UNIVERSIDAD DE EXTREMADURA

TESIS DOCTORAL

CARACTERIZACIÓN DE LA CAPACIDAD TECNOLÓGICA Y FUNCIONAL DE LA FIBRA DIETÉTICA EXTRAÍDA DE SUBPRODUCTOS VEGETALES

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PROGRAMA DE DOCTORADO EN CIENCIA DE LOS ALIMENTOS

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"El logro de grandes cosas se ve facilitado por aquellos que hacen pequeñas cosas con gran amor."

Teresa de Calcuta

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RESUMEN

A nivel mundial, se generan millones de toneladas de **residuos agrícolas** ricos en **fibra dietética** que contienen también otros componentes funcionales como polisacáridos solubles, compuestos fenólicos y ácidos grasos. En Extremadura, estos residuos, provenientes de **industrias como la viticultura, la granada, el tomate y el brócoli**, representan valiosas fuentes de aditivos e ingredientes funcionales. Por ejemplo, el orujo de uva, los subproductos del procesamiento de la granada y del tomate, así como las partes no comerciales del brócoli, como sus tallos, hojas e inflorescencias, son especialmente ricos en fibra dietética y otros compuestos bioactivos como fenoles, vitamina C y glucosinolatos en el caso de los derivados del brócoli. Estos subproductos agrícolas ofrecen oportunidades para aplicarlos en diversas industrias, incluida la alimentaria, donde pueden utilizarse como aditivos e ingredientes funcionales.

Así, el propósito central de esta Tesis Doctoral es la **revalorización** de estos **subproductos agrícolas generados en Extremadura** como una valiosa fuente de **fibra dietética**. Para lograrlo, se evaluará su composición química, estructura y propiedades funcionales y se emplearán diversas tecnologías para tratar la fibra dietética, como fluidos supercríticos y tratamientos enzimáticos, para optimizar su aprovechamiento. Además, se investigará el impacto de la fibra dietética en aspectos fundamentales como el tránsito intestinal, la supervivencia y el crecimiento de cepas probióticas, así como su digestibilidad *in vitro*. Se explorará también su potencial como alternativa para sustituir azúcares añadidos en alimentos, y se buscará desarrollar nuevos ingredientes alimentarios con propiedades prebióticas o simbióticas.

Desde una perspectiva tecnológica y nutricional, se ha estudiado detalladamente la **composición química y las propiedades funcionales** de los **subproductos industriales de la vinificación**, específicamente los hollejos, los raspones y las lías. Este análisis incluyó la evaluación de las características químicas y físicas, así como la capacidad de retención de grasa y agua, de hinchamiento, antioxidante y el efecto prebiótico de la fibra alimentaria presente en estos subproductos. Los hallazgos indicaron que tanto los hollejos, los raspones como las lías son notablemente ricos en fibra, destacándose la fibra presente en los raspones por contener las mayores cantidades de polifenoles no extraíbles

unidos a polisacáridos, los cuales exhiben una alta actividad antioxidante y un efecto prebiótico significativo. Por otro lado, se observó que la fibra proveniente de las lías mostraba la mayor capacidad de retención de agua y aceite.

En lo que se refiere al **efecto de la fibra dietética** obtenida a partir de subproductos estudiados sobre la **supervivencia en tránsito gastrointestinal, el crecimiento y el metabolismo de cepas probióticas**, los hallazgos revelaron que los subproductos analizados presentaban variabilidad en las cantidades de polisacáridos, los cuales influían de manera diversa en el comportamiento de las cepas de microorganismos probióticos. Destacó que la fibra obtenida de la piel del tomate mostró un efecto protector más pronunciado sobre las cepas probióticas en comparación con las demás fuentes de fibra estudiadas. En cuanto al crecimiento, se observó que los raspones de uva generaron los resultados más favorables, promoviendo especialmente el desarrollo de bacterias lácticas. Por último, todas las fibras evaluadas demostraron capacidad para aumentar el contenido de ácidos grasos de cadena corta en condiciones *in vitro*, aunque los tallos de brócoli y la cáscara de granada mostraron un estímulo particularmente destacado en la producción de estos ácidos grasos.

Otro objetivo de este estudio fue **mejorar la composición y propiedades funcionales** de la fibra dietética de subproductos estudiados mediante **diferentes métodos de degradación y modificación** de esta, como fluidos supercríticos, tratamiento enzimático, autoclave y ultrasonidos. Los resultados obtenidos en los subproductos de brócoli (incluyendo hojas, tallos e inflorescencias) revelaron diferencias significativas en su composición química, destacando un mayor contenido de fibra dietética insoluble y soluble en los tallos. Los tratamientos enzimáticos demostraron reducir el contenido de azúcares neutros y aumentar el de ácidos urónicos, lo que se asoció con un incremento en las actividades funcionales específicas. Concretamente, la aplicación de celulasa y el complejo multienzimático visconzima mejoraron la solubilidad y la capacidad de adsorción de glucosa, mientras que el tratamiento con fluidos supercríticos mejoró las capacidades de hinchamiento, retención de agua y aceite. Se observó que el contenido de compuestos fenólicos no extraíbles fue mayor en las inflorescencias, y aumentó con los tratamientos enzimáticos y de fluidos supercríticos, lo que

resultó en una mayor capacidad antioxidante. Además, el tratamiento enzimático demostró un impacto significativo en la estimulación del crecimiento de las bacterias ácido lácticas estudiadas, alcanzando los valores más altos en la producción de los ácidos grasos de cadena corta analizados.

En relación con la **tecnología de fluidos supercríticos**, se llevaron a cabo ajustes en los parámetros de presión, temperatura y tiempo para la extracción de compuestos bioactivos y la mejora de las propiedades de la fibra dietética residual, utilizando como modelos la cáscara de granada y las hojas de brócoli. Se implementó un diseño Box-Behnken en conjunto con la metodología de superficie de respuesta para optimizar la presión de extracción (250-300 bar), la temperatura (45-55 °C) y el tiempo (2-4 h). Los resultados demostraron que las variaciones en estas condiciones de fluidos supercríticos tuvieron un impacto significativo en las respuestas investigadas. En el caso de la cáscara de granada, las actividades antioxidantes de los extractos, la fibra dietética residual y la pectina fueron los parámetros que mejor se adaptaron al modelo cuadrático desarrollado en este estudio. La optimización de la metodología de superficie de respuesta de estos parámetros en este subproducto se llevó a cabo utilizando la metodología de la función deseada de Derringer, con condiciones óptimas estimadas para la presión de extracción (291 bar), la temperatura (46,5 °C) y el tiempo (2,5 h). Respecto a las hojas de brócoli, los resultados indicaron que el tratamiento de la matriz de fibra dietética con temperaturas y presiones adecuadas (55 °C, 150 bar, 1 hora) durante el proceso supercrítico ocasionó la ruptura de las cadenas moleculares de los polisacáridos y la reducción del peso molecular. En términos de composición, se observó que la fibra dietética analizada estaba mayormente compuesta por ácido galacturónico, y los niveles más altos de modificación se alcanzaron a 55 °C, 300 bar durante 1 hora, con el mayor contenido de azúcares neutros encontrados en la glucosa y la fucosa. En ambos casos, los resultados revelaron un aumento de estos compuestos después del tratamiento con fluidos supercríticos en comparación con el control. En cuanto al impacto de las condiciones de fluidos supercríticos sobre las propiedades de la fibra dietética, se observó una mejora en la capacidad de retención de agua, hinchamiento y adsorción de glucosa de la fibra dietética mediante la aplicación de temperatura y presión con fluidos supercríticos, aunque temperaturas y presiones excesivas causaron daños en la

estructura interna, reduciendo estas propiedades. Respecto al efecto sobre la actividad antioxidante de la fibra dietética, la aplicación de temperatura y presión mediante fluido supercrítico, especialmente con tiempos prolongados, resultó en una mejora de esta propiedad.

Tras el estudio y mejora de las propiedades de la fibra dietética obtenida a partir de subproductos estudiados, el siguiente objetivo a abordar fue analizar el impacto de un **proceso simulado de digestión humana** sobre la composición y las propiedades funcionales de esta **fibra dietética**. Para ello, se utilizó un sistema de digestión simulada controlado por ordenador que constaba de tres biorreactores (que simulaban el estómago, el intestino delgado y el colon). Se investigaron los fenoles no extraíbles asociados a la fibra dietética y su influencia en la capacidad antioxidante y la actividad antiproliferativa a lo largo de las fases digestivas simuladas. Además, se examinaron las modificaciones en la composición de oligosacáridos, la población microbiológica y los ácidos grasos de cadena corta producidos en los medios de digestión. El tipo y la composición de cada fibra dietética influyeron significativamente en sus propiedades funcionales y su comportamiento durante el tránsito intestinal. En particular, la fibra dietética de la cáscara de granada mantuvo su alto contenido en fenoles durante la digestión del colon, lo que podría mejorar la salud intestinal debido a su fuerte actividad antioxidante. Del mismo modo, la fibra dietética del brócoli y la cáscara de granada demostraron efectos antiproliferativos tanto en el intestino delgado como en el grueso, provocando modificaciones significativas en la microbiología colónica. Además, estos tipos de fibra fomentaron el crecimiento de las bifidobacterias por encima de las bacterias ácido lácticas. Así pues, estos resultados sugieren que la fibra dietética de la cáscara de granada parece ser un ingrediente alimentario funcional prometedor para mejorar la salud humana.

Habiendo examinado la composición y las propiedades de la fibra dietética obtenida de subproductos como la cáscara de granada, cáscara de tomate, tallos de brócoli y raspones de uva; y considerando el impacto de diversos métodos de modificación, así como el proceso de digestión en sus características tecnológicas y funcionales, el último objetivo abordado en esta tesis consistió en evaluar el **potencial uso de fibra dietética** proveniente de lías de vino como **sustituto del**

azúcar en salsas de tomate. Este análisis abarcó un estudio exhaustivo de sus propiedades fisicoquímicas, nutricionales, funcionales y sensoriales. Para ello, se prepararon nueve tipos de salsa de tomate: una con azúcar completo (FS) y ocho con azúcar reducido (SR). En cuatro de las salsas SR, se sustituyó el azúcar por fibra solubilizada directamente, mientras que en las otras cuatro se utilizó fibra solubilizada y liofilizada a diferentes niveles para lograr reducciones del 25 % (SR25), 50 % (SR50), 75 % (SR75) y 100 % (SR100) del azúcar. Los resultados indicaron que la incorporación de fibra dietética tuvo un impacto significativo en varias características, como el pH, acidez, sólidos solubles, contenido de azúcar y fibra dietética total. También se observaron cambios en la capacidad antioxidante y el perfil de polifenoles. Las salsas demostraron características funcionales prometedoras, como una mejor capacidad de hinchamiento, retención de agua y aceite. La evaluación sensorial reveló una buena aceptación por parte de los consumidores de las salsas modificadas con fibra dietética, especialmente las que presentaban una reducción del 25 % de azúcar (SR25). Estos hallazgos resaltan el potencial del uso de subproductos como las lías de vino para mejorar la calidad nutricional y funcional de los productos alimenticios, mientras se fomenta la sostenibilidad en la industria alimentaria.

En conjunto, estos resultados sugieren que los **subproductos** estudiados representan una **valiosa fuente de fibra dietétic**a con **características tecnológicas y funcionales** destacadas que, bien modificadas o no, presentan un **gran potencial para su aplicación en la industria alimentaria**.

ABSTRACT

Worldwide, millions of tons of **agricultural residues rich in dietary fiber** are generated, which also contain other functional components such as soluble polysaccharides, phenolic compounds, and fatty acids. In Extremadura, these residues, originating from **industries such as viticulture, pomegranate, tomato, and broccoli**, represent valuable sources of additives and functional ingredients. For example, grape pomace, by-products from pomegranate and tomato processing, as well as non-commercial parts of broccoli, such as stems, leaves, and inflorescences, are especially rich in dietary fiber and other bioactive compounds such as phenols, vitamin C, and glucosinolates in the case of broccoli derivatives. These agricultural by-products offer opportunities for application in various industries, including the food industry, where they can be used as additives and functional ingredients.

Thus, the **central purpose** of this doctoral thesis is the **revaluation** of these **agricultural by-products generated in Extremadura** as a valuable source of **dietary fiber**. To achieve this, their chemical composition, structure, and functional properties will be evaluated, and various technologies will be employed to treat dietary fiber, such as supercritical fluids and enzymatic treatments, to optimize their utilization. Furthermore, the impact of dietary fiber on fundamental aspects such as intestinal transit, survival and growth of probiotic strains, as well as *in vitro* digestibility, will be investigated. Their potential as an alternative to substitute added sugars in foods will also be explored, and efforts will be made to develop new food ingredients with prebiotic or symbiotic properties.

From a technological and nutritional perspective, the **chemical composition and functional properties** of **industrial by-products from winemaking**, specifically grape skins, stems, and lees, have been thoroughly studied. This analysis included the evaluation of chemical and physical characteristics, as well as the capacity for fat and water retention, swelling, antioxidant activity, and the prebiotic effect of dietary fiber present in these by-products. The findings indicated that both grape skins, stems, and lees are remarkably rich in fiber, with grape stem fiber standing out for containing the highest amounts of non-extractable polyphenols bound to polysaccharides, which exhibit high antioxidant activity and a significant prebiotic effect. Furthermore, it

was observed that fiber from lees showed the highest oil and water retention capacity.

Regarding the **effect of dietary fiber** obtained from the studied by-products on **survival in gastrointestinal transit, the growth and metabolism of probiotic strains**, the findings revealed that the analyzed by-products presented variability in the amounts of polysaccharides, which influenced the behavior of probiotic microorganism strains differently. It was noteworthy that the fiber obtained from tomato skin showed a more pronounced protective effect on probiotic strains compared to other fiber sources studied. Regarding growth, grape stems yielded the most favorable results, especially promoting the development of lactic acid bacteria. Finally, all evaluated fibers demonstrated the ability to increase the content of short-chain fatty acids under *in vitro* conditions, although broccoli stems and pomegranate peel showed particularly prominent stimulation in the production of these fatty acids.

Another objective of this study was to **improve the composition and functional properties** of dietary fiber from studied by-products through **different degradation and modification methods**, such as supercritical fluids, enzymatic treatment, autoclave, and ultrasound. The results obtained in broccoli by-products (including leaves, stems, and inflorescences) revealed significant differences in their chemical composition, with higher contents of insoluble and soluble dietary fiber in the stems. Enzymatic treatments were shown to reduce the content of neutral sugars and increase uronic acid content, which was associated with an increase in specific functional activities. Specifically, the application of cellulase and the multi-enzyme complex viscozyme improved solubility and glucose adsorption capacity, while treatment with supercritical fluids improved swelling, water, and oil retention capacities. It was observed that the content of non-extractable phenolic compounds was higher in inflorescences and increased with enzymatic and supercritical fluid treatments, resulting in a higher antioxidant capacity. Additionally, enzymatic treatment demonstrated a significant impact on stimulating the growth of the studied lactic acid bacteria, reaching the highest values in the production of analyzed short-chain fatty acids.

Regarding **supercritical fluid technology**, adjustments were made in pressure, temperature, and time parameters for the extraction of bioactive compounds and improvement of residual dietary fiber properties, using pomegranate peel and broccoli leaves as models. A Box-Behnken design was implemented in conjunction with the response surface methodology to optimize the extraction pressure (250-300 bar), temperature (45-55 °C), and time (2-4 h). The results demonstrated that variations in these supercritical fluid conditions had a significant impact on the investigated responses. In the case of pomegranate peel, the antioxidant activities of the extracts, residual dietary fiber, and pectin were the parameters that best fit the quadratic model developed in this study. The optimization of the response surface methodology of these parameters in this by-product was carried out using the desired Derringer function, with optimal conditions estimated for extraction pressure (291 bar), temperature (46.5 °C), and time (2.5 h). Regarding broccoli leaves, the results indicated that treatment of the dietary fiber matrix with appropriate temperatures and pressures (55 °C, 150 bar, 1 hour) during the supercritical process caused the breakdown of molecular chains of polysaccharides and molecular weight reduction. In terms of composition, it was observed that the analyzed dietary fiber was mostly composed of galacturonic acid, and the highest levels of modification were achieved at 55 °C, 300 bar for 1 hour, with the highest content of neutral sugars found in glucose and fucose. In both cases, the results revealed an increase in these compounds after treatment with supercritical fluids compared to the control. Regarding the impact of supercritical fluid conditions on the properties of dietary fiber, an improvement was observed in the water retention capacity, swelling, and glucose adsorption capacity of dietary fiber through the application of temperature and pressure with supercritical fluids, although excessive temperatures and pressures caused damage to the internal structure, reducing these properties. Regarding the effect on the antioxidant activity of dietary fiber, the application of temperature and pressure through supercritical fluid, especially with prolonged times, resulted in an improvement in this property.

After studying and improving the properties of dietary fiber obtained from studied by-products, the next objective was to analyze the impact of a **simulated human digestion process** on the composition and functional properties of this

dietary fiber. For this purpose, a computer-controlled simulated digestion system consisting of three bioreactors (simulating the stomach, small intestine, and colon) was used. The non-extractable phenols associated with dietary fiber and their influence on antioxidant capacity and antiproliferative activity throughout simulated digestive phases were investigated. Additionally, modifications in the composition of oligosaccharides, microbial population, and short-chain fatty acids produced in digestion media were examined. The type and composition of each dietary fiber significantly influenced its functional properties and behavior during intestinal transit. In particular, dietary fiber from pomegranate peel maintained its high phenol content during colon digestion, which could improve intestinal health due to its strong antioxidant activity. Similarly, dietary fiber from broccoli and pomegranate peel demonstrated antiproliferative effects in both the small and large intestines, causing significant modifications in colonic microbiology. Furthermore, these types of fiber promoted the growth of bifidobacteria over lactic acid bacteria. Thus, these findings suggest that dietary fiber from pomegranate peel appears to be a promising functional food ingredient for improving human health.

Having examined the composition and properties of dietary fiber obtained from by-products such as pomegranate peel, tomato peel, broccoli stems, and grape stems; and considering the impact of various modification methods, as well as the digestion process on their technological and functional characteristics, the final objective addressed in this thesis was to evaluate the **potential use of dietary fiber** from wine lees as a **substitute for sugar** in tomato sauces. This analysis included a comprehensive study of their physicochemical, nutritional, functional, and sensory properties. For this purpose, nine types of tomato sauce were prepared: one with full sugar (FS) and eight with reduced sugar (SR). In four of the SR sauces, sugar was replaced by directly solubilized fiber, while in the other four, solubilized and freeze-dried fiber were used at different levels to achieve reductions of 25 % (SR25), 50 % (SR50), 75 % (SR75), and 100 % (SR100) of sugar. The results indicated that the incorporation of dietary fiber had a significant impact on various characteristics, such as pH, acidity, soluble solids, sugar content, and total dietary fiber. Changes in antioxidant capacity and polyphenol profile were also observed. The sauces demonstrated promising

functional characteristics, such as improved swelling capacity, water and oil retention. Sensory evaluation revealed good acceptance by consumers of sauces modified with dietary fiber, especially those with a 25% reduction in sugar (SR25). These findings highlight the potential use of by-products such as wine lees to improve the nutritional and functional quality of food products while promoting sustainability in the food industry.

Overall, these results suggest that the studied **by-products** represent a **valuable source of dietary fiber** with outstanding **technological and functional characteristics** that, whether modified or not, present **great potential for application in the food industry**.

INTRODUCCIÓN

1.- FIBRA DIETÉTICA

En la actualidad, el consumo de fibra dietética está asociado con una alimentación saludable (Thomas, 2023). Ya en la década de los 70 cuando apenas existían estudios sobre los efectos beneficiosos de la fibra dietética, se generó mucho interés sobre su consumo y se creía que existía relación directa entre su inclusión en la dieta y menor incidencia de enfermedades digestivas y cardiacas (Burkitt y col., 1974). Hoy en día son muchas las evidencias de los efectos beneficiosos que tiene una dieta rica en fibra dietética sobre la salud de los seres humanos. La Unión Europea expone que, la fibra dietética tiene "uno o más efectos fisiológicos beneficiosos tales como disminuir el tiempo de tránsito intestinal, aumentar el volumen de las heces, ser fermentables por la microflora colónica, reducir los niveles de colesterol total y lipoproteínas de baja densidad (LDL) en sangre, reducir la post-glucemia prandial y reducción de los niveles de insulina en sangre" en función de su composición y estructura (Directiva 2008/100/CE). Sin embargo, la ingesta de fibra dietética en la mayoría de los países del mundo se encuentra muy por debajo de la cantidad recomendada. Es por ello que la fibra dietética ha sido catalogada como uno de los cinco "nutrientes de interés" por el Comité Asesor de Pautas Alimentarias de Estados Unidos. (McGuire, 2011).

En cuanto al consumo diario, las recomendaciones generales aconsejan una ingesta de entre 20 a 30 gramos al día para una persona adulta y entre 7 a 20 gramos al día para la población infantil en función de su edad (OMS, 2003). Sin embargo, no existen estudios que avalen cantidades óptimas de fibra dietética en la dietas de niños ni ancianos (Korczak y Slavin, 2020) Por otro lado, ingestas diarias superiores a 50 gramos no parecen aportar beneficios adicionales, pudiendo por el contrario provocar problemas de intolerancia (Álvarez y Sánchez, 2006).

Actualmente, no existe una definición única que englobe los distintos componentes de la fibra dietética y sus funciones. Se cree que Hipsley en 1953 fue el primero en utilizar el término "Fibra dietética" para referirse a los constituyentes no digeribles que forman la pared celular de las plantas. Concretamente, celulosa, hemicelulosa y lignina (Hipsley, 1953). Se piensa que fue un intento de diferenciar alguna propiedad o constituyente de los alimentos de lo que entonces se conocía como

fibra bruta. Posteriormente en la década de los 70, otros autores propusieron otras definiciones más completas de fibra dietética. Trowel y col. (1976) consideraron que la fibra dietética se componía por todos los polisacáridos no digeribles y resistentes a la hidrólisis por las enzimas digestivas del ser humano, tales como gomas, mucílagos, pectinas y oligosacáridos, además de lignina. Años más tarde, en 2001 La American Association of Cereal Chemist (AACC) definió la fibra dietética como: "la parte comestible de las plantas o hidratos de carbono análogos que son resistentes a la digestión y absorción en el intestino delgado, con fermentación completa o parcial en el intestino grueso. Actualmente, se siguen formulando nuevas definiciones de fibra dietética, una de las más actuales la define como un gran grupo de componentes de polisacáridos, que juegan un papel importante en la salud gastrointestinal, el mecanismo de nutrientes y diversas enfermedades en el huésped (Li y col., 2022b).

1.1. Polisacáridos que componen la fibra dietética

La fibra dietética está formada en su mayoría por polisacáridos que forman parte de las paredes celulares de las plantas. La pared celular es un conjunto complejo de polisacáridos principalmente, con funciones críticas para las plantas (Chebli y Geitmann, 2017).

La pared celular está formada por la pared primaria, la pared secundaria, la membrana celular y la lámina media (Figura 1). La pared primaria está formada por celulosa, que es el polisacárido encargado de aportar resistencia y rigidez al sistema. Entre la pared primaria y secundaria, presente en los tejidos vegetales de fundamentalmente árboles y compuesta por lignina principalmente, se encuentra la membrana celular. Los polisacáridos pécticos y hemicelulosas son los componentes que forman la membrana celular que aportan flexibilidad al conjunto (Li y col., 2021b). Por último, la lámina media es la que se sitúa en la capa más externa y contiene principalmente pectinas, que son de vital importancia para la resistencia mecánica y la porosidad de la pared, así como para el deslizamiento, extensión y señalización intercelular de la pared celular (Burton y col., 2010).

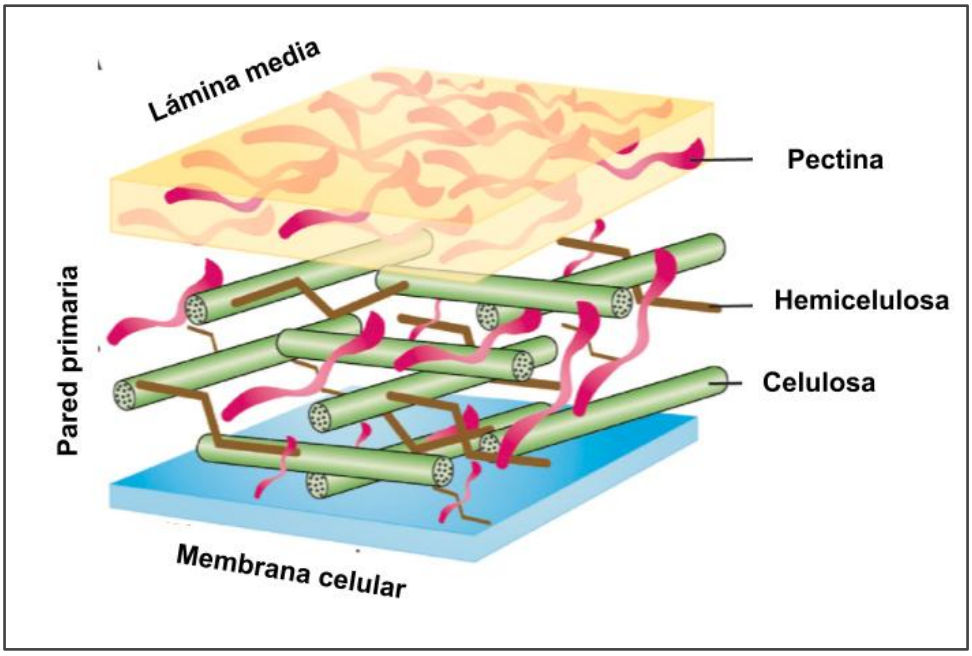


Figura 1. Estructura de la pared celular de las plantas. Fuente: Adaptado de Li y col., 2021b.

1.1.1. Celulosa

La celulosa es el biopolímero más abundante en la Tierra, con una producción anual de unas 50.000 millones de toneladas (Hindi, 2017). Compuesta exclusivamente por moléculas de β-glucosa unidas por enlaces β-1,4-glucosídicos (Figura 2), la celulosa se caracteriza por su capacidad de modificación química e hidrofilia (He y col., 2021).

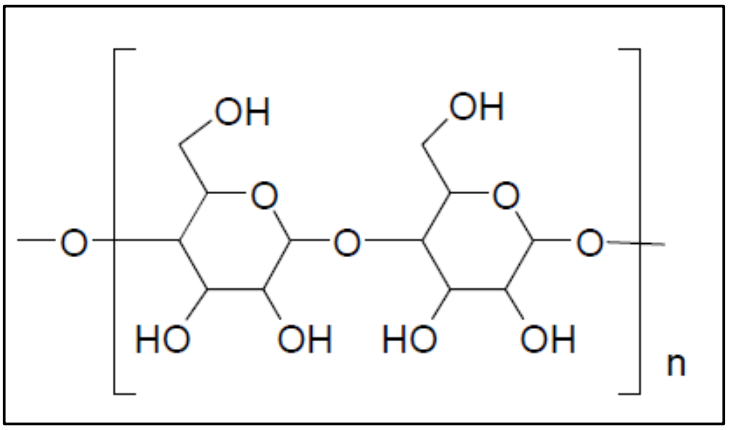


Figura 2. Estructura de la celulosa. Fuente:https://www.eii.uva.es/organica/qoi/tema-03.php

La celulosa se encuentra en diferentes residuos agrícolas, como la piel del ajo (Hernández-Varela y col., 2021), el maíz (Gu y col., 2020), el orujo de uva (Coelho y col., 2018) y la zanahoria (Siqueira y col., 2016). La celulosa tiene múltiples aplicaciones en la industria alimentaria; entre otros, se ha comprobado que su aplicación como sustituto de grasas mejora la textura de los alimentos (Espert y col., 2020; Wang y col., 2018). Además, se ha utilizado ampliamente como película para proteger los alimentos. Riaz y col. (2020) fabricaron recubrimientos a base de celulosa a partir de extracto de raíz de cebollino chino, y los resultados mostraron que los recubrimientos poseían propiedades antioxidantes y antimicrobianas.

1.1.2. Hemicelulosa

La hemicelulosa es un heteropolisacárido, compuesto por un conjunto heterogéneo de polisacáridos, compuesto a su vez por dos tipos de monosacáridos unidos por enlaces β, que forman una cadena lineal ramificada. Es el segundo componente más abundante de los residuos agrícolas, representando aproximadamente el 20-40% (Tatar y col., 2014; Yang y col., 2020). Las hemicelulosas incluyen glucanos, xiloglucanos, mananos, xilanos y β-(1→3,1→4)-glucanos (Scheller y Ulvskov, 2010) (Figura 3). Sin embargo, los polisacáridos que constituyen la mayoría de las hemicelulosas son el manano y el xilano (Naidu y col., 2018).

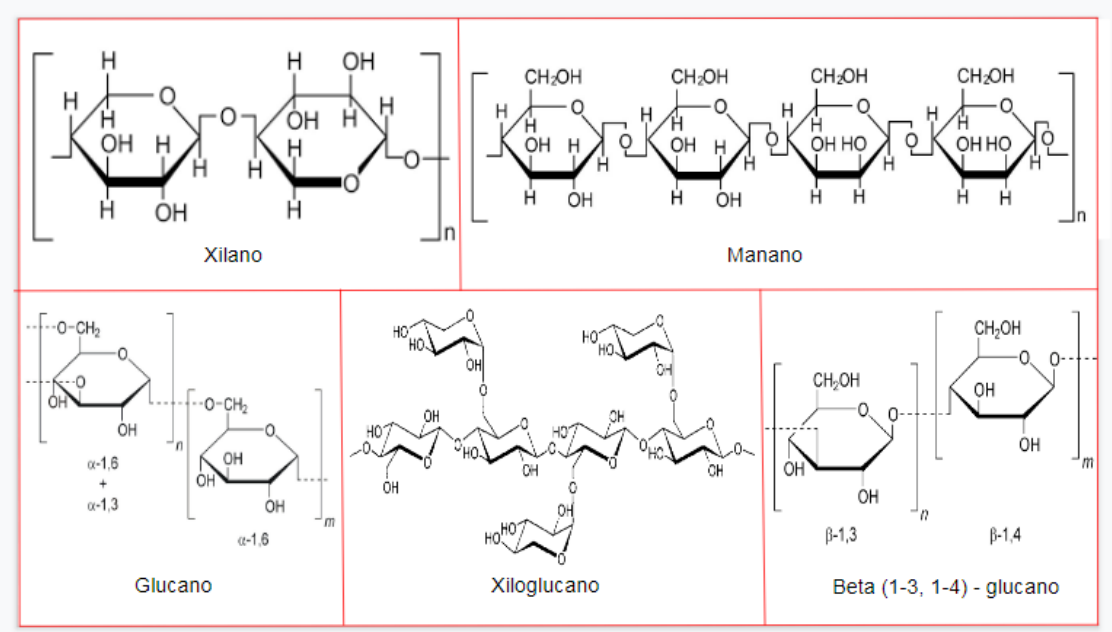


Figura 3. Estructuras de xilano, manano, glucano, xiloglucano y β-(1→3,1→4)-glucano. Fuente: Elaboración propia.

El xilano es la hemicelulosa más abundante que se encuentra en la naturaleza (Fu y col., 2020). El xilano de los residuos agrícolas se puede hidrolizar y convertir en xilosa; además, puede convertirse en xilooligosacáridos (XOS) con diferentes grados de polimerización (Ratnadewi y col., 2016). Aachary y Prapulla. (2011) informaron sobre XOS con un grado de polimerización de 2-3 tienen un potencial prebiótico máximo. Los XOS se extraen industrialmente del maíz y la caña de azúcar (Monteiro y col., 2021; Wu y col., 2019), aunque también se pueden obtener de subproductos agrícolas como la cáscara de piña (Banerjee y col., 2019), la paja de arroz (Gautério y col., 2020) y los tallos de quinoa (Gil-Ramirez y col., 2018).

Otro polisacárido que forma parte de las hemicelulosas es el manano, que está significativamente presente en los residuos agrícolas. Por hidrólisis enzimática, el manano se puede convertir en manooligosacáridos (MOS) (Jana y col., 2018). Los MOS se consideran prebióticos emergentes y se pueden obtener de diferentes residuos agrícolas, como la harina de copra (Jana y Kango, 2020).

