UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

APLICACIÓN DE LA NARIZ ELECTRÓNICA PARA LA EVALUACIÓN OLFATIVA DE ACEITUNAS DE MESA

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ÍNDICE

Listado de Figuras

Listado de Tablas

Abreviaturas y acrónimos

- CICYTEX. Centro de Investigaciones Científicas y Tecnológicas de Extremadura
- COVs. Compuestos Orgánicos Volátiles
- COI. Consejo Oleícola Internacional (International Olive Council o IOC en inglés)
- COVT. Compuestos Orgánicos Volátiles Totales
- DPP. Defecto Predominante Percibido
- F_0. Tiempo de letalidad térmica a 121,1ºC
- GC. Cromatografía de gases
- INTAEX. Instituto Tecnológico Agroalimentario de Extremadura
- LDA. Linear Discriminant Analysis
- MLR. Multiple Linear Regression
- m.o. Microorganismos
- MOS. Óxido metálico semiconductor
- MOx. Óxido metálico
- NIST. National Institute of Standards and Technology
- PCA. Principal Component Analysis
- PLS. Partial Least Squares
- PLS-DA. Partial Least-Squares Discriminant Analysis
- P valor. Probabilidad de un valor estadístico
- SPME. Solid-Phase Micro-Extractión
- UEx. Universidad de Extremadura
- R^2. Coeficiente de determinación
- R_{cv}^2. Coeficiente de determinación para validación cruzada
- R_P^2. Coeficiente de determinación de predicción
- RER. Relación del error del rango
- RMSEE. Error cuadrático medio de la estimación
- RMSECV. Error cuadrático medio de la validación cruzada
- RMSEP. Error cuadrático medio de la predicción

PRESENTACIÓN

I.1 Resumen

La legislación vigente en aceitunas de mesa en España indica que este producto debe estar libres de olores y sabores extraños y sin síntomas de alteración en curso o fermentación anormal (Real Decreto 679/2016, 2016). El panel de cata es el método utilizado para el análisis sensorial de distintos tipos de aceitunas (IOC, 2021) que permite conocer como percibirán los aromas el consumidor final. Las industrias utilizan el panel de cata de forma rutinaria en diversas fases de la elaboración y control, y constituye un instrumento fundamental para determinar la calidad. Para lograr buenos resultados debe haber un número razonable de panelistas capacitados, lo cual resulta costoso y requiere de mucho tiempo de aprendizaje y preparación de muestras. Además, el sistema olfativo humano sufre de agotamiento que limita el número de productos a catar y tiene una importante componente subjetiva que puede dar lugar a resultados no deseados. Las industrias también utilizan los análisis cromatográficos para poder identificar los compuestos volátiles caráterísticos de los diferentes atributos olfativos en las aceitunas de mesa, pero estos resultan ser costosos y requieren de personal cualificado. Por todo esto, es necesario disponer de unos equipos que puedan funcionar de manera similar al olfato humano, pero sin la componente subjetiva de éste que permita automatizar el panel sensorial para obtener mayor rendimiento y mejores resultados. Para avanzar en esta tecnología aplicada a la cata de aceitunas, se ha utilizado un prototipo de *nariz electrónica* de pequeño tamaño y consumo, basados en una matriz de sensores conectados por bluetooth a un dispositivo móvil o Tablet, que podría suponer una disminución en el tiempo y costos del análisis sensorial en el sector de la aceituna de mesa. Por tanto, la presente Tesis Doctoral aborda el desarrollo de un nuevo método de análisis sensorial utilizando la *nariz electrónica* en combinación con algoritmos quimiométricos de calibración multivariante, para la detección y discriminación de defectos olfativos y aromas en aceitunas de mesa y/o sus salmueras, elaboradas al "estilo español" y al "estilo californiano". La metodología que se utilizó durante la presente Tesis Doctoral consistió en estudiar muestras de aceitunas verdes y negras, sanas y con defectos de fermentación o esterilización. También se inocularon mohos alterantes en salmueras, y se aplicaron diferentes

tratamientos térmicos de esterilización a las aceitunas negras. Además, se rellenaron las aceitunas verdes y negras con distintos hidrocoloides aromatizados para enmascarar defectos y mejorar la clasificación y aceptación del consumidor. Todas las muestras fueron analizadas mediante: análisis sensorial olfativo con el panel de cata, análisis cromatográfico para la identificación de los compuestos volátiles y mediante la evaluación de los compuestos volátiles del espacio de cabeza de las muestras con la *nariz electrónica.* Los datos fueron tratados usando herramientas quimiométricas adecuadas. Tanto para el análisis sensorial olfativo como para la aplicación de la *nariz electrónica*, se siguieron las recomendaciones del Consejo Oleícola Internacional (IOC, 2021). Todos los experimentos y sus resultados se han publicado en un total de 7 artículos científicos, que constituyen un aval de la calidad de la tesis presentada.

En la primera publicación "*E-nose* Discrimination of Abnormal Fermentations in Spanish-Style Green Olives. (2021). Molecules, 26(17), 5353", se utilizó la *nariz electrónica* para evaluar los defectos de fermentaciones anormales (*zapatería*, *butírico*, *pútrido* y *mohoso*) de las aceitunas de mesa al "estilo español", previamente clasificados por un panel de cata según el protocolo del COI. Las aceitunas de mesa con los diferentes defectos individuales o combinados fueron claramente discriminadas entre sí mediante la *nariz electrónica*. Un total de 49 compuestos volátiles fueron identificados por cromatografía de gases. Los principales compuestos volátiles encontrados en las aceitunas con *zapatería* fueron el ácido ciclohexanocarboxílico y ácido butanoico, para el defecto *butírico* resultaron ser el ácido butanoico y ácido pentanoico, para el *pútrido* el ácido propanoico y alcohol isopropílico, mientras que para el *mohoso* el 2-metoxi fenol.

En la segunda publicación "Application of electronic nose to discriminate species of mold strains in synthetic brines. (2022). Frontier Microbiology, 13, 897178.", se utilizó la *nariz electrónica* para detectar y discriminar nueve especies de mohos en salmueras sintéticas. Se determinó el efecto del desarrollo microbiano sobre la calidad sensorial, el perfil volátil y la capacidad de la *nariz electrónica* para discriminar las salmueras alteradas de las sanas.

Las nueve cepas estudiadas fueron *Galactomyces geotricum* 2, *Penicillium expansum* 3, 4 y 20, *Penicillium glabrum* 19, *Aspergillus flavus* 9, 18 y 21 y *Fusarium solani* 11. Las salmueras inoculadas con las cepas de moho presentaron atributos negativos relacionados con aromas a *moho*, *madera*, *cuero* o *rancidez*, entre otros. El análisis sensorial nos permitió clasificar las salmueras sintéticas en función del grado de alteración que producen las cepas de moho utilizadas. Se identificaron un total de 19 compuestos volátiles por cromatografía de gases. Además, los datos obtenidos con la *nariz electrónica* permitieron discriminar las salmueras inoculadas, independientemente de la intensidad del defecto.

En la tercera publicación, "Determination of the masking effect of the 'Zapateria' defect in flavoured stuffed olives using E-nose. (2022). Molecules. 27, 4300." la *nariz electrónica* se utilizó para evaluar la capacidad del aroma del relleno para enmascarar el defecto olfativo de *zapatería*. Para ello, las aceitunas de mesa al "estilo español", alteradas con defecto de *zapatería*, se rellenaron con un hidrocoloide aromatizado con aroma de 'Mojo picón' a tres concentraciones diferentes (2, 4 y 8%). El análisis sensorial clasificó las aceitunas de mesa según el defecto predominantemente percibido (DPP) por el panel de cata (IOC, 2021). La muestra control, rellenas sin aroma añadido, presentó una alta concentración de defectos de *zapatería* que se clasificaron en segunda categoría (DPP > 4.5). Cuando se añadió aroma a las aceitunas alteradas, la intensidad del defecto disminuyó. De hecho, las concentraciones de aromas más altas añadidas provocaron que las aceitunas se clasificaran como categoría extra (DPP ≤ 3). Se identificaron un total de 16 compuestos volátiles. Los principales en aceitunas con defecto de *zapatería* fueron el ácido ciclohexanocarboxílico y el ácido pentanoico. La *nariz electrónica* permitió discriminar entre aceitunas rellenas sin aroma añadido y aceitunas con aroma a 'Mojo picón' a diferentes concentraciones. El modelo de Análisis Discriminante de Mínimos Cuadrados Parciales (PLS-DA) arrojó una tasa de aciertos del 93,8%. Finalmente, la regresión PLS permitió establecer un modelo lineal predictivo entre la *nariz electrónica* y los valores del análisis sensorial. Los valores de R^2 fueron 0,74 para el defecto percibido y 0,86 para el aroma percibido.

En el cuarto artículo, "Application of digital olfaction for table olive industry. (2022). Sensors, 22, 5702.", la *nariz electrónica* se utilizó para evaluar aceitunas de mesa al "estilo español" fermentadas en condiciones no controladas, que produjeron alteraciones características en las aceitunas en aroma e intensidad. Las alteraciones principales percibidas por los catadores fueron *zapatería, butírico* y *pútrido*, siendo clasificados, en base a sus atributos sensoriales, en diferentes categorías según los criterios marcados por el Consejo Oleícola Internacional (COI). Estas aceitunas presentaron un perfil de compuestos volátiles característico que hacen que la *nariz electrónica* se utilice con éxito para la discriminación de las aceitunas de mesa sanas y no sanas, siendo esta última también separada entre primera y segunda categoría.

Los siguientes artículos científicos se realizaron con aceitunas negras al "estilo californiano", a las que se estudió el *efecto de cocido* y la formación de acrilamida, que se origina según el tratamiento térmico de esterilización aplicado y el enmascaramiento del defecto a con la adición de rellenos de hidrocoloides con aromas.

En la quinta publicación, "Electronic nose application for the discrimination of sterilization treatments applied to Californian-style black olive varieties. (2021). Journal of the Science of Food and Agriculture, 102(6), 2232-224.", se utilizó la *nariz electrónica* para discriminar los diferentes tratamientos de esterilización aplicados a dos variedades de aceitunas, *'Manzanilla Cacereña'* y *'Hojiblanca'*. Un aumento en la duración del tiempo de esterilización originó un mayor *efecto de cocido*. Los compuestos volátiles encontrados relacionados con el *efecto de cocido* fueron 2,4-dimetilhexano, 3-metilpiridina, benzaldehído, 4-etenilpiridina y α-farnesenol. La *nariz electrónica* diferenció entre muestras de aceitunas de diferentes variedades y, además, discriminó aceitunas sometidas a diferentes tratamientos térmicos de esterilización, independientemente de la variedad de aceituna estudiada. Los resultados obtenidos por el modelo PLS arrojaron un buen coeficiente de correlación de predicción (R^2 = 0,88), entre los valores medidos de la *nariz electrónica* y los parámetros de *efecto de cocido* obtenidos del panel de cata en muestras que habían sufrido diferentes tratamientos térmicos. Por lo tanto, la *nariz electrónica*, permitió estimar cuantitativamente el defecto sensorial del *efecto de cocido*.

En la sexta publicación, "Characterization of Polyphenol and Volatile Fractions of Californian-Style Black Olives and Innovative Application of *nariz electrónica* for Acrylamide Determination. (2021). Foods, 10(12), 2973.", se utilizó la *nariz electrónica* para determinar la concentración de acrilamida en las variedades de aceitunas *'Hojiblanca'* y *'Manzanilla Cacereña'*. Los resultados muestran como la intensidad de esterilización influyó en el contenido final de fenoles, acrilamida y compuestos volátiles. Además, en función del tratamiento térmico aplicado, los grupos de compuestos volátiles con olor desagradable, como el 4-etenil-piridina, benzaldehído y 2,4-dimetil-hexano, aumentaron significativamente sus contenidos. También, se encontró mediante análisis de regresión lineal múltiple (R^2 = 0,9994), como el contenido de acrilamida está correlacionado con los compuestos volátiles. Por lo tanto, se ha podido estimar el contenido de acrilamida mediante el análisis de mínimos cuadrados parciales de los datos de la *nariz electrónica*.

En el séptimo artículo, "Evaluation of the olfactory pattern of black olives stuffed with flavoured hydrocolloids. (2022). LWT-Food Science and Technology, 113556.", se aplicó la *nariz electrónica* para evaluar olfativamente el aroma añadido a los rellenos y su efecto enmascarador del *efecto de cocido*. Para ello, se analizaron aceitunas rellenas de hidrocoloides aromatizados, sometidas a diferentes tratamientos térmicos. El aroma propio de la aceituna percibido por el panel de cata antes de la esterilización se consideró muy intenso. Sin embargo, la aplicación de una sola esterilización causó una disminución del 35-42% de la intensidad del aroma percibido por los catadores. La esterilización también condujo a un aumento del *efecto de cocido* que fue menos detectado por los panelistas cuando se aromatizaron las aceitunas. La *nariz electrónica* mostró como las aceitunas sometidas a esterilización presentaban un perfil aromático diferente. Por lo tanto, este dispositivo pudo discriminar los distintos tratamientos térmicos de esterilizacion aplicados con mayor precisión que el panel de cata.

Finalmente, podemos decir que la *nariz electrónica* utilizada en esta Tesis Doctoral es perfectamente aplicable en las aceitunas verdes al "estilo español", para la detección olfativa de defectos de fermentación y detección precoz de presencia de diferentes cepas de mohos en salmueras, así como por la evaluación olfativa de aceitunas rellenas con distintos aromas añadidos. Con respecto a las aceitunas negras al "estilo californiano", este dispositivo electrónico es adecuado para el control olfativo de las aceitunas sometidas a los diferentes tratamientos de esterilización y es una posible alternativa a la determinación indirecta de una sustancia tóxica, la acrilamida.

I.2 Justificación y objetivos

Esta Tesis Doctoral evalúa un sistema olfativo artificial o *nariz electrónica* para su aplicación en el sector de la aceituna de mesa (aceitunas verdes al "estilo español" y aceitunas negras al "estilo californiano"). La implementación de este sistema de olfato electrónico para discriminar muestras con diferente perfil aromático en aceitunas de mesa y sus salmueras, requiere de un estudio previo de los compuestos volátiles y del perfil sensorial de la matriz objeto de estudio para poder corroborar los cambios de señal obtenidos por los sensores. De manera general, se pretende estudiar en diferentes tipos de muestras, la correlación entre la respuesta de la *nariz electrónica* y su relación con otros métodos tradicionales de análisis como la cromatografía de gases o el panel sensorial, para de esta forma, encontrar un modelo matemático que permita predecir las respuestas de las técnicas tradicionales de análisis de aceituna de mesa y/o salmueras a partir de las respuestas de la *nariz electrónica*.

Las aceitunas de mesa verdes elaboraciones al "estilo español" pueden sufrir perturbaciones durante su fermentación o conservación, que provoquen alteraciones organolépticas y defectos olfativos que ocasionen el rechazo del consumidor. El COI define algunos de estos defectos olfativos como son *zapatería*, *butírico*, *pútrido*, *mohoso*, etc. Se consideró interesante aplicar la *nariz electrónica* para ver si podía discriminar a las aceitunas con estos defectos y clasificarlas en categorías comerciales. Estos defectos también pueden ser causados por mohos, por lo que se aplicó la *nariz electrónica* para evaluar la discriminación de los principales mohos alterantes en salmueras. La detección precoz de defectos provocados por el desarrollo microbiano durante la fermentación y conservación, incluido la proliferación de mohos, podría evitar grandes pérdidas económicas por el rechazo de aceitunas con intensidad de defectos elevada o clasificadas en categorías comerciales inferiores.

Por otra parte, las aceitunas elaboradas al "estilo californiano" son sometidas a un tratamiento de esterilización para su estabilización microbiológica que, de no ser controlado adecuadamente, puede producir el *efecto de cocido* y alteración de los

aromas originales propios de la aceituna. La aplicación de la *nariz electrónica* para clasificar y predecir la intensidad del *efecto de cocido* percibido predominante de las aceitunas de mesa, podría contribuir al control olfativo de las aceitunas que pasan por el proceso de esterilización, ayudando a ajustar los tratamientos térmicos. Al mismo tiempo, durante el tratamiento térmico se eleva la concentración de acrilamida, por lo que se planteó la determinación de este compuesto tóxico con el análisis de la *nariz electrónica* como medida de control que pudieran prevenir problemas de salud en las personas.

Por último, se propone como solución para disminuir la percepción de defectos olfativos de las aceitunas de mesa verdes al "estilo español" y negras al "estilo californiano", el relleno de las aceitunas con hidrocoloides a los que se han añadido aromas naturales. La finalidad es evitar el rechazo del consumidor dando lugar a un producto más atractivo organolépticamente y clasificarlo en una mejor categoría sensorial.

Por lo tanto, los objetivos específicos de la presente Tesis son los siguientes:

Objetivo 1. Aplicar la *nariz electrónica* para desarrollar un método de clasificación sensorial de aceitunas de mesa elaboradas al "estilo español", según la intensidad de los defectos de fermentaciones anormales.

Objetivo 2. Evaluar la capacidad de la *nariz electrónica* para discriminar salmueras de aceitunas de mesa no alteradas de las alteradas con diferentes cepas de mohos, a partir del perfil de compuestos volátiles emanadas al espacio cabeza de las muestras.

Objetivo 3. Evaluar mediante la *nariz electrónica*, la evolución sensorial olfativa de aceitunas de mesa con defecto de zapatería, antes y después de añadir diferentes concentraciones de aroma de 'mojo picón' al relleno de hidrocoloide, para comprobar si el defecto de la zapatería se enmascara o diluye tras una determinada concentración de aroma añadido, mejorando así la aceptación por parte del consumidor.

Objetivo 4. Aplicar la *nariz electrónica* para desarrollar una metodología de control de calidad en la industria, capaz de discriminar muestras sanas y no sanas, además de clasificarlas en las diferentes categorías según sus atributos sensoriales.

Objetivo 5. Evaluar la capacidad de la *nariz electrónica* para ayudar al panel de análisis sensorial a diferenciar entre aceitunas negras al "estilo californiano" de dos variedades distintas y, sometidas a diferentes procesos de esterilización y, confirmar su utilidad cuantitativa para evaluar su respuesta a la intensidad del aroma percibido.

Objetivo 6. Evaluar la composición química de fenoles, acrilamida y compuestos volátiles en las aceitunas negras tratadas térmicamente y, la capacidad de la *nariz electrónica* para predecir el contenido de acrilamida.

Objetivo 7. Estudiar, mediante la *nariz electrónica*, la evolución sensorial olfativa de distintos aromas añadidos en el relleno de aceitunas negras tras ser sometidos a diferentes tratamientos de esterilización.

En definitiva, en la presente Tesis Doctoral se usará un sistema sensorial artificial para la evaluación olfativa de las aceitunas de mesa que se podría integrar en un panel de cata tradicional y, los análisis realizados, serán comparados y correlacionados con las técnicas tradicionales de análisis. Como resultado, se espera poder evaluar el desempeño de este sistema sensorial artificial en paneles de catas en el sector de la aceituna de mesa.

I.3 Contenido

La presente Tesis Doctoral contiene cuatro capítulos que se describen a continuación:

- Capítulo I. Introducción. Se describe la aceituna de mesa, los tipos de elaboraciones y las técnicas de análisis habituales para su control y clasificación. Además, se presentan los antecedentes de los sistemas olfativos artificiales o narices electrónicas en el sector, su descripción, funcionamiento y aplicaciones.

- Capítulo II. Resultados y discusión. Se pone de manifiesto un resumen de los principales resultados y discusión obtenidos en los diferentes ensayos realizados, en función de las publicaciones científicas obtenidas. También se presenta una relación de los artículos científicos originales publicados.

- Capítulo III. Conclusiones. Se recogen las principales conclusiones obtenidas con la realización de esta Tesis Doctoral.

- Capítulo IV. Perspectivas futuras. Se plantea una panorámica futura de las posibles líneas de investigación a continuar trabajando con la *nariz electrónica* en el sector de la aceituna de mesa.

CAPÍTULO I. INTRODUCCIÓN

1.1 Aceituna de mesa

Según se describe en el Real Decreto 679/2016, de 16 de diciembre, por el que se establece la norma de calidad de las aceitunas de mesa, se define "aceituna de mesa" como: "el fruto de determinadas variedades del olivo cultivado (*Olea europaea* sativa Hoffg. Link.), sano, obtenido en el estado de madurez adecuado y de calidad tal que, sometido a las elaboraciones adecuadas, proporcione un producto listo para el consumo y de buena conservación" (R.D. 679/2016, p. 88526-88527).

Las aceitunas de mesa forman parte de un sector con una gran importancia en España, por su carácter estratégico e implantación territorial, siendo el territorio nacional el mayor explotador y productor del mundo (R.D. 679/2016, 2016).

Según su coloración, la normativa nacional cataloga las aceitunas en los siguientes tipos:

> 1. Verdes: son las obtenidas de frutos recogidos en el ciclo de maduración anterior al envero y cuando han alcanzado su tamaño normal. Estas aceitunas serán firmes, sanas, y no tendrán otras manchas distintas de las de su pigmentación natural. La coloración del fruto podrá variar del verde al amarillo paja.
>
> 2. De color cambiante: son las obtenidas de frutos con color rosado, rosa vino o castaño, recogidos durante el envero, antes de su completa madurez.
>
> 3. Negras naturales: son las obtenidas de frutos recogidos en plena madurez o poco antes de ella, pudiendo presentar, según la zona de producción y la época de la recogida, color negro rojizo, negro violáceo, violeta, negro verdoso o castaño oscuro.
>
> 4. Negras: son las obtenidas de frutos que, no estando totalmente maduros, han sido oscurecidos mediante oxidación (R.D. 679/2016, p. 88527).

1.1.1 Situación

Según el Ministerio de Agricultura, Pesca y Alimentación (MAPA, 2021), de los 2,75 millones de hectáreas de olivar en España, 197.335 hectáreas corresponden a olivar cuya aceituna se destina a mesa (77.650 hectáreas de olivar de aceituna de mesa y 119.685 hectáreas de aptitud mixta). España es líder mundial en producción y exportación de aceituna de mesa. La producción en la Unión Europea (UE) es del 62% y a nivel mundial del 17%. La aceituna de mesa es uno de los productos claves de la agricultura española a nivel mundial, debido a la calidad, gran variedad y versatilidad de la producción española.

Las principales variedades cultivadas son: *'Hojiblanca'* con 46% de la producción nacional total, *'Manzanilla'* el 36%, *'Gordal sevillana'* el 7%, *'Manzanilla Cacereña'* el 4% y *'Carrasqueña'* el 3%. La producción se localiza en un 80% en Andalucía y en un 13% en Extremadura. La principal provincia productora, Sevilla, tiene aproximadamente el 58% de la producción nacional total. Las variedades por excelencia de mesa en Extremadura son la *'Manzanilla'* en la provincia de Badajoz y la *'Cacereña'* y *'Hojiblanca'* en la provincia de Cáceres. Estas variedades suponen el 96% de la producción regional (MAPA, 2022).

Según la Agencia de Información y Control Alimentarios, la producción nacional de aceituna de mesa en la campaña 2020/2021 ha sido aproximadamente de 545.950 t. Andalucía alcanza un total de 458.878 t y Extremadura sobre 80.200 t, siendo 42.402 t correspondientes a Badajoz, elaboradas al "estilo español" o "Sevillano" y, 37.797 t en Cáceres elaboradas como aceitunas negras oxidadas al "estilo californiano" (ASEMESA, 2021).

Las industrias de transformación o entamadoras están distribuidas en las dos provincias, aunque el número de instalaciones en Badajoz, donde hay 66, casi duplica al de Cáceres, con 34. A pesar de esta gran diferencia en el número de instalaciones, la producción de aceituna procesada de las dos provincias es muy parecida. La industria de la aceituna de mesa está muy concentrada en la comarca de Tierra de Barros, destacando el municipio de Almendralejo con 26 entamadoras. En el norte de

Cáceres también hay una notable presencia de industrias de procesado, con especial relevancia en la comarca de Las Hurdes.

1.1.2 Tipos de elaboraciones de la aceituna de mesa

El objetivo común de todos los procesados de las aceitunas de mesa es eliminar el sabor amargo natural de las aceitunas crudas, para que puedan ser comestibles. Este amargor se debe a la presencia de compuestos fenólicos en las aceitunas, entre los que destaca la oleuropeína.

Los procesos de elaboración enumerados en el Real Decreto 679/2016, del 16 de diciembre, por el que se establece la norma de calidad de las aceitunas de mesa son:

1. Aderezo: es el proceso por el que las aceitunas verdes, de color cambiante o negras naturales, son sometidas a un tratamiento alcalino para eliminar el principio amargo y posteriormente son acondicionadas en salmuera en la que sufren una fermentación parcial o completa.

2. En salmuera: es el proceso por el que las aceitunas verdes, de color cambiante o negras naturales, son tratadas directamente con una salmuera, donde sufren una fermentación completa o parcial.

3. Aliñado: es el proceso de añadir a la salmuera condimentos o especias, eventualmente vinagre, y cualquier otro producto alimenticio.

4. Oxidación: es el proceso por el cual las aceitunas de los tipos verde y de color cambiante, que en una fase previa se conservan en salmuera, fermentadas o no, son ennegrecidas por oxidación en medio alcalino.

5. Deshidratación: es el proceso por el que las aceitunas pierden parte de su humedad por tratamiento con sal seca, aplicación de calor o cualquier otro proceso tecnológico.

6. Otros procesos de elaboración: las aceitunas pueden elaborarse de formas diferentes o complementarias de las antes indicadas, siempre que los frutos

utilizados respondan a los requisitos establecidas en la presente norma de calidad (p. 88527).

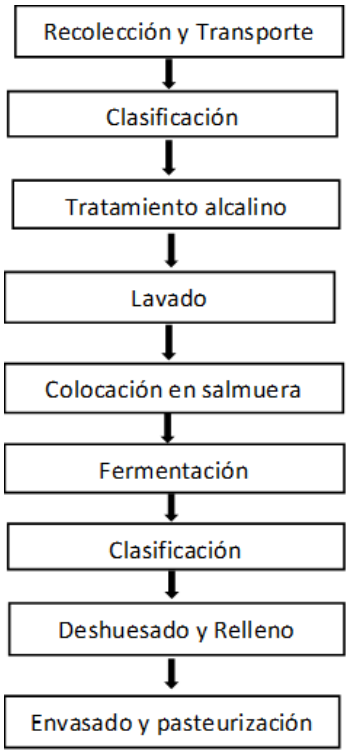
A continuación, se van a describir detalladamente los principales procesos de elaboración a nivel industrial y a los que son sometidas las aceitunas de mesa por su importancia económica.

1.1.2.1 Elaboración al "estilo español"

La elaboración de las aceitunas de mesa verdes al "estilo español" está compuesta por los siguientes procesos: recolección y transporte, clasificación, tratamiento alcalino, lavado, colocación en salmuera, fermentación, acondicionamiento, envasado y pasteurización térmica (Figura 1).

Figura 1.

Etapas del proceso de elaboración de aceitunas de mesa al "estilo español".



Los frutos verdes, tras su recolección, transporte y clasificación, se someten al proceso de cocido o tratamiento con hidróxido sódico (NaOH), que es un proceso esencial en la elaboración de aceitunas aderezadas, cuyo objetivo principal es la hidrólisis del glucósido denominado oleuropeína, causante del característico amargor en la aceituna. Posteriormente, se producen consecutivos lavados de la aceituna con agua, para eliminar el exceso de NaOH. Tras el lavado, las aceitunas se ponen en una salmuera inicial de concentraciones que varían de 10 a 12% (p/v) de cloruro sódico (NaCl), donde se inicia el proceso de fermentación. Durante la primera semana, para evitar el desarrollo de alterantes, la concentración de sal no debe ser inferior a 5%. De esta manera se favorece el crecimiento de *Lactobacillus* sp. y se inhibe el crecimiento de microorganismos alterantes esporulados durante la primera etapa de fermentación, donde todavía existe un pH alto. Se deben hacer controles frecuentes del pH, principalmente al inicio de la fermentación, ya que el hidróxido sódico residual que hay en los frutos, se va dispersando por el medio haciendo que la salmuera pueda alcanzar valores de pH > 10 unidades al inicio de la fermentación. La segunda fase de la fermentación se inicia con el crecimiento de bacterias lácticas, fase que dura hasta que el valor de pH es de 4,5 unidades. Esta fase es de aproximadamente 15-20 días. En esta fase se produce la inhibición de los bacilos Gram negativos y el descenso de cocos lácticos (Rejano et al., 2010; Bautista-Gallego et al., 2011; Ruiz-Barba y Jiménez-Díaz, 2012). En la tercera fase de la fermentación se produce el predominio de las bacterias lácticas siendo responsables de la fermentación de los azúcares y la producción de ácido láctico. En esta fase el valor de pH es igual o inferior a 4 (Rejano et al., 2010; Hurtado et al., 2012; Ruiz-Barba y Jiménez-Díaz, 2012).

Durante este proceso, también se produce una difusión de compuestos orgánicos de la aceituna hacia la salmuera, debido a la concentración de sal presente en la misma. Además, se produce degradación de compuestos fenólicos (Fendri et al., 2013). Al mismo tiempo se produce el crecimiento de levaduras, destacando *Saccharomyces cerevisiae*, el género *Candida* y *Pichia*, aunque aparecen levaduras de otros muchos géneros y especies diferentes (Ruiz-Moyano et al., 2019; Tufariello et al., 2019). Cuando las bacterias lácticas no tienen más sustratos que consumir, finaliza el proceso de fermentación, dándose el inicio a la fase de conservación.

Si no se realiza la conservación adecuadamente se puede empezar una nueva fase de la fermentación, con el crecimiento de bacterias del género *Propionibacterium* que utilizan como sustrato el ácido láctico, dando lugar a una subida del pH y se produce una mezcla de los ácidos acético y propiónico más débiles que el láctico que originan el incremento del pH.

Para evitar alteraciones al final de la fermentación de las aceitunas de mesa, se aumenta la concentración de NaCl hasta 8,5-9,5% y se mantiene el pH por debajo de 4, lo que evitará el desarrollo de bacterias propiónicas.

Las aceitunas elaboradas envasadas (Figura 2), se podrán conservar con la adición de sal, ácidos, especias, con atmósfera protectora, por vacío, por refrigeración y con la aplicación de térmicos como la pasteurización. Durante la pasteurización las aceitunas de mesa se someten a un tratamiento térmico para matar todas las bacterias patógenas y reducir la actividad enzimática.

Estos procesos de conservación pueden aplicarse por separado o conjuntamente y respondiendo a los requisitos de conservación indicados en la norma del Codex para las aceitunas de mesa (CODEX STAN 66-1981).

Figura 2.

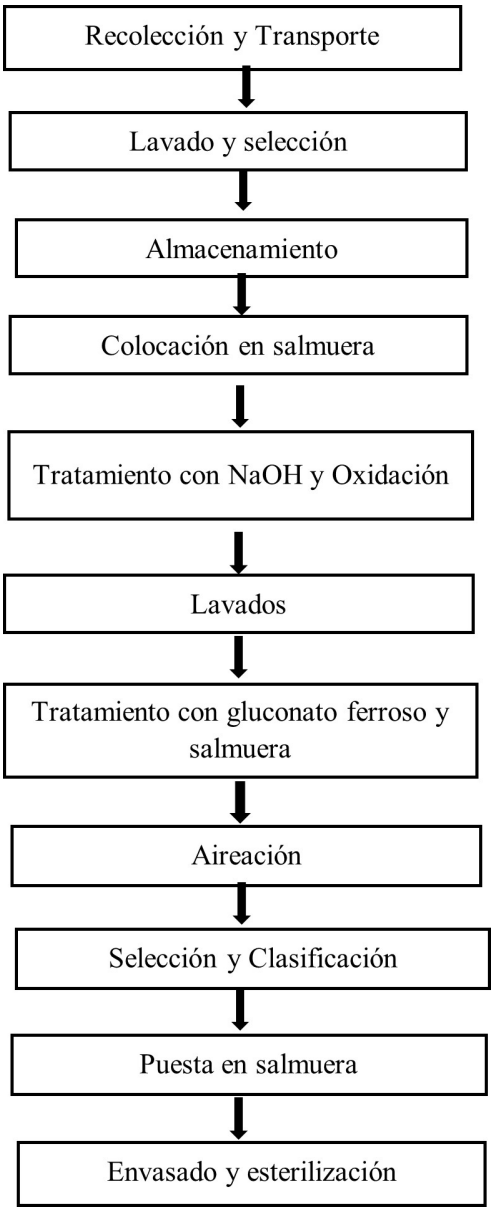
Aceitunas elaboradas al "estilo español"

1.1.2.2 Elaboración al "estilo californiano"

La elaboración de las aceitunas negras al "estilo californiano" está compuesta por los siguientes procesos: recolección y transporte, lavado y selección, almacenamiento, colocación en salmuera y/o ácido, tratamiento alcalino y oxidación con aire, lavado, tratamiento con gluconato ferroso y salmuera, aireación, selección y clasificación, puesta en salmuera, envasado y esterilización (Figura 3).

Figura 3.

Diagrama de elaboración de aceitunas de mesa negras oxidadas al "estilo californiano".



Para la preparación de aceitunas negras oxidadas las aceitunas se cosechan en diferentes estados de maduración, pero por regla general, las industrias recolectan las aceitunas cuando éstas se encuentran en un estado de maduración verde o verde-amarillento (Martín-Vertedor et al., 2021).

Las aceitunas pueden ser almacenadas desde 2 a 6 meses en salmuera con concentración de NaCl de 5 a 10% y/o ácido acético del 1-3%, o por el contrario, las aceitunas pueden ser directamente sometidos a los procesos de oxidación sin conservación previa (Ghanbari et al., 2012), aspecto que requiere un mayor gasto energético en oxidación por parte de las industrias transformadoras.

Las aceitunas tras ser conservadas, se les realiza un lavado previo con agua a presión, y son sometidas a sucesivos tratamientos con hidróxido de sodio (NaOH) hasta eliminar el amargor, durante distintos períodos de tiempo. Este proceso produce una penetración progresiva del NaOH desde la piel a la pulpa y, después al hueso. Mientras las aceitunas se están desamargando, el aire es insuflado a través del líquido de gobierno para oxidar las aceitunas de una forma uniforme y continua (Arroyo-López et al., 2010). La concentración de la solución de NaOH depende del estado de madurez de la aceituna, el cultivar, la temperatura ambiente y la penetración de NaOH que se quiere en el fruto.

Cuando las aceitunas han sido sometidas al tratamiento con NaOH, el pH es neutralizado. Para ello, las aceitunas son tratadas progresivamente con ácido acético/láctico hasta conseguir bajar el pH a aproximadamente 8 unidades en el fruto. Posteriormente, se añade una sal de hierro, gluconato o lactato ferroso para fijar el color negro intenso, que es característico (Figura 4) de este tipo de aceitunas (Pereira, et al., 2006; Arroyo-López et al., 2008).

Por último, las aceitunas se envasan en salmuera en diferentes recipientes, con una pequeña cantidad de la sal de hierro para evitar la degradación del color negro. Para garantizar la conservación, el producto final se somete a esterilización por calor a 120ºC durante 15-30 minutos (Ghanbari et al., 2012).

Figura 4.

Aceitunas negras al "estilo californiano".

Algunos investigadores (Martín-Vertedor et al., 2020) expresan los procesos térmicos de esterilización como letalidad acumulativa análoga expresada como F_0. Esta variable es una integral térmica entre la temperatura y el tiempo de esterilización aplicado sobre el alimento. La F_0 que utilizan las industrias de aceitunas de mesa al "estilo californiano" son igual o superior a 15 min según la antigua normativa (R.D. 1230/2001, 2001). Sin embargo, este Real Decreto fue modificado recientemente (R.D. 679/2016, 2016) en el que se ha suprimido este mínimo F_0, debido a que era excesivo tanto tiempo de tratamiento de esterilización para estabilizar las aceitunas negras elaboradas. Está demostrado que excesivos tiempos de exposición de calor en las aceitunas provoca la aparición de un defecto denominado *efecto de cocido* en las aceitunas y la síntesis de una sustancia tóxica, la acrilamida. Por lo tanto, industrialmente, se deben optimizar los procesos térmicos de esterilización para mejorar la calidad de las aceitunas que se comercializan.

1.1.3 Alteraciones olfativas en las aceitunas de mesa

Durante el proceso de fermentación de la aceituna de mesa se puede dar lugar al crecimiento de microorganismos no deseados que produzcan diferentes tipos de alteraciones. Entre las más importantes, según el origen y fases de la fermentación en que tienen lugar, pueden ser: alambrado, ablandamiento, fermentaciones *butírica* y *pútrida*, *zapatería*, fermentaciones con sabores extraños, sedimentaciones y gases en los envases, cianosis, etc (Lanza, 2013). El Consejo Oleícola Internacional establece

un método para el análisis sensorial en las aceitunas de mesa (IOC, 2021) y describe los atributos negativos relacionados con el olor (Tabla 1).

Tabla 1.

Vocabulario específico de las aceitunas de mesa a efectos del método (COI/OT/MO Nº1/Rev.3, 2021, Junio-2021)

Atributos negativos	
Fermentación anormal	Sensación olfativa percibida directa o retronasalmente, característica de fermentaciones anormales. Tal fermentación puede ser: - *Pútrido*: sensación que recuerda al olor de la materia orgánica en descomposición. - *Butírico*: sensación que recuerda a la mantequilla o al queso. - *Zapatería*: sensación provocada por la combinación de ácidos grasos volátiles que recuerdan al cuero podrido.
Mohoso	Sensación olfativo-gustativa percibida directa o retronasalmente, característica de aceitunas atacadas por moho.
Rancio	Sensación olfativa percibida de forma directa o retronasal, característica de las aceitunas que han sufrido un proceso de enranciamiento.
Efecto de cocido	Sensación olfativa percibida directa o vía retronasal, característica de aceitunas que han sufrido un calentamiento excesivo en temperatura y/o duración durante la pasteurización o esterilización.
Jabonosa	Sensación olfativa-gustativa que recuerda al jabón.
Metálico	Sensación olfativo-gustativa que recuerda a los metales.
Terroso	Sensación olfativo-gustativa que recuerda a tierra o polvo.
Vínico-avinagrado	Olfativo-gustativo Sensación que recuerda al vino o al vinagre.

A continuación se van a comentar las principales alteraciones en aceitunas de mesa:

- Fermentación *butírica* y *pútrida*. La característica principal de estas fermentaciones es el olor desagradable que presenta la salmuera y el sabor que adquieren las aceitunas. Se debe al desarrollo de distintas especies de bacterias del género *Clostridium* durante las primeras fases de la fermentación, cuando los azúcares y otras sustancias fermentables son muy abundantes en la salmuera. El ácido butírico que producen durante su metabolismo altera el olor y el sabor de la aceituna. Inicialmente, la salmuera tiene un olor semejante a manteca rancia, pero a medida que la alteración progresa, el olor se hace más fuerte, parecido a las fermentaciones *pútridas* u olor a materia orgánica en descomposición (De Castro et al., 2018).

El desarrollo de los microorganismos del género *Clostridium* está determinado por la concentración de cloruro sódico en la salmuera y por el valor del pH. Puede suceder que en el fondo de los fermentadores se den las circunstancias favorables para el desarrollo de estos microrganismos y que tenga lugar la fermentación *butírica*. Esto se produce cuando el agua de lavado que arrastran las aceitunas no se ha escurrido bien antes de añadir la salmuera, originando zonas de elevado pH, condiciones de anaerobiosis y sustancias nutritivas en abundancia, aunque en el centro de los depósitos la fermentación es normal (Gómez et al., 2004). Para prevenir o corregir su aparición y desarrollo, se debe mantener la concentración de sal adecuada, nunca inferior a un 5% y seguir unas buenas prácticas de fabricación.

- *Zapatería*. Esta alteración se caracteriza cuando las aceitunas presentan un desagradable olor y sabor, anormales y característicos. Se originan consecuencia del desarrollo de bacterias de los géneros *Clostridium* y *Propionibacterium* (De Castro et al., 2018). Su desarrollo se favorece durante la conservación de las aceitunas, sobre todo cuando esta no es adecuada, provocando que el valor de pH no se mantenga por debajo de 4,2 unidades y la concentración de sal sea inferior al 7%. También se puede presentar en recipientes donde la fermentación no alcanza las condiciones de acidez correctas, es decir al final del proceso de fermentación en aceitunas al "estilo español". La actividad fermentativa de estos microorganismos se puede identificar en la salmuera por la existencia de ciertos compuestos volátiles que producen olores

anormales a los de la fermentación láctica, como lo son el ácido propiónico, ácido ciclohexanocarboxílico, así como otros volátiles minoritarios por transformación del ácido láctico, lo que eleva el pH (Montaño et al., 1992). Se puede prevenir esta alteración aumentando la concentración de ácido de la salmuera y de esta manera, inhibir el crecimiento de los microorganismos responsables y así, estabilizar el valor del pH de conservación.

- Otras fermentaciones con sabores extraños. Alteración poco frecuente que a veces se desarrolla en aceitunas negras envasadas, debido al crecimiento de ciertos microorganismos indeseables durante el lavado, después del tratamiento alcalino. Además de que el sabor se modifique recordando al pescado, existen otros sabores y olores anormales como pueden ser metálico, salado, aceitoso, húmedo, rancio, etc.(Barranco et al., 2007). Mantener un medio ácido es una buena forma de disminuir la aparición de estos sabores y olores.

- Alteraciones por mohos. Las alteraciones debido al desarrollo de mohos alterantes causan deterioro visual y afectan a las propiedades organolépticas de los aceites como son el olor, el sabor y la textura. Algunas especies de mohos también se caracterizan por producir ciertas micotoxinas que podrían presentar una posible amenaza para la salud pública (Leyva et al., 2017). Cuando los mohos se desarrollan durante el proceso de elaboración de las aceitunas de mesa pueden producir una serie de alteraciones que provocan ablandamiento, características negativas de sabor y mala apariencia del producto final. Los microorganismos más representativos son *Aspergillus* y *Penicillium* (Bavaro et al., 2017).

El método del COI (IOC, 2021) clasifica las aceitunas de mesa según los defectos olfativos que presenten, tales como sensaciones *mohosas, rancias* o *avinagradas* (Lanza y Amoruso, 2020; Martín-Vertedor et al., 2021). Las prácticas industriales no controladas podrían facilitar el desarrollo del proceso de fermentación que podría provocar estas alteraciones.

1.2 Análisis sensorial en aceitunas de mesa. El panel de cata

Desde el 21 de noviembre de 2008 el procedimiento para la clasificación de aceitunas de mesa, basadas en parámetros de calidad, se estableció en el: COI/ OT/MO/Doc. Nº1 denominado "Método para el análisis sensorial de aceitunas de mesa". El 25 de noviembre de 2011, el Consejo Oleícola Internacional adoptó la versión revisada del método (IOC, 2011), para finalmente tener una tercera versión en junio de 2021 (IOC, 2021). El método descrito establece los criterios y procedimiento necesarios para el análisis sensorial del olor, sabor y textura de las aceitunas de mesa y establece la sistemática para su clasificación comercial. Es aplicable únicamente al fruto del olivo cultivado (*Olea europaea* L.) que haya sido adecuadamente tratado o transformado y preparado para el comercio o para el consumo final como aceitunas de mesa, de conformidad con la norma comercial aplicable a aceitunas de mesa referenciadas (IOC, 2004). La evaluación sensorial del producto final la realiza un panel de cata formado por 8 a 10 catadores expertos seleccionados en función de sus aptitudes y dirigidos por el responsable. Los catadores son elegidos según un estándar internacional, de acuerdo con su sensibilidad y poder discriminatorio con respecto a las características organolépticas de las aceitunas de mesa (IOC, 2021). A efectos de clasificación, se considera la mediana del defecto predominante percibido (DPP) (Tabla 2), evaluado con una escala de valoración de 1 a 11 puntos, donde 1 equivale a la más baja intensidad y 11 a la intensidad máxima.

Tabla 2.

Clasificación de las aceitunas de mesa según la intensidad del defecto predominante percibido (DPP).

Categorías	Defecto predominante percibido (DPP)
Extra	$1,0 < DPP \leq 3,0$
Primera categoría ó selecta	$3,0 < DPP \leq 4,5$
Segunda categoría ó estándar	$4,5 < DPP \leq 7,0$
No comercializables	$7,0 < DPP \leq 11,0$

El Panel de Aceitunas de Mesa CREA-IT (Consejo de Investigación y Economía Agrícolas—Centro de Investigación de Ingeniería y Procesamiento Agroalimentario) fue reconocido por el Consejo Oleícola Internacional desde 2008 y trabaja en el análisis sensorial de muestras de aceitunas de mesa italianas y europeas (Lanza, 2013). Hasta la fecha, el análisis sensorial se utiliza de forma rutinaria a nivel industrial en diversas fases del proceso de elaboración, y constituye un instrumento fundamental para determinar la calidad de estos alimentos y su puesta en los mercados. En Extremadura, se constituyó un panel de cata formado por un grupo de expertos en análisis de alimentos del Instituto Tecnológico Agroalimentario de Extremadura (INTAEX) (Martín-Vertedor et al., 2020) que analizan de forma rutinaria muestras de aceitunas enmarcadas dentro de los proyectos de investigación de este Instituto (Figura 5). Este panel de cata sigue las pautas legales marcadas por el COI. La norma COI (IOC, 2021) sobre evaluación sensorial de aceitunas de mesa, establece que los paneles de cata existentes en todo el mundo realizarán un análisis sensorial siguiendo patrones estandarizados, con el objeto de obtener unos resultados fiables y robustos, y por lo tanto poder clasificar a las aceitunas de mesa en distintas categorías comerciales.

Figura 5.

Panel de cata del INTAEX.

Para que las aceitunas de mesa puedan ser comercializadas no deben presentar defectos significativos, pero si existen, hay que verificar que no afecten a la integridad de la aceituna. Además, deben tener un buen aspecto físico, y pueden presentar ligeros defectos de color, forma, epidermis o firmeza de la pulpa. Los defectos que pueden presentar las aceitunas y que afectan a la integridad de la epidermis son el arrugado, deformidad, despellejado, alambradas, picadas, etc., mientras que

los defectos que no afectan a la integridad de estas son golpes, manchas, molestado, pintas, puntos, etc. (R. D. 679/2016).

1.3 Parámetros que determinan la calidad

En la actualidad existe una gran preocupación en las industrias aceituneras por la presencia de algunos compuestos indeseables resultantes de las alteraciones de aceitunas de mesa vistas en el apartado anterior (*zapatería, alambrado* o fermentaciones *butíricas* y *pútridas*, entre otras). Los métodos de detección para el control de estas posibles alteraciones en aceitunas de mesa pasan por realizar un análisis microbiológico (Pereira et al., 2008) con el que se determinan las poblaciones de microorganismos tanto deseados como no deseados que están presentes en las salmueras y en los frutos.

No se dispone de criterios microbiológicos oficiales para las aceitunas de mesa, sin embargo, los requisitos mínimos relacionados con la higiene de este producto vienen reflejados en la Norma 66-1981 (Rev. 1-1987, 1987) del Codex Alimentarius (Anónimo, 1987). Esta norma indica que el producto final deberá estar libre de microorganismos y parásitos en cantidades que puedan generar un peligro para la salud y no deben contener ninguna sustancia procedente de microorganismos en cantidades que entrañen un riesgo para la seguridad alimentaria. Según el COI (IOC, 2004), las aceitunas fermentadas con líquido de cobertura pueden contener bacterias lácticas y/o levaduras, resultantes de la fermentación. El número de microorganismos en un medio de cultivo selectivo puede ser, para cada uno, hasta 10^9 unidades formadoras de colonias /mL de salmuera o gramo de pulpa. Por otro lado, las aceitunas conservadas por esterilización por calor (aceitunas al "estilo californiano") deberán pasar por un tratamiento térmico suficiente en tiempo y temperatura como para eliminar, entre otras las esporas de *Clostridium botulinum* (IOC, 2004). Para evaluar la calidad microbiológica de las aceitunas de mesa se suele estudiar la presencia de microorganismos patógenos – *Salmonella* y *Staphylococcus aureus*, – microorganismos indicadores de contaminación microbiana – coliformes totales, *Escherichia coli*, *Streptococcus fecal*, esporas de *Clostridium* reductoras de

sulfito y mohos – y microorganismos implicados en la fermentación del producto – microorganismos mesofílicos y levaduras –.

En la primera fase de fermentación de las aceitunas al "estilo español", prevalecen las bacterias Gram negativas (*Enterobacter, Citrobacter, Aeromonas, Escherichia* y *Klebsiella*). Después de unas 48 h se desarrollan las bacterias ácido láctico. Si la disminución del pH durante los primeros días no es lo suficientemente rápida, las aceitunas pueden deteriorarse rápidamente, debido al desarrollo principalmente de *Enterobacteriaceae* que forman "bolsas de gas", lo que resulta en un ablandamiento y rotura de la cutícula de la aceituna provocando defectos significativos en el producto final (Lanza, 2013).

Un pH alto también puede contribuir al desarrollo de *Clostridium*, dando como resultado a la fermentación *pútrida* (olor que recuerda a la materia orgánica en descomposición) o *butírica* (olor que recuerda a la mantequilla rancia). Esta fermentación maloliente provocada por anaerobios *butíricos* como *Clostridium butyricum* produce aceitunas completamente fisuradas (Gililland y Vaughn, 1943). El gas producido reduce la densidad de la aceituna provocando que flote en la superficie.

El desarrollo de microorganismos pectinolíticos (*Saccharomyces oleaginosus, S. kluyveri, Hansenula anomala, Pichia manshurica, Pichia kudriavzevii, Candida boidinii, Rhodotorula minuta, R. rubra, Rhodotorula glutinis, Aspergillus niger, Penicillium sp. y Fusarium sp.*) y levaduras y mohos celulolíticos (*Cellulomonas sp*.), también se asocian con el *ablandamiento* de la fruta. Esto se debe a la acción de sus enzimas degradantes que actúan sobre las sustancias pécticas y la celulosa (Arroyo-López et al., 2012; Golomb et al., 2013).

En la segunda fase, a los 15 ó 20 días, los microorganismos Gram negativos disminuyen progresivamente, hasta desaparecer. Los azúcares reductores y los glucósidos, fuentes básicas de carbono, necesarias para el desarrollo de los lactobacilos y otros microorganismos, pasan de la pulpa de la aceituna a la salmuera, donde son aprovechados por microorganismos heterofermentadores u homofermentadores que los transforman en ácido láctico. Los microorganismos que crecen en esta segunda fase son *Lactococos* y producen ácido láctico, que contribuye a la reducción adicional del

pH. Esto entonces favorece, en la tercera fase, el crecimiento de los lactobacilos que son acidúricos, siendo su crecimiento óptimo entre pH 5,5 y 5,8. Esta fase se caracteriza por un abundante crecimiento de lactobacilos homofermentativos, con predominio de *Lactobacillus plantarum*. Una de las anormalidades más comunes asociadas con las aceitunas elaboradas al "estilo español" se conoce en la industria como "levadura o manchas blancas". Son pequeñas manchas blancas que se pueden desarrollar entre la piel y la pulpa de la aceituna. Estudios microscópicos y microbiológicos han demostrado que este defecto se debe a colonias de bacterias *L. plantarum* y no a levaduras (Fernández et al., 1997; Kailis and Harris, 2007). Al final de la fermentación láctica, el pH desciende a valores próximos a 4,0, aumentando la acidez y asegurando la conservación del producto. La fermentación láctica termina cuando se agota el suministro de carbohidratos disponibles (p. ej., glucosa de los glucósidos y azúcares reductores).

Al final de esta tercera fase, durante el almacenamiento, si el producto no está pasteurizado, podría sufrir fermentaciones no deseadas con el desarrollo del género *Propionibacterium*, que pueden metabolizar el ácido láctico produciendo ácido acético y propiónico aumentando el pH y la acidez volátil, y provocando una disminución en el ácido láctico. Además, la neoformación de un ácido graso volátil, el ácido ciclohexanocarboxílico (Montaño et al., 1996) y la producción de aminas biogénicas, como la cadaverina y la tiramina, (García et al., 2004), podrían estar relacionadas con el defecto de *zapatería* de las aceitunas. Estas condiciones también fomentan el desarrollo de *Clostridium* (*C. sporogenes, C. bifermentans*), que junto con *Propionibacterium* (*Propionibacterium pentosaceum, Propionibacterium Zeae, Propionibacteriumacnes*) pueden promover el deterioro por *zapatería* (Kawatomari y Vaughn, 1956; Cancho et al., 1980). Para evitar el desarrollo descontrolado y dañino de estas bacterias, el pH debe mantenerse en valores de 4,0 y el NaCl en la salmuera debe elevarse al 8%. Un aumento del pH y las condiciones anaerobias de las aceitunas envasadas también puede promover el crecimiento de *C. botulinum* (Fenicia et al., 1992; Cawthorne et al., 2005). Esta intoxicación es común en aceitunas negras oxidadas al "estilo californiano", sobre todo si no se realiza un tratamiento de esterilización térmica efectiva. Aquí, el pH está entre 5,8 y 7,9, y el NaCl entre 1 y 3%. Debido a estos valores de pH y NaCl, no se garantiza la seguridad de las

aceitunas negras oxidadas y habría que esterilizarlas para evitar el desarrollo de patógenos alimentarios.

Además, podemos decir, que en las aceitunas de mesa existen tres parámetros que influyen mucho en la conservación del producto durante el proceso de elaboración. Estos son: pH, acidez libre y contenido en sal. En las aceitunas de mesa, se pueden usar diferentes métodos para garantizar la conservación durante el tiempo de comercialización. Estos parámetros físico-químicos influyen directamente sobre el desarrollo microbiano, sus propiedades físico-químicas y sobre la conservación de las aceitunas.

Las industrias de aceitunas de mesa deben controlar estos parámetros que le permiten comercializar el producto con calidad y seguridad, con olor y sabor normal, limpias, sanas, sin defectos ni gérmenes patógenos o sus toxinas, etc.

También los laboratorios de análisis disponen de métodos instrumentales por medio de cromatografía de gases o cromatografía de líquidos, HPLC (Bleve et al., 2014), que permite identificar los compuestos presentes en las aceitunas tras ser sometido a un proceso fermentativo con posibles alteraciones. Hoy en día, la microextracción en fase sólida (SPME) seguida de la cromatografía de gases acoplada a la espectrometría de masas (GC-MS) es una de las técnicas más utilizadas para el análisis de compuestos orgánicos volátiles (COVs) en los alimentos (Merkle et al., 2015).

Las características más apreciadas por los consumidores son el sabor y olor de un alimento. Ambas características están estrechamente relacionadas con la composición cualitativa y cuantitativa de los compuestos volátiles y puede verse influenciadas por una serie de factores, que incluyen el tipo de aceituna, el estado de madurez y el método de procesamiento (Sabatini y Marsilio, 2008). El olor es un parámetro olfativo importante que determina la calidad de los productos alimenticios y, por lo tanto, es de interés investigar los compuestos volátiles que contribuyen a los olores característicos que pueden servir como indicadores de evaluación de la calidad del producto final (Panagou et al., 2008).

El espacio de cabeza de la salmuera de la aceituna de mesa ha sido estudiado para identificar compuestos volátiles responsables del olor desagradable de la aceituna con defecto a *zapatería* (Montaño et al., 1992), para comparar los compuestos volátiles en aceitunas verdes al "estilo español", "estilo griego", "estilo Castelvetrano", etc. (Sabatini y Marsilio, 2008).

Un aroma agradable se deriva del equilibrio de un número de sustancias volátiles, tales como hidrocarburos, alcoholes, aldehídos, cetonas, ésteres y otros compuestos.

La tecnología de procesamiento de la aceituna de mesa influye en gran medida en la composición físico-químico de las aceitunas de mesa y el producto final. Los cambios en el aroma de la aceituna nos permiten comparar diferentes cultivares y métodos de procesamiento, y, además, son indicadores de la evolución de la calidad durante el procesamiento y almacenamiento de ésta.

