓ **Atributos globales** (n=2): aristas y persistencia.
- ✓ **Defectos** (n=12): vegetal, manzana podrida, vinagre, pegamento, jabón, azufre, huevo podrido, cebolla, coliflor, caballo, terroso y corcho.

A evaluar usando **copa transparente** normalizada:

- ✓ **Apariencia** (n=3): limpidez, color, e intensidad de color (capa).

Además, para cada uno de estos atributos se desarrollaron *productos de referencia* y se les asignó una puntuación dentro de la escala [que oscila entre 0 (extremadamente baja o no perceptible) y 10 (extremadamente elevada)] utilizada para cuantificar su intensidad. A modo de ejemplo, se puede indicar que para el atributo "color" a cada juez se le proporciona la escala de color mostrada en la **Figura 6**, para que compare y evalúe el color de la muestra bajo estudio.

Figura 6. Escala de referencia usada para la evaluación sensorial del color del Fondillón, según los requerimientos de la DOP Alicante.

En el caso de usar el panel entrenado de la DOP Alicante (organismo acreditado por ENAC según la norma ISO/IEC 17065 "Evaluación de la conformidad – Requisitos para organismos que certifican productos, procesos y servicios"), en cada una de las sesiones de cata se introduce una muestra de repetibilidad, una muestra de reproducibilidad y una muestra con defecto.

La DOP Alicante ha desarrollado perfiles tipo para cada uno de sus vinos, incluyendo el Fondillón y lo que el panel entrenado evalúa es la proximidad de los perfiles sensoriales de los vinos bajo estudio con respecto a estos perfiles tipo. El perfil tipo del Fondillón es el representado en la **Figura 7** y aquellos descriptores marcados con una fecha de color verde son aquellos cuya intensidad está por encima de un umbral de 6,0.

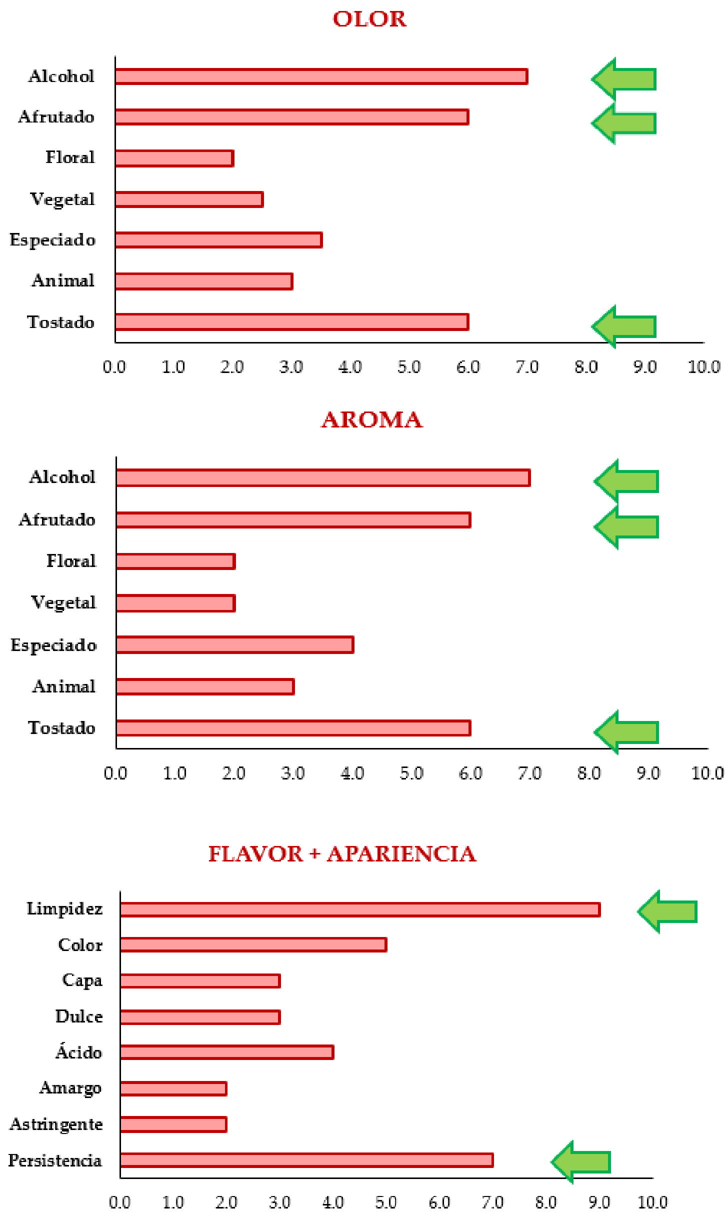


Figura 7. Perfil tipo del vino protegido Fondillón, según los requerimientos de la DOP Alicante.

Perfil sensorial del Fondillón

El perfil "legal" del Fondillón es el siguiente, de acuerdo con el Pliego de Condiciones de la DOP Alicante (Conselleria de Agricultura, Pesca, Alimentación y Agua, 2011):

- ✓ **Color**: Caoba, ámbar y tonalidades cobrizas.
- ✓ **Nariz**: Intensidad aromática, fruta madura, frutos secos, madera bien integrada, torrefactos.
- ✓ **Boca**: Equilibrada, buena estructura, gran volumen, persistencia, un toque ligeramente goloso.

Para cada uno de los atributos del perfil legal del Fondillón hay una tolerancia de ± 2 puntos de intensidad. Esto hace que el perfil sensorial del Fondillón sea muy amplio y englobe a vinos muy distintos entre sí; por supuesto, el principal factor que influye es el tiempo de envejecimiento y también las condiciones higiénicas y ambientales de las barricas en las que este envejecimiento se realiza.

La totalidad de las muestras de Fondillón estudiadas en esta tesis doctoral (publicaciones **1, 2 y 3**) cumplían con el perfil legal establecido. Solamente en algunos casos en los que se han manipulado algunas muestras [dopándolas con los compuestos responsables de algunos de los principales defectos, en concreto, SO_2 (azufre) y etil acetato (pegamento)] las muestras no cumplían con el perfil oficial, tal y como es lógico.

Conclusiones del objetivo 3

El léxico y la ficha de cata desarrollados específicamente para el Fondillón demostraron su validez para la descripción sensorial de este tipo de vino. De especial relevancia es el color tan característico que tiene este vino naturalmente dulce debido a su envejecimiento superior a los 10 años, que abarca tonalidades cobrizas, ámbar y caoba. Una definición, quizás simplista, del Fondillón sería: "vino con dulce, equilibrado (aunque con un marcado contenido alcohólico), con olor y aroma intensos en los que predominan los frutos maduros, los frutos secos, las notas tostadas de café, con madera bien integrada y con una persistencia muy larga".