1.1.3. Polisacáridos pécticos

Las pectinas, en su mayoría consideradas fibra dietética soluble forman parte de la pared celular de las plantas y son polímeros heteropolisacáridos ricos en ácido poligalacturónico que pueden estar compuestos por hasta 17 monosacáridos diferentes (Chan y col., 2017), lo que los convierte en uno de los polisacáridos vegetales más estructuralmente complejos (Mohnen, 2008). Se compone de tres dominios estructuralmente distintos: homogalacturonano (HG), ramnogalacturonano (RG-I) y ramnogalacturonano (RG-II) (Figura 4).

Las pectinas se obtienen tradicionalmente de subproductos agrícolas, como cáscaras de cítricos y orujo de manzana (Elshahed y col., 2021; Putnik y col., 2017). La creciente demanda mundial de este heteropolisacárido debido a los numerosos beneficios para la salud que se le atribuyen (Bayar y col., 2018) hace que se busquen fuentes alternativas de pectina en otros vegetales y subproductos, como la berenjena (Kazemi y col., 2019), la cáscara de tomate (Grassino y col., 2020; Halambek y col., 2020), tallo de brócoli (Petkowicz y col., 2020) y cáscara de granada (Shakhmatov y col., 2019), entre otros.

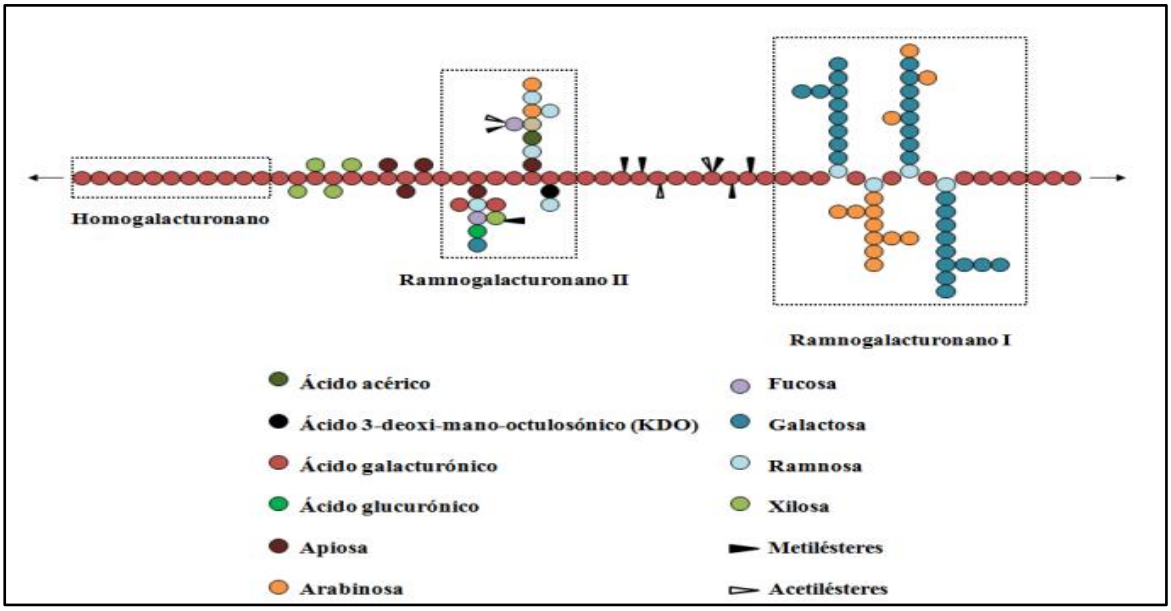


Figura 4. Estructura homogalacturonano, ramnogalacturonano I y ramnogalacturonano II. Adaptado de Willats y col. (2006).

El componente principal de las pectinas es HG, un polímero con una cadena homogénea lineal de α-1,4-glucósido unido al ácido D-galacturónico (Wang y col., 2019). Los ésteres metoxi, ubicados en los grupos carboxilo C6 del ácido D-galacturónico, son sustituciones que generalmente se encuentran en la región HG y juegan un papel importante en las propiedades funcionales y los beneficios para la salud de estos polisacáridos pécticos (Fishman y col., 2015). RG-I es el segundo polisacárido péctico más abundante en la pared celular de las plantas (Lemaire y col., 2020). Se compone de un esqueleto repetitivo de ácido galacturónico y disacárido de ramnosa, generalmente con cadenas laterales neutras (Wu y col., 2020). Se ha demostrado que los polisacáridos RG-I tienen una serie de propiedades bioactivas prometedoras (Hu y col., 2020); su bioactividad se atribuye a su composición y estructura (Mao y col., 2019). Aunque menos comunes en la fracción péctica, los RG-II son polisacáridos con abundantes propiedades bioactivas y muchos beneficios para la salud humana. Su estructura comprende una cadena principal unida al ácido galacturónico y cadenas laterales de oligosacáridos altamente complejos y otros monosacáridos inusuales (Park y col., 2017).

Además, la despolimerización de la pectina libera oligosacáridos pécticos (POS) (Sabater y col., 2021). Los POS se describen actualmente como prebióticos emergentes con numerosos beneficios para la salud (Chung y col., 2017).

1.2. Otros componentes de la fibra

La importancia de considerar no solo los componentes principales de la fibra dietética, como la celulosa, hemicelulosa y la pectina, sino también otros compuestos bioactivos asociados, como polifenoles y proteínas, resalta la complejidad y la diversidad de esta fracción dietética. Aunque presentes en menor medida en los concentrados de fibra dietética, estos compuestos también contribuyen a sus propiedades funcionales. (Doblin y col., 2010; Zhang y col., 2020).

Los compuestos fenólicos constituyen uno de los grupos más extensos de metabolitos secundarios en las plantas, presentes en una variedad de tejidos como frutos, semillas, hojas, tallos y flores, son altamente valorados por sus propiedades saludables (Tanase y col., 2019). Estos polifenoles se caracterizan por tener al menos un

anillo aromático con un grupo hidroxilo en su estructura (Yahia y Carrillo, 2018). Según el número de anillos fenólicos y los elementos estructurales que los unen, se pueden clasificar en diversas subclases, como ácidos fenólicos, flavonoides, estilbenos y lignanos (Li y col., 2014).

Los compuestos fenólicos asociados a las fracciones de fibra dietética, conocidos como compuestos fenólicos no extraíbles, incluyen compuestos de bajo peso molecular, como ácidos fenólicos, y compuestos poliméricos de alto peso molecular, como proantocianidinas y taninos hidrolizables (Fernandes y col., 2023). Los compuestos fenólicos no extraíbles presentan una característica única, no pueden ser extraídos con métodos convencionales ni se liberan durante la digestión en el intestino delgado debido a su interacción con componentes estructurales como la celulosa, hemicelulosa, pectina y lignina (Martins y col., 2022). Es por ello, que la fibra dietética sirve como un vehículo para estos compuestos en el organismo, facilitando su llegada al colon donde pueden ejercer sus efectos beneficiosos (Macagnan y col., 2018).

1.3. Clasificación de la fibra dietética

Las distintas fibras dietéticas, principalmente formadas por polisacáridos, que forman las paredes celulares vegetales, se clasifican fundamentalmente por su capacidad de ser solubles en agua. La fibra dietética soluble puede presentar distintos grados de solubilidad y se compone por β-glucano, gomas, oligosacáridos, glucomanano, arabinoxilano y polisacáridos pécticos, mientras que la fibra dietética insoluble incluye, lignina, hemicelulosa y celulosa (Jakobek y Matić, 2019) (Figura 5).

La fibra dietética soluble en su mayoría no es degradada en su paso por el intestino delgado, llegando intacta al intestino grueso, donde es fácilmente fermentada en el colon por la microbiota (Kang y col., 2022; Bhatt y Gupta, 2022). La fibra dietética soluble es la más utilizada por la industria agroalimentaria debido a sus propiedades fisiológicas, nutricionales y bioactivas (Chen y col., 2020). Por el contrario la fibra dietética insoluble es biológicamente inerte, no forma geles debido a que es insoluble en agua y la fermentación es limitada (Gill y col., 2021). Aunque aporta otro tipos de beneficios como un mayor contenido de agua en las heces lo que proporciona un efecto laxante (McRorie Jr, JW y McKeown, 2017).

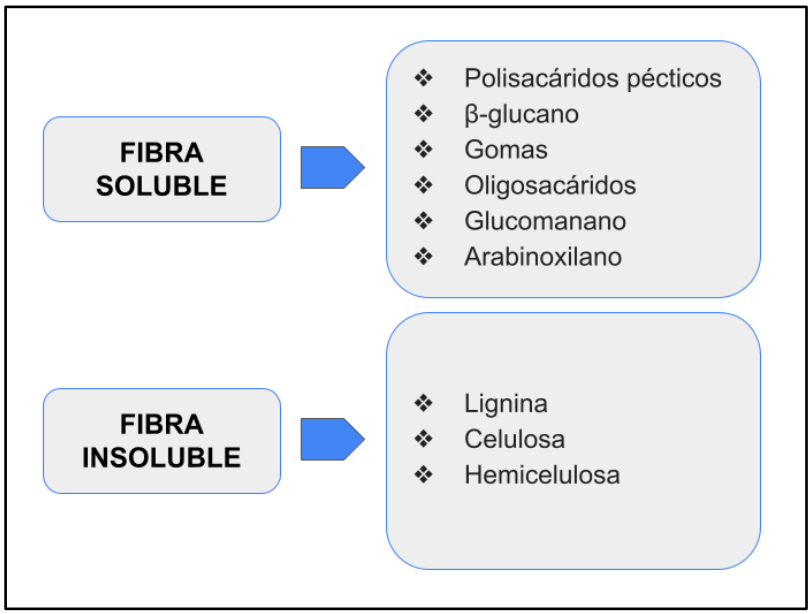


Figura 5. Componentes de la fibra soluble e insoluble. Fuente: Elaboración propia.

2.- PROPIEDADES FÍSICO-QUÍMICAS Y TECNOLÓGICAS DE LA FIBRA DIETÉTICA

Entre las propiedades tecnológicas y físico-químicas más importantes de la fibra dietética se incluyen, su capacidad de hidratación, viscosidad y capacidad para formar geles, capacidad para captar moléculas orgánicas y capacidad de fermentación.

2.1. Capacidad hidratación

Las propiedades de hidratación se definen como la capacidad de la fibra dietética para interactuar y acumular, inmovilizar o retener agua. Se trata de una de las principales propiedades físico-químicas a investigar (Kerr y Wicker, 2000). Las propiedades de hidratación están relacionadas con la estructura química, las propiedades físicas de las partículas de la fibra dietética, las condiciones ambientales de la solución acuosa, tales como el pH, la temperatura, la fuerza iónica, y los procesos mecánicos aplicados a la disolución (Miehle y col., 2022). Las propiedades de hidratación de la fibra dietética principalmente se determinan mediante dos parámetros medibles diferentes, la capacidad de retención de agua, y la capacidad de hinchamiento.

La capacidad de retención de agua se define como la capacidad de los ingredientes alimentarios o alimentos para retener agua durante la aplicación de presión osmótica o centrifugación (Robertson y Eastwood, 1981). Por otro lado, el hinchamiento se define como la tasa de volumen ocupada cuando una porción de fibra dietética se sumerge en agua, en relación al peso real (Raghavendra y col., 2006). Esta propiedad indica la capacidad de hinchamiento que posee la fibra dietética a medida que el agua es absorbida.

Tanto la capacidad de retención de agua como el hinchamiento dependen de varios factores como, el peso molecular, la presión osmótica, la composición y estructura química de las fibras dietéticas (Guillon y Champ, 2000). Además, la fibra dietética soluble suele presentar mayor capacidad de retención de agua y capacidad de hinchamiento, que la fibra dietética insoluble, debido a que la fibra soluble tiene mayor área superficial y mayor número de grupos hidrófilos (Ma y Mu, 2016).

La fibra dietética con buenas propiedades de hidratación al ingresar al sistema gastrointestinal presenta efectos positivos sobre la salud, aumentando el volumen de la fibra dietética tras ser ingerida, lo que mejora la sensación de saciedad, promueve el peristaltismo gastrointestinal y tiene efecto laxante (Maphosa y Jideani, 2016). Por ello es de vital importancia conocer las propiedades tecnológicas de la fibra dietética antes de utilizarla como aditivo o ingrediente funcional para así poder maximizar los beneficios de su aplicación.

2.2. Viscosidad

La viscosidad se describe como la resistencia de una solución al flujo. La viscosidad de una solución de fibra depende de la concentración, del tamaño de las partículas, de la forma de las moléculas y del grado de interacción entre ellas. (Morris y col., 1981).

La viscosidad es promovida por la fibra dietética soluble en mayor medida en comparación con la fibra dietética insoluble (Dikeman y col., 2006). La inclusión de fibra dietética viscosa en la dieta ha demostrado que altera el tiempo de tránsito en el intestino superior, aumenta el tiempo del vaciamiento gástrico y modula el tránsito del intestino delgado (Müller y col., 2018). Además recientemente, estudios revelaron que la viscosidad de la fibra dietética tiene una relación directa en la producción de ácidos grasos de cadena corta (Li y col., 2022d).

2.3. Capacidad para captar moléculas orgánicas

La capacidad de distintas fibras dietéticas para captar e incluso unirse y formar nuevas estructuras a sustancias orgánicas como ácidos biliares, glucosa y sustancias cancerígenas puede tener un papel importante en determinados efectos fisiológicos que se le atribuyen a la fibra dietética.

La capacidad de retención de moléculas orgánicas, en particular ácidos grasos, determina la cantidad de lípidos que pueden ser adsorbidos por la superficie de los polisacáridos que forman la fibra dietética, a este parámetro medible se le conoce como

capacidad de retención de aceite y se define como la capacidad que presenta la fibra dietética para retener aceite tras un proceso de centrifugación (González-Centeno y col., 2010). La fibra dietética con elevada capacidad de retención de aceite permite la estabilización de productos que presentan un elevado contenido en grasa (Elleuch y col., 2011).

2.4.Capacidad de fermentación

La fermentabilidad constituye una de las propiedades más importantes de la fibra dietética, ya que de ella se derivan un gran número de efectos beneficiosos para la salud, tanto locales como sistémicos (do Prado, y col., 2021). Los principales productos de la fermentación de la fibra dietética por los microorganismos del colon son ácidos grasos de cadena corta, principalmente acético, propiónico y butírico (Koh y col., 2016; Louis, y Flint, 2017; Wu y col., 2023b).

El grado y la velocidad de fermentación de la fibra dietética depende de la solubilidad, la estructura de los polisacáridos y de la accesibilidad de los microorganismos a la fibra dietética. La velocidad y el grado de fermentación de la fibra dietética es un parámetro de vital importancia. La fibra dietética con bajo grado de fermentabilidad tendrá como consecuencia una baja tasa de liberación de ácidos grasos de cadena corta (Shuwen y col., 2019). Por el contrario, una fermentabilidad demasiado rápida puede causar una producción de gas demasiado alta en el colon proximal y como consecuencia baja fermentación y producción de ácidos grasos en el colon distal (Yao y col., 2023).

3.- FUNCIÓN BIOACTIVA DE LA FIBRA DIETÉTICA

La fibra dietética tiene un papel clave durante todas las etapas de la digestión, desde la masticación hasta la evacuación de las heces (Figura 6).

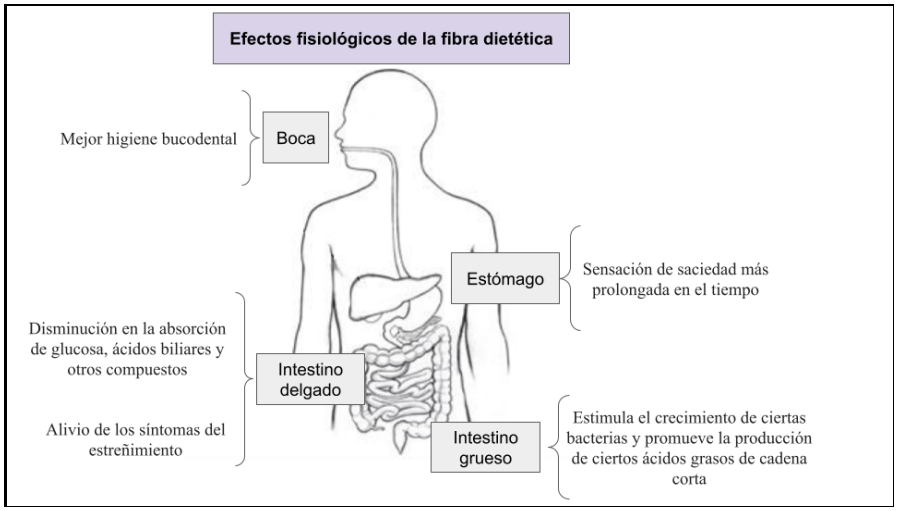


Figura 6. Efectos fisiológicos de la fibra dietética durante las etapas de digestión. Fuente: Elaboración propia

En la primera etapa de la digestión, durante la masticación, las dietas ricas en fibra dietética necesitan más tiempo de procesamiento, debido a que el tiempo de masticación debe de ser mayor, lo que implica una disminución de la velocidad de deglución provocando una mayor salivación, y por lo tanto mejor higiene bucodental (Johnson, 2016).

Posteriormente, la fibra dietética llega al estómago, donde la fibra dietética soluble principalmente forma geles como consecuencia de su viscosidad, lo que produce un vaciamiento gástrico más lento, provocando una sensación de saciedad más prolongada en el tiempo (Li y Uppal, 2010).

Ya en el intestino delgado la fibra dietética soluble fundamentalmente, por su naturaleza viscosa aumenta el tiempo de tránsito lo que provoca el aumento del espesor de la capa de agua, que conlleva con ello una disminución en la absorción de glucosa, ácidos biliares y otros compuestos (Mudgil y Barak, 2013; Li y col., 2022c; Zou y col., 2022). Por el contrario, la fibra dietética insoluble, reduce el tiempo de tránsito en el intestino y aumenta el volumen fecal, aliviando los síntomas del estreñimiento (Lattimer

y Haub, 2010). Por lo tanto, para que la fibra dietética sea beneficiosa para el organismo es fundamental que la relación entre fibra soluble/fibra insoluble sea cercana a 1:2 (Sharoba et al., 2013).

Por último, cuando la fibra dietética llega al intestino grueso es fermentada por la microbiota presente en el colon, estimulando el crecimiento de ciertas bacterias y promoviendo la producción de ciertos ácidos grasos de cadena corta (Hua y col., 2021), ácido láctico (Sun y col., 2019) y gases, tales como dióxido de carbono, metano e hidrógeno gaseoso (Comino y col., 2018), Los ácidos grasos de cadena corta, como el acetato, el butirato y el propionato, son los productos finales mayoritarios de la fermentación tras su paso por el colon y se sabe que pueden tener efectos beneficiosos locales y sistémicos sobre la salud (Rastall y Gibson, 2015).

La Tabla 1 resume las funciones bioactivas de los polisacáridos que forman parte de la fibra dietética obtenidos a partir de subproductos en la industria alimentaria. Además del efecto prebiótico, los polisacáridos han sido estudiados por su actividad antioxidante y antimicrobiana; debido a esta actividad se han reportado efectos importantes: prevención de enfermedades como el cáncer, regulación del síndrome metabólico crónico, aplicación como inmunomoduladores y antiinflamatorios, y actividad antidiabética, anticoagulante y antiviral.

Tabla 1: Tabla resumen: polisacáridos que forman la fibra dietética y sus efectos beneficiosos, procedentes de subproductos agrícolas

Polisacárido	Subproductos	Efectos beneficiosos	Referencias
Celulosa	Piel de ajo, maíz, orujo de uva, zanahoria, extracto de raíz de cebollino chino	Procesos tecnológicos: como sustituto de grasas, para mejorar la textura de los alimentos. Actividad nutricional, antioxidante y antimicrobiana.	Siqueira y col., 2016; Coelho y col., 2018; Wang y col., 2018; Espert y col., 2020; Gu y col., 2020; Riaz y col., 2020; Hernández-Varela y col., 2021

Polisacárido	Subproductos	Efectos beneficiosos	Referencias
Xilano/xiloligosacáridos,metilglucuronoxilano (hemicelulosa)	Maíz (mazorcas de maíz), caña de azúcar, cáscara de piña, paja de arroz, tallos de quinua, soja, semilla de mango, paja de cebada, planta de neem, castaña española	Actividad prebiótica Prevención de enfermedades: prevención del cáncer	Aachary y Prapulla, 2011; Ratnadewi y col., 2016; Gil-Ramirez y col., 2018; Banerjee y col., 2019; Álvarez y col.,, 2020; Gautério y col., 2020; Monteiro y col.,, 2021;
Manano/manooligosacáridos (hemicelulosa)	Harina/sémola de copra, pastel de palmiste, goma guar, cáscara de patata	Actividad prebiótica	Jana y col., 2018; Jana y Kango, 2020
Pectinas, oligosacáridos pécticos (POS), homogalacturonano (HG), ramnogalacturonano (RG-I), ramnogalacturonano (RG-II)	Cáscara de cítricos, cáscara de mandarina, pulpa de manzana, berenjena, cáscara de tomate, tallo de brócoli, cáscara de granada, pulpa de patata	Actividad prebiótica Prevención de enfermedades: prevención del cáncer, regulación del síndrome metabólico crónico, usos inmunomoduladores, antiinflamatorios y probióticos	Grønhaug y col., 2011; Bermudez-Brito y col., 2015; Wang y col., 2015; Khodaei y col., 2016; Chung y col., 2017; Putnik y col., 2017; Prandi y col., 2018; Kazemi y col., 2019; Shakhmatov y col., 2019; Elshahed y col., 2021; Grassino y col., 2020; Halambek y col., 2020; Petkowicz y Williams, 2020; Jin y col., 2021
Heteropolisacáridos, WVP-1 (manosa, glucosa, galactosa y arabinosa) WVP-2 (manosa, ramnosa, ácido glucurónico, ácido galacturónico, glucosa, galactosa y arabinosa)	Orujo de mango, cáscaras de castaña	Prevención de enfermedades: prevención del cáncer, inmunomodulador, antidiabético, anticoagulante, antiviral Actividad biológica: actividad antioxidante	Hu y col., 2018; Zeng y col., 2020

Polisacárido	Subproductos	Efectos beneficiosos	Referencias
Polisacáridos naturales de bajo peso molecular (SLWPP-3)	Subproductos de calabaza	Actividad biológica: actividad antioxidante Prevención de enfermedades: antidiabético	Li y col., 2021
Heteropolisacáridos (ramnosa, glucosa, galactosa, manosa, xilosa, arabinosa y ácido galacturónico)	Casco exterior pistacho	Proceso tecnológico: conservante de carnes Actividad biológica: actividad antioxidante	Hamed y col., 2020
Heteropolisacáridos: PKP-E	Piñas	Actividad biológica: actividad antioxidante	Zhang y col., 2021
glucano, inulina	Cereales	Actividad biológica: actividad inmunomoduladora, antiinflamatoria, antimicrobiana	Kaden-Volynets y col.,, 2019 Hosary y col., 2020

3.1. Actividad prebiótica

La fermentación de ciertos polisacáridos que componen la fibra dietética por bacterias gastrointestinales potencialmente beneficiosas para los seres vivos, puede tener efectos positivos para la salud, ya que provoca modificaciones en la composición de las comunidades bacterianas, convirtiendo por tanto a la fibra dietética en un agente prebiótico. (Lordan y col., 2020). Las fibras dietéticas prebióticas funcionan como un sustrato selectivo para algunos géneros de bacterias potencialmente beneficiosas, como *Lactobacillus* y *Bifidobacterium*, entre otras (Markowiak y Ślizewska, 2017). Importantes investigaciones informaron que los polisacáridos derivados de las paredes celulares de las plantas tienen una alta actividad prebiótica (Baenas y col., 2020; Redondo-Cuenca y col., 2023) y pueden estimular el crecimiento de ciertas bacterias y promover la producción de ciertos ácidos grasos de cadena corta (Ruiz-Moyano y col., 2020).

Numerosos autores han demostrado el efecto prebiótico de diferentes polisacáridos procedentes de residuos agrícolas. Los experimentos *in vitro* realizados con XOS extraídos de paja de cebada demostraron su idoneidad para su uso como

ingrediente prebiótico (Álvarez y col., 2020). Otro tipo de polisacárido hemicelulósico que demuestra un carácter prebiótico es el MOS, ya que resiste la digestión en el tracto gastrointestinal superior (Singh y col., 2018). Los polisacáridos pécticos también han mostrado actividad prebiótica. Se ha demostrado las propiedades prebióticas de RG-I; Khodaei y col. (2016) extrajeron RG-I de papas y reportaron el crecimiento de bacterias beneficiosas (*Bifidobacterium* spp. y *Lactobacillus* spp.). También la fermentación de POS extraídos de remolacha azucarera tuvo como consecuencia un crecimiento selectivo de bacterias del género *Lactobacillus (*Prandi y col., 2018).

3.2. Actividad antioxidante

Hoy en día, existe una creciente evidencia de que muchos tipos de polisacáridos poseen una importante actividad antioxidante. Zeng y col. (2020) investigaron las características estructurales de dos polisacáridos obtenidos de cáscaras de castañas de agua chinas y mostraron que ambos tenían actividad antioxidante potencial. Asimismo, un polisacárido de bajo peso molecular extraído de *Cucurbita moschata* también presento una actividad antioxidante comprobada, exhibiendo una capacidad de eliminación significativa contra los radicales ABTS (2,2′-azinobis (ácido 3-etilbenzotiazolina-6-sulfónico)) y DPPH (2,2-difenil-1-picrilhidrazilo) (Li y col., 2020a). También, heteropolisacáridos compuestos de ramnosa, glucosa, galactosa, manosa, xilosa, arabinosa y ácido galacturónico extraídos de la cáscara del pistacho mostraron un potencial antioxidante significativo (Hamed y col., 2020). En otro estudio cuatro polisacáridos similares a la pectina (PKP-E-1-1, -1-2, -2-1 y -2-2) extraídos de piñas *Pinus koraiensis* mostraron propiedades antioxidantes potenciales prometedoras para aplicaciones alimentarias (Zhang y col., 2021).

El estrés oxidativo, desencadenado por los radicales libres, se ha relacionado con la aparición y desarrollo de varias enfermedades humanas. En algunas de estas enfermedades, se ha observado que las defensas antioxidantes naturales son deficientes. Esta situación ha sugerido la posibilidad de que la progresión de dichas enfermedades pueda ser retardada mediante la suplementación con antioxidantes naturales. La terapia antioxidante podría ser beneficiosa en afecciones como la diabetes mellitus, las lesiones por reperfusión y enfermedades inflamatorias, además de contribuir a prevenir procesos crónicos como la aterosclerosis y la carcinogénesis.

3.3. Actividad antidiabética

La diabetes es un problema global creciente y una pesada carga económica para los servicios de salud. Millones de personas padecen la enfermedad que causa muchas muertes cada año, además de estar asociada con un mayor riesgo de otros problemas de salud. Los polisacáridos tienen un efecto hipoglucemiante significativo y, por lo tanto, pueden ser útiles para prevenir la diabetes mellitus resultante de defectos en la producción o acción de la insulina que causan hiperglucemia. Diferentes polisacáridos derivados de la fibra dietética han ganado popularidad entre los investigadores debido a sus numerosas propiedades bioactivas, incluidos los efectos inhibidores contra las enzimas que hidrolizan el almidón, como la α-amilasa y la α-glucosidasa, lo que destaca su potencial como agentes antidiabéticos en el tratamiento y la prevención de la diabetes mellitus (Zheng y col., 2019a). Li y col. (2021a) informaron de los efectos hipoglucemiantes de los polisacáridos extraídos de subproductos de calabaza.

3.4. Actividad contra el cáncer

Actualmente se está estudiando el efecto anticancerígeno de los polisacáridos naturales de residuos vegetales. Sharma y col. (2020) demostraron en su estudio que XOS extraídos del aserrín de Azadirachta inhibe el crecimiento de células de cáncer colorrectal humano (HT-29). En otro estudio, se aislaron tres polisacáridos principales de pulpa de mango, compuestos por siete monosacáridos (manosa, ramnosa, glucosa, galactosa, xilosa, arabinosa y fucosa) y dos ácidos urónicos, y los polisacáridos aislados mostraron actividad anticancerígena significativa contra HepG2, MCF- 7, A549, HeLa, A2780, HCT-116 y células BGC (Hu y col., 2018). También se ha demostrado que el HG de la extracción de bayas de *Hippophae rhamnoides* tiene un efecto antitumoral (Wang y col., 2015). Asimismo, se ha demostrado que RG-I es capaz de promover la adhesión y migración celular (Li y col., 2018a) y la actividad inmunomoduladora (Grønhaug y col., 2011). Recientemente Wu y col. (2023a) observaron, que un nuevo polisacárido soluble en agua fría aislado de la raíz de *Rhodiola rosea* L. administrado en ratones portadores de tumores H22 a una dosis de 100 y 300 mg/kg consiguió inhibir el crecimiento tumoral de 23,59 % a 45,52 %.

3.5. Actividad antiinflamatoria

Se ha demostrado que los polisacáridos naturales como el glucano (Mikkelsen y col., 2014), la inulina (Kaden-Volynets y col., 2019) y las pectinas (Xiong y col., 2021) tienen una fuerte actividad antiinflamatoria. Hosary y col. (2020) aislaron glucano de la planta *Avena sativa* L. y demostraron su capacidad antiinflamatoria, mostrando su alto potencial para su uso como hidrogel cicatrizante que también mostró actividad antimicrobiana contra *Staphylococcus aureus* y *Micrococcus luteus*. Sin embargo, como lo demuestran Bermúdez-Brito y col. (2015), los polisacáridos pécticos muestran una mayor actividad antiinflamatoria que la inulina y el glucano. Además, los polisacáridos pécticos muestran una alta actividad antiinflamatoria en sus tres dominios (HG, RG-I y RG-II) (Jin y col., 2021).

3.6. Actividad antimicrobiana

Los polisacáridos naturales han sido objeto de varios estudios que han revelado su destacada actividad antimicrobiana. Se ha comprobado que poseen una notable capacidad para inhibir el crecimiento de una amplia variedad de microorganismos infecciosos y causantes de deterioro. Rostami y col. (2017) extrajeron polisacáridos de *Malva sylvestris* y demostraron que las bacterias Gram-positivas (*Bacillus cereus* PTCC 1015 y *Staphylococcus aureus* PTCC 1112) eran menos sensibles que las bacterias Gram-negativas (*Escherichia coli* PTCC 1763 y *Salmonella typhimurium* PTCC 1709) a los diferentes extractos de polisacáridos obtenidos. Sin embargo, Hosary y col. (2020) desarrollaron un hidrogel utilizando polisacáridos derivados de la *Avena sativa* L, el producto desarrollado mostró actividad leve contra *Candida albicans* y actividad alta solo contra las cepas bacterianas Gram positivas *Staphylococcus aureus* ATCC 297373 y *Micrococcus luteus* ATCC 10240, y ninguna contra las dos cepas Gram negativas utilizadas en el estudio, *Escherichia coli* ATCC 10536 y *Pseudomonas aeruginosa* ATCC 25619. Del mismo modo, Hashemifesharaki y col. (2020) obtuvieron polisacáridos por extracción asistida por microondas de raíz de malvavisco y éste mostró actividad antimicrobiana principalmente contra bacterias Gram-positivas, en particular, *Staphylococcus aureus* PTCC 1189 y *Bacillus circulans* ATCC 4516.

4.- MODELOS DE DIGESTIÓN SIMULADA

Comprender los mecanismos de digestión gastrointestinal es fundamental para el diseño de alimentos funcionales. Por esta razón, la digestión gastrointestinal ha sido objeto de numerosos estudios (Krul y col., 2000; Havenaar y col., 2000; McDougall y col., 2005; Delgado y col., 2011; Cian y col., 2015; Ketnawa y col., 2018; Spínola y col., 2019; Pimentel y col., 2020; Ye y col., 2022). Los métodos de digestión gastrointestinal simulados, integran los principales parámetros de la digestión (duración, pH, temperatura, agitación y concentración de líquidos digestivos y enzimas) y abarcan las etapas oral, gástrica e intestinal, y en algunos casos, la fermentación colónica (Makran y col., 2020). De manera general, los estudios de digestión gastrointestinal se pueden clasificar fundamentalmente como estudios *in vivo* e *in vitro.*

4.1. Digestión simulada *in vivo*

Los métodos de digestión simulada *in vivo*, son los más fiables y precisos, sin embargo, son muy costosos y lentos. Además, utilizan animales o seres humanos, por lo que también hay que tener en cuenta ciertas cuestiones éticas. Por estos motivos desde hace varias décadas se ha dedicado mucho tiempo y esfuerzo al desarrollo de procedimientos *in vitro* (Boisen y Eggum, 1991). Las condiciones a simular de los modelos de digestión *in vitro* se basan en las condiciones *in vivo*, es por ello que las correlaciones *in vitro* - *in vivo* en los modelos de digestión son extremadamente importantes (Fatouros y Mullertz, 2008).