La formación del aroma de las aceitunas de mesa es un proceso dinámico que se desarrolla principalmente durante la fermentación de la aceituna por bacterias autóctonas del ácido láctico y levaduras, junto con una variedad de microorganismos contaminantes, que producen compuestos volátiles a partir de los principales constituyentes de la fruta a través de diversas vías bioquímicas. Las bacterias del ácido láctico influyen directamente en el aroma de las aceitunas fermentadas, pero también la presencia de ciertos microorganismos no deseados y competitivos, forman en la salmuera alcoholes, ésteres, aldehídos y cetonas, así como ciertos ácidos (McFeeters, 2004; Fleming et al., 1969). Por lo tanto, es importante determinar el contenido de compuestos volátiles y los cambios de sus perfiles cualitativos y cuantitativos durante todo el proceso de producción (Sabatini y Marsilio, 2008).

1.4 La acrilamida en aceitunas negras al "estilo californiano"

La acrilamida es una sustancia química carcinógena que se genera en algunos alimentos, durante procesos de cocción a partir de 120ºC (Agencia Española de Consumo, Seguridad Alimentaria y Nutrición [AECOSAN], 2016). Principalmente se forman a partir de azúcares y aminoácidos como la asparagina, que

están presentes de forma natural en los alimentos. La reacción de Maillard suele ser una de las vías de formación, originando además un oscurecimiento de los alimentos que afecta al color (AECOSAN, 2016).

Entre los alimentos con mayor contenido de acrilamida tenemos: patatas fritas al estilo tradicional (300 $\mu g.Kg^{-1}$), patatas chips (unos 700 $\mu g.Kg^{-1}$), galletas (350 $\mu g.Kg^{-1}$) y el café (300 $\mu g.Kg^{-1}$). En adultos se ha estimado que la ingesta promedio diaria de esta sustancia está entre 0,3 y 3 $\mu g.Kg^{-1}$ de peso corporal y día, con ingestas máximas de 5,1 $\mu g.Kg^{-1}$ de peso y día. En niños, la ingestión diaria puede llegar a triplicar estos valores, por kg de peso corporal.

Los métodos utilizados en la elaboración de aceitunas negras al "estilo californiano" pueden producir acrilamida (Pérez-Nevado et al., 2018; Casado y Montaño., 2008). Estas aceitunas, en comparación con las aceitunas al "estilo español" o "griego", contienen niveles altos de acrilamida debido a las altas temperaturas requeridas en su esterilización (Pérez-Nevado et al., 2018). Las recientes investigaciones en este tema conducen a multitud de estudios científicos para mitigar la formación de acrilamida en aceitunas negras al "estilo californiano" durante el proceso de elaboración (Casado y Montaño, 2008; Charoenprasert y Mitchell, 2014; Pérez-Nevado et al., 2018; Tang et al., 2016; Martín-Vertedor et al., 2020; 2021).

Dado el peligro para la salud que presenta la acrilamida, los organismos internacionales responsables de la salud, han recomendado que se reduzca el contenido de esta sustancia en los alimentos procesados. El reglamento (EC) 2017/2158 de la Comisión de 20 de noviembre de 2017, es la normativa que establece los límites de referencia para detectar la presencia de acrilamida en algunos productos alimenticios. Por esta razón, la determinación de acrilamida en aceitunas es de suma importancia para la seguridad humana. En este sentido, la Recomendación de la Comisión Europea (EC) 2019/1888, actualizada el 7 de noviembre de 2019, añadió las aceitunas almacenadas en soluciones de salmuera a la lista de alimentos en los que los niveles de acrilamida deben ser controlados por las autoridades de los estados miembros de la Unión Europea.

En los últimos años se han desarrollado métodos de análisis clásicos para la detección de esta sustancia tóxica, basados en la Cromatografía Líquida de Alta Resolución (HPLC) o Cromatográfia de gases (Bologna et al., 1999).

1.5 Nariz electrónica

La *nariz electrónica* ofrece la posibilidad de evaluar la información contenida en el espacio de cabeza en multitud de alimentos. El uso de este dispositivo para la evaluación de la calidad como medio de detección olfativa no destructiva se está generalizando cada vez más. Presenta la ventaja de su bajo costo, buena fiabilidad y alta portabilidad para mediciones *in situ* y en línea, frente a los métodos tradicionales de análisis de laboratorios que resultan laboriosos, de mayor duración y más costosos (Panagou et al., 2008).

1.5.1 Origen

Las primeras investigaciones surgen de dos grupos de investigación en los años 80, en la Universidad de Warwick (UW) en Gran Bretaña y en el Argonne National Laboratory (ANL) en Estados Unidos. Investigadores de la UW liderados por Krishna Persaud y George Dodd, publican sobre un conjunto de sensores de óxidos metálicos (MOx) utilizados en la *nariz electrónica* en 1982 (Persaud y Dodd, 1982). El otro grupo ANL, desarrolló un dispositivo para detectar y medir productos químicos que permitan obtener la "huella digital" olfativa para caracterizar las sustancias.

En 1991 se celebró el primer Congreso Internacional en Narices Electrónicas en Reykiasvik, Islandia, financiado por la OTAN, originando un incremento de su interés. Actualmente, el término *nariz electrónica* se utiliza para referirse a la matriz o array de sensores de gases que miden cambios en la atmósfera gaseosa, que alteran las señales del sensor de una manera determinada. Existen diferentes tipos de sensores con diferentes principios de funcionamiento: resistivos basados en diferentes materiales sensibles (óxidos metálicos, polímeros conductores y polímeros intrínsecamente conductores), sensores ópticos, sensores de ondas acústicas

superficiales, transistores de efecto de campo sensible a los gases y microbalanzas de cuarzo (QMB). Las técnicas más novedosas son los sistemas microelectromecánicos (MEMS) combinados con las nanotecnologías (Loutfi et al., 2015). También se ha utilizado este término para caracterizar sistemas de detección acoplados a cromatógrafos de gases o espectrómetros de masas.

El uso de la *nariz electrónica* en la evaluación de la calidad de los alimentos se basa en que en el alimento se pueden generar metabolitos gaseosos, principalmente compuestos orgánicos volátiles (COVs) presentes en el espacio de cabeza de un producto alimenticio y que podría proporcionar información útil para la determinación de su calidad (Balasubramanian et al., 2007). Al interaccionar los COVs con los sensores de la *nariz electrónica* provocan cambios en la respuesta de cada sensor que son recopilados, y cuyos datos pueden ser interpretados tras ser procesados con algoritmos matemáticos adecuados. Los datos obtenidos pueden caracterizar y discriminar diferentes muestras en función de estos COVs generados.

1.5.2 Descripción y fundamentos generales

La *nariz electrónica* se compone principalmente de dos sistemas, uno de detección o transducción química y otro de reconocimiento de patrones automatizado (Berna, 2010). El sistema de detección puede ser un sensor o matriz de sensores diferentes. Los COVs de la muestra que se pone en contacto con el conjunto de sensores, producen un patrón que es característico del aroma de la muestra. Al presentar muchos productos químicos diferentes a la matriz de sensores, se puede construir una base de patrones. El análisis de datos y reconocimiento de patrones, son partes fundamentales de cualquier sistema de matriz de sensores. Hay una variedad de métodos de reconocimiento de patrones que se pueden clasificar en tres clases dependiendo de los datos disponibles y del tipo de resultado que se requiere. El análisis gráfico con diagramas de barras o radiales son formas sencillas de tratamiento de datos que se pueden utilizar con una *nariz electrónica*. Una segunda forma de analizar las señales de la *nariz electrónica* es por medio del análisis multivariante. Este análisis generalmente

implica la reducción de la alta dimensionalidad o números de variables, lo que permite que la información se muestre en una dimensión más pequeña de variables parcialmente correlacionadas. Entre las técnicas multivariantes más utilizadas se encuentran: Análisis de Componentes Principales (PCA), Análisis Discriminante Lineal (LDA), Mínimos Cuadrados Parciales (PLS), Mínimos Cuadrados Parciales Discriminante (PLS-DA), etc. Una tercera forma de analizar las señales se basa en Redes Neuronales Artificiales (ANN). Una red neuronal consiste en un conjunto de algoritmos de procesamiento interconectados que funcionan simultáneamente. En un nivel muy simplificado y abstracto, ANN se basa en el proceso cognitivo del cerebro humano.

1.5.3 Sensores de gases utilizados

Los sensores de gases más utilizados para las *narices electrónica*s son: resistivos, ondas acústicas superficiales, catalíticas, ópticas y electroquímicas (Park et al., 2019). De acuerdo con la literatura (Borrego et al., 2016), los sensores electroquímicos de gases presentan un buen comportamiento; sin embargo, los sensores de gases resistivos son los más utilizados en dispositivos portátiles y miniaturizados debido a su pequeño tamaño y bajo consumo de energía, en particular, los basados en óxidos metálicos semiconductores (MOS). Los sensores de MOS están formados por una película o filamento de óxido metálico (normalmente dióxido de estaño) dopado con otros compuestos como trióxido de tungsteno u óxido de zinc (tipo n) u óxido de níquel (tipo p). Los semiconductores más ampliamente utilizados de óxidos metálicos tipo n son los de óxido de estaño (SnO_2). Los motivos son su elevada sensibilidad a temperaturas menores a 300ºC y su condición de ser químicamente estable.

La conductividad superficial del SnO_2 se altera por la adsorción superficial de las especies gaseosas y por las reacciones químicas de estas especies en la superficie del semiconductor (Ihokura y Watson, 1994). Por tanto, la concentración de electrones en la superficie del sensor puede ser modificada por procesos de oxidación o reducción. Los gases a los que son sensibles este tipo de sensores son aquellos que

reaccionan con la superficie del sensor implicando un intercambio de carga electrónica en la interacción sólido-gas.

Los mecanismos de interacción entre el semiconductor y la fase gaseosa que son la fisisorción y quimisorción (Sberverglieri, 1992). Las especies de gases pueden clasificarse en reductoras y oxidantes. En la superficie del SnO_2 se ha dado la quimisorción de oxígeno, debido al tratamiento térmico realizado a la superficie, hasta llegar a una situación de equilibrio. Un gas oxidante implica la adsorción o reacción superficial de alguna especie sobre la superficie del semiconductor que provoca un incremento en el vaciamiento de electrones libres en la superficie. El gas oxidante, en su interacción con el SnO_2, actúa como aceptor, disminuyendo la conductividad del semiconductor. Por el contrario, un gas reductor actúa como donador e incrementan la conductividad del SnO_2. Las reacciones que ocurren entre los materiales de detección y los gases se describen en las ecuaciones

(1) y (2): $^1/_2\, O_2 + e^- \rightarrow O^-$ (1) y $R_{(g)} + O^- \rightarrow RO_{(g)}$ (2)

El sensor generalmente comprende un tubo de soporte de cerámica que contiene una bobina de calentamiento de platino sobre la cual se recubre el exterior del tubo con SnO_2 sinterizado con cualquier aditivo catalítico. Las propiedades superficiales del SnO_2 se modifican introduciendo aditivos al SnO_2, usualmente metales nobles. Con esta adición aumenta la sensibilidad y selectividad a determinados COVs.

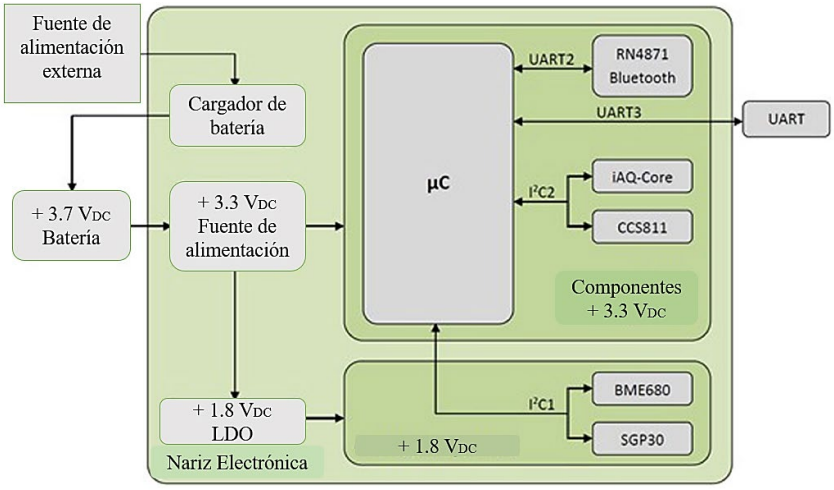
Por tanto, cuando el SnO_2 entra en contacto con los volátiles del aire, se forma una capa de agotamiento electrónico, lo que provoca un cambio en la conductividad de los sensores (Barsan et al., 2007). En los últimos años, han surgido sensores miniaturizados con una unidad de procesamiento integrada en el mismo paquete. Además, en algunos casos, los sensores de temperatura, humedad y presión están integrados junto a los sensores de gases (Arroyo et al., 2020). Estos sensores son de gran novedad e interés en dispositivos electrónicos como el utilizado en el presente trabajo.

1.5.4 Características de la nariz electrónica utilizada en esta tesis doctoral

Las componentes que forman parte de la *nariz electrónica* están constituidas por una fuente de alimentación, una batería de iones de litio de +3,7 VDC, un convertidor DC-DC de +3,3 V y un regulador lineal de baja caída de +1,8 V (Figura 6). El núcleo del sistema es un microcontrolador de 32 bits, Microchip modelo PIC32MM0256GPM048, que realiza las operaciones principales: control de sensores a través de dos interfaces seriales I2C; comunicaciones con dispositivos inteligentes mediante el uso de un pequeño módulo Bluetooth de bajo consumo RN4871 de Microchip; y comunicaciones UART por cable con otros dispositivos. Se ha elegido este microcontrolador por su bajo consumo de corriente gracias a su baja frecuencia de funcionamiento (24 MHz). A pesar de ello, posee una gran potencia de procesamiento debido a su arquitectura de 32 bits, que se complementa con una memoria de programa de 256 KB y una memoria de datos de 32 KB. Se han instalado cuatro chips de sensores de gases digitales. Sin embargo, debido a sus diferentes voltajes de suministro (+1,8 V y +3,3 V), se ha decidido utilizar dos interfaces, I^2C separadas para lograr una mayor simplicidad. En cuanto al consumo de energía del dispositivo, se ha calculado una corriente máxima teórica (según fabricantes) de 412 mA, aunque la corriente de funcionamiento medida experimentalmente fue de hasta 185 mA.

Figura 6.

Diagrama de bloque de la nariz electrónica.



Las dimensiones del dispositivo son 39 mm × 33 mm. Los cuatro chips, que integran sensores de óxido metálico (MOx), son los siguientes: BME680 (Bosch Sensortec, 2019), SGP30 (Sensirion, 2019) y CCS811 (Ams, 2019) e iAQ-Core (Ams, 2019). Todos ellos son sensores de gas inteligentes miniaturizados. Estos dispositivos llevan integrada electrónica analógica y digital combinada en una microplaca caliente con los elementos detectores en un chip. En general, las señales de los sensores resistivos llegan al procesador a través de circuitos analógicos de acondicionamiento. Después, estas señales se procesan con diferentes tipos de algoritmos (compensación de línea base, promedio, corrección de humedad, etc.). Además, algunos de ellos permiten a los usuarios escribir parámetros de calibración. Por último, las señales de salida (calibradas y/o sin procesar) se transmiten a partir de protocolos de comunicación digital. Esta interfaz digital integrada simplifica enormemente la integración de estos sensores en la *nariz electrónica*. La Figura 6 anterior, muestra los principales componentes de los sensores digitales de gases.

Todos los sensores, excepto BME680, incluyen algoritmos inteligentes para procesar las señales sin procesar para generar COVT (compuestos orgánicos volátiles totales) y valores de predicción equivalentes de CO_2 (eCO_2). Además, SGP30 proporciona señales sin procesar para H_2 y etanol. Se ha establecido un protocolo simple basado en ASCII para la comunicación Bluetooth entre el microcontrolador y un dispositivo inteligente externo. Hay algunos comandos para recuperar datos de sensores individuales y algunos para cambiar los parámetros del sensor, configurar los valores de los calentadores, etc.

Para cada tiempo de muestra, el microcontrolador lee los valores de cada sensor y los envía a un dispositivo móvil a través de Bluetooth en un marco formateado. Cada marco de datos se compone de columnas separadas por tabulaciones horizontales y terminadas con caracteres de retorno de acarreo y avance de línea.

En la Tabla 3, se enumeran los sensores que componen la *nariz electrónica*. Para el procesado de datos se usaron los sensores del 1 al 11.

Tabla 3.

Sensores de la nariz electrónica.

Columna	Descripción	Sensor
T	Temperatura (ºC)	BM680
P	Presión (hPa)	BM680
H	Humedad (%RH)	BM680
1	Medición Gas(Ω)	BM680
2	eCO_2 (ppm)	SGP30
3	COVT (ppb)	SGP30
4	H_2	SGP30
5	Etanol	SGP30
6	eCO_2 (ppm)	CCS811
7	COVT (ppb)	CCS811
8	Resistencia Sensor	CCS811
9	(Ω) eCO_2 (ppm)	iAQ-Core
10	COVT (ppb)	iAQ-Core
11	Resistencia Sensor (Ω)	iAQ-Core

Algunos de estos sensores se han utilizado previamente para monitorear la actividad de las plantas (Catini et al., 2019) para estudiar su respuesta individual a los compuestos BTEX (benceno, tolueno, etilbenceno y xilenos) (Yurko, 2019) o para investigar su arquitectura y funcionamiento (específicamente el modelo SGP30 de Sensirion) (Rüffer et al., 2018).

1.6 Antecedentes sobre el uso de narices electrónicas en el sector

El uso de la *nariz electrónica* o sistema de sensores de gas en aceites de oliva es anterior al año 2000 (Ulmer et al., 1997; Martín et al., 1999). Sin embargo, sus primeras aplicaciones en aceitunas de mesa se remontan al 2008 (Panagou et al., 2008), siendo las más actuales en 2020 y 2021 (Martínez et al., 2020; Sánchez et al., 2021a; Sánchez et al., 2021b; Sánchez et al., 2022a). La *nariz electrónica* se presenta como una herramienta capaz de discriminar muestras de aceitunas, aceitunas de mesa y aceites de oliva, en función de su perfil oloroso de manera rápida, objetiva, económica y medioambientalmente sostenible.

La *nariz electrónica* se ha utilizado para clasificar y discriminar de manera rápida entre varios tipos de aceites según su composición, origen del olivar, variedad, grado de adulteración, etc. (González et al., 1999). Estos investigadores utilizaron la *nariz electrónica* con sensores semiconductores de óxido metálico (MOS), para diferenciar 140 muestras de aceites para clasificar entre aceite de oliva virgen, no virgen y aceites de semilla. Autores como James et al. (2004) discriminaron entre muestras de aceite de oliva virgen extra, oliva no virgen y aceite de colza utilizando la *nariz electrónica* basado en sensores piezoeléctricos. Otros autores como Gan et al. (2005) clasificaron 16 aceites comestibles diferentes, incluidos entre aceites de colza, girasol y semilla de uva, en función de la composición volátil de su espacio de cabeza.

La *nariz electrónica* también se ha utilizado para clasificar los aceites según la región de origen. Por ejemplo, para analizar aceite de oliva procedente de varias regiones de Italia y de España (Cosio et al., 2006). También se utilizó la *nariz electrónica* para discriminar el aceite de oliva virgen extra de la región de Liguria (Italia) frente a otros aceites de oliva comerciales (Casale et al., 2010).

Otras aplicaciones que se le ha dado a este dispositivo electrónico son la detección de adulteraciones en los aceites de oliva, ya que algunos fabricantes podrían adulterar sus productos con aceites de inferior calidad (Monfreda et al., 2012). Oliveros et al. (2002) utilizaron la *nariz electrónica* comercial FOX 4000 para detectar adulteraciones de aceite de oliva con aceite de girasol. El mismo modelo de *nariz electrónica* también se utilizó para la detección de mezclas de aceite de oliva con aceite de colza (Mildner-Szkudlarz et al., 2008).

También se pudo usar la *nariz electrónica* con sensores MOS para discriminación de aceites procedentes de diferentes variedades de aceituna (Jolayemi et al., 2017).

Otro ejemplo de aplicación fue el uso de la *nariz electrónica* con sensores MOS para la detección de defectos como el *rancio, mohoso, turbio* y *vinoso* en aceites de oliva (Lerma-García et al., 2010). Recientemente también se usó un dispositivo comercial PEN3 (Airsense) con sensores MOS para detectar defectos en aceites y aceitunas (Martínez et al., 2020), y la EOS835 comercial (Sacmi Industry, S.r.l., Imola, Italy), para el análisis sensorial de la calidad del aceite de oliva virgen (Chacón et al., 2022).

Por otra parte, en la bibliografía también encontramos clasificación de aceitunas de mesa verdes fermentadas con la *nariz electrónica* NST 3320 formadas por 10 sensores MOS-FET (semiconductor de óxido metálico con transistor de efecto de campo), 12 sensores MOS (semiconductores de óxido metálico), y sensor IR de CO_2 junto a un sensor de humedad relativa (Panagou et al., 2008). Martinez et al. (2020) realizaron los análisis para predecir la calidad de la aceituna después de la cosecha utilizando la PEN3 (AirsenseAnalytics GmbH, Schwerin, Alemania).

La primera utilidad que se perseguía con el desarrollo de las *narices electrónicas* era sustituir a los paneles de análisis sensorial en la clasificación de aromas. El panel de expertos tiene un elevado coste, dificultad para unirlos y un cierto error de subjetividad en sus apreciaciones. El cansancio o estado anímico, entre otros factores, influyen en sus valoraciones, comparado con la objetividad de un análisis instrumental.

En ocasiones estos grupos de expertos se han apoyado en los análisis por cromatógrafos de gases para clasificar y/o cuantificar los COVs, aunque esto supone

un elevado coste de tiempo y dinero, así como una mayor dificultad técnica. En esta Tesis Doctoral se pretende discriminar los atributos positivos y negativos de las aceitunas de mesa elaboradas al "estilo californiano" y "estilo español", y desarrollar unos modelos de patrones olfativos que permitan establecer relaciones entre los resultados obtenidos de la *nariz electrónica* y los análisis sensoriales y cromatográficos tradicionales.

1.7 Adquisición y procesado de datos

En primer lugar, se realizan las medidas con la *nariz electrónica* para registrar las señales de respuesta de los sensores. A continuación, se realiza un pretratamiento de estas señales para extraer la información y posteriormente, procesarlos para obtener el patrón de olfativo de la muestra.

1.7.1 Análisis de aceitunas con la nariz electrónica. Adquisición de los datos

Panagou et al. (2008) utilizaron la *nariz electrónica* para diferenciar la calidad de las aceitunas de mesa verdes fermentadas en función de sus huellas dactilares volátiles y clasificarlas en tres clases principales (aceptable, marginal, inaceptable). Estos investigadores utilizaron la *nariz electrónica* NST 3320 Emission Analyzer (Applied Sensors, Linköping, Suecia), equipada con un automuestreador de espacio de cabeza y una unidad detectora que contiene 23 sensores diferentes y un software integrado (NST Senstool) para procesar los datos de los sensores.

El instrumento estaba equipado con 12 sensores MOS (semiconductor de óxido metálico) que funcionan a 250–400ºC, 10 sensores MOSFET (transistor de efecto de campo semiconductor de óxido metálico) que funcionan a 140–170ºC y un sensor de humedad. Para las medidas, estos autores pesaron muestras de aceitunas (2g) y las colocaron en viales de 25ml, sellados con tapones de rosca con septum, e incubados a 37ºC durante 1 h para equilibrar el espacio de cabeza por encima de la muestra. Posteriormente, las muestras se colocaron en el muestreador automático y se bombeó gas de espacio de cabeza sobre los sensores de la *nariz electrónica*. Cada muestra la

analizaron por triplicado. La fase de registro de la línea de base se fijó en 30 s, la fase de muestra en 30 s y la fase de recuperación de los sensores en 200 s, incluido el tiempo de lavado de las líneas de gas (100 s). El tiempo de ciclo total por muestra fue de 4 min y 20 s.

Martínez et al. (2020) realizaron los análisis para predecir la calidad de la aceituna después de la cosecha utilizando un sistema olfativo portátil (92 × 190 × 255 mm) de 2,3 kg, que consta de una unidad de muestreo de gas y una matriz de sensores (MOS). Este equipo disponía de un software (Win Muster v. 1.6.2), el PEN3 que permite la adquisición, visualización y análisis de los datos y funciona utilizando aire ambiente filtrado durante el paso de limpieza y gas diluido durante el muestreo. Para cada lote seleccionó una muestra de 250 g de fruta. Las muestras se colocaron en un recipiente de vidrio (1L), que luego se cubrió con parafilm. Los recipientes se mantuvieron a 25°C durante 8 min. Cada proceso de medición implicó dos etapas. La primera consiste en el proceso de limpieza en el que el aire pasa a través de un filtro de carbón activado antes de entrar en el conjunto de sensores. En la segunda etapa, el aire pasa a través de la muestra.

Las condiciones de funcionamiento de la *nariz electrónica* durante las mediciones se establecieron de la siguiente manera: el tiempo de pre-muestreo fue de 10 s, el tiempo de medición fue de 70 s, el tiempo de muestreo fue de 1 s, el tiempo de lavado fue de 50 s, el tiempo de punto cero fue de 10 s y finalmente la cámara y la inyección el flujo se fijó en 400 mL/min (Martinez et al., 2020).

En nuestro estudio, las mediciones con *nariz electrónica* se realizarán en las condiciones recomendadas por el COI para la cata de aceitunas de mesa (IOC, 2021).

Se colocaban 10 mL de salmuera y de tres a cuatro aceitunas, en función del tamaño, en los vasos de degustación estándar, cubiertos con un vidrio de reloj y colocados en un bloque termostatizado a 25°C durante 15 minutos. Otro vaso de degustación estándar se dejaba sin muestras, solo con aire, donde se producía la desorción o recuperación de los sensores. Se tomaban cinco o más medidas para cada muestra de aceitunas de mesa en función del ensayo (Figura 7).

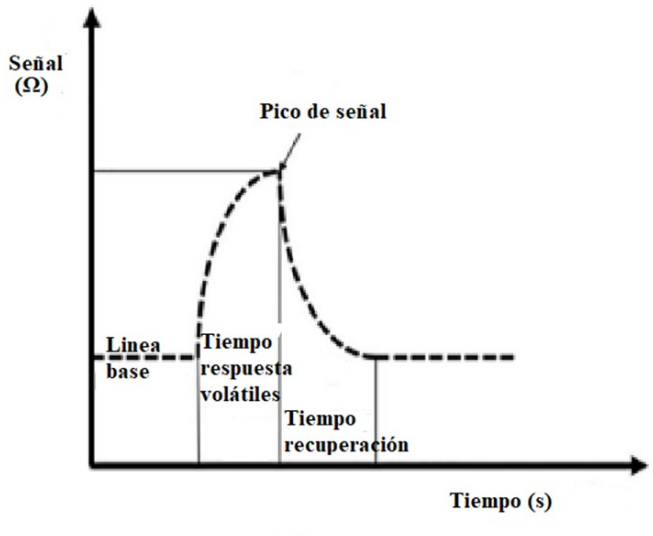
Figura 7.

Medidas realizadas con la nariz electrónica a las aceitunas de mesa.

Cada ciclo de adquisición de datos constaba de dos partes: colocar la *nariz electrónica* encima de la copa de muestra durante 60 s para registrar las señales del sensor a los volátiles y, posteriormente, colocarla en una copa vacía durante 30 s para que los sensores de gas registren la señal del aire como línea base. La curva de respuesta de cada sensor está compuesta por N puntos correspondientes a las mediciones del sensor con el tiempo (Figura 8).

Figura 8.

Curva del registro de la señal de la nariz electrónica.



El algoritmo utilizado para la caracterización de las curvas de respuesta de cada sensor es: valor máximo de la señal menos el valor mínimo de la señal multiplicado por 100 y menos uno ((X_{iMAX}-X_{iMIN}) x 100-1). Como resultado, se obtuvo para cada muestra un vector de datos con 11 filas, uno por cada sensor.

La *nariz electrónica* con la que se realizó esta Tesis Doctoral es un dispositivo portátil miniaturizado, diseñado en la Universidad de Extremadura (Arroyo et al, 2020). Este prototipo, como se comentó en epígrafes anteriores, consta de cuatro chips de sensores de gases digitales con sensores integrados de óxido metálico (MOS): BME680 de Bosch, SGP30 de Sensirion y CCS811 e iAQ-Core de ScioSense. Un microprocesador lee los valores detectados por los sensores y los envía a un dispositivo smartphone a través de bluetooth. Después, los datos resultantes se transfieren a un ordenador para el procesamiento de datos y análisis quimiométricos.

1.7.2 Análisis quimiométricos utilizados. Pretratamiento y procesado de datos

Los datos del dispositivo pueden ser posteriormente procesados mediante análisis quimiométricos. Una de las principales aplicaciones de la quimiometría es la calibración multivariante, para cuantificar uno o más componentes en muestras complejas. Una muestra tendrá múltiples datos instrumentales que serán denominados datos multivariados (Haaland y Thomas, 1988).

El avance en la experimentación instrumental analítica junto con el desarrollo de algoritmos matemáticos han supuesto que la calibración multivariante permita la resolución de problemas analíticos complejos, disminuyendo el tiempo de análisis e incluso eliminando procesos de pretratamiento de las muestras (Carabajal et al., 2017).

El análisis de datos y reconocimiento de patrones, son partes fundamentales de cualquier sistema olfativo. El procesado de señal multivariante utilizado en la *nariz electrónica* se lleva a cabo mediante análisis quimiométricos.

El procesado de los datos comienza cuando los sensores originan señales que son almacenadas en el ordenador. Los datos se presentan como ficheros de texto. Con el procesado de la señal se espera obtener información sobre la respuesta deseada

de la muestra, su clasificación, cuantificación, estimación de parámetros de técnicas de análisis convencionales, etc., minimizando las interferencias que puedan presentar la matriz de sensores. La clasificación puede hacer uso de técnicas no supervisadas, que nos permiten ver la semejanza entre medidas sin establecer ningún criterio, mientras que las técnicas supervisadas, clasifican con criterios previamente impuestos en la fase de entrenamiento. En el caso de la estimación de parámetros de análisis de laboratorio cromatográficos o sensorial, se aplican técnicas de cuantificación multivariantes como la técnica de PLS que relacionan la señal de los sensores generadas por los volátiles de las aceitunas o salmueras con concentraciones de COVs obtenidos por cromatografía y organolépticas de las muestras analizadas.

Los datos recopilados de los sensores individuales de la matriz requieren un procedimiento de procesamiento para analizar y clasificar los datos.

Los tratamientos de datos constan de las siguientes etapas:

1.- Etapa de preprocesado. Es el paso previo a la aplicación de reconocimiento de patrones. En esta etapa la información de los sensores se extrae para procesar su respuesta. Este preprocesado lleva incluido la compensación de la deriva de los sensores, extracción de los parámetros descriptivos a partir de la respuesta temporal de los sensores y preparar el vector de características para los procesados posteriores. El preprocesado se realiza con el programa Matlab. El programa utiliza como entrada el fichero de las medidas con datos temporales. A continuación, calcula el valor correspondiente a la línea base y el valor en equilibrio de los sensores ante el aroma.

2. Reducción de la dimensionalidad. Para la reducción de la dimensionalidad de los datos y reconocimiento de patrones se utilizan modelos matemáticos como los del PCA, el PLS-DA y PLS.

3. Predicción. En esta tesis se ha considerado el algoritmo PLS por ser de los más utilizados y de los que mejores resultados ofrece. El algoritmo PLS intenta maximizar la covarianza (Geladi y Kowalski, 1986) a través de variables latentes. PLS es un método de correlación multivariable entre las señales que provienen de los

sensores y las variables del panel sensorial y/o análisis cromatográfico. Es una técnica supervisada, y en la fase de entrenamiento se origina un modelo que relaciona variables predictoras con variables a predecir maximizando la covarianza. En la fase de evaluación, se usa el modelo construido durante la fase de entrenamiento para a partir de nuevas variables de entrada poder predecir variables de salida. Para determinar la validez de los modelos generados por PLS se ha utilizado la validación cruzada.

4. Validación. Para determinar la validez de los modelos de predicción generados por PLS o clasificación por PLS-DA, se necesitan predicciones de medidas de las que se sabe el valor de salida y así, poder comparar las estimaciones realizadas con el valor real. Este paso final, en el reconocimiento de patrones, permite la estimación de errores o rendimientos del modelo entrenado mediante técnicas de validación. En esta Tesis Doctoral se han utilizado validaciones cruzadas de orden 1 ("leave-one-out").

En las tareas de regresión y clasificación se ha utilizado el Software Matlab R2016b versión 9.1 (The Mathworks Inc. Natick, MA, USA) con la PLS_Toolbox 8.2.1 (Eigenvector Research Inc., Wenatchee, WA, USA).

CAPÍTULO II. RESULTADOS Y DISCUSIÓN

En la presente Tesis Doctoral se evalúa un sistema olfativo artificial (*nariz electrónica*) para la detección y discriminación de diferentes tipos de muestras de aceituna de mesa y sus salmueras en función del perfil sensorial y aromático. Con el conjunto de datos obtenidos se desarrollan modelos matemáticos que permitan una diferenciación de las aceitunas en función de su calidad, variedad o aroma.

En las primeras cuatro publicaciones se estudiaron aceitunas de mesa verdes elaboradas al "estilo español" que presentan fermentaciones anormales, alteraciones por mohos y se ha evaluado el aroma de las aceitunas de mesa verdes rellenas de hidrocoloides con aroma añadido. Además, se han obtenido modelos de clasificación de las aceitunas de mesa verdes en categorías de calidad. Por otra parte, en las tres últimas publicaciones, se estudiaron aceitunas negras elaboradas al "estilo californiano" sometidas a diferentes tratamientos térmicos de esterilización y los efectos olfativos producidos por el exceso de calentamiento, como pueden ser el *efecto de cocido* y la formación de acrilamida. Se ha estimado la cantidad de acrilamida con ayuda de la *nariz electrónica*. Además, se ha evaluado el aroma de aceitunas de mesa negras al "estilo californiano" rellenas con hidrocoloides con diferentes aromas añadidos.

Los resultados y discusión de este trabajo tratan de dar respuesta a todos los objetivos expuestos. Para ello, se ha estructurado la exposición del trabajo realizado en 7 publicaciones científicas indexadas que a continuación resumen los resultados más relevantes en los ensayos realizados.

2.1 Artículo 1. "E-nose discrimination of abnormal fermentations in Spanish-style green olives. (2021). Molecules, 26(17), 5353"

Sánchez, R., Martín-Tornero, E., Lozano, J., Boselli, E., Arroyo, P., Meléndez, F., Martín-Vertedor, D.

2.1.1 Motivación y objetivos

Las aceitunas de mesa al "estilo español" es uno de los procesos de elaboración más comunes a nivel industrial. Los frutos de *Olea europaea* spp. se tratan con sosa cáustica para eliminar su amargor y posteriormente se fermentan en una solución salina durante varios meses. Si se realizan malas prácticas industriales durante el procesado, estas aceitunas pueden sufrir fermentaciones anómalas que faciliten el desarrollo de ciertos microorganismos indeseables, dando lugar a aceitunas defectuosas que presenten olores y sabores no deseables (Lanza, 2013). Estas alteraciones son una de las principales causas de pérdidas económicas para los productores.

La legislación vigente en España (Real Decreto 679/2016) exige que las aceitunas de mesa deben estar libres de malos olores y sabores y estas no deben presentar síntomas de alteración o fermentaciones anormales. A este respecto, el COI desarrolló un método de análisis sensorial para la clasificación de aceitunas de mesa teniendo en cuenta la intensidad del defecto predominante percibido (DPP) por un panel de cata. Las principales alteraciones en fermentaciones anómalas desarrolladas en aceitunas al "estilo español" son: *zapatería* (D1), *butírico* (D2), *pútrido* (D3) y *mohoso* (D4). El defecto *zapatería* es causado por microorganismos del género *Clostridium* que hace que en las aceitunas se produzca una combinación de ácidos grasos volátiles que aporta una sensación a cuero podrido. El defecto *butírico* provoca un olor a rancio, mantequilla o queso, mientras que el *pútrido* recuerda al olor a huevos podridos. Por último, el defecto *mohoso* o de *humedad* se percibe como olor a moho.

El control de los procesos de fermentación puede realizarse a través de enfoques químicos, químico-físicos y microbiológicos, y también a través de la evaluación organoléptica (IOC, 2021). Este último establece los criterios y procedimientos necesarios para el análisis sensorial de las sensaciones negativas, gustativas y olfativas de las aceitunas de mesa, que también pueden atribuirse a una proliferación anormal de microorganismos. Sin embargo, los análisis sensoriales basados en un panel de expertos entrenados y la cromatografía de gases son técnicas más caras, laboriosas y requieren un tiempo considerable de análisis. Por otro lado, se han desarrollado dispositivos electrónicos para la clasificación de aceitunas de mesa verdes fermentadas

(Panagou et al., 2008), para la detección de defectos en aceite de oliva (Lerma-García et al., 2010), siendo los principales defectos que caracterizan el aceite de oliva el *rancio, mohoso, turbio* y *vinoso*, o para detectar otros defectos en aceites y aceitunas (Martínez et al., 2020). Por tanto, se hace necesario para el control de la aceituna de mesa y su clasificación en la industria, desarrollar técnicas de análisis como la *nariz electrónica*, que son objetivas, económicas, rápidas y que podrían realizar una detección precoz de las fermentaciones anormales que sin duda, podrían evitar pérdidas económicas en las industrias.

El objetivo del presente estudio fue desarrollar un protocolo analítico basado en un dispositivo de *nariz electrónica* capaz de clasificar aceitunas de mesa elaboradas al "estilo español" que presenten diferentes defectos olfativos provocado por fermentaciones no controladas.

2.1.2 Diseño experimental

Las aceitunas de la variedad '*'Carrasqueña"* se recolectaron en el estado de madurez verde, dentro de los límites de la zona olivarera "Tierra de Barros" (Badajoz, España), durante la campaña 2019/2020 y fueron procesados al "estilo español", por una empresa ubicada en el suroeste de Extremadura (España). Las aceitunas fueron fermentadas en depósitos con capacidad de 236 L. Una vez finalizado este proceso, las aceitunas se envasaron en latas (150 g cada lata) con una salmuera al 4% p/v NaCl. A las aceitunas se les realizó un análisis sensorial por el panel de cata, con el objeto de detectar los diferentes defectos por fermentaciones anómalas. Los diferentes lotes de aceitunas fueron agrupados según los defectos sensoriales encontrados como *zapatería* (D1), *butírico* (D2), *pútrido* (D3) y *mohoso* (D4). Se utilizaron un lote de aceitunas de mesa sanas como control del proceso (C). Los experimentos se realizaron por duplicado.

Posteriormente, se realizó el análisis con la *nariz electrónica* de las muestras seleccionadas siguiendo las recomendaciones del COI para el análisis sensorial. Para ello, se utilizaron copas normalizadas de cata que se colocaron en un bloque calefactor

a 25ºC. A cada copa se les introdujo 4 aceitunas de cada grupo (C, D1, D2, D3 y D4), 10 mL de salmuera y finalmente se cubrió con un vidrio de reloj. Además, se realizaron combinaciones de dos defectos sensoriales mezclando 2 aceitunas de cada defecto que contenían 5 mL de salmuera de cada muestra con defecto. Las combinaciones que se realizaron fueron: D1 + D2, D2 + D3 y D1 + D3. Para la muestra con combinación de tres defectos, D1 + D2 + D3 se mezclaron tomando 1 aceituna y 3 mL de salmuera de cada muestra.

2.1.3 Resumen de los resultados y discusión

Las muestras de aceitunas de mesa al "estilo español" evaluadas sensorialmente por el panel de cata del INTAEX, fueron clasificados, según recomendaciones del COI, por la intensidad del DPP obtenido de la evaluación del panel de cata, del mismo modo que ya lo habían hecho con anterioridad otros autores como Marx et al. (2017). Los catadores indicaron que los defectos en las aceitunas sin combinar eran de alta intensidad y se clasificaron como de segunda categoría porque el DPP estaba entre $4,5 < DPP < 7,0$. Estas aceitunas podrían ser legalmente comercializadas a pesar de los defectos significativos. Cuando se mezclaban aceitunas con combinación de defectos, el defecto *pútrido* tuvo el mayor impacto sensorial entre todos los defectos, mientras que el defecto *butírico* tuvo el menor. Las intensidades del DPP de las combinaciones fue $\leq 3,5$, debido al efecto dilución que se produce en las combinaciones. Estas aceitunas son clasificadas como de primera categoría.

En cuanto al análisis por cromatografía de gases, se identificaron un total de 49 compuestos volátiles en las aceitunas con distintos defectos. Cada defecto se caracterizó por la presencia de un perfil de compuestos volátiles específico. Se sabe que los microorganismos juegan un papel importante en la formación del perfil volátil de los alimentos fermentados (Sabatini et al., 2009) y en la caracterización del perfil aromático de las aceitunas de mesa.

Los compuestos volátiles identificados se agruparon según su grupo funcional, resultando ser los fenoles el grupo mayoritario en las aceitunas sanas con un 52%,

mientras que los identificados en las muestras de *zapatería* y *butíricas* son principalmente ácidos carboxílicos (83% y 84%, respectivamente). Por otro lado, las muestras *pútridas* y *mohosas* mostraron un mayor contenido de alcoholes. Con respecto a los compuestos individuales, los principales constituyentes volátiles de la matriz en muestras sanas fueron creosol (40,2%), un derivado monoterpénico (15,3%), 2-etilfenol (8,7 %), alcohol feniletílico (7,8 %), ácido benzoico (7,4 %) y ácido acético (7,4 %). La comparación con los datos de la literatura es difícil debido a la gran variabilidad entre los diferentes estudios. Los COVa son, de hecho, fuertemente influenciados por muchos factores, como la variedad, el estado de maduración o las condiciones de procesamiento (Sabatini, et al., 2009; Dabbou, 2012). Sin embargo, estos compuestos disminuyen considerablemente en aceitunas con defectos, y aparecen otros compuestos diferentes. En el presente trabajo, los principales constituyentes de las muestras con defecto de *zapatería* fueron ácido butanóico (37,9%), ácido (E)-3-hexenoico (17,8%), ácido hexanoico (8,5%) y ácido pentanoico (ácido valérico) (5,4%). Estos resultados están de acuerdo con estudios previos, que asociaron estos ácidos grasos de cadena corta con el deterioro de la *zapatería* (Panagou y Tassou, 2006; De Castro et al., 2018). El ácido ciclohexanocarboxílico sólo se encontró en muestras con defecto *zapatería,* aunque no representa una gran proporción del total de volátiles identificados (3,7%), pero se ha identificado como un compuesto clave en las aceitunas con defecto a *zapatería* (Montaño et al., 1992). Los principales COVs responsables del defecto *butírico* fueron el ácido butanoico (55,5%), ácido pentanoico (18,5%), ácido propanoico (4,9%) y butan-2-ol (7,6 %). Este resultado es comparable con estudios previos (de Castro et al., 2018). Por otro lado, el defecto *pútrido* tiene un perfil volátil completamente diferente de los defectos descritos anteriormente, donde los principales COVs fueron alcohol isopropílico (16,0%), alcohol feniletílico (15,9%), ácido propanoico (15,8%), 2,4-dimetil-heptano (14,5%) y 3-metil-butan-1-ol (9,3%). Existen pocos estudios que describan la composición volátil del defecto *pútrido*. Finalmente, el defecto *mohoso* presentaba un perfil volátil cuyos principales compuestos fueron 2-metoxi-fenol (46,2%), seguido de 2,4-dimetil-heptano (23,6%) y estireno (8,7%). Esta es la primera vez que se identifican los COVs del defecto *mohoso* en este tipo de aceitunas.

Por otra parte, se analizaron los datos de la *nariz electrónica* de muestras de aceitunas de mesa con los diferentes defectos mediante PCA, para obtener una mejor interpretación de las interacciones entre las múltiples variables. Los resultados de PCA mostraron que el 59,8% de la varianza total de los datos eran explicados por PC1 y el 28,2% por PC2. El modelo basado en los dos primeros componentes mostró una clara diferenciación de las muestras según sus características olfativas, siendo capaz de separar las aceitunas sanas de aquellas con defectos del proceso de fermentación. Estos resultados son coincidentes con los obtenidos por el panel de cata.

Tras los buenos resultados obtenidos en el PCA, se realizó un análisis de clasificación usando PLS-DA y validación cruzada ("leave-one-out"). Los resultados se mostraron en una matriz de confusión, donde el porcentaje de predicciones correctas obtuvo un éxito de alrededor el 99%. Estos resultados muestran la capacidad y precisión de la *nariz electrónica* para discriminar entre aceitunas sanas y aceitunas con distintos defectos a *zapatería*, *butírico*, *pútrido* y *mohoso*.

Seguidamente, se analizaron con la *nariz electrónica* las muestras de aceitunas con combinaciones de defectos. A pesar del efecto de dilución resultante de la combinación, los dos primeros componentes principales PC1 y PC2, fueron suficientes para visualizar la agrupación de las diferentes combinaciones, ya que la suma explicó el 76% de la varianza total. Posteriormente, se aplicó PLS-DA para construir un modelo de clasificación y la correspondiente matriz de confusión obteniendo un 99% de predicciones correctas, demostrando que la combinación de más de un defecto y el efecto de dilución de la intensidad del patrón de olor no fue un obstáculo para la *nariz electrónica*; de hecho, siempre se evidenció una clara discriminación entre las muestras. No existe mucha literatura científica sobre la discriminación de aceitunas defectuosas con dispositivos electrónicos. Martín-Vertedor et al. (2021) describieron la predicción del *efecto de cocido* producido por la aplicación de diferentes tratamientos de esterilización en aceitunas negras oxidadas de dos variedades de aceituna utilizando una lengua electrónica, mientras que otros investigadores (Martínez et al., 2020) utilizaron la *nariz electrónica* para detectar defectos en aceites y aceitunas.

2.1.4 Conclusiones

- El panel de cata clasificó las aceitunas con defectos a *zapatería*, *butíricas*, *pútridas*, y *mohoso* en distintas categorías comerciales.

- El análisis cromatográfico caracterizó las muestras sanas y con defecto con un perfil de COVs específico para cada uno de ellos.

- La clasificación realizada con la *nariz electrónica* coincidió con los resultados obtenidos por el panel de cata.

- El dispositivo electrónico también discrimina muestras con diferente intensidad de defectos.

- La *nariz electrónica* resultó ser una herramienta útil para el reconocimiento de las percepciones olfativas derivadas de fermentaciones anómalas que se producen en las aceitunas de mesa y, podría desempeñar en el futuro un papel importante en la clasificación por calidad de las aceitunas de mesa a nivel industrial.

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E-Nose Discrimination of Abnormal Fermentations in Spanish-Style Green Olives

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Abstract: Current legislation in Spain indicates that table olives must be free of off-odors and off-flavors and without symptoms of ongoing alteration or abnormal fermentations. In this regard, the International Olive Council (IOC) has developed a protocol for the sensory classification of table olives according to the intensity of the predominantly perceived defect (PPD). An electronic nose (e-nose) was used to assess the abnormal fermentation defects of Spanish-style table olives that were previously classified by a tasting panel according to the IOC protocol, namely zapateria, butyric, putrid, and musty or humidity. When olives with different defects were mixed, the putrid defect had the greatest sensory impact on the others, while the butyric defect had the least sensory dominance. A total of 49 volatile compounds were identified by gas chromatography, and each defect was characterized by a specific profile. The e-nose data were analyzed using principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA). The different defects were clearly separated from each other and from the control treatment, independently of PPD intensity. Moreover, the e-nose differentiated control olives from table olives with combined sensory defects despite the dilution effect resulting from the combination. These results demonstrate that e-nose can be used as an olfactory sensor for the organoleptic classification of table olives and can successfully support the tasting panel.

Keywords: sensory analysis; volatile compounds; defects; e-nose; table olives

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1. Introduction

Spain is the largest producer of table olives in the world, representing a market that generates annual trade valued at 1.7 billion euros worldwide. Olives produced in Spain are present in almost all countries. Moreover, the export of table olives to other countries, such as Morocco, Egypt, Greece, Turkey, Argentina, Peru, and Portugal, has increased in recent years [1].

To produce table olives, the "Spanish style" is the most common process. The fruits of *Olea europaea* spp. are treated with caustic soda to remove their bitterness and then fermented in a salt solution for several months. However, a number of critical points during the elaboration process can facilitate abnormal fermentation, thereby leading to defective olives. These alterations are among the main causes of economic loss for producers.

The current legislation only considers the physical defects of extra or fancy, first choice or selected olives, and second choice or standard olives [2]. However, the regulation indicates that table olives must be free of strange odors and flavors and free of symptoms of ongoing alteration or abnormal fermentation. Therefore, to classify table olive defects according to sensory analysis, producers should analyze samples with a tasting panel trained and validated by the IOC (2011) [3]. However, the protocol established by the IOC is only a recommendation and is not yet in force. The sensory panel should classify table olives according to olfactory defects, including putrid, zapateria, butyric, musty, rancid, or vinegary sensations [4,5]. These defects perceived by tasters are normally present in table olives, in particular the zapateria defect. According to the IOC regulation, the zapateria defect is caused by a combination of volatile fatty acids formed by abnormal fermentation, leading to the sensation of rotten leather. The butyric defect is the off-flavor of rancid butter or cheese. The putrid defect is the odor of decaying organic matter. Finally, the musty or humidity defect produces a smell of mold. These defects are probably caused by bad industrial practices that facilitate uncontrolled development of the fermentation process.

Analysis of volatile compounds by chromatography may also help to identify compounds responsible for abnormal fermentations [6–9]. However, sensory analyses based on a trained expert panel and characterization of the volatile fraction of fermented olives based on gas chromatography are expensive, laborious, and time-consuming procedures requiring sophisticated equipment and/or skilled personnel. Thus, it is important to develop a fast and reliable technique to discriminate table olives according to their sensory characteristics. The same protocol should also be used to identify incipient defects in olives to control the onset of anomalous fermentations.

The electronic nose (e-nose) is an electromechanical powerful sensory device that enables the discrimination of aroma profiles of different matrices, such as wine [10], edible mushrooms [11], cooked chicken [12], edible oils [13], or fresh vegetables [14]. This device is also used to classify olives on olive trees [9] or even to differentiate table olives elaborated according to the Spanish-style protocol [7]. In this respect, the e-nose may be a fast, cheap, and effective alternative to identify different types of fermentation defects in large amounts of olives on an industrial scale. The e-nose is a nondestructive device complementary to the tasting panel. It can be routinely installed to identify early signs of off-flavors during the fermentation of Spanish-style table olives with the aim of correcting them before the olives become unacceptable and unmarketable.

The aim of the present study was to develop an analytical protocol based on an e-nose device to differentiate Spanish-style defective olives according to their sensory attributes. The data were compared with the profile of volatile compounds determined by gas chromatography and with sensory characterization of the olives carried out by a trained tasting panel.

2. Results and Discussion

2.1. Sensory Profile of Table Olives

The selected Spanish-style table olives were sensorially evaluated by a tasting panel in order to classify them according to the predominantly perceived defect (PPD). It should be noted that the quality standards [2] do not include sensory analysis as an evaluation criterion for classifying table olives into different commercial categories. This regulation only takes into account the physical defects in the fruit, such as softness, skin defects, or broken fruits. Therefore, in this research, the sensory evaluation and classification of the olives were obtained according to the IOC regulation based on the evaluation of PPD intensity by the tasting panel.

Healthy table olives were selected for the control treatment (control). However, several other olives presented various sensory defects related to abnormal fermentation. The main defects found in different samples were zapateria (D1), butyric (D2), putrid (D3), and musty or humidity (D4). Similarly, Marx et al. [15] evaluated several table olive samples

using a tasting panel who obtained a similar classification of the samples according to the sensory defect detected (i.e., butyric, putrid, zapateria, musty, and/or winey–vinegary).

The tasters indicated that defects in the olives were of high intensity. For this reason, the olives were classified in the second or standard category because the PPD was higher than 3.5 and less than or equal to 6.0 (Table 1). Thus, all these olives could be legally marketed despite the significant defects [3].

Table 1. Predominantly perceived sensory defects of Spanish-style table olive and of combined samples.

	Control	**D1**	**D2**	**D3**	**D4**
Sensory Evaluation	n.d.	Zapateria 6.0 ± 0.9	Butyric 5.5 ± 0.7	Putrid 5.8 ± 0.8	Musty 6.0 ± 0.9
	Control	**D1 + D2**	**D2 + D3**	**D1 + D3**	**D1 + D2 + D3**
Sensory Evaluation	n.d.	Zapateria 3.5 ± 0.8	Putrid 3.5 ± 0.9	Putrid 3.5 ± 0.8	Putrid 3.0 ± 0.7

n.d., not detected.

Failure to control the product during the fermentation of Spanish-style olives causes an increase in pH, which can contribute to the development of microorganisms that cause abnormal fermentation due to their ability to metabolize lactic acid.

Combinations of the different olive defects were made to verify what the PPD was in the olives and to determine its intensity through the panel (Table 1). Thus, equally combined mixtures of defective olives were made as follows: zapateria + butyric (D1 + D2), butyric + putrid (D2 + D3), zapateria + putrid (D1 + D3), and zapateria + butyric + putrid (D1 + D2 + D3). As can be seen in Table 1, the intensity of the defect was reduced by almost half when the different combinations were made. Thus, a dilution effect was observed as the intensity of the defect decreased. When the defective olives were mixed, their commercial sensory category according to the IOC regulation improved as it went from the second category ($3.5 < \text{PPD} \leq 6.0$) to the first category ($2 < \text{PPD} \leq 3.5$). Mixing olives with different defects in the same package is a commercial strategy that allows companies to reduce the waste of defective olives. This practice is fully legal and allowed as long as the percentage of physical defects complies with the legislation.

When olives with different defects were mixed, one of the defects prevailed over the others (dominance effect). In fact, the putrid defect had the greatest sensory impact on the others, while the butyric defect had the least sensory dominance. Zapateria and musty defects presented an intermediate dominance. This result has interesting consequences for table olives producers. In the case of table olives with some defects developed in the fermentation tanks, an appropriate mixing of the olives according to the dominance of their defect can be useful to market olives of better quality that comply with the current legislation.

2.2. Volatile Compounds of the Pure Defects

Aroma is considered a quality index for olive products [16]. It is known that microorganisms play an important role in the formation of the volatile profile of fermented foods [16] and therefore play a decisive role in the characterization of the flavor profile of table olives.

The volatile compounds were analyzed in the five types of table olives (control, zapateria, putrid, butyric, and musty). The identified volatile compounds listed according to chemical group, odor attributes, and relative content in percentage of intact (control) and defective olives are shown in Table 2.

Table 2. Relative contents of volatile compounds (mean % (n = 3)) obtained from table olives with zapateria, butyric, putrid, and musty defect compared to healthy olives (control). RT, retention time.