Sin embargo, es posible que sea necesario incluir algún atributo adicional para terminar de definir la enorme complejidad de este tipo de vino. Hay algunas sensaciones químicas a petróleo que no quedan completamente recogidos en la versión actual del léxico y la ficha de cata. Por eso, en otros capítulos de esta tesis doctoral se estudian los compuestos volátiles responsables de estas sensaciones (hipotéticamente TDN y vitispirano) y se tratarán de vincular con nuevos descriptores a incluir en el léxico y la ficha de cata. Y también es posible que una nota ligera a ácido acético que aporta carácter a este tipo de vino queda enmascarada en el atributo genérico "acidez" y merezca un estudio detallado.

Objetivo 4.
Establecer el perfil general del consumidor típico de Fondillón.

Los resultados relativos a este objetivo se encuentran recogidos en la publicación 4 e incluyen los siguientes puntos: (*i*) estudio afectivo con un panel de consumidores de vinos naturalmente dulces, y (*ii*) estudio *on-line* para tratar de entender por qué se consume o no este tipo de vino. Las **hipótesis de trabajo** inicial de este estudio fueron: (*i*) que los consumidores no conocían las peculiaridades que hacen tan especial y único al Fondillón, (*ii*) que los consumidores con un elevado poder adquisitivo elevado no estaban interesados por un vino producido en una pequeña DOP y poco popular, y (*iii*) que había **poco soporte científico sobre su especificidad**. Esta situación comportaba que los productores/bodegas perdiesen el interés en producir y vender este vino, especialmente por la gran inversión en tiempo y dinero necesaria para su producción.

Sin embargo, es evidente que en los últimos 5-7 años y debido a las publicaciones científicas que avalan la calidad y especificidad de este vino y a la firme apuesta del Consejo Regulador de la DOP Alicante por convertir a este vino en el núcleo de este organismo, han relanzado las ventas y la imagen de este vino. Este cambio de tendencia, por ejemplo, se ve reflejada en el hecho de que en la campaña 2019-2020 se embotellaron 11.050 botellas de Fondillón comparado con las 5.720 embotelladas en la campaña 2014-2015; cifras que suponen un aumento de casi el 100 %. Otro factor que ha supuesto un relanzamiento importante de este producto es que los vinos Gran Reserva Fondillón Brotons 1964 (Bodega Brotons) y Fondillón 50 años "Siempre te esperaré" (Bodegas Monóvar) fueran galardonados "*ex aequo*" con el Premio Alimentos de España al mejor Vino 2020 del Ministerio de Agricultura, Pesca y Alimentación (MAPA). Es indudable que los premios en el mundo de la Enología fijan la atención de un elevado número de consumidores.

Estudio afectivo

En este estudio se trabajó con 5 muestras de Fondillón, seleccionadas para cubrir los 5 grupos/clústeres de Fondillón identificados empleando para ello la prueba "*napping*" y 20 catadores entrenados. Los 5 vinos empleados fueron:

1. **F1**, Gran Reserva 1987, representativo de muestras caracterizadas por ser dulces, con sabor a Moscatel, pasas y de color ámbar.
2. **F2**, Solera 1948, de gusto dulce y amargo, con notas amaderadas, a cartón y tofe.

3. **F3**, Reserva Especial, de sabor amargo, ácido y con elevada percepción de alcohol y de color caoba.
4. **F4**, Gran Reserva 1964, dulce, amargo, con notas tostadas, a tofe y con un postgusto muy largo.
5. **F5**, Solera 1996, dulce con notas tostadas, a caramelo y vainilla y de color ámbar.

El estudio afectivo se realizó con 100 consumidores habituales de vino dulce u oxidado (Oporto, Madeira, Jerez, Fondillón, …) al menos dos veces por mes y se evaluó el grado de satisfacción: (*i*) global y (*ii*) de los atributos principales: color, alcohol, tostado/caramelo/café, químico, dulzor, acidez y postgusto. El grado de satisfacción se evaluó usando una escala hedónica de 9 puntos, y para evaluar la intensidad de cada atributo se usó una escala JAR (*Just About Right*) también de 9 puntos.

Adicionalmente para identificar los atributos que son claves en la satisfacción de los consumidores se desarrolló una pregunta *Check-All-That-Apply* (CATA) que consistía en 18 atributos sensoriales (9 notas de olor y 9 de sabor: afrutado, vainilla, café, floral, caramelo, fruto seco, bosque mediterráneo, pasas y tofe). Finalmente, se incluyó una pregunta sobre la preferencia del consumidor (después de haber probado estas 5 muestras de Fondillón, ¿qué muestra es la que más te gusta o prefieres?) y otra pregunta sobre la intención de compra (¿estarías dispuesto/a a comprar este vino?).

Tabla 5. Puntuaciones del grado de satisfacción de los consumidores españoles respecto a las muestras de Fondillón analizadas.

Samples	Overall	Color	Alcohol (o)[¥]	Toasted (o)[¥]	Chemical (o)[¥]	Sweetness	Sourness	Alcohol (f)[¥]	Toasted (f)[¥]	Chemical (f)[¥]	Aftertaste
	ANOVA Test[†]										
	***	***	***	***	**	***	***	***	**	**	***
	Tukey Multiple Range Test[‡]										
F1	6.7 a	6.8 ab	6.8 a	6.8 a	5.4 ab	6.2 a	6.6 a	6.5 a	6.8 a	6.0 ab	6.6 ab
F2	5.8 b	6.5 ab	5.6 b	5.9 b	4.6 b	5.2 b	5.6 b	5.5 b	5.5 b	5.0 b	5.6 c
F3	5.9 b	6.9 a	6.4 ab	6.4 ab	4.1 b	5.3 b	5.5 b	5.5 b	6.0 ab	4.5 b	5.4 c
F4	6.9 a	7.0 a	6.0 ab	6.3 ab	6.3 a	6.5 a	6.1 ab	5.9 ab	6.7 a	7.0 a	7.0 a
F5	6.0 b	6.1 b	5.6 b	6.0 b	5.7 ab	5.9 ab	5.9 ab	5.4 b	6.2 ab	6.3 ab	6.2 bc

*[†] **, ***, significant at p < 0.01, and 0.001, respectively. [‡]Values (mean of 100 consumers) followed by the same letter, within the same column, were not significantly different (p > 0.05), according to LSD least significant difference test. [¥](o) = odor; (f) = flavor.*

Las muestras con un mayor valor de satisfacción global fueron la F4 (6,9) y la F1 (6,7) (**Tabla 5**). Es importante resaltar que al estudiar el perfil del consumidor que valoró positivamente estas dos muestras, solo al 5 % de los consumidores les gustaron estas dos muestras simultáneamente. Es decir, cada una de estas muestras es representativa de un tipo de Fondillón diferente y con un tipo de consumidor bien definido y también diferente.