Los roedores son los animales más utilizados para comparar los resultados *in vitro* y las respuestas *in vivo*, ya que comparten una amplia gama de similitudes metabólicas, funcionales y moleculares con la fisiología humana (Mota y col., 2022). Darío Frías y Sgarbieri. (1998) utilizaron la digestión simulada *in vivo* para estudiar el efecto de la adición de diferentes proporciones de goma guar sobre la ingesta de alimentos, niveles de colesterol sérico, triacilgliceroles, glucosa y colesterol en ratas. Los resultados mostraron que la goma guar (10 % y 20 % (p/p)) redujo la ingesta de alimentos y el peso corporal de las ratas. Además, inhibió la digestión de los alimentos en el intestino, y redujo el nivel de colesterol. Estudios más recientes probaron la bioaccesibilidad y transporte de polifenoles de cáscara de lenteja y su biodisponibilidad y metabolismo en ratas, los resultados mostraron que cuando las ratas ingirieron, los polifenoles de la cáscara de lentejas experimentaron extensas reacciones

metabólicas, que incluyeron hidroxilación, metilación, glucuronidación y sulfatación. Con los resultados obtenidos afirmaron que los polifenoles de la cáscara de lentejas son bioaccesibles y biodisponibles (Guo y col., 2023).

4.2. Digestión simulada *in vitro*

Los modelos de digestión *in vitro* se utilizan para estudiar los cambios estructurales, la digestibilidad y la liberación de los componentes de los alimentos en condiciones gastrointestinales simuladas (Hur y col., 2011). En la actualidad, su uso está muy extendido, debido a que son relativamente sencillos, rápidos y seguros. Además, no tienen las restricciones éticas de los métodos *in vivo* (Chen y col., 2011; Li y col., 2020b). Los métodos que simulan la digestión gastrointestinal se pueden dividir en dos categorías: modelos estáticos de digestión *in vitro* y modelos dinámicos de digestión *in vitro*.

4.2.1. Modelos estáticos de digestión in vitro

Los modelos estáticos de digestión *in vitro* son ampliamente utilizados por su gran simplicidad y alto rendimiento. El proceso de digestión simulada ocurre en un solo biorreactor donde se acumulan todos los productos digestivos (Li y col., 2022). Es por ello que presenta algunos inconvenientes ya que no contempla algunos factores importantes, tales como, el flujo de los fluidos digestivos, la estructura espacial del estómago y el vaciado gástrico, entre otros (Mackie y col., 2020). Uno de los modelos de digestión *in vitro* estático más utilizados en la industria alimentaria es el método INFOGEST (Brodkorb y col., 2019). El método INFOGEST fue desarrollado como parte de un consorcio internacional de científicos que trabajaron para identificar y armonizar las condiciones requeridas para simular adecuadamente la boca, el estómago y el intestino delgado humano, incluidas temperaturas, tiempos de incubación, valores de pH, composiciones iónicas, actividades enzimáticas, niveles de bilis y niveles de mucina (Tan y col., 2022).

El modelo INFOGEST se ha aplicado ampliamente para evaluar la digestión de proteínas, almidón y lípidos en alimentos (Zhou y col., 2021; Ariëns y col., 2021; Sousa y col., 2023), también para medir la bioaccesibilidad de compuestos bioactivos (Boyd y col., 2020; Jensen y col., 2022). Sin embargo, estudios informaron de la ineficacia del método INFOGEST como un procedimiento para la digestión de polisacáridos (Gallego-Lobillo y

col., 2021). Sousa y col. (2023) en su trabajo analizaron la digestibilidad de las proteínas de la dieta mediante el modelo estático INFOGEST y lo validaron con datos *in vivo*. Los resultados mostraron que en general, las digestibilidades *in vitro* totales coincidieron con los resultados *in vivo*. También, las digestibilidades *in vitro* de los aminoácidos individuales y aminoácidos indispensables digestibles *in vitro*, estuvieron altamente correlacionadas con los resultados *in vivo*. Además, el modelo estático INFOGEST resultó ser adecuado para el estudio de la bioaccesibilidad de los compuestos fenólicos. Rasera y col. (2023) estudiaron la bioaccesibilidad de los compuestos fenólicos de granos de mostaza negra germinada y sin germinar utilizando el modelo INFOGEST, los resultados obtenidos mostraron que la bioaccesibilidad de los compuestos fenólicos de los granos de mostaza estuvo significativamente influenciada por la digestión gastrointestinal.

4.2.2. Modelos dinámicos de digestión in vitro

Los modelos dinámicos de digestión *in vitro*, son los más fiables y los que mejor simulan la digestión fisiológica humana. Los modelos dinámicos regulan el pH, el flujo del alimento y la incorporación en tiempo real de enzimas digestivas durante el proceso de digestión simulada. Además, los sistemas dinámicos pueden ser monocompartimentales o multicompartimentales. El inconveniente de los modelos dinámicos es que son mucho más complejos, más caros y requieren el uso de equipos altamente especializados en comparación a los modelos estáticos de digestión *in vitro* (Xavier y Mariutti, 2021).

Li y col. (2022e) en su estudio compararon sistemáticamente modelos *in vitro* dinámicos y estáticos, analizando el efecto de la hidrólisis del almidón durante la digestión de canela. Los resultados mostraron una mayor hidrólisis del almidón en las fases digestiva, gástrica e intestinal en el modelo dinámico en comparación con el método estático. Por lo tanto, el estudio reveló que las condiciones fisiológicas dinámicas, incluida la evolución del pH, el vaciamiento gástrico y las secreciones digestivas dinámicas son de gran importancia para predecir los efectos funcionales de la canela.

5.- PROBLEMÁTICA DE LOS RESIDUOS AGRÍCOLAS EN EL MUNDO

Actualmente se generan a nivel mundial 37 millones de toneladas de residuos agrícolas, aunque la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO) estima que este valor es mucho mayor dada la dificultad de medir la totalidad de las pérdidas (FAO, 2020), lo que origina un grave problema económico y ambiental (Wang y col., 2016; Sagar y col., 2018; Sani y col., 2022; Sarfraz y col., 2023). En España, especialmente en regiones como Extremadura, el sector de la horticultura y las industrias de vinificación y procesamiento de tomate, representan elementos fundamentales de la economía. Según los datos proporcionados por el Ministerio de Agricultura, España registró una producción total de vino de 33,5 millones de hectolitros. Dentro de este panorama vitivinícola, Extremadura se posiciona como la tercera productora a nivel nacional. Además, en el ámbito de la fruticultura, Extremadura también ha consolidado su posición como la tercera región en producción de granadas, por detrás de la Comunidad Valenciana y Murcia. En el sector de la agricultura intensiva, Extremadura sobresale como la principal productora de tomate de industria en el país, destacando su liderazgo en este cultivo a nivel nacional. Asimismo, en el cultivo de brócoli, la región extremeña ocupa el cuarto lugar en términos de producción a nivel nacional, demostrando su diversidad y relevancia en el panorama agrícola español. Estos cultivos e industrias representan una parte significativa de la actividad agrícola en la región, generando tanto productos finales como subproductos y residuos. Entre estos residuos se incluyen desechos y subproductos vegetales, como pieles, tallos, hojas y semillas. (Rifna y col., 2021; Kamal y col., 2022; Rivas y col., 2022).

5.1. Subproductos agrícolas como fuentes de fibra dietética

Los subproductos vegetales son fuentes ricas de fibra dietética (Perez-Pirotto y col., 2022; Yin y col., 2022), polisacáridos solubles (Nakamura y col., 2023), compuestos fenólicos (Bondam y col., 2022) y ácidos grasos (Gottardo y col., 2022), lo que los hace particularmente interesantes para su uso como aditivos e ingredientes funcionales (Chamorro y col., 2022; de Oliveira y col., 2022). La fibra dietética forma parte mayoritaria de los residuos de la industria agroalimentaria y constituye uno de los recursos renovables más importantes del planeta. La gran variedad en su composición química y estructura, así como la biodegradabilidad y seguridad de los polisacáridos que la componen, la hacen ideal para su aplicación en diversos campos, como la industria alimenticia, farmacéutica, cosmética, ingeniería de tejidos y biocombustibles, entre otras (Chen y col., 2017; Santagata y col., 2018; Li y col., 2022a; Chockchaisawasdee y col., 2023).

5.1.1. Subproductos procedentes de las industrias de vinificación

La generación de subproductos agrícolas en el sector de la viticultura es especialmente preocupante; se estima que por cada 100 kg de uva se producen aproximadamente 25 kg de residuos. Es por ello, que el enfoque de economía circular tiene un papel importante en este sector. (Bordiga y col., 2015; Angelini y col., 2022). Lo residuos agrícolas procedentes de la actividad vitivinícola fundamentalmente son residuos sólidos, como el orujo de uva, que representan el 60% del total de subproductos vitivinícolas, y se compone principalmente de pieles de uva, restos de pulpa, raspones de los racimos y semillas (González-Ballesteros y col., 2018; Antonić y col., 2020). El orujo de uva está compuesto principalmente por fibra dietética, ésta representa entre el 45% y el 75% del peso seco de los orujos de uva blanca y tinta, respectivamente (Llobera y Canellas 2007; Gül y col., 2013). La fibra dietética del orujo de uva está constituido principalmente por polisacáridos, tales como hemicelulosa, celulosa y pectina; y en menor medida lignina, proteínas estructurales y compuestos fenólicos asociados a las paredes celulares (Beres y col., 2016; Martínez-Meza y col., 2021). Además, cabe señalar que la fibra dietética que forma parte del orujo de uva es considerada fibra dietética antioxidante. El término fibra dietética antioxidante hace referencia a "un concentrado de fibra dietética que contiene cantidades significativas de antioxidantes naturales asociados a compuestos no digeribles" (Bravo y Saura-Calixto, 1998; Quirós-Sauceda y col., 2014).

Además, las industrias de vinificación generan residuos líquidos, el más importante se trata de las lías de vinificación que representan el 25 % de los residuos producidos durante el proceso de elaboración del vino (Bonamente y col., 2015). Las lías de vinificación se generan durante el proceso de fermentación y están formadas principalmente por biomasa microbiana, como levaduras y bacterias; además de carbohidratos insolubles tales como celulosa y hemicelulosa. También, aunque en menor medida contienen compuestos fenólicos, lignina, proteínas y minerales (Dimou y col., 2015; Delgado De La Torre y col., 2015; Fia, 2016). El porcentaje y composición de la fibra dietética de las lías de vinificación genera controversia: algunos autores afirman que las lías de vinificación no contienen una fracción significativa de fibra dietética (Pérez-Bibbins y col., 2015), mientras que otros autores aseguran que el porcentaje de fibra dietética es elevado (Rivas y col., 2021b)

5.1.2. Subproductos procedentes del cultivo de la granada

Otro de los sectores que se enfrenta a grandes problemas y desafíos debido a los residuos que genera es el sector hortofrutícola. En concreto cabe señalar el cultivo de la granada. Actualmente, el cultivo de la granada se está extendiendo por multitud de áreas del mundo (Holland y col., 2009; Ercisli y col., 2011; Sarkhosh y col., 2020; Hooks y col., 2021), como consecuencia de una demanda masiva por sus conocidas características medicinales y su alto valor nutricional (Arun y col., 2022). Esto provoca que las industrias de procesamiento de esta fruta se encuentren en cualquier parte del mundo (Hasnaoui y col., 2014). La producción de granada en España también sigue la tendencia y va en aumento situándose además, como primer productor de granada a nivel Europeo (Alcaraz-Mármol y col., 2017). Sin embargo, tan solo el 50 % del fruto es aprovechado, el 50 % restante lo componen membranas internas y cáscaras (Akhtar y col., 2015). Es por ello que anualmente se generan en el mundo alrededor de 1,6 millones de toneladas de residuos procedentes de las industrias de procesamiento de granadas (Bertolo y col., 2021). Estos subproductos tienen una alta concentración de compuestos bioactivos como fibra dietética y compuestos fenólicos (Hasnaoui y col., 2014; Rivas y col., 2021a) lo que los hace especialmente interesantes para su uso como aditivos o ingredientes funcionales por la industria alimentaria.

5.1.3. Subproductos procedentes del cultivo del tomate

La producción de tomate en el mundo va en aumento, en 2020 se produjeron 186 millones de toneladas frente a las 180 millones de toneladas en 2018 (Kaboré y col., 2022). Además, se estima que la producción crezca hasta alcanzar las 221 millones de toneladas entre 2020 y 2024 (Coelho y col., 2023). Otro aspecto a tener en cuenta es que gran parte de los tomates que se producen en el mundo son destinados a la industria alimentaria para su procesado. En el año 2018 se procesaron 41 millones de toneladas de tomates en el mundo (WPTC, 2018), que son convertidos principalmente, en salsa, pasta, jugo y tomates enlatados (Nour y col., 2018).

El orujo de tomate es el subproducto más abundante, se estima que al producir 1 kilogramo de salsa de tomate se generan alrededor de 200 gramos de éste subproducto (Secondi y col., 2019). El orujo de tomate seco está compuesto por un 33 % de semillas, un 27 % de piel y un 40 % de pulpa (Allison y Simmons, 2017; Vidyarthi y Simmons, 2020). En cuanto a su composición el orujo de tomate contiene hasta un 60 % de fibra dietética, principalmente celulosa y hemicelulosa (Abbasi-Parizad y col., 2020), y en menor medida polisacáridos pécticos (Vidal y col., 2022). Además, en su composición cabe destacar otros compuestos bioactivos dada su importancia, tales como, compuestos fenólicos, vitaminas, licopeno y carotenoides (Azabou y col., 2020; Tuoxunjiang y col., 2020; Reda y col., 2022).

5.1.4. Subproductos procedentes del cultivo del brócoli

El brócoli se consume ampliamente en todo el mundo, es por ello que su producción ha aumentado en aproximadamente 6 toneladas en la última década, llegando a producirse en el mundo 27 toneladas de brócoli en 2019 (FAOSTAT, 2021). Sin embargo, tan solo el 15-20% del peso de la planta representa la parte comestible (Shi y col., 2019). Es por ello que el cultivo del brócoli genera una gran cantidad de residuos y subproductos tanto cuando se consume en fresco como transformado. Los subproductos del cultivo del brócoli incluyen tallos, hojas e inflorescencias. y en cuanto a su composición se sabe que son subproductos ricos en fibra dietética y otros compuestos bioactivos, como fenoles, vitamina C, polifenoles y glucosinolatos (Sánchez-Pujante y col., 2020; Salas-Millán y col., 2022).

6.- EXTRACCIÓN DE POLISACÁRIDOS QUE COMPONEN LA FIBRA DIETÉTICA: MÉTODOS E INFLUENCIA EN LA FUNCIÓN BIOACTIVA

Los diferentes métodos de extracción, el solvente de extracción, el pH, la proporción de materia prima/solvente, la temperatura y el tiempo tienen una influencia significativa en el rendimiento, las propiedades tecnológicas y funcionales de los polisacáridos (Fang y col., 2020; Shang y col., 2018). Cada método de extracción tiene sus ventajas y desventajas; por lo tanto, el método de extracción elegido debe adaptarse al propósito final, la naturaleza del subproducto y el coste del procedimiento. La Tabla 2 muestra una descripción general de los métodos de extracción optimizados y su influencia en la función bioactiva de los polisacáridos obtenidos a partir de subproductos vegetales.

Tabla 2: Tabla resumen: Métodos de extracción de polisacáridos que forman parte de la fibra dietética procedente de subproductos agrícolas y su posible influencia en la función bioactiva

Método de extracción	Compuesto	Subproducto	Influencia en la función bioactiva	Referencias
Extracción con agua caliente	Polisacáridos	Morera blanca	Actividad antidiabética, inmunomodul adora, antiinflamato ria y antioxidante	He y col., 2018
Extracción con agua caliente	Polisacáridos	Cáscaras de sandía	Actividad antihipertensi va y antioxidante	Romdhane y col., 2017
Extracción con agua caliente	Polisacáridos	Frutos de granada	Actividad prebiótica	Khatib y col., 2017
Extracción con agua caliente	Polisacáridos	Fruto olivo silvestre	N.d.*	Sharifian-Nejad y col., 2019
Extracción asistida por ultrasonido	Polisacáridos/Pecti na	Tubérculos de ñame, cáscaras de frutas, procesamiento de tomate, patata	Actividades antioxidantes, anticoagulant es, antitumorales , antiinflamato rias y prebióticas	Cui y Zhu, 2021

Método de extracción	Compuesto	Subproducto	Influencia en la función bioactiva	Referencias
Extracción asistida por ultrasonido	Polisacáridos/Pectina	Cáscaras de: berenjena	Actividad antioxidante	Kazemi y col., 2019
Extracción asistida por ultrasonido	Polisacáridos/Pectina	Piel de granada	N.d.*	Moorthy y col., 2015
Extracción por microondas asistida por ultrasonido	Polisacáridos/Pectina	Piel de tomate	N.d.*	Sengar y col., 2020
Extracción asistida por ultrasonido	Polisacáridos/Pectina	Piel de chirimoya	N.d.*	Shivamathi y col., 2019
Extracción asistida por ultrasonido	Polisacáridos/Pectina	Piel de mango	N.d.*	Guandalini y col., 2019
Alta presión hidrostática y extracción asistida por ultrasonidos	Polisacáridos/Pectina	Piel de tomate	N.d*	Grassino y col., 2020
Extracción asistida por ultrasonido	Polisacáridos de hemicelulosa/ xiloglicanos	Orujo de uva	N.d.*	Minjares-Fuentes y col., 2016
Extracción asistida por ultrasonido	Fructooligosacáridos	Residuos industriales de alcachofa	Actividad prebiótica	Machado y col., 2015
Extracción asistida por microondas	Polisacáridos	Raíces de malvavisco	Actividad antioxidante, antimicrobiana y antitumoral	Hashemifesharaki y col., 2020
Extracción asistida por microondas	Polisacáridos/Pectina	Piel papaya	N.d.*	Maran y Prakash, 2015
Extracción asistida por microondas	Polisacáridos	Kiwi	Actividad antioxidante	Han y col., 2019
Extracción asistida por surfactante y microondas	Polisacáridos/Pectina	Piel de naranja	N.d.*	Su y col., 2019
Extracción asistida por microondas	Polisacáridos/Pectina	Piel de banana	N.d.*	Swamy y Muthukumarappan, 2017

Método de extracción	Compuesto	Subproducto	Influencia en la función bioactiva	Referencias
Extracción por microondas con disolvente caliente	Polisacáridos/Pectina	Piel de pomelo	N.d.*	Chen y col., 2016
Hidrodifusión de microondas y gravedad	Polisacáridos	Subproductos de bróccoli	N.d.*	Ferreira y col., 2018
Extracción asistida por microondas	Polisacáridos	Cáscara de grano de cacao	Actividad antioxidante	Mellinas y col., 2020
Extracción asistida por microondas	Polisacáridos/Pectina	Piel de frutas	N.d.*	Dao y col., 2021
Extracción asistida por microondas	Polisacáridos de hemicelulosa y xiloglicanos	Residuos de plantas de tabaco	N.d.*	Yuan y col., 2019a
Extracción asistida por enzimas	Polisacáridos	Planta de malva silvestre	Actividad antioxidante, antitumoral y antimicrobiana	Rostami y col., 2017
Extracción asistida por enzimas	Polisacáridos	Planta de copa	Actividad antioxidante e hipoglucemiante	Guo y col., 2020
Extracción asistida por enzimas	Polisacáridos/Pectina	Orujo de kiwi	N.d.*	Yuliarti y col., 2015
Extracción asistida por enzimas	Polisacáridos/Pectina	Orujo de manzana	Actividad antioxidante y, anticancerígena	Wikiera y col., 2015
Extracción asistida por enzimas	Polisacáridos/Pectina	Piel de granada	Actividad antioxidante	Li y col., 2018
Extracción de fluidos supercríticos	Inulina y fructooligosacárido	Zumo de manzana	Actividad antioxidante	Silva y col., 2019
Extracción de fluidos supercríticos asistida por enzimas	Polisacáridos	Piel de granada	Actividad antioxidante	Mushtaq y col., 2017
Extracción de fluidos supercríticos	Polisacáridos	Semillas de Artemisia	N.d.*	Chen y col., 2014

Método de extracción	Compuesto	Subproducto	Influencia en la función bioactiva	Referencias
Extracción acelerada por solventes	Polisacáridos	Brotes de bambú	Actividad antioxidante	Chen y col., 2018
Extracción acelerada por solventes	Polisacáridos	Uva japonesa	Actividad antioxidante e hipoglucémica	Yang y col., 2019
Microfluidización dinámica de alta presión	Polisacáridos	hojas de nelumbo	Actividad antioxidante	Zhang y col., 2015
Extracción sinérgica de celulasa ultrasónica	Polisacáridos	Orujo de piña	Actividad hipoglucémica y anticancerígena	Hu y col., 2019
Extracción profunda con disolvente eutéctico	Polisacáridos/Pectina	Piel de pomelo	N.d.*	Liew y col., 2018

***No determinada**

6.1. Extracción de agua caliente (HWE)

HWE es uno de los métodos más utilizados para extraer polisacáridos, siendo convencional, simple y económico. Sin embargo, el uso de HWE es limitado debido a su bajo rendimiento; solo se pueden obtener polisacáridos extracelulares ya que la pared celular no se degrada (Mohan y col., 2020). Se necesitan altas temperaturas y largos tiempos de extracción para lograr altos rendimientos (He y col., 2018), lo que resulta en la degradación de la estructura y una disminución en la calidad y la bioactividad (Gharibzahedi y col., 2019; Dong y col., 2016). Por lo tanto, existe la necesidad de explorar nuevos métodos de extracción de polisacáridos que aseguren un buen rendimiento además de mantener las características bioactivas y funcionales de los polisacáridos.

Sin embargo varios autores han informado sobre la optimización de las condiciones HWE y la garantía de buenos rendimientos. Romdhane y col. (2017) informaron el efecto de la temperatura, el tiempo y la proporción de agua/materia prima, en el rendimiento de la extracción, utilizando la metodología clásica de "un factor a la vez". Estos autores obtuvieron un buen rendimiento de polisacáridos extraídos de la cáscara de sandía, y mostraron buena

actividad funcional y una importante capacidad antioxidante. Khatib y col. (2017) mostraron que el uso de agua caliente maximiza la solubilidad y la capacidad de extracción de los polisacáridos crudos del mesocarpio de granada, que presentaron además, propiedades prebióticas *in vitro* al servir como un excelente sustrato para el crecimiento de bacterias potencialmente probióticas, como *Lactobacillus* y Cepas de *bifidobacterias*. Sharifian-Nejad y Shekarchizadeh, (2019) optimizaron la extracción de polisacáridos de frutos de *oleaster* en función de la temperatura, la relación agua/materia seca, el tiempo y la relación de alcohol y la mayor pureza de polisacáridos a una temperatura de 60 °C; y obtuvo los mejores resultados a una relación agua/materia seca de 53:1 (V / W); tiempo, 5 horas; y relación de alcohol de 2.9 (V / V) con una solubilidad de 67.46%.

6.2. Extracción asistida por ultrasonido (EAU)

UAE se basa en un fenómeno llamado cavitación acústica (Figura 7), que implica la generación y formación de burbujas de vapor de gas en un líquido que se expanden y finalmente colapsan. La cavitación genera corrientes de líquido circulante y turbulencia, así como un aumento de la temperatura y la presión (Cui y Zhu, 2021). Esto conduce a un aumento en el rendimiento total de extracción (Tomke y col., 2017). Entre las ventajas de la UAE está ser una de las técnicas más rentables para la extracción de polisacáridos (Cui y Zhu, 2021), además de ser eficiente, rápida y respetuosa con el medio ambiente.

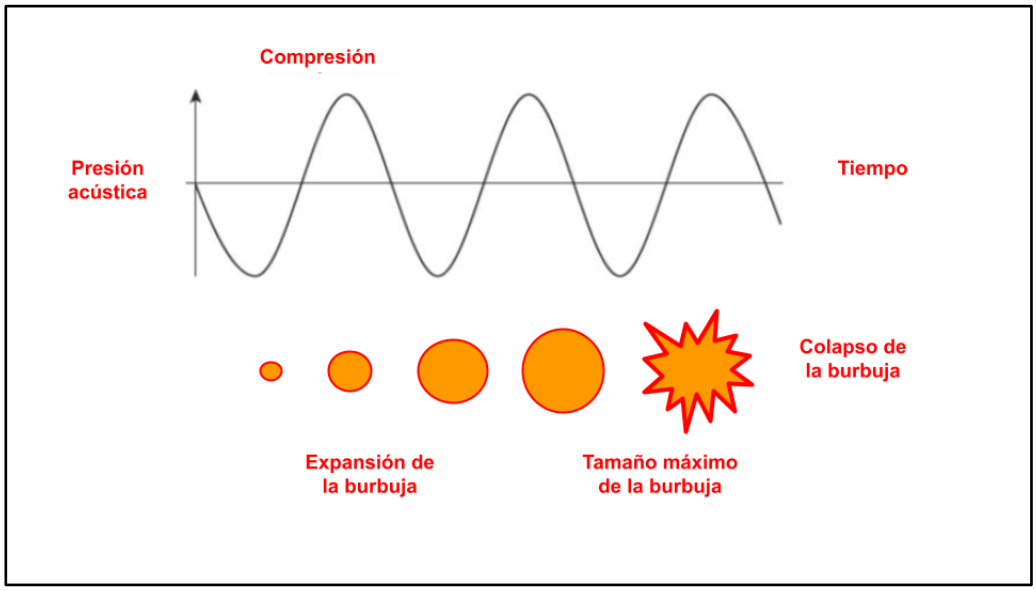


Figura 7. Fenómeno cavitación acústica. Fuente: Elaboración propia.

Los EAU se han utilizado para extraer polisacáridos pécticos de la cáscara de frutas y verduras, como berenjena (Kazemi y col., 2019), granada (Moorthy y col., 2015), tomate (Sengar y col., 2020), chirimoya (Shivamathi y col., 2019) y mango (Guandalini y col., 2019). EAU de cáscara de tomate pudo extraer de manera eficiente dos valiosos ingredientes bioactivos (pectina y polifenoles) simultáneamente, además de reducir el tiempo de extracción con respecto a las técnicas de extracción convencionales (Grassino y col., 2020). También se ha informado que se pueden obtener altos rendimientos de polisacáridos de hemicelulosa con tiempos de extracción cortos, especialmente xiloglicanos por UAE en orujo de uva (Minjares-Fuentes y col., 2016). UAE resulta eficiente para la extracción de azúcares prebióticos a partir de residuos industriales de alcachofa: se extrajeron con éxito 1-kestosa, nistosa, fructofuranosilnistosa y rafinosa, obteniendo un extracto de aproximadamente 9,6 mg de sacáridos prebióticos/g de materia prima seca (Machado y col., 2015).

6.3. Extracción Asistida por Microondas (MAE)

MAE implica la penetración de radiación electromagnética en una matriz sólida. El calentamiento generado se debe a la fricción molecular provocada por la conducción iónica de los iones disueltos y la rotación de los dipolos del disolvente polar, que favorecen la extracción de los compuestos bioactivos. Tanto el calentamiento producido como la presión interna originada provocan la rotura de la celda; como consecuencia, se altera la estructura, lo que facilita la liberación al disolvente de los compuestos bioactivos, mejorando el coeficiente de transferencia (Maran y Prakash, 2015; Han y col., 2019; Hu y col., 2019a) (Figura 8). MAE es una técnica prometedora para la extracción de polisacáridos; tiene ventajas tales como altos rendimientos, menor uso de solventes, tiempos de extracción más cortos y ser amigable con el medio ambiente (Su y col., 2019; Al-Dhabi y Ponmurugan, 2020; Cao y col., 2018).

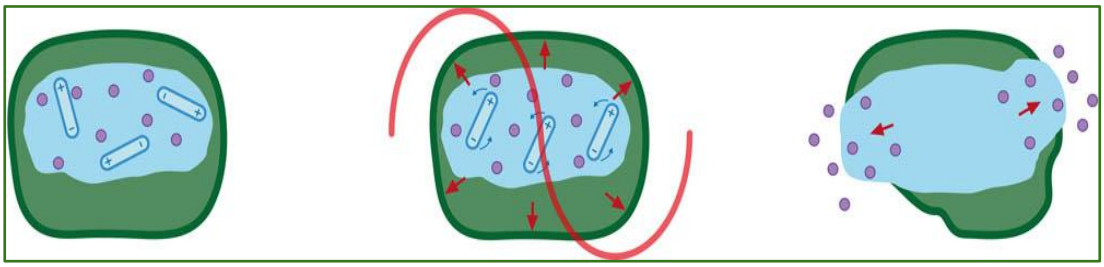


Figura 8. Fenómeno de extracción mediante tecnología microondas. Fuente: https://www.equipar.com.mx/milestone-extraccion

Actualmente, MAE es ampliamente utilizado en la extracción de polisacáridos de diversas fuentes de subproductos, como cáscara de plátano (Swamy y Muthukumarappan, 2017), cáscara de toronja (Chen y col., 2016), subproductos de brócoli (Ferreira y col., 2018) y cáscara de cacao (Mellinas y col., 2020), entre otros. Dao y col. (2021) reportaron mayores rendimientos (18,73 %) y mayor pureza de polisacáridos pécticos extraídos de cáscaras de frutas, como pitaya y maracuyá, en comparación con los métodos convencionales. Además, se acortaron los tiempos de extracción, lo que se tradujo en una reducción del consumo energético. También se han informado altos rendimientos de polisacáridos de hemicelulosa como xilano, glucuronoxilano y xiloxilano extraídos por MAE de residuos de plantas de tabaco (Yuan y col., 2019a).

6.4. Extracción asistida por enzimas (EAE)

Mientras que los métodos EAU y MAE rompen la pared celular, EAE degrada las partes de la célula por hidrólisis enzimática, lo que provoca una mejora en el rendimiento y la actividad biológica de los polisacáridos (Abuduwaili y col., 2019; Nadar y col., 2018). El método es selectivo para los compuestos bioactivos extraídos y respetuoso con el medio ambiente (Hashemifesharaki y col., 2020). El rendimiento de la extracción depende de varios factores, como la relación líquido-sólido, el pH, la cantidad de enzima, la temperatura y el tiempo de extracción (Guo y col., 2020; Poojary y col., 2017).

EAE se ha utilizado eficazmente para la extracción de pectina del orujo de kiwi, demostrando un mayor rendimiento de pectina mediante extracción enzimática con Celluclast (celulasas, poligalacturonasa, pectina liasa y ramnogalacturonano liasa) que mediante extracción ácida con ácido cítrico (Yuliarti y col., 2015). Wikia y col. (2015) también informaron sobre mayores rendimientos de extracción de pectina (15,3 %) del orujo de

manzana mediante EAE en comparación con la extracción ácida con ácido sulfúrico. En otro trabajo EAE logró mayores rendimientos de extracción de polisacáridos de la cáscara de granada que los obtenidos con HWE y UAE y los polisacáridos extraídos mediante EAE mostraron fuertes propiedades antioxidantes (Li y col., 2018b). Asimismo la extracción de polisacáridos de *Dendrobium chrysotoxum* por EAE proporcionó rendimientos 1,25 veces mayores que con HWE (Pan y col., 2015).

6.5. Extracción de fluidos supercríticos (SFE)

SFE es una tecnología emergente en la extracción de compuestos bioactivos que permite conservar las cualidades naturales de los compuestos y garantiza la seguridad alimentaria (Da Silva y col., 2016). El punto crítico del CO_2 (304.15 k y 7,38 MPa) (Figura 9) permite recuperar compuestos bioactivos con un alto grado de pureza y extractos limpios especialmente útiles para alimentos funcionales (Mushtaq y col., 2015). La tecnología SFE se ha utilizado, en gran medida, para sustancias apolares, aunque es posible la extracción selectiva de compuestos polares mediante el uso de modificaciones. Por lo tanto, el uso de codisolventes, como el etanol y el metanol, puede aumentar la eficiencia de la extracción de polisacáridos (Rivas y col., 2021a). Sin embargo, las condiciones de SFE y la información disponible sobre la extracción de polisacáridos y sus propiedades son limitadas. Chen y col. (2014) reportaron un rendimiento de 18,59% para SFE de polisacáridos de plantas de *Artemisia sphaerocephala* con parámetros de extracción de 45 MPa a 45 °C, con un flujo de CO_2 de 20 L/h por 2 h. Asimismo Rivas y col. (2021a) SFE optimizado usando CO_2 para obtener compuestos de alto valor a partir de subproductos de cáscara de granada.