	RT (min)		Content (% of Total Area of Identified Compounds)				
		Attributes	**Control**	**Zapateria**	**Butyric**	**Putrid**	**Musty**
Carboxylic Acids							
Acetic acid	2.7	Pungent, sour	7.4 ± 0.7	2.9 ± 0.9	2.5 ± 0.7	n.d.	n.d.
Propanoic acid	4.9	Rancid, cheesy	n.d.	4.0 ± 0.5	4.9 ± 0.4	15.8 ± 2.5	0.5 ± 0.1
Butanoic acid	8.2	cheesy	n.d.	37.9 ± 4.4	55.5 ± 4.4	n.d.	1.1 ± 0.1
Pentanoic acid	13.5	Pungent, rancid	n.d.	5.4 ± 1.1	18.5 ± 1.7	n.d.	n.d.
Hexanoic acid	18.5	Pungent, rancid	n.d.	8.5 ± 0.9	n.d.	n.d.	n.d.
(E)-3-Hexenoic acid	20.7	Cheesy, green, dairy	n.d.	17.8 ± 2.3	n.d.	n.d.	n.d.
Cyclohexanecarboxylic acid	26.5	Fruity, woody	n.d.	3.7 ± 0.6	n.d.	n.d.	n.d.
Benzoic acid	28.2	Pungent, sour	7.4 ± 0.7	2.9 ± 0.9	2.5 ± 0.7	n.d.	n.d.
Alcohols							
Isopropyl alcohol	1.8	Pungent solvent	n.d.	n.d.	n.d.	16.0 ± 2.8	n.d.
Butan-2-ol	2.4	Winey	n.d.	n.d.	7.6 ± 0.9	n.d.	n.d.
Butan-1-ol	3.1	Fusel, oily	n.d.	n.d.	n.d.	n.d.	2.6 ± 1.0
3-methyl-butan-1-ol	4.7	Woody, whiskey, sweet	n.d.	n.d.	n.d.	9.3 ± 2.1	1.1 ± 0.1
2-methyl-butan-1-ol	4.8	Winey, spicy	n.d.	n.d.	n.d.	5.3 ± 1.6	0.8 ± 0.1
(Z)-3-Hexen-1-ol	9.7	Green, leaf, nuts	0.6 ± 0.1	n.d.	n.d.	n.d.	n.d.
Cyclohexanol	11.3	Camphoreous	n.d.	n.d.	n.d.	0.7 ± 0.1	n.d.
Benzyl alcohol	19.5	Floral, fruity	2.3 ± 0.1	n.d.	n.d.	0.8 ± 0.1	1.0 ± 0.1
Octan-1-ol	21.1	Waxy, green	0.8 ± 0.1	n.d.	n.d.	n.d.	4.6 ± 1
Phenylethyl Alcohol	23.3	Mild rose	7.8 ± 1.0	n.d.	0.9 ± 0.1	15.9 ± 0.6	1.9 ± 0.2
Phenols							
Phenol	17.7	Phenolic, plastic	n.d.	n.d.	n.d.	1.7 ± 0.3	0.6 ± 0.1
2-methoxy-phenol	21.9	Smoky, woody, phenolic	3.2 ± 0.3	1.3 ± 0.1	n.d.	3.3 ± 0.4	46.2 ± 6.5
4-ethyl-phenol	26.7	Wet horse, Phenolic	8.7 ± 1.3	n.d.	n.d.	n.d.	n.d.
Creosol	27.0	Spicy	40.2 ± 4.1	4.9 ± 0.2	3.6 ± 0.1	5.8 ± 0.2	0.7 ± 0.1
2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl) phenol	38.0	n.d.	n.d.	3.6 ± 0.4	n.d.	n.d.	n.d.

Table 2. *Cont.*

	RT (min)		Content (% of Total Area of Identified Compounds)				
		Attributes	**Control**	**Zapateria**	**Butyric**	**Putrid**	**Musty**
Aldehydes							
Octanal	17.3	Fatty, sharp	0.50 ± 0.02	n.d.	n.d.	0.30 ± 0.01	0.40 ± 0.01
Esters							
Propyl propionate	7.3	Fruity, berry	0.60 ± 0.01	n.d.	n.d.	n.d.	n.d.
Methyl pentanoate	7.9	Fruity, sweet	n.d.	1.5 ± 0.1	1.4 ± 0.1	n.d.	n.d.
3-Methylbutyl acetate	10.6	Fruity, sweet	0.40 ± 0.01	n.d.	n.d.	1.3 ± 0.1	n.d.
Ethyl pentanoate	11.8	Fruity, fresh	n.d.	1.0 ± 0.1	0.1 ± 0.1	n.d.	n.d.
Methyl hexanoate	13.1	Fruity, pineapple	n.d.	1.3 ± 0.1	1.1 ± 0.1	n.d.	n.d.
3-Methylbutyl propanoate	15.5	Fruity, apricot	n.d.	n.d.	n.d.	0.50 ± 0.01	n.d.
Propyl pentanoate	17.0	Ethereal, fruity	n.d.	0.7 ± 0.1	0.40 ± 0.01	n.d.	n.d.
Ethyl hexanoate	17.1	Sweet, fruity	n.d.	1.0 ± 0.1	0.10 ± 0.01	n.d.	n.d.
4-Hexen-1-ol, acetate	17.4	Fruity	1.2 ± 0.1	n.d.	n.d.	n.d.	n.d.
Propyl hexanoate	22.1	Berry, fruit	n.d.	n.d.	0.20 ± 0.01	n.d.	n.d.
Methyl benzoate	22.2	Herb, lettuce	n.d.	2.3 ± 0.3	n.d.	n.d.	n.d.
Ethyl cyclohexanecarboxilate	24.0	Aromatic, fruity	n.d.	n.d.	n.d.	0.8 ± 0.1	n.d.
n-Propyl benzoate	30.7	Fruity	n.d.	n.d.	n.d.	0.9 ± 0.2	n.d.
Ketones							
6-methyl-5-hepten-2-one	16.3	Fruity, pungent, green	0.60 ± 0.01	n.d.	n.d.	n.d.	n.d.
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl) (damascenone)	35.5	Floral	0.40 ± 0.01	n.d.	n.d.	n.d.	n.d.

Table 2. *Cont.*

RT (min)			Content (% of Total Area of Identified Compounds)				
		Attributes	**Control**	**Zapateria**	**Butyric**	**Putrid**	**Musty**
Other Compounds							
2,4-dimethyl-heptane	6.7	Unpleasant odor of plastic	1.1 ± 0.1	n.d.	n.d.	14.5 ± 2.6	23.6 ± 3.6
Styrene	11.2	Floral, sweet	0.6 ± 0.1	n.d.	n.d.	n.d.	8.7 ± 1.5
1-chlorooctane	20.3	n.d.	n.d.	n.d.	n.d.	n.d.	2.2 ± 0.1
3-ethyl-4-methyl-pyridine	21.6	Sharp, penetrating, strong aromatic odor	n.d.	n.d.	n.d.	2.1 ± 0.4	n.d.
2-Ethenyl-1,1-dimethyl-3-methylene-cyclohexane	23.0	n.d.	15.3 ± 1.0	n.d.	n.d.	n.d.	n.d.
1,2-dimethoxybenzene	24.8	Vanilla	n.d.	n.d.	n.d.	n.d.	2.0 ± 0.2
3,4-dimethoxytoluene	29.2	n.d.	n.d.	n.d.	0.3 ± 0.1	2.1 ± 0.2	n.d.
Copaene	35.2	Woody, spicy	1.1 ± 0.2	n.d.	0.5 ± 0.1	0.8 ± 0.1	0.4 ± 0.1
α-Muurolene	40.4	Woody	n.d.	n.d.	0.10 ± 0.01	0.10 ± 0.01	n.d.
α-Farnesene	40.7	Soft cooking of vegetables, woody	0.1 ± 0.1	n.d.	n.d.	1.7 ± 0.4	1.5 ± 0.2
sum			100.3	100.7	100.2	99.7	99.9

n.d., less than 0.1%.

A total of 49 volatile compounds were identified. Among them, 20 compounds were found in healthy olives, 17 in zapateria and butyric samples, 20 in putrid samples, and 18 in musty samples.

Figure 1 shows the composition of the five types of samples for each chemical class.

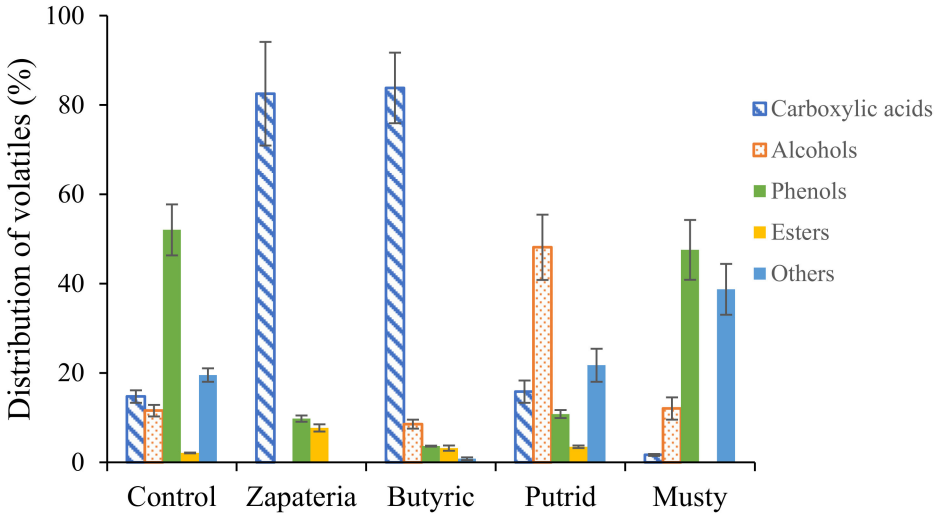

Figure 1. Distribution of chemical families of volatile compounds in healthy and defective olives.

The compounds isolated and identified in healthy control olives were mainly phenols (52%), while those identified in zapateria and butyric samples were mainly carboxylic acids (83% and 84%, respectively). On the other hand, putrid and musty samples showed a higher content of alcohols and other compounds, respectively, with respect to the healthy olives.

Regarding the individual compounds (Table 2), the major constituents of the volatile matrix in healthy samples were creosol (40.2%), a monoterpene derivative (15.3%), 2-ethyl-phenol (8.7%), phenylethyl alcohol (7.8%), benzoic acid (7.4%), and acetic acid (7.4%). Comparison with literature data on the volatile composition of olive fruits is difficult because of the great variability among different studies. Volatile compounds are, in fact, strongly influenced by many factors, such as variety, ripening state, or processing conditions [16–18]. However, these compounds decrease considerably in olives with defects, and other different compounds appear. In the present work, the main constituents of zapateria samples were butanoic acid (37.9%), (E)-3-hexenoic acid (17.8%), hexanoic acid (8.5%), and pentanoic acid (valeric acid) (5.4%). These results are in agreement with previous studies, which associated these short chain fatty acids with zapateria spoilage [8,19,20]. Cyclohexanecarboxylic acid was found only in samples with this defect. It did not represent a large proportion of the total identified volatile compounds (3.7%), but it has been identified as a key compound of zapateria samples in previous studies [19]. In other studies, it has been reported that cyclohexanecarboxylic acid, in combination with other volatile acids, appears to be responsible for the unpleasant smell typical of zapateria olives [21].

The major volatile compounds responsible for the butyric defect were butanoic acid (55.5%), pentanoic acid (18.5%), propanoic acid (4.9%), and butan-2-ol (7.6%). This result is in agreement with previous studies [20].

On the other hand, the putrid defect has a completely different volatile profile from the defects described above, as shown in Figure 1. In this case, the major volatile compounds were isopropyl alcohol (16.0%), phenylethyl alcohol (15.9%), propanoic acid (15.8%), 2,4-dimethyl-heptane (14.5%), and 3-methyl-butan-1-ol (9.3%). As far as we know, there are only very few studies describing the volatile composition of the putrid defect.

Finally, the defect indicated as musty had a volatile profile similar to the putrid defect. The major volatile compounds present in the olives affected by the musty defect were 2-methoxy-phenol (46.2%), followed by 2,4-dimethyl-heptane (23.6%) and styrene

(8.7%). This is the first time that the volatile compounds of the musty defect were isolated and identified.

2.3. Discrimination of Table Olive Defects with the E-Nose

A multisensory system (e-nose) was used to classify table olives according to the sensory defect. The volatile compounds emanated from the samples were put in contact with the sensor array. The response of each sensor was a different instrumental signal with an amplitude that depended on its interaction with the sample. A radial graph (Figure 2) was drawn to show the different amplitude of the responses for the 11 sensors of the e-nose.

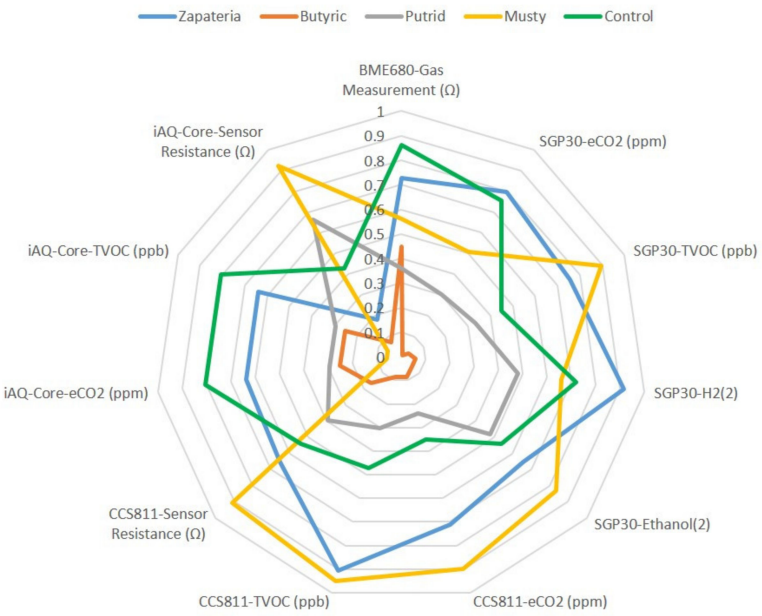

Figure 2. Radial plots for the responses of the sensor array to the control and defective table olives.

For the representation of the radial graph, the extracted features of each sensor were normalized using the criteria established by [7] to have all the data on the same magnitude scale. The radial profile for each sample was different depending on the type of alteration of the olives. The set of sensors gave different signals for the five groups of samples, suggesting that they all contributed to odor discrimination. The complexity of the output data necessitated the use of multivariate analysis methods, such as PCA, as discussed in the next sections.

2.4. Discrimination of Isolated Defects

The e-nose data of table olive samples with different defects were first analyzed by principal component analysis (PCA) (Figure 3) to obtain a better visualization of the interactions between the variables and grouping of the samples.

PCA is a well-known pattern-recognition technique, which returns results as a projection of the data into a reduced hyperspace defined by the principal components [22]. Principal components are linear combinations of the original variables, where the first principal component represents the largest variance, the second principal component accounts for the second largest variance, and so on.

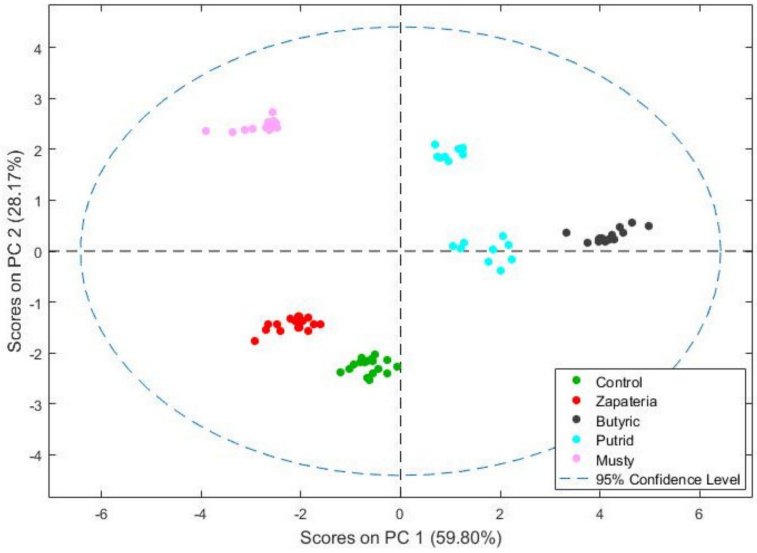

Figure 3. Score plot of the PCA analysis for healthy olives (control) and olives with off-odor of zapateria, butyric, putrid, and musty.

The PCA results showed that 59.8% of the total variance of data was explained by PC1 and 28.2% by PC2. The model based on the first two components showed a clear differentiation of the samples according to their olfactory characteristics and was able to separate healthy olives from those with defect in the fermentation process.

The PCA of the data showed that the e-nose response well fitted with the sensory analysis performed by the trained panel.

After the good results obtained in the PCA, a classification analysis was performed using PLS-DA and leave-one-out cross-validation. The results are shown in Table 3 as a confusion matrix. The sum of the diagonal elements of the confusion matrix gives the percentages of correct predictions. As can be seen, about 99% of correct predictions was obtained.

Table 3. Confusion matrix obtained through PLS-DA for discrimination between control (healthy olives) and isolated defects. Values are expressed in percentage.

	Predicted Class				
Real Class	**Control**	**Zapateria**	**Butyric**	**Putrid**	**Musty**
Control	20	0	0	0	0
Zapateria	0	20	0	0	0
Butyric	0	0	20	0	0
Putrid	0	0	0	19	0
Musty	0	0	0	1	20

These results show the ability and accuracy of e-nose to discriminate between different defects (zapateria, butyric, putrid, and musty) and compare them with the control treatment (healthy olives). Thus, the e-nose is able to discriminate olives according to their quality at an industrial level. This tool can be used to control the fermentation process of olives to ensure their quality.

2.5. Discrimination of Combined Defects

The response of the e-nose to odor patterns resulting from combinations of different fermentation defects was also studied. As in the previous case, the data were first analyzed using PCA. The score plot of the two first principal components for the discrimination of samples with combinations of defects is shown in Figure 4.

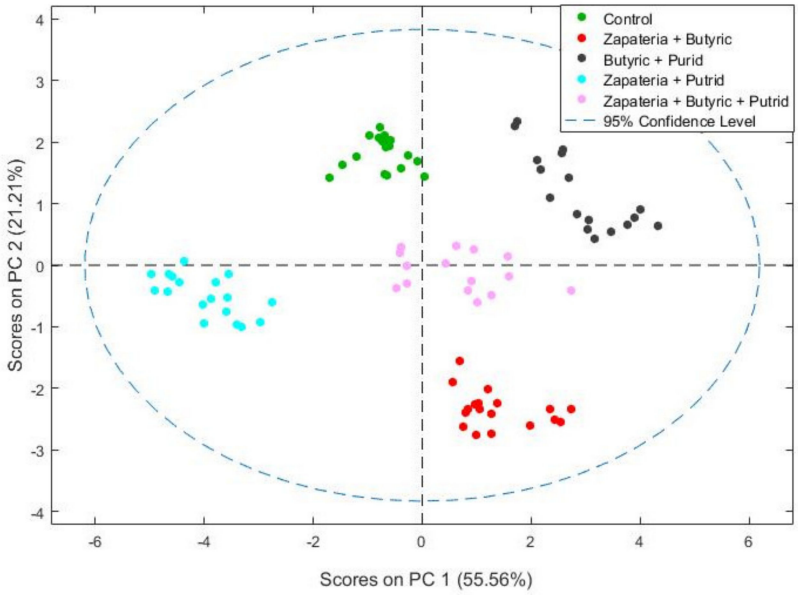

Figure 4. Score plot of the PCA for healthy olives (control) and combinations of zapateria, butyric, and putrid off-odors.

PCA based on e-nose data differentiated control olives from table olives with combined sensory defects despite the dilution effect resulting from the combination. The first and second principal components (PC1 and PC2) were sufficient to visualize the data structure as they explained 76% of the total variance.

Subsequently, PLS-DA was applied to construct a classification model and the corresponding confusion matrix (leave-one-out cross-validation), the results of which are shown in Table 4.

Table 4. Confusion matrix obtained through PLS-DA for discrimination between control (healthy olives) and combined defects. Values are expressed in percentage.

	Predicted Class				
Real Class	**Control**	**Zapateria + Butyric**	**Butyric + Putrid**	**Zapateria + Putrid**	**Zapateria + Butyric + Putrid**
Control	20	0	0	0	0
Zapateria + Butyric	0	20	0	0	0
Butyric + Putrid	0	0	20	1	0
Zapateria + Putrid	0	0	0	19	0
Zapateria + Butyric + Putrid	0	0	0	0	20

The results obtained (99% of correct predictions) showed that the combination of more than one defect and the dilution effect of the intensity of the odor pattern was not an obstacle for the e-nose; in fact, a clear discrimination was always evidenced. Furthermore, the e-nose also showed a clear differentiation between the control and combined defects, whose values of PPD are shown in Table 1 (control: PPD = n.d. (extra category) and defect samples that correspond to the first category ($3 < PPD \leq 4.5$)). To the best of our knowledge, there is not much literature on the discrimination of defective olives with electronic devices. However, a study [23] described the prediction of the cooked defect produced by the application of different sterilization treatments in oxidized black olives from two olive

varieties using an electronic tongue. Therefore, these results highlight the feasibility of these devices as rapid analytical tools to monitor the processing of table olives.

3. Materials and Methods

3.1. Table Olives Elaboration

Olives of the "Carrasqueña" variety were harvested at the green stage of ripeness within the limits of the "Tierra de Barros" olive-growing area (Badajoz, Spain) during the 2019/2020 campaign and were processed according to the Spanish-style protocol [24] by a company located in the southwest of Extremadura (Spain). The product was introduced into fermenters with the capacity of 236 L in three replications. During the fermentation process, aliquots of olives were sampled and a sensory analysis was carried out by a trained tasting panel. After completion of the fermentation process, the olives were covered with brine (4% *w/v* NaCl) and sealed in cans (150 g each can). Each week, the cans of olives were taken and a sensory analysis was performed by the same tasting panel with the aim of identifying Spanish-style table olives with abnormal fermentation defects (D1, D2, D3, and D4). When the olives showed the desired defect, they were kept in a refrigerator (4 °C) until the analysis was carried out. A control sample without fermentation defects was also stored (control).

The experiments were carried out in a standard glass jar containing as many olives as the bottom of the glass could hold and arranged in a single layer. Then, 10 mL of covering liquid was added on top of the olives following the IOC rules. In addition, to obtain the combinations of defects, samples were mixed proportionally. D1 + D2, D2 + D3, and D1 + D3 were mixed in a 50:50 ratio by mixing 2 olives and 5 mL of saline solution from each sample. For the combination of three defects, D1 + D2 + D3 were also mixed equally by taking 1 olive and 3 mL of each sample.

3.2. Analyses

The table olives were subjected to sensory analysis, characterization of volatile compounds, and e-nose measurements as detailed below.

3.2.1. Sensory Analysis

Table olives were evaluated by a sensory panel composed of eight experts from the CICYTEX Research Center (Extremadura, Spain) who were trained according to the IOC recommendations [3]. For this study, the intensity and type of the off-odor perceived by the taster was assessed on a structured scale from 0 to 10. The results were expressed as median values of defects; values were considered valid when the coefficient of variation was less than 20. Finally, table olives were classified according to the quality categories established by the IOC [3].

One-way ANOVA was performed followed by Tukey's multiple range test to establish statistically significant differences between the different samples. Significance was set at $p < 0.05$. SPSS 18.0 software was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Data were expressed as mean and standard deviations (SD).

3.2.2. Analysis of Volatile Compounds

The volatile compounds were analyzed in triplicate with a Bruker Scion 456-GC triple quadrupole gas chromatograph. Pitted olives were crushed and homogenized following the procedure reported in [25]. A 2 g aliquot was mixed with 7 mL of a 30% NaCl solution in a 15 mL glass vial. Volatile components were sampled from the headspace at 40 °C for 15 min using SPME with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex fiber (65 µm, Supelco). After SPME, desorption was carried out at the injection port of the gas chromatograph at 250 °C for 15 min. The components were separated using a VF-5MS capillary column (30 m × 0.25 mm; ID: 0.25 mm). The tentative identification of the analytes was based on comparison of mass fragmentations with the NIST 2.0 MS library.

3.2.3. E-Nose Analysis

The e-nose equipment was a portable miniaturized device designed by the University of Extremadura (Spain) [26]. This prototype consisted of four digital gas sensor chips with integrated metal oxide (MOX) sensors: BME680 from Bosch, SGP30 from Sensirion, and CCS811 and iAQ-Core from ScioSense. The microprocessor read the values detected by the sensors, formatted them, and sent them to an external smart device via Bluetooth. The resulting data were then passed to a computer.

E-nose measurements were performed following IOC recommendations. Specifically, 10 mL of brine containing four olives was introduced into standard tasting glasses, covered with a watch glass, and placed on a block thermostatted at 25 °C. Another standard tasting glass was left without samples to serve as a baseline reference. Five measurements were taken for each table olive sample, and each data acquisition cycle consisted of two parts. First, the e-nose was placed on the sample glass for 60 s and the sensor signals were recorded. Then, the e-nose was moved to the glass without sample to perform desorption with free air for 30 s to bring the gas sensor signal back to the baseline.

Each sensor response curve consisted of N points corresponding to the sensor measurements with time. The features used to characterize the sensor response curves were the maximum signal value minus the minimum signal value multiplied by 100 and subtracted by 1 ((MAX-MIN)$\times$100 $-$ 1). As a result, a data vector with 11 rows (sensors) for each sample was obtained.

3.2.4. Multivariate Data Analysis

The e-nose data consisted of a matrix of 100 rows (10 measurements for each duplicate sample) and 11 columns (sensors). The data were first subjected to principal component analysis (PCA) to perform an exploratory analysis. Subsequently, PLS-DA was applied to build the classification model. As the variables were measured in different units, the original variables were autoscaled. Data analysis was performed using Matlab R2016b version 9.1 (The Mathworks Inc., Natick, MA, USA) with PLS_Toolbox 8.2.1 (Eigenvector Research Inc., Wenatchee, WA, USA).

4. Conclusions

The e-nose proved to be a useful tool for recognizing olfactory sensations derived from abnormal fermentations occurring in table olives, such as zapateria, butyric, putrid, and musty defects. The classification made with the e-nose coincided with the results obtained by the tasting panel. When combined with chemometric tools (PCA and PLS-DA), e-nose provides a rapid and inexpensive method to monitor the occurrence of abnormal fermentations during the processing of table olives. Therefore, the e-nose can play a role in the table olive industry in the future for the rapid in-house classification of commercial table olives according to the criteria set by the IOC or when a tasting panel is not available. In addition, the panel leader can use the e-nose as a support to discriminate samples with different intensity of the predominant defect in order to produce table olives compositions of better quality that comply with current market legislation.

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Sánchez, R., Pérez-Nevado, F., Montero-Fernández, I., Lozano, J., Meléndez, F., Martín-Vertedor, D.

2.2.1 Motivación y objetivos

Durante el proceso de elaboración de aceitunas de mesa al "estilo español", las aceitunas se tratan con hidróxido sódico (NaOH) para eliminar el amargor. Posteriormente, se lavan con agua para eliminar la mayor cantidad posible de NaOH. Una vez terminado el lavado y retirada el agua empleada, las aceitunas se ponen en salmuera de 10-12% donde permanecerán durante la fermentación y posterior conservación.

La fermentación de los frutos es realizada principalmente por bacterias lácticas (IOC, 2020), durante la cual, algunas levaduras también pueden desarrollarse, teniendo efectos positivos en la calidad del producto final, como la producción de compuestos volátiles y metabolitos deseables que mejoran las características organolépticas (Hernández et al., 2007; Nisiotou et al., 2009; Hurtado et al., 2012; Zago et al., 2013; Schaide et al., 2019). Sin embargo, también pueden aparecer defectos organolépticos asociados al crecimiento de hongos cuando las condiciones del medio sean favorables (de Castro et al., 2022), contaminándose las aceitunas, pudiendo incluso producir micotoxinas dañinas en el caso de las especies de *Aspergillus* y *Penicilium* (Ghitakou et al., 2006). En otros casos, la alteración se produce porque las esporas llegan a las salmueras y provocan el desarrollo de hongos durante el almacenamiento de las aceitunas, afectando su apariencia, sabor, gusto y textura (Franzetti et al., 2011). Por todo esto, durante la elaboración de las aceitunas de mesa se debe realizar una correcta manipulación, control de la temperatura de almacenamiento, salinidad y proceso de envasado, para evitar el desarrollo de cepas de hongos y/o micotoxinas (Bavaro et al., 2017).

En las aceitunas de mesa pueden aparecer diferentes tipos de defectos por fermentaciones no deseadas, definidos por el COI como defectos a *pútrido*, *zapatería*, *butíricas*, *mohosas*, *rancias* o *avinagradas*. Según el defecto predominante percibido (DPP) por un catador, las aceitunas pueden clasificarse sensorialmente en diferentes categorías (IOC, 2021). Por lo tanto, es conveniente realizar un control de la fermentación de las aceitunas de mesa, para evitar alteraciones que puedan provocar alteraciones y el rechazo del producto.

Las técnicas analíticas actuales para la detección temprana de defectos durante el proceso de fermentación son complejas, lentas y costosas (Seesaerd et al., 2022). Entre estas técnicas están los análisis microbiológicos, la cromatografía de gases o el análisis sensorial realizado por un panel de cata. Sin embargo, existen otras técnicas como la *nariz electrónica*, que han demostrado ser una potente herramienta para detectar defectos olfativos en matrices alimentarias (Duan et al., 2021). Autores como Sánchez et al. (2021a) utilizaron la *nariz electrónica* para discriminar aceitunas elaboradas al "estilo español" que habían sufrido fermentaciones anormales.

Por lo tanto, el objetivo de este trabajo fue, evaluar la capacidad de la *nariz electrónica* para discriminar entre salmueras sintéticas alteradas con diferentes cepas de mohos, mediante la evaluación olfativa del perfil de COVs que emanan al espacio de cabeza de las muestras.

2.2.2 Diseño experimental

La salmuera sintética se preparó utilizando NaCl al 4,0%, 0.05% de glucosa, 0,05 de extracto de levadura y ajustando el pH a 4,5 con ácido láctico. Para el crecimiento y aislamiento de los mohos alterantes se utilizó agar. Una vez preparada, se dosificaron 40 mL de esta salmuera en tubos para su esterilización en autoclave a 121ºC durante 15 min. Después de esterilizada, se inocularon salmueras con nueve cepas de mohos: una *Galactomyces geotricum* (G.G.2), tres cepas de *Penicillium expansum* (P.E.3, P.E.4, P.E.20), una de *Penicillium glabrum* (P.G.19), tres de *Aspergillus flavus* (A.F.9, A.F.18, A.F.21) y una cepa de *Fusarium solani* (F.S.11). Se dejaron salmueras testigo sin inocular. Tras la inoculación, las salmueras sintéticas se incubaron durante 15 días a 25ºC en un agitador orbital a 100 rpm. Al final de la incubación se realizaron análisis de compuestos volátiles, análisis sensoriales y medidas de espacio de cabeza de las muestras usando la *nariz electrónica*.

2.2.3 Resumen de los resultados y discusión

El perfil sensorial de las salmueras varió en función del tipo de microorganismo inoculado. Las muestras de salmuera sin inocular no presentaron atributos negativos, pero sí positivos posiblemente relacionados con las levaduras, con aromas a almendra u olor dulce. Aunque a bajas intensidades, estos atributos positivos también se encontraron en las salmueras inoculadas por mohos. La disminución de su concentración en salmueras con mohos puede deberse al metabolismo microbiano causado durante su desarrollo. Las salmueras sintéticas inoculadas mostraron claros defectos sensoriales relacionados con alteraciones microbianas. Los atributos a *cuero* y *rancio*, fueron los principales defectos encontrados en las salmueras inoculadas, con valoraciones más altas en A.F.9 y A.F.18 para ambos atributos. Otros atributos negativos presentados en menor cantidad por los microorganismos alteradores fueron el olor *metálico* y *químico* de las cepas P.E.3, P.E.4 y P.E.20. Así, los mayores defectos sensoriales negativos se atribuyeron a las salmueras sintéticas inoculadas con G.G.2, P.E.3, A.F.18, siendo clasificadas en la primera categoría, con una puntuación del DPP de 4 ($3{,}0 < DPP \leq 4{,}5$). Finalmente, A.F.21, F.S.11 y P.G.19 fueron los únicos mohos que se clasificaron en la categoría extra ($DPP \leq 3$) aunque presentaron defectos sensoriales a bajas concentraciones. Todas las salmueras sintéticas inoculadas presentan defectos sensoriales con DPP inferiores a 7 y por tanto, no rechazables sensorialmente.

El perfil de compuestos volátiles, obtenidos por cromatografía, de las salmueras inoculadas dependió del tipo de moho inoculado. Los volátiles se clasificaron en dos grupos: olores positivos y olores negativos. La salmuera control no presentó volátiles relacionados con olores desagradables, como sí ocurrió en las salmueras inoculadas con mohos. Los microorganismos que contribuyeron a una peor intensidad de olor desagradable en la solución de salmuera fueron G.G.2, P.E.3, A.F.9 y A.F.18, mientras que los microorganismos con menor aporte de olor negativo a las salmueras fueron P.E.20, A.F.21, F.S.11 y P.G.19. Estos resultados son coincidentes con los obtenidos del análisis sensorial del panel de cata.

De los COVs encontrados en la salmuera control, el nonanal (44,5%) y el 2-4-dimetil-benzaldehído (33,4%) fueron los principales. El que más contribuye al olor desagradable en salmuera sintética inoculada con G.G.2 y P.E.3 fue el dodecanal con concentraciones de 43,3% y 22,4%, respectivamente. En las salmueras inoculadas con A.F.18 y A.F.9, los compuestos que más contribuyeron al olor desagradable fueron los ácidos carboxílicos, butanoico y propanoico. Los porcentajes encontrados para el ácido butanoico en A.F.18 del 12,3% y para el A.F.9. del 12,4%. Sin embargo, para el ácido propanoico se encontraron una concentración del 10,2% en A.F.18 y 10,5% en el A.F.9. Otro compuesto desagradable sintetizado durante las fermentaciones de los mohos fue el 2-metoxifenol, en mayor concentración en G.G.2 (4,9%) y en las cepas de *Aspergillus* A.F.9 y A.F.8 (3,2%).

Los resultados del modelo PCA de la *nariz electrónica* mostraron una clara diferenciación de la salmuera control y las muestras de salmuera inoculadas con el 95,3% de la varianza total. Seguidamente, con el análisis de clasificación PLS-DA, se obtuvo la matriz de confusión de estas muestras con una tasa de éxito de clasificación del 93,5%. Estos resultados demuestran la capacidad y precisión de la *nariz electrónica* para discriminar entre diferentes salmueras con alteraciones causadas por diferentes cepas de moho y salmuera control sin alteración.

En este estudio se utilizó la *nariz electrónica* para evaluar su capacidad para discriminar muestras de salmuera inoculadas con varias cepas del mismo moho, tres cepas del *A. flavus* y otras tres cepas del *P. expansum*. Con sólo dos componentes principales se pudo visualizar la agrupación de datos en los dos gráficos y, explicaron el 82,2% de la variación total de los mohos de las cepas de *A. flavus* y el 97,4% de los mohos de las cepas de *P. expansum*. Las matrices de confusión obtenidas a partir de aplicar el PLS-DA mostraron una tasa de aciertos del 78,0% para las cepas de *A. flavus* y una tasa de aciertos del 93,0% para las cepas de *P. expansum*.

Por tanto, la *nariz electrónica* fue capaz de discriminar muestras de salmuera inoculadas con grupos de cepas de *A. flavus* y *P. expansum*. Existen pocas referencias sobre la discriminación de aceitunas alteradas por moho utilizando la *nariz electrónica*. Sánchez et al. (2021a) discriminaron los defectos de la aceituna de mesa producidos

en fermentaciones anormales como son los defectos de *zapatería*, *butírico*, *pútrido* y *moho*. Hay estudios que demostraron la utilidad de la *nariz electrónica* para la detección e identificación de especies mohosas (Mota et al., 2021); y detección de hongos (Loulier et al., 2020).

2.2.4 Conclusiones

- Las cepas de mohos inoculadas en las salmueras sintéticas provocaron defectos sensoriales negativos a *moho*, *rancio* y *cuero*, que los clasificaron en la primera categoría sensorial.

- Las cepas de mohos que más efectos olfativos desagradables causaron en las salmueras sintéticas fueron las G.G.2, P.E.3, A.F.9 y A.F.18.

- Los principales compuestos volátiles de olor desagradable responsables de las alteraciones por cepas de mohos de G.G.2 y P.E.3 fue el dodecanal y de las cepas A.F.18 y A.F.9 los ácidos carboxílicos, butanoico y propanoico.

- La *nariz electrónica* combinado con herramientas quimiométricas es capaz de discriminar muestras de salmuera con alteraciones incipientes de mohos, confirmando su idoneidad para distinguir alteraciones durante el procesado industrial.

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Application of Electronic Nose to Discriminate Species of Mold Strains in Synthetic Brines

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The chemical composition of the brine for Spanish-style table olives plays a crucial role during the fermentation process. Traditional laboratory analysis requires a high consumption of reagents, highly qualified personnel, sophisticated equipment, long analysis times, and large amounts of samples. Analysis carried out using an electronic nose (E-nose) offers an alternative, non-destructive technique and is useful in determining alterations in brines caused by microorganisms. In the present research, nine mold strains isolated from spoiled olives were inoculated in synthetic brines to determine the effect of microbial development on sensory quality, volatile profile, and the capacity of E-nose to discriminate altered brines from the healthy ones. The brines inoculated with the mold strains presented negative attributes related to aromas of mold, wood, leather, rancidity and, organic solvents among others. The highest intensity of defect was presented by the brines inoculated with the strains *Galactomyces geotricum* (G.G.2); three *Penicillium expansum* (P.E.3, P.E.4, and P.E.20); one *Penicillium glabrum* (P.G.19); three *Aspergillus flavus* (A.F.9, A.F.18, and A.F.21); and one *Fusarium solani* (F.S.11). A total of 19 volatile compounds were identified by gas chromatography. Sensory analysis allowed us to classify the synthetic brines based on the degree of alteration produced by the mold strains used. Also, the E-nose data were able to discriminate the inoculated brines regardless of the intensity of the defect. These results demonstrate the capacity of the E-nose to discriminate alterations in brines produced by molds, thereby making it a useful tool to be applied during the elaboration process to detect early alterations in table olive fermentation.

Keywords: altering microorganisms, Spanish-style table olives, sensory quality, inoculation, volatile compounds

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INTRODUCTION

Spanish-style table olives are consumed in many countries. During their elaboration process, olives are first treated with alkali, and then passed through a stage of washing and fermentation in brine that gives them unique, organoleptic characteristics. However, this process of fermentation and its subsequent conservation can bring about deterioration defects associated with abnormal

fermentation or environmental conditions (de Castro et al., 2022). The olive fermentation is mainly carried out by lactic acid bacteria (IOC, 2020); however, some yeasts have positive effects, producing volatile compounds and desirable metabolites that improve the sensorial characteristics (Hernández et al., 2007; Nisiotou et al., 2009; Arroyo-López et al., 2012; Hurtado et al., 2012; Zago et al., 2013; Schaide et al., 2019).

On the other hand, the fermentation process can cause the growth of filamentous fungi whenever environmental conditions are favorable, contaminating the olives and producing harmful mycotoxins such as those derived from *Aspergillus* and *Penicilium* species (Ghitakou et al., 2006). In other cases, the alteration occurs because the spores reach the brines and cause fungal growth to develop during the storage of the olives, affecting their appearance, flavor, taste, and texture (Franzetti et al., 2011). For this reason, process control precautions, such as good handling, storage temperature, salinity, and packaging processes should be recommended to avoid the development of fungi and mycotoxin throughout the entire table olive process (Bavaro et al., 2017). In fact, the regulation of International Olive Council (IOC) (2021) classified table olives according to sensory evaluation. Different types of spoilage may appear in table olives, such as putrid, zapateria, butyric, musty, rancid, or vinegary sensations (Lanza and Amoruso, 2020; Martín-Vertedor et al., 2021). Therefore, uncontrolled industrial practices could provoke the development of certain table olives alterations.

The early detection of defects during the fermentation process currently requires complex and/or time-consuming analytical techniques based on microbiological analysis, gas chromatography, or spectroscopic methods, and sensory analysis. The latter is a regularly used method to quickly detect and identify odors and spoilage microorganisms; on the contrary, the detection of volatile organic compounds is limited due to its expensive instruments, and slow, complex and voluminous analysis processes (Seesaerd et al., 2022).

Defects in food matrices are therefore determined by means of electronic devices such as the E-nose, which is used as an olfactory system through a series of sensors (Duan et al., 2021). These sensors mimic the human nose to recognize, classify, and evaluate the different volatile compounds (Siadat et al., 2014). Authors such as Sánchez et al. (2021b) used an E-nose to discriminate anomalous fermentations in Spanish-style table olives. Therefore, the objective of this work was to discriminate altered, synthetic brines inoculated with different mold strains according to their sensory attributes and volatile compound profile with the use of an E-nose.

MATERIALS AND METHODS

Chemical Reagents

For the preparation of the synthetic brine, NaCl (Sharlau, Spain), lactic acid (Sharlau, Spain), glucose (Sharlau, Spain), and yeast extract (Condalab, Spain) were used. YPD agar (Condalab, Spain) was used for the growth of the altering molds. Commercial standard volatile organic compounds (VOC) solutions were used for the volatile organic compounds studied [propanoic acid, butanoic acid, 3,5-dimethyl-benzenemethanol, 2-methoxy-phenol, octanal, dodecanal, 2-methyl-butanoic acid, butyl ester butanoic acid, pentadecane, 3-methyl-1-butanol, 1-ethylpropyl-benzene, hexanal, heptanal, 2-nonanone, benzaldehyde, nonanal, 2,4-dimethyl-benzaldehyde, (E)-2-decenal, and a-murolene]. All these chemical reagents were purchased from Fisher Scientific (Fisher Scientific, MO, United States).

Experiment Design

The experimental design (**Figure 1**) consists of eight samples for each class or species of mold strain and uninoculated synthetic brine. The total amount of samples was 80. A synthetic brine was used in this work and made up of 4% NaCl, 0.5% glucose, 0.05% yeast extract, and adjusting the pH to 4.5 with lactic acid; this brine was prepared according to Tofalo et al. (2014) with some modifications. Next, 40 ml of this brine were dispensed into 50 ml Falcon tubes and then autoclaved at 121°C for 15 min. After sterilization, the synthetic brine was inoculated with nine spoilage mold strains: one *Galactomyces geotricum* (G.G.2); three *Penicillium expansum* (P.E.3, P.E.4, and P.E.20); one *Penicillium glabrum* (P.G.19); three *Aspergillus flavus* (A.F.9, A.F.18, and A.F.21); and one *Fusarium solani* (F.S.11). These molds had been obtained and characterized by Pérez-Nevado et al. (2011) from olives during the table olive process.

Prior to the inoculation, mold strains were grown in YPD agar (Condalab, Spain) at 25°C for 10 days. The mold spores were then collected in distilled water, and the number of spores was determined using a Neubauer improved chamber; this suspension was diluted properly to inoculate a concentration of 10^5 spores/ml in the brine. Control brines were made without inoculation. All the treatments were performed in triplicate.

The inoculated and non-inoculated synthetic brines were incubated in 50 ml Falcon tubes with a loose (air-open) cap for 15 days at 25°C in an orbital shaker at 100 rpm. Volatile compound analysis, sensory analysis and headspace measurements of the samples using an E-nose were performed at the end of the incubation. A diagram of the experiment is shown in **Figure 1**.

Analyses

To carry out this study, three different types of analyses were performed on the synthetic brines inoculated with molds: sensory analysis, volatile profile, and E-nose measurements.

Sensory Analysis

A tasting panel was composed of eight experts from the CICYTEX research center (Extremadura, Spain) and University of Extremadura. Tasters were trained according to the IOC recommendations [International Olive Council (IOC), 2021].

For the sensory analysis of the synthetic brine, a score board was prepared. Inoculated synthetic brines were evaluated by the sensory panel on a structured scale from 0 to 10 points according to positive and negative odors perceived. The assessment of the sensory properties of the brine included aspect related to the aroma perceived to yeast, almond, sweet,

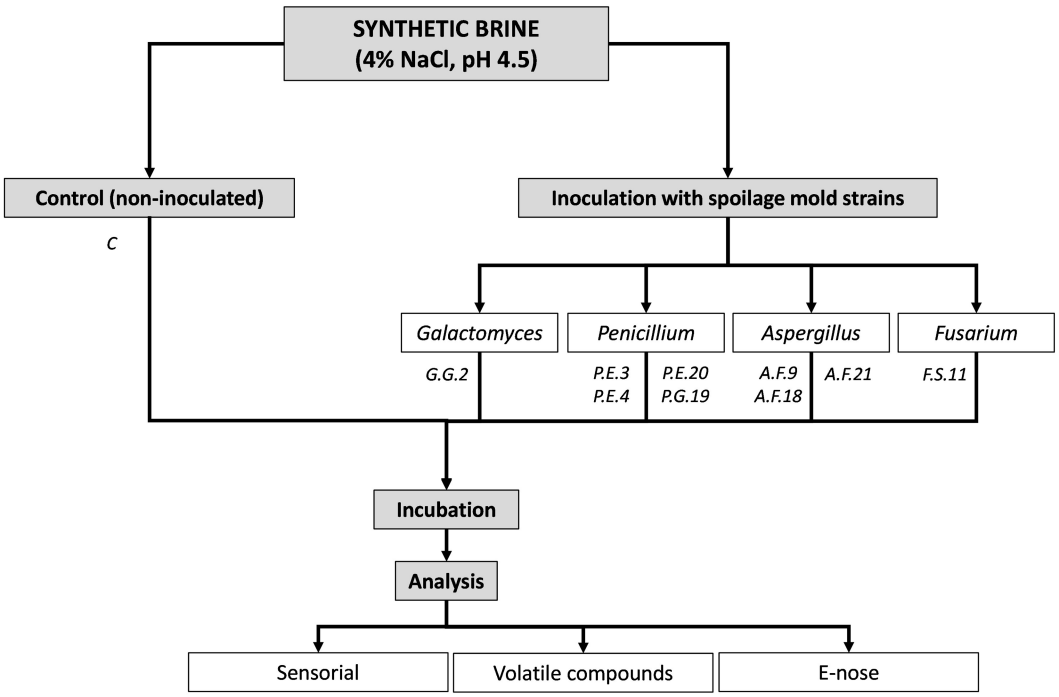

FIGURE 1 | Diagram of the experimental design.

mold, woody, leather, rancid, toasted, metal, and chemical. The intensity of the different odor attributes was evaluated by the trained panel, and the outcomes were expressed as average values. When the coefficient of variation was less than 20, the values were considered valid. Based on the standard International Olive Council (IOC) (2021), brines were classified according to the intensity of the defect predominantly perceived (DPP) as follows: Extra or Fancy (DPP < 3); First, 1st, Choice or Select (3 < DPP < 4.5); Second, 2nd or Standard (4.5 < DPP < 7.0); and Olives that may not be sold as table olives (DPP > 7).

Volatile Compound Analysis

For VOC determination, synthetic brines were filtered, and 2.0 ml were introduced into a vial with 7 ml of NaCl solution (30% p/v). The samples were introduced into polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex fiber (65 µm, Supelco) at 40°C for 30 min according to the methodology described by Sánchez et al. (2021a) and López-López et al. (2019). The determinations were performed using a model 456-GC triple quadrupole gas chromatograph with DB WAXETR capillary column (60 m × 0.25 mm; ID: 0.25 mm) was purchased from Agilent Technologies (Palo Alto, CA, United States). The peaks obtained were compared with the NIST reference spectral library.

E-Nose

The E-nose used was a small (39 mm × 33 mm), lightweight, wireless device with a low power consumption (185 mA), powered by a 3.7 V_{DC} and 2,000 mAh rechargeable LiPo battery. Its size and weight make it easy to handle, and it can be placed directly on the tasting glasses with a 3D printed case. The E-nose board has a +3.3 V_{DC} and +1.8 V_{DC} power supplies, and is governed by a PIC32MM0256GPM048 microcontroller from Microchip Technology Inc. (Chandler, AZ, United States). Among other features, this microcontroller has been chosen because of it has 256 KB of program memory, 32 KB of data memory, and several I^2C and UART modules. The E-nose also has a Microchip's RN4871 Bluetooth module, a UART port serial communication, and a battery charger *via* micro USB-B. The sensors used (Sánchez et al., 2021a) are BME680 from Bosch Sensortech GmbH (Germany), SGP30 from Sensirion AG (Switzerland), and CCS811 and iAQ-Core from ScioSense B.V. (The Netherlands). All of them are Metal Oxide Semiconductor (MOS) sensors, have their microprocessor, analog-to-digital converter, and I^2C bus interface. These sensors send a total of 14 signals, distributed as follows:

- BME680: temperature (°C), pressure (hPa), humidity (% RH), and gas measurement (Ω).
- SGP30: equivalent CO_2 (eCO_2; ppm), total volatile organic compounds (TVOC; ppb), and the raw measurements of H_2 and ethanol.
- CCS811: eCO_2 (ppm), TVOC (ppb), and sensor resistance (Ω).
- iAQ-Core: eCO_2 (ppm), TVOC (ppb), and sensor resistance (Ω).

A block diagram of the E-nose is shown in **Figure 2**. The data obtained are sent *via* Bluetooth to a smartphone using an ASCII based protocol, in which all signals are sent at the same time separated by tabs in a single row, and each

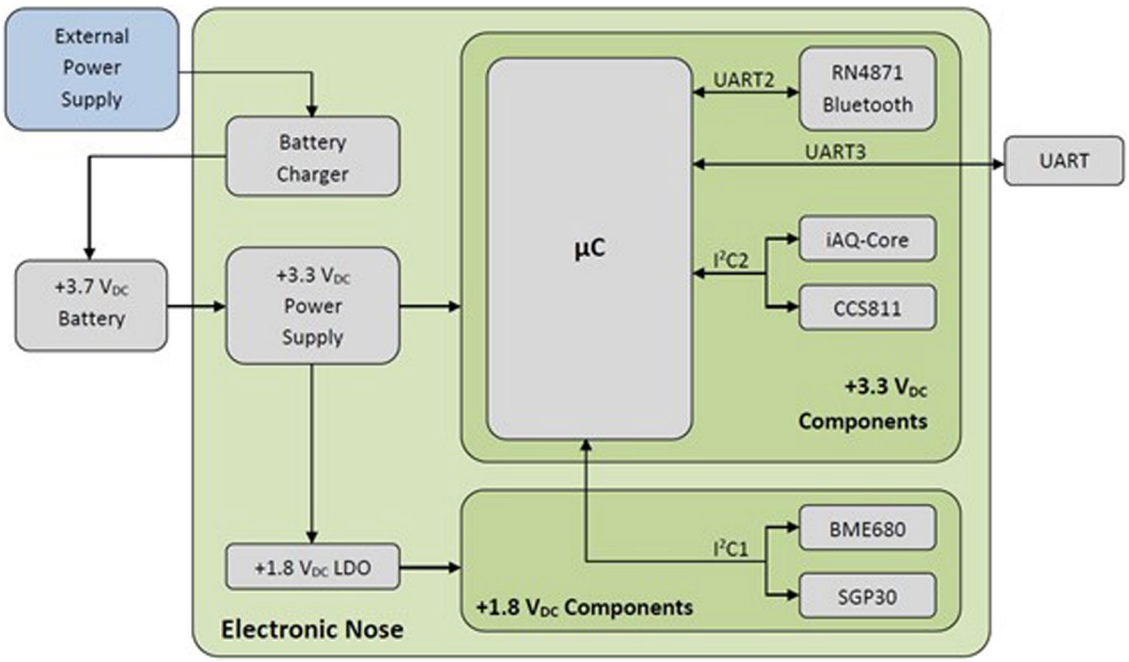

FIGURE 2 | Blocks diagram of the electronic nose (E-nose).

measurement is sent every second, resulting in a table where each column corresponds to a signal and each row to a measurement. Commands to start and stop experiments have also been implemented. Finally, an Android application has been developed to get and save this data for further processing with an easy user interface.

For the aroma analysis measurements of the E-nose, the recommendations of the International Olive Council (IOC) (2021) were followed. The cups were placed in a heating block at 25°C, and 10 ml of brine were placed inside and then covered with a watch glass. The measurements were made by placing the E-nose sensors in contact with the headspace of the brine samples for 60 s and then, in the desorption phase, placing them in contact with the air contained in an empty cup for 30 s. Eight measurements were taken for each brine sample. The data obtained were further processed by the statistical tool MATLAB.

Multivariate Data Analysis

The Principal Component Analysis (PCA) method eliminates any redundancy or correlation in the sensor responses. With the help of graphs, we could plot the principal components of the PCA of the brine samples with different molds. The values obtained from the brine samples with different molds were grouped in the graph according to their volatile compound profile.

The final objective was to automatically recognize patterns whose class we did not know. For this purpose, a supervised classification analysis called partial least squares discriminant analysis (PLS-DA) was applied (Barker and Rayens, 2003). The models were optimized with a number of latent variables equal to 7. These variables were selected through cross-validation with the leave-one-out procedure. For each model, eight samples were used for each class. A supervised classification requires prior knowledge of a group of patterns and their class, in order to subsequently obtain the model. Several PLS-DA models were built with different classification objectives. Specifically, one model was developed to discriminate between brine samples altered by strains of different molds and another to discriminate between brine samples altered by molds of the same species. The confusion matrix of the different models was constructed, and the correct predictions were calculated from the sum of the diagonal elements found in these matrices.

RESULTS

Sensory Aroma of Inoculated Brines

Synthetic brines inoculated with the different mold strains were evaluated by a tasting panel that scored them according to positive and negative attributes (**Figure 3**). The analyzed brines were classified into different sensory categories (extra, first, and second category) according to the predominant perceived defect (PPD) intensity indicated by the International Olive Council (IOC) (2021). Different sensory profiles were obtained depending on the microorganism inoculated in the synthetic brines. The non-inoculated samples (C) did not show negative attributes but presented positive ones related to a yeast, almond, or sweet odor. The intensity of these attributes ranged from 5 to 6 points out of 10. At low concentrations, these positive attributes were also found in the inoculated ones, with the highest scores in brines with P.E.4, P.E.20, F.S.11, and P.G.19;

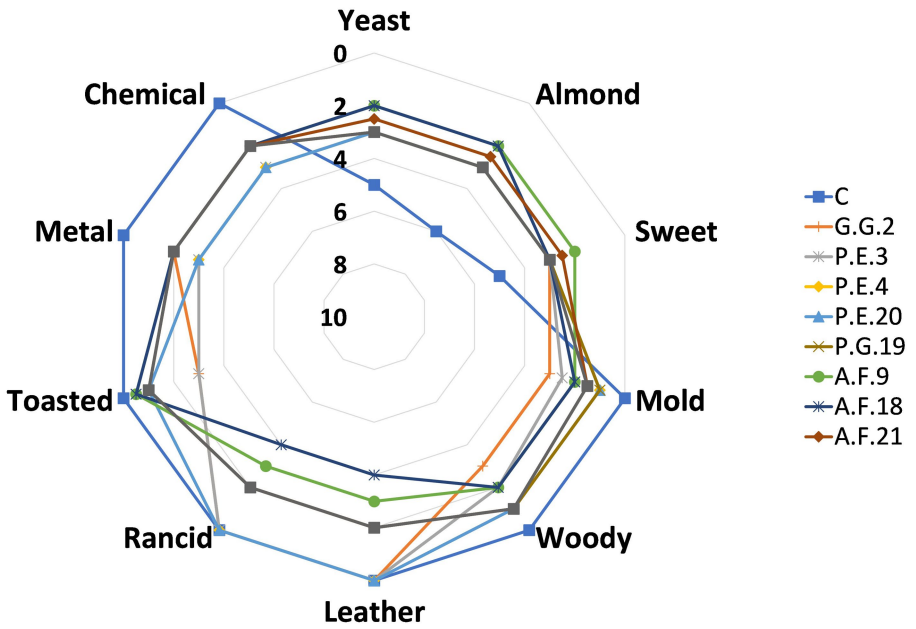

FIGURE 3 | Intensity of the positive and negative attributes (mean ± SD) of synthetic brines inoculated with different mold strains.

and the lowest scores in brines with A.F.9 and A.F.18. However, the inoculated synthetic brines showed clear sensory defects related to microbial alterations. Leather and rancid attributes were the main defects found in the inoculated brines with the highest scores in A.F.9 and A.F.18 for both attributes. Other negative attributes were a metal and chemical odor, albeit in a lower quantity. The mold strains P.E.3, P.E.4, and P.E.20 showed the highest score of these attributes. Moldy, woody, and toasted odors were also detected in the inoculated synthetic brines in different concentrations. On the whole, the greatest negative sensory defects were ascribed to synthetic brines inoculated with G.G.2, P.E.3, A.F.9, and A.F.18, while the lowest values were given to P.E.4 and P.E.20.

Volatile Compounds of Inoculated Brines

The distribution of VOC in the control and in the inoculated brines is shown in **Figure 4**. A total of 19 volatile compounds were identified by gas chromatography. These compounds were classified according to their positive or negative sensory attributes. Statistically significant differences were observed between the distribution of VOCs in inoculated samples. The control (C) did not show VOCs associated with unpleasant odors. On the other hand, in inoculated brines, the microorganisms that contributed to the worst unpleasant odor intensity were G.G.2, P.E.3, A.F.9, and A.F.18, while P.E.20, A.F.21, F.S.11, and P.G.19 had the least negative odor contribution to the brines.

