UNIVERSITAT POLITÈCNICA DE VALÈNCIA

TESIS DOCTORAL
PROGRAMA DE DOCTORADO EN INGENIERÍA Y PRODUCCIÓN INDUSTRIAL

IMPLEMENTACIÓN DE TECNOLOGÍA DE MEMBRANAS PARA LA VALORIZACIÓN DE LOS COMPUESTOS FENÓLICOS PRESENTES EN LAS AGUAS RESIDUALES DE LA INDUSTRIA DE PRODUCCIÓN DE ACEITE DE OLIVA

DIRECTORAS
Silvia Álvarez Blanco
María Cinta Vincent Vela

Carmen M. Sánchez Arévalo
Valencia
Junio, 2023

Agradecimientos

Me hace especial ilusión escribir esta parte. Cuando llegué a la UPV, solo conocía un título de tesis, una temática y una entrevista telefónica en la que mis supervisoras ya aparentaban tan estupendas como luego resultaron ser. Y, al final de estos cuatro años, soy una de esas (pocas) personas que no quiere acabar la tesis. Yo me mantendría siempre con mis (muchísimas) tareas actuales, mis compañeros, mis jefas y mis alumnos de prácticas. Pero al final he llegado hasta aquí, y eso es gracias a mucha gente que me ha ayudado tanto durante el camino.

Me gustaría agradecer enormemente a mis directoras, las profesoras Silvia Álvarez Blanco y Cinta Vincent Vela. Formáis un equipo excelente y es un lujo trabajar con vosotras. Gracias por trabajar tanto, a todas horas, para sacar el máximo partido a todo lo que abordamos; por tener ideas increíbles y ser tan brillantes. Gracias por leerme la mente tantas veces, por entender mi mentalidad analítica y por decirme siempre que sí. He aprendido muchísimo de vosotras, y espero seguir haciéndolo en el futuro.

Gracias a mi familia, a mis padres y a mi abuela, pilares fundamentales de mi vida. Cada uno habéis contribuido de muchas formas a esta tesis. Gracias a mi madre, que cree firmemente que puedo conseguir cualquier cosa y me apoya hasta el final, gracias por quererme tanto, cuidarme y darme impulso cuando lo necesito. Tu frase: "lo que tu decidas estará bien, y nosotros te apoyaremos" me ha traído hasta aquí. Gracias a mi padre, por enseñarme tanto de fontanería, prestarme herramientas y estar disponible para todos los retos. Sin darte cuenta, me fuiste formando como ingeniera desde pequeña y, aunque no lo sepas, me enseñabas también sobre igualdad de género y rebeldía. Y gracias a mi abuela, que es mi refugio más sólido, mi amor y mi ancla. Gracias por escucharme cuando más lo necesito, por valorarme tal como soy y entenderme mejor que nadie. Gracias por pintarme una sonrisa siempre que estamos juntas y por enseñarme a ser mejor persona. Muchas gracias también a todos mis tíos y mis primos, que me arropan tanto y me hacen tan feliz. Gracias a Nina, Fer, Conchita y Santiago, por completar mi familia de la mejor forma posible.

¡Un millón de gracias para mis compañeros de la UPV! A Mariajo, por todo, en realidad; por enseñarme desde el principio, por las risas y por los salseos, y por estar ahí con una sonrisa cuando acudo a ti. A Eva, gracias por alegrarme los días con tu humor, por darme dosis de realidad cuando me ha hecho falta, y, especialmente, gracias por todo tu cariño. A Jose Luis, gracias por estar siempre más que dispuesto a ayudar, y

gracias también por tus ocurrencias, que tantos buenos ratos nos dan. A Magda y Raúl, mis compañeros de viajes, congresos y aventuras científicas. Gracias por estar cerca y por hacerme partícipe de todos los momentos buenos y los no tan buenos, que se han ido superando poco a poco. No habría sido lo mismo sin vosotros. Gracias a Pablo, por contagiarme tu motivación y permitirme aprender contigo sobre naranjas. Gracias a Clara y a Elena, que nos alegran con sus visitas. Un gracias muy especial para Dioni, porque marcaste la diferencia cuando nos conocimos y siempre que estoy contigo vuelvo más contenta. Gracias por tu ayuda, tu energía y por hacerme reír de esa manera. Mil gracias a Sergio, por ayudarme siempre en absolutamente todo, aunque te hagas de rogar. Gracias por avisarme cuando me estoy agobiando por tonterías (como ir a congresos geniales), por tus consejos y por comentar las novedades musicales del panorama *indie*. Otras mil gracias para Laura, por tu ternura y tu cariño, gracias por interceder cuando Sergio nos regaña y comprarme matraces nuevos por Reyes 😊.

Gracias a todos los alumnos y alumnas con los que he podido compartir la realización de su TFG o TFM y las prácticas de laboratorio, sobre todo, gracias a Harry (*thank you, truly*), Álvaro, Isa, Carmen, Pablo, Ana, Víctor, Fausto y Ane.

Gracias a Alicia, por ser una profe y una compañera de laboratorio estupenda. Siempre aportas optimismo y cariño, gracias. Gracias a Isabel Iborra, por estar siempre disponible y ser tan divertida y agradable para todo. Gracias, indudablemente, a Jose Antonio Mendoza, por acogerme desde el primer momento, y por gestionar el grupo con sabiduría y amabilidad. Yo opino que el ambiente de trabajo depende mucho de los que están por encima, y es una suerte tener a jefes como tú. Y gracias al resto de personas del ISIRYM y del Departamento de Ingeniería Química y Nuclear con los que he compartido estos años, como Beatriz, Amparo, M Ángeles, Carlos, Esperanza, María Sancho y Rita. No me quiero olvidar de otros miembros de la UPV que me han ayudado y me han aportado alegría día tras día, como Luismi, Arun y Charo. ¡Gracias!

I would like to give a special thank to Professor Bart Van der Bruggen, for receiving me at KU Leuven and supporting all my ideas and research during my time there. Also, thank you to all the great friends I made in Leuven, especially to Ana, Marcello, Luca, Ziloy, Hande, Giovanni, Nithin, Chiara, Ronald, and Marthe. I did not want to ever leave Leuven, and that was thank to all of you.

Quiero agradecer enormemente a todos mis amigos, que, aunque no entienden bien qué pasa con los horarios del laboratorio, siempre están ahí. A Las Chos, mis amigas de siempre, por alegrarme la vida y acompañarme en este camino, valorando mis logros y apoyándome. Sois las mejores, y yo no sería tan feliz sin vosotras. A Javi, por tu amistad

sincera y tu apoyo incondicional, espero que sigamos así toda la vida. A Jose, por las anécdotas de los Jinetes y por todas las conversaciones y las risas. Gracias a Las Chufis, con quienes descubrí los laboratorios y he vivido tantos buenos ratos desde que empezamos la carrera juntas. Gracias a Irene, por permanecer a mi lado a pesar de la distancia y seguir siendo mi amiga íntima. Gracias también a Alba y Marqués, con los que he compartido el último año y disfrutado tanto. Un gracias enorme para Peiró, por ser un amigo genial, por regalarme aquella caja de clips que me ha acompañado toda la tesis y por alegrarnos los findes con tus visitas. Por supuesto, millones de gracias para María, Noelia, Raquel y Álex, por cambiar mi vida cuando nos encontramos y por todo lo que hacéis por mí cada día, incluyendo estos *tuppers* de comida que me han salvado el final de la tesis. Os quiero mucho a todos.

A María del Mar Aparicio, gracias por tu amistad profunda y sincera. Haber hecho este camino contigo ha sido más fácil y más divertido. Gracias por nuestros audios infinitos, por los consejos, por los abrazos que me das y por estar ahí, simplemente. Gracias también a Jordi y al resto de amigos que hice en el Departamento de Química Analítica de la UGR. Solo tengo buenos recuerdos de aquel tiempo. Gracias a Lucía, por tantas cosas. Por enseñarme tanto desde el principio, por preocuparte por mí y ayudarme siempre, por aquella despedida en la becaría, y porque realmente no lo fue. Te quiero mucho, Luciérnaga. Gracias a Alegría. Desde el primer día que me diste clase, he intentado parecerme más a ti, y te debo gran parte de mis logros. Confío en ti ciegamente, y eso solo me ha traído cosas buenas. Gracias por estar tan presente en mi vida, y por ayudarme tanto, con esa forma tuya de hacerlo todo fácil. Eres mi ejemplo a seguir, mi referente, gracias por todo lo que haces por mí.

Por último, las mayores gracias son para Carlos. A ti te podría agradecer toda una vida, pero hoy en concreto te quiero dar las gracias por acompañarme en cada paso de este camino. Gracias por ser la red, por encargarte de todo para que yo pueda hacer lo que más me gusta, que es estar en el laboratorio. Gracias por traer el coche a la UPV para mis múltiples necesidades de mudanza, ir a la almazara, recoger maderas, etc. Y por leerte documentos importantes y darme tu opinión sincera, aunque siempre positiva. Gracias por subirme los ánimos cuando los tenía enterrados, por aprenderte los nombres de mis amigos del lab y seguir todas las historias. Gracias por venir a comer conmigo los viernes y ser mi ayudante de laboratorio. Contigo trabajar los findes no es tan malo, todo es bueno contigo. Gracias por nuestras cenas en la terraza riéndonos tanto. Gracias por dejarme ser, por respetarlo todo. Espero seguir contagiándome de tu bondad infinita, que tanto admiro. Te quiero.

ÍNDICE

ÍNDICE DE FIGURAS

ESTRUCTURA DE LA TESIS DOCTORAL

INTRODUCCIÓN

CAPÍTULO 1. CHAPTER 1

CAPÍTULO 2. CHAPTER 2

CAPÍTULO 3. CHAPTER 3

CAPÍTULO 4. CHAPTER 4

CAPÍTULO 5. CHAPTER 5

CAPÍTULO 6. CHAPTER 6

CAPÍTULO 7. CHAPTER 7

CAPÍTULO 8. CHAPTER 8

ÍNDICE DE TABLAS

INTRODUCCIÓN

CAPÍTULO 1. CHAPTER 1

CAPÍTULO 2. CHAPTER 2

CAPÍTULO 3. CHAPTER 3

CAPÍTULO 4. CHAPTER 4

CAPÍTULO 5. CHAPTER 5

CAPÍTULO 6. CHAPTER 6

CAPÍTULO 7. CHAPTER 7

CAPÍTULO 8. CHAPTER 8

RESUMEN

SUMMARY

RESUM

El sector agroalimentario constituye una de las industrias con mayor relevancia internacional, ya que se encarga de abastecer a una población mundial en continuo crecimiento. Entre los alimentos mayormente elaborados y comercializados a nivel global, se encuentra el aceite de oliva, que es el mayor exponente de la Dieta Mediterránea. Durante su producción, se generan grandes volúmenes de subproductos, entre los que destaca el alperujo, que contiene todos los restos de la aceituna que permanecen una vez que se ha extraído el aceite. Se trata de un subproducto con una elevada carga orgánica, lo que puede suponer un riesgo ambiental si no se gestiona adecuadamente. Por otro lado, el alperujo es rico en compuestos fenólicos, los cuales tienen un elevado interés industrial, debido, principalmente, a sus potentes propiedades antioxidantes. Este contenido en compuestos bioactivos representa una oportunidad excelente para valorizar el alperujo, recuperando compuestos de interés y, simultáneamente, reduciendo la carga contaminante del residuo.

En la presente Tesis Doctoral, "Implementación de Tecnología de Membranas para la valorización de los compuestos fenólicos presentes en las aguas residuales de la industria de producción de aceite de oliva" se ha abordado este reto, en el marco de la Tecnología de Membranas, contribuyendo a la economía circular de la industria oleícola.

En primer lugar, se llevó a cabo la optimización de un proceso de extracción sólido-líquido asistida por ultrasonidos, para extraer los compuestos fenólicos presentes en el alperujo. Se obtuvieron resultados satisfactorios tanto con mezclas de etanol/agua como con agua pura. Además, empleando técnicas analíticas avanzadas, se llevó a cabo una caracterización detallada de los metabolitos presentes en los extractos derivados del alperujo, de forma que se determinaron más de 50 compuestos, pertenecientes a diferentes familias químicas.

A lo largo de las siguientes etapas del proceso, se emplearon tanto los extractos obtenidos con etanol/agua 50:50 (v/v) como los extractos acuosos. En cuanto a estos últimos, se trataron mediante ultrafiltración, estudiando las membranas UP005, UH030, UH050 y UP150 (Microdyn Nadir) y seleccionando las membranas UP005 y UH030, debido a su adecuada productividad y eficacia en términos de rechazo a la demanda química de oxígeno. Los compuestos fenólicos fueron recuperados con mayor pureza en el permeado de este proceso. A continuación, la corriente de permeado obtenida durante la etapa de ultrafiltración fue sometida a un proceso de nanofiltración, empleando la membrana NF270 (DuPont), para llevar a cabo la concentración de los compuestos fenólicos previamente purificados. Además, se demostró la viabilidad de esta membrana, NF270, para separar compuestos fenólicos de bajo peso molecular y

azúcares, empleando disoluciones modelo con una composición basada en las aguas residuales de la industria oleícola.

Los extractos hidroalcohólicos de alperujo también fueron tratados mediante procesos de membrana. Previamente, se llevó a cabo una revisión exhaustiva de la literatura científica relacionada con la ultrafiltración en medio orgánico, analizando los efectos de los disolventes en las membranas y las estrategias para preservar su integridad y rendimiento. A continuación, se evaluó un proceso de ultrafiltración para purificar los extractos de alperujo preparados con etanol/agua 50:50 (v/v), obteniendo resultados satisfactorios en cuanto a la estabilidad de las membranas (empleando las membranas UF010104 y UF010801v3, de la casa comercial SolSep BV, y UP005, de Microdyn Nadir) y la recuperación de compuestos fenólicos, los cuales se obtuvieron en la corriente de permeado. Para aumentar la pureza de estos compuestos fenólicos y abordar su fraccionamiento, se estudió un proceso de nanofiltración con disolventes orgánicos, empleando una disolución simulada, cuyo disolvente fue etanol/agua 50:50 (v/v), y con una composición basada en el permeado del proceso de ultrafiltración (con la membrana UP005) en medio orgánico. De entre todas las membranas evaluadas (Duramem®150, Duramem®300, Duramem®500 y Puramem®600, de la casa comercial Evonik, NFS y NFX, de la casa comercial Synder, oNF-1 y oNF-2, de la casa GMT-Borsig y NF270, de DuPont) la membrana NF270 fue la más destacada, debido a su notable densidad de flujo de permeado. Además, esta membrana rechazó adecuadamente los compuestos no deseados, como azúcares y ácidos, lo que facilitó la recuperación satisfactoria de los compuestos fenólicos en el permeado, tras su purificación y fraccionamiento. Considerando los resultados obtenidos durante el estudio de los procesos de ultrafiltración y nanofiltración del extracto hidroalcohólico del alperujo, se propuso un proceso integrado basado en la extracción de estos compuestos con etanol/agua 50:50 (v/v), la ultrafiltración de este extracto, con la membrana UP005, para llevar a cabo su purificación, la nanofiltración, con la membrana NF270, de la corriente de permeado obtenida previamente, para aumentar la pureza y fraccionar los compuestos fenólicos y, finalmente, la concentración de la corriente de permeado obtenida durante la nanofiltración, mediante un proceso de ósmosis reversa, empleando la membrana NF90 (DuPont) que rechazó apropiadamente los compuestos fenólicos.

The agri-food sector is one of the industries with higher international relevance, because it is responsible for supplying a world population that keeps increasing. Olive oil, which is the main representative of the Mediterranean Diet, is one of the most produced and commercialized foods in the world. During its production, large volumes of by-products are generated. Among them, the wet olive pomace (or *alperujo*, by its name in Spanish) stands out. It contains all the olive components remaining after the olive oil extraction. This by-product has a high organic load, which may represent an environmental risk if it is not properly disposed. On the other side, the wet olive pomace is rich in phenolic compounds, which have high industrial interest, mainly due to their powerful antioxidant properties. This content in bioactive compounds represents an excellent opportunity to valorize the wet olive pomace, recovering compounds of interest, and, during the same process, reducing the contaminant load of the residue.

In the present Doctoral Thesis, "Implementation of Membrane Technology to valorize the phenolic compounds from the olive mill waste", this challenge has been addressed, in the framework of Membrane Technology, aiming to contribute to the circular economy of the olive oil industry.

First, an ultrasound-assisted solid-liquid extraction was optimized to extract the phenolic compounds from wet olive pomace. Satisfactory results were obtained, both with mixtures of ethanol/water and with pure water. Furthermore, employing advanced analytical methodologies, a detailed characterization of the metabolites present in the extracts derived from wet olive pomace was conducted. Thus, more than 50 compounds, belonging to different chemical families, were determined.

During the following stages of the process, both the extracts obtained with ethanol/water 50:50 (v/v) and the aqueous extracts were considered. The aqueous extracts were treated by ultrafiltration, studying the UP005, UH030, UH050 and UP150 membranes (Microdyn Nadir) and selecting the UP005 and UH030 membranes, due to their suitable productivity and efficacy, in terms of the rejection of the chemical oxygen demand. The phenolic compounds were recovered at a higher purity in the permeate. Afterwards, the ultrafiltration permeate was treated by nanofiltration, employing the NF270 membrane (DuPont), in order to concentrate the previously purified phenolic compounds. Moreover, the feasibility of the NF270 membrane to separate low-molecular-weight phenolic compounds from sugars was demonstrated. To that end, simulated solutions, with a composition based on olive mill wastewaters, were employed.

The hydroalcoholic extracts of wet olive pomace were also treated by membrane processes. Previously, an exhaustive review of the scientific literature related to organic-solvent ultrafiltration was addressed. The solvent effects on the membranes and the possible strategies to preserve their integrity and performance were analyzed. Later, it was evaluated an ultrafiltration process to purify the wet olive pomace extracts prepared with ethanol/water 50:50 (v/v). Satisfactory results were obtained, regarding the membranes stability (UF010104 and UF010801v3, from the manufacturer SolSep BV, and UP005, from Microdyn Nadir) and the recovery of phenolic compounds. These molecules were obtained in the permeate stream. To increase the purity of the phenolic compounds and address their fractionation, an organic-solvent nanofiltration process was studied. A simulated solution, whose solvent was ethanol/water 50:50 (v/v), was employed. The composition of this solution was based on the UP005 permeate obtained in organic media. Among all the evaluated membranes (Duramem®150, Duramem®300, Duramem®500, and Puramem®600, from Evonik, NFS, and NFX, from Synder, oNF-1, and oNF-2, from GMT-Borsig, and NF270, from DuPont) the NF270 membrane stood out due to the high permeate flux. Furthermore, this membrane properly rejected the unwanted compounds, such as sugars and acids. This allowed the satisfactory recovery of phenolic compounds in the permeate stream, after their purification and fractionation. Considering the results obtained during the study of the ultrafiltration and nanofiltration of the hydroalcoholic extract of wet olive pomace, an integrated process was proposed. It was based on the extraction of the compounds of interest with ethanol/water 50:50 (v/v), and a further ultrafiltration, nanofiltration, and reverse osmosis process. The ultrafiltration of the extract was performed with the UP005 membrane to conduct its purification. Then, the UP005 permeate was nanofiltered, employing the NF270 membrane to enhance the purity of the phenolic compounds and fractionate them. Finally, the NF270 permeate was concentrated by means of a reverse osmosis process, employing the NF90 membrane (DuPont), which suitably rejected the phenolic compounds.

El sector agroalimentari constitueix una de les indústries amb major rellevància internacional, ja que s'encarrega de proveir una població mundial en continu creixement. Entre els aliments majorment elaborats i comercialitzats a nivell global, hi ha l'oli d'oliva, que és el major exponent de la Dieta Mediterrània. Durant la seua producció, es generen grans volums de subproductes, entre els quals destaca l'alperujo, que conté totes les restes de l'oliva que romanen una vegada que s'ha extret l'oli. Es tracta d'un subproducte amb una elevada càrrega orgànica, la qual cosa pot suposar un risc ambiental si no es gestiona adequadament. D'altra banda, l'alperujo és ric en compostos fenòlics, els quals tenen un elevat interés industrial, degut, principalment, a les seues potents propietats antioxidants. Aquest contingut en compostos bioactius representa una oportunitat excel·lent per a valorar l'alperujo, recuperant compostos d'interés i, simultàniament, reduint la càrrega contaminant del residu.

En la present Tesi Doctoral, "Implementació de Tecnologia de Membranes per a la valorització dels compostos fenòlics presents a les aigües residuals de la indústria de producció d'oli d'oliva" s'ha abordat aquest repte, en el marc de la Tecnologia de Membranes, contribuint a l'economia circular de la indústria oleícola.

En primer lloc, es va dur a terme l'optimització d'un procés d'extracció sòlid-líquid assistida per ultrasons, per a extraure els compostos fenòlics presents a l'alperujo. Es van obtindre resultats satisfactoris tant amb mescles d'etanol/aigua com amb aigua pura. A més, emprant, tècniques analítiques avançades, es va dur a terme una caracterització detallada dels metabòlits presents en els extractes derivats de l'alperujo, de manera que es van determinar més de 50 compostos, pertanyents a diferents famílies químiques.

Al llarg de les següents etapes del procés, es van emprar tant els extractes obtinguts amb etanol/aigua 50:50 (v/v) com els extractes aquosos. Quant a aquests últims, es van tractar mitjançant ultrafiltració, estudiant les membranes UP005, UH030, UH050 i UP150 (Microdyn Nadir) i seleccionant les membranes UP005 i UH030, a causa de la seua adequada productivitat i eficàcia en termes de rebuig a la demanda química d'oxigen. Els compostos fenòlics van ser recuperats amb major puresa al permeat d'aquest procés. A continuació, el corrent de permeat obtinguda durant l'etapa d'ultrafiltració va ser sotmesa a un procés de nanofiltració, emprant la membrana NF270 (DuPont), per a dur a terme la concentració dels compostos fenòlics prèviament purificats. A més, es va demostrar la viabilitat d'aquesta membrana, NF270, per a separar compostos fenòlics de baix pes molecular i sucres, emprant dissolucions model amb una composició basada en les aigües residuals de la indústria oleícola.

Els extractes hidroalcohòlics d'alperujo també van ser tractats mitjançant processos de membrana. Prèviament, es va dur a terme una revisió exhaustiva de la literatura científica relacionada amb la ultrafiltració al mig orgànic, analitzant els efectes dels dissolvents a les membranes i les estratègies per a preservar la seua integritat i rendiment. A continuació, es va avaluar un procés d'ultrafiltració per a purificar els extractes d'alperujo preparats amb etanol/aigua 50:50 (v/v), obtenint resultats satisfactoris quant a l'estabilitat de les membranes (emprant les membranes UF010104 i UF010801v3, de la casa comercial SolSep BV, i UP005, de Microdyn Nadir) i la recuperació de compostos fenòlics, els quals es van obtindre en el corrent de permeat. Per augmentar la puresa d'aquests compostos fenòlics i abordar el seu fraccionament, es va estudiar un procés de nanofiltració amb dissolvents orgànics, utilitzant una dissolució simulada, que el seu dissolvent va ser etanol/aigua 50:50 (v/v), i amb una composició basada en el permeat del procés d'ultrafiltració (amb la membrana UP005) al mig orgànic. D'entre totes les membranes avaluades (Duramem®150, Duramem®300, Duramem®500 i Puramem®600, de la casa comercial Evonik, NFS i NFX, de la casa comercial Synder, oNF-1 i oNF-2, de la casa GMT - Borsig i NF270, de DuPont) la membrana NF270 va ser la més destacada, a causa de la seua notable densitat de flux de permeat. A més, aquesta membrana va rebutjar adequadament els compostos no desitjats, com a sucres i àcids, la qual va facilitar la recuperació satisfactòria dels compostos fenòlics en el permeat, després de la seua purificació i fraccionament. Considerant els resultats obtinguts durant l'estudi dels processos d'ultrafiltració i nanofiltració de l'extracte hidroalcohòlic de l'alperujo, es va proposar un procés integrat basat en l'extracció d'aquests compostos amb etanol/aigua 50:50 (v/v), la ultrafiltració d'aquest extracte, amb la membrana UP005, per a dur a terme la seua purificació, la nanofiltració, amb la membrana NF270, del corrent de permeat obtinguda prèviament, per augmentar la puresa i fraccionar els compostos fenòlics i, finalment, la concentració del corrent de permeat obtinguda durant la nanofiltració, mitjançant un procés d'osmosi reversa, emprant la membrana NF90 (DuPont) que va rebutjar apropiadament els compostos fenòlics.

Contribuciones Científicas

El trabajo experimental y los resultados alcanzados durante el desarrollo de esta Tesis Doctoral han derivado en distintas contribuciones científicas, entre las que se encuentran diversas publicaciones científicas en revistas de alto impacto, así como comunicaciones presentadas en congresos, todos ellos internacionales, y acciones de divulgación.

PUBLICACIONES EN REVISTAS CIENTÍFICAS

- CM Sánchez-Arévalo, A Iborra-Clar, MC Vincent-Vela, S Álvarez-Blanco. 2022. Exploring the extraction of the bioactive content from the two-phase olive mill waste and further purification by ultrafiltration. LWT Food Science & Technology, 165, 113742. https://doi.org/10.1016/j.lwt.2022.113742

- CM Sánchez-Arévalo, MC Vincent-Vela, S Álvarez-Blanco. 2023. Green management of wet olive pomace by means of ultrafiltration of an aqueous extract of phenolic compounds. Integral characterization of the streams by LC-ESI-QToF-MS. Environmental Technology & Innovation (*Under review*)

- CM Sánchez-Arévalo, A Jimeno-Jiménez, C Carbonell-Alcaina, MC Vincent-Vela, S Álvarez-Blanco. 2021. Effect of the operating conditions on a nanofiltration process to separate low-molecular-weight phenolic compounds from the sugars present in olive mill wastewaters. Process Safety and Environmental Protection, 148, 428–436. https://doi.org/10.1016/j.psep.2020.10.002

- CM Sánchez-Arévalo, A Pérez García-Serrano, MC Vincent-Vela, S Álvarez-Blanco. 2023. Combining ultrafiltration and nanofiltration to obtain a concentrated extract of purified polyphenols from wet olive pomace. Membranes, 13 (2), 119. https://doi.org/10.3390/membranes13020119

- CM Sánchez-Arévalo, MC Vincent-Vela, MJ Luján-Facundo, S Álvarez-Blanco. 2023. Ultrafiltration with organic solvents: a review on achieved results, membrane materials and challenges to face. Process Safety and Environmental Protection, 117, 118-137. https://doi.org/10.1016/j.psep.2023.06.073

- CM Sánchez-Arévalo, A Iborra-Clar, MC Vincent-Vela, S Álvarez-Blanco. 2023. Solvent-resistant ultrafiltration to recover bioactive compounds from wet olive pomace extracts. LWT Food Science & Technology, 186, 115167. https://doi.org/10.1016/j.lwt.2023.115167

- CM Sánchez-Arévalo, T Croes, B Van der Bruggen, MC Vincent-Vela, S Álvarez-Blanco. 2023. Feasibility of several commercial membranes to recover valuable

phenolic compounds from extracts of wet olive pomace through organic-solvent nanofiltration. Separation and Purification Technology, 305, 122396. https://doi.org/10.1016/j.seppur.2022.122396

- CM Sánchez-Arévalo, F Aldegheri, MC Vincent-Vela, S Álvarez-Blanco. Integrated membrane process in organic media: combining organic solvent ultrafiltration, nanofiltration, and reverse osmosis to purify and concentrate the phenolic compounds from wet olive pomace. Food and Bioprocess Technology (*Under review*).

PRESENTACIONES EN CONGRESOS

Presentaciones en formato póster

- CM Sánchez-Arévalo; V Awoyemi; MC Vincent-Vela; S Álvarez Blanco. Utilization of commercial ultrafiltration membranes to recover phenolic compounds from wet olive pomace. EuroMembrane 2022. Naples, Italy.

- CM Sánchez-Arévalo; A Iborra-Clar; MC Vincent-Vela; S Álvarez Blanco. Combining ultrafiltration and organic-solvent nanofiltration for the recovery of hydroxytyrosol from wet olive pomace. 37th EMS Summer School 2022. Alentejo, Portugal.

- CM Sánchez-Arévalo; T Croes; MC Vincent-Vela; S Álvarez Blanco; B Van der Bruggen. Application of organic solvent nanofiltration to the fractionation and purification of phenolic compounds present in a hydroalcoholic extract from wet olive pomace. 8th International Conference on Organic Solvent Nanofiltration (OSN 2021-2022). Rennes, France.

- CM Sánchez-Arévalo; I Casas-Roncero; MC Vincent-Vela; S Álvarez Blanco. Ultrafiltration of two-phase olive mill waste to obtain a permeate enriched in phenolic compounds. EuroMembrane 2021. Copenhagen, Denmark.

- CM Sánchez-Arévalo; I Casas-Roncero; MC Vincent-Vela; S Álvarez Blanco. Ultrafiltration of two-phase olive mill waste to recover phenolic compounds. Comparison of the processing of a solvent based and an aqueous extract. 18th Network Young Membrains Meeting (NYM 2021). Lund, Sweden.

- CM Sánchez-Arévalo; H Ellicott; MC Vincent-Vela; S Álvarez Blanco. Assessment of several organic and inorganic membranes to ultrafilter a phenolic extract from two-phase olive mill wastewater. II International Congress on Water and Sustainability. Terrassa, Spain.

- A Jimeno-Jiménez, CM Sánchez-Arévalo; C Carbonell-Alcaina; MC Vincent-Vela; S Álvarez Blanco. Separation of phenolic compounds from sugars in the nanofiltration of olive mill wastewaters. 12th International Congress on Membranes and Membrane Processes (ICOM 2020). Online.

Presentaciones orales

- CM Sánchez-Arévalo; T Croes; MC Vincent-Vela; S Álvarez Blanco; B Van der Bruggen. Purification and fractionation of polyphenols from wet olive pomace by means of organic-solvent nanofiltration. EuroMembrane 2022. Naples, Italy.

- M Cifuentes-Cabezas, CM Sánchez-Arévalo; C Carbonell-Alcaina; A Bes-Piá, MC Vincent-Vela; JA Mendoza-Roca, S Álvarez Blanco. Recovery of bioactive compounds from food wastes by membrane processes. EuroMembrane 2022. Naples, Italy.

- CM Sánchez-Arévalo; A Sáez-Montesinos; MC Vincent-Vela; S Álvarez Blanco. An integrated membrane process to recover polyphenols from wet olive pomace. 19th Network Young Membrains Meeting (NYM 2022). Naples, Italy.

- CM Sánchez-Arévalo; A Iborra-Clar; MC Vincent-Vela; S Álvarez Blanco. Exploring the performance of organic membranes for the organic-solvent ultrafiltration of an ethanol-based extract from two-phase olive mill wastewater. EuroMembrane 2021. Copenhagen, Denmark.

- M Cifuentes-Cabezas, CM Sánchez-Arévalo; C Carbonell-Alcaina; A Bes-Piá, MC Vincent-Vela; S Álvarez-Blanco, JA Mendoza-Roca. Recuperación de polifenoles de efluentes del procesado de aceituna y de la producción de aceite mediante tecnologías de membrana. 1º Congreso Internacional Virtual de Ingeniería de Alimentos. Online.

- CM Sánchez-Arévalo; MC Vincent-Vela; S Álvarez Blanco. Revalorization of two-phase olive mill wastewater: recovery of antioxidant, bioactive compounds from a phytotoxic residue. II International Congress on Water and Sustainability. Terrassa, Spain.

ACCIONES DE DIVULGACIÓN CIENTÍFICA

- CM Sánchez-Arévalo; MC Vincent-Vela; S Álvarez Blanco. Recuperación de compuestos antioxidantes de los residuos semisólidos de la almazara. I Divulga NextGen. Online.
- CM Sánchez-Arévalo; MC Vincent-Vela; S Álvarez Blanco. Recuperación de compuestos fenólicos presentes en los residuos de la almazara. V Jornadas Divulgativas para Jóvenes Investigadores. Valencia, Spain.

OBJETIVOS

OBJETIVO GENERAL

El objetivo principal de esta Tesis Doctoral consiste en llevar a cabo la recuperación de los compuestos fenólicos presentes en el alperujo, aplicando la Tecnología de Membranas. De esta forma, se pretende disminuir la carga orgánica de este residuo y, además, valorizarlo mediante la obtención de productos de alto valor añadido.

OBJETIVOS ESPECÍFICOS

- Analizar el proceso de extracción sólido-líquido de los compuestos fenólicos del alperujo, con el fin de identificar las condiciones de operación óptimas, así como el disolvente más adecuado (agua o disolventes orgánicos) para la recuperación máxima de compuestos fenólicos y minimización del impacto ambiental del alperujo.

- Realizar una caracterización profunda de los extractos obtenidos y de todas las corrientes obtenidas en los procesos de membranas, llevando a cabo una aproximación metabolómica, para determinar la mayor cantidad posible de compuestos pertenecientes a la fracción minoritaria de la oliva presentes en el alperujo.

- Analizar de forma pormenorizada el efecto de las variables de operación (presión, velocidad tangencial y factor de concentración) y de las características de las membranas (tamaño de poro y material) de ultrafiltración, sobre el ensuciamiento de las mismas, la recuperación de polifenoles y la eliminación de materia orgánica. Se seleccionarán las condiciones de operación y las membranas que permitan la máxima recuperación de compuestos fenólicos en la corriente de permeado, la máxima eliminación de materia orgánica en la corriente de rechazo y la máxima densidad de flujo de permeado.

- Analizar de forma pormenorizada el efecto de las variables de operación (presión, velocidad tangencial y factor de concentración) y de las características de las membranas (tamaño de poro y material) de nanofiltración sobre la recuperación de polifenoles, la eliminación de materia orgánica y la densidad de flujo de permeado. Se seleccionarán las condiciones de operación y las membranas que permitan la máxima recuperación de compuestos fenólicos, bien en la corriente de permeado o en la corriente de rechazo, y la máxima densidad de flujo de permeado.

- Llevar a cabo la concentración de los compuestos fenólicos purificados previamente (mediante ultrafiltración y, en su caso, nanofiltración). Para ello, se estudiará la

aplicación de un proceso de nanofiltración u ósmosis inversa. Se seleccionarán las membranas y las condiciones de operación más adecuadas para alcanzar la máxima concentración de compuestos fenólicos y la mayor productividad.

- Estudiar el resultado de llevar a cabo los procesos de ultrafiltración, nanofiltración y ósmosis reversa en medio orgánico, tras haber determinado el disolvente más adecuado y las condiciones de operación óptimas para realizar la etapa de extracción sólido-líquido. Para ello, se estudiará el efecto del disolvente sobre la densidad de flujo de permeado y la selectividad de las membranas

ESTRUCTURA DE LA TESIS

En la presente Tesis Doctoral, "Implementación de Tecnología de Membranas para la valorización de los compuestos fenólicos presentes en las aguas residuales de la industria de producción de aceite de oliva" se ha explorado el potencial de la Tecnología de Membranas en el tratamiento de subproductos derivados del olivo, con el objetivo de recuperar compuestos de alto valor añadido, como son los compuestos fenólicos.

Así, esta memoria contiene una sección inicial, la **Introducción**, que presenta el contexto en el que se encuadra este trabajo, aportando una descripción general de la industria oleícola y las posibles contribuciones de la Tecnología de Membranas en cuanto a la gestión de los subproductos derivados. Además, la introducción contiene una breve reseña final en la que se ponen en valor determinadas técnicas analíticas avanzadas, pertenecientes al ámbito de la Metabolómica, para comprender y mejorar los procesos de membrana aplicados al tratamiento de corrientes de origen vegetal.

A continuación de esta primera sección, se presentan los capítulos que contienen la **Metodología Experimental, Resultados y Discusión** correspondientes a esta Tesis Doctoral. Dichos capítulos están agrupados en tres secciones independientes, atendiendo a las técnicas empleadas y a la naturaleza química de las corrientes estudiadas.

La **primera sección** trata sobre la **extracción de los compuestos fenólicos** presentes en el alperujo. Esta sección incluye el **Capítulo 1**, en el que se optimizó un proceso de extracción sólido-líquido asistida por ultrasonidos. Se estudió la efectividad del agua como agente extractante, así como diferentes mezclas de etanol/agua. También se evaluó el efecto de la temperatura y tiempo de extracción sobre el rendimiento de la extracción.

Además, en este capítulo se presenta una caracterización detallada de todos los metabolitos procedentes de la fracción minoritaria de la oliva que permanecen en el alperujo tras la elaboración del aceite de oliva. Para ello, se empleó la cromatografía líquida acoplada a espectrometría de masas, desarrollando un método analítico multiclase y no dirigido, que permitió determinar más de 50 compuestos en los perfiles cromatográficos. Esta potente metodología analítica y aquellas derivadas de sus modificaciones han permitido la caracterización del metaboloma del resto de muestras analizadas en esta Tesis Doctoral.

La **segunda sección** de la memoria contiene tres capítulos que están relacionados con **procesos de membrana en medio acuoso**.

El **Capítulo 2** contiene el estudio de un proceso de ultrafiltración para purificar el extracto acuoso de alperujo obtenido de acuerdo al Capítulo 1. Para ello, se evaluaron cuatro membranas comerciales, orgánicas, con un amplio rango de corte molecular (5 - 150 kDa) y se estudiaron numerosas condiciones de operación, en el rango 0.75 - 5.5 bar (correspondientes a la presión transmembranal) y 1.5 – 3.5 m/s (correspondientes a la velocidad tangencial).

En el **Capítulo 3**, se presenta el estudio de un proceso de nanofiltración para separar, por un lado, los compuestos fenólicos de bajo peso molecular y, por otro lado, los azúcares presentes en los residuos de la almazara. Así, se empleó la membrana NF270 (de la casa comercial DuPont) para tratar disoluciones modelo con diferentes composiciones de tirosol y sacarosa.

El **Capítulo 4** contiene el desarrollo de un proceso integrado, basado en los resultados obtenidos en los Capítulos 1, 2 y 3. Así, se llevó a cabo la combinación de una etapa de extracción sólido-líquido, empleando agua como agente extractante para extraer los compuestos fenólicos del alperujo; una etapa de ultrafiltración, destinada a purificar los compuestos fenólicos procedentes de un extracto acuoso de alperujo, y una etapa de nanofiltración, para concentrar la corriente de permeado (enriquecida en compuestos fenólicos) obtenida durante la ultrafiltración. Durante la ultrafiltración se estudió el comportamiento de las membranas UP005 y UH030 (de la casa comercial Microdyn Nadir), que habían ofrecido los mejores resultados en los estudios previos. Durante la nanofiltración, se empleó eficazmente la membrana NF270.

La **tercera sección** de esta Tesis Doctoral contiene cuatro capítulos relacionados con **procesos de membrana en presencia de un disolvente orgánico**.

El **Capítulo 5** presenta una revisión pormenorizada de la técnica de ultrafiltración en medio orgánico, analizando los efectos que puede tener la presencia de un disolvente orgánico sobre las membranas de ultrafiltración y el proceso del filtrado. Además, se hace énfasis sobre las diferentes estrategias posibles para acondicionar la membrana y prepararla para el contacto prolongado con un disolvente orgánico, de forma que se preserve su integridad y su rendimiento.

En el **Capítulo 6** se encuentra el estudio de un proceso de ultrafiltración con disolventes orgánicos, empleando diversas membranas poliméricas de diversos materiales y umbral de corte molecular, para tratar un extracto hidroalcohólico de alperujo, obtenido

mediante la metodología optimizada en el Capítulo 1. El reto de este procedimiento recayó en obtener resultados satisfactorios en cuanto a la estabilidad de las membranas, así como valores adecuados de flujo de permeado y rechazos a los diferentes componentes del extracto, para retener toda la materia orgánica no deseada y obtener los compuestos fenólicos en el permeado de las membranas.

El **Capítulo 7** contiene el estudio de un proceso de nanofiltración con disolventes orgánicos para abordar la purificación y fraccionamiento de los polifenoles procedentes de un extracto hidroalcohólico de alperujo, una vez que han sido purificados parcialmente mediate ultrafiltración. Para llevar a cabo este estudio, se tomó como referencia el permeado obtenido tras tratar un extracto hidroalcohólico de alperujo con la membrana UP005 (de acuerdo con los resultados presentados en el Capítulo 6). Teniendo en cuenta la composición de este permeado, se preparó una disolución modelo que contenía uno o varios representantes de cada familia química presente en el permeado real (fenoles simples, ácidos fenólicos, secoiridoides, flavonoides, ácidos orgánicos, ácidos grasos libres y azúcares). Esta disolución se empleó para estudiar el comportamiento de un total de 9 membranas orgánicas, de diversos materiales y tamaño de poro. Así, se estudió la estabilidad de estas membranas en presencia de la mezcla etanol/agua 50:50 (v/v) y los valores de densidad de flujo de permeado, así como su selectividad en el fraccionamiento los compuestos fenólicos y su separación del resto de componentes de la disolución.

En el **Capítulo 8**, se presenta un proceso integrado, en medio orgánico, consistente en un proceso de ultrafiltración, seguido de nanofiltración y ósmosis inversa. Tras el estudio independiente de la etapa de ultrafiltración (Capítulo 6) y de la etapa de nanofiltración (Capítulo 7) de un extracto hidroalcohólico del alperujo, se abordó la combinación de ambas etapas para purificar los compuestos fenólicos extraídos del alperujo de acuerdo con las mejores condiciones de operación identificadas en el Capítulo 1, es decir, en presencia de la mezcla etanol/agua 50:50 (v/v). Además, se llevó a cabo la concentración del permeado obtenido en el proceso de nanofiltración. Para ello, se propuso un proceso de ósmosis inversa, empleando la membrana NF90 (de la casa comercial DuPont).

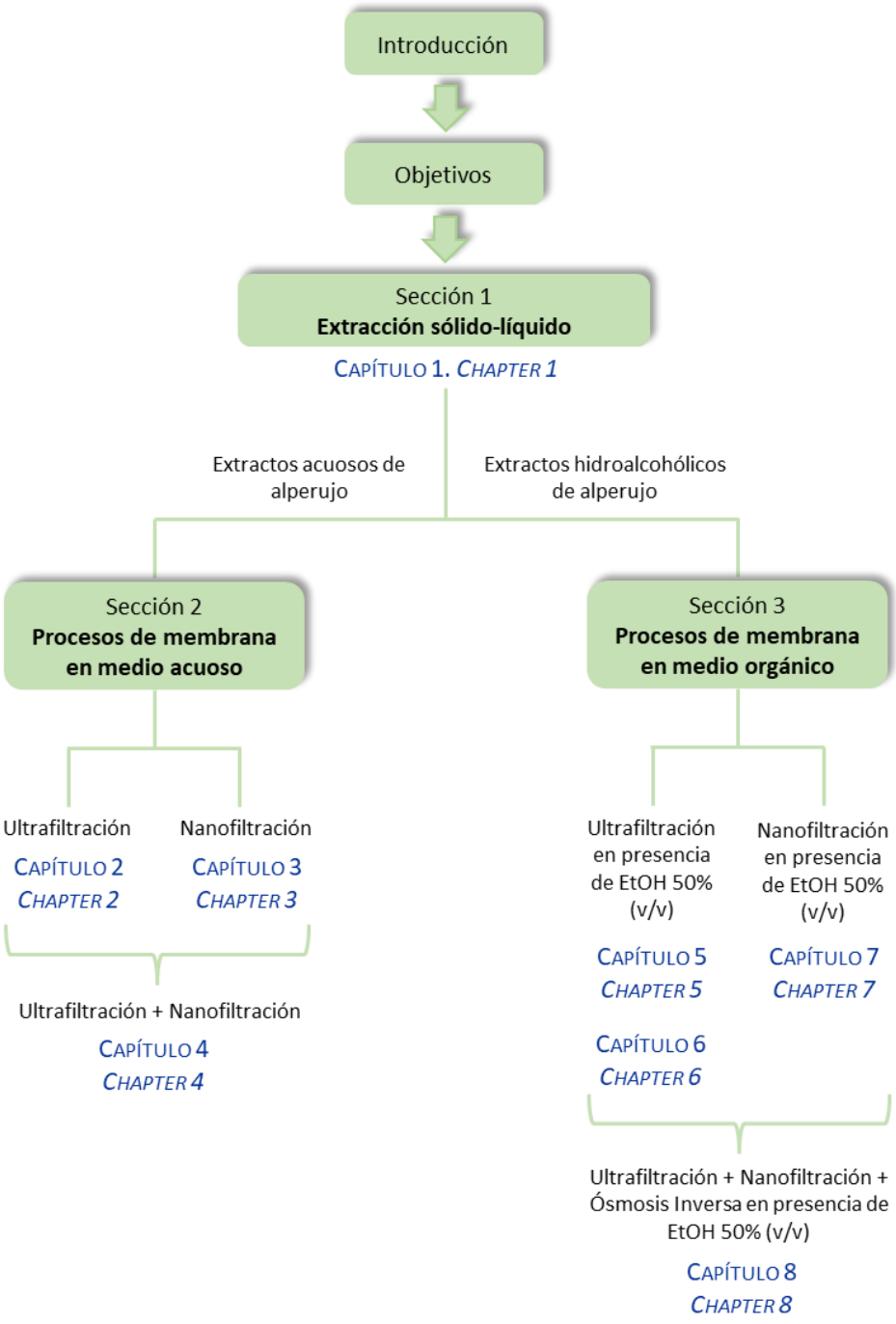


Figura E1. Esquema representativo de la estructura de la presente Tesis Doctoral.

INTRODUCCIÓN

1. INTERÉS DE LOS PRODUCTOS DERIVADOS DEL OLIVAR

El árbol del olivo (*Olea europaea L.*) es uno de los más apreciados desde los inicios de la Historia. Las primeras civilizaciones ya empleaban los productos derivados del olivar en la nutrición, la construcción e, incluso, la fabricación de bálsamos y ungüentos [1,2]. Todos estos usos y aplicaciones han llegado hasta nuestros días. En la actualidad, el cultivo del olivo, la olivicultura, tiene una enorme importancia a nivel económico, ecológico y cultural. Las aceitunas y el aceite de oliva se consideran una de las bases de la dieta Mediterránea, reconocida mundialmente por su carácter saludable, así como por su influencia en el aumento de la longevidad y la reducción del riesgo de enfermedades coronarias y cáncer [3,4].

Indudablemente, el principal producto obtenido del olivar es el aceite de oliva. Este alimento ha estado presente en la alimentación de los habitantes del área Mediterránea desde el Paleolítico [5]. Hoy en día, se comercializa de manera global, considerándose una fuente de grasa saludable. Incluso, se le ha atribuido el carácter de alimento funcional, capaz de aportar beneficios para el organismo a través de su composición única. Durante los últimos cinco años, el consumo de aceite de oliva a nivel mundial ha superado los tres millones de toneladas, y, además, los datos reflejan una tendencia creciente de este consumo [6]. Para cubrir esta alta demanda de aceituna y aceite de oliva, aproximadamente 12 millones de hectáreas de olivar se cultivan cada año en el planeta [7]. El cultivo del olivar está extendido por todo el mundo, sin embargo, la mayoría de las explotaciones se concentran en países de Europa, concretamente, en España, Grecia e Italia. Así, España es el primer país productor y exportador de aceite de oliva, liderando el mercado mundial [8].

Estos datos subrayan la importancia de la industria oleícola a nivel mundial y, especialmente, en los países de la cuenca mediterránea. En el contexto de una industria de tal magnitud y relevancia internacional, es esencial contribuir a su mejora y evolución, para impulsar el aumento de su rendimiento y sostenibilidad.

2. EL FRUTO DE LA OLIVA

2.1. Anatomía y composición del fruto

La aceituna, u oliva, es una drupa ovalada (Figura I.1), en la que es posible diferenciar tres partes: el pericarpio, que comprende la piel de la aceituna; el mesocarpio, que corresponde a la pulpa carnosa y supone entre el 70% y el 80% del fruto; y el endocarpio, que contiene el hueso y la semilla.

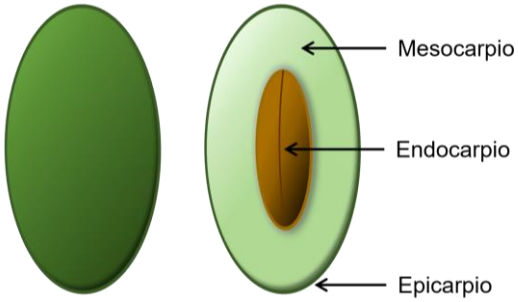


Figura I.1. Esquema del fruto de la aceituna, con sus tres tejidos diferenciados.

En cuanto a su composición, los componentes de la aceituna pueden clasificarse en compuestos mayoritarios, es decir, aquellos que suponen una mayor proporción de la composición, y compuestos minoritarios, que se encuentran en menor cantidad. Entre los compuestos mayoritarios de la aceituna se encuentran los triglicéridos. Un elevado porcentaje de los triglicéridos de la oliva contiene ácidos grasos monoinsaturados. Así, uno de los componentes distintivos de los productos derivados de la oliva es el ácido oleico, al que se atribuyen efectos cardioprotectores [9,10]. La fracción minoritaria de la oliva incluye compuestos fenólicos, triterpenos, tocoferoles y pigmentos [11]. La proporción de estos compuestos en la aceituna es dinámica, y está muy influenciada por la variedad del cultivo, el índice de maduración, las prácticas agronómicas y las condiciones climáticas de la zona de crecimiento del olivo [12,13].

2.2. Compuestos bioactivos derivados de la oliva

Los compuestos bioactivos son un grupo de moléculas, presentes en diferentes alimentos, cuyos beneficios para el organismo van más allá de sus propiedades meramente nutricionales [14]. Estos compuestos proceden generalmente de alimentos vegetales, donde se encuentran en bajas concentraciones. El potencial de estos compuestos se debe a sus interesantes actividades biológicas, entre las que destaca la actividad antioxidante, antiinflamatoria o antimicrobiana, entre otras. Por supuesto, todos estos beneficios para la salud deben estar apoyados con estudios *in vivo* que establezcan los detalles de su biodisponibilidad y mecanismos de acción. En cualquier caso, existen numerosas evidencias que indican que el consumo de alimentos ricos en determinados compuestos bioactivos resulta en una reducción del riesgo de envejecimiento celular, enfermedades coronarias o determinadas infecciones [15,16].

Por lo tanto, estos compuestos se han convertido en el centro de interés de muchas industrias, ya que se trata de ingredientes de origen natural que pueden elevar enormemente la calidad de preparados farmacéuticos, cosméticos y alimentarios.

El fruto de la oliva es una de las fuentes más conocidas de compuestos bioactivos de interés [17]. Estos compuestos incluyen compuestos fenólicos, triterpenos y tocoferoles.

2.2.1. Compuestos fenólicos procedentes de la aceituna

Los compuestos fenólicos son el grupo más numeroso y común de fitoquímicos. Se encuentran en la mayoría de los vegetales, incluyendo el olivo y la aceituna. Se generan como consecuencia del metabolismo secundario de las plantas, a través de la vía del ácido shikímico y el ácido acético [18]. Este grupo de moléculas es muy diverso y heterogéneo. Sin embargo, todos los compuestos fenólicos se caracterizan por contener en su estructura química un anillo bencénico con, al menos, un grupo hidroxilo. Aquellos compuestos que contienen más de un anillo fenólico se incluyen en el grupo de los polifenoles. Además, estas sustancias naturales pueden sufrir modificaciones en su estructura, debidas a esterificaciones, glucosilaciones u oxidaciones propias del metabolismo vegetal. Por lo tanto, es posible encontrar compuestos fenólicos ligados a residuos de glucosa, lípidos o a otros fenoles [19,20].

Los compuestos fenólicos representan una de las categorías más importantes entre los compuestos minoritarios de la oliva. En el aceite de oliva, son responsables de aportar el sabor amargo y picante, contribuyendo a las singulares características organolépticas de este alimento. Además, se trata de potentes antioxidantes naturales, que permiten alargar el período de conservación del aceite. Esta capacidad antioxidante no solo confiere interés a los compuestos fenólicos en relación con la vida útil del aceite de oliva, sino que esta propiedad puede aportar beneficios en el organismo de los consumidores de productos derivados del olivar. En presencia de especies oxidantes, los compuestos fenólicos actúan como reductores, evitando la oxidación de otras moléculas. Así, numerosos estudios han demostrado que determinados compuestos fenólicos presentes en la aceituna pueden prevenir algunas afecciones, sobre todo las relacionadas con el estrés oxidativo, como las enfermedades cardiovasculares y las alteraciones metabólicas. Entre los principales efectos atribuidos a estos compuestos se encuentran el efecto antioxidante, antiinflamatorio, inmunomodulador, antimicrobiano y antiangiogénico.

Todo ello confiere a los compuestos fenólicos importantes aplicaciones en farmacia, en cosmética, para prevenir el envejecimiento de la piel, y en nutrición, a través de la alimentación funcional y personalizada. Actualmente, determinados fenoles

procedentes de matrices derivadas del olivo (tales como la pulpa de la aceituna o las hojas de olivo) se extraen y comercializan en el mercado en forma de preparados enriquecidos en estos extractos vegetales. Algunos de estos productos se encuentran en el mercado bajo el nombre de Alyvium® [21], Mygrium® [22] u OliPhenolia® [23].

En la aceituna y sus derivados, los principales compuestos fenólicos pueden clasificarse en seis familias químicas: fenoles simples (o alcoholes fenólicos), ácidos fenólicos, secoiridoides, flavonoides y lignanos [24].

Los fenoles simples, o alcoholes fenólicos, están constituidos por un anillo aromático con uno o varios alcoholes (-OH) como sustituyentes. Entre los fenoles simples de la oliva destacan el tirosol y el hidroxitirosol (y sus posibles formas modificadas), los cuales son dos de los compuestos fenólicos más conocidos y estudiados en las muestras procedentes del olivo. En concreto, el hidroxitirosol es uno de los compuestos seleccionado por la *European Food Safety Authority* (EFSA) para determinar el carácter saludable del aceite de oliva [25]. Además, existe una enorme cantidad de estudios científicos que avalan su poder cardioprotector [26], antidiabético y antioxidante [27,28].

Los ácidos fenólicos contienen un anillo aromático que, en este caso, contiene uno o varios grupos carboxilo (-COOH) como sustituyentes. Algunos ácidos fenólicos procedentes de la oliva tienen un elevado interés en la industria cosmética por su alto poder antioxidante. Por ejemplo, el ácido ferúlico se emplea en *serums* de tratamiento facial como un valioso activo para neutralizar los radicales libres producidos por la radiación solar [29]. Además, otros ácidos fenólicos, como el ácido cafeico o el ácido *p*-coumárico, han demostrado favorecer la vasodilatación y mejorar el estrés oxidativo [30,31].

Los secoiridoides, flavonoides y lignanos son familias químicas más complejas que implican la presencia de varios anillos bencénicos. Uno de los secoiridoides más característicos de la aceituna es la molécula de oleuropeína. Este compuesto contiene en su estructura una molécula de hidroxitirosol, una molécula de ácido elenólico y, generalmente, un residuo de glucosa (Figura I.2). Su capacidad antioxidante y antiinflamatoria ha sido puesta de manifiesto en numerosos estudios [27,28,32]. Entre los flavonoides y lignanos, destacan las moléculas de luteolina y pinoresinol, respectivamente, debido a sus propiedades antioxidantes [33].

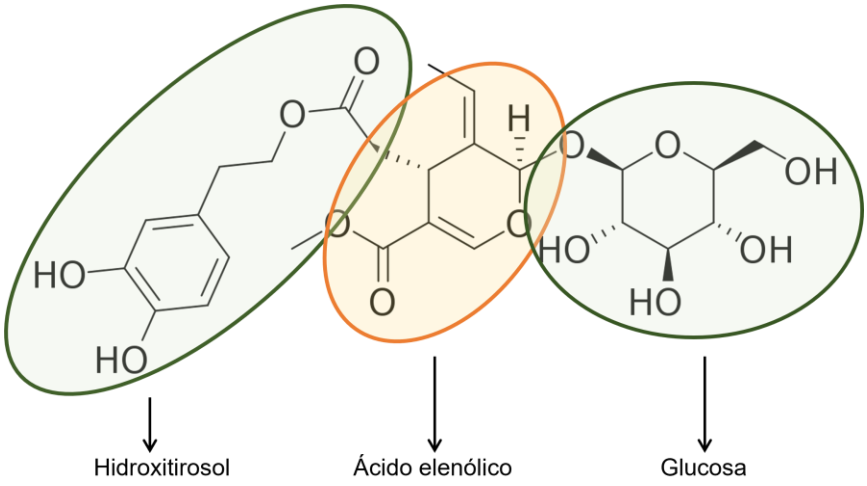


Figura I.2. Estructura de la molécula de oleuropeína.

A pesar del amplio abanico de beneficios relacionados con los compuestos fenólicos y polifenoles, existe un aspecto ambiental que debe considerarse. Los compuestos fenólicos son fitotóxicos, y su contacto con la flora de un ecosistema puede producir daños en las plantas que lo habitan [34]. Además, su carácter antimicrobiano, comentado anteriormente, dificulta el tratamiento biológico de estos compuestos [35]. Por lo tanto, los compuestos fenólicos son compuestos de elevado interés industrial, pero deben ser también objeto de vigilancia, para evitar consecuencias no deseadas sobre el entorno.

2.2.2. Otros compuestos bioactivos presentes en la aceituna

Además de los compuestos fenólicos, la aceituna posee otros compuestos bioactivos de interés, como son los triterpenos pentacíclicos y los tocoferoles. Los triterpenos pentacíclicos son moléculas grandes (450 – 500 g/mol) que incluyen cinco o seis ciclos en su estructura química. Algunos de los triterpenos más estudiados en el olivo y sus derivados son el ácido maslínico, el ácido oleanólico y el ácido betulínico [36,37]. El interés de los compuestos triterpénicos reside en los efectos antitumorales, antivirales o analgésicos, entre otros. Los tocoferoles son un grupo de compuestos que, junto con los tocotrienoles, constituyen la vitamina E. Aunque estas moléculas únicamente son sintetizadas por organismos fotosintéticos, son también esenciales para el correcto funcionamiento fisiológico de los organismos animales [38,39]. Por tanto, su consumo a través de productos vegetales es fundamental.

3. ACEITE DE OLIVA

3.1. Denomicación y clasificación del aceite de oliva

De acuerdo con el Consejo Oleícola Internacional, el aceite de oliva es aquel aceite extraído de la fruta del olivo únicamente mediante métodos físicos, bajo unas condiciones, particularmente condiciones de temperatura, tales que no generen alteraciones en el aceite. El aceite de oliva es aquel que no sufre ningún otro tratamiento más allá del lavado, decantación, centrifugación y filtración. Según esta definición, es posible obtener diferentes categorías de aceite de oliva, entre las que se encuentran el aceite de oliva virgen (que, además, puede ser aceite de oliva virgen extra si se cumplen unos estándares determinados de calidad), el aceite de oliva refinado y la mezcla del aceite de oliva virgen y refinado, que se conoce como aceite de oliva puro [40].

3.2. Producción del aceite de oliva

En el fruto de la oliva, el aceite se almacena en gotas dentro de las vacuolas de la célula vegetal. La obtención del aceite de oliva consiste en su extracción desde la pulpa de la aceituna.

La campaña de recolección de aceituna se lleva a cabo anualmente entre los meses de noviembre y febrero. Una vez que se recoge la aceituna, se lleva a cabo su procesamiento en una planta de producción de aceite que recibe el nombre de almazara. En la almazara, las aceitunas se someten a un control de calidad previo, para descartar aquellos frutos dañados o en mal estado. También se eliminan restos de hojas, ramas y piedras. A continuación, las aceitunas se lavan para eliminar polvo o pesticidas residuales. A partir de esta fase, comienza la extracción del aceite. A lo largo de los años, la producción del aceite de oliva ha evolucionado desde el sistema tradicional (basado en un proceso discontinuo y la utilización de molinos de piedra) a un moderno sistema continuo que es más productivo y requiere menos mano de obra y menos espacio. Actualmente, este sistema comprende las siguientes etapas [41]:

- Molienda: esta fase permite la disgregación de la pulpa de la aceituna y la ruptura de la célula vegetal, para liberar las gotas de aceite. Además, se generan restos sólidos y se libera el agua contenida en la oliva. Como resultado, se obtiene una pasta oleosa, mezcla del aceite extraído, el agua de vegetación de la aceituna y los restos sólidos.

- Batido: consiste en la agitación lenta de la pasta oleosa, para aglutinar las gotas de aceite y desintegrar la emulsión de aceite y agua.

- Decantación o centrifugación: en esta fase se produce la separación del aceite y el resto de componentes de la pasta, como el agua y los restos sólidos de la aceituna. En esta etapa, existen dos variantes principales, ya que la decantación puede llevarse a cabo en una centrífuga (o decánter) de tres fases o de dos fases. El empleo de un tipo y otro de centrifugación es primordial, ya que determina el flujo de trabajo y los residuos generados en el proceso productivo. Así, es posible distinguir entre el método de producción de aceite de dos fases y el de tres fases (Figura I.3)

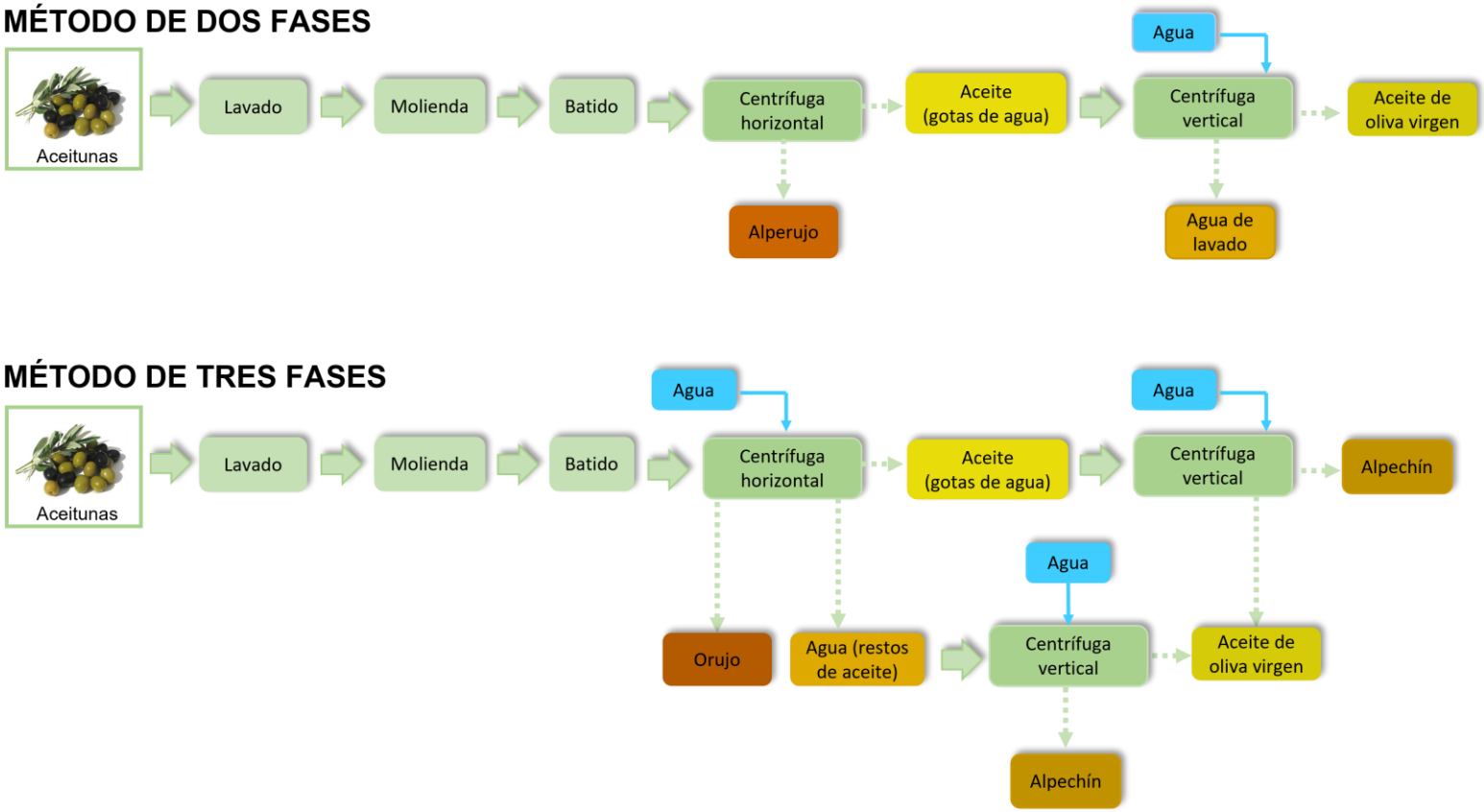


Figura I.3. Diagrama de bloques para los métodos de dos y tres fases en la producción de aceite de oliva. Información adaptada de [7,42].

3.2.1. Método de tres fases

Cuando la etapa de batido finaliza, la pasta se dirige a un decánter horizontal de tres fases. Antes de comenzar la centrifugación, se añade agua a la pasta para facilitar la separación de las diferentes fracciones. En la centrífuga, se generan tres corrientes (tres fases): una corriente de aceite de oliva que aun contiene una pequeña cantidad de agua e impurezas sólidas, una corriente muy abundante de agua residual, denominada alpechín, y una corriente sólida denominada orujo.

El aceite obtenido en esta primera centrifugación horizontal debe ser centrifugado de nuevo para purificarlo. En este caso, se emplea una centrífuga vertical que permite la obtención del aceite de oliva listo para su comercialización. El alpechín también debe ser centrifugado en un decánter vertical para separar los restos de aceite de esta corriente acuosa.

3.2.2. Método de dos fases

Según este método, la centrífuga horizontal da lugar únicamente a dos fases. Una de esas fases es el aceite de oliva con algunos restos de agua. Al igual que en el método de tres fases, el aceite obtenido puede someterse a un lavado en una centrífuga vertical, de la que se obtiene el aceite de oliva virgen y una corriente minoritaria de agua residual con restos sólidos. La otra fase obtenida en la centrifuga horizontal es el principal subproducto del proceso productivo. Se trata de un residuo semisólido denominado alperujo.

De acuerdo con este método, no es necesario añadir agua antes de realizar la centrifugación horizontal y, además, no se genera la corriente de alpechín, que es el residuo más voluminoso del método de tres fases. De hecho, el método de dos fases fue diseñado como una mejora frente al método de tres fases, para disminuir el gasto de agua y energía. Con este método, se produce un ahorro del 80% de agua y un 20% de energía, en comparación con el método de tres fases [42,43].

En año 2022, España contaba con 1830 almazaras en activo [44]. Más del 90% de la producción de estas almazaras se lleva a cabo mediante el método de dos fases [45,46]. Dado que, como se ha comentado anteriormente, España es el principal productor global de aceite de oliva, el método de dos fases se sitúa como el procedimiento más empleado del mundo para producir dicho alimento. En este contexto, el control de este proceso adquiere una dimensión mundial, con lo que se vuelve esencial la optimización del rendimiento y la gestión de los residuos generados.

4. SUBPRODUCTOS DE LA ALMAZARA

Para producir un litro de aceite de oliva, se necesitan entre 5 y 8 kilogramos de aceituna, dependiendo de la variedad del olivar y el momento de la recolección [47]. Por lo tanto, todo el resto de componentes de la aceituna, como la piel, la pulpa, el hueso o la semilla, quedan como subproducto en la almazara. Así, los residuos de la producción de aceite de oliva representan un importante problema ambiental. Se trata de corrientes con una elevada carga orgánica, generados de forma muy abundante y de manera concentrada en el tiempo, ya que se producen únicamente durante los meses en los que tiene lugar la campaña de aceituna. Por lo tanto, su gestión y/o eliminación debe llevarse a cabo con rigor, para evitar vertidos indeseados o contaminaciones del ecosistema.

Por otro lado, al margen de este riesgo ecológico, los subproductos de la almazara pueden considerarse como una fuente de riqueza en cuanto a compuestos químicos de interés. Algunos de los compuestos más interesantes de la aceituna, como son los componentes de la fracción minoritaria (en la que se incluyen los compuestos fenólicos), no se transfieren por completo al aceite de oliva. Por el contrario, un porcentaje considerable permanece en los subproductos [48–50]. Esto se produce porque aquellos compuestos de mayor polaridad (con respecto al resto de componentes de la fracción minoritaria de la oliva) son más hidrofílicos, y presentan, por tanto, mayor afinidad por el agua de vegetación de la aceituna que por el aceite [51,52]. Como consecuencia, un elevado porcentaje de compuestos fenólicos y triterpenos pentacíclicos escapan del aceite de oliva, ya que migran a la fracción acuosa que se genera durante la molienda de la aceituna y, por tanto, permanecen en los residuos generados. Así, tanto el alperujo como las aguas residuales de la almazara contienen compuestos interesantes que juegan un papel esencial en su valorización.

Dado que el alperujo es el residuo mayoritario del proceso de dos fases, este es el principal subproducto de la producción de aceite de oliva. A continuación, se detallan sus características y las de otros residuos generados en el proceso.

4.1. Residuos distintos al alperujo generados en las almazaras de dos fases

En primer lugar, cuando la materia prima llega a la almazara, se eliminan todas aquellas ramas, palos y hojas que han sido recolectadas junto con las aceitunas. Estas hojas son una fuente de compuestos bioactivos aprovechables [53,54]. A continuación, se realiza el lavado de las aceitunas, para eliminar suciedad y restos de polvo. Esta agua de lavado de las aceitunas se suele reutilizar durante varios ciclos de lavado, ya que

únicamente entra en contacto con el fruto de la oliva, y en ningún momento puede contaminar el producto final, que es el aceite de oliva.

Una vez que se produce el batido de las aceitunas y se extrae el aceite en la centrífuga horizontal, se lleva a cabo un lavado de ese aceite en una centrífuga vertical, añadiendo agua potable a la pasta. Como se detalló en la sección 3.2, en esta etapa se retiran el agua y algunos restos sólidos. Como resultado, se obtiene un residuo conocido como agua de lavado del aceite. Esta agua contiene una carga orgánica elevada, incluyendo también compuestos fenólicos de interés.

4.2. Alperujo

El alperujo es un residuo semisólido, de color oscuro, que está formado por restos de la pulpa, agua de vegetación, piel, hueso y semillas de la aceituna. En definitiva, contiene todos los componentes residuales de la oliva, una vez que se ha producido la molienda de este fruto y se ha separado el aceite. Como se ha comentado anteriormente, este subproducto se produce en la centrífuga horizontal, en las almazaras que aplican el método productivo de dos fases [46]. Por cada tonelada de aceitunas procesadas, se generan 800 kilogramos de alperujo, lo que resulta en la producción mundial de más de 4 millones de toneladas de alperujo al año [55].

El alperujo tiene una humedad elevada, así como un pH ligeramente ácido [49,56]. Además, posee un alto contenido en materia orgánica (Tabla I.1). Entre esta materia orgánica se encuentran también los compuestos fenólicos procedentes de la aceituna.

Tabla I.1. Características del alperujo. Contenido elaborado a partir de [57].

Parámetro	Contenido
Humedad (%)	63.6
pH[a]	5.1
Materia orgánica total (g/kg)	947.7
Lignina (g/kg)	369.0
Celulosa (g/kg)	206.0
Hemicelulosa (g/kg)	380.6
Contenido graso (g/kg)	101.9
Nitrógeno (g/kg)	11.4
Fósforo (g/kg)	0.9
Potasio (g/kg)	22.8
Ratio C/N	47.2
Azúcares (g/kg)	5 - 6
Compuestos fenólicos (g/kg)	1 - 11

[a]extracto acuoso, de proporción alperujo/disolvente 1:10

Debido a su elevada carga orgánica, el vertido inadecuado del alperujo puede generar daños en el ecosistema receptor, afectando a aguas y suelos. Además, su contenido en compuestos fenólicos le aporta un carácter dual (Figura I.4). Por un lado, los compuestos fenólicos pueden impedir o disminuir el crecimiento de los microorganismos, alterando el microbioma y dificultando la degradación biológica de este residuo. Además, los compuestos fenólicos le aportan al alperujo carácter fitotóxico, por lo que supone un riesgo para los organismos vegetales del entorno [56]. Sin embargo, estas moléculas poseen también todas las propiedades interesantes detalladas en la sección 2.2.1. Entre ellas, destacan los beneficios para la salud derivados de la capacidad antioxidante, antiinflamatoria y antiproliferativa de estas moléculas.

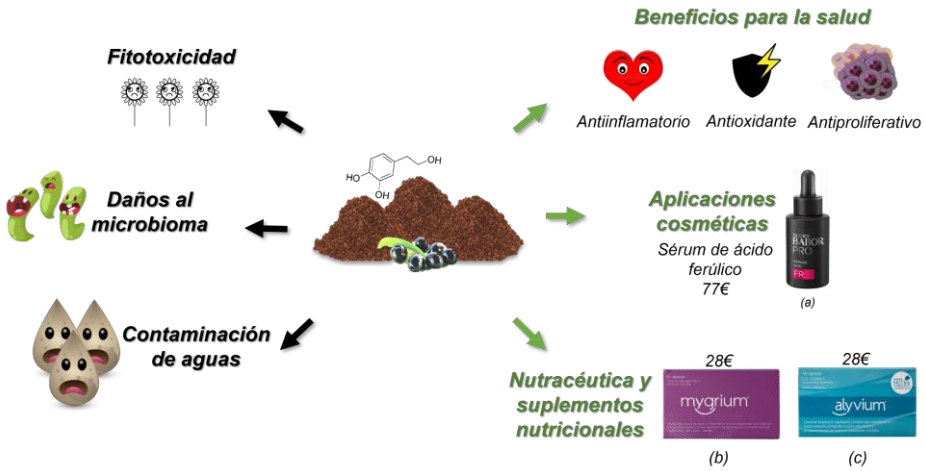


Figura I.4. Características de los compuestos fenólicos presentes en el alperujo.[1]

Estas propiedades resultan en aplicaciones farmacéuticas, cosméticas y alimentarias (a través de la nutracéutica), por lo que la recuperación de los compuestos fenólicos presentes en el alperujo es una estrategia interesante para prevenir el daño ecológico y explotar los beneficios derivados de su riqueza en compuestos fenólicos.

4.3. Tratamiento del alperujo

Debido a la abundancia de este subproducto y a sus características composicionales, su tratamiento no es directo. Además de las prácticas habitualmente utilizadas para

[1]Fuente: (a) https://es.babor.com/products/babor-pro/60958-ferulic-acid-concentrate.html; (b) https://www.mygrium.com/; (c) https://www.alyvium.com/es/

gestionar este residuo, existen también metodologías alternativas que han adquirido relevancia en los últimos años para abordar la valorización del alperujo.

4.3.1. Metodologías generales/tradicionales para el tratamiento del alperujo

El contenido graso del alperujo es varía entre un 2% y un 5% Por tanto, este subproducto se utiliza de nuevo para extraer el aceite residual que contiene [58]. Dicha extracción se lleva a cabo en plantas orujeras, que reciben el alperujo en grandes balsas donde éste se acumula hasta su procesado. A pesar de que las almazaras productoras de aceite de oliva están activas durante aproximadamente 4 meses, las plantas orujeras pueden extender su actividad hasta 10 meses [59]. Por lo tanto, el alperujo permanece acumulado en estas grandes balsas durante largos períodos de tiempo.

La extracción de aceite a partir de alperujo puede llevarse a cabo mediante una nueva centrifugación o a través de secado y extracción química mediada por disolventes. En este último caso, el secado del alperujo conlleva altas demandas energéticas y puede generar diversos problemas técnicos en la maquinaria empleada, a través de la adhesión y aglomeración de los azúcares que contiene [60]. A partir del alperujo se obtiene un aceite de oliva de menor calidad, denominado aceite de orujo de oliva crudo. Dependiendo de sus características, este aceite puede contener compuestos indeseados (como hidrocarburos aromáticos policíclicos, entre otros), y podrá refinarse (aceite de orujo refinado) o mezclarse con aceite de oliva virgen para su comercialización [61,62]. Los residuos secos obtenidos en esta etapa suelen quemarse para producir energía para la propia planta orujera, con la consecuente emisión de CO_2 [56].

Otro aspecto a considerar con respecto a las orujeras es su posible colapso. Esto puede ocurrir cuando la campaña anual de aceituna es especialmente productiva. En este caso, es posible sobrepasar la capacidad de la planta orujera para almacenar y procesar todo el alperujo entrante, generándose un excedente de este residuo que, incluso, puede solapar con el alperujo de la siguiente campaña [59].

En este contexto, varias metodologías adicionales para tratar el alperujo se han propuesto en la última década. Entre ellas, se encuentra el compostaje [56,57], la digestión anaerobia [63], la aplicación en alimentación animal o la generación de biocombustibles [46].

4.3.2. Tecnología de membranas para el tratamiento del alperujo

Además de los tratamientos del alperujo detallados anteriormente, uno de los enfoques más interesantes es el empleo del alperujo como fuente de compuestos de alto

valor añadido, como son los compuestos fenólicos presentes en este subproducto. De hecho, varios destinos finales del alperujo (como el compostaje o el tratamiento biológico) requieren la retirada previa de los compuestos fenólicos, por lo que su recuperación es una estrategia excelente para valorizar este residuo y detoxificar la matriz residual.

La tecnología de membranas ha resultado eficaz en la recuperación de compuestos de interés a partir de productos y subproductos vegetales. De hecho, las aplicaciones de estos procesos en la industria agroalimentaria han experimentado una gran expansión en las últimas décadas. Por lo tanto, esta tecnología es una alternativa prometedora para valorizar el alperujo.

5. TECNOLOGÍA DE MEMBRANAS

5.1. Conceptos y parámetros específicos de los procesos de membrana

Una membrana es una barrera semipermeable que permite el paso selectivo de determinadas sustancias, rechazando otras. De acuerdo con su composición, existen membranas inorgánicas (compuestas por metales u óxidos metálicos) y membranas orgánicas. Éstas últimas están compuestas por un polímero entrecruzado que da lugar a la estructura de la membrana. La mayoría de membranas sintéticas (orgánicas e inorgánicas) están formadas por varias capas. La primera de ellas es una capa fina, de grosor bajo, denominada capa activa, que posee las características necesarias (composición, tamaño de poro, etc.) para llevar a cabo la separación de interés. Bajo la capa activa existe una capa de soporte, cuya única función es sujetar la capa activa y proporcionar resistencia mecánica a la membrana. El tamaño de poro y la composición del soporte deben ser tales que no influyan en el transporte a través de la membrana, sino que la capa activa es la única que determina el paso de unos compuestos u otros.

Cuando una corriente (corriente de alimentación) es tratada mediante un proceso de membrana, se obtendrán como resultado otras dos corrientes. Una de esas corrientes es el resultado de todas las sustancias que permean a través de la membrana (permeado), mientras que la otra contiene aquellas sustancias que no atraviesan la membrana (rechazo). Para que esta separación tenga lugar, debe existir una fuerza impulsora que permita el transporte de moléculas a través de la membrana. Esta fuerza impulsora puede ser una diferencia de presión, de concentración o de potencial eléctrico.

Para evaluar la productividad y la efectividad de un proceso de membranas, se consideran principalmente dos parámetros:

- **Densidad de flujo de permeado:** representa el volumen de fluido que atraviesa la membrana, por unidad de tiempo y área. Por tanto, indica la productividad del proceso. Este parámetro (J_p) se define mediante la Ley de Darcy:

$$J_p = \frac{\Delta P}{\mu \cdot R_m} = L_p \cdot \Delta P \quad (1)$$

donde ΔP corresponde a la presión transmembranal, μ es la viscosidad del permeado, R_m es la resistencia hidráulica de la membrana y L_p corresponde a la permeabilidad hidráulica de la membrana.

- **Rechazo:** representa la eficiencia de la membrana para retener un determinado compuesto. El rechazo (R) se define mediante la siguiente ecuación:

$$R\ (\%) = 1 - \frac{C_p}{C_a} \cdot 100 \quad (2)$$

donde C_p corresponde a la concentración de un compuesto determinado en el permeado y C_R es la concentración de ese compuesto en la corriente de alimentación de la membrana.

Tanto la densidad de flujo de permeado como el rechazo de la membrana frente a determinados compuestos puede verse afectado por la polarización por concentración y el ensuciamiento. La polarización por concentración es el resultado de la acumulación de los sólidos retenidos en el área cercana a la superficie de la membrana [64], donde aumenta la concentración de estos solutos a medida que avanza el proceso, hasta que se alcanza el estado estacionario [65]. El ensuciamiento de la membrana (conocido como *fouling* en inglés) es un fenómeno causado por la deposición de solutos y partículas en la superficie de la capa activa y en los poros de la membrana [66]. Los principales factores que influyen en el ensuciamiento están relacionados con las propiedades de la membrana (dependientes del material, principalmente), las características de la alimentación (composición, concentración de solutos, pH, etc.) y las condiciones de operación aplicadas [67]. El ensuciamiento constituye el principal inconveniente de los procesos mediados por membranas, ya que puede derivar en marcados descensos en la densidad de flujo de permeado. La selectividad de la membrana también puede verse afectada. Si el ensuciamiento es muy severo, es necesario detener el proceso para llevar a cabo la limpieza de la membrana (mediante métodos físicos o químicos) o, en el peor de los casos, ésta tendrá que reemplazarse totalmente, aumentando los costes del procedimiento.

5.2. Procesos de membrana gobernados por la presión

5.2.1. Clasificación

Estos procesos pueden clasificarse de acuerdo al rango de presión aplicado en cada caso. Además, también tendrá gran relevancia el umbral de corte molecular de la membrana (*Molecular Weight Cut Off*, MWCO, en inglés), el cual indica el peso molecular de la sustancia más pequeña cuyo rechazo por parte de la membrana es un 90%.

- ***Microfiltración***

Las membranas de microfiltración suelen tener un tamaño de poro que varía entre 0.1 y 10 µm, lo que indica la versatilidad de esta técnica. Todas aquellas sustancias por encima de este tamaño, como bacterias grandes y partículas coloidales, se obtendrán en el rechazo de la membrana, mediante el mecanismo de exclusión molecular [68]. Para llevar a cabo esta separación, la microfiltración requiere presiones bajas, inferiores a 2 bares [69].

- ***Ultrafiltración***

El umbral de corte molecular de las membranas de ultrafiltración se sitúa en 1 – 500 kDa. Al igual que en la microfiltración, el mecanismo de transporte a través de las membranas de ultrafiltración es la exclusión molecular. Así, estas membranas son capaces de rechazar bacterias, moléculas grandes, como proteínas, y la mayoría de los virus [68]. Para ello, es necesario aplicar una presión de 0.5 – 8 bares.

- ***Nanofiltración***

En los procesos de nanofiltración, se retienen aquellas sustancias con un peso molecular entre 100 y 1000 Da, ya que sobrepasan el tamaño de poro de las membranas [70]. Esto determina mayores requerimientos de presión que en el caso de la ultrafiltración, alcanzando los 5-50 bar. En los procesos de nanofiltración, el mecanismo de disolución-difusión a través de la membrana también influye en el transporte de las sustancias, además de la exclusión molecular y la interacción electrostática entre los solutos cargados y la membrana [71]. Aquellas especies rechazadas por las membranas de nanofiltración comprenden moléculas de menor tamaño, como algunos azúcares e iones polivalentes.

- ***Ósmosis inversa***

Las membranas de ósmosis inversa son capaces de rechazar incluso iones monovalentes disueltos en la disolución de alimento [72]. Únicamente el disolvente

(normalmente agua) atraviesa la membrana, mientras que los sólidos se retienen [73]. Por ello, la ósmosis inversa es ampliamente utilizada a nivel industrial en operaciones de desalación. Se emplean membranas densas, que carecen de poros. Por lo tanto, el transporte a su través es debido únicamente al mecanismo de disolución-difusión. En los procesos de ósmosis reversa, es necesario aplicar presiones más elevadas, entre 20 y 80 bar [74].

5.2.2. Modos de operación en los procesos de membrana

En una planta de membranas, existen diferentes configuraciones dependiendo de la dirección del flujo de la corriente de alimento con respecto a la membrana. La selección de un modo de operación u otro es relevante, especialmente en determinadas aplicaciones como el tratamiento de subproductos y aguas residuales.

Así, pueden distinguirse dos modos de operación principales (Figura I.5):

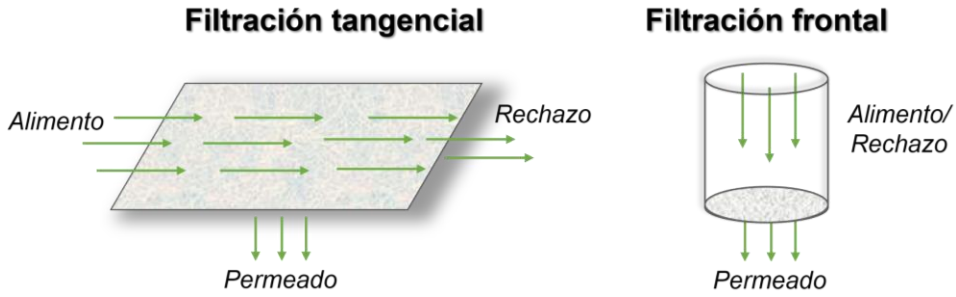


Figura I.5. Representación esquemática de la filtración tangencial y la filtración frontal en un proceso de membrana.

- Filtración de flujo tangencial a la membrana: en esta configuración, la corriente a tratar se alimenta tangencialmente a la cara activa de la membrana. De esta forma, se reduce el depósito de sólidos sobre la superficie de la membrana, lo que minimiza su ensuciamiento [75]. Además, en las instalaciones de flujo tangencial (o *cross-flow*, en inglés) en las que se tratan subproductos y aguas residuales, la corriente de rechazo se retira de forma continua del módulo de membranas y se suele recircular. Esto puede derivar en mayores valores de flujo de permeado, con lo que se aumenta la productividad del proceso.

- Filtración de flujo perpendicular a la membrana: en este caso, la alimentación se aplica perpendicularmente a la membrana. Por tanto, puede producirse la acumulación de solutos en las inmediaciones de la capa activa [75]. A nivel industrial, estos procesos se llevan a cabo en módulos de membranas. Por el contrario, a escala de laboratorio, esta filtración suele llevarse a cabo en celdas (conocidas con el

término *dead-end* en inglés), en las que se produce una agitación para reducir el ensuciamiento de la membrana. Normalmente, la entrada de la corriente de alimentación y la salida de la corriente de rechazo no se producen de forma progresiva, a diferencia de la filtración tangencial. Por el contrario, estas celdas se cargan previamente con todo el fluido a tratar, y este se mantiene en contacto con la membrana hasta que finaliza el proceso. En consecuencia, con el tiempo, los sólidos presentes en el alimento pueden generar una torta sobre la superficie de la membrana, afectando al flujo y a los rechazos de la membrana. No obstante, las instalaciones de flujo frontal presentan menores requerimientos energéticos, y pueden ser útiles para tratar corrientes con bajo contenido en sólidos.

5.3. Procesos de membranas en medio acuoso vs. medio no acuoso

La gran mayoría de procesos de membrana tienen lugar en medio acuoso, ya que la tecnología de membranas encuentra una de sus mayores aplicaciones en el tratamiento de aguas y en la producción de agua potable [76,77]. La aportación de los procesos de membrana a estos ámbitos es muy valiosa, debido a los bajos requerimientos energéticos correspondientes a la mayoría de procesos, moderadas condiciones de operación, la posibilidad de automatización, etc. Todos los procesos de membrana descritos anteriormente (microfiltración, ultrafiltración, nanofiltración y ósmosis inversa) están ampliamente extendidos en medios acuosos.

Sin embargo, los procesos de membrana en medios orgánicos se encuentran aún en desarrollo. El interés de la tecnología de membranas para tratar corrientes en las que existe uno o varios disolventes orgánicos es innegable. Industrialmente, los disolventes orgánicos están presentes en numerosos procesos productivos, como la síntesis de determinados compuestos [78], la extracción de moléculas de interés o la producción y desgomado de aceites vegetales [79]. Los procesos de membrana tienen aplicaciones en todos estos campos, tanto en el mismo proceso productivo como en el tratamiento de los efluentes y residuos generados. No obstante, la presencia de disolventes dificulta el desempeño de las membranas, por lo que esta área de trabajo requiere más esfuerzos para perfeccionar los procesos y favorecer su expansión industrial. A nivel científico, la mayoría de estudios se centran en la nanofiltración con disolventes orgánicos, u *organic solvent nanofiltration*, en inglés [80–84]. Otros procesos de membrana menos estudiados, como la ultrafiltración con disolventes orgánicos o la ósmosis inversa con disolventes orgánicos son objeto de una atención significativamente menor en la literatura científica, no obstante, el interés de estos procedimientos es también elevado, y su desarrollo puede tener un alto impacto en los actuales procesos productivos.

6. IMPORTANCIA DE LA METABOLÓMICA EN LA VALORIZACIÓN DE SUBPRODUCTOS ALIMENTARIOS

La Metabolómica es el estudio, a nivel cualitativo y cuantitativo, del conjunto de metabolitos de bajo peso molecular presentes en un determinado organismo, célula o tejido, en un determinado estado biológico [85]. Cuando esta tecnología se aplica al ámbito vegetal o alimentario, permite conocer de forma detallada, en un mismo análisis, la composición de un alimento o matriz vegetal derivada [86].

El estudio metabolómico de muestras biológicas reales (es decir, muestras no simuladas ni dopadas artificialmente) no es directo. En concreto, las matrices vegetales aúnan una amplia variedad de compuestos, de muy diversa naturaleza química. Además, dentro del metaboloma de una muestra, es posible encontrar compuestos pertenecientes a un amplio rango de concentraciones, desde moléculas prevalentes, muy concentradas, a simples trazas. En consecuencia, la determinación simultánea, en un mismo análisis, de todos estos metabolitos es una tarea exigente.

A pesar de la complejidad que puede derivarse de una aproximación metabolómica, se trata de una estrategia altamente poderosa, ya que la información que se obtiene sobre la matriz de estudio es exhaustiva y pormenorizada. En comparación con estrategias de análisis total, que únicamente ofrecen un dato global para un conjunto de moléculas, un estudio metabolómico permite determinar de forma individual cada uno de los compuestos de interés de una matriz.

Para poder abordar estas estrategias, se emplean generalmente potentes herramientas analíticas que permiten la separación y detección de los compuestos de una muestra. Entre las técnicas separativas empleadas mayoritariamente se encuentran la cromatografía de líquidos de alta eficacia (HPLC, por sus siglas en inglés, correspondientes a *high performance liquid chromatography*) y la cromatografía de gases, ambas acopladas a distintos detectores, especialmente espectrómetros de masas, pero también otros como fluorímetros o redes de diodos (*diode-array),* dependiendo de la aplicación concreta.

Así, un estudio metabolómico requiere de una preparación adecuada de la muestra, el análisis de esta, el preprocesamiento de los abundantes datos obtenidos, el análisis riguroso de dichos datos y, finalmente, la interpretación de los resultados y/o tendencias observadas [87]. Este flujo de trabajo puede aplicarse eficazmente para evaluar las corrientes generadas mediante tecnología de membranas. De hecho, el conocimiento profundo de la identidad y concentración de cada uno de los metabolitos presentes en las corrientes de alimento, rechazo y permeado de una membrana permite determinar

el porcentaje de rechazo individual de cada compuesto. Esto brinda una información extremadamente útil sobre el comportamiento de una membrana y el transporte a su través.

Para analizar corrientes vegetales derivadas de procesos de membrana, es especialmente útil el empleo de la cromatografía de líquidos acoplada a espectrometría de masas, que ofrece alta sensibilidad y permite detectar la mayoría de los compuestos orgánicos, incluso en muestras complejas [86,88,89]. Por tanto, se trata de una excelente técnica para evaluar los extractos procedentes del alperujo tras su tratamiento mediante ultrafiltración, nanofiltración y/u ósmosis inversa.

7. REFERENCIAS

[1] D. Kaniewski, E. Van Campo, T. Boiy, J.F. Terral, B. Khadari, G. Besnard, Primary domestication and early uses of the emblematic olive tree: palaeobotanical, historical and molecular evidence from the Middle East, Biol. Rev. 87 (2012) 885–899. https://doi.org/10.1111/J.1469-185X.2012.00229.X.

[2] C. Peri, The Extra-Virgin Olive Oil Handbook, John Wiley & Sons, Ltd, 2014.

[3] A. Trichopoulou, D. Corella, M.A. Martinez-Gonzalez, F. Soriguer, J.M. Ordovas, The Mediterranean Diet and Cardiovascular Epidemiology, Nutr. Rev. 64 (2006) S13–S19. https://doi.org/10.1111/J.1753-4887.2006.TB00258.X.

[4] G.B. Lim, Mechanistic insights into the cardiovascular benefits of a Mediterranean diet, Nat. Rev. Cardiol. 2014 118. 11 (2014) 433–433. https://doi.org/10.1038/nrcardio.2014.92.

[5] J. Lozano, A. Fernández Gutiérrez, A. Segura-Carretero, Composición del aceite de oliva, in: Aceite de Oliva Virgen Extra. Tesoro de Andalucía, Servicio de Publicaciones de la Fundación Unicaja: Málaga, 2009: pp. 197–224.

[6] I.O. Council, IOC Olive Oil Dashboard. Consumption, 2023. https://www.internationaloliveoil.org/wp-content/uploads/2021/12/IOC-Olive-Oil-Dashboard-1.html#consumption-1.

[7] D. Urieta, A. Menor, S. Caño, J. Barreal, M. del M. Velasco, R. Puentes, La olivicultura internacional: difusión histórica, análisis estratégico y visión descriptiva, 1ª Edición, Fundación Caja Rural de Jaén, 2018.

[8] International Olive Council, IOC Olive Oil Dashboard. Production, 2023. https://www.internationaloliveoil.org/wp-content/uploads/2021/12/IOC-Olive-Oil-Dashboard-1.html#production-2.

[9] M.I. Covas, V. Ruiz-Gutierrez, R. de la Torre, A. Kafatos, R.M. Lamuela-Raventos, J. Osada, R.W. Owen, F. Visioli, Minor Components of Olive Oil: Evidence to Date of Health Benefits in Humans, Nutr. Rev. 64 (2006) S20–S31.

https://doi.org/10.1301/nr.2006.oct.S20.