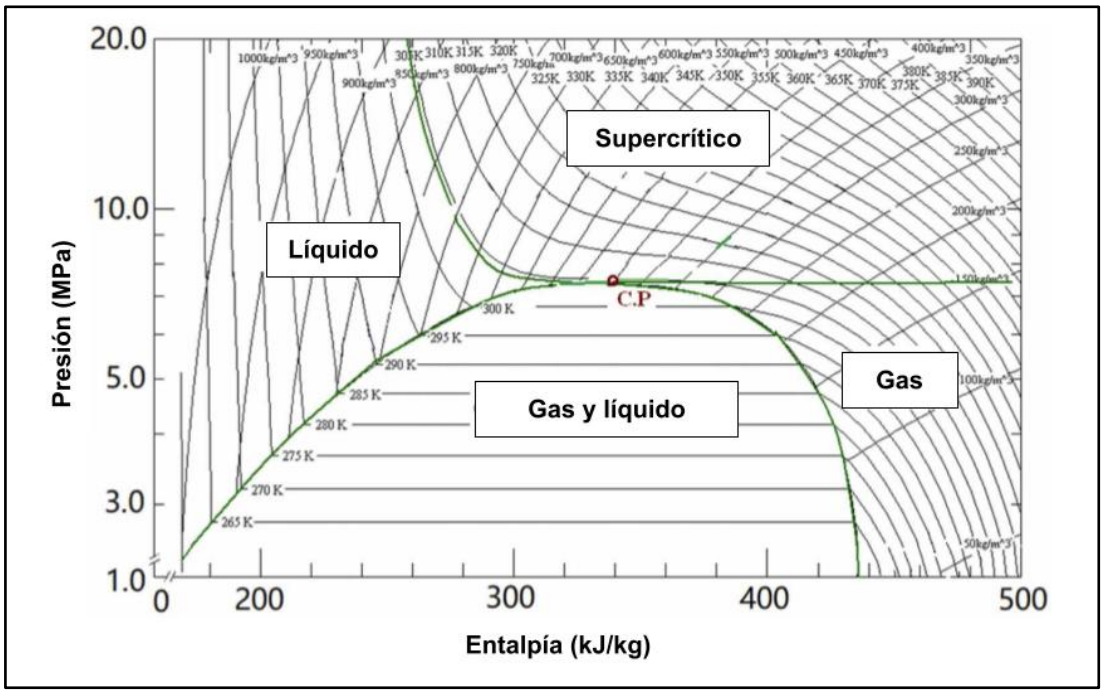


Figura 9. Diagrama de fases de dioxide de carbono. Fuente: Adaptado de Chen y col. (2022).

6.6. Otros métodos de extracción

Se están desarrollando nuevos métodos de extracción de polisacáridos, como la extracción profunda con disolventes eutécticos (Shang y col., 2021), la extracción acelerada con disolventes (Chen y col., 2018; Liew y col., 2018) y la microfluidización dinámica a alta presión (Zhang y col., 2015). Además, los métodos mencionados anteriormente también se pueden combinar para mejorar los rendimientos y las funciones bioactivas de los polisacáridos.

Estudios anteriores informaron de polisacáridos compuestos por manosa, ramnosa, glucosa, galactosa, xilosa, arabinosa, fucosa, ácido glucurónico y ácido galacturónico, se extrajeron del orujo de piña combinando UAE y EAE para proporcionar una extracción sinérgica de celulasa ultrasónica; los polisacáridos extraídos demostraron la capacidad de inhibir el desarrollo de células HepG2 resistentes a la insulina y, por lo tanto, pueden considerarse ingredientes potenciales para desarrollar un nuevo alimento beneficioso (Hu y col., 2019b). Además, Liew y col. (2018) informó un rendimiento de 1,5 a 3,5 veces mayor para la extracción de polisacáridos pécticos que combinaban UAE y EAE en comparación con los métodos de extracción por separado.

7.- ESTRATEGIAS PARA PURIFICAR E INCREMENTAR LA ACTIVIDAD BIOLÓGICA DE LA FIBRA DIETÉTICA

La bioactividad de la fibra dietética depende de su estructura, unidades de monosacáridos, tipo de enlace, configuración espacial, distribución de cadenas ramificadas y peso molecular (Huang y col., 2020; Gao y col., 2017). El alto peso molecular de los polisacáridos en la naturaleza hace que sea necesario modificarlos y degradarlos para mejorar su solubilidad y hacerlos más accesibles a las células (Liu y col., 2017; Tang y col., 2014). Se utilizan métodos químicos (Zheng y col., 2019b), físicos (Hashemifesharaki y col., 2020) y biológicos (Li y col., 2020a; Liu y col., 2021) para purificar y modificar los polisacáridos que forman la fibra dietética, reduciendo así su tamaño y peso molecular para mejorar su bioactividad.

7.1. Modificación enzimática

La degradación enzimática mejora las propiedades y la bioactividad de los polisacáridos modificando su estructura y reduciendo el peso molecular (Ma y col., 2018). La principal ventaja es la alta selectividad, que permite modificar los polisacáridos de forma personalizada, obteniendo estructuras bien definidas (Karaki y col., 2016). La modificación enzimática, además de mejorar las propiedades de los polisacáridos y la producción de oligosacáridos (Ma y col., 2018), también aumenta la posibilidad de extraer polifenoles no extraíbles asociados con los polisacáridos de la pared celular (Radenkovs y col., 2018; Kitrytė y col., 2017; Esparza-Martínez y col., 2016).

Se ha reportado la obtención de POS con un grado específico de polimerización por tratamiento enzimático de polisacáridos pécticos extraídos de la piel de cebolla. El tratamiento de pectina se realizó con dos enzimas (Endo-Poligalacturonasa M2 (EPG-M2) y pectinasa) y un complejo multienzimático llamado Viscozyme (una mezcla de enzimas que contiene actividades de xilanasa, arabanasa, β-glucanasa, hemicelulosa y celulasa) (Nolasco-Arroyo y col., 2019), siendo la enzima EPG-M2 la que mejores resultados mostró para la producción de POS (Babbar y col., 2016). En otro trabajo Mateo y col. (2017) purificaron mediante tratamiento enzimático el polisacárido soluble arabinoxilano extraído del salvado de trigo mediante la acción de enzimas (endoxilanasas GH10 y GH11), logrando producir XOS y

arabino-xilooligosacáridos con potencial prebiótico. Asimismo se demostró que el tratamiento enzimático con α-amilasa y amiloglucosidasa de los polisacáridos inmunoestimuladores aislados del ginseng rojo mejora la actividad moduladora del sistema inmunitario intestinal en comparación con los polisacáridos no tratados enzimáticamente (Kim y col., 2019).

7.2. Modificación mediante ultrasonido

La degradación por ultrasonido es un método físico que permite la operación a alta o baja frecuencia dependiendo del propósito previsto (Cui y Zhu, 2021). La degradación por ultrasonido modifica el peso molecular y la solubilidad y mejora las propiedades y la bioactividad de los polisacáridos (Wang y col., 2021; Xu y col., 2019). Además, el tratamiento con ultrasonido es un método rápido y ecológico que permite ahorrar energía (Chemat y col., 2017).

Chen y col. (2019a) demostraron en su estudio el efecto del tratamiento con ultrasonidos sobre la digestibilidad de cinco fracciones de polisacáridos extraídos de brotes de bambú (*Chimonobambusa quadrangularis*). Los resultados indicaron que hubo una mejora en el potencial prebiótico de los polisacáridos. Del mismo modo, Zeaiter y col. (2019) reportaron una mayor actividad prebiótica en polisacáridos de alcachofa extraídos mediante la técnica de ultrasonido en comparación con otros tratamientos. Además de mejorar la actividad prebiótica, se ha demostrado que la modificación por ultrasonido mejora otras propiedades bioactivas, como la actividad antioxidante. Otro estudio también informó que el tratamiento ultrasónico mejoró la actividad antioxidante de los polisacáridos pécticos extraídos del espino blanco (Chen y col., 2019b). Asimismo se ha informado que la modificación por ultrasonido mejora la bioactividad de los polisacáridos, como las propiedades antiinflamatorias (Nuerxiati y col., 2019) y antitumorales (Hu y col., 2017).

7.3. Modificación por tecnología de microondas

El tratamiento de polisacáridos por tecnología de microondas es una vía prometedora para obtener polisacáridos con propiedades y bioactividad adecuadas. La bioactividad de los polisacáridos de origen natural está condicionada por su estructura, composición y número de monosacáridos, peso molecular, tipo de enlace y grado de polimización (Mirzadeh y col., 2020). Por lo tanto, a pesar de ser un enfoque prometedor, es una técnica novedosa para la

mejora de polisacáridos, y el efecto sobre la bioactividad debe investigarse y estudiarse más a fondo. Sin embargo, existe evidencia de que los polisacáridos obtenidos y tratados con tecnología de microondas tienen actividad antioxidante (Yuan y col., 2019b), antifúngica (Bhatia y col., 2015), antiviral (Boulho y col., 2017), antitumoral (Hashemifesharaki y col., 2020) y antibacteriana (Alboofetileh y col., 2019).

Investigaciones han evaluado las concentraciones inhibitorias mínimas de patógenos como *E. coli*, *B. subtilis* y *S. aureus* en presencia de polisacáridos de ginseng tratados con microondas y mediante métodos convencionales. Se observó una mayor actividad antibacteriana en los polisacáridos tratados con microondas en comparación con aquellos tratados mediante tecnología convencional (Zhao et al., 2019). Asimismo Hashemifesharaki y col. (2020) demostraron en su estudio que el tratamiento con microondas es capaz de purificar las fracciones homogéneas de polisacáridos extraídos de la raíz de malvavisco, y estos mostraron una mejora significativa en su actividad antirradical, antioxidante, antimicrobiana y antitumoral.

7.4. Otros tratamientos

Se están investigando otras técnicas para la modificación y purificación de polisacáridos, como el procesamiento de plasma frío (Jiang y col., 2020; Zhu, 2017), procesamiento de campo eléctrico pulsado (Zhu, 2018), alta presión (Mohan y col., 2020), UAE de alta presión (Yang y col., 2019; Pereira y col., 2019), fluidos supercríticos (Escobar y col., 2020; Barbosa y col., 2020) y degradación con ácido ascórbico (Zou y col., 2020; Lin y col., 2019).

El plasma frío es una tecnología emergente en el procesamiento de compuestos biológicos sensibles a la temperatura (Segat y col., 2016), y también se considera una tecnología respetuosa con el medio ambiente (Pankaj y col., 2018). Se ha utilizado para modificar las propiedades y la bioactividad de los polisacáridos (Pankaj y col., 2015). El polisacárido galactomanano se extrajo de las semillas de leguminosas y se modificó por degradación con plasma frío; los resultados mostraron propiedades funcionales y reológicas mejoradas del polisacárido (Rashid y col., 2020).

La tecnología de campo eléctrico pulsado es una tecnología no térmica respetuosa con el medio ambiente (Yang y col., 2016). Se utiliza para la extracción y purificación de polisacáridos y oligosacáridos, como la pectina (Saberian y col., 2017; de Oliveira y col., 2015; Almohammed y col., 2017) y la inulina (Khuenpet y col., 20174), mejorando sus propiedades.

La alta presión es un método no térmico cada vez más popular en el procesamiento de compuestos bioactivos. Se ha utilizado para la modificación de macromoléculas, mejorando significativamente sus propiedades funcionales y bioactividad. Actúa sobre los polisacáridos provocando cambios en su estructura, unión y composición y número de monosacáridos (Huang y col., 2018). Porfiri y col. (2017) mostraron el alto potencial de la alta presión para modificar polisacáridos, logrando la solubilización de ciertos compuestos de soja considerados polisacáridos insolubles.

8.- APLICACIONES DE FIBRA DIETÉTICA EN LA INDUSTRIA ALIMENTARIA

La incorporación de fibra dietética en determinados alimentos puede provocar cambios en la textura, en las características organolépticas y en la vida útil de los alimentos. Además, la aplicación de la fibra dietética en los alimentos puede atender a diversas razones, tales como, su uso como sustituto de otros compuestos menos saludables (grasa o azúcares, fundamentalmente), o simplemente para suplementar alimentos que contienen poca fibra dietética o que carecen de ella.

Tabla 3: Tabla resumen: Aplicaciones de la fibra dietética procedente de subproductos agrícolas y su efecto sobre el alimento

Efecto de la aplicación	Tipo de fibra y alimento sobre el que se aplica	Referencias
Mejora de la textura	Se mejoraron las propiedades texturales en salchichas de Frankfurt tras la adición de fibra dietética extraída de piña. Se observó una reducción en los parámetros de dureza, masticabilidad, gomosidad y elasticidad.	Henning y col., 2016
Mejora de la textura	Se encontraron cambios significativos en los parámetros texturales tras la aplicación de FOS en salchichas de Frankfurt. Los valores de fuerzas de penetración y compresión fueron superiores en las muestras con FOS.	Felisberto y col., 2015
Mejora de la textura	La aplicación de fibra dietética procedente de brotes de bambú a un pudin de leche, mejoró las propiedades texturales y aumentó la dureza y elasticidad.	Zheng y col., 2017
Mejora de la textura	Se observó una mejora en la viscosidad del producto tras enriquecer mermelada con fibra dietética del fruto del melocotón.	Dalal y col., 2020
Mejora de la vida útil	La fibra dietética adicionada a la pasta consiguió una mayor vida útil y mejor calidad del producto final.	Bustos y col., 2015
Mejora de la vida útil	Aumento de la vida útil del yogur al añadir fibra dietética	Mohamed y col., 2014
Mejora de la vida útil	Se observó una inhibición en la oxidación de lípidos durante el almacenamiento tras incorporar fibra dietética rica en pectina a nuggets de carne de ovino	Verma y col., 2013
Sustitutivo de compuestos poco saludables	Se añadió celulosa como sustituto de la grasa en carne emulsionada y cocida, obteniendo una reducción significativa de grasa sin cambios en las propiedades tecnológicas.	Schmiele y col., 2015
Sustitutivo de compuestos poco saludables	Se logró una reducción del 70% de grasa sin cambios en las propiedades organolépticas y texturales, sustituyendo la grasa en helados con fibra dietética extraída de subproductos de la naranja.	Moraes Crizel y col., 2013
Como suplemento nutricional	Galletas de harina de trigo suplementadas con polvo de orujo de zanahoria rico en fibra dietética mostraron que el contenido de fibra dietética total aumentó respecto al control. Además, las propiedades antioxidantes y sensoriales también se vieron mejoradas.	Ahmad y col., 2016
Como suplemento nutricional	Agregar huesos de dátiles ricos en fibra a las hamburguesas hizo que el producto final fuera más saludable, con mayor contenido en fibra dietética. Además, logró mantener sus propiedades organolépticas y texturales.	Sayas-Barberá y col., 2020

8.1. Mejora de la textura

Entre las distintas aplicaciones que tiene la fibra dietética, cabe destacar su uso como agente texturizante. Por este motivo la fibra dietética es adicionada a multitud de alimentos como productos lácteos, productos cárnicos, jaleas, helados y productos de panadería. Henning y col. (2016) informaron de la mejora de las propiedades texturales en salchichas de Frankfurt tras la adición de fibra dietética previamente extraída de piña. Observaron que los parámetros de dureza, masticabilidad, gomosidad y elasticidad se redujeron con la adición de fibra dietética de piña. En otro trabajo se informó sobre el efecto de los FOS cuando se adicionaron a salchichas de Frankfurt y los resultados del análisis textural mostraron cambios significativos. Se observaron valores superiores en las fuerzas de penetración y compresión en el control en comparación con las salchichas previamente adicionadas con FOS, lo que se atribuyó a la capacidad de formación de geles de los FOS (Felisberto y col., 2015). También en productos lácteos los resultados de algunos trabajos resultaron prometedores. Zheng y col. (2017) adicionaron fibra dietética procedente de brotes de bambú a un pudin de leche y sus resultados sugieren que su aplicación mejoró las propiedades texturales y fortaleció la dureza y elasticidad. Del mismo modo, Dalal y col. (2020) enriquecieron mermelada con fibra dietética del fruto del melocotón y ésta exhibió una mejor viscosidad (Dalal et al., 2020).

8.2. Mejora de la vida útil

Actualmente es poco lo que se conoce sobre la mejora de la vida útil de los alimentos tras la aplicación de fibra dietética y se trata de un uso poco extendido. Sin embargo, son muchos los trabajos que confirman el aumento de la vida útil de diversos productos tras la adición de fibra dietética a los mismos. Bustos y col. (2015) añadieron fibra dietética a la pasta y como resultado obtuvieron fideos con una mayor vida útil y mejor calidad. También otros autores confirmaron un aumento de la vida útil en productos lácteos como el yogur al añadir fibra dietética, el exceso de agua del producto es absorbido por la fibra dietética y de ésta forma la vida útil del yogur se ve prolongada (Mohamed y col., 2014). También en la industria cárnica la aplicación de fibra dietética a la carne consigue aumentar la vida útil. Verma y col. (2013) incorporaron fibra dietética rica en pectina procedente de polvo de guayaba a nuggets de carne de ovino y observaron que la oxidación de lípidos se inhibió en los nuggets durante el almacenamiento en comparación con las muestras de control sin fibra

dietética añadida. Además, las muestras control recibieron una puntuación sensorial más baja, en los parámetros de sabor y olor, como resultado de una mayor oxidación de lípidos.

8.3. Sustitutivo de compuestos poco saludables

El marco actual de salud pública promueve el aumento de la ingesta de fibra dietética y la disminución de los azúcares simples y grasas como parte de una dieta saludable (Organización Mundial de la Salud, 2015; Bates y col., 2020). Es por ello, que la sustitución de las grasas y azúcares por fibra dietética en los alimentos podría ser una solución (Cotton y col., 1996).

El proceso de sustitución de la grasa de los alimentos consiste en la eliminación de la grasa y su sustitución por otros elementos, como agua, aire, proteínas o carbohidratos (Mikkelsen y col., 2000). Las fibras dietéticas cada vez son más utilizadas como sustitutos de grasas, ya que su uso reduce el contenido de la misma a la vez que aumenta el de fibra dietética de los alimentos (Sahan y col., 2008). Debido a la textura de la fibra dietética, sustituir el contenido total de grasa sigue siendo un tema a investigar (Zamora y col., 2023). Es por ello que el reemplazo de grasas es parcial y los investigadores buscan el equilibrio entre grasa y fibra dietética (Scheuer y col., 2014).

Schmiele y col. (2015) analizaron el uso de la celulosa como sustituto de la grasa en un sistema modelo de carne emulsionada y cocida, utilizando una Metodología Superficie de Respuesta. Los resultados mostraron una reducción de grasa del 50 % con respecto al lote control. Además, no se modificaron de forma significativa las propiedades organolépticas, por lo que la celulosa demostró ser un ingrediente funcional prometedor como sustituto de la grasa. Otros autores utilizaron fibra dietética para reemplazar la grasa de otros alimentos, como el helado. En otro trabajo de Moraes Crizel y col. (2013) sustituyeron la grasa en helados con fibra dietética extraída de los subproductos de la naranja. Los resultados obtenidos mostraron una reducción del 70 % de la grasa sin ocasionar cambios en las propiedades organolépticas y texturales.

Los polisacáridos procedentes de la fibra dietética también son utilizados como sustitutos de azúcares simples por sus propiedades como edulcorantes (Gerschenson y col., 2017). Rodriguez-Garcia y col. (2022) en su estudio el objetivo fue comparar la funcionalidad de cuatro fibras solubles, dos de inulina y dos de dextrosa como posibles sustitutos de la sacarosa en galletas de masa corta evaluando su efecto sobre la reología de la masa, las dimensiones de la galleta, la textura, el color y el perfil sensorial. Los resultados mostraron que las fibras solubles pueden reemplazar con éxito el azúcar en galletas de masa corta reduciendo de manera significativa el contenido de sacarosa con respecto al control. Sin embargo, se encontraron cambios poco importantes en las propiedades organolépticas, todas las galletas reducidas en azúcar fueron significativamente más firmes y crujientes en el perfil sensorial. Carcelli y col. (2021), en su estudio desarrollaron y caracterizaron tres recetas de salsas de fresa reducidas en azúcar (30%, 50% y 70%), sustituyéndola por un jarabe semisólido de fibra y los resultados mostraron que además de reducir el contenido de azúcares reductores, aumentó el contenido de fibra dietética desde 0 g/100 g de salsa hasta 31,5 g/100 g de salsa, lo que les permitió informar en la etiqueta del producto "Reducido en azúcar" y "alto en fibra dietética".

8.4. Como suplemento

La fibra dietética procedente de subproductos agrícolas como frutas y hortalizas puede utilizarse para aumentar el valor nutricional del producto final (Twarogowska y col., 2022). Son muchos los trabajos donde se suplementan determinados alimentos como productos cárnicos, lácteos y de panadería con concentrados de fibra dietética, con el fin de enriquecerlos o mejorar sus propiedades funcionales y bioactivas (Heo y col., 2019; Yadav y col., 2020; Santos y col., 2022).

Se incorporó polvo de orujo de zanahoria rico en fibra dietética a galletas elaboradas con harina de trigo, los resultados mostraron que el contenido de fibra dietética total aumentó respecto al control, además, las propiedades antioxidantes y sensoriales también se vieron mejoradas (Ahmad y col., 2016). Sayas-Barberá y col. (2020) utilizaron huesos de dátiles ricos en fibra dietética como aditivo para mejorar su contenido en hamburguesas y conseguir un producto más saludable. Los resultados mostraron que el producto final al que se le añadió el extracto de los huesos de dátiles tenía mayor contenido de fibra dietética, manteniendo además, sus propiedades organolépticas y texturales.

OBJETIVOS

El **objetivo general** de esta Tesis Doctoral es la revalorización de subproductos agrícolas como fuente de fibra dietética, mediante la evaluación detallada de su composición química, estructura y propiedades funcionales. Se emplearán diversas tecnologías de tratamiento de la fibra dietética, como fluidos supercríticos, enzimático, autoclave y ultrasonido, con el fin de maximizar su aprovechamiento. Además, se investigará el impacto de la fibra dietética en aspectos como el tránsito intestinal, la supervivencia y el crecimiento de cepas probióticas, así como su digestibilidad *in vitro*. Se explorará también su potencial como sustituto de azúcares añadidos en alimentos y se buscará desarrollar nuevos ingredientes alimentarios prebióticos o simbióticos.

Para la consecución de este objetivo general son necesarios los siguientes **objetivos específicos**:

1. Analizar la composición química, las propiedades funcionales y tecnológicas de la fibra dietética procedente de subproductos agrícolas.

2. Investigar los efectos de la fibra dietética de los subproductos agrícolas en la supervivencia, crecimiento y metabolismo de cepas con propiedades probióticas durante el tránsito intestinal.

3. Evaluar la mejora de las propiedades funcionales y tecnológicas de la fibra dietética de los subproductos agrícolas después de someterlos a postratamientos de modificación con diferentes tecnologías: fluidos supercríticos, tratamiento enzimático, autoclave y ultrasonido.

4. Evaluar las propiedades funcionales y tecnológicas de la fibra dietética de los subproductos agrícolas tras un pretratamiento con diferentes condiciones de fluidos supercríticos

5. Modelizar las condiciones de fluidos supercríticos como postratamiento de modificación de la fibra dietética para la optimización de sus propiedades funcionales y tecnológicas.

6. Estudiar la digestibilidad *in vitro* de la fibra dietética de diversos subproductos agrícolas utilizando un equipo de digestión simulada automatizado, con el fin de

analizar su composición, estructura y propiedades funcionales durante las distintas etapas de la digestión.

7. Analizar el efecto de la adición de fibra dietética obtenida a partir de subproductos agrícolas como sustituto de los azúcares añadidos, sobre los aspectos fisicoquímicos, nutricionales y sensoriales de ciertos alimentos.

MATERIAL Y MÉTODOS

Para el desarrollo de los diferentes trabajos se utilizaron 8 subproductos agrícolas (Figura 10) y bacterias con propiedades probióticas (Figura 11).

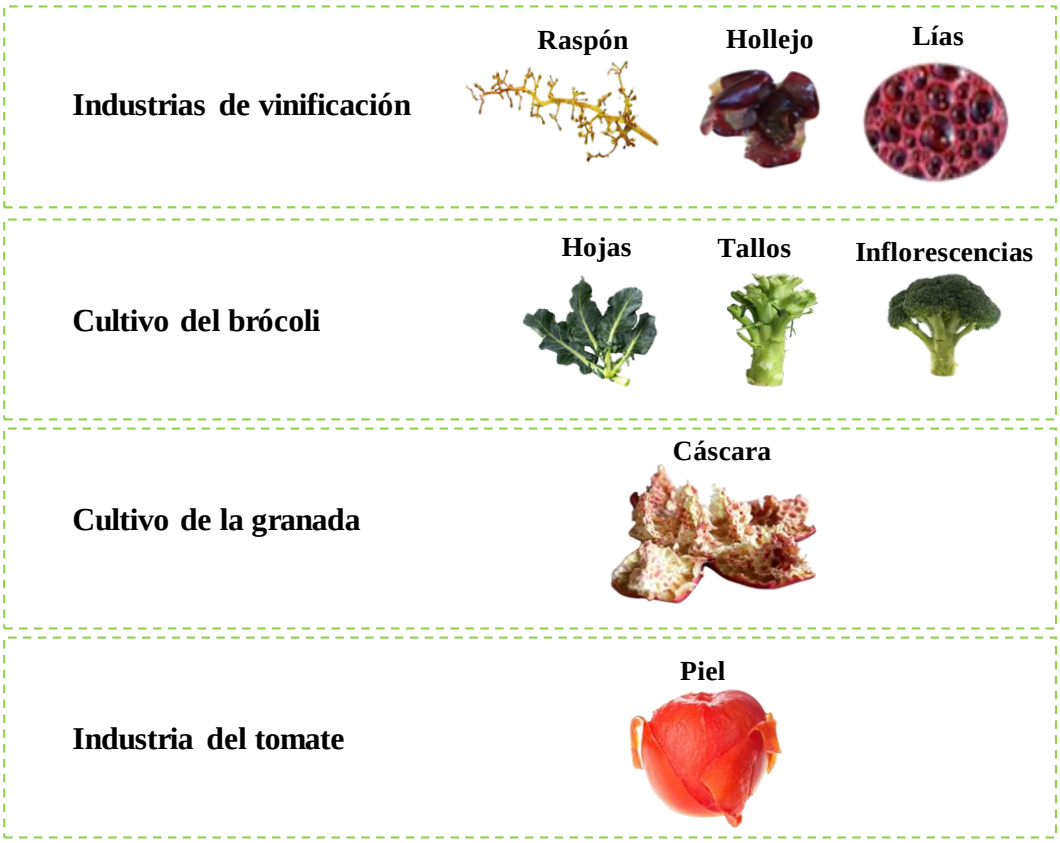


Figura 10. Subproductos agrícolas.

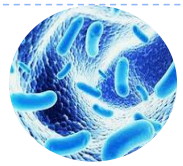
Lactococcus lactis CECT 188 *Lactobacillus curvatus CECT 904* *Lactobacillus sakei CECT 5765 y CECT 980* *Lactobacillus brevis CECT 815* *Lactobacillus plantarum G1LB5* *Lactobacillus casei HL 245 y HL 233* *Enterococcus faecium SE 906 y SE 920*		**Capítulo 1**
Lactobacillus casei HL 245 y HL 233 *Lactobacillus reuteri PL 503 y PL 519* *Enterococcus faecium SE 906 y SE 920*		**Capítulo 2**
Lactobacillus sakei CECT 5765 *Lactobacillus brevis CECT 815* *Lactobacillus plantarum AB572C* *Lactobacillus casei HL233* *Enterococcus faecium SE920*		**Capítulo 3**

Figura 11. Especies de microorganismos utilizados durantes el estudio.

CAPÍTULO 1

Para el desarrollo del **Capítulo 1** de la tesis doctoral se realizó el siguiente diseño experimental (Figura 12). Para llevarlo a cabo se eligieron los subproductos más relevantes de la industria vitivinícola como raspón, hollejo y lías de vinificación.

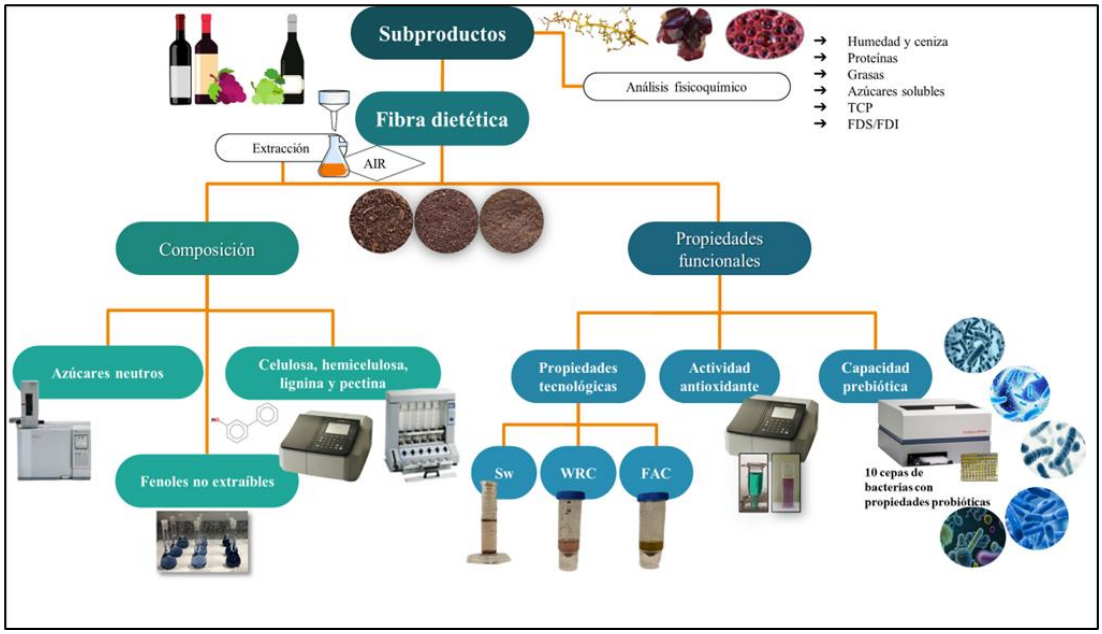


Figura 12. Diseño experimental del capítulo 1.

En primer lugar, se caracterizaron fisicoquímicamente los subproductos para conocer su contenido de humedad, cenizas, proteínas, grasas y fibra dietética soluble (FDS) e insoluble (FDI). En la siguiente etapa se extrajo la fibra dietética total de los subproductos siguiendo el método del residuo insoluble en alcohol (AIR). Una vez extraída la fibra dietética se analizó su composición. En segundo lugar, se determinó el contenido de celulosa, hemicelulosa y lignina mediante el uso del equipo dosi-fiber. Posteriormente, se analizó el perfil de azúcares neutros, mediante cromatografía de gases. Asimismo, el contenido de pectina y compuestos fenólicos no extraíbles asociados a la fibra dietética fueron determinados espectrofotométricamente utilizando los métodos m-hidroxidifenilo y Folin-Ciocalteure, respectivamente. En la última etapa se realizó el estudio de las propiedades funcionales de la fibra dietética de los subproductos. La capacidad antioxidante se midió por dos métodos, DPPH (2,2-difenil-1-picrilhidrazilo) y ABTS (2,2′-azinobis (ácido 3-etilbenzotiazolina-6-sulfónico)). La capacidad prebiótica fue determinada con la ayuda de un lector de microplacas de

fluorescencia. Además, fueron analizadas las propiedades tecnológicas, la capacidad de hinchamiento (Sw), la capacidad de retención de agua (WRC) y capacidad de retención de aceite (FAC).

Análisis estadístico del Capítulo 1:

Todos los experimentos se realizaron por triplicado.