Las muestras con un mayor grado de satisfacción están asociadas a notas a café y frutos secos (F4) y a caramelo y bosque mediterráneo (F1). Sin embargo, los consumidores valoran negativamente intensidades excesivas de alcohol, acidez, amargor e incluso de notas a tostado. Por tanto, se puede concluir que el perfil sensorial ideal del Fondillón se caracteriza por **un equilibrio entre alcohol, dulzor, acidez y amargor, con notas florales y afrutadas intensas, y con un prolongado y agradable postgusto**.

Los resultados de la prueba de preferencia y de la intención de compra fueron los siguientes:

- F1: 28 % de los consumidores prefirió esta muestra, y el 83 % de los consumidores estaban dispuestos a comprarla.
- F2: 10 % (preferencia) y 53 % (disponibilidad a comprar), respectivamente.
- F3: 13 % y 60 %, respectivamente.
- **F4: 30 % y 100 %, respectivamente.** Es la muestra con los valores más elevados de estos dos parámetros afectivos.
- F5: 18 % y 60 %, respectivamente.

<u>Perfil de consumidor, estudio *on-line*</u>

Se realizó una encuesta on-line a 294 consumidores (38 % mujeres) de las comunidades autónomas de Murcia y Valencia, seleccionados bajo dos criterios: (*i*) beber vino de la DOP Alicante al menos dos veces por semana, o (*ii*) beber vinos dulces u oxidados (Oporto, Madeira, Jerez, Fondillón, etc.) al menos una vez al mes. Por tanto, los participantes deberían tener, al menos, nociones básicas sobre el Fondillón o vinos similares. El cuestionario empleado tiene preguntas relacionadas con los hábitos de consumo de Fondillón y demográficas (género, edad, ingresos y educación).

Los resultados obtenidos indican que solo el 50 % de los participantes en este estudio consumían Fondillón, a pesar de vivir en regiones muy vinculadas a este tipo de vino, y beber vino de la DOP Alicante o vinos dulces/oxidados.

El perfil de un consumidor tipo de Fondillón (**Figura 8**) (basado en los datos de aquellos consumidores que consumen regularmente este tipo de vino) es el siguiente: **hombre** (68 %), **de entre 42 y 52 años** (41 %), **con una educación elevada**, consistente en bachiller o superior (51 %), **y con un nivel de ingresos anuales entre 25.000 y 50.000 euros** (33 %). Adicionalmente es importante indicar que el 37 % de los consumidores solo bebe Fondillón en ocasiones especiales y el 23 % solo lo bebe 2-3 veces al año. Es decir, **el consumo de Fondillón se limita generalmente a ocasiones especiales**.