[10] S. Lopez, B. Bermudez, S. Montserrat-De La Paz, S. Jaramillo, L.M. Varela, A. Ortega-Gomez, R. Abia, F.J.G. Muriana, Membrane composition and dynamics: A target of bioactive virgin olive oil constituents, Biochim. Biophys. Acta - Biomembr. 1838 (2014) 1638–1656. https://doi.org/10.1016/J.BBAMEM.2014.01.007.

[11] I. Gouvinhas, N. Machado, C. Sobreira, R. Domínguez-Perles, S. Gomes, E. Rosa, A.I.R.N.A. Barros, Critical review on the significance of Olive phytochemicals in plant physiology and human health, Molecules. 22 (2017). https://doi.org/10.3390/molecules22111986.

[12] S. Theodosi, I.S. Kosma, A. V. Badeka, Quality characteristics of Koroneiki olive oil from Zakynthos island (Greece) and differentiation depending on the altitude level, Eur. Food Res. Technol. 247 (2021) 1235–1248. https://doi.org/10.1007/S00217-021-03705-1.

[13] Â.G. Batista, J.K. da Silva-Maia, M.R. Maróstica, Generation and alterations of bioactive organosulfur and phenolic compounds, Chem. Chang. Dur. Process. Storage Foods Implic. Food Qual. Hum. Heal. (2021) 537–577. https://doi.org/10.1016/B978-0-12-817380-0.00011-7.

[14] D.I. Santos, J.M.A. Saraiva, A.A. Vicente, M. Moldão-Martins, Methods for determining bioavailability and bioaccessibility of bioactive compounds and nutrients, Innov. Therm. Non-Thermal Process. Bioaccessibility Bioavailab. Nutr. Bioact. Compd. (2019) 23–54. https://doi.org/10.1016/B978-0-12-814174-8.00002-0.

[15] P. Kanakis, A. Termentzi, T. Michel, E. Gikas, M. Halabalaki, A.L. Skaltsounis, From olive drupes to olive Oil. An HPLC-orbitrap-based qualitative and quantitative exploration of olive key metabolites, Planta Med. 79 (2013) 1576–1587. https://doi.org/10.1055/s-0033-1350823.

[16] N. Siriwardhana, N.S. Kalupahana, M. Cekanova, M. LeMieux, B. Greer, N. Moustaid-Moussa, Modulation of adipose tissue inflammation by bioactive food compounds, J. Nutr. Biochem. 24 (2013) 613–623. https://doi.org/10.1016/J.JNUTBIO.2012.12.013.

[17] L. Olmo-García, R.P. Monasterio, C.M. Sánchez-Arévalo, A. Fernandez-Gutierrez, J.M. Olmo-Peinado, A. Carrasco-Pancorbo, Characterization of new olive fruit derived products obtained by means of a novel processing method involving stone removal and dehydration with zero waste generation, J. Agric. Food Chem. 67 (2019) 9295–9306. https://doi.org/10.1021/acs.jafc.9b04376.

[18] M. de L.R. Giada, M. de L.R. Giada, Food Phenolic Compounds: Main Classes, Sources and Their Antioxidant Power, Oxidative Stress Chronic Degener. Dis. - A Role Antioxidants. (2013). https://doi.org/10.5772/51687.

[19] E. Ramírez, E. Medina, M. Brenes, C. Romero, Endogenous Enzymes Involved in the Transformation of Oleuropein in Spanish Table Olive Varieties, J. Agric. Food Chem. 62 (2014) 9569–9575. https://doi.org/10.1021/jf5027982.

[20] F. Shahidi, M. Naczk, Biosynthesis, Classification, and Nomenclature of Phenolics in Food and Nutraceuticals, in: Food Phenolics, CRC Press, 2003: pp. 17–32. https://doi.org/10.1201/9780203508732.ch1.

[21] Solvitae Medical, Alyvium®, (2023). https://www.alyvium.com/es/.

[22] Solvitae Medical, Mygrium®, (2023). https://www.mygrium.com/.

[23] J.D. Roberts, J. Lillis, J.M. Pinto, A.G.B. Willmott, L. Gautam, C. Davies, Á. López-Samanes, J. Del Coso, H. Chichger, The Impact of a Natural Olive-Derived Phytocomplex (OliPhenolia®) on Exercise-Induced Oxidative Stress in Healthy Adults, Nutrients. 14 (2022) 5156. https://doi.org/10.3390/nu14235156.

[24] M. Servili, B. Sordini, S. Esposto, S. Urbani, G. Veneziani, I. Di Maio, R. Selvaggini, A. Taticchi, Biological Activities of Phenolic Compounds of Extra Virgin Olive Oil, Antioxidants. 3 (2014) 1. https://doi.org/10.3390/ANTIOX3010001.

[25] M. Bellumori, L. Cecchi, M. Innocenti, M.L. Clodoveo, F. Corbo, N. Mulinacci, The EFSA Health Claim on Olive Oil Polyphenols: Acid Hydrolysis Validation and Total Hydroxytyrosol and Tyrosol Determination in Italian Virgin Olive Oils, Mol. 2019, Vol. 24, Page 2179. 24 (2019) 2179. https://doi.org/10.3390/MOLECULES24112179.

[26] L. Wu, Y. Xu, Z. Yang, Q. Feng, Hydroxytyrosol and olive leaf extract exert cardioprotective effects by inhibiting GRP78 and CHOP expression, J. Biomed. Res. 32 (2018) 371–379. https://doi.org/10.7555/JBR.32.20170111.

[27] H. Jemai, A. El Feki, S. Sayadi, Antidiabetic and Antioxidant Effects of Hydroxytyrosol and Oleuropein from Olive Leaves in Alloxan-Diabetic Rats, J. Agric. Food Chem. 57 (2009) 8798–8804. https://doi.org/10.1021/jf901280r.

[28] J. Madureira, F.M.A. Margaça, C. Santos-Buelga, I.C.F.R. Ferreira, S.C. Verde, L. Barros, Applications of bioactive compounds extracted from olive industry wastes: A review, Compr. Rev. Food Sci. Food Saf. 21 (2022) 453–476. https://doi.org/10.1111/1541-4337.12861.

[29] S. Ou, K.C. Kwok, Ferulic acid: Pharmaceutical functions, preparation and applications in foods, J. Sci. Food Agric. 84 (2004) 1261–1269. https://doi.org/10.1002/jsfa.1873.

[30] Y. Yue, P. Shen, Y. Xu, Y. Park, p-Coumaric acid improves oxidative and osmosis stress responses in Caenorhabditis elegans, J. Sci. Food Agric. 99 (2019) 1190–1197. https://doi.org/10.1002/JSFA.9288.

[31] H. Silva, N.M.F. Lopes, Cardiovascular Effects of Caffeic Acid and Its Derivatives: A Comprehensive Review, Front. Physiol. 11 (2020) 1453.

https://doi.org/10.3389/FPHYS.2020.595516/BIBTEX.

[32] J. Park, J.S. Min, U. Chae, J.Y. Lee, K.S. Song, H.S. Lee, H.J. Lee, S.R. Lee, D.S. Lee, Anti-inflammatory effect of oleuropein on microglia through regulation of Drp1-dependent mitochondrial fission, J. Neuroimmunol. 306 (2017) 46–52. https://doi.org/10.1016/j.jneuroim.2017.02.019.

[33] O. Tebboub, R. Cotugno, F. Oke-Altuntas, M. Bouheroum, Í. Demirtas, M. D'Ambola, N. Malafronte, A. Vassallo, Antioxidant potential of herbal preparations and components from galactites elegans (All.) nyman ex soldano, Evidence-Based Complement. Altern. Med. 2018 (2018). https://doi.org/10.1155/2018/9294358.

[34] A. Pinho, D. V Lopes, R.C. Martins, M.J. Quina, Phytotoxicity assessment of olive mill solid wastes and the in fl uence of phenolic compounds, Chemosphere. 185 (2017) 258–267. https://doi.org/10.1016/j.chemosphere.2017.07.002.

[35] D. Richard, M.D.L. Delgado-Nuñez, Kinetics of the degradation by catalytic hydrogenation of tyrosol, a model molecule present in olive oil waste waters, J. Chem. Technol. Biotechnol. 78 (2003) 927–934. https://doi.org/10.1002/jctb.876.

[36] L. Olmo-García, K. Wendt, N. Kessler, A. Bajoub, A. Fernández-Gutiérrez, C. Baessmann, A. Carrasco-Pancorbo, Exploring the capability of LC-MS and GC-MS multi-class methods to discriminate virgin olive oils from different geographical indications and to identify potential origin markers, Eur. J. Lipid Sci. Technol. 121 (2019) 1–13. https://doi.org/10.1002/ejlt.201800336.

[37] Y. Allouche, A. Jiménez, M. Uceda, M.P. Aguilera, J.J. Gaforio, G. Beltrán, Triterpenic content and chemometric analysis of virgin olive oils from forty olive cultivars, J. Agric. Food Chem. 57 (2009) 3604–3610. https://doi.org/10.1021/JF803237Z/ASSET/IMAGES/LARGE/JF-2008-03237Z_0003.JPEG.

[38] L. Bermúdez, T. Del Pozo, B. Silvestre Lira, F. de Godoy, I. Boos, C. Romanò, V. Previtali, J. Almeida, C. Bréhélin, R. Asis, L. Quadrana, D. Demarco, S. Alseekh, R. Salinas Gamboa, L. Pérez-Flores, P.G. Dominguez, C. Rothan, A.R. Fernie, M. González, A. Stocker, A. Hemmerle, M.H. Clausen, F. Carrari, M. Rossi, A Tomato Tocopherol-Binding Protein Sheds Light on Intracellular α-Tocopherol Metabolism in Plants, Plant Cell Physiol. 59 (2018) 2188–2203. https://doi.org/10.1093/PCP/PCY191.

[39] J. Falk, S. Munné-Bosch, Tocochromanol functions in plants: antioxidation and beyond, J. Exp. Bot. 61 (2010) 1549–1566. https://doi.org/10.1093/JXB/ERQ030.

[40] International Olive Council, DESIGNATIONS AND DEFINITIONS OF OLIVE OILS, (2023). https://www.internationaloliveoil.org/.

[41] R. Aparicio, J. Harwood, Handbook of Olive Oil, Lavoisier S.A.S., 2013.

https://doi.org/10.1007/978-1-4757-5371-4.

[42] S. Dermeche, M. Nadour, C. Larroche, F. Moulti-Mati, P. Michaud, Olive mill wastes: Biochemical characterizations and valorization strategies, Process Biochem. 48 (2013) 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010.

[43] N. Azbar, A. Bayram, A. Filibeli, A. Muezzinoglu, F. Sengul, A. Ozer, A Review of Waste Management Options in Olive Oil Production, Http://Dx.Doi.Org/10.1080/10643380490279932. 34 (2010) 209–247. https://doi.org/10.1080/10643380490279932.

[44] P. y A. Subdirección General de Cultivos Herbáceos, Industriales y Aceite de Oliva. Dirección General de Producciones y Mercados Agrarios. Ministerio de Agricultura, Informe cierre de campaña de la situación de mercado del sector del aceite de oliva, 2022. https://www.mapa.gob.es/es/agricultura/temas/producciones-agricolas/aceite-oliva-y-aceituna-mesa/.

[45] J.A. Alburquerque, J. Gonzálvez, D. García, J. Cegarra, Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction, Bioresour. Technol. 91 (2004) 195–200. https://doi.org/10.1016/S0960-8524(03)00177-9.

[46] S. Dermeche, M. Nadour, C. Larroche, F. Moulti-Mati, P. Michaud, Olive mill wastes: Biochemical characterizations and valorization strategies, Process Biochem. 48 (2013) 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010.

[47] M.G. Rubio, A.R. Medina, A.M. Díaz, M.J.A. Cañada, Influence of harvesting method and washing on the presence of pesticide residues in olives and olive oil, J. Agric. Food Chem. 54 (2006) 8538–8544. https://doi.org/10.1021/JF0615911/ASSET/IMAGES/LARGE/JF0615911F00001.JPEG.

[48] G.E. Maraulo, C. dos Santos Ferreira, M.F. Mazzobre, β-cyclodextrin enhanced ultrasound-assisted extraction as a green method to recover olive pomace bioactive compounds, J. Food Process. Preserv. 45 (2021). https://doi.org/10.1111/jfpp.15194.

[49] M.Á. Fernández-Prior, J.C.P. Fatuarte, A.B. Oria, I. Viera-Alcaide, J. Fernández-Bolaños, G. Rodríguez-Gutiérrez, New Liquid Source of Antioxidant Phenolic Compounds in the Olive Oil Industry: Alperujo Water, Foods. 9 (2020). https://doi.org/10.3390/foods9070962.

[50] N. Caporaso, D. Formisano, A. Genovese, Use of phenolic compounds from olive mill wastewater as valuable ingredients for functional foods, Crit. Rev. Food Sci. Nutr. 58 (2018) 2829–2841. https://doi.org/10.1080/10408398.2017.1343797.

[51] M. Servili, R. Selvaggini, S. Esposto, A. Taticchi, G.F. Montedoro, G. Morozzi, Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic

and technological aspects of production that affect their occurrence in the oil, J. Chromatogr. A. 1054 (2004) 113–127. https://doi.org/10.1016/J.CHROMA.2004.08.070.

[52] J.S. Torrecilla, J.C. Cancilla, Phenolic compounds in olive oil mill wastewater, Olives Olive Oil Heal. Dis. Prev. (2020) 693–700. https://doi.org/10.1016/B978-0-12-819528-4.00051-1.

[53] W. Medfai, M.D.M. Contreras, A. Lama-Muñoz, R. Mhamdi, I. Oueslati, E. Castro, How Cultivar and Extraction Conditions Affect Antioxidants Type and Extractability for Olive Leaves Valorization, ACS Sustain. Chem. Eng. 8 (2020) 5107–5118. https://doi.org/10.1021/acssuschemeng.9b07175.

[54] M. Contreras, A. Lama-muñoz, F. Espínola, I. Romero, E. Castro, M. Moya, Valorization of olive mill leaves through ultrasound-assisted extraction, Food Chem. 314 (2020) 126218. https://doi.org/10.1016/j.foodchem.2020.126218.

[55] J.M. Moreno-Maroto, M. Uceda-Rodríguez, C.J. Cobo-Ceacero, M.C. de Hoces, M.Á. MartínLara, T. Cotes-Palomino, A.B. López García, C. Martínez -García, Recycling of 'alperujo' (olive pomace) as a key component in the sintering of lightweight aggregates, J. Clean. Prod. 239 (2019) 118041. https://doi.org/10.1016/j.jclepro.2019.118041.

[56] G. Tortosa, J.A. Alburquerque, G. Ait-Baddi, J. Cegarra, The production of commercial organic amendments and fertilisers by composting of two-phase olive mill waste ("alperujo"), J. Clean. Prod. 26 (2012) 48–55. https://doi.org/10.1016/j.jclepro.2011.12.008.

[57] J.A. Alburquerque, J. Gonzálvez, G. Tortosa, G.A. Baddi, J. Cegarra, Evaluation of "alperujo" composting based on organic matter degradation, humification and compost quality, Biodegradation. 20 (2009) 257–270. https://doi.org/10.1007/s10532-008-9218-y.

[58] D.F. Tirado, E. de la Fuente, L. Calvo, A selective extraction of hydroxytyrosol rich olive oil from alperujo, J. Food Eng. 263 (2019) 409–416. https://doi.org/10.1016/j.jfoodeng.2019.07.030.

[59] J. Vilar, S. Caño, I. Raya, L. Moreno, M. del M. Velasco, Alperujo processing sector. Possibilities for a potential financial and operational collapse., Sevilla, 2020. https://www.aneorujo.es/documentacion/.

[60] A. Lama-Muñoz, F. Rubio-Senent, A. Bermúdez-Oria, J. Fernández-Bolaños, Á.F. Prior, G. Rodríguez-Gutiérrez, The use of industrial thermal techniques to improve the bioactive compounds extraction and the olive oil solid waste utilization, Innov. Food Sci. Emerg. Technol. 55 (2019) 11–17. https://doi.org/10.1016/j.ifset.2019.05.009.

[61] Ministerio de Agricultura Pesca y Alimentación. Gobierno de España., Cadena de valor del aceite de oliva virgen extra. Campañas 2018-2019 y 2019-2020, 2021.

https://www.mapa.gob.es/es/alimentacion/temas/observatorio-cadena/estudiodecadenadevalordelaovecampanas2018-20192019-2020_tcm30-563323.pdf.

[62] C.M. Sánchez-Arévalo, L. Olmo-García, J.F. Fernández-Sánchez, A. Carrasco-Pancorbo, Polycyclic aromatic hydrocarbons in edible oils: An overview on sample preparation, determination strategies, and relative abundance of prevalent compounds, Compr. Rev. Food Sci. Food Saf. 19 (2020) 3528–3573. https://doi.org/10.1111/1541-4337.12637.

[63] R. Borja, F. Raposo, B. Rincón, Treatment technologies of liquid and solid wastes from two-phase olive oil mills, Grasas y Aceites. 57 (2006) 32–46. https://doi.org/10.3989/gya.2006.v57.i1.20.

[64] R. Field, Fundamentals of Fouling, in: K.-V. Peineman, S. Pereira Nunes (Eds.), Membr. Water Treat., Weinheim, 2010: pp. 1–23.

[65] H. Koseoglu, E. Guler, B.I. Harman, E. Gonulsuz, Water flux and reverse salt flux, Elsevier B.V., 2018. https://doi.org/10.1016/B978-0-444-63961-5.00002-X.

[66] H. Sari Erkan, N. Bakaraki Turan, G. Önkal Engin, Membrane Bioreactors for Wastewater Treatment, Compr. Anal. Chem. 81 (2018) 151–200. https://doi.org/10.1016/bs.coac.2018.02.002.

[67] H. Li, V. Chen, Membrane Fouling and Cleaning in Food and Bioprocessing, First Edit, Elsevier Ltd, 2010. https://doi.org/10.1016/B978-1-85617-632-3.00010-0.

[68] A. Cassano, A. Basile, Integrating different membrane operations and combining membranes with conventional separation techniques in industrial processes, Handb. Membr. React. 2 (2013) 296–343. https://doi.org/10.1533/9780857097347.1.296.

[69] A. Bardhan, A. Akhtar, S. Subbiah, Microfiltration and ultrafiltration membrane technologies, Adv. Polym. Membr. Water Remediat. (2022) 3–42. https://doi.org/10.1016/B978-0-323-88514-0.00001-2.

[70] J. Gadkari, A. Shrivastav, S. Bhattacharya, Membrane bioreactors for wastewater treatment, Membr. Hybrid Process. Wastewater Treat. (2021) 423–443. https://doi.org/10.1016/B978-0-12-823804-2.00017-3.

[71] E. Nagy, Nanofiltration, Basic Equations Mass Transp. through a Membr. Layer. (2012) 249–266. https://doi.org/10.1016/B978-0-12-416025-5.00010-7.

[72] J.M. Ochando-Pulido, S. Rodriguez-Vives, G. Hodaifa, A. Martinez-Ferez, Impacts of operating conditions on reverse osmosis performance of pretreated olive mill wastewater, Water Res. 46 (2012) 4621–4632. https://doi.org/10.1016/j.watres.2012.06.026.

[73] H.T. El-Dessouky, H.M. Ettouney, Reverse Osmosis, Fundam. Salt Water Desalin. (2002) 409–437. https://doi.org/10.1016/B978-044450810-2/50009-9.

[74] A.A. Khan, A. Chandra, Hybrid membrane technique: A technological advancement of textile waste effluent treatment, in: Membr. Hybrid Process. Wastewater Treat., Elsevier, 2021: pp. 313–340. https://doi.org/10.1016/B978-0-12-823804-2.00014-8.

[75] A.G. Fane, C.Y. Tang, R. Wang, Membrane Technology for Water: Microfiltration, Ultrafiltration, Nanofiltration, and Reverse Osmosis, Treatise Water Sci. 4 (2011) 301–335. https://doi.org/10.1016/B978-0-444-53199-5.00091-9.

[76] D.L. Oatley-Radcliffe, M. Walters, T.J. Ainscough, P.M. Williams, A.W. Mohammad, N. Hilal, Nanofiltration membranes and processes: A review of research trends over the past decade, J. Water Process Eng. 19 (2017) 164–171. https://doi.org/10.1016/J.JWPE.2017.07.026.

[77] S.K. Sikdar, A. Criscuoli, Sustainability and How Membrane Technologies in Water Treatment Can Be a Contributor, (2017) 1–21. https://doi.org/10.1007/978-981-10-5623-9_1.

[78] J. Yeo, L. Peeva, S. Chung, P. Gaffney, D. Kim, C. Luciani, S. Tsukanov, K. Seibert, M. Kopach, F. Albericio, A. Livingston, Liquid Phase Peptide Synthesis via One-Pot Nanostar Sieving (PEPSTAR), Angew. Chemie Int. Ed. 60 (2021) 7786–7795. https://doi.org/10.1002/ANIE.202014445.

[79] M.H. Abdellah, C.A. Scholes, L. Liu, S.E. Kentish, Efficient degumming of crude canola oil using ultrafiltration membranes and bio-derived solvents, Innov. Food Sci. Emerg. Technol. 59 (2020) 102274. https://doi.org/10.1016/j.ifset.2019.102274.

[80] G. Ignacz, C. Yang, G. Szekely, Diversity matters: Widening the chemical space in organic solvent nanofiltration, J. Memb. Sci. 641 (2022) 119929. https://doi.org/10.1016/j.memsci.2021.119929.

[81] R.B. Merlet, M.A. Pizzoccaro-Zilamy, A. Nijmeijer, L. Winnubst, Hybrid ceramic membranes for organic solvent nanofiltration: State-of-the-art and challenges, J. Memb. Sci. 599 (2020). https://doi.org/10.1016/j.memsci.2020.117839.

[82] A. Oxley, A.G. Livingston, Anti-fouling membranes for organic solvent nanofiltration (OSN) and organic solvent ultrafiltration (OSU): graft modified polybenzimidazole (PBI), J. Memb. Sci. 662 (2022) 120977. https://doi.org/10.1016/j.memsci.2022.120977.

[83] J. Campbell, J.D.S. Burgal, G. Szekely, R.P. Davies, D.C. Braddock, A. Livingston, Hybrid polymer/MOF membranes for Organic Solvent Nanofiltration (OSN): Chemical modification and the quest for perfection, J. Memb. Sci. 503 (2016) 166–176. https://doi.org/10.1016/J.MEMSCI.2016.01.024.

[84] P. Vandezande, L.E.M. Gevers, I.F.J. Vankelecom, Solvent resistant nanofiltration: Separating on a molecular level, Chem. Soc. Rev. 37 (2008) 365–405. https://doi.org/10.1039/b610848m.

[85] O. Fiehn, Metabolomics - The link between genotypes and phenotypes, Plant Mol. Biol. 48 (2002) 155–171. https://doi.org/10.1023/A:1013713905833.

[86] L. Olmo-García, A. Carrasco-Pancorbo, Chromatography-MS based metabolomics applied to the study of virgin olive oil bioactive compounds: Characterization studies, agro-technological investigations and assessment of healthy properties, TrAC Trends Anal. Chem. 135 (2021) 116153. https://doi.org/10.1016/J.TRAC.2020.116153.

[87] S. Sethi, M.A.F. Hayashi, B.S. Barbosa, J.G.M. Pontes, L. Tasic, E. Brietzke, Metabolomics: From Fundamentals to Clinical Applications, 965 (2017). https://doi.org/10.1007/978-3-319-47656-8.

[88] L. Cui, H. Lu, Y.H. Lee, Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases, Mass Spectrom. Rev. 37 (2018) 772–792. https://doi.org/10.1002/MAS.21562.

[89] D.S. Wishart, Metabolomics: applications to food science and nutrition research, Trends Food Sci. Technol. 19 (2008) 482–493. https://doi.org/10.1016/J.TIFS.2008.03.003.

METODOLOGÍA EXPERIMENTAL, RESULTADOS Y DISCUSIÓN

SECCIÓN 1

Extracción sólido-líquido

Esta sección incluye el estudio de la etapa de extracción sólido-líquido, recogida en el Capítulo 1, que presenta la optimización de esta extracción, así como el desarrollo de un método analítico para determinar los metabolitos extraídos.

CAPÍTULO 1

Exploring the extraction of the bioactive content of two-phase olive mill waste and further purification by ultrafiltration

Carmen M. Sánchez-Arévalo[1], Alicia Iborra-Clar[1,2], María Cinta Vincent-Vela[1,2], Silvia Álvarez-Blanco[1,2]

[1]Research Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

[2]Department of Chemical and Nuclear Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

✉carsana5@upv.es

LWT - Food Science and Technology, 165 (2022) 113742

https://doi.org/10.1016/j.lwt.2022.113742

Abstract: The two-phase olive mill waste is enormously produced in the Mediterranean area. This major waste is significantly rich in bioactive compounds that are highly valued by industry, such as phenolic and triterpenic compounds. Here, a thorough study of the most suitable solvent, extraction time, and temperature for the large-volume, solid-liquid extraction of bioactive compounds has been made, in order to achieve maximum concentrations of the target compounds. Ultrasound effect has been considered. A deep characterization of the extracts by high-performance liquid chromatography coupled with electrospray-quadrupole-time of flight-mass spectrometry (LC-ESI-qToF-MS) has contributed to evaluate the effect of the operational parameters on the extraction performance. Forty-four compounds have been found and classified in their corresponding chemical families. At the optimum experimental conditions (EtOH 50% (v/v), 40°C, ultrasound-assisted), more than 6.8 mg/g of bioactive content was recovered, and it was later purified by means of ultrafiltration. The membrane UP005 retained a significant percentage of the organic matter, whereas most of the bioactive compounds were recovered in the permeate. This contributed not only to revalorize this waste, but also to reduce its organic load and phytotoxicity, thus protecting the ecosystem of the final disposal zone of the residue.

Keywords: two-phase olive mill waste, phenolic compounds, ultrasound-assisted extraction, ultrafiltration, LC-MS.

1. INTRODUCTION

The vast majority of the olive mills use the two-phase process to produce olive oil, which requires a lower water consumption than the three-phase process [1]. Together with the olive oil, the two-phase process results in a by-product that consists of a combination of the widely known olive mill wastewater (OMWW) and olive pomace. This residue is entitled by different names in literature, but an accepted designation is two-phase olive mill waste (TPOMW) [2]. The TPOMW, also known as "*alperujo*" by its name in Spanish, is a wet, semisolid paste, with remnants of olive pulp and stone and a moisture content of 55-75%. It is highly enriched in organic matter, including phenolic compounds. Additionally, other molecules from the minor fraction of olives can be found [3,4].

The environmental impact of TPOMW is undeniable, especially in the Mediterranean area, where the production of olive oil (and the subsequent residues) is concentrated during a few months of the annual campaign. Every year, between November and March, 4-10 million tons of TPOMW are generated [5,6], and the risk of discharging it without any previous treatment increases exceptionally. Some biological treatments have been

suggested as a strategy to dispose of the TPOMW, such as composting and biodegradation [3,7–11]. However, the antibiotic and phytotoxic character of the phenolic compounds may hinder the growth of the microorganisms implied in the process. Thus, the extraction of the polyphenols before any management is recommended.

Still, the biophenols and other compounds present in the olive-derived products, such as TPOMW, are associated with effective antioxidant properties. Their antiaging effect [12] and their associated prevention against cardiovascular diseases and neoplasia processes have also prompted a high interest in applying these bioactive compounds in the pharmaceutical and cosmetic fields. Thus, the recovery of phenolic compounds from the residues originated in the olive mills represents a double benefit. On one side, high-value products are obtained at an affordable cost and, additionally, the organic load and phytotoxicity of the by-product are reduced.

As the TPOMW is a solid waste, it requires additional extraction steps to recover these compounds from the solid matrix, as opposed to the more widely studied OMWW liquid effluent. Ultrasound-assisted extraction (UAE) has proven to be a proper strategy to extract phenolic compounds from a variety of alimentary matrices, including olive-derived products [4,13–15]. As a consequence of the molecular turbulence and the cavitation phenomenon that takes place in the ultrasound bath, better extractions of interesting compounds can be reached in lower times, and usually lower volumes of solvents are required [16]. Several parameters have to be optimized in order to enlarge the efficiency of the process, such as the selected temperature, time of extraction, and solvent of choice. In this contribution, it has been developed and optimized a methodology to extract from TPOMW a wide range of bioactive compounds belonging to the minor fraction of olives (including phenolic compounds, triterpenic acids, and fatty acids). The large-scale implementation has been considered through two premises: high volumes of the sample have been managed during the study and, also, the single-step strategy has been preferred. Those considerations are not widely found in literature, but are very desirable to avoid future irreproducibility during the potential industrial application.

In order to evaluate the extraction procedure, a multi-family method based on liquid chromatography coupled to mass spectrometry (LC-MS) has been employed. Afterwards, the extract obtained with the selected parameters has been treated by an ultrafiltration process, aiming to remove the concomitant organic matter that accompanies the

phenolic compounds in the extract. This technique has proven to be highly relevant when recovering bioactive molecules from agrofood residues. Furthermore, it offers important advantages such as high selectivity, feasible membrane reutilization, large-scale applications, etc [17].

Considering the literature gap regarding the fruitful and scalable utilization of this by-product, this work provides an efficient strategy to reuse such a significant residue. The scientific community has, of course, gained awareness in this regard and there are several investigations that have demonstrated the bioactive richness of the TPOMW [18–20]. However, the green approach has not always been contemplated.

Moreover, an absolute quantification of total phenolic content has been the prevailing trend [21–23]. This approach is highly valid, but it could be less descriptive than an individual determination of each compound. The latter allows a better understanding of the matrix content and permits the identification of the most interesting and potential molecules. In some cases, the recovery of some specific molecules is aimed, attending to commercial or industrial requirements, and, in that scenario, the importance of knowing the type and identifying each biophenol is undeniable. In this study, we have combined a bio-compatible treatment of the promising sub-product TPOMW with a membrane-technology procedure and a robust LC-MS methodology to effectively understand the efficiency of the process. The preparation, comprehension, and primary purification of the extracts obtained here is a baseline that allows the exploitation of this contaminant olive mill residue. Moreover, the orientation to a large-scale production that has been considered is quite desirable to fulfill industrial necessities.

2. MATERIAS AND METHODS

2.1. Materials

Samples of TPOMW were kindly provided by San Isidro Cooperative (Segorbe, Valencia, Spain). They were obtained during the campaign of 2020/2021. Before being processed in the laboratory, samples were stored at 5°C and 50% humidity to avoid the proliferation of microorganisms. To prepare mobile phases for LC-MS, acetonitrile (Honeywell, USA), acetic acid (Honeywell, USA) and ultrapure water were employed. Water was obtained from a Direct-Q®, 3UV system (Merck Millipore, USA). Pure standards of caffeic acid, luteolin, and p-coumaric acid were purchased from Sigma-Aldrich (USA). Hydroxytyrosol and oleuropein were obtained from BioNova Científica (Spain) and PanReac Applichem (Spain), respectively. Standard solutions were prepared

in the appropriate solvent, diluted to the desired concentrations and stored at -20°C prior to their utilization. Depending on the sample to be evaluated, standards were diluted in pure ethanol (VWR, USA), pure water, or EtOH/water 50:50, in a volume:volume basis (v/v).

2.2. Extraction of phenolic compounds

Two different strategies of solid-liquid extraction were evaluated for the recovery of interesting compounds from TPOMW: conventional extraction and UAE. Moreover, the type of solvent (water or EtOH and their mixtures), the temperature (20, 30 and 40°C) and the duration of the extraction (5, 15, 30, 60, 90 and 120 minutes) were studied.

For the conventional extraction, a constant stirring at 200 rpm was performed by an overhead stirrer (Heidolph, Instruments, Germany) and a pitched-blade impeller. In the case of the UAE, a temperature-controlled Elmasonic P 70 H ultrasound bath (Elma, Germany) was employed. The frequency and power of the applied ultrasounds were 37 kHz and 220 W, respectively. Between the two possible frequency values supported by the equipment (37 and 80 kHz), 37 kHz was chosen according to the manufacturer's recommendations for mixtures and dispersions. Ultrasonic power was set to the maximum to obtain a maximum amplitude, which influences the cavitation process. Indeed, some authors have achieved satisfying extractions with similar ultrasound powers and agro-alimentary matrices [24–26]. As the large-scale aspect was one of the crucial aspects of this work, 900 g of TPOMW were weighted to be extracted with 9 liters of solvent. A sample/solvent ratio of 1:10 (on a mass/volume basis) was considered. Once the extraction process was initiated, 40 mL aliquots were collected at the different time points set before. Extracts were then centrifuged (ThermoFisher, USA) at 17200 RCF for 6 min. The resulting supernatants were filtered using SFMC-245-100 0.5 µm filters (ThermoFisher, USA) before their characterization.

2.3. Statistical analysis

The software Statgraphics Centurion 18 and Microsoft Excel 365 were employed to assess the analysis of the obtained results. The standard deviations reported in the Results section correspond to the deviation among experimental replicates. One-way analysis of variance (ANOVA) was applied to the results to evaluate the statistical differences, which were considered when the P-value from the Tukey's test was lower than 0.05. The data derived from the extraction experiments were subjected to a response surface analysis, in order to maximize the concentration of polyphenols in the extract, by means of the variation of the independent variables (temperature, ethanol

concentration and time). To evaluate the goodness of the model fit, the values of R^2 and adjusted R^2 were considered.

2.4. Ultrafiltration procedure

The optimum extract in terms of phenolic content was subjected to an ultrafiltration process in a solvent-resistant, dead-end XFUF 076 01 stirred cell (Merck Millipore, USA). The membrane was selected according to previous results of our research group [27], pursuing the lowest rejection to the phenolic compounds and the highest rejection to the rest of the organic matter. Thus, the membrane UP005 (Microdyn Nadir, Germany) was employed. It is composed of polyethersulfone and exhibits a molecular weight cut-off of 5 kDa. Before its utilization, the membrane was immersed in the pure solvent (EtOH/water 50:50 (v/v)) for 2 hours to condition the polymer and prepare it for the contact with the ethanolic sample. Moreover, it was subjected to a compaction step at a transmembrane pressure of 5 bar and a stirring speed of 400 rpm.

The ultrafiltration experiments were carried out at 2 bar and 400 rpm and were conducted until a volume reduction factor (VRF) of at least 2.5 was achieved. Samples of the extract, final retentate, global permeate (recovered during the whole process) and instantaneous permeate collected at each time point were characterized to calculate the rejection values. They were calculated by means of equation (1), which results from the material balance applied to the solute during a concentration experiment (assuming that rejection is constant and not dependent on the concentration in the retentate) [28]:

$$C_r = C_0 \cdot VRF^R \quad (1)$$

where C_r is the concentration in the retentate, C_0 is the concentration in the feed solution (TPOMW extract), VRF is the volume reduction factor, and R is the rejection coefficient. Additionally, the reduction observed for the color and conductivity values was calculated according to:

$$Elimination = 1 - \frac{C_p}{C_0} \cdot 100 \quad (2)$$

where C_p is the concentration in the ultrafiltration permeate.

2.5. Characterization of TPOMW, phenolic extracts and UF streams

2.5.1. Measurement of phenolic and triterpenic content in the extracts

To obtain preliminary results in a short time, the Folin-Ciocalteu methodology was conducted to assess the concentration of total phenolic content [29], which was

expressed as milligrams of tyrosol equivalents per gram of TPOMW (mgTYeq/g of wet material).

Once the general results of the different extractions were evaluated, a single time of extraction was chosen. With the variable of time fixed, the rest of the extracts corresponding to all combinations of solvent types, temperature and type of extraction (UAE or simple agitation) were subjected to LC-MS. Also, the ultrafiltration streams were analyzed using this methodology. A 1260 Infinity II LC system coupled to a 6546 quadrupole-time-of-flight (qToF) mass analyzer (Agilent Technologies, USA) was employed. Electrospray was used as an interface. To develop the multi-class LC-MS methodology that was required to understand the whole content of the extracts, previous works about excellent characterizations of olive matrices were revised and taken as a reference [30,31].

After an injection of 5 µL, analytes were separated throughout an InfinityLab Poroshell 120 EC-C18 column (3.0 x 100 mm, 2.7 µm particle size) (Agilent Technologies, USA), operating at a temperature of 40 °C. Elution of compounds was performed by using water as phase A and acetonitrile as phase B. Both mobile phases were acidified with 0.5% of acetic acid (v/v) and the gradient was as follows: 5% (0-0.5 min), 11% (achieved at 2.5 min), 20% (at 7 min), 35% (at 18 min), 95% (at 22 min). After 24 min of total analysis time, a post-time of 3 minutes was dedicated to the column equilibration. Flow rate was 0.5 microliters/minute. Mass spectrometer worked on negative polarity and full scan mode (30 – 1000 mass/charge ratio (*m/z*)). The main parameters to be optimized for the ionization source were drying gas temperature and flow (200°C and 8 L/min, respectively), nebulizer pressure (30 psi) and capillary voltage (3500 V). To perform ion mass corrections, a calibrant solution was employed. It provided the *m/z* values of 112.9856, 966.0007, and 1033.9881 as references.

The identification of the peaks, obtained by LC-ESI-qToF-MS, was achieved by the study of mass spectrometry data of the samples and the corresponding pure standards. Additionally, previously reported information [14,31–33] and a self-created database of olive-derived compounds were considered. The software MassHunter (Agilent, USA), in its Qualitative and Quantitative versions, was used to explore the chromatograms. For quantitation purposes, peaks were integrated and the obtained areas were interpolated in the corresponding external calibration curve of caffeic acid (y = 18150033x – 587113 in EtOH/water 50:50 (v/v); (y = 6073596x – 485768 in water; y = 19739706 – 816146 in pure EtOH), hydroxytyrosol (y = 18579981x – 1272129 in EtOH/water 50:50 (v/v);

y = 10404695x – 3868981 in water; y = 27772509x – 886432 in pure EtOH), luteolin (y = 20409358x + 7314701 in EtOH/water 50:50 (v/v); y = 19299x - 83897 in water; y = 209173844x – 1429030 in pure EtOH), *p*-coumaric acid (y = 1576099x + 10829796 in EtOH/water 50:50 (v/v); 1872460x – 2645579 in water; y = 24961035x + 479131 in pure EtOH) or oleuropein (y = 6209163.2x – 538759 in EtOH/water 50:50 (v/v); y = 3755542.9x – 2315616.2 in water; y = 10674099.3x – 228475.4 in pure EtOH). In all cases, the concentration of the standard analyte was the independent variable, whereas peak intensity was the dependent variable. All regression coefficients (r^2) were above 0.9934. When several isomers belonging to the same compound were found, the sum of their areas was considered, and that data was employed to obtain one value of concentration. That procedure was proven to be valid before [34]. As will be described in the Results section, the number of found compounds surpassed by far the number of standards. However, a semi-quantitative analysis was considered sufficient to compare the results obtained with the different treatments and is also very usual when such number of molecules are determined [35,36].

To establish the limit of detection (LOD) and the limit of quantification (LOQ) of the method, it was calculated the analyte concentration that gave a signal to noise ratio of 3 and 10, respectively. To evaluate the precision, intra-day repeatability was assessed through the relative standard deviation (RSD) of three different injections (within the same LC-MS sequence) of an ultrasound-assisted extract obtained with EtOH/water 50:50 (v/v), at 40°C.

2.5.2. Other techniques applied for the characterization of the extracts

To fully characterize the extracts, pH (pHmeter GLP31+, Crison, Spain), electric conductivity (Conductimeter GLP31+, Crison, Spain), and total solid content were measured. The total sugars content was determined using the anthrone method [37,38]. Color was determined according to ISO 7887:2011, method B [39]. Absorbance (A) was measured at three different wavelengths (436 nm, 525 nm, and 620 nm) using a UV-VIS DR 6000 spectrophotometer (Hach Lange, Germany). The color coefficient was given by the following formula:

$$Colour = \frac{(A_{436}^2 + A_{525}^2 + A_{620}^2)}{(A_{436} + A_{525} + A_{620})} \quad (3)$$

2.6. Evaluation of the one-step extraction

Once the best conditions regarding time, temperature, and ultrasounds application were determined, the recovery capacity of the technique was evaluated. To that end, the

sediment that remained after the centrifugation stage was re-extracted (by applying the same process as before). In order to maintain the same ratio of sample/solvent, the sediment was weighted to adjust the needed volume of extractant. This cycle was repeated once again with the sediment of the second extraction, to ensure that the percentage of residual olive minor fraction in the sample (the percentage that was not extracted) was sufficiently low to qualify the extraction as truthful. The supernatants obtained by each of the three extraction cycles were analyzed through the procedures detailed in sections 2.5.1 and 2.5.2.

3. RESULTS AND DISCUSSION

3.1. Total phenolic content

Figure 1.1 shows the results of the applied UAE and agitation-mediated extraction. According to the figure, the highest efficiency of extraction was obtained with EtOH/water 50:50 (v/v) in all cases, regardless of the extraction time, temperature, or presence of ultrasounds. Water was the following solvent in terms of extractant power, whereas pure ethanol presented the worst results. The selection of the solvent to extract phenolic compounds is not trivial. Babbar et al. compared the effect of using methanol, ethyl acetate, chloroform, and hexane as solvents in the extraction of phenolic compounds from a range of vegetable wastes. In all cases, methanol proved to be the most effective solvent [40].

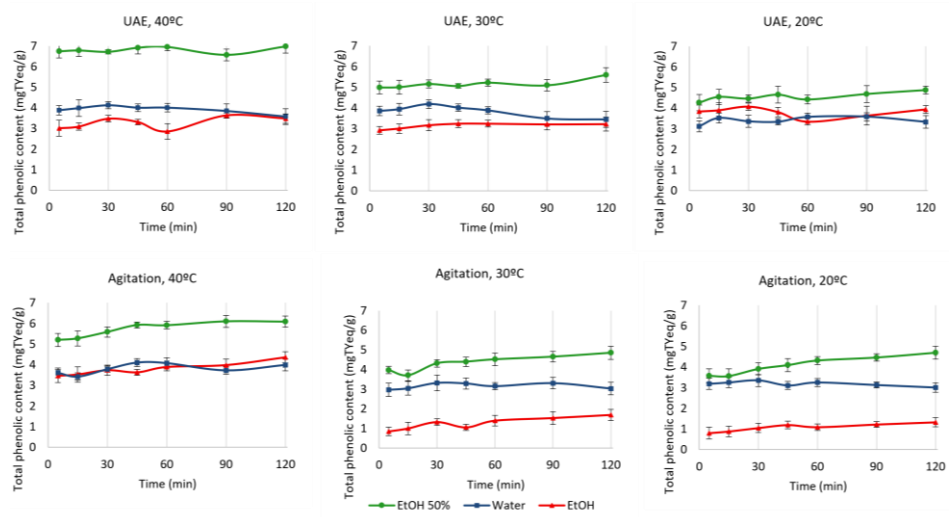


Figure 1.1. Total phenolic content of the whole set of extracts obtained from ultrasound-assisted extraction (UAE) and agitation-mediated extraction, at the different temperatures and time points evaluated. Error bars indicate the standard deviation of the experimental replicates.

Other solvents, such as N,N-dimethylformamide, mixtures of tetrahydrofuran/water or even tensioactive species have been employed too for the isolation of phenolic fractions [41]. However, other relevant considerations should be made when it comes to selecting the solvent, not only its recovery potential. Nowadays, environmentally friendly solvents (such as ethanol (EtOH) and even water) should be preferentially employed. Zagklis and Paraskeva considered this during their study on the extraction of phenolic compounds from grape marc, opting to use EtOH/water mixtures as a solvent due to environmental considerations and suitability with respect to the food industry [42].

In this study, the presence of ethanol in the solvent mixture (ethanol/water) contributed to obtain an adequate polarity to recover phenolic compounds. The optimum results obtained with the mixture of 50:50 (v/v) were coherent and in accordance with previous findings [43]. The results obtained with pure ethanol can be attributed to a fast dehydration of vegetable cells, which may result in the aggregation of microcellular material (such as proteins, cell wall components, etc.) and hinders the diffusion of compounds to the solvent [44]. Considering the variable of time, most of the phenolic content was extracted rapidly, especially in the UAE cases. Nevertheless, a small increment of the extraction yield can be observed in the first minutes and during the first hour for most of the experimental conditions tested. This can also be revised in Figure 1.4 (panel A), which contains the surface response analysis of the total phenolic content with the variation of time and ethanol concentration at a constant temperature of 40°C. According to the ANOVA data for the fitting of the response surface model, the plots presented in Figure 1.4A and 1.4B correspond to models of statistical significance. This was supported by high F values (higher than 6.38 for the graph in Figure 1.4A and higher than 59.89 for the model in Figure 1.4B) and P values lower than 0.0429 (Figure 1.4A) and 0.0002 (Figure 1.4B) for the considered effects. Additionally, the model adequacy was endorsed by a R2 of 0.9446 and 0.9758 and an adjusted R2 of 0.9138 and 0.9677 for figures 1.4A and 1.4B, respectively. Those results motivated the selection of an extraction time of 60 minutes for the following studies, in order to ensure that maximum phenolic content was extracted. Regarding the temperature, an increment higher than 3 mg/g in the recovery of phenolic compounds can be observed as this variable increases from 20°C to 40°C. The data displayed in Figure 1.1 suggested that higher temperatures and the application of ultrasounds were best for the extraction performance. To confirm these results and to better assess the effect of the sonication, all extracts (at the time of 60 minutes) were also analyzed by LC-MS.

3.2. Quantification of the compounds identified by LC-MS

After the characterization by LC-MS of all the obtained extracts (at the three temperatures, three types of solvent and both conditions of sonication or agitation), 41 compounds were identified and classified in their corresponding chemical family. Table .1 contains a thorough description of the composition of the analyzed samples. Compound identification, retention time (Rt), *m/z,* and assigned chemical class are described. Three additional species were detected, but their identification was not possible. In those cases, a molecular formula was proposed after the study of spectral data with MassHunter. Those formulas and the software score assigned to each labeling are available in Table 1.1.

Table 1.1. Retention time (R_t), mass/charge ratio (*m/z*) and assigned identity and chemical class of the compounds detected by LC-ESI-qTOF-MS. Scores of the molecular formulas achieved with MassHunter when identification was not possible are indicated in brackets.

Rt	Compound Identity	m/z	Chemical family
1.001	Quinic acid	191.0555	Organic acids
1.051	Malic acid	133.0150	Organic acids
2.538	Vanillic acid	167.0352	Phenolic acids and aldehydes
3.173	Hydroxytyrosol	153.0551	Simple phenols
3.641	Acyclodihydroelenolic acid hexoside	407.1560	Secoiriodoids
3.942	Hydroxy-decarboxymethyl elenolic acid	199.0607	Secoiriodoids
4.443	$C_7H_{12}O_5$ (score: 86.96%)	175.0614	Unknowns
5.061	Vanillin	151.0396	Phenolic acids and aldehydes
5.279	Caffeic acid	179.0347	Phenolic acids and aldehhydes
5.429	Gallocatechin	305.0702	Flavonoids
5.913	Hydroxyelenolic acid	257.0669	Secoiriodoids
5.930	Tyrosol	137.0608	Simple phenols
6.114	Decarboxymethyl elenolic acid	183.0658	Secoiriodoids
6.164	Elenolic acid glucoside	403.1246	Secoiriodoids
6.281	$C_{16}H_{26}O_{10}$ (score: 96.93%)	377.1453	Unknowns
6.899	*p*-Coumaric acid	163.0397	Phenolic acids and aldehydes
6.999	Aldehydic form of Decarboxymethyl Elenolic acid	215.0925	Secoiriodoids
7.250	Phenylethyl primeveroside	415.1612	Secoiriodoids
8.042	$C_{11}H_{16}O_6$ (score: 99.12%)	243.0876	Unknowns
8.136	Dehydro-oleuropein aglycone	375.1087	Secoiriodoids
8.186	Hydroxyoleuropein	555.1717	Secoiriodoids
8.603	Luteolin rutinoside	593.1516	Flavonoids

Rt	Compound Identity	m/z	Chemical family
9.489	Luteolin 7-O-glucoside	447.0933	Flavonoids
9.697	Oleuropein	539.1769	Secoiriodoids
9.772	Elenolic acid	241.0720	Secoiriodoids
10.391	Ferulic acid	193.0503	Phenolic acids and aldehydes
10.508	Hydroxytyrosol acyclodihydroelenolate	381.1560	Secoiriodoids
11.761	Decarboxymethyl oleuropein aglycone	319.1187	Secoiriodoids
11.811	Pinoresinol	357.1337	Lignans
11.827	Ligstroside	523.1820	Secoiriodoids
13.048	Luteolin	285.0405	Flavonoids
14.083	Oleuropein aglycone	377.1242	Secoiriodoids
16.306	Diosmetin	299.0558	Flavonoids
19.931	Dihydroxy-hexadecanoic acid	287.2230	Fatty acids derivatives
20.511	Ligstroside aglycone	361.1293	Secoiriodoids
20.783	Trihydroxy-octadecenoic acid	329.2335	Fatty acids derivatives
21.485	Gingerol	293.1759	Flavonoid
21.619	Betulinic acid	455.3538	Triterpenic acids
22.654	Hydroxy-octadecatrienoic acid	293.2122	Fatty acids derivatives
22.655	Dihydroxy-octadecanoic acid	315.2516	Fatty acids derivatives
22.989	Hydroxy-octadecadienoic acid	295.2277	Fatty acids derivatives
23.189	Maslinic acid	471.3488	Triterpenic acids
23.406	Hydroxy-octadecenoic acid	297.2435	Fatty acids derivatives
23.590	Hydroxy-octadecanoic acid	299.2591	Fatty acids derivatives

In the case of the *m/z* 175.0613, such formula may correspond to the isopropyl malic acid. Other unknown compounds corresponded to *m/z* 377.1451 and *m/z* 243.0876. They have been already found in olive flours [14] and other samples related to the olive pulp [35]. Proposed empirical formulas were coincident with those in literature, even when a different software was employed. The applied LC-MS methodology permitted the evaluation of more than 40 compounds (from different chemical families) within a single run, which facilitated the study of the phenolic profile. The latter was considered very promising for future applications, as many of the most valuable polyphenols in the industry were present in the extracts. Although the non-targeted approach has not been commonly applied during the analysis of TPOMW, the composition of the samples in this study was consistent with relevant previous literature [45,46].

Figure 1.2 contains two chromatograms in which peaks distribution can be explored. As the figure shows, there were some peaks with a notably large area, in contrast with other shorter peaks that are less visible in the chromatogram because of the figure scale. The first chromatogram corresponds to an ultrasound-assisted extraction, whereas the second was obtained by simple agitation. As can be seen, some differences stand out. Both chromatograms are from samples obtained with the same solvent (EtOH/water 50:50 (v/v)), at the same temperature (40°C). Comparison of peak height within the same chromatogram could be misleading, because different compounds might display different response factors in the mass spectrometer. Therefore, the visual inspection of the figure should be done by comparing the behavior of the same peak in each sample. Several compounds that were better extracted by means of ultrasounds application have been marked with colored arrows.

Some of the most relevant peaks in the upper chromatogram (UAE) were much shorter or even absent in the bottom one (agitation). This fact contributed to enlarge the concentration of phenolic compounds in the sonicated sample. The 44 detected compounds were found in both cases, which set the agitation-mediated strategy as a proper methodology to easily recover the olive minor-fraction. However, it is undeniable that the sonication contributed to increase the phenolic content of the final extract and its application was very desirable to get the most out of the TPOMW, in terms of phenolic compounds.

Analytical parameters of the LC-ESI-qToF methodology that allowed these findings can be revised in Supplementary Table 1.1. The table shows the obtained values for LOD, LOQ, and method repeatability. LODs were found to be below 0.098 ppm in all cases and LOQs were in the range of 0.002-1.217 ppm. These results were satisfactory, as they allowed the quantification of all the compounds detected. All calculated concentrations were analytically valid, considering the parameters of Supplementary Table 1.1. The results for the RSD of three injections of the same sample were very low, which indicated a good repeatability of the analytical strategy developed.

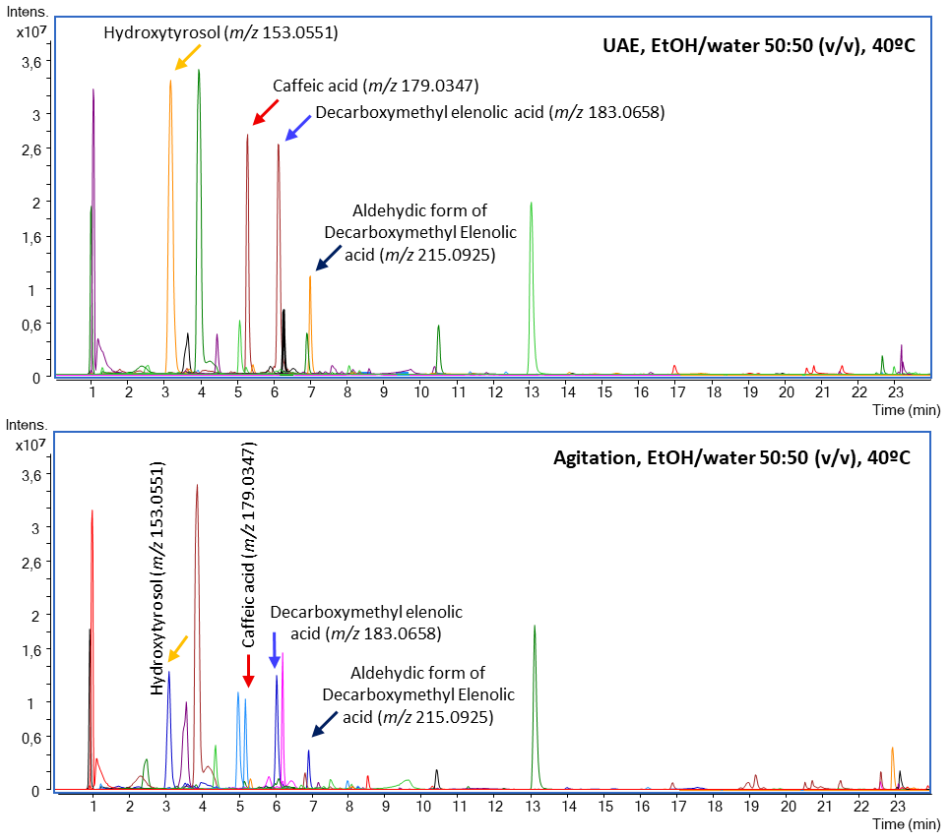


Figure 1.2. Extracted ion chromatograms of two samples of two-phase olive mill waste (TPOMW), extracted by sonication (upper chromatogram) and simple agitation (lower chromatogram). Arrows of the same color indicate the peaks corresponding to the same compound in each one of the extraction studies.

3.3. Selection of best conditions to recover the olive minor fraction

The samples of TPOMW contained organic acids, simple phenols, phenolic acids and aldehydes, flavonoids, a lignan (pinoresinol), secoiridoids (the most abundant chemical class, by a notorious difference), triterpenic acids and fatty acid derivatives. The distribution of these families for the studied strategies of agitation and ultrasound-assisted extraction is presented in Figure 1.3.

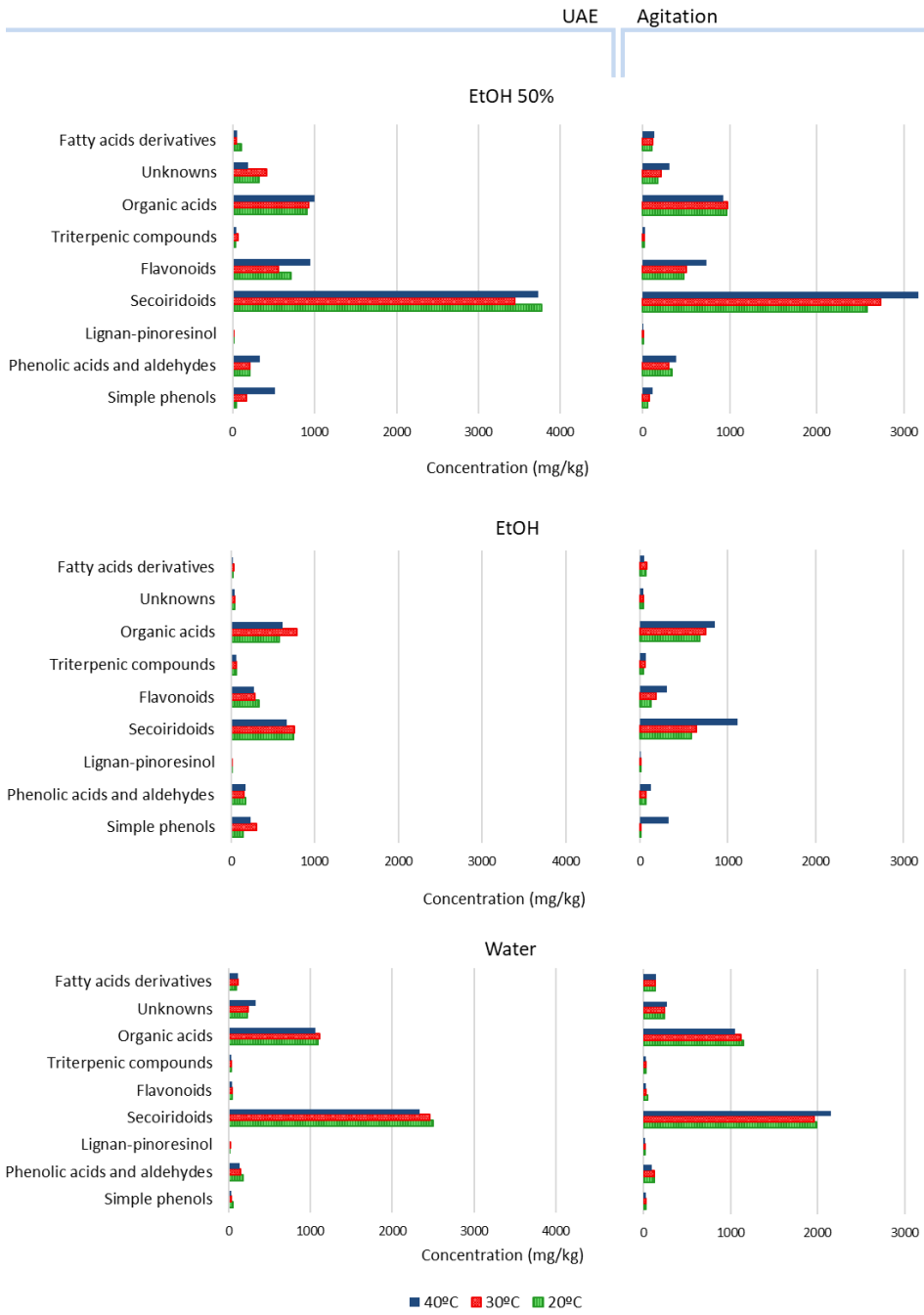


Figure 1.3. Obtained concentration of phenolic compounds and other chemical families after the ultrasound-assisted (left graphs) and agitation-mediated (right graphs) extractions, with the three solvents tested, at different temperatures.

As it was observed in the quantification of total phenols, EtOH/water 50:50 (v/v) performed remarkably. This can also be observed in Figure 1.4B, which shows the surface response analysis with ethanol concentration and temperature as independent variables, at an extraction time of 60 minutes. Water was also a good alternative, as the obtained concentration levels were interesting too, but the lower efficiency for flavonoids was noticeable in Figure 1.3. Considering the concentration values and the correct extraction of all chemical families present in the samples of TPOMW, EtOH/water 50:50 (v/v) was confirmed as the best solvent.

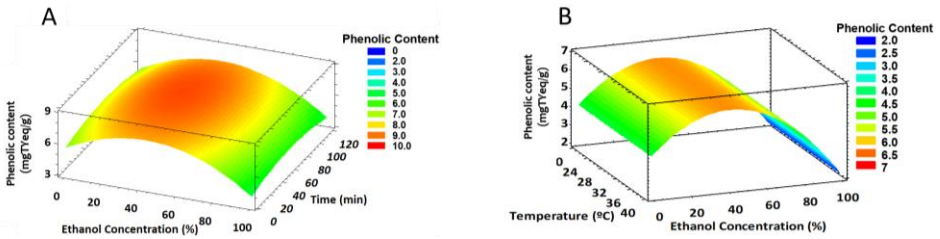


Figure 1.4. Response surface analysis for the total phenolic content obtained with the different extraction variables. Data from plot A correspond to a temperature of 40°C. Data from plot B correspond to an extraction time of 60 minutes.

Regarding the temperature, Figure 1.4B does not indicate any preferential temperature when the total phenolic content at the three tested temperatures is considered. However, if some chemical families, such as simple phenols and flavonoids, are analyzed individually after the extraction with EtOH/water 50:50 (v/v), 40°C stands out as more efficient. These chemical classes are of special interest because many biological positive effects have been attributed to their principal representatives [47,48]. In fact, the health claim approved by the European Food Safety Authority about olive oil phenolic compounds was based in the hydroxytyrosol content. According to the ANOVA applied to the concentration values obtained at the different temperatures for the extraction with EtOH/water 50:50 (v/v), the individual family of simple phenols (which include hydroxytyrosol) displayed differences of statistical significance (P-value <0.05, according to F test) among the values obtained at each temperature. This also occurred for the phenolic acids, which include relevant compounds such as ferulic and caffeic acid. In this case, the concentration obtained at 20°C was not significantly different from the concentration obtained at 30°C, but both mean values were statistically different from the concentration of phenolic acids obtained at 40°C. The specific concentration of simple phenols and phenolic acids can be found in Supplementary Figure 1.1. As the discussed molecules were preferentially extracted at 40°C, it seems pertinent to apply this temperature.

Global results of total phenolic content (Figure 1.1) already suggested that ultrasounds application would contribute to the extraction of the olive minor fraction from TPOMW. Indeed, LC-MS data showed that higher levels of these phytochemicals were obtained by the UAE approach. This was evident for flavonoids, simple phenols, and phenolic acids and aldehydes. However, the most evident results were once again those for secoiridoids, which surpassed 3.5 g/kg when sonication was applied and stayed at 3 g/kg in the cases of simple agitation. As has been already commented, the cavitation bubbles formed during sonication can damage the vegetal tissue [24,49]. As a consequence, solvent penetration within the sample is increased, and thus, compounds transfer and recovery are enhanced. Since the objective of this work was to extract the maximum levels of antioxidants and other interesting molecules to further exploit their properties, the UAE strategy was preferred against agitation. More details regarding color, pH, and conductivity of the solutions obtained through the UAE process, with ethanol/water 50:50 (v/v), at 40°C have been provided in Supplementary Figure 1.2. Those results reflect that pH stayed essentially constant during the extraction stages. Conductivity and color followed a similar trend to total phenolic compounds, which was shown in Figure 1.1. That distribution supports our initial reasoning about a rapid liberation of the majority of the compounds from the matrix.

Table 1.2 displays a comparison of the concentration ranges achieved in the extract during the sonication-mediated extraction and the extractions performed by agitation only. Results have been specified for every chemical family detected in our analysis.

The results of the concentration of each chemical class contained in Figure 1.3 indicated that secoiridoids were the most concentrated group. This is corroborated by the concentration ranges in Table 1.2. The molecule of hydroxy-decarboxymethyl elenolic acid was always the most concentrated secoiridoid, no matter the conditions applied during the sample treatment, and it was better extracted at 40°C, when ultrasounds were applied (by a difference of more than 150 mg/kg).

Table 1.2. Concentration ranges for the compounds that belong to the chemical families detected by LC-ESI-qTOF-MS. Results correspond to two extracts obtained with EtOH/water 50:50 (v/v), at the condition of sonication or agitation and the three tested temperatures. Relative standard deviation was in the range of 0.11%-13.23% for all the concentrations included in the table. Evaluation of the efficiency of the extraction at the selected conditions

Chemical family	Concentration ranges for the individual compounds (mg/kg)					
	UAE, EtOH/water 50:50 (v/v)			Agitation, EtOH/water 50:50 (v/v)		
	40°C	30°C	20°C	40°C	30°C	20°C
Secoiridoids	2.61-1982.57	0.59-1206.74	1.97-1761-55	2.50-1814.44	2.25-1605.56	2.22-1555.57
Organic acids	307.05-690.42	293.44-631.46	270.30-640.58	267.68-659.74	319.58-657.58	319.89-643.23
Flavonoids	5.70-832.31	5.06-454.40	4.65-619.10	3.92-710.20	3.58-488.51	3.54-456.32
Phenolic acids and aldehydes	6.93-193.70	1.48-116.51	1.87-138	2.50-299.41	2.24-241.51	2.30-284.56
Simple phenols	5.76-511.37	6.52-163.80	10.23-34.77	7.57-105.53	5.89-70.08	5.05-51.88
Fatty acids derivatives	1.99-27.60	0.58-26.15	2.38-63.01	2.54-69.50	2.64-54.43	2.86-48.09
Triterpenic acids	1.85-46.78	0.46-60.96	1.86-30.31	1.85-31.28	1.84-18.32	1.84-20.63
Lignan-pinoresinol	6.48	2.04	2.57	3.71	3.10	3.13
Unknowns	25.17-89.25	12.18-213.03	24.84-217.50	16.79-208.02	14.30-136.48	15.14-99.56

To evaluate the efficiency of the process, the remaining sediment obtained after a first UAE cycle was successively re-extracted. Sample re-extraction was preferred over its external fortification, because the solid character of TPOMW may hinder the uniform incorporation of new molecules to the matrix.

From Figure 1.5, it is possible to conclude that the developed one-step extraction is sufficient to recover almost all the phenolic content of the TPOMW samples, either after the extraction with ethanol at 50% or water (which were the most promising solvents, as explained before).

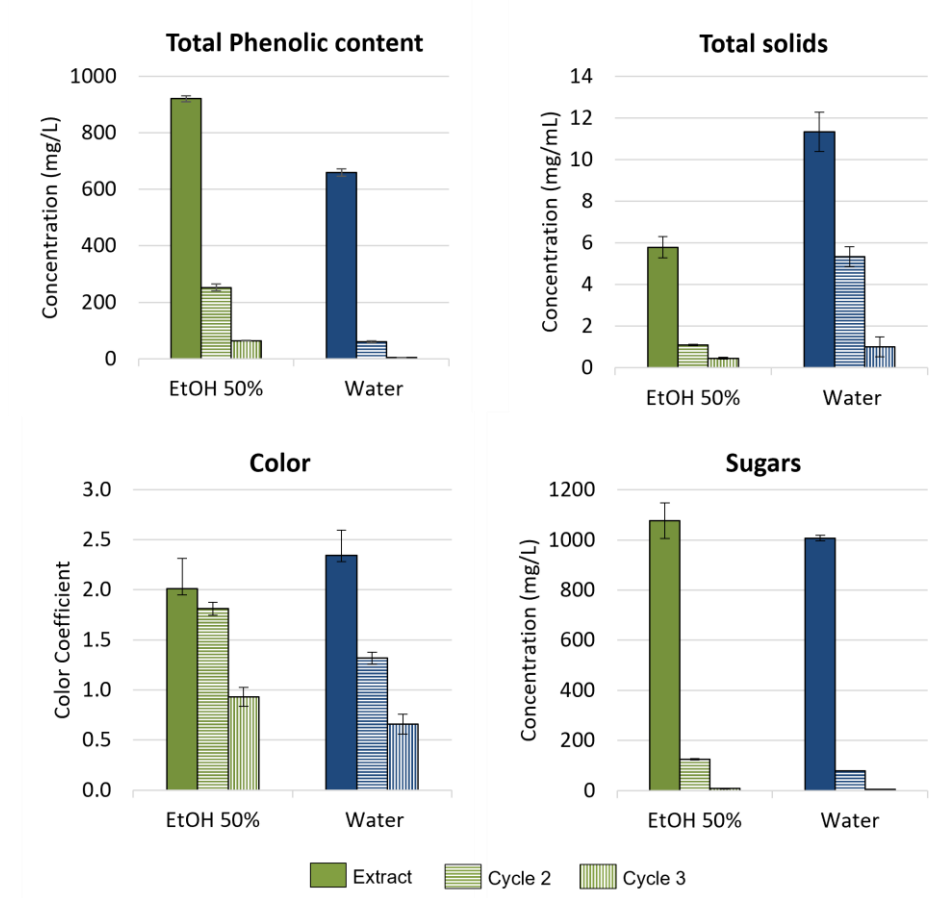


Figure 1.5. Achieved concentration values of total phenolic content, total solids, and sugars after each extraction cycle. The obtained color coefficient is also shown. Ultrasound-assisted extraction was performed with a solvent to sample ratio of 1:10 and 40ºC. Error bars refer to the standard deviation of experimental replicates.

After the second extraction, less than 27% of the phenolic content determined in the first cycle was detected. The residual biophenols after this second extraction were already limited, and after the third cycle, only 7% of the initially extracted phenolic content was recovered. Most of the sugars were also retrieved after the first extraction. These results confirmed the high efficiency of the UAE procedure corresponding to the first cycle. Considering that this was a large-volume extraction, the recovery of the highest bioactive percentage in a single step is of relevance. This avoids using more solvent and simplifies the process, contributing to its large-scale adaptation. In the case of the aqueous extraction, the obtained concentration of polyphenols was lower (as expected, considering Figures 1.1 and 1.3). Still, the efficiency was satisfactory, because the sample was already quite exhausted after the first cycle of extraction. A similar trend can be observed for the total solids. A disadvantage that can be attributed to the aqueous extraction is the proportion of extracted polyphenols with respect to the total solids (these may include undesired molecules, such as sugars). This proportion was more than three times higher in the extraction with EtOH at 50%, which was remarkable. Regarding the color determined in the extracted fractions, this was the only parameter that behaved differently. The first extract did not display a much higher color coefficient than those from the subsequent cycles. However, the lower capacity of the solvents to transport pigments from the sample was a positive situation, as the final product would be clearer.

Considering these aspects, the extraction with EtOH/water 50:50 (v/v) can be again confirmed as a powerful procedure. It is important to highlight that the results achieved with water were also of interest, as high concentrations of phenolic compounds were acquired. However, as this study pursued the recovery of most of the bioactive content from TPOMW and ethanol is also considered a green solvent, the ethanolic mixture was selected.

3.4. Ultrafiltration of the most favorable extract

The permeate flux experienced a decline from 4.5 L/h·m^2, obtained at the beginning of the process, to 2.6 L/h·m^2, which was registered at a VRF of 2.8 and 2 bar of transmembrane pressure. This decrease resulted from the sample concentration in the membrane module and, consequently, the increasing concentration of the solutes. Figure 1.6 exposes the fraction of organic matter retained by the membrane. Two axes have been provided, as the results about color and conductivity refer to elimination (equation 1) and the results about total solid content, total sugars content and olive minor fraction refer to rejection (equation 2). To calculate the rejection to the olive

minor fraction, the total concentration of the molecules corresponding to each chemical family was considered.

As shown in the Figure 1.6, the membrane retained almost all the total solids and sugars of the TPOMW extract. The color coefficient was also reduced, whereas the majority of the olive minor fraction was recovered in the permeate.

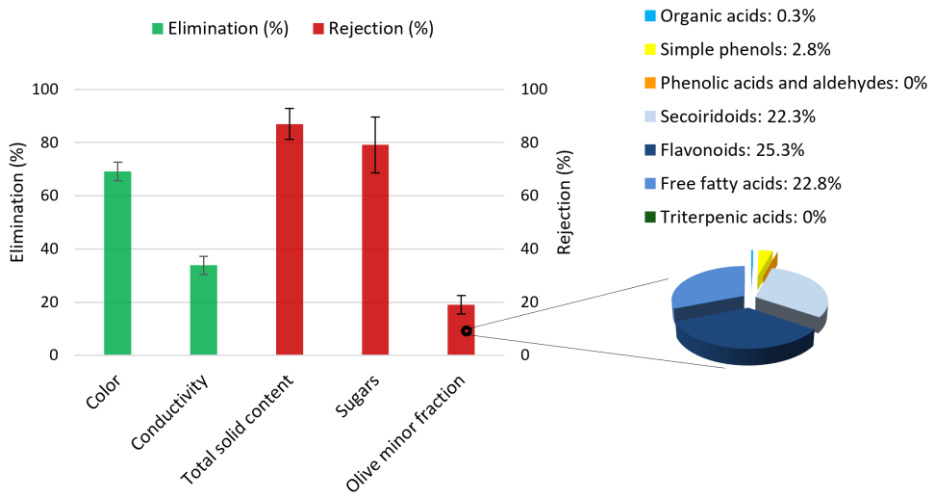


Figure 1.6. Performance of the UP005 membrane in terms of elimination of color and conductivity and rejection of compounds (total solid content and olive minor fraction) after the ultrafiltration process conducted at 2 bar. Error bars indicate the standard deviation observed for experimental replicates. The sector diagram illustrates the specific rejection for each chemical class of phenolic compounds and triterpenes.

These results were very promising, as very valued compounds have been recovered in the permeate, while other existent molecules (such as sugars, pigments and other solutes) remained in the membrane retentate. Regarding the olive minor fraction, none of the chemical classes were highly retained, but it is especially remarkable that some of them were not rejected at all. That is the case of phenolic acids and aldehydes, which include molecules so highly appreciated by industry as ferulic acid, vanillin or *p*-coumaric acid [50]. Similarly, simple phenols (including a high proportion of hydroxytyrosol) were almost completely recovered in the permeate, at a much higher purity than in the initial extract. The individual concentration of each compound can be revised in Table 1.3.

Table 1.3. Concentrations in ultrafiltration feed and permeate, for every compound present in the samples.

Compound Identity	Concentration in the UF Feed (ppm)	Concentration in the UF Permeate (ppm)
Quinic acid	11.32 ± 0.07	11.30 ± 0.05
Malic acid	4.86 ± 0.06	4.85 ± 0.03
Vanillic acid	0.107 ± 0.002	0.132 ± 0.002
Hydroxytyrosol	44.46 ± 0.03	45.1 ± 0.4
Acyclodihydroelenolic acid hexoside	189.2 ± 0.2	63.9 ± 0.5
Hydroxy-decarboxymethyl elenolic acid	651 ± 1	635 ± 2
$C_7H_{12}O_5$ (score: 86.96%)	3.194 ± 0.006	2.17 ± 0.01
Vanillin	1.174 ± 0.006	1.778 ± 0.001
Caffeic acid	112.26 ± 0.08	115.3 ± 0.1
Gallocatechin	1.630 ± 0.004	0.304 ± 0.004
Hydroxyelenolic acid	11.06 ± 0.03	4.48 ± 0.01
Tyrosol	2.11 ± 0.02	0.723 ± 0.009
Decarboxymethyl elenolic acid	185.3 ± 0.9	185.8 ± 0.8
Elenolic acid glucoside	1.862 ± 0.009	1.91 ± 0.02
$C_{16}H_{26}O_{10}$ (score: 96.93%)	15.01 ± 0.03	8.00 ± 0.05
p-Coumaric acid	2.764 ± 0.005	3.31 ± 0.01
Aldehydic form of Decarboxymethyl Elenolic acid	10.10 ± 0.06	4.81 ± 0.02
Phenylethyl primeveroside	1.68 ± 0.01	1.21 ± 0.01
$C_{11}H_{16}O_6$ (score: 99.12%)	2.0 ± 0.4	2.163 ± 0.006
Dehydro-oleuropein aglycone	0.231 ± 0.003	0.125 ± 0.002
Hydroxyoleuropein	0.91 ± 0.03	0.207 ± 0.002
Luteolin rutinoside	2.30 ± 0.06	1.35 ± 0.01
Luteolin 7-O-glucoside	0.44 ± 0.02	0.21 ± 0.07
Oleuropein	0.93 ± 0.01	0.95 ± 0.01
Elenolic acid	21.60 ± 0.09	21.67 ± 0.05
Ferulic acid	1.5 ± 0.03	1.514 ± 0.001
Hydroxytyrosol acyclodihydroelenolate	8.4 ± 0.6	8.98 ± 0.03
Decarboxymethyl oleuropein aglycone	13.86 ± 0.02	8.88 ± 0.04
Pinoresinol	0.422 ± 0.01	0
Ligstroside	1.146 ± 0.007	1.855 ± 0.003
Luteolin	10.55 ± 0.05	10.57 ± 0.09
Oleuropein aglycone	8.54 ± 0.04	8.833 ± 0.004
Diosmetin	0.075 ± 0.002	0

Compound Identity	Concentration in the UF Feed (ppm)	Concentration in the UF Permeate (ppm)
Dihydroxy-hexadecanoic acid	0.9 ± 0.3	0.435 ± 0.003
Ligstroside aglycone	0.242 ± 0.002	0.246 ± 0.004
Trihydroxy-octadecenoic acid	0.95 ± 0.09	1.40 ± 0.02
Gingerol	0.25 ± 0.02	0.170 ± 0.003
Betulinic acid	0.1981 ± 0.0005	0.246 ± 0.001
Hydroxy-octadecatrienoic acid	0.387 ± 0.002	0.440 ± 0.001
Dihydroxy-octadecanoic acid	1.380 ± 0.009	1.016 ± 0.005
Hydroxy-octadecadienoic acid	0.775 ± 0.003	0.440 ± 0.007
Maslinic acid	1.67 ± 0.08	1.86 ± 0.02
Hydroxy-octadecenoic acid	0.100 ± 0.002	0.088 ± 0.001
Hydroxy-octadecanoic acid	0.054 ± 0.002	0.0530 ± 0.0004

4. CONCLUSIONS

The process described here permitted to isolate a numerous variety of biophenols, which were obtained at high concentration (near 10 mg/g TPOMW). The detailed characterization of the extracts by LC-ESI-qToF-MS revealed that more than 40 compounds were obtained, from eight different chemical classes, in only one step of mixing and sonication. A large-scale, industrial application is possible. Moreover, the only organic solvent employed was ethanol, which has been recognized as a low-toxicity, environmentally friendly solvent. Afterward, the purification of the extract can be achieved by means of ultrafiltration. The selected membrane and operational parameters allowed the removal of almost the entire solids and sugars content, whereas the bioactive compounds were recovered in the permeate at high purity.

The optimum extraction was procured with ethanol/water 50:50 (v/v), at 40°C and with ultrasound application. The developed strategy allowed the recovery of a considerable proportion of phenolic and triterpenic compounds from a major waste from the olive industry, as it is TPOMW. This by-product has not been extensively utilized, but its bioactive content can be greatly exploited if it is properly extracted from the semisolid mixture of olive pulp, skin, and stones. Additionally, the withdrawal of these species from the residue contributes to its detoxification and favors the future stages of composting.

Funding: Grant CTM2017-88645-R, funded by MCIN/AEI/ 10.13039/501100011033 and by ERDF A way of making Europe. Additionally, the grant PRE2018-08524 was funded by

MCIN/AEI/ 10.13039/501100011033 and by ESF Investing in your future. Funding for open access charge: CRUE-Universitat Politècnica de València.

5. REFERENCES

[1] I.E. Kapellakis, K.P. Tsagarakis, J.C. Crowther, Olive oil history, production and by-product management, Rev. Environ. Sci. Bio/Technology. 7 (2008) 1–26. https://doi.org/10.1007/s11157-007-9120-9.

[2] P.M. Ahmed, P.M. Fernández, L.I.C. Figueroa, H.F. Pajot, Exploitation alternatives of olive mill wastewater: production of value-added compounds useful for industry and agriculture, Biofuel Res. J. 6 (2019) 980–994. https://doi.org/10.18331/brj2019.6.2.4.

[3] R. Borja, F. Raposo, B. Rincón, Treatment technologies of liquid and solid wastes from two-phase olive oil mills, Grasas y Aceites. 57 (2006) 32–46. https://doi.org/10.3989/gya.2006.v57.i1.20.

[4] E. Medina, C. Romero, M. Brenes, Residual Olive Paste as a Source of Phenolic Compounds and Triterpenic Acids, Eur. J. Lipid Sci. Technol. 120 (2018) 1–6. https://doi.org/10.1002/ejlt.201700368.

[5] J.A. Alburquerque, J. Gonzálvez, D. García, J. Cegarra, Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction, Bioresour. Technol. 91 (2004) 195–200. https://doi.org/10.1016/S0960-8524(03)00177-9.

[6] J. Vilar, S. Caño, I. Raya, L. Moreno, M. del M. Velasco, Alperujo processing sector. Possibilities for a potential financial and operational collapse., Sevilla, 2020. https://www.aneorujo.es/documentacion/.

[7] I. Sampedro, E. Aranda, G. Rodríguez-Gutiérrez, A. Lama-Muñoz, J.A. Ocampo, J. Fernández-Bolaños, I. García-Romera, Effect of a new thermal treatment in combination with saprobic fungal incubation on the phytotoxicity level of alperujo, J. Agric. Food Chem. 59 (2011) 3239–3245. https://doi.org/10.1021/jf2003305.

[8] Y. Bouhia, K. Lyamlouli, L. El Fels, Z. Youssef, Y. Ouhdouch, M. Hafidi, Effect of Microbial Inoculation on Lipid and Phenols Removal During the Co-composting of Olive Mill Solid Sludge with Green Waste in Bioreactor, Waste and Biomass Valorization. 12 (2021) 1417–1429. https://doi.org/10.1007/s12649-020-01077-3.

[9] E. Papadaki, M.Z. Tsimidou, F.T. Mantzouridou, Changes in Phenolic Compounds and Phytotoxicity of the Spanish-Style Green Olive Processing Wastewaters by Aspergillus niger B60, J. Agric. Food Chem. 66 (2018) 4891–4901. https://doi.org/10.1021/acs.jafc.8b00918.

[10] G. Koutrotsios, E. Larou, K.C. Mountzouris, G.I. Zervakis, Detoxification of olive

mill wastewater and bioconversion of olive crop residues into high-value-added biomass by the choice edible mushroom Hericium erinaceus, Appl. Biochem. Biotechnol. 180 (2016) 195–209. https://doi.org/10.1007/s12010-016-2093-9.

[11] J.A. Alburquerque, J. Gonzálvez, G. Tortosa, G.A. Baddi, J. Cegarra, Evaluation of "alperujo" composting based on organic matter degradation, humification and compost quality, Biodegradation. 20 (2009) 257–270. https://doi.org/10.1007/s10532-008-9218-y.

[12] N. Sabatini, E. Perri, D. Rongai, Olive Oil Antioxidants and Aging, Elsevier Inc., 2018. https://doi.org/10.1016/B978-0-12-811442-1.00004-3.

[13] T.W. Caldas, K.E.L. Mazza, A.S.C. Teles, G.N. Mattos, A.I.S. Brígida, C.A. Conte-Junior, R.G. Borguini, R.L.O. Godoy, L.M.C. Cabral, R. V. Tonon, Phenolic compounds recovery from grape skin using conventional and non-conventional extraction methods, Ind. Crops Prod. 111 (2018) 86–91. https://doi.org/10.1016/j.indcrop.2017.10.012.

[14] L. Olmo-García, R.P. Monasterio, C.M. Sánchez-Arévalo, A. Fernandez-Gutierrez, J.M. Olmo-Peinado, A. Carrasco-Pancorbo, Characterization of new olive fruit derived products obtained by means of a novel processing method involving stone removal and dehydration with zero waste generation, J. Agric. Food Chem. 67 (2019) 9295–9306. https://doi.org/10.1021/acs.jafc.9b04376.

[15] P. Gogoi, P. Chutia, P. Singh, C.L. Mahanta, Effect of optimized ultrasound-assisted aqueous and ethanolic extraction of Pleurotus citrinopileatus mushroom on total phenol, flavonoids and antioxidant properties, J. Food Process Eng. (2019) 1–12. https://doi.org/10.1111/jfpe.13172.

[16] F. Chemat, N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review, Ultrason. Sonochem. 34 (2017) 540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035.

[17] S. Álvarez-Blanco, J.-A. Mendoza-Roca, M.-J. Corbatón-Báguena, M.-C. Vincent-Vela, Valuable Products Recovery from Wastewater in Agrofood by Membrane Processes, in: A. Figoli, A. Criscuoli (Eds.), Sustain. Membr. Technol. Water Wastewater Treat. Green Chem. Sustain. Technol., Springer Nature Singapore, 2017: pp. 295–318. https://doi.org/10.1007/978-981-10-5623-9_11.

[18] M. del M. Contreras, I. Gómez-Cruz, I. Romero, E. Castro, Olive Pomace-Derived Biomasses Fractionation through a Two-Step Extraction Based on the Use of Ultrasounds: Chemical Characteristics, Foods. 10 (2021) 111. https://doi.org/10.3390/foods10010111.

[19] R. Japón Luján, F.P. Capote, A. Marinas, M.D. Luque de Castro, Liquid chromatography/triple quadrupole tandem mass spectrometry with multiple

reaction monitoring for optimal selection of transitions to evaluate nutraceuticals from olive-tree materials, Rapid Commun. Mass Spectrom. 22 (2008) 855–864. https://doi.org/10.1002/rcm.3423.

[20] P. Tapia-Quirós, M.F. Montenegro-Landívar, M. Reig, X. Vecino, T. Alvarino, J.L. Cortina, J. Saurina, M. Granados, Olive mill and winery wastes as viable sources of bioactive compounds: A study on polyphenols recovery, Antioxidants. 9 (2020) 1–15. https://doi.org/10.3390/antiox9111074.

[21] G.E. Maraulo, C. dos Santos Ferreira, M.F. Mazzobre, β-cyclodextrin enhanced ultrasound-assisted extraction as a green method to recover olive pomace bioactive compounds, J. Food Process. Preserv. 45 (2021). https://doi.org/10.1111/jfpp.15194.

[22] J.C. Martínez-Patiño, I. Gómez-Cruz, I. Romero, B. Gullón, E. Ruiz, M. Brnčićc, E. Castro, Ultrasound-Assisted Extraction as a First Step in a Biorefinery Strategy for Valorisation of Extracted Olive Pomace, Energies. 12 (2019) 2679. https://doi.org/10.3390/en12142679.

[23] R. Wang, R. He, Z. Li, S. Li, C. Li, L. Wang, Tailor-made deep eutectic solvents-based green extraction of natural antioxidants from partridge leaf-tea (Mallotus furetianus L.), Sep. Purif. Technol. 275 (2021) 119159. https://doi.org/10.1016/j.seppur.2021.119159.

[24] A.B. Das, V. V. Goud, C. Das, Extraction of phenolic compounds and anthocyanin from black and purple rice bran (Oryza sativa L.) using ultrasound: A comparative analysis and phytochemical profiling, Ind. Crops Prod. 95 (2017) 332–341. https://doi.org/10.1016/j.indcrop.2016.10.041.

[25] N.A. Al-Dhabi, K. Ponmurugan, P. Maran Jeganathan, Development and validation of ultrasound-assisted solid-liquid extraction of phenolic compounds from waste spent coffee grounds, Ultrason. Sonochem. 34 (2017) 206–213. https://doi.org/10.1016/j.ultsonch.2016.05.005.

[26] R.S. Rabelo, M.T.C. Machado, J. Martínez, M.D. Hubinger, Ultrasound assisted extraction and nanofiltration of phenolic compounds from artichoke solid wastes, J. Food Eng. 178 (2016) 170–180. https://doi.org/10.1016/j.jfoodeng.2016.01.018.

[27] M. Cifuentes-Cabezas, C. Carbonell-Alcaina, M.C. Vincent-Vela, J.A. Mendoza-Roca, S. Álvarez-Blanco, Comparison of different ultrafiltration membranes as first step for the recovery of phenolic compounds from olive-oil washing wastewater, Process Saf. Environ. Prot. 149 (2021) 724–734. https://doi.org/10.1016/j.psep.2021.03.035.

[28] B. Díaz-Reinoso, A. Moure, H. Domínguez, J.C. Parajó, Ultra- and nanofiltration of aqueous extracts from distilled fermented grape pomace, J. Food Eng. 91 (2009) 587–593. https://doi.org/10.1016/j.jfoodeng.2008.10.007.

[29] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics acids with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.

[30] L. Olmo-García, J.J. Polari, X. Li, A. Bajoub, A. Fernández-Gutiérrez, S.C. Wang, A. Carrasco-Pancorbo, Deep insight into the minor fraction of virgin olive oil by using LC-MS and GC-MS multi-class methodologies, Food Chem. 261 (2018) 184–193. https://doi.org/10.1016/j.foodchem.2018.04.006.

[31] L. Olmo-García, K. Wendt, N. Kessler, A. Bajoub, A. Fernández-Gutiérrez, C. Baessmann, A. Carrasco-Pancorbo, Exploring the capability of LC-MS and GC-MS multi-class methods to discriminate virgin olive oils from different geographical indications and to identify potential origin markers, Eur. J. Lipid Sci. Technol. 121 (2019) 1–13. https://doi.org/10.1002/ejlt.201800336.

[32] L. Saftić, Ž. Peršurić, E. Fornal, T. Pavlešić, S. Kraljević Pavelić, Targeted and untargeted LC-MS polyphenolic profiling and chemometric analysis of propolis from different regions of Croatia, J. Pharm. Biomed. Anal. 165 (2019) 162–172. https://doi.org/10.1016/j.jpba.2018.11.061.

[33] F. Rubio-Senent, S. Martos, A. Lama-Muñoz, J.G. Fernández-Bolaños, G. Rodríguez-Gutiérrez, J. Fernández-Bolaños, Isolation and identification of minor secoiridoids and phenolic components from thermally treated olive oil by-products, Food Chem. 187 (2015) 166–173. https://doi.org/10.1016/j.foodchem.2015.04.022.

[34] B. Gilbert-López, Z.L. Valencia-Reyes, V.M. Yufra-Picardo, J.F. García-Reyes, N. Ramos-Martos, A. Molina-Díaz, Determination of Polyphenols in Commercial Extra Virgin Olive Oils from Different Origins (Mediterranean and South American Countries) by Liquid Chromatography–Electrospray Time-of-Flight Mass Spectrometry, Food Anal. Methods. 7 (2014) 1824–1833. https://doi.org/10.1007/s12161-014-9825-7.

[35] L. Olmo-García, N. Kessler, H. Neuweger, K. Wendt, J.M. Olmo-Peinado, A. Fernández-Guti rrez, C. Baessmann, A. Carrasco-Pancorbo, Unravelling the distribution of secondary metabolites in olea europaea l.: exhaustive characterization of eight olive-tree derived matrices by complementary platforms (LC-ESI/APCI-MS and GC-APCI-MS), Molecules. 23 (2018) 1–16. https://doi.org/10.3390/molecules23102419.

[36] A.D. Troise, R. Ferracane, M. Palermo, V. Fogliano, Targeted metabolite profile of food bioactive compounds by Orbitrap high resolution mass spectrometry: The "FancyTiles" approach, Food Res. Int. 63 (2014) 139–146. https://doi.org/10.1016/j.foodres.2014.01.001.

[37] T.G. Ludwig, H.J.V. Goldberg, The Anthrone method for the determination of carbohydrates in foods and in oral rinsing, J. Dent. Res. 35 (1956) 90–94. https://doi.org/10.1177/00220345560350012301.

[38] R. Dreywood, Qualitative Test for Carbohydrate Material, Ind. Eng. Chem. - Anal. Ed. 18 (1946) 499. https://doi.org/10.1021/I560156A015.

[39] ISO 7887:2011, Water Quality. Examination and Determination of Colour. Method B, https://www.iso.org/standard/46425.html, 2011. https://www.iso.org/standard/46425.html.

[40] N. Babbar, H.S. Oberoi, S.K. Sandhu, V.K. Bhargav, Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants, J. Food Sci. Technol. 51 (2014) 2568–2575. https://doi.org/10.1007/s13197-012-0754-4.

[41] A. Carrasco-Pancorbo, L. Cerretani, A. Bendini, A. Segura-Carretero, T. Gallina-Toschi, A. Fernández-Gutiérrez, Analytical determination of polyphenols in olive oils, J. Sep. Sci. 28 (2005) 837–858. https://doi.org/10.1002/jssc.200500032.

[42] D.P. Zagklis, C.A. Paraskeva, Purification of grape marc phenolic compounds through solvent extraction, membrane filtration and resin adsorption/desorption, Sep. Purif. Technol. 156 (2015) 328–335. https://doi.org/10.1016/j.seppur.2015.10.019.

[43] L.G. D'Alessandro, K. Dimitrov, P. Vauchel, I. Nikov, Kinetics of ultrasound assisted extraction of anthocyanins from Aronia melanocarpa (black chokeberry) wastes, Chem. Eng. Res. Des. 92 (2014) 1818–1826. https://doi.org/10.1016/j.cherd.2013.11.020.

[44] E.M. Garcia-Castello, A.D. Rodriguez-Lopez, L. Mayor, R. Ballesteros, C. Conidi, A. Cassano, Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (Citrus paradisi L.) solid wastes, LWT - Food Sci. Technol. 64 (2015) 1114–1122. https://doi.org/10.1016/j.lwt.2015.07.024.

[45] Á. Peralbo-Molina, F. Priego-Capote, M.D. Luque de Castro, Tentative Identification of Phenolic Compounds in Olive Pomace Extracts Using Liquid Chromatography–Tandem Mass Spectrometry with a Quadrupole–Quadrupole-Time-of-Flight Mass Detector, J. Agric. Food Chem. 60 (2012) 11542–11550. https://doi.org/10.1021/jf302896m.

[46] I. Cea-Pavez, J. Lozano-Sánchez, I. Borrás-Linares, H. Nuñez, P. Robert, A. Segura-Carretero, Obtaining an Extract Rich in Phenolic Compounds from Olive Pomace by Pressurized Liquid Extraction, Molecules. 24 (2019) 3108. https://doi.org/10.3390/MOLECULES24173108.

[47] European Commission, COMMISSION REGULATION (eu) No 432/2012 of May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health, Brussels, Belgium, 2012. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02012R0432-20170822&from=EN (accessed November 20, 2020).

[48] M. Robles-Almazan, M. Pulido-Moran, J. Moreno-Fernandez, C. Ramirez-Tortosa, C. Rodriguez-Garcia, J.L. Quiles, Mc. Ramirez-Tortosa, Hydroxytyrosol: Bioavailability, toxicity, and clinical applications, Food Res. Int. 105 (2018) 654–667. https://doi.org/10.1016/j.foodres.2017.11.053.

[49] T.J. Mason, A.J. Cobley, J.E. Graves, D. Morgan, New evidence for the inverse dependence of mechanical and chemical effects on the frequency of ultrasound, Ultrason. Sonochem. 18 (2011) 226–230. https://doi.org/10.1016/j.ultsonch.2010.05.008.