El análisis estadístico de los datos se realizó utilizando SPSS para Windows, versión 21.0 (IBM Corp., Armonk, NY, EE. UU.). Se determinaron estadísticas descriptivas de los datos y las diferencias dentro y entre los grupos se estudiaron mediante análisis de varianza unidireccional (ANOVA) y se separaron mediante la prueba honesta de diferencias significativas de Tukey ($p \leq 0,05$).

Se realizó análisis de componentes principales (PCA) sobre la matriz de correlación de las variables.

CAPÍTULO 2

Para el desarrollo del **Capítulo 2** se llevó a cabo el diseño experimental que aparece en la siguiente Figura 13.

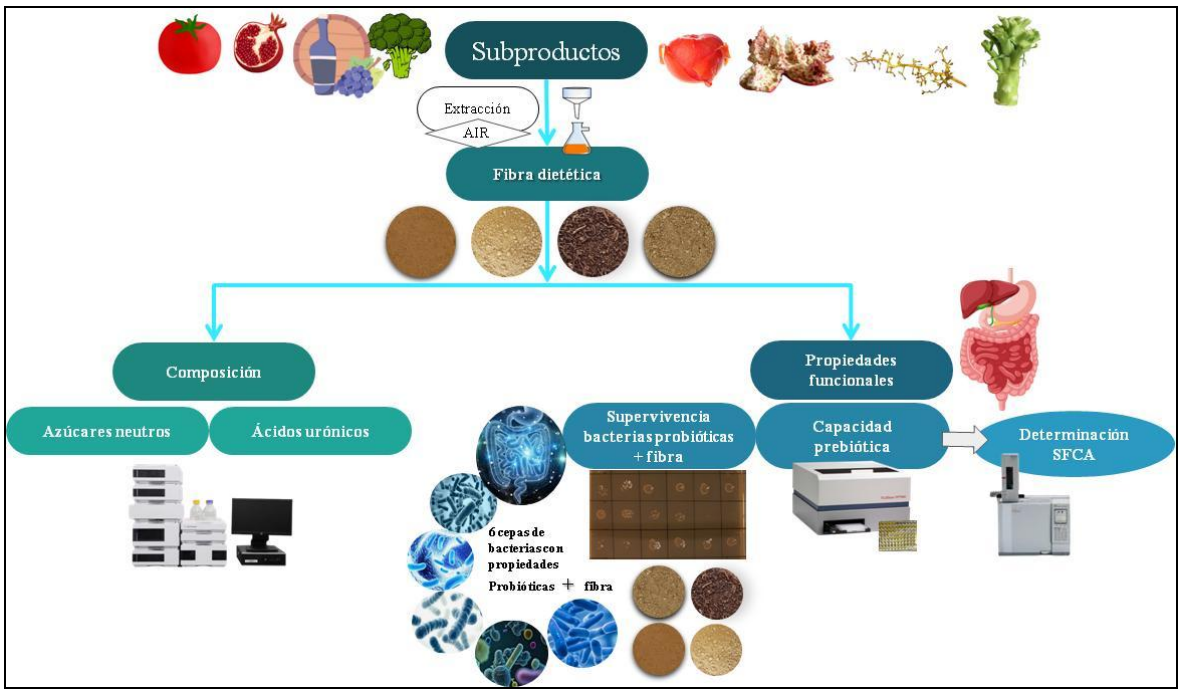


Figura 13. Diseño experimental del capítulo 2.

Para desarrollarlo, se eligieron subproductos procedentes del tomate, granada, brócoli e industrias de vinificación. En primer lugar, se extrajo la fibra dietética mediante el método del residuo insoluble en alcohol (AIR), procedente de la cáscara de tomate, cáscara de granada, tallos de uva y tallos de brócoli. En la etapa siguiente, se analizó la composición de cada uno de los extractos de fibra dietética obtenidos, para conocer su perfil de azúcares neutros y su contenido en ácido urónico mediante cromatografía líquida de alta presión (HPLC). En la última etapa, se estudió la supervivencia a través del tránsito gastrointestinal de 6 cepas de bacterias con propiedades probióticas en presencia de los extractos de fibra dietética. Por último, se estudió la capacidad prebiótica de la fibra dietética de los 4 subproductos, analizando el crecimiento de estas bacterias probióticas cuando crecieron en su presencia como única fuente de carbono, y su crecimiento fue medido en un turbidómetro. Finalmente, se determinó la producción de ácidos grasos de cadena corta (SFCA) de dichas bacterias probióticas cuando crecieron en presencia de los extractos de fibra dietética.

Análisis estadístico del Capítulo 2:

El análisis estadístico de los datos se realizó utilizando SPSS para Windows, versión 21.0 (IBM Corp., Armonk, NY, EE. UU.). Se determinaron estadísticas descriptivas de los datos y las diferencias dentro y entre los grupos se estudiaron mediante análisis de varianza unidireccional (ANOVA) y se separaron mediante la prueba de diferencias honestamente significativas de Tukey ($p \leq 0,05$). Se realizó análisis de componentes principales (PCA) sobre la matriz de correlación de las variables. Se trabajó con tres réplicas biológicas y cada una de ellas fue analizada por triplicado.

CAPÍTULO 3

Para desarrollar **el Capítulo 3**, se diseñó el experimento detallado en la Figura 14.

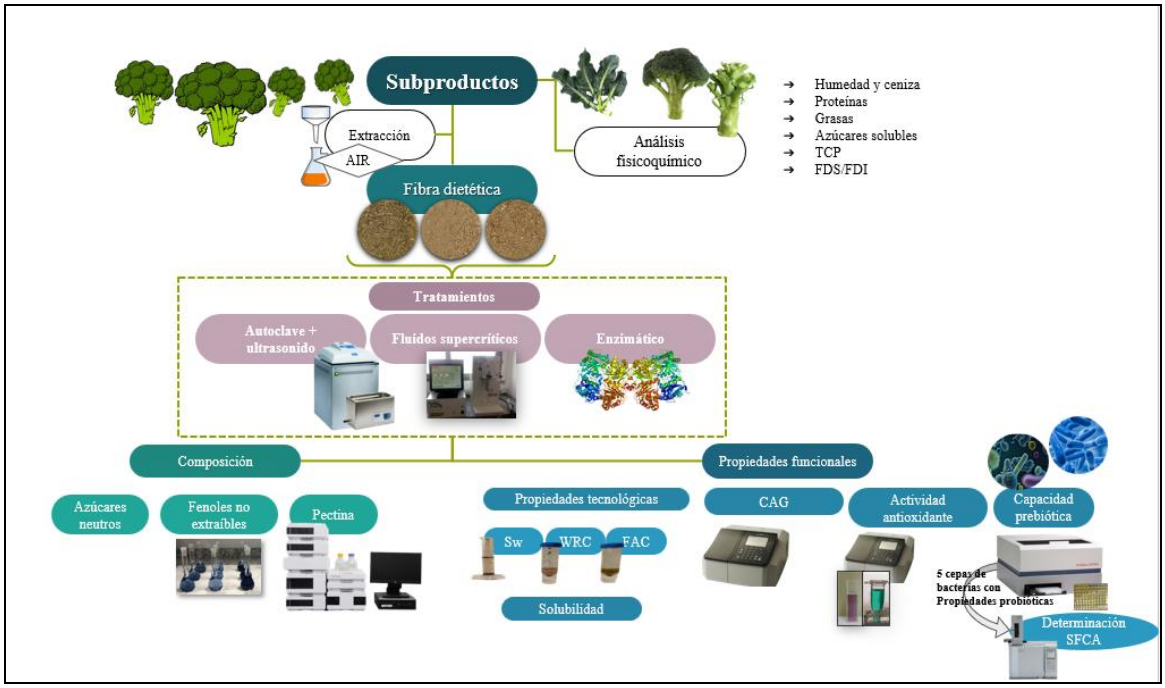


Figura 14. Diseño experimental del capítulo 3.

Los subproductos seleccionados fueron subproductos procedentes del cultivo de brócoli, como tallos, hojas e inflorescencias. En primer lugar, se llevó a cabo la caracterización fisicoquímica de los tres subproductos, evaluando parámetros como humedad, cenizas, proteínas, grasas, azúcares solubles, compuestos fenólicos totales (TCP) y fibra dietética soluble (FDS) e insoluble (FDI). Posteriormente, se extrajo la fibra dietética de los subproductos y los concentrados resultantes se sometieron a diferentes tratamientos, incluyendo tratamientos enzimáticos, con tecnología supercrítica, y con la combinación de autoclave y ultrasonido. Después de aplicar estos tratamientos, se analizó la composición y las propiedades funcionales de los concentrados de fibra dietética tratados, en comparación con un grupo de control al que no se le aplicó tratamiento. Para estudiar la composición de los concentrados, se analizaron los azúcares neutros y el contenido de pectina mediante cromatografía líquida de alta eficiencia (HPLC), y el contenido de fenoles no extraíbles se analizó utilizando un método colorimétrico. Entre las propiedades funcionales analizadas se

incluyeron las propiedades tecnológicas, como la capacidad hinchamiento (Sw), la capacidad de retención de agua (WRC) y de aceite (FAC). Además, se evaluó la solubilidad y la capacidad de absorción de glucosa (CAG), y se midió la actividad antioxidante mediante dos métodos: DPPH (2,2-difenil-1-picrilhidrazilo) y ABTS (2,2′-azinobis (ácido 3-etilbenzotiazolina-6-sulfónico)). La capacidad prebiótica se determinó utilizando un lector de microplacas de fluorescencia. Por último, se determinó la producción de ácidos grasos de cadena corta (SFCA) cuando las bacterias crecieron en presencia de los extractos de fibra dietética tratados y sin tratar como única fuente de carbono.

Análisis estadístico del Capítulo 3:

Se utilizó SPSS para Windows, versión 21.0 (SPSS Inc., Chicago, IL, EE. UU.) para el análisis estadístico de los datos. Se determinaron estadísticas descriptivas de los datos y las diferencias dentro y entre los grupos se analizaron mediante análisis de varianza unidireccional y bidireccional (ANOVA) y se separaron mediante la prueba honesta de diferencias significativas de Tukey ($p \leq 0,05$). Se realizó análisis de componentes principales (PCA) sobre la matriz de correlación de las variables. Se trabajaron tres réplicas biológicas y cada réplica se analizó por triplicado.

CAPÍTULO 4

Para llevar a cabo el diseño experimental del **Capítulo 4**, se siguió el plan de trabajo ilustrado en la Figura 15.

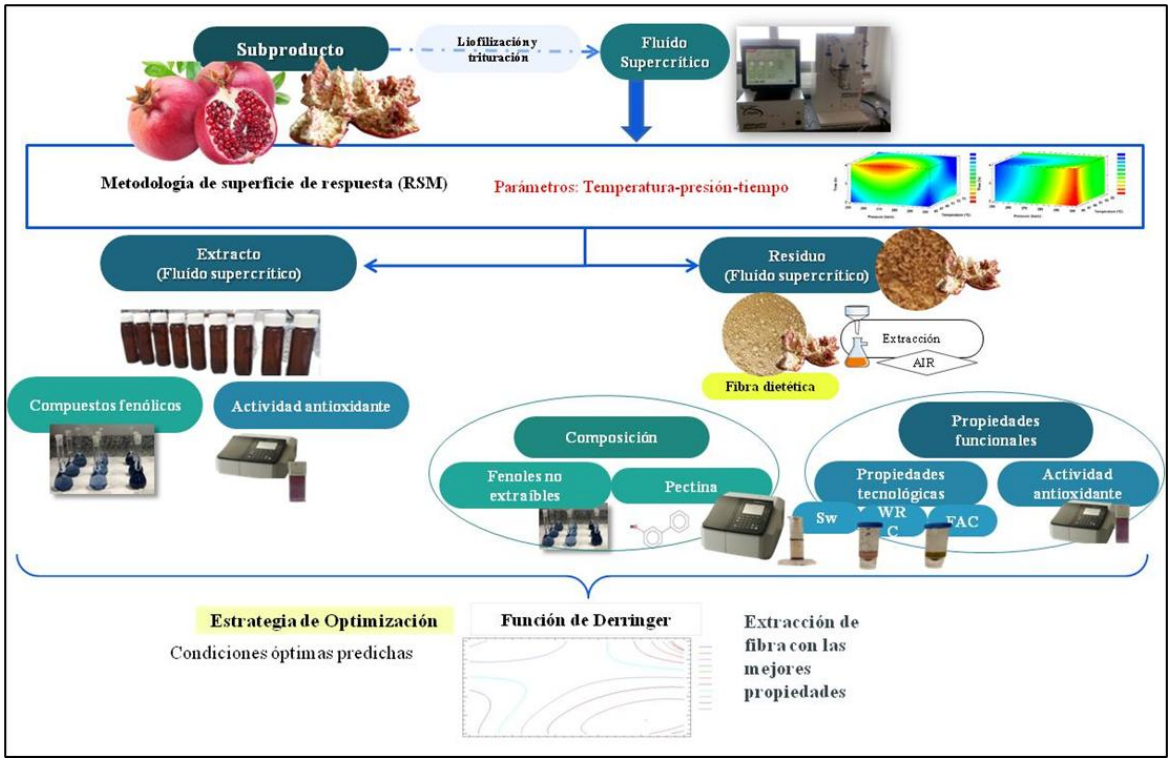


Figura 15. Diseño experimental del capítulo 4.

Para su desarrollo se eligió la cáscara de granada como el subproducto a estudiar. En primer lugar, la cáscara de granada fue sometida a procesos de liofilización y trituración para su preparación. Posteriormente se llevó a cabo la extracción de compuestos bioactivos mediante tecnología supercrítica utilizando un modelo de superficie de respuesta con las condiciones de temperatura, presión y tiempo. Después, se determinó el contenido de compuestos fenólicos totales en los extractos obtenidos y se evaluó su capacidad antioxidante utilizando el método DPPH (2,2-difenil-1-picrilhidrazilo). Simultáneamente, se realizó la extracción de la fibra dietética del residuo resultante de la extracción supercrítica utilizando el método del residuo insoluble en alcohol (AIR). La fibra dietética obtenida se caracterizó en términos de su composición y propiedades funcionales. En cuanto a la composición de la fibra dietética, se analizó su contenido de pectina y compuestos fenólicos no extraíbles

espectrofotométricamente utilizando los métodos m-hidroxidifenilo y Folin-Ciocalteure, respectivamente. Se realizaron pruebas para evaluar las propiedades funcionales de la fibra, incluyendo su capacidad de hinchamiento (Sw), capacidad retención de agua (WRC) y capacidad retención de aceite (FAC). Además, se investigó la capacidad antioxidante de la fibra dietética mediante el método DPPH.

Análisis estadístico del Capítulo 4:

Se aplicó un Box-Behnken (BBD) de tres factores, tres niveles y dos bloques con 15 ejecuciones experimentales por bloque (12 en puntos factoriales y 3 en el centro) combinado con la metodología de superficie de respuesta (MSR) para determinar los efectos. de las condiciones de extracción de fluidos supercrítico sobre las características tanto del extracto como de la fibra dietética residual de la cáscara de granada. MSR se realizó empleando el software StatGraphics Centurion XVI Versión 8.0. El modelo cuadrático fue el siguiente:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \varepsilon$$

donde Y es la variable de respuesta predicha por el modelo; β0 es un valor de compensación; β1, β2 y β3 son los coeficientes de regresión para los términos principales (lineales); β11, β22 y β33 son efectos cuadráticos; β12, β3 y β23 son efectos de interacción; X1, X2 y X3 son las variables independientes; y ε es el error experimental. Para cada factor experimental, el software generó un análisis de varianza (ANOVA), estableciendo la significancia estadística al nivel de confianza del 95%. Con el mismo programa estadístico también se obtuvieron gráficos de superficie de respuesta, el nivel óptimo para cada variable analizada y la optimización de respuestas múltiples mediante la función de deseabilidad de Derringer.

Además, se realizó ANOVA unidireccional para la comparación de muestras de punto central y la extracción con fluidos supercríticos del BBD.

CAPÍTULO 5

Para desarrollar el **Capítulo 5**, llevamos a cabo el experimento detallado en la Figura 16.

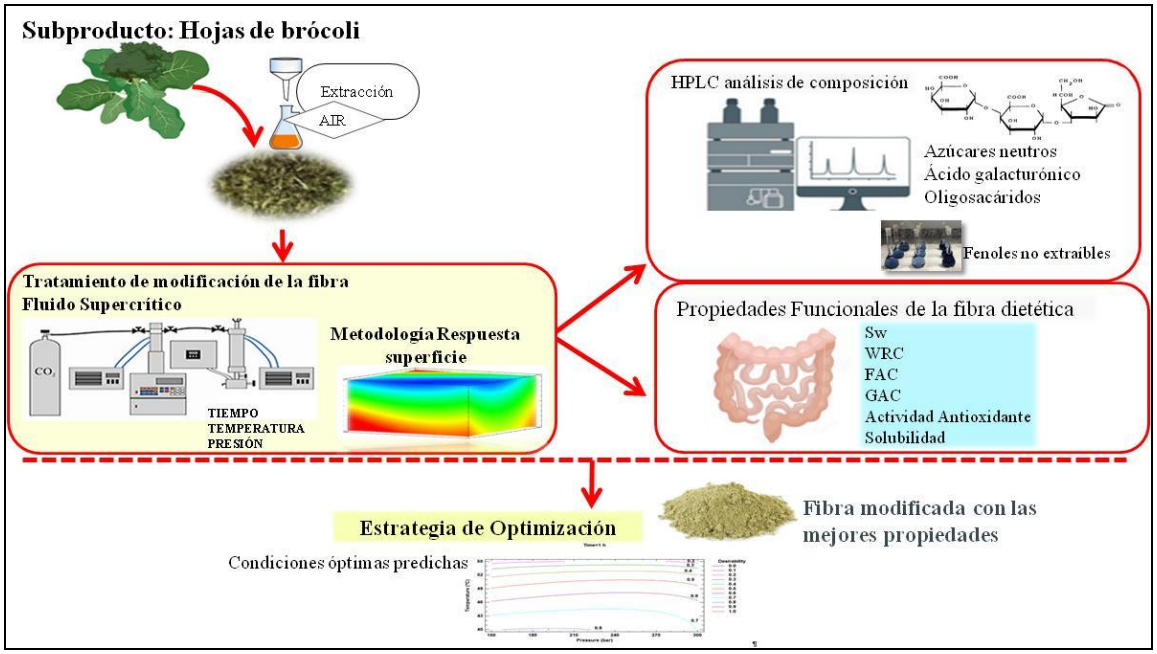


Figura 16. Diseño experimental de la segunda parte del capítulo 5.

Se eligieron las hojas del brócoli como subproducto a investigar. En primer lugar, se extrajo la fibra dietética de las hojas mediante el método del residuo insoluble en alcohol (AIR) y el concentrado resultante se sometió a un proceso de modificación mediante tecnología supercrítica con CO_2. Este proceso se llevó a cabo siguiendo un modelo de superficie respuesta, ajustando las condiciones de temperatura, presión y tiempo. Después de aplicar el tratamiento de modificación, analizamos la composición y las propiedades funcionales de los concentrados de fibra dietética, comparándolos con un grupo de control sin tratar. Para estudiar la composición y estructura de la fibra dietética, utilizamos técnicas de cromatografía líquida de alta eficiencia (HPLC) para determinar el perfil de azúcares neutros, el contenido de ácido galacturónico y la concentración de oligosacáridos y su grado de polimerización. El contenido de fenoles no extraíbles se evaluó espectrofotométricamente utilizando el método Folin-Ciocalteure. Entre las propiedades funcionales analizadas, consideramos propiedades tecnológicas como la capacidad de hinchamiento (Sw), la capacidad de retención de

agua (WRC) y de aceite (FAC). Además, evaluamos la solubilidad y la capacidad de absorción de glucosa (CAG). La actividad antioxidante se midió utilizando dos métodos: DPPH (2,2-difenil-1-picrilhidrazilo) y ABTS (2,2'-azinobis (ácido 3-etilbenzotiazolina-6-sulfónico)).

Análisis estadístico del Capítulo 5:

Se aplicó un diseño Box-Behnken (BBD) de dos bloques, tres niveles y tres factores, con 15 ejecuciones experimentales por bloque (12 en los puntos de los factores y 3 en el medio), combinado con la metodología de superficie de respuesta (MSR), para determinar los efectos de las condiciones de tratamiento con fluido supercrítico en la composición y propiedades funcionales de la fibra dietética modificada de hojas de brócoli. Se utilizó el software StatGraphics Centurion XVI Versión 8.0 para realizar el MSR. El modelo cuadrático fue el siguiente:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \varepsilon$$

donde Y es la variable de respuesta predicha por el modelo; β0 es un valor de compensación; β1, β2 y β3 son los coeficientes de regresión para los términos principales (lineales); β11, β22 y β33 son efectos cuadráticos; β12, β13 y β23 son efectos de interacción; X1, X2 y X3 son las variables independientes; y ε es el error experimental. Para cada factor experimental, el software generó un análisis de varianza (ANOVA), estableciendo la significancia estadística al nivel de confianza del 95%. Con el mismo programa estadístico también se obtuvieron gráficos de superficie de respuesta, el nivel óptimo para cada variable analizada y la optimización de respuestas múltiples mediante la función de deseabilidad de Derringer.

Además, se realizó un ANOVA de una vía para comparar el punto central del tratamiento con fluido supercrítico y el control.

CAPÍTULO 6

Para llevar a cabo el **Capítulo 6**, se siguió el diseño experimental detallado en la Figura 17 con el fin de analizar el efecto de la fibra dietética extraída de distintos subproductos agrícolas durante un proceso de digestión humana simulado.

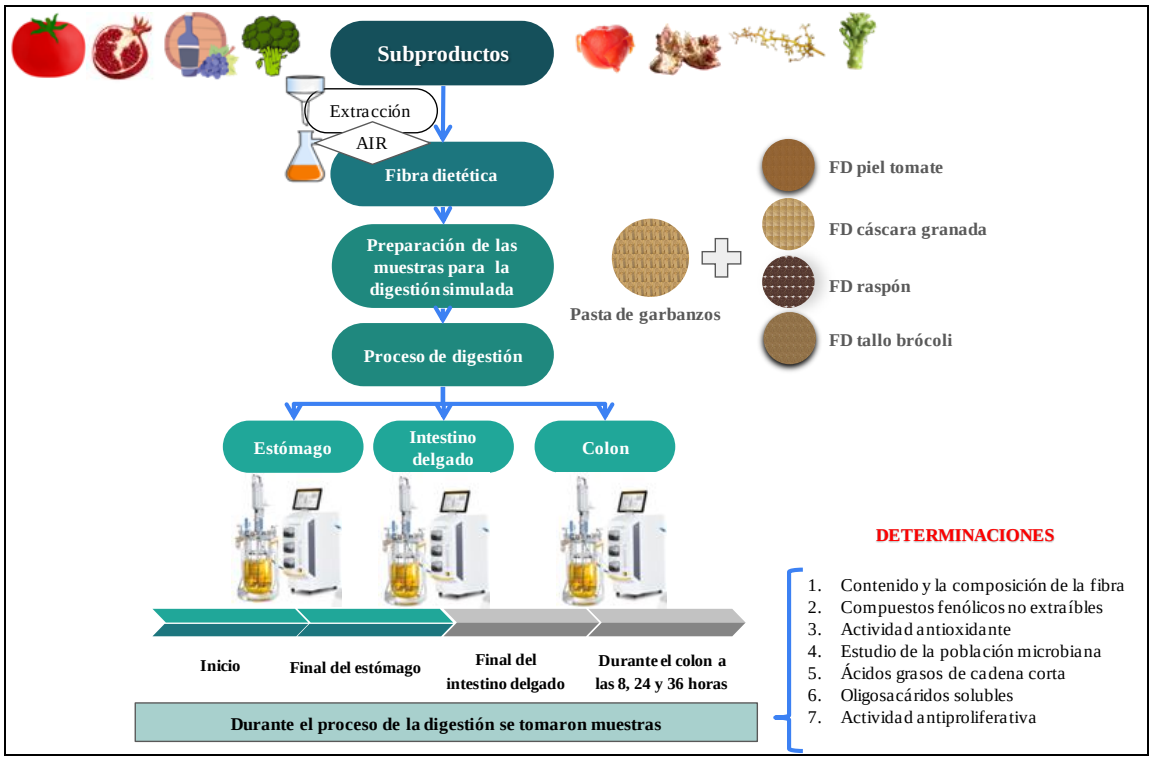


Figura 17. Diseño experimental del capítulo 6.

Los subproductos utilizados incluyeron cáscara de granada, piel de tomate, tallos de brócoli y tallos de uva, proporcionados por industrias de Extremadura, España. En primer lugar, la fibra dietética se extrajo de los subproductos siguiendo el método de residuos insolubles en alcohol (AIR). Para preparar el suplemento alimenticio que posteriormente se someterá a la digestión simulada, los concentrados de fibra dietética procedente de los subproductos se mezclaron con agua estéril y se homogeneizaron con puré de garbanzos cocidos. Los suplementos alimenticios resultantes fueron 5: Control (pasta de garbanzos), cáscara de granada (pasta de garbanzos más concentrado de fibra de cáscara de granada), piel de tomate (pasta de garbanzos más concentrado de fibra de piel de tomate), tallo de brócoli (pasta de garbanzos más concentrado de fibra de tallo de brócoli) y tallo de uva (pasta de garbanzos más concentrado de fibra de tallo de uva). La digestión simulada se realizó utilizando un modelo in vitro dinámico del sistema digestivo humano, que consta de tres bioreactores conectados en serie para simular el

estómago, intestino delgado y colon. Se controlaron varios parámetros como pH, temperatura y presión (O_2). Durante el proceso de digestión, se agregaron al bioreactor diferentes componentes, incluyendo el suplemento alimenticio, HCl, pepsina, NaHCO3, fluidos pancreáticos, biliares e inóculo fecal (voluntario sano). Se utilizaron seis puntos de muestreo: fase inicial, final del estómago, final del intestino delgado y en el colon a 8 horas, 20 horas y 36 horas. Se realizaron en cada uno de los 6 puntos de muestreo determinaciones de fibra dietética para evaluar el contenido y la composición de la fibra en diferentes etapas de la digestión, así como el contenido de compuestos fenólicos no extraíbles y capacidad antioxidante. Además, se caracterizaron los extractos de digestión mediante la evaluación de la población microbiana, ácidos grasos de cadena corta, oligosacáridos solubles y actividad antiproliferativa en células HT-29 de adenocarcinoma colorrectal humano.

Análisis estadístico del Capítulo 6:

Los datos se analizaron utilizando SPSS para Windows (versión 21.0, IBM Corp.) y se calcularon estadísticas descriptivas. Las diferencias dentro y entre los grupos se evaluaron con un análisis de varianza unidireccional (ANOVA) seguido de la prueba honesta de diferencias significativas de Tukey ($p \leq 0,05$) para comparaciones entre grupos. Además, se realizó un análisis de componentes principales (PCA) sobre la matriz de correlación de las variables.

CAPÍTULO 7

Para desarrollar el **Capítulo 7**, se llevó a cabo el diseño experimental de la Figura 18.

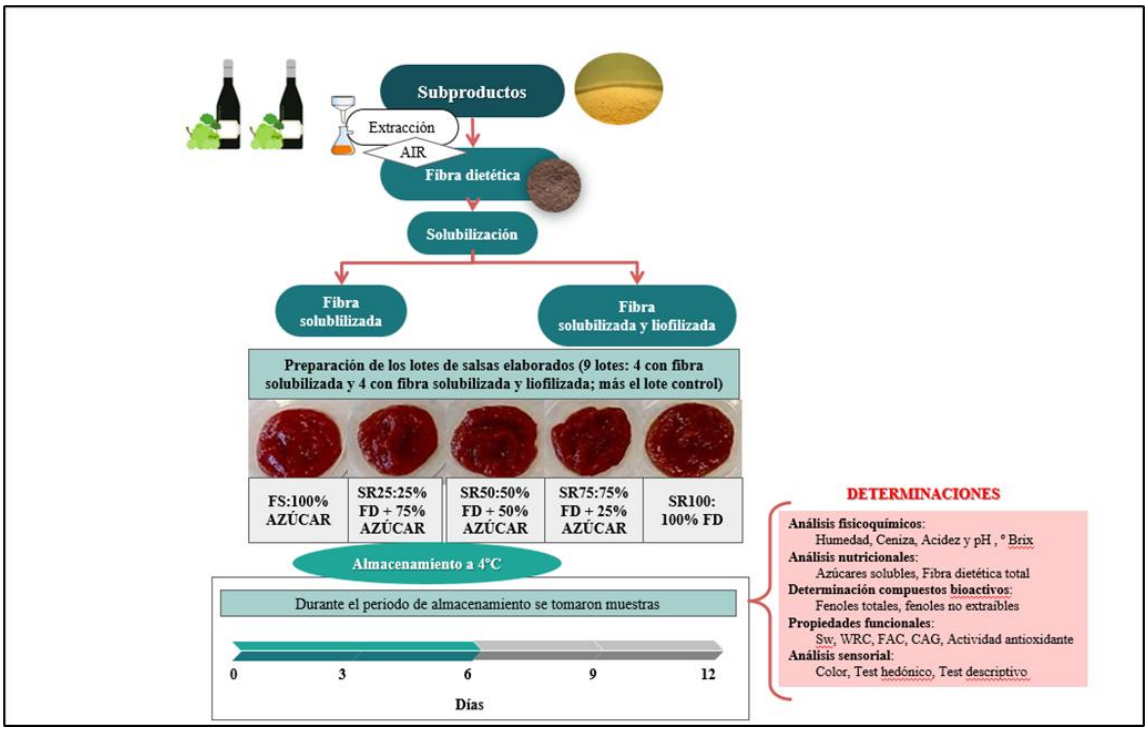


Figura 18. Diseño experimental del capítulo 7.

Se utilizaron lías de la primera fermentación del vino blanco y concentrados de tomate suministrados por industrias en la región de Extremadura, España. Para la preparación de los extractos de fibra dietética, se utilizó el método de Residuo Insoluble en Alcohol (AIR) para extraer los polisacáridos de la pared celular de los residuos de vinificación. Posteriormente, el AIR la fibra se solubilizó y se aplicó tanto directamente como liofilizada. En la preparación de la salsa de tomate, se realizaron nueve lotes en total, una salsa con azúcar, sin adición de fibra y las otras ocho salsas con diferentes porcentajes de fibra solubilizada y solubilizada y liofilizada. Estas salsas se almacenaron a 4°C durante 12 días y se sometieron a análisis cada 3 días. Los análisis incluyeron determinaciones de humedad, cenizas, pH, acidez total, contenido de sólidos solubles, azúcares solubles y reductores, compuestos fenólicos y actividad antioxidante de las salsas. Además, se caracterizó la fibra dietética de las salsas mediante análisis de azúcares neutros y contenido de ácido galacturónico, así como propiedades funcionales como capacidad de hinchamiento (Sw), capacidad de retención de agua (WRC),

capacidad de retención de aceite (FAC) y capacidad de adsorción de glucosa (CAG). Finalmente, se evaluó el color de las salsas utilizando un colorímetro y se realizó un análisis sensorial mediante una escala de 0 a 10 para atributos visuales, gustativos y aromáticos, así como una prueba hedónica para evaluar la aceptabilidad de las muestras. Este diseño experimental permitió investigar el impacto completo de la adición de fibra dietética en las características de las salsas de tomate, desde su composición química hasta su aceptabilidad sensorial.

Análisis estadístico del Capítulo 7:

El análisis estadístico de los datos se realizó utilizando SPSS para Windows, versión 21.0 (IBM Corp., Armonk, NY, EE. UU.). Se determinaron estadísticas descriptivas de los datos y las diferencias dentro y entre los grupos se estudiaron mediante análisis de varianza unidireccional (ANOVA) y se separaron mediante la prueba honesta de diferencias significativas de Tukey ($p \leq 0,05$). Se realizó análisis de componentes principales (PCA) sobre la matriz de correlación de las variables.

RESULTADOS Y DISCUSIÓN

CAPÍTULO 1

foods

MDPI

Article

Chemical Composition and Functional Properties of Dietary Fibre Concentrates from Winemaking By-Products: Skins, Stems and Lees

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Abstract: The objective of this study was to evaluate, from a technological and nutritional point of view, the chemical composition and functional properties of the industrial winemaking by-products, namely skins, stems and lees. The chemical and physical characteristics, as well as the functional properties (fat and water retention and swelling capacity, antioxidant capacity, and their prebiotic effect), of the dietary fibre of these by-products were studied. The results showed that the skins, stems, and lees are rich in fibre, with the stem fibre containing the highest amounts of non-extractable polyphenols attached to polysaccharides with high antioxidant activity and prebiotic effect. Lee fibre had the highest water retention capacity and oil retention capacity. The results reveal that winemaking by-products could be used as a source of dietary fibre with functional characteristics for food applications.