The VOC that contributed more to the unpleasant odor in synthetic brine inoculated with G.G.2 and P.E.3 was dodecanal, with concentrations of 23.2 and 22.4%, respectively (**Table 1**). In the brines inoculated with A.F.18 and A.F.9, the VOCs that contributed more to the unpleasant odor were carboxylic acids, butanoic acids (12.2 and 12.4%, respectively), and propanoic acid (10.2 and 10.5%, respectively). Other acids such as butanoic acid and propane acid were found in A.F.18 (12.3%) and in A.F.9. (12.4%). However, propane acid presented a concentration of 10.5% in A.F.18 and 10.5% in A.F.9. Another unpleasant compound synthesized during the mold's fermentation was 2-methoxy-phenol. This was detected in higher concentrations in G.G.2 (4.9%) and in *A. flavus* strains A.F.9 and A.F.8 (3.2%). Moreover, different VOCs were detected in C brines. Nonanal (44.5%) and 2-4-dimethyl-benzaldehyde (33.4%) were the main VOCs found but other compounds such as 1-ethylpropyl-benzene (7.5%) and 2-nonanone (7.0%) were also detected. It should be noted that the percentage of these compounds decreased when the brine was inoculated by the different mold strains and in some of them, they were not detected.

Discrimination of Inoculated Synthetic Brines With E-Nose

Brine samples inoculated with different mold strains were analyzed with an E-nose and data obtained were analyzed using a PCA (**Figure 5**). A separation between C and inoculated brines could be observed. The PCA results showed that 87.60% of the total variance of the data was explained by PC1 and 7.75% by PC2. The model based on the first two components showed a clear differentiation of C and the inoculated brine samples.

After the application of a PLS-DA classification analysis and leave-one-out cross-validation of the data, we obtained the confusion matrix shown in **Table 2**. The sum of the diagonal elements of the confusion matrix gave a classification success rate of 93.5%. These results prove the ability and accuracy of the E-nose to discriminate between different brines with alterations caused by different mold strains.

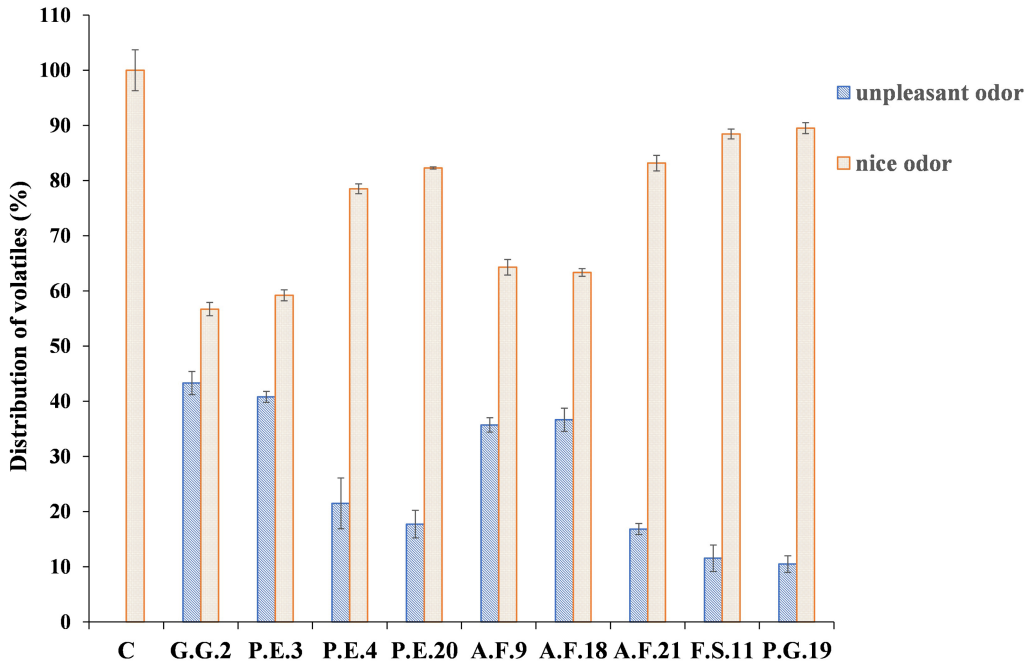

FIGURE 4 | Chemical distribution of volatile compounds in synthetic brines inoculated with different mold strains.

TABLE 1 | Content of volatile compounds (mean %, $n = 3$) obtained from brines of olives inoculated compared with olive tables (control).

	Volatile compounds	R.T (min)	C	G.G.2	P.E.3	P.E.4	P.E.20	P.G.19	A.F.9	A.F.18	A.F.21	F.S.11
Unpleasant odor	Propanoic acid	3.9	0.0	4.5	6.2	5.5	4.7	2.4	10.2	10.5	3.9	2.5
	Butanoic acid	8.2	0.0	5.4	7.1	6.2	4.8	3.5	12.4	12.2	5.6	4.0
	3,5-dimethyl-benzenemethanol	29.9	0.0	3.2	1.5	1.6	1.7	1.3	3.4	3.4	1.7	1.4
	2-methoxy-phenol	21.3	0.0	4.9	2.4	2.5	2.6	2.1	3.2	3.2	2.6	2.2
	Octanal	17.0	0.0	0.0	0.0	2.7	0.0	0.0	3.4	3.5	0.0	0.0
	Dodecanal	36.4	0.0	23.2	22.4	1.7	3.1	0.3	0.1	0.3	0.4	0.4
	2-methyl-butanoic acid	10.1	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.7	1.8	0.0
	butyl ester butanoic acid	34.8	0.0	2.1	0.8	1.1	0.4	0.4	0.2	0.7	0.7	0.5
	Pentadecane	36.0	0.0	0.0	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.6
Positive odor	3-methyl-1-Butanol	4.4	0.0	3.1	4.3	3.8	3.2	3.7	10.3	10.0	3.6	3.7
	1-ethylpropyl-benzene	30.6	7.5	2.7	1.1	1.2	0.0	0.0	0.0	0.0	0.0	0.0
	Hexanal	6.7	1.0	0.0	0.0	1.0	0.2	0.2	0.4	0.9	1.0	1.1
	Heptanal	11.6	1.1	0.0	0.0	0.2	0.9	0.6	0.1	0.3	0.0	0.4
	2-Nonanone	13.1	7.0	5.0	5.8	3.4	6.9	6.1	3.5	3.3	3.1	7.2
	Benzaldehyde	14.8	5.5	0.0	0.0	0.0	0.8	3.4	0.0	0.7	0.9	3.2
	Nonanal	22.3	44.5	20.3	20.8	42.3	44.6	38.7	23.5	23.6	44.1	44.4
	2,4-dimethyl-benzaldehyde	27.8	33.4	20.6	21.3	22.2	21.5	34.2	22.9	20.5	27.0	25.6
	(E)-2-Decenal	30.0	0.0	3.0	4.1	3.8	3.2	1.6	3.5	3.0	2.7	1.7
	α-muurolene	40.0	0.0	2.0	1.8	0.7	1.1	1.0	0.1	1.0	1.0	1.0

RT, retention time.

In addition, the response of the E-nose to brine samples non-inoculated and inoculated with several mold strains of *A. flavus* and *P. expansum* was analyzed using a PCA (**Figure 6**). In this figure, it is possible to differentiate non-inoculated brines from inoculated ones. The first and second principal components (PC1 and PC2) were sufficient to visualize the clustering of data and explained 82.2 and 97.4% of the total variance of *A. flavus* and *P. expansum*, respectively. Subsequently, the results obtained after applying the PLS-DA (**Table 3**) showed a 78% hit rate for *A. flavus* strains and a 93% hit rate for *P. expansum*.

DISCUSSION

According to the PPD established by the International Olive Council [International Olive Council (IOC), 2021], the

TABLE 2 | Confusion matrix obtained from eight samples of each class through partial least squares discriminant analysis (PLS-DA) for discrimination between different synthetic brine inoculated by molds.

	Predicted class (%)					
Real class	**C**	**G.C.2**	**P.E.3**	**F.S.11**	**A.F.18**	**P.G.19**
C	16.6	0	0	0	0	0
G.C.2	0	16.6	0	0	0	0
P.E.3	0	0	16.6	0	0	0
F.S.11	0	0	0	16.6	0	0
A.F.18	0	0	0	0	12.5	2.0
P.G.19	0	0	0	0	4.2	14.6

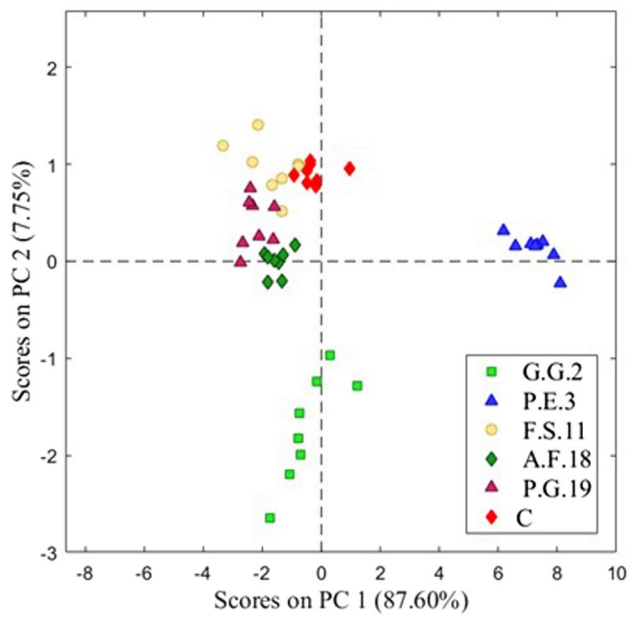

FIGURE 5 | Score plot of the Principal Component Analysis (PCA) analysis for control inoculated by different mold strains.

metabolism of mold strains could have caused sensory defects in the synthetic brines. In contrast, when evaluated by the tasting panel, the non-inoculated synthetic brines presented positive sensory attributes that could be due to the yeast extract added to the brine to improve the microbial growth. It should be noted that these positive attributes were also present in inoculated brines but in all cases to a lesser extent. The decrease in their concentration in brines with molds may be due to the microbial metabolism caused during their development.

Tasters detected many negative attributes in different concentrations in the inoculated brines. In this sense, most of the molds (G.G.2, P.E.3, P.E.4, P.E.20, A.F.9, and A.F.18) provoked some alteration in the brine and were classified into the first category as the DPP had a score of 4 ($3 < DPP \leq 4.5$). The rest of the mold strains (A.F.21, F.S.11, and P.G.19) were the only mold strains classified into the extra category ($PPD \leq 3$) although they presented sensory defects at low concentrations. Furthermore, the PPD in the synthetic brines inoculated was less than 6, thus it could be marketed.

In the Spanish-style table olive process, the sensorial properties can be modified by the formation of volatile and non-volatile aromatic compounds (Mas et al., 2014). These include peptides, amino acids, vitamins, minerals, and polyphenols, all of which can contribute to the sensory aroma. The non-inoculated brines (C) presented VOCs responsible for positive aromas. This is the case of 3-methyl-1-butanol, a product formed in alcoholic fermentations by microorganisms (Pietruska et al., 2010), and with a fruity aroma such as apples and bananas (Shen et al., 2022). The carbonyl compounds found in the non-inoculated samples are thought to be related to the commercial yeast extract added during the preparation of the brines. The prominent compound was Nonanal whose concentration varied depending on the microorganism inoculated in the brine; P.E.4, P.E.20, A.F.21, and F.S.11 presented similar concentrations to C; in the rest of the mold strains, the concentration of nonanal was reduced by half compared to C. This compound, like other aromatic aldehydes found in essential oils of various plants, has antifungal properties such as the inhibition of *A. flavus* (Li et al., 2021). A further compound that provides positive attributes is 2,4-dimethyl-benzaldehyde, related to aroma of cherry, almond, and vanilla (Zhang et al., 2021). Overall, the concentrations in inoculated brines were lower compared to the control, except for P.G.19, which increased. In the case of aromatic ketones such as 2-nonanone, only its concentration in F.S.11 increased in the brines in relation to C. This aromatic carbonyl compound also exhibits antimicrobial properties (Mahmound et al., 2020).

On the other hand, VOCs associated with unpleasant aromas were found in the brines inoculated by different mold strains. The microorganisms produced two short-chain carboxylic acids, namely propanoic acid and butanoic acid. The first has a cheesy odor and the second produces a buttery and cheesy odor (Liu et al., 2022). A.F.9 and A.F.18 were the main contributors to the formation of these carboxylic acids. In general, molds generate undesirable products during fermentation processes (Sánchez et al., 2021b), and at the same time cause serious problems to food safety and human health (Gong et al., 2019). Two derivatives of butanoic acid found in our study were 2-methyl-butanoic acid and butyl ester-butanoic acid. These are associated with unpleasant odors in brines; the first derivative has a cheesy, sweaty, and sharp odor (Tachihara et al., 2006). The butyl ester-butanoic acid is a compound synthesized by microorganisms through enzymes acyl transferases and lipases

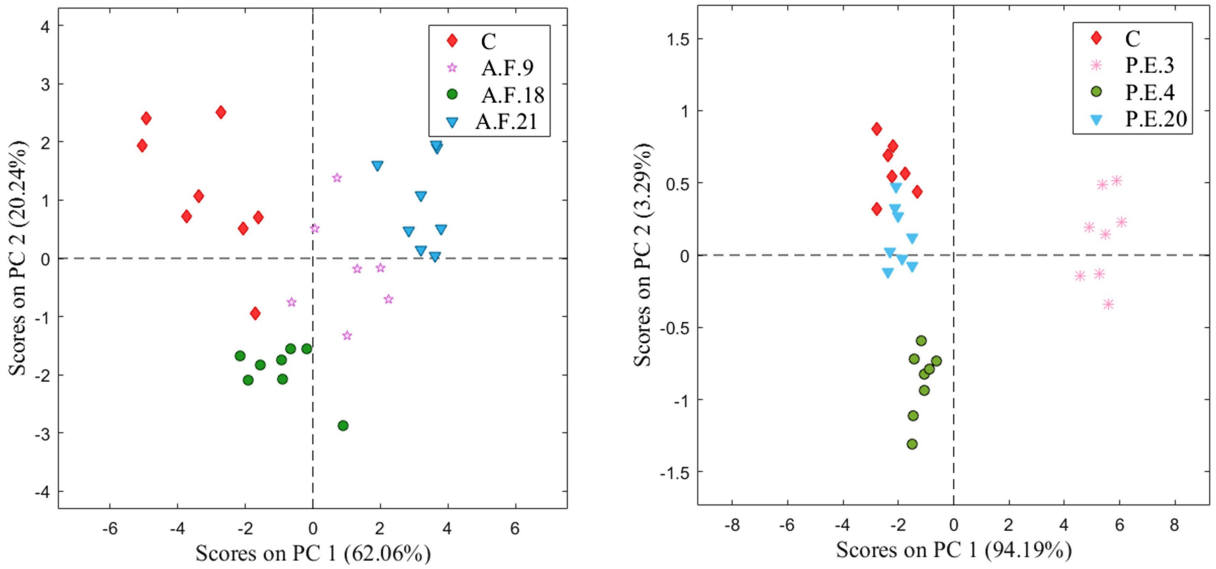

FIGURE 6 | Score plot of the PCA for brine inoculated by the *A. flavus* species (left), and the score plot of the PCA for brine inoculated by *P. expansum* species (right).

TABLE 3 | Confusion matrix obtained from eight samples of each class through PLS-DA for discrimination between synthetic brine inoculated by *Aspergillus flavus* and *Penicillium expansum* strains.

					Predicted class (%)				
	A. flavus					***P. expansum***			
Real class	**C**	**A.F.9**	**A.F.18**	**A.F.21**	**Real class**	**C**	**P.E.3**	**P.E.4**	**P.E.8**
C	18.7	3.1	3.1	0	C	21.9	0	0	3.1
A.F.9	3.1	15.6	3.1	0	P.E.3	0	25.0	0	0
A.F.18	3.1	3.1	18.7	0	P.E.4	0	0	25.0	0
A.F.21	0	3.1	0	25.0	P.E.8	3.1	0	0	21.9

that play an important role in butanol esterification (Noh et al., 2019). In our study, these compounds were synthesized by the strain of *G. galactomyces* G.G.2. In other studies (Eliskases-Lechner et al., 2011), *Geotrichum* was heavily involved in the flavor and aroma of cheese, and was responsible for the deterioration and reduction of the shelf-life of fermented products derived from milk. The dodecanal aldehyde compound was found in different concentrations in all the inoculated brines. G.G.2 and P.E.3 were the microorganisms capable of synthesizing the highest amounts of this compound. Microorganisms could be used to synthesize aldehydes as an alternative to traditional synthetic routes (Kunjapur and Prather, 2015).

In this way, the different mold strains used to inoculate synthetic brines were discriminated with the E-nose. This discrimination could be due to the different aroma profiles present in the inoculated brine. This electronic tool was even able to discriminate brine samples inoculated with several strains of *A. flavus* and *P. expansum*. Few references exist on the discrimination of olives altered by mold using an E-nose. Sánchez et al. (2021b) discriminated table olive defects produced by different microorganisms, such as zapateria, butyric, putrid, and mold during the fermentation period of Spanish-style table olives. There are studies that demonstrated the usefulness of sensory-based E-nose systems to the fungal detection and identification of associated species (Mota et al., 2021); and to develop a detection system to differentiate fungus using an E-nose device (Loulier et al., 2020). Therefore, the results obtained in this study prove that an E-nose, combined with chemometric analysis, is a powerful tool with an analytical capacity to discriminate VOCs produced by different mold strains. Moreover, it is a fast, precise and low-cost method compared to classic analysis techniques since it is non-destructive and does not require highly qualified personnel (Nishi et al., 2015).

CONCLUSION

The inoculated mold strains produced negative sensory defects in the synthetic brines. The main defects detected were mold, rancid, and leather, most of which were classified into the first category established by the IOC. The inoculated brines showed a different profile of volatile compounds associated

with unpleasant aromas that depended on the inoculated mold strain; dodecanal was the VOC with the highest content, followed by butanoic acid and propanoic acid. Therefore, taking into account the sensory analysis and VOC, the mold strains that caused the most unpleasant effects in the synthetic brines were G.G.2, P.E.3, A.F.9, and A.F.18. These defects were also discriminated by using an E-nose, a powerful tool capable of differentiating the aroma of the synthetic brines inoculated with different mold strains. Combined with other chemometric tools, this easy-to-use, low-cost device can discriminate samples according to incipient alterations caused by molds, thereby confirming its suitability to detect different alterations during the fermentation process in Spanish-style table olives at industrial level, although further studies would be required to prove this.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RS, FP-N, IM-F, and DM-V contributed to the conceptualization of the study. RS, JL, and FM contributed to data curation and methodology. Formal analysis was performed by RS, IM-F, and DM-V. DM-V and FP-N acquired the funding. DM-V carried out the project administration. RS, FP-N, and DM-V performed the investigation and organized the resources. JL and DM-V carried out the supervision. JL, RS, FM, and DM-V contributed to the validation. RS and DM-V contributed to the visualization. RS, IM-F, FM, JL, and DM-V wrote the original draft. RS, IM-F, JL, and FP-N contributed to the writing—review and editing of the final manuscript. All authors contributed to the article and approved the submitted version.

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2.3 Artículo 3. "Determination of the masking effect of the 'Zapateria' defect in flavoured stuffed olives using E-nose. (2022). Molécules, 27, 4300."

Sánchez, R., Boselli, E., Fernández, A., Arroyo, P., Lozano, J., Martín-Vertedor, D.

2.3.1 Motivación y objetivos

Una de las elaboraciones más comunes de aceitunas verdes es al "estilo español" en la que las aceitunas en salmuera son fermentadas varios meses con bacterias lácticas (Sánchez et al., 2006; Schaide, et al., 2019). Cuando en la fermentación de las aceitunas no se llevan a cabo buenas prácticas se pueden producir alteraciones como la *zapatería*.

Entre los compuestos volátiles responsables del olor desagradable del defecto a *zapatería* se encuentra el ácido ciclohexanocarboxílico (Montaño et al., 1992). Otros compuestos químicos relacionados con el defecto a *zapatería* y que se forman en las aceitunas verdes fermentadas durante el almacenamiento son el ácido propiónico, fórmico, butírico, succínico, isobutírico y n- ácido valérico, así como acetaldehído, metanol, etanol, 2-butanol y n-butanol (García et al., 2004).

El uso de hidrocoloides en alimentos es cada vez más común. Pueden influir en el procesamiento de los alimentos, propiedades nutricionales y sensoriales, lo que genera beneficios en el producto final (Lu et al., 2020). Los hidrocoloides son además utilizados en el relleno de aceitunas de mesa para abaratar costes en otros ingredientes.

Recientemente, la *nariz electrónica* se ha utilizado para evaluar defectos anormales de fermentación en aceitunas de mesa "estilo español", incluidos los defectos a *zapatería, butírico, pútrido* y *mohoso* (Sánchez et al., 2021a). Los principales compuestos volátiles encontrados del defecto a *zapatería* son ácido butanoico, ácido (E)-3-hexenoico, ácido hexanoico, ácido pentanoico y el ácido ciclohexanocarboxílico que ha sido identificado como un compuesto clave de las muestras de *zapatería* en estudios previos (Montaño et al., 1992).

La *nariz electrónica* con su matriz de sensores es capaz de discriminar muestras con diferentes perfiles de aroma en diferentes alimentos. En la matriz del vino se ha estudiado su uso para evitar el desarrollo de sabores y aromas desagradables (Gonzalez y Fuentes, 2022); en aceites comestibles se ha utilizado además en la cuantificación e identificación del período de almacenamiento (Jiang et al., 2021); en cítricos en la detección de infecciones (Wen et al., 2019) y en ajo en la detección

temprana y clasificación de infecciones fúngicas (Makarichian et al., 2022). Estos dispositivos también se utilizaron en la clasificación aceitunas procedentes de variedades de olivos (Martínez et al., 2020) o incluso para diferenciar aceitunas de mesa con fermentaciones anormales (Sánchez et al., 2021a; Sánchez et al., 2022a).

El objetivo principal de este trabajo fue estudiar la evolución olfativa de las aceitunas de mesa con defecto de *zapatería* mediante *nariz electrónica*, antes y después de la adición de diferentes concentraciones de aroma de 'mojo picón' al relleno. El objetivo fue mostrar que el defecto de *zapatería* queda enmascarado a una cierta concentración de aroma añadido, mejorando la aceptación del consumidor.

2.3.2 Diseño Experimental

Una empresa del suroeste de España suministró aceitunas de mesa verdes al "estilo español" fueron identificadas y clasificadas sensorialmente por el panel de cata en aceitunas con defecto a *zapatería.* Seguidamente, las aceitunas se deshuesaron para su posterior relleno.

Los rellenos de diferentes hidrocoloides se prepararon con una base de agua al que se le añadieron 2% de alginato de sodio, 1% de goma guar y 2, 4 y 8% de aroma 'mojo picón' (Neroliane, Grasse, Francia) según Sánchez et al. (2022b). Este aroma es una salsa picante preparada con ajo, comino, pimentón dulce, guindilla, sal marina, de oliva y vinagre (Weichselbaum et al., 2009).

100 aceitunas se rellenaron manualmente con una jeringa y se dividieron en cuatro grupos de muestras: i) aceitunas de mesa rellenas con el hidrocoloide, pero sin aroma 'mojo picón' a las que se denominaron control (C); ii) aceitunas rellenas con 2% de aroma 'mojo picón' (M2); iii) aceitunas rellenas con 4% de aroma 'mojo picón' (M4); iv) aceitunas rellenas con 8% de aroma 'mojo picón' (M8). A continuación, las aceitunas se maceraron durante 24 horas en una solución de $CaCl_2$ al 0,25%. Posteriormente, las aceitunas de cada grupo se colocaron en frascos que contenían 25 aceitunas rellenas y 100 mL de salmuera (3% NaCl, p/v). Los frascos se pasteurizaron

a 80ºC durante 20 min. Finalmente, las muestras fueron analizadas por el panel de cata, cromatografía de gases y *nariz electrónica*.

2.3.3 Resumen de los resultados y discusión

Las aceitunas se clasificaron en diferentes categorías sensoriales según la evaluación de defectos predominantemente percibidos (DPP) por el panel de cata (IOC, 2021). Las muestras control se clasificaron en segunda categoría debido al defecto de *zapatería* presente, por lo que se buscó enmascarar este defecto mediante la adición de concentraciones diferentes de aroma.

Los resultados mostraron que la concentración de aroma de 'mojo picón' del relleno de aceitunas de mesa influyó significativamente en la intensidad del aroma y la percepción de defectos de las aceitunas estudiadas (P valor < 0,05). Los catadores pudieron discriminar las diferentes muestras según la cantidad de aroma agregado. La intensidad del olor a 'mojo picón' osciló entre 3,1 (M2) y 7,2 (M8). Este fue un buen resultado, ya que la aplicación de esta concentración de aroma a los hidrocoloides fue suficiente para obtener un aroma positivo muy intenso en las aceitunas.

La percepción de la intensidad del defecto por parte de los catadores disminuyó a medida que aumentaba la concentración del aroma. El defecto osciló entre 3,5 (M2) hasta no ser detectado (M8). Por tanto, las aceitunas con relleno M4 y M8 se clasificaron dentro de la mejor categoría sensorial denominada categoría extra con DPP ≤ 3. Finalmente, las aceitunas sin aroma añadido presentaron alta intensidad del defecto, clasificándose como de segunda categoría. Por lo tanto, se puede afirmar que la intensidad del aroma del relleno provocó una disminución en la percepción del defecto a *zapatería* y esto, podría servir para enmascarar el defecto y comercializar las aceitunas dentro de una mejor categoría.

En cuanto a los compuestos volátiles analizados, fueron identificados un total de 16. Los principales volátiles encontrados en el aroma de 'mojo picón' fueron beta-pineno (19,7%), p-cimeno (18,7%), gamma-terpineno (24,7%) y disulfuro de dialilo (14,6%). El aroma de estos compuestos está relacionado con atributos positivos como frutas

frescas, cítricos, hierbas y cebollas verdes. Los principales constituyentes de las aceitunas verdes control con defecto de *zapatería* fueron creosol (25,0%), propilenglicol (19,8%), ácido 2,4-hexadienoico, éster etílico (14,5%), ácido ciclohexancarboxílico (12,7%) y ácido 2,4-hexadienoico metil éster (11,2%). Los compuestos volátiles responsables de los malos olores fueron el propilenglicol, el ácido ciclohexanocarboxílico, el ácido pentanoico y el (E)-2-decenal. Estos compuestos no se encontraron en altas concentraciones, pero están relacionados con el defecto de *zapatería* (Wen et al., 2019; Makarichian et al., 2022). El ácido ciclohexanocarboxílico y el ácido pentanoico han sido descritos por Sanchez et al. (2021) como las moléculas responsables del olor desagradable de la *zapatería* de las aceitunas de mesa al "estilo español". Cabe señalar que, el olor del ácido pentanoico también está relacionado con el defecto *butírico* (Sánchez et al., 2021a).

El relleno de aceitunas defectuosas con hidrocoloide provocó la dilución de los compuestos volátiles relacionados con los defectos de la aceituna, independientemente de la concentración utilizada. Este efecto se observó claramente incluso en el aroma a baja concentración.

El PCA exploratorio no supervisado, basado en los dos primeros componentes, logró diferenciar entre aceitunas rellenas sin aroma añadido y aceitunas con aroma de 'mojo picón' a diferentes concentraciones. PC1 explicó el 64,0% de la varianza total de los datos, mientras que PC2 explicó el 15,1%. Estos resultados son comparables a los obtenidos por Sánchez et al. (2022b), donde se discriminó entre aceitunas negras rellenas con y sin aroma añadido.

Posteriormente, se realizó un análisis de clasificación mediante PLS-DA utilizando una validación cruzada de exclusión. Se utilizaron ocho muestras de cada clase para construir el modelo. En la matriz de confusión, el 3,1% de las muestras pertenecientes a los grupos C y M2 se clasificaron erróneamente entre los dos grupos. Esto puede deberse a que la cantidad de aroma a 'mojo picón' añadida al relleno de M2 demasiado baja para clasificar correctamente todas las muestras, como sí lo hizo con los grupos M4 y M8.

Para estudiar la correlación entre los datos obtenidos de la *nariz electrónica* y el panel de cata se implementó un análisis quimiométrico PLS. Este modelo de regresión permitió cuantificar y establecer modelos de predicción, es decir, a partir de la *nariz electrónica* y el modelo quimiométrico, poder obtener valores de análisis sensorial equivalentes a los proporcionados por un panel de catadores de aceitunas. En este estudio se utilizaron los descriptores sensoriales del defecto a *zapatería* y aroma percibido.

Para obtener el modelo PLS, el conjunto de muestras se dividió en un conjunto de calibración del 70%, que se utilizó para calibrar y realizar una validación cruzada de los modelos. Además, un conjunto de validación con las muestras restantes (30%) se utilizó para probar la robustez y precisión de los modelos desarrollados. Se construyeron dos modelos PLS diferentes, uno para cada parámetro. El R^2_{CV} de los modelos desarrollados para el defecto percibido y la calificación general fueron 0.83 y 0.88, respectivamente. También se estimaron los valores de RMSECV (0,78 para el defecto a *zapatería* y 1,10 para el aroma percibido).

2.3.4 Conclusiones

- Las aceitunas de mesa verdes al "estilo español" rellenas de hidrocoloide aromatizado con 'mojo picón' mejoran la categoría sensorial de las aceitunas con *zapatería*.

- Las altas concentraciones de aroma de 'mojo picón' agregado hicieron que el defecto de la aceituna fuera casi imperceptible para los catadores.

- Los principales volátiles del defecto a *zapatería* fueron el ácido ciclohexanocarboxílico y el ácido pentanoico que disminuyeron a medida que aumentaba el porcentaje de aroma añadido.

- La *nariz electrónica* discrimina aceitunas con defecto a *zapatería* de aquellas rellenas con diferentes concentraciones de aroma añadido.

- El algoritmo PLS permite predecir cuantitativamente el defecto a *zapatería* percibido por el panel de cata.

- La *nariz electrónica* es una herramienta de discriminación útil que se puede aplicar en aceitunas rellenas con hidrocoloides aromatizados.

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Determination of the Masking Effect of the 'Zapateria' Defect in Flavoured Stuffed Olives Using E-Nose

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Abstract: Spanish-style table olives are one of the most common processed foods in the Mediterranean countries. Lack of control during fermentation can lead to one of the main defects of the olive, called 'Zapateria', caused by the combination of volatile fatty acids reminiscent of rotten leather. In this study, table olives altered with 'Zapateria' defect were stuffed with a hydrocolloid flavoured with the aroma 'Mojo picón' to improve consumer acceptance. Sensory analysis, determination of volatile compounds and electronic nose (E-nose) were used to evaluate the quality of the olives. The control samples had a high concentration of the defect 'Zapateria' and were classified in the second commercial category, while higher 'Mojo picón' flavour concentrations resulted in these olives being classified as 'extra category' (a masking effect). The main volatile compounds in olives with 'Zapateria' defect were cyclohexanecarboxylic acid and pentanoic acid. E-nose allowed discrimination between stuffed olives without added flavouring and olives with 'Mojo picón' flavouring at different concentrations. Finally, PLS regression allowed a predictive linear model to be established between E-nose and sensory analysis values. The R_P^2 values were 0.74 for perceived defect and 0.86 for perceived aroma. The E-nose was successfully applied for the first time to classify Spanish-style table olives with 'Zapateria' defect intensity and with the addition of the 'Mojo picón' aroma masking the defect.

Keywords: sensory analysis; stuffed olives; electronic nose; 'Zapateria' defect; 'Mojo picón' flavour; aromas

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1. Introduction

According to the International Olive Oil Council [1], world table olive production is expected to increase by 7% in the 2021/2022 season, reaching 2,846,500 tonnes. Spain stands out as the world's largest producer and one of the countries with the highest increase in production (18%), reaching 645,000 tonnes, compared to the average production of 551,000 tonnes. Regarding consumption for 2021/2022, the IOC forecasts a 1.2% increase compared to 2020/2021. Table olives are widely consumed in the Mediterranean coast and worldwide and are elaborated according to different styles.

One of the most common products is known as "green olives treated in brine in the Sevillian or Spanish style" [2,3]. Spanish-style processing consists of an alkaline treatment with NaOH (1.5–4.5%) to remove bitterness, followed by several washes with water to remove residual lye, and then fermentation in brine to improve the nutritional and

sensory quality of the product. With the alkaline treatment, the pH of the olive pulp reaches values of 11.0–13.0, which decrease to 8.0–9.0 after repeated washings. After washing, the olives are immersed in 6–10% NaCl [4]. At the end of the lactic fermentation (when glucose and reducing sugars are exhausted), the pH drops to 4.0 or less; this increase in acidity ensures the preservation of the product. At the end of this phase, if the product is not pasteurised, it can undergo undesirable fermentation during storage, which can lead to an increase in pH and volatile acidity, a decrease in lactic acid, the formation of cyclohexanecarboxylic acid [5] and the production of biogenic amines, such as cadaverine and tyramine [6]. In order to avoid uncontrolled and harmful microbial growth, the pH must be kept <4.0 and the NaCl in the brine must be raised to >8% [4].

During the elaboration, optimal processing conditions preventing microbial spoilage must be applied. Numerous works have been published on olives processed according to the "Spanish style" [7–10]. When fermented olives are not produced according to the best practices, an outbreak of putrefaction called 'Zapateria' can occur. Among the volatile compounds responsible for the unpleasant smell of the 'Zapateria' off-flavour is cyclohexanecarboxylic acid [5]. Other chemical compounds related to the 'Zapateria' defect and formed in fermented green table olives during storage are propionic acid (from microorganisms of the genus *Propionibacterium*), volatile acids such as formic, butyric, succinic, isobutyric and n-valeric acid, as well as acetaldehyde, methanol, ethanol, 2-butanol and n-butanol. *Clostridium* species related to the final steps of 'Zapateria' deterioration have also been found [6].

For the present study, the raw material used were "Spanish-style" green olives affected by 'Zapateria' defect and filled with hydrocolloids to which 'Mojo picón' flavouring was added ('Mojo picón' is the flavour of a hot sauce prepared with garlic, cumin, sweet paprika, chilli pepper, sea salt, olive oil and vinegar). The green olives are those obtained from fruits harvested before veraison when they have reached their final size. In the table olives industry, stuffed table olives can be prepared in different ways. After being pitted, olives can be stuffed with pepper, onion, almonds, celery, anchovy, orange or lemon peel, hazelnut, caper or with their pastes prepared for stuffing [11].

For decades, stuffing has usually consisted of a paste with an indeterminate amount of flavouring ingredients or additives, such as a piece of anchovy and pepper. This is an expensive and very laborious procedure, so stuffed olives may also contain spices and aromatic herbs or their natural extracts and authorised additives, including flavourings, thickeners and binders for food use as defined by the Codex Alimentarius for this product and with limited use according to Good Manufacturing Practices [12,13]. The filling consists of a gelled mass based on alginate, a stabiliser that holds the different components of the mass together and prevents them from escaping from the inside of the olives. It should be noted that the use of hydrocolloids in food is becoming increasingly common. They can influence food processing, nutritional and sensory properties, leading to benefits in the final product [14]. In this regard, new electronic and digital technologies that aim to better understand different food matrices should be considered.

Recently, an electronic nose has been used to evaluate abnormal fermentation defects in "Spanish-style" table olives, including the defects of 'Zapateria', 'Butyric', 'Putrid' and 'Mould' [15,16]. The "Spanish style" processed green olives used in this study had the 'Zapateria' defect. Inoculating the brine with selected starter cultures (lactic acid bacteria and yeasts) reduces the probability of spoilage and helps to achieve a better and more predictable fermentation process [4]. Therefore, the control of fermentation processes can be performed by monitoring the pH and NaCl concentration of the brine, chromatographic detection of volatile organic compounds (VOC), microbiological analysis and also organoleptic evaluation [1]. According to the IOC method, table olives are to be commercially graded taking into account the intensity of any defects determined by a panel of 8 to 12 tasters, based on smell, taste and texture. At the same time, the IOC method defines the characteristic olfactory sensations of abnormal fermentations in table olives. These

include the negative attribute of 'Zapateria', caused by the combination of volatile fatty acids reminiscent of rotten leather.

The electronic nose (E-nose) is a low-cost device made from a combination of sensors that allows samples with different aroma profiles to be discriminated in different types of matrices. In the wine matrix, its use has been studied to avoid the development of unpleasant flavours and aromas in final wines [17]; in edible oils, it has been used for the qualitative evaluation of the storage period [18]; in citrus fruits, for the detection of infections [19]; in garlic, for the early detection and classification of fungal infections [20]. This device has also been used to classify the quality of olives from different olive trees [21] or even to differentiate table olives with abnormal fermentations [15,16]. In this sense, the E-nose, due to its speed and low cost, can be an alternative to chromatography and sensory panel to assess the presence of defects. This is an important aspect for the subsequent classification of the product according to its flavour profile.

For these reasons, the main objective of this work was to study the olfactory evolution of table olives with a 'Zapateria' defect by means of E-nose, before and after the addition of different concentrations of 'Mojo picón' aroma to the filling. The aim was to show that the 'Zapateria' defect can be masked by a certain concentration of added flavours, which improve consumer acceptance.

2. Results and Discussion

2.1. Sensory Aroma of Hydrocolloid-Filled Olives

A trained panel was used to determine the 'Mojo picón' aroma intensity and the negative odour related to 'Zapateria' of 'Spanish style table olives (variety 'Manzanilla de Sevilla') stuffed with different concentration of hydrocolloids (M2, M4 and M8) (Table 1).

Firstly, it should be noted that the control sample had a high concentration of 'Zapateria' defect. Therefore, according to the IOC regulations, from the results obtained, the olives can be classified into different sensory categories based on the evaluation of the defect predominantly perceived (DPP) by the tasting panel [1]. Therefore, the control samples were classified in the second category (Table 1). This defect is characteristic of this type of table olive processing, so an attempt was made to see how this defect was masked by the addition of different flavour concentrations. Flavouring was added at different concentrations in the hydrocolloids, except in the control samples, where no aroma was added.

The results showed that the aroma concentration of the table olive stuffing significantly influenced the aroma intensity and defect perception of the olives studied (p-value < 0.05).

Table 1. Predominantly perceived sensory defects (mean ± standard deviation) of Spanish-style table olives stuffed with flavoured hydrocolloids. Different lowercase letters indicate statistically significant differences according to the intensity of the added flavour between each treatment (Tukey's Test, p-value < 0.05).

	'Mojo picón' Aroma	**C**	**M2**	**M4**	**M8**
Positive attribute					
'Mojo picón'	9.7 ± 0.8 a	n.d.	3.1 ± 0.6 d	5.3 ± 0.4 c	7.2 ± 0.6 b
Negative attribute					
'Zapatería'	n.d.	5.1 ± 0.5 a	3.5 ± 0.4 b	1.1 ± 0.2 c	n.d.
Commercial classification	Extra	2nd. Category	1st. Category	Extra	Extra

C: Spanish-style green table olives without aroma; M2: Spanish-style green table olives with 'Mojo picón' aroma at 2%; M4: Spanish-style green table olives with 'Mojo picón' aroma at 4%; M8: Spanish-style green table olives with 'Mojo picón' aroma at 8%. n.d.: not detected.

In other words, the tasters were able to discriminate the different samples according to the intensity of the flavour added (Table 1). The intensity of the 'Mojo picón' flavour

ranged from 3.1 (M2) to 7.2 (M8). This was a good result since the application of this flavour concentration to the hydrocolloids was sufficient to obtain a very intense aroma in whole olives.

On the other hand, the perception of the intensity of the defect by the tasters decreased as the concentration of 'Mojo picón' flavouring increased. However, the defective olives were flavoured with different concentrations which resulted in a decrease in the intensity of the 'Zapateria' defect in the flavoured stuffed olives. The defect ranged from 3.5 (M2) until not being detected (M8). Therefore, these olives were classified in the best commercial category called 'extra category' or 'fancy' (DPP ≤ 3) when the olives were stuffed with M4 and M8. It should be noted that the defect was perceived more in this type of olives than in those with a lower concentration of aroma (M2), classified as 'first category' or 'selected' (3 < DPP ≤ 4.5). Finally, the olives without aroma had a high defect intensity, being classified as 'second category' or 'standard'. It can, therefore, be stated that the intensity of the aroma of the filling caused a decrease in the perception of the 'Zapateria' defect in the studied samples and this could serve to mask the defect and market the olives filled with hydrocolloids in a better commercial category.

It must be considered that the olives without flavouring presented a significantly more intense defect, corresponding to a worse category, than the olives stuffed with the flavoured hydrocolloids. Furthermore, the olives with the highest flavour concentration presented a significantly lower defect intensity, being classified as olives with a higher commercial category (DPP ≤ 3). The results show that stuffing the olives with different concentrations of flavoured hydrocolloids could be a strategy to mask the 'Zapateria' defect, making Spanish-style green olives more attractive to the consumer. This aroma of 'Mojo picón' was previously used in the stuffed black olives by Sánchez et al. [22]. This flavour had a good aromatic intensity compared to other flavourings used and it was enough to mask the "cooked effect" in "California-style" black olives. In addition, this filling presented an intense orange colour that stands out with the black and green table olives.

2.2. Volatile Compounds of Hydrocolloid-Filled Olives

Volatile compounds were analysed in table olives stuffed with the aromatic hydrocolloid at different concentrations (M2–M8). The identified volatile compounds listed and their percentage content of the odor are shown in Table 2. Of all the volatile compounds detected by gas chromatography, the 16 most representative volatile compounds of table olives were determined. Of these, 8 compounds were found in the pure flavour, 8 in table olive altered with 'Zapateria' defect (C) and 9 in table olives stuffed with different concentrations of 'Mojo picón' flavour (M2, M4 and M8).

Table 2. Content of volatile compounds (mean%, n = 3) of stuffed olive added with flavoured hydrocolloids. Different lowercase letters indicate statistically significant differences according to experimental treatment for each volatile compound (Tukey´s Test, p-value < 0.05).

CAS Number	Volatile Compound	T.R. (min.)	'Mojo picón' Aroma	C	M2	M4	M8
64-19-7	Acetic acid	2.6	2.6 ± 0.2 a	n.d.	n.d.	n.d.	n.d.
57-55-6	Propylene glycol	5.8	n.d.	19.8 ± 4.5 a	n.d.	n.d.	n.d.
109-52-4	Pentanoic acid	12.1	n.d.	3.1 ± 0.7 a	n.d.	n.d.	n.d.
127-91-3	beta-pinene	15.5	19.7 ± 2.7 b	n.d.	11.8 ± 2.1 a	13.2 ± 2.1 a	11.3 ± 1.3 a
1515-80-6	2,4-Hexadienoic acid, methyl ester	17.8	n.d.	11.2 ± 2.5 a	n.d.	n.d.	n.d.
99-87-6	p-cymene	18.1	18.7 ± 3.4 ns	n.d.	19.2 ± 2.2 ns	21.0 ± 3.2 ns	19.0 ± 2.4 ns
99-85-4	Gamma-terpinene	19.9	24.7 ± 3.5 ns	n.d.	27.4 ± 3.8 ns	27.3 ± 2.7 ns	21.7 ± 3.5 ns
2179-57-9	Diallyl disulphide	21.1	14.6 ± 2.2 b	n.d.	12.1 ± 2.5 a	11.6 ± 1.5 a	14.4 ± 2.4 b
2396-84-1	2,4-Hexadienoic acid, ethyl ester	22.0	n.d.	14.5 ± 3.2 a	n.d.	n.d.	n.d.
98-89-5	Cyclohexanocarboxylic acid	26.5	n.d.	12.7 ± 2.2 c	5.2 ± 1.1 b	2.0 ± 0.5 a	n.d.
93-51-6	Creosol	26.7	n.d.	25.0 ± 3.4 d	5.0 ± 0.8 c	2.4 ± 0.7 a	3.6 ± 0.6 b
88973-62-0	Propyl 2,4-hexadienecarboxylate	26.9	n.d.	9.8 ± 1.5 a	n.d.	n.d.	n.d.
122-03-2	Cuminaldehyde	29.1	6.2 ± 0.8 a	n.d.	9.8 ± 1.1b	9.7 ± 0.9 b	14.3 ± 2.1 c
3913-81-3	2-Decenal, (E)-	29.9	n.d.	3.8 ± 0.5 a	n.d.	n.d.	n.d.
1197-15-5	alpha-terpinen-7-al	31.2	6.8 ± 0.9 b	n.d.	3.5 ± 0.5 a	3.7 ± 0.2 a	6.1 ± 0.4 b
2050-87-5	Allyl trisulfide	31.8	6.8 ± 0.8 a	n.d.	6.1 ± 0.8 a	9.1 ± 0.4 b	9.5 ± 1.3 b

C: Spanish-style green table olives without aroma; M2: Spanish-style green table olives with 'Mojo picón' aroma at 2%; M4: Spanish-style green table olives with 'Mojo picón' aroma at 4%; M8: Spanish-style green table olives with 'Mojo picón' aroma at 8%. RT = retention time. n.d.: not detected.

The main constituents of the volatile matrix in pure aroma were beta-pinene (19.7%), p-cymene (18.7%), gamma-terpinene (24.7%) and diallyl disulphide (14.6%). The aroma of these compounds is related to positive attribute such as fresh fruit, citrus, herbs and green onions. The main constituents of the green olives with 'Zapateria' defect were creosol (25.0%), propylene glycol (19.8%), 2,4-hexadienoic acid, ethyl ester (14.5%), cyclohexancarboxylic acid (12.7%) and 2.4-hexadienoic acid methyl ester (11.2%). The volatile compounds responsible for unpleasant odors were propylene glycol, cyclohexanecarboxylic acid, pentanoic acid and 2-decenal, (E)-. These compounds were not found at high concentrations, but they are related to 'Zapateria' spoilage [8,19,20]. Cyclohexanecarboxylic acid and pentanoic acid have been described by Sanchez et al. [15,22] as the molecules responsible for the unpleasant odor of 'Zapateria' of Spanish-style table olives. It should be noted that the odor of pentanoic acid is also related to the butyric defect as reported by previous studies [15].

However, these compounds decreased considerably in the altered olives, and other compounds appeared. Filling of defective olives with hydrocolloid caused the dilution of the aroma compounds linked to the olive defects, regardless of the concentration used. This effect was clearly observed even in the aroma at low concentration. The predominant volatile compounds in olives filled with aromatised hydrocolloid were practically the same as those present in the pure flavour of 'Mojo picón', but these aromas were at different concentrations.

2.3. Application of E-nose for the Discrimination of Stuffed Olives

The information obtained from the E-nose has multiple variables and, in order to be able to interpret them, it is necessary to reduce them as much as possible. Using Principal Component Analysis (PCA) we can go from 11 variables, one for each sensor, to two or three principal components (linear combination of variables). The Principal Components allow the graphical representation of the grouping of data with similar values.

PCA in E-nose applications has been widely used in food quality control [23], classification and quality control of edible oils [24] and the half-life of vegetable oils [25]. The unsupervised exploratory PCA, based on the first two components, was able to differentiate between stuffed olives without added flavouring and olives added with 'Mojo picón' flavouring at different concentrations (Figure 1). PC1 explained 64.0% of the total variance of the data, while PC2 explained 15.1%. These results coincide with those obtained by Sánchez et al. [22], who were able to separate stuffed black olives with and without added flavouring. In Figure 1, the scores close to each other represent observations with similar characteristics. There is proximity of the red points (C) and green points (M2). This indicates that 'Mojo picón' aroma added at a low concentration (2%) is not enough to fully achieve the objective of masking the initial aroma of table olives with a predominant 'Zapateria' defect. On the contrary, the light blue points (M8) with high concentration (8%) are further away from the control (C, red points) showing that the defect was efficiently masked by 8% 'Mojo picón' aroma. This evidence could be interesting for table olives producers to obtain a better product without organoleptic defects.

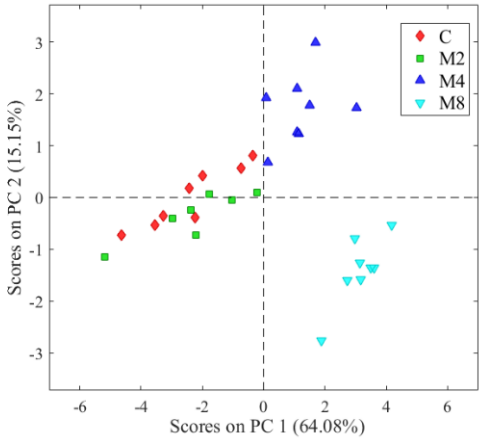

Figure 1. Score plot obtained from the PCA of olives stuffed with flavoured hydrocolloids at different concentrations. C: Spanish-style green table olives without aroma; M2: Spanish-style green table olives with 'Mojo picón' flavour at 2%; M4: Spanish-style green table olives with 'Mojo picón' flavour at 4%; M8: Spanish-style green table olives with 'Mojo picón' flavour at 8%. n.d.: not detected.

Subsequently, a classification analysis was performed by PLS-DA using leave-one-out cross-validation. The PLS-DA algorithm has previously been used to classify post-harvest olive fruit quality [21] and discrimination of extra virgin olive oils [26]. The confusion matrix of the PLS-DA model (Table 3) shows that the sum of the diagonal elements gave a hit rate of 93.8%.

Table 3. Confusion matrix obtained through PLS-DA for discrimination between stuffed olives with flavoured hydrocolloids. Values are expressed in percentage.

	Predicted Class			
Real Class	**C**	**M2**	**M4**	**M8**
C	21.9	3.1	0	0
M2	3.1	21.9	0	0
M4	0	0	25.0	0
M8	0	0	0	25.0

Eight samples from each class were used to construct the model. In the confusion matrix, 3.1% of the samples belonging to groups C and M2 were wrongly classified between the two groups. This may be due to the fact that the amount of 'Mojo picón' flavour

added to the filling of M2 was too low for all samples to be classified correctly. On the other hand, groups M4 and M8 were successfully predicted with an accuracy of 100%.

2.4. Quantification of Sensory Parameters Using E-nose

One way to study the correlation between the data obtained from the E-nose and the tasting panel is Partial Least Squares (PLS) chemometric analysis. This regression model made it possible to quantify and establish prediction models, i.e., from the E-nose and the chemometric model, to obtain sensory analysis values equivalent to those provided by a panel of olive tasters. In this study, the sensory descriptors of the 'Zapateria' defect were used.

To obtain the PLS model, 70% of the samples set was split into a calibration set, which was used to calibrate and cross-validate the models. Furthermore, a validation set with the remaining samples (30%) was only used to test the robustness and accuracy of the developed models. Two different PLS models were built, one for each parameter. The R^2_{CV} for the models developed for the perceived defect and the overall rating were 0.83 and 0.88, respectively. The RMSECV values (0.78 for the 'Zapateria' defect and 1.10 for perceived aroma) were also estimated.

The cross-validation shown in Figure 2 represents the experimental values of the sensory parameters against the predicted values.

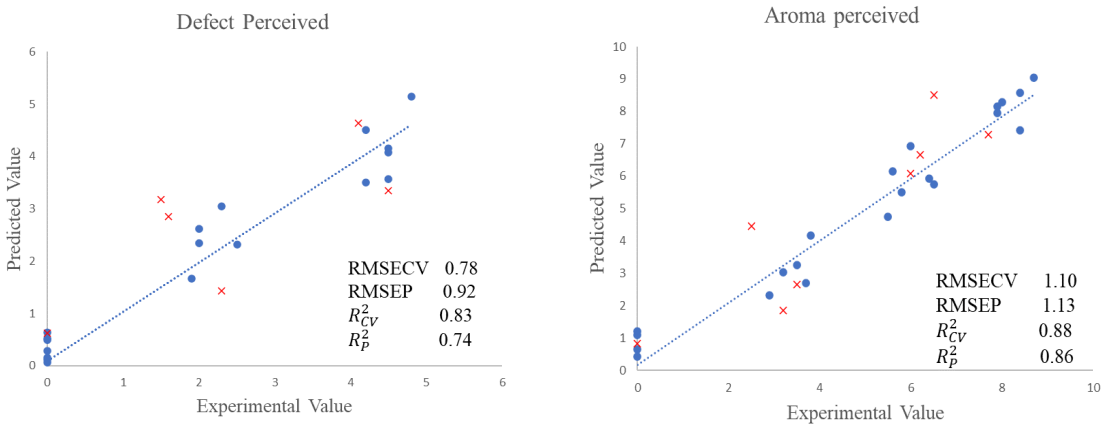

Figure 2. Experimental values against PLS cross-validation predictions (●) and validation set predictions (x) for defect and aroma perceived.

In the PLS graph corresponding to the perceived defect, samples with experimental values of zero can be observed. This is due to the fact that at higher concentrations of added 'Mojo picón' aroma, the 'Zapateria' defect was completely masked. With regard to the graph of perceived 'Mojo picón' aroma, the experimental values of zero correspond to the control group samples, to which no aroma was added to the filling.

The predictive ability of a calibration model must be validated with samples not included in the initial calibration. The validation results are also presented in Figure 2 and were very acceptable. The R^2_P values were 0.74 for perceived defect and 0.86 for perceived aroma, while the RMSEP values were 0.92 and 1.13 for perceived defect and perceived aroma, respectively.

The application of this type of PLS algorithm, in which the E-nose values of perceived defects and aromas in table olives samples are predicted, has been described before: the impact of sterilisation treatments on the effect of cooking in table olives was studied [27].

3. Materials and Methods

3.1. Experimental Design and Sample Preparation

The experimental design is illustrated in Figure 3.

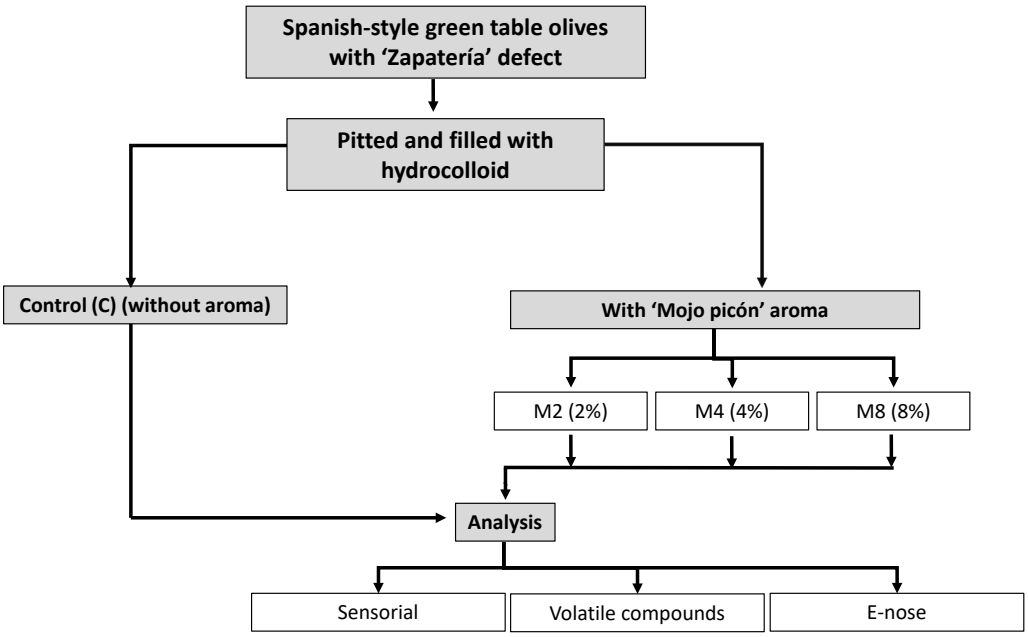

Figure 3. Diagram of the experimental design.

Spoilt Spanish-style green table olives were supplied by a company in southwest Spain. The table olives were first identified and sensory classified by the tasting panel. Then, the olives were pitted for the subsequent stuffing. The different hydrocolloid fillings were prepared with a water base added with 2% sodium alginate, 1% guar gum and 2, 4 and 8% 'Mojo picón' commercial flavouring (Neroliane, Grasse, France) according to Sánchez et al. [22]. This flavour is a hot sauce prepared with garlic, cumin, sweet paprika, chilli pepper, sea salt, olive oil and vinegar [28]. The additives were added with 50 mL of water and mixed. All the filling ingredients were food grade. In total, 100 olives were filled manually with a syringe. Four groups of samples were made: (i) table olives stuffed with the hydrocolloid but no 'Mojo picón' flavouring has been added. (C); (ii) olives stuffed with 2% of 'Mojo picón' flavour (M2); (iii) olives stuffed with 4% of 'Mojo picón' flavour (M4); (iv) olives stuffed with 8% of 'Mojo picón' flavour (M8). The olives were then soaked for 24 h in a 0.25% $CaCl_2$ solution. Subsequently, the olives for each group were placed in jars containing 25 stuffed olives and 100 mL of brine (3% NaCl, *w*/*v*). Finally, the jars were pasteurised at 80 °C for 20 min.