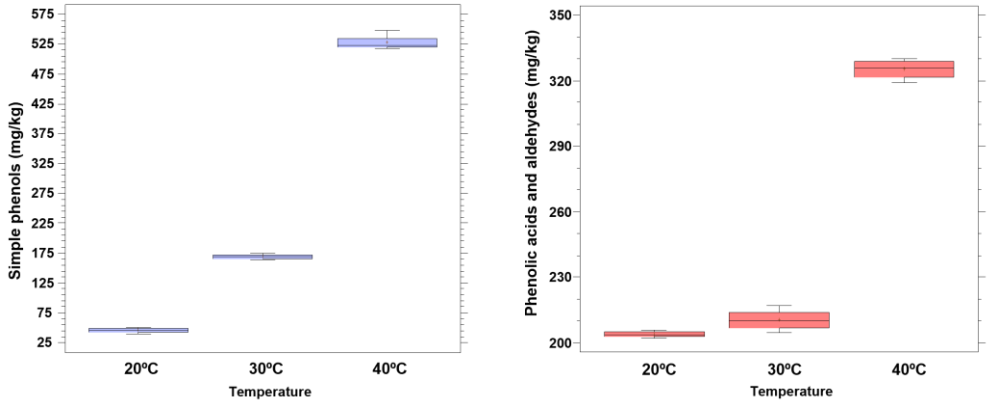
[50] E. Valanciene, I. Jonuskiene, M. Syrpas, E. Augustiniene, P. Matulis, A. Simonavicius, N. Malys, Advances and prospects of phenolic acids production, biorefinery and analysis, Biomolecules. 10 (2020) 1–41. https://doi.org/10.3390/biom10060874.

Supplementary Table 1.1. Analytical parameters of the developed liquid chromatography coupled to mass spectrometry (LC-MS) multi-class methodology. Intra-day repeatability was calculated by the relative standard deviation (%) of three independent injections (carried out in the same sequence) of an extract obtained with the mixture of EtOH/water 50:50 (v/v), at 40°C.

[a]: Limit of detection; [b]: Limit of quantification

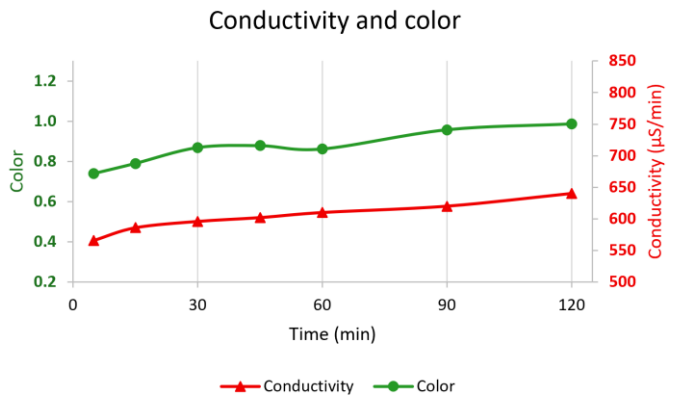
	Hydroxytyrosol	Caffeic acid	*p*-coumaric acid	Luteolin	Oleuropein
Intra-day repeatability (%)	0.121	0.834	0.786	0.697	0.778
EtOH/water 50:50 (v/v)					
LOD[a] (ppm)	0.021	0.002	0.001	0.021	0.015
LOQ[b] (ppm)	0.068	0.008	0.004	0.075	0.051
Water					
LOD (ppm)	0.023	0.003	0.001	0.361	0.098
LOQ (ppm)	0.077	0.011	0.003	1.217	0.328
Pure ethanol					
LOD (ppm)	0.005	0.003	0.002	0.004	0.003
LOQ (ppm)	0.017	0.009	0.006	0.010	0.011

Supplementary Figure 1.1. Boxplots of the concentration of simple phenols (left) and phenolic acids and aldehydes (right) with the applied temperatures for their extraction from two-phase olive mill waste.



Supplementary Figure 1.2. Complementary analyzed parameters for the TPOMW extract obtained at the best conditions (ultrasound assisted extraction, 40°C, ethanol/water 50:50 (v/v)). In the graph, left axis (green) corresponds to color and right axis (red) corresponds to conductivity values.

pH Values							
Time (min)	5	15	30	45	60	90	120
	5,72	5,79	5,72	5,70	5,68	5,63	5,62



SECCIÓN 2

Procesos de membrana en medio acuoso

Esta sección contiene el tratamiento de los extractos acuosos del alperujo mediante tecnologías de membrana, tales como la ultrafiltración (Capítulo 3), la nanofiltración (Capítulo 4) y la combinación de ambos procesos (Capítulo 5) para recuperar los compuestos fenólicos contenidos en esta matriz.

CAPÍTULO 2. CHAPTER 2

Green management of wet olive pomace by means of ultrafiltration of an aqueous extract of phenolic compounds. Integral characterization of the streams by LC-ESI-QToF-MS

Carmen M. Sánchez-Arévalo[1], María Cinta Vincent-Vela[1,2], Silvia Álvarez-Blanco[1,2]

[1]Research Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

[2]Department of Chemical and Nuclear Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

✉carsana5@upv.es

Environmental Technology & Innovation *(Under review)*

Abstract: Wet olive pomace is a major by-product generated by olive mills. To contribute to the circular economy of the olive industry, the recovery of interesting compounds from wet olive pomace was assessed. To that end, a previously optimized solid-liquid extraction, only employing water as the extractant, was first applied to the wet olive pomace. Afterwards, an ultrafiltration process to treat the obtained extract was developed. Several membranes (UP005, UH030, UH050, and UP150, from Microdyn Nadir) were studied, in a wide range of cross-flow velocities (1.5 - 3.5 m/s) and transmembrane pressures (0.75 - 5.5 bar). By a thorough characterization of the ultrafiltration streams by LC-ESI-QToF-MS, it was possible to describe the evolution of the rejection of 29 phenolic compounds. Some adsorption processes were also observed in the ultrafiltration process. The UP005 and UH030 membranes displayed satisfactory values of permeate flux and rejection. Both membranes efficiently retained a high fraction of the total solids, chemical oxygen demand, and color. On the contrary, the phenolic compounds were obtained in the ultrafiltration permeate, which constitutes a source of antioxidant molecules with applications in cosmetics, pharmacy, and nutraceutics.

Keywords: ultrafiltration, phenolic compounds, wet olive pomace, aqueous extract, olive waste.

1. INTRODUCTION

The current worldwide situation regarding water shortage along with the vast, daily generation of residues, underlines more than ever the necessity of implementing the principles of circular economy in all industries. In particular, the ecological footprint of the agri-food industry is highly relevant [1,2], considering the spread of intensive agriculture in order to supply the growing population. One of the most important food industries is related to the production of virgin olive oil. According to the estimations of the International Olive Council, the world production of olive oil surpassed 3 million tons during the crop year 2021/2022 [3].

In the Mediterranean area, the majority of the olive mills apply the two-phase methodology, which is known for lower water consumption and higher extraction efficiency than the alternative method, which is the three-phase method [4]. According to the two-phase method, the olive fruits undergo several stages, including washing, crushing, a first centrifugation in a horizontal decanter, and a second centrifugation in a vertical decanter. In the horizontal centrifuge, the olive oil is separated from the rest of the olive components, which constitute the wet olive pomace.

Thus, wet olive pomace is a semi-solid past containing all the remnants of the olive drupes after the olive oil extraction. This includes the olive pulp, skin, stone, seeds, and vegetation water. As a result, the organic load of this by-product is very high. Interestingly, many phenolic compounds from the olive fruit remain in the wet olive pomace [5]. This residue has a dual character. On one side, the phytotoxicity of the biophenols is a threat for the environment if the disposal of wet olive pomace is not taken seriously [6]. On the other side, wet olive pomace can be considered a source of antioxidant molecules with applications in cosmetics, pharmacy, and nutrition [6–8].

Therefore, the recovery of phenolic compounds from wet olive pomace permits its valorization and contributes to the circular economy of the olive oil sector. This objective can be addressed by membrane technology. Several research groups have demonstrated the feasibility of membrane processes to purify or concentrate phenolic compounds from olive residues [9–12]. However, the results available in the literature are mainly referred to liquid streams, both from the two-phase and the three-phase methodology. Therefore, the conclusions achieved by these valuable contributions cannot be directly correlated with the treatment of wet olive pomace.

Wet olive pomace is the most challenging olive waste when it comes to the treatment by membrane technology. The two-phase process is the most popular methodology for producing olive oil and its implementation increases every year [13]. However, the research about the most abundant olive mill by-product is by far less common than the studies dealing with the olive mill wastewater obtained in the three-phase process, or with olive oil washing wastewater. To recover the phenolic compounds from wet olive pomace, a solid-liquid extraction has to be performed in advance and, as a result, a liquid extract with dark color and high organic load is obtained. This extraction can be performed using an organic solvent (such as ethanol, and mixtures of ethanol and water) as the extractant agent, obtaining satisfactory concentrations of phenolic compounds. Also, the extraction of phenolic compounds can be addressed only with water [4,14]. In this case, the recovery can be slightly lower, but important advantages are derived instead. First of all, avoiding the organic solvent means a lower environmental impact. Secondly, the cost of the process is reduced if only water is needed. This premise is especially relevant if an industrial approach is considered, as large volumes of the extractant will be needed. And thirdly, obtaining an aqueous extract may imply a benefit for the subsequent treatment of the stream. In the case of a membrane process, the presence of an organic solvent in the feed solution may represent an additional challenge to consider, due to a possible modification of the

membrane structure, affecting its permeation characteristics. Therefore, employing water as the extractant agent during the solid-liquid extraction is an efficient strategy to simplify the process and reduce its repercussion.

The result of the mentioned extraction is a highly foulant stream, containing the polyphenols of interest and many other molecules that are inevitably extracted as well, hindering the purification of the target molecules [14]. In this context, the selection of the most appropriate membrane is not direct, because the severe fouling that may affect the membrane will influence its productivity and the rejection values [15]. The fabrication of new membranes less affected by fouling is essential, and the scientific community is becoming more and more aware of the importance of this task [16–19]. However, the new membranes that are currently being developed at a laboratory scale are still not available for the industry and cannot be considered yet for their implementation in the circular economy of the olive mills. Thus, it is essential to study the commercial membranes that are right now in the market and investigate their viability to work with a problematic residue such as the wet olive pomace.

In this work, several commercial ultrafiltration membranes have been studied. The productivity and efficiency of the membranes have been assessed, in order to select the most appropriate strategy to purify the phenolic compounds present in aqueous extracts of wet olive pomace. Furthermore, the cleaning of the membranes has been studied, to explore their reusability.

2. MATERIAS AND METHODS

2.1. Extract of wet olive pomace

The samples of wet olive pomace were collected from the olive mill San Isidro de Segorbe (Castellón, Spain), which applies the two-phase methodology. Samples were stored at 5˚C before their processing. In order to extract the phenolic content from wet olive pomace, an ultrasound-assisted solid-liquid extraction was accomplished for 45 minutes at 40˚C. The sonication was performed at 37 kHz and 220 W. 600 g of wet olive pomace were treated, applying a solid/solvent ratio of 1:10. Only osmotized water was employed as a solvent. Later, the obtained extracts were centrifuged at 17200 RCF for 6 min to remove the olive stone and seed remnants. This procedure was developed in a previous work [14]

2.2. Ultrafiltration

The ultrafiltration plant was equipped with a Rayflow membrane module (Orelis Environnement, France), where a flat sheet membrane was placed. The membrane area was 129 cm^2. Four commercial membranes (Microdyn Nadir, Germany) were tested, at three different cross-flow velocities (1.5, 2.5 and 3.5 $m \cdot s^{-1}$) and a wide range of transmembrane pressure (0.75 – 5.5 bar). Two of the tested membranes were made of polyethersulfone (UP005, with a 5 kDa molecular weight cut off (MWCO), and UP150, with a 150 kDa MWCO) and two of them were made of hydrophilic polyethersulfone (UH030, with a 30 kDa MWCO, and UH050, with a 50kDa MWCO). Before the processing of the wet olive pomace extracts, the membranes were immersed in osmotized water overnight, to hydrate them and remove the residual conservatives. Afterwards, they were compacted (at the highest transmembrane pressure of the applied operating conditions) and characterized through the determination of their water permeability [15].

Six liters of wet olive pomace extract were treated with each membrane. Both the retentate and the permeate streams were recirculated to the feed tank, to keep the concentration in the feed solution constant. Samples of 50 mL were collected from the feed and the permeate streams to be analyzed and determine the rejection of solutes. This rejection (R) was calculated according to the following formula:

$$R = 1 - \frac{C_p}{C_f} \quad (1)$$

where C_p refers to the concentration in the permeate and C_f refers to the concentration in the feed.

To clean the membranes, several cleaning protocols were employed, as reflected in Table 2.1. A solution of P3 Ultrasil 115 (Ecolab, USA) at 1% (v/v) was applied for 1 hour after a first rinsing (15 minutes) with tap water, at ambient temperature. Afterwards, a water rinsing was again applied, at ambient temperature, to remove the detergent from the ultrafiltration plant. Finally, the membrane was rinsed with osmotized water. In order to reduce the water consumption during this study, the water employed for the second membrane rinsing was kept and employed during the following cleaning cycle in the plant, as the water for the first rinsing.

Table 2.1. Cleaning protocol applied to each membrane employed in this work.

Membrane	Cleaning protocol	
	Cleaning agent	Temperature
UP005 (after working at 0.75, 1.5 and 2.5 bar)	P3 Ultrasil 115 at 1% (v/v)	25˚C
UP005 (after working at 3.5, 4.5 and 5.5 bar)	P3 Ultrasil 115 at 1% (v/v)	35˚C
UH030	P3 Ultrasil 115 at 1% (v/v)	40˚C
UH050	P3 Ultrasil 115 at 1% (v/v)	35˚C
UP150	P3 Ultrasil 115 at 1% (v/v)	40˚C
	P3 Ultrasil 110 at 1% (v/v)	40˚C
	NaClO at 200 $mg \cdot L^{-1}$ (v/v)	40˚C

During the application of Ultrasil, the temperature had to be raised in some cases, in order to increase the cleaning efficiency. The membranes were considered to be clean when a water permeability recovery higher than 90% was obtained. In the case of the UP150 membrane, the water permeability was not recovered with the detergent solution, at any of the tested temperatures. Other alternatives were tested, such as a solution of P3 Ultrasil 110 (Ecolab, USA) at 1% (v/v) and a solution of sodium hypochlorite at 200 mg·L-1. However, the UP150 membrane could not be reused.

2.3. Analytical methods

2.3.1. Analysis of the olive minor fraction

The analytes corresponding to the olive minor fraction were determined by liquid chromatography coupled to mass spectrometry (LC-MS), employing an Agilent 1290 Infinity II liquid chromatograph (Agilent Technologies, USA). The mass spectrometer was equipped with electrospray ionization (ESI) and a 6546 quadrupole-time-of-flight (QToF) mass analyzer (Agilent Technologies, USA). The injection volume was 4 µL. To separate the compounds, a Zorbax Extend C18 column (4.6 x 100 mm, 1.8 µm) (Agilent Technologies, USA) was used, working at 40˚C and a flow rate of 0.9 $mL \cdot min^{-1}$. Ultrapure water and acetonitrile (Honeywell, USA) were the mobile phases, both acidified with 0.5% of acetic acid (v/v). The LC gradient and MS conditions (with a negative ionization) were adapted from a previous work [14].

2.3.2. Analysis of organic matter

Chemical oxygen demand (COD) of the samples was assessed through commercial kits (Merck, Germany). The analysis of total solids was performed by evaporating a determined volume of the samples and calculating the difference in the weight of the container before and after the evaporation. Finally, to determine the color, the

absorbance of the samples at different wavelengths was measured with a UV-VIS DR 6000 spectrophotometer (Hach Lange, Germany). The color coefficient was given by the following formula (UNE-EN ISO 7887:2012 Method B):

$$Colour\ coefficient = \frac{(A_{436}^2 + A_{525}^2 + A_{620}^2)}{(A_{436} + A_{525} + A_{620})} \quad (2)$$

where A_{436} is the absorbance of the sample at 436 nm, A_{525} is the absorbance at 525 nm and A_{620} is the absorbance at 620 nm.

3. RESULTS AND DISCUSSION

3.1. Composition of the extracts of wet olive pomace

After the ultrasound-assisted solid-liquid extraction, the obtained extracts were characterized in terms of phenolic content (which are the compounds of interest) and amount of organic matter, which should be reduced as much as possible during the following ultrafiltration process. In order to avoid the degradation of the different compounds in the extracts or their biological contamination, the extract (ultrafiltration feed) was periodically renovated. In total, three extractions were performed. The composition of each extract and the corresponding operating conditions of its processing are detailed in Table 2.2. As shown in Table 2.2, the organic load of the extracts of wet olive pomace is high. This supports the application of an ultrafiltration process for retaining a high percentage of the organic matter, while phenolic compounds are aimed to be recovered in the permeate stream.

1.1. Permeate flux

Once the membranes were compacted, their water permeability was calculated, obtaining 13 ± 2 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$, 168 ± 12 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$, 213 ± 18 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ and 228 ± 14 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ for the UP005, UH030, UH050, and UP150 membranes, respectively. This data was consistent with the MCWO of the membranes, as the permeability increased with higher values of MCWO. Also, the obtained permeabilities were in line with the manufacturers' specifications and previous works dealing with some of these membranes [11,15].

Table 2.2. Characterization of the organic matter present in the aqueous extracts of wet olive pomace employed as feed for the ultrafiltration process. The operating conditions at which each extract was treated are also detailed.

Parameter	Value	Operating conditions	Employed membranes
Phenolic content (mg/L extract)	523 ± 3	1.5 m·s^{-1} 0.75 – 5.5 bar	UP005, UH030, UH050, UP150
COD[1] (mg/L)	9200 ± 364		
Total solids (mg/mL)	7.6 ± 0.5		
Color coefficient	1.7 ± 0.5		
Conductivity (µS/cm)	1675 ± 17		
pH	5.13 ± 0.07		
Phenolic content (mg/L extract)	633 ± 22	2.5 m·s^{-1} 0.75 – 4.5 bar	UP005, UH030
COD[1] (mg/L)	14985 ± 2019		
Total solids (mg/mL)	7 ± 1		
Color coefficient	2.42 ± 0.02		
Conductivity (µS/cm)	1721 ± 62		
pH	5.04 ± 0.02		
Phenolic content (mg/L extract)	637 ± 24	3.5 m·s^{-1} 0.75 – 5.5 bar	UP005, UH030
COD[1] (mg/L)	11688 ± 548		
Total solids (mg/mL)	7.3 ± 0.3		
Color coefficient	2.484 ± 0.003		
Conductivity (µS/cm)	1699 ± 32		
pH	5.02 ± 0.01		

Parameter	Mean Values
Phenolic content (mg/L extract)	598 ± 65
COD (mg/L)	11957 ± 2902
Total solids (mg/mL)	7.2 ± 0.5
Color coefficient	2.2 ± 0.4
Conductivity (µS/cm)	1698 ± 23
pH	5.06 ± 0.06

[1]Chemical oxygen demand

Afterwards, the aqueous extract of wet olive pomace was treated in the ultrafiltration plant. The results regarding the permeate flux obtained at the different operating conditions are shown in Figure 2.1.

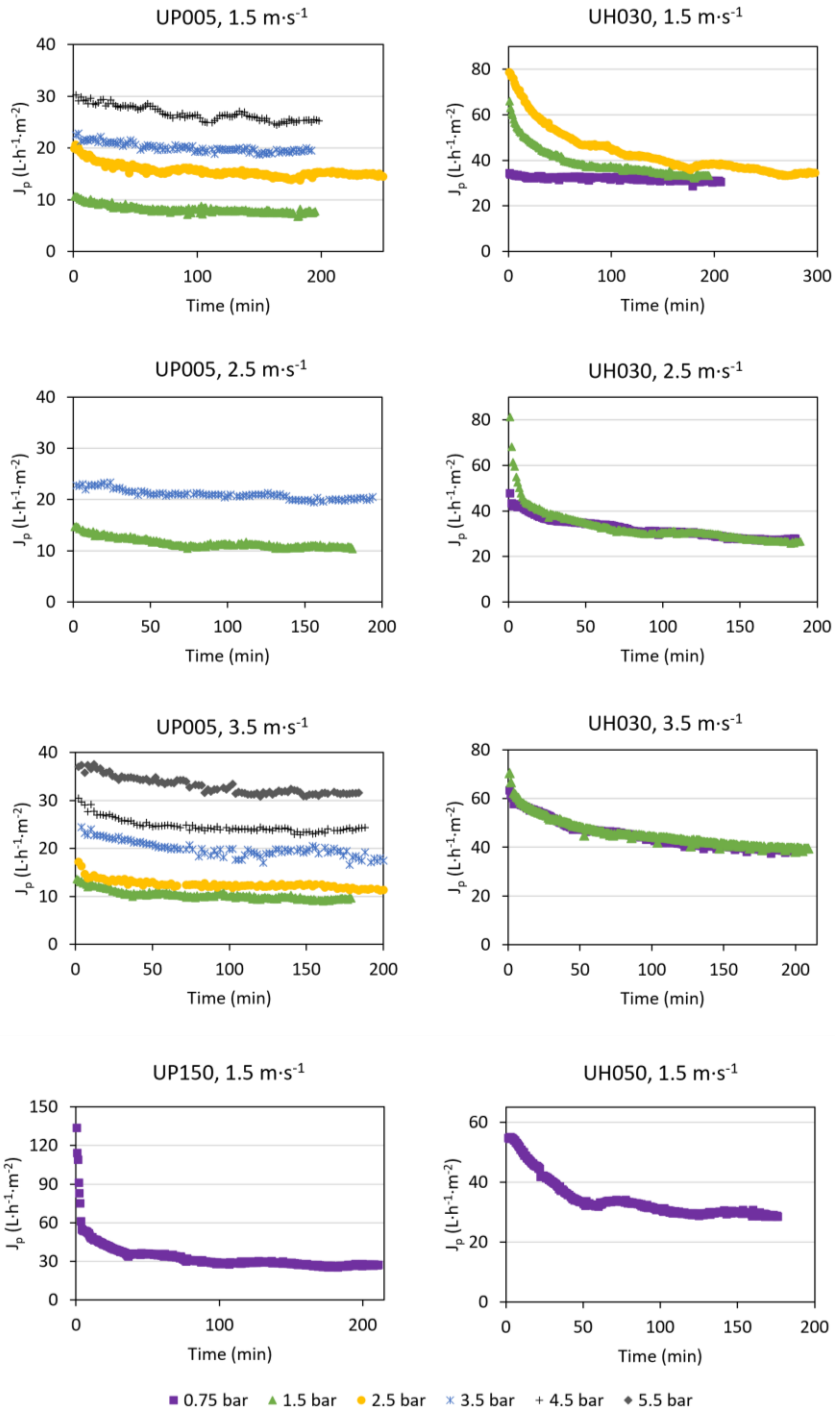


Figure 2.1. Evolution of permeate flux with time, at the different operating conditions, for the UP005, UH030, UH050, and UP150 membranes.

When comparing the tests performed at the same pressure, it can be observed that the membranes with larger pore size (UH030, UH050, and UP150) presented greater permeate flux at the beginning of the process. However, these membranes displayed a more drastic drop of the permeate flux with time. After a certain time period, the permeate flux decreased slowly until a steady state was reached. The difference in flux drop is especially notable if the flux of the UH030 and UP005 membranes is compared at 1.5 and 2.5 bar (Figure 2.1). This trend in the evolution of permeate flux with time is normally found in ultrafiltration processes [22] and can be attributed to cake layer formation and pore blocking. As the UP005 membrane is tight, the initial permeate flux is low and the convective transport of solutes towards the membrane surface is smaller than for the rest of the membranes. Therefore, it was less affected by concentration polarization and fouling. In consequence, permeate flux decline was less pronounced. Another reason that prevented the fouling of the UP005 membrane is the polarity of the active layer. It has been demonstrated that membranes with lower hydrophilicity are less fouled when feeds containing polyphenols are treated [15,23]. Then, the UP005 membrane combined a small pore size and a less hydrophilic active layer, reducing the fouling of this membrane. Figure 2.1 suggests that fouling was more severe for the membranes with larger MWCO and it was more relevant at higher pressures, because a faster drop of the flux was displayed. This was prompted by the higher concentration of solutes at the membrane surface occurring at higher pressures. Considering the pore size of the UH050 and UP150 membranes and the permeate flux that was observed at the steady state, a severe fouling can be inferred for these membranes. Corbatón-Báguena et al. and Qu et al. found that membranes with large pore diameter and close to that of the solutes present in the feed solution, suffered a more severe fouling and poor recyclability due to pore constriction, as a result of the solutes penetrating the membrane pores and blocking them [24,25]. Therefore, no further transmembrane pressures were tested for the UH050 and UP150 membranes, as the fouling was expected to increase at higher pressures. In the particular case of the UH050 membrane, the rejection values that were obtained for this membrane also discouraged its utilization, as will be commented in section 3.3.

When the flux at the steady state was plotted against the transmembrane pressure (Figure 2.2), the low fouling of the UP005 membrane was confirmed. When the transmembrane pressure was increased, the permeate flux exhibited by this membrane increased linearly too, even at 3.5, 4.5, and 5.5 bar, which can be considered high pressures for an ultrafiltration process. However, the opposite was found for the UH030 membrane. When the pressure was increased from 0.75 to 1.5 bar, the flux at the steady

state remained constant, suggesting the formation of a gel layer on the surface of the UH030 membrane. For this membrane, the limiting pressure was 0.75 bar [26,27] and, beyond that value, the applied TMP did not influence the permeate flux.

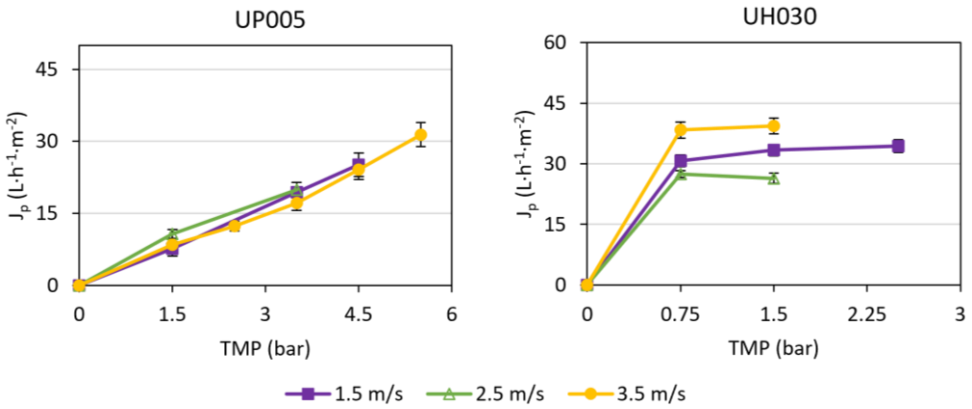


Figure 2.2. Permeate flux at the steady state for the UP005 and UH030 membranes, at the different operating conditions.

The effect of the cross-flow velocity was also evaluated. However, the influence of this parameter on the permeate flux was much less important than the transmembrane pressure. Regarding the UP005 membrane, the modification of the cross-flow velocity did not derived in an increase of the permeate flux. This can be attributed to the low fouling that was suffered by this membrane. In that situation, the softer turbulence achieved by the lowest cross-flow velocity (1.5 m·s^{-1}) was already sufficient and the increment of the tangential velocity did not lead to any benefit in the productivity of the process. In fact, at 2.5 m·s^{-1}, pressures higher than 2.5 bar were not tested, because the obtained permeate flux was very similar to the permeate flux obtained at 1.5 m·s^{-1} and the increase in energy investment was not considered to be necessary. In the case of the UH030 membrane, the fouling was more relevant. As expected, the greatest permeate flux was achieved at the highest cross-flow velocity (3.5 m·s^{-1}). At this high velocity, the diffusive transport from the cake layer back to the bulk solution is enhanced, leading to a higher permeate flux [28]. However, at 2.5 m·s^{-1}, the permeate flux displayed by the UH030 membrane was lower than that observed at 1.5 m·s^{-1}, despite of the increase in the turbulence in the module. The reason for this lower permeate flux is the higher value of COD in the extract of wet olive pomace that was treated at 2.5 m·s^{-1} (see Table 2.2). In consequence, a greater fouling was expected for the experiments carried out at this velocity and, therefore, a lower flux was obtained. In any case, an increment in the cross-flow velocity did have a beneficial effect on the permeate flux for the UH030 membrane.

1.2. Rejection values

1.2.1. Rejection of the organic matter

Figure 2.3 and Figure 2.4 show the rejection of the organic matter (except phenolic compounds) achieved with each tested membrane. As can be observed, the rejection values regarding the UP005 membrane increased with the pressure, at all cross-flow velocities, which is in line with a higher water flux. At the highest pressures, interesting rejection values (greater than 50%) were obtained with the UP005 membrane, because a high withdrawal of the organic matter was pursued, in order to recover the polyphenols at higher purity in the permeate. In the case of the UH030 membrane, the gel layer formation (Figure 2.2) determined that the permeate flux did not increase with transmembrane pressure. In consequence, the rejection was only slightly increased by increasing the pressure. In the case of the UH050 (Figure 2.4), the higher MWCO of the membrane determined lower rejection values for color, total solids, and COD. This was not interesting, considering the aim of the work. As the purification of polyphenols was not favored by this membrane and the obtained permeate flux (Figure 2.1) was not higher than the permeate flux obtained with other membranes more effective in terms of rejections, the UH050 membrane was not tested at any further conditions of transmembrane pressure or cross-flow velocity.

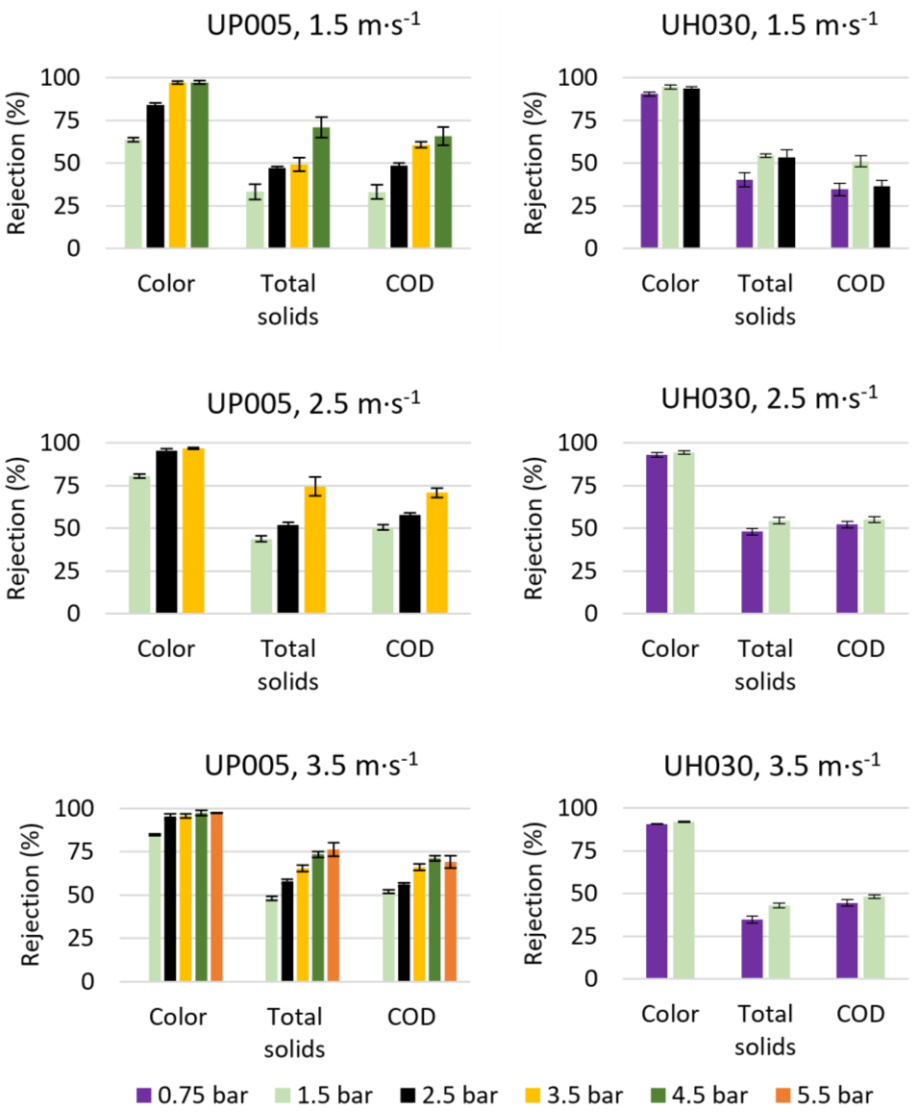


Figure 2.3. Rejection of the color, total solids and COD achieved with the UP005 and UH030 membranes at all tested operating conditions.

On the contrary, the high rejection values (for all three parameters of color, total solids, and COD) obtained with the UP150 membrane were not expected, considering the MWCO of the membrane. Interestingly, the rejections achieved with this membrane were higher than the rejection values obtained with the UP005 membrane at 1.5 $m \cdot s^{-1}$ and 0.75 bar, even when the UP150 membrane has a pore size 30 times larger than that of the UP005 membrane. The strong cake layer formed on the surface of this membrane can act as a secondary membrane, increasing the rejection values [29,30].

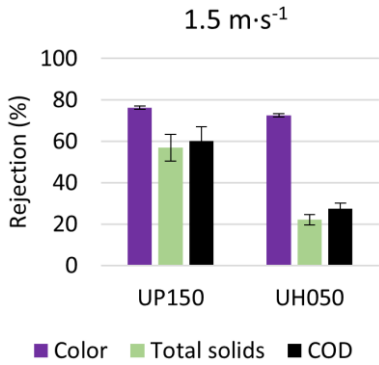


Figure 2.4. Rejection of color, total solids and COD achieved with the UP150 and UH050 membranes at 1.5 $m \cdot s^{-1}$ and 0.75 bar.

1.2.2. Rejection of the phenolic compounds

The rejection of phenolic compounds and its variation with the transmembrane pressure are shown in Figure 2.5 and Figure 2.6. In order to determine the individual rejection of each chemical family of polyphenols, the permeate and feed streams were analyzed by LC-ESI-QToF-MS. Then, each compound was quantified and assigned to a chemical class, according to their structure. This analytical strategy allowed a deep understanding of the transport of the target compounds throughout the membranes. All the compounds identified in the aqueous extracts of wet olive pomace and the permeates of the employed membranes are listed in Table 2.3. The samples were analyzed by means of a non-targeted methodology, which allowed the determination of all the phenolic compounds present in a sample within the same run. This powerful strategy allowed the identification and individual quantification of 29 phenolic compounds, belonging to four different chemical families, namely simple phenols, phenolic acids, secoiridoids, and flavonoids. This bioactive content was extremely interesting, considering the aim of valorizing the wet olive pomace and its derived extracts.

Table 2.3. Compounds determined by LC-ESI-QToF in the aqueous extracts of wet olive pomace and derived ultrafiltration streams, listed according to their retention time in the chromatographic column. The m/z and chemical family of each analyte have been provided.

Compound Identity	*m/z*	Concentration in the extract of wet olive pomace (mg/kg)[a]	Chemical family
Vanillic acid	167.0352	94 ± 2	Phenolic acids and aldehydes
Hydroxytyrosol	153.0551	18.07 ± 0.14	Simple phenols
Hydroxytyrosol glucoside	315.1081	27.14 ± 0.21	Simple phenols
Acyclodihydroelenolic acid hexoside	407.1560	161 ± 1	Secoiriodoids
Hydroxy-decarboxymethyl elenolic acid	199.0607	1630 ± 20	Secoiriodoids
Vanillin	151.0396	41.62 ± 0.51	Phenolic acids and aldehydes
Caffeic acid	179.0347	42 ± 1	Phenolic acids and aldehydes
Gallocatechin	305.0702	104 ± 5	Flavonoids
Tyrosol	137.0608	20.45 ± 0.40	Simple phenols
Hydroxyelenolic acid	257.0669	405.80 ± 29.96	Secoiriodoids
Decarboxymethyl elenolic acid	183.0658	970 ± 10	Secoiriodoids
Elenolic acid glucoside	403.1246	166 ± 6	Secoiriodoids
p-Coumaric acid	163.0397	54 ± 2	Phenolic acids and aldehydes
Aldehydic form of Decarboxymethyl Elenolic acid	215.0925	313 ± 21	Secoiriodoids
Phenylethyl primeveroside	415.1612	53.97 ± 0.76	Secoiriodoids
Hydroxyoleuropein	555.1717	37.8 ± 0.9	Secoiriodoids
Luteolin 7-O-glucoside	447.0933	38 ± 1	Flavonoids
Dehydro-oleuropein aglycone	375.1087	18.7 ± 0.1	Secoiriodoids
Elenolic acid	241.0720	657 ± 28	Secoiriodoids
Hydroxytyrosol acyclodihydroelenolate	381.1560	21.0 ± 0.4	Secoiriodoids
Ferulic acid	193.0503	36.11 ± 0.01	Phenolic acids and aldehydes
Luteolin rutinoside	593.1516	17.94 ± 0.03	Flavonoids
Oleuropein	539.1769	19.65 ± 0.19	Secoiriodoids
Luteolin	285.0405	21 ± 2	Flavonoids
Decarboxymethyl oleuropein aglycone	319.1187	0.050 ± 0.007	Secoiriodoids
Ligstroside	523.1820	19.52 ± 0.56	Secoiriodoids
Diosmetin	299.0558	17.39 ± 0.17	Flavonoids
Oleuropein aglycone	377.1242	22 ± 2	Secoiriodoids

[a]Calculated as the mean values of the three aqueous extracts employed in this study.

As shown in Figure 2.5, the UP005 membrane allowed the passage of a large proportion of simple phenols and phenolic acids, because these two chemical families were poorly rejected. Both chemical classes include the smallest molecules present in the feed stream, as reflected in Table 2.3. This was an essential outcome, because greatly valuable molecules, such as ferulic acid, *p*-coumaric acid, tyrosol, and hydroxytyrosol [31–33] were recovered in the permeate at a higher purity. The greater rejection values for this membrane corresponded to larger molecules, such as flavonoids and secoiridoids, which, at the highest pressures (2.5 – 5.5 bar) were rejected at 40% approximately. At all cross-flow velocities, the rejection of biophenols decreased when the transmembrane pressure was increased for the UP005 membrane. According to the results presented in Figure 2.1 and Figure 2.2, the UP005 membrane did not suffer an intense fouling. Therefore, it is reasonable to conclude that this phenomenon did not largely influence the observed tendency regarding the rejection values. On the contrary, this effect can be attributed to a slight enlargement of the membrane pores at high pressures, enabling a higher flux of solutes and the consecutive lowering of the rejection. This phenomenon was also described by Arkhangelsky and Gitis during the ultrafiltration of viruses. These authors found a relevant increment in the presence of viruses in the permeate of a polyethersulfone membrane (which is the same material as that of the membranes tested in this work) as a result of pressure increase. When they measured the rejection of PEG and PEO in the range 0.3-600 kDa, the MWCO varied from 28 kDa to 35 kDa as a result of a pressure increment from 1 bar to 5 bar. Therefore, these authors attributed the lowering of the rejection of viruses to the enlargement of the membrane pores [34]. In this work, the pore enlargement benefited the recovery of phenolic compounds, decreasing their rejection, because the molecular size of these compounds is similar to the pore size. However, the macromolecules (proteins, polysaccharydes, etc.) and organic matter with large molecular weight were effectively rejected (as shown in Figure 2.3), as their size was much higher than the size of the membrane pores and they were not affected by the pore enlargement.

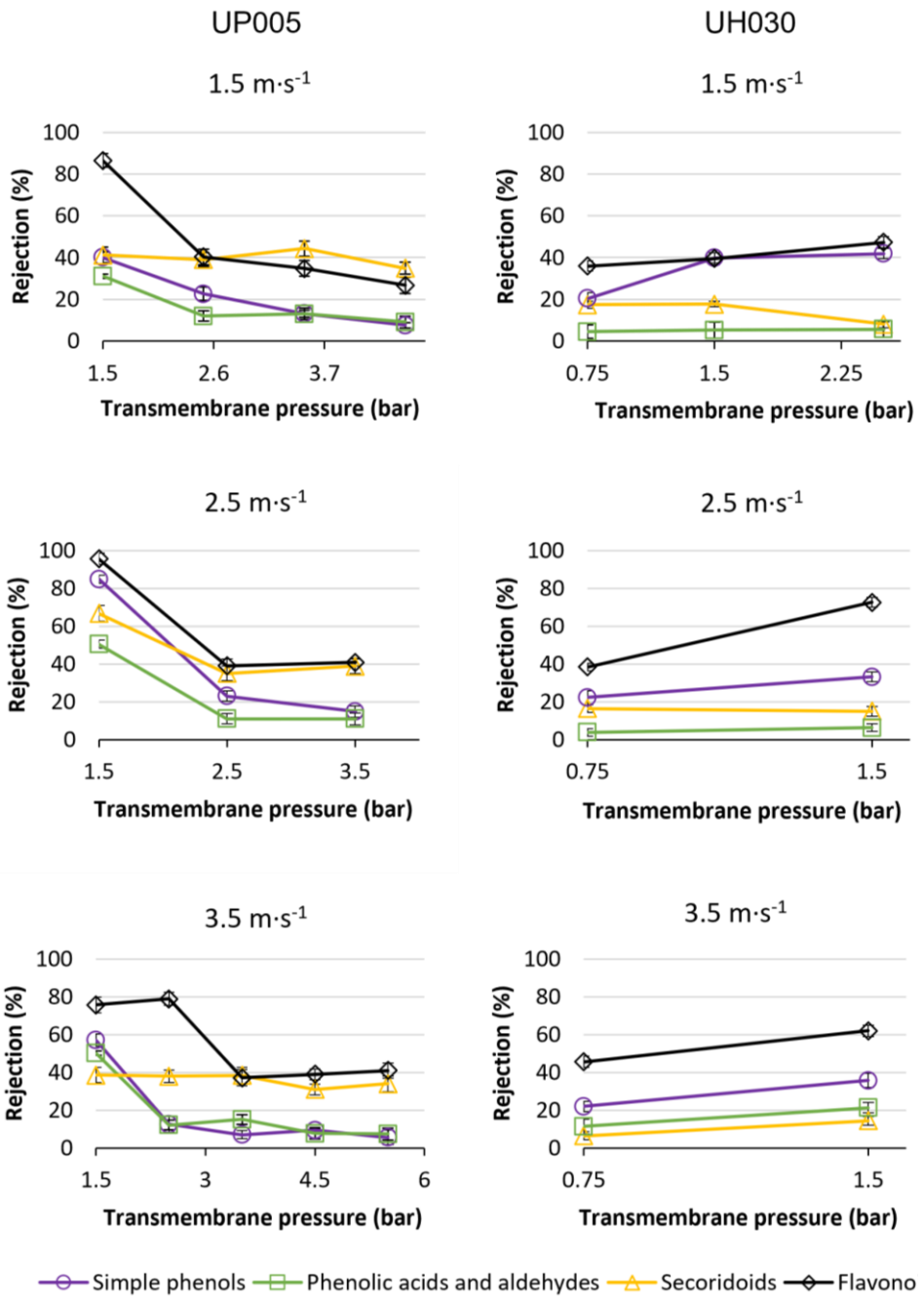


Figure 2.5. Rejection of phenolic compounds achieved with the UP005 and UH030 membranes at all the tested operating conditions.

In the case of the UH030, the rejection of phenolic compounds was also low, achieving the recovery of these valuable compounds in the permeate stream as well. Due to the gel layer formation (as commented in section 3.2), the transmembrane pressure was not increased beyond 1.5 bar. Again, the flavonoids were the most rejected molecules, whereas phenolic acids and aldehydes, secoiridoids, and simple phenols were successfully obtained in the permeate. Interestingly, the simple phenols, which include small compounds such as tyrosol (138 g/mol) and hydroxytyrosol (154 g/mol) were rejected at higher percentages than larger compounds, such as oleuropein (540 g/mol) or ligstroside (524 g/mol), belonging to the family of secoiridoids. This was more notable at the lowest cross-flow velocity (1.5 $m \cdot s^{-1}$), and can be explained by an adsorption process, favored by the hydrophilic character of the UH030 membrane, in comparison with the UP005 membrane. In fact, the adsorption of phenolic compounds on the UH030 membrane has been previously reported [15]. Virtanen et al., analyzed by computational molecular dynamics simulation the possible interactions between the UH004 membrane (which is a hydrophilic PES membrane, the same as the UH030, and is provided by the same manufacturer) and vanillin, which is a phenolic aldehyde. They described hydrophobic interactions as the main mechanism of the interaction and, additionally, they reported a significant number of hydrogen bonds occurring between the hydroxyl group of the vanillin molecules and the PES membrane, both at the ether group and the sulfur oxygen group of the PES polymer [35]. The molecule of vanillin only contains one hydroxyl group available for a hydrogen bond. However, the simple phenols present in the extract of wet olive pomace (tyrosol, hydroxytyrosol, and hydroxytyrosol derivatives) have several hydroxyl groups in their chemical structure. This suggests a high relevance of the hydrogen bonding between the simple phenols and the membrane surface, which supports the adsorption of this chemical family and, therefore, the observed rejection.

Regarding the UH050 and UP150 membranes, which had the highest MWCO, the rejection of phenolic compounds was higher for those chemical families involving molecules of larger size. Therefore, phenolic acids and aldehydes were poorly rejected, and the rejection value increased for secoiridoids and flavonoids. Still, no rejection surpassed 25% for the UH050 membrane. For the UP150 membrane, secoiridoids and flavonoids were rejected in a higher percentage. Even though the MWCO of this membrane is high, the severe fouling hindered the passage of larger compounds. Nevertheless, the purification of phenolic compounds achieved by the UP150 membrane was much more relevant than in the case of the UH050. This is because, even though both membranes displayed low rejections of the interesting biophenols, the UH050 membrane also displayed low rejections of the COD, as shown previously in Figure 2.4.

Therefore, no separation was provided by this membrane. Furthermore, the rejection of simple phenols achieved by the UH050 was expected to be lower, considering its MWCO. As occurred with the UH030 membrane, which was made of the same material as the UH050 membrane, an adsorption process is a plausible explanation for the increment in the rejection of simple phenols. Cifuentes-Cabezas et al. demonstrated the significant adsorption of tyrosol (one of the main simple phenols) and catechin (a flavonoid) onto the surface of the UH050 membrane [11].

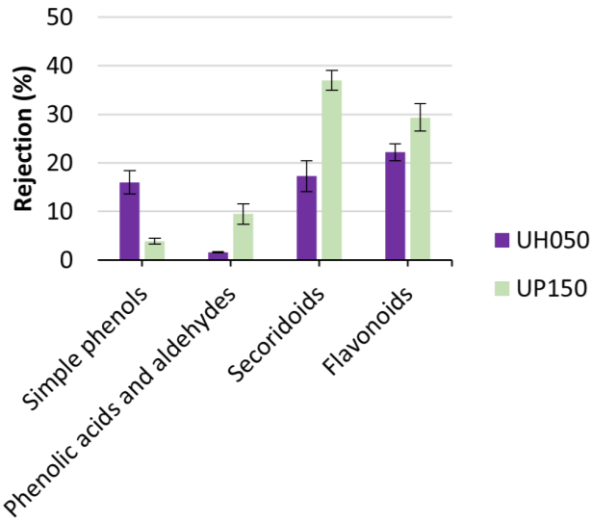


Figure 2.6. Rejection of the phenolic compounds achieved with the UH050 and UP150 membranes at 1.5 $m \cdot s^{-1}$ and 0.75 bar.

2. CONCLUSIONS

The phenolic content of the wet olive pomace was recovered by means of a simple, aqueous extraction, followed by a purification through an ultrafiltration process. According to our results, the UP005 and UH030 membranes displayed the best performance. Both membranes allowed the recovery of phenolic compounds in the permeate, and rejected unwanted species, as reflected by the high rejections of color, total solids, and COD. The selection between these two membranes is dependent on the final application. If the productivity of the process is to be pursued, the UH030 membrane displayed higher values of permeate flux (at low transmembrane pressures, when the gel layer was not so significant). On the contrary, if a finer purification of phenolic compounds is aimed, the UP005 membrane displayed higher rejections of the concomitant organic matter. In both cases, the ultrafiltration of the aqueous extracts of

wet olive pomace has demonstrated to greatly contribute to the reutilization of a concerning by-product, obtaining high added-value compounds in the process.

Funding: This work was supported by the research project CIAICO/2021/333, funded by the Regional Government of Valencia (Generalitat Valenciana). The predoctoral grant PRE2018-08524 was funded by MCIN/AEI/ 10.13039/501100011033 and by ESF Investing in your future. Funding for open access charge: CRUE-Universitat Politècnica de València.

3. REFERENCES

[1] I. Cazcarro, R. Duarte, J. Sánchez-Chóliz, C. Sarasa, A. Serrano, Environmental Footprints and Scenario Analysis for Assessing the Impacts of the Agri-Food Industry on a Regional Economy: A Case Study in Spain, J. Ind. Ecol. 19 (2015) 618–627. https://doi.org/10.1111/JIEC.12209.

[2] S. Naresh Kumar, B. Chakabarti, Energy and Carbon Footprint of Food Industry, Environ. Footprints Eco-Design Prod. Process. (2019) 19–44. https://doi.org/10.1007/978-981-13-2956-2_2/FIGURES/10.

[3] International Olive Council, DESIGNATIONS AND DEFINITIONS OF OLIVE OILS, (2023). https://www.internationaloliveoil.org/.

[4] V. Sygouni, A.G. Pantziaros, I.C. Iakovides, E. Sfetsa, P.I. Bogdou, E.A. Christoforou, C.A. Paraskeva, Treatment of two-phase olive mill wastewater and recovery of phenolic compounds using membrane technology, Membranes (Basel). 9 (2019) 27. https://doi.org/10.3390/membranes9020027.

[5] F. Sciubba, L. Chronopoulou, D. Pizzichini, V. Lionetti, C. Fontana, R. Aromolo, S. Socciarelli, L. Gambelli, B. Bartolacci, E. Finotti, A. Benedetti, A. Miccheli, U. Neri, C. Palocci, D. Bellincampi, Olive Mill Wastes: A Source of Bioactive Molecules for Plant Growth and Protection against Pathogens, Biol. 2020, Vol. 9, Page 450. 9 (2020) 450. https://doi.org/10.3390/BIOLOGY9120450.

[6] N. Caporaso, D. Formisano, A. Genovese, Use of phenolic compounds from olive mill wastewater as valuable ingredients for functional foods, Crit. Rev. Food Sci. Nutr. 58 (2018) 2829–2841. https://doi.org/10.1080/10408398.2017.1343797.

[7] P. Miralles, A. Chisvert, A. Salvador, Determination of hydroxytyrosol and tyrosol by liquid chromatography for the quality control of cosmetic products based on olive extracts, J. Pharm. Biomed. Anal. 102 (2014) 157–161. https://doi.org/10.1016/j.jpba.2014.09.016.

[8] E. Roselló-Soto, M. Koubaa, A. Moubarik, R.P. Lopes, J.A. Saraiva, N. Boussetta, N. Grimi, F.J. Barba, Emerging opportunities for the effective valorization of wastes and by-products generated during olive oil production process: Non-conventional methods for the recovery of high-added value compounds, Trends Food Sci. Technol. 45 (2015) 296–310. https://doi.org/10.1016/j.tifs.2015.07.003.

[9] A. Bottino, G. Capannelli, A. Comite, A. Jezowska, M. Pagliero, C. Costa, R. Firpo, Treatment of Olive Mill Wastewater through Integrated Pressure-Driven Membrane Processes, Membr. 2020, Vol. 10, Page 334. 10 (2020) 334. https://doi.org/10.3390/MEMBRANES10110334.

[10] C. Saf, M. Villain-Gambier, M. Belaqziz, I. Ziegler-Devin, D. Trebouet, N. Ouazzani, Fouling control investigation by pH optimization during olive mill wastewater ultrafiltration, Process Saf. Environ. Prot. 164 (2022) 119–128. https://doi.org/10.1016/J.PSEP.2022.06.010.

[11] M. Cifuentes-Cabezas, C. Carbonell-Alcaina, M.C. Vincent-Vela, J.A. Mendoza-Roca, S. Álvarez-Blanco, Comparison of different ultrafiltration membranes as first step for the recovery of phenolic compounds from olive-oil washing wastewater, Process Saf. Environ. Prot. 149 (2021) 724–734. https://doi.org/10.1016/j.psep.2021.03.035.

[12] A. Cassano, C. Conidi, L. Giorno, E. Drioli, Fractionation of olive mill wastewaters by membrane separation techniques, J. Hazard. Mater. 248–249 (2013) 185–193. https://doi.org/10.1016/j.jhazmat.2013.01.006.

[13] L. Silvestri, A. Forcina, G. Di Bona, C. Silvestri, Circular economy strategy of reusing olive mill wastewater in the ceramic industry: How the plant location can benefit environmental and economic performance, J. Clean. Prod. 326 (2021) 129388. https://doi.org/10.1016/J.JCLEPRO.2021.129388.

[14] C.M. Sánchez-Arévalo, A. Iborra-Clar, M.C. Vincent-Vela, S. Álvarez-Blanco, Exploring the extraction of the bioactive content from the two-phase olive mill waste and further purification by ultrafiltration, LWT - Food Sci. Technol. 165 (2022) 113742. https://doi.org/10.1016/j.lwt.2022.113742.

[15] C.M. Sánchez-Arévalo, A. Pérez García-Serrano, M.C. Vincent-Vela, S. Álvarez-Blanco, Combining Ultrafiltration and Nanofiltration to Obtain a Concentrated Extract of Purified Polyphenols from Wet Olive Pomace, Membranes (Basel). 13 (2023) 119. https://doi.org/10.3390/membranes13020119.

[16] Y. Wibisono, D. Alvianto, B.D. Argo, M.B. Hermanto, J.E. Witoyo, M.R. Bilad, Low-Fouling Plate-and-Frame Ultrafiltration for Juice Clarification: Part 1—Membrane Preparation and Characterization, Sustain. 15 (2023). https://doi.org/10.3390/su15010806.

[17] B. Eryildiz, B. Keskin, M.E. Pasaoglu, T. Turken, V. Vatanpour, I. Koyuncu, Preparation and characterization of polyvinyl chloride membranes and their fouling behavior in water purification, J. Appl. Polym. Sci. 140 (2023) e53496. https://doi.org/10.1002/APP.53496.

[18] C. Wang, M.J. Park, H. Yu, H. Matsuyama, E. Drioli, H.K. Shon, Recent advances of nanocomposite membranes using layer-by-layer assembly, J. Memb. Sci. 661 (2022) 120926. https://doi.org/10.1016/J.MEMSCI.2022.120926.

[19] X. You, M. Wang, G. Jiang, X. Zhao, Z. Wang, F. Liu, C. Zhao, Z. Qiu, R. Zhao, Multifunctional porous nanofibrous membranes with superior antifouling properties for oil-water separation and photocatalytic degradation, J. Memb. Sci. 668 (2023) 121245. https://doi.org/10.1016/J.MEMSCI.2022.121245.

[20] UNE-EN ISO 7887:2012 Method B, Water Quality. Examination and Determination of Colour, 2012.

[21] B. Díaz-Reinoso, A. Moure, H. Domínguez, J.C. Parajó, Ultra- and nanofiltration of aqueous extracts from distilled fermented grape pomace, J. Food Eng. 91 (2009) 587–593. https://doi.org/10.1016/j.jfoodeng.2008.10.007.

[22] M.J. Luján-Facundo, J.A. Mendoza-Roca, B. Cuartas-Uribe, S. Álvarez-Blanco, Ultrasonic cleaning of ultrafiltration membranes fouled with BSA solution, Sep. Purif. Technol. 120 (2013) 275–281. https://doi.org/10.1016/j.seppur.2013.10.018.

[23] M. Cai, Y. Lv, S. Luo, Y. Liu, P. Sun, Fouling Behavior of Polyphenols during Model Juice Ultrafiltration: Effect of Membrane Properties, Food Bioprocess Technol. 11 (2018) 1787–1793. https://doi.org/10.1007/S11947-018-2110-9/TABLES/3.

[24] M.J. Corbatón-Báguena, S. Álvarez-Blanco, M.C. Vincent-Vela, Cleaning of ultrafiltration membranes fouled with BSA by means of saline solutions, Sep. Purif. Technol. 125 (2014) 1–10. https://doi.org/10.1016/j.seppur.2014.01.035.

[25] F. Qu, H. Liang, J. Zhou, J. Nan, S. Shao, J. Zhang, G. Li, Ultrafiltration membrane fouling caused by extracellular organic matter (EOM) from Microcystis aeruginosa: Effects of membrane pore size and surface hydrophobicity, J. Memb. Sci. 449 (2014) 58–66. https://doi.org/10.1016/J.MEMSCI.2013.07.070.

[26] P. Aimar, R. Field, Limiting flux in membrane separations: A model based on the viscosity dependency of the mass transfer coefficient, Chem. Eng. Sci. 47 (1992) 579–586. https://doi.org/10.1016/0009-2509(92)80008-Z.

[27] R.W. Field, P. Aimar, Ideal limiting fluxes in ultrafiltration: comparison of various theoretical relationships, J. Memb. Sci. 80 (1993) 107–115. https://doi.org/10.1016/0376-7388(93)85136-K.

[28] M.C. Vincent-Vela, S. Álvarez-Blanco, J. Lora-García, E. Bergantiños-Rodríguez, Estimation of the gel layer concentration in ultrafiltration: Comparison of different methods, Desalin. Water Treat. 3 (2009) 157–161. https://doi.org/10.5004/dwt.2009.454.

[29] I.S. Chang, S.O. Bag, C.H. Lee, Effects of membrane fouling on solute rejection during membrane filtration of activated sludge, Process Biochem. 36 (2001) 855–860. https://doi.org/10.1016/S0032-9592(00)00284-3.

[30] M.E. Ersahin, Y. Tao, H. Ozgun, H. Spanjers, J.B. Van Lier, Characteristics and role of dynamic membrane layer in anaerobic membrane bioreactors; Characteristics and role of dynamic membrane layer in anaerobic membrane bioreactors,

Biotechnol. Bioeng. 113 (2016) 761–771. https://doi.org/10.1002/bit.25841/abstract.

[31] S. Ou, K.C. Kwok, Ferulic acid: Pharmaceutical functions, preparation and applications in foods, J. Sci. Food Agric. 84 (2004) 1261–1269. https://doi.org/10.1002/jsfa.1873.

[32] M. Robles-Almazan, M. Pulido-Moran, J. Moreno-Fernandez, C. Ramirez-Tortosa, C. Rodriguez-Garcia, J.L. Quiles, Mc. Ramirez-Tortosa, Hydroxytyrosol: Bioavailability, toxicity, and clinical applications, Food Res. Int. 105 (2018) 654–667. https://doi.org/10.1016/j.foodres.2017.11.053.

[33] M.Á. Fernández-Prior, J.C.P. Fatuarte, A.B. Oria, I. Viera-Alcaide, J. Fernández-Bolaños, G. Rodríguez-Gutiérrez, New Liquid Source of Antioxidant Phenolic Compounds in the Olive Oil Industry: Alperujo Water, Foods. 9 (2020). https://doi.org/10.3390/foods9070962.

[34] E. Arkhangelsky, V. Gitis, Effect of transmembrane pressure on rejection of viruses by ultrafiltration membranes, Sep. Purif. Technol. 62 (2008) 619–628. https://doi.org/10.1016/j.seppur.2008.03.013.

[35] T. Virtanen, P. Parkkila, A. Koivuniemi, J. Lahti, T. Viitala, M. Kallioinen, M. Mänttäri, A. Bunker, Characterization of membrane–foulant interactions with novel combination of Raman spectroscopy, surface plasmon resonance and molecular dynamics simulation, Sep. Purif. Technol. 205 (2018) 263–272. https://doi.org/10.1016/j.seppur.2018.05.050.

CAPÍTULO 3. CHAPTER 3

Effect of the operating conditions on a nanofiltration process toseparate low-molecular-weight phenolic compounds from the sugarspresent in olive mill wastewaters

Carmen M. Sánchez-Arévalo[1], Álvaro Jimeno-Jiménez[1], María Cinta Vincent-Vela[1,2], Silvia Álvarez-Blanco[1,2]

[1]Research Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

[2]Department of Chemical and Nuclear Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

✉sialvare@iqn.upv.es

Process Safety and Environmental Protection, 148 (2021) 428-436

https://doi.org/10.1016/j.psep.2020.10.002

Abstract: The efficiency of nanofiltration to purify the tyrosol present in the olive mill wastewaters (OMWWs) has been studied. The similar molecular weight of tyrosol and the sucrose existing in this kind of by-products restricts the discrimination between both molecules through a membrane process, but the interest of phenolic compounds to be applied in cosmetics and pharmacology greatly motivates its recovery at the highest purity possible. Thus, two different simulated OMWWs composed of tyrosol and mixtures of tyrosol and sucrose, respectively, were nanofiltered using the NF270 membrane. Three transmembrane pressures (TMPs) and three cross-flow velocities were tested. The optimum results were obtained at 0.5 $m \cdot s^{-1}$ and 15 bar. The rejections of the chemical oxygen demand (COD) were above 78%, whereas phenolic compounds were barely retained. This indicates that the sugar was accurately separated from tyrosol, which was recovered in the permeate stream at a high purity.

Keywords: nanolfiltration; olive mill wastewater; phenolic compounds; sucrose; separation.

1. INTRODUCTION

For some years now, phenolic compounds from the olive fruit have called particular attention. Apart from being responsible for the sensorial characteristics and stability of virgin olive oil, their principal meaning relies on their antioxidant and anti-inflammatory properties, that have been widely related with potential health benefits, including preventing the risk of suffering some heart and neurological diseases and even cancer. As a consequence of these outstanding properties, food, pharmaceutical and cosmetic industries have shown a great interest in these compounds [1–4].

Phenolic compounds are present in every product derived from the olive grove, including olive leaves, stems, seeds, fruit skin, fruit pulp and obviously olive oil [5]. As a result, these molecules can be found in the residues obtained after olive processing too. A considerable percentage of the phenolic compounds of the olive drupe is transferred to the wastewaters obtained in the olive mills (olive mill wastewaters, OMWWs) [6], and they are also present in the brines derived from the production of table olives, where tyrosol and hydroxytyrosol stand out by their high concentration [7].

However, despite the beneficial effect for human health, the reducing power of phenolic compounds implies a huge environmental impact if these products are directly discharged to the medium. Even at low concentrations, phenolic compounds are able to negatively impact the viability of microorganisms, plants and small vertebrates [8,9]. For these reasons, the treatment of olive mill wastewaters before their discharge to the

environment is mandatory, in order to reduce the high content in organic matter and the presence of phenolic compounds, which could damage the normal plant growth and the aquatic ecosystem [10].

In consequence, the recovery of phenolic compounds from OMWW results in a reduction of the toxicity of these streams (improving their further applicability as fertilizers, compost etc) as well as a corresponding collection of high-added value products with commercial or pharmaceutical applications. In this context, membrane technology appears as a relevant approach. In the recent years, the application of membrane processes to separate bioactive compounds has emerged as a satisfactory strategy [11]. The low requirements of energy, the environmental safety, the high selectivity and the easy scale up of the process make membrane technology very appropriate to selectively separate and purify phenolic compounds from the rest of species present in these by-products. To achieve this objective, some aspects regarding the membrane of choice and the sample to be treated should be considered. The efficiency of the process will highly depend on the proper choice of the membrane material, interactions among the solutes present in the feed solution and the applied operational parameters [12].

Also, the combination of different techniques and membrane procedures may be of interest. Some reported approaches to recover and purify phenolic compounds from OMWW are based in sequential membrane processes. For instance, Cassano and co-workers designed a process based on two ultrafiltration procedures followed by a nanofiltration step to fractionate OMWW from the three-phase olive oil production process [13]. In other cases, nanofiltration was carried out after one ultrafiltration operation [14], or even after a microfiltration process, obtaining satisfying results in terms of COD rejection and recovery of polyphenols as well [15,16].

The ultrafiltration of OMWW allows the removal of suspended solids and organic compounds of relatively high molecular weight. The corresponding permeate stream that is obtained is rich in phenolic compounds, as demonstrated by Carbonell-Alcaina et al. when wastewaters from the production of table olives were treated [17]. However, a subsequent nanofiltration step is essential to remove other species of lower molecular weight that remain in the ultrafiltration permeate. Enlarging the purity of the phenolic extract highly benefits its further applicability in the industry, as they may be incorporated in cosmetic preparations, food supplements etc.

In this work, the viability of nanofiltration to recover tyrosol from OMWW has been investigated using as feed a model solution composed of tyrosol and sucrose. The effect of the operating conditions on the recovery has been considered. Tyrosol was selected as it is a major constituent of OMWW and it has been widely accepted as one of the principal representatives of phenolic compounds from olive-derived matrices [18–21]. Additionally, low molecular weight phenolic compounds have been reported to have greater antioxidant activity than polymeric ones [22]. Thus, this phenolic compound attracts special interest for the cosmetic and pharmaceutical industries [23], being its recovery a great opportunity to revalue the residues from olive mills. In the case of sucrose, it was chosen as representative of the sugars present in OMWWs.

The molecular weights of tyrosol (138.18 $g \cdot mol^{-1}$) and sucrose (342.30 $g \cdot mol^{-1}$) are quite similar, which hinder a proper purification of the phenolic extract. Kontos and co-workers specifically targeted the separation of tyrosol and sucrose from a synthetic solution, but it was not through a membrane process, but a cooling crystallization treatment [24]. In fact, there are not many papers addressing the partition of these two compounds and, to our knowledge, their separation by membrane processes has not been tackled in the literature despite its interest.

2. MATERIALS AND METHODS

2.1. Feed solutions

Simulated feed solutions were prepared trying to obtain a concentration of phenolic compounds and COD similar to the actual content of real olive mill wastewaters. An olive vegetation wastewater whose characterization was previously published [25] was simulated. Two synthetic OMWWs were prepared: one of them only contained tyrosol (OMWW I); the second solution reproduced more accurately the actual content of OMWW, containing both tyrosol and sucrose (OMWW II). The sucrose content was the only difference between both solutions. Thus, it was possible to compare the behaviour of tyrosol by itself and that for tyrosol in the presence of the sugar. The influence of sucrose in the results and the study of its separation from the phenolic alcohol were then investigated.

According to the reference sample, the concentration of tyrosol (Maybridge, United Kingdom) was near to 373 $mg \cdot L^{-1}$ for both OMWW I and OMWW II. The COD of the OMWW II was required to be 2.875 g of $oxygen \cdot L^{-1}$, which corresponded to a concentration of 2.61 $g \cdot L^{-1}$ of sucrose (Panreac, Spain). Chlorhydric acid, supplied by

J.T. Baker (The Netherlands) was employed to adjust the pH to 5.3, which is the typical pH of OMWW.

2.2. Nanofiltration plant and experimental procedure

To conduct the nanofiltration tests, a pilot plant was designed. It has been schematized in Figure 3.1. The utilized feed tank had a capacity of 10 L. A NF270 membrane (Dow Chemical, EEUU), with an active area of 0.00472 m^2, was located in a flat module, which was preceded by a plunger pump. Some specifications about the membrane can be found in Table 3.1.

Table 3.1. NF270 membrane specifications provided by the manufacturer (Dow, EEUU).

Characteristics	Data
Membrane material	Thin-film polyamide composite
Minimum salt rejection[a] (%)	>97
Permeate flow rate[a]	52.93 $L \cdot h^{-1} \cdot m^{-2}$
Cl^- tolerance	<0.1 ppm
Maximum operating pressure	41 bar
Maximum operating temperature	45 °C
Operating pH range	2-11

[a]Data reported by the manufacturer, based on the following conditions MgSO4 (2000 ppm) rejection, at 25ºC, 4.8MPa and recovery of 15%.

Considering the molecular weight of the target molecule (tyrosol) and that of sucrose, the MWCO of the membrane was judged adequate to separate it from sucrose. A flowmeter and two manometers were employed. Each manometer was situated at the inlet and outlet side of the membrane module. The permeate stream was collected in a recipient placed onto a balance (PKP Balance, Kern & Sohn GmbH, Germany) which measured the permeate mass every 5 seconds. The plant was operated in a total recycle mode; therefore, the collected permeate was periodically flowed back to the feed tank and the retentate stream was continuously recycled back to the feed tank. Temperature was controlled by means of an electric resistance, that allowed the heating of the feed solution, and a cooling coil to refrigerate it. Before the conduction of any experiment, the NF membrane was washed with osmotized water (with a conductivity of 6.5 $\mu S \cdot m^{-1}$) to remove the preservative agent. After that, the membrane was immersed in an osmotized-water bath during 24 h, in order to hydrate it and remove impurities that might reduce its functionality. The compactation of the membrane was addressed afterwards. To this end, osmotized water was nanofiltrated during 4h, at 1 m/s and 18 bar. This pressure was higher than the largest pressure applied during the experiments (15 bar), to ensure that the membrane is adapted and resists this value.

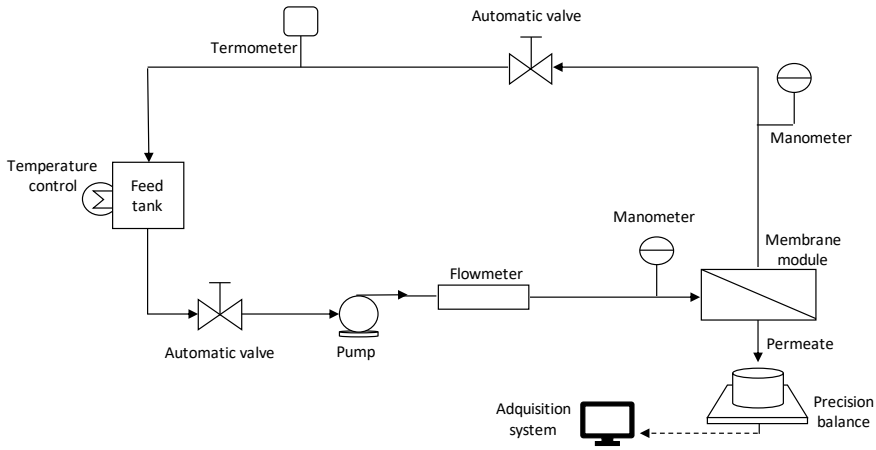


Figure 3.1. Diagram of the nanofiltration plant.

Before the filtration experiments, pure water flux was measured at a crossflow velocity of 1 m·s^{-1} and different transmembrane pressures (5, 10 and 15 bar) with osmotized water in order to determine the hydraulic permeability of the membrane (L_p) ($L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$), according to the following equation:

$$L_p = \frac{J_p}{\Delta P} \quad (1)$$

where J_p ($L \cdot h^{-1} \cdot m^{-2}$) is the permeate flux and ΔP (bar) is the transmembrane pressure.

The simulated OMWWs were nanofiltered at different transmembrane pressures (5, 10 and 15 bar) and cross-flow velocities of 0.5, 1 and 1.5 m·s^{-1} until stable permeate flux and retention values were reached (1 hour). Temperature was maintained at 25°C.

The tested values of cross-flow velocity were decided after performing a nanofiltration experiment with type II simulated OMWW at a wide range of cross-flow velocities (0.25, 0.5, 0.75, 1, 1.25 and 1.5 m·s^{-1}) and a TMP of 15 bar, as that is the tested TMP that was expected to cause the greatest membrane fouling. The indicated values of 0.5, 1 and 1.5 m·s^{-1} were selected as appropriate for the subsequent experiments.

The membrane was conveniently cleaned after each run by flushing the pilot plant with different solutions at 1 m·s^{-1}. First, tap water was flushed through the system without recirculation for 10 min. Then, P3 Ultrasil 115 (Ecolab, Barcelona, Spain) was used to remove the solutes adsorbed on the membrane surface and embedded inside the membrane pores. Six litres of an aqueous solution of this detergent at pH 11 were recirculated for 1 h. Finally, the membrane was rinsed with tap water (10 min, without

recirculation) and then with osmotized water (5 min, without recirculation and pressure, and then at 1 bar for 30 min). Water permeability was again determined after each cleaning cycle, in order to corroborate that the membrane cleaning was efficient. The cleaning process was repeated if needed until at least a 90% of the initial membrane permeability was recovered.

2.3. Streams characterization

The synthetic solutions were characterized in order to measure the concentration of phenolic compounds and the COD. Moreover, 20 mL aliquots of the permeate streams were taken at time-points of 10, 30 and 55 minutes, in order to characterize them and evaluate the rejection of the solutes and the efficiency of the process to separate them. The Folin-Ciocalteu method was conducted to determine the concentration of total phenolic compounds [26]. The COD (mg · L^{-1}) was measured by means of the LCK 014 kits supplied by Hach Lange (Germany). Then, rejection (*R*) of the membrane towards tyrosol or COD was calculated as:

$$R = \left(1 - \frac{C_p}{Cf}\right) \cdot 100 \quad (2)$$

where C_p (mg · L^{-1}) and C_f (mg · L^{-1}) are the solute concentration in the permeate and feed solution, respectively.

In order to estimate the percentage of the COD that corresponded only to the oxidation of tyrosol (COD_{TY}), the following calculation was performed:

$$COD_{TY} = \frac{TPC \cdot COD_{TY}^{+}}{COD} \cdot 100 \quad (3)$$

TPC (mg · L^{-1}) corresponds to the value of total phenolic content. COD_{TY}^{+} (mg oxygen · mg $tyrosol^{-1}$) is the theoretical consumption of oxygen that is necessary to oxidize one gram of tyrosol, and that is given by the chemical formula of the molecule:

$$C_8H_{10}O_2 + 9.5\ O_2 \rightarrow 8\ CO_2 + \ H_2O$$

Those 9.5 mol of oxygen that are needed to oxidize one mol of tyrosol correspond to 2.20 grams of oxygen per gram of tyrosol, which is the value of COD_{TY}^{+}.

2.4. Kedem-Spiegler model

In order to theoretically predict the values of permeate flux obtained for the type II model solution, which is more similar to a real OMWW, the KSM was applied [27]:

$$J_w = J_p = L_p \cdot (\Delta P - \sigma \Delta \pi) \quad (4)$$

being $\Delta\pi$ (bar) the osmotic pressure and σ the reflection coefficient, which is the maximal retention that is possible for a given solute [28].

The concentration of tyrosol was much lower than that of sucrose. Moreover, as the molecular weight of tyrosol is lower than that of sucrose, it was expected that its rejection was lower too. Therefore, taking into account both aspects, it can be considered that the osmotic pressure gradient is mainly due to sucrose. On the other hand, considering that the MWCO of the membrane is 300 Da [29] and the molecular weight of sucrose, it was supposed that sucrose did not cross the membrane, being the reflection coefficient, σ, equal to 1. As a consequence, according to the KSM, the water flux (J_w) can be defined as follows:

$$J_w = J_p = L_p \cdot (\Delta P - \Delta \pi) \quad (5)$$

The osmotic pressure of a sucrose solution was determined according to the following expression [30]:

$$\Delta\pi = -\frac{R_g \cdot T}{V_w} \cdot \ln\left(\frac{\frac{100 - C_m}{M_w} - \frac{4 \cdot C_m}{M_s}}{\frac{100 - C_m}{M_w} - \frac{3 \cdot C_m}{M_s}}\right) \quad (6)$$

where R_g (J mol^{-1} K^{-1}) is the ideal gas constant, T is the solution temperature (K), V_w is the partial molar volume of water (it was assumed as the partial molar volume for pure water, which is 18.07 · 10^{6} m^3·mol^{-1}), C_m (kg·m^{-3}) is the concentration of sucrose on the membrane surface and M_s (kg·mol^{-1}) and M_w (kg·mol^{-1}) are the molecular weight of sucrose and water, respectively.

C_m was calculated according to the film theory, which defines J_w as:

$$J_w = k \cdot ln\left(\frac{C_m - C_p}{C_f - C_p}\right) \quad (7)$$

In this expression, k (m·s^{-1}) is the mass transfer coefficient and C_f and C_p are solute concentrations in the feed and in the permeate, respectively. When considering that sucrose does not cross the membrane, $C_p \approx 0$ and then

$$C_m = C_f \cdot e^{\frac{J_w}{k}} \quad (8)$$

k was determined by means of semiempirical correlations of dimensionless numbers that were particularized for spacer-filled flat sheet and spiral wound membrane modules at turbulent flow conditions (Schock and Miquel, 1987):

$$Sh = 0.065 \cdot Re^{0.875} \cdot Sc^{0.25} \quad (9)$$

The Sherwood number (*Sh*) is a function of *k*, the hydraulic diameter of the membrane (d_h) (m) and the diffusion coefficient (D_{AB}) ($m^2 \cdot s^{-1}$):

$$Sh = \frac{k \cdot d_h}{D_{AB}} \quad (10)$$

The Reynolds number (Re) is defined as function of the density of the solution (ρ) ($kg \cdot m^{-3}$), the cross flow velocity (u) ($m \cdot s^{-1}$), d_h and the viscosity (μ) (Pa·s):

$$Re = \frac{\rho \cdot u \cdot d_h}{\mu} \quad (11)$$

And finally, the Schmidt number (*Sc*) is defined as function of μ, ρ and D_{AB}:

$$Sc = \frac{\mu}{\rho \cdot D_{AB}} \quad (12)$$

Grouping the expressions (8, 9 and 10), the following equation is obtained:

$$k = 0.065 \cdot \rho^{0.625} \cdot \mu^{-0.625} \cdot D_{AB}^{0.75} \cdot d_h^{-0.125} \cdot \mu^{0.875} \quad (13)$$

The parameters ρ, μ and D_{AB} were determined as previously reported [30], using empirical equations. The equation to determine ρ was the following:

$$\rho = \frac{100}{\frac{100 - C_f}{\rho_w} + \overline{v} \cdot C_f} \quad (14)$$

where ρ_w is the water density and $\overline{v}$ ($kg \cdot m^{-3}$) is the partial specific volume of sucrose.

Viscosity was calculated by the following expression, where μ_w corresponds to water viscosity.

$$\mu = \mu_w \cdot e^{\frac{2.61 \cdot C_f}{100 - C_f}} \quad (15)$$

Finally, the diffusion coefficient was determined as follows:

$$D_{AB,in\ mix} = D_{0,s} \cdot \left(\frac{\mu_w}{\mu}\right)^{0.45} \quad (16)$$

being $D_{0,s}$ the diffusion coefficient of a diluted solution of sucrose ($5.24 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$).

Taking into account all these correlations (equations 9 to 16), the simultaneous resolution of equations 5 (KSM) and 8 (film theory) allow the estimation of the concentration on the membrane surface and the permeate flux.

3. RESULTS AND DISCUSSION

3.1. Characterization of the feed solutions

The synthetic OMWWs were characterized right before the nanofiltration experiments, in order to know the real concentration of the analytes, COD and pH. The obtained results can be reviewed in Table 3.2.

Table 3.2. Characterization results for the simulated OMWWs, in terms of concentration of phenolic compounds, COD and pH.

	Simulated OMWW I	Simulated OMWW II
Phenolic compounds (mg/L)	330.41 ± 32.23[a]	318.44 ± 20.93
COD (mg/L)	727.67 ± 93.49	3116.00 ± 369
pH	5.34 ± 0.04	5.28 ± 0.03

[a]Standard deviation of the measurement.

3.2. Membrane characterization

The water permeability of the membrane was determined to be 15.73 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$. This value was the resulting slope of the linear fitting when permeate flux was expressed as a function of TMP. The value of permeability obtained is similar to those obtained by other authors for this membrane [32].

3.3. Variation of permeate flux with cross-flow velocity and transmembrane pressure

In Figure 3.2, the variation of permeate flux with the cross-flow velocity for the type II model solution at a TMP of 15 bar is presented. A wide range of cross-flow velocities was contemplated. In the figure, the results of permeate flux are presented in different colours and shapes for each of the tested velocities.

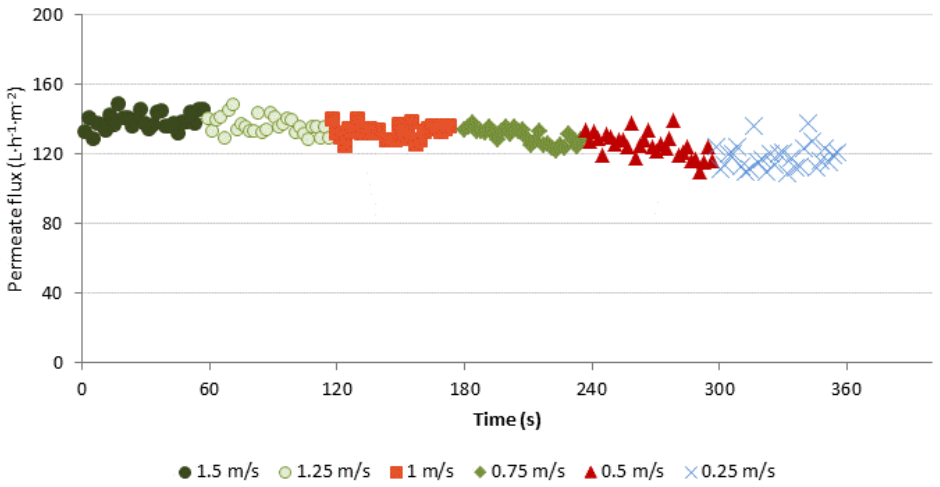


Figure 3.2. Mean values of permeate flux obtained at different cross-flow velocities, during the nanofiltration of OMWW II, at 15 bar.

It can be noted that permeate flux increased with the cross-flow velocity within the considered range. However, the differences observed were not remarkable, thus indicating that concentration polarization and fouling were not severe. From these results, it was decided to continue the following set of experiments at the highest cross-flow velocity tested (1.5 $m \cdot s^{-1}$) and at two cross-flow velocities within the tested range (1 and 0.5 $m \cdot s^{-1}$).

Figure 3.3 shows the obtained results for the nanofiltration of the two simulated OMWWs at the tested conditions of TMP and cross-flow velocity. Experiments conducted at the same cross-flow velocity have been plotted in the same graphic, in order to facilitate the interpretation and comparison of the results. The three graphics reflect a substantial increment of permeate flux as TMP increases. Moreover, no noticeable decline of permeate flux with time was appreciated and the values of the relative flux (compared to the flux obtained during the nanofiltration of pure water) were always above 77%, even in the case of the lowest cross-flow velocity tested. These aspects indicate that membrane fouling was not significant.

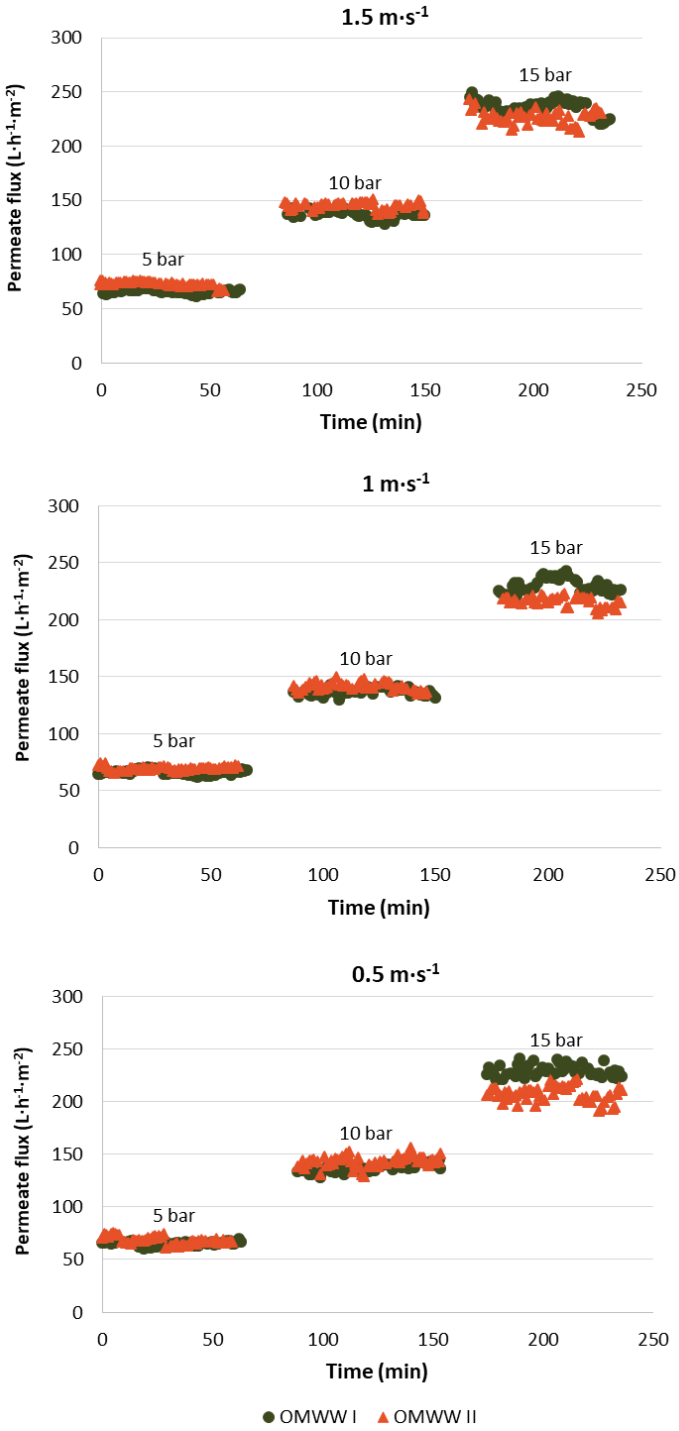


Figure 3.3. Evolution of permeate flux with time at different cross-flow velocities and transmembrane pressures.

At 5 and 10 bar, the values of the permeate flux were very similar for both types of solutions, I and II, for all the cross-flow velocities tested. Considering the composition of each simulated solution, some differences in the values of the permeate flux could be expected. However, this variation was probably reduced because, at those TMPs, the concentration polarization phenomenon was not relevant. On the contrary, at 15 bar, the simulated solution II showed lower values of permeate flux than those observed for the solution I (only containing tyrosol). The relative flux of OMWW II ranged between 89 - 98% (depending on the cross-flow velocity applied), whereas OMWW I presented a relative flux of 99-100%. The higher pressure applied in this case contributed to the concentration polarization phenomenon, which was logically more significant for the simulated solution II. Similar conclusions can be reached from Figure 3.4, which contains the stationary permeate flux observed at every pressure and cross-flow velocity studied. The values of permeability for the three tested feeds (deionized water, OMWW I and OMWW II) were very similar, being that of deionized water greater. However, at 15 bar, the lower permeate flux of OMWW II was more noticeable. It can also be observed that as cossflow velocity increases, the difference between the permeability for OMWW I and OMWW II decreases.

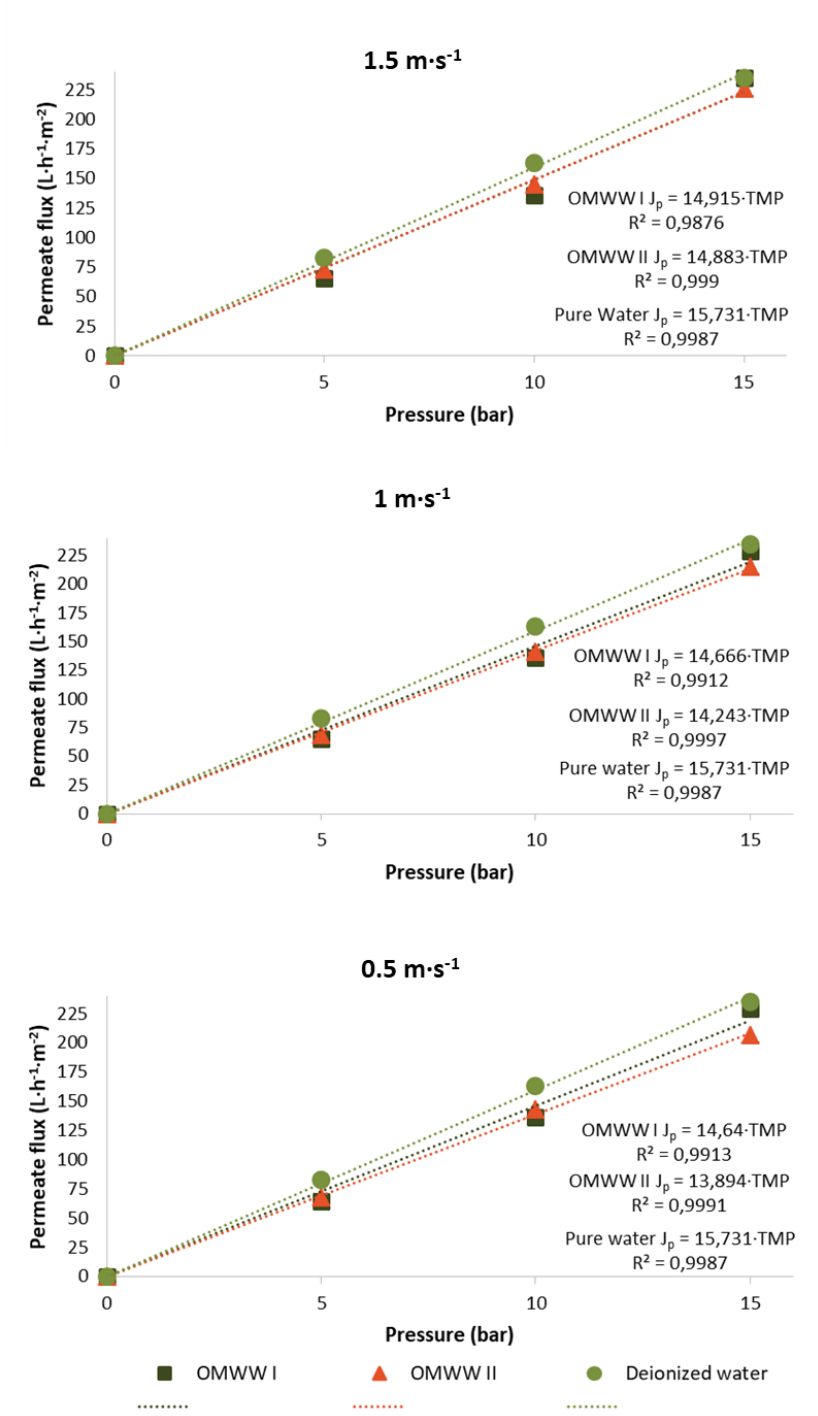


Figure 3.4. Stationary permeate flux obtained at the different pressures and cross-flow velocities applied during the nanofiltration.

3.4. Rejection of solutes

The just discussed difference in the values of permeate flux observed at 15 bar occurred mainly at the cross-flow velocities of 0.5 and 1 $m \cdot s^{-1}$. However, at 1.5 $m \cdot s^{-1}$ the values of permeate flux for both solutions were much closer. Higher values of cross-flow velocity provided a major turbulence inside the membrane module that contributed to the back diffusion of solute from the membrane surface, thus reducing the concentration polarization phenomenon, featuring higher values of permeate flux when compared with lower velocities. The highest influence of cross-flow velocity on permeate flux was observed at the largest TPM tested (15 bar), as at this TMP the convective transport of solutes towards the membrane is greatest and, therefore, the concentration polarization phenomenon is more significant. Figure 3.5A shows the rejection of phenolic compounds at different operating conditions. The data presented in the figure corresponds to steady state rejection.

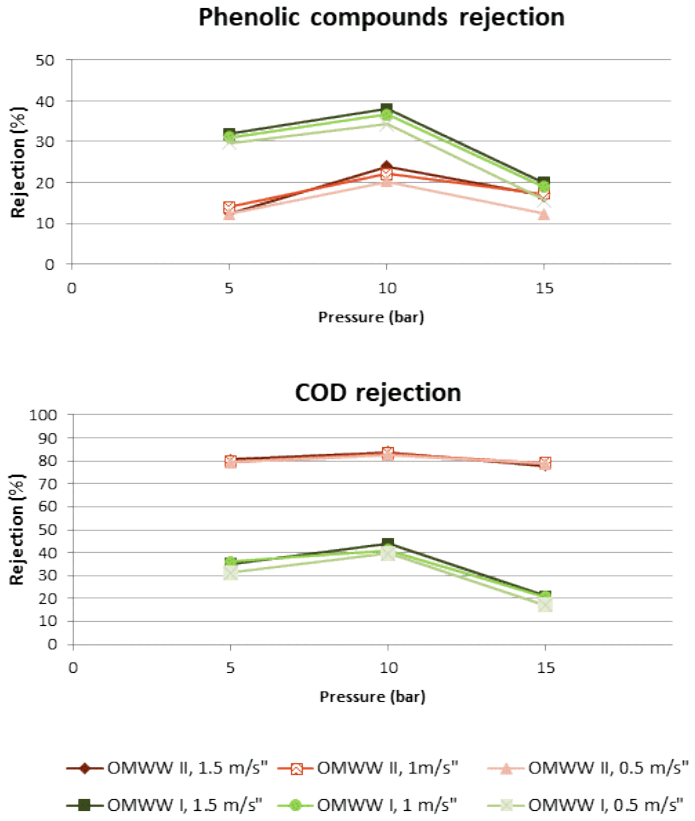


Figure 3.5. Steady state rejection of phenolic compounds (A) and COD (B) obtained for the simulated OMWW I and II at different pressures and crossflow velocities.

As it can be observed, low rejection values of tyrosol were obtained. The NF270 membrane hardly retained the phenolic compound, which facilitated its recovery in the permeate stream. In contrast, Avram and co-workers reported a complete rejection of phenolic compounds from hot water extracts of blueberry pomace, using the same membrane [32]. This discrepancy is explained by the MWCO of the membrane and the substantial difference between the molecular weight of tyrosol (present in the simulated OMWWs) and the large-size polyphenols from the blueberry pomace (mainly anthocyanins). Also, some polymerizations may have occurred among the molecules of the extract, thus increasing the rejection. In our case, the molecular weight of tyrosol was considered and the membrane NF270 was carefully chosen in order not to retain the compound of interest, but to separate it from the sugar present in the feed solution. Regarding the potential electrostatic interactions between the membrane and the compound of interest, the isoelectric point of the NF270 membrane has been described to be in the range of 3.3-5.2 [33,34]. Being the pH of the OMWWs around 5.3, it is reasonable to assume that the membrane surface may be negatively charged, at least partially. However, this scenario did not conflict with the permeation of tyrosol, because the molecule was neutral at the working pH, since it has been observed by several authors that pH values greater than 9 have to be reached in order to obtain tyrosol in its deprotonated form (Vulcano et al., 2015; Carrasco-Pancorbo et al., 2006). This facilitated the diffusion of tyrosol to the membrane and the subsequent low rejection that was observed.