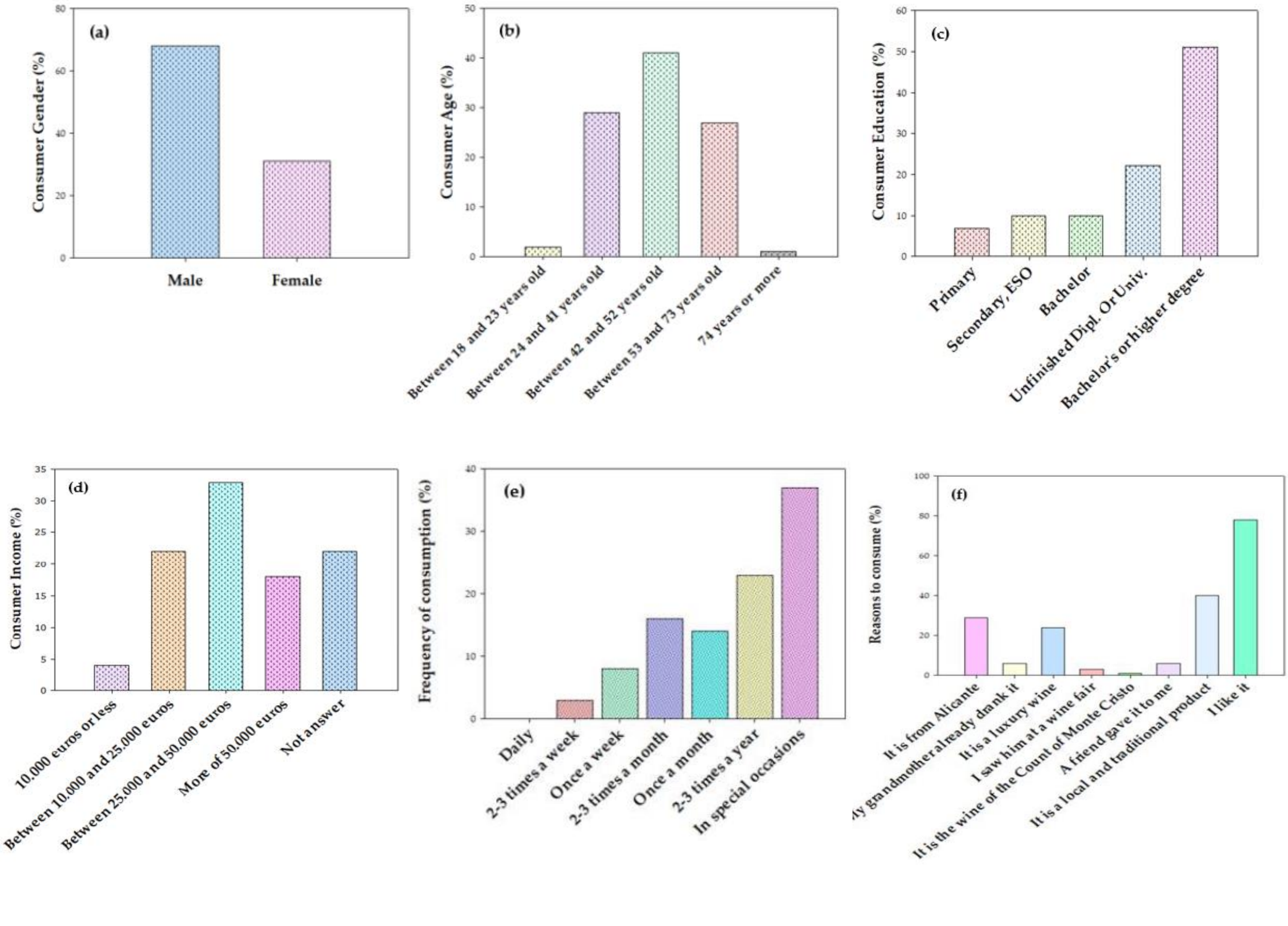


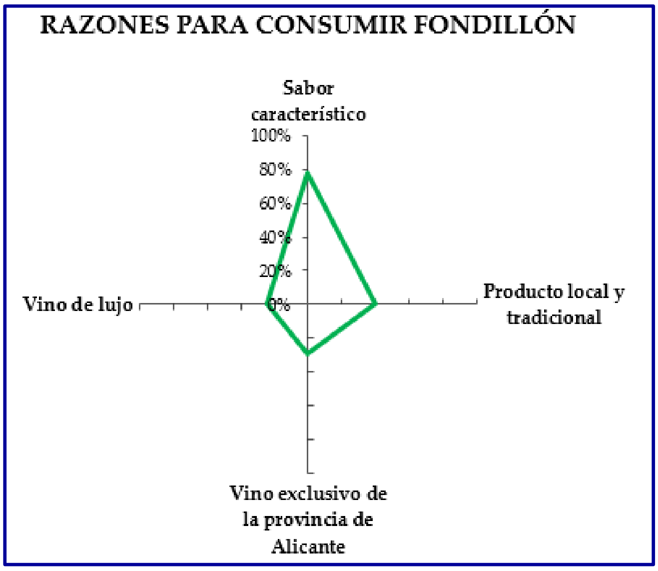
Figura 8. Perfil del consumidor de Fondillón afectado por (a) género, (b) edad, (c) nivel educativo y (d) ingresos anuales, junto con hábitos de consumo clave: (e) frecuencia de consumo y (f) principales razones para comprar y consumir este vino.

Con respecto a las razones para consumir Fondillón, destacan: les gusta el sabor característico (78 %), es un producto local y tradicional (40 %), es un vino exclusivo de la provincia de Alicante (29 %) y es un vino de lujo (24 %).

Con respecto a los atributos sensoriales que controlan la satisfacción del consumidor hay que destacar: dulzor, aroma tostado/caramelo, a pasas, a frutos secos, postgusto largo y buen balance.

Con respecto al lugar habitual de consumo, el 77 % de los consumidores lo beben **en casa**, y solo el 16 % en restaurantes, principalmente por el elevado precio de este vino en estos establecimientos y el hecho de no encontrarse en un elevado porcentaje de las cartas de vinos.

Entre las razones principales para NO consumir Fondillón cabe destacar que el 27 % de los participantes indican que no está disponible en los lugares habituales de compra de bebidas alcohólicas, el 14 % es difícil de encontrar, el 9 % porque es un producto caro, y lo que resulta más frustrante **el 18 % de los casi 300 entrevistados NO conoce qué es el Fondillón** (**Figura 9**).



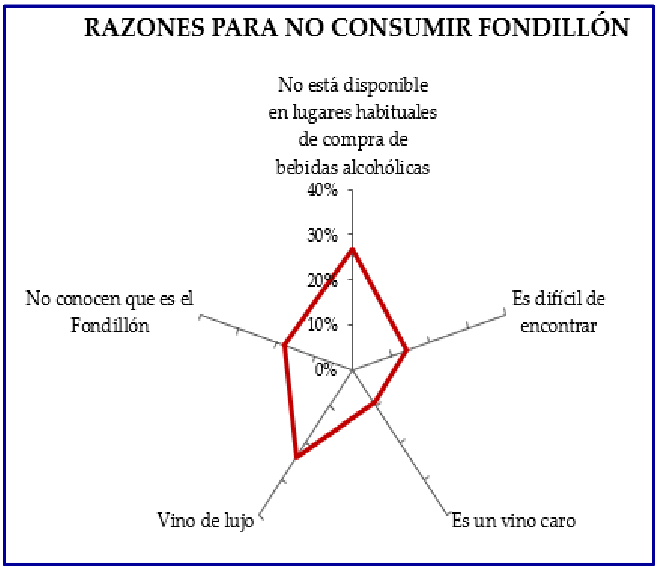


Figura 9. Razones para consumir o no Fondillón

Conclusiones del objetivo 4

El perfil sensorial tipo de Fondillón más valorado/apreciado por los consumidores es: **buen equilibrio entre alcohol, dulzor y amargor, con notas florales y afrutadas intensas, y con un prolongado y agradable postgusto**.

El consumidor actual de Fondillón es un **hombre de entre 42 y 52 años, con un elevado nivel cultural/educativo, y con unos ingresos anuales entre 25.000 y 50.000 euros, y que bebe Fondillón principalmente en casa y en ocasiones especiales**.

Las principales razones para consumir Fondillón es que un vino tradicional de Alicante y con un sabor característico y distintivo. Sin embargo, el 50 % de los consumidores no lo consumen porque: (*i*) no lo conocen, (*ii*) es muy caro, o (*iii*) no es fácil de encontrar. En este sentido es importante reseñar el elevadísimo precio de este vino en los restaurantes y el hecho de que no está en muchas de las cartas de vino.

Las bodegas, el Consejo Regulador y las distribuidoras/comercializadoras de Fondillón deben mejorar sus campañas de comunicación y distribución como parte de sus estrategias de marketing para dar a conocer el valor real del Fondillón y ponerlo a disposición de los consumidores en un elevado porcentaje de bares y restaurantes, inicialmente de la Comunidad Valenciana y posteriormente del país.

Objetivo 5.
Estudiar el efecto del envejecimiento y potencial adulteración sobre los principales parámetros fisicoquímicos, composición volátil y perfil sensorial del Fondillón.

Los resultados relativos a este objetivo se encuentran recogidos en la publicación 5 e incluyen los siguientes puntos: (*i*) azúcares y ácidos orgánicos, (*ii*) actividad antioxidante e índice de polifenoles totales, (*iii*) características cromáticas, (*iv*) minerales, y (*v*) composición volátil.

Azúcares y ácidos orgánicos

El envejecimiento del vino Monastrell en barricas de roble necesario para alcanzar la categoría de Fondillón comporta la reducción significativa del contenido de los siguientes ácidos orgánicos: cítrico, tartárico y málico. Estos cambios con el tiempo comportan una reducción media de casi el 70 % de las concentraciones de estos tres ácidos orgánicos. Los valores de referencia para los ácidos cítrico, tartárico y málico en el Fondillón son: 0,8, 2,5 y 1,9 g/L, respectivamente.

Sin embargo, el ácido acético aumenta ligeramente durante el envejecimiento de este vino elaborado con uva sobremadura. De hecho, la presencia de bajas concentraciones de ácido acético en vinos oxidados no se considera un aroma indeseado (*off-flavor*) sino un atributo característico y positivo (Caldeira *et al.*, 2016). El valor de referencia del ácido acético en el Fondillón es: 1,3 g/L.