3.2. Analyses

3.2.1. Sensory Analysis

A tasting panel formed according to IOC recommendations [1] consisted of eight experts from the research centre CICYTEX (Extremadura, Spain) and the University of Extremadura. For the sensory analysis of the table olives, an evaluation score board was prepared on a structured scale from 1 to 11 points based on perceived positive ('Mojo picón') and negative ('Zapateria') odors. The used standard of 'Zapateria' was cyclohexanecarboxylic acid at different concentrations. The tasting panel was also trained with defected table olives samples given by IOC interlaboratory exchange. The commercial pure aroma of 'Mojo picón' was also used as a standard for panel training. The trained panel rated the intensity of the aroma attributes. The results were expressed as average values.

For the sensory analysis, the samples were placed in standard glasses according to IOC recommendations [1]. Each sample consisted of three stuffed olives and 10 mL brine. Eight sample replicates were prepared for each group. In total, 32 samples were analysed.

3.2.2. Analysis of Volatile Compounds

Stuffed green olives were analysed by chromatography for the determination of volatile organic compounds (VOC). The olive samples were crushed and homogenised. A 2.0 g aliquot of each sample was placed in a vial, to which 7 mL of NaCl solution (30% *w*/*v*) was added. A polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex fibre (65 µm, Supelco) was used to sample the volatile compounds. The vials were closed and placed at 40 °C for 30 min according to the methodology described by Sánchez et al. [15] and López-López et al. [29]. Determinations were performed using a gas chromatograph with a triple quadrupole mass spectrometry detector model 456-GC, using a capillary column Agilent DB WAXetr (60 m × 0.25 mm; DI: 0.25 mm). Peaks were identified with the help of the NIST 2.0 MS reference spectral library.

3.2.3. E-nose System

The E-nose used for this work was designed by the research team in Sensory Systems from the University of Extremadura. It consists of a combination of 11 commercially available metal oxide semiconductor (MOX) sensors with global selectivity from the following manufacturers: (i) Bosch BME680: temperature (°C), pressure (hPa), humidity (%RH) and gas measurement (Ω); (ii) Sensirion SGP30: eCO_2 (ppm), TVOC (ppb), H_2(2) and ethanol; (iii) ScioSense CCS811: eCO_2 (ppm), TVOC (ppb) and sensor resistance (Ω); and (iv) ScioSense iAQ-Core: eCO_2 (ppm), TVOC (ppb) and sensor resistance (Ω). These devices are characterised by the integration of analogue and digital electronics combined with a hot microboard and with the sensing elements on a single chip. The power supply consisted of a +3.7 V lithium battery and communicated via Bluetooth with a mobile phone application. The E-nose measurements took place in two phases: an adsorption phase, in which the sensors are placed in contact with the headspace of the samples for 60 s, and a 30 s desorption phase, in which the sensors are only placed in contact with air, which serves as a reference signal.

3.3. Multivariate Data Analysis

The measurements obtained with E-nose had to be processed with chemometric tools for their interpretation. To identify outliers and study the discrimination of potential groups, an exploratory data analysis was first performed using Principal Component Analysis (PCA). This type of analysis reduces the information provided by the E-nose to the minimum number of variables, called Principal Components. Principal Components are linear combinations of the original response vectors. Then, Partial Least Squares Discriminant Analysis (PLS-DA) [30] was performed to identify the components or latent variables (LV) discriminating the most between the different sample groups. A confusion matrix was constructed to deduce cross-validation predictions. From this matrix, the percentage of correct predictions of the sum of the diagonal elements found was calculated.

Another chemometric tool used was Partial Least Squares Regression (PLS), to build quantification models and assess the correlation between E-nose measurements and tasting panel parameters [31]. For this purpose, samples were randomly divided between two sets, one for calibration containing 70% of all samples and a second set for validation containing 30% of the remaining samples. The validation set was used to test the accuracy of the developed models.

The parameters used to assess the accuracy of the models were the root mean square error of calibration (RMSEC), cross-validation (RMSECV) and prediction (RMSEP) and the coefficient of determination for cross-validation (R^2_{CV}) and prediction (R^2_P). Data analysis was

performed using Matlab version R2016b, version 9.1 (The Mathworks Inc., Natick, MA, USA) with PLS_Toolbox 8.2.1 (Eigenvector Research Inc., Wenatchee, WA, USA).

4. Conclusions

Spanish-style green table olives stuffed with 'Mojo picón' flavoured hydrocolloid improve the commercial category of olives with the 'Zapateria' defect. The high concentrations of added flavour meant that the defect was almost imperceptible to the tasters. The main volatile compounds of the 'Zapateria' defect, which are cyclohexanecarboxylic acid and pentanoic acid, decreased as the percentage of added flavouring increased. The E-nose discriminates between olives with 'Zapateria' defect and stuffed olives with different concentrations of added 'Mojo picón' flavouring. The PLS algorithm shows that there is a good prediction between E-nose and sensory analysis. This makes it possible to predict the 'Zapateria' defect perceived by the tasting panel.

In the present study, E-nose was successfully used for qualitative purposes and quantitative analysis. Therefore, E-nose can be considered a useful discrimination tool that can be applied to olives stuffed with flavoured hydrocolloids. This device can be used as an aid to the tasting panel and combined with chemometric analysis, can be used to perform rapid, inexpensive, non-destructive and environmentally friendly qualitative analysis.

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2.4 Artículo 4. "Application of digital olfaction for table olive industry. (2022). Sensors, 22, 5702."

Sánchez, R., Fernández, A., Martín-Tornero, E.; Meléndez, F., Lozano, J., Martín-Vertedor, D.

2.4.1 Motivación y objetivos

El estilo de elaboración más frecuente es el "estilo español", que consiste en tratar la aceitunas con sosa cáustica para eliminar el amargor. A continuación, se colocan en una solución de NaCl donde durante varios meses tiene lugar un proceso de fermentación. Durante la fermentación existen algunos puntos críticos en los que pueden ocurrir anomalías, dando lugar a aceitunas de mesa defectuosas que pueden provocar pérdidas económicas elevadas para los productores.

Hoy en día solo las aceitunas con defectos físicos se clasifican como aceitunas extra, de primera o de segunda categoría (R.D. 679/2016, 2016). Si bien, la obligatoriedad de ausencia de sabores, olores extraños o síntomas de fermentaciones anormales o alteraciones en curso está presente en la normativa vigente, por lo que el análisis sensorial debería ser obligatorio para la clasificación de las aceitunas de mesa. Sin embargo, el protocolo de análisis sensorial para aceitunas de mesa del COI no es obligatorio sino solo una recomendación (IOC, 2021). Las malas prácticas industriales son las causas más habituales de estos defectos, facilitando el desarrollo de procesos de fermentación descontrolados. Por esa razón, ciertos defectos olfativos como la *zapatería,* las sensaciones *mohosas, butíricas, rancias* o *avinagradas* deben ser clasificados por el panel sensorial (Lanza y Amoruso, 2020; Martín-Vertedor et al., 2021). Según el COI, el defecto de *zapatería* recuerda olor a cuero podrido, el defecto *mohoso* o de humedad genera olor a moho, el *butírico* recuerda al olor de la mantequilla o queso rancios y finalmente, el defecto *pútrido* es responsable del olor a materia orgánica en descomposición.

El análisis cromatográfico de los COVs también puede ayudar a identificar los compuestos responsables de la fermentación anormal (Xu et al., 2021). Sin embargo, el análisis sensorial basado en un panel de expertos y la caracterización de la fracción volátil de las aceitunas fermentadas en base a la cromatografía de gases son procesos laboriosos, costosos y lentos que requieren equipos complejos y/o personal cualificado. Por tanto, es relevante desarrollar una técnica rápida y fiable para diferenciar las aceitunas de mesa en función de sus propiedades organolépticas.

Las fermentaciones anormales deben ser identificadas para percibir el defecto temprano y tener la oportunidad de controlarlas.

En la actualidad, la instrumentación electrónica está surgiendo para proponer nuevas formas de medición no destructivas, rápidas, de bajo costo y respetuosas con el medio ambiente. En ese sentido, la *nariz electrónica* es un poderoso dispositivo sensorial capaz de discriminar perfiles de aroma de diferentes matrices (Martínez-García et al., 2021). Este dispositivo también se ha utilizado para clasificar aceitunas frescas (Martínez et al., 2020) y, puede ser una alternativa al panel de cata, eficaz para la detección temprana de olores desagradables originados por este tipo de fermentaciones anormales, sobre todo a escala industrial. La identificación temprana de olores desagradables puede ser útil para corregirlos antes de que las aceitunas se vuelvan inaceptables y no comercializables.

Por lo tanto, el objetivo del presente estudio fue desarrollar una metodología funcional con la *nariz electrónica* para discriminar muestras sanas y no sanas (este último grupo dividido en primera y segunda categoría) de aceitunas de mesa al "estilo español" según sus atributos sensoriales.

2.4.2 Diseño experimental

Se recolectaron aceitunas de la variedad *'Carrasqueña'*, recolectadas en estado verde de maduración durante la campaña 2021/2022. Se procesaron en una planta piloto según el protocolo al "estilo español" (Schaide et al., 2019). Las aceitunas se introdujeron en un fermentador con capacidad de 236 L. Las aceitunas se sometieron a tratamiento con disolución de NaOH y después, se lavaron con agua. Finalmente, se sumergieron en pequeños fermentadores de 10 L de capacidad con salmuera al 5,5% de NaCl. Se realizó una fermentación espontánea durante 121 días para estimular alteraciones en las aceitunas (Schaide et al., 2019). Cada semana se tomaron muestras de aceitunas y se realizó un análisis sensorial por el panel de cata con el objetivo de identificar aceitunas con fermentación anormal. Cuando las aceitunas presentaron algún defecto, se mantuvieron en refrigeración (4°C) hasta la realización del análisis.

También se almacenaron muestras con fermentación controlada (control). A las 81 muestras seleccionadas previamente mediante el análisis sensorial, se les realizó al análisis de compuestos volátiles, y por último, el análisis con la *nariz electrónica*.

2.4.3 Resumen de los resultados y discusión

Las aceitunas de mesa al "estilo español" fueron clasificadas sensorialmente (IOC, 2021) en diferentes categorías según el defecto predominante percibido (DPP) por el panel de cata. Las muestras de aceitunas de mesa sin ninguna alteración sensorial se clasificaron como categoría extra. Sin embargo, otras mostraron varios defectos que están relacionados con una fermentación anormal. El primer lote de muestras tenía un rango de concentración de defectos de 3,8-4,1 y se clasificaron en la primera categoría sensorial ($3,0 < DPP \leq 4,5$). Los principales defectos descritos en diferentes muestras por panelistas fueron *zapatería, butírico* y *pútrido*. Posteriores lotes presentaron un rango de defecto entre 5,9 a 6,2, con defecto a *zapatería* y *pútrido*. Estas aceitunas se clasificaron en la segunda categoría ($4,5 < DPP \leq 7,0$).

Cabe destacar que las aceitunas estudiadas podrían comercializarse legalmente a pesar de las importantes alteraciones sensoriales (IOC, 2021). Por tanto, estas aceitunas que no fueron controladas durante la fermentación presentaron fermentaciones anómalas que provocaron un perfil sensorial marcado con defectos de diferente intensidad. En este sentido, los investigadores Marx et al. (2017) evaluaron aceitunas de mesa de diferentes empresas mediante un panel de cata, detectando defectos de diferente intensidad como *butírico, pútrido, zapatería, moho* o *avinagrado*.

En las muestras analizadas se identificaron diferentes COVs. Los principales en las aceitunas sanas fueron creosol (48,1%), 2-Etenil-1,1-dimetil-3-metileno-ciclohexano (18,0%), ácido acético (9,6%), alcohol feniletílico (9,7%) y ácido benzoico (8,6%). No obstante, algunos de estos compuestos volátiles, como el ácido acético, creosol o ácido benzoico, disminuyeron considerablemente, especialmente en aceitunas con alta intensidad de defecto. El alcohol feniletílico es un producto que suele formarse en fermentaciones alcohólicas (Fischer y Pietruszka, 2010), presentando fragancias que recuerdan a

manzanas o plátanos (Wang et al., 2022). Estos compuestos aromáticos también aparecen en aceitunas alteradas, pero en menor proporción.

Los principales constituyentes de las muestras con *zapatería* fueron ácido butanoico (14,6-22,8%), propilenglicol (9,6-15,9%), ácido (E)-3-hexenoico (8,2-14,4%), ácido hexanoico (1,8-5,5%), ácido ciclohexanocarboxílico (1,8-7,4%) y ácido pentanoico (ácido valérico) (3,2-4,5%). El defecto *pútrido* también presentó un perfil volátil particular. El principal constituyente presente en las aceitunas de mesa fue el ácido propanoico (17,2-23,4%), alcohol isopropílico (17,2-21,3%), 2,4-dimetilheptano (15,4-17,9%) y alcohol feniletílico (12,0-21,3%). 19,6%). Los COVs responsables del defecto *butírico* fueron el ácido butanoico (40,7%), ácido pentanoico (11,8%), ácido propanoico (3,6%) y butan-2-ol (4,6%). Estos compuestos presentaron una mayor proporción en las muestras clasificadas en la segunda categoría. Cabe reseñar que determinados COVs sólo se detectan en algunos de los defectos estudiados. Es el caso del (Z)-3-hexen-1-ol y el 2-etenil-1,1-dimetil-3-metileno-ciclohexano en aceitunas sanas, el propilenglicol o el ácido (E)-3-hexenoico en aceitunas con *zapatería*, alcohol isopropílico en aceitunas *pútridas* o el butan-2-ol en aceitunas de mesa con defecto a *butírico*. Estos resultados están de acuerdo con estudios previos (Panagou et al., 2008; Montaño et al., 1992; de Castro et al., 2018). El ácido butanoico, pentanoico, propanoico y el butan-2-ol son COVs presentes en el defecto *butírico* y *zapatería* y el ácido propanoico incluso en el defecto *pútrido*. Estos ácidos carboxílicos están asociados con el olor a *rancio* (ácido propanoico) y el olor a mantequilla y queso (ácido butanoico) (Liu et al., 2022).

Los datos del perfil de volátiles de muestras de aceitunas de mesa con diferentes defectos se analizaron mediante PCA. El modelo basado en los dos primeros componentes explicó el 66,4% de la varianza total de las muestras, y representó una clara diferenciación de las muestras según sus características olfativas, y separando aceitunas sanas de aquellas con alteraciones durante el proceso de fermentación. Se utilizó la *nariz electrónica* para clasificar las aceitunas de mesa previamente analizadas por el panel de cata. La respuesta de los sensores a

la misma muestra fue diferente, por lo se puede describir un patrón olfativo con la combinación de las 11 señales. Para representar los patrones de respuesta de los sensores a las categorías extra, primera y segunda, se dibujó un gráfico radial donde pudo verse claramente que la amplitud de las señales de las aceitunas de mesa extra son generalmente de mayor magnitud que las señales de las aceitunas clasificadas en primera y segunda categoría. Las curvas que representan las categorías son claramente diferentes entre sí.

Los datos de la *nariz electrónica* de las tres categorías también se analizaron mediante PCA. Los dos primeros componentes principales fueron suficientes para explicar el 77% de la varianza total de los datos. Los gráficos mostraron una clara discriminación entre aceitunas extra y, de primera y segunda categoría. Tras los reveladores resultados obtenidos en el PCA, se realizó un análisis de clasificación supervisado mediante PLS-DA y validación cruzada "leave-one-out". Los resultados representados en la matriz de confusión dieron una tasa de éxito del modelo de clasificación del 100%.

Existen pocas referencias sobre la discriminación de aceitunas sometidas a fermentaciones anormales utilizando la *nariz electrónica*. Algunos investigadores han discriminado con la *nariz electrónica* aceitunas de mesa al "estilo español" inoculadas con diferentes mohos alteradores (Sánchez et al. 2022c) y aquellas con fermentación anormal con defecto sensorial a *zapatería, butíricas* y *pútridas* (Sánchez et al., 2021a). Otros autores presentaron un sistema de *nariz electrónica* capaz de discriminar diferentes especies de hongos (Loulier et al., 2020; Mota et al., 2021). Por lo tanto, este equipo podría implementarse en la industria de la aceituna de mesa debido a su fácil instalación, pequeño tamaño, rapidez, precisión y bajo costo de análisis.

2.4.4 Conclusiones

- Las alteraciones sensoriales percibidas por los catadores fueron *zapatería, butírico* y *pútrido*, siendo las muestras clasificadas en diferentes categorías comerciales según los criterios establecidos por el COI.

- Algunos de los volátiles presentes en las aceitunas de categoría extra como el ácido acético, creosol o ácido benzoico, disminuyen considerablemente en las aceitunas con alta intensidad de defecto.

- El perfil de volátil característico para cada defecto son el propilenglicol, el ácido 2,4-hexadienoico metil ester, ácido hexanoico, el ácido (E)-3-hexanoico y el ácido ciclohexanocarboxílico en el defecto a *zapatería* y el 2-butanol en el defecto *butírico* y en el alcohol isopropílico en el *pútrido*.

- La clasificación realizada quimiométiricamente con las medidas de la *nariz electrónica* coincidió con la clasificación obtenida por los panelistas y fue coherente con la clasificación realizada quimiométricamente de los volátiles detectados.

- Aplicar este dispositivo en la industria para el control precoz de alteraciones durante la fermentación, sería una estrategia eficaz para mejorar la calidad de las aceitunas de mesa.

sensors

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Article

Application of Digital Olfaction for Table Olive Industry

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Abstract: The International Olive Council (IOC) established that olives must be free of odors, off-flavors, and absent of abnormal ongoing alterations or fermentations. The use of electronic devices could help when classifying defects in a fast, non-destructive, cheap, and environmentally friendly way. For all of that, table olives were evaluated according to IOC regulation in order to classify the defect predominant perceiving (DPP) of the table olives and their intensity. Abnormal fermentation defects of Spanish-style table olives were assessed previously by an IOC-validated tasting panel. 'Zapateria', 'Putrid', and 'Butyric' were the defects found at different concentrations. Different volatile compounds were identified by gas chromatography in altered table olives. The same samples were measured with an electronic nose device (E-nose). E-nose data combined with chemometrics algorithms, such as PCA and PLS-DA, were able to successfully discriminate between healthy and non-healthy table olives, being this last one also separated between the first and second categories. Volatile compounds obtained with gas chromatography could be related to the E-nose measuring and sensory analysis, being capable of matching the different defects with their correspondents' volatile compounds.

Keywords: E-nose; digital olfaction; volatile compounds; sensory analysis; table olives

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1. Introduction

Spain is the leading country in table olive production, being present in almost all countries such as Argentina, Peru, Portugal, Egypt, Morocco, and Turkey. The table olive sector is highly important to the agri-food industry due to job generation and production volume, transformation, commercialization, and exportation activities [1].

The most frequent elaboration style is "Spanish-style", which consists in treating *Olea europea* spp. Fruits with caustic soda in order to eliminate bitterness. After that, table olives will be fermented for several in a NaCl solution. As the fermentation goes by, there are some critical points in months which abnormalities could happen, leading to defective table olives. These unexpected processes are one of the main causes of high economic loss for table olive producers.

Nowadays, only physical defects are categorized as extra, first, or second category olives [2]. Although, the absence of strange flavors, odors, or symptoms of abnormal fermentations or ongoing alterations is a compulsory statement present in the current regulation. For that reason, the performance of sensory analysis by a well-trained tasting panel and validated by IOC [3] should be accomplished for classification purposes. Nevertheless, the IOC protocol is not mandatory but only a recommendation. Certain olfactory defects such as zapateria, musty, butyric, rancid, or vinegary sensations should be classified

by the sensory panel [4,5]. Zapateria defect is the predominant sensation perceived by the tasters when present in table olives. A combination of volatile fatty acids generated during abnormal fermentations is responsible for this particular rotten leather sensation, according to IOC. Musty or humidity defect generates mold smell. The butyric defect is a butter or cheese off-flavor. Finally, the putrid defect is responsible for the decaying organic matter odor. Bad industrial practices are the most usual causes of these defects, facilitating uncontrolled fermentation process development.

Chromatographic analysis of volatile compounds can also assist in identifying responsible compounds for abnormal fermentation [6–9]. However, sensory analysis based on an expert panel and volatile fraction characterization of fermented olives based on gas chromatography are laborious, costly, and time-consuming processes requiring complex equipment and/or qualified personnel. Therefore, it is relevant to develop a fast and reliable technique to differentiate table olives based on their organoleptic properties. Abnormal fermentations should be identified by this protocol in order to perceive the early defect and have the opportunity of controlling them.

Nowadays, electronic instrumentation is emerging to propose new ways of non-destructive, fast, low-cost, and environmentally friendly measurement. In that sense, the electronic nose (E-nose) is a powerful sensory device that is able to discriminate aroma profiles of different matrices [10–13]. This device has also been used to classify olives on olive trees [14]. In this respect, the E-nose may be an effective alternative to identify different types of abnormal fermentations in table olives on an industrial scale. Non-destructive E-nose could be complementary to the tasting panel. Early off-flavor identification can be useful to correct them before the olives become unacceptable and unmarketable.

The development of a functional E-nose methodology to discriminate between healthy and non-healthy samples (this last group is divided into first and second categories) of Spanish-style table olives according to their sensory attributes has been performed. Data were contrasted with gas chromatography and sensory analysis of the table olives by the testing panel. The novelty that this work is the portability and adaptation of the E-nose to the standard olive tasting cups, which allow the reproduction of the evaluation protocol of sensory attributes recommended by the IOC of which table olives can be classified into quality categories. Having an objective instrument to classify in categories accessible to the table olive industry could lead to the consequent improvement in table olives quality. Thus, the aim of this work was to develop a quality control methodology in the industry capable of discriminating between healthy and unhealthy samples, as well as classifying them into different categories according to their sensory attributes with a digital olfaction device.

2. Materials and Methods

2.1. Experimental Design

Olives (*Olea europaea* L.) of the "Carrasqueña" variety were obtained in a research field of the CICYTEX research center (Badajoz, Spain). Olives were harvested at the green stage of maturation during the 2021/2022 campaign. They were processed in a semi-scale station according to the Spanish-style protocol [15].

The product was introduced into a fermenter with the capacity of 236 L. Olives were submitted to lye treatment, and after all, the product was washed with water and immersed into small fermenters of 10 L of capacity with brine at 5.5% NaCl (Sigma-Aldrich, St. Louis, MO, USA). Spontaneous microbiological fermentation was carried out for 121 days [15]. Each week, the tasting panel in charge of the assay performed a sensory analysis to discriminate between olives without abnormal fermentations of the ones that have them. Samples that did not present a clear defect were discarded by the panelists. Thus, the number of samples previously selected by the tasting panel with natural microbiological alteration was 81.

The samples were stored in refrigeration (4 °C) when the target defect was perceived until the analysis was performed. A sample with controlled fermentation was also stored (control). A diagram of the overall experimental design is shown in Figure 1.

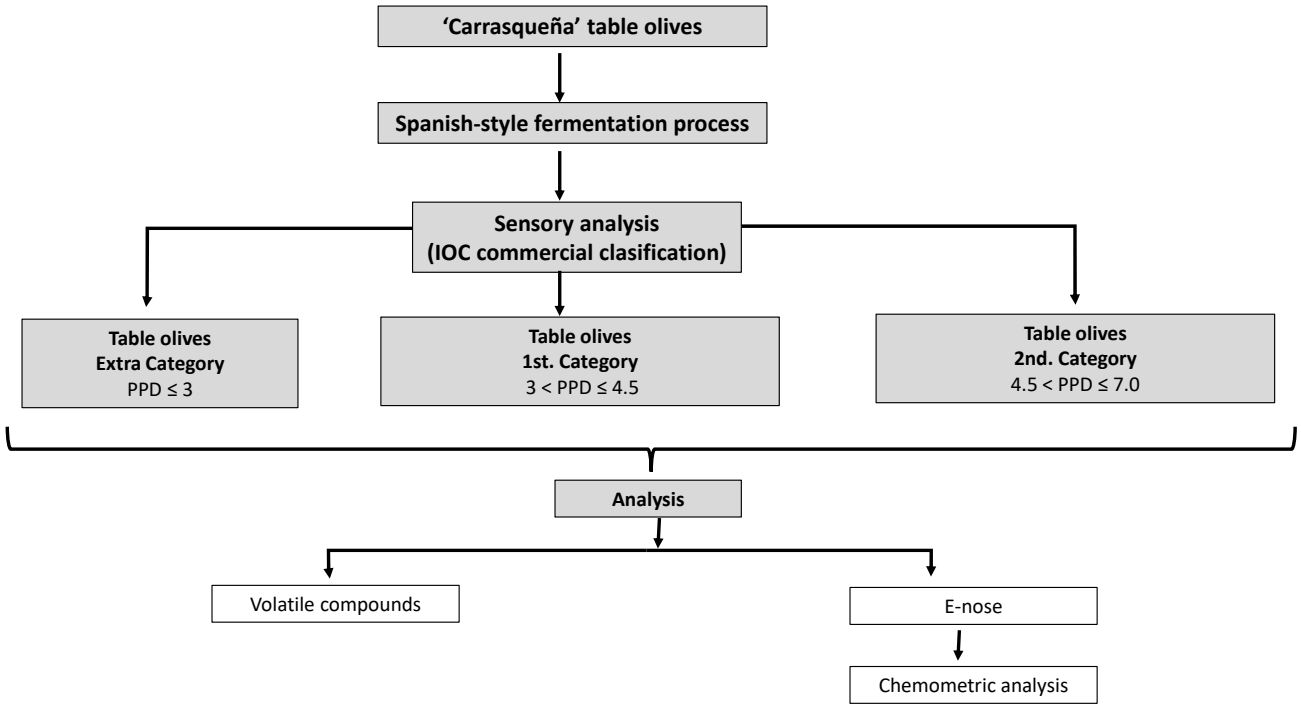

Figure 1. Diagram of the overall experiment design.

2.2. Analyses

2.2.1. Sensory Analysis

The variety used for this assay was 'Carrasqueña', a typical Spanish table olive. These samples were analyzed by a well-trained sensory panel from CICYTEX-INTAEX Research Center (Extremadura, Spain). A standard glass jar was used to place table olives inside in at the same height, filling them with 10 mL of brine. On a well-defined scale from 1 to 11, abnormal fermentations perceived by the panelists were evaluated in terms of intensity and off-odor perception. The results were displayed as mean defect values, considering them acceptable when the variation coefficient was less than 20. Finally, table olives were categorized according to the quality classification elaborated by the IOC [3]. A total of 81 samples were studied.

For the statistical analysis, a one-way ANOVA was performed plus Tukey's multiple range test to establish statistically significant differences between the different samples. Significance was set at $p < 0.05$. The software used was SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean and standard deviations (SD).

2.2.2. Analysis of Volatile Compounds

A triple quadrupole gas chromatograph (Scion 456-GC, Bruker, Madrid, Spain) followed the method described by López-López et al. [7] and was used to analyze volatile compounds by triplicate. SPME was used to sample from the headspace (40 °C for 15 min) with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex fiber (65 µm, Supelco, Madrid, Spain). Once desorption occurred at the injection port of the gas chromatograph (250 °C for 15 min), the components were divided using a capillary column (30 m × 0.25 mm, ID: 0.25 mm, VF5MS, Agilent, Madrid, Spain). NIST 2.0 MS library was used to analytes identification through mass fragmentation comparative analysis.

2.2.3. E-Nose Analysis

The E-nose used in this work was designed by the University of Extremadura and consisted of an array of 11 metal oxide (MOX) sensors. These sensors are distributed on four chips previously explained in previous research [13]. The microprocessor receives the signal from the sensors, processes them, and sends the data to a smartphone via Bluetooth.

A response value of the sensor array is obtained every second. Thus, to characterize the sensor response curves, the formula of the maximum signal value minus the minimum signal value multiplied by 100 and subtracted by: (MAX − MIN) × 100 − 1) was used. As a result, a data vector with 11 rows (sensors) was obtained for each sample. Finally, they are transferred to the computer for chemometric analysis. The measurements and data obtaining with the electronic devise are shown in https://susy.mdpi.com/user/submission/video/5f6c41e532625088e8bf165b38a5ce18.

This device described in more detail by Arroyo et al. [13] has low power consumption and is the size of a hockey puck, making it easy to transport. This array of sensors has been used previously by the authors [13,16,17]. The measurements with the E-nose were performed following the same protocol as the table olive tasting panel that is based on IOC recommendations [3]. Three olives with about 10 mL of brine were placed in standard tasting glasses, covered with a watch glass, and placed in a block thermostat at 25 °C. One tasting cup remained empty. Each data acquisition cycle consisted of two parts, one adsorption, and one desorption. Alternately, the E-nose was placed in the cups with samples and air. First, 30 s in the cup with air to achieve desorption of volatiles on the sensors and return the signal to baseline. Next, the E-nose measured the volatiles in the headspace of the samples for 60 s, and the signals from the sensors were recorded.

2.2.4. Multivariate Data Analysis

E-nose data were first analyzed using principal component analysis (PCA) to perform exploratory analysis. This algorithm minimizes the dimensionality of the variables to a smaller number, denominated principal components. The aim of this unsupervised method was to detect outliers and recognize patterns or cluster formation. As the variables were measured in different units, the original variables were autoscaled. Subsequently, the supervised classification analysis called partial least squares discriminant analysis (PLS-DA) [18] was applied to build a classification model. This algorithm identifies the components or latent variables (LV) that most discriminate between the different groups of samples. These variables were selected by cross-validation with the leave-one-out procedure. A supervised classification requires prior knowledge of the class of each sample, and that was obtained with the previous classification of the tasting panel. Specifically, a model was developed to discriminate between table olive samples belonging to three different categories. The confusion matrix of the model was constructed to represent the prediction success. The proportion of the correct predictions was calculated from the sum of the diagonal elements found in the confusion matrices.

Data analysis was performed using Matlab R2016b version 9.1 (The Mathworks Inc., Natick, MA, USA) with PLS_Toolbox 8.2.1 (Eigenvector Research Inc., Wenatchee, WA, USA).

3. Results

First, the organoleptic profile and the volatile compounds of the samples studied are described. Next, the results of E-nose technology are shown to distinguish between table olives' health and those with different defects. Finally, an experimental model was developed with the data provided with E-nose to classify olives according to their quality.

3.1. Sensory Analysis of Spanish-Style Table Olives

Spanish-style table olives were sensorially classified into different categories according to the defect predominant perceived (DPP) by the tasting panel (Table 1). Table olives samples without any sensory alteration were classified as the "Extra" category. However, other samples showed several defects that are related to abnormal fermentation. The first batch of samples had a defect concentration range of 3.8–4.1. The main defects described in different samples by panelists were 'Zapateria', 'Butyric', and 'Putrid'. These olives were classified in the first commercial category ($3 < DPP \leq 4.5$). As can be seen, other samples presented a range of defects between 5.9 and 6.2 being the DPP 'Zapateria' and 'Putrid'. These olives were classified into a second category ($4.5 < DPP \leq 7.0$).

Table 1. Defect predominantly perceived (DPP) of Spanish-style table olive. Different lowercase letters mean a statistically significant difference between altered olives within the same category (one-way ANOVA followed by Tukey's test, $p < 0.05$). Different uppercase letters mean a statistically significant difference between each altered olive in different categories (one-way ANOVA followed by Tukey's test, $p < 0.05$).

	Extra $\mathbf{DPP \leq 3}$	**1st Category** $\mathbf{3 < DPP \leq 4.5}$			**2nd Category** $\mathbf{4.5 < DPP \leq 7.0}$	
Sensory evaluation	'No defect' n.d. $n = 18$	'Zapateria' 4.1 ± 0.9 ns A $n = 15$	'Putrid' 3.8 ± 0.7 ns A $n = 15$	'Butyric' 3.9 ± 0.8 ns $n = 15$	'Zapateria' 6.2 ± 0.8 ns B $n = 9$	'Putrid' 5.9 ± 0.9 ns B $n = 9$

n.d., not detected; ns: not significant differences.

3.2. Volatile Profile of Spanish-Style Table Olives

Table olives altered with different types and intensities of defects were analyzed to determine the volatile compounds profile (Table 2). Different volatile compounds were identified in altered table olives. The main volatiles compounds in healthy table olives were creosol (48.1%), 2-Ethenyl-1,1-dimethyl-3-methylene-cyclohexane (18.0%), acetic acid (9.6%), phenylethyl alcohol (9.7%), and benzoic acid (8.6%). Nevertheless, some of these volatile compounds decreased considerably in altered olives. That is the case of acetic acid, creosol, or benzoic acid, whose concentration decreases, especially in olives with a high intensity of defect. Regardless of the intensity of the defect found, other different compounds appear. The main constituents of 'Zapateria' samples were butanoic acid (14.6–22.8%), propylene glycol (9.6–15.9%), (E)-3-hexenoic acid (8.2–14.4%), hexanoic acid (1.8–5.5%), cyclohexanecarboxylic acid (1.8–7.4%) and pentanoic acid (valeric acid) (3.2–4.5%). The 'Putrid' defect also presented a particular volatile profile. The major constituent present in table olives were propanoic acid (17.2–23.4%), isopropyl alcohol (17.2–21.3%), 2,4-dimethyl-heptane (15.4–17.9%), and phenylethyl alcohol (12.0–19.6%). The volatile compounds responsible for the 'Butyric' defect were butanoic acid (40.7%), pentanoic acid (11.8%), propanoic acid (3.6%), and butan-2-ol (4.6%). These compounds presented a higher content in samples classified in the second category.

Table 2. Relative percentage of volatile compounds obtained from altered Spanish-style table olives classified into different commercial categories. RT, retention time.

RT (min)	**Volatile Compounds**	**Extra**	**1st. Category**			**2nd. Category**	
			'Zapateria'	**'Putrid'**	**'Butyric'**	**'Zapateria'**	**'Putrid'**
1.8	Isopropyl alcohol			17.2 ± 1.3 *			21.3 ± 4.2 *
2.4	Butan-2-ol				4.5 ± 0.6 *		
2.7	Acetic acid	9.6 ± 1.5	8.3 ± 1.2	5.4 ± 0.7	7.5 ± 1.2	1.9 ± 0.5	
4.8	2-methyl-butan-1-ol			5.2 ± 0.8			6.5 ± 2.2
4.9	Propanoic acid		0.9 ± 0.2	17.2 ± 7.4	3.6 ± 0.8	2.6 ± 0.6	23.4 ± 8.7
5.8	Propylene glycol		9.6 ± 1.1 *			15.9 ± 5.8 *	
6.7	2,4-dimethyl-heptane	1.3 ± 0.5		15.4 ± 5.7			17.9 ± 8.6
8.2	Butanoic acid		14.6 ± 4.6		40.7 ± 8.4	22.8 ± 9.5	
9.7	(Z)-3-Hexen-1-ol	0.7 ± 0.1 *					
11.2	Styrene						
13.5	Pentanoic acid		3.2 ± 0.6		11.8 ± 2.7	4.5 ± 2.4	
17.8	2,4-Hexadienoic acid, methyl ester		3.2 ± 0.7 *			9.6 ± 3.7 *	
18.5	Hexanoic acid		1.8 ± 0.6 *			5.5 ± 1.1 *	
20.7	(E)-3-Hexenoic acid		8.2 ± 1.2 *			14.4 ± 6.7 *	
21.9	2-methoxy-phenol	4.0 ± 0.4	2.6 ± 0.7			0.8 ± 0.2	4.1 ± 0.6

Table 2. *Cont.*

RT (min)	Volatile Compounds	Extra	1st. Category			2nd. Category	
			'Zapateria'	'Putrid'	'Butyric'	'Zapateria'	'Putrid'
22.0	2,4-Hexadienoic acid, ethyl ester		3.1 ± 0.6 *			9.5 ± 2.1 *	
23.0	2-Ethenyl-1,1-dimethyl-3-methylene-cyclohexane	18.0 ± 2.6 *					
23.3	Phenylethyl Alcohol	9.7 ± 1.5		12.0 ± 4,4			19.6 ± 8.9
26.5	Cyclohexanecarboxylic acid		1.8 ± 0.4 *			7.4 ± 2.1 *	
27.0	Creosol	48.1 ± 6.8	35.2 ± 9.4	27.5 ± 9.6	25.4 ± 10.4	3.2 ± 0.5	7.2 ± 0.8
28.2	Benzoic acid	8.6 ± 1.3	7.5 ± 5.9		6.5 ± 3.6	1.9 ± 0.4	

*: Compound unique to a concrete defect; empty cells correspond to non-detected measures.

On the other hand, it should be noted that certain volatile compounds identified are only detected in some of the defects studied. It is the case of (Z)-3-hexen-1-ol and 2-ethenyl-1,1-dimethyl-3-methylene-cyclohexane in healthy olives, propylene glycol, or (E)-3-hexenoic acid in 'Zapateria' table olives, isopropyl alcohol in 'Putrid' olives or butan-2-ol in 'Butyric' table olives.

A principal component analysis (PCA) was performed to observe the interaction of the variables and grouping of the samples originated from the profile data of table olive analysis (Figure 2). The total variance of data was explained a 35.73% by PC1 and 30.65% by PC2.

A clear sample differentiation according to their olfactory characteristics was shown by the two components-based models. It can be seen that the exploratory analysis was able to discriminate healthy olives from those with a defect in the fermentation process.

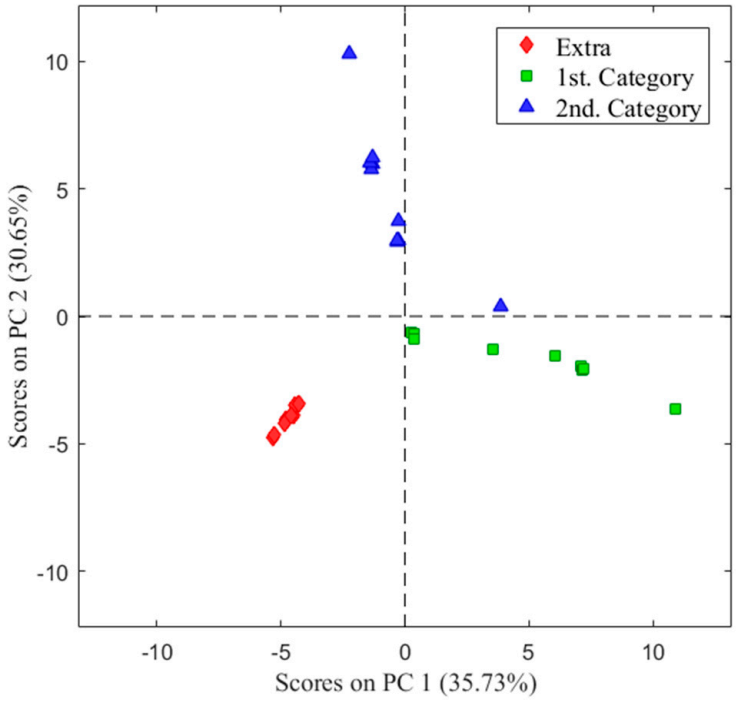

Figure 2. Score plot of the principal component analysis (PCA) analysis for healthy (extra) and defective olives (1st and 2nd categories).

3.3. E-Nose Classification of Spanish-Style Table Olives

The E-nose was used to classify the table olives previously analyzed by the tasting panel. The olives classified into three different categories were placed in the tasting cups. The E-nose was placed on top of the cups to evaluate the headspace of the samples. The E-nose consists of 11 sensors that emit an electrical signal in the presence of volatile compounds in the samples. The response of each sensor to the same sample is different, so

we can describe an olfactory pattern with the combination of the 11 signals. To represent the response patterns of the sensors to the extra, first, and second categories, a radial plot was drawn (Figure 3). For it, the data of E-nose obtained for each category were first normalized according to the formula $(X_i - X_{MIN})/(X_{MAX} - X_{MIN})$; where X_i is the experimental value measured for sample i; X_{MIN} is the minimum experimental value of the data series and X_{MAX} is the maximum experimental value of the data series. Afterward, the average values of the data series for each category were obtained, and finally, they were represented in the radial graph. It can be clearly seen in Figure 3 that the amplitude of the signals from extra table olives is generally of greater magnitude than the signals from olives classified in the first and second categories. The curves representing the categories are clearly different from each other.

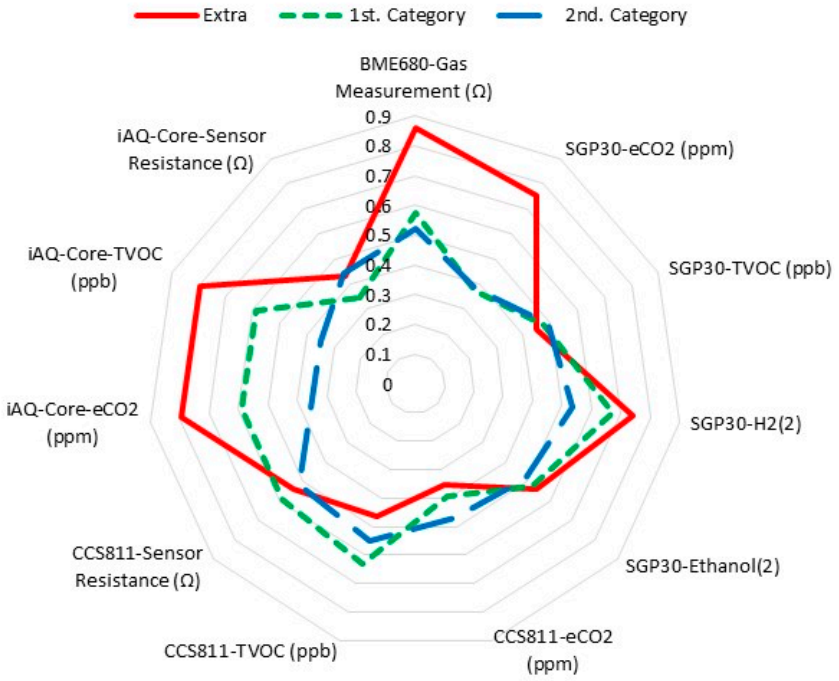

Figure 3. Radial plots of the sensor's responses to Spanish-style table olives of different categories.

The E-nose data of table olive samples from the three categories were first analyzed by principal component analysis (PCA). Scores values of the two first principal components are plotted against each other in Figure 4. It can be observed by grouping the samples into three categories. The first two principal components are enough to explain 77% of the total variance of the data. There is clear discrimination between extra olives and the first and second categories.

Following the revealing results obtained in the PCA, a supervised classification analysis was performed using PLS-DA and leave-one-out cross-validation. The results are shown in Table 3 as a confusion matrix, where the diagonal represents the number of samples that have been correctly assigned. As we can see, all the samples were correctly classified in their category, being the success rate of the classification model of 100%. The 100% correct classification result is consistent with the clear difference in aroma of the sensory analysis samples of each category. The extra category has a DPP value of 0, and the first one has a minimum difference with the extra of 3.8 points and 1.8 with the second category. Therefore, the E-nose can be a useful tool to develop a classification and quality control methodology accessible for routine use in any industry, capable of discriminating between healthy and unhealthy samples, as well as classifying them into different categories according to their sensory attributes.

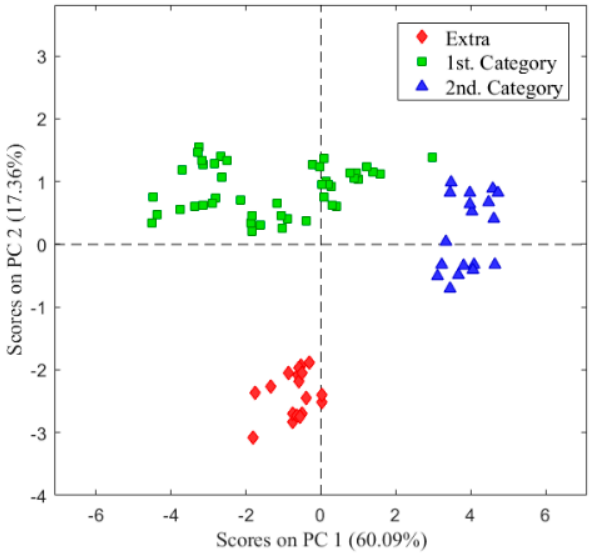

Figure 4. Score plot of the principal component analysis (PCA) analysis for table olives classified in different categories.

Table 3. Confusion matrix obtained through PLS-DA for discrimination between control (healthy olives) and isolated defects. Values are expressed in number of samples.

	Predicted Class		
Real Class	**Extra**	**1st Category**	**2nd Category**
Extra	18	0	0
1st Category	0	45	0
2nd Category	0	0	18

4. Discussion

When the chemical conditions are not appropriate during the elaboration process of Spanish-style table olives, undesirable alterations occur. This was the case in this study when olives were fermented with low concentrations of salt after lye and washing treatments. Thus, different kinds and intensities of defects in Spanish-style table olives were found. Table olives were sensory analyzed by a tasting panel following the methodology established by the International Olive Council [3]. When industrialists realize that certain batches of olives show alterations, the intensity of the defect is usually too high. This causes olives to have defects that, depending on their intensity, make the product belong to one commercial category or another, which could negatively affect the business economy. Normally, samples are taken from fermenters with possible abnormal fermentation and should be analyzed by a professional tasting panel to assess their quality. Industrial should control the chemical composition of the fermenters to avoid alterations. The lack of monitoring of the product during olives fermentation causes a pH modification, which can contribute to abnormal fermentation. In contrast, some fermenters studied presented any kind of alterations that make olives present a characteristic aromatic pattern. Some of these samples were classified into the extra category (DPP < 3), although they did not present sensory defects. Note that these are olives of the highest commercial category. On the other hand, tasters detected some negative attributes in olives that were analyzed by the defect predominant perceived (DPP). Some groups of samples were classified into the first category because the DPP was higher than 3 and less than or equal to 4.5. 'Zapateria', 'Butyric', and 'Putrid' were the main defects found in these samples. Furthermore, tasters indicated some defects such as 'Zapateria' and 'Putrid' in high intensity. Thus, these olives were classified in the second category because the DPP was higher than 4.5 and less than or equal to 7.0 (Table 1). We have to highlight that the olives studied could be legally marketed

despite the significant sensory alterations [3,19]. Therefore, these olives that were not controlled during fermentation presented anomalous fermentations that caused a marked sensory profile with defects with different intensities. In this sense, researchers [20] assessed table olives from different companies by a tasting panel detecting different intensity defects such as butyric, putrid, zapateria, musty, or winey–vinegary.

In the same way, Spanish-style table olives, after finishing the fermentation process, present particular volatile compounds and non-volatile aromatic compounds that contribute to the sensory aroma [21]. These olives were classified as 'Extra' category presented volatile organic compounds (VOCs) responsible for positive aromas. Creosol, acetic acid, or 2-ethenyl-1,1-dimethyl-3-methylene-cyclohexane are the main VOCs presented in healthy olives. Other alcohols compound such as (Z)-3-hexen-1-ol or phenylethyl alcohol are products formed in alcoholic fermentations [22], presenting a fruity fragrance to apples or bananas [23]. These aromatic compounds also appear in altered olives but in lower concentrations. Defected olives are characterized by an increase in certain volatile compounds characteristic of the defect. That is the case of propylene glycol, 2,4-hexadienoic acid, methyl ester, or (E)-3-hexenoic acid in 'Zapateria' alteration, or isopropyl alcohol in 'Putrid' defect, and butan-2-ol in 'Butyric'. These outcomes are in agreement with previous studies [8,24,25]. Cyclohexanecarboxylic acid also appears in the 'Zapateria' defect at low concentrations, but it has been identified as a key compound of this alteration in some studies [25]. Other researchers [26] indicated that this compound, in combination with other VOCs, is responsible for the characteristic unpleasant odor in the 'Zapateria' defect. Butanoic acid, pentanoic acid, propanoic acid, and butan-2-ol are VOCs presented in 'Butyric' and 'Zapateria' defect and propanoic acid even in 'Putrid' defect. These carboxylic acids are associated with cheesy odor (propanoic acid) and buttery and cheesy odor (butanoic acid) [27]. This result is in agreement with previous studies [25]. Furthermore, certain molds generate undesirable products during Spanish-style elaboration processes [28], producing musty aromas. In general, the VOCs of the samples studied allowed to classify the samples according to their commercial category. Clearly, the PCA of the VOCs classified olives according to the intensity and the type of defect, which play an important role in their commercial classification.

On the other hand, the radial profile of the response of each sensor of the E-nose to the aromatic profile of the olives shows that each sensor reacts in a certain way to the VOCs of the headspace of the samples. The radial figure was different from one commercial category to another. Next, the PCA model was performed to discriminate table olives categories with the E-nose. This electronic device was able to discriminate olives based on their commercial category. This discrimination could be due to the particular aroma profiles of the VOCs present in the different samples. E-nose was even able to discriminate samples with different defects in each category. Few references exist on the discrimination of olives submitted to abnormal fermentation using an E-nose. Researchers have discriminated by E-nose Spanish-style table olives inoculated with different altering [29] and those with abnormal fermentation with sensory defects to zapateria, butyric and putrid [28]. Other researchers presented an E-nose system able to discriminate different fungal species [30,31]. Other researchers have also established a PLS-DA model in order to produce a predictive classification model capable of separating the table olives varieties [32] and table olives stuffed with flavored hydrocolloids [33,34] that can be used as a rapid and inexpensive screening method for discrimination of standard characteristic aroma in Spanish-style and Californian-style table olives. All in all, the outcomes presented in our research prove that this electronic device is a powerful tool to discriminate VOCs produced by Spanish-style table olives. This equipment could be easily implemented in the table olive processing industry due to its installation simplicity, small size, fast measurement, precision, and low cost of analysis.

5. Conclusions

Spanish-style table olives fermented under uncontrolled chemical conditions showed characteristic alterations in aroma and intensity. 'Zapateria', 'Butyric', and 'Putrid' sensory alterations were the main defect perceived by tasters being classified in different commercial categories according to the criteria set by the IOC. These olives presented a volatile compounds profile characteristic that made the E-nose successfully used for discriminating olives from different commercial categories. Thus, this device could be useful for detecting olfactory sensations provoked by abnormal fermentation. The commercial classification made for E-nose matched the results obtained by the panelists. Therefore, this electronic device is complementary to the panel test and, combined with chemometric analysis, can be used to perform rapid, inexpensive, non-destructive, and environmentally friendly qualitative analysis for the table olives industry. Installing this device in the industry would be an effective strategy to detect incipient alterations during the fermentation process. Therefore, table olives quality could be improved thanks to these emergent electronic olfactive technologies. With this study, it has been put into account that a simple design can incorporate electronic devices as an alternative to conventional methods to assure food quality during its industrial processing, avoiding important economic losses.

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2.5 Artículo 5. "Electronic nose application for the discrimination of sterilization treatments applied to Californian-style black olive varieties, (2021). Journal of the Science of Food and Agriculture, 102(6), 2232-2241."

Sánchez, R., Martín-Tornero, E., Lozano, J., Fernández, A., Arroyo, P., Meléndez, F., Martín-Vertedor, D.

2.5.1 Motivación y objetivos

Las aceitunas negras al "estilo californiano" se elaboran sometiendo a las aceitunas a un tratamiento alcalino con hidróxido sódico y simultáneamente se le somete a una oxidación continua con aire hasta que el color del fruto se va ennegreciendo. Posteriormente, se realizan los lavados para eliminar el exceso de NaOH y se neutraliza hasta alcanzar un pH de 7. Las aceitunas resultantes son envasadas con una solución salina y gluconato ferroso, y son sometidas a un tratamiento de esterilización térmica. Las altas temperaturas de esterilización, además de provocar la degradación de vitaminas y otros compuestos bioactivos, estimulan la degradación oxidativa induciendo a la generación de aromas y flavores desagradables en el producto final. Cada industria de aceitunas de mesa aplica sus propios tratamientos térmicos, lo que conlleva a productos con diferentes perfiles sensoriales. Estos tratamientos modifican ciertos atributos de la aceituna, pudiendo incluso aparecer algunos atributos negativos, como es el *efecto de cocido*.

Una de las funciones del panel de cata es diferenciar los atributos positivos y negativos en las aceitunas de mesa para clasificarlas en las diferentes categorías de calidad según las recomendaciones del Consejo Oleícola Internacional (IOC, 2011). El análisis organoléptico también puede utilizarse como apoyo complementario al análisis cromatográfico de los compuestos volátiles de las aceitunas de mesa. El problema real, es que la industria no siempre puede disponer de un panel de cata o equipos cromatográficos por su elevado coste y la dificultad del manejo de estos equipos. En los últimos años, la tecnología de *nariz electrónica* se ha implantado como una poderosa herramienta que permite discriminar muestras de alimentos en función de las características del perfil aromático que posea. En la bibliografía se han encontrado resultados que demuestran la capacidad de la *nariz electrónica* para discriminar ciertos COVs responsables del grado de madurez en muestras de uva y sus variedades (Aleixandre et al., 2015) o de las bayas (Aghilinategh et al., 2020).

También existen trabajos dónde estos dispositivos detectan los COVs que producen los tomates infestados de áfidos y moscas blancas en comparación con un grupo de tomates sanos (Cui et al., 2019). Otros investigadores evalúan la composición y perfil aromático de variedades de tomates (Paolo et al., 2019) e identifican diferentes

variedades de patatas (Khorramifar et al., 2021). Además, el análisis mediante *nariz electrónica* permite analizar un gran número de muestras al día, por lo que podría utilizarse para mejorar la calidad del producto elaborado a nivel industrial en los puntos críticos del proceso. Por lo tanto, el objetivo del presente estudio fue, evaluar la capacidad de la *nariz electrónica*, para ser usado con el análisis sensorial del panel de cata, para diferenciar aceitunas negras al "estilo californiano" de dos variedades de aceitunas de mesa sometidas a diferentes procesos de esterilización térmica y, confirmar su utilidad cuantitativa para evaluar la intensidad del aroma percibido.

2.5.2 Diseño experimental

La materia prima utilizada en este trabajo son aceitunas de la variedad *'Hojiblanca'* y *'Manzanilla Cacereña'*. Tras cuatro meses de almacenamiento en ácido acético al 1,5% (v/v) y NaCl al 8% (p/v), se procedió a la elaboración de las aceitunas al "estilo californiano" mediante oxidación con aire forzado. Una vez finalizada la elaboración, las aceitunas se envasaron en latas con NaCl al 2% (p/v) y 0,015% de gluconato ferroso (p/v). Las latas se introdujeron en autoclave y se sometieron a diferentes ciclos de esterilización: F_0= 10 min (T1), F_0 = 14 min (T2), F_0 = 18 min (T3), F_0 = 22 min (T4) y F_0 =26 min (T5), dónde F_0 corresponde a la letalidad acumulada y se expresa, como la duración equivalente del tratamiento térmico necesario para reducir la carga microbiana inicial. Las muestras de aceitunas se analizaron por el panel de cata, se determinó el perfil de COVs por cromatografía y se midieron con *nariz electrónica.*

Las medidas del aroma de las aceitunas se realizaron en las copas de cata normalizadas en las que se introdujeron cuatro aceitunas y 5 mL de salmuera, se taparon con un vidrio reloj y se colocaron en un bloque calefactor a 25ºC. Se tomaron 5 medidas por cada muestra. Las muestras se realizaron por triplicado.