According to the figure 3.5A, when the TMP was increased from 5 to 10 bar, rejection raised too, which is in accordance with the KSM for a nanofiltration process. However, when the TMP increased from 10 to 15 bar, phenolic compounds rejection decreased, which may be explained by a fouling phenomenon, prompted by the concentration polarization that was favoured at this pressure level. The effect of cross-flow velocity was barely observed, as the differences in rejection with the variations of the velocity were very small. Nevertheless, rejection did slightly increase with the cross-flow velocity, as expected. As can be seen in the figure, phenolic compounds rejections for the simulated OMWW II were lower than those for the solution I. This effect was not related with a reduction in the permeate flux, as could be expected [35], because, according to Figure 3.3 and Figure 3.4, the values of permeate flux were similar for both types of simulated solutions. As explained before, the fouling of the membrane was not harsh, thus, the observed values of permeate flux were very similar for both solutions. Instead, the decrease in the rejection for the simulated solution II may be explained by an increase in the viscosity of OMWW II (prompted by the presence of sucrose). In that case,

the mass transfer coefficient of tyrosol, *k*, would be affected too. This parameter is a diffusion rate constant related to the back diffusion of tyrosol from the membrane surface towards the bulk solution. If *k* is lower, the concentration of tyrosol on the membrane surface, C_m, is higher, which results in an increase of the concentration in the permeate and the subsequent decrease of the rejection.

Additionally, Figure 3.6 shows the values of rejection obtained at the different operation times. In general, the rejection values observed for a given pressure and cross-flow velocity scarcely varied with time, which confirms that the occurring fouling of the membrane was low. However, at the highest TMP tested (15 bar), the rejection slightly increased with time, what indicates that fouling is more noticeable at this TMP.

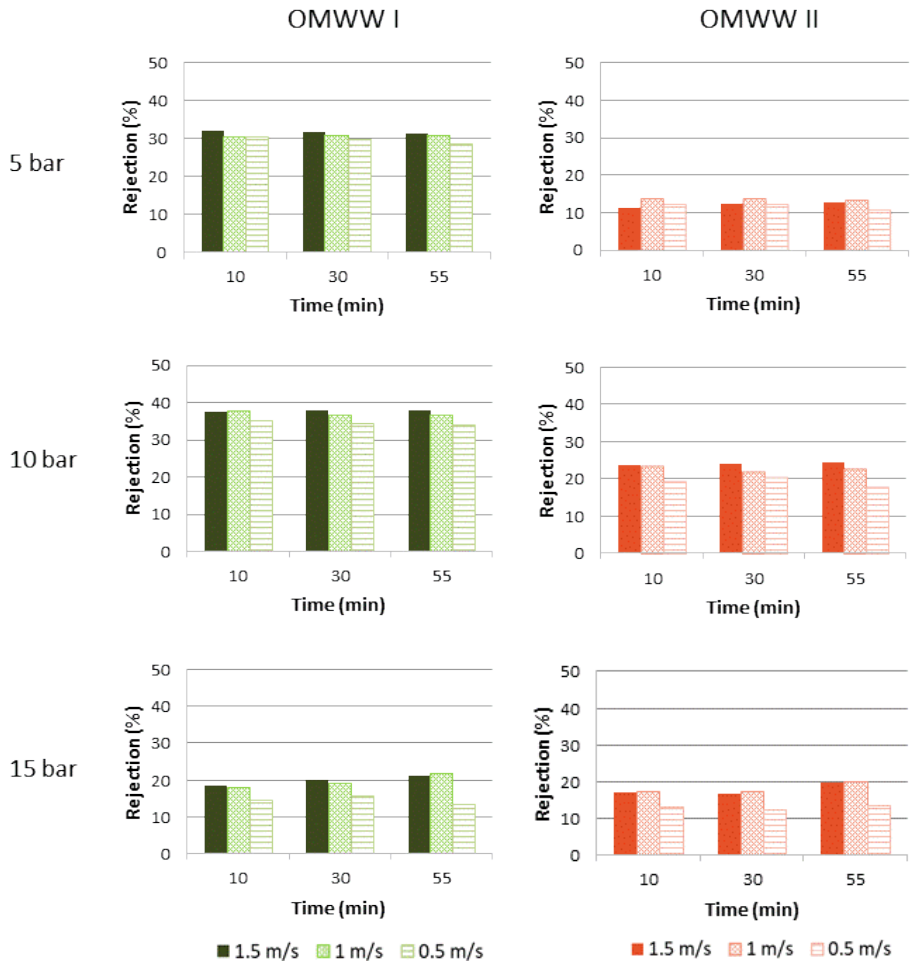


Figure 3.6. Variation of the rejection of phenolic compounds at different time-points (10, 30 and 55 minutes) during the nanofiltration experiments.

COD rejection for both simulated solutions at the different operating conditions that were tested is shown in Figure 3.5B. The data refer to steady state rejection. The same as it was observed for the rejection of phenolic compounds, the differences of COD rejection observed during the time were very small. The variation of COD rejection with TMP and cross-flow velocity followed the same trend that was already commented for the phenolic compounds. Nevertheless, the simulated OMWW II displayed much higher COD rejections than the simulated OMWW I, what indicates that sucrose rejection was very high, as sucrose is only present in OMWW II and not in OMWW I. As tyrosol rejection was observed to be low and sucrose is the only additional component in OMWW II, it is assumable that sucrose was being rejected in a high percentage. These rejection values were indicative of the selectivity of the process, which leaded to a permeate stream enriched in tyrosol and purified from the rest of components of the feed solution. Indeed, the percentage of COD in the permeate stream that corresponded only to tyrosol (calculated according to equation 3), which is shown in Figure 3.7, was above 90% for all the operating conditions tested. This indicated that practically the whole organic matter that was present in the permeate was tyrosol itself. The initial objective of the study (based on the separation of tyrosol from the sugars of the OMWW) was then satisfactorily achieved. On the other hand, the assumption of a value of σ equal to 1 for sucrose that was made at the beginning to predict the values of the permeate flux was confirmed to be correct.

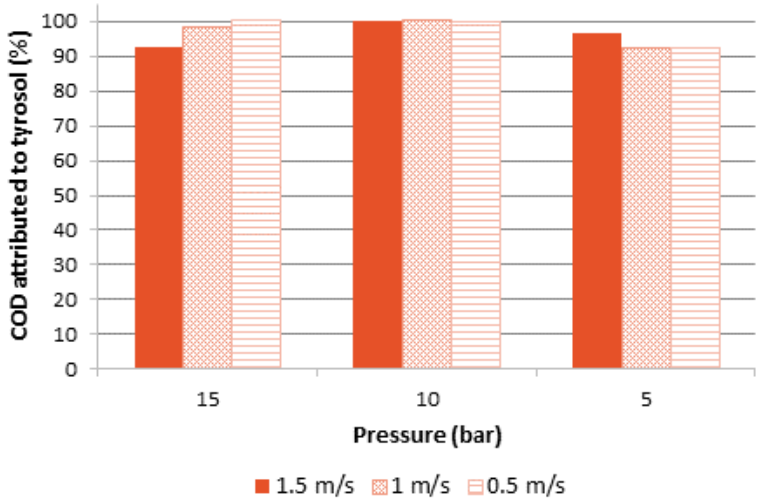


Figure 3.7. Fraction of COD in the permeate attributed to tyrosol, for the OMWW II at the different transmembrane pressures and cross-flow velocities tested. Data have been obtained by applying equation 3 to the results obtained at the operating time of 30 min.

According to these results, a TMP of 15 bar and a cross-flow velocity of 0.5 $m \cdot s^{-1}$ were selected as optimum for the separation of tyrosol and sucrose. The observed fouling was considered minor, whereas the highest permeate flux (above 225 $L \cdot h^{-1} \cdot m^{-2}$) (Figure 3.3) and the lowest phenolic compounds rejection were obtained (12.3% ± 0.2%, for OMWW II) (Figure 3.5). All these parameters should be further studied with real OMWW. Ongoing experiments are being conducted in our lab in this regard. In any case, at these conditions, it is possible to obtain a pure product, perfectly able to be incorporated in other preparations, as cosmetic or nutraceutical formulas. The format of the final product will determine if more treatments are needed, as drying, encapsulation etc. However, as the tyrosol is recovered in an aqueous phase, the solution is biocompatible, safe and easy to handle.

3.5. Predictions of Kedem-Spiegler Model

KSM was initially conceived for reverse osmosis operations; nevertheless, it has been successfully applied to the nanofiltration of uncharged molecules in previous reports [36,37]. The values of different parameters estimated by means of the combination of the KSM and the film theory for the solution II are listed in Table 3.3.

Table 3.3. Results predicted by the combination of the Kedem-Spiegler Model and the film theory and comparison with the steady state experimental permeate flux.

Pressure (bar)	Cross-flow velocity (m/s)	Re	Osmotic pressure gradient (bar)	Sucrose concentration on membrane surface (kg/m^3)	Predicted permeate flux ($L/h \cdot m^2$)	Experimental permeate flux ($L/h \cdot m^2$)	Error (%)
5	0.5	1003.3	0.376	5.221	68.324	67.507	1.210
	1	2006.6	0.309	4.297	69.532	69.155	0.546
	1.5	3009.8	0.293	4.069	73.378	72.691	0.945
10	0.5	1012.3	0.492	6.827	146.060	144.828	2.465
	1	2024.6	0.346	4.804	146.426	140.451	4.254
	1.5	3036.9	0.307	4.266	150.771	142.546	4.104
15	0.5	1021.2	0.615	8.514	209.935	205.429	2.193
	1	2042.4	0.385	5.338	223.075	215.037	3.738
	1.5	3063.6	0.323	4.492	238.231	227.586	4.677

This Table also contains the calculated Reynolds numbers, according to equation 11. All values of Re were above 1000. Schock and Miquel demonstrated that Re values above 400 correspond to turbulent flow when working with spacer-filled spiral wound or flat sheet elements [31]. This conclusion supports the application of equation 9.

To facilitate the comparison with the experimental results, only one value of experimental permeate flux has been given. This is the media of each registered value after 30 minutes of operation, where the steady state was achieved and the flux was

constant. The table reflects the phenomenon that has been commented in the previous sections: an increase in the cross-flow velocity is related with an increase in the turbulence inside the membrane module, which contributes to increase the back-diffusion of the solute towards the bulk solution and produces a decline in the osmotic pressure gradient due to the lower concentration of sucrose on the membrane surface. The theoretical values of permeate flux predicted by the model were accurately confirmed by the experimental values. According to Figure 3.8, good agreement was achieved. The difference between estimated and experimental data was lower than 5% in all cases (Table 3.3). The values of J_p experimentally observed were lower than the predicted ones, as a consequence of membrane fouling, which is not contemplated by the KSM and causes the corresponding resistance to the permeation through the membrane [38].

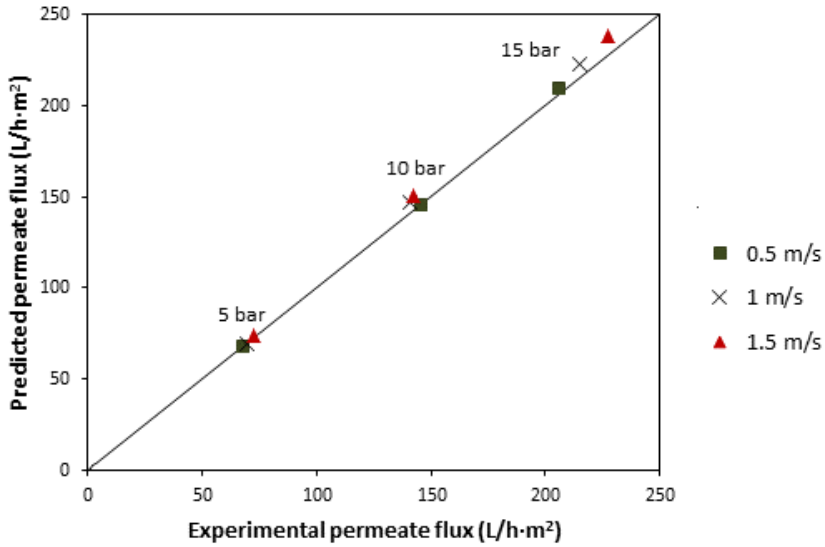


Figure 3.8. Comparison between predicted values of permeate flux according to Kedem-Spiegler model and the experimental values obtained during the nanofiltration experiments.

As the discrepancies between experimental and theoretical flux were very small, membrane fouling can be considered to be small too. Additionally, differences between predicted and experimental values of permeate flux can be also justified by the small fraction of sucrose that was not retained by the membrane. The consideration of σ equal to 1 was indeed reasonable, but, as the sucrose rejection did not achieved the 100%, it could contribute to some discrepancies between the model and the experimental results.

4. CONCLUSIONS

An efficient nanofiltration process to purify phenolic compounds from OMWWs has been developed. Special attention has been given to their separation from sugars due to the similar molecular weight. The increase of TMP resulted in higher permeate fluxes. Similarly, higher values of cross-flow velocity contributed to remove the solutes from the membrane surface and reduce the concentration polarization effect.

Tyrosol was observed to be recovered in the permeate stream, as the values of rejection ranged between 12.3% ± 0.2% and 23.9% ± 0.7%. COD rejection ranged between 77.8% and 83.9%. From the low values of COD that were determined in the permeate, more than 90% of the permeate COD can be attributed to tyrosol. Thus, it can be concluded that sucrose was highly retained by the membrane. Rejection to both tyrosol and COD increased with the increment of TMP and cross-flow velocity, but the effect of TMP was more significative.

The obtained values of permeate flux were accurately predicted by the KSM. The error was always under 4.7%, which demonstrates that the KSM was an appropriate model to predict the effect of the operating conditions on the permeate flux.

The results presented here demonstrate the suitability of membrane technology and, specifically, nanofiltration, to recover valued bioactive compounds from olive mill wastes. The retirement of the phenolic compounds from the by-products generated during the olive oil campaign contributes to their decontamination and also constitutes their revalorization, through the future industrialization of the obtained beneficial molecules.

Funding: This work was supported by the Spanish Ministry of Economy, Industry and Competitiveness through the project CTM2017-88645-R, and by the Spanish Ministry of Science, Innovation and Universities through the PRE2018-085245 pre-doctoral grant.

5. REFERENCES

[1] I. Casaburi, F. Puoci, A. Chimento, R. Sirianni, C. Ruggiero, P. Avena, V. Pezzi, Potential of olive oil phenols as chemopreventive and therapeutic agents against cancer: A review of in vitro studies, Mol. Nutr. Food Res. 57 (2013) 71–83. https://doi.org/10.1002/mnfr.201200503.

[2] J. López-Miranda, F. Pérez-Jiménez, E. Ros, R. De Caterina, L. Badimón, M.I. Covas, E. Escrich, J.M. Ordovás, F. Soriguer, R. Abiá, C. Alarcón de la Lastra, M. Battino, D. Corella, J. Chamorro-Quirós, J. Delgado-Lista, D. Giugliano, K. Esposito, R. Estruch, J.M. Fernandez-Real, J.J. Gaforio, C. La Vecchia, D. Lairon, F.

López-Segura, P. Mata, J.A. Menéndez, F.J. Muriana, J. Osada, D.B. Panagiotakos, J.A. Paniagua, P. Pérez-Martinez, J. Perona, M.A. Peinado, M. Pineda-Priego, H.E. Poulsen, J.L. Quiles, M.C. Ramírez-Tortosa, J. Ruano, L. Serra-Majem, R. Solá, M. Solanas, V. Solfrizzi, R. de la Torre-Fornell, A. Trichopoulou, M. Uceda, J.M. Villalba-Montoro, J.R. Villar-Ortiz, F. Visioli, N. Yiannakouris, Olive oil and health: Summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain) 2008, Nutr. Metab. Cardiovasc. Dis. 20 (2010) 284–294. https://doi.org/10.1016/j.numecd.2009.12.007.

[3] R. Ghanbari, F. Anwar, K.M. Alkharfy, A.H. Gilani, N. Saari, Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)-A review, 2012. https://doi.org/10.3390/ijms13033291.

[4] N.B. Ray, K.D. Hilsabeck, T.C. Karagiannis, D.E. McCord, Bioactive Olive Oil Polyphenols in the Promotion of Health, in: Role Funct. Food Secur. Glob. Heal., Elsevier Inc., 2019: pp. 623–637. https://doi.org/10.1016/b978-0-12-813148-0.00036-0.

[5] L. Olmo-García, N. Kessler, H. Neuweger, K. Wendt, J.M. Olmo-Peinado, A. Fernández-Guti rrez, C. Baessmann, A. Carrasco-Pancorbo, Unravelling the distribution of secondary metabolites in olea europaea l.: exhaustive characterization of eight olive-tree derived matrices by complementary platforms (LC-ESI/APCI-MS and GC-APCI-MS), Molecules. 23 (2018) 1–16. https://doi.org/10.3390/molecules23102419.

[6] M.D. Di Mauro, R.C. Giardina, G. Fava, E.F. Mirabella, R. Acquaviva, M. Renis, N. D'Antona, Polyphenolic profile and antioxidant activity of olive mill wastewater from two Sicilian olive cultivars: Cerasuola and Nocellara etnea, Eur. Food Res. Technol. 243 (2017) 1895–1903. https://doi.org/10.1007/s00217-017-2893-3.

[7] I. Fendri, M. Chamkha, M. Bouaziz, M. Labat, S. Sayadi, S. Abdelkafi, Olive fermentation brine: Biotechnological potentialities and valorization, Environ. Technol. (United Kingdom). 34 (2013) 183–193. https://doi.org/10.1080/09593330.2012.689364.

[8] A. Pinho, D. V Lopes, R.C. Martins, M.J. Quina, Phytotoxicity assessment of olive mill solid wastes and the in fl uence of phenolic compounds, Chemosphere. 185 (2017) 258–267. https://doi.org/10.1016/j.chemosphere.2017.07.002.

[9] S. Babic, O. Malev, P. Maryline, A.T. Lebedev, D.M. Mazur, A. Ku, Č. Rozelindra, P. Treb, Toxicity evaluation of olive oil mill wastewater and its polar fraction using multiple whole-organism bioassays, Sci. Total Environ. 686 (2019) 903–914. https://doi.org/10.1016/j.scitotenv.2019.06.046.

[10] S. Dermeche, M. Nadour, C. Larroche, F. Moulti-Mati, P. Michaud, Olive mill wastes: Biochemical characterizations and valorization strategies, Process Biochem. 48 (2013) 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010.

[11] R. Castro-Muñoz, V. Fíla, Membrane-based technologies as an emerging tool for

separating high-added-value compounds from natural products, Trends Food Sci. Technol. 82 (2018) 8–20. https://doi.org/10.1016/j.tifs.2018.09.017.

[12] E. Díaz-Montes, R. Castro-Muñoz, Metabolites recovery from fermentation broths via pressure-driven membrane processes, Asia-Pacific J. Chem. Eng. 14 (2019). https://doi.org/10.1002/apj.2332.

[13] A. Cassano, C. Conidi, L. Giorno, E. Drioli, Fractionation of olive mill wastewaters by membrane separation techniques, J. Hazard. Mater. 248–249 (2013) 185–193. https://doi.org/10.1016/j.jhazmat.2013.01.006.

[14] A. Alfano, L. Corsuto, R. Finamore, M. Savarese, F. Ferrara, S. Falco, G. Santabarbara, M. De Rosa, C. Schiraldi, Valorization of Olive Mill Wastewater by Membrane Processes to Recover Natural Antioxidant Compounds for Cosmeceutical and Nutraceutical Applications or Functional Foods, Antioxidants. 7 (2018) 72. https://doi.org/10.3390/antiox7060072.

[15] F. Bazzarelli, E. Piacentini, T. Poerio, R. Mazzei, A. Cassano, L. Giorno, Advances in membrane operations for water purification and biophenols recovery/valorization from OMWWs, J. Memb. Sci. 497 (2016) 402–409. https://doi.org/10.1016/j.memsci.2015.09.049.

[16] E. Garcia-Castello, A. Cassano, A. Criscuoli, C. Conidi, E. Drioli, Recovery and concentration of polyphenols from olive mill wastewaters by integrated membrane system, Water Res. 44 (2010) 3883–3892. https://doi.org/10.1016/j.watres.2010.05.005.

[17] C. Carbonell-Alcaina, S. Álvarez-Blanco, M.A. Bes-Piá, J.A. Mendoza-Roca, L. Pastor-Alcañiz, Ultrafiltration of residual fermentation brines from the production of table olives at different operating conditions, J. Clean. Prod. 189 (2018) 662–672. https://doi.org/10.1016/j.jclepro.2018.04.127.

[18] R. Borja, C.J. Banks, R. Maestro-Durán, J. Alba, The effects of the most important phenolic constituents of olive mill wastewater on batch anaerobic methanogenesis, Environ. Technol. (United Kingdom). 17 (1996) 167–174. https://doi.org/10.1080/09593331708616373.

[19] W. Najjar, S. Azabou, S. Sayadi, A. Ghorbel, Catalytic wet peroxide photo-oxidation of phenolic olive oil mill wastewater contaminants. Part I. Reactivity of tyrosol over (Al-Fe)PILC, Appl. Catal. B Environ. 74 (2007) 11–18. https://doi.org/10.1016/j.apcatb.2007.01.007.

[20] D. Richard, M.D.L. Delgado-Nuñez, Kinetics of the degradation by catalytic hydrogenation of tyrosol, a model molecule present in olive oil waste waters, J. Chem. Technol. Biotechnol. 78 (2003) 927–934. https://doi.org/10.1002/jctb.876.

[21] A. Cassano, C. Conidi, E. Drioli, Comparison of the performance of UF membranes

in olive mill wastewaters treatment, Water Res. 45 (2011) 3197–3204. https://doi.org/10.1016/j.watres.2011.03.041.

[22] U.T. Syed, C. Brazinha, J.G. Crespo, J.M. Ricardo-da-Silva, Valorisation of grape pomace: Fractionation of bioactive flavan-3-ols by membrane processing, Sep. Purif. Technol. 172 (2017) 404–414. https://doi.org/10.1016/j.seppur.2016.07.039.

[23] P. Miralles, A. Chisvert, A. Salvador, Determination of hydroxytyrosol and tyrosol by liquid chromatography for the quality control of cosmetic products based on olive extracts, J. Pharm. Biomed. Anal. 102 (2014) 157–161. https://doi.org/10.1016/j.jpba.2014.09.016.

[24] S.S. Kontos, F.K. Katrivesis, T.C. Constantinou, C.A. Zoga, I.S. Ioannou, P.G. Koutsoukos, C.A. Paraskeva, Implementation of membrane filtration and melt crystallization for the effective treatment and valorization of olive mill wastewaters, Sep. Purif. Technol. 193 (2018) 103–111. https://doi.org/10.1016/j.seppur.2017.11.005.

[25] J.M. Ochando-Pulido, S. Rodriguez-Vives, G. Hodaifa, A. Martinez-Ferez, Impacts of operating conditions on reverse osmosis performance of pretreated olive mill wastewater, Water Res. 46 (2012) 4621–4632. https://doi.org/10.1016/j.watres.2012.06.026.

[26] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Methods Enzymol. 299 (1999) 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

[27] K.S. Spiegler, O. Kedem, Thermodynamics of hyperfiltration (reverse osmosis): Criteria for efficient membranes, Desalination. 1 (1966) 311–326.

[28] B. Van der Bruggen, Microfiltration, ultrafiltration, nanofiltration, reverse osmosis, and forward osmosis, in: P. Luis (Ed.), Fundam. Model. Membr. Syst., Elsevier Inc., 2018: pp. 25–70. https://doi.org/10.1016/b978-0-12-813483-2.00002-2.

[29] M.J. López-Muñoz, A. Sotto, J.M. Arsuaga, B. Van der Bruggen, Influence of membrane, solute and solution properties on the retention of phenolic compounds in aqueous solution by nanofiltration membranes, Sep. Purif. Technol. 66 (2009) 194–201. https://doi.org/10.1016/j.seppur.2008.11.001.

[30] H. Nabetani, M. Nakajima, A. Watanabe, S.I. Nakao, S. Kimura, Prediction of the flux for the reverse osmosis of a solution containing sucrose and glucose, J. Chem. Eng. Japan. 25 (1992) 575–580. https://doi.org/10.1252/jcej.25.575.

[31] G. Schock, A. Miquel, Mass transfer and pressure loss in spiral wound modules, Desalination. 64 (1987) 339–352. https://doi.org/10.1016/0011-9164(87)90107-X.

[32] A.M. Avram, P. Morin, C. Brownmiller, L.R. Howard, A. Sengupta, S.R. Wickramasinghe, Concentrations of polyphenols from blueberry pomace extract using nanofiltration, Food Bioprod. Process. 106 (2017) 91–101. https://doi.org/10.1016/j.fbp.2017.07.006.

[33] M. Mänttäri, A. Pihlajamäki, M. Nyström, Effect of pH on hydrophilicity and charge and their effect on the filtration efficiency of NF membranes at different pH, J. Memb. Sci. 280 (2006) 311–320. https://doi.org/10.1016/j.memsci.2006.01.034.

[34] L.D. Nghiem, A.I. Schäfer, M. Elimelech, Pharmaceutical retention mechanisms by nanofiltration membranes, Environ. Sci. Technol. 39 (2005) 7698–7705. https://doi.org/10.1021/es0507665.

[35] C. Bellona, J.E. Drewes, P. Xu, G. Amy, Factors affecting the rejection of organic solutes during NF/RO treatment - A literature review, Water Res. 38 (2004) 2795–2809. https://doi.org/10.1016/j.watres.2004.03.034.

[36] B. Cuartas-Uribe, M.C. Vincent-Vela, S. Álvarez-Blanco, M.I. Alcaina-Miranda, E. Soriano-Costa, Application of nanofiltration models for the prediction of lactose retention using three modes of operation, J. Food Eng. 99 (2010) 373–376. https://doi.org/10.1016/j.jfoodeng.2010.03.023.

[37] B. Cuartas-Uribe, M.C. Vincent-Vela, S. Álvarez-Blanco, M.I. Alcaina-Miranda, E. Soriano-Costa, Nanofiltration of sweet whey and prediction of lactose retention as a function of permeate flux using the Kedem-Spiegler and Donnan Steric Partioning models, Sep. Purif. Technol. 56 (2007) 38–46. https://doi.org/10.1016/j.seppur.2007.01.006.

[38] A. Giacobbo, A.M. Bernardes, M.J.F. Rosa, M.N. De Pinho, Concentration polarization in ultrafiltration/nanofiltration for the recovery of polyphenols from winery wastewaters, Membranes (Basel). 8 (2018). https://doi.org/10.3390/membranes8030046.

CAPÍTULO 4. CHAPTER 4

Combining ultrafiltration and nanofiltration to obtain a concentrated extract of purified polyphenols from wet olive pomace

Carmen M. Sánchez-Arévalo[1], Ane Pérez García-Serrano[1], María Cinta Vincent-Vela[1,2], Silvia Álvarez-Blanco[1,2]

[1]Research Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

[2]Department of Chemical and Nuclear Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

✉sialvare@iqn.upv.es

Membranes 2023, 13, 119

https://doi.org/10.3390/membranes13020119

Abstract: Despite the environmental concerns raised every year by the generation of high volumes of wet olive pomace, it contains valuable phenolic compounds that are essential for the valorization of this by-product. In this work, an integrated process to recover phenolic compounds from wet olive pomace is proposed. It consists of ultrasound-assisted solid-liquid extraction, followed by ultrafiltration and nanofiltration. Several commercial membranes were studied at different operational conditions. The ultrafiltration stage allowed the purification of biophenols, which were obtained in the permeate stream. Regarding organic matter, satisfactory rejection values were obtained with both commercial UH030 and UP005 membranes (Microdyn Nadir), but the latter provided more efficient purification and higher values of permeate flux, above 18 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ at 2.5 bar and 1.5 $m \cdot s^{-1}$. Later, this permeate stream was concentrated by means of a nanofiltration process, obtaining polyphenol rejection values that surpassed 85% with the commercial NF270 membrane (DuPont), then achieving the concentration of the previously purified polyphenols.

Keywords: ultrafiltration; nanofiltration; phenolic compounds; wet olive pomace; integrated process; rejection.

1. INTRODUCTION

Annual production of virgin olive oil ends with the generation of this valuable product and, inevitably, tons of residues derived from the processing of olives [1]. As a result of the application of the two-phase methodology in an olive mill, wet olive pomace is produced. It is a semi-solid by-product, containing the remains of olive pulp, stone, skin and vegetation water. It comprises a concerning residue due to its phytotoxic character and high organic load [2]. Therefore, its treatment and detoxification preceding its disposal is of high importance.

Additionally, the principles of circular economy that have gained relevance in recent years motivate the valorization of this by-product in order to incorporate it back into the consumption chain [3]. In the context of the olive mill, wet olive pomace can be employed as a source of high-added-value compounds due to its high content of phenolic compounds. These molecules are able to reduce oxidant chemical species (reactive oxygen species, for instance), then preventing the oxidation of other compounds, such as essential biomolecules. Apart from their powerful antioxidant capacity, several authors have described their antiproliferative, anti-inflammatory and antibiotic effects, among others [4–6]. This repertoire of significant properties determines the application of polyphenols in the pharmaceutical, cosmetic and nutraceutical fields [7].

The content of phenolic compounds can be retrieved from wet olive pomace by means of solid–liquid extraction. Several techniques have been investigated with this objective, such as agitation, maceration, pressurized liquid extraction and ultrasound-assisted extraction [8–11]. In most cases, the extraction is efficient, but the obtained polyphenols are either not highly pure or very diluted. The combination of both scenarios is possible too. For these reasons, the implementation of membrane technology is an excellent strategy. It allows the possibility of working in mild operating conditions, with low energy consumption, excellent separation efficiency and control over this separation efficiency [12,13]. Furthermore, this technology permits working continuously and at smaller facilities, being environment-friendly and based on nonharmful materials [14].

The efficiency of membrane processes to treat and valorize agri-food residues has already been demonstrated. Ultrafiltration has been effectively applied to recover phenolic compounds from Eucalyptus bark [15], olive oil washing wastewater [16] or grape pomace [17]. Furthermore, a sequential process can be designed, combining ultrafiltration and nanofiltration [18], or microfiltration and nanofiltration [19]. These integrated processes logically require operating in concentration mode. Despite what it may seem, this is not trivial, because the constant increment of feed concentration greatly affects membrane fouling and, consequently, the permeate flux. Studies in recirculation mode are enormously useful during the membrane selection stage, as the concentration in the feed tank is kept constant, but assessing membrane performance in concentration mode during extended periods is mandatory if the industrial application is to be considered. Some scientific contributions applying membrane technology and working in concentration mode to recover polyphenols from wet olive pomace are summarized in Table 4.1.

Table 4.1. Description of scientific contributions about the application of membrane technology to recover polyphenols from wet olive pomace.

Process	Working mode	Permeate flux ($L \cdot h^{-1} \cdot m^{-2}$)	Polyphenols concentration	Reference
UF[1]-NF[2]-RO[3]	Concentration	n.d.[4]	200 mg GAE[5]/L	[20]
UF-NF-RO	Concentration	n.d.	32.9 mg/L flavonoids	[21]
NF	Concentration	15 (20 bar)	1234.3 ± 54.0 mg GAE/L	[22]
UF-NF	Concentration	UF: 18 (2.5 bar); NF: 47 (9 bar)	882 mg TY[6]/L	This work

[1]Ultrafiltration; [2]Nanofiltration; [3]Reverse osmosis; [4]Not detailed; [5]Gallic acid equivalents; [6]Tyrosol equivalents.

This premise regarding the concentration mode was considered in this work. In this study, an integrated process consisting of ultrafiltration and subsequent nanofiltration of an aqueous extract of wet olive pomace was investigated. A double aim was pursued. On one side, the reduction of the environmental impact of a major residue from an extended industry, as it is the olive oil sector; and, on the other side, the recovery of valuable compounds such as olive-derived polyphenols.

To that end, polymeric commercial membranes were employed to treat aqueous extracts of wet olive pomace through ultrafiltration and subsequent nanofiltration. The extracts of wet olive pomace are brown liquids, rich in sugars, phenolic compounds, triterpenes, organic acids and free fatty acids [8,23]. Considering this composition, an ultrafiltration process can be implemented to remove the undesired compounds and purify the polyphenols of interest. The obtained stream can be later concentrated by means of a nanofiltration process, obtaining a profitable product out of a challenging residue.

2. MATERIAS AND METHODS

2.1. Reagents and raw material

Wet olive pomace was obtained from the two-phase olive mill San Isidro Cooperative (Segorbe, Castellón, Spain) during the olive campaign of 2021/2022. After their collection, samples were refrigerated at 5 °C to preserve them. The Folin–Ciocalteu reagent was provided by MP Biomedicals (Ilkirch, France). To prepare the mobile phases for chromatography, acetonitrile was purchased from Honeywell (Charlotte, North Carolina, USA), and osmotized water was obtained from a Direct-Q®, 3UV system (Burlington, Massachusetts, Merck Millipore, USA). Pure standards of tyrosol, hydroxytyrosol and oleuropein were purchased from Bionova Científica (Madrid, Spain). Sigma-Aldrich (Saint Louis, Missouri, USA) provided the standards for caffeic acid, luteolin and *p*-coumaric acid.

2.2. Extractions of polyphenols

The phenolic content from the wet olive pomace was extracted according to a previously optimized methodology [8]. Briefly, 600 g of wet olive pomace were subjected to ultrasound-assisted extraction (UAE), employing osmotized water as the extractant. The UAE was performed at 40 °C for 45 min. Afterwards, the sample was centrifuged at 17,200 RCF for 6 min, and the resulting extract was vacuum filtered with a 60 µm filter (Fanoia, Barcelona, Spain) and subsequently treated by membrane technology.

2.3. Membrane processes

A simplified scheme of the proposed procedure to purify phenolic compounds from wet olive pomace can be found in Figure 4.1.

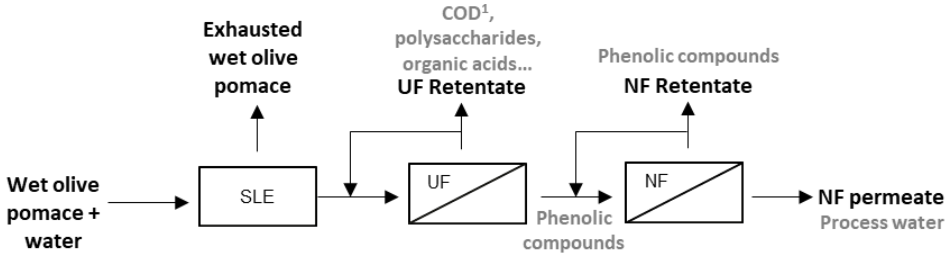


Figure 4.1. Schematic diagram of the recovery of phenolic compounds from wet olive pomace by the pro-posed integrated process consisting of solid–liquid extraction (SLE), ultrafiltration (UF) and nan-ofiltration (NF). [1]COD: chemical oxygen demand.

2.3.1. Ultrafiltration process

The aqueous extract of wet olive pomace was ultrafiltered in an ultrafiltration cross-flow plant (Orelis Environnement, Salindres, France). A Rayflow membrane module (Orelis Environnement, Salindres, France) contained two ultrafiltration membranes (Microdyn-Nadir, Wiesbaden, Germany) working in series. Each membrane was tested in a different run, in order to control the variation of the permeate flux with the volume reduction factor (VRF). The information about the tested membranes is given in Table 4.2. Microscopic characterization of the considered ultrafiltration membranes can be found in [24–26].

Table 4.2. Characteristics of the employed membranes.

Parameter	UH030	UP005	NF270
MWCO (kDa)[1]	30	5	0.3–0.4
Material	HPES [2]	PES [3]	Polyamide
Contact angle	56° ± 3° [24]	54.27° ± 3.48° [25]	15.9° ± 1.3° [26]
Manufacturer	Mycrodin Nadir	Mycrodin Nadir	DuPont
Process	UF	UF	NF

[1]Molecular weight cut off; [2]Hydrophilic polyethersulfone; [3]Polyethersulfone.

Prior to their utilization, the membranes were immersed in osmotized water overnight in order to hydrate them and remove any conservative remnants. Then, they

were compacted with osmotized water at cross-flow velocity of 1.5 m·s^{-1} and transmembrane pressure (TMP) of 3 bar until stable permeate flux was observed. The hydraulic permeability (L_w) of the membranes was also determined in the range of 1 - 2.5 bar at 1 m·s^{-1} according to the following equation:

$$L_w = \frac{J_w}{TMP} \quad (1)$$

where J_w represents water permeate flux.

Afterwards, the extract was processed at 2.5 bar and 1.5 m·s^{-1}. These conditions were selected according to previous experience from our research group [16,27,28] and preliminary experiments (manuscript in preparation). Each membrane had an area of 129 cm^2. This process was carried out in concentration mode in order to collect the ultrafiltration permeate to be treated in a subsequent nanofiltration stage. The permeate was collected until a VRF of at least 2 was achieved. In consequence, these experiments were continued for several weeks. At the end of each working day, the feed solution was removed from the ultrafiltration plant, and the membrane was rinsed for 15 min with water to reduce the accumulation of residues in the plant and avoid the development of organic fouling during the night. The next working day, the procedure was resumed. When necessary, chemical cleaning had to be done during the ultrafiltration process, as detailed in Section 2.3.3.

2.3.2. Nanofiltration process

The ultrafiltration permeate was then treated by nanofiltration in an HP4750 bench-top cell (Sterlitech**,** Auburn, Washington, USA), with a membrane area of 14.6 cm^2. The NF270 membrane (DuPont-Filmtec, Wilmington, Delaware, USA) was employed. It was previously immersed in osmotized water (for at least 12 h) and compacted at 9.5 bar. The hydraulic permeability of the membrane was tested in the range of 5 - 9 bar. Afterwards, several TMPs (5, 7, and 9 bar) were tested to treat the ultrafiltration permeate, until a VRF of 2.5 was achieved. The feed solution was constantly stirred at 500 rpm.

2.3.3. Membrane cleaning

To clean the ultrafiltration membranes, the first rinsing with tap water was followed by cleaning with a solution of P3 Ultrasil (Ecolab, Saint Paul, Minnesota, USA) at 1% (v/v) and 35°C. The chemical cleaning was maintained for 1 h at 1.5 bar and 1.5 m·s^{-1}. Afterwards, the membranes were again rinsed with tap water until the detergent was totally withdrawn from the module. This was monitored by measuring the pH of the

permeate and comparing it with the pH of tap water. The membranes were cleaned after each filtration experiment with the wet olive pomace. Additionally, a cleaning step had to be introduced during the long-term ultrafiltration operation with the UH030 membrane.

The cleaning for the nanofiltration step was dependent on the feed that was treated. Thus, the membrane was cleaned by a simple rinse with tap water, without the application of any temperature or pressure, after the treatment of the permeate from the UP005 membrane. When the permeate from the UH030 membrane was treated by nanofiltration, a solution of P3 Ultrasil at 1% (v/v) and 35°C was employed to clean the membrane. This cleaning consisted of filtration of 200 mL of the chemical solution at 2.5 bar, followed by rinsing with tap water.

2.4. Characterization of the streams

Samples from the feed solution, retentate, global permeate (corresponding to the global product obtained during the whole process) and instantaneous permeate (corresponding to the permeate obtained during the final minutes of the process) were characterized. All samples were analyzed at least in duplicates. Rejection of the solutes (R) was calculated according to the following equation:

$$R = \left(1 - \frac{C_p}{C_r}\right) \cdot 100 \quad (2)$$

where C_p is the concentration in the instantaneous permeate and C_r is the concentration in the retentate stream.

The chemical oxygen demand (COD) of the samples was analyzed by means of the commercial Spectroquant® COD Test Cells (Merck, Darmstadt, Germany). The total solids content was assessed through evaporation of a determined volume and consecutive weighing of the dry sample. pH (Crison, Barcelona, Spain) of the samples was also monitored. The Folin–Ciocalteu methodology was employed to determine the total phenolic content of the analyzed streams [29]. A pure standard of tyrosol was used to perform the external calibration of the analysis, then expressing the results as mg tyrosol/L. Additionally, the phenolic profile of the streams derived from the UP005 membrane was assessed through liquid chromatography coupled to mass spectrometry (LC-MS). To that end, a previously optimized methodology was applied [8]. Shortly, the samples were filtrated using 0.2 µm filters (ThermoFisher, Waltham, Massachusetts, USA), and the analytes were separated employing a 1260 Infinity II LC system equipped

with a Zorbax Extend C18 column (4.6 × 100 mm, 1.8 µm) (Agilent Technologies, Santa Clara, California, USA). Acidified acetonitrile and acidified water (containing 0.5% (v/v) of acetic acid) were employed to perform the gradient of the mobile phases. This instrument was coupled to a 6546 quadrupole-time-of-flight (QToF) mass analyzer (Agilent Technologies, Santa Clara, California, USA), working in negative polarity. Electrospray (ESI) was employed as the interface. Samples were injected at least in duplicates and quantified by external calibration.

3. RESULTS AND DISCUSSION

3.1. Aqueous extract of wet olive pomace

The characterization of the aqueous extract of wet olive pomace, employed as a feed solution for the process, is shown in Table 4.3. The study and optimization of the extraction stage were previously published [8]. As reflected, the COD and total solids content of the extract are considerable, making necessary the application of an ultrafiltration process to purify the extracted phenolic content. A relevant concentration of phenolic compounds is present in the wet olive pomace, enabling this residue as a source of high-added-value molecules.

Table 4.3. Characteristics of the aqueous extract of wet olive pomace.

Parameter	Concentration
COD[1] (mg/L)	8290 ± 548
Total solids (g/L)	9.05 ± 0.05
Total phenolic content (mg tyrosol/kg)	3970 ± 80
pH	5.4 ± 0.1
Conductivity (µS/cm)	1642 ± 18

[1]Chemical oxygen demand.

3.2. Performance of ultrafiltration

3.2.1. Permeate flux

After the compaction, the hydraulic permeability of each membrane was investigated, obtaining 85.7 ± 0.9 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ for the UH030 membrane and 15 ± 1 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ for the UP005 membrane. The permeate flux obtained with the UH030 and UP005 membranes when the extract was treated can be found in Figure 4.2. As can be seen in Figure 4.2, seventeen ultrafiltration stages were needed to achieve a VRF of 2 with the UH030 membrane. In the first stage, a sharp decline of the permeate flux occurred due to severe membrane fouling [30,31].

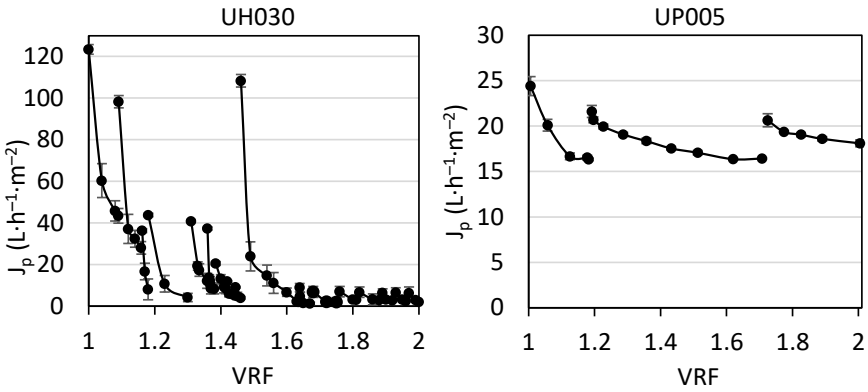


Figure 4.2. Evolution of permeate flux with the volume reduction factor (VRF) for the UH030 membrane (left) and the UP005 membrane (right) when the extract was treated.

At the beginning of the next stage, the permeate flux started at higher values with respect to the end of the first stage, and a sharp flux decline was again exhibited. This was observed because the membrane rinsing (performed at the end of each working day, as detailed in Section 2.3.1) was able to remove the incipient cake layer that was formed from the beginning of the process. The restoring of the permeate flux contributed to maintaining the efficiency of the procedure as the VRF increased, whereby the aqueous rinsing was considered adequate and it was implemented at the end of every working day. From the third stage, the initial value of the permeate flux decreased. Even though a similar curve for the flux decline was obtained every working day, the high initial values observed during the first and second stages were not obtained anymore. This was an indication of the thickening and tightening of the cake layer, which occurred from the VRF 1.15 until the VRF 1.5. The higher concentration of the feed solution (at higher VRF values) prompted a more severe concentration polarization that maximized the effect of the cake layer and membrane fouling [32,33]. As can be seen in Figure 4.2, the initial flux of the ninth ultrafiltration stage was more than four times lower than the initial permeate flux at VRF 1. In consequence, a chemical cleaning with P3 Ultrasil 115 1% (v/v) at 35 °C was performed (Section 2.3.3). Two cleaning cycles were needed to recover the hydraulic permeability of the UH030 membrane due to the severe fouling, accumulated during the extended operation time of the ultrafiltration. The permeability of the cleaned UH030 membrane was 85.2 L·h^{-1}·m^{2}·bar^{-1}. At the beginning of the tenth stage, the high initial permeate flux was restored; however, the flux decrease was again fast, as in the first and second working cycles, when the membrane was not fouled yet. In this case, membrane rinsing did not lead to satisfactory values of permeate flux, even at the beginning of the next stages, because a strong cake layer was formed from the VRF of 1.6 henceforth.

The right panel of Figure 4.2 shows the permeate flux obtained with the UP005 membrane, which was less affected by fouling. If the flux of both membranes is compared, at the beginning of the process (VRF near 1), the permeate flux was much higher for the UH030 membrane. Therefore, during the first working stage, the UH030 membrane was considered to be more productive. This was expected because at this low concentration level, the concentration polarization and fouling were still low. Then, the membrane with the highest MWCO (see Table 4.2) exhibited the highest permeate flux. However, as the ultrafiltration progressed, severe fouling was suffered by the UH030 membrane, in contrast with the UP005 membrane. This discrepancy can be attributed to several different characteristics of the membranes. First, the UH030 membrane has a larger pore size, more likely to suffer from pore blocking. This phenomenon was quite plausible here, considering the complexity of agri-food samples, such as the aqueous extract of wet olive pomace, which contains numerous organic molecules from different chemical families and a wide range of molecular weights. Other authors have also described the pore blocking of membranes with higher MWCO in comparison with tighter ultrafiltration membranes. Lujan-Facundo et al. found that bovine serum albumin blocked the pores of the UH030 membrane, leading to significant fouling [27]. Similarly, Corbatón-Báguena et al. [34] and Qu et al. [35] described that solutes with a similar size to the membrane pores can penetrate inside them and reduce the flux, obtaining better results with membranes of smaller pore size, whose pores cannot be blocked by larger molecules. Furthermore, it has been described that membranes with higher permeability (such as the UH030 membrane in this case) are more likely to suffer from gel layer formation [16], which determines a reduction in the permeate flux and greatly hinders membrane cleaning, as was commented before. Another parameter that influenced the behavior of these membranes regarding the permeate flux is the polarity of the membrane surface. In this regard, the contact angle of the UH030 and UP005 membranes is reported in Table 4.2. Both membranes are made of polyethersulfone. The contact angle of both membranes is similar and lower than 90°, which indicates a hydrophilic character. According to the manufacturer, the UH030 membrane has been modified to increase its hydrophilicity. Therefore, lower values of contact angle for this membrane could be expected. However, the roughness of the UH030 membrane is 12.12 ± 3.16 nm, whereas the UP005 membrane presents a roughness of 1.59 ± 0.20 nm [25]. According to several authors [25,36,37], rougher membranes can display higher contact angles than more hydrophobic membranes with lower roughness. Furthermore, several authors have demonstrated that membranes with higher roughness suffer from more severe fouling [38,39], as was the case for the UH030 membrane in this work. The difference in hydrophilicity between the UP005 and UH030

membranes can be essential when the feed solution includes foulants such as phenolic compounds. Phenolic compounds have been demonstrated to contribute highly to irreversible fouling of ultrafiltration membranes due to an adsorption process [40]. The affinity between those compounds and the membrane surface can determine to a great extent their adsorption [41] and, consequently, membrane fouling. Cifuentes-Cabezas et al. determined that the adsorption of phenolic compounds on the surface of the UP005 membranes was 0.349 ± 0.014 $mg \cdot m^{-2}$. These authors found higher adsorption of phenolic compounds (0.465 ± 0.037 $mg \cdot m^{-2}$) on the active layer of the UH050 membrane, whose material is the same as that of the UH030 membrane [16]. As described by Cassano et al. [42] the higher polarity of the hydrophilic polyethersulfone (as in the UH030 membrane) determines a stronger interaction with the polyphenols from wet olive pomace, leading to stronger fouling.

The less hydrophilic active layer and the lower MWCO of the UP005 membrane led to reduced fouling and therefore, flux decline was much lower for this membrane. In consequence, fewer working stages were needed to achieve a VRF of 2. This is in agreement with the work of Cifuentes-Cabezas et al., who also observed that the UP005 did not suffer from severe fouling during ultrafiltration of olive oil washing wastewater [16]. As can be seen in Figure 4.2, a simple rinsing with osmotized water at the end of every working day was effective enough to remove the fouling layer from the UP005 membrane and restore a high permeate flux, which was maintained until the end of the ultrafiltration procedure, despite the progressive concentration of the feed solution.

From the third stage until the end of the global process (Figure 4.2), very similar values of permeate flux were exhibited by the UH030 membrane at the end of every stage. Each decline curve ended in low values of permeate flux, in the range of 1.3 - 5.4 $L \cdot h^{-1} \cdot m^2$, irrespective of the VRF. The concentration factor and applied rinsing or cleaning only influenced the capacity of removing the existent cake layer (in order to address the following stage), but the final value of the permeate flux was inevitably low. This, along with the results presented in Figure 4.3, motivated the selection of the UP005 membrane for the integrated process.

3.2.2. Rejection values

The rejection values obtained with both ultrafiltration membranes are presented in Figure 4.3. The effect of the size exclusion phenomenon can be observed in the graphs presented in Figure 4.3. The UH030 membrane (Figure 4.3A) barely retained the phenolic content from the wet olive pomace because of its larger pore size.

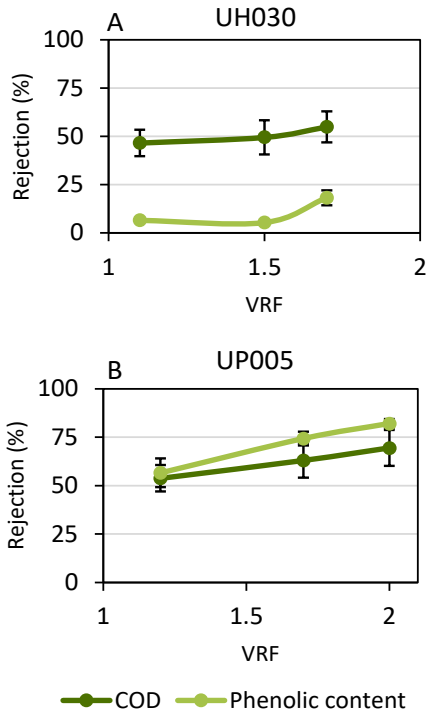


Figure 4.3. Rejection values obtained at the different volume reduction factors (VRFs) for the UH030 membrane (A) and the UP005 membrane (B).

On the contrary, the obtained values for the rejection of the COD were higher, reaching 55 ± 8%. The increment in the rejection of the COD with the VRF occurred due to the strong fouling of the membrane that was previously commented on during the discussion of Figure 4.2. The high concentration of solutes near the membrane surface prompted the formation of an additional layer which contributed to the retention of large solids, such as proteins or polysaccharides.

In the case of the UP005 membrane, a gradual increment could also be observed for COD rejection as an effect of membrane fouling. For this membrane, COD rejection reached 70 ± 2%, which was considered to be sufficient. The rejection of phenolic compounds obtained with the UP005 membrane was unexpectedly high, considering the results reported in the literature dealing with olive-derived wastewater [16,30]. Therefore, a more detailed analysis of the phenolic content of the streams derived from the UP005 was performed. These results are shown in Figure 4.4. The permeate stream obtained with the UP005 membrane was analyzed by LC-ESI-QTOF-MS in order to identify the individual compounds present in the sample and the assessed rejection for each of them. This characterization provided more detailed information than the Folin–Ciocalteu methodology, which only rendered a global rejection value.

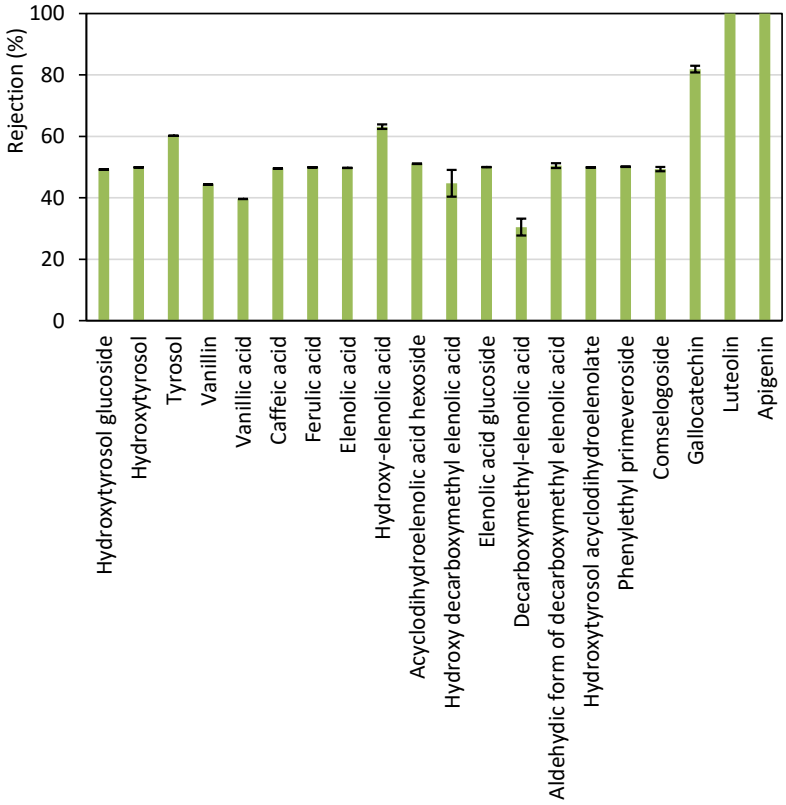


Figure 4.4. Rejection of each phenolic compound detected in the aqueous extract of wet olive pomace, obtained with the UP005 membrane at a volume reduction factor of 2. The operational conditions were 1.5 $m \cdot s^{-1}$ and 2.5 bar.

Four chemical families of phenolic compounds were found in the samples. These were simple phenols (including tyrosol, hydroxytyrosol and hydroxytyrosol glucoside), phenolic acids and aldehydes (including vanillin, vanillic acid, caffeic acid and ferulic acid), secoiridoids (elenolic acid, hydroxy-elenolic acid, acyclodihydroelenolic acid, hydroxy-decarboxymethyl elenolic acid, elenolic acid glucoside, decarboxymethyl elenolic acid, aldehydic form of decarboxymethyl elenolic acid, hydroxytyrosol acyclodihydroelenolate, phenylethyl primeveroside and comselpogoside) and flavonoids (gallocatechin, luteolin and apigenin). As can be seen in Figure 4.4, the rejection of flavonoids by the UP005 membrane was considerably high, above 80%, in comparison with that of the rest of the compounds. Therefore, the UP005 membrane achieved fractionation of polyphenols from wet olive pomace. Flavonoids were kept in the retentate (along with a high proportion of COD from the initial extract), whereas simple phenols, phenolic acids and secoridoids were recovered in the permeate. These

molecules attract great interest nowadays because of their applications in nutraceutics, pharmacy and cosmetics [43,44]. Vanillic acid and decarboxymethyl elenolic acid were the less-rejected compounds, as shown in Figure 4.4. The obtention of a permeate enriched in these two compounds is of high interest due to the bioactive properties attributed to these molecules. Vanillic acid is a hydroxybenzoic acid that has shown interesting pharmacological effects, such as antiviral effects against the Epstein–Barr virus [45]. Furthermore, it has been effectively used to enhance the growth of microalgae, which can be later applied as a source of nutrients [46]. In the case of decarboxymethyl elenolic acid, this molecule has demonstrated antimicrobial effects [47,48], which suggests potential applications related to these antibiotic properties.

Then, the results derived from the LC-MS confirmed the satisfying performance of the UP005 membrane, because it allowed the purification of valuable phenolic compounds by retaining the concomitant organic matter.

3.3. Concentration of phenolic compounds by means of nanofiltration

3.3.1. Permeate flux in the nanofiltration step

The permeate streams obtained with the UH030 and UP005 membranes were enriched in polyphenols with a potential application in industry. Therefore, the concentration of this extract was pursued by means of a nanofiltration process, employing the commercially available NF270 membrane. After its compaction, this membrane was characterized by testing its hydraulic permeability, obtaining 9 ± 1 L·h^{-1}·m^{-2}·bar^{-1}. Subsequently, the ultrafiltration permeates were submitted to nanofiltration. Figure 4.5 shows the obtained permeate flux at each transmembrane pressure applied after the treatment of both streams.

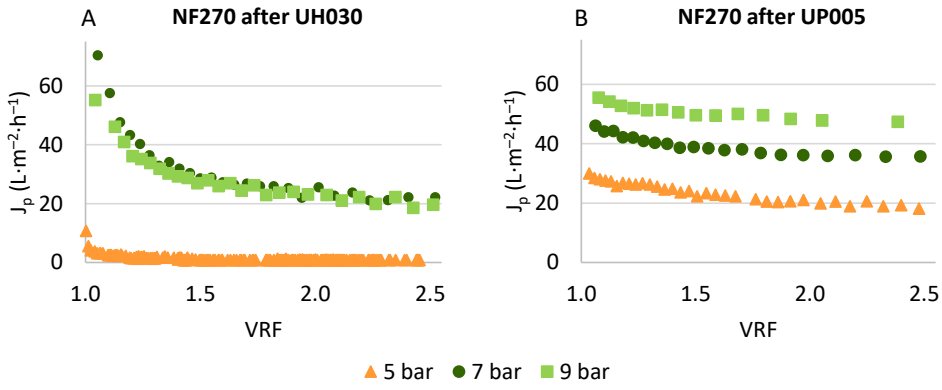


Figure 4.5. Permeate flux obtained with the NF270 membrane after the ultrafiltration of the extract with the UH030 membrane (A) and the UP005 membrane (B).

Even though the results presented in both graphs of Figure 4.5 correspond to the same membrane (NF270), the evolution of permeate flux was very different in each case. This is explained by the different compositions of the feed solutions, which corresponded to the ultrafiltration permeate obtained with each ultrafiltration membrane. The objective of the ultrafiltration process was to purify the phenolic compounds (recovered in the permeate of the ultrafiltration) and reject as much organic matter as possible. As reflected in Figure 4.3, the rejection of COD achieved by the UH030 membrane was lower than the rejection obtained with the UP005 membrane. In consequence, the NF270 membrane suffered from higher fouling during the nanofiltration of the UH030 permeate (Figure 4.5A). An increment of the permeate flux can be observed in Figure 5A when the transmembrane pressure was increased from 5 to 7 bar. However, no increase in the permeate flux was observed with pressures higher than 7 bar. At 9 bar, a slight increment of the flux is shown in Figure 4.5A, but the evolution of the permeate flux was very similar to the one obtained at 7 bar, especially at high values of the VRF. For both pressures, stable values of permeate flux of 20 ± 0.2 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ were obtained at a VRF of 2.5. As reported previously [19,26], higher pressures lead to higher deposition of solutes on the membrane surface, contributing to concentration polarization and fouling. The constant behavior of the permeate flux with the variation of transmembrane pressure is an indicator of the formation of a fouling layer on the membrane surface [49,50], which hindered solute diffusion throughout the membrane and reduced water transport too. When the pressure was increased up to 9 bar, this layer was compacted, contributing to flux reduction. This finding was also confirmed during the cleaning stage. The cleaning of the NF270 membrane after the processing of the UH030 permeate was more difficult than after treating the UP005 permeate. The detergent P3 Ultrasil (Ecolab, Spain) at 1% (v/v) had to be employed at 35°C in order to recover the initial permeability of the NF270 membrane when the UH030 permeate was treated.

When the NF270 membrane was employed to treat the UP005 permeate, the permeate flux obtained increased linearly with transmembrane pressure, which indicated low fouling. This reduced fouling was in accordance with the high rejection of the organic matter that was observed during the ultrafiltration stage (Figure 4.3B), which led to a purified extract. In fact, the cleaning of the membrane after each experiment was performed simply by rinsing with tap water, and 100% of hydraulic permeability was recovered. This allowed the recycling of the membrane, which was reused during the whole process. The permeate flux obtained at all pressures was satisfactory. However, the membrane was more productive at 9 bar. At this pressure, a high permeate flux of 44.24 ± 0.08 $L \cdot h\text{-}1 \cdot m^{-2} \cdot bar^{-1}$ was achieved at a VRF of 2.5. Therefore, this pressure was

selected at the most convenient for the concentration of the previously purified extract of wet olive pomace.

3.3.2. Rejection values in nanofiltration

In order to evaluate the concentration of the aimed compounds, the rejection values obtained for total solids, COD and phenolic content were studied. These values are reflected in Figure 4.6.

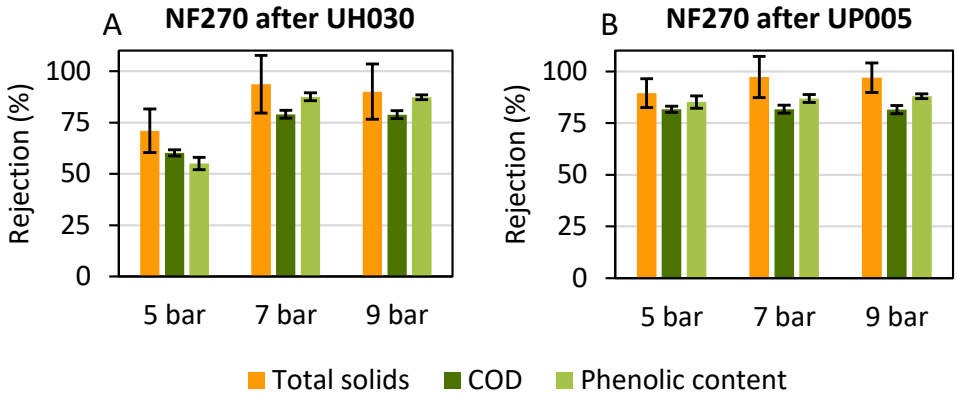


Figure 4.6. Rejection values obtained with the NF270 membrane at a volume reduction factor of 2.5 after the treatment of the permeate obtained with the UH030 membrane (A) and the UP005 membrane (B).

The NF270 membrane was able to retain most of the solutes present in the permeate of the previous ultrafiltration. As already commented, the polyphenols present in the nanofiltration feed (that is the ultrafiltration permeate) were already highly purified. This means that the majority of the organic molecules present in this stream were phenolic compounds, especially in the stream corresponding to the permeate obtained with the UP005 membrane. For this reason, the rejection of total solids, COD and phenolic compounds was very similar, because the measurement of total solids and COD included the polyphenols.

At all studied pressures, the rejection values were high, in line with those from previous works [51,52]. During the treatment of the UH030 permeate, an increment in the rejection of total solids, COD and phenolic compounds could be observed when the pressure was increased from 5 bar to 7 bar (Figure 4.6A). In this case, this effect cannot be attributed to the compaction of the membrane with the pressure increment, because it was initially submitted to high pressure (see Section 2.3.2) to avoid further inconsistencies during the study. Instead, the increment in the rejection can be explained by an increase in the flux of water, which was not coupled to an increment in the flux of

solutes. In an NF process, the diffusion of solutes plays an essential role in solutes transport [53]. However, this diffusion is not affected by TMP [54,55], whereas the flux of the solvent (water in this case) is highly dependent on the applied pressure. Then, increasing the pressure resulted in a high increment of the flux of water, whereas the flux of solutes did not suffer such a high rise. Figure 5A supports this reasoning, as a high increment in the permeate flux was observed when the pressure increased from 5 bar to 7 bar. In consequence, at 7 bar, the rejection of total solids, COD and phenolic compounds was higher than the values observed for 5 bar. At 9 bar, the rejection values were very similar to those at 7 bar. For instance, the rejection of total phenols was 87 ± 2% at 7 bar and 87 ± 1% at 9 bar. According to Figure 4.5, permeate flux did not increase either. As can be inferred from Figure 6A, this last increment in pressure did not derive any improvement for the membrane performance due to membrane fouling. On the contrary, the pressure increment from 5 bar to 7 bar enhanced the permeate flux and the rejection values, leading to a more efficient process, with a higher concentration of the desired compounds.

When the UP005 permeate was nanofiltered with the NF270 membrane, high rejections of COD, total solids and phenolic compounds were obtained at all pressures. High, satisfactory rejection values were achieved even when applying the lowest pressure. A slight increment in the rejection of the polyphenols of interest, COD and total solids can be observed in Figure 6B as TMP increased. Even though the selection of 5 bar to concentrate the purified extract of wet olive pomace could be suggested, high values of permeate flux and higher rejection of biophenols obtained at 9 bar should be taken into account too. Considering that the process was more productive at the highest pressure and that no fouling was observed, the application of 9 bar was selected for the concentration stage.

4. CONCLUSIONS

Ultrafiltration is a suitable membrane process to perform the purification of phenolic compounds from wet olive pomace. The UH030 membrane suffered from severe fouling that led to low values of permeate flux, whereas this fouling was not observed for the UP005 membrane. The UP005 membrane led to satisfactory results of permeate flux. Regarding rejections, this membrane rejected 70% of COD, allowing the passage of molecules of interest, such as several families of phenolic compounds of high added value. These could be later concentrated by a nanofiltration process by employing the NF270 membrane, which rejected more than 80% of the phenolic content at 9 bar and provided a high permeate flux. Then, the combination of ultrasound-assisted solid-liquid

extraction with water (at 40°C, for 45 min), ultrafiltration with the UP005 membrane (2.5 bar, 1.5 $m \cdot s^{-1}$) and nanofiltration with the NF270 membrane (9 bar) allowed the obtention of concentrated phenolic compounds at high purity.

Author contributions: Conceptualization, C.M.S.-A., M.C.V.-V. and S.A.-B.; methodology, C.M.S-A. and A.P.G.-S.; software, C.M.S.-A.; validation, C.M.S-A.; formal analysis, C.M.S-A., M.C.V.-V. and S.A.-B.; in-vestigation, C.M.S.-A., M.C.V.-V. and S.A.-B.; resources, M.C.V.-V. and S.A.-B.; data curation, C.M.S.-A.; writing—original draft preparation, C.M.S-A.; writing—review and editing, M.C.V.-V. and S.A.-B.; visualization, C.M.S.-A., M.C.V.-V. and S.A-B.; supervision, M.C.V.-V. and S.A.-B.; project administration, M.C.V.-V. and S.A.-B.; funding acquisition, M.C.V.-V. and S.A.-B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by MCIN/AEI/ 10.13039/501100011033 and by ERDF A way of making Europe, grant number CTM2017-88645-R. Additionally, a predoctoral grant PRE2018-08524 was funded by MCIN/AEI/ 10.13039/501100011033 and by ESF Investing in your future.

5. REFERENCES

[1] F. Sciubba, L. Chronopoulou, D. Pizzichini, V. Lionetti, C. Fontana, R. Aromolo, S. Socciarelli, L. Gambelli, B. Bartolacci, E. Finotti, A. Benedetti, A. Miccheli, U. Neri, C. Palocci, D. Bellincampi, Olive Mill Wastes: A Source of Bioactive Molecules for Plant Growth and Protection against Pathogens, Biol. 2020, Vol. 9, Page 450. 9 (2020) 450. https://doi.org/10.3390/BIOLOGY9120450.

[2] S. Dermeche, M. Nadour, C. Larroche, F. Moulti-Mati, P. Michaud, Olive mill wastes: Biochemical characterizations and valorization strategies, Process Biochem. 48 (2013) 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010.

[3] M. Donner, Y. Erraach, F. López-i-Gelats, J. Manuel-i-Martin, T. Yatribi, I. Radić, F. El Hadad-Gauthier, Circular bioeconomy for olive oil waste and by-product valorisation: Actors' strategies and conditions in the Mediterranean area, J. Environ. Manage. 321 (2022) 115836. https://doi.org/10.1016/J.JENVMAN.2022.115836.

[4] J. Quero, L.F. Ballesteros, P. Ferreira-Santos, G.R. Velderrain-Rodriguez, C.M.R. Rocha, R.N. Pereira, J.A. Teixeira, O. Martin-Belloso, J. Osada, M.J. Rodríguez-Yoldi, Unveiling the Antioxidant Therapeutic Functionality of Sustainable Olive Pomace Active Ingredients, Antioxidants 2022, Vol. 11, Page 828. 11 (2022) 828. https://doi.org/10.3390/ANTIOX11050828.

[5] M. Robles-Almazan, M. Pulido-Moran, J. Moreno-Fernandez, C. Ramirez-Tortosa, C. Rodriguez-Garcia, J.L. Quiles, Mc. Ramirez-Tortosa, Hydroxytyrosol: Bioavailability, toxicity, and clinical applications, Food Res. Int. 105 (2018) 654–

667. https://doi.org/10.1016/j.foodres.2017.11.053.

[6] J. Park, J.S. Min, U. Chae, J.Y. Lee, K.S. Song, H.S. Lee, H.J. Lee, S.R. Lee, D.S. Lee, Anti-inflammatory effect of oleuropein on microglia through regulation of Drp1-dependent mitochondrial fission, J. Neuroimmunol. 306 (2017) 46–52. https://doi.org/10.1016/j.jneuroim.2017.02.019.

[7] N. Malekjani, S.M. Jafari, Valorization of olive processing by-products via drying technologies: a case study on the recovery of bioactive phenolic compounds from olive leaves, pomace, and wastewater, Crit. Rev. Food Sci. Nutr. (2022). https://doi.org/10.1080/10408398.2022.2068123.

[8] C.M. Sánchez-Arévalo, A. Iborra-Clar, M.C. Vincent-Vela, S. Álvarez-Blanco, Exploring the extraction of the bioactive content from the two-phase olive mill waste and further purification by ultrafiltration, LWT - Food Sci. Technol. 165 (2022) 113742. https://doi.org/10.1016/j.lwt.2022.113742.

[9] O. Zannou, H. Pashazadeh, S.A. Ibrahim, I. Koca, C.M. Galanakis, Green and highly extraction of phenolic compounds and antioxidant capacity from kinkeliba (Combretum micranthum G. Don) by natural deep eutectic solvents (NADESs) using maceration, ultrasound-assisted extraction and homogenate-assisted extraction, Arab. J. Chem. 15 (2022) 103752. https://doi.org/10.1016/J.ARABJC.2022.103752.

[10] I. Cea-Pavez, J. Lozano-Sánchez, I. Borrás-Linares, H. Nuñez, P. Robert, A. Segura-Carretero, Obtaining an Extract Rich in Phenolic Compounds from Olive Pomace by Pressurized Liquid Extraction, Molecules. 24 (2019) 3108. https://doi.org/10.3390/MOLECULES24173108.

[11] B. Díaz-Reinoso, A. Moure, H. Domínguez, J.C. Parajó, Ultra- and nanofiltration of aqueous extracts from distilled fermented grape pomace, J. Food Eng. 91 (2009) 587–593. https://doi.org/10.1016/j.jfoodeng.2008.10.007.

[12] S.P. Bera, M. Godhaniya, C. Kothari, Emerging and advanced membrane technology for wastewater treatment: A review, J. Basic Microbiol. 62 (2022) 245–259. https://doi.org/10.1002/JOBM.202100259.

[13] S.K. Sikdar, A. Criscuoli, Sustainability and How Membrane Technologies in Water Treatment Can Be a Contributor, (2017) 1–21. https://doi.org/10.1007/978-981-10-5623-9_1.

[14] E. Pekgenc, B. Yavuzturk Gul, V. Vatanpour, I. Koyuncu, Biocatalytic membranes in anti-fouling and emerging pollutant degradation applications: Current state and perspectives, Sep. Purif. Technol. 282 (2022) 120098. https://doi.org/10.1016/J.SEPPUR.2021.120098.

[15] P.R. Pinto, I.F. Mota, C.M. Pereira, A.M. Ribeiro, J.M. Loureiro, A.E. Rodrigues, Separation and recovery of polyphenols and carbohydrates from Eucalyptus bark

extract by ultrafiltration/diafiltration and adsorption processes, Sep. Purif. Technol. 183 (2017) 96–105. https://doi.org/10.1016/j.seppur.2017.04.003.

[16] M. Cifuentes-Cabezas, C. Carbonell-Alcaina, M.C. Vincent-Vela, J.A. Mendoza-Roca, S. Álvarez-Blanco, Comparison of different ultrafiltration membranes as first step for the recovery of phenolic compounds from olive-oil washing wastewater, Process Saf. Environ. Prot. 149 (2021) 724–734. https://doi.org/10.1016/j.psep.2021.03.035.

[17] S. Yammine, R. Rabagliato, X. Vitrac, M.M. Peuchot, R. Ghidossi, Selecting ultrafiltration membranes for fractionation of high added value compounds from grape pomace extracts, OENO One. 53 (2019) 487–497. https://doi.org/10.20870/OENO-ONE.2019.53.3.2343.

[18] A. Alfano, L. Corsuto, R. Finamore, M. Savarese, F. Ferrara, S. Falco, G. Santabarbara, M. De Rosa, C. Schiraldi, Valorization of Olive Mill Wastewater by Membrane Processes to Recover Natural Antioxidant Compounds for Cosmeceutical and Nutraceutical Applications or Functional Foods, Antioxidants. 7 (2018) 72. https://doi.org/10.3390/antiox7060072.

[19] D. Tamires Vitor Pereira, G. Vollet Marson, G. Fernández Barbero, A. Gadioli Tarone, C. Baú Betim Cazarin, M. Dupas Hubinger, J. Martínez, Concentration of bioactive compounds from grape marc using pressurized liquid extraction followed by integrated membrane processes, Sep. Purif. Technol. 250 (2020). https://doi.org/10.1016/j.seppur.2020.117206.

[20] V. Sygouni, A.G. Pantziaros, I.C. Iakovides, E. Sfetsa, P.I. Bogdou, E.A. Christoforou, C.A. Paraskeva, Treatment of two-phase olive mill wastewater and recovery of phenolic compounds using membrane technology, Membranes (Basel). 9 (2019) 27. https://doi.org/10.3390/membranes9020027.

[21] P. Tapia-Quirós, M.F. Montenegro-Landívar, M. Reig, X. Vecino, J. Saurina, M. Granados, J.L. Cortina, Integration of membrane processes for the recovery and separation of polyphenols from winery and olive mill wastes using green solvent-based processing, J. Environ. Manage. 307 (2022). https://doi.org/10.1016/J.JENVMAN.2022.114555.

[22] M.A. Nunes, S. Pawlowski, A.S.G. Costa, R.C. Alves, M.B.P.P. Oliveira, S. Velizarov, Valorization of olive pomace by a green integrated approach applying sustainable extraction and membrane-assisted concentration, Sci. Total Environ. 652 (2019) 40–47. https://doi.org/10.1016/j.scitotenv.2018.10.204.

[23] I. Criado-Navarro, C.A. Ledesma-Escobar, M.J. Parrado-Martínez, R.M. Marchal-López, J.M. Olmo-Peinado, J.A. Espejo-Calvo, F. Priego-Capote, Monitoring the partition of bioactive compounds in the extraction of extra virgin olive oil, LWT - Food Sci. Technol. 162 (2022). https://doi.org/10.1016/j.lwt.2022.113433.

[24] S. Metsämuuronen, M. Nyström, Enrichment of α-lactalbumin from diluted whey with polymeric ultrafiltration membranes, J. Memb. Sci. 337 (2009) 248–256.

https://doi.org/10.1016/J.MEMSCI.2009.03.052.

[25] M.J. Luján-Facundo, J.A. Mendoza-Roca, B. Cuartas-Uribe, S. Álvarez-Blanco, Evaluation of cleaning efficiency of ultrafiltration membranes fouled by BSA using FTIR-ATR as a tool, J. Food Eng. 163 (2015) 1–8. https://doi.org/10.1016/j.jfoodeng.2015.04.015.

[26] G.S. Vieira, F.K.V. Moreira, R.L.S. Matsumoto, M. Michelon, F.M. Filho, M.D. Hubinger, Influence of nanofiltration membrane features on enrichment of jussara ethanolic extract (Euterpe edulis) in anthocyanins, J. Food Eng. 226 (2018) 31–41. https://doi.org/10.1016/J.JFOODENG.2018.01.013.

[27] M.J. Luján-Facundo, J.A. Mendoza-Roca, B. Cuartas-Uribe, S. Álvarez-Blanco, Ultrasonic cleaning of ultrafiltration membranes fouled with BSA solution, Sep. Purif. Technol. 120 (2013) 275–281. https://doi.org/10.1016/j.seppur.2013.10.018.

[28] C. Carbonell-Alcaina, J.L. Soler-Cabezas, A. Bes-Piá, M.C. Vincent-Vela, J.A. Mendoza-Roca, L. Pastor-Alcañiz, S. Álvarez-Blanco, Integrated membrane process for the treatment and reuse of residual table olive fermentation brine and anaerobically digested sludge centrate, Membranes (Basel). 10 (2020) 1–14. https://doi.org/10.3390/membranes10100253.

[29] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics acids with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.

[30] C. Carbonell-Alcaina, S. Álvarez-Blanco, M.A. Bes-Piá, J.A. Mendoza-Roca, L. Pastor-Alcañiz, Ultrafiltration of residual fermentation brines from the production of table olives at different operating conditions, J. Clean. Prod. 189 (2018) 662–672. https://doi.org/10.1016/j.jclepro.2018.04.127.

[31] E.A. Baptista, P.C.R. Pinto, I.F. Mota, J.M. Loureiro, A.E. Rodrigues, Ultrafiltration of ethanol/water extract of Eucalyptus globulus bark: Resistance and cake build up analysis, Sep. Purif. Technol. 144 (2015) 256–266. https://doi.org/10.1016/j.seppur.2015.02.036.

[32] V. Contreras-Jácquez, U. Valenzuela-Vázquez, D.A. Grajales-Hernández, J.C. Mateos-Díaz, M. Arrellano-Plaza, M.E. Jara-Marini, A. Asaff-Torres, Pilot-Scale Integrated Membrane System for the Separation and Concentration of Compounds of Industrial Interest from Tortilla Industry Wastewater (Nejayote), Waste and Biomass Valorization. 13 (2022) 345–360. https://doi.org/10.1007/s12649-021-01530-x.

[33] A. Cassano, L. Donato, C. Conidi, E. Drioli, Recovery of bioactive compounds in kiwifruit juice by ultrafiltration, Innov. Food Sci. Emerg. Technol. 9 (2008) 556–562. https://doi.org/10.1016/J.IFSET.2008.03.004.

[34] M.J. Corbatón-Báguena, S. Álvarez-Blanco, M.C. Vincent-Vela, Cleaning of ultrafiltration membranes fouled with BSA by means of saline solutions, Sep. Purif. Technol. 125 (2014) 1–10. https://doi.org/10.1016/j.seppur.2014.01.035.

[35] F. Qu, H. Liang, J. Zhou, J. Nan, S. Shao, J. Zhang, G. Li, Ultrafiltration membrane fouling caused by extracellular organic matter (EOM) from Microcystis aeruginosa: Effects of membrane pore size and surface hydrophobicity, J. Memb. Sci. 449 (2014) 58–66. https://doi.org/10.1016/J.MEMSCI.2013.07.070.

[36] D. Hou, G. Dai, H. Fan, J. Wang, C. Zhao, H. Huang, Effects of calcium carbonate nano-particles on the properties of PVDF/nonwoven fabric flat-sheet composite membranes for direct contact membrane distillation, Desalination. 347 (2014) 25–33. https://doi.org/10.1016/J.DESAL.2014.05.028.

[37] Q. Li, X. Pan, Z. Qu, X. Zhao, Y. Jin, H. Dai, B. Yang, X. Wang, Understanding the dependence of contact angles of commercially RO membranes on external conditions and surface features, Desalination. 309 (2013) 38–45. https://doi.org/10.1016/j.desal.2012.09.007.

[38] S.R. Panda, N. Bhandaru, R. Mukherjee, S. De, Ultrafiltration of oily waste water: Contribution of surface roughness in membrane properties and fouling characteristics of polyacrylonitrile membranes, Can. J. Chem. Eng. 93 (2015) 2031–2042. https://doi.org/10.1002/CJCE.22313.

[39] C. Hobbs, S. Hong, J. Taylor, Effect of surface roughness on fouling of RO and NF membranes during filtration of a high organic surficial groundwater, J. Water Supply Res. Technol. 55 (2006) 559–570. https://doi.org/10.2166/AQUA.2006.038.

[40] M. Cai, Y. Lv, S. Luo, Y. Liu, P. Sun, Fouling Behavior of Polyphenols during Model Juice Ultrafiltration: Effect of Membrane Properties, Food Bioprocess Technol. 11 (2018) 1787–1793. https://doi.org/10.1007/S11947-018-2110-9/TABLES/3.

[41] D. Li, X. Yang, Z. Zhou, B. Jiang, A. Tawfik, S. Zhao, F. Meng, Molecular traits of phenolic moieties in dissolved organic matter: Linkages with membrane fouling development, Environ. Int. 133 (2019) 105202. https://doi.org/10.1016/J.ENVINT.2019.105202.

[42] A. Cassano, C. Conidi, E. Drioli, Comparison of the performance of UF membranes in olive mill wastewaters treatment, Water Res. 45 (2011) 3197–3204. https://doi.org/10.1016/j.watres.2011.03.041.

[43] J. Madureira, F.M.A. Margaça, C. Santos-Buelga, I.C.F.R. Ferreira, S.C. Verde, L. Barros, Applications of bioactive compounds extracted from olive industry wastes: A review, Compr. Rev. Food Sci. Food Saf. 21 (2022) 453–476. https://doi.org/10.1111/1541-4337.12861.

[44] N. Caporaso, D. Formisano, A. Genovese, Use of phenolic compounds from olive mill wastewater as valuable ingredients for functional foods, Crit. Rev. Food Sci.

Nutr. 58 (2018) 2829–2841. https://doi.org/10.1080/10408398.2017.1343797.

[45] T. Akihisa, J. Zhang, H. Tokuda, Potentially Chemopreventive Triterpenoids and Other Secondary Metabolites From Plants and Fungi, 2016. https://doi.org/10.1016/B978-0-444-63932-5.00001-2.

[46] X. Tan, J. Zhu, M. Wakisaka, Effect of phytochemical vanillic acid on the growth and lipid accumulation of freshwater microalga Euglena gracilis, World J. Microbiol. Biotechnol. 37 (2021) 1–9. https://doi.org/10.1007/s11274-021-03185-1.

[47] E. Medina, M. Brenes, C. Romero, A. García, A. De Castro, Main antimicrobial compounds in table olives, J. Agric. Food Chem. 55 (2007) 9817–9823. https://doi.org/10.1021/jf0719757.

[48] E. Medina, C. Romero, M. Brenes, P. García, A. De Castro, A. García, Profile of anti-lactic acid bacteria compounds during the storage of olives which are not treated with alkali, Eur. Food Res. Technol. 228 (2008) 133–138. https://doi.org/10.1007/S00217-008-0916-9/FIGURES/7.

[49] S.K. Zaidi, A. Kumar, Effects of ethanol concentration on flux and gel formation in dead end ultrafiltration of PEG and dextran, J. Memb. Sci. 237 (2004) 189–197. https://doi.org/10.1016/j.memsci.2004.03.017.

[50] S.-C. Tu, V. Ravindran, W. Den, M. Pirbazari, Predictive membrane transport model for nanofiltration processes in water treatment, AIChE J. 47 (2001) 1346–1362. https://doi.org/10.1002/aic.690470612.

[51] A.M. Avram, P. Morin, C. Brownmiller, L.R. Howard, A. Sengupta, S.R. Wickramasinghe, Concentrations of polyphenols from blueberry pomace extract using nanofiltration, Food Bioprod. Process. 106 (2017) 91–101. https://doi.org/10.1016/j.fbp.2017.07.006.

[52] R. Erragued, M.E.M. Braga, M. Bouaziz, L.M. Gando-Ferreira, Integration of solvent extraction and membrane processes to produce an oleuropein extract from olive leaves, Sep. Purif. Technol. 299 (2022) 121751. https://doi.org/10.1016/J.SEPPUR.2022.121751.

[53] A.J.C. Semião, M. Foucher, A.I. Schäfer, Removal of adsorbing estrogenic micropollutants by nanofiltration membranes: Part B—Model development, J. Memb. Sci. 431 (2013) 257–266. https://doi.org/10.1016/j.memsci.2012.11.079.

[54] A. Imbrogno, A.I. Schäfer, Micropollutants breakthrough curve phenomena in nanofiltration: Impact of operational parameters, Sep. Purif. Technol. 267 (2021) 1–13. https://doi.org/10.1016/j.seppur.2021.118406.

[55] Z. Wang, G. Liu, Z. Fan, X. Yang, J. Wang, S. Wang, Experimental study on treatment of electroplating wastewater by nanofiltration, J. Memb. Sci. 305 (2007) 185–195. https://doi.org/10.1016/j.memsci.2007.08.011.

SECCIÓN 3

Procesos de membrana en medio orgánico

Esta sección contiene el tratamiento de los extractos hidroalcohólicos del alperujo mediante tecnologías de membrana. El Capítulo 5 contiene una revisión bibliográfica detallada acerca de la ultrafiltración con disolventes orgánicos.

A continuación, los siguientes capítulos tratan sobre la ultrafiltración (Capítulo 6), la nanofiltración (Capítulo 7) y la combinación de ambos procesos (Capítulo 8) para recuperar los compuestos fenólicos presentes en los extractos de alperujo obtenidos con etanol/agua 50:50 (v/v).

CAPÍTULO 5. CHAPTER 5

Ultrafiltration with organic solvents: a review on achieved results, membrane materials and challenges to face

Carmen M. Sánchez-Arévalo[1], María José Luján-Facundo[1,2], María Cinta Vincent-Vela[1,2], Silvia Álvarez-Blanco[1,2]

[1]Research Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

[2]Department of Chemical and Nuclear Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

✉sialvare@iqn.upv.es

Process Safety and Environmental Protection, 177, 118-137

https://doi.org/10.1016/j.psep.2023.06.073

Abstract: Among all the available membrane processes, ultrafiltration is one of the most commonly used and industrially adapted. Apart from aqueous filtrations, the ultrafiltration of solvent-based solutions has found various applications. Some of them are the recovery of valuable compounds from agro-food industries (olive oil, wine, etc.) and the separation of solvents during edible oil production. However, the contact of the membrane (especially polymeric membranes) with an organic solvent still brings different challenges regarding permeate fluxes, rejection values and the long-term stability of the membrane. In this review, the results achieved by research works dealing with organic solvent ultrafiltration have been examined, analyzing the effects of the solvent on the process. Additionally, special attention has been paid to the pre-treatment of the membrane. All the applied strategies to pre-condition the membrane have been reported and discussed here. For the first time, all these relevant data have been formally structured and studied in-depth, aiming to gain more knowledge about organic solvent ultrafiltration.

Keywords: ultrafiltration, solvent, organic solvent ultrafiltration, ethanol, membrane.

1. INTRODUCTION

The benefits of membrane technology are undeniable nowadays. Membrane-based processes comprise a potent separation tool for a wide range of compounds and contaminants in aqueous streams and wastewater [1]. Among their main advantages, they include a significant reduction of the energy and chemicals invested in the process, adjustable selectivity, mild requirements of applied conditions (temperature, pressure, etc.), and easy automation, escalation, and combination with other processes in line. These attributes endorse the use of membranes in a wide range of applications, such as wastewater treatment, water reclamation, medicine, chemistry, food processing, concentration of high-added value compounds, valorization of residues, etc. [2]. The advantages of membrane processes to recover and concentrate target compounds have been disclosed by several authors [3–5]. In particular, ultrafiltration is a powerful technology for removing impurities from a sample, separating large molecules of interest or even concentrating them [6–8].

In general, the vast majority of the pressure-driven membrane processes that are in use nowadays are performed over an aqueous medium. This is also the case for ultrafiltration. However, rapid industrialization and technological development has resulted in high demand for cheap and eco-friendly separation methods applicable to organic media [9]. Many applications (such as petrochemical, pharmaceutical, and fine

chemical industries) unquestionably require using solvents upstream of the membrane process [10,11]. For instance, they are employed in many reactions of organic synthesis, extraction of some vegetable oils, and recovery of interesting compounds to be later applied in the industry (such as phenolic compounds, triterpenes, fatty acids, vitamins, etc.). Also, most processes consisting of extracting organic molecules from their original matrices include an organic solvent. Despite the plethora of advantages that solvent-based membrane processes can offer, the treatment of non-aqueous mediums through ultrafiltration is a challenge yet to be overcome. This is because the nature of a non-aqueous feed solution deeply affects some important operational aspects, such as the permeate flux, membrane stability, rejection, selectivity, or reproducibility of the procedures. Some challenges related to the use of ultrafiltration membranes with organic solvents have been summarized in Figure 5.1.

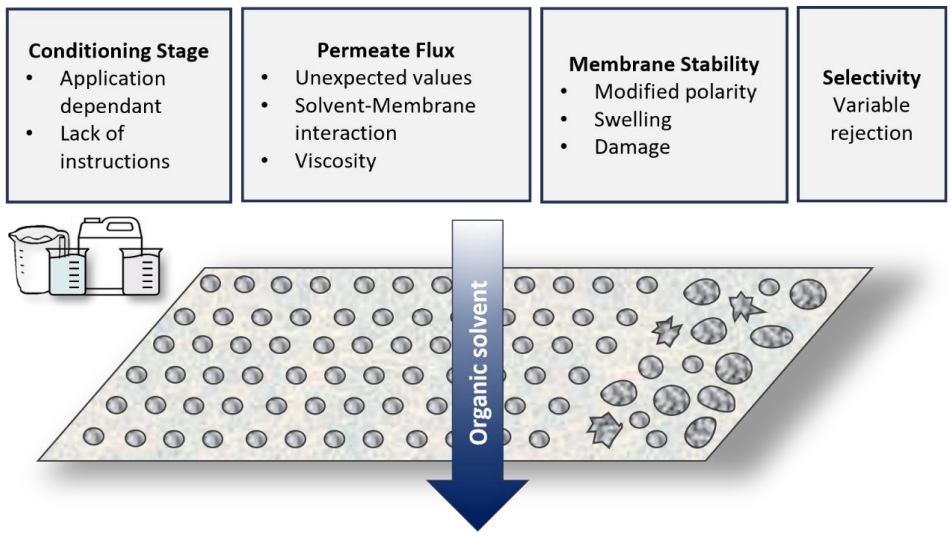


Figure 5.1. Characteristics of an ultrafiltration process dealing with organic solvents.

Generally, researchers working on organic solvent ultrafiltration face four main problems: membrane conditioning, possible alteration of the chemical and mechanical stability of the membrane (which may compromise the polymer structure), atypical low fluxes, and variation of the membrane selectivity. Manufacturers do not usually include solvents' effect on membrane performance in the specification sheet. This fact forces researchers to investigate a repertoire of different pre-treatments and strategies first to test the suitability of the membrane to work with solvents. Also, the conditioning stage aims to prepare the membrane surface and increase the low permeate flux values generally obtained in the subsequent permeance experiments. In many cases, selecting

the adequate membrane for a specific application is difficult, considering that numerous factors may influence solvent permeation and solute retention. Some of these aspects are related to the structure of the active layer, the distribution of the pore size, the thickness, and the density of the membrane, etc. [12]. Furthermore, it is unknown if the membrane will resist the solvent contact. This aspect involves an additional challenge to the inherent complexity of the process, as opposed to the filtration of aqueous solutions. Despite previous stages of membrane testing when implementing an organic solvent in the feed solution, many existing reports related to this field describe the observance of membrane disruption as a consequence of the interaction with the solvent for a considerable time [13,14]. In addition, the use of organic solvents can increase membrane fouling (in comparison to aqueous filtration) and, consequently, the adequacy of the cleaning procedure gains relevance [11]. Even when the filtration process is carried out in an aqueous medium, fouling is the major disadvantage of membrane-based processes. This phenomenon reduces the permeation rate and causes higher operational and maintenance costs [15]. However, membrane fouling can be even more severe when organic solvents are used. The characteristics of the organic solvents, such as polarity and viscosity, and their impact on membrane permeability, affect the tendency of foulants to deposit onto the membrane surface [15,16]. In addition, due to the characteristics of the organic solvents, the foulants could present different physical behaviors in organic solvents in comparison to aqueous mediums. The chemical interactions between the membrane and the foulants can also be altered in an organic medium. This affects parameters like zeta potential or settling capacity, among others, which can directly alter the membrane fouling behavior [17]. In this way, Yin et al., [18] reported that solvents with higher polarity present better anti-fouling properties due to the higher repulsive foulant-membrane interfacial interactions.

Overcoming the mentioned experimental obstacles is of high interest, but the combination of solvents and ultrafiltration still remains underinvestigated. Authors working in this scene have made essential discussions in their research articles, but, as stated by some researchers before [19], there is a critical lack of a systematic study that collects and discusses the relevant data to make progress in this area. Organic-solvent nanofiltration, which has been more often practiced, was excellently covered by Van der Bruggen et al. (2008). However, despite the obvious similarities between organic-solvent nanofiltration and organic-solvent ultrafiltration, there are several aspects that are not shared by both techniques. Among the main differences between organic-solvent nanofiltration and organic-solvent ultrafiltration, it is possible to find the membrane type (in terms of material, pore size, etc.), the organic solvents more commonly used, and the

final application of the process. Whereas nanofiltration membranes can offer much higher purification efficiency, an ultrafiltration process requires lower energy consumption and provides higher values of permeate flux. In addition, the membrane material condition the permitted organic solvent to be used, as will be thoroughly discussed in this work. For all these reasons, the area of application of organic-solvent nanofiltration and organic-solvent ultrafiltration is different. The main applications for organic-solvent nanofiltration rely on the petrochemical industry, food industry and byproducts, bulk industry, fine chemistry, and pharmaceutical industry (Priske et al., 2016). Organic-solvent ultrafiltration, as will be exposed in section 2, has been mostly proposed in the food industry sector, especially for the recovery of beneficial compounds from industrial wastes and in the edible oil industry. Some applications in biorefineries have also been proposed [4,21–23].