Keywords: soluble and insoluble dietary fibre; non-extractable phenolic compounds; antioxidant capacity; neutral sugar; in vitro fermentation

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1. Introduction

Waste from the agri-food industries is a current matter of global concern, with the generation of around 37 million tons of agricultural residues in the world during 2017 [1]. The generation of agricultural waste is particularly of concern in viticulture; it is estimated that 25 kg of waste is produced for every 100 kg of grapes [2]. This involves an economic and ecological problem in the management of the winemaking industries [3,4]. The recovery process plays an important role in the circular economy approach. It improves biomass value where the biorefinery acts as a platform that includes several conversion technologies [5,6].

Industrial winemaking activities produce solid residues such as grape pomace (60% of total wine by-products), which is mainly made up of grape skins (50%), residual pulp and stalks (25%) and seeds (25%) [7]. In addition, another by-product to highlight is the wine lees that are produced during fermentation [8] and account for 25% of the waste produced [9–11]. These by-products are rich in dietary fibre (DF), being an important source of soluble polysaccharides [12–15] as well as antioxidant compounds [16–20]. Therefore, by-products of winemaking can be used to produce ingredients with suitable functional properties for the development of new food products. These by-products were used for novel biscuit formulation as an alternative to DF and phenolic compounds [21] and showed reduced oxidation of seafood and meats [22,23]. In addition, insoluble dietary fibre from grape pomace decreased tannins in red wine by up to 38%, showing its efficacy as a clarifying agent in wines [24].

The functional properties of dietary fibre—including the water retention capacity (WRC), the swelling capacity (Sw), the fat retention capacity (FAC), antioxidant activity and prebiotic activity—are associated with the physicochemical characteristics of cell wall polysaccharides, varying according to their composition [25]. WRC, mainly related to insoluble dietary fibre (IDF), prevents and treats different intestinal disorders by increasing faecal bulk and reducing the gastrointestinal transit time. In food-technological terms, dietary fibre with high WRC can be used as a functional ingredient to avoid syneresis and to modify the viscosity and texture of some formulated foods, whereas dietary fibre with high FAC allows stabilisation of fat in emulsion-based products [26]. Sw in the stomach and an increase in viscosity of the digesta is associated with soluble dietary fibre (SDF), which slows down the absorption of nutrients from the intestinal mucosa and lowers the postprandial blood glucose and insulin responses [27].

The prebiotic effect of dietary fibre is probably the most important functional property. Dietary fibre reaches the colon, where it is fermented by the intestinal microbiota, generating short-chain fatty acids (SCFA), such as butyric, propionic, and acetic acids [28]. These compounds are associated with a wide range of physiological properties, including the improvement of digestive tract disorders [29–31] and anticancer activity [32,33].

In this context, the objective of this study was to analyse the chemical composition and the functional properties of skins, stems, and lees as by-products of industrial winemaking and thus offer new opportunities for waste use in the wine industry.

2. Materials and Methods

2.1. Plant Material

Winemaking by-products used in this work were provided by wineries from the Region of Extremadura, Spain. Winemaking by-products studied included red grape skins, stems, and wine lees from grapes of the Tempranillo variety. Grape skin and stem samples were taken after pressing the grapes. In the case of lees, samples were taken at the end of the fermentation. The samples were freeze-dried (LyoBeta, Telstar, Barcelona, Spain). The parameters of the freeze-drying process were freezed for 4 h at −40 °C and primary drying (8.5 h at −20 °C and 6.5 h at 20 °C) at 400 µbar. The samples were then ground with a grinder and sieved with a fine mesh (max 1 mm). Finally, the samples were vacuum-packed using a vacuum packing machine (Model SAMMIC SV-420, Gipuzkoa, Spain) and stored at room temperature until use. All determinations (Figure 1) were done in triplicate.

2.2. Chemical Composition

2.2.1. Moisture and Ash

The moisture and ash determinations were based on methods from AOAC International [34]. Moisture and ash content was determined by drying the samples at 105 and 500 °C, respectively, until a constant weight was achieved.

2.2.2. Crude Protein and Total Fat

Crude protein was determined following the Kjeldahl method [35]. Fat content was determined gravimetrically by extraction with diethyl ether using a Soxhlet apparatus [36].

2.2.3. Determination of Soluble Sugars

Total soluble sugars (TSS) were extracted with distilled water and determined using the sulfuric acid-ultraviolet (UV) method proposed by Albalasmeh et al. [37]. Reducing sugars (RS) were determined by the dinitrosalicylic acid (DNS) method [38]. Calibration was performed with standard solutions of glucose. The results were expressed as g/100 g dry sample.

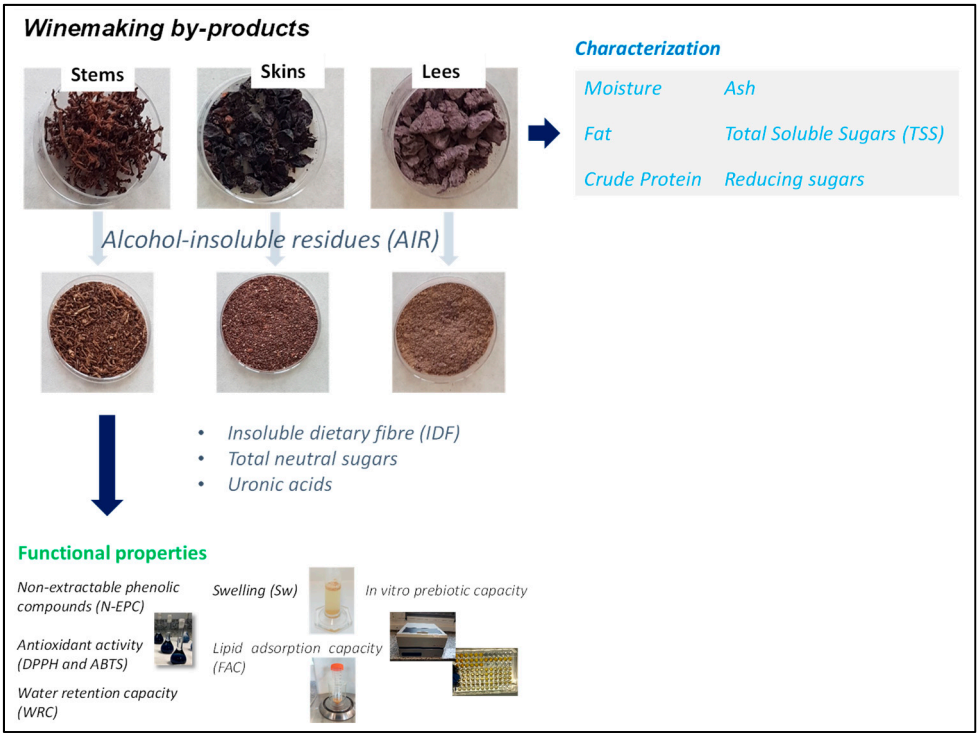

Figure 1. Graphic diagram of the study design.

For the characterisation of soluble sugars, the carbohydrates in 1 mL of solution were converted into alditol acetates and quantified by gas chromatography (Shimadzu 2010 Plus) following the method described by Bastos et al. [39]. A capillary column, DB-225 (30 m × 0.25 mm i.d.; 0.15 µm; Agilent, Santa Clara, CA, USA) and auto-injector (Shimadzu AOC-20i) were used. The temperatures of the FID detector and injector were 240 and 230 °C, respectively. The oven temperature was initially held at 140 °C for 2.5 min, then increased to 200 °C at a rate of 20 °C/min and held for 4.5 min, after which it increased at a rate of 30 °C/min to a final temperature of 220 °C, at which it was held for 18 min. The injection volume was 1 µL with a split ratio 1:10. Helium was used as a carrier gas at a flow rate of 1.49 mL/min. Components were identified by comparing their retention times with those of derivatised standards and quantified using 2-deoxyglucose as an internal standard. The results were expressed as mg/g dry samples.

2.2.4. Determination of Total, Soluble, and Insoluble Dietary Fibre

The total dietary fibre (TDF) of winemaking by-products was measured following the standard enzymatic-gravimetric method [40]. First, 1 g of dry sample was mixed into 50 mL of distilled water. Then, samples were digested with 200 µL of α-amylase (Sigma-Aldrich, St. Louis, MO, USA) at 80 °C for 1 h with constant agitation. After digestion, 100 µL of amyloglucosidase solution (50 mg/mL) (Sigma-Aldrich) was added and the mixture was kept at 60 °C for 3 h. Next, the pH was adjusted to 7.0 with NaOH 10% (*w*/*v*), followed by incubation with 200 µL of protease (Sigma-Aldrich) at 80 °C for 1 h. Finally, digested samples were vacuum filtered with cellulose-free filters (Whatman glass microfiber filters, 934-AHTM). The solid fraction contained in the filter represented insoluble dietary fibre (IDF). To precipitate the soluble dietary fibre (SDF), 4 volumes of 96% ethanol were added to the filtrate at 60 °C. Both fractions of fibre were dried overnight at 45 °C in an oven and were then weighed. The TDF was calculated as the sum of IDF and SDF. The results were expressed as g/100 g dry sample. All experiments were conducted in triplicate.

2.3. Characterisation of Insoluble Fibre: Cellulose, Hemicellulose, and Lignin

The determination of cellulose, hemicellulose and lignin was carried out by calculating the fractions of neutral detergent fibre (NDF) and acid detergent fibre (ADF) [41] in a fibre extractor (Dosi-Fiber, J.P. Selecta, Abrera, Spain). The ADF determination consisted of boiling the sample with cetyltrimethylammonium bromide in an acid medium with subsequent filtration and washing of the residue. This method resulted in a good estimate for cellulose and lignin. For NDF determination, the sample was treated with a hot solution of sodium lauryl sulphate with a subsequent gravimetric determination of the residue. This method gives a good estimate of insoluble fibre (cellulose, hemicellulose, and lignin). The difference between NDF and ADF was the hemicellulose content. The cellulose, hemicellulose, and lignin contents were expressed as mg/100 g of fibre.

2.4. Characterisation of Soluble Fibre: Neutral Sugar and Pectins

Extraction of dietary fibre from the winemaking by-products was carried out using a modification of the double residue method in alcohol to determine the alcohol-insoluble residue (AIR) described by Femenia et al. [42]. Briefly, 3 replicates (5 g each) of the dry sample were homogenised with 85% (*v*/*v*) ethanol. The mixture was boiled on a shaker for 10 min, and then the solid residue was collected using a Büchner funnel with cellulose-free filters (Whatman, 934-AHTM glass microfiber filters). This process was repeated twice, the last time with absolute ethanol. Finally, the insoluble solid residue was washed with acetone, and the excess solvent was removed after 24 h at room temperature.

Total neutral sugars (TNS) were released through a process of hydrolysis of the fibre using 12 M sulfuric acid (3 h at room temperature and 100 °C for 1 h) and were determined, as D-glucose equivalent, with the anthrone method proposed by Van Handel [43].

For the characterisation of neutral sugars (rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose), neutral sugars were converted into their alditol acetates and quantified by gas chromatography as described in Section 2.2.2. The content of each individual neutral sugar was expressed as mg/g.

The uronic acid content was determined spectrophotometrically by the m-hydroxydiphenyl method [44] with galacturonic acid as standard and 3,5-dimethylphenol as reagent. The results were expressed as mg galacturonic acid/g AIR.

2.5. Functional Properties of the Fibre

2.5.1. Swelling, Water Retention Capacity and Fat Retention Capacity

Sw, WRC and FAC determinations were carried out following the method described by Garau et al. [45]. For Sw, 0.1 g of AIR was mixed with 10 mL of distilled water on a calibrated cylinder. After 24 h incubation at room temperature (20–25 °C), the increase in volume was measured, and the results were expressed as mL water/g AIR. For WRC, 0.2 g of AIR was hydrated in 10 mL of distilled water and left for 24 h at room temperature (20–25 °C). The next day, the sample was centrifuged at 2000× *g* for 25 min. After centrifugation, the supernatant was decanted, and the resulting solid residue was weighed. WRC was expressed as g water/g AIR. For FAC, the process was the same as for WRC determination, using 5 mL of sunflower oil in place of the 10 mL of water. The results were expressed as g oil/g AIR.

2.5.2. Non-Extractable Polyphenols and Antioxidant Activity

The determination of the antioxidant capacity of non-extractable polyphenols bound to AIR was achieved according to the method described by Arranz et al. [46] with some modifications. A total of 0.5 g of AIR was mixed with 20 mL of a methanol/water solution (50: 50) acidified with hydrochloric acid to pH 2. The mixture was incubated with stirring for 1 h and subsequently centrifuged for 20 min at 2500× *g*. This process was repeated with an acetone/water solution (70: 30). The excess solution was removed by heating at 37 °C in a rotary evaporator under vacuum (Hei-VAP Precision, Heidolph, Germany). The resultant residue was resuspended in 30 mL of distilled water. The total non-extractable

polyphenols bound to AIR were determined using the Folin-Ciocalteure agent [47] in a UV-1800 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA). Gallic acid was used as the standard. The results were expressed as mg gallic acid equivalent (GAE)/100 g AIR.

The antioxidant capacity of the samples was evaluated by 2 antioxidant assay methods: the 2,2-diphenyl-1-picrilhydrazyl (DPPH) depletion method according to the procedure of Teixeira et al. [48], and the capacity to remove the 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical according to the method of Re et al. [49]. Trolox was used as the standard, and the results were expressed as mg Trolox equivalent/100 g AIR.

2.5.3. In Vitro Prebiotic Capacity

The prebiotic capacity of the soluble dietary fibre extracts on lactic acid bacteria (LAB) associated with fermented food products was determined using the method described previously by Ruiz-Moyano et al. [50]. For this study *Lactococcus lactis* (CECT 188), *Lactobacillus curvatus* (CECT 904), *Lactobacillus sakei* (CECT 5765 and CECT 980), *Lactobacillus brevis* (CECT 815), *Lactobacillus plantarum* (G1LB5; [46]), *Lactobacillus casei* (HL 245 and HL 233; [51]) and *Enterococcus faecium* (SE 906 and SE 920; [52]) were used. Prior to the assay, LAB were grown in de Man–Rogosa–Sharpe broth (MRS; Condalab, Madrid, Spain) at 37 °C for 24 h. The strains were tested for growth in the presence of solubilised AIR (autoclaved at 121 °C for 16 min followed by an ultrasound treatment for 1 h). The absence of free sugars in each solubilised AIR was previously checked. For each bacterial strain, 5 µL of suspension was inoculated in 200 µL of semi-solid MRS medium containing 0.125 g/L agar without glucose and supplemented with 2 g/L of each sterile filtered dietary fibre extract as the sole carbohydrate source. The positive control for growth consisted of semi-solid MRS supplemented with 2 g/L glucose, whereas the negative control was a carbohydrate-free semi-solid MRS. Turbidity was measured in a fluorescence microplate reader (FLUOstar OPTIMA F, BMG Labtech, Ortenberg, Germany), growth was carried out for 48 h at a temperature of 37 °C, with readings at a wavelength of 570 nm every 1 h. For each strain, the ability to grow was evaluated by comparing the percentage growth in each extract with the positive control.

2.6. Statistical Analysis

Statistical analysis of the data was carried out using SPSS for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics of the data were determined, and the differences within and between groups were studied by one-way analysis of variance (ANOVA) and separated by Tukey's honest significant difference test ($p \leq 0.05$). Principal component analysis (PCA) was performed on the correlation matrix of the variables.

3. Results and Discussion

3.1. General Chemical Composition

The results of the chemical composition of winemaking by-products (skins, stems and lees) are shown in Table 1. The stem samples showed higher moisture values ($p \leq 0.05$) than the skin and the lee samples, reaching 16.26%. The lowest moisture value corresponded to the skin samples, with a value of 7.66%. However, the ash content of the lee samples was 13.18%, which was significantly higher than the amount found in other samples, ranging from 6.08% for stems up to 8.12% for the skin. The samples with the highest crude protein content were lees (22.32%), followed by skin and stem samples, with values of 12.24% and 7.94%, respectively. The fat content ranged from 1.95 to 4.85% (Table 1), not showing significant differences between samples (skins, stems and lees). The fact that the lees were the by-product with the highest percentage of proteins was explained by the nature of the residue; lees were mainly made up of the remains of the yeast autolysis during the process of fermentation. In the same way, Rubio et al. [53] also found higher protein content in lees, followed by skin and stems. The values obtained from skin samples agreed with those published by Deng et al. [54]. However, the stem and lees values differed from those found

by González-Centeno et al. [55] and Bordiga [56]. The composition depended on numerous parameters related to the types of grapes and in the case of the lees the type of yeast used, and to the vinification method, resulting in a wide compositional heterogeneity, as shown by data reported in the literature [57].

Table 1. Chemical composition of skins, stems, and lees (g/100 g dry weight).

Parameters	Skins	Stems	Lees
	Mean SD [1]	Mean SD	Mean SD
Moisture	7.66 ± 0.15 [a]	16.26 ± 0.16 [c]	9.09 ± 0.27 [b]
Ash	8.12 ± 1.40 [a]	6.08 ± 1.18 [a]	13.18 ± 0.92 [b]
Protein	12.24 ± 0.88 [b]	7.94 ± 0.35 [a]	20.32 ± 0.75 [c]
Fat	4.24 ± 0.93	1.95 ± 0.39	4.85 ± 1.15
TSS	3.65 ± 0.35 [a]	22.01 ± 1.56 [b]	1.63 ± 0.09 [a]
Reducing sugars	1.78 ± 0.08 [a]	19.60 ± 2.37 [b]	0.49 ± 0.05 [a]
Glucose	0.90 ± 0.73 [a]	10.62 ± 11.22 [b]	<0.5 *[a]
Fructose	0.79 ± 0.54 [a]	11.71 ± 12.01 [b]	<0.5 *[a]
TDF	82.30 ± 2.71 [b]	71.39 ± 1.01 [a]	82.32 ± 1.69 [b]
IDF	78.18 ± 2.91 [b]	67.68 ± 0.95 [a]	78.43 ± 1.32 [b]
SDF	4.13 ± 0.20	3.71 ± 0.06	3.90 ± 0.37

TSS, total soluble sugars; TDF, total dietary fibre; IDF, insoluble dietary fibre; SDF, soluble dietary fibre. [1] SD, standard deviation. * The limit of detection was 0.5 mg/g. [a,b,c] Values with different superscripts are significantly different ($p \leq 0.05$) between samples.

3.1.1. Total Soluble Sugars

Table 1 also shows the TSS content of the winemaking by-products. The values of total and reducing sugars (consisting of similar amounts of glucose and fructose) were highest in stem samples, with 22.01 and 19.60 g/100 g, respectively, because this by-product does not undergo fermentation [53]. On the contrary, the lees extracted when the malolactic fermentation had finished contained the lowest TSS content because they were transformed into alcohol.

3.1.2. Total, Insoluble and Soluble Dietary Fibre

The samples of skin, stem and lees studied were rich in TDF (71.39–82.32 g/100 g) and in IDF (Table 1). IDF has been described as the predominant dietary fibre fraction for winemaking by-products [58]. The IDF content from samples ranged from 67.68 to 78.43 g/100 g, showing higher values for lees and skin (Table 1). The content of SDF was similar for each of the by-products studied, with values between 3.71 and 4.13 g/100 g. The values obtained from TDF agreed with those published by other authors for skin and stem samples [59,60]. However, the composition of dietary fibre in winemaking lees continued to create controversy: some authors affirm that lees do not contain a significant fraction of dietary fibre [61], while other authors state that the content is around 22–50% [53]. The high dietary fibre content found in this work for lees has not been reported in previous publications. The high variability of these data was probably due to the different recovery methods, winemaking procedures applied, and varieties of grape.

3.2. Fibre Constituents: Cellulose, Hemicellulose, Lignin, Neutral Sugars, and Pectins

In skin, stems and lee samples, insoluble dietary fibre is mainly lignin (47.31, 29.83, and 44.41 g/100 g, respectively), followed by hemicellulose and cellulose (Table 2). The values obtained for lignin, cellulose and hemicellulose in the skin, stems and lee samples were similar to those reported in the literature [53,62–64].

Table 2. Fibre constituents of skins, stems and lees.

Parameters	Skins	Stems	Lees
	Mean SD [1]	Mean SD	Mean SD
IDF (g/100 g)			
Hemicellulose	22.57 ± 2.16	17.18 ± 0.22	28.79 ± 9.25
Cellulose	7.24 ± 0.04 [a]	14.55 ± 3.59 [a]	30.03 ± 0.54 [b]
Lignin	47.31 ± 3.61 [b]	29.83 ± 3.27 [a]	44.41 ± 5.91 [b]
Total neutral sugars of AIR (mg/g)	114.71 ± 19.04 [b]	84.82 ± 16.76 [a]	156.04 ± 8.82 [c]
Rhamnose	<0.5 *[a]	<0.5 *[a]	50.33 ± 0.95 [b]
Fucose	32.37 ± 2.51 [b]	12.40 ± 11.31 [ab]	2.32 ± 0.47 [a]
Xylose	11.76 ± 1.15 [b]	<0.5 *[a]	<0.5 *[a]
Mannose	19.61 ± 0.77 [a]	17.94 ± 4.42 [a]	35.63 ± 1.32 [b]
Glucose	26.07 ± 1.06 [a]	28.07 ± 5.30 [a]	78.30 ± 1.45 [b]
Galactose	7.56 ± 1.97 [b]	<0.5 *[a]	<0.5 *[a]
Arabinose	17.90 ± 0.66 [b]	4.08 ± 0.57 [a]	5.30 ± 1.76 [a]
Uronic acids of AIR (mg/g)			
Uronic acids (pectin)	31.37 ± 1.47 [b]	31.09 ± 3.17 [b]	12.37 ± 0.53 [a]

IDF, insoluble dietary fibre; AIR, alcohol-insoluble residue. [1] SD, standard deviation. * The limit of detection was 0.5 mg/g. [a,b,c] Values with different superscripts are significantly different ($p \leq 0.05$) between samples.

The most abundant sugars in the AIR of the skin and the stems (Table 2) were uronic acids (which indicate the pectin content), followed by glucose and fucose in the case of the skin, and mannose in the stems, while arabinose, xylose, galactose and rhamnose were the minority sugars. In the case of lees, glucose was the major monosaccharide, followed by rhamnose and mannose, with concentrations around 78, 50 and 35 g/100 g, respectively. Uronic acids, fucose and arabinose values were lower in the lees than in skin and stems (Table 2). The profile found for the sugar composition of skin and stem agreed in general with the results obtained by other authors [55,65], although other authors have described pectin (measured as galacturonic acid equivalent) and glucose as the major skin and stem cell wall components [66]. High glucose levels could be related to high glucan and xyloglucan values [66–68].

With respect to xylose and fucose, higher amounts were found in skin samples than those described by other authors for these winemaking by-products [67,69,70]. The presence of xylose and fucose could indicate a higher amount of hemicellulosic polysaccharides in grape skins [71]. Ortega-Regules et al. [72] showed that the neutral sugar profile of the vinification by-products was highly variable, depending on the cultivar used. The degree of maturation of the grapes, their geographical origin and oenological techniques were also responsible for the differences found in the chemical composition of the by-products [66,73].

On the other hand, there were few studies on the profile of neutral sugars in winemaking lees, although some authors have reported that the main monosaccharides of lees were glucose, mannose and rhamnose [74,75], which agreed with the data of our study.

3.3. Functional Properties of the Fibre

3.3.1. Swelling, Water Retention Capacity and Oil Retention Capacity

The functional properties of dietary fibres (Sw, WRC and FAC) were important in determining suitability for application as functional ingredients in foods. The results of these functional property analyses of winemaking by-products are shown in Table 3.

Table 3. Functional properties and non-extractable phenolic compounds (N-EPC) of dietary fibre from skins, stems and lees.

Parameters	Skins	Stems	Lees
	Mean SD [1]	Mean SD	Mean SD
Sw (mL water/g)	6.55 ± 0.03 [a]	7.76 ± 0.54 [ab]	8.43 ± 0.47 [b]
WRC (g water/g)	4.57 ± 0.21	4.61 ± 0.34	9.11 ± 2.39
FAC (g oil/g)	3.76 ± 0.27	5.48 ± 0.51	5.48 ± 0.88
N-EPC (mg GAE/100 g)	92.83 ± 15.76 [b]	138.63 ± 28.68 [c]	44.64 ± 4.35 [a]
DPPH (mg Trolox/100 g)	3823.68 ± 63.65 [b]	6093.01 ± 376.40 [c]	2049.23 ± 33.73 [a]
ABTS (mg Trolox/100 g)	4205.82 ± 307.11 [b]	5682.74 ± 308.97 [c]	2395.50 ± 671.69 [a]

Sw, swelling water capacity; WRC, water retention capacity; FAC, oil retention capacity; GAE, gallic acid equivalents; DPPH and ABTS, antioxidant capacity. [1] SD, standard deviation. [a,b,c] Values with different superscripts are significantly different ($p \leq 0.05$) between samples.

The Sw values, related to the porosity of the fibre [76], were significantly higher for the lee samples than for skin samples. However, the values of WRC and FAC did not show differences between the three by-products studied (Table 3). These values were similar to those published by other authors for winemaking by-products [55]. However, the stem samples showed lower values of WRC than those reported in previous studies, ranging from 5.5 to 10.7 g water/g AIR [55]. These differences may be due to the nature of the plant material used, in particular the IDF content. The structure and chemical composition of the fibre were known to play an important role in the kinetics of water absorption [77,78]. The high FAC values obtained in the stem and lee samples are beneficial because they are associated with oil retention during the digestion of food and reduce serum cholesterol levels [79].

In summary, the lee samples exhibited the best characteristics for use as a functional food ingredient, although these properties not only depend on the type of soluble fibre, but also on the particle size distribution and the characteristics of the surface [80]. In addition, the particle size may cause changes in the functional properties of dietary fibre, thus smaller particle sizes showed higher values of WRC and Sw [80].

3.3.2. Non-Extractable Polyphenols and Antioxidant Activity

The phenolic compound content in AIR (N-EPC) and the antioxidant capacity, determined by two methods (DPPH and ABTS), are also shown in Table 3. Among the three AIRs, stem AIR presented the highest N-EPC value of 138.63 mg GAE/100 g, whereas lees AIR had the lowest N-EPC value of 44.64 mg GAE /100 g. This difference may be due to the lignin content of each of the samples (Table 2). Bender et al. [62] observed that the concentration of phenolic compounds increased with reduced lignin content because of the breakdown of the lignin structure. Changes in the structure of the fibre affected the non-extractable phenolic compound content by loosening the hydrogen bonds and causing delignification [81].

Regarding DPPH and ABTS values, significant differences were found between the samples of by-products studied (Table 3). The stem samples showed the highest antioxidant activity both by the DPPH method (6093.01 mg Trolox/100 g) and ABTS (5682.74 mg Trolox/100 g), followed by skin and lee samples in agreement with the total phenolic content observed in samples (Table 3). In fact, there was a highly significant correlation ($r = 0.961$; $p < 0.01$) between the content of phenolic compounds of the fibre and its antioxidant activity by the DPPH method. The results reflect that the increase in antioxidant activity was promoted by the non-extractable total phenolic content. This mechanism has been corroborated by previous studies [82–84]. Ferri et al. [82] reported that seeds and raw skin extracts of several grape cultivars exhibited a high antioxidant capacity measured by the ABTS method, which was positively correlated with the total polyphenol content.

3.3.3. In Vitro Prebiotic Capacity

Table 4 shows the capacity of the 10 LAB strains tested for in vitro growth on three extracts of dietary fibre, observing differences in the growth of the strains according to the fibre used. Even though we expected higher bacterial growth on culture media with the skin fibre, due to the presence of fermentable fibre components such as pectins and hemicelluloses, we only observed low growth (Table 4). This growth was similar to growth on culture media with the lees fibre. The culture media enriched with the dietary fibre extracted from the stem samples presented the highest percentage of growth for all strains studied. The superior growth of bacteria with stem fibre could be related to the presence of the non-extractable polyphenols (Table 3). The data obtained could suggest that the studied strains use some phenols for their metabolism. This finding agrees with the study by Landete et al. [85]. The authors demonstrated that bacteria such as *Lactobacillus* spp. can degrade phenolic compounds to other molecules with high added value. Nonetheless, more studies would be needed in relation to the structures of the oligosaccharides present to know the potential of the grape stem as prebiotics. *E. faecium* SE 906E had the highest growth percentage (76.79%), followed by *L. sakei* CECT 5765 (67.11%) and *E. faecium* SE 920 (62.46%) with moderate growth, showing differences from the rest of the strains ($p \leq 0.05$).

Table 4. Percentage of growth of lactic acid bacteria (LAB) strains, with respect to the positive control, on soluble dietary fibre extracts of skins, stems and lees.

Microorganisms	Skins	Stems	Lees
	Mean SD *	**Mean SD**	**Mean SD**
Lactobacillus curvatus CECT 904	$11.04 \pm 0.86^{a1,2}$	25.83 ± 0.77^{b1}	$11.04 \pm 0.86^{a1,2}$
Lactococcus lactis CECT 188	9.28 ± 0.76^{b1}	26.74 ± 0.06^{c1}	6.00 ± 0.34^{a1}
Lactobacillus sakei CECT 5765	$28.74 \pm 2.59^{a4,5}$	$67.11 \pm 0.85^{b3,4}$	27.11 ± 0.05^{a4}
L. sakei CECT 980	$20.08 \pm 1.08^{b2,3,4}$	44.61 ± 1.58^{c2}	$14.08 \pm 0.57^{a2,3}$
Lactobacillus brevis CECT 815	9.42 ± 0.64^{a1}	29.90 ± 0.58^{c1}	$14.12 \pm 0.43^{b2,3}$
Lactobacillus plantarum G1LB5	$10.58 \pm 0.89^{a1,2}$	$33.50 \pm 8.62^{b1,2}$	$8.17 \pm 0.54^{a1,2}$
Lactobacillus casei HL 245	$10.30 \pm 0.42^{a1,2}$	$39.65 \pm 3.51^{b1,2}$	$12.19 \pm 4.08^{a1,2,3}$
L. casei HL 233	$15.52 \pm 6.92^{a1,2,3}$	47.39 ± 0.08^{b2}	$13.28 \pm 4.65^{a1,2,3}$
Enterococcus faecium SE 906	30.46 ± 4.58^{a5}	76.79 ± 0.48^{b4}	23.02 ± 3.79^{a4}
E. faecium SE 920	$22.49 \pm 0.68^{b3,4,5}$	62.46 ± 3.11^{c3}	$12.11 \pm 0.17^{a1,2,3}$

* SD, standard deviation. ^{a,b,c} Values with different superscripts are significantly different ($p \leq 0.05$) between samples. ^{1,2,3,4,5} Values with different subscripts are significantly different ($p \leq 0.05$) between strains in one sample. Negative growth (<20%). Slight growth (20–40%), Moderate growth (40–70%). High growth (>70%).

3.4. Multivariate Analysis of the Parameters Related to Dietary Fibre Extracted from Skin, Stems and Lees

PCA was carried out for the entire set of dietary fibre data to obtain an interpretable overview of the main information. Figure 2 shows the two-way loadings and score plots, where PC2 was plotted against PC1, explaining more than 90% of the total variance. Higher values for AIR, N-EPC, antioxidant activity (DPPH and ABTS) and bacterial growth were clearly correlated and explained the positive axis of PC1, which was related to the stem samples. Therefore, the principal component analysis confirmed the results shown previously, indicating that the increase in antioxidant activity was promoted by the N-EPC content and that the culture media enriched with the dietary fibre extracted from the stem samples showed the highest percentage of growth. On the contrary, TNS and functional properties (WRC and FAC) explained the negative axis of PC1 and were associated with lee samples. The second PC was mainly explained by neutral sugars (galactose, arabinose, and xylose) located in the extreme of the negative axis, relating to high values in skin samples.