2.5.3 Resumen de los resultados y discusión

Los resultados del panel de cata mostraron que la intensidad del *efecto de cocido* y, la evaluación global del aroma de las dos variedades de aceituna de mesa, fueron significativamente influenciados por los tratamientos de esterilización térmica. El *efecto de cocido* aumentó significativamente al aumentar los tiempos de esterilización (T1 hasta T5). De acuerdo con la normativa del COI, las aceitunas del presente estudio se pudieron clasificar en distintas categorías sensoriales en función del defecto predominante percibido (DPP) y su intensidad. El principal defecto detectado por los catadores fue el *efecto de cocido*. Las aceitunas con tratamiento térmico más suave (T1) fueron clasificadas en la máxima categoría comercial, extra (DPP ≤ 3,0); T2 en la primera categoría (3,0 < DPP ≤ 4.5) y T3 en la segunda categoría (4,5 < DPP ≤ 7,0). Sin embargo, las aceitunas sometidas a tratamientos térmicos más agresivos (T4 y T5), fueron clasificadas como no aptas para ser comercializadas como aceitunas de mesa (DPP > 7,0). Por lo tanto, y de acuerdo con las recomendaciones del COI, las aceitunas sometidas a tratamientos térmicos más suevas (F_0 = 10 min) se clasificaron en la categoría comercial de la más alta calidad. Por otro lado, las aceitunas sometidas a un tratamiento de esterilización más agresivo, como T4 y T5 (F_0 = 22 y 25 min, respectivamente), se clasificaron como de baja calidad debido a la alta intensidad de *efecto de cocido* detectado por el panel de cata. Así, los resultados revelan que, no sólo es importante controlar la temperatura durante el proceso de esterilización térmica en las latas de aceitunas, sino que la duración del tratamiento también es decisiva para conseguir minimizar la degradación oxidativa que puede influir en las características sensoriales del producto final.

Otro aspecto de interés es que para tratamientos térmicos iguales o superiores a 22 min (T4 y T5), el *efecto de cocido* fue tan intenso que dominó las percepciones olfativas de los catadores obteniéndose valores similares del defecto para ambos tratamientos. Estos resultados sugieren que, a partir de un determinado tiempo de esterilización ya no se detectan mayores defectos sensoriales en el producto. Similares resultados han sido encontrados por Martín-Vertedor et al. (2020) con *lengua electrónica*, donde con F_0 = 25 min, se encontró un *efecto de cocido* similar al revelado en el presente estudio. Teniendo en cuenta estos hallazgos, es importante para las industrias, optimizar los

tratamientos de esterilización térmica, manteniendo F_0 cerca de 10 min para conseguir obtener aceitunas esterilizadas de la máxima categoría comercial.

Los análisis cromatográficos identificaron 34 compuestos volátiles diferentes. Los principales compuestos volátiles de las aceitunas de mesa con la máxima categoría comercial (F_0 = 10 min) fueron el benzaldehído (21.7–21.1%), creosol (20.0–20.7%), y ciclohexanocarboxilato de etilo (17.5-19.5%). El contenido de algunos compuestos volátiles como benzaldehído, 4-etenilpiridina y 2,4-dimetilhexano, aumentaron cuando el tratamiento de esterilización térmica fue más agresivo. Estos compuestos están relacionados con el olor desagradable a *cocido*. Por otro lado, otros compuestos volátiles aromáticos disminuyeron su concentración a medida que aumentaba el tiempo de esterilización, como fue el caso del creosol, el etil ciclohexanocarboxilato, copaeno y estireno.

Por otro lado, se utilizó el PCA para visualizar los grupos de aceitunas con diferentes perfiles volátiles de las muestras estudiadas. Los resultados mostraron que el benzaldehído y 4-etenilpiridina fueron los compuestos volátiles más influyentes, ya que permitió discriminar entre los diferentes tratamientos de esterilización estudiados. Estos resultados están de acuerdo con autores como López-López et al. (2019), quienes demostraron que estos compuestos son formados como resultado de la esterilización a 121°C. Los resultados obtenidos por el PCA de los compuestos volátiles de las muestras de las dos variedades son comparables a los obtenidos en el panel de cata, llevándonos a la conclusión de que, a partir de una F_0 de más de 22 min, el perfil volátil de la aceituna no se ve modificado significativamente, y es cuando presentan la mayor intensidad del defecto sensorial de *cocido*.

Se utilizó la *nariz electrónica* para clasificar las dos variedades de aceitunas de mesa tras su exposición a diferentes ciclos de esterilización. El PCA realizado para cada una de las variedades de aceitunas mostró una buena separación entre las muestras sometidas a los tratamientos térmicos más suaves (T1 y T2) de los tratamientos más agresivos (T3-T5). Sin embargo, la *nariz electrónica* mostró menor discriminación entre las muestras T3 y las muestras T4 y T5, que parecen superponerse. Hay que indicar además que el análisis discriminante PLS-DA aplicado a las muestras de la

variedad *'Hojiblanca'*, obtuvo el 93,7% de las predicciones correctas y, para las muestras de *'Manzanilla Cacereña'* fue del 84,6%.

Finalmente, se realizó un modelo PLS para poder hacer predicciones cuantitativas de los atributos sensoriales de las aceitunas relativos a la evaluación global y, del *efecto de cocido* percibido por el panel de cata en función del tiempo e intensidad de la esterilización térmica. En ambos casos, los índices de correlación del modelo de regresión fueron 0,78 y 0,88, respectivamente. Existe escasa literatura científica sobre la discriminación de defectos sensoriales en aceitunas de mesa con la utilización de dispositivos electrónicos. Sin embargo, existen estudios (Martín-Vertedor et al., 2020) que describen la predicción de los defectos de *cocido* producidos por la aplicación de diferentes tratamientos de esterilización en dos variedades de aceitunas negras oxidadas mediante la utilización de una *lengua electrónica*. Estos investigadores, también encontraron que ciertas variedades de aceitunas sometidas a distintos tratamientos de esterilización tienen un impacto en la intensidad del *efecto de cocido* encontrado, debido a su relación con una sustancia tóxica (acrilamida) en las aceitunas negras al "estilo californiano" (Martín-Vertedor et al., 2020).

2.5.4 Conclusiones

- El aumento de la duración de la esterilización provocó mayores percepciones de *efecto de cocido*; sin embargo, cuando se aplicó un F_0 superior a 22 minutos, el perfil de volátiles de las aceitunas no se modificó significativamente.

- Los volátiles característicos de la aparición del *efecto de cocido* fueron benzaldehído, 4-etenilpiridina y 2,4-dimetilhexano.

- La *nariz electrónica* demostró ser una herramienta de discriminación útil para diferenciar entre muestras sometidas a diferentes tratamientos térmicos, independientemente de la variedad de aceituna estudiada.

- Los resultados obtenidos por los modelos PLS sugirieron que la *nariz electrónica* podría utilizarse para estimar cuantitativamente parámetros olfativos del análisis sensorial, como el *efecto de cocido* y la evaluación global.

- La *nariz electrónica* resultó ser un potente instrumento de análisis cuantitativo y cualitativo, que podría ser utilizado para el control de calidad de las aceitunas de mesa al "estilo californiano" en la industria.

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Electronic nose application for the discrimination of sterilization treatments applied to Californian-style black olive varieties

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Abstract

BACKGROUND: Olive oil continues to be the main destination for olives. The production of table olives is increasing. 'Californian-style' processes are among the most frequently employed to produce oxidized olives. Sensory evaluation requires the development of an instrumental detection method that can be used as an adjunct to traditional tasting panels.

RESULTS: An electronic nose (E-nose) was used to classify two varieties of olives following exposure to different sterilization. Principal component analysis (PCA) revealed that both varieties had different volatile profiles. Sensory panel evaluations were similar for both. Partial least squares-discriminant analysis (PLS-DA) obtained from the E-nose was able to separate the two varieties and explained 82% of total variance. Moreover, volatile profiles correctly classified olives according to sterilization times recorded up to 121 °C . The only exception was at $F_0 \geq 22$ min, at which a plot of PCA outcomes failed to differentiate scores. E-nose data showed similar results to those produced from the volatile analysis when grouping samples were sterilized to $F_0 \geq 18$ min, at the same time distinguishing these samples from those subjected to less intense thermal treatments. A partial least squares (PLS) chemometric approach was evaluated for quantifying important olive quality parameters. With regards to validation parameters, R_P^2 pertaining to perceived defect was 0.88, whilst R_P^2 pertaining to overall assessment was 0.78.

CONCLUSIONS: E-nose offers a fast, inexpensive and non-destructive method for discriminating between varieties and thermal treatments up to a point at which cooking defects are highly similar (from $F_0 = 18$ onwards).

Keywords: sterilization treatment; sensory analysis; cooking defect; E-nose; olives

INTRODUCTION

Spain is the world's leading table olive producer, with a large number of olive trees being found in the Mediterranean region. The olive sector is hugely important to the national agri-food industry for two main reasons. First, it generates a large number of jobs and, second, it places Spain as a leading producer due to its production volume, and transformation, commercialization, and export activities. Californian-style black olives are destined mainly for export and constitute 45% of all exported olives.[1] These table olives are elaborated by submitting them to lye treatment to eliminate bitterness and to force air oxidation to obtain their black color. Finally, sterilization processes are mandatory to ensure that microbiologically stable olives are obtained and to guarantee food safety of the oxidized black olive.[2]

To achieve this high temperature, induced thermal treatment is needed. This type of process provokes the degradation of vitamins and other components and stimulates oxidative olive degradation, leading to the generation of non-characteristic aromas and flavors (off flavors) in the final product.[3,4] In Spain, it was previously mandatory for a minimum amount of heat to be applied (Royal Decree 1230/2001) in olive sterilization processes ($F_0 \geq 15$ min). However, the requirement to apply such a severe heat treatment was removed by a later Royal Decree (679/2016).

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As a result, each table olive industry applies different thermal treatments that led to products with different sensory profiles which may produce different negative attributes such as cooking effects.[4,5]

When used in combination with specialized tasting panels, conventional analytical techniques such as gas chromatography are able to detect these negative attributes in olives.[2,6,7] These panels are able to differentiate between positive and negative attributes in olives and classify them according to different quality categories as laid out in the International Olive Council (IOC) regulations.[1] These types of analyses are more difficult to carry out and, in many cases, industries do not have the resources to carry them out due to their high cost.[8]

In recent years, electronic nose (E-nose) technology has emerged as an powerful electromechanical sensory device, which allows, for example, the quality assessment of rice based on variation in aroma profiles.[9] Such devices have also been used to classify sparkling wines according to the yeast and temperature used in processing,[10] and to evaluate the flavor properties of cooked chicken drumsticks in response to different sugar smoking durations.[11]

The most commonly used sensors in E-noses are metal oxide semiconductors (MOS). Metal oxide semiconductor-field effect transistors (MOSFET), piezoelectric sensors, and conducting polymer sensors (CP) are also sometimes used.[12] Some E-noses are able to detect volatile compounds via a circuit that conducts the chemical compound into a chamber.[13] Other small E-noses measure volatile compounds directly from the sample without an air conduction circuit– for example, via passive sampling.[14] With regards to edible oils, an E-nose has been used to classify different types of oils according to different varieties, to detect adulteration, and to evaluate the extent of oxidation.[12] This technology has also been applied to the detection of edible mushrooms,[15] and to the assessment of the quality and olfactory profiles of fresh-cut stored carrots.[16]

Research using E-nose technology has been conducted to classify green fermented table olives as acceptable, unacceptable, or marginal, prior to making them available on the market.[8] More recently, this technology was used to predict fresh olive fruit quality prior to the industrial manufacturing process.[17]

For these reasons, the aim of the present study was to evaluate the performance of an E-nose system in aiding a trained sensory panel to differentiate Californian-style black olives from other varieties and olives subjected to different sterilization processes, according to their sensory attributes. Furthermore, its quantitative usefulness was verified for the first time in order to evaluate its response to different sensory parameters. The E-nose was designed to provide a simple and easy-to-use tool with a low analytical burden. Its short analysis times also allow a large number of samples to be analyzed per day. As a result, this fast and non-destructive technique for the sensory evaluation of olives can be implemented at industrial level to improve the quality of this product.

MATERIAL AND METHODS

Samples

The raw material (*Olea europaea* L.) used to elaborate the present table olives came from the 'Vegas Bajas del Guadiana' zone. From this, two different varieties of olive trees ('Hojiblanca' and 'Manzanilla Cacereña') were harvested at the first stage of maturation (when they are green in color) during the 2020/21 season. Olives were transported to a company located in the northwest of Extremadura (Spain) and were immediately stored in triplicate in industrial tanks. Following 4 months of being stored with acetic acid (Panreac Applichem, Darmstadt, Germany) at 1.5% v/v and NaCl (Valdequímica, Barcelona, Spain) at 8% p/v, Californian-style black olive processing was initiated.[18] Olives were packed (150 g) in cans with a solution containing 2% of NaCl (p/v) and 0.015% (10–40 ppm p/v) of ferrous gluconate (Sigma Aldrich, Saint Louis, MO).

Thermal sterilization experimental treatments

An industrial autoclave was used to conduct the sterilization process.[2] Olive cans were submitted to five different thermal treatments which corresponded to the following F_0 values (accumulated lethality): F_0 = 10 min (T1), F_0 = 14 min (T2), F_0 = 18 min (T3), F_0 = 22 min (T4) and F_0 = 26 min (T5). The F_0 values were calculated following the method described by Martín-Vertedor *et al.*[2] The time–temperature profile was recorded by placing Pt100 temperature sensors (Uxbridge, United Kingdom) in the middle of the can. Sensors were connected to a computer and it was ensured that the required heat penetration for reducing the corresponding microbial population was achieved. The F_0 value was calculated using recorded heat penetration data and expressed as the equivalent treatment duration required to reduce the initial microbial load.[3] Cans were stored at room temperature until analysis. All experiments were performed in triplicate.

Analyses

Three analyses were carried out on the table olives subjected to the thermal treatments mentioned above: sensory analysis, chromatographic analysis of volatiles, and E-nose measurements.

Sensory analysis

The sensory panel was set up at the Scientific and Technological Research Center of Extremadura (CICYTEX) and evaluations were carried out in an individual testing cabin inside one of the tasting rooms (ISO 8589). Analysis was performed by a trained panel of eight table olive experts as described in the IOC regulations.[1] For the sensory analysis, a scoreboard with a 10 cm structured scale was designed to evaluate cooking effects, aroma intensity, and overall aroma evaluations. Sensory evaluation outcomes were expressed as average values. Values were considered to be valid when the coefficient of variation was less than 20.

One-way ANOVA was used followed by Tukey's multiple range test to establish statistically significant differences between different thermal treatments within each of the table olive varieties. Significance was set at $P < 0.05$. SPSS 18.0 software was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Data were expressed as means and standard deviations (SD).

Volatile compound analysis

Volatile compounds were determined using gas chromatography following the same procedure reported in the literature.[19] Approximately 100 g of black olives were pitted and homogenized. Paste (2 g) was placed in a 15 mL glass vial with 7.0 mL of 30% (w/v) NaCl. A polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex fiber (65 μm, Supelco) was used for headspace extraction of the sample at 40 °C for 30 min. Following extraction, the fiber was inserted into the injection port of the gas chromatography for desorption at 250 °C for 15 min. Analyses were performed by a Bruker Scion 456-GC triple quadrupole gas

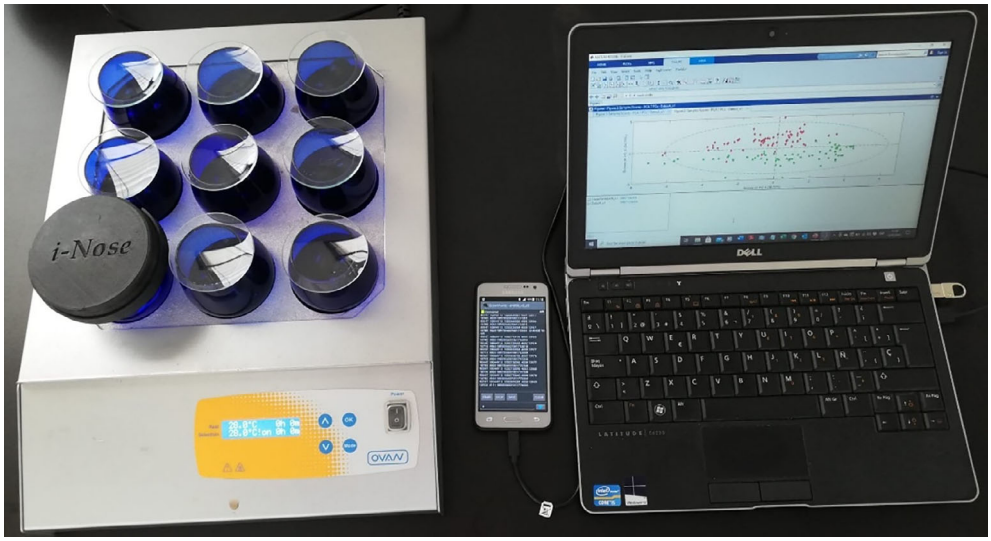

Figure 1. E-nose device and technology needed to measure samples of Californian-style black olive in a standard glass and heating block.

Table 1. E-nose sensors

Sensor code	Description	References
T	Temperature (°C)[a]	BME680
P	Pressure (hPa)[a]	BME680
H	Humidity (%RH)[a]	BME680
1	Gas measurement (Ω)	BME680
2	$_eCO_2$ (ppm)	SGP30
3	TVOC (ppb)	SGP30
4	H_2[b]	SGP30
5	Ethanol[b]	SGP30
6	$_eCO_2$ (ppm)	CCS811
7	TVOC (ppb)	CCS811
8	Sensor resistance (Ω)	CCS811
9	$_eCO_2$ (ppm)	iAQ-Core
10	TVOC (ppb)	iAQ-Core
11	Sensor resistance (Ω)	iAQ-Core

[a] Constant values during our measurements that were not used in statistical analysis.
[b] Concentration can be computed from measurement with a reference.

chromatograph with an Agilent DB WAXetr capillary column (60 m × 0.25 mm; ID: 0.25 mm). Compound identification was based on mass spectra matching with the standard National Intitute Standards and Technology (NIST) 2.0 MS library.

E-nose

A miniaturized portable (39 × 33 mm) E-nose designed by the University of Extremadura (Spain)[14] was used. The E-nose developed is shown in Fig. 1. This device consists of four digital gas sensor chips with integrated metal oxide (MOX) sensors: BME680 from Bosch, SGP30 from Sensirion, and CCS811 and iAQ-Core from ScioSense. Table 1 contains information about the E-nose sensors.

Data provided by the resistive sensing elements were processed using different algorithms (averaging, humidity correction, baseline compensation, etc.). Sampling times were configured in line with ASCII-based protocols. A microprocessor read the values detected by the sensors, formatted them and sent them to an external smart device via Bluetooth. The resulting data were then passed to a computer and organized into columns in a spreadsheet.

Olive aroma measurements were performed according to standard tasting panel techniques, which adhered to recommendations laid out by the International Olive Council.[1] Specifically, standard glasses containing the samples were placed on a heating block and covered with a watch-glass. The purpose of this assay was to perform a sensory classification of the table olives according to potential defects.

Olives were fully thawed 1 day prior to analysis, and 5 mL of brine containing four olives was introduced into standard tasting glasses and covered with a watch glass. The tasting glasses were then placed on a block that was heated to 25 °C. Another standard tasting glass was left in the same conditions at 25 °C to serve as a baseline reference.

Each data acquisition cycle consisted of two parts. The first part corresponded to a 30 s desorption period during which free air was brought into contact with the sensors. The system took a reading of the resistive value supplied by each sensor at 1 s intervals. This provided baseline reference values. In the second part of the cycle, the absorption of table olive volatiles was analyzed at 1 s intervals for 60 s. Five measures were taken for each sample of table olives.

Multivariate data analysis

Initially, E-nose data were analyzed using principal component analysis (PCA). The aim of this was to detect outliers and evaluate potential cluster formation (exploratory data analysis) in relation to the two examined varieties and thermal treatments.[20] Principal component analysis is a powerful unsupervised pattern recognition technique, which serves to express information provided by the E-nose according to a smaller number of variables. These are denominated principal components. Principal components are linear combinations of original response vectors. Analysis was set to consider maximum data variance and was orthogonal. Given that study variables were measured according to different units, original variables were autoscaled.

Next, partial least square discriminant analysis (PLSDA)[21] was applied to develop a predictive classification model. This analysis applies an algorithm that identifies the components or latent variables (LV) which most discriminate between two different groups of samples. It examines the X matrix developed from the data according to maximum covariance with a target class defined in the Y matrix.

Several PLSDA models were built with different classification purposes. Specifically, one model was developed to discriminate between olive varieties and another to discriminate between different thermal treatments.

A confusion matrix was constructed for the deduction of cross-validation predictions. The proportion of correct predictions was calculated from the sum of the diagonal elements found in the confusion matrices.

The partial least squares (PLS) method was also used to build quantification models for the evaluation of parameters by the tasting panel.[22] Samples were divided into two sets and each parameter was modeled individually. The first set (the calibration set) contained 70% of all samples and was used to calibrate and cross-validate the models. The second set (the validation set) contained all remaining samples (30%) and was only used to test the robustness and accuracy of developed models. Samples were divided randomly between the two sets.

Partial least squares model optimization enables an optimal number of latent variables (LV) to be selected through a cross-validation procedure (leave-one-out) conducted with the calibration set described above. Selection of the optimal number of LV for each parameter is based on calculation of the minimum root mean square error.

Validation test samples were used to validate the model. The parameters used to evaluate the accuracy of the models were root mean square error of calibration (RMSEC), cross-validation (RMSECV), and prediction (RMSEP), and coefficients of determination for cross-validation (R^2_{CV}) and prediction (R^2_P).

RMSEC, RMSECV, and RMSEP were calculated according to Eqn (1):

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} \left(\hat{Y}_i - Y_i\right)^2}{N}} \quad (1)$$

where, Y_i is the experimental measurement for sample *i*; $\hat{Y}$ is the corresponding value obtained for calibration (RMSEC), cross-validation (RMSECV), and prediction (RMSEP), and *N* is the number of samples.

Data analysis was performed using the Matlab R2016b version 9.1 (The Mathworks Inc. Natick, MA, USA) with the PLS_Toolbox 8.2.1 (Eigenvector Research Inc., Wenatchee, WA, USA).

RESULTS AND DISCUSSION

Outcomes of the analysis conducted by the tasting panel and gas chromatography will first be described. Then, the application of E-nose technology will be presented together with its use to distinguish between olive varieties and between different thermal treatments. Finally, the capacity of the E-nose to quantify specific attributes with regard to the tasting panel will be explained.

Sensory aroma and volatile compounds in table olives

A trained panel conducted a sensory analysis of 'Manzanilla Cacereña' and 'Hojiblanca' varieties of Californian-style black olives, which had been subjected to different thermal treatments (T1–T5). Outcomes from the one-way ANOVA (Table 2) showed that the intensity of cooking effects and overall aroma evaluations of both table olive varieties in their respective brine solutions were significantly influenced by thermal sterilization treatments (*P*-value <0.05). The main defect found in olives subjected to different treatments pertained to cooking.

Greater defects were found as the F_0 of the sterilization treatment increased (T1-T5). According to IOC regulations, results obtained in the present study can be classified into different sensory categories following consideration of the predominantly perceived defect (PPD) and intensity attributes.[1] As can be seen, T1 was classified into a category denominated as 'extra' or 'fancy' (PPD≤2), T2 into the 'first' or 'select' category (2 < PPD≤3.5), and T3 into the 'second' or 'standard' category (3.5 < PPD≤6.0). T4 and T5 olives were classified as not fit to be sold as table olives (PPD > 6.0). According to IOC stipulations, only olives subjected to F_0 = 10 min can be placed into the highest commercial category and deemed to be of the highest quality. On the other hand, olives subjected to a more aggressive sterilization treatment, such as T4 and T5 (F_0 = 22 and 25 min, respectively), were classified as too poor quality to be marketed due to the high intensity of defects detected by the tasting panel. Thus, the outcomes reveal that it is not only important to control the temperature inside the can during the thermal process but also the duration of the sterilization process, to minimize oxidative degradation, which can influence the final sensory characteristics of the product. In addition, overall assessments of the final product were worse when it had been submitted to more aggressive thermal treatments. As a consequence, consumers are less likely to opt to purchase olives subjected to more intense thermal sterilization treatments.

Another aspect of interest is that, for thermal treatments ≥ T4, the cooking effect was so intense that it overpowered taste perceptions and made it impossible to differentiate T4 from T5. This suggested that, even when more intense thermal treatments are applied, greater sensory defects are not necessarily noticed. Indeed, perceptible defects stabilized at F_0 = 22 min. Similar results have been found by Martín-Vertedor *et al.*[2] In their study, olive discrimination occurred at F_0 = 25 min, with a similar defect profile being found to that revealed in the present study. In the same way, researchers have examined the application of thermal treatments to oxidized black olives, observing that longer and more aggressive sterilization treatments led to a deeper and more unpleasant olive taste.[2] Given these findings, it is important for industries to optimize thermal sterilization treatments, keeping F_0 close to 10 min to obtain the highest quality sterilized olives. Other researchers have reported that the application of thermal treatments caused olive quality to deteriorate in terms of color and texture.[4,23]

'Manzanilla Cacereña' table olives also had a significantly higher cooking defect than 'Hojiblanca' olives, specifically, when more intense thermal treatments (F_0 > 10 min) were applied. This was despite both varieties being elaborated according to the Californian-style. However, the sensory attributes considered were higher in 'Hojiblanca' variety. Significant differences in terms of sensory aroma for both olive cultivars may also be attributed to the genetic factors inherent in each variety. This conclusion is supported by the findings of other researchers.[4]

As another aspect of the present study, gas chromatography (GC) was used to analyze the volatile profile of the olives. The identified volatile compounds listed and the relative content in percentage are shown in Table 3. A total of 34 individual volatile

Table 2. Sensory aroma attributes of Californian-style black olives ('Manzanilla Cacereña' and 'Hojiblanca') submitted to different thermal sterilization treatments (T1-T5) evaluated by a trained panel. Results are expressed as means ± standard deviations of three sample replicates. For the same cultivar, different lower case letters mean a statistical significant difference between thermal treatment (one-way ANOVA followed by Tukey's test, $P < 0.05$). For the same thermal treatment, different upper case letters mean a statistical significant difference between olive cultivar (one-way ANOVA followed by Tukey's test, $P < 0.05$)

Variety	Thermal treatment	DPP	Global aroma assessment
'Hojiblanca'	T1	1.8 ± 0.3a ^{NS}	8.2 ± 0.3d ^{NS}
	T2	2.9 ± 0.4b ^{NS}	7.1 ± 0.4c ^{NS}
	T3	4.6 ± 0.7c ^{A}	6.3 ± 0.3b ^{NS}
	T4	6.3 ± 0.7d ^{A}	5.6 ± 0.2a ^{NS}
	T5	6.6 ± 1.9d ^{A}	5.5 ± 0.3a ^{NS}
'Manzanilla Cacereña'	T1	1.9 ± 0.2a	7.8 ± 0.4d
	T2	2.9 ± 0.7b	6.8 ± 0.3c
	T3	5.2 ± 0.3c ^{B}	5.9 ± 0.5b
	T4	7.0 ± 0.4d ^{B}	5.3 ± 0.4a
	T5	7.1 ± 0.5d ^{B}	5.3 ± 0.4a

DPP, median of the defect perceived; T1: F_0 = 10 min; T2: F_0 = 14 min; T3: F_0 = 18 min; T4: F_0 = 22 min; T5: F_0 = 26 min.

compounds were identified. The major constituents of the volatile matrix in table olives with the highest commercial category (F_0 = 10 min) were benzaldehyde (21.7–21.1%), creosol (20.0–20.7%), and ethyl cyclohexanecarboxylate (17.5–19.5%). However, it was observed that the content of some volatile compounds such as 2,4-dimethylhexane, 3-methylpyridine, benzaldehyde, 4-ethenylpyridine, and α-farnesene increased as thermal sterilization treatment became more aggressive. These compounds were related to an unpleasant and cooked odor. On the other hand, other aromatic volatile compounds decreased in concentration as sterilization time increased, as was the case with creosol, ethyl cyclohexanecarboxylate, copaene, and styrene. Thus, these results revealed that the main odors detected by the tasting panel were relate, with unpleasant aromas.

Principal component analysis was also used to visualize whether differences existed between the volatile profiles of examined samples (Fig. 2). Outcomes showed that benzaldehyde and 4-ethenylpyridine were the most influential volatile compounds when distinguishing between different sterilization treatments. These outcomes are in accordance with those reported by López-López *et al.*,[19] who showed that these compounds are formed as a result of sterilization at 121 °C.

The first two principal components accounted for 92% of the variation seen in the data, with principal component 1 (PC1) accounting for 89% and principal component 2 (PC2) explaining 3%. Figure 2 shows the score plot produced for PC1 versus PC2. As can be seen, the 'Hojiblanca' variety is located in the positive part of PC2, and the 'Manzanilla Cacereña' variety occupies the negative zone. Although both varieties were elaborated following the same procedure, they had different profiles. With regards to the thermal treatment applied, it can be observed in both varieties that, as F_0 increases, samples in the plot edge further to the right into PC1. In this plot, olives subjected to T1, T2, and T3 are distributed separately, whereas T4 and T5 appear together.

These results are consistent with those produced in the panel test, leading us to the conclusion that when an F_0 of more than 22 min is applied, the olive's volatile profile is not significantly modified. Nonetheless, these table olives presented the greatest sensory defects of all those examined.

Variety discrimination using an E-nose

The potential use of E-nose technology for distinguishing between different variety olives was studied. This device detects some volatile differences, distinguishing two different varieties from the response curve produced by each of its sensors. To examine variety differentiation, preprocessed signals (x_i) were autoscaled using the algorithm $(x_i - x_{Min}) / (x_{Max} - x_{Min})$, where, x_i is the experimental measurement for sample i. This was then represented in a radial diagram (Fig. 3) to reveal the aroma fingerprint of the table olives that were studied. This type of representation has been applied previously to mushrooms[15] using a triangular test procedure.

It is also noteworthy that the E-nose performance was found to be stable and, moreover, humidity and temperature conditions were analogous, assuring the quality and reliability of the outcomes. When examining the radial representations, it can be seen that some sensors, such as sensors 2, 3, 4, 7, 9, and 10, demonstrated a greater discernment response than others. The electronic device can, therefore, discriminate qualitatively between 'Hojiblanca' and 'Carrasqueña' varieties due to their different aroma fingerprints. It was also observed that the 'Manzanilla Cacereña' variety had a wider response to all of the sensors than the 'Hojiblanca' variety. This could be the result of the higher quantity and concentration of the volatile compounds present in this variety.

Signals produced by the E-nose (11 sensors) were employed to perform exploratory analysis via PCA. The first and second principal components explained 70% and 12% of variance, respectively. Thus, data for these two components is enough to differentiate between the two varieties given that they explain 82% of total variance. Values for the first two components were plotted versus the other components. Following examination of the score plot (Fig. 4), a clear grouping of samples can be observed according to olive variety. Data from the 'Manzanilla Cacereña' samples are found in the left hand-side of the plot, corresponding to negative PC1 scores. On the other hand, data from 'Hojiblanca' olives occupy the right hand-side of the plot, corresponding to positive PC1 scores. These results were very similar to those obtained by the PCA of volatile compounds. Figure 4 also shows the loading

Table 3. Relative contents of volatile compounds (mean %, $n = 3$) obtained from Californian-style black olives ('Manzanilla Cacereña' and 'Hojiblanca') submitted to different thermal sterilization treatments (T1-T5). RT, retention time

	RT (min)	Content (% of total area of identified compounds)									
		'Hojiblanca'					'Manzanilla Cacereña'				
		T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
2,4-Dimethylhexane	6.7	0.4 ± 0.0	2.3 ± 0.6	4.3 ± 0.1	6.1 ± 0.6	8.5 ± 0.4	0.7 ± 0.2	2.3 ± 0.1	5.4 ± 0.1	6.6 ± 0.4	7.6 ± 0.1
3-Methylpyridine	10.0	n.d.	n.d.	n.d.	1.9 ± 0.1	2.2 ± 0.3	n.d.	2.0 ± 0.0	3.3 ± 0.7	3.9 ± 0.0	3.8 ± 0.0
1,4-Xylene	10.2	1.6 ± 0.2	7.2 ± 2.4	1.5 ± 0.0	1.4 ± 0.1	n.d.	3.2 ± 0.7	n.d.	0.7 ± 0.2	n.d.	0.6 ± 0.9
Styrene	11.3	4.4 ± 0.1	3.5 ± 0.0	3.7 ± 0.3	2.9 ± 0.2	2.6 ± 0.1	5.7 ± 0.4	2.6 ± 0.0	1.2 ± 0.2	1.0 ± 0.0	1.3 ± 0.4
3-Ethylpyridine	14.9	n.d.	n.d.	1.2 ± 0.5	1.3 ± 0.3	1.7 ± 0.1	1.5 ± 0.2	3.4 ± 0.0	4.4 ± 0.2	5.1 ± 0.1	5.5 ± 0.2
Hept-2-yn-1-ol	15.0	1.2 ± 0.0	n.d.	n.d.	1.2 ± 0.1	1.2 ± 0.0	n.d.	n.d.	n.d.	1.0 ± 0.0	1.0 ± 0.0
Benzaldehyde	15.3	21.7 ± 0.1	24.0 ± 2.1	32.4 ± 0.7	41.1 ± 0.8	39.6 ± 1.2	21.1 ± 1.1	34.9 ± 0.0	38.8 ± 0.6	40.6 ± 0.1	42.6 ± 1.1
4-Ethenylpyridine	15.6	1.3 ± 0.1	5.7 ± 1.6	12.0 ± 0.4	13.3 ± 0.5	12.8 ± 1.8	5.1 ± 0.1	10.1 ± 1.1	15.9 ± 0.5	16.9 ± 1.0	16.3 ± 0.8
Butyl butanoate	16.2	0.8 ± 0.1	0.4 ± 0.6	0.6 ± 0.2	0.3 ± 0.4	0.2 ± 0.0	n.d.	n.d.	n.d.	n.d.	n.d.
Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	17.0	0.6 ± 0.1	n.d.	n.d.	0.3 ± 0.4	0.3 ± 0.4	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl hexanoate	17.2	1.5 ± 0.1	1.4 ± 0.3	0.8 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	1.4 ± 0.3	0.9 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Octanal	17.4	0.2 ± 0.0	0.6 ± 0.1	0.8 ± 0.1	1.3 ± 0.2	1.9 ± 0.1	n.d.	0.7 ± 0.0	0.8 ± 0.2	1.2 ± 0.0	1.3 ± 0.0
[(Z)-Hex-3-enyl] acetate	17.5	3.4 ± 0.3	2.1 ± 0.1	1.6 ± 0.0	1.3 ± 0.1	1.3 ± 0.1	3.6 ± 0.2	1.8 ± 0.0	1.4 ± 0.1	1.2 ± 0.0	1.1 ± 0.1
Hexyl acetate	17.9	1.8 ± 0.0	1.6 ± 0.1	1.2 ± 0.0	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.3	0.6 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
2,3-Dihydro-1H-indene	18.5	0.3 ± 0.1	0.4 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Ethylhexan-1-ol	19.0	0.6 ± 0.2	0.4 ± 0.2	0.5 ± 0.1	0.2 ± 0.3	0.4 ± 0.2	1.3 ± 0.2	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
(3E)-3,7-Dimethylocta-1,3,6-triene	19.7	0.6 ± 0.1	1.2 ± 0.3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-ol	20.0	n.d.	0.2 ± 0.3	2.1 ± 0.1	2.3 ± 0.0	2.4 ± 0.1	n.d.	0.4 ± 0.0	3.2 ± 0.1	3.3 ± 0.0	3.3 ± 0.1
2-Ethyl-1,4-dimethylbenzene	20.8	n.d.	1.1 ± 0.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Octan-1-ol	21.2	1.4 ± 0.2	n.d.	n.d.	n.d.	n.d.	2.0 ± 0.4	n.d.	n.d.	n.d.	n.d.
5-Ethenyl-2-methylpyridine	21.7	n.d.	2.5 ± 1.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nonanal	22.7	4.3 ± 0.3	4.2 ± 0.1	2.9 ± 0.4	3.4 ± 0.1	2.1 ± 0.0	6.1 ± 1.3	4.1 ± 0.1	3.2 ± 0.2	3.2 ± 0.0	2.0 ± 0.2
(6E)-7,11-Dimethyl-3-methylidenedodeca-1,6,10-triene	23.1	1.3 ± 0.1	2.2 ± 0.1	4.2 ± 0.1	4.3 ± 0.2	6.1 ± 0.7	1.3 ± 0.4	2.9 ± 0.1	4.0 ± 0.0	5.2 ± 0.1	6.0 ± 0.0
Ethyl cyclohexanecarboxylate	24.1	17.5 ± 1.4	11.1 ± 7.3	14.9 ± 3.7	1.0 ± 0.3	1.0 ± 0.7	19.5 ± 0.4	12.2 ± 2.2	6.0 ± 0.8	n.d.	n.d.
4-Hydroxy-3-methylbenzaldehyde	24.3	1.0 ± 0.0	0.6 ± 0.4	0.1 ± 0.0	0.1 ± 0.0	0.9 ± 0.0	0.9 ± 0.3	1.2 ± 0.0	n.d.	n.d.	0.8 ± 0.1
Ethyl benzoate	26.1	1.3 ± 0.1	0.6 ± 0.1	n.d.	n.d.	0.5 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.
2-Methoxy-4-methylphenol	27.2	20.0 ± 0.9	13.9 ± 2.3	10.4 ± 1.9	7.0 ± 1.9	7.5 ± 0.5	20.7 ± 0.2	15.6 ± 0.3	6.6 ± 1.4	6.9 ± 0.5	2.1 ± 0.1
Cyclohexasiloxane, dodecamethyl-	31.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl 3-phenylpropanoate	34.4	1.0 ± 0.1	1.2 ± 0.0	0.5 ± 0.2	0.5 ± 0.2	0.3 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
Copaene	35.3	6.2 ± 1.4	9.6 ± 0.6	2.6 ± 1.3	5.4 ± 3.8	3.3 ± 0.2	2.0 ± 0.0	n.d.	n.d.	n.d.	1.0 ± 0.3
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	35.6	1.7 ± 0.3	0.3 ± 0.4	0.6 ± 0.2	0.4 ± 0.0	0.3 ± 0.1	n.d.	n.d.	n.d.	n.d.	n.d.
α-Muurolene	40.6	1.6 ± 0.3	1.0 ± 0.0	0.3 ± 0.2	0.6 ± 0.4	0.2 ± 0.2	n.d.	n.d.	n.d.	n.d.	n.d.
α-Farnesene	40.8	0.8 ± 0.4	n.d.	n.d.	n.d.	0.3 ± 0.0	0.9 ± 0.3	1.5 ± 0.0	1.6 ± 0.1	1.5 ± 0.1	1.3 ± 0.4
β-Bisabolene	41.6	0.4 ± 0.1	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.0	n.d.	n.d.	n.d.	0.4 ± 0.1

n.d. not detected.

plot of the PC1. This plot allows separation of the two varieties. As can be seen, all variables apart from variable 11 (sensor resistance iAQ-Core) have a large influence on discrimination of the two varieties. Furthermore, from this graph it can be deduced that variables 2, 3, 6, 7, 9, and 10 have more influence on the 'Hojiblanca' variety, whilst the other variables are more relevant to the 'Manzanilla Cacereña' variety. These variables correspond to carbon dioxide (CO_2) and the total volatile organic compounds of the four sensors. Given that exploratory analysis produced good sample discrimination, a classification model was built. In this sense, PLSDA was applied in order to produce a predictive classification model capable of separating the two varieties.

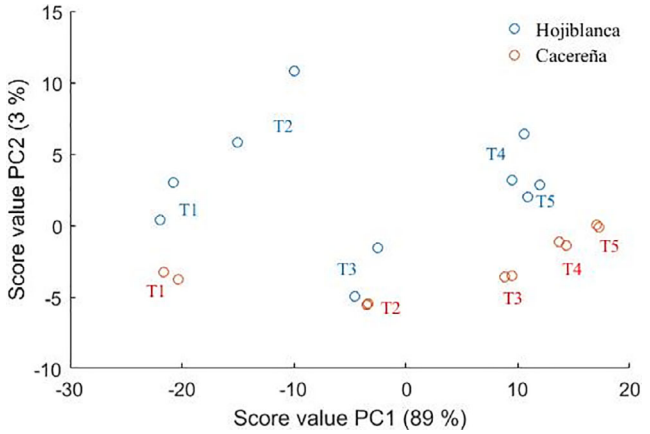

Figure 2. Principal component analysis (PCA) of the volatile compounds of Californian-style black olives from 'Hojiblanca' and 'Manzanilla Cacereña' by thermal sterilization treatment (T1: F_0 = 10 min; T2: F_0 = 14 min; T3: F_0 = 18 min; T4: F_0 = 22 min; T5: F_0 = 26 min, at 121 ± 3 °C).

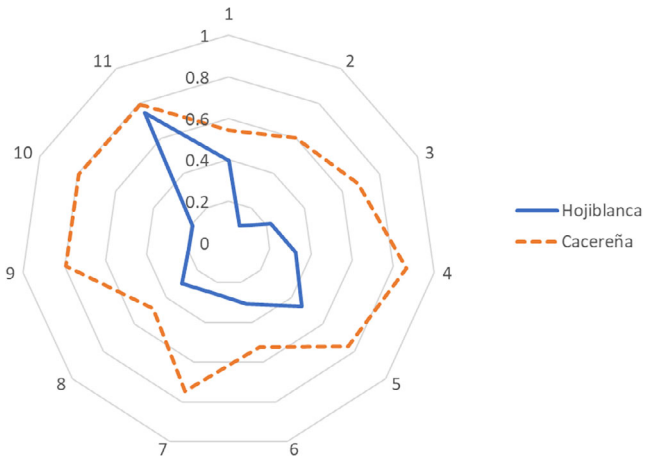

Figure 3. Radial representation of the table olive aroma fingerprints obtained from averaged preprocessed sensor signals.

The confusion matrix obtained using leave-one-out cross-validation (Table 4) revealed that almost all samples had been correctly classified, with 98.8% of predictions being correct.

These findings are consistent with those obtained from the panel test and volatile profile analysis, differentiating between both varieties and confirming the suitability of the E-nose for distinguishing between different table olive varieties. This may be due to the fact that the volatile compounds in the sample react with the sensor surface, adsorb its molecules, and oxidize them, which, in turn, reduces the resistance of the sensor.[17,24,25]

Thermal treatment discrimination using an E-nose

In this analysis, data for each variety were treated independently to visualize thermal treatment discrimination outcomes better. As was the case in the previous analysis, first, PCA was conducted on the measures produced by the E-nose to see whether trends emerged or samples could be grouped. Figure 5 presents the score and loadings plots of the first two principal components for 'Hojiblanca' (A) and 'Manzanilla Cacereña' (B) varieties.

Table 4. Confusion matrix obtained for the PLS-DA cross validation for olive variety discrimination. Values are expressed in percentages considering total samples

	Predicted variety	
Real variety	'Hojiblanca' (%)	'Manzanilla Cacereña' (%)
'Hojiblanca'	**53.5**	1.2
'Manzanilla Cacereña'	0	**45.3**

Diagonal bold values contains the percentage of correct assignments.

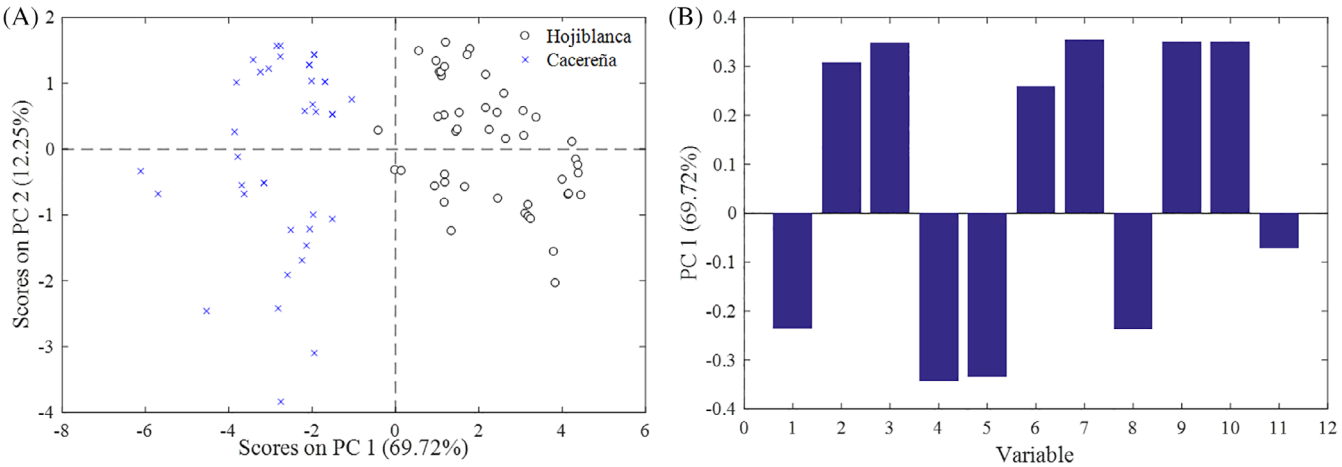

Figure 4. E-nose PCA score plot (A) and loading (B) obtained for the discrimination of Californian-style black olives from the 'Hojiblanca' and 'Manzanilla Cacereña' varieties.

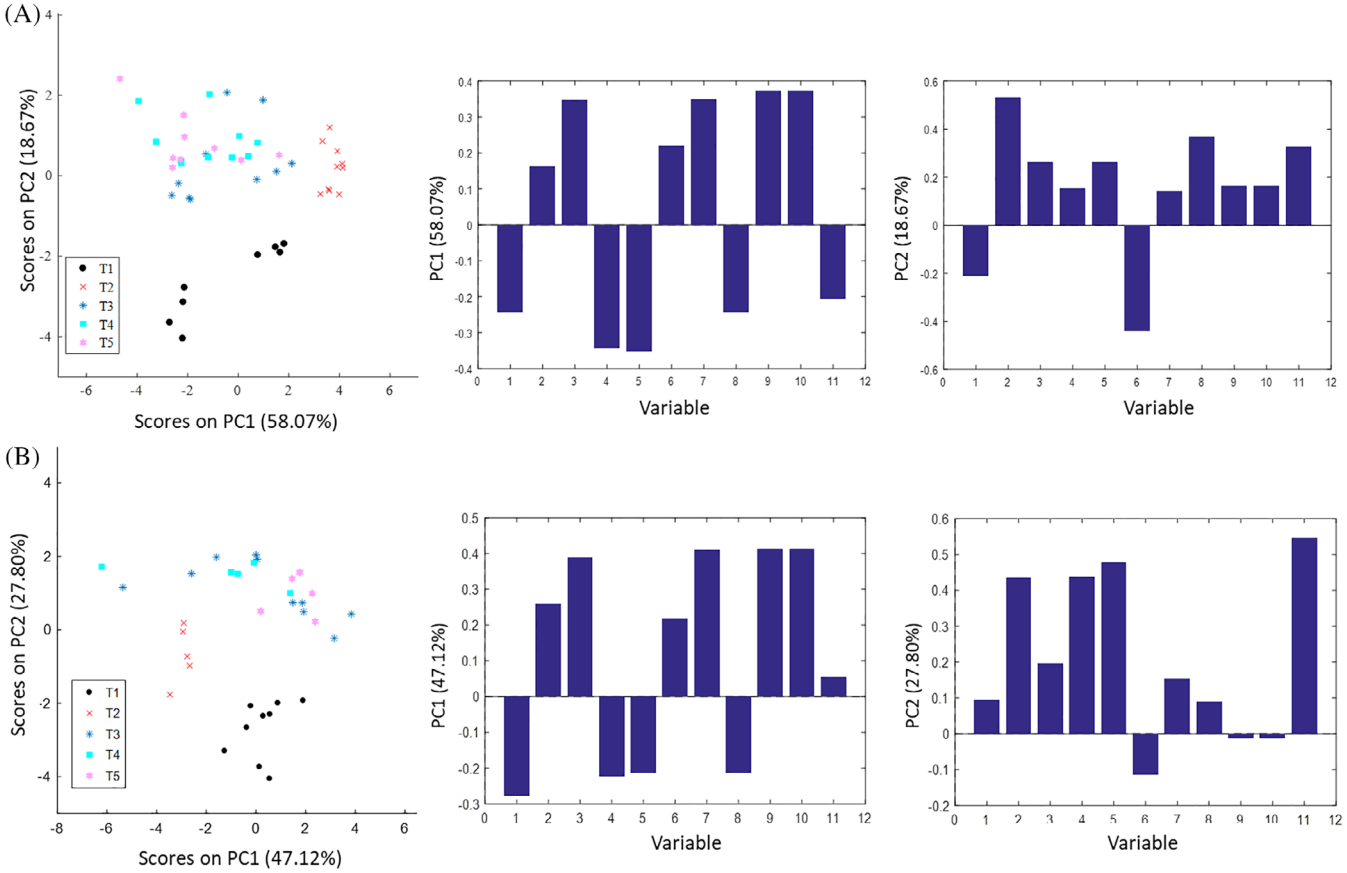

Figure 5. E-nose PCA score plots of the two first components and loading plots obtained for the discrimination of 'Hojiblanca' (A) and 'Manzanilla Cacereña' (B) by thermal sterilization treatment (T1: F_0 = 10 min; T2: F_0 = 14 min; T3: F_0 = 18 min; T4: F_0 = 22 min; T5: F_0 = 26 min, at 121 ± 3 °C).

Table 5. Confusion matrix obtained for a PLS-DA model for thermal treatment discrimination in each variety. Values are expressed in percentages

	Predicted							
	'Hojiblanca' (%)				'Manzanilla Cacereña' (%)			
Real	T1	T2	T3,T4,T5	Not assigned	T1	T2	T3,T4,T5	Not assigned
T1	**16.3**	0	0	2.1	**15.4**	0	0	7.7
T2	0	**18.3**	0	2.1	0	**10.3**	0	2.6
T3,T4,T5	0	0	**59.1**	2.1	0	0	**58.9**	5.1

Diagonal bold values contains the percentage of correct assignments.

In the score plot, three groups were observed in both varieties. Good separation is seen between the T1 and T2 samples, and between these samples and the other three. However, the T3, T4 and T5 samples appear to overlap in the score plot. These results confirm that volatile compounds are modified as sterilization time increases. Thus, these results are in line with those obtained previously from the panel test and volatile profile analysis, in which samples with a F_0 of 22 or more were not differentiated. However, the E-nose demonstrated lower discrimination capacity with T3 (F_0 = 18 min) samples not being separated from T4 and T5 samples.

With regard to loadings, for PC2 (the component responsible for discriminating between T1 and the other thermal treatments) the most relevant variables for discrimination were variables 2 and 6 (Table 1) for 'Hojiblanca' variety olives and variables 2, 4, 5 and 11 for 'Manzanilla Cacereña' variety olives. In both cases, T1 was observed to be more strongly influenced by variable 6 (CO_2 of CCS811 sensor). On the other hand, loadings in PC1 (the one which allows discrimination between T2 and T3, T4 and T5) were very similar to those obtained in the discrimination of varieties discussed above.

To characterize table olive samples according to the thermal treatment they are subjected to, a supervised classification model, PLSDA, was again applied. With regards to model classification performance, T3, T4, and T5 samples were placed into the same category. Confusion matrices produced in the cross-validation conducted on 'Hojiblanca' and 'Manzanilla Cacereña' samples (with four LV) are shown in Table 5.

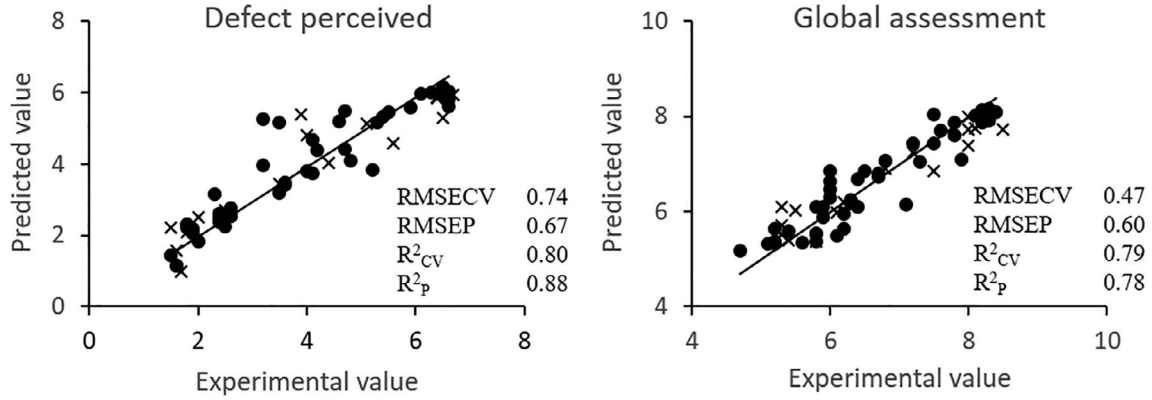

Figure 6. Experimental values against PLS cross-validation predictions (●) and validation set predictions (x) for defect perceived and global assessment.

Almost all samples were appropriately classified. With regards to 'Hojiblanca' samples, 93.7% of predictions were correct, whilst the remaining samples were not assigned to a group, and 84.6% of predictions were correct for the 'Manzanilla Cacereña' variety. The remaining samples for both varieties were not assigned to any group. These outcomes suggest that this technology was able to discriminate between samples with F_0 = 10 min (T1), F_0 = 14 min (T2) and F_0 values higher than 14 min.

Quantification of sensory parameters using an E-nose

With the aim of evaluating the use of the E-nose to quantify important quality parameters in table olives, a partial least squares (PLS) chemometric approach was evaluated. Partial least square regression was used to establish prediction models from the evaluations carried out by the sensory panel and data produced by E-nose signals. Specifically, perceived defect and overall assessment values from the tasting panel were used.

The PLS models were optimized using the calibration set discussed above. Two different PLS models were built, one for each parameter. Five LV were selected as optimum for both models. The R^2_{CV} values for the models developed for perceived defect and overall assessment were 0.8 and 0.79, respectively. Low RMSECV values were also estimated (0.74 for perceived defect and 0.47 for overall assessment). Cross-validation is presented in Fig. 6(A) through a graphical representation of the experimental parameter values versus the predicted values.

It is well known that the true predictive ability of a calibration model cannot be judged by using internal validation alone but must be validated with samples that were not included in the calibration test. To test the quality of the proposed models, they were applied to predict outcomes within the validation set. Outcomes of the validation are also presented in Fig. 6. Validation parameters pertaining to the PLS models were also very acceptable. R^2_P values were 0.88 for perceived defects and 0.78 for overall assessment, whilst the RMSEP values were 0.67 and 0.60 for perceived defects and overall assessment, respectively. As far as we know, little literature exists on the discrimination of defective olives with electronic devices. However, one study[2] has described the prediction of cooking defects produced by the application of different sterilization treatments to two varieties of oxidized black olives using an electronic tongue. These researchers also found that olive cultivar and sterilization treatment has a significant impact on the amount of cooking defect found due to its relationship with a toxic substance (acrylamide) in Californian-style black olives.[26] Other researchers[27] have argued that electronic noses can be used as olfactory sensors to detect abnormal fermentation (such as *zapateria*, *butyric*, *putrid*, and *musty*) in table olive defects in order to ensure their quality at industrial level. In this way these results indicated the feasibility of these devices as rapid analytical tools for monitoring the elaboration process of table olives. The results obtained demonstrate that the E-nose can determine tasting panel parameters such as perceived defect or overall assessment.

CONCLUSIONS

The E-nose was shown to be a useful discrimination tool for application with table olive varieties subjected to different sterilization treatments. The results suggest that, when combined with chemometric tools, this device (PCA, PLSDA, and PLS) may be used as a rapid and inexpensive screening method for the discrimination of standard characteristic aromas in table olives. Increasing the duration of thermal sterilization caused greater cooking effect sensations in the olives; however, when an F_0 higher than 22 min was applied, olive volatile profile was not significantly modified. All these outcomes were demonstrated through analysis of the volatile profile of olives both via traditional techniques, such as a tasting panel and gas chromatography, and via results obtained from the E-nose. Shorter sterilization periods therefore led to better quality characteristics in olives. Outcomes produced by the PLS models also suggest that the E-nose may be used to estimate taste parameters, such as cooking effect and overall assessments, with the advantage of being a 100% objective method. In summary, the E-nose is a powerful instrument that, when used with adequate chemometric analysis tools, can perform fast, inexpensive, non-destructive and environmentally friendly qualitative and quantitative analysis. In this way, the present study demonstrates the capability of this technology for use in the quality control of table olives at an industrial scale.