More recently, Merlet and co-workers (2020) examined hybrid ceramic membranes whose surfaces were submitted to organic functionalization to be applied in organic-solvent nanofiltration. Additionally, Ren et al. published a remarkable review about new advances in solvent-resistant materials and their implementation in different membrane processes [25]. However, to the best of our knowledge, a critical review specifically dedicated to organic solvent ultrafiltration processes is not yet available in the literature. Taking into account the existing gap within the written knowledge on this topic and the procedures that are mainly performed in membrane technology, the present contribution will discuss the reported performance of several types of membranes during the ultrafiltration of solvent-based solutions. Additionally, the industrial interest in organic solvent ultrafiltration and the future directions of this field will be discussed. Although the intention of this review was not to restrict the study to only polymeric membranes, the use of ceramic membranes (made of alumina, titania, zirconia, or hafnia) for this application is hardly available in the literature. This fact can be attributed to the observed values of permeate flux, which have been reported to be low, due to the highly hydrophilic character of ceramic membranes, in comparison with less polar solvents, such as hexane, for instance [26]. In consequence, details about inorganic membranes are less abundant (but not absent) in this review. Furthermore, polymeric materials have lower fabrication costs, higher flexibility, and ease of processing compared to inorganic materials [27]. In addition, the structure of polymeric membranes is more sensitive to being altered by the solvent than ceramic ones.

The most common alterations of membrane morphology and the observed effects on permeate flux will also be described. Additionally, a specific section will be devoted

to the different conditioning strategies, which are a crucial stage to ultrafilter non-aqueous mediums. Finally, some of the most solvent-resistant polymer materials to be applied in this area will be described in detail. Although not exclusively, the focus will be placed on alcohols. Especially, the effect of ethanol on the membrane performance will be commented, because it is one of the main and more widely used extractant agents in laboratories and industries. The main findings and methodologies of this promising research area will be provided.

2. AREAS OF APPLICATION FOR ORGANIC SOLVENT ULTRAFILTRATION

Annual use of organic solvents across the world is calculated to be around 30 million tons [28]. As commented before, many industries applying organic solvents in their process can benefit from implementing organic solvent ultrafiltration. The following sub-sections include those industrial applications that have been more widely supported by research studies. Mainly, these applications include the recovery of interesting compounds from industrial waste (such as olive pomace, olive mill wastewater, winery sludge, grape seeds, bark, and lignocellulosic residues), the recovery of solvents, the degumming stage in the edible oil industry and several separations in biorefineries.

2.1. Recovery of valuable compounds from industrial waste

The fabrication of many commercial products implies the generation of vast amounts of residues during the production process. In many cases, those industrial by-products still contain valuable compounds. These compounds can be of interest due to their nutritional value or because of their bioactive and functional properties [4]. In order to extract the valuable compounds from the sample that contains them, a solvent-mediated extraction is the most preferred methodology.

In fact, ethanol as a solvent is commonly selected. According to the Registration, Evaluation, Authorisation, and Restriction of Chemicals regulation (REACH), under the European Regulation (EC) Nº 1907/2006, the ethanolic extraction of organic molecules is a highly preferred option, due to the biocompatible character of ethanol, its low toxicity and easy handling [7,29–33]. This has been regulated by the European Chemicals Agency (ECHA), which is the agency responsible for the implementation of the European Union's chemicals legislation to protect the environment and human health. Apart from the consistently employed alcohols (especially ethanol, as mentioned), there is a growing tendency towards the implementation of the denominated green solvents (cyrene, methyltetrahydrofuran, ethyl lactate, etc.), both in academia and at the industrial level [34,35]. These are less toxic solvents, sometimes biodegradable and renewable, and

ultimately safer than conventional solvents for the operators and for the environment [36].

In this way, interesting molecules can be extracted from the residues generated by industries such as winery, olive oil production, and pulp and paper fabrication. Once this solvent-based extract is generated, the target compounds must be purified. Traditionally, the extracted compounds have been separated from the co-extracted solutes by chromatographic techniques, e.g., adsorption chromatography [32]. Nevertheless, chromatography requires large volumes of eluents and solvents to condition the stationary phase. In this context, organic solvent ultrafiltration appears as a more effective alternative to be implemented in the circular economy of the previously mentioned industries.

2.1.1. Winery industry

The winery sludge, which is the solid by-product obtained after the decanting stage, contains a high concentration of phenolic compounds (up to 19 $g \cdot kg^{-1}$) [37]. These molecules can be extracted with ethanol, and they can be further purified by organic solvent ultrafiltration. This technique allows the separation of polyphenols and pectins, which are also extracted along with the biophenols [38]. Grape seeds are another fruitful source of phenolic compounds. After an extraction with ethanol at 50 % (v/v), the polyphenols can be concentrated by an organic solvent ultrafiltration process [7].

2.1.2. Olive oil industry

The by-products generated by the olive oil industry are concerning because of their high organic load. However, membrane technology has been demonstrated to contribute greatly to their valorization. A mixture of ethanol at 50 % (v/v) allows the extraction of a plethora of phenolic compounds with interesting bioactivities [39]. These molecules can be later purified by organic solvent ultrafiltration [40]. Olive leaves are another interesting residue from the olive oil industry. They can be employed as a source of oleuropein, which is a powerful antioxidant [41]. After an extraction with a hydroalcoholic mixture, the olive leaves extract can be treated by organic solvent ultrafiltration in order to remove suspended solids and reduce the fouling of a subsequent organic solvent nanofiltration process to assess the concentration of this valuable molecule [42].

2.1.3. Pulp and paper industry

Bark is a lignocellulosic residue generated by the pulp industry. Apart from lignin, cellulose, and hemicellulose, bark contains phenolic compounds of interest [43]. The phenolic content of bark can be extracted with hydroalcoholic mixtures and later concentrated by organic solvent ultrafiltration, generating a natural product rich in polyphenols [29,44,45]. Furthermore, the molecules released during the degradation of polymeric lignin, such as dilignols and trilignols, can be recovered by means of an ethanolic extraction, followed by organic solvent ultrafiltration (as a pre-treatment of the extract to remove suspended solids) and organic solvent nanofiltration [30]

2.2. Applications in the edible oil industry

Organic solvent ultrafiltration also finds its application in the separation of vegetable oils from the solvents employed for their extraction from seeds or fruits. This mixture of solvent and extracted oil is known as miscella. Traditionally, oil purification from the extractant solvents has been performed by distillation, generating significant energy costs [46]. Another disadvantage of distillation is the potential alteration of the oil quality and nutritional properties due to the high temperatures applied during this process [47].

This could be hugely reduced with its substitution by a membrane process, replacing a thermal separation process with a non-thermal process [22]. Organic solvent ultrafiltration offers the possibility to recycle the solvent and reuse it in a following round of the process. In fact, this technology has been proposed in the literature to perform the separation of mixtures of soybean oil and hexane [48–50]. Vegetable oil degumming is also an appropriate field for the application of organic solvent ultrafiltration. After the extraction of edible oils, entailing organic solvents, and the separation of the oil and the solvent, vegetable oils undergo a degumming stage [51]. The aim of this process is to remove the phospholipid content, as they affect the organoleptic properties of the final product [52]. In this case, a membrane process is a more eco-friendly solution than traditional methods such as filtering, settling or centrifugal action, since these conventional techniques generate large amounts of wastewater and require high energy consumption [52]. For example, Ochoa et al., [23] studied the membrane degumming of crude soybean oil with four polymeric (made of PVDF, PES, and PSf) ultrafiltration membranes. Results demonstrated that PVDF membranes were stable in contact with hexane and phospholipid retention values were over 98%.

Thus, it is evident that the implementation of membrane processing exhibits enormous potential in several lines of work, but more efforts are required to improve

the current limitations and insufficient chemical resistance of most of the available ultrafiltration membranes. In addition, only a few organic solvent ultrafiltration membranes are commercially available. These membranes must have long-term chemical and physical stability to resist the contact with the solvent during the operation time [53]. An important manufacturer in this field is SolSep BV (Apeldoorn, The Netherlands), also known for the fabrication of organic solvent nanofiltration membranes. Also, several commercial membranes from Microdyn Nadir (Wiesbaden, Germany) and Alfa Laval (Lund, Sweden) have demonstrated to resist the contact with ethanol and mixtures of ethanol and water, even though these membranes are, in principle, conceived for aqueous separations [22,46,53,54]. Thus, solvent-resistant membranes are not abundant in the market. Similarly, accurate information about their properties and pre-treatment guidelines is not abundant in the literature [12,50,55].

2.3. Applications in biorefineries

Separation and purification processes, such as membrane separations, have paramount importance in biorefineries [56]. In particular, microfiltration and ultrafiltration are the pressure-driven membrane processes most used in this field [57], considered as a white biotechnology. Conventional refineries include biofuel production, biochemicals production, and water and wastewater treatment. In the case of lignocellulosic biorefineries, agricultural residues and woods are used as raw materials. In both cases, membrane separation processes can be applied before and after the fermentation stage. In the case of organic-solvent ultrafiltration, it finds an application during the production of bioethanol. Aqueous microfiltration and ultrafiltration techniques can be used as pretreatment, in combination with a decanter, to purify the raw material after the hydrolysis process and before the fermentation step. However, if the combined hydrolysis and fermentation is preferred, an organic-solvent ultrafiltration can be employed to remove the biofuel from the fermenter [21,58]. This avoids the inhibition of the yeast activity by the ethanol molecules and improves the productivity of the process.

3. POSSIBLE EFFECTS OF ORGANIC SOLVENTS ON MEMBRANE STRUCTURE

Organic solvents are carbon-based compounds capable of dispersing or dissolving one or more substances. Generally, organic solvents can be divided into non-polar and polar solvents. The molecules of non-polar solvents (e.g., toluene, hexane, and benzene) present atoms with very similar electronegativities, and the charge is distributed symmetrically around the molecule. By contrast, polar organic solvents (e.g., acetonitrile,

methanol, and ethanol) present higher dipole moments due to the different electronegativities of their atoms [18,59]. In addition to polarity, there are other physical properties of the organic solvents that should be considered in order to select the most suitable one. These include surface tension and viscosity [60,61]. Surface tension is a phenomenon at the surface of a liquid medium caused by intermolecular forces. It is related to the tendency of a fluid to occupy the lowest possible area. Therefore, liquids with strong intermolecular forces present a high surface tension. Takeuchi and Takahashi (1987) explained that, for the membrane to be properly wetted by the solvent, the surface tension of the organic solvent should be lower than the critical surface tension of the membrane material. On the other hand, viscosity reflects the flow resistance of the liquid, and it is related to the forces of intermolecular attraction. In the following section, the relation between viscosity and permeate flux will be discussed. Figure 5.2 contains a classification of the main organic solvents used in ultrafiltration in terms of polarity, surface tension, and viscosity, since these are the most important solvent characteristics to consider for organic solvent ultrafiltration.

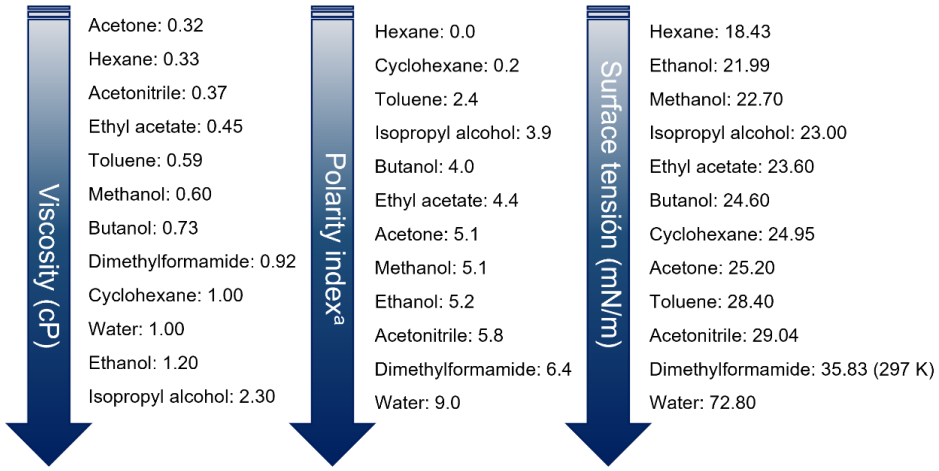


Figure 5.2. Viscosity (293K), polarity and surface tension (293K) of the main organic solvents (and water) used in ultrafiltration (DDB, 2001; Sadek, 2002; STD, 2017). [a]: according to (Sadek, 2002).

Regarding the membrane materials, polyimide (PI), polycarbonate (PC), polysulfone (PSf), cellulose acetate, polyethersulfone (PES), polyvinyl chloride, and polyvinylidene fluoride (PVDF) are the most common synthetic polymers for the manufacturing of ultrafiltration organic membranes [36]. However, there are other membrane materials with good solvent-resistant properties, such as polyketone (PK), polybenzimidazole (PBI), and sulfonated poly (ether ether ketone) (SPEEK) [63–65]. In order to use these membranes at an industrial scale, the mentioned materials should have high selectivity and rejection, high permeability, good mechanical and thermal stability, low fabrication

cost, and high surface area [66]. One of the key challenges of organic solvent ultrafiltration is that polymer membranes tend to swell or dissolve in organic solvents, and the pressure and temperature applied can also affect the membrane's long-term stability [67]. Therefore, the study and selection of the correct material are of paramount importance in order to minimize the possible effect of the organic solvent.

A considerable percentage of the organic solvents involved in ultrafiltration experiments are alcohols, primarily ethanol. The exact mechanisms underlying their observed effects on ultrafiltration membranes are still unknown, but some theories have emerged up to date. The chemical structure of alcohols, $(CH_3)_n$-OH, may explain the uncommon permeate fluxes that have been observed by some authors (references in this regard will be given in the following sections of the present review). It has been suggested that the solvent molecules will adopt one disposition or another depending on the relative polarity between the membrane and the solvent. Thus, the hydrophilic section of the solvent molecules will approach the most polar sector, and the hydrophobic tail will be headed to the section with lower polarity. In the study performed by De Melo et al. [49], the conditioning of α-alumina oxide/zirconium ceramic membranes with n-butanol ensured a notable improvement in hexane permeate flux. As the membrane of this study was inorganic, any change in the ceramic structure can be excluded. However, the modulation of the properties of the active layer is plausible and was supported by the results of these authors. A possible explanation was related to the interaction of the hydroxyl radical of n-butanol with the membrane surface, whereas the carbon tail would be oriented toward the bulk solution, then reducing the high hydrophilicity of the membrane and making its polarity more similar to that of hexane. Additionally, Van der Bruggen and co-workers reported the modulation of the top layer polarity of polymeric membranes due to a clustering phenomenon occurring on the membrane's active layer. Their findings suggested that, after an immersion in ethanol, the original polarity of the membrane could be modified upwards for less polar, hydrophobic membranes. Alternatively, the polarity of hydrophilic, polar membranes could be reduced as a result of the solvent contact [68].

Regarding the study of Argyle and co-workers [55], they employed ethanol to condition PSf membranes. It was suggested a possible preferential adhesion to the membrane pores of the polar hydroxyl radical of the molecules of ethanol, while the hydrophobic part would be oriented to the pore space. Thus, the membrane's charge and polarity decrease, lowering the adhesion of water to the pore wall. Charge reduction

was confirmed by zeta potential measurements. Consequently, these authors reported a larger water flux through the membranes after ethanol conditioning.

Solvents might also affect the chemical nature of the polymer that entitles the membrane, causing a phenomenon of swelling [25]. The expansion of the membrane material is a recurrent event when working with organic solvents and has been reported since the first papers dealing with this topic were published [69]. It consists of a dissolution of the polymer and modification of its morphology because of the alteration (by the solvent molecules) of the forces that enable the crosslinking of the membrane. This is commonly traduced in a thickening of membrane pores, which can affect both the active layer's micropores and the support layer's macropores. In consequence, expansion and distension of the structure and lowering of the rejection performance of the membrane are possible. It is possible that only one of these situations can be observed, but also both cases may occur simultaneously. The nature of the membrane polymeric material plays an important role in the degree of swelling. Hydrophilic polymers are more vulnerable to swelling during solvent exposure. By contrast, hydrophobic polymers have a higher solvent resistance, but a relevant disadvantage is that, due to their insolubility in most of solvents, they cannot be manufactured applying phase inversion techniques [70]. Several authors have reported some findings related to the observance of the swelling phenomenon during the use of polymeric membranes for organic solvent applications. Krupková et al. [71] studied the performance of commercial cellulose membranes with a MWCO between 1 and 5 kDa, treating organic markers dissolved in methanol and dichloromethane (DCM). Methanol has a polar character, whereas DCM is nonpolar. Their results confirmed that, in the case of DCM, due to its nonpolar character, swelling was more pronounced. However, due to the robustness of the membrane support layer (made with PP) swelling was not significant. Depending on the percentage of rupture of polymer-polymer forces and the type of pores affected, different scenarios can be favored, leading to the different results observed by some authors. Even though the expansion of membrane pores may lead to higher permeate fluxes, swelling should be taken cautiously, because a great drop of the rejection is possible too, affecting membrane selectivity [19,55,72].

Additionally, the eventual bonds established between the polymer and the solvent molecules may result in membrane rupture, as the microstructure of the polymer is chemically altered. Even small fissures in the polymer chain are possible and, logically, they are able to impact the performance of the membrane [19]. As reported in the literature, when membranes are disrupted by solvents, their tensile resistance is modified and they are more sensitive to pressure increments [13]. As a result, when

certain pressure values are achieved, the linear relation between pressure and flux is no longer applicable and membrane deformations, such as swelling or shrinking, are possible. This repertoire of effects varies depending on polymer composition, type of solvent, range of applied pressure, etc.

The variety of events that can take place at the membrane surface underlines the importance of performing a visual analysis of the membrane itself. Microscopic/spectroscopic technologies can bring a lot of benefits to evaluate differences in polymer structure before and after membrane conditioning or ultrafiltration experiments. Giorno and co-workers [73] provided a very valuable set of Scanning Electron Microscopy (SEM) images during their study about the preparation of oil-in-water emulsions. They employed 10 kDa and 50 kDa polyamide membranes and reported the effect of iso-propanol and isooctane, used as conditioning solvents. SEM images of the membrane cross-section revealed detailed information about the pores. Swelling and structural modification were noticeable, although the ultrafiltration performance was, in the end, stable and reproducible. Argyle et al. [74] studied the effect of an ethanol-based pre-treatment on the mechanical structure and surface modification of PSf ultrafiltration membranes (with polypropylene support) with a MWCO of 50 kDa. After Fourier Transform Infrared (FTIR) analysis, they observed slight differences between both spectra (before and after treatment) and reported the disappearance of some spectral bands (at 920 and 1040 cm^{-1}) after the pre-treatment. According to Belfer et al. [75], these bands can be attributed to membrane preservatives. Weis et al. [76] described that the band at 1040 cm^{-1} may correspond to an aliphatic alcohol, such as glycerol. Therefore, the FTIR analysis performed by Argyle et al. [74] revealed that a simple treatment of PSf membranes with ethanol can remove preservatives and modify the characteristics of the active layer. Microscopy observations are included in other articles, with the aim of studying possible membrane structure modifications after the use of organic solvents. This is the case of De Souza-Araki et al. [13], who studied the membrane structural stability after hexane filtration with several commercial ultrafiltration polymeric membranes: 30 kDa and 50 kDa PVDF, 10 kDa PES, 0.05 µm PC, and 0.05 µm and 0.025 µm mixed cellulose esters (CME). SEM images confirmed that there were no apparent visual morphological changes before and after the treatment with hexane. Some other research works included a visual interpretation of microscopy data [14,18,19]. As stated, image confirmation of solvent effects over the membrane is highly recommended, in order not to attribute to the solvent some abnormal results that may come from other experimental aspects. In many cases, potential morphological

alterations, such as swelling, clustering, wrinkling, or fracture, can be visually detected and used to complete the data of the study.

4. PERMEATE FLUX OF ORGANIC SOLVENTS IN ULTRAFILTRATION

As it was commented before, organic solvents have a fundamental role in many industries. The use of these solvents implies a further separation, recovery, or disposal. Recently, membrane processes have become one of the most promising and emerging technologies for this purpose [28].

Ultrafiltration membranes are porous mediums, with pore sizes in the range of 0.02 to 0.05 µm. In consequence, the corresponding permeate flux (*J*) through the membrane is described by the Darcy's law (equations 1 and 2):

$$J = \frac{\Delta P}{\mu \cdot R} \quad (1)$$

or

$$J = \frac{L \cdot \Delta P}{\mu} \quad (2)$$

where ΔP corresponds to the transmembrane pressure, R is the membrane resistance, L is the membrane permeability, and µ is the viscosity of the fluid permeating across the membrane pores. As it is obvious in (1) and (2), the viscosity of any solvent other than water will influence the value of the permeate flux. In this way, Jaffrin and co-workers [77] already reported in 1994 that the viscosity of ethanol was mainly responsible for the flux reduction that they observed during the ultrafiltration of ethanol/water solutions of albumin, using PSf (with a molecular weight cut-off (MWCO) of 10 kDa) and cellulose acetate (with a MWCO of 20 kDa) membranes. As reflected in Figure 5.2, some alcohols display higher viscosity than water [78], but the differences in this parameter are not enough to justify the values of permeate flux that have been reported by other authors [13,45], because some findings are substantially distant from the observed water flux using the same membranes. Baptista et al. found that hydraulic permeability was always higher (even more than twice) than the permeability of an ethanol-based feed [29]. As an example, a PVDF membrane with a MWCO of 30 kDa displayed a hydraulic permeability of 91.2 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, whereas the permeability of an 80% (v/v) ethanol solution was 35.5 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. This variation could be expected according to the differences in the medium viscosity, as water viscosity is half of the viscosity of the hydroalcoholic solution. Interestingly, Pinto et al. [44] also studied the

same membrane and observed a decrease in permeability (from 50 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ to approximately 36 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$) when increasing the ethanol percentage from 52% to 80% (v/v). At 35ºC, the viscosity of ethanol 80% (v/v) is lower than that of ethanol 52% (v/v) [79]. If viscosity was the only parameter affecting the process, an increase in permeability would be expected, but the opposite effect was observed. Thus, these results demonstrated that solvent viscosity was not the only variable involved and other factors, like the polarity of the solution or membrane-solvent interactions, should be considered too. In fact, it is possible to find membranes with similar MWCO and very different permeabilities to a given solvent, depending on the material of the polymer and its affinity regarding the ultrafiltration feed.

To better understand the results that have been reported when using organic solvents during ultrafiltration experiments, it is convenient to distinguish between processes dealing only with solvent solutions and those with solutes dissolved in the samples.

4.1. Permeate flux when only solvent solutions are treated

Some authors have performed the ultrafiltration of feeds containing only the solvent or mixtures of water and solvent, at different percentages. These works allow the study of the modifications in the permeate flux that are only attributed to the effect of the solvent, and not to other molecules present in the feed solution.

De Souza-Araki et al. [13] performed a complete study in which they tested six flat sheet polymeric membranes, composed of materials such as mixed cellulose esters (MCE), PC, PVDF, and PES. The permeate flux of water, ethanol, and hexane was studied for each membrane. The most noteworthy results were obtained for the PVDF and MCE membranes. In the case of PVDF, the water flux was significantly higher than for the MCE membrane. Both solvents displayed almost half of the flux obtained with water. MCE membranes did not resist the contact with ethanol above 3 bar. For this material, they observed that the permeate flux of hexane was higher than the water flux, suggesting a better affinity of hexane for the membrane material due to its hydrophobic character. However, when a membrane of the same material and larger pore size was evaluated, the permeate flux did not increase. The authors then proposed a possible solvent immobilization on the membrane surface, derived from a strong interaction. In such event, a contraction of the pores can occur, thus blocking the migration of solvent molecules into them and across the membrane [80]. Oxley et al. (2022) recently published an interesting work about the preparation of crosslinked (with two grafting

agents, elastamine and 2-methoxyethylamine), organic-solvent-resistant PBI ultrafiltration membranes [81]. Acetonitrile was the solvent with the highest permeate flux values (in the range of 33-68 $L \cdot m^{-2} \cdot h^{-1}$), whereas toluene provided the lowest values, around 10 $L \cdot m^{-2} \cdot h^{-1}$. The MWCO of the membranes was between 2,000-20,000 $g \cdot mol^{-1}$, therefore being in the limit between an ultrafiltration and a nanofiltration membrane. Savaş-Alkan et al. [28] also studied the preparation of solvent-resistant ultrafiltration membranes in order to recover the solvent, which was propylene glycol methyl ether acetate (PGMEA). They fabricated cellulose acetate UF membranes by alkaline hydrolysis, varying the precursor solution (dimethyl sulfoxide (DMS), acetone and polyethylenglycol (PEG). When the organic solvent (dimethylformamide (DMF), DMSO, methanol and PGMEA) permeability was measured, the two tested membranes (membrane C20P10, made with 20 and 70 wt% of CA and DMS, respectively and, membrane C25P10A10-AN, made with 25 and 55 wt% of CA and DMS, respectively) obtained the highest permeability with methanol (values of 32 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ and 2.4 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, respectively). These two membranes were also tested with PGMEA, whose molar volume is 137 cm^3/mol, and the permeability using this solvent were much lower for both membranes, with values of 0.5 and 0.1 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ for the C20P10 and C25P10A10-AN membranes, respectively.

In addition to polymeric membranes, the solvent flux behavior of ceramic ultrafiltration membranes was studied by Buekenhoudt et al. [82]. In this work, three ceramic ultrafiltration membranes (with a pore diameter of 3, 5, and 100 nm) were evaluated in terms of pure water and organic solvent (methanol, dimethylformamide, tetrahydrofuran, toluene, and hexane) permeability. Results demonstrated that pure water flux was higher than any organic solvent flux, for all the membranes. According to their results, lower values of permeate flux were obtained for solvents with lower polarity, being toluene and hexane the solvents leading to the lowest flux for the tested three membranes.

4.2. Permeate flux when solutes are present in the feed solution

If the feed contains solutes, they can be involved in the concentration polarization phenomenon and contribute to the fouling of the membrane. Thus, the obtained results can be influenced not only by the organic solvent, but also by the presence of these dissolved compounds in the feed solution. In organic-solvent membrane filtration, physicochemical membrane properties, such as polarity and functional groups, have a great impact on the interaction between the membrane and the solutes and, logically, on the solute rejection [83]. If an unexpected permeate flux is obtained, it should not be

directly attributed solely to the solvent. The combined effect of the solutes (if present) should be observed too. In this context, Ma and co-workers (2021) applied molecular dynamics simulations to understand the adsorption process of dextran onto a polyacrylonitrile (PAN) membrane. They studied it in an aqueous medium, but also in formamide. The authors found that the solvent-solute and solvent-membrane interactions were different in each case. In water, the dextran-membrane interaction was more relevant, leading to more severe fouling and a higher rejection of the dextran molecules. On the contrary, the dextran-solvent interaction was higher in formamide, which generated a solvation phenomenon that reduced membrane fouling and procured lower rejection values. Figure 5.3 illustrates the importance of the solute-solvent-membrane interaction and its possible influence on membrane fouling.

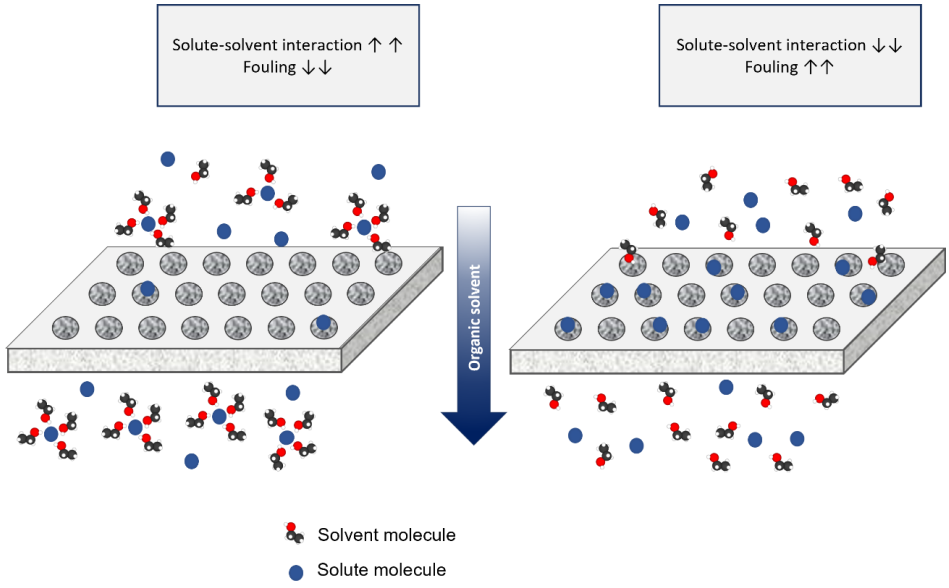


Figure 5.3. Potential effect of solute-solvent-membrane interaction on membrane fouling.

A different solvent, in contact with the same solute and the same membrane, determined a specific fouling tendency and permeate flux, which underlines the importance of the solvent environment during ultrafiltration. Viscosity (293K), polarity, and surface tension (293K) of the main organic solvents (and water) used in ultrafiltration [84–86]. [a]: according to [84]. Yin et al. [87] also studied PAN ultrafiltration membranes to check the membrane fouling after a series of experiments with three colloidal foulants (SiO_2, TiO_2, and Al) dissolved in methanol, ethyl acetate, and acetonitrile. When only the pure organic solvent was present in the feed solution, methanol exhibited the greatest permeate flux, followed by ethyl acetate. For the three foulants dissolved in organic

mediums, aluminium was the one causing the lowest fouling. The behaviour of the other two foulants suggested a higher resistance of the cake layer that varied with the different solvents. Jaffrin et al. (1997) published an interesting work on the ultrafiltration of ethanol-based albumin solutions. PSf hollow-fiber membranes with a MWCO of 30 kDa were employed. As it was reported, ethanol contributed, not only to the medium viscosity, but also to a progressive membrane fouling that continued increasing for many hours and was partly responsible for the low permeate flux. Albumin diffusivity was reduced in the ethanolic medium. Consequently, its transport to the bulk solution was disfavoured and its accumulation at the membrane surface enhanced the fouling. As these results suggest, there are many aspects, not excluding viscosity, that influence the flux through the ultrafiltration membranes. Some of the events (e.g. pore modification, swelling, etc.) that have been explained in the previous section may participate in flux modification. Thus, the prediction of ultrafiltration flux when working with organic solvents must take into account other mechanisms apart from the simple permeation, especially when polymeric membranes are involved [89]. The degree of compatibility between the polarity of the solvent and the membrane surface can influence the tendency of the solvent to permeate. In the case of water and hydrophilic membranes, the permeance is enhanced by the hydrogen bonds that are formed between the molecules of water and the polymer [44,89]. However, if water is substituted by an organic solvent (even if it is a mixture of miscible solvent and water), the capacity to establish these bonds will be different and generally lower [90], which considerably affects the transport through the membrane. In the case of ethanol, the transport through a hydrophilic membrane would be less favored than water transport, due to the lower polarity of this alcohol (see Figure 5.2). Nevertheless, it should not be concluded that the higher the hydrophobicity of the membrane, the higher the permeate flux of an organic solvent, because potential hydrophobic interactions and adsorption issues are possible too and must be considered. Each case should be studied carefully in order to reach a compromise solution between the polarities of both mediums and the overall process efficiency.

5. CONDITIONING OF ULTRAFILTRATION MEMBRANES

During the ultrafiltration of organic solvents, unexpected low fluxes may be obtained. Thus, prior to apply a solvent-based ultrafiltration, it is recommended to condition the membranes to achieve an effective process [91]. The conditioning procedure consists of a previous contact between the membrane polymer and the organic solvent to ensure the membrane stability during organic solvent ultrafiltration

and to prepare it to operate without any interference from the organic solvent [92]. Figure 5.4 shows a proposed workflow for solvent-based UF process.

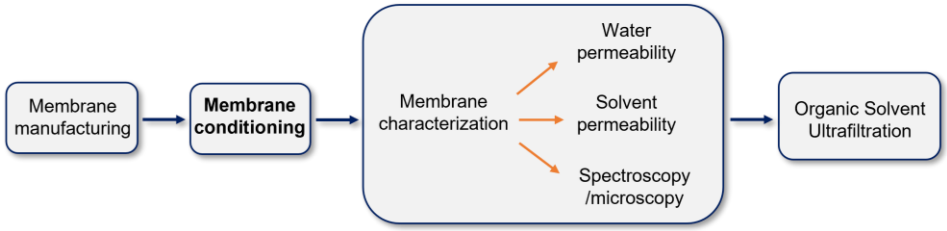


Figure 5.4. Proposed workflow during solvent-based UF process.

As shown in Figure 5.4, it is essential to first condition the membrane, not only to enhance its resistance to the solvent and extend its lifetime, but also to ensure an adequate performance. Possibly, this operation is the most important step of the whole procedure, as the success of the ultrafiltration depends to a large extent on an optimal design of the pre-treatment of the membrane. For ceramic membranes, conditioning plays a crucial role in regulating their high hydrophilicity. Similarly, polymeric membranes strongly depend on the conditioning methodology to maintain their integrity and display proper operation performance [93]. In this section, an analytical description of the conditioning strategies that have been preferentially adopted by researchers is exposed. No similar examination has been done before in the literature, but the importance of this topic makes it greatly interesting to be discussed. Table 5.1 can be helpful to explore the information contained in this review. The table includes data about the membrane material of each study, conditioning strategy (or strategies) and derived results about permeate flux of the tested solvent or any other corresponding feed solution, as well as the observed rejection values. Every article described in this section has been included and carefully detailed in Table 5.1. The conceivable combinations of membrane materials, pore size, and type of solvents are very extensive, so it is very probable that this conditioning stage needs to be optimized for each work case. Be that as it may, membrane conditioning usually consists of introducing it in non-aqueous solvent blends (with a particular percentage of water) during a variable period of time, which ranges from 30 minutes to 24 hours.

Among the related literature in this area, it is possible to distinguish two different lines in the use of organic solvents to pretreat a membrane. One of these strategies has already been introduced and aims to prepare the membrane for the subsequent ultrafiltration of non-aqueous solutions. Interestingly, there are authors who also employ

solvents to improve the behavior of the membrane when ultrafiltering aqueous mediums. Both possibilities and the procured outcomes will be commented on hereafter.

5.1. Organic solvent applied as pre-treatment and permeation solvent

As already stressed, membrane conditioning is unavoidable when the solvent is intended to be present in the feed solution. Immediately after the conditioning, there is a common practice of testing the hydraulic permeability of the membrane [30]. Indeed, it seems very accurate to compare the membrane performance just after the conditioning and at the end of the ultrafiltration experiment, in order to study possible changes in membrane performance to the pressure variation, temperature, etc., and, consequently, the suitability of the conditioning step. Similarly, it is advised to check water and solvent permeability after each experiment of organic solvent ultrafiltration, to gather information related to fouling, membrane-solvent interactions, and overall process efficiency.

Three experimental approaches have been proposed up to date: membrane immersion in the same solvent as in the feed solution, membrane immersion in solutions of increasing percentage of the same solvent, and membrane immersion in different solvents. Some authors [55,93–95] have performed the conditioning directly in the ultrafiltration equipment, circulating the solvent and applying pressure. Those cases have been indicated in the corresponding sub-section.

Table 5.1. Detailed description of each work described in this review, including the employed membranes, conditioning solvent, and procedure. Derived results of permeability and rejection are provided too.

Ref.	Memb.	Feed solution	Feed solvent	Conditioning procedure	Before conditioning	After conditioning			
					Water perm. ($L/m^2 \cdot h \cdot bar$)	Water permeability ($L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$)	Solvent perm. ($L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$)	Permeate flux (feed solution) ($L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$)	Rejection
[53]	PBI-M0[a]	PEG solution	Acetonitrile, MeOH[b], DMF[c], toluene	-	n.s.	n.s.	68 (acetonitrile) 39 (MeOH) 32 (dimethylformamide) 10 (toluene)	n.s	Rejection of PEG 2000 $g \cdot mol^{-1}$ 32% (acetonitrile) 58% (MeOH) 68% (dimethylformamide) 56% (toluene)
	PBI-M90[a]						65 (acetonitrile) 29 (MeOH) 18 (dimethylformamide) 10 (toluene)		Rejection of PEG 2000 $g \cdot mol^{-1}$ 50% (acetonitrile) 62% (MeOH) 75% (dimethylformamide) 56% (toluene)
	PBI-M97.5[a]						42 (acetonitrile) 25 (MeOH) 12 (dimethylformamide) 9.5 (toluene)		Rejection of PEG 2000 $g \cdot mol^{-1}$ 65% (acetonitrile) 76% (MeOH) 95% (dimethylformamide) 58% (toluene)
	PBI-M100[a]						33 (acetonitrile) 18 (MeOH) 10 (dimethylformamide) 10 (toluene)		Rejection of PEG 2000 $g \cdot mol^{-1}$ 90% (acetonitrile) 90% (MeOH) 96% (dimethylformamide) 58% (toluene)
[28]	Cellulose C20 (15 kDa)[d]	DMF, DMSO[e], MeOH, PGMEA[f]		Storing in EtOH 20% until use	n.s.	18	n.s.	-	n.s.

	Cellulose C20P10 (14 kDa)[d]					24	14 (DMF), 1 (DMSO), 32 (MeOH), 0.5 (PGMEA)		Rejection of SU-8 42%
	Cellulose C25P10 (13 kDa)					7	n.s.		n.s.
	Cellulose C25P10-AN (13.5 kDa)[d]					8	n.s.		n.s.
	Cellulose C25P10A10 (5.8 kDa)[d]					3.5	n.s.		Rejection of SU-8 82%
	Cellulose C25P10A10-AN (3.5 kDa)[d]					3	1.2 (DMF), 0.2 (DMSO), 2.4 (MeOH), 0.1 (PGMEA)		Rejection of SU-8 84% Rejection of PEG 1000 Da 90% (DMSO), 20% (MeOH), 40% (water)
	Cellulose C30P10A10 (4 kDa)[d]					1	n.s.		Rejection of SU-8 82%
[16]	PAN (20 kDa)	Dextran solution (0.2 $g \cdot L^{-1}$) (Water, formamide)	Water, formamide	n.s.	n.s.	n.s.	n.s.	Water: 750-433 Formamide: 180-160	~65% (water), 0% (formamide)
[51]	Ceramic: Al_2O_3/ZrO_2 (5 kDa)	Canola oil/solvent mixtures	*p*-cymene, EtOH, limonene, hexane	1. 3 hours immersion in EtOH (pressurization) 2. Overnight immersion in EtOH (pressurization)	-	-	16.7 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (EtOH); 20 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (*p*-cymene); 35 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (hexane); 18 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (limonene); 12 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (pinene);	Hexane/oil 90:10 (v/v): 13 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ *p*-Cymene/oil 90:10 (v/v): 11 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$; Limonene/oil 90:10 (v/v): 10 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ Pinene/oil 90:10 (v/v): 7 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	Rejection of phospholipids hexane/oil 90:10 (v/v): 95% *p*-cymene/oil 90:10 (v/v): 97%; limonene/oil 90:10 (v/v): 92%; pinene/oil 90:10 (v/v): 92%
	PES (10 kDa)						33 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (EtOH); 27 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (*p*-cymene);	Hexane/oil 90:10 (v/v): 25 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	n.s.

							67 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (hexane); 8 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (limonene); 3 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (pinene)	*p*-Cymene/oil 90:10 (v/v): 7 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ Limonene/oil 90:10 (v/v): 3 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ Pinene/oil 90:10 (v/v): 9 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	
	PSf (90 kDa)						55 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (EtOH); 38 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (*p*-cymene); 82 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (hexane); 23 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (limonene); 8 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (pinene)	Hexane/oil 90:10 (v/v): 19 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ *p*-Cymene/oil 90:10 (v/v): 21 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ Limonene/oil 90:10 (v/v): 9 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ Pinene/oil 90:10 (v/v): 3 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	n.s.
[19]	PSf (100 kDa)	Dextran solution (1 $g \cdot L^{-1}$), BSA solution (1 $g \cdot L^{-1}$), pepsin solution (1 $g \cdot L^{-1}$)	Water	Immersion in hot water (70ºC), afterwards: <u>Studied strategies</u> A. EtOHᵍ 60%: 35ºC, 75 min, 130 rpm B. EtOH 100%: 50ºC, 120 min, 130 rpm C. NaOH treatment (0.55 M and 1 M)	110	After treatment A: 600 After treatment B: ~840 After treatment C: 205-250	-	<u>After treatment B</u> Dextran: 600-750 BSA: 150-350 Pepsin: 225-350	<u>Rejections after treatment B</u> Dextran 70 kDa: 0.84%; 100 kDa: 2.31%; 229 kDa: 11.62; 2000kDa: 49.32% BSA: ~20% Pepsin: ~75%
[14]	PVDF (7 kDa)	Biodiesel	EtOH	Overnight immersion in feed solvent	12.4	-	24.1	1.9	<u>Rejection of total glycerol</u>
									23.7%
	PSf (5 kDa)				9	-	1.7	0.38	13.7%

[55]	PSf (50 kDa,)	Black tea liquor	Water	1. Immersion in hot water (temperature n.s.) 2. Overnight immersion in EtOH 50% or 100% (pressurization)	Relative water flux 1	Relative increase of water flux 1.32 ± 0.01 (EtOH 50%), 2.32 ± 0.02 (EtOH 100%)	-	Relative filtration flux 0.7-0.4 (EtOH 50%); 0.6-0.25 (EtOH 100%)	Rejection of TPC ~0.4 (EtOH 50% and EtOH 100%)
	PSf (100 kDa)				1	3.75 ± 0.11 (EtOH 50%), 3.8 ± 0.06 (EtOH 100%)	-	0.4-0.2 (EtOH 50%); 0.3-0.15 (EtOH 100%)	~0.3 (EtOH 50% and EtOH 100%)
	PSf (0.1 µm)				1	2.09 ± 0.04 (EtOH 50%), 2.13 ± 0.04 (EtOH 100%)	-	0.35-0.15 (EtOH 50%); 0.32-0.12 (EtOH 100%)	~0.28 (EtOH 50% and EtOH 100%)
[29]	Polyamide composite (1 kDa)	*Eucaliptus globulus* bark extract (EtOH 80%)	EtOH/water 80%	Increasing EtOH proportion by 10% until 80%	3.9	-	1.7	1.67	Rejection of TPC 49.4%
	PES (5 kDa)				72.4	-	25	2	49.4%
	PVDF (30 kDa)				91.2	-	35.5	3.33	61.1%
	PSf (60 kDa)				191.3	-	57.2	13.33	13.9%
[49]	Ceramic: Single channel, ZrO_2/ Al_2O_3 active layer (20 kDa)	Synthetic and real soybean oil/solvent mixtures (EtOH, propanol, hexane)	Hexane	1. Overnight immersion in EtOH 2. Overnight immersion in n-propanol 3. Overnight immersion in iso-propanol 4. Overnight immersion in butanol	192.5	After 1: 120.5 After 2: 76.1 After 3: 70.4 After 4: 60.6	After 1: 9.2 After 2: 4.8 After 3: 172.4 After 4: 313.8	Real soybean oil/hexane mixture 14 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	Rejection of soybean oil 4-8%[h]
[44]	Polyamide composite (1 kDa)	*Eucaliptus globulus* bark extract (EtOH 52% and EtOH 80%)	EtOH/water 52%, EtOH/water 80%	Increasing EtOH proportion by 10% until 52% or 80%	4	-	<2.5	n.s.	15-20% (gallic acid); 20-25% (maltose); 45-50% (tannic acid)
	PES (5 kDa)				70	-	25-40	1.5	n.s.
	PVDF (30 kDa)				90	-	35-50	3	n.s.
	PSf (60 kDa)				190	-	40-60	n.s.	n.s.
[82]	3 nm ZrO_2	MeOH, DMF, THF[i], toluene, hexane		n.s.	50	-	38.2 (MeOH), 21.6 (DMF), 28 (THF), 9.6 (toluene)	-	-

	5 nm TiO_2				120	-	70 (MeOH), 46 (DMF), 53 (THF), 14.8 (toluene)	-	-
	100 nm TiO_2				700	-	225 (MeOH), 159 (DMF), 220 (THF), 420 (hexane)	-	-
[30]	SelRO™ MPS-U20S (20 kDa)	Degraded straw extract (EtOH 75%)	EtOH/water 75%	1.Overnight immersion in water 2. Overnight immersion in feed solvent	42.88	-	41.50	4-11	n.s.
[48]	PES/PVP (50 kDa)	Refined soybean oil/n-hexane mixtures	Hexane	1. Overnight immersion in EtOH 2. Overnight immersion in n-propanol 3. Overnight immersion in butanol	120	-	After 1: 0.02 $g \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ After 2: 36 $g \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ After 3: <26 $g \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	13-60 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$[h]	Rejection of soybean oil 10-30%[h]
[13]	PC (0.05 µm),	Water, EtOH, hexane		30 min immersion in hexane	40	35	50 (EtOH), 40 (hexane)		-
	MCE (0.5 µm)				-	95	210 (hexane); flux for EtOH n.s		-
	MCE (0.025 µm)				90[j]	30	30 (EtOH), 200 (hexane)		-
	PVDF (30 kDa)				-	150	150 (EtOH), 1700 (hexane)		-
	PVDF (50 kDa)			1. Overnight immersion in water 2. Overnight immersion in EtOH 3. Overnight immersion in hexane	-	45-65[k]	25-35 (EtOH), 17-32 (hexane)		-
	PES (10 kDa)				-	20	35 (EtOH), 1.7-4 (hexane)		-

[96]	PES (MWCO n.s.)	Water	Acetone, EtOH, iso-propanol	2 h immersion in feed solvent	1055	2853 (acetone), 2211 (EtOH), 2192 (iso-propanol)	-	-	-
		Sludge supernatant			-	-	-	Data for EtOH n.s., 2000-10 (iso-propanol), 1350-5 (virgin)	-
	PSf (MWCO n.s.)	Water			339 (EtOH), 339 (iso-propanol). Data for acetone n.s.	1170 (EtOH), 1004 (iso-propanol)	-	-	-
		Sludge supernatant			-	-	-	1100-86 (EtOH), 1050-82 (iso-propanol), 350-58 (virgin)	-
		BSA solution			-	-	-	1100-90 (EtOH), 1000-90 (iso-propanol), 340-140 (virgin)	-
		Dextran solution			-	-	-	1100-50 (EtOH) 1000-60 (iso-propanol) 350-40 (virgin)	-
[97]	Ceramic: 19-channels, Al_2O_3 active layer (0.1 µm)	Miscella: Soybean oil in hexane (32% m/m)	Water, EtOH, hexane	1. Increasing EtOH proportion (30%, 50%, 70% and 100%). 2. Increasing hexane proportion 30%, 50%, 70% and 100%)	-	225	EtOH: 198 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ Hexane: 215 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$	28	Rejection of phosphorous content 99.7%
[94]	SelRO™ MPS-U20T (20 kDa)	SLE of corn xantophiles (EtOH 85%)	EtOH/water 85%	Increasing EtOH proportion by 10% until 85%	-	-	2.54	0.32	74%

	RTM-PX (n.s.)						3.7	0.05	91.4%
[52]	PES-ES404 (4 kDa)	Crude oil	Hexane	-	n.s.	14.1	9.7	8	Rejection of phospholipids 94.6%
	PES-ES209 (9 kDa)			-	n.s.	47.6	17	13.8	96.5%
[26]	PES (4 kDa)	Hexane		Studied strategies A. Immersion in iso-propanol 50% (24h), iso-propanol/hexane 50:50 (24h), hexane (24h) B. Overnight immersion in hexane	n.s.	After treatment A 14.1	9.7		-
	Ceramic membranes (ZrO_2; 1 kDa)				n.s.	n.s.	2.5-1.5		-
	Ceramic membranes (ZrO_2; 5 kDa)				29.1	n.s.	5		-
[73]	Polyamide (10 kDa)	Isooctane		Studied strategies A. Water (4h), iso-propanol 50% (3h), isooctane (12h) B. Water (4h), iso-propanol 80% (12h), iso-propanol:isooctane 50:50 (12h), iso-propanol:isooctane 20:80 (12h), isooctane (12h) C. Water (4h), iso-propanol 80% (12h), isooctane:isooctane 50:50 (3h), iso-	180	After Treatment C 600			-
	Polyamide (50 kDa)				320	700-525 Rest of pre-treatments: n.s.			-

				propanol:isooctane 20% (12h), isooctane (3h) D. Water (4h), iso-propanol:isooctane 50:50 (1.5h), isooctane (3h)					
[93]	Cellulose acetate (10 kDa)	Zein ethanolic solution	EtOH/water (0-70%)	<u>Studied strategies</u> A. Increasing EtOH proportion by 10% until 70% B. First treatment with water. Second one with EtOH 70% C. Direct treatment with EtOH 70% D. First treatment with EtOH 100%. Second one with EtOH 70%		-	<u>Results for EtOH 70%, after Treatment A</u>		<u>After Treatment A</u>
					49.5	-	18.2	n.s.	94
	Cellulose ester (10 kDa)				8.7	-	8		97
	Regenerated cellulose (10 kDa)				107.1[j]	-	0.014		95
	Prop. Composite (5 kDa)				8.2	-	8.5		86
	PAN (25 kDa)				57.5	-	27.9		99
	PES (10 kDa) – UFC				24	-	10.9		n.s.
	PES (10 kDa) – PES4H				8.2	-	2.3		95
	Modified PES (10 kDa) – Omega				54.5	-	19.8		98
	Modified PES (10 kDa) – Alpha				138.2	-	54.5		78
	PSf (10 kDa)				32.7	-	16.1		n.s.
	PSf (30 kDa)				167.3	-	33		n.s.
	PVDF (25 kDa)				n.s.	-	0.011		51
	SelRO™ MPS-U20S (20 Kda)				See [30]	-	3.6		93

	SelRO™ MPS-U20T (20 Kda)				n.s.	-	9.1		n.s.
(Ochoa et al. 2001	PVDF 15%-AM 1 (22 KDa)	Crude soybean oil	Hexane	-	n.s.	660.9	95.9		Rejection of phospholipids
	PES 17%-AM 2 (10 KDa)			-	n.s.	291.6	55.4		98.8%
									83.6%
	PVDF 17%-AM 3 (6 KDa)			-	n.s.	180.8	110.3		99.3%
	PSf 17%-AM 4 (10 KDa)			-	n.s.	374.4	222.3		60%

For the conditioning description, the capital letters (A, B, C…) indicate the different strategies evaluated; the numbers (1, 2, 3…) denote the order of the stages within a single treatment. When several treatments were applied, the results for the optimum conditioning have been reported here. As the values reported here have been retrieved from variable sources (graphs, tables, paper plain text…), some decimals may vary. The units of those values which are different from those specified in the Table heading have been detailed in the corresponding cell of the Table. When not specified, all solvent percentages are expressed on a v/v basis.

[a]PBI-based membranes prepared with different grafting conditions; [b]MeOH: methanol; [c]DMF: dimethylformamide; [d]cellulose membranes prepared with different casting solutions; [e]DMSO: dimethyl sulfoxide; [f]PGMEA: propylene glycol methyl ether acetate; [g]EtOH: ethanol; [h]Depending on the mass ratio of the mixture oil/solvent; [i]THF: tetrahydrofuran; [j]Data retrieved directly from the manufacturer; [k]depending on the feed temperature

5.1.1. *Membrane immersion in the same solvent as in the feed solution*

One of the first proposed methodologies to prepare ultrafiltration membranes to work with organic solvents replicates the general protocol followed when treating aqueous mediums. Membranes were immersed in the same solvent as would be found in the feed tank [98]. Torres et al. [14] let a PSf membrane in contact with pure ethanol during 24h. After that, they proceeded to measure ethanol permeate flux and they observed a low permeability (5.2 $L \cdot m^{-2} \cdot h^{-1}$), which was attributed to a shrinkage of the dense layer, leading to a structural alteration of the pore size. A similar approach was implemented by Koncsag and Kirwan [30]. These authors tested the SelRO™ MPS-U20S 20 kDa composite membrane from Koch Separations (Germany). The material of this membrane is crosslinked PAN [99] and the polymer was reported to be hydrophilic and solvent-stable. After an overnight immersion in water, they soaked the membrane in an ethanol/water (75:25 v/v) solution during 24h. Afterwards, the membrane was again flushed with the fresh solvent mixture and employed to ultrafilter an alcoholic extract of straw, obtained with the same proportion of ethanol and water mixture. Water flux at the selected operating conditions (8 bar and 22°C) was 343 $L \cdot m^{-2} \cdot h^{-1}$, while the flux of the ethanol/water mixture was 332 $L \cdot m^{-2} \cdot h^{-1}$. When the straw extract was treated, permeate fluxes were in the range of 35-87 $L \cdot m^{-2} \cdot h^{-1}$, varying according to the organic load of the sample. These authors did not find a significant decrease in permeate flux at the beginning of the process, which suggests that the cake layer was not formed or, conversely, the nature of the ethanolic feed prompted a pore blocking at the beginning of the operation. After performing a cleaning stage consisting of two cycles of rinsing with the working solvent, the membrane permeability (both to water and to the hydroalcoholic pure solvent) was restored, indicating that the fouling was reversible. As detailed in section 4.2, Ma et al. [16] also observed a low fouling tendency of a formamide solution of dextran during an ultrafiltration process with a 20 kDa PAN membrane from the company SolSep BV. They obtained permeate fluxes of 180 - 160 $L \cdot m^{-2} \cdot h^{-1} \cdot$bar and indicated the importance of the interactions occurring within the trio PAN-formamide-dextran.

5.1.2. *Membrane immersion in solutions of increasing percentage of the same solvent*

In this section, several studies in which the conditioning procedure includes a gradual exposure to organic solvents are presented. Shukla & Cheryan published in 2002 an outstanding paper in which several conditioning alternatives were discussed [93]. In order to condition different types of polymeric membranes for a posterior ultrafiltration of a 70% ethanolic extract, several possibilities were studied. They tested three different

pre-treatments: a conditioning with 70% ethanol solution, a transition from 100% ethanol to 70% ethanol and a gradual change from 10% to 70% ethanol, increasing the ethanol proportion by 10% each time. This last approach proved to be the most respectful with the membrane, whereas direct exposure to a high percentage of ethanol resulted in membrane damage. Thus, they demonstrated that abrupt changes in the polarity of the solvent were not advisable. The long-term stability of polymeric membranes in the presence of ethanol was checked in a posterior contribution of the same authors [100], providing useful data about the most resistant materials. The optimal conditions in terms of pre-treatment and membrane selection were reflected in successive works [94,95], in which the conditioning always consisted of setting the membrane in the ultrafiltration device, application of pressure to remove any remaining chemicals from the pores and posterior treatment with different solutions, increasing the percentage of ethanol by a 10% until reaching the ethanol proportion of the feed solution. This strategy has been adopted by other authors, who were able to suitably employ the membranes conditioned with this methodology [29,44]. See Table 5.1 (and section 4) for more details about these contributions.

5.1.3. Membrane immersion in different solvents

A similar concept underlies the soaking of the membrane in different solvents before its contact with the solvent of the feed. The intention is to gradually modify the polarity of the medium and slowly adapt the membrane. Instead of increasing the ratio of solvent/water (as presented before), the whole solvent is substituted by another one with higher or lower polarity, depending on the final objective. Following this strategy, Savaş-Alkan and Çulfaz-Emecen (2022) proposed a simple immersion of polymeric membranes in ethanol/water 20:80 (v/v) until use. This article was widely commented in Section 4.1. De Souza-Araki and co-workers (2010) soaked their membranes in water, ethanol, and, finally, hexane, which was the solvent aimed to be ultrafiltered. More details about their work can be found in section 4.2 and in Table 5.1. García and co-workers [26] also investigated the effect of the pre-treatment in the hexane permeate flux. They conditioned some organic (PES, 4 kDa) and ceramic (ZrO_2; 1 and 5 kDa) membranes by successive immersion in solutions of water/iso-propanol 50:50 (v/v), iso-propanol/hexane 50:50 (v/v) and, finally, pure hexane. Each immersion lasted 24h. This strategy was compared to a different pre-treatment, consisting of direct immersion in hexane (as in section 5.1.1). For the organic membranes, the sequential modification of the solvent polarity gave satisfactory results. Hexane permeate flux reached 120 $L \cdot m^{-2} \cdot h^{-1}$ at 1.2 MPa in this case, whereas the flux was almost zero when the conditioning consisted only of an immersion in hexane. On the contrary, ceramic membranes did not benefit

from any of the strategies and always displayed a downwards flux, as if hexane fouled the membrane. Badan Ribeiro et al. conditioned a ceramic membrane with mixtures of water/ethanol and ethanol/hexane [97] and achieved an evident improvement in hexane permeate flux. First, they moderately increased the proportion of ethanol until 70% was reached, and then, they slowly changed hexane abundance in the medium until the membrane was in contact with pure hexane. Before the membrane preparation, hexane permeate flux was nearly negligible, but after the conditioning, it reached values of 215 $kg \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ (around 328 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$). In a later work, De Melo et al. [49], whose work has been already introduced in previous sections, tested several solvents (water, ethanol, n-propanol, iso-propanol, and n-butanol) to pre-condition a 20 kDa ceramic membrane (α-Al_2O_3 /ZrO_2) before the ultrafiltration of an hexane solution. When the membrane was not pretreated, they found an extremely low permeability for hexane, lower than 0.25 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. It was reported to be higher for water, because its polarity is more similar to that of the membrane, supporting the influence of the solvent/membrane hydrophobicity or hydrophilicity that was commented in section 3. They obtained a large difference in solvent permeability after the conditioning. In the best case, hexane permeability was converted into 313.8 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$. N-butanol, whose polarity was the most similar to hexane, was chosen as the best medium to treat the membrane. Also, n-butanol molecules have the longest carbon chains, which is in accordance with the theory of the molecular organization already described in section 3. In a similar study, the same range of solvents was examined to condition a 50 kDa polyethersulfone/polyvinylpyrrolidone (PES/PVP) organic membrane. In contrast, n-butanol was not the best option to improve the permeate flux, but n-propanol [48]. These results again remark the complexity of the polymer-solvent interactions and the need to adapt the pre-treatment stage to the own characteristics of the membrane and solvent considered.

5.2. Organic solvent applied only as a pre-treatment agent

There are studies in which ethanol (or other organic solvent) has been used to condition the membrane prior to the permeance of aqueous mediums. They show a certain increase in water flux. Normally, this is due to the enlargement of pores and membrane layers, which facilitates the crossing of water molecules from one side to another of the membrane. Again, this pre-treatment could improve the accomplishment of the ultrafiltration, but also, it could negatively impact the results if the obtained rejections are not satisfactory.

In this context, a two-fold increment in pure water permeability was reported by Kochan and co-workers, who treated a PSf membrane with 80% ethanol solution as a wetting agent [96]. When they ultrafiltered protein and dextran solutions, alcoholic pre-treatment was reported to not affect the final permeability. However, the membranes wetted with 80% ethanol suffered a faster decline in the permeate flux during the filtration of the feed solutions. This may be attributed to the enlargement of the polymer pores, as a result of the solvent contact. Thicker pores could be susceptible of being blocked by larger molecules, which normally would remain in the cake layer, but in this case, the pores would be big enough to accommodate them. Argyle et al. also conditioned several PSf membranes with ethanolic solutions [55] (section 3). The feed solution in this study was black tea liquor, whose ultrafiltration was favored in terms of permeate flux. The authors observed increases in the relative flux that ranged between 1.32 and 2.13, depending on the membrane MWCO. An impressive 600% increment in water permeability was found by Ji et al. [19] after an ethanolic pre-conditioning of PSf membranes. However, the expansion of pores had some disadvantages too. It reduced rejection values and promoted the adsorption of proteins coming from the feed (pepsin and BSA solutions) on the membrane surface. As commented by these authors, the contact with the organic solvent may favor the adsorption of larger molecules from the feed inside the pores, thus, fouling should always be considered.

6. MOST RESISTANT MEMBRANES TO WORK WITH ORGANIC SOLVENTS

At this point, it seems pertinent to study the most suitable membrane materials when ultrafiltrating organic solvents. The selection of the appropriate polymer can be crucial in the success of the process. During the careful optimization that is required to find the adequate membrane, it is also reasonable to consider the polymer stability and break strength in the presence of the organic solvent. In-advance knowledge of potential interactions between solvent molecules and the membrane polymer could prevent some undesired phenomena, such as membrane damage, fouling, or insufficient separation efficiency in terms of solute rejection and permeate flux. This task is not effortless, because the membrane material is not the only variable able to influence the results available in the literature. In contrast, other specific characteristics of each study (feed solvent, viscosity, operation time, applied pressure, etc.) may be considered. All of these aspects have already been disclosed above.

However, previous results obtained in this area are very useful information to rely on. In this section, the results obtained with the most common membrane materials have been summarized and classified according to the organic solvent of choice. Such

classification aims to provide a guide about the polymer selection once the solvent to use has been determined. The use of ethanol and hexane (two of the organic solvents most commonly considered in the literature) for membrane filtration has been detailed.Ethanol is a colorless liquid, volatile, and flammable organic compound that belongs to the group of alcohols. It is used in the industry as raw material for the production of alcoholic drinks. Besides, it is present in pharmaceutical preparations and in the chemical industry (for cosmetics or perfume production). In addition, as it was mentioned in section 2, ethanol is one of the most eco-friendly organic solvents due to its biocompatible character, its low toxicity, and easy handling [7,29–33]. Hexane is an organic compound, colorless and volatile, with six carbon atoms forming a straight-chain alkane. Despite its toxicity [101], this organic solvent is broadly employed in many industries, such as chemical industries and edible oil industries.

6.1. Organic solvent: ethanol

In order to compare the results from different research works dealing with ethanol and the same membrane material but different MWCO, Table 5.2 has been provided. This table contains information about those references that included the water and the solvent permeability of the employed membranes. These data (included in Table 5.1) allowed the calculation of the deviation of the solvent permeability with respect to the water permeability of each membrane. This parameter was calculated following Equation 3:

$$\text{Water to solvent permeability ratio} = \frac{Solvent\ permeability}{Water\ permeability} \quad (3)$$

It should be noted that, for this calculation, the water permeability of the membranes without any conditioning or contact with an organic solvent has been considered. These water permeability values can be consulted in the sixth column of Table 5.1. Therefore, the reported ratio allows the comparison of the membrane performance in an aqueous environment and an organic medium.

As can be inferred from Table 5.1 and Table 5.2, PSf is one of the most used materials in membrane technology. Many PSf membranes are currently available for aqueous ultrafiltration. For that reason, there is a high interest in the design of a strategy that allows its application with organic solvents. PSf membranes of a wide range of MWCO have been tested with this objective.

Table 5.2. Ratio between ethanol and water permeability of the membranes described in this review and employed to treat hydroalcoholic solutions.

Membrane material	Proportion (ethanol/water)[a]	Ratio ethanol/water permeability	Reference
PVDF	100:0	1.94	[14]
PSf	100:0	0.19	
Polyamide	80:20	0.44	[29]
PES	80:20	0.35	
PVDF	80:20	0.39	
PSf	80:20	0.3	
Polyamide	52:48	0.63	[44]
	80:20	0.40	
PES	52:48	0.36	
	80:20	0.57	
PVDF	52:48	0.39	
	80:20	0.56	
PSf	52:48	0.21	
	80:20	0.32	
SelRO™ MPS-U20S	75:25	0.97	[30]
PC	100:0	1.32	[13]
MCE	100:0	0.33	
Cellulose acetate	70:30	0.37	[93]
Cellulose ester	70:30	0.92	
Regenerated celullose	70:30	0	
Prop. Composite	70:30	1.04	
PAN	70:30	0.49	
PES	70:30	0.45	[93]
PES	70:30	0.28	
Modified PES	70:30	0.36	
Modified PES	70:30	0.39	
PSf	70:30	0.49	
PSf	70:30	0.20	

[a]ethanol proportion is expressed in a v/v basis.

According to Table 5.2, the solvent-to-water permeability ratio for PSf membranes was 0.2 - 0.5 when they are used with ethanol. Shukla and Cheryan compared PSf membranes of different MWCOs (10 and 30 kDa) and configurations (flat sheet or hollow fiber) and obtained solvent permeabilities (ethanol/water 70:30 (v/v)) of 20 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ and 33 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, respectively, with flat sheet membranes [93]. Apart from PSf, Shukla and Cheryan studied cellulose-based membranes, PAN, PES, and PVDF polymers. The PAN (25 kDa) membrane was also a good alternative (with a solvent permeability of 27.9 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$), despite an observed ratio between solvent and water permeability of 0.49. When PES membranes were tested (with a MWCO of 10 kDa), permeability values of 2 - 11 $L \cdot m^{-2} \cdot h^{-1} \cdot bar$ were obtained for ethanol at 70% (v/v) [93]. In those cases, the rejection values to zein protein were high, above 78% at 1.38 bar. Among all the tested membranes, which were equally conditioned (section 5.1.2), these authors found the highest values of permeability for a modified-PES membrane (purchased from Pall Filtron) with the same MWCO of 10 kDa and subjected to the same pre-treatment (55 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$). In this work, a ratio between solvent and water permeability of 0.39 was obtained for the same membrane. These results suggest that the modified PES polymer displayed a lower tendency to get fouled by the solvent molecules, in comparison with the PSf membranes evaluated. The swelling phenomenon cannot be used here to explain the higher permeabilities of this PES membrane, because the corresponding rejection value was 78% when a zein protein (whose molecular weight is around 22-27 kDa [102]) solution was ultrafiltered with this set of membranes. PSf and PES polymers also yielded significant permeabilities in the study of Kochan et al., but the largest values were obtained for PES again [96].

Membranes of PVDF, which is a solvent-resistant polymer [103], have been another common alternative. In Table 5.2, the highest ratio between solvent and water permeability (1.94) was observed for a PVDF membrane after the filtration of pure ethanol. It indicates a considerably higher solvent flux than the water flux of the membrane. This fact can be attributed to a high affinity between the organic solvent and the PVDF material, which are both hydrophilic [14]. Furthermore, this membrane was prepared using a casting solution of low viscosity, which led to a finger-like substructure under the top layer that may have improved the solvent permeate flux. The works of Pinto and Baptista also dealt with PVDF membranes. For a 30 kDa MWCO membrane, they obtained similar permeabilities to ethanol at 52% (v/v), around 35.5 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$, corresponding to a ratio between solvent and water permeability of 0.39 [29,44]. The same polymer, with equal MWCO, was tested by De Souza Araki and co-workers. As they performed a different conditioning and selected pure ethanol as the feed solution, their

results are not comparable, but the high permeability that they obtained (more than 150 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$) is very promising and sets PVDF as an interesting polymer in this area.

Among those works in which the membrane was pretreated by overnight immersion in the feed solvent (such as ethanol or mixtures of ethanol/water), Koncsag and Kirwan [30] obtained a high permeability (343 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ and 332 $L \cdot m^{-2} \cdot h^{-1} \cdot bar^{-1}$ when they used water or mixture ethanol/water 75:25 (v/v), respectively) with the composite membrane MPS-U2OS, from Koch [30], already introduced in section 5.1.1. Torres and co-workers also applied this conditioning strategy to membranes made of different materials [14]. They reported higher permeability values for the membrane of PVDF versus the PSf membrane (see Table 5.1). The PVDF membrane had a slightly higher MWCO (7 kDa with respect of 5 kDa), however, the rejection of total glycerol was higher for this membrane than in the case of the PSf membrane.

6.2. Organic solvent: hexane

When the solvent to be processed was hexane, PES membranes were employed in several studies. Tres et al. employed a 50 kDa PES/PVP membrane to perform the separation of mixtures of soybean oil and hexane. To enhance the flux of hexane, several solvents were tested to condition the membranes. An overnight immersion in n-propanol gave the best results in this case, leading to a hexane flux of 36 $g \cdot m^{-2} \cdot h^{-1} \cdot bar$ [48], which corresponds to 0.055 $L \cdot m^{-2} \cdot h^{-1} \cdot bar$, considering that hexane density is 654.8 $kg \cdot m^{-3}$. De Souza-Araki also evaluated the permeability of hexane with a PES membrane (after a progressive conditioning with water, ethanol, and hexane (section 5.1.3), nevertheless, the contrasting of the results should be done carefully here, because the MWCO of the membrane was 10 kDa and so the differences in the permeate flux were expected. Still, the hexane permeability found in this work was around 3 $L \cdot m^{-2} \cdot h^{-1} \cdot bar$, which is larger than the value reported by Tres et al., despite working with a tighter membrane. These results underline the importance of a correct pre-conditioning of the membrane, especially when solvents of low polarity are employed.

In the study of De Souza-Araki, other polymers, such as MCE, PC, and PVDF were tested, with the latter being the most permeable to hexane. The major values of permeate flux registered were 25-30 $L \cdot m^{-2} \cdot h^{-1}$ (at 1.5 bar). This data was achieved with a 30 kDa PVDF membrane, whereas a 50 kDa PVDF provided a permeate flux of 13 $L \cdot m^{-2} \cdot h^{-1}$ (at 1.5 bar) approximately. The authors explained these results by a different morphology of the pores, according to their SEM analysis. For the membranes whose water permeability was reported, the ratio between solvent and water permeability (calculated

according to Equation 3) has been detailed in Table 5.3. As can be seen, solvent flux was not lower than the flux obtained with water for polymeric membranes (ratios higher than 1), suggesting that PC and MCE are also valid materials to work with hexane. By contrast, for ceramic membranes, in the study published by Buekenhoudt et al. [82], hexane permeability was lower than water permeability (ratio lower than 1), which can be attributed to the high hydrophilic character of the membrane surface and low polarity of the solvent.

Table 5.3. Ratio between hexane and water permeability of the membranes described in this review and employed to treat pure hexane.

Membrane material	Ratio hexane and water permeability	Reference
PC	1.05	[13]
MCE	2.22	
100 nm TiO_2	0.6	[82]

Several authors selected ceramic membranes to deal with hexane, and their works have been discussed in section 5.1.3. De Melo et al. and Badan-Ribeiro et al. found satisfactory values of permeate flux after a proper conditioning of the membranes [49,97]. Despite their higher price and a lower ratio of membrane area with respect to the module volume, the high chemical stability of inorganic membranes makes them a suitable option to work with organic solvents.

6.3. Other organic solvents

Even though the most prevalent organic solvents regarding membrane technology are ethanol (and its corresponding mixtures with water) and hexane, some applications require the use of other solvents less extended in the literature, such as acetone, heptane, isooctane, and iso-propanol. One of these applications is membrane emulsification [104]. According to this technique, the membrane acts as a barrier between the phase that will form the drops (dispersed phase) and the phase that will contain those droplets. By applying pressure, the dispersed phase will be forced through the membrane, creating drops when entering in contact with the continuous phase. One of the challenges of this procedure relies on the achievement of an adequate flow of the dispersed phase. Thus, the compatibility of the membrane and the organic solvent that may act as this dispersed phase is essential to overcome this limitation. To investigate this, Giorno et al. tested the flow of isooctane (a potential dispersion phase in membrane emulsification) with polyamide membranes [73]. After a treatment with water, iso-propanol at 80%, iso-propanol at 20%, and, finally, isooctane, they obtained isooctane permeate fluxes of 600 $L \cdot m^{-2} \cdot h^{-1} \cdot bar$ and 700 $L \cdot m^{-2} \cdot h^{-1} \cdot bar$ for a 10 kDa membrane and

50 kDa membrane, respectively. Then, the applied conditioning allowed the obtention of high fluxes to form oil-in-water emulsions with polymeric membranes.

7. CONCLUSIONS

Ultrafiltration is a commonly practiced technique, both at the industrial level and within research laboratories. It finds many applications in the food industry, for protein concentration, lactose concentration, separation of nutrients, etc. Wastewater treatment is also one of the main uses of this technology, including oil removal, reduction of the organic load, etc. Even the pharmaceutical industry has benefitted from ultrafiltration, performing blood plasma purification, for instance. All these uses disclose the versatility and advantages of this membrane process. However, the implementation of ultrafiltration membranes to treat solvent-based solutions is a less explored area, despite all the advantages that are derived from this technique. Among the applications of solvent-based ultrafiltration, it is possible to mention the recovery of valuable compounds from the agri-food and the pulp and paper industry, and the applications in the edible oil industry (during the step of recovering the solvent and the degumming process) and biorefineries.

Other membrane technologies, such as organic-solvent nanofiltration, have a well-established research community (from the academic and industry fields) aiming to advance in the knowledge and application of the process. In contrast, the investigation of solvent-based ultrafiltration still finds some room for growth. Researchers on the topic and membrane manufacturers have some work ahead to improve the performance of this procedure. The most commonly employed solvents in this area are ethanol (and mixtures of ethanol and water), and hexane. As reported in many studies, these solvents may interfere with the membrane structure, causing swelling and even membrane rupture. Therefore, investigating the interactions between the solvent and the polymer is still a pending task that will for sure allow the development of better, more resistant polymers. Furthermore, it is necessary to gain more knowledge regarding the solute-solvent, solute-membrane, and solvent-membrane interactions in organic media, because these three pairs of interactions may influence the fouling tendency and the transport of solutes during the ultrafiltration process.

To contribute to this effort, this review presents an overview of relevant data, regarding available values of permeate flux, rejection and selectivity, and variations of polymer morphology after the contact with the solvent. The importance of membrane pre-conditioning has also been highlighted. According to the literature, the main

strategies to condition an ultrafiltration membrane prior to its utilization in organic media are, on one side, its immersion in the same solvent as will be later employed in the feed stream, and, on the other side, the application of solvent solutions with increasing concentrations. Alternatively, the combination of different solvents with decreasing polarity has also been proposed.

As has been commented, some of the most preferred materials for organic solvent ultrafiltration are PA, PVDF, PES, PSf, PC, and cellulose derivatives. The values of permeability and permeate flux that have been presented here may imply a double interpretation when deciding if a given polymer is adequate or not for a specific application. This aspect has been highlighted during the whole extension of this review. In some scenarios, the highest permeate flux can be desirable, to enhance the efficiency of the process. However, if the rejection of solutes is a priority, membranes which had displayed a lower permeability after the conditioning could be preferable. In those cases, the organic solvent could generate swelling events and, even if satisfactory permeate fluxes are obtained, the solutes of interest may not be retained. Then, the conditioning of the membrane polymer should be optimized, and adjusted to each application, because its impact on the membrane performance is not trivial. With a correct proceeding, the pre-treatment of the membrane may allow the tuning of its properties, then tailoring the polymer according to the application. The MWCO should be carefully selected in those contexts and, even in some cases, a lower MWCO could be worthy of testing, in order to anticipate the potential swelling of the membrane and reduce its impact on the rejection.

The literature gap regarding solvent-based ultrafiltration indicates the challenges of this topic. However, the possibilities of this technique are an excellent incentive to increase the research efforts, improve the current understanding of the process and broaden its applications.

Funding: MCIN/AEI/ 10.13039/501100011033 and ERDF A way of making Europe funded the Grant CTM2017-88645-R. The grant PRE2018-08524 was funded by MCIN/AEI/ 10.13039/501100011033 and by ESF Investing in your future.

8. REFERENCES

[1] S. Al Aani, T.N. Mustafa, N. Hilal, Ultrafiltration membranes for wastewater and water process engineering: A comprehensive statistical review over the past decade, J. Water Process Eng. 35 (2020) 101241. https://doi.org/10.1016/j.jwpe.2020.101241.

[2] W. Shi, C. Yang, M. Qiu, X. Chen, Y. Fan, A new method for preparing α-alumina ultrafiltration membrane at low sintering temperature, J. Memb. Sci. 642 (2022) 119992. https://doi.org/10.1016/j.memsci.2021.119992.

[3] F. Bazzarelli, E. Piacentini, T. Poerio, R. Mazzei, A. Cassano, L. Giorno, Advances in membrane operations for water purification and biophenols recovery/valorization from OMWWs, J. Memb. Sci. 497 (2016) 402–409. https://doi.org/10.1016/j.memsci.2015.09.049.

[4] S. Álvarez-Blanco, J.-A. Mendoza-Roca, M.-J. Corbatón-Báguena, M.-C. Vincent-Vela, Valuable Products Recovery from Wastewater in Agrofood by Membrane Processes, in: A. Figoli, A. Criscuoli (Eds.), Sustain. Membr. Technol. Water Wastewater Treat. Green Chem. Sustain. Technol., Springer Nature Singapore, 2017: pp. 295–318. https://doi.org/10.1007/978-981-10-5623-9_11.

[5] R. Castro-Muñoz, C. Conidi, A. Cassano, Membrane-based technologies for meeting the recovery of biologically active compounds from foods and their by-products, Crit. Rev. Food Sci. Nutr. 59 (2019) 2927–2948. https://doi.org/10.1080/10408398.2018.1478796.

[6] A. Nazir, K. Khan, A. Maan, R. Zia, L. Giorno, K. Schroën, Membrane separation technology for the recovery of nutraceuticals from food industrial streams, Trends Food Sci. Technol. 86 (2019) 426–438. https://doi.org/10.1016/j.tifs.2019.02.049.

[7] H. Nawaz, J. Shi, G.S. Mittal, Y. Kakuda, Extraction of polyphenols from grape seeds and concentration by ultrafiltration, Sep. Purif. Technol. 48 (2006) 176–181. https://doi.org/10.1016/j.seppur.2005.07.006.

[8] R. Castro-Muñoz, G. Boczkaj, E. Gontarek, A. Cassano, V. Fíla, Membrane technologies assisting plant-based and agro-food by-products processing: A comprehensive review, Trends Food Sci. Technol. 95 (2020) 219–232. https://doi.org/10.1016/j.tifs.2019.12.003.

[9] S. Mohammed, Graphene oxide: A mini-review on the versatility and challenges as a membrane material for solvent-based separation, Chem. Eng. J. Adv. 12 (2022) 100392. https://doi.org/10.1016/j.ceja.2022.100392.

[10] S. Widodo, Khoiruddin, D. Ariono, Subagjo, I.G. Wenten, Membrane separation for non-aqueous solution, IOP Conf. Ser. Mater. Sci. Eng. 285 (2018). https://doi.org/10.1088/1757-899X/285/1/012008.

[11] A. Oxley, A.G. Livingston, Anti-fouling membranes for organic solvent nanofiltration (OSN) and organic solvent ultrafiltration (OSU): graft modified polybenzimidazole (PBI), J. Memb. Sci. 662 (2022) 120977. https://doi.org/10.1016/j.memsci.2022.120977.

[12] H. Susanto, M. Ulbricht, Characteristics, performance and stability of polyethersulfone ultrafiltration membranes prepared by phase separation method using different macromolecular additives, J. Memb. Sci. 327 (2009) 125–135. https://doi.org/10.1016/J.MEMSCI.2008.11.025.

[13] M. de Souza Araki, C. de Morais Coutinho, L.A.G. Gonçalves, L.A. Viotto, Solvent permeability in commercial ultrafiltration polymeric membranes and evaluation of the structural and chemical stability towards hexane, Sep. Purif. Technol. 71 (2010) 13–21. https://doi.org/10.1016/j.seppur.2009.10.005.

[14] J.J. Torres, N.E. Rodriguez, J. Toledo Arana, Ochoa, N. Ariel, J. Marchese, C. Plagiero, Ultrafiltration polymeric membranes for the purification of biodiesel from ethanol, J. Clean. Prod. 141 (2017) 641–647. http://dx.doi.org/10.1016/j.jclepro.2016.09.130.

[15] M.B. Tanis-Kanbur, N.R. Tamilselvam, J.W. Chew, Membrane fouling mechanisms by BSA in aqueous-organic solvent mixtures, J. Ind. Eng. Chem. 108 (2022) 389–399. https://doi.org/10.1016/j.jiec.2022.01.017.

[16] Y. Ma, S. Velioğlu, Z. Yin, R. Wang, J.W. Chew, Molecular dynamics investigation of membrane fouling in organic solvents, J. Memb. Sci. 632 (2021) 119329. https://doi.org/10.1016/J.MEMSCI.2021.119329.

[17] H.T. Lay, J.W. Chew, Critical flux of colloidal foulant in microfiltration: Effect of organic solvent, J. Memb. Sci. 616 (2020) 118531. https://doi.org/10.1016/j.memsci.2020.118531.

[18] Z. Yin, Y. Ma, B. Tanis-Kanbur, J.W. Chew, Fouling behavior of colloidal particles in organic solvent ultrafiltration, J. Memb. Sci. 599 (2020) 19–22. https://doi.org/10.1016/j.memsci.2020.117836.

[19] M. Ji, P.K. Roy, J. Barman, K. Khare, Commercial polysulfone membranes pretreated with ethanol and NaOH: Effects on permeability, selectivity and antifouling properties, Langmuir. 35 (2019) 7672–7679. https://doi.org/10.1021/acs.langmuir.9b00483.

[20] B. Van der Bruggen, M. Mänttäri, M. Nyström, Drawbacks of applying nanofiltration and how to avoid them: A review, Sep. Purif. Technol. 63 (2008) 251–263. https://doi.org/10.1016/j.seppur.2008.05.010.

[21] F. Lipnizki, J. Thuvander, G. Rudolph, Membrane processes and applications for biorefineries, in: Curr. Trends Futur. Dev. Membr. Membr. Environ. Appl., 2019: pp. 283–301. https://doi.org/10.1016/B978-0-12-816778-6.00013-8.

[22] P. Ramesh, W.L. Xu, M. Sorci, C. Trant, S. Lee, J. Kilduff, M. Yu, G. Belfort, Organic

solvent filtration by brush membranes: Permeability, selectivity and fouling correlate with degree of SET-LRP grafting, J. Memb. Sci. 618 (2021) 118699. https://doi.org/10.1016/j.memsci.2020.118699.

[23] N. Ochoa, C. Pagliero, J. Marchese, M. Mattea, Ultrafiltration of vegetable oils degumming by polymeric membranes, Sep. Purif. Technol. 22–23 (2001) 417–422. https://doi.org/10.1016/S1383-5866(00)00178-7.

[24] R.B. Merlet, M.A. Pizzoccaro-Zilamy, A. Nijmeijer, L. Winnubst, Hybrid ceramic membranes for organic solvent nanofiltration: State-of-the-art and challenges, J. Memb. Sci. 599 (2020). https://doi.org/10.1016/j.memsci.2020.117839.

[25] D. Ren, S. Ren, Y. Lin, J. Xu, X. Wang, Recent developments of organic solvent resistant materials for membrane separations, Chemosphere. 271 (2021) 129425. https://doi.org/10.1016/J.CHEMOSPHERE.2020.129425.

[26] A. García, S. Álvarez, F. Riera, R. Álvarez, J. Coca, Water and hexane permeate flux through organic and ceramic membranes: Effect of pretreatment on hexane permeate flux, J. Memb. Sci. 253 (2005) 139–147. https://doi.org/10.1016/j.memsci.2004.11.030.

[27] H. Alhweij, E.A. Carolina Emanuelsson, S. Shahid, J. Wenk, High performance in-situ tuned self-doped polyaniline (PANI) membranes for organic solvent (nano)filtration, Polymer (Guildf). 245 (2022) 124682. https://doi.org/10.1016/j.polymer.2022.124682.

[28] A. Savaş-Alkan, P.Z. Çulfaz-Emecen, Solvent recovery from photolithography wastes using cellulose ultrafiltration membranes, J. Memb. Sci. 647 (2022) 120261. https://doi.org/10.1016/j.memsci.2022.120261.

[29] E.A. Baptista, P.C.R. Pinto, I.F. Mota, J.M. Loureiro, A.E. Rodrigues, Ultrafiltration of ethanol/water extract of Eucalyptus globulus bark: Resistance and cake build up analysis, Sep. Purif. Technol. 144 (2015) 256–266. https://doi.org/10.1016/j.seppur.2015.02.036.

[30] C.I. Koncsag, K. Kirwan, A membrane screening for the separation/concentration of dilignols and trilignols from solvent extracts, Sep. Purif. Technol. 94 (2012) 54–60. https://doi.org/10.1016/j.seppur.2012.04.009.

[31] M. Loginov, N. Boussetta, N. Lebovka, E. Vorobiev, Separation of polyphenols and proteins from flaxseed hull extracts by coagulation and ultrafiltration, J. Memb. Sci. 442 (2013) 177–186. https://doi.org/10.1016/j.memsci.2013.04.036.

[32] J.G. Crespo, C. Brazinha, Membrane processing: Natural antioxidants from winemaking by-products, Filtr. Sep. 47 (2010) 32–35. https://doi.org/10.1016/S0015-1882(10)70079-3.

[33] J.Y. Tovar-Sánchez, M.B. de la Mora, T. García-Fernández, M.A. Valverde-Alva, C. Sánchez-Aké, R. García-Contreras, R. Castañeda-Guzmán, M. Villagrán-Muniz,

Biocompatible POSS-gold nanocomposites synthesized by laser ablation in ethanol, Opt. Laser Technol. 147 (2022). https://doi.org/10.1016/j.optlastec.2021.107650.

[34] S.H. Chang, Utilization of green organic solvents in solvent extraction and liquid membrane for sustainable wastewater treatment and resource recovery—a review, Environ. Sci. Pollut. Res. 2020 2726. 27 (2020) 32371–32388. https://doi.org/10.1007/S11356-020-09639-7.

[35] G. Szekely, M.F. Jimenez-Solomon, P. Marchetti, J.F. Kim, A.G. Livingston, Sustainability assessment of organic solvent nanofiltration: From fabrication to application, Green Chem. 16 (2014) 4440–4473. https://doi.org/10.1039/c4gc00701h.

[36] S.A. Naziri Mehrabani, V. Vatanpour, I. Koyuncu, Green solvents in polymeric membrane fabrication: A review, Sep. Purif. Technol. 298 (2022) 121691. https://doi.org/10.1016/j.seppur.2022.121691.

[37] M.A. Bustamante, R. Moral, C. Paredes, A. Pérez-Espinosa, J. Moreno-Caselles, M.D. Pérez-Murcia, Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry, Waste Manag. 28 (2008) 372–380. https://doi.org/10.1016/j.wasman.2007.01.013.

[38] C.M. Galanakis, E. Markouli, V. Gekas, Recovery and fractionation of different phenolic classes from winery sludge using ultrafiltration, Sep. Purif. Technol. 107 (2013) 245–251. https://doi.org/10.1016/J.SEPPUR.2013.01.034.

[39] P. Tapia-Quirós, M.F. Montenegro-Landívar, M. Reig, X. Vecino, T. Alvarino, J.L. Cortina, J. Saurina, M. Granados, Olive mill and winery wastes as viable sources of bioactive compounds: A study on polyphenols recovery, Antioxidants. 9 (2020) 1–15. https://doi.org/10.3390/antiox9111074.

[40] C.M. Sánchez-Arévalo, A. Iborra-Clar, M.C. Vincent-Vela, S. Álvarez-Blanco, Exploring the extraction of the bioactive content from the two-phase olive mill waste and further purification by ultrafiltration, LWT - Food Sci. Technol. 165 (2022) 113742. https://doi.org/10.1016/j.lwt.2022.113742.

[41] H. Jemai, A. El Feki, S. Sayadi, Antidiabetic and Antioxidant Effects of Hydroxytyrosol and Oleuropein from Olive Leaves in Alloxan-Diabetic Rats, J. Agric. Food Chem. 57 (2009) 8798–8804. https://doi.org/10.1021/jf901280r.

[42] R. Erragued, M.E.M. Braga, M. Bouaziz, L.M. Gando-Ferreira, Integration of solvent extraction and membrane processes to produce an oleuropein extract from olive leaves, Sep. Purif. Technol. 299 (2022) 121751. https://doi.org/10.1016/J.SEPPUR.2022.121751.

[43] P.C.R. Pinto, G. Sousa, F. Crispim, A.J.D. Silvestre, C.P. Neto, Eucalyptus globulus bark as source of tannin extracts for application in leather industry, ACS Sustain. Chem. Eng. 1 (2013) 950–955. https://doi.org/10.1021/sc400037h.

[44] P.C.R. Pinto, I.F. Mota, J.M. Loureiro, A.E. Rodrigues, Membrane performance and application of ultrafiltration and nanofiltration to ethanol/water extract of Eucalyptus bark, Sep. Purif. Technol. 132 (2014) 234–243. https://doi.org/10.1016/j.seppur.2014.04.042.

[45] P.R. Pinto, I.F. Mota, C.M. Pereira, A.M. Ribeiro, J.M. Loureiro, A.E. Rodrigues, Separation and recovery of polyphenols and carbohydrates from Eucalyptus bark extract by ultrafiltration/diafiltration and adsorption processes, Sep. Purif. Technol. 183 (2017) 96–105. https://doi.org/10.1016/j.seppur.2017.04.003.

[46] L.A. Johnson, E.W. Lusas, Comparison of alternative solvents for oils extraction, J. Am. Oil Chem. Soc. 60 (1983) 229–242. https://doi.org/10.1007/BF02543490.

[47] E. Reverchon, I. De Marco, Supercritical fluid extraction and fractionation of natural matter, J. Supercrit. Fluids. 38 (2006) 146–166. https://doi.org/10.1016/J.SUPFLU.2006.03.020.

[48] M. V. Tres, R. Nobrega, R.B. Carvalho, J. V. Oliveira, M. Di Luccio, Solvent recovery from soybean oil/n-hexane mixtures using hollow fiber membrane, Brazilian J. Chem. Eng. 29 (2012) 577–584. https://doi.org/10.1590/S0104-66322012000300015.

[49] J.R.M. de Melo, M. V. Tres, J. Steffens, J. Vladimir Oliveira, M. Di Luccio, Desolventizing organic solvent-soybean oil miscella using ultrafiltration ceramic membranes, J. Memb. Sci. 475 (2015) 357–366. https://doi.org/10.1016/j.memsci.2014.10.029.

[50] A.P. Badan Ribeiro, J.M.L.N. de Moura, L.A.G. Gonçalves, J.C.C. Petrus, L.A. Viotto, Solvent recovery from soybean oil/hexane miscella by polymeric membranes, J. Memb. Sci. 282 (2006) 328–336. https://doi.org/10.1016/j.memsci.2006.05.036.

[51] M.H. Abdellah, C.A. Scholes, L. Liu, S.E. Kentish, Efficient degumming of crude canola oil using ultrafiltration membranes and bio-derived solvents, Innov. Food Sci. Emerg. Technol. 59 (2020) 102274. https://doi.org/10.1016/j.ifset.2019.102274.

[52] A. García, S. Álvarez, F. Riera, R. Álvarez, J. Coca, Sunflower oil miscella degumming with polyethersulfone membranes: Effect of process conditions and MWCO on fluxes and rejections, J. Food Eng. 74 (2006) 516–522. https://doi.org/10.1016/j.jfoodeng.2005.03.038.

[53] A. Oxley, P.R.J. Gaffney, D. Kim, P. Marchetti, A.G. Livingston, Graft modification of polybenzimidazole membranes for organic solvent ultrafiltration with scale up to spiral wound modules, J. Memb. Sci. 647 (2022) 120199. https://doi.org/10.1016/j.memsci.2021.120199.

[54] D.R. Machado, D. Hasson, R. Semiat, Effect of solvent properties on permeate flow through nanofiltration membranes. Part II. Transport model, J. Memb. Sci. 166 (2000) 63–69. https://doi.org/10.1016/S0376-7388(99)00251-3.

[55] I.S. Argyle, A. Pihlajamäki, M.R. Bird, Black tea liquor ultrafiltration: Effect of ethanol pre-treatment upon fouling and cleaning characteristics, Food Bioprod. Process. 93 (2015) 289–297. https://doi.org/10.1016/j.fbp.2014.10.010.

[56] S. Ramaswamy, H.J. Huang, B. V. Ramarao, Separation and Purification Technologies in Biorefineries, Sep. Purif. Technol. Biorefineries. (2013) 1–584. https://doi.org/10.1002/9781118493441.

[57] A.A. Kiss, J.P. Lange, B. Schuur, D.W.F. Brilman, A.G.J. van der Ham, S.R.A. Kersten, Separation technology–Making a difference in biorefineries, Biomass and Bioenergy. 95 (2016) 296–309. https://doi.org/10.1016/J.BIOMBIOE.2016.05.021.

[58] M. Chadni, M. Moussa, V. Athès, F. Allais, I. Ioannou, Membrane contactors-assisted liquid-liquid extraction of biomolecules from biorefinery liquid streams: A case study on organic acids, Sep. Purif. Technol. 317 (2023) 123927. https://doi.org/10.1016/j.seppur.2023.123927.

[59] A.P. Richez, H.N. Yow, S. Biggs, O.J. Cayre, Dispersion polymerization in non-polar solvent: Evolution toward emerging applications, Prog. Polym. Sci. 38 (2013) 897–931. https://doi.org/10.1016/j.progpolymsci.2012.12.001.

[60] T. Wongsawa, T. Koonsang, N. Kunthakudee, T. Prapasawat, K. Maneeintr, U. Pancharoen, The experimental investigations on viscosity, surface tension, interfacial tension and solubility of the binary and ternary systems for tributyl phosphate (TBP) extractant in various organic solvents with water: Thermodynamic NRTL model and molecular inte, J. Mol. Liq. 251 (2018) 229–237. https://doi.org/10.1016/j.molliq.2017.12.074.

[61] R. Haghbakhsh, M. Taherzadeh, A.R.C. Duarte, S. Raeissi, A general model for the surface tensions of deep eutectic solvents, J. Mol. Liq. 307 (2020) 112972. https://doi.org/10.1016/J.MOLLIQ.2020.112972.

[62] H. Takeuchi, K. Takahashi, W. Goto, Some observations on the stability of supported liquid membranes, J. Memb. Sci. 34 (1987) 19–31. https://doi.org/10.1016/S0376-7388(00)80018-6.

[63] R.S.L. Yee, K. Zhang, B.P. Ladewig, The effects of sulfonated poly(ether ether ketone) ion exchange preparation conditions on membrane properties, Membranes (Basel). 3 (2013) 182–195. https://doi.org/10.3390/membranes3030182.

[64] L. Zhang, Y. Lin, L. Cheng, Z. Yang, H. Matsuyama, A comprehensively fouling-and solvent-resistant aliphatic polyketone membrane for high-flux filtration of difficult oil-in-water micro-and nanoemulsions, (2019). https://doi.org/10.1016/j.memsci.2019.03.090.