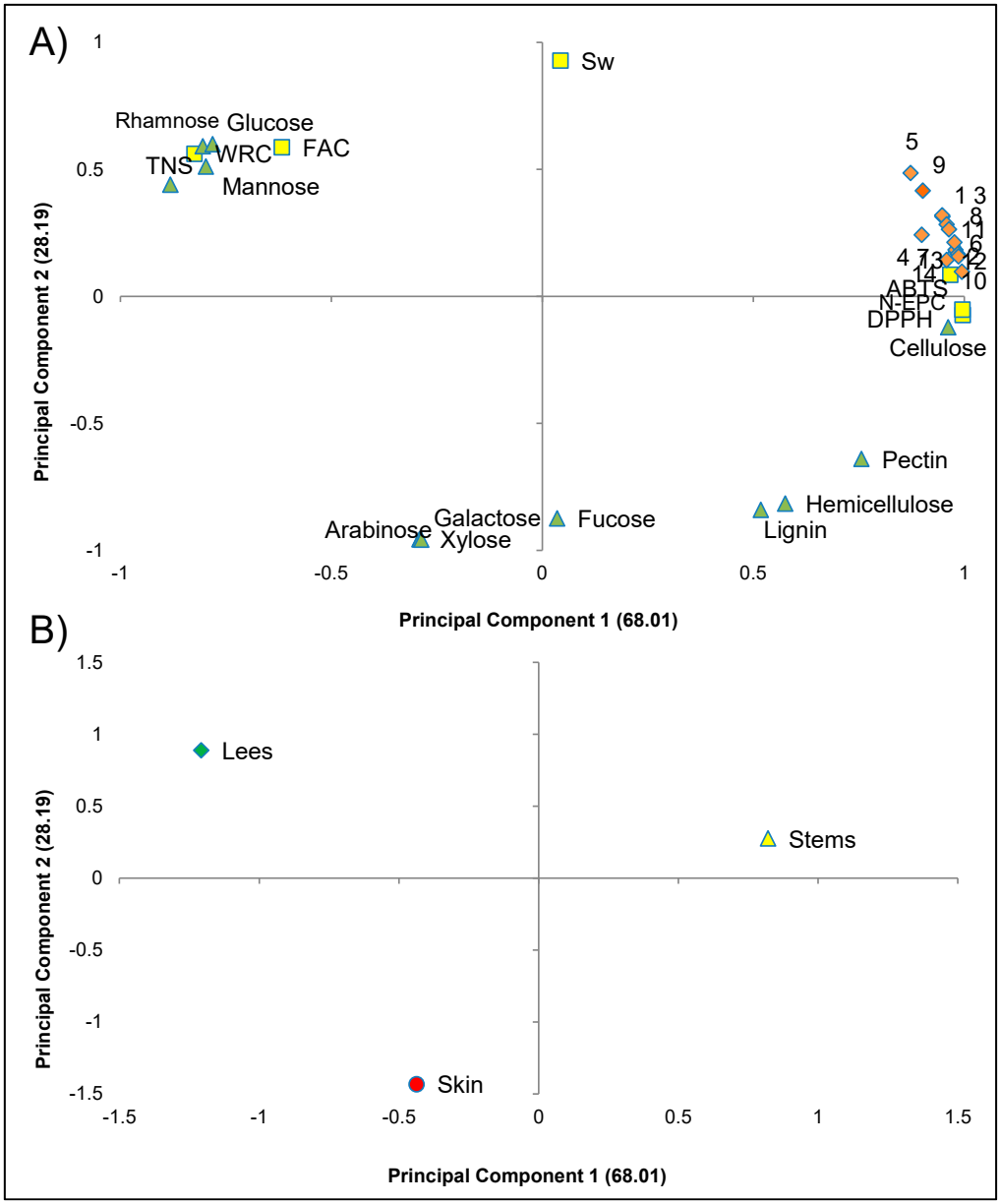

Figure 2. Principal component analysis of the analytical results of dietary fibre from skin, stems and lee samples. Loading plot (**A**). TNS, total neutral sugar; N-EPC, non-extractable phenolic compounds; DPPH and ABTS, antioxidant activity; Sw, swelling; WRC, water retention capacity; FAC, fat retention capacity; Bacterial growth: 1, *Lactobacillus curvatus* CECT 904; 2, *Lactococcus lactis* CECT 188; 3, *Lactobacillus sakei* CECT 5765; 4, *Lactobacillus sakei* CECT 980; 5, *Lactobacillus brevis* CECT 815; 6, *Lactobacillus plantarum* G1LB5; 7, *Lactobacillus casei* HL 245; 8, *L. casei* HL 233; 9, *Enterococcus faecium* SB 906; 10, *E. faecium* SB 920. Score plot (**B**). Skin, stem, and lees sample.

4. Conclusions

This study presents a complete physical and chemical characterisation of three winemaking by-products: skins, stems and lees. These by-products, rich in fibre, were demonstrated to have useful functional properties and a high non-extractable concentration of phenolic compounds. The stems have a high concentration of non-extractable polyphenols attached to polysaccharides, high antioxidant activity, and are a good substrate for bacterial growth. However, lees showed a high concentration of total neutral sugar and fibre with a good aptitude for the functional properties WRC and FAC. The skins gave high concentrations of galactose, arabinose and xylose. The results reveal that winemaking by-products can be considered sources of high-quality dietary fibre for food applications

with good functional characteristics. However, further studies on the structures of the oligosaccharides involved would be necessary for a better understanding of grape stems as prebiotics.

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CAPÍTULO 2

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Article

Improving the Viability and Metabolism of Intestinal Probiotic Bacteria Using Fibre Obtained from Vegetable By-Products

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Abstract: This study evaluated the effect of dietary fibre obtained from pomegranate, tomato, grape and broccoli by-products on the gastrointestinal transit survival, growth, and metabolism of six probiotic strains. The results showed that the studied by-products contained variable amounts of polysaccharides that affected the six probiotic microorganisms in different ways. In addition, the protective effect of the fibre obtained on the probiotic strains was more effective in the case of the fibre obtained from tomato peel. In terms of growth, grape stems showed the best results, favouring the growth of lactic acid bacteria. Finally, all fibres were able to increase the content of short-chain fatty acids in the in vitro test, but broccoli stems and pomegranate peel stimulated higher production of short-chain fatty acids. The results of this study demonstrate that plant by-product fibres can improve survival, growth, and metabolism in terms of the fatty acid profiles of probiotic strains, highlighting the desirability of harnessing these by-product fibres to develop new high-value-added ingredients as probiotic carriers.

Keywords: dietary fibre; by-product valorisation; probiotics; prebiotic

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1. Introduction

Plant-based food production generates large amounts of food waste during processing and storage, which are referred to as by-products that could be reutilized to avoid environmental risks and economic losses [1]. These residues comprise vegetable waste and by-products such as skins, stems, and seeds. Vegetable by-products are rich sources of dietary fibre (DF) and an important source of soluble polysaccharides. Polysaccharides from agro-food industry residues constitute one of the most important renewable resources. The wide variety in their chemical composition and structure, as well as their biodegradability and safety, make them suitable for applications in many fields such as food, pharmaceuticals, cosmetics, tissue engineering, biofuels, and others [2,3]. These are, therefore, bioactive compounds that are gaining increasing interest in their use as food additives or supplements, focusing efforts on improving their biological activity by new extraction methodologies [4].

Polysaccharides from agricultural residues are part of plant cell walls, which are highly diverse in their structure and composition [5]. In general, cell walls are constituted by high-molecular-weight polysaccharides, which are mainly lignin, cellulose, hemicelluloses, pectins, and other non-starch polysaccharides such as inulin and oligosaccharides [6]. Cell-wall-forming polysaccharides are classified into insoluble and soluble polysaccharides based on their solubility in water. Insoluble polysaccharides are lignin, cellulose, hemicelluloses, and pectins (insoluble); the soluble polysaccharide group consists of other pectins, and hemicelluloses [7].

Pectins are mostly considered as soluble fibre and are a polygalacturonic acid-rich part of the plant cell wall that can be composed of up to 17 different monosaccharides [8]. They are also the most structurally complex natural plant polysaccharides [9], and numerous health benefits are attributed to them [10]. They can be obtained from many vegetable sources, primarily citrus peel and apple pomace, but are also present in tomato peel [11,12], pomegranate peel [13], grape skin [14], and broccoli stems [15].

One of the most important activities of DF is its prebiotic activity, which can selectively stimulate the activity or growth of probiotic microorganisms in the colon [16]. In this regard, research efforts have been directed towards the inclusion of these prebiotics in daily dietary foods, most notably in dairy products [17], but also in beverages, processed fruits and vegetables, bakery, confectionery, snacks, sweeteners, and baby formula [18]. Due to this growth-stimulating activity of probiotic microorganisms, the addition of prebiotics and probiotics together to foods is more attractive because it promotes the viability and stability of the microorganisms [19]. Probiotics, aided by prebiotics, have a beneficial effect on consumer health by improving microbial balance at the gut level [20]. To provide these benefits, probiotics must remain viable, with a total number of live organisms above 10^6 cfu/g of food. The survival of these microorganisms during food processing and storage as well as during intestinal transit is clearly compromised on many occasions [21]. Several technologies have been employed to improve the viability of these microorganisms exposed to environmental stress and intestinal transit, such as encapsulation, in which different methodologies have been used depending on the drying temperature: high temperature drying (spray drying and fluid bed drying) and low temperature drying (ultrasonic vacuum spray drying, spray cooling, electrospinning, supercritical technique, freeze drying, extrusion, emulsion, enzymatic gelation, and impact spray technique) [22].

An alternative method is to combine probiotic cells with fibrous materials where probiotic cells can be stuck to or even form biofilms on the surface. This would confer high viability after gastrointestinal digestion [23,24]. Only a few studies are available in the literature on the use of fibre-rich agronomic by-products as protectors of probiotics as well as prebiotics for growth and metabolism stimulation [25].

The aim of this work was to characterize the fibre obtained from tomato, pomegranate, grape, and broccoli by-products and to investigate the effects of this fibre on the intestinal transit survival, growth, and metabolism of six strains with probiotic properties in order to develop new prebiotic or symbiotic food ingredients.

2. Materials and Methods

2.1. Plant Material

In this study, four agro-industrial by-products were used, including pomegranate peel, tomato peel, grape stalk, and broccoli stem. The vegetable by-products used in this work were provided by horticultural plants from the Extremadura region of Spain. All samples were dried in a forced-air oven at 45 °C for 24–48 h, ground, packed in vacuum bags, and stored at room temperature until use.

2.2. Sample Preparation

DF concentrates were prepared using the alcohol-insoluble residue method described by Femenia et al. [26], with modifications. Briefly, three replicates (5 g each) of dry samples were homogenized with 85% (*v*/*v*) ethanol. The mixture was boiled on a shaker for 10 min, and then the solid residue was collected using a Büchner funnel with cellulose-free filters (Whatman, 934-AH ™ glass microfiber filters, Sigma Chemical Co., St Louis, MO, USA). This process was repeated twice, the final time with absolute ethanol. Finally, the insoluble solid residue was washed with acetone, and the excess solvent was removed after 24 h at room temperature.

2.3. Determination of Neutral Sugar and Uronic Acid

To determine the content of uronic acids and the profile of neutral sugars, the DF was previously solubilized (autoclave treatment at 121 °C for 16 min followed by an ultrasound treatment for 1 h), and the soluble content of the DF extract was precipitated by adding to the supernatant 3 times its volume of absolute ethanol at 60 °C. Then, it was centrifuged, and the supernatant was removed. The solid residue was dried in an oven at 45 °C. Once dry, the soluble residue of the DF extract was subjected to a hydrolysis process with 12 M sulfuric acid (3 h at room temperature and 100 °C for 1 h). Finally, the monosaccharides and galacturonic acids released were determined by HPLC. HPLC analyses in this study were conducted using a 1260 Infinity II LC Agilent HPLC System (Waters, Milford, MA, USA), which consisted of a separation module, RI detector. The HPLC system was equipped with a Rezex-ROA column (7.8 mm internal diameter × 150 mm; Phenomenex, Torrance, CA, USA). In isocratic mode, the mobile phase was water, at a flow rate of 0.6 mL/min. In elution mode, the sample injection volume was 10 µL, the column temperature was 80 °C and the detector temperature was 40 °C.

2.4. Survival of Probiotics in the Presence of DF Extracts

Co-incubation of probiotics and DF extracts was performed in MRS broth supplemented with 1% DF extracts. The bacterial strains used were *Lactobacillus casei* (HL 245, HL 233) (Spanish type culture collection), *Lactobacillus reuteri* (PL503, PL519), *Enterococcus faecium* (SE 906, SE 920) [27,28]. Then, 10 mg of DF was weighed out and mixed with 900 µL of (de Man, Rogosa and Sharpe) MRS broth, which was sterilised at 121 °C for 15 min, cooled, and inoculated with 100 µL of MRS broth sterile with probiotic inoculum content of 10% (10^9 cfu/mL) in aseptic conditions. Co-incubation was carried out over 18 h at 37 °C at 180 rpm with orbital shaking to ensure a homogeneous distribution of the DF extract supply. As a blank, probiotics incubated without DF extracts were used.

A standardised in vitro static digestion method was performed for simulated gastrointestinal digestion [29]. Simulated gastric juice was mixed with an equal volume of probiotic suspensions. A 1 M HCl solution was used to adjust the pH of these samples to pH 2 prior to gastric digestion at 37 °C over 2 h. Following gastric digestion, the gastric mixtures were then mixed with an equal volume of simulated intestinal juice and adjusted to pH 7 with a solution of 1 M NaOH prior to intestinal digestion at 37 °C for 6 h. When gastric and intestinal digestion were completed, viable bacteria were counted in MRS medium in the digested samples after 0, 2 and 6 h. The data were expressed in logarithmic reduction of cfu/mL with respect to the initial inoculum (Time 0 h: 8 Log cfu/mL).

2.5. In Vitro Prebiotic Capacity

The prebiotic capacity of the soluble DF extracts was assessed using the method described previously by Ruiz-Moyano et al. [30]. Inoculum preparations consisted of aliquots of stock cultures of the bacteria strains *L. casei* (HL 245, HL 233) (Spanish type culture collection), *L. reuteri* (PL 503, PL 519), and *E. faecium* (SE 906, SE 920) (Ruiz-Moyano et al. [27,28] grown in Man–Rogosa–Sharpe (MRS; Scharlab, Barcelona, Spain) broth over 24 h at 37 °C. The strains were tested for growth in the presence of solubilized DF (autoclave treatment at 121 °C for 16 min followed by an ultrasound treatment for 1 h). Five microlitres of each bacterial suspension strain was inoculated into 200 µL of semi-solid MRS medium which contained 0.125 g/L agar, glucose-free and supplemented with 2 g/L of each sterile-filtered extract as the exclusive carbohydrate source. Semi-solid MRS supplemented with 2 g/L glucose was used as a positive control for growth, and other DF control was made with short-chain fructo-oligosaccharide (FOS), whereas the negative control was a carbohydrate-free semisolid MRS. Turbidity was measured in a fluorimeter (FLUOstar OPTIMA F), growth was carried out for 48 h at a temperature of 37 °C and readings were taken at a wavelength of 570 nm at 1-h intervals. The ability of each strain to grow was evaluated by comparing, in percentage, the growth in each extract with that in the control.

2.6. Determination of Short-Chain Fatty Acids Produced in the Presence of Fibre Extracts

To determine the ability to produce short-chain fatty acids (SCFAs), lactic acid bacteria (LAB) strains that were grown with the improved fibre extracts were selected. They were grown in modified MRS broth at 37 °C. The MRS was prepared as a commercial MRS without glucose and sodium acetate and complemented with 2 g/L of carbohydrate source (improved fibre extracts). Culture supernatants were obtained by centrifugation of the medium at 8000× *g* for 5 min before filtration through 0.22-µm filters (Thermo Fisher Scientific, Waltham, MA, USA).

To measure the amount of SCFAs, 500 µL of supernatant was mixed with 500 µL of ultrapure water and 100 µL of internal standard (2-ethylbutyric acid). Then 0.5 µL was injected into a gas chromatograph with a split/split-less injector and a flame ionisation detector (Shimadzu 2010 Plus). SCFAs were separated on a DB-FFAP capillary column (30 m × 0.25 mm id; 0.25 µm). The initial oven temperature was maintained at 80 °C for 2 min, and then increased to 200 °C at 20 °C/min and maintained for 12 min. The injector and detector were set at 250 °C. Helium at 1.8 mL/min was the carrier gas. Individual SCFAs were determined by comparing their retention times with those of the reference standard mixtures from Sigma (Sigma Chemical Co., St Louis, MO, USA). SCFA concentrations were determined as the ratio of the peak area of the analyte to the internal standard (2-ethylbutyric acid), according to Brighenti [31].

2.7. Statistical Analysis

Statistical analysis of the data was carried out using SPSS for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics of the data were determined, and the differences within and between groups were studied by one-way analysis of variance (ANOVA) and separated by Tukey's honestly significant difference test ($p \leq 0.05$). Principal component analysis (PCA) was performed on the correlation matrix of the variables. We worked with three biological replicates and each of them were analysed in triplicate.

3. Results and Discussion

3.1. Fibre Constituents: Neutral Sugars and Pectins

The results of the DF composition of by-products of tomato and pomegranate peels, and grape and broccoli stems are shown in Table 1.

The most abundant components of the DF extracts were uronic acids (which indicate the pectin content), and their values ranged from 581.24 to 934.72 mg of galacturonic acid per gram of fibre extract. Tomato peel exhibited the highest values, 934.72 mg/g, showing significant differences with the rest of the by-product extracts studied. Regarding the neutral sugar profile, the main monosaccharides were glucose and fucose in tomato and pomegranate peel, mannose in the case of grape stems, showing significant differences with the rest of the by-products, and arabinose and xylose in broccoli stems. In general, it was observed that galactose and rhamnose were the minority sugars in all analysed samples.

The results obtained for pectin concentration were higher than those found by other authors. Depending on the extraction method, Sengar et al. [32] obtained between 675.8 and 913.3 g/kg of galacturonic acid in tomato peel. Pomegranate peel was described with a lower amount of pectins, between 377 and 755 mg/g uronic acids [33]. Lower amounts of pectins were also found in grape and broccoli stems, ranging from 31.09 mg/g in grape stems [14] to 159.5 mg/g in broccoli stems [34].

The profile of the majority of neutral sugars was variable depending on the by-product. Glucose levels in tomato and pomegranate skins were significantly higher than those in grape and broccoli stems. This glucose may come from non-pectic polysaccharides or be a remnant of soluble sugars that were not completely removed during the extraction procedure, and other sugars identified in other studies coincide, although in different amounts, with those found in our study [14,32–34].

Table 1. Neutral sugar and uronic acid profile of dietary fibre from vegetable by-products (mg/g dry weight).

Parameters	Tomato Peel			Pomegranate Peel			Grape Stems			Broccoli Stems		
	Mean		SD [1]	Mean		SD	Mean		SD	Mean		SD
Neutral sugar (mg/g)												
Glucose	6.04	±	1.37 ^{b}	4.7	±	1.00 ^{b}	1.35	±	0.01 ^{a}	0.56	±	0.00 ^{a}
Rhamnose	0.26	±	0.00 ^{c}	0.12	±	0.04 ^{b}		<0.1 ^{a}		0.34	±	0.00 ^{d}
Xylose		<0.1 *^{a}		2.17	±	0.46 ^{b}		<0.1 ^{a}		2.37	±	0.02 ^{b}
Mannose	0.97	±	0.31 ^{a}	0.48	±	0.12 ^{a}	2.33	±	0.01 ^{b}	0.62	±	0.00 ^{a}
Fucose	3.09	±	0.70 ^{b}	3.09	±	0.03 ^{b}	1.65	±	0.01 ^{a}	2.61	±	0.18 ^{ab}
Galactose	1.54	±	0.53 ^{b}	0.84	±	0.22 ^{ab}		<0.1 ^{a}		0.69	±	0.00 ^{ab}
Arabinose	1.33	±	0.00 ^{a}	1.47	±	0.15 ^{a}	1.20	±	0.01 ^{a}	3.40	±	0.02 ^{b}
Uronic acids (pectin) (mg/g)												
Galacturonic acid	934.72	±	45.81 ^{b}	581.24	±	53.91 ^{a}	655	±	0.32 ^{a}	657.51	±	4.58 ^{a}

[1] SD, standard deviation; * The limit of detection was 0.1 mg/g; ^{a,b,c} Values with different superscripts are significantly different ($p \leq 0.05$) between samples.

3.2. Survival of Probiotics in the Presence of DF Extracts

Table 2 shows the results obtained from the assay performed to check the ability of the DF extracts to protect the probiotic strains used from gastrointestinal tract transit. The data are expressed as Log cfu/mL reduction with respect to the initial inoculum (Time 0 h: 8 Log cfu/mL).

In the case of *E. faecium* SE 920, it was observed that tolerance to low pH and gastrointestinal transit was superior in the presence of the different DF extracts, and the best behaviour was observed in the presence of tomato peel extract, although it improved in all cases with respect to the control without fibre. In the same way, tomato peel extracts were able to improve survival through the gastrointestinal tract in all strains of *L. casei* and *L. reuteri*. In addition, the survival of all strains was improved more or less in the presence of DF, except that *E. faecium* SE 906 did not tolerate the low pH and gastrointestinal transit in vitro in the absence of extract, in the control or in the presence of the different DF extracts. Tomato DF extract presented the highest pectin concentration, which may be related to its protective activity. Anal and Singh [35] studied several biopolymers generally recognized as safe as encapsulation materials, including gelatine, pectin, and alginate, for their ability to improve the viability and shelf life of probiotic bacteria. Other authors have shown that multi-layered emulsions including pectins extracted from citrus peels can be used to incorporate probiotics into various products and improve their viability during processing, storage, and after ingestion [36]. Blaiotta et al. [37] demonstrated that chestnut fibre was able to greatly improve the tolerance of *Lactobacillus* to simulated gastric and bile juice.

Soluble DF can create a viscous environment for probiotic bacteria, providing a protective effect and enhancing cell adhesion or biofilm formation in the fibre matrix [38].

3.3. In Vitro Prebiotic Capacity

Table 3 shows the capacity of the six probiotic bacterial strains tested for in vitro growth on four DF extracts. The results showed that the DF extracts from the grape stem sample achieved the highest growth values for all the strains studied, *E. faecium* SE 920 showing the highest growth (107.29%). In addition, significant differences were found in the growth of the *E. faecium* SE 906, *E. faecium* SE 920, *L. casei* HL 245, and *L. casei* HL 233 strains in the presence of the grape stem fibre extracts with respect to the rest of the extracts. However, no significant differences were found in the growth of the two *L. reuteri* strains. On the one hand, pomegranate peel extracts achieved moderate growth of *L. casei* HL 233 and *E. faecium* strains (48.35% and 44.49%, respectively). On the other hand, all the strains studied showed a slight growth in the presence of tomato peel and broccoli stem extracts.

Table 2. Tolerance of bacterial strains to low pH, and complete gastrointestinal transit. Data are expressed in Log cfu/mL reduction with respect to the initial inoculum (Time 0 h: 8 Log cfu/mL).

Strains	Extracts	pH Tolerance (2.5)	Tolerance to Gastointestinal Transit
		2 h	6 h
E. faecium SE 906	Control	8	8
	Tomato peel	8	8
	Pomegranate peel	8	8
	Broccoli stems	8	8
	Grape stems	8	8
E. faecium SE 920	Control	3	8
	Tomato peel	0	1
	Pomegranate peel	1	4
	Broccoli stems	1	2
	Grape stems	2	2
L. casei HL 245	Control	2	5
	Tomato peel	2	4
	Pomegranate peel	2	2
	Broccoli stems	2	2
	Grape stems	2	2
L. casei HL 233	Control	2	2
	Tomato peel	0	1
	Pomegranate peel	2	2
	Broccoli stems	1	2
	Grape stems	2	2
L. reuteri PL 503	Control	1	2
	Tomato peel	0	0
	Pomegranate peel	1	2
	Broccoli stems	1	1
	Grape stems	0	1
L. reuteri PL 519	Control	3	5
	Tomato peel	2	2
	Pomegranate peel	3	5
	Broccoli stems	4	4
	Grape stems	3	4

Table 3. Percentage growth of lactic acid bacteria (LAB) strains, with respect to the positive control, on soluble dietary fibre extracts.

	***E. faecium* SE 906**		***E. faecium* SE 920**		***L. casei* HL 245**		***L. casei* HL 233**		***L. reuteri* PL 503**		***L. reuteri* PL 519**	
Extracts	Mean	SD [1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control (FOS [2])	7.21	± 0.53 [a]	27.97	± 0.65 [a]	68.14	± 0.78 [c]	28.75	± 0.77 [a]	29.05	± 0.06 [ab]	7.60	± 10.4 [a]
Tomato peel	8.88	± 0.19 [a]	23.29	± 4.35 [a]	14.16	± 6.44 [a]	23.95	± 3.07 [a]	31.21	± 0.50 [ab]	25.38	± 1.28 [ab]
Pomegranate peel	10.34	± 0.96 [a]	44.49	± 0.45 [b]	38.23	± 1.25 [b]	48.35	± 1.66 [b]	21.52	± 1.55 [a]	16.06	± 0.46 [ab]
Broccoli stems	15.53	± 0.82 [b]	26.84	± 0.03 [a]	30.36	± 1.94 [b]	24.09	± 6.95 [a]	26.95	± 6.55 [ab]	21.81	± 0.22 [ab]
Grape stems	42.31	± 2.71 [c]	107.29	± 1.32 [c]	58.12	± 4.58 [c]	82.90	± 2.26 [c]	34.27	± 0.45 [b]	37.58	± 9.94 [b]

[1] SD, standard deviation; [2] FOS, fructo-oligosaccharides; [a,b,c] Values with different superscripts are significantly different ($p \leq 0.05$) between samples; Negative growth (≤20%); Slight growth (>20%–≤40%); Moderate growth (>40%–≤70%); High growth (>70%).

Polysaccharides such as pectin are formed by groups of complex structure, which are metabolised more slowly. Therefore, the ability of bacteria to metabolize fibres of this subgroup as an energy source will be influenced by the degree of polymerization, molecular weight, chain size, and the presence of branching in the molecule [39]. The speed and rate of prebiotic DF fermentation by the gut microbiota depends on several factors such as solubility, chain size, porosity, total particle surface area, and the structure and organization

of the fibre cell wall. Furthermore, the fermentation outcome of a fibre mixture is not the same as that of each individual DF [40]. In addition, other dietary components such as proteins, lipids, and phenolic compounds can affect the fermentability and prebiotic effect of DF [41].

3.4. Short-Chain Fatty Acids Produced in the Presence of Fibre Extract Production

Table 4 shows the production of short-chain fatty acids of the probiotic bacteria grown in the presence of the different DF extracts studied. No significant differences were found in the production of the different fatty acids at the strain level. However, significant differences were found according to the type of extract used. Acetic acid was the major acid in all cases, and the values ranged from 14.39 to 355.59 mM. The highest production of acetic acid was observed in the presence of broccoli stem extract, which showed significant differences with respect to the other extracts, followed by grape stem and pomegranate peel samples. By contrast, tomato skin showed the lowest value for acetic acid production. Overall, it can be stated that pomegranate skin achieved the highest values for the other minority fatty acids, followed by the broccoli stem and grape stem samples; however, as in the case of acetic acid, the tomato skin DF extracts showed the lowest values. The glucose and FOS controls obtained average values for all the fatty acids studied.

Baenas et al. [42] observed that the production of fatty acids in the presence of DF extracted from raspberry was generally increased during fermentation. Similar to the results obtained by us, previous studies revealed that DF extracted from potato residue could promote the production of acetic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid [43].

3.5. Multivariate Analysis of the Parameters Related to DF Extract Studied from Different Subproducts

PCA was carried out for the entire set of DF data to obtain an interpretable overview of the main information. Figure 1 shows the two-way loadings and score plots, where PC2 was plotted against PC1, explaining 90% of the total variance. According to Figure 1A, PC1 best explains the difference between the variables of bacterial survival to gastrointestinal transit and the production of short-chain fatty acids such as propionic acid, while PC2 shows the variability between the neutral sugars present in the soluble fibre extract.

With regard to the factors studied, principal component analysis clearly shows how the fibre extracts obtained from the different by-products behave differently with respect to the variables studied. The fibre extracted from grape stems was positively correlated with the percentage of lactic acid bacterial growth with respect to the positive control, while the fibre extracted from broccoli stems and pomegranate peels was correlated with the production of short-chain fatty acids. On the other hand, tolerance to low pH and gastrointestinal transit was superior when tomato peel extract was present.

Table 4. Production of short-chain fatty acids in mM of lactic acid bacteria (LAB) in the presence of different soluble fibre extracts.

	Acetic Acid		Propionic Acid		Isovaleric Acid		Butyric Acid		Isocaproic Acid		Isobutyric Acid		Valeric Acid		Caproic Acid	
	Mean	**SD** [1]	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Extracts (E)																
Control (Glucose)	70.90 ±	57.00 ^{b}	0.51 ±	0.34 ^{abc}	0.37 ±	0.21 ^{bc}	0.06 ±	0.03 ^{ab}	0.69 ±	1.46	0.08 ±	0.06 ^{ab}	0.40 ±	0.21 ^{bc}	0.24 ±	0.17 ^{ab}
Control (FOS [2])	85.15 ±	26.30 ^{b}	0.41 ±	0.07 ^{ab}	0.35 ±	0.11 ^{abc}	0.04 ±	0.01 ^{a}	0.18 ±	0.07	0.06 ±	0.01 ^{ab}	0.30 ±	0.13 ^{b}	0.17 ±	0.06 ^{ab}
Tomato peel	14.39 ±	10.70 ^{a}	0.08 ±	0.03 ^{a}	0.11 ±	0.03 ^{a}	0.01 ±	0.00 ^{a}	0.00 ±	0.00	0.02 ±	0.00 ^{a}	0.00 ±	0.00 ^{a}	0.03 ±	0.04 ^{a}
Pomegranate peel	221.40 ±	27.27 ^{c}	1.13 ±	0.45 ^{d}	0.83 ±	0.30 ^{d}	0.13 ±	0.05 ^{c}	0.72 ±	0.37	0.22 ±	0.12 ^{d}	0.91 ±	0.29 ^{d}	0.61 ±	0.28 ^{c}
Broccoli stems	355.59 ±	150.39 ^{d}	1.06 ±	0.47 ^{cd}	0.76 ±	0.31 ^{d}	0.13 ±	0.00 ^{c}	0.64 ±	0.35	0.19 ±	0.09 ^{c}	0.82 ±	0.39 ^{d}	0.59 ±	0.30 ^{c}
Grape stems	256.37 ±	52.94 ^{c}	0.89 ±	0.22 ^{bcd}	0.61 ±	0.12 ^{cd}	0.09 ±	0.02 ^{bc}	0.42 ±	0.15	0.13 ±	0.03 ^{bc}	0.63 ±	0.17 ^{cd}	0.41 ±	0.12 ^{bc}
Strains (S)																
E. faecium SE 906	161.49 ±	110.94	0.61 ±	0.49	0.51 ±	0.33	0.07 ±	0.05	0.36 ±	0.35	0.12 ±	0.12	0.47 ±	0.37	0.33 ±	0.27
E. faecium SE 920	153.71 ±	116.08	0.63 ±	0.49	0.45 ±	0.31	0.07 ±	0.05	702 ±	1.36	0.11 ±	0.09	0.47 ±	0.37	0.32 ±	0.27
L. casei HL 245	184.06 ±	212.62	0.67 ±	0.58	0.49 ±	0.39	0.08 ±	0.07	0.37 ±	0.39	0.11 ±	0.11	0.53 ±	0.46	0.34 ±	0.34
L. casei HL 233	163.86 ±	137.21	0.62 ±	0.42	0.46 ±	0.28	0.07 ±	0.05	0.34 ±	0.31	0.10 ±	0.08	0.45 ±	0.36	0.31 ±	0.27
L. reuteri PL 503	136.30 ±	106.83	0.72 ±	0.63	0.43 ±	0.29	0.06 ±	0.05	0.31 ±	0.29	0.09 ±	0.08	0.44 ±	0.34	0.27 ±	0.25
L. reuteri PL 519	149.20 ±	89.38	0.81 ±	0.54	0.46 ±	0.30	0.07 ±	0.05	0.34 ±	0.30	0.11 ±	0.08	0.96 ±	0.37	0.31 ±	0.26
Values P																
Pe	0.000		0.000		0.000		0.000		0.031		0.000		0.000		0.000	
Ps	0.230		0.850		0.952		0.957		0.576		0.960		0.952		0.983	
*Pe*s*	0.000		0.929		0.876		0.960		0.796		0.995		0.982		1.000	

[1] SD, standard deviation; [2] FOS, fructo-oligosaccharides; ^{a,b,c,d} Values with different superscripts are significantly different ($p \leq 0.05$) between type of extracts or strains. *Pe*: *p*-value of the extracts; *Ps*: *p*-value of the strains; *Pe*s*: *p*-value of the interaction between extracts and strains.