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COMPLIANCE WITH ETHICS REQUIREMENTS

This research does not include any experiment using animal and/or human subjects.

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2.6 Artículo 6. "Characterization of polyphenol and volatile fractions of Californian-Style black olives and innovative application of E-nose for acrylamide determination, (2021). Foods, 10(12), 2973."

Martín-Tornero, E., Sánchez, R., Lozano, J., Martínez, M., Arroyo, P., Martín-Vertedor, D.

2.6.1 Motivación y objetivos

La aceituna negra al "estilo californiano" es uno de los procesos de elaboración con más relevancia en el sector de las aceitunas de mesa. Una vez elaboradas las aceitunas con tratamiento alcalino y oxidación forzada con aire, requieren de tratamientos térmicos de esterilización para su conservación. A temperaturas por encima de 120ºC se promueve la síntesis de acrilamida, una sustancia tóxica cuyo proceso parece ser iniciado a través de la reacción química de Maillard y/o por rutas lipídicas (Casado y Montaño, 2008; Pérez-Nevado et al., 2018; Pan et al., 2020). La intensidad del proceso de esterilización térmica aplicados en este tipo de industrias, pueden producir concentraciones de acrilamida que oscilan entre 30 y 1050 $ng.g^{-1}$ (Casado y Montaño, 2008; Pérez-Nevado et al., 2018). Este rango de concentraciones se debe a que cada industria aplica unas condiciones diferentes durante el tratamiento de esterilización. Por lo tanto, durante el proceso de elaboración se debe controlar la intensidad de los tratamientos de esterilización para lograr un producto microbiológicamente estable y con la mínima cantidad de acrilamida.

La Autoridad Europea de Seguridad Alimentaria considera este tipo de aceitunas como fuente potencial de acrilamida (EFSA, 2015; Delatour et al., 2004) ya que estas aceitunas de mesa contienen niveles del compuesto que asemejan su concentración con los de otros alimentos como patatas fritas, pan, galletas, café, chocolate o cacao. Por esta razón, el conocimiento del contenido de acrilamida en las aceitunas de mesa al "estilo californiano" es importante para que se produzcan aceitunas de calidad para ser comercializadas sin riesgos para la salud humana.

En estudios previos sobre la composición de compuestos volátiles de aceitunas negras (López-López et al., 2019), el proceso de oscurecimiento o maduración en las aceitunas tuvo un impacto notable en el perfil de volátiles original del espacio de cabeza de las aceitunas, formándose compuestos como el 2-metilbutanal, 3-metilbutanal, 3-etilpiridina, 3-etil-4-metilpiridina, octano y benzaldehído. Alrededor del 70% de los componentes identificados en los productos finales correspondían a compuestos que se formaron probablemente debido a diferentes reacciones químicas que se produjeron durante el tratamiento de esterilización. Entre estos compuestos

volátiles, los predominantes fueron benzaldehído, sulfuro de dimetilo y acetato de etilo.

La cuantificación de acrilamida y compuestos volátiles requieren de métodos previos de extracción, purificación y detección mediante cromatografía, resultando ser costosos, complejos y que requieren mucho tiempo de análisis. Por otra parte, existen dispositivos de bajo coste, como la *nariz electrónica* que son capaces de evaluar los aromas de los COVs característico de los alimentos de manera rápida. Por lo tanto, estos dispositivos se podrían utilizar para establecer alguna relación entre el perfil volátil y el contenido de acrilamida en las aceitunas negras elaboradas al "estilo californiano".

Por lo tanto, este trabajo tuvo como objetivo evaluar la composición química referida al contenido de fenoles, acrilamida y compuestos volátiles en las aceitunas negras elaboradas al "estilo californiano", así como evaluar la capacidad de la *nariz electrónica* para predecir de manera indirecta el contenido de acrilamida.

2.6.2 Diseño experimental

Aceitunas de las variedades *'Manzanilla Cacereña'* y *'Hojiblanca'*, elaboradas al "estilo californiano", fueron envasadas y esterilizadas. Las latas fueron sometidas a cinco tratamientos de esterilización diferentes en función del parámetro de letalidad acumulada (F_0) que se calculó integrando el perfil de tiempo-temperatura registrado para cada tratamiento aplicado. El resultado fue la aplicación de los siguientes tratamientos: (i) F_0 = 10 min (T1), (ii) F_0 = 14 min (T2), (iii) F_0 = 18 min (T3), (iv) F_0= 22 min (T4), (v) F_0 = 26 min (T5). Las latas esterilizadas se almacenaron a temperatura ambiente hasta el momento del análisis. Los experimentos se realizaron por cuadruplicado.

2.6.3 Resumen de los resultados y discusión

La intensidad de los tratamientos térmicos de esterilización en ambas variedades tuvo una influencia significativa en las concentraciones finales de compuestos fenólicos, acrilamida y compuestos volátiles en general. De hecho, el aumento en la intensidad de esterilización (F_0 desde 10 a 26 minutos) redujo la concentración de fenoles y aumentó la síntesis de acrilamida. Esto puede ser debido a que los fenoles son termosensibles (Savini et al., 2017) y, por lo tanto, a una mayor F_0, se produciría una mayor reducción del contenido de estos compuestos.

En cuanto a los compuestos volátiles, las aceitunas negras al "estilo californiano" tras ser esterilizadas presentan un olor característico al *efecto de cocido* (Sánchez et al., 2021b). Este defecto provoca que las aceitunas presenten un perfil volátil determinado. Entre los compuestos volátiles identificados, los grupos éster y fenol, disminuyeron su concentración al aumentar el tratamiento de esterilización; sin embargo, aldehídos y otros grupos de compuestos volátiles aumentaron significativamente. Compuestos identificados que se asocian con olores desagradables son el 4-etil-piridina, benzaldehído y 2,4-dimetil-hexano, que aumentaron en sus concentraciones tras la aplicación de los tratamientos térmicos y que están relacionados con el *efecto de cocido* (López-López et al., 2019). Hay que destacar que la variedad *'Manzanilla Cacereña'* presentó la mayor cantidad de compuestos fenólicos y el menor contenido de acrilamida.

Por último, se construyó un modelo de Regresión Lineal Múltiple (MLR) entre el contenido relativo de todos los compuestos volátiles identificados y la acrilamida experimental obtenida por el método de referencia. Los resultados mostraron una buena correlación ($R^2 = 0,994$) entre la concentración de acrilamida cuantificada por el método HPLC tradicional y la concentración predicha obtenida por el modelo. De estos resultados se podría decir que es posible predecir el contenido de acrilamida a partir del perfil de compuestos volátiles. Sin embargo, la cromatografía sigue siendo una técnica costosa que requiere de mucho tiempo de análisis y de equipos costosos.

Por otro lado, se realizó el análisis con la *nariz electrónica* para evaluar el perfil aromático de las aceitunas e intentar correlacionarla con el contenido en acrilamida.

Para ello, se realizó un modelo PLS para relacionar las mediciones del perfil aromático de la *nariz electrónica* y los valores experimentales de la concentración de acrilamida. El modelo PLS se optimizó utilizando un conjunto de muestras de calibración y fue probado con otras muestras para la validación. El modelo mostró una alta linealidad con R_c^2= 0,85, y R_{CV}^2= 0,79 y R_P^2= 0,78, respectivamente. Además, demostró ser un método preciso para la cuantificación indirecta de esta sustancia tóxica ya que presentó una relación de errores del rango (RER > 10) y una desviación residual predictiva (RPD > 2,5) adecuadas. En la misma línea de resultados, Martín-Vertedor et al. (2020) determinaron el contenido en acrilamida con el uso de *lengua electrónica*. Rief et al. (2021) realizaron un análisis multivariante PLS para predecir indirectamente el contenido de acrilamida producido durante el tueste del café a partir de espectros de resonancia magnética de protones.

2.6.4 Conclusiones

- Las aceitunas con un alto contenido en compuestos fenólicos presentaron menor contenido de acrilamida.

- El contenido de fenoles, alcoholes, aldehídos y ésteres disminuyó a medida que aumenta la F_0.

- Algunos compuestos volátiles como benzaldehído, 4-etenilpiridina o 2,4-dimetilhexano están relacionados con el *efecto de cocido*, olor desagradable en las aceitunas, sintetizadas por la aplicación de tratamientos térmicos de esterilización.

- Los tratamientos de esterilización térmica más agresivos provocaron el característico *efecto de cocido*.

- La evaluación cromatográfica de los volátiles de las aceitunas tratadas térmicamente establecieron una buena correlación con la concentración de acrilamida.

- La *nariz electrónica* se podría utilizar a nivel industrial para predecir el contenido en acrilamida en aceitunas negras al “estilo californiano”.

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Characterization of Polyphenol and Volatile Fractions of Californian-Style Black Olives and Innovative Application of E-Nose for Acrylamide Determination

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Abstract: Californian-style black olives require a sterilization treatment that produces a carcinogenic contaminant, acrylamide. Thus, this compound was evaluated in two different olive cultivars using an electronic nose (E-nose). The sterilization intensity had a significant influence on the final phenol concentrations, acrylamide content, and volatile compounds. Increasing the sterilization intensity from 10 to 26 min (F0) reduced the phenol content, but it promoted acrylamide synthesis, leading to a wide range of this toxic substance. The Ester and phenol groups of volatile compounds decreased their content when the sterilization treatment increased; however, aldehyde and other volatile compound groups significantly increased their contents according to the thermal treatments. The compounds 4-ethenyl-pyridine, benzaldehyde, and 2,4-dimethyl-hexane are volatile compounds with unpleasant odours and demonstrated a high amount of influence on the differences found after the application of the thermal treatments. The "Manzanilla Cacereña" variety presented the highest amount of phenolic compounds and the lowest acrylamide content. Finally, it was found that acrylamide content is correlated with volatile compounds, which was determined using multiple linear regression analysis (R^2 = 0.9994). Furthermore, the aroma of table olives was analysed using an E-nose, and these results combined with Partial Least Square (PLS) were shown to be an accurate method (range to error ratio (RER) > 10 and ratio of performance to deviation (RPD) > 2.5) for the indirect quantification of this toxic substance.

Keywords: table olives; sterilization treatments; volatile compounds; acrylamide; electronic nose

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1. Introduction

Olive tree cultivation mainly extends throughout the Mediterranean area, with the commercial uses of olives mainly being the production of oil and table olives. Table olive production exceeded 2.9 million tons during the 2019–2020 season, with the main producers being Spain, Egypt, Turkey, Algeria, Italy, Greece, and Portugal [1,2]. The nutritional relevance of table olives is widely known. Olives are a good source of good monounsaturated fat, vitamin e, polyphenols, and flavonoids (antioxidants with anti-inflammatory benefits).

Californian-style black olive is one of the most important elaboration processes in the table olive sector. These olives are often consumed on pizzas, and canned olives are preserved and are used in other ways. The popularity of Mediterranean cuisine also

contributes to sustained consumption. The main variety used in this process is "Hojiblanca", a variety that is produced in Andalusia, followed by "Manzanilla Cacereña" or "Manzanilla Sevillana" [3,4]. To produce ripe black olives, the olives are harvested when they are green (unripe), and they are then cured through exposure to a series of air oxidation and lye treatments to obtain debittered black olives. The olives are placed in cans and are subjected to a thermal sterilization treatment.

High temperatures above 120 °C promote acrylamide synthesis, a process that seems to be initiated through a chemical reaction such as the Maillard reaction, which is related to carbohydrates and amino acids (asparagine) [4–6]. However, the synthesis of this toxic substance has also recently been related to fat-rich table olives [7]. The intensity of the thermal sterilization process applied in each industry produces different acrylamide concentrations in oxidized olives ranging from 30 to 1050 ng g^{-1} [4,6]. This wide range in acrylamide content is mainly due to the fact that each industry applies a different thermal sterilization treatment. Thus, industries should control sterilization treatments to apply the minimum amount of heat necessary to achieve a microbiologically stable product with the least amount of acrylamide.

This chemical molecule is classified as toxic and carcinogenic and has demonstrated adverse health effects [5–8]. Furthermore, the European Food Safety Authority considers this kind of olives as a potential source of acrylamide since these table olives contain higher levels of the compound compared to other foods such as chips, bread, cookies, coffee, chocolate, or cocoa [9,10]. For this reason, knowledge of the acrylamide content in table olives is important for human health. A European recommendation [11] indicates that authorities must control the acrylamide level in olives stored in brine solutions on an industrial scale. In this sense, several results in the literature apply different mitigation strategies to reduce acrylamide levels during the industrial elaboration process of Californian-style black [4,5,12,13] and green olives [14].

Acrylamide and volatile compound quantification require long-term extraction and purification methods [6,15]. Moreover, analyses are carried out using expensive equipment, such as with mass spectrometry detectors that are similar to those used for gas [4,16] or liquid chromatography [6,12,17]. They turn out to be expensive, complex, and time-consuming techniques that require trained personnel.

Electronic devices are able to evaluate the volatile organic compounds that are responsible for the characteristic odour patterns of the food. These devices commonly called electronic noses (E-nose) and consist of an array of gas sensors and a data acquisition block, and the application of data processing techniques has proven to be a useful tool for the evaluation of olives. However, electronic nose devices have not been used to predict the acrylamide content in foods or Californian-style black olives as of yet. In the literature, we were only able to find other devices with voltammetric biosensors for detecting this toxic substance in foods [18,19]. Recently, studies have been carried out using electronic noses to predict the quality of the oil to be processed from the measurements obtained in freshly harvested olives [20] and to evaluate the odours produced in anomalous fermentations of Spanish-style green olives [15]. The variation of the volatile organic compounds generated in food after thermal treatments has also been studied to monitor the evaluation of the quality of the oil used for frying [21], to identify the olfactory profile of rapeseed oil as a function of heating time [22], or in the sensory evaluation of Californian-style black olive sterilization treatments [23].

Thus, this work aimed to assess the chemical composition in terms of phenol profile, antioxidant activity, acrylamide content, and volatile compounds in Californian-style black olives ("Manzanilla Cacereña" and "Hojiblanca" varieties) that have been submitted to different thermal treatments (10–26 min at 121 ± 3 °C) to find out whether the E-nose can be used to determine acrylamide content.

2. Materials and Methods

2.1. Experimental Design

Two olives varieties ("Hojiblanca" and "Manzanilla Cacereña") were harvested at the green stage of maturation during the 2020/21 season in the "Vegas Bajas del Guadiana" area. The olives were stored for 4 months with acetic acid (1.5% *v*/*v*) and salt (8% *p*/*v*) in industrial tanks in triplicate. The olive comprised Californian-style black olives, and the olives were packed into cans with 2% NaCl and 0.015% ferrous gluconate [13].

The cans were sterilized at a specific temperature (121 ± 3 °C) with the different equivalent treatment durations (in minutes) required to reduce the initial microbial load. Cans were submitted to five sterilization treatments using the accumulated lethality for reducing the microbial population, which was calculated as F0 values by integrating the time–temperature profile recorded for each treatment that was studied: (i) F0 = 10 min (T1), (ii) F0 = 14 min (T2), (iii) F0 = 18 min (T3), (iv) F0 = 22 min (T4), and (v) F0 = 26 min (T5). All of the cans were stored at room temperature. The experiments were performed four times.

2.2. Chemical and Reagents

For Californian-style black olive elaboration, an acetic acid solution was purchased from Panreac Applichem® (Darmstadt, Germany), ferrous gluconate and sodium chloride were supplied by Sigma–Aldrich (St. Louis, MO, USA), and calcium chloride was obtained by Tetra Chemicals (Helsingborg, Sweden).

The following analytical standards were used for phenolic profile analysis: hydroxytyrosol, procyanidin B1 (PB1), procyanidin B2 (PB2), apigenin-7-O-glucoside, oleuropein, and verbascoside were supplied by Extrasynthése (Genay, France), and tyrosol, vanillic acid, epicatechin, luteolin-7-O-glucosidem and p-coumaric acid were supplied by Sigma-Aldrich Chemie (Steinheim, Germany). P.A. grade formic acid was purchased from PANREAC (Barcelona, Spain), and sodium fluoride was supplied by Sigma-Aldrich Chemie (Steinheim, Germany). HPLC grade acetonitrile and methanol were provided from Fisher chemical (Loughborough, UK). For the antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazyl) and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were supplied by Alfa-Aesar (Kandel, Germany) and Sigma-Aldrich (Steinheim, Germany), respectively.

Acrylamide and 2,3,3-D3-acrylamide were acquired from Fluka (Buchs SG, Switzerland) and Cambridge Isotope Laboratories (Andover, MA, USA), respectively. Analytical grade methanol was obtained from Merck (Darmstadt, Germany).

A nylon syringe filter was purchased from FILTER-LAB (Barcelona, Spain). Isolute Multimode (300 mg, 6 mL) and ENV+ (200 mg, 3 mL) Solid Phase Extraction (SPE) columns were obtained from IST (Hengoed, Mid-Glamorgan, UK). Nylon and nitrocellulose syringe filters (0.45 μm) were purchased from Tracer Analytical Technologies (Madrid, Spain). Water was purified with an Elix/Milli-Q water purification system (Millipore, Bedford, MA, USA). For volatile compound analysis, stableflex polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre was acquired from Supelco. The DB WAXETR capillary column (60 m × 0.25 mm; DI: 0.25 mm) was purchased from Agilent. For the electronic device (E-nose), the commercial sensor array of the oxide semiconductor (MOX) was procured from different manufacturers: i) Bosch BME680: temperature (°C), pressure (hPa), humidity (% RH), and gas measurement (Ω); (ii) Sensirion SGP30: eCO_2 (ppm), TVOC (ppb), H_2 (2), and ethanol; (iii) ScioSense CCS811: eCO_2 (ppm), TVOC (ppb), and sensor resistance (Ω); and iv) ScioSense iAQ-Core: eCO_2 (ppm), TVOC (ppb), and sensor resistance (Ω).

2.3. Analyses

Different analyses were carried out on Californian-style black olives subjected to thermal treatments. Specifically, phenol chromatographic analysis, antioxidant activity, acrylamide content, volatile compound, and E-nose measurements were performed.

2.3.1. HPLC Analysis of the Phenolic Profile of the Olives

The phenolic extraction and characterization of the phenolic profile were performed according to the methodology described by Cabrera-Bañegil et al. [24] via chromatographic separation. A 2 g sample of table olives was crushed, mixed with a solution of 2 mM NaF in 10 mL of methanol, and homogenized. It was placed in an ultrasonic bath (P-Selecta, mod 513) for 30 min. The extracts were centrifuged at 1677× *g* at 4 °C for 10 min. Finally, the supernatant extracts were filtered through a 0.22 mm nylon syringe filter (FILTER-LAB) before injection into the HPLC system [8,24,25]. Samples were analysed on an Agilent 1100 series HPLC system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detector (DAD) and a fluorescence detector (FLD). The analytical column that was employed was a Phenomenex Gemini-NX C18 column (Phenomenex, Torrance, CA, USA), (150 mm × 4.6 mm, 3 μm). The column temperature was set at 40 C. The injection volume was 10 μL, and the flow rate was 1 mL min^{-1}. The mobile phases were 0.1% (*v*/*v*) formic acid in water (eluent A) and 0.1% (*v*/*v*) formic acid in acetonitrile (eluent B). The gradient used was as follows: 0–1 min, 3% B in isocratic mode; 1–30 min, linear gradient from 3% to 35% B; 30–33 min, linear gradient from 35% to 50% B; 33–34 min, linear gradient from 50% to 100%; and 34–50 min, 100% B in isocratic mode. The gradient was then returned to 3% eluent B, and this composition was held for 3 min to re-equilibrate the column. Quercetin and oleuropein were monitored by DAD and were quantified at 255 nm; the benzoic acids were quantified at 280 nm; the cinnamic acids were quantified at 320 nm; flavones and quercetin-3-rutin were quantified at 350 nm; and anthocyanins were quantified at 515 nm. Fluorescence detection at 275/315 nm was used for the analysis of hydroxytyrosol, tyrosol, PB1, catechin, PB2, and epicatechin

2.3.2. DPPH Antioxidant Activity

The phenolic extract obtained from the olive matrix explained in Section 2.3.1 was used to carry out the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical method. An amount of 300 μL of the phenol extract sample was mixed with 2.7 mL of DPPH methanolic solution. The corresponding mixture was kept in darkness for 1 h, and the absorbance was then measured at 517 nm against a blank (MeOH) in a J.P Selecta, S.A spectrophotometer (Madrid, Spain). The results were expressed as Trolox equivalent values (mg Trolox·100 mL^{-1} extract).

2.3.3. Acrylamide Determination

Acrylamide determination was carried out following the method described by Pérez-Nevado et al. [6] and that had been validated by Fernández et al. [26]. An amount of 2 g of olives was crushed, homogenized, and shaken with 10 mL of Milli-Q water for 60 min. Afterwards, the sample was centrifuged for 30 min at 4 °C at 1677 g. The aqueous phase was filtered through a 0.45 μm nylon syringe filter and was cleaned through a Telos PCX (200 mg/3 mL) solid-phase extraction cartridge. The cartridge was conditioned with 4 mL of methanol followed by 4 mL of Milli-Q water, and 3 mL of the sample was injected into the column and eluted with 3 mL of Milli-Q water. The eluate was introduced in another Telos PRP cartridge (60 mg/3 mL), which had been conditioned in the same way and eluted with 3 mL of Milli-Q water. The quantification of the acrylamide content was carried out by the standard addition method using a standard acrylamide solution (50–150 ng mL^{-1}).

Samples were analysed on an Agilent 1290 Infinity II liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a degasser, quaternary pump, column

oven, and autosampler, and the Chemstation software was used for instrument control, data acquisition, and data analysis. The analytical column used was a Zorbax Eclipse XDB-C18 column (150 mm × 2.1 mm, 3.5 μm) (Agilent technologies), the temperature of which was set at 30 ◦C. The mobile phase was composed of 95% solvent A (0.1% formic acid in Milli-Q water) and 5% solvent B (0.1% of formic acid in methanol). The flow rate was 0.25 mL/min, and the injection volume was 3 μL.

Detection was performed with a mass spectrometer Agilent Technologies 6460 triple quadrupole equipped with an electrospray ion source operating in positive ion mode. The ion source parameters were set as follows: gas temperature: 340 °C, gas flow: 12 L h^{-1}, nebuliser: 40 psi, sheath gas temp: 400 °C, sheath gas flow: 12 L h^{-1}, capillary voltage: +2.5 kV, nozzle voltage: 300 V, and delta EMV: 300. The fragmentary voltage was 50 V, and the collision energy was 9 V and 20 V, respectively.

2.3.4. Volatile Compound Analysis

A paste was obtained from the previously deboned and homogenized black olives. An aliquot of 2.0 g of paste was mixed with 7.0 mL of 30% NaCl (*w*/*v*) in a 15 mL glass vial. The volatile compounds were analysed with a Bruker Scion 456-GC triple quadrupole gas chromatograph following a procedure reported in the literature [27]. They were sampled from the headspace at 40 °C for 15 min using an SPME with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) StableFlex fiber (65 μm, Supelco). After SPME, desorption was carried out in the injection port of the gas chromatograph at 250 °C for 15 min. The components were separated using a VF-5MS capillary column (30 m × 0.25 mm; ID: 0.25 mm). The different volatile compounds were identified by comparison with the NIST 2.0 MS library. A representative chromatogram is shown in Figure 1.

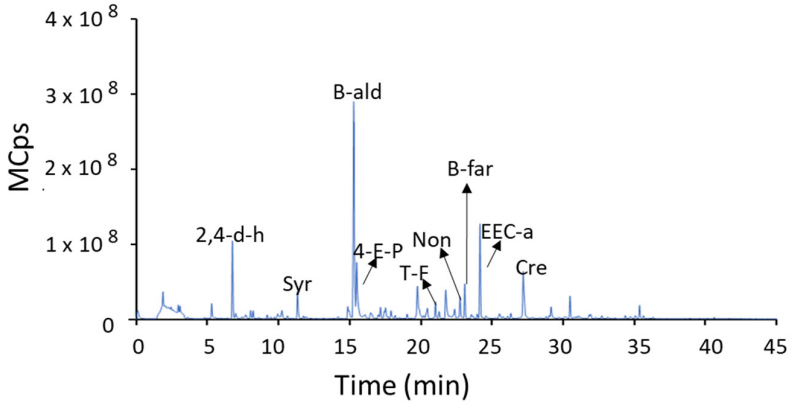


Figure 1. Representative chromatogram of the aromatic profile of thermal sterilized California-style black olives. Syr: styrene; 4-E-P: 4-ethenyl-pyridine; t-F: trans-farnesol; B-ald: benzaldehyde; Non: nonanal; EEC-a: ethyl ester cyclohexanecarboxylic acid; Cre: creosol; 2,4-d-h: 2,4-dimethyl-hexane; b-far: beta-farnesene.

2.3.5. E-nose Determination

The E-nose device that was used was small in size (39 mm × 33 mm), portable, low-energy and could be connected to a smartphone via Bluetooth protocol. It was designed by the Research Group on Perception and Intelligent Systems at the University of Extremadura. The E-nose consists of four gas sensor chips with integrated metal oxide (MOX) sensors: BME680, SGP30, CCS811, and iAQ-Core. The microcontroller read the values detected by the sensors, formatted them, and sent them to an external smart device

via Bluetooth. All of the sensors, except BME680, include intelligent algorithms to process the raw signals to output TVOC (Total Volatile Organic Compounds) and equivalent CO2 (eCO2) prediction values. Additionally, SGP30 provides raw signals for H_2 and ethanol. The BME680 also includes temperature, relative humidity, and atmospheric pressure sensors.

This type of sensor matrix has been successfully used for the evaluation of trichloroanisole (TCA) in cork stoppers [28], air quality measurement [29], or the discrimination of anomalous fermentation defects in table olives [15]. The odour profile measurements of the olive and brine samples were carried out following the recommendations of the International Olive Council [30] for the olive tasting panel.

Samples of olives with brine were placed in the tasting glasses, covered with a clock glass, and placed in a heating block at 25 °C. Each data acquisition cycle consisted of two parts after an initial period of sensor stabilization. The first part corresponded to a 30 s desorption period, during which the sensors were placed in an empty cup. In the second part of the cycle, the volatile absorption achieved by the table olives was analysed at one-second intervals for 60 s. Five measurements were taken for each table olive sample.

2.4. Data Analysis

Two-way ANOVA was performed to determine the significant differences between olive varieties and the sterilization treatments on the different chemical compounds being studied. One-way ANOVA and Tukey's test were conducted when the interaction effect was not statistically significant at a level of $p < 0.05$. SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used to perform the analysis. The results were expressed as mean values ± standard deviation.

2.5. Chemometric Analysis

Chemometric analysis was performed using Matlab 2016b version 9.1 (Math-Works, Natick, MA, USA) with the PLS-toolbox 8.2.1 (Eigenvector Research, Inc., Wenatchee, WA, USA).

Multiple Linear Regression (MLR) was used to identify the relationship between the percentage of the volatile compounds and the acrylamide concentration. The coefficient of determination (R^2) was applied to evaluate the performance of the applied model.

The partial least squares (PLS) method [31] was used to build a model for the quantification of acrylamide from the E-nose data. The data set was split into a calibration set (70% of the samples), which was used to calibrate and cross-validate the models, and a validation set (30%), which was only used to test the robustness and accuracy of the developed models. The samples were divided randomly between the two sets. The optimal number of latent variables (LV) was optimized based on the calculation of the minimum root mean square error.

The model performance was estimated using the following statistic parameters: the coefficient of determination for calibration (R^2_{cal}), cross-validation (R^2_{CV}), and prediction (R^2_P) (Equation (1)); the root mean square error for calibration (RMSEC), cross-validation (RMSECV), and prediction (RMSEP) (Equation (2)); and the ratio of performance to deviation (RPD) (Equation (3)) and the range to error ratio (RER) (Equation (4)).

$$R^2 = 1 - \frac{\sum(y_{pred} - y_{exp})^2}{\sum(y_{pred} - y_{mean})^2} \quad (1)$$

$$RMSE = \sqrt{\sum \frac{(y_{pred} - y_{exp})^2}{N}} \quad (2)$$

$$RPD = \frac{SD}{RMSEP} \quad (3)$$

$$\mathrm{RER} = \frac{(y_{max} - y_{min})}{\mathrm{RMSEP}} \quad (4)$$

where y_{exp} is the experimental value; y_{pred} is the corresponding value obtained for the calibration (R^2_{cal} and RMSEC), cross-validation (R^2_{CV} and RMSECV), and prediction (R^2_P and RMSEP); N is the number of samples; and SD is the standard deviation of the experimental values.

The PLS model can be regarded as an acceptable model if it has a low number of PCs, low RMSE values, high R^2, an RPD value greater than 2, and a low gap between the two sets (i.e., calibration and validation) [32]. RER values above 10 indicate that the models were adequately identified [33].

3. Results

3.1. Effect of Thermal Treatments on the Chemical Properties

The chemical properties studied in Californian-style table olives submitted to different thermal treatments are reported in Table 1. A two-way ANOVA analysis was computed to assess the effect of the olive varieties and sterilization treatments. A significant interaction was found in almost all of the variables that were studied, so data are shown for each olive variety and thermal treatment studied. Thus, the phenolic compounds profile and antioxidant activity were performed after each thermal treatment and at the same time as when the acrylamide formation and the volatile compounds were being analysed

Table 1. Chemical composition of Californian-style black olives ("Manzanilla Cacereña" and "Hojiblanca") submitted to different thermal sterilization treatments. Results are expressed as mean ± SD of three sample replicates. For the same cultivar, different lowercase letters mean a statistically significant difference between thermal treatment (one-way ANOVA followed by Tukey's test, $p < 0.05$).

'Manzanilla Cacereña'										
	T1		***T2***		**T3**		**T4**		**T5**	
Phenolic profile (mg·100g^{-1})										
Hydroxytyrosol	1226.6 ± 69.8	e B	1004.5 ± 32.8	d B	920.1 ± 14.5	c B	680.5 ± 25.8	b B	551.8 ± 26.2	a B
Tyrosol	312.1 ± 6.4	d B	205.4 ± 10.5	c B	196.6 ± 16.1	c B	143.7 ± 5.7	b B	127.0 ± 6.3	a B
PB1	41.4 ± 3.5	d B	40.6 ± 3.6	d B	27.5 ± 5.5	c B	19.1 ± 1.6	b NS	15.5 ± 1.0	a B
Vanillic acid	6.8 ± 0.5	b B	3.8 ± 0.2	a NS	4.1 ± 0.2	a NS	3.7 ± 0.1	a NS	3.7 ± 0.1	a NS
Epicatechin	4.3 ± 0.1	c NS	4.8 ± 0.7	d B	3.9 ± 0.5	b NS	3.8 ± 0.5	b NS	3.2 ± 0.1	a NS
Oleuropein	239.4 ± 10.9	e B	206.8 ± 4.8	d B	155.0 ± 7.4	c B	125.8 ± 3.4	b B	99.6 ± 1.5	a B
Luteolin-7-O-glucoside	6.1 ± 0.6	e B	4.9 ± 0.1	d B	4.1 ± 0.2	c NS	1.8 ± 0.2	b B	1.4 ± 0.1	a NS
Apigenin-7-O	7.8 ± 0.6	c B	6.1 ± 1.4	b B	6.2 ± 0.2	b NS	2.7 ± 0.2	a B	2.5 ± 0.1	a B
Verbascoside	8.9 ± 1.1	d B	9.6 ± 0.7	d B	7.4 ± 0.3	c B	1.9 ± 0.1	b B	1.3 ± 0.1	a B
p-coumaric	21.6 ± 1.4	e B	18.7 ± 0.5	d B	16.4 ± 0.7	c B	3.4 ± 0.4	b B	2.4 ± 0.1	a B
Σ phenols	1874.9 ± 77.9	e B	1505.2 ± 47.4	d B	1341.2 ± 32.7	c B	986.4 ± 28.6	b B	808.5 ± 30.4	a B
Antioxidant properties (mgTrolox·g extrac^{-1})										
DPPH	2.7 ± 0.1	d B	2.6 ± 0.2	d B	2.5 ± 0.3	c B	2.2 ± 0.1	b B	1.9 ± 0.1	a B
Toxic substance (ng·g^{-1})										
Acrylamide	105.4 ± 3.4	a A	137.7 ± 3.1	b A	188.7 ± 5.2	c A	312.4 ± 6.0	d A	383.5 ± 8.9	e A
'Hojiblanca'										
	T1		***T2***		**T3**		**T4**		**T5**	
Phenolic profile (mg·100g^{-1})										
Hydroxytyrosol	911.2 ± 9.9	e A	839.0 ± 15.6	d A	793.4 ± 14.6	c A	526.4 ± 3.0	b A	425.8 ± 6.0	a A
Tyrosol	194.0 ± 7.8	e A	164.4 ± 5.3	d A	152.2 ± 4.1	c A	104.9 ± 5.2	b A	89.6 ± 3.5	a A
PB1	30.9 ± 1.2	d A	23.3 ± 1.1	c A	20.2 ± 2.4	c A	17.3 ± 2.6	b NS	9.7 ± 0.5	a A
Vanillic acid	5.0 ± 0.1	c A	4.1 ± 0.2	b NS	3.9 ± 0.3	b NS	3.1 ± 0.1	a NS	3.1 ± 0.1	a NS
Epicatechin	3.7 ± 0.3	ns NS	3.2 ± 0.1	ns A	3.9 ± 0.4	ns NS	4.2 ± 0.1	ns NS	2.8 ± 0.6	ns NS
Oleuropein	173.2 ± 9.4	e A	150.8 ± 8.4	d A	141.6 ± 9.8	c A	113.8 ± 3.0	b A	91.9 ± 6.5	a A
Luteolin-7-O-glucoside	5.2 ± 0.2	c A	4.4 ± 0.1	b A	4.3 ± 0.3	b NS	1.3 ± 0.2	a A	1.3 ± 0.1	a NS
Apigenin-7-O	6.1 ± 0.1	c A	5.1 ± 0.2	c A	6.2 ± 0.6	c NS	2.2 ± 0.1	b A	1.2 ± 0.1	a A
Verbascoside	4.6 ± 1.0	b A	5.4 ± 0.2	b A	4.9 ± 0.1	b A	1.2 ± 0.2	a A	1.1 ± 0.1	a A
p-coumaric	16.5 ± 1.1	c A	16.6 ± 0.5	c A	10.7 ± 0.5	b A	2.7 ± 0.2	a A	1.9 ± 0.1	a A
Σ phenols	1350.3 ± 20.6	e A	1216.4 ± 24.7	d A	1141.3 ± 10.6	c A	777.1 ± 9.4	b A	628.5 ± 12.2	a A
Antioxidant properties (mgTrolox·g extrac^{-1})										
DPPH	1.0 ± 0.1	d A	1.0 ± 0.1	d A	0.9 ± 0.1	c A	0.8 ± 0.1	b A	0.6 ± 0.1	a A
Toxic substance (ng·g^{-1})										
Acrylamide	136.7 ± 4.4	a B	172.9 ± 2.8	b B	244.0 ± 5.8	c B	362.8 ± 4.3	d B	446.1 ± 12.9	e B

F0 = 10 min; T2: F0 = 14 min; T3 F0 = 18 min; T4: F0 = 22 min; and T5: F0 = 26 min; ns or NS means no significant differences.

3.1.1. Effect of the Sterilization Treatments on the Antioxidant Properties

The phenolic profiles of the table olives allowed for the separation and identification of 10 phenols. Qualitative differences in these compounds were found after the application of different sterilization treatments on the studied varieties. Regardless of the intensity of the thermal treatment applied, hydroxytyrosol was the main phenolic compound recorded in the table olives that were studied, followed by tyrosol and oleuropein. The other minor phenolic compounds were PB1, p-coumaric, verbascoside, apigenin-7-*O*-glucoside, vanillic acid, luteolin-7-*O*-glucoside, and epicatechin.

The application of different intensities of thermal treatments in the present study had a significant influence on the final phenol concentrations. Thus, the increase of the sterilization intensity from F0 values of 10 to 26 min reduced the phenol contents in Californian-style black olives in both of the studied varieties. In fact, when the sterilization treatment was more aggressive, the total content of phenolic compounds decreased by more than 50% in both varieties. Although almost all of the phenolic compounds significantly decreased their initial content; the phenols that were the most sensitive to sterilization treatments were verbascoside and p-coumaric, the contents of which decreased by more than 80%, while the least sensitive were epicatechin and vanillic acid, which decreased by 20%. Hydroxytyrosol, oleuropein, p-coumaric, or the sum of the phenol profiles (Σ phenols) that were analysed decreased significantly with increasing amount of sterilization treatments; however, other phenols such as tyrosol, PB1, or vanillic acid were more or less affected depending on the intensity of the thermal treatment.

Furthermore, differences related to phenolic compounds were found when comparing different Californian-style black olive varieties. The highest concentrations were found in the "Manzanilla Cacereña" variety in all of the sterilization treatments that were studied. The total phenol profiles (Σ phenols) were more than 20% higher in "Manzanilla Cacereña" than they were in "Hojiblanca" regardless of the thermal treatment applied. The phenols with the least varietal influence were vanillic acid and epicatechin, especially when the sterilization treatments were greater.

Concerning the antioxidant profile of the two varieties considered herein, the results are depicted in Table 1. The DPPH antioxidant method showed significant differences between the thermal treatment applied and the table olive varieties studied. The preliminary results suggested that the strongest antioxidant activity was found when the thermal treatments were less aggressive in both varieties. The variety that showed the highest anti-radical activity was "Manzanilla Cacereña".

3.1.2. Effect of Thermal Treatments on Acrylamide Content

The acrylamide levels that were analysed by HPLC-MS-QQQ in olives submitted to different sterilization treatments are shown in Table 1. According to the results, there is a significant interaction effect ($p \leq 0.05$) between the olive cultivar and the thermal sterilization treatments. Thus, the results are presented individually for each olive variety that was studied and the thermal treatments that were applied.

The sterilization treatments that were used in the last step of the elaboration process of the Californian-style black olive had a huge influence on the acrylamide synthesis. This led to a wide range of concentrations of this toxic substance (105.4 to 446.1 $ng \cdot g^{-1}$) depending on the applied time-period treatment and olive variety used. In fact, when the applied thermal intensity was too aggressive (T5), the acrylamide content increased by more than 70% with respect to treatment T1. Thus, with the gentler thermal treatments are used (T1–T3), the increase in acrylamide content is not as high.

In addition, the olive variety also was determined to influence acrylamide levels. The variety with the highest acrylamide content was "Hojiblanca", while the lowest level of this toxic substance was observed in the "Manzanilla Cacereña" variety. In both varieties, the acrylamide content increased with the cumulative sterilization treatments. In fact, the

"Hojiblanca" variety presented higher acrylamide levels in a range of 14 to 23% depending on the thermal treatment applied.

Finally, the correlation between the level of acrylamide and phenols was carried out by taking into account the high influence of phenols on the formation of this toxic substance. Depending on the phenolic compound that was studied, the Pearson correlation varied its significance level. A negative correlation was observed for most of the phenols analysed and for the sum of the total phenols (Σ phenols) with an acrylamide concentration. The compounds with the highest correlation were hydroxytyrosol (R^2 = 0.90), tysosol (R^2 = 0.73), PB1 (R^2 = 0.78), oleuropein (R^2 = 0.82), luteolin-7-*O*-glucoside (R^2 = 0.91), apigenin-7-*O* (R^2 = 0.86), verbascoside (R^2 = 0.77), *p*-coumaric (R^2 = 0.93), and Σ phenols (R^2 = 0.88).

3.1.3. Effect of Thermal Treatments on the Volatile Compounds

The volatile compounds of Californian-style black olive from both varieties submitted to different thermal treatments were classified into six types according to chemical group (Table 2). The group of volatile compounds with the highest representation in this elaboration process was aldehydes, followed by esters, phenols, aromatics, other compounds, and alcohols.

Table 2. Distribution (%) of chemical families of volatile compounds in Californian-style black olives ("Manzanilla Cacereña" and "Hojiblanca") submitted to sterilization treatments (T1–T5).

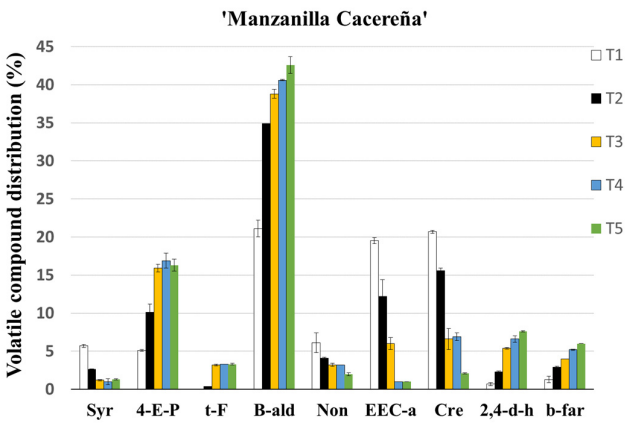
	'Manzanilla Cacereña'					**'Hojiblanca'**				
	T1	**T2**	**T3**	**T4**	**T5**	**T1**	**T2**	**T3**	**T4**	**T5**
Aromatics	15.5	18.1	25.5	26.9	27.5	7.6	14.2	18.4	20.8	19.3
Alcohols	3.3	2.0	3.7	4.7	4.8	3.2	4.3	4.6	3.7	4.0
Aldehydes	28.1	40.9	42.8	45	46.7	27.2	32.4	37.2	45.9	44.5
Esters	26.1	15.8	8.7	2.3	2.2	27.9	22.4	15.6	5.1	5.2
Phenols	20.9	15.6	6.6	6.9	2.1	20.6	15.9	11.4	7.0	7.5
Others	6.1	7.6	12.7	14.2	16.7	13.5	10.8	12.8	17.5	19.5

T1: F0 = 10 min; T2: F0 = 14 min; T3 F0 = 18 min; T4: F0 = 22 min; and T5: F0 = 26 min.

The thermal treatments caused a variation in the volatile compound profile of the treated olives. Aromatics, aldehydes, and other volatile compound groups increased their content according to the thermal treatment. The alcohol group was under-represented in this type of olives and did not present a clear trend regarding the effect on the thermal treatments that were applied. On the other hand, the volatile compound groups comprising esters and phenols decreased their content when the sterilization treatment increased. Moreover, the volatile compounds in each group stabilized their contents with the most aggressive sterilization treatments (T4–T5).

Furthermore, although almost 50 volatile compounds were identified, the major constituents in the olive matrix are shown in Figure 2 The unpleasant odour aromas such as 4-ethenyl-pyridine (aromatics), benzaldehyde (aldehydes), and 2,4-dimethyl-hexane (others), which had a high influence after the application of the thermal treatments, increased in content. Moreover, other fruity aromas, such as ethyl ester cyclohexanecarboxylic acid (esters) and creosol (phenols), decreased their content [23,34,35].

(A) 'Manzanilla Cacereña' variety



(B) 'Hojiblanca' variety

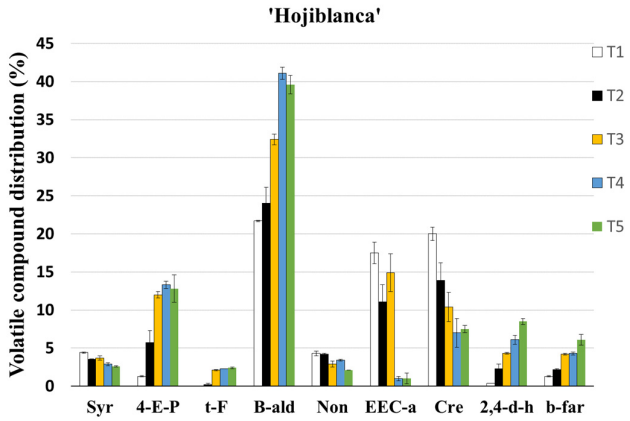


Figure 2. Relative contents of volatile compounds (mean% (n = 4)) obtained from Californian-style black olives from "Manzanilla Cacereña"(**A**) and "Hojiblanca"(**B**) varieties and submitted to five different sterilization treatments: T1: F0 = 10 min; T2: F0 = 14 min; T3 F0 = 18 min; T4: F0 = 22 min; and T5: F0 = 26 min. Syr: styrene; 4-E-P: 4-ethenyl-pyridine; t-F: trans-farnesol; B-ald: benzaldehyde; Non: nonanal; EEC-a: ethyl ester cyclohexanecarboxylic acid; Cre: creosol; 2,4-d-h: 2,4-dimethyl-hexane; b-far: beta-farnesene.

The behaviour of the volatile compounds determined to be present in the volatile profiles of both of the olive varieties that were studied followed a similar synthesis and degradation pattern. However, some groups of volatile compounds were synthesized more in one variety than they were in the other. Such is the case of the aromatic group in which the "Manzanilla Cacereña" variety presented higher values. This is due to the higher content of the 4-ethenyl-pyridine. The rest of the volatile compounds present similar values when soft and more aggressive thermal treatments were applied (T1 and T5).

3.2. Relationship between Volatile Compounds and Acrylamide Content

As seen in Section 3.1.3, the volatile compound profile varied with the thermal treatment, just as the concentration of acrylamide increased with the increasing thermal treatments.

To determine whether certain volatile compounds could be correlated with the acrylamide content to some extent, a correlation study with the individual volatile

compounds was carried out. The results showed that many variables were positively or negatively correlated with acrylamide, demonstrating R^2 values above 0.6. Acrylamide content waspositively correlated with the percentage of 2,4-dimethyl-hexane, ($Rr^2 = 0.87$), 2-heptyn-1-ol ($R^2 = 0.88$), benzaldehyde ($R^2 = 0.81$), 4-ethenyl-pyridine ($R^2 = 0.67$), octanal ($R^2 = 0.88$), trans-farnesol ($R^2 = 0.81$), and beta-farnesene ($R^2 = 0.84$). Moreover, a negative correlation was found with ethyl ester hexanoic acid ($R^2 = 0.61$), nonanal ($R^2 = 0.71$), ethyl ester cyclohexanecarboxylic acid ($R^2 = 0.72$), and creosol ($R^2 = 0.89$).

Based on these results, we studied the possibility of finding a regression model between these two types of variables. An MLR model was built between the relative contents of all of the volatile compounds that were found and the experimental acrylamide content obtained by the reference method. The result is shown in Figure 3, where we can observe the good correlation ($r^2 = 0.994$) between the acrylamide concentration quantified by the traditional HPLC method and the predicted concentration obtained by the model.

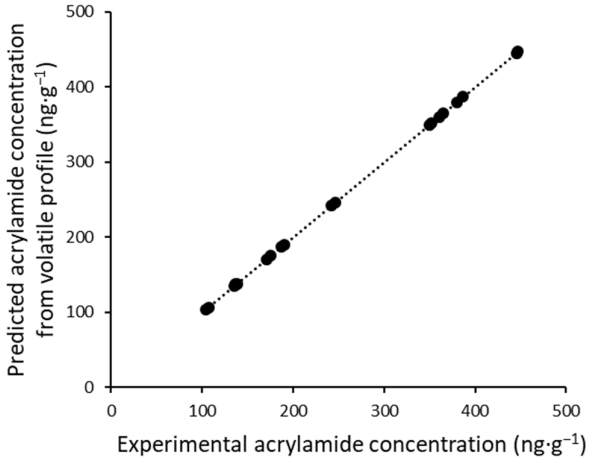


Figure 3. Predicted acrylamide concentration by volatile profile vs. experimental value.

3.3. Acrylamide Quantification by Using the E-nose

From the results of the previous section, it could be said that it is possible to predict the acrylamide content from the volatile compound profile. However, it is still a very expensive and time-consuming technique. Therefore, the use of the E-nose for the indirect determination of acrylamide was studied.

E-nose signals were obtained from the measurements of Californian-style black olives submitted to different thermal treatments. Before performing the quantitative model, feature extraction was conducted in order to characterize the sensor response curves. The feature that was selected was the maximum signal value minus the minimum signal value multiplied by 100 and subtracted by 1. These data were used to perform a partial least squares (PLS) model to evaluate the relationship between the E-nose measurements and the experimental values of the acrylamide concentration.

The PLS model was optimized using the calibration set. The number of LVs used in the calibration was selected using the leave-one-out cross-validation by the lowest RMSECV and the higher R^2cal. The best number of LVs was 4. The resulting PLS model was then tested with the validation set. Based on this model, the calculation of the merit figures was performed, and the results are shown in Table 3 The model shows high linearity with R^2cal, which has a value of 0.85, and R^2cv and R^2pred, which show values of 0.79 and 0.78, respectively. The root mean square error in both the cross-validation and validation were considered low. Moreover, from this table, it was also observed that it is possible to determine the acrylamide content with relatively high accuracy (RER and RPD above 10 and 2.5, respectively).

Table 3 Parameters and figures of merit of the PLS model for the determination of acrylamide through the use of E-nose.

LVs	R^2_{cal}	R^2_{CV}	R^2_P	RMSEC	RMSECV	RMSEP	RPD	RER
4	0.85	0.79	0.78	35.24	41.48	37.07	2.63	10.61

R^2_{cal}: coefficient of calibration; R^2_{CV}: coefficient of cross-validation; R^2_{pred}: coefficient of prediction; RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross-validation; RMSEP: root mean square error of prediction; RPD: ratio of performance to deviation; RER: range error ratio.

These results are corroborated by the graph of predicted values obtained by the PLS model versus the experimental values obtained by the reference method. As shown in Figure 4, there is a solid correlation between predicted and measured values over the calibration and validation sets. As such, the results of this PLS model are efficient and point out the fact that this technology can be used to estimate the acrylamide content in Californian-style black olives with no sample preparation.

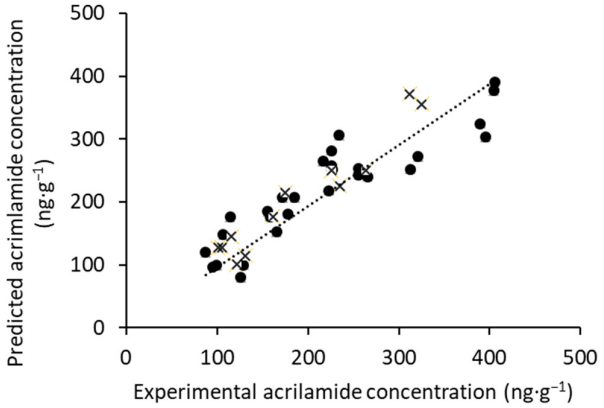


Figure 4. Correlation plot of calibration (•) and prediction (×) sets for the determination of acrylamide concentration.

4. Discussion

The chemical composition of California-style table olives varies according to the intensity of the thermal treatments used. Phenols are known to be thermosensitive [6,36], which is confirmed in the present study. The application of different intensities of thermal treatments in Californian-style black olives resulted in a significant reduction of these compounds. Moreover, it should be noted that the phenolic profile of this type of olive elaboration is quite poor in terms of the number of identified and quantified phenolic compounds. In other types of table olive production, a much broader phenolic profile appears. For instance, unfermented table olives from different varieties were studied by Franco et al. [37] and showed a phenolic profile of more than 24 phenols. Lodolini et al. [8] studied the phenolic profile of different table olive elaboration processes and showed that the highest contents of these compounds were found in the natural style. Pistarino et al. [38] found lower hydroxytyrosol concentrations in fermented table olives of the "Taggiasca" variety, whereas similar tyrosol concentrations were reported. Furthermore, all the elaboration processes where lye and thermal treatments are applied provoke a decrease in phenolic compound content [24].

Despite this, thermal sterilization is necessary for this type of table olives to ensure food safety by the inactivation of the bacteria spores. However, the acrylamide concentration was affected by the intensity of the thermal treatments. It should be noted that an F0

value lower than 18 min resulted in acrylamide synthesis below 250 ng g^{-1}. On the contrary, sterilization treatments longer than 22 min clearly exceed this proposed limit concentration, which could have a negative effect on people's health [5,8,9]. Therefore, it should be noted that the industries producing this type of olive must regulate the intensity of the thermal treatments that are applied to sterilize the olives in order to increase the quality of the final product. Acrylamide generation in table olives is more pronounced in Californian-style black ripe olives. This elaboration process involves oxidation with air, treatment with sodium hydroxide, washing phases, the addition of ferrous gluconate, and thermal sterilization (121 °C during 20–30 min). In this sense, different strategies have been proposed to mitigate the presence of this toxic substance during its industrial processing [13,14]. These strategies are based on the reduction of the precursor of this pollutant. The different phases in which some improvements have been proposed are (a) table olive maturation stage; (b) length of the storage period; (c) types and times of washing treatments before oxidation; (d) table olive presentation formats; (e) NaCl addition; and (f) CaCl2 addition. Thus, the minimum levels of acrylamide in table olives marketed in Europe should be set at values closer to 300 μg/kg, thus guaranteeing the health of consumers of table olives at an international level. In this way, the application of mitigation measures and good practices at the industrial level is promoted to meet the levels stipulated by the regulation. The intake of Californian-style table olives with high acrylamide content could cause serious health problems.

In our results, it is shown that those olives with a high phenolic compound content were those that presented the least amount of acrylamide. Different studies indicate that phenols can inhibit acrylamide synthesis and that sometimes, the results that are obtained by different research groups are contradictory. In fact, the olives with the highest hydroxytyrosol content presented higher acrylamide reduction [39]. However, other phenolic compounds could also act against acrylamide syntheses such as hydroxytyrosol, tyrosol, and oleuropein [40], which also appears in oxidized olives. In addition, certain natural additives such as blanched garlic, oregano, or rosemary showed a significant reduction in acrylamide [39].

Pérez Nevado et al. [6] found that the presence of antioxidant compounds in olives is directly proportional to the mitigation of acrylamide levels when the olives are both fresh and after having undergone some thermal treatment. As a result of this observation, it the addition of antioxidant compounds to the olive, in form of phenolic compounds, to check if there is any contaminant mitigation has been studied [12]. After inoculation with these bioactive compounds, these researchers showed that acrylamide formation decreased by 35% for the olive leaf extracts and by 54% for the mixture of the extract and the hydroxytyrosol.

Regarding the volatile compounds, Californian-style black olives have a characteristic odour of a cooked sensory note [15,23,41] that is mainly provoked by the thermal treatment that was used to stabilize these types of olives. Following IOC regulations, the results obtained by these researchers can classify table olives that have been submitted to different sterilization treatments into different sensory categories after considering the predominantly perceived defect (PPD) and intensity attributes. Thus, olives that are subjected to a more aggressive sterilization treatment are classified as low quality, leading to there being a greater cooking effect sensation in the olives. Therefore, the results show that the odorous compounds are directly related to the intensity of the applied thermal treatments. The behaviour of the aroma is similar to that of phenols, as these compounds are strongly influenced by the heat applied. Some volatile compound groups increase considerably in table olives with the application of different thermal sterilization treatments, while other different groups decrease. For example, the content of phenols, alcohols, and esters decreased as the F0 increased. However, the aldehyde content decreased, probably due to oxidative processes [42], with the aldehydes being the main products of lipid oxidation and amino acid degradation [43].

Some the volatile compounds such as benzaldehyde, 4-ethenylpyridine or 2,4-dimethylhexane are related to the cooking effect and an unpleasant odour. López-López et al. [27] showed that the volatile compounds with an unpleasant odour are synthesized with the application of temperature higher than 121 °C. In the literature, it is widely described that thermal sterilization treatments cause this characteristic cooking effect that is seen in olives and that this effect is caused by long exposure to heat in the olive cans. In addition, some researchers [13] have described that this cooking effect increases with the applied thermal intensity. Therefore, it is once again of interest, at an industrial level, to regulate the intensity of thermal treatments to obtain olives with a fruitier aromatic profile and less unpleasant aromas.

The olive aroma provided by increased thermal treatments may correlate with increased acrylamide content in both varieties. Several volatile compounds were positively or negatively correlated, with an $r^2 > 0.6$, with the acrylamide content. Moreover, an MLR model showed that it would be possible to determine the acrylamide content through the volatile profile.

Therefore, as this linear correlation has been found, the next step was to evaluate the volatile compounds using an electronic nose to indirectly determine the acrylamide content. In this case, a PLS was performed to establish a standard model that served as an indirect method for the quantification of this toxic substance. A linear relationship was established between the measurements predicted by the PLS model and the experimental acrylamide content. Moreover, good quality parameters were obtained in the developed model. Taking the obtained results into account, we can state that it could be possible to predict the acrylamide concentration using the electronic nose.