[65] D. Chen, C. Yan, X. Li, L. Liu, D. Wu, X. Li, A highly stable PBI solvent resistant nanofiltration membrane prepared via versatile and simple crosslinking process,

Sep. Purif. Technol. 224 (2019) 15–22. https://doi.org/10.1016/j.seppur.2019.04.077.

[66] N. Nasrollahi, L. Ghalamchi, V. Vatanpour, A. Khataee, M. Yousefpoor, Novel polymeric additives in the preparation and modification of polymeric membranes: A comprehensive review, J. Ind. Eng. Chem. 109 (2022) 100–124. https://doi.org/10.1016/j.jiec.2022.02.036.

[67] M.A. Abdulhamid, G. Szekely, Organic solvent nanofiltration membranes based on polymers of intrinsic microporosity, Curr. Opin. Chem. Eng. 36 (2022) 100804. https://doi.org/10.1016/j.coche.2022.100804.

[68] B. Van der Bruggen, J. Geens, C. Vandecasteele, Influence of organic solvents on the performance of polymeric nanofiltration membranes, Sep. Sci. Technol. 37 (2002) 783–797. https://doi.org/10.1081/SS-120002217.

[69] Q.T. Nguyen, P. Aptel, J. Neel, Characterization of ultrafiltration membranes, J. Memb. Sci. 5 (1979) 235–251. https://doi.org/10.1016/s0376-7388(00)80451-2.

[70] E. Gungormus, S. Alsoy Altinkaya, A new-generation poly (ether imide sulfone) based solvent resistant ultrafiltration membrane for a sustainable production of silica nanopowder, Sep. Purif. Technol. 304 (2023) 122351. https://doi.org/10.1016/j.seppur.2022.122351.

[71] A. Krupková, M. Müllerová, R. Petrickovic, T. Strašák, On the edge between organic solvent nanofiltration and ultrafiltration: Characterization of regenerated cellulose membrane with aspect on dendrimer purification and recycling, Sep. Purif. Technol. 310 (2023) 123141. https://doi.org/10.1016/j.seppur.2023.123141.

[72] S. Zeidler, U. Kätzel, P. Kreis, Systematic investigation on the influence of solutes on the separation behavior of a PDMS membrane in organic solvent nanofiltration, J. Memb. Sci. 429 (2013) 295–303. https://doi.org/10.1016/J.MEMSCI.2012.11.056.

[73] L. Giorno, R. Mazzei, M. Oriolo, G. De Luca, M. Davoli, E. Drioli, Effects of organic solvents on ultrafiltration polyamide membranes for the preparation of oil-in-water emulsions, J. Colloid Interface Sci. 287 (2005) 612–623. https://doi.org/10.1016/j.jcis.2005.02.015.

[74] I.S. Argyle, C.J. Wright, M.R. Bird, The effect of ethanol pre-treatment upon the mechanical, structural and surface modification of ultrafiltration membranes, Sep. Sci. Technol. 52 (2017) 2040–2048. https://doi.org/10.1080/01496395.2017.1310234.

[75] S. Belfer, R. Fainchtain, Y. Purinson, O. Kedem, Surface characterization by FTIR-ATR spectroscopy of polyethersulfone membranes-unmodified, modified and protein fouled, J. Memb. Sci. 172 (2000) 113–124. https://doi.org/10.1016/S0376-7388(00)00316-1.

[76] A. Weis, M.R. Bird, M. Nyström, The chemical cleaning of polymeric UF membranes fouled with spent sulphite liquor over multiple operational cycles, J. Memb. Sci. 216 (2003) 67–79. https://doi.org/10.1016/S0376-7388(03)00047-4.

[77] M.Y. Jaffrin, J.P. Charrier, Optimization of ultrafiltration and diafiltration processes for albumin production, J. Memb. Sci. 97 (1994) 71–81. https://doi.org/10.1016/0376-7388(94)00148-R.

[78] M.L. Huber, A.H. Harvey, Fluid Properties. Viscosity of Liquids, in: J.R. Rumble (Ed.), Handb. Chem. Phys., 99th ed., Taylor & Francis, Boca Ratón, Florida, 2018: pp. 231–235.

[79] I.S. Khattab, F. Bandarkar, M.A.A. Fakhree, A. Jouyban, Density, viscosity, and surface tension of water+ethanol mixtures from 293 to 323K, Korean J. Chem. Eng. 29 (2012) 812–817. https://doi.org/10.1007/s11814-011-0239-6.

[80] D.R. Paul, O.M. Ebra-Lima, The mechanism of liquid transport through swollen polymer membranes, J. Appl. Polym. Sci. 15 (1971) 2199–2210. https://doi.org/10.1002/app.1971.070150912.

[81] A. Oxley, P.R.J. Gaffney, D. Kim, P. Marchetti, A.G. Livingston, Graft modification of polybenzimidazole membranes for organic solvent ultrafiltration with scale up to spiral wound modules, J. Memb. Sci. 647 (2022) 120199. https://doi.org/10.1016/j.memsci.2021.120199.

[82] A. Buekenhoudt, F. Bisignano, G. De Luca, P. Vandezande, M. Wouters, K. Verhulst, Unravelling the solvent flux behaviour of ceramic nanofiltration and ultrafiltration membranes, J. Memb. Sci. 439 (2013) 36–47. https://doi.org/10.1016/j.memsci.2013.03.032.

[83] G. Ignacz, G. Szekely, Deep learning meets quantitative structure–activity relationship (QSAR) for leveraging structure-based prediction of solute rejection in organic solvent nanofiltration, J. Memb. Sci. 646 (2022) 120268. https://doi.org/10.1016/J.MEMSCI.2022.120268.

[84] P.C. Sadek, The HPLC Solvent Guide, 2nd ed., John Wiley & Sons, Ltd, Michigan, 2002.

[85] STD, Surface Tension Database, (2017). http://surface-tension.de/.

[86] DDB, Dortmund Data Bank, Explorer (2001). http://www.ddbst.com/ddb.html.

[87] Z. Yin, R.J.E. Yeow, Y. Ma, J.W. Chew, Link between interfacial interaction and membrane fouling during organic solvent ultrafiltration of colloidal foulants, J. Memb. Sci. 611 (2020) 118369. https://doi.org/10.1016/j.memsci.2020.118369.

[88] M.Y. Jaffrin, L.H. Ding, C. Couvreur, P. Khari, Effect of ethanol on ultrafiltration of bovine albumin solutions with organic membranes, J. Memb. Sci. 124 (1997) 233–241. https://doi.org/10.1016/S0376-7388(96)00241-4.

[89] C. Guizard, A. Ayral, A. Julbe, Potentiality of organic solvents filtration with ceramic membranes. A comparison with polymer membranes, Desalination. 147 (2002) 275–280. https://doi.org/10.1016/S0011-9164(02)00552-0.

[90] D. Ballal, W.G. Chapman, Hydrophobic and hydrophilic interactions in aqueous mixtures of alcohols at a hydrophobic surface, J. Chem. Phys. 139 (2013). https://doi.org/10.1063/1.4821604.

[91] M. Razali, C. Didaskalou, J.F. Kim, M. Babaei, E. Drioli, Y.M. Lee, G. Szekely, Exploring and Exploiting the Effect of Solvent Treatment in Membrane Separations, ACS Appl. Mater. Interfaces. 9 (2017) 11279–11289. https://doi.org/10.1021/acsami.7b01879.

[92] A.R.L. Fraga, J.C. Quintana, G.L. Destri, N. Giamblanco, R.G. Toro, F. Punzo, Polymeric membranes conditioning for sensors applications: Mechanism and influence on analytes detection, J. Solid State Electrochem. 16 (2012) 901–909. https://doi.org/10.1007/s10008-011-1456-y.

[93] R. Shukla, M. Cheryan, Performance of ultrafiltration membranes in ethanol-water solutions: Effect of membrane conditioning, J. Memb. Sci. 198 (2002) 75–85. https://doi.org/10.1016/S0376-7388(01)00638-X.

[94] E.M. Tsui, M. Cheryan, Membrane processing of xanthophylls in ethanol extracts of corn, J. Food Eng. 83 (2007) 590–595. https://doi.org/10.1016/j.jfoodeng.2007.03.041.

[95] E.M. Tsui, M. Cheryan, Characteristics of nanofiltration membranes in aqueous ethanol, J. Memb. Sci. 237 (2004) 61–69. https://doi.org/10.1016/j.memsci.2004.02.026.

[96] J. Kochan, T. Wintgens, R. Hochstrat, T. Melin, Impact of wetting agents on the filtration performance of polymeric ultrafiltration membranes, Desalination. 241 (2009) 34–42. https://doi.org/10.1016/j.desal.2008.01.056.

[97] A.P. Badan Ribeiro, N. Bei, L.A. Guaraldo Gonçalves, J.C. Cunha Petrus, L.A. Viotto, The optimisation of soybean oil degumming on a pilot plant scale using a ceramic membrane, J. Food Eng. 87 (2008) 514–521. https://doi.org/10.1016/j.jfoodeng.2008.01.003.

[98] R.W. Lencki, S. Williams, Effect of nonaqueous solvents on the flux behavior of ultrafiltration membranes, J. Memb. Sci. 101 (1995) 43–51. https://doi.org/10.1016/0376-7388(94)00282-4.

[99] P. Vandezande, L.E.M. Gevers, I.F.J. Vankelecom, Solvent resistant nanofiltration: Separating on a molecular level, Chem. Soc. Rev. 37 (2008) 365–405. https://doi.org/10.1039/b610848m.

[100] R. Shukla, M. Cheryan, Stability and Performance of Ultrafiltration Membranes in Aqueous Ethanol, Sep. Sci. Technol. 38 (2003) 1533–1547.

https://doi.org/10.1081/SS-120019091.

[101] M.J. Morrow, Encyclopedia of the Neurological Sciences (Second Edition), Encycl. Neurol. Sci. (Second Ed. (2014) 892–893. http://www.sciencedirect.com:5070/referencework/9780123851581/encyclopedia-of-the-neurological-sciences (accessed March 15, 2023).

[102] A.O. Elzoghby, M.M. Elgohary, N.M. Kamel, Implications of Protein- and Peptide-Based Nanoparticles as Potential Vehicles for Anticancer Drugs, in: Adv. Protein Chem. Struct. Biol., Academic Press, 2015: pp. 169–221. https://doi.org/10.1016/bs.apcsb.2014.12.002.

[103] S. Ebnesajjad, Introduction to Fluoropolymers, in: Appl. Plast. Eng. Handb., William Andrew Publishing, 2011: pp. 49–60. https://doi.org/10.1016/B978-1-4377-3514-7.10004-2.

[104] E. Piacentini, E. Drioli, L. Giorno, Membrane emulsification technology: Twenty-five years of inventions and research through patent survey, J. Memb. Sci. 468 (2014) 410–422. https://doi.org/10.1016/J.MEMSCI.2014.05.059.

CAPÍTULO 6. CHAPTER 6

Solvent-resistant ultrafiltration to recover bioactive compounds from wet olive pomace extracts

Carmen M. Sánchez-Arévalo[1], Alicia Iborra Clar[1,2], María Cinta Vincent-Vela[1,2], Silvia Álvarez-Blanco[1,2]

[1]Research Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

[2]Department of Chemical and Nuclear Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

✉sialvare@iqn.upv.es

LWT - Food Science and Technology, 186 (2023) 115167

Abstract: To contribute to the circular economy in olive mills, wet olive pomace can be employed as a source of valuable phenolic compounds. These compounds are efficiently extracted with ethanol/water 50:50 (v/v), at 40˚C. Here, the ultrafiltration membranes UF010104, UF010801v3 (from SolSep BV), and UP005 (Microdyn Nadir) have been tested to purify the phenolic compounds present in the hydroalcoholic extracts of the pomace. Several membrane conditioning protocols were explored, being the most adequate a short pre-treatment consisting of soaking the membrane in the working solvent for two hours. All the considered membranes permitted the recovery of the biophenols of interest in the permeate stream, being most of the organic matter retained. A powerful analytical methodology based on liquid chromatography coupled to mass spectrometry (LC-ESI-QToF-MS) allowed the evaluation of the individual rejection of 43 compounds. The UF010104 membrane displayed the highest permeate flux (15 $L \cdot h^{-1} \cdot m^{-2}$, at 2.5 bar) and high rejection of the total solids (67 ± 3%, at 2.5 bar), thus achieving the purification of the phenolic compounds in the permeate stream. The cleaning of the membranes used in organic solvent media was also investigated.

Keywords: solvent-resistant ultrafiltration, ultrafiltration, ethanol, phenolic compounds, wet olive pomace.

1. INTRODUCTION

The increasing recognition of olive oil as a beneficial fat has resulted in great production rates over the last years. This extended processing of olives ends with tons of appreciated olive oil, but also, large amounts of by-products are derived from the process. The majority of the olive oil produced in the world is generated by the two-phase methodology [1]. According to this method, vast amounts of wet olive pomace are produced. This is a semi-solid residue, which contains the remains of the olive drupe after its crushing, malaxation, and centrifugation to extract the oil. In consequence, it is a mixture of olive pulp, stones, seeds, and vegetation water. The enormous and periodic generation of a residue with such a high organic load can raise some concerns about the environmental effects to be expected if the wet olive pomace is not properly disposed of. Some of the derived risks are water contamination, microbiome damage and alteration of the autochthonous flora and fauna of the affected area [2,3].

One of the reasons for the phytotoxicity attributed to the wet olive pomace is its high content of phenolic compounds. However, these compounds also represent a source of wealth, due to their potent antioxidant properties, which enable wide applications in cosmetics, pharmaceutics and nutraceuticals [1,4]. As a consequence, the utilization of the wet olive pomace as a source of phenolic compounds implies the

obtainment of high-added value compounds and reduces the risk of inappropriate dumping into the environment. Furthermore, the large production volumes of wet olive pomace make this by-product a low-cost raw material.

All these reasons motivate its use to recover bioactive compounds, including polyphenols. To that end, a solid-liquid extraction (SLE) needs to be performed [5,6]. As a result, the phenolic content of the sample is retrieved, but some concomitant organic matter is extracted too. To perform the purification of the compounds of interest, membrane technology can be applied. It offers high selectivity, scalability, and the possibility of reusing the membranes. Ultrafiltration has given excellent results when it comes to treating agro-food effluents, both for the purification and the concentration of interesting compounds [7,8]. In this case, the ultrafiltration of the obtained extract contributes to remove polysaccharides, pigments, and other solutes, reducing the total solids content and the chemical oxygen demand of the samples. The polyphenols are then recovered in the permeate stream, at a higher purity.

Different solvents can be considered in the SLE stage to increment the extraction yield. It has been described that mixtures of ethanol and water are very effective for this purpose [6] and high recoveries of polyphenols have been obtained when ethanol/water 50:50 (v/v) was employed as extractant. However, the purification of an ethanolic extract from wet olive pomace by means of ultrafiltration is absent in the literature. To the best of our knowledge, there are no contributions on this matter. This is also applicable to other agri-food matrices. In this context, the membrane processing of solvent-based extracts is very scarce [9]. Regarding membrane processes, and especially ultrafiltration, aqueous filtration has been extensively studied, however, filtration in non-aqueous media is a more recent research field. In this context, the structure of the membrane may undergo shrinkage, swelling, and pore modification, which ultimately may alter the rejection values and permeate flux. Indeed, most of the contributions dealing with the ultrafiltration of olive-derived wastes have been based on aqueous mediums [10–12]. Again, this premise can be extended to a wide range of agri-food products (and by-products), which are normally treated in aqueous medium [7,13,14].

However, the greater recovery rates that are achieved after the non-aqueous extraction of biophenols cannot be ignored. There is a great benefit in using biocompatible ethanolic mixtures to recover the bioactive content from the wet olive pomace [6,15]. The installation of a wet olive pomace processing plant, entailing an extraction and subsequent ultrafiltration, in the surroundings of the two-phase olive mills, could contribute to transform a concerning residue into a resource with the

potential of supplying high-added-value compounds with a wide range of applications in the market. Thus, it seems pertinent to first study the ultrafiltration of these extracts in order to purify them, even though the solvent may entail additional challenges. Logically, the main limitation is the presence of the organic solvent, which can affect the membrane performance and increase the cost of the process. However, the non-aqueous extraction of phenolic compounds also generates a higher recovery, which increases the overall efficiency of the process. The proposed strategy would allow combining the advantages of both a more efficient extraction stage and a membrane process that is selective, scalable, and operated at mild conditions.

In order to develop a feasible ultrafiltration process with a hydroalcoholic feed solution, the preconditioning of the membrane is an essential stage [16], which is not commonly assessed in the literature [17]. This procedure influences the membrane stability after its contact with the solvent and also affects the permeate flux and rejection.

This contribution explores the organic-solvent ultrafiltration of a hydroalcoholic extract of wet olive pomace, by means of polymeric membranes. The pretreatment of the membranes has been carefully considered to ensure their stability and satisfactory performance. Additionally, the purification of the phenolic compounds retrieved from the wet olive pomace has been pursued, aiming to valorize the by-product.

2. MATERIAS AND METHODS

2.1. Solid-liquid extraction

Samples of olive minor fraction were kindly provided by the cooperative San Isidro de Segorbe (Castellón, Spain), which is a two-phase olive mill. They were immediately stored at 5^{O}C before their processing. The olive minor fraction from the wet olive pomace was extracted according to a methodology described in a previous work of these authors [6]. Briefly, the samples were extracted during 45 min, with a solution of ethanol/water 50:50 (v/v), in an ultrasound bath (Elma, Germany) working at 37 kHz, 220 W, and 40^{O}C. The ratio of sample/solvent was 1:10. Apart from the sonication, agitation was provided by a pitched-blade impeller installed in an overhead stirrer (Heidolph, Instruments, Germany). The obtained extracts were then centrifuged (Sigma, USA) at 17200 RCF for 6 min and used as a feed for the ultrafiltration process.

2.2. Ultrafiltration experiments

The experiments were conducted in a bench-top XFUF 076 01 stirred cell (Merck Millipore, USA), whose material is resistant to organic solvents. The cell has an active filtration area of 33.2 cm^2. The stirring was set at 500 rpm. The transmembrane pressure (TMP) in the module was achieved by means of an inert nitrogen stream. The specifications of the polymeric membranes employed can be revised in Table 6.1. The membranes from the company SolSep BV were selected because of their solvent-resistant nature. Moreover, their application in the recovery of valuable compounds has not been studied before, despite the promising character of these membranes. The UP005 membrane was also tested because this is a commonly studied membrane in ultrafiltration processes. It has proven to resist ethanol/water 50:50 (v/v) [6] and its performance in the purification of olive-derived phenolic compounds in aqueous solutions has been reported to be very successful in comparison with other membranes [12,18]. However, until now, its use in organic media has been scarce. After the conditioning stage, the membranes were compacted.

Table 6.1. Specifications of the membranes

Membrane	MCWO (kDa)[a]	Material	Expected permeability[a]	Maximum Temp. (°C)	Supplier
UP005	5[b]	PES[c]	10 $L{\cdot}h^{-1}{\cdot}m^{-2}{\cdot}bar^{-1}$ (in water)	95	Microdyn-Nadir
UF010104	20[d]	PAN[e] [19,20]	100 $L{\cdot}h^{-1}{\cdot}m^{-2}{\cdot}bar^{-1}$ (in ethanol)	95	SolSep BV
UF010801v3	20[b]	Proprietary	100-200 $L{\cdot}h^{-1}{\cdot}m^{-2}{\cdot}bar^{-1}$ (in water)	80	SolSep BV

[a]Data provided by the manufacturer; [b]Determined in water; [c]PES: Polyethersulfone; [d]Determined in hexane; [e]PAN: Polyacrylonitrile; Temp.: temperature.

Afterwards, the initial permeability of the 50 % (v/v) ethanol solution (L_p) was determined by the slope of the straight line resulting from plotting the permeate flux (J_p) against the transmembrane pressure (TMP), according to the Darcy's law:

$$J_p = L_p \cdot TMP = \frac{TMP}{\mu \cdot R_m} \quad (1)$$

where μ is the dynamic viscosity of the medium ($kg{\cdot}m^{-1}{\cdot}s^{-1}$) and R_m is the membrane resistance (m^{-1}).

Then, the extract of wet olive pomace was ultrafiltrated, in a range of 1.5-4 bar, depending on the membrane.Experiments were conducted until a volume reduction factor (VRF) of 3 was achieved. VRF is defined by the following formula:

$$VRF = \frac{V_f}{V_r} \quad (2)$$

where V_f is the initial feed volume and V_r is the volume of the resulting retentate in the cell. As the cell was initially filled with 300 mL of the extract of wet olive pomace, 200 mL of permeate and 100 mL of retentate were collected when the VRF of 3 was reached.

2.2.1. Conditioning of the membranes

Before using the membranes, they were conditioned to prepare them for the contact with the ethanol present in the feed solution. Two conditioning strategies were studied. One of them was a progressive pretreatment, consisting of a gradual increment of the ethanol percentage in a solution the membrane was soaked in, as suggested by Cheryan and co-workers [16]. Thus, the membrane was first immersed in osmotized water during 24 h. Next, it was immersed in a solution of ethanol at 10%, for 24 h. This sequence continued until the membrane was soaked in a 50% ethanol solution during 24 h. The other studied pre-treatment consisted of a direct immersion of the membrane in the working solvent (ethanol at 50%) for two hours prior to its use.

2.2.2. Membrane cleaning

After every ultrafiltration test with wet olive pomace extract, the membranes were cleaned, in order to reuse them. Numerous cleaning strategies were studied to recover the initial permeability of the solvent. The membranes were considered to be clean if at least 90% of the initial permeability of ethanol at 50% (v/v) was recovered. A decision diagram about the development of the cleaning protocol can be found in Figure 6.1. The first cleaning procedure to be tested was a chemical cleaning with P3 Ultrasil 110 (Ecolab, Spain), diluted in water at 1% (v/v) and performed at 1.5 bar and 35^{O}C, during 1.5 hours. P3 Ultrasil 110 is a detergent containing NaOH, ethylenediaminetetraacetate (EDTA), which is a chelating agent, sodium cumene sulfonate, which acts as a surfactant and wax remover, and sodium dodecylbenzene sulfonate, which is an anionic surfactant. If the membrane was not cleaned by this process, but a permeability recovery of at least 50% was achieved with this methodology, the same detergent solution was applied, with a temperature increment of 10^{o}C. When this strategy was not sufficient to clean the membrane, another temperature increment of 10^{o}C was applied if an improvement in

the permeability recovery was observed after the process. This premise was repeated until reaching a temperature of 55°C.

However, if the permeability recovery was lower than 50%, the cleaning agent was changed. As an alternative, P3 Ultrasil 115 (Ecolab, Spain), at 1% ((v/v), in water), was employed, at the same operating conditions. P3 Ultrasil 115 contains NaOH, KOH, EDTA, and alkylsulfonate as the anionic surfactant. The same procedure was followed with this cleaning agent. If the membrane was not cleaned, but an increment of permeability recovery was obtained, the same protocol was employed at a higher temperature, never surpassing 55°C to protect the membrane polymer. Otherwise, the detergent was changed to a solution of NaOH at 0.4% (v/v) diluted in the working solvent (ethanol/water 50:50 (v/v)). This cleaning was performed for 30 minutes.

1.1.1. Determination of the contact angle

The contact angle of the employed membranes was measured after the two-hours conditioning. To that end, a DataPhysics OCA instrument (DataPhysics Instruments GmbH, Germany) was employed. Pure water drops of 5 µL were delivered on the membrane surface and the contact angle was determined. This was repeated 10 times, in different sections of the same membrane, and the average contact angle was considered.

1.1. Analysis of streams

The total solid content was analyzed by evaporating a known volume of the sample and measuring the weight of the dry residue. The color coefficient of each stream was calculated by the following formula (UNE-EN ISO 7887:2012 Method B):

$$Colour\ coefficient = \frac{(A_{436}^2 + A_{525}^2 + A_{620}^2)}{(A_{436} + A_{525} + A_{620})} \quad (3)$$

which includes the absorbance of the sample at 436 nm (A_{436}), 525 nm (A_{525}) and 620 nm (A_{620}). These absorbances were measured in a UV-VIS DR 6000 spectrophotometer (Hach Lange, Germany). Additionally, conductivity was measured by means of a GLP31+ conductivity meter (Crison, Spain). All samples were characterized at least in duplicates.

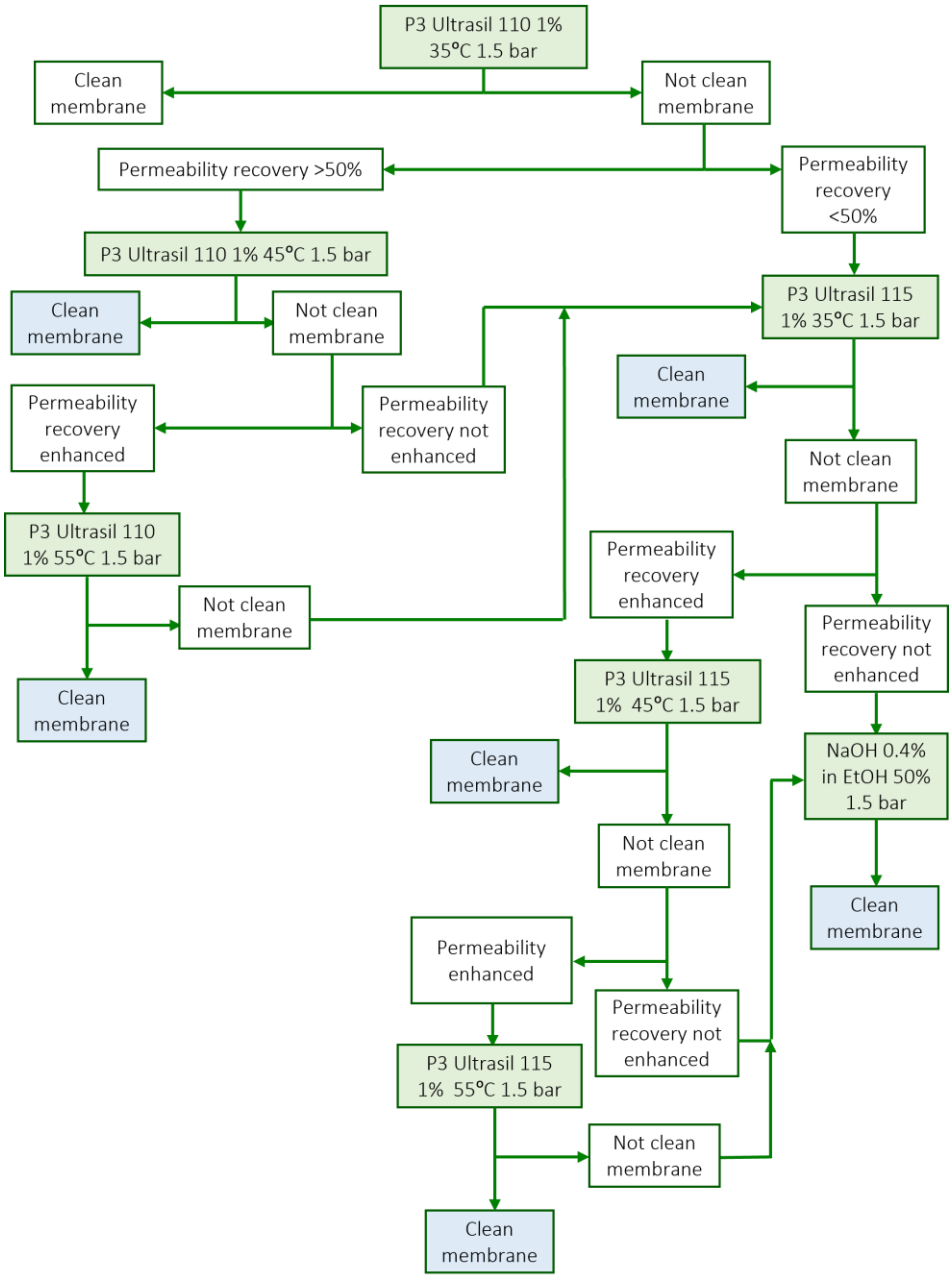


Figure 6.1. Decision diagram about the cleaning procedure applied to recover the initial membrane permeability to the solvent. The solution percentages refer to v/v proportions.

The bioactive content of the samples was determined by liquid chromatography (LC) coupled to mass spectrometry (MS). To that end, an Agilent 1260 Infinity II liquid chromatograph was coupled to a 6546 quadrupole-time-of-flight (QToF) mass analyzer, applying electrospray ionization (ESI) (Agilent Technologies, USA). 4 µL of the sample were injected and the compounds were separated throughout a Zorbax Extend C18 column (4.6 x 100 mm, 1.8 µm) (Agilent Technologies, USA) at 40^{O}C and a flow rate of 0.9 mL/min. The mobile phases were ultrapure water (as phase A) and acetonitrile (as phase B), both acidified with 0.5% of LC-MS grade acetic acid. The initial conditions of the analysis were 95% A and 5% B. Afterwards, the following gradient was applied: 35% B was achieved at minute 12, 80% B at 14 min and 100% B at 18 min. This percentage was maintained for 3 minutes and then the initial conditions were restored in 2.5 minutes. The column was again re-equilibrated for 3 minutes. The specific parameters for the mass spectrometer, working in negative polarity, were selected according to a previous study [6]. To perform the quantification of compounds, an external calibration was conducted, employing pure standards of citric acid (VWR, USA), tyrosol (VWR, USA), hydroxytyrosol (Sigma Aldrich, USA), caffeic acid (VWR, USA), *p*-coumaric acid (Sigma Aldrich, USA), oleuropein (Sigma Aldrich, USA), luteolin (VWR, USA), decarboxymethyl oleuropein aglycone (Sigma Aldrich, USA) and hydroxy-octadecanoic acid (Sigma Aldrich, USA) as standard for organic acids, simple phenols, phenolic acids and aldehydes, secoiridoids, flavonoids and free fatty acids, respectively. The range for the standard solutions was 0.1 – 100 mg·L^{-1}. All samples were injected in duplicates or triplicates. Additionally, the total phenolic content was determined by the Folin-Ciocalteu methodology [22]. In this case, the quantification was performed according to a calibration curve of tyrosol, diluted in ethanol/water 50:50 (v/v), in the range 1 - 500 mg·L^{-1}.

The instantaneous rejection of the different compounds was determined by the following formula:

$$Rejection = 1 - \frac{C_p}{C_r} \cdot 100 \quad (4)$$

where C_p is the concentration in the ultrafiltration permeate and C_r is the concentration in the retentate. To calculate the rejection, the samples taken at the end of each experiment were characterized, in order to analyze the instantaneous rejection at the end of the process.

2. RESULTS AND DISCUSSION

2.1. Characterization of the feed stream

The feed stream consisted of a hydroalcoholic extract of wet olive pomace, obtained through the methodology optimized previously [6]. As can be seen in Table 6.2, the concentration of phenolic compounds in the extracts of wet olive pomace is relevant. A detailed description of each phenolic compound present in the hydroalcoholic extract of wet olive pomace is shown in our previous work [6]. Considering the concomitant content of total solids, the ultrafiltration of the extract can be performed in order to reduce the proportion of other compounds and recover the polyphenols at a higher purity in the permeate stream.

Table 6.2. Characterization of the hydroalcoholic extract of wet olive pomace. The standard deviation corresponding to each data has been included.

Parameter	Total solids (g/L)	Total phenolic content (mg tyrosol/kg)	Conductivity (µS/cm)	pH
Concentration	8.1 ± 0.2	8090 ± 850	569 ± 35	5.9 ± 0.3

2.2. Selection of the conditioning strategy for the ultrafiltration membranes

In order to evaluate the effect of the two pre-treatments that were conducted, the solvent flux through the membranes was studied. As shown in Figure 6.2A, 6.2B, and 6.2C, the membranes from SolSep BV, UF0101104 and UF010801v3, displayed a much higher permeate flux than UP005, irrespective of the applied preconditioning. This was expected, considering the lower molecular weight cut-off (MWCO) of the UP005 membrane. After the 2 h conditioning, the flux of ethanol/water 50:50 (v/v) for the UF0101104 membrane became stable after 3 hours of filtration. During that time, the feed cell had to be refilled several times to continue the experiment. This implied depressurizing the system to be able to open the feed chamber. When the UF0101104 membrane was progressively conditioned (see section 2.2.1), the permeate flux showed a decreasing tendency, however, when the system was depressurized to refill the feed solvent, the permeate flux started again from its initial value. This phenomenon was attributed to the compaction of the membrane, which occurred during the pressurization at 3 bar. When the pressure application stopped, the membrane polymer restored its initial state, and a new compaction cycle started when the pressure was again provided to the system. Constriction of ultrafiltration polymeric membranes as a result of high-pressure application in a dead-end cell has been described before [23,24]. The final value

of the membrane permeability to the ethanol at 50% (v/v) after the progressive pretreatment was 82.1 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$, which was higher than the obtained solvent permeability after the 2 h conditioning (64.5 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$). This difference suggested that the membrane conditioned for 2 hours was better adapted to the solvent. It is also possible that the membrane progressively conditioned underwent a swelling phenomenon. This consists of a modification of the membrane structure. The reaction between the solvent molecules and the polymer components normally ends with the expansion of the polymer and the enlargement of the pores, which results in a higher permeate flux. This effect was also observed for the UF010801v3 membrane. As can be observed in Figure 6.2B, the permeate flux was much higher in this case (the membrane permeability was 41.61 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$), in comparison with the 2 hours pretreatment (the membrane permeability was 32.63 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$).

The progressive conditioning intended to gently modify the polarity of the medium in contact with the membrane, in order to protect the polymer structure and prevent any damage, as suggested by Shukla and Cheryan [16]. However, the observed effect in the case of the UF0101104 and UF010801v3 membranes was the contrary. Considering that these membranes were developed to be specifically applied with organic solvents, it is possible that their polymers were selected because of their resistance to solvents of low polarity. However, the first stages of the progressive conditioning of the membranes entailed more polar mediums, such as pure water or hydroalcoholic solutions of a low percentage of ethanol. The results from Figure 6.2A and 6.2B suggest that the polymer of the membranes was affected during this pre-treatment. Shukla and Cheryan measured the swelling of several membranes in water, ethanol at 70% (v/v), and ethanol at 100% (v/v) [16]. They discussed that polar solvents and water produced a more relevant swelling, due to a higher dielectric constant. They also observed that PAN membranes suffered a higher swelling in water. As the UF0101104 membrane is also made of PAN, certain swelling and pore enlargement can be suggested here after the progressive conditioning of the membrane. Regarding the UP005 membrane, whose active layer is made of PES, it displayed a solvent permeability of 6.55 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ after the progressive conditioning, whereas this value was 4.96 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ when the membrane was pretreated during two hours in ethanol/water 50:50 (v/v). A slight swelling could also be suggested, however, it was not very relevant in this case. Shukla and Cheryan also observed a low degree of swelling of a PES membrane in water [16].

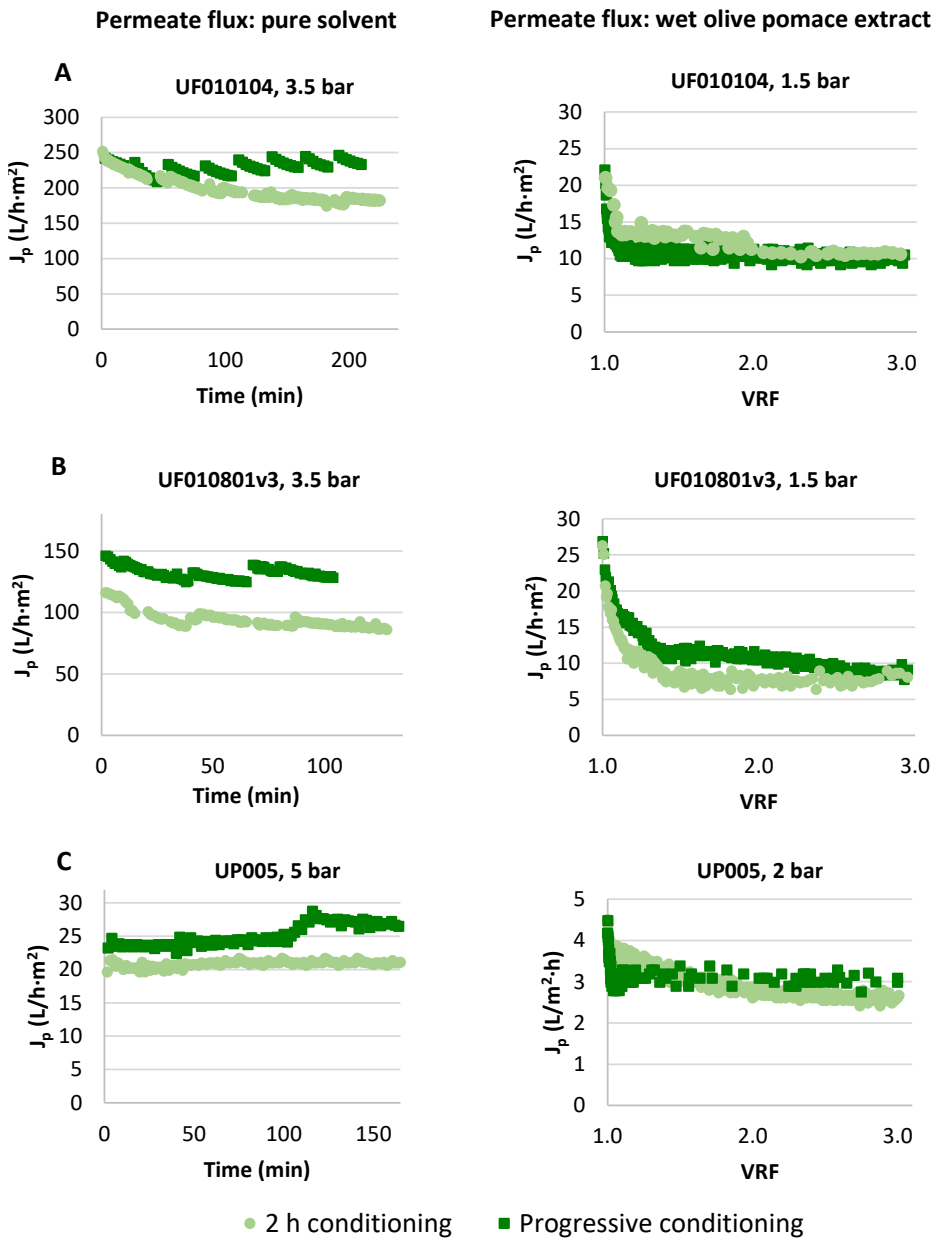


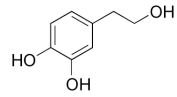
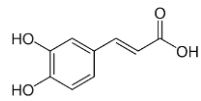
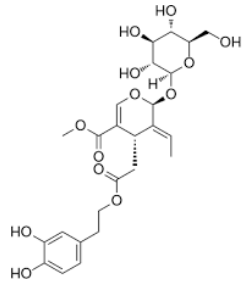
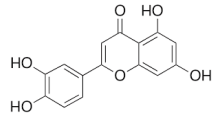
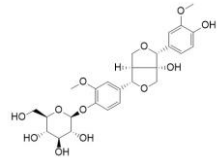
Figure 6.2. Permeate flux obtained with the pure solvent and the extract of wet olive pomace after each membrane pre-treatment. The results for the UF010104 (A), UF010801v3 (B), and UP005 membranes (C) are shown.

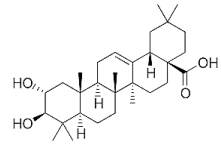
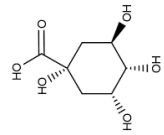
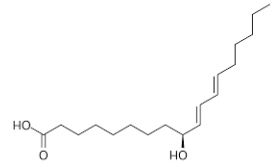
In any case, to select a conditioning, not only the solvent flux was evaluated. Also, the permeate flux during the ultrafiltration of the real extract of wet olive pomace after each membrane conditioning was compared. These results are shown in Figure 6.2D,

6.2E, and 6.2F. A high reduction in the permeate flux was observed when the extract of wet olive pomace was ultrafiltered instead of the pure solvent. This was due to the great accumulation of solutes at the membrane surface, which contributed to the formation of the filtration cake [25]. The progressive conditioning (which lasted 5 days) did not induce any benefit regarding the efficiency of the ultrafiltration process. The permeate flux was slightly higher after this treatment for the UP005 and UF010801v3 membranes, but the observed differences were not notable enough to apply a five-days pretreatment, in contrast with a two-hours, single-step conditioning. At this stage of the study, the data from Figure 6.2 already indicated that the short pre-treatment was a more suitable strategy. Even though the progressive conditioning resulted in higher values of permeate flux, the results indicated that the membranes swelled. Although a higher permeate flux is indeed more beneficial for the productivity of the process, the difference in terms of permeate flux (employing the extracts of wet olive pomace as the feed) was always below 15% when the steady state was reached. Therefore, the more extended time and the higher consumption of reagents and water for the progressive pre-treatment discouraged its application.

The rejection values were considered too. As displayed in Figure 6.3 and Table 6.3, eight chemical families were determined by means of the LC-ESI-QToF-MS methodology detailed in section 2.3. These included organic acids, simple phenols, phenolic acids and aldehydes, secoiridoids, flavonoids, and lignans. More details about the individual molecules that were found in the extract of wet olive pomace are shown in Table 6.3, which includes all the compounds identified by means of LC-MS and classified according to their chemical class. Furthermore, Supplementary Table 6.1 contains the *m/z* and retention time for each analyte. The elution order within the compounds of the same chemical family has been maintained and, additionally, an example of each group has been provided to illustrate the chemical structure of those molecules. In order to identify each compound, a thoughtful study of the obtained chromatograms was conducted. The retention time of each peak, as well as the derived *m/z* was examined and compared with pure standards and with previous reports [6,26–28].

Table 6.3. Detailed composition of the metabolites present in the extract of wet olive pomace. The corresponding m/z for each compound has been indicated in brackets.

Chemical Family	Identified Compounds	Example
Simple phenols	Hydroxytyrosol (153.0551), Hydroxytyrosol glucoside (315.1085), Hydroxytyrosol derivative (323.1504), Tyrosol (137.0608)	Hydroxytyrosol 
Phenolic acids and aldehydes	Vanillic acid (167.0345), Vanillin (151.0396), Caffeic acid (179.0347), *p*-Coumaric acid (163.0397)), Ferulic acid (193.0503), Ferulic acid methyl ester (207.0668)	Caffeic acid 
Secoiridoids	Acyclodihydroelenolic acid hexoside (407.1560), Hydroxy-decarboxymethyl elenolic acid (199.0607), Hydroxy-elenolic acid (257.0669), Elenolic acid glucoside (403.1246), Decarboxymethyl elenolic acid (183.0658), Aldehydic form of decarboxymethyl elenolic acid (215.0925), Phenylethyl primeveroside (415.1612), Hydroxy-oleuropein (555.1717), Hydrogenated elenolic acid (243.0876), Dehydro-oleuropein aglycone (375.1087), Verbascoside, Cafselogoside (623.1981), Elenolic acid (241.0720), Comselogoside (535.1457), Oleuropein (539.1769), Decarboxy-methyl oleuropein aglycone (319.1187), Ligstroside (523.1820), Oleuropein aglycone (377.1242), Oleuropein aglycone derivative (377.1453)	Oleuropein 
Flavonoids	Gallocatechin (305.0702), Luteolin (285.0405)	Luteolin 
Lignans	Hydroxypinoresinol (373.1294)	Hydroxypinoresinol 

Chemical Family	Identified Compounds	Example
Triterpenic acids	Maslinic acid (471.3488), Betulinic acid (455.3538)	Maslinic acid 
Organic acids	Quinic acid (191.0555), Malic acid (133.0150), Isopropyl-malic acid (175.0614), Citric acid (191.0191)	Quinic acid 
Free fatty acids	Trihydroxy-octadecadienoic acid (327.2180), Trihydroxy-octadecenoic acid (329.2335), Dihydroxy-hexadecanoic acid (287.2230), Hydroxy-octadecatrienoic acid (293.2122), Hydroxy-octadecadienoic acid (295.2277)	Hydroxy-octadecadienoic acid 

As shown in Table 6.3, the hydroalcoholic extract of wet olive pomace is a source of prized molecules. The interest of hydroxytyrosol and tyrosol (included in the category of simple phenols) to be applied in cosmetic products relies on their powerful antioxidant capacity [29], which set them as the most recognized polyphenols and enables their incorporation in cosmetic products that are nowadays being commercialized [30]. The phenolic acids and aldehydes from this residue include interesting compounds such as caffeic, ferulic, and *p*-coumaric acid, which are employed as key ingredients in the nutraceutical and cosmetic industry [31,32]. The chemical family of secoiridoids contained several glycosylated and hydroxylated forms of elenolic acid and oleuropein. These modifications may be due to partial degradation of the molecules during the storage period of the wet olive pomace in the olive mill, before its collection. Nevertheless, some of these derivatives have also been related to the health benefits associated to olive extracts [27,33]. Luteolin, belonging to the chemical class of flavonoids, is also a highly appreciated molecule that has been correlated with positive bioactive potential [34]. Hydroxypinoresinol, which was the only lignan present in the extract, has been related to a strong reduction of peroxide levels, providing a high antioxidant activity [35]. The family of triterpenic acids does not comprise any phenolic compounds, but the antiproliferative properties that have been demonstrated *in-vitro*

for maslinic acid are also worth noting [36]. Finally, the organic and free fatty acids listed in Table 6.3 are a group of compounds that are inevitably retrieved with the rest of the bioactive content and are also part of the vegetal extract.

The application of the short conditioning was also supported by the rejection results obtained during the ultrafiltration of the wet olive pomace extract after each membrane pre-treatment. The results regarding the rejection of phenolic compounds, free fatty acids, organic acids, color, total solids and conductivity have also been plotted in Figure 6.3. As can be seen, both the gradual and direct (two hours) pre-treatment of the membranes conducted to similar results in terms of rejection of compounds. Again, the progressive conditioning did not contribute positively to the performance of the membranes. It is also of interest that the rejections observed for the UP005 membrane after the progressive conditioning were, in most cases, lower than the rejections achieved after the two hours pretreatment. This supports the possibility of a swelling phenomenon, because the structure of the swelled polymer would be more relaxed, allowing a higher passage of the compounds [37].

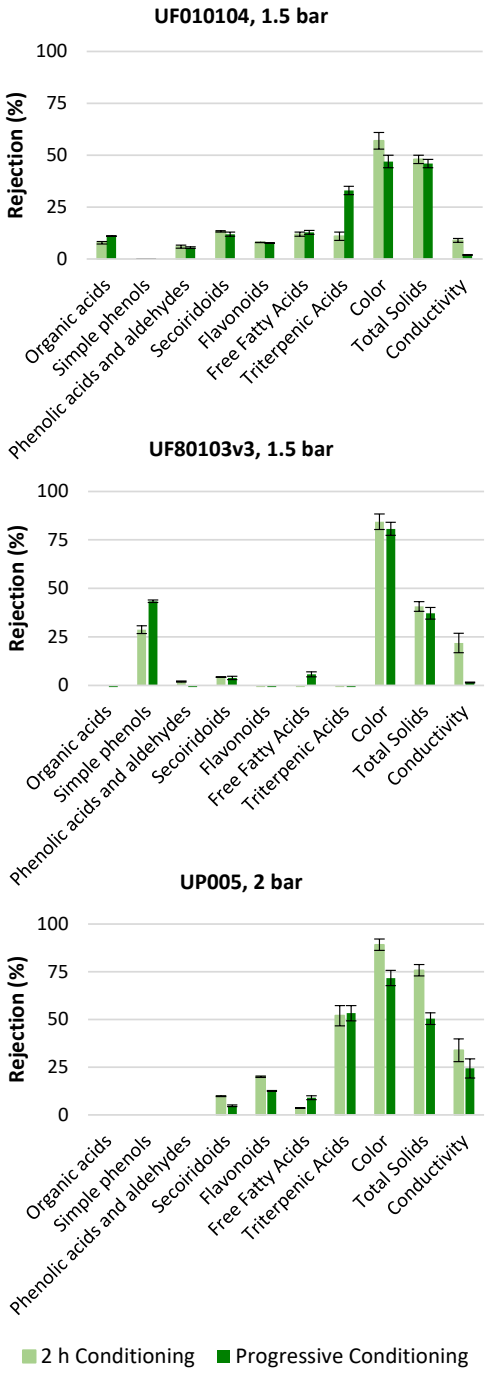


Figure 6.3. Rejection values achieved after the ultrafiltration of the extract of wet olive pomace with the studied membranes, pretreated by the progressive and direct conditioning. The results correspond to a volume reduction factor of 3. Error bars refer to the standard deviation.

2.3. Permeate flux

Once the direct, two-hours conditioning was selected as the more suitable option to pretreat the membranes, the three membranes were conditioned in this way and subjected to the ultrafiltration process at several values of TMP. The values of permeate flux that were obtained are shown in Figure 6.4.

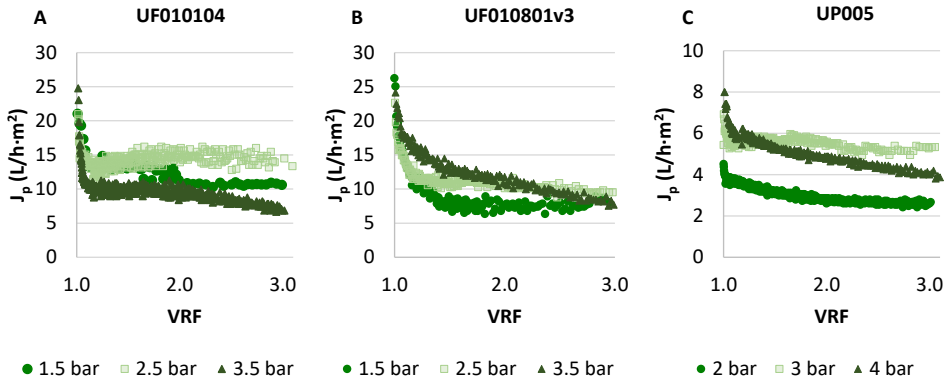


Figure 6.4. Permeate flux obtained at each transmembrane pressure, during the ultrafiltration of a hydroalcoholic extract of wet olive pomace, after the direct conditioning of the membranes by a two-hours immersion in ethanol at 50% (v/v). The results for the UF010104 (A), UF010101v3 (b), and UP005 (C) are shown.

As observed for the pure solvent (Figure 6.2A, 6.2B, and 6.2C), the UF010104 and UF010801v3 membranes (Figure 5.4A and 5.4B) displayed much higher values of permeate flux than the UP005 membrane (Figure 6.4C). It has to be noted that the MWCO of these membranes is greater than that of the UP005 (5 kDa). The permeate flux registered for the UF010801v3 membrane was always lower in comparison with the UF010104 membrane. Even though the composition of the SolSep BV polymers is not public, the mentioned differences in the permeate flux of the UF010104 and UF010801v3 membranes can be attributed to the membrane material. According to Yin and co-workers, the UF010104 membrane is made of polyacrylonitrile (PAN) [19], however, the composition of the UF010801v3 is unknown. The application of these membranes is quite new in the literature, which hinders the comparison of the results. Nevertheless, the variation in the composition of the polymer could explain a different interaction of the membrane active layer with the solvent molecules [23,38]. In order to consider the polarity of the employed membranes, the contact angle was measured. The results are shown in Table 6.4. As can be seen in Table 6.4, the UF010104 membrane was the most hydrophilic one after the conditioning. The polarity index of the solvent mixture (ethanol/water 50:50 (v/v)) is not far from the polarity index of water [39,40]. Then, the more hydrophilic membrane displayed the highest values of permeate flux.

Table 6.4. Contact angle of the membranes after a 2 hours immersion in ethanol/water 50:50 (v/v). Standard deviation of each determination has been indicated.

Membrane	Contact angle
UF010104	40˚ ± 1˚
UF010801v3	50˚ ± 4˚
UP005	60˚ ± 5˚

According to the results shown in Table 6.4, the UF010801v3 membrane is less hydrophilic than the UF010104 one. The orientation of the molecules of ethanol and water and their interaction with the walls of the membrane pores may impact the permeate flux, leading to the results from Figure 6.4A and 6.4B. Regarding the UP005 membrane, other authors have also employed this membrane to treat aqueous effluents from the olive industry. Carbonell-Alcaina et al. obtained a permeate flux of 15 $L \cdot h^{-1} \cdot m^{-2}$ (working at 3 bar, at a VRF of 2.5) during the aqueous ultrafiltration of fermented brines from the processing of table olives [18]. Those results were obtained in a cross-flow plant, which makes it difficult to compare the permeate flux. Despite the different mode of operation, 15 $L \cdot h^{-1} \cdot m^{-2}$ is three times higher than the flux reported here. In a cross-flow plant, a lower membrane fouling can be expected, therefore, a higher permeate flux than in a dead-end cell is plausible. However, the great difference that was observed with respect to the results obtained by Carbonell-Alcaina and co-workers indicates that the organic nature of the medium (and not only the frontal filtration) also influenced the membrane performance. The working solvent, ethanol at 50% (v/v), has a higher viscosity than water [41], which substantially influences the permeate flux, as described by de Darcy's law. However, the viscosity is not the only characteristic of the solvent that may influence the results. Organic solvents may increase the hydrophobicity of the membrane active layer if the polymer is hydrophilic [42], as is the case of the UP005 membrane [43]. Two different regions can be differentiated in the ethanol molecules. One of them is comprised by the hydroxyl group, which constitutes a polar head, whereas the other region is the non-polar, carbon tail. When these molecules approach a hydrophilic polymer (such as the active layer of the UP005 membrane) the interaction between the hydroxyl groups and the membrane surface is favored [44]. Consequently, the polar head of the molecules is faced to the membrane, exposing the carbon chain to the bulk solution, as described by several authors [38,45]. In fact, after the membrane immersion in ethanol/water 50:50 (v/v), the observed contact angle was 60˚ ± 5˚. In its native form, this membrane has been reported to have a contact angle of 54.3˚ ± 3.5˚ [46]. This phenomenon may have contributed to decrease the affinity of the feed solution and the membrane and, in consequence, led to a decline of the permeate flux.

Additionally, the features of the feed solution should be considered. The extract of wet olive pomace is highly foulant, as it contains a high organic load coming from the fresh by-product. According to a previous work by these authors, and, as reflected in Table 6.2, the total solids content can reach 8100 $mg \cdot L^{-1}$ [6], which can be remarkably increased as the concentration procedure progresses and the VRF is enhanced. Considering the large difference observed between the permeate flux of the pure solvent and the flux during the ultrafiltration of the wet olive pomace extract, a high accumulation of solutes on the surroundings of the membrane surface and the subsequent fouling occurred. Other authors have also described significant fouling during the cross-flow ultrafiltration of real extracts of agro-alimentary products [47,48]. Indeed, the presence of polysaccharides, vegetable proteins, and, in this case, polyphenols, may induce the membrane fouling, due to adsorption onto the membrane surface, gel layer formation and the subsequent increment of membrane resistance [49–52] and this can be expected to occur to a higher extent in dead-end mode. In this case, the fast decline in permeate flux observed in Figure 6.4 is indicative of fouling phenomenon. Moreover, although permeate flux increased with TMP, all the membranes displayed a reduction of flux at the highest TPM with respect to the previous ones. This suggests the formation of a gel layer on the membrane surface. When comparing the permeate flux observed at the two lowest pressures (below 3.5 bar), it can be noticed that the flux increment was much lower for the SolSep BV membranes than for the UP005 membrane. In general, the SolSep BV membranes displayed higher values of permeate flux in comparison with the UP005 membrane. Then, the concentration of solutes at the proximity of the SolSep BV membranes was higher, favoring the development of a gel layer. Moreover, the gel layer formation was especially noticeable for the UF010104 membrane, whose mean value of permeate flux at the highest pressure was even lower than the mean values registered at the lowest TMP. This can be explained by the rejection of compounds displayed by this membrane, which will be presented in section 3.4. The UF010104 membrane provided a higher rejection of total solids (Figure 6.5). Therefore, the thickness of the gel layer was higher for this membrane, leading to the reduction of the permeate flux.

2.4. Rejection of compounds

The rejection of total solids, color, and conductivity is presented in Figure 6.5. In order to purify the bioactive compounds of interest, the rejection of total solids is intended to be maximum, as this parameter indicates the organic load of the sample. Logically, this value can never reach 100%, because the phenolic compounds, which are intended to be recovered in the permeate stream, are also included in the total solids.

The UP005 membrane displayed a satisfactory, high rejection of total solids, reaching 75 ± 3 %. Additionally, the color of the sample was notably reduced, and the elimination of conductivity reached 46 ± 5 % (at 4 bar). These results were expected, as this is a tight ultrafiltration membrane that does not allow the permeation of large solutes. On the contrary, the UF010801v3 membrane only rejected the total solids in a maximum percentage of 41 ± 3, obtained at 1.5 bar, because of a higher MWCO (Table 6.1). This membrane efficiently reduced the color of the samples, displaying a rejection of more than 80%, which may be due to an adsorption process of vegetal pigments onto the polymer surface.

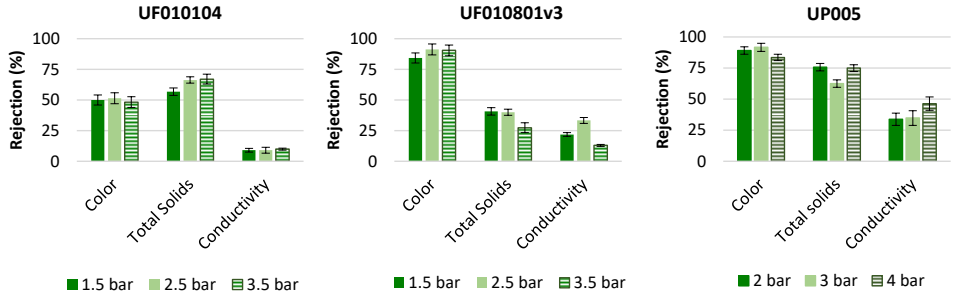


Figure 6.5. Rejections of color, total solids and conductivity achieved with the tested membranes during the ultrafiltration of a hydroalcoholic extract of wet olive pomace, at several pressures and a volume reduction factor of 3. Error bars refer to the standard deviation.

According to several authors studying the filtration of organic solvents through polymeric membranes, the pore size is not always the only phenomenon dominating the transport. The importance of other effects, such as solute-membrane interaction, as well as solvent clustering onto the membrane surface, has been described before [43,53]. The rejection of total solids obtained with the UF010104 membrane was also very high, despite a higher MWCO (Table 6.1) in comparison with the UP005 membrane. According to the information provided by the manufacturer, the MWCO of the UF010104 membrane was measured in hexane, whereas the MWCO of the UP005 and UF010801v3 membranes refers to water. Therefore, the comparison of the pore size of both membranes is not direct. If the UF010104 membrane swelled in hexane or had its structure altered in any way during the determination of its MWCO, it is possible that the MCWO of the UF010104 membrane in water is smaller than 20 kDa. This means that higher rejections are expected for this membrane in comparison with the UF010801v3 membrane. Also, as indicated in section 3.3, this membrane appeared to suffer from a severe gel layer formation. This could have contributed to a higher rejection of the total solids, as a thick gel layer hinders their transport throughout the membrane and, additionally, may promote the interaction of compounds. Considering the composition

of the feed solution, including molecules with available carboxyl and hydroxyl groups, chemical reactions leading to the complexation of the macromolecules are reasonable [54,55].

The rejection of bioactive compounds, and especially phenolic compounds, is an essential aspect to evaluate the operating conditions and the suitability of the membranes, because the aim of the process is to purify the polyphenols and separate them from the concomitant organic matter present in the hydroalcoholic extract. As can be observed in Figure 6.6, all membranes displayed a higher rejection of phenolic compounds as the TMP increased. This is consistent with the occurring concentration of solutes at the membrane surface and the formation of a filter cake [25,56]. This layer may interfere with the transport of compounds through steric hindrance or repulsive forces. Figure 6.6 shows the rejection of each chemical family determined by LC-ESI-QToF-MS (for more details, see section 2.3). The individual rejection of each compound can be inspected in Table 6.5, which also contains the concentration of each compound in the permeate of the membranes. The concentration in the retentate stream can be found in Supplementary Table 6.1. Among all the compounds belonging to the olive minor fraction, secoiridoids and triterpenic acids were the most rejected compounds by the UP005 membrane. These chemical families include the largest molecules of the extract of wet olive pomace. For instance, the molecular weight of oleuropein (the main representative of secoiridoids) is 540 $g \cdot mol^{-1}$, which hinders its passage through the pores of this tight membrane. In fact, the individual rejection of oleuropein by the UP005 membrane was 52% at 3 bar and VRF 3.

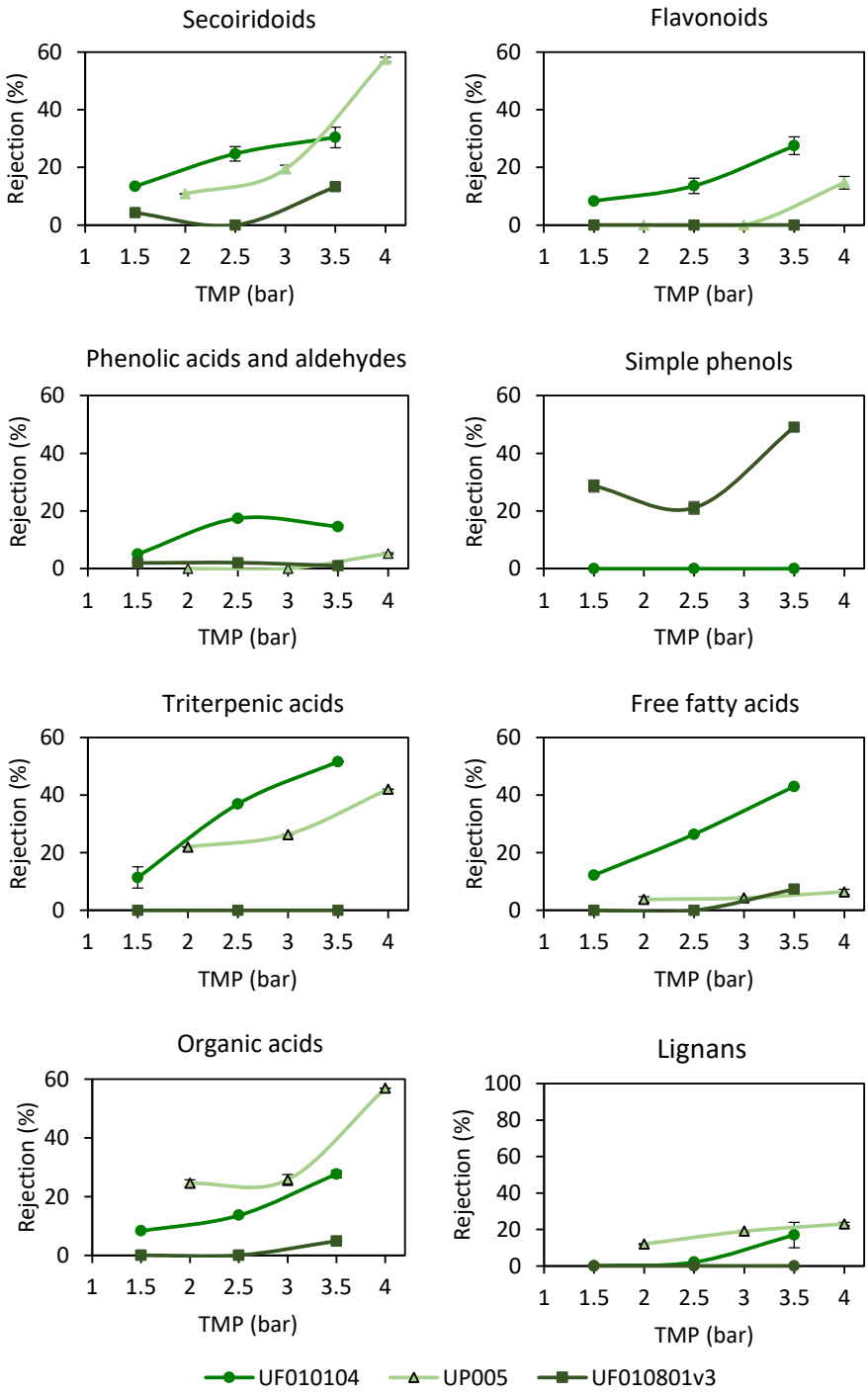


Figure 6.6. Rejection of the chemical families belonging to the olive minor fraction during the ultrafiltration of a hydroalcoholic extract of wet olive pomace. Results achieved with the tested membranes at several pressures and a volume reduction factor of 3.

The same reasoning is applicable to the triterpenic acids. The most concentrated triterpene in this sample is maslinic acid, whose molecular weight is 473 $g \cdot mol^{-1}$ [6]. The chemical structure of triterpenes, as well as secoiridoids, comprise non-linear structures, with cyclic moieties that may also influence the transport across the membrane pores. According to the figure, the rejection of organic acids by the UP005 membrane was also high, especially at the highest pressure. Fei et al. described that phenolic compounds can interact with organic membranes through hydrogen bonds, π - π interactions and electrostatic interactions [57]. The organic acids, similarly to phenolic compounds, exhibit hydroxyl groups and, logically, carboxyl groups that could interact either with the polymer surface or with the solvent molecules adhered to the membrane active layer, resulting in an adsorption process that prevents the permeation of this chemical class. The high rejection of these compounds is desirable, because the UP005 exhibited an enhanced flux of more interesting solutes (such as simple phenols or phenolic acids) over the transport of organic acids, which increases the purity of polyphenols in the permeate stream.

The UF010801v3 membrane allowed the permeation of most of the compounds belonging to the olive minor fraction. Even at the highest pressure applied, the rejection values were extremely low, resulting in an efficient recovery of polyphenols (among others) in the permeate stream. The only chemical class that was retained in a higher percentage was the family of simple phenols, which, in this sample, include the molecules of hydroxytyrosol (154 $g \cdot mol^{-1}$), hydroxytyrosol glucoside (316 $g \cdot mol^{-1}$) and tyrosol (154 $g \cdot mol^{-1}$) (Table 6.3). It is evident that the pore size (corresponding to a MWCO of 20 kDa) is not responsible of the rejection of these compounds, because larger molecules (secoiridoids, flavonoids, etc.) were obtained in the permeate. In contrast, an adsorption process may be the reason for the retention of these compounds. Cifuentes-Cabezas et al. already reported the adsorption of tyrosol on the surface of ultrafiltration membranes made of different polymers [12].

Table 6.5. Rejection of each compound belonging to the olive minor fraction corresponding to the wet olive pomace-derived samples. The final concentration in the permeate of the tested membranes is also provided. All values correspond to a volume reduction factor of 3. Relative standard deviation was always below 13%.

Compound	UF010104		UF010801v3		UP005		Standard for quantification
	Rej (%)	Conc. in the permeate ($mg \cdot kg^{-1}$)	Rej (%)	Conc. in the permeate ($mg \cdot kg^{-1}$)	Rej (%)	Conc. in the permeate ($mg \cdot kg^{-1}$)	
Hydroxytyrosol	0.2	205.04	33.9	128.44	0.4	243.69	Hydroxytyrosol
Hydroxytyrosol glucoside	0.2	15.87	38.9	6.79	7.0	15.52	Hydroxytyrosol
Hydroxytyrosol derivative	0.2	18.36	40.1	19.65	0.2	45.06	Hydroxytyrosol
Tyrosol	0.6	331.60	18.9	270.50	0.3	309.19	Tyrosol
Vanillic acid	0.7	1.82	0.5	2.19	0.0	1.81	Caffeic acid
Vanillin	0.5	18.55	0.7	14.22	2.3	11.46	Caffeic acid
Caffeic acid	22.0	66.85	1.7	70.67	0.0	70.92	Caffeic acid
***p*-Coumaric acid**	14.3	20.00	3.1	28.85	0.0	23.89	*p*-Coumaric acid
Ferulic acid	30.0	6.04	2.1	13.78	0.0	14.23	Caffeic acid
Ferulic acid methyl ester	0.30	24.92	3.2	29.71	2.1	24.53	Caffeic acid
Acyclodihydroelenolic acid hexoside	32.5	207.28	0.3	361.98	0.3	300.56	Oleuropein
Hydroxy-decarboxymethyl elenolic acid	27.4	1452.92	0.1	1975.11	4.0	1718.94	Oleuropein
Hydroxy-elenolic acid	18.0	201.32	4.7	197.17	6.9	196.13	Oleuropein
Elenolic acid glucoside	27.0	6.79	0.2	9.03	17.8	10.37	Oleuropein
Decarboxymethyl elenolic acid	26.0	598.89	0.4	1358.23	6.8	1121.44	Oleuropein
Aldehydic form of decarboxymethyl elenolic acid	9.6	129.20	7.8	135.53	27.0	73.71	Oleuropein
Phenylethyl primeveroside	31.0	4.47	0.2	2.92	21.3	5.34	Oleuropein
Hydroxy-oleuropein	n.f.	0.00	3.0	2.95	41.9	1.86	Oleuropein
Hydrogenated elenolic acid	41.7	11.82	0.4	15.39	4.3	17.40	Oleuropein
Dehydro-oleuropein aglycone	n.f.	0.00	0.3	4.74	54.1	1.90	Oleuropein
Verbascoside	52.4	1.15	0.6	2.22	39.7	1.26	Oleuropein
Cafselogoside	21.0	33.69	0.9	17.86	36.9	26.33	Oleuropein
Elenolic acid	11.8	26.32	0.02	21.67	10.4	20.76	Oleuropein
Comselogoside	12.3	53.97	0.2	43.98	14.4	45.86	Oleuropein
Oleuropein	59.0	0.54	0.3	1.99	52.1	0.71	Oleuropein
Decarboxy-methyl oleuropein aglycone	15.3	10.81	0.2	12.64	27.4	8.30	Oleuropein
Ligstroside	72.2	0.66	0.3	1.44	43.1	0.98	Oleuropein
Oleuropein aglycone	1.0	35.52	0.7	29.56	36.4	18.79	Oleuropein
Oleuropein aglycone derivative	2.6	147.99	0.2	106.91	1.7	177.65	Oleuropein
Luteolin	0.0	7.48	0.0	4.73	19.0	4.45	Luteolin
Gallocatechin	12.0	46.68	0.4	57.90	0.4	49.83	Luteolin
Hydroxypinoresinol	37.4	5.72	1.1	10.85	1.5	8.91	Oleuropein

Compound	UF010104		UF010801v3		UP005		Standard for quantification
	Rej (%)	Conc. in the permeate ($mg \cdot kg^{-1}$)	Rej (%)	Conc. in the permeate ($mg \cdot kg^{-1}$)	Rej (%)	Conc. in the permeate ($mg \cdot kg^{-1}$)	
Maslinic acid	35.5	11.20	0.4	9.67	19.7	14.62	Maslinic acid
Betulinic acid	49.0	0.20	1.0	2.15	11.6	1.60	Maslinic acid
Quinic acid	13.4	236.37	0.0	489.72	15.9	246.59	Citric acid
Malic acid	33.5	97.18	2.9	253.08	33.6	98.70	Citric acid
Isopropyl-malic acid	8.7	37.32	7.0	30.52	34.9	20.79	Citric acid
Citric acid	13.2	1398.78	0.0	1587.60	27.0	1050.46	Citric acid
Trihydroxy-octadecadienoic acid	n.f.	0.00	0.0	0.67	6.1	0.28	Citric acid
Trihydroxy-octadecenoic acid	28.2	1.97	0.1	4.87	2.2	3.36	Citric acid
Dihydroxy-hexadecanoic acid	24.9	9.28	0.3	11.72	6.1	12.97	Citric acid
Hydroxy-octadecatrienoic acid	48.9	0.51	0.0	0.64	3.3	0.71	Citric acid
Hydroxy-octadecadienoic acid	27.7	4.69	0.0	9.47	2.2	7.58	Citric acid

Rej: rejection; Conc: concentration; n.f.: not found in the feed solution.

Regarding the UF010104 membrane, the obtained rejection values did not surpass 20% at the lowest pressures for all the families included in Figure 6.6. This was expected, considering the MWCO and the size of the polyphenols present in the hydroalcoholic extract of wet olive pomace. Taking into account the evolution of permeate flux with TMP for this membrane (Figure 6.4), the formation of a fouling layer onto the membrane surface is plausible. This also explains the evolution of the rejection with the TMP. A high rejection was only observed for triterpenic acids (whose large, cyclic structure has already been discussed) and free fatty acids. The fatty acids from the feed solution (reported in Table 6.3) contain a long, hydrophobic carbon chain. On the other side, the UF010104 membrane displayed the lowest contact angle of the three tested membranes (Table 6.3). Therefore, this membrane was the most hydrophilic one, which suggests some repulsion forces with respect to the non-polar chain of free fatty acids, increasing their rejection.

2.5. Membrane cleaning

Figure 6.7 illustrates the obtained solvent permeability after each cleaning cycle. To recover the membrane permeability after the ultrafiltration of the extracts, the decision diagram detailed in Figure 6.1 was followed.

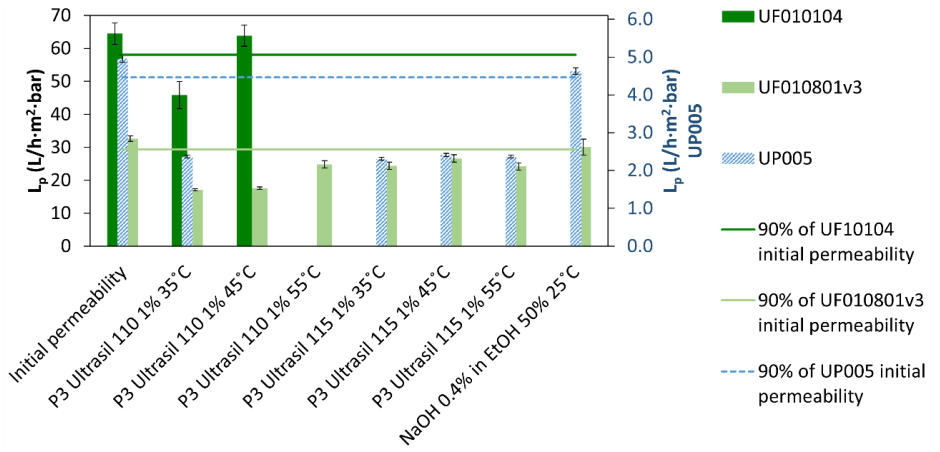


Figure 6.7. Solvent permeability obtained with each membrane after the tested cleaning procedures. Horizontal lines correspond to 90% of the initial permeability of the solvent. Error bars are referred to the standard deviation.

The cleaning of the membranes was not straightforward. As commented in section 3.3, significant fouling affected the polymers. Ji et al. tested several polysulfone membranes in a dead-end cell and described that fouling was more severe for the membranes treated with ethanol (at different percentages and temperatures) as a pretreatment, in comparison with hot water-treated membranes. They hypothesized that the high affinity between the ethanol-treated membranes and the solutes from the feed solution (dextran, in their case) determined a hindering of the back-diffusion of the solutes, with the subsequent formation of a fouling layer [49]. This hypothesis is also applicable to the results presented here, regarding the interaction between the solutes from the feed solution and the membranes.

The UF010104 membrane was efficiently cleaned with P3 Ultrasil 110 at 1% (v/v), but some temperature had to be applied. Regarding the UF010801v3 and the UP005 membranes, none of the Ultrasil detergents were effective enough to recover 90% of the solvent permeability. Nevertheless, a solution of NaOH diluted in the working solvent (and not water) displayed satisfactory results. Possibly, the utilization of ethanol/water 50:50 (v/v) as a solvent for the cleaning agent contributed to lowering the layer of solutes accumulated on the membrane surface. If fact, this solvent mixture was the one selected to perform the extraction of phenolic compounds from the wet olive pomace as a result of its high extraction yield. In contrast, the aqueous solution of P3 Ultrasil (in its two different versions) did not reduce the interactions that maintained the foulant adhered to the polymer, except in the case of the UF010104 membrane. This indicates that the fouling layer was not so strongly attached to the surface of this membrane.

Other authors have suggested the use of NaOH to clean polymeric membranes, such as UP005, used to treat different feed streams. Carbonell-Alcaina and co-workers combined NaOH and citric acid during the cleaning protocol of the UP005 membrane used to treat wastewaters from table olive production in cross-flow mode [18], whereas Luján-Facundo et al. cleaned the UP005 membrane (tested in cross-flow mode) fouled by whey proteins with a solution of NaOH and the simultaneous application of ultrasounds [58].

3. CONCLUSIONS

Wet olive pomace is a source of valuable compounds with numerous applications. These compounds can be extracted by conducting an ultrasound-assisted extraction at 40˚C, employing ethanol/water 50:50 (v/v) as a solvent. The application of the organic solvent during the extraction ensures a high recovery of biophenols, which enriches the efficiency of the process. The extracted phenolic content can be later purified by solvent-resistant ultrafiltration, but some challenges derived from the use of polymeric membranes with organic solvents have to be overcome. Among them, it has to be noted a potential alteration of the membrane performance, derived from a modification of the membrane structure as a result of the solvent contact (swelling, shrinkage, etc.). This may end in unexpected values of permeate flux and rejection.

In this context, the membranes used in this work were first pretreated to prepare them to the solvent exposure. The short protocol was selected. Regarding the performance of the membranes, all three tested membranes allowed the recovery of valuable phenolic compounds with considerable demand in the market. The selection of one of the membranes should be contingent upon the specific application. The UF010801v3 membrane allowed the production of a vegetal extract, rich in secoiridoids, flavonoids, phenolic acids, triterpenes, organic acids, and free fatty acids. The UF010104 membrane permitted the recovery of all these compounds and, additionally, the simple phenols were also present in the permeate. Moreover, the high rejection of total solids that was observed ensured an effective purification of the compounds of interest. Finally, if the non-phenolic species are to be retired from the permeate, the UP005 membrane achieved high rejection values of triterpenic acids, fatty acids, and organic acids. However, the lower values of permeate flux (in comparison with the SolSep BV membranes) should also be considered.

Funding: This work was supported by MCIN/AEI/ 10.13039/501100011033 and by ERDF A way of making Europe, grant number CTM2017-88645-R. The predoctoral grant

PRE2018-08524 was funded by MCIN/AEI/ 10.13039/501100011033 and by ESF Investing in your future. Funding for open access charge: CRUE-Universitat Politècnica de València.

4. REFERENCES

[1] J. Madureira, F.M.A. Margaça, C. Santos-Buelga, I.C.F.R. Ferreira, S.C. Verde, L. Barros, Applications of bioactive compounds extracted from olive industry wastes: A review, Compr. Rev. Food Sci. Food Saf. 21 (2022) 453–476. https://doi.org/10.1111/1541-4337.12861.

[2] A.S. Stasinakis, I. Elia, A. V. Petalas, C.P. Halvadakis, Removal of total phenols from olive-mill wastewater using an agricultural by-product, olive pomace, J. Hazard. Mater. 160 (2008) 408–413. https://doi.org/10.1016/J.JHAZMAT.2008.03.012.

[3] S. Dermeche, M. Nadour, C. Larroche, F. Moulti-Mati, P. Michaud, Olive mill wastes: Biochemical characterizations and valorization strategies, Process Biochem. 48 (2013) 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010.

[4] M.Á. Fernández-Prior, J.C.P. Fatuarte, A.B. Oria, I. Viera-Alcaide, J. Fernández-Bolaños, G. Rodríguez-Gutiérrez, New Liquid Source of Antioxidant Phenolic Compounds in the Olive Oil Industry: Alperujo Water, Foods. 9 (2020). https://doi.org/10.3390/foods9070962.

[5] M. del M. Contreras, I. Gómez-Cruz, I. Romero, E. Castro, Olive Pomace-Derived Biomasses Fractionation through a Two-Step Extraction Based on the Use of Ultrasounds: Chemical Characteristics, Foods. 10 (2021) 111. https://doi.org/10.3390/foods10010111.

[6] C.M. Sánchez-Arévalo, A. Iborra-Clar, M.C. Vincent-Vela, S. Álvarez-Blanco, Exploring the extraction of the bioactive content from the two-phase olive mill waste and further purification by ultrafiltration, LWT - Food Sci. Technol. 165 (2022) 113742. https://doi.org/10.1016/j.lwt.2022.113742.

[7] D.P. Zagklis, C.A. Paraskeva, Purification of grape marc phenolic compounds through solvent extraction, membrane filtration and resin adsorption/desorption, Sep. Purif. Technol. 156 (2015) 328–335. https://doi.org/10.1016/j.seppur.2015.10.019.

[8] R. Castro-Muñoz, G. Boczkaj, E. Gontarek, A. Cassano, V. Fíla, Membrane technologies assisting plant-based and agro-food by-products processing: A comprehensive review, Trends Food Sci. Technol. 95 (2020) 219–232. https://doi.org/10.1016/j.tifs.2019.12.003.

[9] P.G. Lim, C.Y. Cheok, J.Y. Sum, M.K.H. Ko Ko, Concentrating Garcinia mangostana L. rind extract using sequential ultrafiltration and nanofiltration, Biomass Convers. Biorefinery. (2023). https://doi.org/10.1007/s13399-023-04129-5.

[10] A. Cassano, C. Conidi, L. Giorno, E. Drioli, Fractionation of olive mill wastewaters

by membrane separation techniques, J. Hazard. Mater. 248–249 (2013) 185–193. https://doi.org/10.1016/j.jhazmat.2013.01.006.

[11] B. Díaz-Reinoso, A. Moure, H. Domínguez, J.C. Parajó, Ultra- and nanofiltration of aqueous extracts from distilled fermented grape pomace, J. Food Eng. 91 (2009) 587–593. https://doi.org/10.1016/j.jfoodeng.2008.10.007.

[12] M. Cifuentes-Cabezas, C. Carbonell-Alcaina, M.C. Vincent-Vela, J.A. Mendoza-Roca, S. Álvarez-Blanco, Comparison of different ultrafiltration membranes as first step for the recovery of phenolic compounds from olive-oil washing wastewater, Process Saf. Environ. Prot. 149 (2021) 724–734. https://doi.org/10.1016/j.psep.2021.03.035.

[13] A. Mir-Cerdà, I. Carretero, J.R. Coves, A. Pedrouso, C.M. Castro-Barros, T. Alvarino, J.L. Cortina, J. Saurina, M. Granados, S. Sentellas, Recovery of phenolic compounds from wine lees using green processing: Identifying target molecules and assessing membrane ultrafiltration performance, Sci. Total Environ. 857 (2023) 1–7. https://doi.org/10.1016/j.scitotenv.2022.159623.

[14] H. Arslan, E. Ondul Koc, Y. Ozay, O. Canli, S. Ozdemir, G. Tollu, N. Dizge, Antimicrobial and antioxidant activity of phenolic extracts from walnut (Juglans regia L.) green husk by using pressure-driven membrane process, J. Food Sci. Technol. 60 (2023) 73–83. https://doi.org/10.1007/s13197-022-05588-w.

[15] V. Sygouni, A.G. Pantziaros, I.C. Iakovides, E. Sfetsa, P.I. Bogdou, E.A. Christoforou, C.A. Paraskeva, Treatment of two-phase olive mill wastewater and recovery of phenolic compounds using membrane technology, Membranes (Basel). 9 (2019) 27. https://doi.org/10.3390/membranes9020027.

[16] R. Shukla, M. Cheryan, Performance of ultrafiltration membranes in ethanol-water solutions: Effect of membrane conditioning, J. Memb. Sci. 198 (2002) 75–85. https://doi.org/10.1016/S0376-7388(01)00638-X.

[17] M. Razali, C. Didaskalou, J.F. Kim, M. Babaei, E. Drioli, Y.M. Lee, G. Szekely, Exploring and Exploiting the Effect of Solvent Treatment in Membrane Separations, ACS Appl. Mater. Interfaces. 9 (2017) 11279–11289. https://doi.org/10.1021/acsami.7b01879.

[18] C. Carbonell-Alcaina, J.L. Soler-Cabezas, A. Bes-Piá, M.C. Vincent-Vela, J.A. Mendoza-Roca, L. Pastor-Alcañiz, S. Álvarez-Blanco, Integrated membrane process for the treatment and reuse of residual table olive fermentation brine and anaerobically digested sludge centrate, Membranes (Basel). 10 (2020) 1–14. https://doi.org/10.3390/membranes10100253.

[19] Z. Yin, Y. Ma, B. Tanis-Kanbur, J.W. Chew, Fouling behavior of colloidal particles in organic solvent ultrafiltration, J. Memb. Sci. 599 (2020) 19–22. https://doi.org/10.1016/j.memsci.2020.117836.

[20] Y. Ma, S. Velioğlu, Z. Yin, R. Wang, J.W. Chew, Molecular dynamics investigation

of membrane fouling in organic solvents, J. Memb. Sci. 632 (2021) 119329. https://doi.org/10.1016/J.MEMSCI.2021.119329.

[21] UNE-EN ISO 7887:2012 Method B, Water Quality. Examination and Determination of Colour, 2012.

[22] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics acids with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.

[23] I.S. Argyle, C.J. Wright, M.R. Bird, The effect of ethanol pre-treatment upon the mechanical, structural and surface modification of ultrafiltration membranes, Sep. Sci. Technol. 52 (2017) 2040–2048. https://doi.org/10.1080/01496395.2017.1310234.

[24] M. de Souza Araki, C. de Morais Coutinho, L.A.G. Gonçalves, L.A. Viotto, Solvent permeability in commercial ultrafiltration polymeric membranes and evaluation of the structural and chemical stability towards hexane, Sep. Purif. Technol. 71 (2010) 13–21. https://doi.org/10.1016/j.seppur.2009.10.005.

[25] V. Calabrò, A. Basile, Fundamental membrane processes, science and engineering, Adv. Membr. Sci. Technol. Sustain. Energy Environ. Appl. (2011) 3–21. https://doi.org/10.1533/9780857093790.1.3.

[26] L. Olmo-García, R.P. Monasterio, C.M. Sánchez-Arévalo, A. Fernandez-Gutierrez, J.M. Olmo-Peinado, A. Carrasco-Pancorbo, Characterization of new olive fruit derived products obtained by means of a novel processing method involving stone removal and dehydration with zero waste generation, J. Agric. Food Chem. 67 (2019) 9295–9306. https://doi.org/10.1021/acs.jafc.9b04376.

[27] I. Cea-Pavez, J. Lozano-Sánchez, I. Borrás-Linares, H. Nuñez, P. Robert, A. Segura-Carretero, Obtaining an Extract Rich in Phenolic Compounds from Olive Pomace by Pressurized Liquid Extraction, Molecules. 24 (2019) 3108. https://doi.org/10.3390/MOLECULES24173108.

[28] L. Olmo-García, N. Kessler, H. Neuweger, K. Wendt, J.M. Olmo-Peinado, A. Fernández-Guti rrez, C. Baessmann, A. Carrasco-Pancorbo, Unravelling the distribution of secondary metabolites in olea europaea l.: exhaustive characterization of eight olive-tree derived matrices by complementary platforms (LC-ESI/APCI-MS and GC-APCI-MS), Molecules. 23 (2018) 1–16. https://doi.org/10.3390/molecules23102419.

[29] M. Robles-Almazan, M. Pulido-Moran, J. Moreno-Fernandez, C. Ramirez-Tortosa, C. Rodriguez-Garcia, J.L. Quiles, Mc. Ramirez-Tortosa, Hydroxytyrosol: Bioavailability, toxicity, and clinical applications, Food Res. Int. 105 (2018) 654–667. https://doi.org/10.1016/j.foodres.2017.11.053.

[30] P. Miralles, A. Chisvert, A. Salvador, Determination of hydroxytyrosol and tyrosol by liquid chromatography for the quality control of cosmetic products based on

olive extracts, J. Pharm. Biomed. Anal. 102 (2014) 157–161. https://doi.org/10.1016/j.jpba.2014.09.016.

[31] V. Contreras-Jácquez, U. Valenzuela-Vázquez, D.A. Grajales-Hernández, J.C. Mateos-Díaz, M. Arrellano-Plaza, M.E. Jara-Marini, A. Asaff-Torres, Pilot-Scale Integrated Membrane System for the Separation and Concentration of Compounds of Industrial Interest from Tortilla Industry Wastewater (Nejayote), Waste and Biomass Valorization. 13 (2022) 345–360. https://doi.org/10.1007/s12649-021-01530-x.

[32] S. Ou, K.C. Kwok, Ferulic acid: Pharmaceutical functions, preparation and applications in foods, J. Sci. Food Agric. 84 (2004) 1261–1269. https://doi.org/10.1002/jsfa.1873.

[33] I. Kalaboki, D. Koulougliotis, D. Kleisiari, E. Melliou, P. Magiatis, A. Kampioti, E. Eriotou, A. Destouni, A study on the clustering of extra virgin olive oils extracted from cultivars growing in four ionian islands (Greece) by multivariate analysis of their phenolic profile, antioxidant activity and genetic markers, Foods. 10 (2021) 3009. https://doi.org/10.3390/FOODS10123009/S1.

[34] C. Zhang, J. Zhang, X. Xin, S. Zhu, E. Niu, Q. Wu, T. Li, D. Liu, Changes in Phytochemical Profiles and Biological Activity of Olive Leaves Treated by Two Drying Methods, Front. Nutr. 9 (2022) 899. https://doi.org/10.3389/fnut.2022.854680.

[35] O. Tebboub, R. Cotugno, F. Oke-Altuntas, M. Bouheroum, Í. Demirtas, M. D'Ambola, N. Malafronte, A. Vassallo, Antioxidant potential of herbal preparations and components from galactites elegans (All.) nyman ex soldano, Evidence-Based Complement. Altern. Med. 2018 (2018). https://doi.org/10.1155/2018/9294358.

[36] F.J. Reyes-Zurita, E.E. Rufino-Palomares, J.A. Lupiáñez, M. Cascante, Maslinic acid, a natural triterpene from Olea europaea L., induces apoptosis in HT29 human colon-cancer cells via the mitochondrial apoptotic pathway, Cancer Lett. 273 (2009) 44–54. https://doi.org/10.1016/J.CANLET.2008.07.033.

[37] J.P. Robinson, E.S. Tarleton, The removal of solutes from organic solvents using nanofiltration, in: Innov. Filtr. Sep. Conf., The Filtration Society, Loughborough, 2004.

[38] I.S. Argyle, A. Pihlajamäki, M.R. Bird, Black tea liquor ultrafiltration: Effect of ethanol pre-treatment upon fouling and cleaning characteristics, Food Bioprod. Process. 93 (2015) 289–297. https://doi.org/10.1016/j.fbp.2014.10.010.

[39] P.C. Sadek, The HPLC Solvent Guide, 2nd ed., John Wiley & Sons, Ltd, Michigan, 2002.

[40] V.M. Pappas, D. Palaiogiannis, V. Athanasiadis, T. Chatzimitakos, E. Bozinou, D.P. Makris, S.I. Lalas, Optimization of Pulsed Electric-Field-Based Total Polyphenols'

Extraction from Elaeagnus pungens 'Limelight' Leaves Using Hydroethanolic Mixtures, Oxygen. 2 (2022) 537–546. https://doi.org/10.3390/oxygen2040035.

[41] M.L. Huber, A.H. Harvey, Fluid Properties. Viscosity of Liquids, in: J.R. Rumble (Ed.), Handb. Chem. Phys., 99th ed., Taylor & Francis, Boca Ratón, Florida, 2018: pp. 231–235.

[42] B. Van der Bruggen, J. Geens, C. Vandecasteele, Influence of organic solvents on the performance of polymeric nanofiltration membranes, Sep. Sci. Technol. 37 (2002) 783–797. https://doi.org/10.1081/SS-120002217.

[43] F.M. Penha, K. Rezzadori, M.C. Proner, V. Zanatta, G. Zin, D.W. Tondo, J. Vladimir de Oliveira, J.C.C. Petrus, M. Di Luccio, Influence of different solvent and time of pre-treatment on commercial polymeric ultrafiltration membranes applied to non-aqueous solvent permeation, Eur. Polym. J. 66 (2015) 492–501. https://doi.org/10.1016/j.eurpolymj.2015.03.010.

[44] C.M. Sánchez-Arévalo, T. Croes, B. Van der Bruggen, M.C. Vincent-Vela, S. Álvarez-Blanco, Feasibility of several commercial membranes to recover valuable phenolic compounds from extracts of wet olive pomace through organic-solvent nanofiltration, Sep. Purif. Technol. 305 (2023). https://doi.org/10.1016/j.seppur.2022.122396.

[45] J.R.M. de Melo, M. V. Tres, J. Steffens, J. Vladimir Oliveira, M. Di Luccio, Desolventizing organic solvent-soybean oil miscella using ultrafiltration ceramic membranes, J. Memb. Sci. 475 (2015) 357–366. https://doi.org/10.1016/j.memsci.2014.10.029.

[46] M.J. Luján-Facundo, J.A. Mendoza-Roca, B. Cuartas-Uribe, S. Álvarez-Blanco, Evaluation of cleaning efficiency of ultrafiltration membranes fouled by BSA using FTIR-ATR as a tool, J. Food Eng. 163 (2015) 1–8. https://doi.org/10.1016/j.jfoodeng.2015.04.015.

[47] C. Carbonell-Alcaina, S. Álvarez-Blanco, M.A. Bes-Piá, J.A. Mendoza-Roca, L. Pastor-Alcañiz, Ultrafiltration of residual fermentation brines from the production of table olives at different operating conditions, J. Clean. Prod. 189 (2018) 662–672. https://doi.org/10.1016/j.jclepro.2018.04.127.

[48] J.A.A. Mejia, A. Ricci, A.S. Figueiredo, A. Versari, A. Cassano, M.N. de Pinho, G.P. Parpinello, Membrane-based Operations for the Fractionation of Polyphenols and Polysaccharides From Winery Sludges, Food Bioprocess Technol. 15 (2022) 933–948. https://doi.org/10.1007/s11947-022-02795-3.

[49] M. Ji, P.K. Roy, J. Barman, K. Khare, Commercial polysulfone membranes pretreated with ethanol and NaOH: Effects on permeability, selectivity and antifouling properties, Langmuir. 35 (2019) 7672–7679. https://doi.org/10.1021/acs.langmuir.9b00483.

[50] A. Sotto, J.M. Arsuaga, B. Van der Bruggen, Sorption of phenolic compounds on

NF/RO membrane surfaces: Influence on membrane performance, Desalination. 309 (2013) 64–73. https://doi.org/10.1016/j.desal.2012.09.023.