En el caso de los azúcares quizás la evolución esperada durante el proceso de envejecimiento fuera que su concentración total disminuyera progresivamente; sin embargo, en el caso del Fondillón se ha observado un aumento significativo especialmente de la fructosa. Este aumento puede deberse a una transferencia de ciertos compuestos de la madera de la barrica (por ejemplo, pentosas, hexosas y polisacáridos de la hemicelulosa) al vino (Valcárcel *et al.*, 2022). Los valores de referencia para glucosa y fructosa en el Fondillón son: 7,1 y 6,0 g/L, respectivamente.

Actividad antioxidante y contenido total de polifenoles

La bibliografía muestra un comportamiento diverso de la actividad antioxidante durante el envejecimiento del vino, algunos investigadores observaron un aumento de este parámetro (e.g., de Villiers *et al.*, 2012) mientras que otros observaron un descenso (e.g., Coelho *et al.*, 2018). Esta controversia puede deberse, entre otros factores, al estado inicial de las uvas, al proceso de vinificación y sobre todo al distinto tipo y tiempo de envejecimiento. En el caso concreto de este estudio se observó, usando tres métodos distintos ($ABTS^{•+}$, FRAP and $DPPH^{•}$), que un envejecimiento corto comportó un aumento de la actividad antioxidante, pero un envejecimiento prolongado (más de 10 años) supuso una reducción significativa en este parámetro. En el Fondillón, este descenso llega a suponer hasta un 53 % con respecto al valor máximo alcanzado en la actividad antioxidante. Los valores de referencia para la actividad antioxidante ($ABTS^{•+}$, FRAP and $DPPH^{•}$) en el Fondillón son: 1,3, 3,5 y 1,5 mmol Trolox/L, respectivamente.

En general, en el vino suele haber una correlación positiva entre la actividad antioxidante y el contenido total de compuestos fenólicos. Este comportamiento fue el observado en el caso del presente estudio, de modo que al disminuir el valor de la actividad antioxidante con el tiempo de envejecimiento también se observó una disminución en el índice fenólico total (TPI), llegando a reducirse su valor ~23 %. El valor de referencia del TPI en Fondillón es de 221 mg ácido gálico/100 mL.

Características cromáticas

Durante el envejecimiento de vinos tintos Monastrell, el vino incrementó su intensidad de la componente amarilla mientras que bajó la de las componentes roja y azul. En definitiva, el vino adquiere un color más marronáceo. Esta tendencia es general y no admite lugar a dudas y es aplicable a la totalidad de vinos oxidados, incluyendo el Fondillón.

Minerales

Los elementos minerales pueden provenir de tres fuentes principales: naturales, antropogénicas o enológicas. Es decir, pueden provenir de la uva (por acumulación natural desde el suelo), las "impurezas" debidas a un proceso de vinificación, o de los distintos procesos de elaboración del vino.

En general se observó que el proceso de envejecimiento, necesario para la elaboración del Fondillón, comportó un aumento significativo de varios micronutrientes (Fe, Cu, Mn y Zn) junto con dos macronutrientes (Mg y Na); mientras que solo se observó un descenso ligero, aunque significativo del contenido de Ca.

Compuestos volátiles

El perfil volátil de un vino está constituido por compuestos provenientes directamente de la uva, del proceso de fermentación realizado por las levaduras y/o del proceso de envejecimiento (Cozzolino, 2015). Durante el envejecimiento, diversos compuestos de la madera de las barricas pueden ser transferidas al vino y otros compuestos pueden formarse a partir de sus precursores, produciendo un perfil aromático final muy complejo (Pohl, 2007).

Para ser capaces de detectar un fraude en la venta de Fondillón, es muy importante que se identifiquen **marcadores de envejecimiento** que no estén presentes o lo estén en concentraciones muy bajas en otros vinos, especialmente los de la variedad Monastrell. Estos compuestos garantizarán la autenticidad de este vino de la DOP Alicante.

Se detectaron 95 compuestos volátiles en el espacio de cabeza de las muestras de vino Monastrell estudiadas. De estos casi cien compuestos, se han identificado 16 que pueden jugar un papel fundamental en la detección de adulteraciones en la venta de Fondillón (**Tabla 6**).

Algunos de estos compuestos, concretamente el furfural, el guaiacol y el eugenol han sido previamente identificados como marcadores de envejecimiento en madera (Perestrelo *et al.*, 2011). Estos mismos autores concluyeron que el **furfural** es un buen marcador del envejecimiento del vino ya que se forma por: (*i*) deshidratación de los azúcares por la reacción de Maillard, (*ii*) pirólisis de los carbohidratos, y/o (*iii*) caramelización.

Otro compuesto de interés es el **benzaldehído**, ya que se encontraron concentraciones relativamente elevadas en Fondillón, comparado con los otros vinos Monastrell estudiados. Por tanto, se puede considerar que el benzaldehído es un aroma típico de vino tinto envejecido.

En general, los norisoprenoides, tales como el **vitispirano** y el **TDN**, aumentan durante el proceso de envejecimiento de un vino y han sido identificados como compuestos odoríferos activos previamente en esta tesis doctoral. El TDN es de especial interés ya que aporta a los vinos en los que está presente una nota química a "queroseno" característica de los vinos Weisser Riesling (Castro-Váquez *et al.*, 2011) y que también juega un papel importante en el perfil aromático del Fondillón.

Tabla 6. Concentración (mg/L) de compuestos volátiles clave en la identificación de un posible fraude o adulteración en la comercialización de Fondillón.

Compuesto volátil	**Joven**	**Reserva**	Fondillón
		(mg L^{-1})	
1,1-dietoxietano	nd[†]	nd	0.13
Furfural	nd	0.09	0.60
1,1-dietoxi-2-metilpropano	nd	nd	0.02
Benzaldehído	nd	0.01	0.28
1-(1-etoxietoxi)-pentano	nd	nd	0.03
Isoamil butirato	nd	nd	0.04
Guaiacol	nd	nd	0.01
Etil sorbato	nd	nd	0.13
Nonanal	nd	nd	0.11
Dietil butanedioato	1.86	1.90	2.80
4-Etillguaiacol	nd	nd	0.02
Vitispirano	0.12	0.22	0.37
trans-Whiskey lactona	0.02	0.05	0.06
cis-Whiskey lactona	0.11	0.09	0.17
Eugenol	nd	0.01	0.01
TDN	0.00	0.04	0.35

[†]*nd: no detectado.*

Para poder disponer de mayores evidencias en la posible adulteración del Fondillón, este vino se mezcló con vino Monastrell joven y envejecido/reserva en proporciones 30, 50 y 70 % para poder construir modelos de Análisis de Compuestos Principales (PCA). Se construyeron 3 modelos distintos de PCA: (*i*) muestras puras 100 % (Fondillón, vino reserva y vino joven, todos ellos Monastrell); (*ii*) Fondillón + vino envejecido/reserva en proporciones 100, 70, 50 y 30 %; y, (*iii*) Fondillón + vino joven en proporciones 100, 70, 50 y 30 %. Con este diseño estadístico se consiguió identificar 8 compuestos que caracterizan las diferencias del Fondillón (puro, sin adulterar) y el resto de las muestras estudiadas; estos compuestos son: **furfural, benzaldehído, etil hexanoato, feniletil alcohol, dietil butanodiato, vitispirano, TDN y etil decanoato**.