In the literature, several works establish direct and indirect relationships between other devices and acrylamide content, but this studied represented the first time that an electronic nose was used for this purpose. PLS multivariate analysis has previously been used in the literature to indirectly predict acrylamide content produced during coffee roasting from proton magnetic resonance (NMR) spectra that had already been recorded for other coffee control purposes. In the NMR spectrum, acrylamide is not directly quantifiable, so these researchers established a correlation between the reference value and the corresponding NMR spectrum using a partial least squares (PLS) regression [44]. Furthermore, Martín-Vertedor et al. [41] established a direct linear model between acrylamide content and potentiometric electronic tongue to monitor the content of this toxic substance in Californian-style black olive. Therefore, the use of electronic devices for acrylamide detection is of interest since industries will be able to determine the content of this toxic substance quickly, efficiently, and reliably.

5. Conclusions

Phenols, acrylamide, and volatile compound concentration depends on the intensity of the sterilization treatments and on the table olive varieties used as Californian-style black olives. Furthermore, phenolic compounds can have a positive effect on the formation of acrylamide. In fact, olives with a high phenolic compound content had a lower content of this toxic substance. The thermal treatments that were applied caused a change in the phenol and aromatic profile of the olives, which is directly related to the acrylamide content. Thus, the aromatic profile of the olives was also detected by the E-nose, establishing a relationship with the acrylamide content. In this way, this portable device is faster than the conventional method and could be interesting for industries to control the amount of this toxic substance before bringing it to the market.

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2.7.1 Motivación y objetivos

Hoy en día es habitual encontrar en las estanterías de los supermercados aceitunas verdes al "estilo español" rellenas con diferentes aromas naturales y artificiales. Sin embargo, las aceitunas negras al "estilo californiano" se comercializan generalmente enteras, deshuesadas o picadas sin aromatizar, a pesar de que la legislación actual permite la posibilidad de rellenar ambos tipos de aceitunas. Entre los aditivos alimentarios y coadyuvantes tecnológicos permitidos en este sector, tenemos aromatizantes, espesantes y aglutinantes de uso alimentario, definidos por el Codex Alimentarius y limitado legalmente por las buenas prácticas de fabricación. A nivel industrial es cada vez más frecuente el uso de hidrocoloides (polisacáridos, proteínas o lípidos) en el relleno de aceitunas de mesa porque este supone un coste menor en comparación con el uso de rellenos con pasta de alimentos naturales.

Las aceitunas negras al "estilo californiano" se elaboran sometiendo a las aceitunas a un tratamiento alcalino (1,5-3,5% p/v NaOH) y a una oxidación forzada con aire hasta que el fruto se va ennegreciendo. Posteriormente, se realizan lavados y la neutralización de la solución alcalina para ser envasadas con una solución salina y gluconato ferroso, para finalmente ser sometidas a un tratamiento de esterilización térmica que hace que el producto sea microbiológicamente estable. Este tratamiento térmico puede eliminar o modificar la intensidad de los aromas añadidos en las aceitunas. Además, si la esterilización es prolongada, puede aparecer el *efecto de cocido* en el producto final. Los cambios que produce la esterilización en el perfil aromático de este tipo de aceitunas, podrían ser el motivo por el que no se comercializan actualmente rellenas aromatizadas. En este punto, las evaluaciones sensoriales de un panel de cata serían de mucha importancia en este tipo de elaboraciones, para predecir la aceptación final del consumidor.

El panel de análisis sensorial en aceitunas de mesa sigue el método establecido por el Consejo Oleícola Internacional (COI) para determinar cómo el consumidor percibirá los aromas para su clasificación sensorial. Para lograr buenos resultados, debe haber un número razonable de panelistas formados. El análisis sensorial por parte de un panel de cata suele ser costoso y requiere mucho tiempo para la preparación de muestras e

interpretación de resultados. Así que, la idea es poder disponer de un instrumento de análisis rápido y eficaz, como la *nariz electrónica*, para clasificar las aceitunas en función de su calidad. Estos dispositivos electrónicos son un potente dispositivo sensorial electromecánico que ha permitido a distintos investigadores discriminar los perfiles aromáticos de diferentes matrices alimentarias como son el vino (Martínez-García et al., 2021), setas comestibles (Portalo-Calero et al., 2019), pollo cocinado (Zhang et al., 2021), aceites comestibles (Majchrzak et al., 2018) e incluso en frutas frescas (Cozzolino et al., 2021). Este dispositivo también se utiliza para clasificar las aceitunas de mesa recolectadas en el olivo (Martínez et al., 2020) o incluso diferenciar distintos tipos de aceitunas de mesa elaboradas al "estilo español" (Panagou et al., 2008). Por lo tanto, el objetivo principal de este trabajo fue estudiar, mediante *nariz electrónica*, la evolución sensorial olfativa de distintos aromas añadidos en el relleno de aceitunas negras tras ser sometidos a diferentes tratamientos de esterilización térmica.

2.7.2 Diseño experimental

Las aceitunas de la variedad *'Hojiblanca'* fueron elaboradas al "estilo californiano". Posteriormente se deshuesaron y se rellenaron con una pasta de hidrocoloide constituida por dos partes de alginato de sodio, una de goma guar y adición de aromas de uso alimentario de 'berenjena', 'mojo picón', 'tomillo', 'orégano' y 'sangría'. Las aceitunas se rellenaron de forma manual con ayuda de una jeringa con la pasta hidrocoloide elaborada y se introdujeron en una disolución de calcio diluida. Las aceitunas rellenas se metieron en frascos termorresistentes con su correspondiente líquido de gobierno para posteriormente ser sometidas a tres tratamientos distintos de esterilización en función de los valores F_0 (letalidad acumulada): F_0= 9 min (T1), F_0= 12 min (T2) y F_0= 15 min (T3). Tras la esterilización, las muestras se almacenaron a temperatura ambiente hasta el momento de su análisis. Estas muestras de aceitunas rellenas con los diferentes aromas fueron analizadas por la *nariz electrónica* y el panel de cata siguiendo las recomendaciones establecidas por el COI (IOC, 2011). Las muestras se analizaron por quintuplicado.

2.7.3 Resumen de los resultados y discusión

El panel de cata identificó los diferentes aromas de las aceitunas negras rellenas tratadas térmicamente con distintos ciclos de esterilización. Obviamente, la mayor intensidad de los aromas lo presentaron aquellas muestras que no fueron sometidas a un tratamiento térmico de esterilización. El aroma que presentó menor intensidad fue 'mojo picón', mientras que los aromas con mayor intensidad fueron 'sangría' y 'orégano'. Tras la esterilización más suave (T1), se observó una disminución de la percepción de la intensidad del aroma del 35-42% y, tras los tratamientos más agresivos (T2 y T3) disminuyó la intensidad aromática entre un 57-73% con respecto a las muestras no esterilizadas. Hay que destacar que los panelistas no detectaron diferencias significativas en la percepción de los aromas añadidos entre los tratamientos T2 y T3. Por lo tanto, estos resultados nos indican que la aplicación de los tratamientos térmicos provoca una pérdida significativa del perfil aromático. Resultados similares fueron obtenidos por otros investigadores en aceitunas y aceites (Messina et al., 2015). Esta pérdida significativa del perfil aromático puede ser una de las razones por la que no se comercializan aceitunas negras rellenas aromatizadas al "estilo californiano". Sin embargo, en nuestro estudio se pudo comprobar que con tratamientos de esterilización suaves y una buena formulación del relleno con hidrocoloide, se conservaba una buena intensidad de los aromas.

Por otro lado, los catadores detectaron que las aceitunas tratadas térmicamente presentaban un aumento en el *efecto de cocido*. Este defecto sensorial se vio enmascarado con el tratamiento T1 en el que la intensidad del defecto era tan pequeña que pareció ser disimulado con el aroma añadido al relleno, siendo las aceitunas clasificadas en la mejor categoría comercial (extra) debido a que la percepción principal del defecto (DPP) fue menor o igual a 3,0. Cabe señalar que este defecto se percibía con menor intensidad en este tipo de aceitunas que en aquellas aceitunas que no tenían ningún aroma, por lo que se clasificaron en una peor categoría comercial (categoría primera) ($3{,}0 < DPP \leq 4{,}5$). Así, se puede afirmar que el aroma del relleno provocó una disminución de la percepción del *efecto de cocido*. Este efecto se observó en las aceitunas rellenas aromatizas con todos los aromas estudiados. Las aceitunas sometidas a los tratamientos térmicos más agresivos presentaron una intensidad de

efecto de cocido significativamente más alta, siendo clasificadas como aceitunas que no pueden venderse como aceituna de mesa por la alta intensidad de defecto (DPP ≥ 7,0). Diferentes investigadores han estudiado la aplicación de diferentes tratamientos de esterilización en aceitunas al "estilo californiano" observándo que los tratamientos de esterilización más prolongados y agresivos conducen a una intensidad aromática negativa de la aceituna más profunda y desagradable (Casado y Montaño, 2008; Charoenprasert y Mitchell, 2014; Tang et al., 2016; Pérez-Nevado et al., 2018; Martín -Vertedor et al., 2020). El proceso de esterilización al que se somete a este tipo de aceitunas, además de provocar cambios en las cualidades olfativas como el color y la textura, disminuye la calidad del producto final (Abriouel et al., 2014).

Los datos obtenidos por la *nariz electrónica* consiguieron discriminar las muestras de aceitunas de mesa rellenas con aromas y sometidas a los distintos tratamientos térmicos de esterilización. Para entender qué relaciones existen entre las variables medidas, el primer paso fue reducir al máximo estas variables mediante la realización de un PCA. Con las aceitunas rellenas con los distintos aromas, los dos primeros componentes principales fueron suficientes para separar claramente las aceitunas rellenas esterilizadas de las no esterilizadas.

Los resultados del PLS-DA confirmaron la buena clasificación de los diferentes tratamientos térmicos en todas las muestras estudiadas: el 100% de las predicciones fueron correctas en los modelos rellenos de 'sangría' y 'orégano', y el 98,6%, 94,6%, 93,3% y 92,7 % de las predicciones fueron correctas para aceitunas rellenas 'sin aroma', y con aromas de 'berenjena', 'tomillo' y 'mojo picón', respectivamente. En estas muestras, los resultados también demostraron que ninguna muestra de T1 se asignó a T2 o T3 y viceversa.

Las pocas predicciones incorrectas fueron solo dentro de las muestras T2 y T3 para estos aromas.

Por lo tanto, la *nariz electrónica* fue capaz de discriminar las aceitunas aromatizadas en función de los tratamientos térmicos de esterilización (T1-T3). Este es un buen resultado ya que se podría decir que el dispositivo electrónico tuvo mayor poder de discriminación que aquellos realizados por el panel de cata que interpretaban los

tratamientos T2 y T3 como si tuviesen una intensidad del aroma similar. Esto puede ser debido a que el aroma del relleno enmascara la percepción del catador, haciendo que éste no encuentre diferencias entre los tratamientos de esterilización.

2.7.4 Conclusiones

- El panel de cata y la *nariz electrónica* discriminaron aceitunas rellenas aromatizadas que no fueron tratadas térmicamente de aquellas sometidas a esterilización.

- El incremento de los tratamientos de esterilización provocaba que la intensidad de los aromas disminuyera, mientras que el *efecto de cocido* aumentaba.

- La *nariz electrónica* resultó ser más efectiva y precisa que el panel de cata, ya que pudo diferenciar los grupos de aceitunas según la intensidad del tratamiento térmico aplicado.

- La *nariz electrónica* es una herramienta rápida, eficaz y efectiva para clasificar aceitunas aromatizadas sometidas a distintos tratamientos de esterilización, por lo que podría utilizarse como apoyo al panel de cata.

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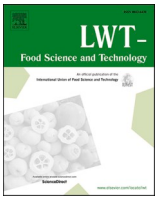

Evaluation of the olfactory pattern of black olives stuffed with flavored hydrocolloids

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ABSTRACT

A sensory panel and an electronic nose (*E-nose*) were used to discriminate olives stuffed with flavored hydrocolloids submitted to different thermal treatments. The aroma perceived by the tasting panel before the sterilization was considered to be highly intense. However, the application of a single sterilization caused a 35–42% decrease in aroma intensity perceived by tasters. The aroma intensity also decreased when the number of sterilizations increased, but the tasting panel did not detect differences between them. The sterilization led to an increase in the 'cooking effect' that was less detected by panelists when the olives were flavored. The *E-nose* showed that the olives subjected to sterilization presented a different aromatic profile. Thus, the *E-nose* was able to discriminate the three treatments applied with greater precision than the tasting panel. *E-nose* technology offers a fast, inexpensive and non-destructive method for discriminating between olives stuffed with flavored hydrocolloids submitted to sterilization.

1. Introduction

According to the International Olive Council (IOC), the average consumption of table olives over a five-season period (2015–2020) amounted to 2,814,100 tons. Consumption was distributed in countries such as Egypt, Turkey, Algeria, the United States and Spain, with the latter consuming 187,500 tons. In addition, as per the Food Consumption Panel of the Ministry of Agriculture, Food and Environment of Spain (MAGRAMA), the consumption of table olives in Spain increased by 10.3% in 2020.

Currently, the olives most commonly found in Spain are stuffed green olives. Black olives are generally sold whole, pitted or chopped, and there are no stuffed oxidized black olives without covering liquid. Fillings of green table olives tend to be peppers, onions, almonds, celery, anchovies, capers or their paste, spices and aromatic herbs or their natural extracts and authorized additives, including flavorings. The use of hydrocolloids (polysaccharides, proteins or lipids) to fill olives with different natural or artificial aromas is widespread in the table olive industry.

These hydrocolloids can influence the food processing, sensory aspects and nutritional benefits of the final product (Lu, Nishinari, Matsukawa, & Fang, 2020). The use of flavored hydrocolloids to fill olives is therefore of great interest to the industry, as it leads to lower costs compared to using fillings with natural food paste.

Black table olives, also called 'Californian style', are subjected to an alkaline (NaOH) and air treatment until the fruit is blackened by oxidation and polymerization of phenolic compounds. They are then generally packaged with a saline solution. For the product to be microbiologically stable, it requires a sterilization phase (121–126 °C for at least 15–30 min), unlike green olives which are pasteurized (Martín-Vertedor, Fernández, et al., 2020; Fernández et al., 2020). The sterilization in table olives is calculated as the 'accumulated sterility value' which is the sum of the partial lethality values reached during the sterilization process and is expressed in terms of exposure times at a reference temperature (IOC, 2004).

The thermal sterilization treatment can change the aromas of the olives. In addition, prolonged sterilization may bring about the 'cooking effect' (Martín-Vertedor, Rodrigues, et al., 2020). Changes in the odor

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profile of table olives after sterilization treatments could be why black olives filled with added food flavorings are not currently marketed. For this reason, it is necessary to study the combination of black olives with food fillings and flavors that are both stable at the sterilization temperature and pleasant for the consumer.

Since aroma is such a necessary attribute for food acceptability, it is important to obtain sensory evaluations from a tasting panel. This method is used for the sensory analysis of different types of olives (IOC, 2011) and is the only method to determine how the consumer will perceive the aromas and rate them sensually. However, sensory analysis by a tasting panel is often expensive and time-consuming (Panagou, Sahgal, Magan, & Nychas, 2008). In addition, a good result is dependent on a reasonable number of trained panelists, the availability of which is not always possible. The use, therefore, of an analytical instrument that functions as that of human smell, such as the *E-nose*, would be very interesting.

The *E-nose* is an instrument that combines a series of gas sensors and pattern processing techniques to detect odors that have been proven by different researchers (Radi et al., 2021). The sensor array defines an odor made up of a large number of different volatile compounds in the headspace of a sample, providing its odor pattern. The *E-nose* can be used as a rapid and automated method and alternative technique to determine food additive concentrations (Qiu & Wang, 2017). The *E-nose* designed at the University of Extremadura is able to detect changes in the intensity or concentration of food odors through a series of metal oxide sensors (Arroyo et al., 2020; Sánchez et al., 2021; Sánchez, Martín-Tornero, et al., 2022). Thus, when the volatile compounds emanating from the sample react with the sensor surface, an oxygen exchange occurs with a decrease in electrical conductivity (Martínez Gila, Gámez García, Bellincontro, Mencarelli, & Gómez Ortega, 2020; Sánchez et al., 2021).

This work presents a double novelty; on the one hand, a new table olive product has been elaborated to be marketed and, on the other hand, the *E-nose* technique has been applied to discriminate table olives according to the different aromas studied. The new product consists of Californian-style black olives filled with a consistent and economical hydrocolloid with various natural aromas added. The aromas are used to make the product more attractive to the consumer and, at the same time, mask the possible cooking effect that can be produced during sterilization. The absence of brine solution means the product can be packaged as a snack, easily consumed on the go. Moreover, the application of the *E-nose* technique is novel since it could be used in the industry for table olive discrimination.

For that reason, the main objective of this work was to study the olfactory sensory evolution of black olives stuffed with different flavored hydrocolloids and submitted to different sterilization treatments using a tasting panel and an *E-nose*.

The underlying hypothesis is that an *E-nose* discriminates samples according to the sterilization treatment received, regardless of the aroma added to the filling. At the same time, it is expected that all the aromas used can mask the cooking effect that arises after a certain sterilization time.

2. Materials and methods

2.1. Experimental design and sample preparation

Olives (*Olea europaea*) of the 'Hojiblanca' variety were elaborated as Californian-style black olives by a company located in the northwest of Spain (Martín-Vertedor, Fernández, et al., 2020). The following substances were used for the Californian-style black olive elaboration: a solution of acetic acid (Panreac Applichem, Darmstadt, Germany), calcium chloride (Tetra Chemicals, Helsingborg, Sweden), ferrous gluconate and sodium chloride (Sigma-Aldrich, St. Louis, MO, USA). After the elaboration process, table olives were pitted and placed into jars (250 mL) with a solution containing 2% NaCl and 0.015% (10–40 ppm) ferrous gluconate. Next, the solution was removed, and the olives were stuffed with flavored hydrocolloids with edible aromas of 'eggplant', 'mojo picón', 'thyme', 'oregano' or 'sangaree', or 'without aroma'.

The hydrocolloids were prepared using the following food-grade ingredients: two parts of sodium alginate (Saporepuro, Torino, Italy), one part of guar gum (Saporepuro, Torino, Italy) and one part of the corresponding natural aroma (Neroliane, Grasse, France) were mixed with distilled water to obtain a volume of 100 mL. The additives were mixed using a commercial mixer for 1 min. The final mixture resembled snail mucus. One batch of table olives was filled with an unflavored hydrocolloid. Olives were manually filled using a syringe. The stuffed olives were placed into a 7.5% calcium chloride solution for 30 s and, later, into another 0.25% calcium chloride solution for 24 h. This liquid was then removed, and the jars were prepared to be sterilized. Five replicates per sample were prepared for the *E-nose* analysis and eight replicates were prepared for the tasting panel. Jars containing the control stuffed olives were kept in the refrigerator until the analysis was done. The other jars were subjected to different sterilization cycles as indicated in the following section.

2.2. Thermal sterilization treatments

Olive jars were submitted to three different sterilization treatments which corresponded to the following F_0 values (accumulated lethality): $F_0 = 9$ min (T1), $F_0 = 12$ min (T2) and $F_0 = 15$ min (T3) following the method proposed by Martín-Vertedor, Fernández, et al. (2020). All experiments were performed in quintuplicate. Sterilization was carried out in a laboratory autoclave (JP Selecta model Presoclave III 80, Barcelona, Spain). Two Pt100 temperature sensors (TC, United Kingdom) were placed in the middle of the jar connected to a computer in order to monitor the F_0 value calculated according to Martín-Vertedor, Fernández, et al. (2020). Jars were stored at room temperature until analysis.

2.3. Analyses

The following analyses were carried out on the stuffed black olives subjected to sterilization treatments. Specifically, sensory analysis and *E-nose* measurements were performed.

2.3.1. Sensory analysis

The sensory analysis was carried out by a group of table olive panelists located in the Technological Institute of Food and Agriculture (CICYTEX-INTAEX) facilities, following the recommendations for tasting room conditions (ISO, 2007) and the indications established by the IOC (2011). A scoreboard was designed for the purpose of this experiment to evaluate the intensity of the aroma and the defects according to a 10 cm structured scale. Sensory evaluation outcomes were expressed as average values. Values were considered reliable when CV $<$ 20% (IOC, 2011).

A one-way ANOVA was used followed by Tukey's multiple range test to establish statistically significant differences between different thermal treatments within each of the table olive varieties. Significance was set at $p < 0.05$. SPSS 18.0 software was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Data were expressed as means and standard deviations (SD).

2.3.2. E-nose system

The *E-nose* device was designed by the Research Group on Perception and Intelligent Systems of the University of Extremadura. It contains a novel chip with 11 types of metal oxide semiconductor (MOX) commercial gas sensors purchased from different manufacturers which exhibit global selectivity: i) BME680 (Bosch Sensortech GmbH, Reutlingen, Germany): temperature (°C), pressure (hPa), humidity (%RH) and gas measurement (Ω); ii) SGP30 (Sensirion AG, Stäfa, Switzerland): eCO_2 (ppm), TVOC (ppb), H_2(2) and ethanol; iii) CCS811 (ScioSense B. V., Eindhoven, The Netherlands): eCO_2 (ppm), TVOC (ppb) and sensor

resistance (Ω); and iv) iAQ-Core (ScioSense B.V., Eindhoven, The Netherlands): eCO_2 (ppm), TVOC (ppb) and sensor resistance (Ω). Odor evaluation using an *E-nose* provides an odor pattern for each sample. This device has low power consumption and is the size of a hockey puck, thereby making it easily portable and convenient (Arroyo et al., 2020). This type of *E-nose* sensor has been studied on previous occasions and can be found in bibliographic references such as Lozano, Santos, and Horrillo (2016); Arroyo, Lozano, and Suárez (2018); Portalo-Calero, Arroyo, Suárez, and Lozano (2019); Arroyo et al. (2020).

Olive measurements were carried out according to the recommendations established by the International Olive Council for sensory analysis (IOC, 2011). Standard glasses containing three olives were placed on a heating block at 25 °C and covered with a watch glass. Another glass without olives was left on the block to measure the air inside and to serve as a reference. The *E-nose* was located on the sample glass for 60 s and the sensor signals were recorded. Next, the *E-nose* was moved to the glass without samples to perform desorption with free air for 30 s to return the gas sensor signal to the baseline. The system took a reading of the resistive value provided by each sensor at 1 s intervals. Five measurements were taken for each sample.

Each sensor response curve was composed of N points corresponding to the measurements of the sensor with the time. The features used to characterize the sensor response curves were maximum signal value less minimum one, plus 100, less one in our case. As a result, a vector of data with 11 rows (sensors) for each sample was obtained.

2.4. Multivariate data analysis

Data provided by the *E-nose* were analyzed using multivariate algorithms. First, a principal component analysis (PCA) was applied (Wold, Esbensen, & Geladi, 1987) to explore the main variation among the four groups of samples. This is an unsupervised method and was used to determine whether there were clusters between the samples without using membership information. The data for each of the fillings were analyzed independently.

Next, a partial least square discrimination analysis (PLS-DA) (Barker & Rayens, 2003) was used as a classification algorithm in order to evaluate the possibility of sample discrimination according to the thermal treatment applied. Full cross-validation was performed to evaluate the performance of the model. Data were autoscaled before multivariate analysis in order to avoid the effect of the different dimensions.

Data analysis was carried out in MATLAB version R2016b (The MathWorks Inc., Natick, MA, USA) and PLS Toolbox version 8.2.1 (Eigenvector Research Inc., Wenatchee, WA, USA).

3. Results and discussion

The results were analyzed by a tasting panel to classify the different aromas in the stuffed black olives. Next, outcomes were checked by applying *E-nose* technology to distinguish between the olives stuffed with different flavored hydrocolloids and to discriminate between different sterilization treatments.

3.1. Sensory analysis of flavored stuffed olives with hydrocolloid

Sensory analysis was carried out by a trained panel to determine the aroma intensity and cooking effect in Californian-style black olives ('Hojiblanca' variety) submitted to different sterilization treatments (T1, T2 and T3). The results from the one-way ANOVA showed that the intensity of the thermal treatment significantly affected the aroma intensity and perception of the cooking effect in table olives (P-value < 0.05) (Table 1).

The tasting panel evaluated their perception of the different aromas applied to the olives filled with flavored hydrocolloids as being highly intense. In fact, before being thermally processed, the olives presented an aromatic intensity greater than 5 for all the aromas used. This is a good result since applying this amount of aroma to the hydrocolloids is enough to achieve a highly intense aroma in the whole olives. The stuffed table olives with the lowest intensity were those with 'mojo picón' aroma, while the olives with the greatest intensity were those with 'sangaree' and 'oregano' aromas. These black table olives must be sterilized in order to ensure microbiological stability. The range of thermal applications in different industries is variable, thus the effect of different thermal treatments on the intensity of the perceived aroma was proved (T1, T2 and T3). The results showed that the application of a single thermal sterilization treatment (T1) caused a 35–42% decrease in aroma intensity perceived by the tasters. The application of thermal treatments causes a modification of the volatile compounds present in olives and olive oils (Messina, Sancho, & Walsöe de Reca, 2015), causing a significant loss of aromas. This may be one of the reasons why this type of olive product does not exist on the market. However, with the application of a low-intensity thermal treatment, we were able to obtain flavored hydrocolloid-stuffed olives with a good aroma intensity.

In addition, the tasting panel detected a more pronounced decrease in aroma intensity when the number of thermal treatments was increased (T2 and T3). However, the tasting panel did not detect significant differences in aroma intensity between T2 and T3 thermal treatments; the aroma intensity was approximately 57%–73% less than that for the control olives. The values for aroma intensity were less than

Table 1
Sensory evaluation of stuffed olives with flavour hydrocolloids. Results are expressed as mean ± SD of samples. Different lowercase letters mean a statistical significant difference between sterilization treatment (one-way ANOVA followed by Tukey's test, $P < 0.05$). For the same sterilization treatment, different uppercase letters mean a statistical significant difference between aromas (one-way ANOVA followed by Tukey's test, $P < 0.05$).

	Flavoring															
Hidrocoloids	C				T1				T2				T3			
'Without aroma'	–				–				–				–			
'Mojo picón'	5.1	±	0.78	c A	3.1	±	0.46	b NS	1.7	±	0.59	a A	1.4	±	0.70	a A
'Thyme'	6.1	±	0.93	c B	3.8	±	0.62	b NS	2.6	±	0.22	a B	2.7	±	0.55	a B
'Aubergine'	6.4	±	0.30	c B	4.1	±	0.24	b NS	2.7	±	0.32	a B	2.0	±	0.64	a B
'Sangaree'	7.1	±	0.36	c C	4.7	±	0.37	b NS	2.3	±	0.31	a C	2.9	±	0.61	a B
'Oregano'	7.7	±	0.34	c D	4.4	±	0.34	b NS	2.5	±	0.20	a C	2.7	±	0.34	a B
	Cooked effect															
Hidrocoloids	C				T1				T2				T3			
'Without aroma'	0.0	±	0.30	a NS	3.5	±	0.45	b B	4.7	±	0.35	c C	6.5	±	0.57	d D
'Mojo picón'	0.1	±	0.29	a NS	2.0	±	0.24	b A	3.3	±	1.11	c B	3.5	±	0.79	c C
'Thyme'	0.0	±	0.46	a NS	2.0	±	0.10	b A	3.5	±	0.51	c B	3.5	±	0.58	c C
'Aubergine'	0.0	±	0.54	a NS	2.0	±	0.60	b A	2.8	±	0.55	c A	3.2	±	0.39	c B
'Sangaree'	0.0	±	0.21	a NS	1.7	±	0.36	b A	2.5	±	0.40	c A	2.6	±	0.38	c A
'Oregano'	0.1	±	0.11	a NS	1.9	±	0.36	b A	2.3	±	0.23	c A	2.5	±	0.31	c A

3 points. Most of the aromas studied showed a similar decrease with the application of the thermal treatments. The 'mojo picón' aroma had the most pronounced losses since the rest of the aromas presented similar values for sterilized olives. The table olives stuffed with the unscented hydrocolloid did not present any aroma after thermal treatments were applied. Therefore, it should be noted that it is not advisable to apply very severe thermal treatments since they contribute to a significant loss of aromas that affects the sensory perception of olives by consumers.

The thermal treatment applied to the Californian-style olives after the production process led to an increase in the cooking effect in the olives (Table 1), except for the control olives which did not show this defect. Thus, according to IOC regulations, the olives could be classified into different sensory categories following consideration of the defect predominantly perceived (DPP) by the tasting panel (IOC, 2011). However, even though the olives were flavored, the application of a thermal treatment (T1) caused an increase in the intensity of the 'cooked' defect in the flavored stuffed olives. Thus, these olives could be classified into the best commercial category denominated as 'extra category' or 'fancy' (DPP $\leq$2). The defect was perceived less in this type of olives than in those 'without aroma' which were classified as 'first category' or 'select' (2 < DPP $\leq$3.5). Therefore, it can be stated that the aroma of the filling caused a decrease in the perception of the cooked defect, with the intensity of this defect being similar in olives stuffed with the different flavored hydrocolloids.

When the intensity of the thermal treatment was more severe (T2 and T3), an increase in the intensity of the defect was observed. However, no significant differences in the cooked defect in the stuffed flavored olives were shown between the two treatments. The intensity of the defect was less than 3.5 points for all the aromas; therefore, these table olives could be classified into a category denominated as 'first category' or 'select'.

It should be taken into account that olives 'without aroma' presented a significantly more intense cooked defect, which corresponds to a worse category than olives stuffed with flavored hydrocolloids. In addition, olives subjected to the most aggressive thermal treatment presented the highest defect intensity, thereby classifying them as unsellable (DPP >6.0).

Researchers have studied the application of different sterilization treatments to Californian-style olives, observing that longer and more aggressive sterilization treatments lead to a deeper and more unpleasant taste (Casado & Montaño, 2008; Charoenprasert & Mitchell, 2014; Tang et al., 2016; Pérez-Nevado, Cabrera-Bañegil, Repilado, Martillanes, & Martín-Vertedor, 2018; Martín-Vertedor, Fernández, et al., 2020). The olive sterilization process decreases the product quality in addition to causing changes in olfactory qualities such as color and texture. (Abriouel, Benomar, Gálvez & Pérez, 2014; Pérez-Nevado et al., 2018). Thus, industries need to optimize thermal sterilization treatments, keeping F_0 as low as possible in order to obtain the highest quality sterilized olives.

The results show that stuffing olives with flavored hydrocolloids could be a strategy to mask the cooking effect caused by the application of thermal treatments, thereby making Californian-style black olives more palatable to the consumer.

3.2. *E-nose discrimination between sterilized and non-sterilized stuffed olives*

First, the use of the *E-nose* to discriminate between samples that had or had not been subjected to thermal treatments was studied. To determine whether there were differences between the two groups, PCA models were developed considering each type of stuffed olive individually. PCA analysis also allowed the detection of potential outliers and systematic artifacts in the samples. A total of six PCA models were developed: one for the stuffed olives 'without aroma' and five for the stuffed olives with aroma (five different aromas). Fig. 1 shows the score plots of the first two principal components of the PCA model for stuffed olives 'without aroma' and stuffed olives with 'oregano' aroma, as this is similar in behavior to the rest of the aromas used.

As can be seen, these two components suffice to verify that samples are clustered according to the thermal treatment. These two components explain 71.76% and 78.39% of the total variance in stuffed olives 'without aroma' and with 'oregano' aroma, respectively. Component 1 allows differentiation of the samples since higher scores were obtained for this component in the experiments with thermal treatments than for the control without sterilization.

It can also be observed in both types of samples that unsterilized stuffed olives presented scores closer together while those for olives submitted to thermal treatments were more dispersed and had a linear trend. This may be due to different sterilization treatments being applied, causing the values to be more dispersed.

The promising exploratory analysis suggests the use of a supervised classification method aiming to discriminate between unsterilized and sterilized samples. The supervised PLS-DA model was selected. Table 2 shows the correct prediction (for cross-validation using the leave-one-

Table 2
Confusion matrices obtained through PLS-DA for the discrimination between control samples and samples summited to thermal treatments. Values are expressed in percentage.

	Predicted class			
	Without aroma		Oregano	
Real class	Control	Thermal treatment	Control	Thermal treatment
Control	25.8	0	24.5	0
Thermal treatment	0	74.2	0	75.5

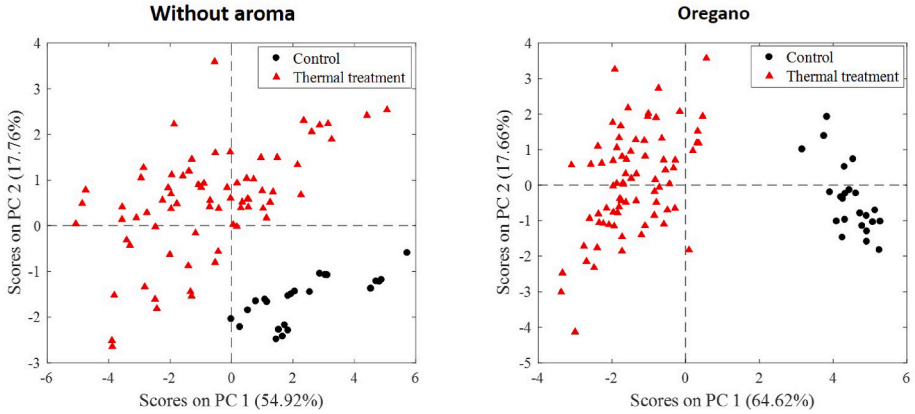

Fig. 1. PCA score plots of the two first principal components obtained from *E-nose* data for discriminating between control and sterilization treatments.

out approach) for both types of samples. The diagonal elements sum of the confusion matrices gives the percentages of correct predictions. In both cases, 100% of the predictions were correct, indicating that the model is suitable for use in stuffed olives.

Thus, the *E-nose* showed that the olives subjected to thermal treatments presented a different aromatic profile than those that had not undergone sterilization. As commented in the previous section, in stuffed olives 'without aroma', the tasting panel indicated that the aromatic sensation perceived was due to the cooking effect that was absent in the control samples. However, for stuffed olives with aroma, the differentiation was due to a simultaneous effect of the aroma and the cooking effect. Thus, in this case, the *E-nose* discriminated the whole aromatic profile detected in the sample. Sánchez et al. (2021), Sánchez, Martín-Tornero, et al. (2022) and Sánchez, Pérez-Nevado, et al. (2022) discriminated anomalous fermentations in Spanish-style green table olives by using *E-nose*.

3.3. *E-nose discrimination between sterilized stuffed olives submitted to different sterilization treatments*

Once it had been verified that the *E-nose* was capable of accurately differentiating between samples that had or had not been subjected to sterilization treatments, the *E-nose* was used to differentiate between different thermal treatments. As above, data from each type of stuffed olive were first processed by PCA. The corresponding score plots of the first two principal components (PC1 and PC2) are shown in Fig. 2.

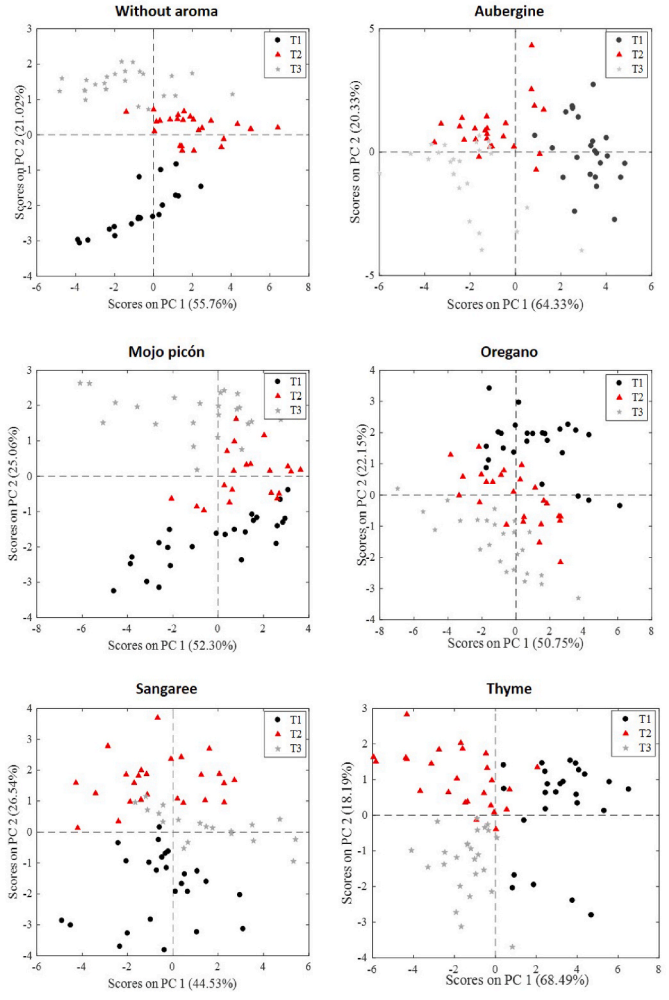

Fig. 2. PCA score plots of the two first principal components obtained from *E-nose* data for different sterilization treatments.

The first two principal components represented total variance values of around 80%, ranging from 71.1% for the filling with 'sangaree' aroma to 86.7% for the filling with 'thyme' aroma. Examining these plots, some grouping of samples according to thermal treatment was observed for each flavored stuffed olive. The scores are seen to progress with the intensity of the sterilization. For the olives stuffed 'without aroma' and with 'mojo picón' and 'sangaree' aromas, the score values for PC2 are higher as the sterilization time increases. However, for 'eggplant', 'oregano' and 'thyme', discrimination is along PC1, with the contribution of the first component being lower as the sterilization time increases. Therefore, the *E-nose* shows a different aromatic trace that classifies the sterilization treatments, which is influenced by the intensity of the perceived aroma and the aromas from the cooking defect. In the specific case of the filling 'without aroma', it can be stated that the *E-nose* classifies it, as does the tasting panel, depending on the cooking defect brought about by the different sterilization treatments.

Furthermore, a classificatory analysis was performed for each stuffing employing the PLS-DA; the results are shown as confusion matrices (Table 3). The models were cross-validated using the leave-one-out approach. The PLS-DA results confirmed the good classification of the different thermal treatments in all the samples studied: 100% of the predictions were correct in the 'sangaree' and 'oregano' stuffed models, and 98.6%, 94.6%, 93.3% and 92.7% of the predictions were correct for olives filled 'without aroma', and with 'eggplant', 'thyme' and 'mojo picón' aromas, respectively. In these samples, the results also demonstrated that no samples of T1 were assigned to T2 or T3 and vice versa. The few incorrect predictions were only within the T2 and T3 samples for these aromas.

As can be seen, the *E-nose* was able to discriminate between the three thermal treatments applied with greater precision than the tasting panel. In this sense, the panel sensorially interpreted T2 and T3 treatments as if they were of similar sterilization intensity, while the *E-nose* was able to differentiate them into different groups. This may be due to the aroma of the filling masking the perception of the tasters, causing them to find no differences between the sterilization treatments. In fact, the panelists indicated similar values between these sterilization treatments for both aroma intensity and cooking effect.

Therefore, the *E-nose* is an effective and more precise tool than a sensory panel since it allows the differentiation of olives subjected to different thermal sterilization treatments regardless of the aroma of the filling contained in the olives.

Table 3
Confusion matrix for the discrimination of thermal treatments applied to the olives based on PLS-DA and using leave one out cross-validation approach. Values are expressed in percentages.

		Predicted class		
	Real class	T1	T2	T3
Without aroma	T1	28.6	0	0
	T2	0	35.7	0
	T3	0	1.4	34.3
Aubergine	T1	32.9	0	0
	T2	0	30.2	2.7
	T3	0	2.7	31.5
Mojo picón	T1	33.8	1.5	0
	T2	0	26.5	2.9
	T3	0	2.9	32.4
Oregano	T1	33.3	0	0
	T2	0	33.3	0
	T3	0	0	33.3
Sangaree	T1	36.2	0	0
	T2	0	36.2	0
	T3	0	0	27.6
Thyme	T1	33.3	0	0
	T2	0	26.7	6.7
	T3	0	0	33.3

4. Conclusions

The sensory analysis and the *E-nose* showed different olfactory profiles of stuffed black olives submitted to sterilization treatments. Therefore, the temperature and time of accumulated lethality need to be controlled by the industry. The tasting panel results showed that the flavored black olives stuffings were able to mask the intensity of the cooked defect caused by sterilization treatments. In fact, the sensory panel classified these olives into a higher category than they really would be. However, the *E-nose* discriminated flavored olives according to the intensity of the thermal sterilization applied, independently of the aroma used. Thus, the *E-nose* is a useful discrimination tool that can be applied in olives stuffed with flavored hydrocolloids submitted to different sterilization treatments. This device can be used to support the tasting panel and combined with chemometric analysis, can be used to perform fast, inexpensive, non-destructive and environmentally-friendly qualitative analysis.

Author Contributions

Conceptualization, R.S., E.-M.T., and D.-M.V.; Data curation, R.S., P.A., F.M., and J.L.; Formal analysis, R.S., E.-M.T., and D.-M.V.; Funding acquisition, D.-M.V.; Investigation, R.S., E.-M.T., and D.-M.V.; Methodology, P.A., F.M., and J.L.; Project administration, D.-M.V.; Resources, R.S., E.-M.T., and D.-M.V.; Supervision, J.L., and D.-M.V.; Validation, J.L., R.S., and D.-M.V.; Visualization, R.S. and D.-M.V.; Writing – original draft, R.S., E.-M.T., and D.-M.V.; Writing – review & editing, R.S., E.-M.T., J.L. and D.-M.V. All authors have read and agreed to the published version of the manuscript.

Compliance with ethics requirements

This research does not include any experiment using animal and/or human subjects.

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.

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CAPÍTULO III. CONCLUSIONES

Podemos concluir de manera general, que la *nariz electrónica* es perfectamente aplicable para la evaluación de los compuestos volátiles de las aceitunas de mesa y/o salmueras. Los sensores utilizados en el dispositivo del tipo MOx (óxidos metálicos) reaccionan, en mayor o menor medida, a casi cualquier tipo de compuestos volátiles presentes en el espacio de cabeza de las aceitunas y/o salmueras, reduciendo u oxidando el material sensible que provoca un cambio en la resistencia de estos. El hecho de que cada uno de los 11 sensores reaccionen de forma distinta a los volátiles presentes en el espacio de cabeza, y con la ayuda de técnicas de análisis quimiométricas, ha permitido discriminar los diferentes patrones olfativos en las aceitunas de mesa y/o sus salmueras. La *nariz electrónica* podría desempeñar un papel importante en la industria de las aceitunas de mesa en la clasificación interna, según los criterios fijados por el COI (IOC, 2021) y cuando no se disponga de panel de cata, para monitorizar la aparición de alteraciones olfativas durante la elaboración o en el producto final, proporcionando un método rápido, económico y respetuoso con el medio ambiente.

A continuación, se enumeran las conclusiones siguiendo el orden de los objetivos específicos planteados.

Objetivo 1. Aplicar la *nariz electrónica* para desarrollar un método de discriminación sensorial de aceitunas de mesa elaboradas al "estilo español", según la intensidad de los defectos de fermentaciones anormales.

1.1 El análisis cromatográfico caracterizó un perfil de compuestos volátiles específico para las aceitunas sanas, y aquellas con defectos por fermentación anómala. Los principales compuestos volátiles de las aceitunas con defecto *zapatería* fueron el ácido ciclohexanocarboxílico y ácido butanoico, para el *butírico* el ácido butanoico y ácido pentanoico, para el *pútrido* el ácido propanoico y alcohol isopropílico y para el *mohoso* el 2-metoxi fenol.

1.2 Las mediciones de la *nariz electrónica* resultaron ser útil para la detección y discriminación de patrones olfativos de aceitunas de mesa al "estilo español" sanas y de aquellas que han sufrido fermentaciones anormales con defectos olfativos a *zapatería*, *butírico*, *pútrido* y *mohoso*. La discriminación proporcionada con este

dispositivo es coincidente con las diferencias de perfiles de aroma encontradas por el panel de cata y perfil de volátiles obtenido por cromatografía.

Objetivo 2. Evaluar la capacidad de la *nariz electrónica* para discriminar las salmueras de aceitunas de mesa no alteradas de las alteradas con diferentes cepas de mohos, a partir del perfil de volátiles emanadas al espacio cabeza de las muestras.

2.1 Las cepas de mohos inoculadas en las salmeras sintéticas produjeron defectos sensoriales negativos a *moho, rancio* o *cuero.*

2.2 Las muestras con diferentes mohos inoculados presentaron un perfil de COVs característicos. De hecho, las que más efectos desagradables (*moho, rancio* y cuero) causaron en las salmueras sintéticas fueron las cepas inoculadas G.G.2, P.E.3, A.F.9 y A.F.18.

2.3 Los principales COVs de olor desagradable característicos de las alteraciones por cepas de mohos de G.G.2 y P.E.3 fueron el dodecanal, mientras que con A.F.18 y A.F.9 los COVs encontrados fueron los ácidos carboxílicos, butanoico y el propanoico.

Objetivo 3. Evaluar mediante la *nariz electrónica*, la evolución sensorial olfativa de aceitunas de mesa con defecto de *zapatería,* antes y después de añadir diferentes concentraciones de aroma de 'mojo picón' al relleno, para comprobar si el defecto de la *zapatería* se enmascara o diluye tras una determinada concentración de aroma añadido, mejorando la aceptación por parte del consumidor.

3.1 Las aceitunas de mesa verdes al "estilo español", con defecto a *zapatería* y rellenas con hidrocoloide aromatizado de 'mojo picón', mejoraron su categoría comercial al enmascarar el defecto. Las altas concentraciones de aroma de 'Mojo picón' agregado hicieron que el defecto fuera casi imperceptible para los catadores.

3.2 Los principales volátiles del defecto a *zapatería* fueron el ácido ciclohexanocarboxílico y el ácido pentanoico que disminuyeron a medida que aumentaba el porcentaje de aroma añadido.

3.3 La *nariz electrónica* discriminó entre aceitunas con defecto a *zapatería* y aceitunas rellenas con diferentes concentraciones de aroma añadido de 'mojo picón'. El algoritmo PLS muestra que existe una buena correlación entre la *nariz electrónica* y el análisis sensorial que permite predecir el defecto a *zapatería* percibido por el panel de cata.

Objetivo 4. Aplicar la *nariz electrónica* para desarrollar una metodología de control de calidad en la industria, capaz de discriminar muestras sanas y no sanas, además de clasificarlas en las diferentes categorías según sus atributos sensoriales.

4.1 Las alteraciones sensoriales percibidas por los catadores, originadas durante la fermentación no controlada, fueron *zapatería, butírico* y *pútrido*. Las muestras se clasificaron en diferentes categorías comerciales según los criterios establecidos por el COI. Se demuestra la necesidad de control de los parámetros durante la fermentación si se quieren aceitunas de mesa de la máxima calidad.

4.2 Algunos de los volátiles encontrados en las aceitunas de la categoría extra fueron el ácido acético y creosol o ácido benzoico, que disminuyeron su cantidad considerablemente en las aceitunas con alta intensidad de defecto. El perfil de compuestos volátiles es característico para cada defecto como el propilenglicol, el ácido 2,4-hexadienoico metil ester, ácido hexanoico, el ácido (E)-3-hexenoico y el ácido ciclohexanocarboxílico que están presentes en el defecto a *zapatería*, el 2-butanol en el defecto a *butírico* y en el alcohol isopropílico en el defecto a *pútrido*.

4.3 La aplicación de este dispositivo en la industria para el control precoz de alteraciones durante la fermentación, sería una estrategia eficaz para mejorar la calidad de las aceitunas de mesa.

Objetivo 5. Evaluar la capacidad de la *nariz electrónica* para ayudar al panel de análisis sensorial a diferenciar entre aceitunas negras al "estilo californiano" de dos variedades distintas y sometidas a diferentes procesos de esterilización y, confirmar su utilidad cuantitativa para evaluar su respuesta a la intensidad del aroma y *efecto de cocido* percibido.

5.1 Un aumento en la duración del tiempo de esterilización, provocó un mayor *efecto de cocido*.

5.2 El aumento de la intensidad de los tratamientos térmicos incrementó los volátiles tales como el 2,4-dimetilhexano, 3-metilpiridina, benzaldehído, 4-etenilpiridina y α-farnesenol. Estos volátiles se relacionan con el olor desagradable a *efecto de cocido*.

5.3 La *nariz electrónica* fue capaz de diferenciar entre muestras de aceitunas de diferentes variedades y además, discriminar aceitunas sometidas a diferentes tratamientos térmicos de esterilización, independientemente de la variedad de aceituna estudiada.

5.4 Los resultados obtenidos por el modelo PLS ofrecieron una buena correlación de los valores medidos de la *nariz electrónica* y los parámetros de *efecto de cocido*, obtenidos del panel de cata. Esto permitió estimar cuantitativamente el defecto sensorial de cocido, con lo que de este modo se evitaría la subjetividad del panel de cata.

Objetivo 6. Evaluar la composición química de fenoles, acrilamida y compuestos volátiles en las aceitunas negras tratadas térmicamente y, la capacidad de la *nariz electrónica* para predecir el contenido de acrilamida.

6.1 Las aceitunas con un alto contenido en compuestos fenólicos presentaron menor contenido de acrilamida.

6.2 Los compuestos fenólicos junto a los alcoholes, aldehídos y ésteres disminuyeron a medida que aumenta la intensidad de los tratamientos de esterilización (F_0).

6.3 Los tratamientos de esterilización térmica más agresivos provocaron mayor intensidad de *efecto de cocido*.

6.4 Los COVs benzaldehído, 4-etenilpiridina o 2,4-dimetilhexano, resultaron estar relacionados con el *efecto de cocido*, de olor desagradable en aceitunas y que que están sintetizadas por la aplicación de los tratamientos térmicos de esterilización.

6.5 La evaluación de los volátiles de las aceitunas tratadas térmicamente estableció una buena correlación con la concentración de acrilamida detectada por cromatografía líquida. Por consiguiente, como la *nariz electrónica* tiene la capacidad de evaluar el perfil de compuestos volátiles de las aceitunas esterilizadas, este dispositivo se podría utilizar a nivel industrial para predecir el contenido en acrilamida en aceitunas negras al "estilo californiano".

Objetivo 7. Estudiar, mediante *nariz electrónica*, la discriminación sensorial olfativa de distintos aromas añadidos en el relleno de aceitunas negras y evaluar la percepción del *efecto de cocido* tras ser sometidos a diferentes tratamientos de esterilización.

7.1 El incremento en la intensidad de los tratamientos de esterilización provocó una disminución de la intensidad de los aromas positivos en las aceitunas, mientras que el *efecto de cocido* aumentaba.

7.2 Las aceitunas al "estilo californiano" rellenas con hidrocoloide aromatizado presentaron una buena intensidad de aroma tras los tratamientos de esterilización.

7.3 La adición de distintos tipos de rellenos de hidrocoloide aromatizados a las aceitunas de mesa enmascaró el *efecto de cocido*, además de provocar un aroma agradable detectado por el panel de cata. De hecho, las aceitunas con alta intensidad de *efecto de cocido* pasaban de la primera categoría comercial a la categoría extra, con la simple adición del relleno aromatizado.

7.4 La *nariz electrónica* discriminó aceitunas rellenas con aromas añadidos sometidas a distintas intensidades de tratamientos de esterilización, presentando una mayor precisión que los obtenidos por el panel de cata.

CAPÍTULO IV. PERSPECTIVAS FUTURAS

El campo de aplicación de la presente Tesis Doctoral corresponde al sector de la industria de la aceituna de mesa. Este se considera uno de los sectores más fuertes y reconocidos de la economía extremeña que contribuye a la mejora de la evaluación de la calidad y diferenciación que aporta un valor añadido en la comercialización de productos de máxima calidad. La originalidad de esta propuesta radica en que se ha profundizado en el conocimiento de COVs y aspectos sensoriales olfativos de las aceitunas de mesa al "estilo español" y "estilo californiano", de los que, hasta la fecha, poco se conocían a nivel científico. Por lo tanto, una de las líneas de investigación futuras sería realizar una cuantificación mediante el uso de patrones de referencia de los principales COVs identificados en la presente Tesis Doctoral que produzcan alteraciones en las aceitunas para conocer su concentración en relación con los atributos sensoriales evaluados del producto.

El grado de innovación de esta investigación radica en el desarrollo de una técnica portátil, rápida y económica que facilita la evaluación sensorial de la aceituna de mesa. Esto se conseguirá sin un tratamiento previo de la muestra, evitando el uso de productos contaminantes, puesto que, bastará con colocar varias aceitunas y su líquido de gobierno en una copa estándar de cata, y ésta podrá ser evaluada mediante el uso del dispositivo (*nariz electrónica*). Es por ello, que se abre la posibilidad de comenzar a utilizar las tecnologías de análisis basadas en una *nariz electrónica* sin necesidad de un gran conocimiento por parte del empresario. De hecho, los resultados de este trabajo serán transferidos al sector de la aceituna de mesa ya que les permitirá a las industrias evaluar la calidad de sus aceitunas de una forma rápida y eficaz. Así, dado que Extremadura se sitúa como una de las principales regiones productoras de aceitunas de mesa, con cualidades organolépticas excepcionales, el hecho de poder disponer de una herramienta rápida y de bajo coste que permita diferenciar y clasificar sensorialmente a las aceitunas de mesa, supondrá una ayuda para la comercialización de productos de calidad. Hasta el momento, este dispositivo consta de una batería de larga duración, sin embargo, para algún tipo de medidas en determinadas matrices sería interesante la instalación de corriente eléctrica en continuo que permita medir durante un largo periodo de tiempo, por ejemplo, durante el proceso de conservación post-cosecha ó durante el proceso de fermentación de la aceituna verde al "estilo español".

Además, la novedad y originalidad de nuestra propuesta se basa también en que se utilizan dispositivos electrónicos olfativos que son complementarios a los órganos de los sentidos de un panel de cata de aceitunas de mesa que valoran los atributos positivos y negativos de las aceitunas elaboradas a nivel industrial en el sector extremeño, y por tanto servirán como una herramienta útil para la clasificación de las aceitunas en sus distintas categorías comerciales. La implementación de estos sistemas digitales servirá para anteponerse a los futuros requisitos exigidos por la Unión Europea en cuanto a la obligatoriedad de evaluar sensorialmente estos productos antes de su puesta en los mercados. Sería interesante continuar con el entrenamiento y perfeccionamiento del panel de cata implantado en las instalaciones del CICYTEX mediante el desarrollo de proyectos de investigación en esta línea de trabajo.

Se considera que la información que aporta esta Tesis Doctoral, supondrá un avance tecnológico para las industrias del sector ya que tendrán información de una herramienta útil y rápida para evaluar sensorialmente aceitunas de mesa durante todo el proceso de elaboración, sobre todo durante la fermentación y su conservación, antes de su puesta en los mercados, permitiendo el cumplimiento de las recomendaciones de la Unión Europea para la mejora de la calidad del producto final elaborado. Una de las futuras líneas de investigación a desarrollar sería la instalación de estos dispositivos electrónicos en los tanques de fermentación de las aceitunas al "estilo español" para monitorizarlas durante el periodo de fermentación láctica para conseguir detectar alteraciones microbiológicas incipientes durante todo el proceso. De esta forma se conseguiría detectar la alteración y poder corregirla mediante la estabilización química del producto con sal y/o ácido. De este modo estos equipos electrónicos servirán como herramientas rápidas, útiles y no destructivas para discriminar aceitunas de mesa de distintas calidades en función del proceso de elaboración aplicado. Por todo esto, se hace necesario el estudio y mejora de los sistemas de *nariz electrónica* y desarrollar nuevos dispositivos con nuevos sensores más específicos.

Finalmente, hay que destacar que una de las limitaciones actuales de la *nariz electrónica* es la inherente deriva de los sensores de gases, que dan lugar a una aleatoria variación temporal de las respuestas de los sensores cuando están expuestos a los

mismos aromas bajo condiciones idénticas. Esto ocasiona que los patrones previamente aprendidos para generar el modelo queden obsoletos al poco tiempo, perdiendo el sistema electrónico la capacidad de identificar los olores conocidos. Habría que desarrollar en futuros trabajos protocolos y modelos matemáticos que corrijan esta deriva.

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