[51] S. Yammine, R. Rabagliato, X. Vitrac, M.M. Peuchot, R. Ghidossi, Selecting ultrafiltration membranes for fractionation of high added value compounds from grape pomace extracts, OENO One. 53 (2019) 487–497. https://doi.org/10.20870/OENO-ONE.2019.53.3.2343.

[52] D. Li, X. Yang, Z. Zhou, B. Jiang, A. Tawfik, S. Zhao, F. Meng, Molecular traits of phenolic moieties in dissolved organic matter: Linkages with membrane fouling development, Environ. Int. 133 (2019) 105202. https://doi.org/10.1016/J.ENVINT.2019.105202.

[53] X.J. Yang, A.G. Livingston, L. Freitas Dos Santos, Experimental observations of nanofiltration with organic solvents, J. Memb. Sci. 190 (2001) 45–55. https://doi.org/10.1016/S0376-7388(01)00392-1.

[54] B. Yang, C. Wang, X. Cheng, Y. Zhang, W. Li, J. Wang, Z. Tian, W. Chu, G. V. Korshin, H. Guo, Interactions between the antibiotic tetracycline and humic acid: Examination of the binding sites, and effects of complexation on the oxidation of tetracycline, Water Res. 202 (2021) 117379. https://doi.org/10.1016/J.WATRES.2021.117379.

[55] K. Xiao, Y. xiao Shen, S. Liang, P. Liang, X. mao Wang, X. Huang, A systematic analysis of fouling evolution and irreversibility behaviors of MBR supernatant hydrophilic/hydrophobic fractions during microfiltration, J. Memb. Sci. 467 (2014) 206–216. https://doi.org/10.1016/J.MEMSCI.2014.05.030.

[56] E. Nagy, Membrane Materials, Structures, and Modules, Basic Equations Mass Transp. Through a Membr. Layer. (2019) 11–19. https://doi.org/10.1016/B978-0-12-813722-2.00002-9.

[57] F. Fei, H.A. Le Phuong, C.F. Blanford, G. Szekely, Tailoring the Performance of Organic Solvent Nanofiltration Membranes with Biophenol Coatings, ACS Appl. Polym. Mater. 1 (2019) 452–460. https://doi.org/10.1021/acsapm.8b00161.

[58] M.J. Luján-Facundo, J.A. Mendoza-Roca, B. Cuartas-Uribe, S. Álvarez-Blanco, Ultrasonic cleaning of ultrafiltration membranes fouled with BSA solution, Sep. Purif. Technol. 120 (2013) 275–281. https://doi.org/10.1016/j.seppur.2013.10.018.

Supplementary Table 1. Analytical data corresponding to the retention time (t_R), mass/charge relation (*m/z*), and concentrations of each compound determined in the retentate streams of the UF010104, UF01801v3, and UP005 membranes. The compounds have been grouped according to their chemical class to facilitate the inspection of the Table. Relative standard deviation was always below 14%.

Compound	t_R (min)	*m/z* $[M-H]^-$	UF010104, 2.5 bar, VRF 3	UF01801v3, 2.5 bar, VRF 3	UP005, 2.5 bar, VRF 3
			mg/kg in the retentate	mg/kg in the retentate	mg/kg in the retentate
Hydroxytyrosol	3.09	153.0551	205.47	194.31	244.64
Hydroxytyrosol glucoside	2.80	315.1085	15.90	11.11	16.7
Hydroxytyrosol derivative	8.64	323.1504	18.39	32.80	45.14
Tyrosol	5.57	137.0608	333.50	333.70	310.12
Vanillic acid	2.70	167.0345	1.83	2.20	1.81
Vanillin	4.83	151.0396	18.65	14.32	11.73
Caffeic acid	4.92	179.0347	85.71	71.89	70.92
***p*-Coumaric acid**	6.45	163.0397	23.33	29.76	23.89
Ferulic acid	9.53	193.0503	8.65	14.07	14.23
Ferulic acid methyl ester	11.93	207.0668	24.99	30.69	25.06
Acyclodihydroelenolic acid hexoside	3.28	407.1560	306.94	362.92	301.44
Hydroxy-decarboxymethyl elenolic acid	3.77	199.0607	2002.10	1976.10	1790.19
Hydroxy-elenolic acid	5.59	257.0669	245.51	206.90	210.6
Elenolic acid glucoside	5.65	403.1246	9.30	9.05	12.62
Decarboxymethyl elenolic acid	5.90	183.0658	809.31	1363.70	1203
Aldehydic form of decarboxymethyl elenolic acid	6.45	215.0925	142.87	147.00	101.01

Compound	t_R (min)	*m/z* $[M-H]^-$	UF010104, 2.5 bar, VRF 3	UF01801v3, 2.5 bar, VRF 3	UP005, 2.5 bar, VRF 3
			mg/kg in the retentate	mg/kg in the retentate	mg/kg in the retentate
Phenylethyl primeveroside	6.56	415.1612	6.47	2.92	6.78
Hydroxy-oleuropein	6.57	555.1717	0.00	3.04	3.21
Hydrogenated elenolic acid	7.47	243.0876	20.27	15.45	18.18
Dehydro-oleuropein aglycone	7.77	375.1087	0.00	4.75	4.14
Verbascoside	7.56	623.1981	2.41	2.23	2.09
Cafselogoside	9.06	551.1409	42.64	18.03	41.74
Elenolic acid	9.06	241.0720	29.83	21.67	23.18
Comselogoside	8.39	535.1457	61.56	44.07	53.58
Oleuropein	9.64	539.1769	1.31	2.00	1.49
Decarboxy-methyl oleuropein aglycone	10.41	319.1187	12.76	12.66	11.44
Ligstroside	10.90	523.1820	2.37	1.44	1.71
Oleuropein aglycone	13.95	377.1242	35.88	29.77	29.54
Oleuropein aglycone derivative	5.67	377.1453	151.96	107.07	180.7
Gallocatechin	5.18	305.0702	53.02	58.12	50.01
Luteolin	11.03	285.0405	7.48	4.73	5.49
Hydroxypinoresinol	9.43	373.1294	9.14	10.97	9.05
Maslinic acid	16.24	471.3488	17.36	9.71	16.74
Betulinic acid	18.06	455.3538	2.35	2.17	1.99
Quinic acid	1.02	191.0555	272.94	489.72	293.21
Malic acid	1.09	133.0150	146.13	260.64	148.64
Isopropyl-malic acid	4.30	175.0614	40.87	32.82	31.94
Citric acid	1.16	191.0191	1611.50	1587.60	1439.59

Compound	t_R (min)	*m/z* $[M-H]^-$	UF010104, 2.5 bar, VRF 3	UF01801v3, 2.5 bar, VRF 3	UP005, 2.5 bar, VRF 3
			mg/kg in the retentate	mg/kg in the retentate	mg/kg in the retentate
Trihydroxy-octadecadienoic acid	13.32	327.2180	0.00	0.67	0.30
Trihydroxy-octadecenoic acid	13.97	329.2335	2.75	4.88	3.43
Dihydroxy-hexadecanoic acid	14.15	287.2230	12.36	11.75	13.81
Hydroxy-octadecatrienoic acid	15.66	293.2122	1.00	0.64	0.74
Hydroxy-octadecadienoic acid	15.96	295.2277	6.48	9.47	7.75

CAPÍTULO 7. CHAPTER 7

Feasibility of several commercial membranes to recover valuable phenolic compounds from extracts of wet olive pomace through organic-solvent nanofiltration

Carmen M. Sánchez-Arévalo[1], Tim Croes[2], Bart Van der Bruggen[2], María Cinta Vincent-Vela[3,4], Silvia Álvarez-Blanco[3,4]

[1]Research Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

[2]Department of Chemical Engineering (CIT), ProcESS-Process Engineering for Sustainable System, KU Leuven, Celestijnenlaan 200f - Box 2424, 3001 Leuven, Belgium

[3]Department of Chemical and Nuclear Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

✉sialvare@iqn.upv.es

Separation and Purification Technology, 305 (2023) 122396

https://doi.org/10.1016/j.seppur.2022.122396

Abstract: Organic-solvent nanofiltration (OSN) has been applied to purify and fractionate the phenolic compounds present in wet olive pomace, which is the main by-product of olive mills. Nine commercial OSN membranes have been tested: DuraMem® 150, DuraMem® 300, DuraMem® 500, PuraMem® 600 (Evonik), NFS, NFX (Synder), oNF-1 and oNF-2 (Borsig) and NF270 (FilmTec). Their stability in ethanol/water 50:50 (v/v) and their effectiveness to treat a model solution of a solvent-based extract of wet olive pomace have been studied. To that end, a METcell cross-flow system (Evonik) has been utilized. DuraMem® 500, NFX and NF270 membranes displayed satisfactory values of permeate flux (10-100 $L \cdot h^{-1} \cdot m^2$) compared to the other tested membranes. Measurements of the contact angle of the membranes after their conditioning and after the nanofiltration process allowed the comprehension of the interaction between the ethanol/water 50:50 (v/v) solution and the membrane. The solvent contact angle was also examined. AFM was employed to understand the modification of membrane morphology. To characterize the samples, liquid chromatography coupled to mass spectrometry (and/or refractive index detector) was employed. The selected membranes exhibited low rejection values for the aimed phenolic compounds (less than 10% for hydroxytyrosol) and high rejection (50-100%) of the undesired compounds, such as sugars and organic acids. Therefore, the purification of the target phenolic compounds was accomplished.

Keywords: organic-solvent nanofiltration, phenolic compounds, sugars, wet olive pomace, rejection.

1. INTRODUCTION

Every year, the olive oil campaign ends with the generation of enormous volumes of by-products. Depending on the methodology applied by the olive mill, there are different types of residues that can be produced. In the Mediterranean area, the three-phase methodology and the two-phase methodology are highly widespread for the production of extra virgin olive oil [1]. However, as Spain is the first world producer and the two-phase method is preferred by the olive mills from this zone, the two-phase procedure is considered to be the most common.

According to this methodology, the main residue derived from olives processing is wet olive pomace (or *alperujo*, by its name in Spanish). This by-product consists of a semi-solid combination of the olive epicarp, mesocarp and endocarp. In consequence, considerable fractions of the olive skin, pulp and stone are part of the residue. The olive fruit is rich in bioactive compounds, including phenolic compounds and vitamins [2,3]. Polyphenols are of high interest for their applications in functional foods, cosmetics and

pharmacy [4]. Then, the recovery of phenolic compounds from the wet olive pomace allows the utilization of an environmentally concerning residue and the retrieval of valuable compounds that otherwise would be discarded with the by-product.

By means of an ultrasound-assisted solid-liquid extraction, it is possible to extract high concentrations of polyphenols from the wet olive pomace. It has been described previously that a mixture of ethanol/water 50:50 (v/v) can be very efficient in this context [5–7]. However, the obtained extract contains other organic compounds that are coextracted with the biophenols and should be then removed. The purification of the polyphenols from the hydroalcoholic extract can be achieved by membrane technology. High rejections of the organic matter have been obtained by solvent-based ultrafiltration [8,9]. However, several unwanted compounds, such as sugars and organic acids are still present in that permeate. In this context, organic-solvent nanofiltration (OSN) could allow the separation of phenolic compounds from the concomitant undesired molecules present in the extract of wet olive pomace. Furthermore, OSN could be applied to perform the fractionation of the recovered polyphenols.

The growing interest of the scientific community in OSN has logically resulted in new methods for membrane synthesis. Current commercial membranes mainly include polyamide, polyimide, polysulfone, polydimethylsiloxane, polybenzimidazole and polyacrylonitrile as a polymer. Moreover, the development of novel materials that improve OSN procedures has become very relevant [10,11]. In the recent years, carbon organic frameworks (COFs) and metal-organic frameworks (MOFs) have emerged as porous materials with tunable pore size and high chemical resistance, which opens a new range of opportunities regarding molecular separations [12–14]. In fact, membrane preparation is the main topic of the research in this membrane technology field [15].

However, thoughtful and further research about the real application of the recent solvent-resistant polymers that are being produced and commercialized is needed. The latter has been more common regarding aqueous nanofiltration [1,16–18], but it is still a growing area when it comes to OSN.

In this contribution, several OSN membranes, as well as conventional ones, have been tested to study their stability and performance regarding the purification and fractionation of the phenolic compounds present in the wet olive pomace. For that purpose, a model solution of a hydroalcoholic extract of wet olive pomace has been employed. Its pre-treatment by ultrafiltration has also been considered when preparing the simulated solution. The rejection of undesired compounds was not the only

objective. The separation of the polyphenols of interest was also pursued, in order to obtain them in fractions of individual or very similar molecules, which will enhance their potential industrial application.

Among the membranes tested in this study, a wide range of pore sizes was contemplated, including membranes with reduced values of molecular weight cut-off (MWCO), such as the NFS membrane (100-250 Da), and also loose nanofiltration membranes with larger pores, as the oNF-1 membrane (600 Da). Additionally, several manufacturers were considered, namely FilmTec, Evonik, Synder and GMT-Borsig, in order to study a diverse repertoire of the current commercial catalog. Most of them are acknowledged producers of OSN membranes. Then, the selection of different commercially available membranes, resistant to ethanol/water 50:50 (v/v) (which is the working solvent in this work) and provided by the main OSN manufacturers was intended. Additionally, the NF270 membrane, which is a conventional membrane (not specifically designed to work with organic solvents) was included in the study. This membrane is extensively employed in the literature, and it has proven to be effective for the recovery of valuable compounds from agro-food samples. The separation of phenolic compounds and sugars in an aqueous medium has already been described with this membrane [17]. Then, its performance in a hydroalcoholic environment was considered of interest.

2. MATERIALS AND METHODS

2.1. Solvents and reagents

Pure ethanol and LC-MS grade acetonitrile were purchased from VWR (USA); extra-pure sulfuric acid, LC-MS grade formic acid and LC-MS grade acetic acid were obtained from Fisher (Fisher Scientific, USA). Ultrapure water was obtained from an Arium® system (Sartorius, Germany). The pure standards of phenolic compounds and triterpenic and fatty acids were purchased from VWR (in the case of tyrosol, luteolin, caffeic acid and citric acid) and Sigma Aldrich (USA) in the case of hydroxytyrosol, oleuropein, decarboxymethyl-oleuropein aglycone, *p*-coumaric acid and hydroxy-stearic acid. Sigma Aldrich also provided the standards for sucrose, D-glucose, and D-fructose.

2.2. Feed solution

A model solution of the permeate obtained in the ultrafiltration of a hydroalcoholic extract of wet olive pomace was prepared. This model solution corresponded to the permeate obtained in an XFUF 076 01 bench-top ultrafiltration cell (Merck Millipore, USA) with the UP005 membrane (Microdyn Nadir, Germany) at 2 bar. This

permeate was analyzed as detailed in section 2.5.1. The determined concentrations of each chemical class present in the ultrafiltration permeate can be revised in Table 7.1.

The analysis through liquid chromatography coupled to mass spectrometry (LC-MS) allowed the determination of seven chemical families, including simple phenols, secoiridoids, flavonoids, phenolic acids (all of them being phenolic compounds), triterpenic acids, and free fatty acids. Sugars were also detected by global analysis (Section 2.5.1). To simulate this content, not only one compound was included, but all the chemical families present in the real stream. Thus, at least one representative for all the chemical classes of the ultrafiltered extract of wet olive pomace was added to the solution. To select one compound as a family representative, the most concentrated one was chosen. When the most concentrated molecule of one chemical class was not commercially available, then the following compound in abundance was selected.

The concentration of the analytes was set according to the actual concentrations present in the real sample. The representative of each chemical family was added at the total concentration of the entire chemical class. For example, the representative for the class of flavonoids (luteolin, in this case) was added at 15 ppm, because this was the sum of concentrations of all the flavonoids present in the sample. Logically, the total concentration of each family was not an exact number. Thus, the concentration value was rounded to the most proximate multiple of 5, in order to facilitate the preparation of samples. Two compounds from the same family were added to the solution in some cases. This occurred when both of them were of high importance because of their chemical structure or economic implications. Then, their relative concentration in the real stream was maintained in the solution. The selection of the representative compounds was made according to their presence in the real sample, their industrial and scientific relevance, and their commercial availability.

In the case of sugars, the ripening stage of the olive fruits (as a prime matter for the generation of wet olive pomace) was taken into account. The extract of wet olive pomace would contain a different concentration of soluble sugars depending on the moment of the olives' harvesting and the time between their harvesting and their processing. As the olive ripening occurs, the polysaccharides from the vegetal cell (including cellulose and hemicellulose, among others) suffer a degradation that results in the release of some of their monomers, increasing the concentration of soluble sugars [19,20]. Then, to better simulate the sugar content of the ultrafiltered hydroalcoholic extract of wet olive pomace, a higher concentration of soluble sugars was contemplated, although the

relative concentration of glucose, fructose and sucrose [21] was maintained. The detailed composition of the model solution is shown in Table 7.1. Information about the molecular weight, concentration in the real sample, and concentration in the model solution are displayed. Additionally, the Table shows the chemical class that is represented by these compounds, its total concentration, and the justification of the representative selection.

The three-dimension structure of the analytes present in the feed solution was analyzed with the software Jmol, which is an open-source Java viewer for chemical structures in 3D (http://www.jmol.org). This software also allowed the calculation of the distance between each atom of the molecules.

Table 7.1. Composition of the model solution employed as feed for the organic solvent nanofiltration process.

Compound	Molecular weight (g/mol)	Concentration in *alperujo* UF Permeate (ppm)[a]	Chemical Family	Total concentration of the chemical family (ppm)	Concentration in the model solution (ppm)	Justification
Tyrosol	138.2	0.728	Simple phenols	49.403	25	Both of them are key representatives of simple phenols. HTY is extremely valued by industry.
Hydroxytyrosol	154.2	45.113			25	
Oleuropein	540.5	0.950	Secoiridoids	958.549	150	Main representative of secoiridoids in literature. High industrial relevance.
Decarboxymethyl oleuropein aglycone	320.3	8.040			10	As a representative of secoiridoids.
Luteolin	286.2	10.570	Flavonoids	14.411	15	As a representative of flavonoids.
Caffeic acid	180.2	115.304	Phenolic acids	122.045	120	As a representative of phenolic acids.
***p*-Coumaric acid**	164.1	3.317			5	As a representative of phenolic acids. High industrial relevance.
Hydroxy-stearic acid	300.5	1.400	Free fatty acids	3.543	5	Free fatty acids are present in the real sample and hinder the purification of polyphenols.
Citric acid	191.1	310.820	Organic acids	326.973	350	Organic acids hinder the purification of polyphenols from the sample.
Sucrose	342.3	300	Sugars	300	50	Sugars hinder the purification of polyphenols from the sample.
Fructose	180.2				300	
Glucose	180.2				1500	

[a]UF Permeate obtained with the UP005 membrane, at 2 bar.

2.3. Organic solvent nanofiltration set-up and experimental procedure

All experiments were carried out in a *METcell* Cross-Flow System (Evonik Industries, Germany), with two membrane modules set in series. The effective area of each module was 14.6 cm^2. In total, 9 commercially available, organic membranes, of which the specifications can be found in Table 7.2, were tested.

Table 7.2. Specifications of the membranes employed in this work. The data have been retrieved from the manufacturers.

Membrane	MWCO[a] (Da)	Material	Manufacturer	Maximum operating pressure (bar)
NF270	300-400	Polyamide	FilmTec	41
Dm150[b]	150	P84® polyimide	Evonik	60
Dm300[c]	300			
Dm500[d]	500			20
Pm600[e]	600	Silicone-coated polyimide		60
NFS	100-250	Proprietary polyamide	Synder	41
NFX	150-300			
oNF-1	600	Polydimethylsiloxane	GMT-Borsig	40
oNF-2	350			

[a]MWCO: molecular weight cut off; [b]Dm150: DuraMem®150; [c]Dm300: DuraMem®300; [d]Dm500: DuraMem®500; [e]Pm600: PuraMem®600;

A new membrane coupon was employed for each experiment. Prior to their utilization, the membranes were immersed in ethanol/water 50:50 (v/v) for at least 12 hours, in order to precondition them. Moreover, a compaction stage was carried out before running any experiment. Thus, the permeate flux, J_p $(L \cdot h^{-1} \cdot m^{-2})$, was monitored until a stable permeate flux was observed, at a transmembrane pressure (TMP) of 38 bar, or 20 bar, in the case of the Dm500 membrane. Apart from the obtention of a compacted separation layer for the membranes, their stability in the presence of the organic solvent was tested. A stable permeate flux during that time determined that the polymer was not damaged by the solvent and, furthermore, it confirmed the removal of any remaining preservative agent. To characterize the membranes, the solvent permeability was calculated according to the equation:

$$L_p = \frac{J_p}{TMP} \quad (1)$$

Afterwards, the synthetic feed solution was nanofiltered at 36 bar (or 20 bar, in the case of the Dm500 membrane). The permeate flux was monitored and permeate samples

were taken at a volume reduction factor (VRF) of 3 for their analysis. When some membranes were considered best in terms of performance, they were further investigated at other values of TMP (15 and 25 bar) and the rejection was evaluated at different values of VRF. The experiments were carried out in duplicates, with a maximum relative standard deviation of 13.5% (intra-day repeatability).

The morphology of the active layer of each membrane was studied before and after their immersion in the solvent. To that end, atomic force microscopy (AFM) was employed, using a MultiMode 8 AFM instrument (Bruker, Germany), equipped with a ScanAsyst-Air probe (Bruker, Germany). The working methodology was Quantitative Nanomechanical Mapping. The obtained images were processed with the software NanoScope Analysis 1.8.

The cleaning of the most promising membrane was also studied. After its utilization, the NF270 membrane was conveniently cleaned with an Ultrasil 1% (v/v) solution. The recovery of the initial solvent permeability of the membrane surpassed 98%. Then, the membrane could be recycled in subsequent experiments.

2.4. Adsorption

To evaluate the adsorption of phenolic compounds on the membrane surface, an HP4750 dead-end filtration cell (Sterlitech, USA) was used. The olive-pomace model solution was placed in the cell to be in contact with the membrane surface for 24 h, in agitation mode. No pressure was applied. The membranes were previously preconditioned as explained in section 2.3, in order to obtain adsorption results comparable to those occurring during the nanofiltration process. All experiments were carried out in duplicate.

The concentration of the compounds in the solution before and after this period was compared and used to calculate the adsorption, as in the following formula:

$$Q = \frac{C_0 - C_f}{A} \cdot V \quad (2)$$

where Q represents the mass of the adsorbed compound per membrane surface unit ($mg \cdot m^{-2}$), C_0 ($mg \cdot L^{-1}$) is the initial concentration of the compound in the solution, C_f ($mg \cdot L^{-1}$) is the final concentration of the compound after the adsorption test, A (m^2) is the membrane area and V (L) is the volume of the solution employed during the experiment. This methodology was developed by Arsuaga and co-workers [22,23] and has been later reproduced [24].

2.5. Sample characterization

2.5.1. Analysis of ultrafiltration permeate to establish the composition of the model solution

The individual composition of the ultrafiltration permeate, intended to be the feed for the nanofiltration process of this work, was determined by LC-MS. The applied methodology was developed in an earlier work [25], using a 1260 Infinity II LC system coupled to a quadrupole-time-of-flight (QToF) mass spectrometer (Agilent Technologies, USA). Shortly, a Zorbax Extend C18 column (4.6 x 100 mm, 1.8 µm) (Agilent Technologies, USA) was employed for the separation of the compounds, operating at 40°C. Injection volume was 4 µL. Analytes elution was conducted with a gradient of acetonitrile and water, both acidified with a 0.5% acetic acid, and a flow rate of 0.8 m/min. Acquisition of MS data was performed in negative ionization mode. Total soluble sugars were analyzed by the Anthrone methodology [26,27].

2.5.2. Characterization of the organic solvent nanofiltration streams

2.5.2.1. Analysis of phenolic compounds and triterpenic and free fatty acids

To characterize the OSN streams, compounds were individually determined by means of a 1100 Agilent liquid chromatograph coupled to a 6110 single-quadrupole (Agilent Technologies, USA). After a 5 µL injection, analytes were eluted throughout the column mentioned in Section 2.5.1, at a flow rate of 0.5 mL/min. To that end, the following gradient was applied: 5% B at initial conditions, 20% B at 1 min, 85% B at 7 min, 100% B at 7.5 min, where water acidified with a 0.5% of formic acid was the solution A and acetonitrile was the solution B. 100% of solution B was maintained until minute 10 and then the gradient went back to the initial conditions. The MS acquisition was set in negative ion mode and single ion monitoring, with the [M-H] value of *m/z* of each compound. All samples were injected at least twice.

Data analysis was performed with the software ChemStation B.04.02 (Agilent Technologies, USA). All compounds were quantified by integrating the peaks obtained in the base peak chromatogram provided by the mass spectrometer. Only the peak of tyrosol was integrated through the diode-array (DAD) signal (also provided by the instrument), because the DAD signal for this compound was considered to be more reliable, considering the injection solution and the applied gradient.

Solutions of pure standards were prepared in ethanol/water 50:50 (v/v), in the range 0.1 - 100 ppm, and used for the external calibration.

2.5.2.2. Determination of sugars

Sucrose, glucose, and fructose concentrations were determined by an Agilent 1200 liquid chromatograph coupled to a refractive index detector (Agilent Technologies, USA). The instrument was equipped with the column Aminex HPX-87H (Bio-rad, Belgium), kept at 40°C during the analysis and protected with a Micro-Guard cation H guard column (Bio-rad, Belgium). 5 mM sulfuric acid was used as a mobile phase, at a flow rate of 0.6 mL/min. The injection volume was 20 µL. All vials were injected in duplicates. For data processing, ChemStation B.04.02 was employed.

2.6. Contact angle measurements

To assess the hydrophilic or hydrophobic character of the membranes surface, a contact angle analyzer Krüss DSA10 Mk2 (Krüss Optronics, Germany) was employed. Water droplets of 20 µL were gently delivered on the membrane surface by a microsyringe. Then images of the drop were taken during 3 seconds and the contact angle was measured. This process was repeated 10 times on different sections of the membrane surface, and the mean values have been reported.

3. RESULTS AND DISCUSSION

3.1. Synthetic ultrafiltration permeate of wet olive pomace

The composition of the feed solution (Table 7.1) was carefully designed to include all the chemical families present in the permeate resulting from the ultrafiltration of an extract from wet olive pomace obtained with ethanol/water 50:50. In the ultrafiltration step, a high proportion of organic matter was already removed. However, some unwanted species were still present in the permeate stream. Therefore, it is key to consider these compounds too, in order to evaluate the effectiveness of the OSN to purify the phenolic compounds. Among these concomitant, undesired molecules, there are sugars (including sucrose, glucose and fructose), organic acids (represented by citric acid in this feed solution) and free fatty acids (represented by hydroxy-stearic acid). Regarding the biophenols, compounds from all the chemical classes present in the real sample were added to the solution. This aspect is important, since the different phenolic families differ in size, chemical structure and polarity, and this may lead to different solute-membrane interactions, as demonstrated in the literature for different solvents, solutes and membrane materials [10,22,28]. Figure 7.1 shows a chromatogram obtained after the analysis of the feed solution by LC-MS (as detailed in section 2.5.2.1).

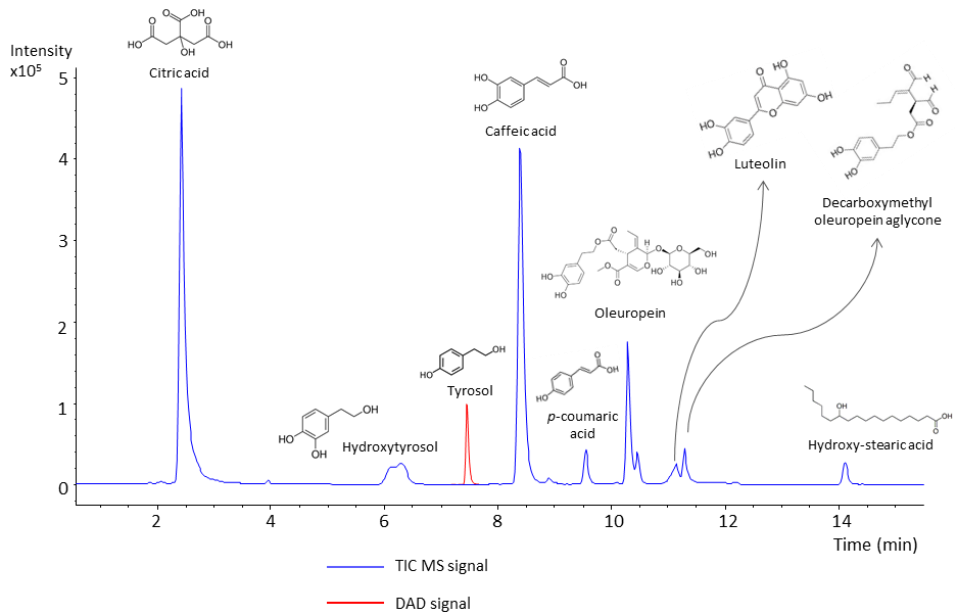


Figure 7.1. Total ion chromatogram (TIC) of the simulated wet olive pomace extract. All compounds were determined by liquid chromatography coupled to mass spectrometry, except for tyrosol, which was detected by means of a diode-array (DAD) detector. The absolute scale for that compound is not shown in the figure.

As can be seen, the composition of the feed solution covers a wide range of chemical structures. These compounds were at high concentration in the sample that has been simulated. In consequence, the content of the simulated wet olive pomace extract was considered to properly reproduce the characteristics of the original sample. The pH of the model solution was 4.4 ± 0.3, which also was in accordance with the real stream, which had a pH of 5.2 ± 0.3.

To better assess the size and atoms distribution of the compounds from the simulated wet olive pomace solution, their three-dimension molecular structure and the distance between the atoms have been presented in Figure 7.2. As shown in Figure 7.2, citric acid is the smallest compound in the solution. It has a non-linear conformation, with several polar end-groups, such as -CO and -OH. Regarding the phenolic compounds, the characteristic benzenic ring can be observed in all molecules. The distance between the carbon atoms of this benzenic structure is 0.28 nm. Several functional groups and side chains can be bonded to the benzene in the different molecules, and the position, number and length of these radicals determine the identity of the compound, its polarity and its molecular width. Therefore, the rejection during the nanofiltration process will be affected too.

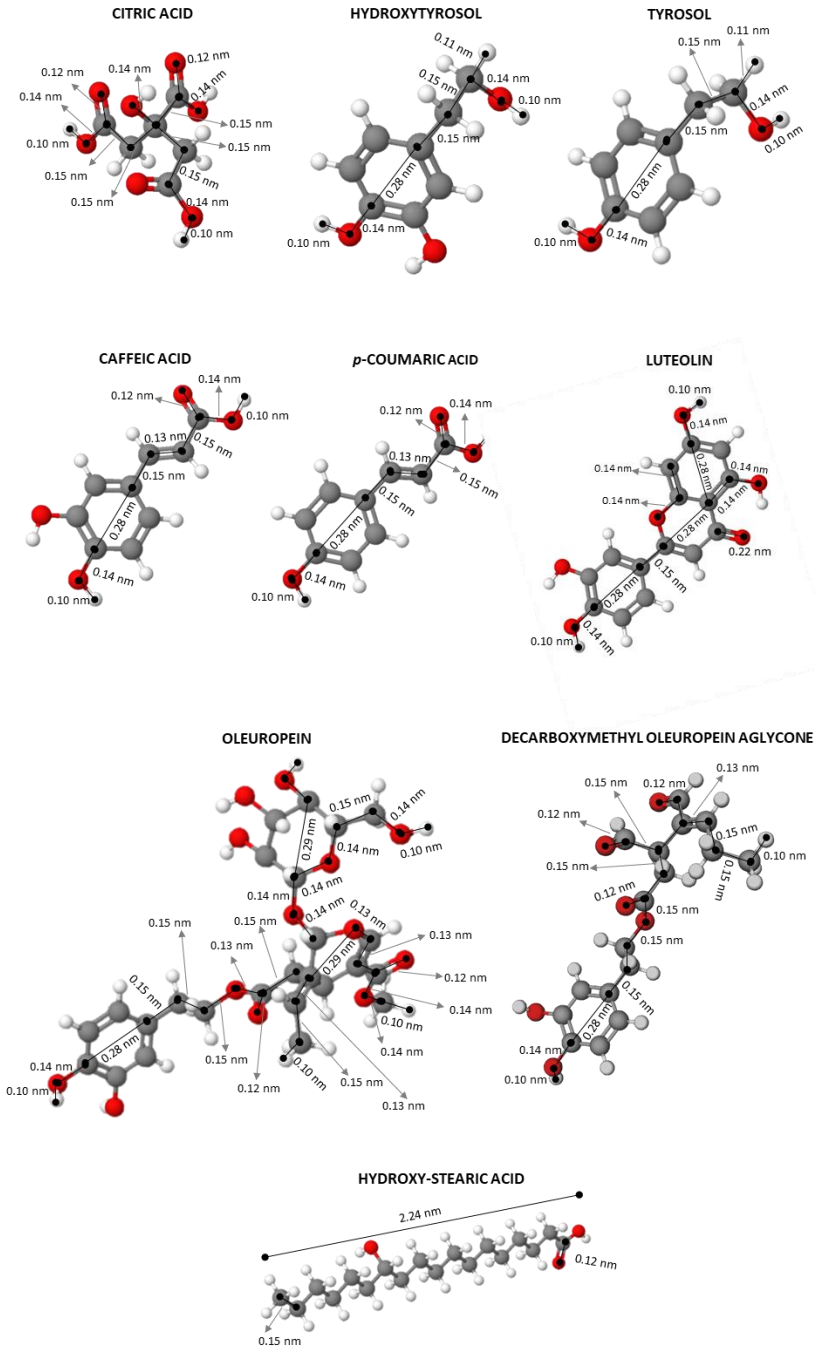


Figure 7.2. Three-dimension structure of the compounds present in the simulated solution of wet olive pomace. The distance between the atoms has also been provided. Oxygen has been presented in red color, carbon corresponds to grey color and hydrogen has been presented in white color.

Among the phenolic compounds, two groups can be differentiated. On one side, tyrosol, hydroxytyrosol, caffeic acid and *p*-coumaric acid only contain one phenolic ring. Their structure differs in the number of hydroxyl radicals (the distance between a carbon from the phenol ring and a bonded oxygen is 0.14 nm) and the composition of the side chain, which influences the molecular length.

On the other side, the molecules of luteolin, decarboxymethyl oleuropein aglycone and oleuropein contain more than one ring in their structure. As shown in Figure 7.2, this determines a larger, more ramified structure in comparison to the previously cited compounds. Finally, hydroxy-stearic acid is a linear fatty acid with a distance of 2.24 nm between the first and the last carbon of the chain. The relative disposition of this molecule in the surrounding of the membrane pores will influence its rejection.

3.2. Evaluation of permeate flux

Figure 7.3 shows the permeate flux obtained at 36 bar (20 bar in the case of Dm500) with the different membranes tested. The permeate flux obtained when the pure solvent and the model solution were nanofiltered can be observed. Logically, flux values were lower in the case of the model solution.

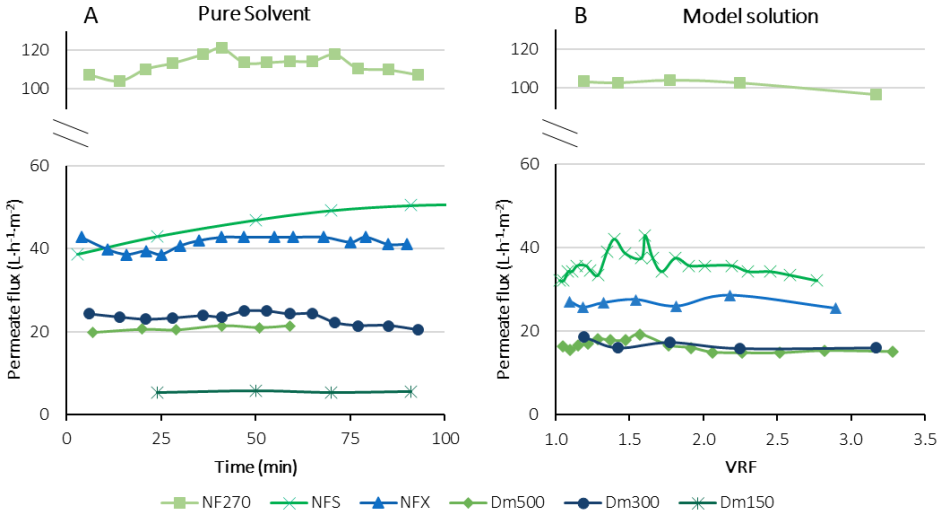


Figure 7.3. Values of permeate flux obtained with the membranes tested. Panel A shows the results for the pure solvent (ethanol/water 50:50 (v/v)) and panel B refers to the wet olive pomace extract model solution. Nanofiltration was carried out at 36 bar for all membranes except for DuraMem®500 (20 bar).

According to the figure (Figure 7.3), three groups can be differentiated regarding the permeate flux of ethanol/water 50:50 (v/v) and the model solution. The lowest fluxes were obtained with the membranes Dm150, Dm300, and Dm500. The solvent

permeability of the Dm150 membrane was 0.15 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$. Thus, this membrane was not considered for the nanofiltration of the synthetic extract of wet pomace, because the solvent flux was already not sufficient. Low differences were found between the solvent permeability of Dm300 (0.70 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$) and Dm500 (0.95 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$) membranes, even though their MWCO is not similar. Considering the higher MWCO of the Dm500 membrane and its thinner active layer, a higher permeability was expected. However, this was not observed. A similar phenomenon was reported by Peshev et al. [29], who obtained lower values of permeate flux for the Dm500 membrane (with respect to the Dm300 membrane) during the nanofiltration of a solution of caffeic and rosmarinic acid in ethanol. Also, Tylkowski and co-workers [30] observed a similar permeate flux for both membranes. These authors determined by scanning electron microscopy that these two membranes presented a different structure. According to them, the active layer of the Dm300 membrane is denser, whereas the Dm500 membrane displays a finger-like structure. Due to the different structure, these authors observed that the thickness of the active layer of the Dm300 membrane remained unchanged after filtration at 50 bar, while it showed a decrease of about 31% in the case of the Dm500 due to compaction. These authors attributed the unexpected, low permeability of the Dm500 membrane to the compaction of the active layer at high pressure, which was not observed for the Dm300.

The NFS and NFX membranes can also be paired in the discussion, because both reported similar values of permeate flux and solvent permeability (1.2 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ for NFS and 1.1 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$ for NFX). However, the highest jump in permeate flux was featured by the NF270 membrane (with a solvent permeability of 3.3 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$). This high flux could be due to the high hydrophilicity of the membrane material (see Table 7.4). The NFX membrane was also highly hydrophilic, but its lower MWCO led to a lower permeate flux. Also, both the NFX and the NF270 membranes are composed of polyamide, however, the manufacturer of the NFX membrane reports the material as “proprietary polyamide”, which suggests the implementation of modifications to the polymer. This can explain the lower flux, along with the smaller pore size.

Considering the outstanding values of permeate flux obtained with this membrane and the interesting rejection values that will be exposed in section 3.3, this membrane was further investigated at the TMPs of 15 and 25 bar (Figure 7.3) to treat the simulated extract of wet olive pomace. An increment in the permeate flux with TMP was observed, which indicated that no significant membrane fouling occurred. In view of the outstanding values of permeate flux obtained at 36 bar, these nanofiltration tests were

conducted until higher VRF values were achieved. This also permitted to explore the rejection at a larger concentration level.

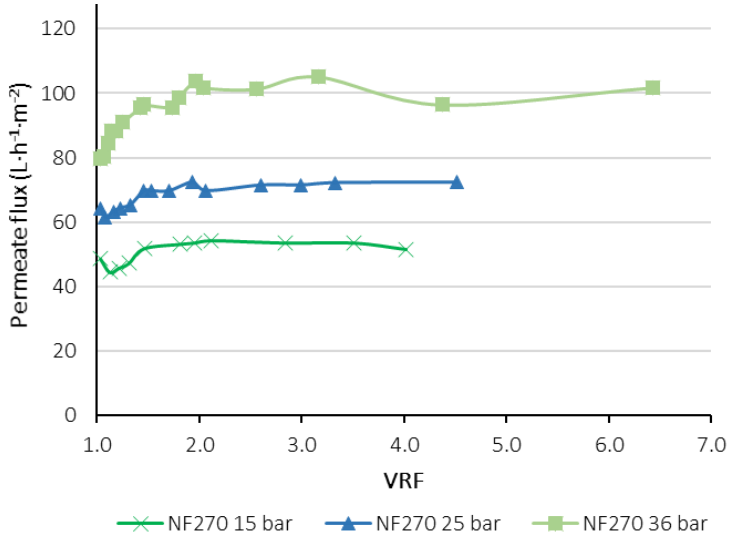


Figure 7.4. Values of permeate flux for the NF270 membrane, at the transmembrane pressures of 15 bar, 25 bar and 36 bar.

Regarding the membranes from GMT-Borsig, no permeation at all was obtained when treating the pure solvent, at any pressure in a range of 8 - 40 bar. This was observed for the oNF-2 membrane, but also for the oNF-1 membrane, in spite of having a very high MWCO (600 Da). No further pressure could be applied, as the manufacturer reported 40 bar as the maximum permitted pressure for this membrane (see Table 7.2). According to [30], the contact angle value of the membrane oNF-2 was 87°, which can be related to a low polarity. Therefore, considering the composition of the working solvent, the solvent-membrane interaction was not favored. In fact, the solvent was unable to wet the polymers and formed drops that later slipped off the membrane surface, as can be seen in Supplementary Figure 1. Previously reported results [28,31] showed that the permeability to ethanol was very low for this membrane (near 0.15 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$), while water permeability was zero. These authors related the solvent permeability to the difference between the solubility parameter of the PDMS polymer and that of the solvent, being greater if the difference is small and extremely low or even zero if the difference is large [28,31].

Similarly, the Pm600 membrane produced no permeate at all, irrespective of the applied pressure. According to the manufacturer, the membrane material is silicone-coated polyimide. Silicones are highly hydrophobic materials. The contact angle of

silicones has been reported to be greater than 100° [32], while the solvent (ethanol/water 50:50 (v/v)) is highly polar. Then, the same as in the case of GMT-Borsig membranes, the solvent was not able to wet the Pm600 membrane, as can be observed in the supplementary information, where it can be appreciated (Supplementary Figure 1) that the membrane repelled the solvent. The manufacturer recommends using the membrane in non-polar solvents. As a consequence, the oNF-1, oNF-2 and Pm600 membranes were not employed during the nanofiltration of the wet olive pomace solution.

3.3. Fractionation of phenolic compounds

The objective of the nanofiltration process that has been developed here is to enhance the purity of the polyphenols. Apart from sugars rejection (which will be commented in Section 3.5), the separation of the phenolic compounds from other organic molecules is relevant. In this case, citric acid and hydroxy-stearic acid were present in the feed solution as undesired compounds. As can be observed from Figure 7.5, most of the membranes performed satisfactorily in terms of rejection values. All of them rejected the free fatty acid (hydroxy-stearic acid) almost completely (Figure 7.5). Citric acid was better rejected by the Dm300, Dm500 and NFX membranes, even though the molecular weight of this compound (192 g/mol) was below the MWCO of all the membranes. Several authors have discussed that the molecular weight of a compound is not sufficient to understand other properties such as hydrogen bonding capacity, hydrophilicity, etc. In fact, it is not rare to find in the literature rejection values of 100% for compounds that should permeate if only their molecular weight is considered. For example, Peshev et al. found rejection values of 82 – 97.4% for phenolic acids around 180 g/mol after an ethanolic OSN process with the membranes DuraMem® 300 (MWCO of 300 Da) and DuraMem® 500 (MWCO of 500 Da) [29]. Vieira and co-workers also found that cyanidin-3-glucoside and cyanidin-3-rutinoside, which were below the MWCO of the NP010 membrane, exhibited a rejection larger than 50% [33]. This is another indicative of many other characteristics of the molecules being relevant in their rejection. Ignacz and Szekely measured the rejection values of 336 different molecules using three commercial DuraMem® polyimide membranes with different molecular weight cut-off values in methanol [34]. They demonstrated that the chemical structure of the compounds affected solute rejection. For instance, they related the presence of carboxylic groups to substantially increased values of rejection, and citric acid presents three carboxylic groups. Thus, functional groups, atoms disposition and chemical structure of the compounds may also influence this phenomenon [28,34]. These authors

also observed that rejection was more dependent on the molecular weight if the solvent presents a higher affinity towards the membrane [34]. As commented in section 3.1, the presence of different radicals and electronegative atoms in the molecule will determine its charge and polarity, influencing the affinity between a compound and the membrane. Moreover, the relative conformation of the atoms within the molecule can determine its shape, which is also very relevant when it comes to its transport throughout the membrane pores.

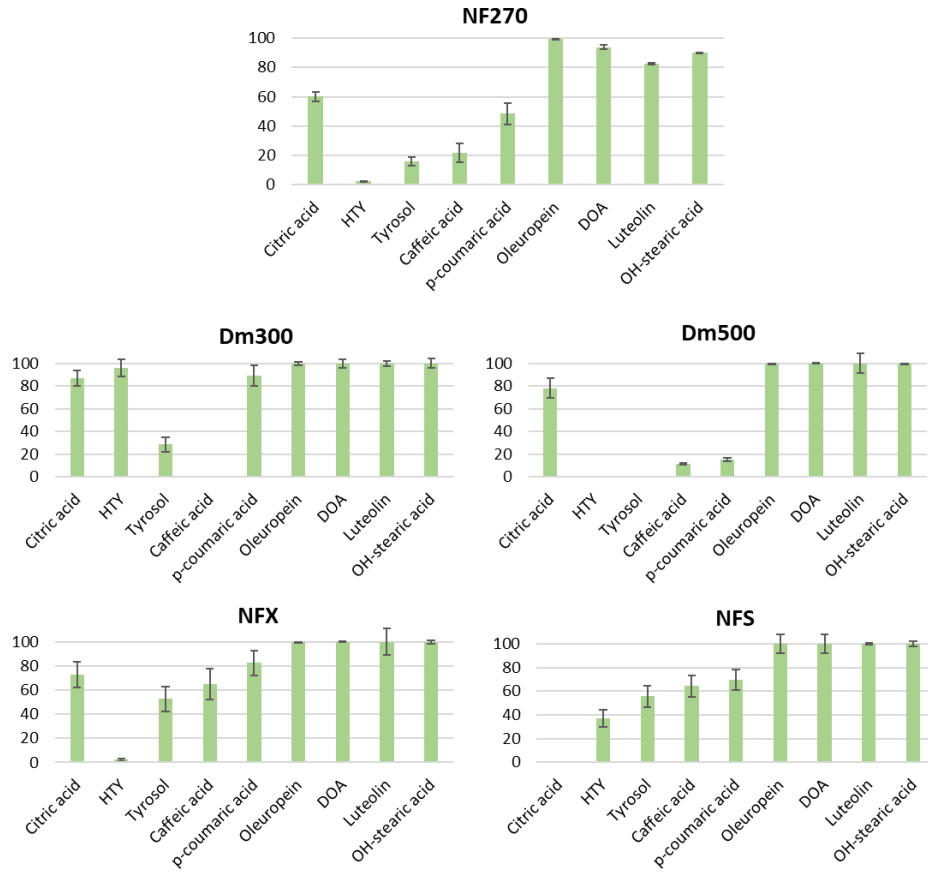


Figure 7.5. Rejection values achieved by the different membranes tested, at 36 bar.

Only the NFS membrane was unable to reject citric acid. To the best of our knowledge, this is the first time that the NFS membrane has been tested in this context. Its performance has only been reported twice in the literature, through the study of its surface modification [35] and the treatment of industrial wastewaters [36]. Table 7.4 indicates that the NFS membrane had an initial contact angle of 49.79° [35] and it changed to 26 ± 2° after its preconditioning. This variation corresponds to the relative

orientation of the ethanol molecules on the membrane surface. In comparison with this membrane, the NFX displayed a similar contact angle (with respect to NFS) after its immersion in ethanol at 50% (v/v) and the NFX membrane did reject citric acid (72.7 ± 10.7 %). However, the contact angle of the NFX membrane increased after the conditioning. This indicated that the reorganization of the membrane surface structure after the overnight immersion in ethanol/water 50:50 (v/v) could have occurred differently for these two membranes. Van der Bruggen and co-workers already reported that a modification of the membrane structure can alter its polarity [37]. Considering the chemical structure of the ethanol molecules present in the solution, it can be inferred that the more polar hydroxyl radical would be oriented to the feed side, whereas the rest of the molecule (with a lower relative polarity) would preferentially interact with the pore walls of the NFS membrane, because of a higher affinity between the membrane and the more hydrophobic carbon tail. This effect led to a greater exposition of polar groups towards the bulk solution, increasing the hydrophilicity of the active layer. In the case of the NFX membrane, the opposed orientation of the ethanol molecules could be suggested. This effect has been observed before [38–40] for different membrane materials and solvents and could explain the modulation of the membrane contact angle, which was reduced almost by half for the NFS membrane. For instance, de Melo et al. observed that hexane flux through ceramic membranes, which are highly hydrophilic, increased to a large extent after pre-treatment with n-butanol. They attributed the flux enhancement to the interaction of the polar head with the polar pore surface of the membrane and the non-polar tail would then be oriented to the bulk solution [38]. These interactions would also explain the higher permeate flux observed for the NFS membrane in comparison with the NFX despite of having lower MWCO. In such a scenario, the affinity between the citric acid and the rearranged NFS membrane surface (with more hydroxyl groups available to interact with the solute) could have been increased, leading to a higher passage. However, the opposed orientation of the ethanol molecules in the case of the NFX membrane did not favor the permeation of citric acid.

In addition to the polarity, the presence of several electronegative atoms in the structure of citric acid (Figure 7.2) suggests the interaction with the NFS membrane, considering its low water contact angle. Additionally, some electrostatic interactions can be also considered. This is the compound in the feed solution with the lowest pK_a, corresponding to 2.79. Being the pH of the model solution around 4.4, it is reasonable to accept that the molecule of citric acid was negatively charged. The pK_a of the rest of the compounds is above 4, indicating that the molecules are neutral. Regarding the membranes, most of them exhibit an isoelectric point close to the pH of the feed

solution. The isoelectric point of the NF270 membrane has been reported to be between 3 and 5.2 [41,42]. The Dm300 and Dm500 membranes have an isoelectric point of 4.8 [43] and for the NFX and NFS membranes, this value is 3.2 and 3.8, respectively [35,44]. According to these values, only the NF270 and NFX are expected to be (at least partially) negatively charged. Then, an electrostatic repulsion can be related to the high rejection of citric acid observed with the NFX membrane in comparison with the NFS membrane, despite a similar contact angle between both membranes.

Finally, the chemical structure of citric acid may have also influenced its rejection. As mentioned, other characteristics of the molecules apart from the molecular weight (such as the size of the compound or the molecular width) are better related with steric interaction between the compound and the membrane pores [45]. Being NFS the tighter membrane tested here and citric acid the smallest and more polar molecule from the feed solution, the obtained rejection values were considered to be acceptable. These results suggest the possibility of applying this membrane to remove citric acid (which could even be recovered from the permeate, if desired) and obtain a retentate enriched in phenolic compounds. Considering the chemical structure of this molecule, it could be concluded that other organic acids that also occur in olive-derived wastes at high concentrations [25], such as malic acid and quinic acid, could be removed too by means of this membrane.

The Dm300 membrane rejected the compounds of high molecular weight. Among the smaller molecules, a marked specificity for caffeic acid was observed, despite what could be expected due to its molecular weight. In comparison with the molecules of hydroxytyrosol and tyrosol, this compound can be considered relatively less polar, because it contains an additional atom of carbon in the side chain and, furthermore, its elution from a C18 non-polar column takes place later (see Figure 7.1). According to different authors, the permeation trough the Dm300 membrane is highly dependent on the physicochemical solute properties causing interactions with both membrane and solvent [34,46]. Thiermeyer et al. concluded that solute transport through this membrane is affected by the affinity between the solute and the membrane [47]. They demonstrated that, in polar solvents such as ethanol and isopropanol, polar solutes showed a tendency to higher rejections than moderately polar solutes. Therefore, considering these results, the less polar character of the caffeic acid favored its permeation through the membrane.

Figure 7.5 clearly shows that the largest compounds did not pass any of the membranes, because luteolin, decarboxymethyl oleuropein aglycone and oleuropein

were always rejected in high percentages. These results allowed the fractionation of the polyphenols according to their molecular weight, which was also one of the objectives of this work. In most of the cases, small-size phenolic compounds (in the range of 138 - 180 g/mol), which were below the MWCO of the membranes, were recovered in the permeate. This trend was very marked for the Dm500 membrane, which allowed the purification of hydroxytyrosol, tyrosol, caffeic acid and *p*-coumaric acid, all of them considered very valuable compounds [48,49]. In contrast, Peshev et al. found high rejections of caffeic acid when these membranes were used to nanofilter a rosemary extract [29]. Nevertheless, those extracts were prepared in pure ethanol, and the membrane conditioning was conducted with this solvent too. On the other hand, caffeic acid concentration was significantly smaller than the one considered in our work and their tests were performed at constant volume operating mode, by recirculation of the permeate to the feed tank. The duration of their tests was also much shorter than in this work. According to several authors [50–52], solute adsorption has a relevant role in nanofiltration rejection. As a result, a breakthrough curve is formed in nanofiltration processes due to the adsorption of solutes on the membrane at low concentrations. Consequently, rejection is high at the beginning of the process, when adsorption is dominant, and it decreases when all the available sites are occupied. The high rejections found by Peshev et al. mean that, due to the low concentrations considered and the total recirculation operating mode, caffeic acid adsorption was the dominant mechanism. However, in this work, as a result of the higher concentrations considered, as well as the continuous concentration of the feed solution during the process, the adsorption sites became occupied by the solute and its passage through the membrane was favored.

The NF270 membrane also presented similar results, because hydroxytyrosol, tyrosol and caffeic acid showed a passage of almost 100%, whereas citric acid and hydroxy-stearic acid (as well as larger phenolic compounds) were retained. Furthermore, these results are even more interesting if the permeate flux values are considered. As reported in Figure 7.3 and Figure 7.4, the flux achieved with the NF270 membrane was six times higher than the permeate flux observed with the Dm500 membrane.

As commented in section 3.2, a good compromise between the rejection of high-molecular weight biophenols and low-molecular-weight biophenols was observed for the NF270 membrane. This high purification capacity as well as the high permeate flux values obtained with this membrane motivated a deeper insight into its performance at different transmembrane pressures. Figure 7.6 displays the rejection obtained with this membrane at the TMPs of 15, 25 and 36 bar. The experiment at 36 bar was conducted

until a higher concentration level was achieved (VRF of 4.5) to confirm the declining tendency in the rejection of *p*-coumaric acid, tyrosol and caffeic acid that was observed.

As can be seen in Figure 7.6, those compounds which surpassed the membrane MWCO were rejected in high percentages at all pressures. Thus, oleuropein, decarboxymethyl oleuropein aglycone and luteolin displayed high rejection values that were almost constant from the beginning of the OSN process until the highest VRF was achieved.

In contrast to the largest molecules, all the compounds below the MWCO exhibited a clear variation of their rejection with the increase in the VRF and all of them behaved equally. Furthermore, the tendency was drastically different at the lowest pressure applied (15 bar) with respect to the results at 25 and 36 bar.

As many authors have explained, the transport of solutes in nanofiltration processes is not only governed by convection, but also by diffusion across the polymer [23,53,54]. In that case, the initial adsorption of the solutes into the membrane surface is a fundamental stage to promote its interaction with the membrane and, eventually, its permeation. The adsorption phenomenon is also supported by the data discussed in section 3.4. It has been previously described [23,50,51] and it is appreciable in the results at 25 and 36 bar from Figure 7.6, that tyrosol, hydroxytyrosol, caffeic acid and *p*-coumaric acid suffered a rapid adsorption on the membrane surface. Accordingly, the rejection values for these compounds were higher at the beginning of the OSN procedure (low VRF), because the highest pressures were responsible for a higher concentration of solutes at the membrane surface, which is consistent with the literature [50]. This situation favoured a rapid occupation of all the saturation sites located on the membrane surface. This fast interaction and adsorption at high pressures occurred until a VRF of 2 was completed.

It has been previously demonstrated [50] that, once saturation is completed, convection dominates the transport, while diffusion is not significant. Indeed, these results suggest that, after the adsorption stage, the transport of the compounds was enhanced, then leading to an increase in the permeate concentration and the subsequent decrease in the rejection, as also observed in other works [50–52].

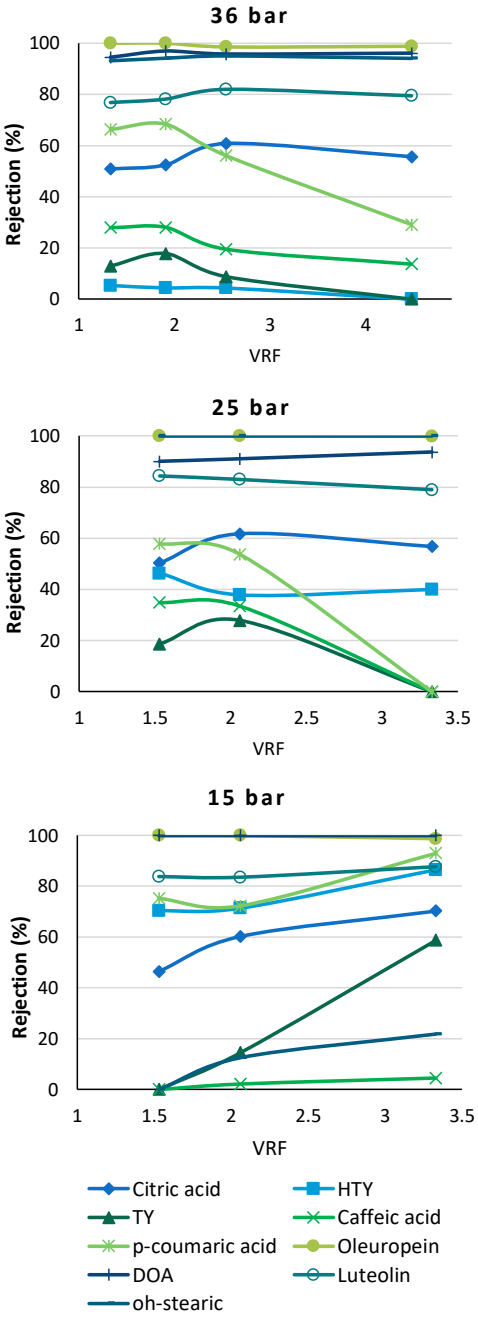


Figure 7.6. Evolution of the rejection of each compound from the model solution with the VRF, using the NF270 membrane at 15 bar, 25 bar and 36 bar. The abbreviations of HTY, TY, DOA and OH-stearic acid correspond to hydroxytyrosol, tyrosol, decarboxymethyl oleuropein aglycone and hydroxy stearic acid, respectively. Relative standard deviation between experimental replicates was always below 13%.

As previously commented, Williams et al. and Imbrogno and Schafer demonstrated that a breakthrough curve is formed in nanofiltration processes when the adsorption of solutes on the membrane occurs at low concentrations [50-51]. Thus, rejection is high at the beginning of the process, when adsorption is dominant, and it decreases when all the available sites are occupied until a steady state is reached. As will be shown in section 3.4, the interaction of phenolic acids and the membrane surface was higher in comparison with simple phenols. Then, the rejection of caffeic and *p*-coumaric acid did decrease with the VRF, but, at 36 bar, the final value was still higher than the rejection of tyrosol and hydroxytyrosol.

At 36 bar, the importance of the size exclusion process was also notable. As can be seen, the order of less retained compounds was the following: hydroxytyrosol (154 g/mol) and tyrosol (138 g/mol) < caffeic acid (180 g/mol) < p-coumaric acid (164 g/mol) < citric acid (191 g/mol) < luteolin (286 g/mol) < hydroxy-stearic acid (300 g/mol) < luteolin (286 g/mol) < oleuropein (540 g/mol). This was another indicative of the saturation of the membrane surface, since other authors have reported that steric hindrance becomes more relevant when adsorption stops increasing [23,52,55].

At 25 and 36 bar, which could be considered high pressures, a high concentration polarization was expected during the experiment, then contributing to the transport across the membrane and the decline of the rejection as FRV increased. However, the results obtained at 15 bar (which were obtained after duplicated experiments, as well as all the results from this work) showed a different tendency. In this case, the rejection of the compounds kept increasing during the process, and only those compounds of larger molecular weight displayed a constant rejection. These results could not be explained by a fouling phenomenon, because the permeate flux did not decrease (Figure 7.3). Considering that the flux of solvent was almost invariable during the whole experiment, the reduction in the permeate concentration was attributed to the flux of solutes. Possibly, the TMP of 15 bar did not generate the drastic adsorption that was observed for 25 and 36 bar. In contrast, the concentration of compounds at the membrane surface was lower at this lower pressure [50]. Then, it is expected that the interactions with the active layer were not as promoted as at the highest pressures and so they would be more extended in the time. The progressive entrapment of the molecules on the membrane surface could explain the rejection tendency at 15 bar, because the adsorption equilibrium was not observed, and so the diffusion of the compounds towards the permeate side and subsequent desorption in the permeate stream were not fostered.

Additionally, the accumulation of compounds in the retentate (at increasing concentrations) could increase the interactions among them. Some authors have discussed the interactions that can take place between phenolic compounds and other organic molecules [56,57].

In any case, the results obtained at 36 bar were preferred, because the operating conditions permitted a high recovery of low-molecular-weight phenolic compounds, whereas the largest compounds, as well as the sugars (see section 3.5) were obtained in the retentate.

3.4. Adsorption of compounds onto the surface of the NF270 membrane

As explained above, the NF270 membrane was considered the most promising due to its high efficiency and interesting rejection values. To that end, the possible adsorption of phenolic compounds onto its surface was investigated. In every nanofiltration process, the interaction of the solutes and the membrane is an essential aspect to understand the membrane transport. The compounds are transported not only because of their size (sieving mechanism), but also due to a solution-diffusion mechanism. The initial interaction of the molecules can be a decisive stage for its permeance. However, if the interaction with the membrane surface is too strong, the transport of the compound may be hindered [58], and it could remain bound to the chemical groups of the membrane surface rather than crossing to the permeate side. This phenomenon was investigated here, by evaluating the concentration of the molecules of interest after their contact with the active layer of the membrane. Table 7.3 shows the adsorption values that were observed after the contact of the conditioned membranes with the model solution (according to section 2.4).

Figure 7.3. Adsorption of the phenolic compounds and organic acids present in the feed solution on the NF270 membrane. Adsorption experiments were conducted during 24 h.

Compound	Adsorption values (mg/m²)
Citric acid	2.4 ± 0.5
Hydroxytyrosol	0.27 ± 0.04
Tyrosol	-
Caffeic acid	4.7 ± 0.1
p-Coumaric acid	10 ± 1
Oleuropein	8.0 ± 0.1
Decarboxymethyl oleuropein aglycone	-
Luteolin	0.35 ± 0.02
Hydroxy-stearic acid	12.2 ± 0.6

The simple phenols were poorly adsorbed (hydroxytyrosol) or not adsorbed at all (tyrosol) (Figure 7.5). Their low molecular weight as well as the low contact angle that was observed for the NF270 membrane (indicative of a high hydrophilicity) favored the permeation of these compounds. Regarding the phenolic acids present in the model solution, caffeic acid and *p*-coumaric acid were more adsorbed onto the surface of the NF270 membrane, especially *p*-coumaric acid. This finding contributed to the understanding of the relative rejection of *p*-coumaric acid, which was higher than the rejection of caffeic acid, despite of belonging to the same chemical family and having similar values of molecular weight. In fact, the molecular weight of *p*-coumaric acid (164 g/mol) is lower than the molecular weight of caffeic acid (180 g/mol), which again confirms that other phenomena (not only size-exclusion) influenced their transport across the membrane. Being adsorption a key factor, this molecule could be driven by the solvent throughout the membrane to a larger extent than *p*-coumaric acid, which, in comparison, showed a major affinity for the polymer. Contreras-Jácquez and co-workers also found that phenolic acids (including *p*-coumaric acid) were adsorbed and retained by polyamides in reverse osmosis membranes [59].

For the other compounds (oleuropein, luteolin, decarboxymethyl oleuropein aglycone and hydroxy-stearic acid), the results from Figure 7.5 clearly indicate that size exclusion dominated their transport. However, a high contribution of the adsorption process was found for oleuropein and hydroxy-stearic acid. In a number of works, the correlation between solute hydrophobicity and adsorption onto polyamide membranes has been demonstrated [22,59,60]. Thus, the several aromatic rings present in the oleuropein molecule, and the hydrophobic character of the hydroxy-stearic acid favor the hydrophobic interactions with the membrane and explain their large adsorption.

3.5. Separation from sugars

One of the objectives of this work was to identify membranes able to reject the sugars that are normally found in olive-derived waste. Thus, they could be separated from other compounds of high value in order to recover them with high purity. Three carbohydrates were present in the feed solution: sucrose, glucose and fructose. Figure 7.7 shows the achieved rejections of these three compounds, after the nanofiltration at 36 bar.

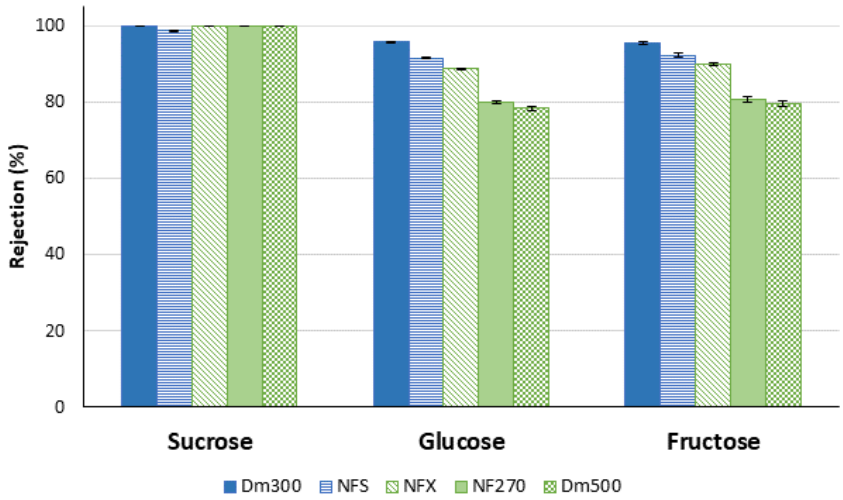


Figure 7.7. Permeate flux obtained with the NF270 membrane after the ultrafiltration of the extract with the UH030 membrane (A) and the UP005 membrane (B).

The results reflected in Figure 7.7 were highly satisfactory. One of the major challenges for the purification of phenolic compounds from agrofood matrices is the presence of sugars. Many carbohydrates are often coextracted with polyphenols and, usually, sugars are not removed after membrane processes such as ultrafiltration. In fact, whereas polymeric carbohydrates can be rejected by ultrafiltration membranes, monomers and dimers can be easily found in ultrafiltration permeates [17]. Thus, the identification of nanofiltration membranes able to reject the carbohydrates that would be present in these ultrafiltration streams is of high interest.

Taking into account the MWCO of the employed membranes and the molecular weight of sucrose, the observed high rejection is reasonable. Furthermore, this performance has been previously reported for the NF270 membrane after an aqueous nanofiltration [17]. The rejection values that were observed for glucose and fructose were very high, considering the molecular weight of these compounds (180 g/mol). Muñoz and co-workers also reported a complete rejection of glucose by the membrane NFX in a hydroalcoholic ambient [61]. Additionally, several authors have described high rejections of glucose and fructose during nanofiltration processes, even when the MWCO of the membranes was far from the molecular weight of the monosaccharides [62,63]. This suggests an additional effect (apart from steric aspects) of the membrane-sugar interactions. As previously commented, Ignacz and Szekely measured the rejection values of 336 different molecules using three commercial DuraMem® polyimide membranes with different molecular weight cut-off values in methanol [35]. For these

membranes the presence of aliphatic rings (not aromatic ones) and hydroxyl groups was observed to improve the rejection, yielding values higher than expected if the molecular weight was only taken into account, while aromatic groups usually negatively affected the rejection value. These results explain the high rejection of sugars observed for the Dm300 and 500 membranes as they present both, rings and hydroxyl groups in their structure. The NF270, NFS and NFX membranes have a polyamide active layer, but, from Figure 7.7, a similar contribution of these functional groups to an increase in the rejection value can be inferred.

These results demonstrated the feasibility of the tested membranes for purifying the studied polyphenols. Considering the rejection values from Figure 7.5 and Figure 7.7, several membranes allowed the recovery of low-molecular-weight biophenols, which were efficiently separated from other phenolic compounds and from the unwanted sugars present in the wet olive pomace.

3.6. Contact angle

For the most promising membranes in terms of permeate flux and rejections, the contact angle was determined (Table 7.4). For the virgin membranes, their water contact angle was retrieved from the literature, whereas it was experimentally determined for the conditioned membranes (after immersion in ethanol/water 50:50 (v/v)) and after the nanofiltration of the simulated wet olive pomace extract. Additionally, the contact angle of a droplet of ethanol/water 50:50 (v/v) was determined.

The water contact angle of the pristine membranes allowed the evaluation of the polarity of the polymers. A higher value of the contact angle indicated a lower hydrophilicity [35,66,67], which was observed for the Dm300 and Dm500 membranes. This was in line with their material (modified polyimide). The rest of the membranes were based on polyamide polymers and were considered more hydrophilic.

Table 7.4. Water contact angle and ethanol/water 50:50 (v/v) contact angle for the NF270, Dm300, Dm500, NFX and NFS membranes. The values for the pristine membrane, the membrane after the conditioning and the membrane after the nanofiltration process are reported.

Membrane	Water contact angle			Ethanol 50% (v/v) contact angle
	Native	After immersion in working solvent	After nanofiltration	After immersion in working solvent
NF270	15.9 ± 1.3° [33]	33.5 ± 0.6°	64 ± 5°	[d]
Dm300[a]	59° [30][c]	69 ± 2°	59 ± 5°	12 ± 4°
Dm500[b]	67.1 ± 0.8° [64]	76 ± 1°	64 ± 4°	36 ± 4°
NFS	49.79° [35][c]	26 ± 2°	-	19 ± 3°
NFX	17.6 ± 2.8° [65]	24 ± 2°	-	11 ± 3°

[a]Dm300: DuraMem®300; [b]Dm500: DuraMem®500; [c]Measured deviation was not reported; [d]Measurement was not possible due to the very small contact angle of the solvent drop

In order to assess the relation of the polarity of the membrane active layer and the observed permeate flux, the water contact angle and the ethanol/water 50:50 (v/v) contact angle of the conditioned membranes was studied. This was considered to be more reliable, because the utilization of the membrane was performed after its overnight immersion in the working solvent. Van der Bruggen et al. demonstrated that the polarity of organic membranes can be modified after their contact with organic solvents such as ethanol [37]. Then, this strategy allowed the evaluation of the real hydrophilic or hydrophobic character of the membranes, right before the OSN process. As can be seen in Table 7.4, a general increase in the water contact angle was observed, prompted by the presence of ethanol in the solvent mixture, except in the case of the NFS membrane, as its water contact angle decreased after immersion in the working solvent (some insights about the contact angle of the NFS membrane have been provided in section 3.3). Still, the Dm300 and Dm500 membranes continued to be the least hydrophilic membranes, which was in line with their values of permeate flux. As summarized in Figures 7.3 and 7.4, the DuraMem® membranes displayed the lowest flux, both in the case of the nanofiltration of the pure solvent and model solution.

Regarding the rest of the membranes, their water contact angle reached a value between 24-34°. Despite of their close polarity, a large difference between their values of permeate flux was found. This can be explained by the MWCO, which was higher for

the NF270 membrane. Zyłła and co-workers also described that the NF270 membrane stood out with respect to NFX and other polymeric membranes in terms of permeate flux, despite close values of the contact angle [65].

The ethanol/water 50:50 (v/v) contact angle of the conditioned membranes was also evaluated. In the case of the NF270 membrane, the solvent droplet was almost immediately dispersed throughout the active layer, hindering the evaluation of the contact angle. The quick filtration of the solvent indicated a high affinity between this membrane and the ethanol/water 50:50 (v/v). This was already suggested by the results related to the permeate flux presented in section 3.2. For the rest of membranes, the droplet was stable enough to perform the measurement. In general, the observed values were lower than the reported water contact angle for each membrane. This was expected, as the DuraMem® membranes and the NFS and NFX membranes are specific for organic solvents. Then, a higher affinity for ethanol/water 50:50 (v/v) than for water is reasonable. Furthermore, it should also be noted that the superficial tension of the solvent is lower than the superficial tension of water, then contributing to the immediate filtration of the solvent droplet throughout the membrane surface [68]. These results are consistent with the values of permeate flux (Figure 7.3A) reported for each membrane. The membrane with the highest permeate flux (NF270) displayed the lowest solvent contact angle, followed by the NFX and NFS membranes, which comprised the second group in terms of permeate flux. Finally, the lowest permeate flux was observed DuraMem® membranes, as well as the highest values of ethanol/water 50:50 (v/v) contact angle.

After the nanofiltration of the hydroalcoholic model solution (containing phenolic compounds, organic and fatty acids and sugars) the water contact angle for all the membranes became much closer, in the range of 59-64°. This was another indicative of the adsorption of compounds onto the membrane, as discussed in section 3.3. Sotto et al. also reported an adsorbed layer on the membrane surface formed after solutes adsorption [23]. In the case of the current study, this layer always led to an increase of the hydrophobicity of the membranes, independently of their initial polarity. In the case of the DuraMem® membranes, this modulation of the active layer was less notable, as those polymers were already highly hydrophobic prior to their contact with the solvent.

3.7. Atomic force microscopy characterization

The effect of the working solvent on the active layer of the tested membranes was investigated by AFM. This allowed studying possible modifications of the surface structure.

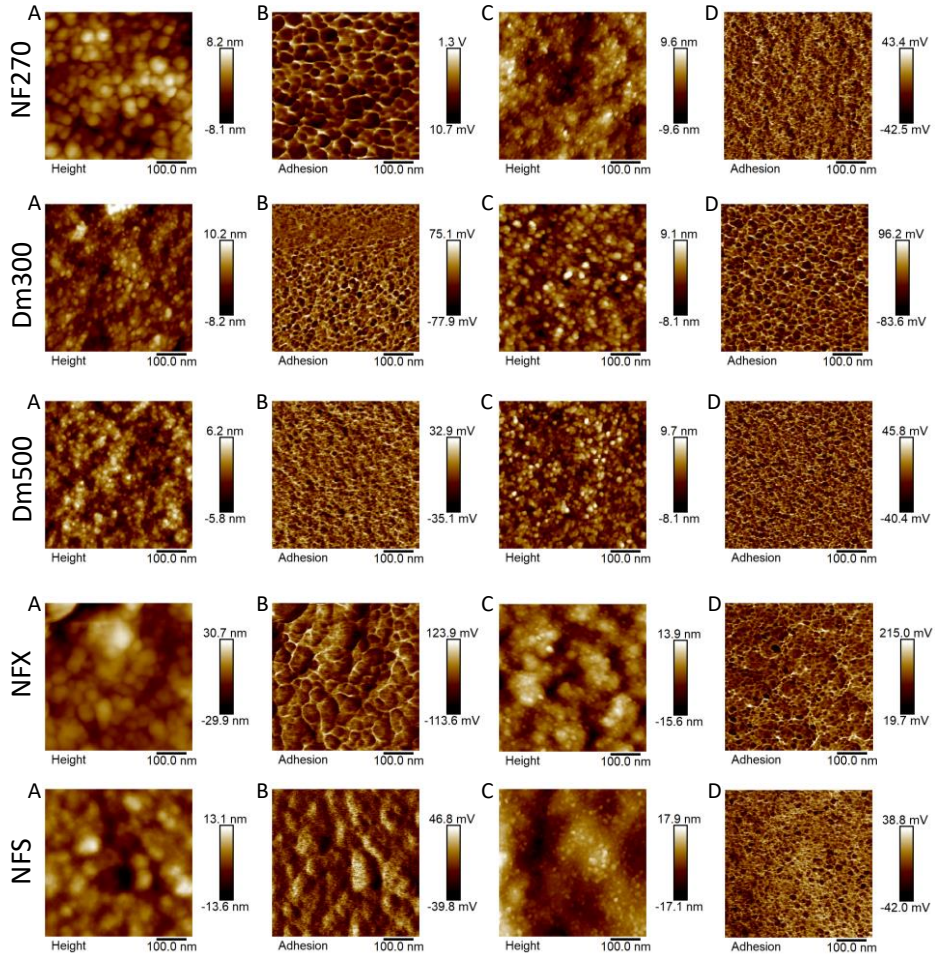


Figure 7.8. AFM characterization of the tested membranes. A and B images correspond to the native membranes, monitoring the height (A) and the adhesion signal (B). C and D images correspond to the conditioned membrane with ethanol/water 50:50 (v/v), monitoring the height (C) and adhesion signal (D).

The height images in Figure 7.8A show that the polymers corresponding to all membranes display a nodular structure. This is in line with previous works reporting a microscopy-based characterization of nanofiltration membranes [69–71]. The membrane pores correspond to the interstitial space between the polymer fibers, which can be packed in nodules or not. A complementary image to those in column A is given in column B. The adhesion signal reflects the different interaction forces that are established between the membrane material and the AFM probe [72]. Those forces were stronger when the probe approached a nodule, and they were less relevant when the probe went over the pores. In consequence, the images in Figure 7.8B correspond to the

flip side of the height images, as in a picture negative. The holes observed in Figure 7.8B are complementary to the nodules in Figure 7.8A and the space among the nodules, correspond to the protrusions reflected in Figure 7.8B. By studying these images, both in the native and conditioned state of the membranes, interesting conclusions can be reached regarding the modification of the polymer structure.

The NF270 membrane exhibited well-defined nodules in its native form (Figure 7.8A and 7.8B). According to Boo et al., the pore size of the native membrane is near 0.8 nm [73]. After its immersion in ethanol/water 50:50 (v/v), a drastic change can be observed on its surface. Zhang et al., observed similar results with a polyamide membrane and explained this variation by the hydrolysis of the polyamide chains, prompted by the solvent [74].

The nodules present in the height images of the DuraMem® membranes (Dm300 and Dm500) were smaller than those in the NF270 membrane. This suggests a smaller pore size, as thinner fibers generate smaller holes among them. By observing the height and adhesion images it is possible to detect a slight thickening of the nodules of both membranes, which can be attributed to a swelling phenomenon. However, the effect of the solvent was not so relevant for these membranes, thus suggesting a more stable structure of the active layer. This stability was expected, as the DuraMem® membranes are recommended for their use with organic solvents.

The Synder membranes (NFX and NFS) presented large nodules (Figure 7.8A), similarly to the NF270, which also contains polyamide in its active layer. The nodules from the NFX and NFS were thick and highly packed. As reflected by the adhesion signal (Figure 7.8B), the valleys were less marked in these membranes. The effect of the membrane immersion in ethanol/water 50:50 (v/v) was highly intense, as in the case of the NF270 membrane. The diameter of the nodules was drastically reduced, leading to a granular structure, reflecting fiber aggregates (Figure 7.8C).

Interestingly, all membranes displayed a similar topology after their conditioning, as it is shown in Figure 7.8D. Due to the effect of the solvent, the polymer suffer a reorganization that resulted in the substitution of the nodular structure by a spongier structure. This has been related to the clustering of hydrophilic and hydrophobic groups from the membrane surface [75–77].

4. CONCLUSIONS

Several OSN membranes have been studied for the recovery of phenolic compounds from a hydroalcoholic extract of wet olive pomace. Some of the tested membranes, such

as oNF-1, oNF-2 and DuraMem® 150, produced extremely low values of permeate flux, because of a poor interaction between the active layer and the solvent molecules. On the contrary, the Dm300, Dm500, NFX, NFS and NF270 membranes displayed acceptable flux. The NF270 membrane stood out due to high values of permeate flux, near 100 $L \cdot h^{-1} \cdot m^{-2}$. Regarding the rejection values, the selected membranes permitted the fractionation of the phenolic compounds, as well as their purification, within the same OSN procedure. Thus, the compounds of interest were recovered in the permeate, whereas some unwanted compounds such as the sugars, free fatty acids and organic acids were rejected. These results suggest that a hydrophilic membrane, with an active layer based on cross-linked polyamide (as in the case of the NF270 or NFX membrane) can be effective to recover phenolic compounds from the wet olive pomace, after a hydroalcoholic extraction. Furthermore, a MWCO of 300 - 400 Da allowed the fractionation of the biophenols, achieving the separation the high-molecular-weight polyphenols from those of low-molecular-weight, which are highly valuable.

The optimization of membrane processes in the presence of organic solvents permits the utilization of these organic solvents in previous stages, such as solid-liquid extraction. This lead, in most cases, to higher efficiency in terms of recovery of bioactive compounds than the utilization of water as solvent. The results presented here indicate that OSN is an effective strategy for obtaining highly pure phenolic compounds from vegetable residues such as wet olive pomace. This generates a source of income from an environmentally concerning by-product and contributes to recycle it.

Aknowledgements: The authors would like to thank Laura Teruel Biosca for her technical support. Additionally, Electron Microscopy Service of the Polytechnic University of Valencia is gratefully acknowledged for help with AFM characterization.

Funding: The grant CTM2017-88645-R was funded by MCIN/AEI/ 10.13039/501100011033 and by ERDF A way of making Europe. Additionally, the grant PRE2018-08524 was funded by MCIN/AEI/ 10.13039/501100011033 and by ESF Investing in your future.

5. REFERENCES

[1] V. Sygouni, A.G. Pantziaros, I.C. Iakovides, E. Sfetsa, P.I. Bogdou, E.A. Christoforou, C.A. Paraskeva, Treatment of two-phase olive mill wastewater and recovery of phenolic compounds using membrane technology, Membranes (Basel). 9 (2019) 27. https://doi.org/10.3390/membranes9020027.

[2] E. Roselló-Soto, M. Koubaa, A. Moubarik, R.P. Lopes, J.A. Saraiva, N. Boussetta,

N. Grimi, F.J. Barba, Emerging opportunities for the effective valorization of wastes and by-products generated during olive oil production process: Non-conventional methods for the recovery of high-added value compounds, Trends Food Sci. Technol. 45 (2015) 296–310. https://doi.org/10.1016/j.tifs.2015.07.003.

[3] P. Tapia-Quirós, M.F. Montenegro-Landívar, M. Reig, X. Vecino, T. Alvarino, J.L. Cortina, J. Saurina, M. Granados, Olive mill and winery wastes as viable sources of bioactive compounds: A study on polyphenols recovery, Antioxidants. 9 (2020) 1–15. https://doi.org/10.3390/antiox9111074.

[4] O.C. Adebooye, A.M. Alashi, R.E. Aluko, A brief review on emerging trends in global polyphenol research, J. Food Biochem. 42 (2018) 1–7. https://doi.org/10.1111/jfbc.12519.

[5] V. Sant'Anna, A. Brandelli, L.D.F. Marczak, I.C. Tessaro, Kinetic modeling of total polyphenol extraction from grape marc and characterization of the extracts, Sep. Purif. Technol. 100 (2012) 82–87. https://doi.org/10.1016/j.seppur.2012.09.004.

[6] T.W. Caldas, K.E.L. Mazza, A.S.C. Teles, G.N. Mattos, A.I.S. Brígida, C.A. Conte-Junior, R.G. Borguini, R.L.O. Godoy, L.M.C. Cabral, R. V. Tonon, Phenolic compounds recovery from grape skin using conventional and non-conventional extraction methods, Ind. Crops Prod. 111 (2018) 86–91. https://doi.org/10.1016/j.indcrop.2017.10.012.

[7] C.M. Sánchez-Arévalo, M.C. Vincent-Vela, S. Álvarez-Blanco, Revalorization of two-phase olive mill wastewater: Recovery of antioxidant, bioactive compounds from a phytotoxic residue, in: Int. Conf. Water Sustain., Barcelona, 2021: pp. 32–33.

[8] C.M. Sánchez-Arévalo, H. Ellicott, M.-C. Vincent-Vela, S. Álvarez-Blanco, Assesment of several organic and inorganic membranes to ultrafilter a phenolic extract from two-phase olive mill wastewater, in: II Int. Congr. Water Sustain., Barcelona, 2021.

[9] R. Shukla, M. Cheryan, Stability and Performance of Ultrafiltration Membranes in Aqueous Ethanol, Sep. Sci. Technol. 38 (2003) 1533–1547. https://doi.org/10.1081/SS-120019091.

[10] P. Marchetti, M.F. Jimenez Solomon, G. Szekely, A.G. Livingston, Molecular Separation with Organic Solvent Nanofiltration: A Critical Review, Chem. Rev. 114 (2014) 10735–10806. https://doi.org/10.1021/CR500006J.

[11] A.M. Tandel, W. Guo, K. Bye, L. Huang, M. Galizia, H. Lin, Designing organic solvent separation membranes: Polymers, porous structures, 2D materials, and their combinations, Mater. Adv. 2 (2021) 4574–4603. https://doi.org/10.1039/d1ma00373a.

[12] R. Shevate, D.L. Shaffer, Large-Area 2D Covalent Organic Framework Membranes

with Tunable Single-Digit Nanopores for Predictable Mass Transport, ACS Nano. 16 (2022) 2407–2418. https://doi.org/10.1021/acsnano.1c08804.

[13] X.Y. Gong, Z.H. Huang, H. Zhang, W.L. Liu, X.H. Ma, Z.L. Xu, C.Y. Tang, Novel high-flux positively charged composite membrane incorporating titanium-based MOFs for heavy metal removal, Chem. Eng. J. 398 (2020) 125706. https://doi.org/10.1016/J.CEJ.2020.125706.

[14] J. Campbell, J.D.S. Burgal, G. Szekely, R.P. Davies, D.C. Braddock, A. Livingston, Hybrid polymer/MOF membranes for Organic Solvent Nanofiltration (OSN): Chemical modification and the quest for perfection, J. Memb. Sci. 503 (2016) 166–176. https://doi.org/10.1016/J.MEMSCI.2016.01.024.

[15] U. Beuscher, E.J. Kappert, J.G. Wijmans, Membrane research beyond materials science, J. Memb. Sci. 643 (2022) 119902. https://doi.org/10.1016/J.MEMSCI.2021.119902.

[16] B. Díaz-Reinoso, A. Moure, H. Domínguez, J.C. Parajó, Ultra- and nanofiltration of aqueous extracts from distilled fermented grape pomace, J. Food Eng. 91 (2009) 587–593. https://doi.org/10.1016/j.jfoodeng.2008.10.007.

[17] C.M. Sánchez-Arévalo, Á. Jimeno-Jiménez, C. Carbonell-Alcaina, M.C. Vincent-Vela, S. Álvarez-Blanco, Effect of the operating conditions on a nanofiltration process to separate low-molecular-weight phenolic compounds from the sugars present in olive mill wastewaters, Process Saf. Environ. Prot. 148 (2021) 428–436. https://doi.org/10.1016/j.psep.2020.10.002.

[18] A. Alfano, L. Corsuto, R. Finamore, M. Savarese, F. Ferrara, S. Falco, G. Santabarbara, M. De Rosa, C. Schiraldi, Valorization of Olive Mill Wastewater by Membrane Processes to Recover Natural Antioxidant Compounds for Cosmeceutical and Nutraceutical Applications or Functional Foods, Antioxidants. 7 (2018) 72. https://doi.org/10.3390/antiox7060072.

[19] A. Jiménez, R. Rodríguez, I. Fernández-Caro, R. Guillén, J. Fernández-Bolaños, A. Heredia, Olive fruit cell wall: Degradation of cellulosic and hemicellulosic polysaccharides during ripening, J. Agric. Food Chem. 49 (2001) 2008–2013. https://doi.org/10.1021/jf000809v.

[20] J. Yu, K. Wang, D.M. Beckles, Starch branching enzymes as putative determinants of postharvest quality in horticultural crops, BMC Plant Biol. 21 (2021) 479. https://doi.org/10.1186/s12870-021-03253-6.

[21] C. Ghilardi, P. Sanmartin Negrete, A.A. Carelli, V. Borroni, Evaluation of olive mill waste as substrate for carotenoid production by Rhodotorula mucilaginosa, Bioresour. Bioprocess. 7 (2020) 52. https://doi.org/10.1186/s40643-020-00341-7.

[22] J.M. Arsuaga, M.J. López-Muñoz, A. Sotto, Correlation between retention and

adsorption of phenolic compounds in nanofiltration membranes, Desalination. 250 (2010) 829–832. https://doi.org/10.1016/j.desal.2008.11.051.

[23] A. Sotto, J.M. Arsuaga, B. Van der Bruggen, Sorption of phenolic compounds on NF/RO membrane surfaces: Influence on membrane performance, Desalination. 309 (2013) 64–73. https://doi.org/10.1016/j.desal.2012.09.023.

[24] M. Cifuentes-Cabezas, C. Carbonell-Alcaina, M.C. Vincent-Vela, J.A. Mendoza-Roca, S. Álvarez-Blanco, Comparison of different ultrafiltration membranes as first step for the recovery of phenolic compounds from olive-oil washing wastewater, Process Saf. Environ. Prot. 149 (2021) 724–734. https://doi.org/10.1016/j.psep.2021.03.035.

[25] C.M. Sánchez-Arévalo, A. Iborra-Clar, M.C. Vincent-Vela, S. Álvarez-Blanco, Exploring the extraction of the bioactive content from the two-phase olive mill waste and further purification by ultrafiltration, LWT - Food Sci. Technol. 165 (2022) 113742. https://doi.org/10.1016/j.lwt.2022.113742.

[26] R. Dreywood, Qualitative Test for Carbohydrate Material, Ind. Eng. Chem. - Anal. Ed. 18 (1946) 499. https://doi.org/10.1021/I560156A015.

[27] T.G. Ludwig, H.J.V. Goldberg, The Anthrone method for the determination of carbohydrates in foods and in oral rinsing, J. Dent. Res. 35 (1956) 90–94. https://doi.org/10.1177/00220345560350012301.

[28] S. Zeidler, U. Kätzel, P. Kreis, Systematic investigation on the influence of solutes on the separation behavior of a PDMS membrane in organic solvent nanofiltration, J. Memb. Sci. 429 (2013) 295–303. https://doi.org/10.1016/J.MEMSCI.2012.11.056.

[29] D. Peshev, L.G. Peeva, G. Peev, I.I.R. Baptista, A.T. Boam, Application of organic solvent nanofiltration for concentration of antioxidant extracts of rosemary (Rosmarinus officiallis L.), Chem. Eng. Res. Des. 89 (2011) 318–327. https://doi.org/10.1016/j.cherd.2010.07.002.

[30] M. Razali, C. Didaskalou, J.F. Kim, M. Babaei, E. Drioli, Y.M. Lee, G. Szekely, Exploring and Exploiting the Effect of Solvent Treatment in Membrane Separations, ACS Appl. Mater. Interfaces. 9 (2017) 11279–11289. https://doi.org/10.1021/acsami.7b01879.

[31] S. Blumenschein, Application of Organic Solvent Nanofiltration for Multi-Purpose Production, Tecnical University of Dortmund, 2017.

[32] K. Grundke, S. Michel, G. Knispel, A. Grundler, Wettability of silicone and polyether impression materials: Characterization by surface tension and contact angle measurements, Colloids Surfaces A Physicochem. Eng. Asp. 317 (2008) 598–609. https://doi.org/10.1016/J.COLSURFA.2007.11.046.

[33] G.S. Vieira, F.K.V. Moreira, R.L.S. Matsumoto, M. Michelon, F.M. Filho, M.D. Hubinger, Influence of nanofiltration membrane features on enrichment of

jussara ethanolic extract (Euterpe edulis) in anthocyanins, J. Food Eng. 226 (2018) 31–41. https://doi.org/10.1016/J.JFOODENG.2018.01.013.

[34] G. Ignacz, G. Szekely, Deep learning meets quantitative structure–activity relationship (QSAR) for leveraging structure-based prediction of solute rejection in organic solvent nanofiltration, J. Memb. Sci. 646 (2022) 120268. https://doi.org/10.1016/J.MEMSCI.2022.120268.

[35] S. Jafarinejad, H. Park, H. Mayton, S.L. Walker, S.C. Jiang, Concentrating ammonium in wastewater by forward osmosis using a surface modified nanofiltration membrane, Environ. Sci. Water Res. Technol. 5 (2019) 246–255. https://doi.org/10.1039/C8EW00690C.

[36] A. Premachandra, S. O'Brien, N. Perna, J. McGivern, R. LaRue, D.R. Latulippe, Treatment of complex multi-sourced industrial wastewater — New opportunities for nanofiltration membranes, Chem. Eng. Res. Des. 168 (2021) 499–509. https://doi.org/10.1016/j.cherd.2021.01.005.

[37] B. Van der Bruggen, J. Geens, C. Vandecasteele, Influence of organic solvents on the performance of polymeric nanofiltration membranes, Sep. Sci. Technol. 37 (2002) 783–797. https://doi.org/10.1081/SS-120002217.

[38] J.R.M. de Melo, M. V. Tres, J. Steffens, J. Vladimir Oliveira, M. Di Luccio, Desolventizing organic solvent-soybean oil miscella using ultrafiltration ceramic membranes, J. Memb. Sci. 475 (2015) 357–366. https://doi.org/10.1016/j.memsci.2014.10.029.

[39] I.S. Argyle, A. Pihlajamäki, M.R. Bird, Black tea liquor ultrafiltration: Effect of ethanol pre-treatment upon fouling and cleaning characteristics, Food Bioprod. Process. 93 (2015) 289–297. https://doi.org/10.1016/j.fbp.2014.10.010.

[40] A. García, S. Álvarez, F. Riera, R. Álvarez, J. Coca, Water and hexane permeate flux through organic and ceramic membranes: Effect of pretreatment on hexane permeate flux, J. Memb. Sci. 253 (2005) 139–147. https://doi.org/10.1016/j.memsci.2004.11.030.

[41] M. Mänttäri, A. Pihlajamäki, M. Nyström, Effect of pH on hydrophilicity and charge and their effect on the filtration efficiency of NF membranes at different pH, J. Memb. Sci. 280 (2006) 311–320. https://doi.org/10.1016/j.memsci.2006.01.034.

[42] L.D. Nghiem, A.I. Schäfer, M. Elimelech, Pharmaceutical retention mechanisms by nanofiltration membranes, Environ. Sci. Technol. 39 (2005) 7698–7705. https://doi.org/10.1021/es0507665.