Conclusiones del objetivo 5

El Fondillón está caracterizado por los siguientes contenidos "simultáneos":

- Contenidos relativamente elevados de Cu (~10 µg/L), Zn (~40 µg/L) y Na (~4,5 g/L).
- Contenidos relativamente elevados de fructosa (~6,0 g/L) y ácido acético (~1,0 g/L).
- Contenidos relativamente elevados de los siguientes compuestos volátiles:
  - 1,1-dietoxietano (~10 mg/L),
  - furfural (~0,6 mg/L),
  - benzaldehído (~0,3 mg/L),
  - vitispirano (~0,3 mg/L) y
  - TDN (~0,3 mg/L).
- Contenidos relativamente bajos de los siguientes compuestos volátiles:
  - etil octanoato (≤ 2,5 mg/L) y
  - etil decanoato (≤ 2,0 mg/L).

Por tanto, en muestras potencialmente calificadas como Fondillón, todos aquellos perfiles químicos que no cumplan "simultáneamente" con estos requisitos anteriores pueden ser sospechosos de ser muestras adulteradas por mezclas con otros tipos de vino.

9. CONCLUSIONES

1. Los parámetros que se consideran **marcadores** importantes del Fondillón son:

    - actividad antioxidante medida mediante el método DPPH•;
    - compuestos volátiles relacionados con el envejecimiento: TDN, vitispirano, furfural, benzaldehído;
    - compuestos volátiles que determinan el olor y aroma (*odor-active compounds*): alcohol feniletílico, etil lactato, dietil succinato y dietil malato),

- perfil polifenólico: ácidos fenólicos, destacando el ácido protocatecuico y el ácido gálico.

2. Casi el 90 % del contenido total de **compuestos volátiles** está constituido por ésteres y alcoholes; sin embargo, compuestos minoritarios como los norisoprenoides pueden jugar un papel clave en el olor y aroma del Fondillón. Se han identificado, por primera vez, 22 **compuestos odoríferos activos** que aportan notas florales, afrutadas y tostadas, mediante cromatografía de gases acoplada a un puerto de olfatometría.

3. La **huella digital del Fondillón** está conformada por constituyentes que lo hacen único, y que incluye concentraciones determinadas de Cu, Zn, Na, fructosa, ácido acético, furfural, benzaldehído, vitispirano, TDN, etil octanoato y etil decanoato. Este perfil único permite identificar muestras susceptibles de estar adulteradas y de suponer un riesgo para el prestigio y la reputación del Fondillón y por extensión de los vinos amparados bajo la DOP Alicante.

4. Se ha desarrollado un **léxico y** una **ficha de cata** específicos para este vino de la DOP Alicante, que han demostrado su validez. Sin embargo, hay dos descriptores que todavía pensamos que no están completamente reflejados en estos documentos y que merecen un trabajo adicional: (*i*) una nota química única (definida como queroseno/petróleo y posiblemente vinculada con el TDN) y (*ii*) una nota a ácido acético, que aporta frescura.

5. La definición sensorial del Fondillón es:

    - Según los expertos "*vino dulce, equilibrado (aunque con un marcado contenido alcohólico), con olor y aroma intensos en los que predominan los frutos maduros, los frutos secos, las notas tostadas de café, con madera bien integrada y con una persistencia muy larga*"

- ✦ Según los consumidores "*vino con buen equilibrio entre alcohol, dulzor y amargor, con notas florales, afrutadas y tostadas intensas, y con un prolongado y agradable postgusto*".

6. El **consumidor** actual de Fondillón es un hombre de entre 42 y 52 años, con un elevado nivel cultural/educativo, y con unos ingresos anuales entre 25.000 y 50.000 euros, y que bebe este vino principalmente en casa y en ocasiones especiales. Las principales razones para no consumir Fondillón son: (*i*) el enorme desconocimiento del producto por parte del consumidor, (*ii*) su elevado precio, y (*iii*) que no es fácil de encontrar (ni en la cadena habitual de compra ni en los restaurantes).

10. FUTURAS INVESTIGACIONES

Las bodegas, las distribuidoras/comercializadoras y el Consejo Regulador de la DOP Alicante podrán utilizar la información generada en esta tesis doctoral para completar y dar una solidez científica a sus campañas de comunicación y distribución como parte de sus estrategias de marketing para dar a conocer el valor real del Fondillón (basándose en un soporte científico sólido y objetivo que certifique su especificidad). Sin embargo, todavía son varios los puntos a mejorar dentro de esta línea de investigación. A continuación, voy a describir brevemente las líneas futuras que me gustaría abordar para dar continuación a la investigación realizada en esta tesis doctoral:

- Una vez realizado el esfuerzo inicial para conocer las bases de la composición fisicoquímica, el perfil polifenólico, el perfil sensorial y el tipo de consumidor del Fondillón, hemos de seguir profundizando y generalizando el conocimiento adquirido para una colección de vinos procedentes principalmente de dos bodegas, al mayor número de muestras procedentes de la totalidad de las bodegas que comercializan Fondillón.
- Una vez generado esta base de datos amplia y completa, se podrán desarrollar modelos matemáticos que nos permitan de un modo casi automático y con un elevado grado de seguridad: (*i*) establecer la edad de un vino bajo estudio; (*ii*) determinar la vida útil y/o periodo óptimo de envejecimiento de un vino determinado; y, (*iii*) determinar posibles adulteraciones del producto y, por tanto, evitar fraudes en la comercialización y venta de vinos etiquetados como Fondillón.
- Estudiar comparativamente los factores "solera" y "añada" para determinar si un método de crianza es superior al otro en el caso concreto del Fondillón.
- Avanzar en cuáles son los parámetros fisicoquímicos, aromáticos y sensoriales claves que convierten a vinos similares al Fondillón (por ejemplo, Oporto, Madeira y Jerez) en vinos exitosos a escala mundial. Y a su vez determinar el perfil de este consumidor, para saber cuál es el consumidor objetivo para la compra de Fondillón.
- Desarrollar estrategias para convencer a los consumidores de la Comunidad Valenciana (y posteriormente de España) que es imprescindible que reclamen vinos de la DOP

Alicante en sus puntos habituales de compra y consumo, para así fomentar la sostenibilidad de la viticultura y enología de nuestra región.

- Implementar estrategias para poner el Fondillón a disposición de los consumidores en un elevado porcentaje de tiendas/supermercados/hipermercados, bares y restaurantes, inicialmente de la Comunidad Valenciana y posteriormente del país.

11. REFERENCIAS

Alonso A., Vázquez-Araújo L., García-Martínez S., Ruiz J.J., and Carbonell-Barrachina A.A. 2009. Volatile compounds of traditional and virus-resistant breeding lines of Muchamiel tomatoes. *Eur. Food Res. Technol*. Vol. 230 (2), https://doi.org/10.1007/s00217-009-1173-2

Bendicho, V. 1991. *Chronica de la muy ilustre, noble y leal ciudad de Alicante*. Ayuntamiento de Alicante, Alicante, España, pp 1584-1642.

Benzie I.F.F., Strain J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem*. 239(1): 70–76, https://doi.org/10.1006/abio.1996.0292

Caldeira I., Santos R., Ricardo-da-Silva J.M., Anjos O., Mira H., Belchior A.P., Canas S. 2016. Kinetics of odorant compounds in wine brandies aged in different systems. *Food Chem.* 211: 937–946, https://doi.org/10.1016/j.foodchem.2016.05.129

Campello-Quereda A., Amores-Liza J.J., Sánchez-Campos J., Martín-Martínez E., Bodewig-Belmonte R. 2009. *Relatos de una ciudad dormida*. Editorial club universitario, Alicante, España, pp 189.

Carmona-Escribano F.J. 2015. *Fondillón Thesis. From a Glorius Past to an Uncertain Future*. http://universofondillon.com/fondillon-thesis-from-a-glorious-past-to-a-uncertain-future/ [Visitada 02 de julio de 2023]

Castro-Vázquez L., Alañón M.E., Calvo E., Cejudo M.J., Díaz-Maroto M.C., Pérez-Coello M.S. 2011. Volatile compounds as markers of ageing in Tempranillo red wines from La Mancha D.O. stored in oak wood barrels. *J. Chromatogr.* 1218(30): 4910–4917, https://doi.org/10.1016/j.chroma.2010.12.094

Cavanilles, A. J. 1797. *Observaciones sobre la historia natural, geografia, agricultura, poblacion y furtos del reyno de Valencia*. Vol. 2. Imprenta real, Madrid, España, pp 236.

Celestino-Pérez S., Blánquez-Pérez J. 2013. *Patrimonio cultural de la vid y el vino Vine and wine cultural heritage*. 2013. UAM, Almendralejo, Madrid, España, pp 426.

Cernuda-Juan E., Marhuenda-Verdejo R., Vidal-Togores J.L., Zugasti, G. 1975. *Aspectos técnicos enológicos de los vinos Alicantinos. Mistelas y Anisados*. Publicaciones del Instituto de Estudios Alicantinos, Alicante, España, pp 142.

5 barricas amantes del vino. 2023. *La historia de un tesoro llamado Fondillón.* https://5barricas.valenciaplaza.com/la-historia-de-un-tesoro-llamado-fondillon/ [Visitada 29 de mayo de 2023]

Coelho E.M., da Silva-Padilha C.V., Miskinis G.A., Barroso de Sá A.G., Pereira G.E., Cavalcanti de Azevêdo L., dos Santos-Lima M. 2018. Simultaneous analysis of sugars and organic acids in wine and grape juices by HPLC: Method validation and characterization of products from northeast Brazil. *J. Food Compost. Anal.* 66: 160–167, https://doi.org/10.1016/j.jfca.2017.12.017

Cozzolino, D. 2015. Cap. 20. Elemental composition in grapes and wine: Role, analytical methods and their use, 473-487. EN: *Handbook of Mineral Elements in Food*; de la Guardia M., Garrigues S. Chichester, West Sussex, United Kingdom: John Wiley & Sons.

Desde 1510, la historia más bonita jamás servida. 2022. Alicante 1510. *La Junta d'Inhibició del Vi d'Alacant, gemen de la DO más antigua del mundo*. https://vinosalicantedop.org/wp-content/uploads/2022/11/ALICANTE-1510-maqueta-defini-1.pdf?cv=1 [Visitada 12 de junio de 2023]

Dion Roger. 2010. *Histoire de la vigne et du vin en France, des origines au XIXe siècle*. Cnrs Eds, Francia, pp 770.

de Saint Laumer J.Y., Cicchetti E., Merle P., Egger J., Chaintreau A. (2010). Quantification in gas chromatography: Prediction of flame ionization detector response factors from combustion enthalpies and molecular structures. *Anal. Chem.* 82(15), 6457–6462, https://doi.org/10.1021/ac1006574

de Villiers A., Alberts P., Tredoux A.G.J., Nieuwoudt H.H. 2012. Analytical techniques for wine analysis: An African perspective; a review. *Anal. Chim.* 730: 2–23, https://doi.org/10.1016/j.aca.2011.11.064

DOP Alicante. 2019. Normativa de elaboración "Fondillón" Alicante Denominación de Origen. https://fondillon.org/wp-content/uploads/2021/03/manual-de-elaboracio%cc%81n-fondillo%cc%81n-alicante-julio-19.pdf [Visitada 10 de junio de 2023]

DOP Alicante. 2022. Normativa de elaboración "Fondillón" Alicante Denominación de Origen. Documento cedido por DOP Alicante

Fundación MARQ. 2014. *El vino en Alicante.* Fundación MARQ y Diputación de Alicante, Alicante, España, pp 189.

G. Valenciana, Orden 5/2011, de 16 de noviembre, de la Conselleria de Agricultura, Pesca, Alimentación y Agua, Por la que se Aprueba el Texto del Reglamento y Pliego de Condiciones de la Denominación de Origen Protegida Alicante y su Consejo Regulador, Diario Oficial de la Comunitat Valenciana, Valencia, España, 2011.

G. Valenciana, Resolución de 4 de agosto de 2021, de la Consellera de Agricultura, Desarrollo Rural, Emergencia Climática y Transición Ecológica, por la que se adopta y hace pública la decisión favorable a la modificación del pliego de condiciones de la Denominación de Origen Protegida Alicante.

Giménez-López E. 1981. *Alicante en el siglo XVIII. Economía de una ciudad portuaria en el antiguo régimen*. Diputación Provincial de Valencia, Institució «Alfons El Magnánim», Valencia, España, pp 449.