[43] A. Böcking, Membrane Transport Properties and Process Design in Nanofiltration with Organic Solvents and Aqueous Solvent Mixtures, Aachen University, 2020.

[44] T. Roncal, L. Lorenzo, S. Prieto-Fernández, J.R. Ochoa-Gómez, Purification and

concentration of formic acid from formic acid/gluconic acid mixtures by two successive steps of nanofiltration and reactive liquid-liquid extraction, Sep. Purif. Technol. 286 (2022). https://doi.org/10.1016/j.seppur.2022.120492.

[45] Y. Kiso, Y. Sugiura, T. Kitao, K. Nishimura, Effects of hydrophobicity and molecular size on rejection of aromatic pesticides with nanofiltration membranes, J. Memb. Sci. 192 (2001) 1–10. https://doi.org/10.1016/S0376-7388(01)00411-2.

[46] B. Tylkowski, I. Tsibranska, R. Kochanov, G. Peev, M. Giamberini, Concentration of biologically active compounds extracted from Sideritis ssp. L. by nanofiltration, Food Bioprod. Process. 89 (2011) 307–314. https://doi.org/10.1016/j.fbp.2010.11.003.

[47] Y. Thiermeyer, S. Blumenschein, M. Skiborowski, Fundamental insights into the rejection behavior of polyimide-based OSN membranes, Sep. Purif. Technol. 265 (2021) 118492. https://doi.org/10.1016/J.SEPPUR.2021.118492.

[48] L. Wu, Y. Xu, Z. Yang, Q. Feng, Hydroxytyrosol and olive leaf extract exert cardioprotective effects by inhibiting GRP78 and CHOP expression, J. Biomed. Res. 32 (2018) 371–379. https://doi.org/10.7555/JBR.32.20170111.

[49] Z. Zhao, T. Sun, Y. Jiang, L. Wu, X. Cai, X. Sun, X. Sun, Photooxidative damage in retinal pigment epithelial cells via GRP78 and the protective role of grape skin polyphenols, Food Chem. Toxicol. 74 (2014) 216–224. https://doi.org/10.1016/J.FCT.2014.10.001.

[50] A. Imbrogno, A.I. Schäfer, Micropollutants breakthrough curve phenomena in nanofiltration: Impact of operational parameters, Sep. Purif. Technol. 267 (2021) 1–13. https://doi.org/10.1016/j.seppur.2021.118406.

[51] M.E. Williams, J.A. Hestekin, C.N. Smothers, D. Bhattacharyya, Separation of Organic Pollutants by Reverse Osmosis and Nanofiltration Membranes: Mathematical Models and Experimental Verification, Ind. Eng. Chem. Res. 38 (1999) 3683–3695. https://doi.org/10.1021/IE990140L.

[52] L.D. Nghiem, A.I. Schäfer, M. Elimelech, Removal of Natural Hormones by Nanofiltration Membranes: Measurement, Modeling, and Mechanisms, Environ. Sci. Technol. 38 (2004) 1888–1896. https://doi.org/10.1021/ES034952R.

[53] S. Darvishmanesh, J. Degrève, B. Van der Bruggen, Comparison of pressure driven transport of ethanol/n-hexane mixtures through dense and microporous membranes, Chem. Eng. Sci. 64 (2009) 3914–3927. https://doi.org/10.1016/j.ces.2009.05.032.

[54] A.J.C. Semião, M. Foucher, A.I. Schäfer, Removal of adsorbing estrogenic micropollutants by nanofiltration membranes: Part B—Model development, J. Memb. Sci. 431 (2013) 257–266. https://doi.org/10.1016/j.memsci.2012.11.079.

[55] E.A. McCallum, H. Hyung, T.A. Do, C.H. Huang, J.H. Kim, Adsorption, desorption, and steady-state removal of 17β-estradiol by nanofiltration membranes, J.

Memb. Sci. 319 (2008) 38–43. https://doi.org/10.1016/J.MEMSCI.2008.03.014.

[56] A. Giacobbo, A.M. Bernardes, M.J.F. Rosa, M.N. De Pinho, Concentration polarization in ultrafiltration/nanofiltration for the recovery of polyphenols from winery wastewaters, Membranes (Basel). 8 (2018). https://doi.org/10.3390/membranes8030046.

[57] C.M. Galanakis, E. Markouli, V. Gekas, Recovery and fractionation of different phenolic classes from winery sludge using ultrafiltration, Sep. Purif. Technol. 107 (2013) 245–251. https://doi.org/10.1016/J.SEPPUR.2013.01.034.

[58] H. Xu, K. Xiao, X. Wang, S. Liang, C. Wei, X. Wen, X. Huang, Outlining the Roles of Membrane-Foulant and Foulant-Foulant Interactions in Organic Fouling During Microfiltration and Ultrafiltration: A Mini-Review, Front. Chem. 8 (2020) 417. https://doi.org/10.3389/FCHEM.2020.00417/BIBTEX.

[59] V. Contreras-Jácquez, U. Valenzuela-Vázquez, D.A. Grajales-Hernández, J.C. Mateos-Díaz, M. Arrellano-Plaza, M.E. Jara-Marini, A. Asaff-Torres, Pilot-Scale Integrated Membrane System for the Separation and Concentration of Compounds of Industrial Interest from Tortilla Industry Wastewater (Nejayote), Waste and Biomass Valorization. 13 (2022) 345–360. https://doi.org/10.1007/s12649-021-01530-x.

[60] L. Braeken, R. Ramaekers, Y. Zhang, G. Maes, B. Van Der Bruggen, C. Vandecasteele, Influence of hydrophobicity on retention in nanofiltration of aqueous solutions containing organic compounds, J. Memb. Sci. 252 (2005) 195–203. https://doi.org/10.1016/J.MEMSCI.2004.12.017.

[61] P. Muñoz, K. Pérez, A. Cassano, R. Ruby-Figueroa, Recovery of anthocyanins and monosaccharides from grape marc extract by nanofiltration membranes, Molecules. 26 (2021) 1–12. https://doi.org/10.3390/molecules26072003.

[62] J.A.A. Mejia, A. Ricci, A.S. Figueiredo, A. Versari, A. Cassano, M.N. de Pinho, G.P. Parpinello, Membrane-based Operations for the Fractionation of Polyphenols and Polysaccharides From Winery Sludges, Food Bioprocess Technol. 15 (2022) 933–948. https://doi.org/10.1007/s11947-022-02795-3.

[63] S. Yammine, R. Rabagliato, X. Vitrac, M.M. Peuchot, R. Ghidossi, The use of nanofiltration membranes for the fractionation of polyphenols from grape pomace extracts, OENO One. 53 (2019) 11–26. https://doi.org/10.20870/OENO-ONE.2019.53.1.2342.

[64] U.T. Syed, C. Brazinha, J.G. Crespo, J.M. Ricardo-da-Silva, Valorisation of grape pomace: Fractionation of bioactive flavan-3-ols by membrane processing, Sep. Purif. Technol. 172 (2017) 404–414. https://doi.org/10.1016/j.seppur.2016.07.039.

[65] R. Zyłła, M. Foszpanczyk, I. Kaminska, M. Kudzin, J. Balcerzak, S. Ledakowicz,

Impact of Polymer Membrane Properties on the Removal of Pharmaceuticals, Membranes (Basel). 12 (2022). https://doi.org/https://doi.org/10.3390/membranes12020150.

[66] K.Y. Law, Definitions for hydrophilicity, hydrophobicity, and superhydrophobicity: Getting the basics right, J. Phys. Chem. Lett. 5 (2014) 686–688. https://doi.org/10.1021/jz402762h.

[67] Y. Kaya, H. Barlas, S. Arayici, Nanofiltration of Cleaning-in-Place (CIP) wastewater in a detergent plant: Effects of pH, temperature and transmembrane pressure on flux behavior, Sep. Purif. Technol. 65 (2009) 117–129. https://doi.org/10.1016/J.SEPPUR.2008.10.034.

[68] M. Sharma, P.K. Roy, J. Barman, K. Khare, Mobility of Aqueous and Binary Mixture Drops on Lubricating Fluid-Coated Slippery Surfaces, Langmuir. 35 (2019) 7672–7679. https://doi.org/10.1021/ACS.LANGMUIR.9B00483/SUPPL_FILE/LA9B00483_SI_005.AVI.

[69] V. Freger, Swelling and morphology of the skin layer of polyamide composite membranes: An atomic force microscopy study, Environ. Sci. Technol. 38 (2004) 3168–3175. https://doi.org/10.1021/es034815u.

[70] X. Lu, S. Nejati, Y. Choo, C.O. Osuji, J. Ma, M. Elimelech, Elements Provide a Clue: Nanoscale Characterization of Thin-Film Composite Polyamide Membranes, ACS Appl. Mater. Interfaces. 7 (2015) 16917–16922. https://doi.org/10.1021/acsami.5b05478.

[71] J.J. Torres, N.E. Rodriguez, J. Toledo Arana, Ochoa, N. Ariel, J. Marchese, C. Plagiero, Ultrafiltration polymeric membranes for the purification of biodiesel from ethanol, J. Clean. Prod. 141 (2017) 641–647. http://dx.doi.org/10.1016/j.jclepro.2016.09.130.

[72] J. Llanos, P.M. Williams, S. Cheng, D. Rogers, C. Wright, Á. Pérez, P. Cañizares, Characterization of a ceramic ultrafiltration membrane in different operational states after its use in a heavy-metal ion removal process, Water Res. 44 (2010) 3522–3530. https://doi.org/10.1016/j.watres.2010.03.036.

[73] C. Boo, Y. Wang, I. Zucker, Y. Choo, C.O. Osuji, M. Elimelech, High Performance Nanofiltration Membrane for Effective Removal of Perfluoroalkyl Substances at High Water Recovery, Environ. Sci. Technol. 52 (2018) 7279–7288. https://doi.org/10.1021/acs.est.8b01040.

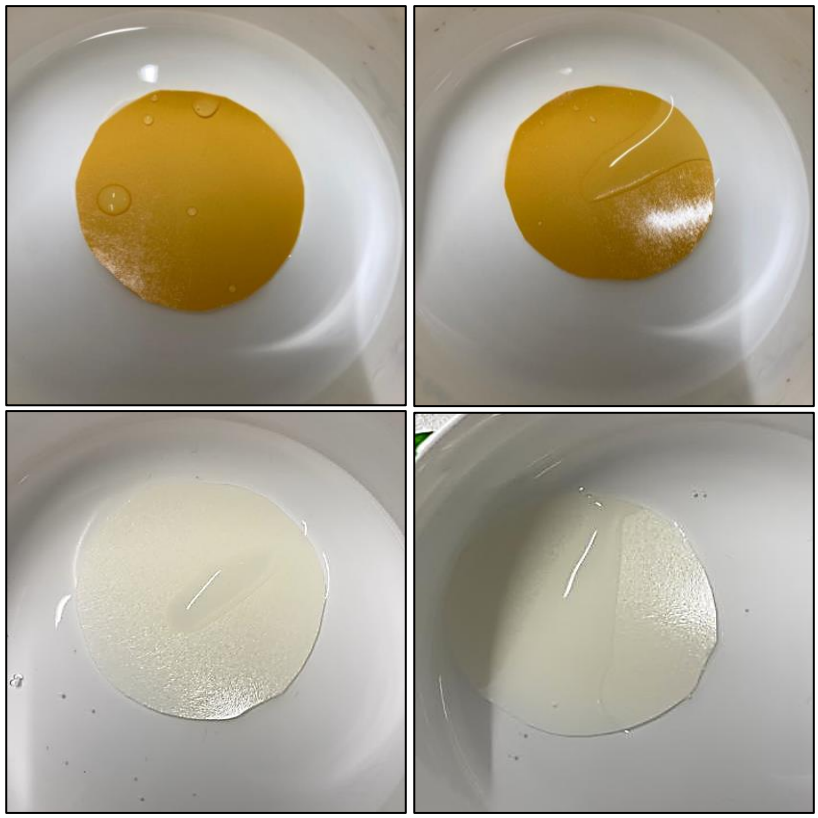
[74] R. Zhang, S. Su, S. Gao, J. Tian, Reconstruction of the polyamide film in nanofiltration membranes via the post-treatment with a ternary mixture of ethanol-water-NaOH: Mechanism and effect, Desalination. 519 (2022). https://doi.org/10.1016/j.desal.2021.115317.

[75] J. Geens, B. Van der Bruggen, C. Vandecasteele, Characterisation of the solvent

stability of polymeric nanofiltration membranes by measurement of contact angles and swelling, Chem. Eng. Sci. 59 (2004) 1161–1164. https://doi.org/10.1016/J.CES.2004.01.003.

[76] B. Van der Bruggen, J. Geens, C. Vandecasteele, Fluxes and rejections for nanofiltration with solvent stable polymeric membranes in water, ethanol and n-hexane, Chem. Eng. Sci. 57 (2002) 2511–2518. https://doi.org/10.1016/S0009-2509(02)00125-2.

[77] S.B. Sigurdardóttir, M.S.M. Sueb, M. Pinelo, Membrane compaction, internal fouling, and membrane preconditioning as major factors affecting performance of solvent resistant nanofiltration membranes in methanol solutions, Sep. Purif. Technol. 227 (2019) 1–8. https://doi.org/10.1016/j.seppur.2019.115686.



Supplementary Figure 7.1. Pictures of the membranes Pm600, oNF-1 and oNF-2 after their immersion in ethanol/water 50:50 (v/v). The solvent drops from the membranes surface can be appreciated.

CAPÍTULO 8. CHAPTER 8

Integrated membrane process in organic media: combining organic solvent ultrafiltration, nanofiltration, and reverse osmosis to purify and concentrate the phenolic compounds from wet olive pomace

Carmen M. Sánchez-Arévalo[1], Fausto Aldegheri[1,2], María Cinta Vincent-Vela[1,2], Silvia Álvarez-Blanco[1,2]

[1]Research Institute for Industrial, Radiophysical and Environmental Safety (ISIRYM), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

[2]Department of Chemical and Nuclear Engineering, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

✉sialvare@iqn.upv.es

Food and Bioprocess Technology (*Under review*)

Abstract: The phenolic compounds from a hydroalcoholic extract of wet olive pomace have been purified and concentrated by an integrated membrane process in organic media. First, the UF010104 (Solsep BV) and UP005 (Micrdyn Nadir) membranes were tested to be implemented during the ultrafiltration stage. Despite the high flux observed with the UF010104 membrane (20.4 ± 0.7 $L \cdot h^{-1} \cdot m^{-2}$, at 2 bar), the UP005 membrane was selected because of a more suitable selectivity. The permeate stream obtained with this membrane was enriched in phenolic compounds, whereas sugars and macromolecules were retained. Then, the ultrafiltration permeate was subjected to a nanofiltration step, employing the NF270 membrane (DuPont), for a further purification and fractionation of the phenolic compounds. The permeate flux was 50.2 ± 0.2 $L \cdot h^{-1} \cdot m^{-2}$, working at 15 bar. Hydroxytyrosol and some phenolic acids were recovered in the permeate, which was later concentrated by reverse osmosis, employing the NF90 membrane. The permeate flux obtained with this membrane was 15.3 ± 0.3 $L \cdot h^{-1} \cdot m^{-2}$.

Keywords: phenolic compounds, organic solvent ultrafiltration, organic solvent nanofiltration, reverse osmosis, integrated process, ethanol.

1. INTRODUCTION

Antioxidant compounds attract an enormous attention nowadays, because of their numerous applications in the pharmacological industry, the food industry and even in the beauty market [1–3]. They can be found in natural sources, such as fruits and vegetables [4]. Interestingly, these valuable compounds are also present in many byproducts generated by the agri-food sector [5]. Employing these by-products as a source of high added-value compounds can contribute to the circular economy of many industries, increasing their sustainability. Furthermore, it may transform a residue into a resource, susceptible of providing a profit for industrialists. With this practice, the incorrect disposal of the residues and the subsequent environmental impact, could eventually be avoided.

In the context of the olive oil industry, which is one of the most important food industries in the Mediterranean Area [6], wet olive pomace is an excellent candidate to be used as a natural and cheap source of antioxidant molecules. This by-product is abundantly generated during olive oil production, when the so-called two-phase methodology is applied [7]. Wet olive pomace contains the remnants of the olive fruit after extracting the oil, therefore, it consists of the olive skin, pulp, stones, and seeds. In consequence, a considerable proportion of the olive minor fraction [8] (which entails a group of compounds that are not abundant in the olive fruit, but they are very significant due to their bioactivities) remains in the wet olive pomace [9]. Thus, it is rich in the

appreciated phenolic compounds, which have been related to powerful antioxidant, antimicrobial, and antiinflammatory capacities [10–13].

The recovery of the phenolic compounds from wet olive pomace can be accomplished by solid-liquid extraction, to extract the compounds from the solid matrix, followed by the application of membrane technology, in order to purify and, when necessary, concentrate the molecules. The efficiency of the extraction highly influences the overall recovery of the process, as it defines the initial concentration. During the following membrane processes, a proportion of the target compounds will inevitably be lost. Therefore, maximizing the initial concentration of compounds in the extract to be treated is of relevance.

When the extraction of compounds is performed with a mixture of ethanol/water 50:50 (v/v), a higher concentration of biophenols is obtained, in comparison with a water-based extraction [14]. However, the membrane processes downstream the hydroalcoholic extraction are normally not much more challenging, because the presence of an organic solvent in the feed solution introduces an additional feature to be considered. The permeate flux might be reduced, the rejection values might be unexpected and even the membrane integrity can be compromised [15,16]. For those reasons, despite the benefits in terms of concentration, when valuable compounds are recovered from an agri-food residue by membrane technology, the previous extraction of such compounds is typically water-based. This is the case of Nunes et al., who applied nanofiltration and reverse osmosis to an aqueous extract of wet olive pomace [17], and Tapia-Quirós et al., who studied several membrane processes, including microfiltration, ultrafiltration, nanofiltration and reverse osmosis to recover polyphenols from olive pomace after an extraction with water [18]. Sygouni and co-workers concluded that the solvent-extraction of the biophenols from wet olive pomace was the most efficient, however, they proceeded to the membrane process with the aqueous extract [19].

Only a few contributions dealing with the membrane filtration of a hydroethanolic extract of wet olive pomace have been published up to date. In a previous study by these authors, the potential of ultrafiltration to reduce the organic load of the hydroalcoholic wet olive pomace extract was demonstrated, while the phenolic compounds were purified in the permeate [14]. Later, these authors also studied the performance of several nanofiltration membranes to treat a hydroalcoholic model solution, whose composition was based on the extract of wet olive pomace obtained with ethanol/water 50:50 (v/v) [20]. However, the development of a solvent-mediated,

integrated process, covering all the steps from the extraction of the phenolic compounds to their purification by membrane technology, and their final concentration, is missing in the literature.

Therefore, in this contribution, a combination of an ultrasound-assisted solid-liquid extraction (with the solvent ethanol/water 50:50 (v/v)) with ultrafiltration, nanofiltration, and reverse osmosis has been proposed for the first time, aiming to obtain a final concentrate of purified phenolic compounds from wet olive pomace.

2. MATERIALS AND METHODS

2.1. Extraction of phenolic compounds from wet olive pomace

The wet olive pomace employed in this study was obtained from a two-phase olive mill in Segorbe (Castellón, Spain). The phenolic compounds were extracted from wet olive pomace using a 50% (v/v) mixture of ethanol/water as solvent. The applied methodology was previously optimized [14]. It entailed an ultrasound-assisted extraction (UAE), performed at 40°C for 45 min. In short, 800 g of wet olive pomace were dissolved in a 1:10 proportion with the organic solvent and subjected to UAE. Afterwards, the sample was centrifuged at 17200 RCF for 6 min, and the extract was vacuum filtered with a 60 µm filter (Fanola, Barcelona, Spain). Afterwards, the resulting extract was treated by ultrafiltration.

2.2. Integrated membrane process

The membrane process that has been proposed to purify and concentrate the phenolic compounds from the hydroalcoholic extracts of wet olive pomace is reflected in Figure 8.1. It entails an ultrafiltration process, to withdraw a high proportion of total solids and sugars, a nanofiltration process, to increment the purity of phenolic compounds and fractionate them, and a reverse osmosis process, to concentrate the permeate stream obtained in the nanofiltration step. As the organic solvent was present throughout the whole process, it entailed an additional challenge, regarding the performance of the membranes.

It should be noted that the retentate obtained during the nanofiltration process is also a valuable stream. As it will be explained, the nanofiltration retentate contains a significant concentration of phenolic compounds. It was also a concentrated stream, enriched in high added-value compounds.

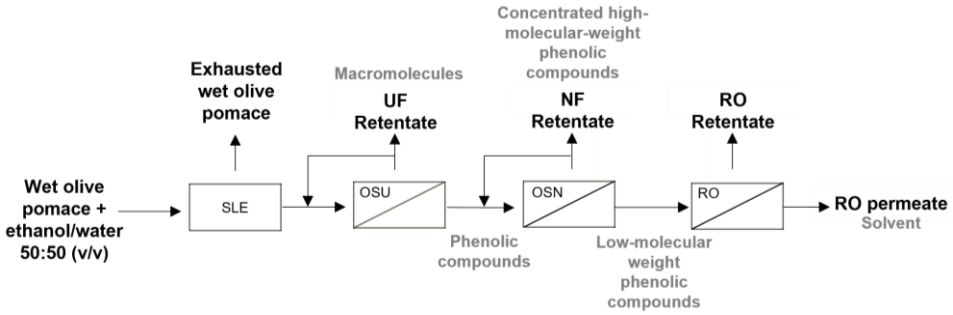


Figure 8.1. Solvent-based, integrated membrane process to purify and concentrate phenolic compounds from the hydroalcoholic extract of wet olive pomace. SLE: solid-liquid extraction; UF: ultrafiltration; OSU: organic solvent ultrafiltration; NF: nanofiltration; OSN: organic solvent nanofiltration; RO: reverse osmosis.

2.3. Solvent-resistant ultrafiltration

The obtained hydroalcoholic extract of wet olive pomace was subjected to an ultrafiltration process, employing an ultrafiltration cross-flow plant (Orelis Environment, Salindres, France), equipped with a Rayflow membrane module (Orelis Environment, Salindres, France). The module contained two ultrafiltration membranes working in series. The area for each membrane was 129 cm^2. The information regarding the membranes tested in this work can be found in Table 8.1.

Table 8.1. Characteristics of the membranes employed in this study.

Membrane	MWCO (kDa)[1]	Material	Manufacturer	Process
UF010104	20[2]	Proprietary	SolSep BV	UF
UP005	5	PES[3]	Microdyn Nadir	UF
NF270	0.3 - 0.4	Polyamide	DuPont	NF
NF90	0.2	Polyamide	DuPont	RO

[1]Molecular weight cut-off; [2]Determined in hexane; [3]Polyethersulfone

Prior to the ultrafiltration of the hydroalcoholic extract, the membranes were immersed for two hours in a solution of ethanol/water 50:50 (v/v) to hydrate them and remove any conservative remnants. Afterwards, the UF010104 and the UP005 membranes were compacted (using a solution of ethanol/water 50:50 (v/v) as feed) at a transmembrane pressure (TMP) of 2.2 bar and 2.7 bar, respectively. The solvent permeability (L_p) of the membranes was investigated, in the range of 0.75 – 2 bar, for the UF010104 membrane, and 1 – 2.5 bar for the UP005 membrane.

The solvent permeability was calculated in terms of the permeate flux (J_p) and the transmembrane pressure according to the equation:

$$L_p = \frac{J_p}{TMP} \quad (1)$$

Subsequently, the extract was ultrafiltered at 2 bar, for the UF010104 membrane, and at 2.5 bar for the UP005 membrane, with a cross-flow velocity of 1.8 m·s^{-1} and 25˚C. These operating conditions were selected according to previous studies from our research group [14,21]. The process was carried out in concentration mode, until a volume reduction factor (VRF) of at least 2 was achieved. Samples from the retentate and permeate streams were collected at different VRF, to analyze the process and calculate rejection values.

If the ultrafiltration process was not completed within the same working day, the membrane was rinsed and the process was resumed the next day. This rinsing consisted of flushing the ultrafiltration plant with a solution of ethanol/water 50:50 (v/v), during 15 minutes. The solvent employed daily for the membrane rinsing was not directly discarded. On the contrary, the solution was recovered from the plant and reused during three days, in order to contribute to the sustainability of the process and reduce the costs.

When necessary, the UP005 membrane had to be cleaned. Then, an Ultrasil 110, at 1% in water (v/v), was employed after rinsing the membrane with the solvent mixture for 15 minutes. The cleaning solution was recirculated through the plant for 1 hour, at 35˚C, working at 0.7 bar and 1.8 m·s^{-1}. Later, the membrane was rinsed with tap water to remove the Ultrasil solution. Finally, a rinsing with ethanol/water 50:50 (v/v) was applied. In the case of the UF010104, the cleaning step was not needed.

2.4. Solvent-resistant nanofiltration

The ultrafiltration permeate was treated in a nanofiltration cross-flow plant, equipped with a stainless steel module specifically designed for the plant [22]. The membrane area on the module was 72 cm^2 and a NF270 (Microdyn Nadir) membrane was used for this process.

Prior to the nanofiltration stage, the membrane was immersed overnight in ethanol/water 50:50 (v/v) as a preconditioning treatment. Afterwards, a compaction stage was carried out at 22 bar and a cross-flow velocity of 1 m·s^{-1}, until a stable permeate flux was observed. The solvent permeability was calculated using equation (1) in a range of 5-20 bar, at 1 m·s^{-1}.

Afterwards, the ultrafiltered extract was nanofiltered at 15 bar and 1 $m \cdot s^{-1}$, in concentration mode, as in the ultrafiltration stage (section 2.2). Samples of both the retentate and permeate streams were collected at different VRF values for further characterization.

2.5. Solvent-resistant reverse osmosis

The previously obtained nanofiltered permeate was treated as the feed in a reverse osmosis process, which served as a final stage to concentrate the phenolic compounds. The set-up implemented was similar to the nanofiltration plant (section 2.3), but, in this case, a NF90 membrane was selected for the process.

As in the previous stages of the integrated process, a preconditioning and compaction of the membrane were necessary before the experiment. The NF90 membrane was compacted at 22 bar and 1 $m \cdot s^{-1}$ and the solvent permeability was studied under the same conditions as in the nanofiltration step. The reverse osmosis process was carried out with the nanofiltered permeate, at 20 bar and 1 $m \cdot s^{-1}$.

2.6. Analysis of the streams

2.6.1. Analysis of organic matter

Samples obtained from the feed extract, retentate, global and instantaneous permeates of every separation process were characterized in terms of color, total solids, total sugars, and total phenolic content, as well as pH and electric conductivity. Each sample was analyzed at least in duplicates and the instantaneous rejection of each solute (R) was calculated according to equation (2):

$$R = \left(1 - \frac{C_p}{C_r}\right) \cdot 100 \quad (2)$$

where C_p is the concentration in the permeate and C_r represents the concentration in the retentate stream.

Color was assessed according to [23], measuring the absorbance (A) at 436 nm, 525 nm, and 620 nm with a UV-VIS DR 6000 spectrophotometer (Hach Lange, Germany). The color coefficient was calculated with the following formula:

$$Colour = \frac{A_{436}^2 + A_{525}^2 + A_{620}^2}{A_{436} + A_{525} + A_{620}} \quad (3)$$

The Folin-Ciocalteu methodology was applied to determine the concentration of total phenolic compounds [24]. To quantify the analytes, tyrosol (VWR International, USA) was employed to prepare the calibration curve, diluted in ethanol/water 50:50 (v/v), in the range of 1 – 500 $mg \cdot L^{-1}$. The total sugar content was determined via the anthrone method [25], employing a glucose (Roche, Switzerland) calibration curve, prepared in ethanol/water 50:50 (v/v), in the range of 1 – 125 $mg \cdot L^{-1}$. Total solids were obtained by evaporation of a known aliquot of the sample. A pHmeter (GLP21+, Crison, Spain) and a conductimeter (GLP31+, Crison, Spain) were used to measure the pH and conductivity of the samples.

2.6.2. Determination of the olive minor fraction

To determine the analytes belonging to the olive minor fraction, an analytical methodology based on liquid chromatography (LC) coupled to mass spectrometry (MS) was applied. The employed instrument was an Agilent 1260 Infinity II liquid chromatograph, coupled to a 6546 quadrupole-time-of-flight (QToF) mass analyzer, equipped electrospray ionization (ESI) (Agilent Technologies, USA). A Zorbax Extend C18 column (4.6 x 100 mm, 1.8 µm) (Agilent Technologies, USA) was used to separate the compounds, operating at 40°C and a flow rate of 0.9 mL/min, after a sample injection of 4 µL. Ultrapure water (Direct-Q® 3UV system, Merck Millipore, USA) and acetonitrile (Honeywell, USA) were employed as phase A and phase B, respectively. Both phases were acidified with 0.5% pure acetic acid (VWR International, USA). The separative conditions and the specific parameters for the mass spectrometry were previously developed [14]. A semi-quantitative approach was performed, conducting an external calibration with pure standards of citric acid (VWR International, USA), tyrosol (VWR International, USA), hydroxytyrosol (Sigma Aldrich, USA), caffeic acid (VWR International, USA), *p*-coumaric acid (Sigma Aldrich, USA), oleuropein (Sigma Aldrich, USA), luteolin (VWR International, USA), decarboxymethyl oleuropein aglycone (Sigma Aldrich, USA) and hydroxy-octadecanoic acid (Sigma Aldrich, USA). Standard solutions were prepared in the range of 0.1 – 100 $mg \cdot L^{-1}$.

3. RESULTS AND DISCUSSION

3.1. Characterization of the hydroalcoholic extract of wet olive pomace

The extract of wet olive pomace was obtained with ethanol/water 50:50 (v/v), according to a previously investigated process [14]. It was characterized prior to its utilization in the ultrafiltration plant. The characteristics of this extract can be found in Table 8.2.

Table 8.2. Characterization of the extract of wet olive pomace obtained with ethanol/water 50:50 (v/v).

Parameter	Determined concentration
Total phenolic content (mg/L)	737 ± 6
Total sugar content (mg/L)	663 ± 18
Total solids (g/L)	7.78 ± 0.02
Color coefficient	2.3 ± 0.1
pH	5.9 ± 0.2
Conductivity (µS/cm)	679 ± 30

As can be seen, the extract contains a remarkable concentration of valuable phenolic compounds, which were the molecules of interest in this work. Apart from those compounds, a high concentration of organic matter was present in the extract, as reflected by the high sugar and total solids content. Therefore, the application of an integrated membrane process to separate the phenolic compounds from the rest of the undesirable matter of the extract has been proposed in this work.

3.2. Organic solvent ultrafiltration

3.2.1. Productivity of the process

To assess the productivity of the ultrafiltration step, the permeate flux was measured. The UF010104 membrane displayed a solvent permeability of 72.2 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$, whereas the solvent permeability of the UP005 membrane was 8.0 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$. This difference was expected, based on the distant MWCO of both membranes. The values of permeate flux obtained when the aqueous extract of wet olive pomace was ultrafiltered are reflected in Figure 8.2. Again, the UF010104 membrane exhibited much higher values of permeate flux than the UP005 membrane, because of the higher pore size. The high, initial permeate flux experimented a progressive decline that continued until a VRF of 1.7 was achieved. At the beginning of the process, flux decline was sharper, prompted by membrane fouling. After the VRF of 1.7, a steady state was reached, and the permeate flux stabilized at 20.4 ± 0.7 $L \cdot h^{-1} \cdot m^{-2}$. The feed solution was a real vegetable extract, with a high organic load and high content of total solids (Table 8.2). Furthermore, an organic solvent, whose greater viscosity (in comparison with water) reduces the flux, was present. Therefore, the performance of the UF010104 membrane was considered very productive.

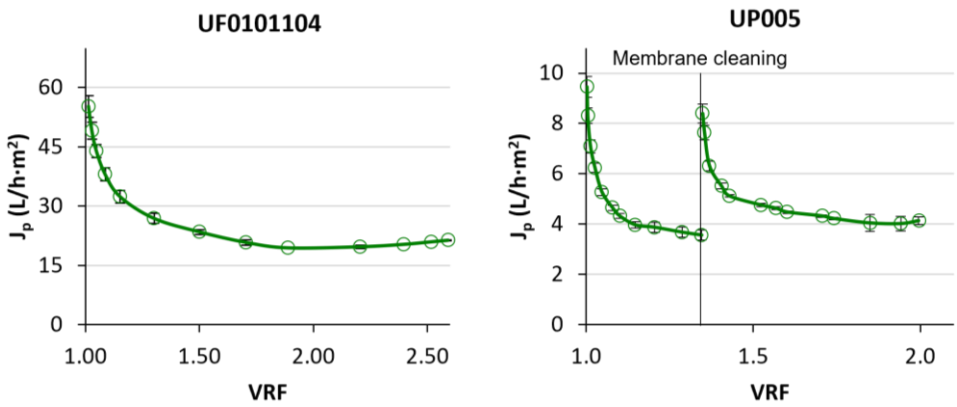


Figure 8.2. Permeate flux values obtained when a hydroalcoholic extract of wet olive pomace was treated with the UF010104 and UP005 membranes, at 1.8 m/s and 2 bar, and 1.8 m/s and 2.5 bar, respectively.

In the case of the UP005 membrane, the permeate flux was logically lower. An initial decrease was also observed for this membrane. Due to the lower values of permeate flux displayed by this membrane, the ultrafiltration of the total volume of the hydroalcoholic extract of wet olive pomace could not be completed within the same working day. Therefore, at the end of each working day, the membrane was rinsed (as commented in section 2.2) and the process was resumed the next day. Also, at the end of the third working day, the UP005 membrane was submitted to a cleaning process, aiming to recover the higher permeate flux that was obtained at the beginning of the ultrafiltration process. After the Ultrasil cleaning (see section 2.2), the recovery of the solvent permeability was only 70%, in comparison with that of the new membrane, which indicates the presence of residual fouling. For that reason, the permeate flux at a VRF of 1.35 was lower than at the beginning of the process (8.4 ± 0.4 $L \cdot h^{-1} \cdot m^{-2}$). Then, the cake layer formation started again, motivating the shape of the curve that is reflected in Figure 8.2. After a VRF of 1.7, the permeate flux of the UP005 membrane became more stable and, finally, it was constant at a VRF of 1.8 until the end of the process.

In the current literature, there are no previous studies dealing with the ultrafiltration of a solvent-based extract of wet olive pomace in cross-flow mode. To the best of our knowledge, the only related work regarding to this topic is a previous study from our research group, consisting of a screening of ultrafiltration membranes, in a bench-top, stirred cell, to treat the hydroalcoholic extract of wet olive pomace [21]. In that study, the UF010104 and UP005 membranes were also tested, but they displayed lower values of permeate flux than those obtained in this work. In fact, in this work, a permeate flux increment of 43% and 24% was obtained for the UF010104 and UP005, respectively, with

respect to the values obtained in the stirred cell. This was due to the effect of the tangential flow, which contributed to reduce the concentration polarization in the membrane module. The flux increment for the UP005 membrane was not so significant as in the case of the UF010104 membrane, because, in the stirred cell, the fouling suffered by this membrane was not as severe as the fouling of the UF010104 membrane. Therefore, although a higher permeate flux was obtained for the UP005 membrane in the cross-flow plant, the increment with respect to the bench-top cell was not so relevant, in comparison with the UF010104 membrane.

3.2.2. Rejection values obtained in the ultrafiltration stage

3.2.2.1. Rejection of undesired organic matter

In order to purify the extracted phenolic compounds, a high rejection of the concomitant organic matter was intended. Figure 8.3 contains the rejection of color, total solids, and sugars obtained with the tested ultrafiltration membranes.

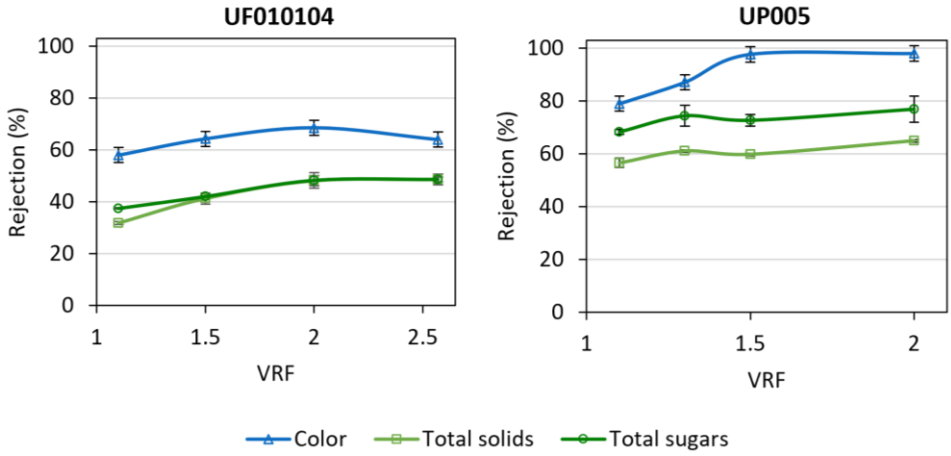


Figure 8.3. Evolution of the rejection of color, total solids, and total sugars with VRF during the ultrafiltration of a hydroalcoholic extract of wet olive pomace. On the left side: results for the UH010104 membrane, at 1.8 m/s and 2 bar. Right side: results for the UP005 membrane, at 1.8 m/s and 2.5 bar.

For both membranes, the rejection increased slightly with VRF, as a result of membrane fouling. More stable values were observed when the steady state was achieved. As commented in section 3.2.1, the steady state was achieved around a VRF of 1.7, both for the UF010104 and the UP005 membranes. Both membranes presented high rejections of color, total solids, and total sugars. However, the lower MWCO of the UP005 membrane favored higher rejections. At a VRF of 2, the UP005 membrane displayed a

rejection of 98 ± 3%, 64.9 ± 0.4%, and 77 ± 5%, for color, total solids, and total sugars, respectively.

Despite the lower values of permeate flux achieved by the UP005 membrane (in comparison with the UF010104 membrane), the selectivity was considered a priority. In that scenario, the UP005 membrane was considered to be more suitable for the purpose of purifying the biophenols. Therefore, the determination of the phenolic compounds in the streams derived from the UP005 membrane was conducted.

3.2.2.2. Rejection of phenolic compounds

According to our preliminary studies [14], a significant proportion of the phenolic content of the extract of wet olive pomace is recovered in the permeate of the UP005 membrane. To achieve a thoughtful knowledge of the rejection of each individual compound, the samples were characterized by LC-ESI-QToF-MS. This allowed to determine 36 compounds, belonging to the olive minor fraction. Among them, eight chemical families were identified. Based on their elution order from the chromatographic column, these families were organic acids, simple phenols, phenolic acids and aldehydes, secoiridoids, lignans, flavonoids, triterpenic acids, and free fatty acids. The organic acids included quinic acid, citric acid, malic acid, and isopropyl malic acid. These are compounds usually found in vegetal extracts. In this case, they were non-desired, as they contributed to reduce the purity of phenolic compounds. This was also applicable to free fatty acids, which resulted from the degradation of the olive triacylglycerides. They included trihydroxy-octadecadienoic acid, trihydroxy-octadecenoic acid, dihydroxy-hexadecanoic acid, hydroxy-octadecatrienoic acid, and hydroxy-octadecadienoic acid. The chemical class of simple phenols contained tyrosol, hydroxytyrosol, and hydroxytyrosol derivatives (glucosides, mainly). Among the phenolic acids and aldehydes, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, ferulic acid methyl ester and vanillin were found. The secoiridoids acids entailed compounds such as acyclodihydroelenolic acid hexoside, hydroxy decarboxymethyl elenolic acid, hydroxy elenolic acid, elenolic acid glucoside, oleuropein aglycone derivative, decarboxymethyl elenolic acid and its aldehydic form, hydrogenated elenolic acid, comselogoside, elenolic acid, hydroxypinoresinol, oleuropein and decarboxymethyl oleuropein aglycone. Only a lignan, hydroxypinoresinol, was found. Among the flavonoids, luteolin, apigenin, and diosmetin were present in the samples. Finally, the chemical family of triterpenic acids included maslinic acid and betulinic acid. The individual rejection of all these compounds is presented in Figure 8.4.

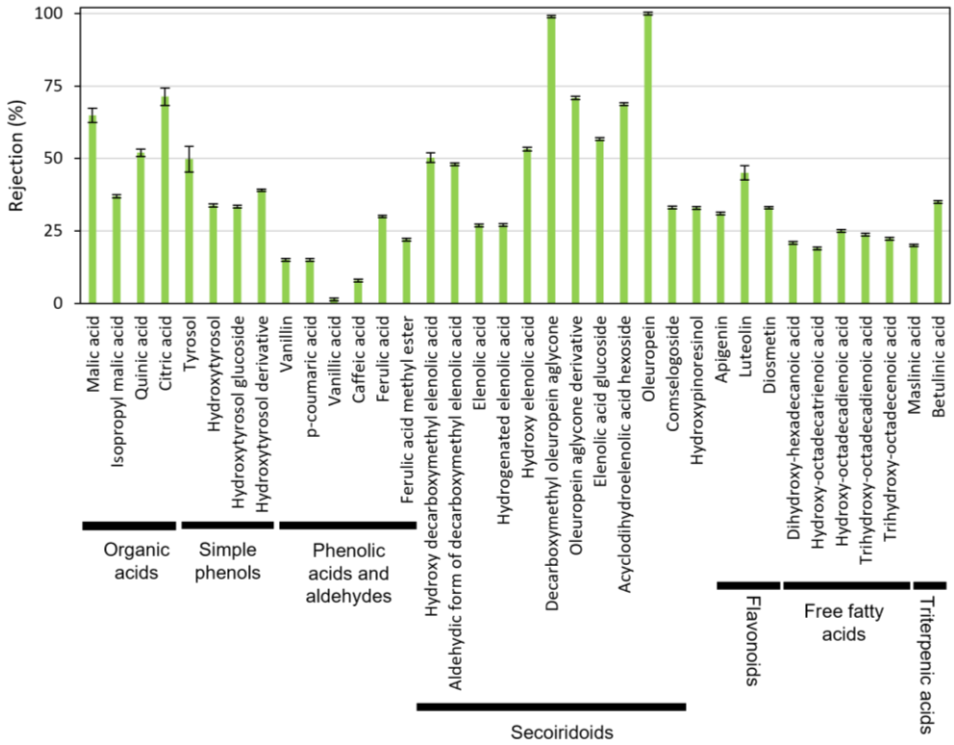


Figure 8.4. Individual rejection of each metabolite detected by LC-MS after the ultrafiltration of the hydroalcoholic extract of wet olive pomace with the UP005 membrane, at a volume reduction factor of 2. The operating conditions were 1.8 m/s and 2.5 bar.

In general, the UP005 membrane displayed a low rejection of the phenolic compounds of interest. Only some phenolic compounds were rejected above 50%, and all of them belonged to the chemical family of secoiridoids. Among them, there were glycosylated compounds and other derivatives with a higher molecular weight. This is the case of elenolic acid glucoside, acyclodihydroelenolic acid hexoside, oleuropein (which contains a residue of glucose in its structure), and the derivative of oleuropein aglycone. In a previous work by the authors, the rejection of phenolic compounds during the ultrafiltration of aqueous extracts (without ethanol) of wet olive pomace was reported, employing the same membrane (UP005) in cross-flow mode [26]. If those results are compared with this work, it is noticeable that lower rejections of biophenols are obtained when the extract is solvent-based. In a non-aqueous, organic media, as it is the hydroalcoholic extract of wet olive pomace, the polymers of ultrafiltration membranes can suffer a pore enlargement as a result of the solvent contact, leading to a decrease in rejection [15,27], as was observed here.

Regarding the compounds from the olive minor fraction analyzed by LC-MS that were not polyphenols, the organic acids were more efficiently rejected, contributing to the adequacy of the purification process. On the contrary, triterpenes and free fatty acids passed through the UP005 membrane, and their retrieval had to be addressed in the subsequent nanofiltration stage.

As exposed, the ultrafiltration process permitted the elimination of unwanted organic matter. However, the permeate stream still contained total solids and sugars susceptible to be withdrawn. Furthermore, the fractionation of the wide range of the obtained phenolic compounds was intended. Therefore, the following stage of the process was implemented.

3.3. Organic solvent nanofiltration

The permeate stream obtained during the ultrafiltration process was submitted to a nanofiltration process, employing the NF270 membrane, whose solvent permeability was 5.08 $L \cdot h^{-1} \cdot m^{-2} \cdot bar^{-1}$. In a previous study from our research group, a screening of numerous commercial membranes to assess the organic solvent nanofiltration of a synthetic extract of wet olive pomace was performed [20]. In that study, the NF270 membrane stood out because of a high permeate flux and an efficient fractionation of phenolic compounds, as well as their separation from sugars.

The permeate flux obtained during the nanofiltration of the hydroalcoholic permeate obtained during the ultrafiltration process is reflected in Figure 8.5A.

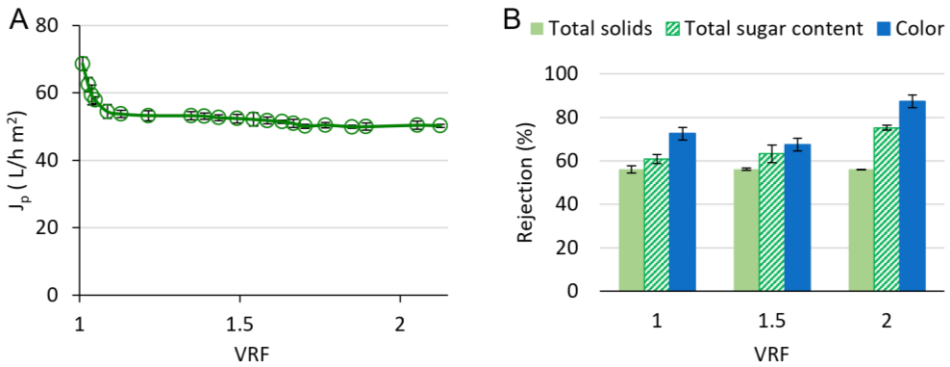


Figure 8.5. Permeate flux (A) and rejection of total sugar content, total solids and color (B) obtained with the NF270 membrane, at 1 m/s and 15 bar.

The flux decline observed during the nanofiltration stage was much softer than that observed during the ultrafiltration process. This is because the feed solution in this case was cleaner. After the treatment of the extract of wet olive pomace by ultrafiltration, the

total solids content was largely reduced (Figure 8.3B), which diminished the concentration polarization in the nanofiltration module. Thus, permeate flux reached a stabilized performance faster. The initial flux decline took place until a VRF of 1.13 was achieved. Afterwards, only a small decrease in the permeate flux occurred, until a steady state was reached at a VRF of 1.7, with a permeate flux of 50.2 ± 0.2 $L \cdot h^{-1} \cdot m^{-2}$.

Figure 8.5B shows the rejection values, regarding the unwanted organic matter, achieved with the NF270 membrane. The unwanted organic matter present in the hydroalcoholic extract of wet olive pomace included the total sugar content, total solids content and pigments responsible for the color of the stream. The rejection of total solids was very stable during the whole process, surpassing 50%. When it comes to the specific determination of sugars, the rejection of the total sugars content increased with the VRF, reaching a value of 75 ± 1 %. Considering the aim of this work, consisting of the recovery of purified phenolic compounds, this high rejection value was greatly satisfactory. One of the main challenges of isolating phenolic compounds from agri-food products (and by-products) is their separation from sugars. The molecular weight of some carbohydrates, especially monosaccharides, such as glucose and fructose, is not far from the molecular weight of some phenolic compounds [28]. Therefore, it is a challenge to procure the operating conditions (and select the proper membrane) to achieve this separation. Regarding color rejection, it also increased during the process, until a rejection value of 87 ± 3% was achieved. As a result, a clean, transparent stream was obtained as the permeate of the nanofiltration process.

To address the rejection of phenolic compounds by the NF270 membrane, Figure 8.6 is provided. As commented, the aim of this work was to recover the highest possible proportion of purified phenolic compounds. This implied the reduction of the organic load, as reflected in Figure 8.3B, but also, the elimination of organic acids, triterpenes and free fatty acids. Some of these compounds, mainly triterpenes and free fatty acids could not be eliminated by ultrafiltration. As can be seen in Figure 8.6, the rejection of these undesired chemical families surpassed 75% after the nanofiltration process.

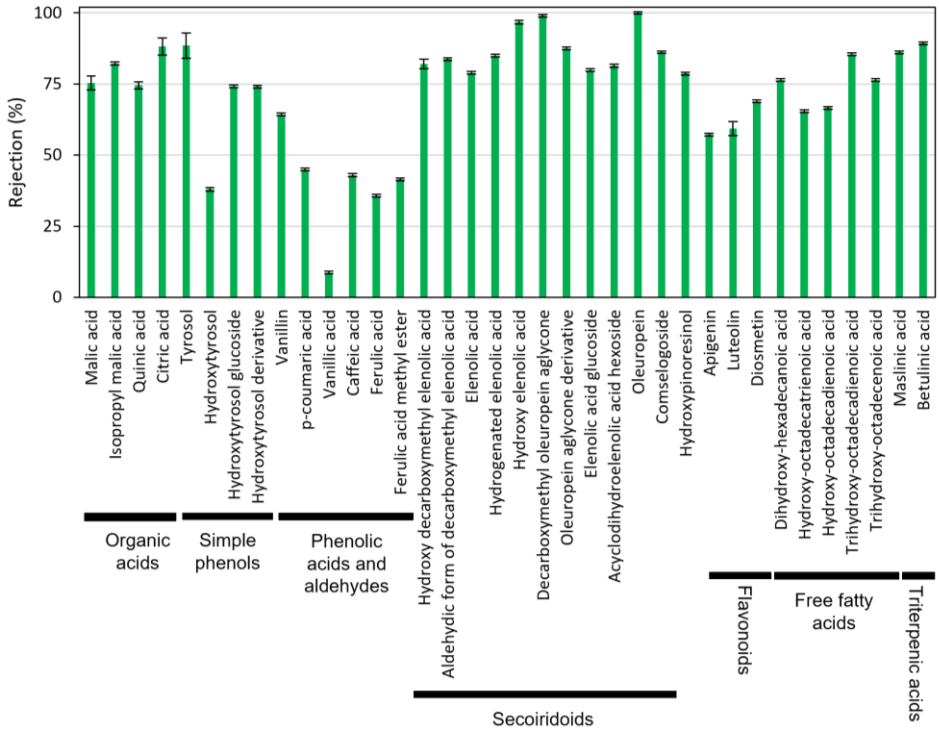


Figure 8.6. Individual rejection of each metabolite detected by LC-MS after the nanofiltration of the hydroalcoholic ultrafiltration permeate with the NF270 membrane, at a volume reduction factor of 2. The operating conditions were 1 m/s and 15 bar.

Furthermore, the NF270 membrane provided an efficient fractionation of phenolic compounds. The compounds of larger size, including secoiridoids, lignans (hydroxypinoresinol), and flavonoids, were rejected in higher percentages, even reaching 100% for some compounds, and surpassing 80% in many cases. Vieira et al. also reported an almost complete retention of high-molecular-weight anthocyanins during the nanofiltration (with the NF270 membrane) of a jussara extract containing a 70% (v/v) of ethanol.

On the contrary, Figure 8.6 shows that the family of phenolic acids and aldehydes was rejected to a lesser extent. The low rejection of the phenolic acids constituted one of the main achievements of this work. A complete passage of these compounds could not be expected, because of the adsorption on the membrane surface as demonstrated in a previous work by the authors [20]. In any case, the results indicate an efficient recovery of phenolic acids, being the molecules of vanillic and ferulic acids rejected in 8.8 ± 0.5% and 35.7 ± 0.5%, respectively. Regarding the simple phenols, most of them were obtained in the retentate stream. However, the observed rejection of

hydroxytyrosol was 38.6 ± 0.6 %, being preferentially recovered in the permeate, together with phenolic acids. This was a favorable result, considering that hydroxytyrosol is one of the most notable phenolic compounds from the olive fruit, due to its high antioxidant power [29]. According to these results, the fractionation of the phenolic compounds was achieved, obtaining a permeate stream enriched in phenolic acids and hydroxytyrosol. Afterwards, this stream was concentrated by reverse osmosis.

The interest of the NF270 permeate is undeniable, considering its composition. However, the importance of the retentate stream should not be underestimated. After the nanofiltration process, simple phenols, secoiridoids, lignans, and flavonoids are concentrated in the retentate. Even though the purity of this stream is not as high as the NF270 permeate, its utilization is still possible in a final application in which the presence of sugars, triterpenic acids, and fatty acids is not detrimental. This could be the case of animal feeds. The enriching of animal feeds with a vegetable concentrated stream such as the NF270 retentate could contribute to preserve the quality of the product, as the phenolic compounds reduce the oxidation of nutrients [7]. Also, some benefits in animal health derived from the antimicrobial and antioxidant character of the phenolic compounds can be derived. In fact, the use of olive pomace for poultry feeding has recently been proposed [30].

3.4. Reverse osmosis

Once the phenolic compounds were purified from sugars, organic acids, free fatty acids, and triterpenes, the permeate stream (after the nanofiltration process with the NF270 membrane) was concentrated. To that end, it was submitted to a reverse osmosis process, employing the NF90 membrane. This membrane was selected because its MWCO is in the middle of a nanofiltration and a reverse osmosis process. Considering the presence of the organic solvent in the feed, the highest MWCO possible was aimed, without ignoring the high retention requirements. According to the literature, its pore size was tight enough to display high rejections of phenolic compounds [18,31]. Therefore, a high flux, as well as high retention of solutes was intended to be obtained with the NF90 membrane. It presented an ethanol/water 50:50 (v/v) permeability of 0.87 $L \cdot h^{-1} \cdot m^{-2} \cdot bar$. The permeate flux displayed when the permeate of the NF270 membrane was treated is shown in Figure 8.7A.

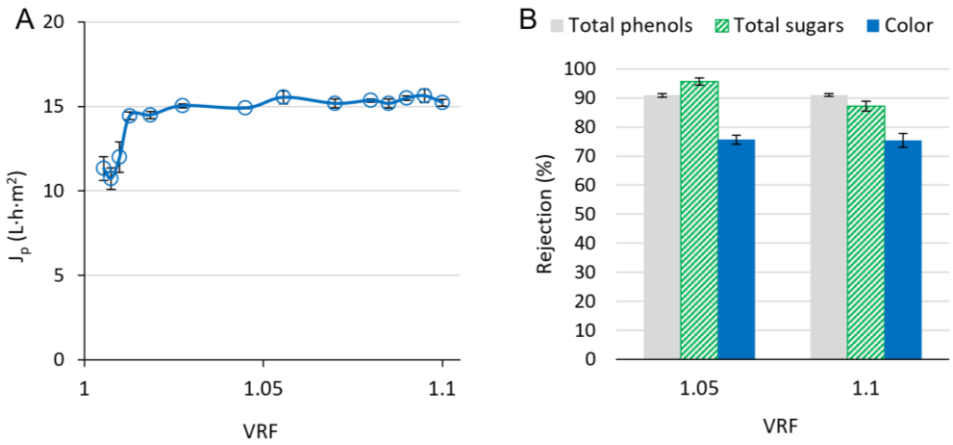


Figure 8.7. Permeate flux (A) and rejection of total sugar content, total solids and color (B) obtained with the NF90 membrane, at 1 m/s and 20 bar.

The curve described by the permeate flux suggested that fouling was not remarkable, because flux did not decrease. After being treated by ultrafiltration and nanofiltration, the hydroalcoholic extract of wet olive pomace had a low total solids content. Therefore, concentration polarization phenomena was not relevant. Instead of decreasing with the VRF, the permeate flux increased during the first moments of the reverse osmosis process, until a VRF of 1.02 was achieved, reaching 15.3 ± 0.3 $L \cdot h^{-1} \cdot m^{-2}$. This indicates the swelling of the membrane, as a result of solvent contact. Several authors have reported the swelling of the NF90 during the filtration of an organic solvent [32,33]. In fact, Tamires Vitor Pereira et al., employed the NF90 membrane to treat a hydroalcoholic extract of grape marc, after a pretreatment by microfiltration. They also observed an initial increment of the permeate flux and attributed it to the polymer swelling, due to the contact with ethanol [33]. As a result of the membrane swelling, the cross-linking of the polymer might be affected, resulting in a higher distance between the polymer chains and, in consequence, a higher pore size [34].

Regarding the retention of solutes performed by the NF90 membrane, satisfactory, high values were achieved. As can be seen in Figure 8.7B, the membrane was able to retain the phenolic compounds above 90%. Thus, the concentration of these compounds was accomplished, after their purification by ultrafiltration and their fractionation by nanofiltration. The hydroalcoholic permeate stream obtained with this membrane can be reused for the rinsing of the ultrafiltration membranes, during the second stage of the integrated process.

4. CONCLUSIONS

An integrated membrane process to purify and concentrate the phenolic compounds present in the hydroalcoholic extracts of wet olive pomace was developed. First, the phenolic compounds were extracted by an ultrasound-assisted, solid-liquid extraction, performed with ethanol/water 50:50 (v/v). Therefore, all the subsequent stages occurred in organic media. The extract was treated by ultrafiltration, in order to reduce the total solids content and separate the phenolic compounds from sugars. For this ultrafiltration, the UF010104 and the UP005 membranes were tested. The UP005 membrane displayed a higher rejection of the unwanted compounds, whereas the passage of phenolic compounds was high. Therefore, the UP005 membrane was preferred. Once the phenolic compounds were purified during the ultrafiltration stage, they were fractionated by means of a nanofiltration process (employing the NF270 membrane), which permitted to recover hydroxytyrosol and phenolic acids (such as vanillic and ferulic acids) in the nanofiltration permeate. Furthermore, additional sugars and total solids were largely retained, enlarging the purity of the permeate stream. Selecting the stream of interest during the nanofiltration process will also be dependent on the final application of the compounds. The permeate stream contains the purest molecules (phenolic acids, in this case), which should be later concentrated, but the retentate contains a wide range of already concentrated compounds (simple phenols, secoiridoids, lignans, and flavonoids) whose antioxidant properties are not minor.

Finally, to concentrate the permeate of the nanofiltration stage, reverse osmosis was proposed, employing the NF90 membrane. This process allowed to recover the purified phenolic compounds at a higher concentration in the retentate, which constituted a valuable stream, enriched in high added-value compounds.

Funding: This research was funded by Generalitat Valencia, grant number CIAICO/2021/333. Additionally, a predoctoral grant PRE2018-08524 was funded by the Spanish “Ministerio de Ciencia e Innovación (MCIN)”/Agencia Estatal de Investigación (AEI)/ 10.13039/501100011033 and by European Social Fund (ESF) Investing in your future.

5. REFERENCES

[1] M.S. Brewer, Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications, Compr. Rev. Food Sci. Food Saf. 10 (2011) 221–247. https://doi.org/10.1111/j.1541-4337.2011.00156.x.

[2] V. Sindhi, V. Gupta, K. Sharma, S. Bhatnagar, R. Kumari, N. Dhaka, Potential applications of antioxidants – A review, J. Pharm. Res. 7 (2013) 828–835. https://doi.org/10.1016/J.JOPR.2013.10.001.

[3] P. Darvishzadeh, V. Orsat, J.L. Martinez, Correction to: Process Optimization for Development of a Novel Water Kefir Drink with High Antioxidant Activity and Potential Probiotic Properties from Russian Olive Fruit (Elaeagnus angustifolia) (Food and Bioprocess Technology, (2021), 14, 2, (248-260), Food Bioprocess Technol. 14 (2021) 261. https://doi.org/10.1007/s11947-021-02583-5.

[4] L. Olmo-García, N. Kessler, H. Neuweger, K. Wendt, J.M. Olmo-Peinado, A. Fernández-Guti rrez, C. Baessmann, A. Carrasco-Pancorbo, Unravelling the distribution of secondary metabolites in olea europaea l.: exhaustive characterization of eight olive-tree derived matrices by complementary platforms (LC-ESI/APCI-MS and GC-APCI-MS), Molecules. 23 (2018) 1–16. https://doi.org/10.3390/molecules23102419.

[5] L. Panzella, F. Moccia, R. Nasti, S. Marzorati, L. Verotta, A. Napolitano, Bioactive Phenolic Compounds From Agri-Food Wastes: An Update on Green and Sustainable Extraction Methodologies, Front. Nutr. 7 (2020) 60. https://doi.org/10.3389/FNUT.2020.00060/BIBTEX.

[6] A. Tresserra-Rimbau, R.M. Lamuela-Raventós, Olives and Olive Oil: A Mediterranean Source of Polyphenols, in: Olives Olive Oil as Funct. Foods, John Wiley & Sons, Ltd, Chichester, UK, 2017: pp. 417–434. https://doi.org/10.1002/9781119135340.ch22.

[7] S. Dermeche, M. Nadour, C. Larroche, F. Moulti-Mati, P. Michaud, Olive mill wastes: Biochemical characterizations and valorization strategies, Process Biochem. 48 (2013) 1532–1552. https://doi.org/10.1016/j.procbio.2013.07.010.

[8] L. Olmo-García, K. Wendt, N. Kessler, A. Bajoub, A. Fernández-Gutiérrez, C. Baessmann, A. Carrasco-Pancorbo, Exploring the capability of LC-MS and GC-MS multi-class methods to discriminate virgin olive oils from different geographical indications and to identify potential origin markers, Eur. J. Lipid Sci. Technol. 121 (2019) 1–13. https://doi.org/10.1002/ejlt.201800336.

[9] H. Jebabli, H. Nsir, A. Taamalli, I. Abu-Reidah, F.J. Álvarez-Martínez, M. Losada-Echeberria, E.B. Catalán, R. Mhamdi, Industrial-Scale Study of the Chemical Composition of Olive Oil Process-Derived Matrices, Process. 2020, Vol. 8, Page 701. 8 (2020) 701. https://doi.org/10.3390/PR8060701.

[10] M. Moorthy, N. Chaiyakunapruk, S.A. Jacob, U.D. Palanisamy, Prebiotic potential of polyphenols, its effect on gut microbiota and anthropometric/clinical markers: A systematic review of randomised controlled trials, Trends Food Sci. Technol. 99 (2020) 634–649. https://doi.org/10.1016/j.tifs.2020.03.036.

[11] M. Robles-Almazan, M. Pulido-Moran, J. Moreno-Fernandez, C. Ramirez-Tortosa, C. Rodriguez-Garcia, J.L. Quiles, Mc. Ramirez-Tortosa, Hydroxytyrosol:

Bioavailability, toxicity, and clinical applications, Food Res. Int. 105 (2018) 654–667. https://doi.org/10.1016/j.foodres.2017.11.053.

[12] J. Madureira, F.M.A. Margaça, C. Santos-Buelga, I.C.F.R. Ferreira, S.C. Verde, L. Barros, Applications of bioactive compounds extracted from olive industry wastes: A review, Compr. Rev. Food Sci. Food Saf. 21 (2022) 453–476. https://doi.org/10.1111/1541-4337.12861.

[13] R. Avila-Sosa, M.G. Gastélum-Franco, A. Camacho-Dávila, J.V. Torres-Muñoz, G.V. Nevárez-Moorillón, Extracts of Mexican oregano (Lippia berlandieri Schauer) with antioxidant and antimicrobial activity, Food Bioprocess Technol. 3 (2010) 434–440. https://doi.org/10.1007/s11947-008-0085-7.

[14] C.M. Sánchez-Arévalo, A. Iborra-Clar, M.C. Vincent-Vela, S. Álvarez-Blanco, Exploring the extraction of the bioactive content from the two-phase olive mill waste and further purification by ultrafiltration, LWT - Food Sci. Technol. 165 (2022) 113742. https://doi.org/10.1016/j.lwt.2022.113742.

[15] C.M. Sánchez-Arévalo, M.C. Vincent-Vela, M.J. Luján-Facundo, S. Álvarez-Blanco, Ultrafiltration with organic solvents: a review on achieved results, membrane materials and challenges to face, Process Saf. Environ. Prot. (2023). https://doi.org/10.1016/J.PSEP.2023.06.073.

[16] P. Vandezande, L.E.M. Gevers, I.F.J. Vankelecom, Solvent resistant nanofiltration: Separating on a molecular level, Chem. Soc. Rev. 37 (2008) 365–405. https://doi.org/10.1039/b610848m.

[17] M.A. Nunes, S. Pawlowski, A.S.G. Costa, R.C. Alves, M.B.P.P. Oliveira, S. Velizarov, Valorization of olive pomace by a green integrated approach applying sustainable extraction and membrane-assisted concentration, Sci. Total Environ. 652 (2019) 40–47. https://doi.org/10.1016/j.scitotenv.2018.10.204.

[18] P. Tapia-Quirós, M.F. Montenegro-Landívar, M. Reig, X. Vecino, J. Saurina, M. Granados, J.L. Cortina, Integration of membrane processes for the recovery and separation of polyphenols from winery and olive mill wastes using green solvent-based processing, J. Environ. Manage. 307 (2022). https://doi.org/10.1016/J.JENVMAN.2022.114555.

[19] V. Sygouni, A.G. Pantziaros, I.C. Iakovides, E. Sfetsa, P.I. Bogdou, E.A. Christoforou, C.A. Paraskeva, Treatment of two-phase olive mill wastewater and recovery of phenolic compounds using membrane technology, Membranes (Basel). 9 (2019) 27. https://doi.org/10.3390/membranes9020027.

[20] C.M. Sánchez-Arévalo, T. Croes, B. Van der Bruggen, M.C. Vincent-Vela, S. Álvarez-Blanco, Feasibility of several commercial membranes to recover valuable phenolic compounds from extracts of wet olive pomace through organic-solvent nanofiltration, Sep. Purif. Technol. 305 (2023).

https://doi.org/10.1016/j.seppur.2022.122396.

[21] C.M. Sánchez-Arévalo, A. Iborra-Clar, M.C. Vincent-Vela, S. Álvarez-Blanco, Solvent-resistant ultrafiltration to recover bioactive compounds from wet olive pomace extracts, LWT - Food Sci. Technol. Under Rev. (2023).

[22] A. Santafé-Moros, J.M. Gonzálvez-Zafrilla, D. Valencia, Design of a Flat Membrane Module for Fouling and Permselectivity Studies, COMSOL Conf. 2010 Paris. (2010). http://www.comsol.asia/papers/8300/download/gozalvez-zafrilla_paper.pdf.

[23] UNE-EN ISO 7887:2012 Method B, Water Quality. Examination and Determination of Colour, 2012.

[24] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics acids with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.

[25] R. Dreywood, Qualitative Test for Carbohydrate Material, Ind. Eng. Chem. - Anal. Ed. 18 (1946) 499. https://doi.org/10.1021/I560156A015.

[26] C.M. Sánchez-Arévalo, A. Pérez García-Serrano, M.C. Vincent-Vela, S. Álvarez-Blanco, Combining Ultrafiltration and Nanofiltration to Obtain a Concentrated Extract of Purified Polyphenols from Wet Olive Pomace, Membranes (Basel). 13 (2023) 119. https://doi.org/10.3390/membranes13020119.

[27] M. Ji, P.K. Roy, J. Barman, K. Khare, Commercial polysulfone membranes pretreated with ethanol and NaOH: Effects on permeability, selectivity and antifouling properties, Langmuir. 35 (2019) 7672–7679. https://doi.org/10.1021/acs.langmuir.9b00483.

[28] C.M. Sánchez-Arévalo, Á. Jimeno-Jiménez, C. Carbonell-Alcaina, M.C. Vincent-Vela, S. Álvarez-Blanco, Effect of the operating conditions on a nanofiltration process to separate low-molecular-weight phenolic compounds from the sugars present in olive mill wastewaters, Process Saf. Environ. Prot. 148 (2021) 428–436. https://doi.org/10.1016/j.psep.2020.10.002.

[29] D.F. Tirado, E. de la Fuente, L. Calvo, A selective extraction of hydroxytyrosol rich olive oil from alperujo, J. Food Eng. 263 (2019) 409–416. https://doi.org/10.1016/j.jfoodeng.2019.07.030.

[30] C.O. de Oliveira, A.A.P. Roll, F.M. Medeiros Gonçalves, D.C.N. Lopes, E.G. Xavier, Olive pomace for the feeding of commercial poultry: effects on performance, meat and eggs quality, haematological parameters, microbiota and immunity, Worlds. Poult. Sci. J. 77 (2021) 363–376. https://doi.org/10.1080/00439339.2021.1894409.

[31] F. Bazzarelli, E. Piacentini, T. Poerio, R. Mazzei, A. Cassano, L. Giorno, Advances in membrane operations for water purification and biophenols recovery/valorization from OMWWs, J. Memb. Sci. 497 (2016) 402–409.

https://doi.org/10.1016/j.memsci.2015.09.049.

[32] B.M. Xaba, S.J. Modise, B.J. Okoli, M.E. Monapathi, S. Nelana, Characterization of Selected Polymeric Membranes Used in the Separation and Recovery of Palladium-Based Catalyst Systems, Membr. 2020, Vol. 10, Page 166. 10 (2020) 166. https://doi.org/10.3390/MEMBRANES10080166.

[33] D. Tamires Vitor Pereira, G. Vollet Marson, G. Fernández Barbero, A. Gadioli Tarone, C. Baú Betim Cazarin, M. Dupas Hubinger, J. Martínez, Concentration of bioactive compounds from grape marc using pressurized liquid extraction followed by integrated membrane processes, Sep. Purif. Technol. 250 (2020). https://doi.org/10.1016/j.seppur.2020.117206.

[34] K. Cliff, A study of membrane swelling and transport mechanisms in solvent resistant nanofiltration, Loughborough University, 2011. https://repository.lboro.ac.uk/articles/thesis/A_study_of_membrane_swelling_and_transport_mechanisms_in_solvent_resistant_nanofiltration/9238769/2.

CONCLUSIONES

CONCLUSIONS

En la presente Tesis Doctoral se ha investigado la valorización de uno de los principales subproductos de la industria oleícola, como es el alperujo, a través de la recuperación de los compuestos fenólicos contenidos en esta matriz, mediante la combinación de extracción sólido-líquido y Tecnologías de Membrana. A continuación, se recogen las principales conclusiones alcanzadas:

- Se ha optimizado un proceso de extracción sólido-líquido asistida por ultrasonidos, para extraer los compuestos fenólicos presentes en el alperujo, en una sola etapa. La mayor cantidad de compuestos fenólicos extraídos, una media de 9204 ± 110 $mg \cdot kg^{-1}$, se obtuvo cuando se empleó una mezcla etanol/agua 50:50 (v/v), en una proporción 1:10 (gramos de residuo sólido/mL de disolvente) y se aplicó una temperatura de 40˚C, durante 45 – 60 minutos. Además, también se extrajo una elevada cantidad de compuestos fenólicos, 6588 ± 126 $mg \cdot kg^{-1}$, cuando se empleó agua como agente extractante, manteniendo el resto de condiciones de operación.

- Se ha desarrollado una metodología analítica multiclase, basada en cromatografía líquida acoplada a espectrometría de masas, que permite la determinación simultánea de más de 50 compuestos, pertenecientes a la fracción minoritaria de la oliva, en un mismo análisis. Así, se ha llevado a cabo una caracterización profunda y detallada de todos los extractos obtenidos, así como de las corrientes derivadas de todos los procesos de membrana empleados. Los compuestos determinados pertenecen a 8 familias químicas diferentes, entre las que se encuentran los fenoles simples (o alcoholes fenólicos), los ácidos fenólicos y aldehídos, los secoiridoides, los lignanos, los flavonoides, los ácidos triterpénicos, los ácidos orgánicos y los ácidos grasos libres. Entre estos analitos, se detectaron compuestos de elevado interés, como el hidroxitirosol, el ácido ferúlico, la oleuropeína y la luteolina.

- La composición, en cuanto a sólidos totales y azúcares totales, del extracto hidroalcohólico, fue de 7 ± 1 $g \cdot L^{-1}$ y 870 ± 29 $mg \cdot L^{-1}$, respectivamente. En el caso del extracto acuoso, se determinó una concentración de sólidos totales y azúcares totales de 9 ± 1 $g \cdot L^{-1}$ y 1007 ± 70 $mg \cdot L^{-1}$, respectivamente. En ambos extractos, los secoiridoides fueron los compuestos fenólicos mayoritarios. Comparando ambos extractos, el extracto hidroalcohólico presentó una mayor proporción de fenoles simples y flavonoides, mientras que en el extracto acuoso se determinó una mayor proporción de ácidos orgánicos y ácidos grasos libres. En ambos casos, los compuestos fenólicos no fueron los únicos solutos extraídos. Por lo tanto, fue

necesario tratar ambos extractos en etapas posteriores, para aumentar la pureza de los compuestos fenólicos de interés.

- La extracción llevada a cabo con el disolvente orgánico logró una mayor recuperación de compuestos fenólicos, lo que resultó más ventajoso, considerando la potencial aplicación de estas moléculas. Sin embargo, el procesamiento de los extractos hidroalcohólicos mediante tecnología de membranas es más complejo, debido a que el disolvente puede modificar el comportamiento de las membranas orgánicas, afectando a la densidad de flujo de permeado y a los rechazos obtenidos. Por el contrario, la extracción realizada con agua como extractante resultó en una concentración de compuestos fenólicos más baja, pero el medio acuoso favorece el posterior tratamiento del extracto a través de procesos de membrana. Por lo tanto, debido al interés de ambas estrategias, no se descartó ninguna de las alternativas y se desarrollaron dos procedimientos completos, independientes, para tratar tanto los extractos hidroalcohólicos de alperujo como los extractos acuosos de alperujo.

Las siguientes conclusiones se derivan del tratamiento, mediante tecnología de membranas, de los **extractos acuosos de alperujo**, obtenidos en presencia de ultrasonidos, durante 45 minutos, a 45˚C, con una proporción muestra/agua de 1:10 (gramos de residuo sólido/mL de disolvente).

- El estudio inicial de la ultrafiltración de los extractos acuosos de alperujo se realizó manteniendo constante la concentración de los compuestos presentes en la corriente de alimento. Las membranas UH050 y UP150 (Microdyn Nadir) fueron descartadas, debido a su baja selectividad y a su elevado ensuciamiento, respectivamente. Por el contrario, las membranas UP005 y UH030 (Microdyn Nadir) ofrecieron los mejores resultados, ya que ambas demostraron un alto rechazo a la materia orgánica no deseada, tal y como reflejaron los elevados rechazos al color, sólidos totales y demanda química de oxígeno. En el caso de la membrana UH030, estos rechazos fueron 90 ± 1% – 95 ± 1%, 34.8 ± 0.1% – 55 ± 1% y 34 ± 1% – 52 ± 1%, respectivamente. En el caso de la membrana UP005, estos rechazos fueron 64 ± 1% – 97.5 ± 0.2%, 26 ± 4% - 76 ± 4 % y 33 ± 1% - 71 ± 2%, respectivamente. Los compuestos fenólicos fueron obtenidos en el permeado de ambas membranas, ya que los rechazos a estas moléculas fueron bajos. De hecho, el rechazo a los fenoles simples y a los ácidos fenólicos y aldehídos alcanzado con la membrana UP005 no superó el 23 ± 2% (a partir de 2.5 bar). En el caso de la membrana UH030, los secoiridoides, junto con los ácidos fenólicos y aldehídos fueron los compuestos fenólicos menos rechazados (5 ± 1% - 21 ± 3%). La membrana UH030 presentó una

mayor productividad, a través de mayores valores de densidad de flujo de permeado (31 ± 2 $L \cdot h^{-1} \cdot m^{-2}$ – 39 ± 2 $L \cdot h^{-1} \cdot m^{-2}$), que la membrana UP005 (8 ± 2 $L \cdot h^{-1} \cdot m^{-2}$ – 31 ± 2 $L \cdot h^{-1} \cdot m^{-2}$). No obstante, los resultaros indicaron un mayor ensuciamiento de la membrana UH030 y, además, la selectividad de la membrana UP005 fue mayor, resultando en una purificación más eficiente de los compuestos fenólicos.

- Con el objetivo de examinar la viabilidad de la nanofiltración para tratar las corrientes derivadas de los subproductos de la almazara, se evaluó el comportamiento de la membrana NF270 (DuPont) para separar los compuestos fenólicos de bajo peso molecular de los azúcares presentes en una disolución simulada, conteniendo sacarosa y tirosol. Este último se obtuvo en el permeado (con rechazos inferiores al 23.9 ± 0.7%), mientras que la sacarosa fue retenida, como reflejó el rechazo a la demanda química de oxígeno, que alcanzó el 89.9 ± 0.5%, a las condiciones de operación de 10 bar y 1.5 $m \cdot s^{-1}$. Además, se aplicó con éxito el modelo de Kedem-Spiegler para predecir la densidad de flujo de permeado, la cual alcanzó 227.6 ± 0.7 $L \cdot h^{-1} \cdot m^{-2}$, a 1.5 $m \cdot s^{-1}$ y 15 bar. El error entre los valores predichos por el modelo y los valores experimentales no superó el 4.7%.

- Teniendo en cuenta los resultados alcanzados durante la etapa de ultrafiltración y el proceso de nanofiltración estudiado, se propuso un proceso integrado, consistente en la extracción sólido-líquido (asistida por ultrasonidos) de los compuestos fenólicos del alperujo en medio acuoso, su purificación mediante ultrafiltración y su posterior concentración mediante nanofiltración. Para la etapa de ultrafiltración, que se llevó a cabo a 1.5 $m \cdot s^{-1}$ y 2.5 bar, se emplearon las membranas UH030 y UP005, que habían demostrado previamente resultados satisfactorios. Sin embargo, a medida que aumentaba la concentración en la alimentación, la membrana UH030 sufrió un ensuciamiento severo, por lo que se seleccionó la membrana UP005. Con esta membrana se obtuvieron valores adecuados en cuanto a la densidad de flujo de permeado (18.1 ± 0.4 $L \cdot h^{-1} \cdot m^{-2}$) y a los rechazos. La membrana UP005 rechazó más del 70 ± 8% de la demanda química de oxígeno, mientras que en el permeado se obtuvieron compuestos fenólicos de alto valor añadido, entre los que destacó el ácido vanílico, el ácido cafeico o el hidroxitirosol. Esta corriente de permeado se trató a continuación con la membrana NF270. Se obtuvieron rechazos a los compuestos fenólicos superiores al 88%, trabajando a 9 bar, por lo que se llevó a cabo la concentración efectiva de los compuestos fenólicos previamente purificados.

Las siguientes conclusiones se derivan del tratamiento, mediante tecnología de membranas, de los **extractos hidroalcohólicos de alperujo**, obtenidos en presencia de ultrasonidos, durante 45 minutos, a 45˚C, con una proporción muestra/(etanol/agua 50:50 (v/v)) de 1:10 (gramos de residuo sólido/mL de disolvente).

- Se estudió, en primer lugar, el pretratamiento de las membranas UF010104, UF010801v3 (SolSep BV) y UP005, seleccionando, como mejor acondicionamiento, su inmersión directa en etanol/agua 50:50 (v/v) durante dos horas. A continuación, se estudió la viabilidad de las tres membranas para recuperar, con mayor pureza, los compuestos fenólicos presentes en los extractos hidroalcohólicos del alperujo, mediante ultrafiltración en medio orgánico. El ensuciamiento de las membranas fue notable, especialmente en el caso de la membrana UF010104. A la vista de los resultados, se formó una capa gel sobre está membrana que impidió aumentar la densidad de flujo de permeado por encima de 10 ± 2 $L \cdot h^{-1} \cdot m^{-2}$ (a 2.5 bar), a pesar de aumentar la presión transmembranal. Las tres membranas permitieron recuperar los compuestos fenólicos en el permeado, sin embargo, el mayor grado de purificación se alcanzó con la membrana UP005, que rechazó los sólidos totales en un 76 ± 3%, mientras que los compuestos fenólicos fueron obtenidos en el permeado de la membrana.

- Para abordar el fraccionamiento y la purificación de los compuestos fenólicos obtenidos en el permeado de la ultrafiltración, se estudió un proceso de nanofiltración en medio orgánico, empleando una disolución modelo, basada en el permeado obtenido con la membrana UP005. Las membranas oNF-1, o-NF-2 (GMT-Borsig), Puramem®600 y Duramem®150 (Evonik) fueron descartadas, debido a los bajos valores de densidad de flujo de permeado que fueron obtenidos. Entre el resto de membranas, Duramem®300, Duramem®500 (Evonik), NFX, NFS (Synder) y NF270, destacó la membrana NF270, debido a su elevada densidad de flujo de permeado (96 ± 4 $L \cdot h^{-1} \cdot m^{-2}$, a 36 bar) y a los rechazos obtenidos. Esta membrana permitió una mayor purificación de los compuestos fenólicos, ya que posibilitó la separación de azúcares y otros ácidos no deseados en la corriente de rechazo. Además, se llevó a cabo el fraccionamiento de los compuestos fenólicos, debido a que las moléculas de menor peso molecular (tirosol, hidroxitirosol y ácido cafeico) se recuperaron en el permeado (fueron rechazados en un 2.25 ± 0.09% - 22 ± 6%), mientras que los polifenoles de mayor tamaño (oleuropeína, decarboximetil oleuropeína aglicona y luteolina) fueron rechazados por encima del 82% y se obtuvieron en el rechazo de la membrana.

De acuerdo con los resultados obtenidos previamente, se propuso un proceso integrado en medio orgánico, consistente en la extracción sólido-líquido (asistida por ultrasonidos) de los compuestos fenólicos del alperujo, empleando etanol/agua 50:50 (v/v) como disolvente, seguida de una etapa de ultrafiltración, nanofiltración y ósmosis reversa. Durante la etapa de ultrafiltración, se seleccionó la membrana UP005 (1.8 $m \cdot s^{-1}$, 2.5 bar), que rechazó eficazmente el color (97 ± 3%), los azúcares totales (79.2 ± 0.4%) y los sólidos totales (60 ± 1%). La mayoría de los compuestos fenólicos fueron recuperados en el permeado de la membrana, ya que fueron rechazados en un 1.4 ± 0.2% - 50 ± 5%, exceptuando a algunos secoiridoides, cuyo rechazo superó el 53.3 ± 0.6%. Posteriormente, el permeado obtenido con la membrana UP005 fue tratado mediante nanofiltración, empleando la membrana NF270, a 1 $m \cdot s^{-1}$ y 15 bar. Se obtuvo una densidad de flujo de permeado de 50.2 ± 0.2 $L \cdot h^{-1} \cdot m^{-2}$, así como unos valores de rechazo satisfactorios. La membrana permitió el fraccionamiento de los compuestos fenólicos. Algunos ácidos fenólicos de interés, así como el hidroxitirosol, fueron obtenidos en el permeado (fueron rechazados en un 8.8 ± 0.5% – 45 ± 1%), mientras que los compuestos fenólicos de mayor tamaño se concentraron en la corriente de rechazo (rechazados en un 57.3 ± 0.5% – 99 ± 1%). A continuación, el permeado de la nanofiltración fue sometido a un proceso de ósmosis inversa, empleando la membrana NF90 (DuPont), a 1 $m \cdot s^{-1}$ y 20 bar. La densidad de flujo de permeado obtenida fue 15.3 ± 0.3 $L \cdot h^{-1} \cdot m^{-2}$. La membrana rechazó los compuestos fenólicos por encima del 90%, favoreciendo su concentración en la corriente de rechazo, una vez que habían sido previamente purificados.

In the Doctoral Thesis reported in this dissertation, the valorization of one of the main by-products from the olive industry, such as wet olive pomace (or *alperujo*, by its name in Spanish), was investigated. To that end, the recovery of phenolic compounds by means of the combination of solid-liquid extraction and Membrane Technology was explored. This section contains the main conclusions reached:

- An ultrasound-assisted solid-liquid extraction process was optimized, in order to extract the phenolic compounds from wet olive pomace in a single step. Most of the extracted phenolic compounds, 9204 ± 110 $mg \cdot kg^{-1}$ on average, were obtained when a mixture of ethanol/water 50:50 (v/v) was employed, in a 1:10 proportion (grams of solid residue/mL of extractant) and a temperature of 40˚C was applied during 45 - 60 minutes. Furthermore, a high average amount of phenolic compounds, 6588 ± 126 $mg \cdot kg^{-1}$, was extracted when water was employed as the extractant agent, maintaining the rest of the operating conditions.

- A multi-class analytic methodology, based on liquid chromatography coupled to mass spectrometry was developed. It allows the simultaneous determination of more than 50 compounds, belonging to the olive minor fraction, within the same analysis. Thus, a deep and thoughtful characterization of all the obtained extracts and all the streams derived from the applied membrane processes was conducted. The determined compounds belong to 8 different chemical families, including simple phenols (or phenolic alcohols), phenolic acids and aldehydes, secoiridoids, lignans, flavonoids, triterpenic acids, organic acids, and free fatty acids. Among these analytes, compounds of high interest were detected, such as hydroxytyrosol, ferulic acid, oleuropein, and luteolin.

- The composition of the hydroalcoholic extract, regarding total solids and total sugars, was 7 ± 1 $g \cdot L^{-1}$ y 870 ± 29 $mg \cdot L^{-1}$, respectively. In the case of the aqueous extract, a concentration of total solids and total sugars of 9 ± 1 $g \cdot L^{-1}$ and 1007 ± 70 $mg \cdot L^{-1}$, respectively, was determined. In both extracts, the secoiridoids were the predominant phenolic compounds. Comparing both extracts, the hydroalcoholic extract presented a higher proportion of simple phenols and flavonoids, whereas the aqueous extract contained a higher proportion of organic acids and free fatty acids. In both cases, the phenolic compounds were not the only extracted solutes. Therefore, it was necessary to treat both extracts in subsequent stages, in order to increase the purity of the phenolic compounds of interest.

- The organic-solvent extraction procured a higher recovery of phenolic compounds, which was more convenient, considering the potential application of these

molecules. Nevertheless, the processing of the hydroalcoholic extract by membrane technology is more complex, because the solvent may modify the performance of the membranes, altering the permeate flux and the obtained rejection values. On the contrary, the extraction performed with water as extractant resulted in a lower concentration of phenolic compounds, but the aqueous medium favors the consecutive treatment of the extract by means of membrane processes. Accordingly, due to the interest of both strategies, any of these alternatives was discarded and two complete and independent processes were developed, in order to treat both the hydroalcoholic and the aqueous extracts of wet olive pomace.

The following conclusions are derived from the treatment, by means of membrane technology, of the **aqueous extracts of wet olive pomace**, obtained by sonication, during 45 minutes at 45˚C, and a sample/solvent proportion of 1:10 (grams of solid residue/mL of solvent).

- During the initial study of the ultrafiltration of the aqueous extracts of wet olive pomace, the concentration of compounds in the feed stream was maintained constant. The UH050 and UP150 membranes (Microdyn Nadir) were discarded, due to their low selectivity and high fouling, respectively. On the contrary, the UP005 and UH030 membranes (Microdyn Nadir) displayed the best results, because both membranes exhibited a high rejection of the unwanted organic matter, as demonstrated by the high rejection values of the color, total solids, and chemical oxygen demand. In the case of the UH030 membrane, these rejection values were 90 ± 1% – 95 ± 1%, 34.8 ± 0.1% - 55 ± 1%, and 34 ± 1% – 52 ± 1%, respectively. In the case of the UP005 membrane, these rejection values were 64 ± 1% – 97.5 ± 0.2%, 26 ± 4% - 76 ± 4 %, and 33 ± 1% - 71 ± 2%, respectively. The phenolic compounds were obtained in the permeate of both membranes, because the rejection of these molecules was low. In fact, the rejection of simple phenols and phenolic acids and aldehydes achieved with the UP005 membrane did not surpass 23 ± 2% (from 2.5 bar). In the case of the UH030 membrane, the secoiridoids and the phenolic acids and aldehydes were the compounds rejected to a lesser extent (5 ± 1% - 21 ± 3%). The UH030 membranes showed a higher productivity, because of higher values of permeate flux (31 ± 2 $L \cdot h^{-1} \cdot m^{-2}$ – 39 ± 2 $L \cdot h^{-1} \cdot m^{-2}$) in comparison with the UP005 membrane (8 ± 2 $L \cdot h^{-1} \cdot m^{-2}$ – 31 ± 2 $L \cdot h^{-1} \cdot m^{-2}$). However, the results indicated a more severe fouling for the UH030 and, furthermore, the selectivity of the UP005 membrane was greater, resulting in a more efficient purification of phenolic compounds.

- Aiming to examine the feasibility of nanofiltration to treat the streams derived from the by-products from the olive mill, it was evaluated the performance of the NF270 membrane (DuPont) to separate the low-molecular-weight phenolic compounds from the sugars present in a simulated solution, containing sucrose and tyrosol. The latter was obtained in the permeate (with rejection values below 23.9 ± 0.7%), whereas sucrose was retained, as reflected by the rejection of the chemical oxygen demand, which reached 89.9 ± 0.5%, at the operating conditions of 10 bar and 1.5 $m \cdot s^{-1}$. Moreover, the Kedem-Spiegler model was successfully applied to predict the permeate flux, which reached 227.6 ± 0.7 $L \cdot h^{-1} \cdot m^{-2}$ at 1.5 $m \cdot s^{-1}$ and 15 bar. The error between the predicted values and the experimental data did not surpass 4.7%.

- Considering the results achieved during the ultrafiltration stage and the studied nanofiltration process, an integrated process was proposed. It consisted of an aqueous ultrasound-assisted solid-liquid extraction of the phenolic compounds from wet olive pomace, followed by their purification by ultrafiltration and their subsequent concentration by nanofiltration. For the ultrafiltration stage, which was conducted at 1.5 $m \cdot s^{-1}$ and 2.5 bar, the UH030 and UP005 membranes were employed, as they had previously displayed satisfactory results. Nevertheless, as the concentration in the feed stream increased, the fouling of the UH030 membrane became more severe. Therefore, the UP005 membrane was selected. With this membrane, suitable values of permeate flux (18.1 ± 0.4 $L \cdot h^{-1} \cdot m^{-2}$) and rejection were obtained. The UP005 membrane rejected more than 70 ± 8% of the chemical oxygen demand, whereas high-added-value compounds, such as vanillic acids, caffeic acid, and hydroxytyrosol were obtained in the permeate. Later, this permeate stream was treated with the NF270 membrane. The obtained rejection of the phenolic compounds exceeded 88%, at 9 bar, then achieving an effective concentration of the previously purified phenolic compounds.

The following conclusions are derived from the treatment, by means of membrane technology, of the **hydroalcoholic extracts of wet olive pomace**, obtained by sonication, during 45 minutes at 45˚C, and a sample/solvent proportion of 1:10 (grams of solid residue/mL of solvent).

- First, the pretreatment of the UF010104, UF010801v3 (SolSep BV) and UP005 membranes was studied. The direct immersion of the membranes in ethanol/water 50:50 (v/v), during 2 hours was selected as the best conditioning. Then, the viability of the membranes to perform an organic-solvent ultrafiltration of the hydroalcoholic extracts of wet olive pomace was studied, aiming to recover the

phenolic compounds at a higher purity. The membrane fouling was notable, especially in the case of the UF010104 membrane. The obtained results indicate that a gel layer was formed on the membrane surface, hindering the increment of the permeate flux above 10 ± 2 $L \cdot h^{-1} \cdot m^{-2}$ (at 2.5 bar), despite increasing the transmembrane pressure. The three tested membranes allowed the recovery of the phenolic compounds in the permeate, nevertheless, the highest purification grade was achieved with the UP005 membrane, which displayed a rejection of total solids of 76 ± 3%, whereas the phenolic compounds were obtained in the permeate.

- To assess the fractionation and purification of the phenolic compounds obtained in the ultrafiltration permeate, an organic-solvent nanofiltration was studied, employing a simulated solution based on the permeate obtained with the UP005 membrane. The oNF-1, o-NF-2 (GMT-Borsig), Puramem®600, and Duramem®150 (Evonik) membranes were discarded due to the low permeate flux values obtained. Among the rest of the membranes, Duramem®300, Duramem®500 (Evonik), NFX, NFS (Synder), and NF270, the NF270 membrane stood out, due to the high permeate flux (96 ± 4 $L \cdot h^{-1} \cdot m^{-2}$, at 36 bar) and the obtained rejection values. This membrane permitted a high purification of the phenolic compounds, as it allowed the separation of sugars and other undesired acids in the retentate stream. Moreover, the fractionation of phenolic compounds was conducted, as the molecules of lower molecular weight (tyrosol, hydroxytyrosol, and caffeic acid) were recovered in the permeate (they were rejected in the range 2.25 ± 0.09% - 22 ± 6%), whereas the polyphenols of high molecular weight (oleuropein, decarboxymethyl oleuropein aglycone, and luteolin) were rejected above 82% and they were obtained in the retentate stream.

- According to the previous results, an integrated process in organic medium was proposed. It consisted of an ultrasound-assisted solid-liquid extraction of the phenolic compounds from wet olive pomace, employing ethanol/water 50:50 (v/v) as solvent. This stage was followed by an ultrafiltration, nanofiltration, and reverse osmosis step. During the ultrafiltration stage, the UP005 membrane (1.8 $m \cdot s^{-1}$, 2.5 bar) was selected. It efficiently rejected the color (97 ± 3%), total sugars (79.2 ± 0.4%), and total solids (60 ± 1%). Most of the phenolic compounds were recovered in the membrane permeate, as their rejection values were 1.4 ± 0.2% - 50 ± 5%, except for some secoiridoids, whose rejection surpassed 53.3 ± 0.6%. Afterwards, the UP005 permeate was treated by nanofiltration, employing the NF270 membrane, at 1 $m \cdot s^{-1}$ y 15 bar. A permeate flux of 50.2 ± 0.2 $L \cdot h^{-1} \cdot m^{-2}$ was obtained, as well as satisfactory rejection values. The membrane allowed the

fractionation of phenolic compounds. Some phenolic acids of interest, as well as hydroxytyrosol, were obtained in the permeate (their rejection was 8.8 ± 0.5% – 45 ± 1%), whereas the phenolic compounds of higher molecular weight were concentrated in the retentate stream (their rejection was 57.3 ± 0.5% – 99 ± 1%). Subsequently, the nanofiltration permeate was submitted to a reverse osmosis process, employing the NF90 membrane (DuPont), at 1 $m \cdot s^{-1}$ and 20 bar. The obtained permeate flux was 15.3 ± 0.3 $L \cdot h^{-1} \cdot m^{-2}$. The phenolic compounds, which had already been purified, were rejected by the membrane above 90%, favoring their concentration in the retentate stream.