Glories Y. 1984. *The color of red wines*. Connaissance Vignevini. Vol 18: 253–271, Talense, Francia, pp 23.

Gómez-Bellard C., Guérin-Fockedey P. 1995. *Los lagares del Alt de Benimaquia (Denia): en los inicios del vino ibérico*. Simposio Arqueología del Vino, Jerez de la Frontera, pp 241-270.

ISO. 1977. *ISO 3591:1977, Sensory Analysis. Apparatus. Wine Tasting Glass*. ISO, Geneva, Switzerland

ISO (International Organization of Standardization). 2012a. ISO 8586:2012. Sensory Analysis—General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors, https://www.iso.org/standard/45352.html [Visitada 10 de junio de 2023]

ISO (International Organization of Standardization). 2012b. ISO/IEC 17065:2012. Conformity Assessment—Requirements for Bodies Certifying Products, Processes and Services, https://www.iso.org/standard/46568.html [Visitada 10 de junio de 2023]

Katalinic V., Milos M., Kulisic T., Jukic M. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem*. 94(4): 550–557, https://doi.org/10.1016/j.foodchem.2004.12.004

Kelebek, H. 2016. LC-DAD–ESI-MS/MS characterization of phenolic constituents in Turkish black tea: Effect of infusion time and temperature. *Food Chem.*, 204: 227–238, https://doi.org/10.1016/j.foodchem.2016.02.132

Luna J.M., Garau M.C., Negre A., March J., Martorell A. 2010. Composición Fenólica y Actividad Antioxidante de Variedades Minoritarias de Vid de las Islas Baleares. EN: *Proceedings of the VII Foro Mundial del Vino*, Logroño, La Rioja, España. 12–14 May 2010.

Mark O´Neill. 2018.Fondillón, *La historia de un tesoro.* http://merkoneill.es/2023. [Visitada 29 de mayo de 2023].

Márquez-Villora J.C. 1999. *El comercio romano en el Portus Ilicitanus. El abastecimiento exterior de productos alimentarios (siglos I aC-V dC)*. Universidad de Alicante, Alicante, España, pp 537.

Martín J.C. 2011. *Valencians contra la Filoxera.* Anaconda Editions, Barcelona, España, pp 184.

Ministerio de agricultura, pesca y alimentación. 2023. https://www.mapa.gob.es/app/materialVegetal/fichaMaterialVegetal.aspx?lng=es&IdFicha=489 [Visitada 29 de mayo de 2023].

Montojo-Montojo V. 2007. *El comercio de Alicante en los reinados de Felipe II y Felipe III: una contrucción desde la cooperación.* Cuadernos de historia moderna, vol (32), pp 87-111.

Münzer J. *Viaje por España y Portugal en los años 1494-1495.* 1991. Ediciones Polifemo, Madrid, España, pp 325.

OIV (International Organization of Vine and Wine). 2020. *Compendium of International Methods of Wine and Must Analysis*. Vol 1, Paris, France.

OIV (International Organization of Vine and Wine). 2022. *Compendium of International Methods of Wine and Must Analysis.* Vol 2, Paris, France.

Perestrelo R., Barros A.S., Câmara J.S, Rocha S.M. 2011. In-depth search focused on furans, lactones, volatile phenols, and acetals as potential age markers of Madeira wines by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry combined with solid phase microextraction. *J. Agric. Food Chem.* 59(7): 3186–3204, https://doi.org/10.1021/jf104219t

Piqueras J. 2014. *La vid y el vino en España.* Publicacions de la Universitat de València, España, pp 468.

Pohl P. 2007. What do metals tell us about wine? *TrAC Trends Analyt Chem.* 26(9): 941–949, https://doi.org/10.1016/j.trac.2007.07.005

Pozo-Bayón M.Á, Moreno-Arribas M.V. 2011. Sherry wines. *Adv. Food Nutr. Res.* 63: 17–40, https://doi.org/10.1016/B978-0-12-384927-4.00002-6

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 26(9-10): 1231–1237, https://doi.org/10.1016/S0891-5849(98)00315-3

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

Robinson F., Johnson H. 2003. *El vino: nuevo atlas mundial.* Blume, Barcelona, España, pp 352.

Robinson A.L., Boss P.K., Solomon P.S., Trengove R.D., Heymann H., Ebeler S.E. 2013. Origins of grape and wine aroma. Part 1.Chemical components and viticultural impacts. *Am. J. Enol. Vitic.* 65: 1–24, doi.org/10.5344/ajev.2013.12070

Rocco D.S, Cravero M.C., Nadia G. 1989. *Metodi per lo studio dei polifenoli dei vini*. L'Enotecnico, Maggio, Asti, Italia, 83–89.

Selli S., Cabaroglu T., Canbas A., Erten H., Nurgel C., Lepoutre J. P., Gunata Z. 2004. Volatile composition of red wine from cv. Kalecik Karası grown in central Anatolia. *Food Chem*. 85(2): 207–213, https://doi.org/10.1016/j.foodchem.2003.06.008

Selli S., Kelebek H., Turan Ayseli M., Tokbas H. 2014. Characterization of the most aroma-active compounds in cherry tomato by application of the aroma extract dilution analysis. *Food Chem*. 165: 540–546, https://doi.org/10.1016/j.foodchem.2014.05.147

Sonmezdag A.S. 2018. Characterization of aroma and aroma-active composition of Gaziantep cheese by solvent-assisted flavor evaporation (SAFE) and aroma extract dilution analysis (AEDA). *J. Food Process. Preserv.* 43(6): e13840, https://doi.org/10.1111/jfpp.13840

Valcárcel-Muñoz M.J., Guerrero-Chanivet M., Rodríguez-Dodero M.C., García-Moreno M.V., Guillén-Sánchez D.A. 2022. Analytical and Chemometric Characterization of Fino and Amontillado Sherries during Aging in Criaderas y Solera System. *Molecules*. 27(2): 365, https://doi.org/10.3390/molecules27020365

Vinos Alicante DOP. 2021. Fondillón, https://fondillon.org [Visitada 9 de junio de 2023].

VitiEno. 2016. La cultura del vino. http://www.vitieno.es/2016/03/28/la-vid-y-el-vino-en-la-espana-del-al-andalus/?cv=1 [Visitada 12 de junio de 2023]

Wine and other Stories. 2023. https://wineandotherstories.com/jerez-and-sherry-p2-sherry-winemaking-and-styles/ [Visitada 29 de mayo de 2023]

Winterhalter P., Sefton M.A., Williams P.J. 1990. Volatile C13-Norisoprenoid Compounds in Riesling Wine Are Generated FromMultiple Precursors. *Am. J. Enol. Vitic.* 41: 277–283, https://doi.org/10.5344/ajev.1990.41.4.277