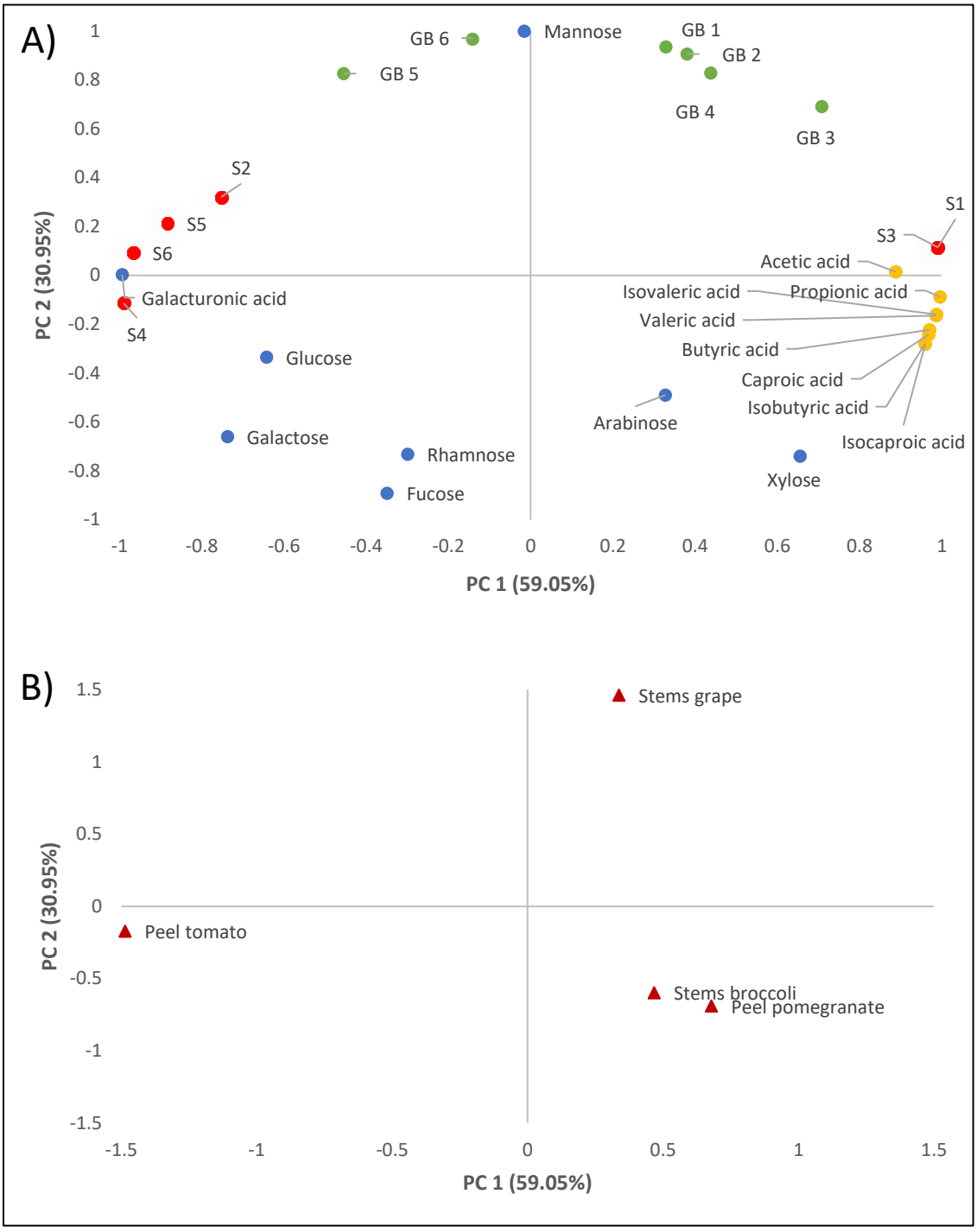

Figure 1. Principal component analysis of the analytical results of the dietary fibre extracts studied. Loading plot (**A**). GB1: growth of *E. faecium* SE 906; GB2: growth of *E. faecium* SE 920; GB3: growth of *L. casei* HL 245; GB4: growth of *L. casei* HL 233; GB5: growth of *L. reuteri* PL 503; GB6: growth of *L. reuteri* PL 519; S1: survival in the gastrointestinal tract of *E. faecium* SE 906; S2: survival in the gastrointestinal tract of *E. faecium* SE 920; S3: survival in the gastrointestinal tract of *L. casei* HL 245; S4: survival in the gastrointestinal tract of *L. casei* HL 233; S5: survival in the gastrointestinal tract of *L. reuteri* PL 503; S6: survival in the gastrointestinal tract of *L. reuteri* PL 519. Score plot (**B**). Grape stem, broccoli stem, pomegranate peel, and tomato peel samples.

4. Conclusions

This study presents a characterization of the effect of DF obtained from pomegranate, tomato, grape, and broccoli by-products on the gastrointestinal transit survival, growth, and metabolism of six probiotic strains. These by-products were shown to have high concentrations of polysaccharides that affected the probiotic microorganisms studied in different ways. The protective effect on intestinal transit of the fibre obtained from tomato skin was superior to that of the other extracts of pomegranate, grape, and broccoli. The fibre extracted from grape stems favoured the growth of lactic acid bacteria, and the fibre extracted from broccoli stems and pomegranate peel stimulated the increased production of short-chain fatty acids. The results reveal that these by-products can be considered as high-quality sources of DF for joint applications with probiotic microorganisms by aiding and stimulating their survival, growth, and metabolism, and their use as prebiotics in the food industry would help to develop new high value-added ingredients.

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CAPÍTULO 3

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Improve the functional properties of dietary fibre isolated from broccoli by-products by using different technologies

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ABSTRACT

The aim of this study was to analyze the chemical composition of leaves, stems and inflorescences obtained from broccoli by-products in order to develop the improvement of the composition and functional properties of DF from broccoli by-products through treatment with different degradation methods, such as modifications with supercritical fluids, enzymatic, autoclave and ultrasound treatment. The results showed that the chemical composition of the different parts of the broccoli by-products, leaves, stems and inflorescences was significantly different, highlighting that the content of insoluble and soluble dietary fibre was higher in the stem. The enzymatic treatments decreased the content of neutral sugars and increased the content of uronic acids, which was related to the increase of specific functional activities. Cellulase and the multi-enzymatic visconzyme complex improved the solubility and glucose adsorption capacity, whereas supercritical fluids treatment improved the swelling, water retention and lipid absorption capacities. The value of non-extractable phenolic compounds was higher in the inflorescences and increased with enzyme and supercritical fluid treatments, as well as the antioxidant capacity. Enzyme treatment also had a greater effect on the stimulation of the growth of the lactic acid bacteria studied and, therefore, reached the highest values in the production of all short-chain fatty acids analysed. Therefore, the use of enzyme treatments of dietary fibre obtained from broccoli by-products improves the functional properties of broccoli fibre.

1. Introduction

Broccoli (*Brassica oleracea* L. var. Italica) is widely consumed worldwide, playing a major economic role in the food industry (Loi et al., 2019). In fact, global production of broccoli and cauliflower has increased by about 6 million tonnes in the last 10 years, with approximately 27 million tonnes produced in 2019 (FAOSTAT, 2021). Their growing popularity is due to the health benefits of their chemical composition (Ares, Nozal, & Bernal, 2013). They are rich in vitamins (Rybarczyk-Plonska et al., 2014), polyphenols (Sánchez-Pujante et al., 2020), glucosinolates (Rybarczyk-Plonska et al., 2016; Yu et al., 2018), carbohydrates and dietary fibre (DF) (Busato, de Almeida Abreu, de Oliveira Petkowicz, Martinez, & Noleto, 2020; Petkowicz & Williams, 2020; Urai, Kataoka, Nishida, & Sekimizu, 2017; Xu, Cao, & Chen, 2015).

Research on broccoli has mainly focused on its edible part, which represents about 10–15% of the plant's weight (Shi et al., 2019). The remaining parts are by-products that include stems, leaves and inflorescence (Ferreira, Passos, Cardoso, Wessel, & Coimbra, 2018). Broccoli by-products are well known to provide an important source of DF and bioactive compounds (Domínguez-Perles, Martínez-Ballesta, Carvajal, García-Viguera, & Moreno, 2010; Schäfer, Stanojlovic, Trierweiler, & Bunzel, 2017; Thomas, Badr, Desjardins, Gosselin, & Angers, 2018), making them suitable for use as a functional ingredient (Hwang & Lim, 2015).

The bioactive activities of DF depend on its composition and solubility (Huang, Chen, Cheng, & Huang, 2020). Soluble dietary fibre (SDF) is known to present better bioactive properties than insoluble dietary fibre (IDF) due to its fermentability and viscosity (Ma & Mu, 2016a, 2016b). The DF of plant by-products is mainly composed of IDF (Chitrakar, Zhang, Zhang, & Devahastin, 2020; He, Sampers, & Raes, 2021;), so it is necessary to modify and degrade it to improve its activities and functional properties. Chemical, biological and physical methods have been developed to modify DF and increase SDF content, such as

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enzymatic degradation (Kim, Kim, Yu, & Suh, 2019), ultrasound degradation (Xu et al., 2019), microwave degradation (Hashemifesharaki, Xanthakis, Altintas, Guo, & Gharibzahedi, 2020) and supercritical fluid degradation (Escobar, Da Silva, Pirich, Corazza, & Ramos, 2020).

Despite the results obtained in previous research with other raw materials, the improvement of DF in broccoli by-products has not been explored. Putrino, Tedesco, Bodini, and de Oliveira (2020) reported that treatment with supercritical fluids caused changes in the structure of coconut DF, increasing porosity and loosening hydrogen bonds causing delignification. Enzymatic treatment also proved to be effective for improving DF. Liu, Zhang, Yi, Quan, and Lin (2021) used cellulase enzyme to treat rice bran DF, which caused changes in the chemical composition and an increased porosity of the DF resulting in an improvement of the physicochemical and functional properties.

Probably the major functional property of DF is its prebiotic activity due to the fermentation of DF in the colon by the intestinal microbiota. Short-chain fatty acids (SCFA), such as butyric, propionic and acetic acids are produced (Baenas et al., 2020), which are associated with a wide range of physiological properties. Significant effects such as improvement of digestive tract disorders (López-Oliva, Agis-Torres, Goni, & Muñoz-Martínez, 2010; Pozuelo et al., 2012; Sun, Ma, Pan, Du, & Sun, 2019) and anticancer activity (Sánchez-Tena et al., 2013; Yusefi, Shameli, Ali, Pang, & Teow, 2020) have been described in the literature. Cell wall polysaccharide characteristics are associated with the functional properties of DF. Water retention capacity (WRC) prevents and treats intestinal disorders by increasing fecal bulk and reducing gastrointestinal transit time and is mainly related to insoluble dietary fibre (IDF). Swelling capacity (Sw) in the stomach and an increase in the viscosity of intake are associated with soluble dietary fibre (SDF), which slows nutrient absorption from the intestinal mucosa and decreases postprandial blood glucose and insulin responses (Mehta, Ahlawat, Sharma, & Dabur, 2015). On the other hand, dietary fibre with high fat absorption capacity (FAC) is associated with a significant reduction in plasma cholesterol levels by the ability to absorb or bind bile acids and increase their excretion (Schneeman, 1999). In addition, these properties, WRC, Sw and FAC, also have technological effects on food products (bakery products, dairy products, jams, meats, soups) as they can modify textural properties, prevent syneresis (the separation of liquid from a gel caused by shrinkage), stabilize fat-rich foods and emulsions, and improve shelf life (Elleuch et al., 2011).

Therefore, the objective of this work was to analyze the chemical composition of leaves, stems and inflorescences obtained from broccoli by-products in order to develop the improvement of the composition and functional properties of DF from broccoli by-products through treatment with different degradation methods, such as modifications with supercritical fluids, enzymatic, autoclave and ultrasound treatment.

2. Materials and methods

2.1. Plant material

The broccoli by-products used in this work were derived from cultivar Parthenon in the region of Extremadura, Spain. The broccoli by-products considered included leaves, stems and inflorescence remains. The three parts of the plant were separated by hand and the samples were dried in a forced air oven at 45 °C for 24–48 h, ground with a mincer, packed in vacuum bags and stored at room temperature until use. All the experiments were performed in triplicate.

2.2. Chemical composition

2.2.1. Moisture and ash

Determinations of moisture and ash were based on the Association of Official Analytical Chemists' (AOAC) Official Methods of Analysis (AOAC, 2005).

2.2.2. Crude protein and total fat

Crude protein and fat content were determined according to the Kjeldahl method (AOAC, 2000a, 2000b) and ISO 1444 (1996).

2.2.3. Determination of soluble sugars

Total soluble sugars (TSS) were extracted with distilled water and determined by the method proposed by Albalasmeh, Berhe, and Ghezzehei (2013) and reducing sugars (RS) following the method of Miller (1959). The results were reported as g/100 g of dry sample.

2.2.4. Total phenolic content (TPC)

Phenolic compounds from 10 g of dried samples were extracted with ethanol/water as described by Casquete et al. (2015, 2018). TPCs of aqueous extracts were determined using Folin-Ciocalteu reagent (Wettasinghe & Shahidi, 1999) and gallic acid was used as standard. The results were given as mg gallic acid equivalents (GAE)/100 g of extract.

2.2.5. Determination of total, soluble and insoluble dietary fibre

Total dietary fibre (TDF) of broccoli by-products was determined according to the standard enzymatic-gravimetric method (AOAC, 2000a, 2000b), digesting 1 g of dry sample with 200 μL of α-amylase (Sigma-Aldrich, St. Louis, USA) at 80 °C for 1 h under continuous shaking, and subsequently with amyloglucosidase followed by protease (Rivas et al., 2021). The digested samples were filtered, and insoluble dietary fibre (IDF) was obtained from the solid fraction while soluble dietary fibre (SDF) was obtained by ethanol precipitation. TDF was calculated as the sum of IDF and SDF. Data were reported in g/100 g of dry sample.

2.3. Total dietary fibre extraction

The dietary fibre concentrates from broccoli by-products (leaves, stems and inflorescences) were extracted by the alcohol insoluble residues (AIR) method, which is a suitable method for plant matrices with low starch content. Cell wall polysaccharides were extracted using the protocol described by Femenia, García-Pascual, Simal, and Rosselló (2003) and modified by Rivas et al. (2021). Briefly, the dried samples were homogenized with 85% (*v*/v) ethanol and boiled for 10 min, then vacuum filtered through cellulose-free filters (Whatman glass microfiber filters, 934-AH™). This process was repeated with absolute ethanol. The solid residue was washed with acetone and the excess solvent was removed after 24 h at a temperature of 23 ± 1 °C. Once the dietary fibre concentrates were extracted from the by-products, treatments (enzymatic, autoclave, ultrasound, supercritical fluids) were applied to modify and improve the structure and functional properties of dietary fibre concentrates. Additionally, the dietary fibre extracts obtained from the by-products were subjected to an analysis to verify that they were free of reducing sugars, fats and proteins by the procedures described in Sections 2.2.2 and 2.2.3.

Once the dietary fibre concentrates were extracted from the by-products, different treatments (enzymatic, with autoclave, ultrasound, supercritical fluids) were applied to modify and improve the structure and functional properties of the dietary fibre concentrates. In addition, untreated fibre concentrates from each of the samples were used as a control to be able to determine the effect of the treatments on the different parameters.

2.3.1. TDF enzymatic treatment

TDF treatment was carried out by the method of Yu et al. (2018) with modifications. One g of AIR was dissolved in citric acid and sodium citrate buffer (50 mL). A total of 800 μL of cellulase enzyme (90 U/g) (Sigma-Aldrich, Madrid, Spain) was added, the solution was adjusted to pH 5 and stirred in a 50 °C bath for 2 h. The solution was cooled to room temperature and filtered under vacuum. A 4-fold volume of absolute ethanol was added to the supernatant and allowed to precipitate for 12 h. The precipitate was collected and dried in the oven together with the

Table 1
Leaves, stems and inflorescences chemical composition.

Parameters	Leaves			Stems			Inflorescences		
	Mean		SD[1]	Mean		SD	Mean		SD
Moisture	12.23	±	1.61	10.55	±	0.42	9.61	±	0.38
Components (g/100 g dry weight)									
Ash	12.68	±	0.51^{b}	17.81	±	0.55^{c}	8.28	±	0.32^{a}
Protein	21.26	±	1.51^{a}	19.44	±	1.25^{a}	28.67	±	1.14^{b}
Fat	3.34	±	0.54	3.17	±	0.11	2.48	±	0.76
TSS	25.39	±	1.59^{b}	18.58	±	0.16^{a}	24.61	±	0.51^{b}
Reducing sugars	7.07	±	0.03^{c}	2.72	±	0.01^{a}	4.07	±	0.12^{b}
TPC	0.70	±	0.03^{b}	0.17	±	0.03^{a}	0.79	±	0.03^{c}
TDF	62.22	±	1.05^{a}	77.28	±	1.39^{b}	64.42	±	1.20^{a}
IDF	56.27	±	1.24^{a}	66.18	±	0.88^{b}	58.36	±	1.38^{a}
SDF	5.94	±	0.20^{a}	11.10	±	0.50^{b}	6.06	±	0.18^{a}

TSS: total soluble sugars; TPC: total phenolic compounds TDF: total dietary fibre; IDF: insoluble dietary fibre; SDF: soluble dietary fibre.[1]SD: standard deviation. [a.b.c] Values with different superscripts are significantly different ($p \leq 0.05$) between samples.

solid residue, both were dried in the oven at 45 °C. The enzyme-enhanced fibre concentrate with cellulase (EFCC) was stored in vacuum bags until use.

A similar procedure was carried out with the multi-enzyme viscoenzyme complex, an enzyme cocktail containing xylanase, arabanase, β-glucanase, hemicellulose and cellulase activities (Sigma-Aldrich, Madrid, Spain), varying the pH of the solution to 4.5 and shaking time of 4 h to obtain the enzyme-enhanced fibre concentrate with viscoenzymes (EFCV).

2.3.2. TDF treatment with autoclave and ultrasound

The AIR was mixed with distilled water in a 1/50 (p/v) ratio, the mixture was treated in an autoclave (121 °C for 16 min), subsequently an ultrasound treatment was applied for 1 h by ultrasonic irradiation using an ultrasonic cleaning bath (360 W, J.P. Selecta, s.a. Barcelona, Spain), operating at a frequency of 50/60 Hz and power of 220 V. Finally, 4 times its volume of absolute ethanol was added to the supernatant, and it was allowed to precipitate for 12 h. The precipitate was collected, and oven dried together with the solid residue, both of which were oven dried at 45 °C. The autoclaved and ultrasound enhanced fibre concentrate (AUEFC) was stored in vacuum bags until use.

2.3.3. TDF treatment with supercritical fluids

Modification of AIR was carried out by supercritical fluid technology (SFE) using CO_2. The treatment was carried out in a dynamic extraction unit in a Speed SFE System 96 (HELIX applied separations). 20 g of AIR were used, inside a 100 mL stainless steel column. The solvent flow rate was set at 2 L/min. The conditions studied were 300 bar with 55 °C of temperature and 150 bar with 45 °C during 2 h. The enhanced fibre concentrate with supercritical fluid treatment (SF300/55EFC and SF150/45EFC) was vacuum packed until use.

2.4. Characterisation of the treated fibre neutral sugar and pectins

Total neutral sugars (TNS) and uronic acid were obtained by a fibre hydrolysis process and characterisation was performed by HPLC using neutral sugar standards and pectins as described by Rivas, Casquete, Córdoba, et al. (2021). The content was expressed in mg/g of fibre.

2.5. Functional properties of the treated fibre

2.5.1. Water solubility (WS)

Water solubility of samples was determined following the method described by Wan et al. (2015) with minor modifications. A mixture of 0.1 g of TDF sample with 5 mL of distilled water was prepared. Subsequently, it was incubated at 90 °C for 30 min in a thermostatically controlled water bath, followed by centrifugation at 2000*g* for 15 min. The supernatant was collected, dried and weighed. WS was expressed as a percentage and performed in triplicate.

2.5.2. Swelling (Sw), water retention capacity (WRC) and fat adsorption capacity (FAC)

Sw, WRC and FAC of alcohol insoluble residue (AIR) were determined following the methodology described by Garau, Simal, Rossello, and Femenia (2007). Sw results were expressed in mL water/g AIR, WRC was expressed in g water/g AIR and FAC results were expressed in g oil/g AIR.

2.5.3. Glucose absorption capacity (GAC)

The GAC of dietary fibre was estimated according to the method described by Niu, Li, Xia, Hou, and Xu (2018) with some modifications. Specifically, 0.1 g of SDF sample was mixed with 25 mL of a 50 mM glucose solution. It was incubated at 37 °C for 6 h with constant shaking and then centrifuged at 4500*g* for 10 min. The supernatant was analysed for reducing sugars with the Miller method as described in a previous section. GAC was expressed in mg of glucose retained/ mg of SDF.

2.5.4. Antioxidant activity (non-extractable polyphenols N-EPC)

N-EPC bound to AIR was achieved according to the method described by Arranz, Saura-Calixto, Shaha, and Kroon (2009) with modifications by Rivas, Casquete, Córdoba, et al. (2021). The total non-extractable polyphenols bound to AIR was determined using the Folin-Ciocalteu reagent (Wettasinghe & Shahidi, 1999) and the antioxidant capacity of the samples was evaluated by the DPPH (2, 2-diphenyl-1-picrylhydrogen oxide depletion) method (Teixeira, Canelas, do Canto, Teixeira, & Dias, 2009) and the ability to scavenge the ABTS (2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical (Re et al., 1999).

2.5.5. Growth of LAB on soluble dietary fibre

The growth capacity of lactic acid bacteria (*Lactobacillus sakei* (CECT5765), *Lactobacillus brevis* (CECT815), *Lactobacillus plantarum* (AB572C; Casquete et al., 2012), *Lactobacillus casei* (HL233; Ruiz-Moyano et al., 2009) and *Enterococcus faecium* (SE920; Ruiz-Moyano et al., 2009) on soluble dietary fibre extracts was carried out as described by Rivas, Casquete, Córdoba, et al. (2021). LAB were grown prior to the assay on de Mann Rogosa Sharpe (MRS) broth (Condalab, Madrid, Spain) at 30 °C for 24 h. After, LAB were tested for growth in the presence on soluble dietary fibre from different part of broccoli plant (leaves, stems and inflorescence) without treatment and after five treatments (EFCC, EFCV, AUEFC, SF300/55EFC and SF150/45EFC). Growth on glucose was used as positive control. The growth capacity was evaluated by comparing the percentage of growth in each extract with the positive control.

2.5.6. Determination of short chain fatty acids (SCFA) produced in the presence of fibre extracts

The production of SCFA of BAL strains at the end of growth on different fibre extracts was measured in a gas chromatograph with a split/split-less injector and a flame ionization detector (Shimadzu 2010 Plus) by the method described by Rivas et al. (2021). SCFA concentrations were determined as the ratio of the peak area of the analyte to the internal standard (2-ethylbutyric acid) according to Brighenti (1997).

2.6. Statistical analysis

SPSS for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of the data. Data descriptive statistics were determined, and differences within and between groups were analysed by one-way and two-way analysis of variance (ANOVA) and separated by Tukey's honest significant difference test ($p \leq 0.05$). Principal component analysis (PCA) was performed on the correlation matrix of the variables. Three biological replicates were worked and each replicate was analysed in triplicate.

3. Results and discussion

3.1. Chemical composition

The results of the chemical composition of broccoli by-products (leaves, stems and inflorescences) are shown in Table 1. Stem samples presented higher moisture values (12.23%) although no significant differences were found between the different parts of the plant. As for ash content, significant differences were observed among the different samples ($p \leq 0.05$), stem samples presented the highest value (17.81%), followed by leaves and inflorescences (12.68% and 8.28%, respectively). The samples with the highest crude protein content were inflorescences (28.67%) and leaves (21.26%), which showed statistically higher differences compared to stem samples (19.44%). Fat content ranged from 2.48% to 3.34%, with no significant differences between plant parts. Li et al. (2021) obtained percentages of ash, protein and fat in leaves, stems and inflorescences very similar to those obtained in our study. However, in other research Shi, Ying, Ye, Sanguansri, and Augustin (2020) reported slightly higher values for protein (27.55%) and fat (5.48%) than ours, although their experiment was done by mixing broccoli leaves and stems, freeze-drying them and analyzing the mixture, and our study was done with leaves and stems separately. However, the protein results obtained by Ferreira et al. (2018) in broccoli by-products were lower compared to our results.

Table 1 also shows the TSS and reducing sugars content of the broccoli by-products. The values of TSS and reducing sugars were highest in the leaf samples, with values of 25.39 and 7.07 g/100 g, respectively. The stem samples showed the lowest values of soluble sugars and significant differences in both parameters with respect to the other parts of the plant. Other authors have found a sugar content of glucose, fructose and sucrose, i.e. reducing sugars, of 3.5% for broccoli stems (Landin-Sandoval et al., 2020).

Regarding the TPC content, the inflorescence samples presented the highest values ($p \leq 0.05$) compared to the rest of the samples, reaching a mean value of 0.79 g GAE/100 g. This was followed by leaf and stem samples (0.70 and 0.17 g GAE/100 g, respectively). Previous studies reported a higher content of phenolic compounds in broccoli inflorescences compared to other parts of the plant (Thomas et al., 2018).

The leaf, stem and inflorescence samples studied were found to be rich in TDF (62.22–77.28 g/100 g) (Table 1). The IDF content of the samples ranged between 56.27 and 66.18 g/100 g, with higher values for the stem samples, which showed significant differences with respect to the rest of the broccoli by-products studied. Similarly, the SDF content was higher in the stem samples (11.10 g/100 g), also showing significant differences to the leaf and inflorescence samples, whose mean values were 5.94 and 6.06 g/100 g, respectively. TDF values published by other

Table 2
Uronic acid and neutral sugar profile (mg/g fibre concentrate) of leaves, stems and inflorescences dietary fibre.

	Uronic acid (pectin)			Glucose			Xylose			Arabinose			Fucose			Rhamnose			Manose			Galactose		
	Mean		SD[1]	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD
Parts of the plant (P)																								
Leaves	657.60	±	55.68	7.45	±	2.44^{b}	2.32	±	0.49^{b}	2.52	±	0.73^{b}	2.39	±	0.16^{a}	0.24	±	0.12^{b}	0.6	±	0.15^{a}	0.67	±	0.18^{a}
Stems	634.11	±	21.49	9.24	±	3.41^{c}	2.72	±	0.69^{c}	2.01	±	0.41^{a}	2.54	±	0.27^{b}	0.16	±	0.07^{a}	0.73	±	0.21^{b}	0.82	±	0.09^{b}
Inflorescences	636.18	±	36.12	5.90	±	2.30^{a}	0.82	±	0.20^{a}	2.81	±	1.00^{b}	2.35	±	0.27^{a}	0.21	±	0.12^{ab}	0.89	±	0.26^{c}	1.48	±	0.47^{c}
Treatments (T)																								
Control	599.40	±	12.88^{a}	9.38	±	2.66^{b}	2.34	±	1.19^{c}	2.77	±	0.54^{c}	2.49	±	0.17^{bc}	0.25	±	0.04^{bc}	0.88	±	0.13^{bc}	1.17	±	0.37^{c}
EFCC	662.00	±	20.47^{bc}	4.74	±	1.99^{a}	1.54	±	0.79^{b}	2.08	±	0.75^{b}	2.16	±	0.14^{a}	0.16	±	0.13^{b}	0.55	±	0.13^{a}	0.71	±	0.29^{a}
EFCV	668.56	±	55.67^{c}	4.45	±	1.73^{a}	1.43	±	0.77^{a}	1.39	±	0.80^{a}	2.23	±	0.19^{ab}	0.05	±	0..01^{a}	0.49	±	0.11^{a}	0.62	±	0.22^{a}
AUEFC	620.77	±	42.77^{ab}	9.75	±	2.91^{b}	2.35	±	1.17^{c}	2.86	±	0.47^{c}	2.58	±	0.24^{c}	0.27	±	0.09^{c}	0.93	±	0.26^{c}	1.27	±	0.58^{c}
SF150/45EFC	656.98	±	22.59^{bc}	8.77	±	1.88^{b}	2.11	±	0.83^{c}	2.95	±	1.01^{c}	2.61	±	0.17^{c}	0.27	±	0.09^{c}	0.87	±	0.26^{bc}	1.20	±	0.64^{c}
SF300/55EFC	648.05	±	12.89^{bc}	8.1	±	2.17^{b}	1.96	±	0.91^{c}	2.62	±	0.50^{c}	2.48	±	0.17^{bc}	0.23	±	0.04^{bc}	0.73	±	0.09^{b}	0.98	±	0.34^{b}
P Values																								
Pp	0.078			<0.001			<0.001			<0.001			0.020			0.005			<0.001			<0.001		
Pt	0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001		
*Pp*t*	0.027			0.002			0.007			0.001			0.349			0.016			0.004			<0.001		

EFCC: cellulase-enhanced dietary fibre; EFCV: dietary fibre enhanced with Viscozyme; AUEFC: improved dietary fibre with ultrasound treatment and autoclave; SF150/45EFC: dietary fibre enhanced with supercritical fluids (150/45/2); SF300/55EFC: dietary fibre enhanced with supercritical fluids (300/55/2). [1]SD: standard deviation. [abc] Values with different superscripts are significantly different ($p \leq 0.05$) between parts of the plant or treatments.

Table 3
Functional properties of leaves, stems and inflorescences dietary fibre.

	Ws (%)		Sw (mL/g)		WRC (g/g)		FAC (g/g)		GAC (g/g)		N-EPC (mg/100 g)		DPPH (mg Trolox/100 g)		ABTS (mg Trolox/100 g)	
	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Parts of the plant (P)																
Leaves	27.34 ±	19.94^{a}	8.50 ±	3.12^{a}	9.52 ±	3.07^{a}	6.64 ±	0.41	0.71 ±	0.17^{a}	291.55 ±	65.43^{b}	106.49 ±	35.95^{b}	449.28 ±	111.66^{b}
Stems	29.75 ±	23.16^{b}	10.48 ±	3.88^{b}	11.50 ±	3.84^{b}	6.67 ±	1.38	0.81 ±	0.22^{b}	192.24 ±	137.96^{a}	82.60 ±	48.21^{a}	331.86 ±	101.78^{a}
Inflorescences	29.29 ±	21.11^{b}	11.66 ±	0.72^{b}	10.67 ±	4.27^{b}	7.00 ±	1.72	0.66 ±	0.16^{a}	341.23 ±	121.99^{c}	132.51 ±	47.29^{c}	564.91 ±	160.47^{c}
Treatments (T)																
Control	4.40 ±	0.67^{a}	11.79 ±	1.87^{b}	12.52 ±	1.87^{b}	4.79 ±	1.29^{a}	0.45 ±	0.04^{a}	230.50 ±	113.02^{c}	113.94 ±	54.47^{b}	565.40 ±	162.12^{d}
EFCC	53.74 ±	5.26^{c}	4.71 ±	1.24^{a}	5.80 ±	1.12^{a}	7.72 ±	0.96^{bc}	0.88 ±	0.16^{e}	158.29 ±	14.71^{a}	80.72 ±	18.51^{a}	391.37 ±	92.27^{b}
EFCV	56.21 ±	3.16^{c}	5.42 ±	2.04^{a}	6.38 ±	1.96^{a}	7.29 ±	0.85^{bc}	0.87 ±	0.16^{de}	344.63 ±	101.75^{e}	140.66 ±	48.51^{c}	544.09 ±	216.38^{d}
AUEFC	4.84 ±	1.22^{a}	11.50 ±	2.15^{b}	12.50 ±	2.14^{b}	6.03 ±	1.43^{ab}	0.75 ±	0.74^{c}	207.37 ±	110.44^{b}	118.99 ±	47.93^{b}	447.08 ±	140.29^{c}
SF150/45EFC	25.37 ±	1.80^{b}	13.24 ±	2.87^{c}	14.24 ±	2.87^{c}	8.26 ±	1.74^{c}	0.77 ±	0.12^{cd}	291.93 ±	124.03^{d}	64.43 ±	36.28^{a}	325.09 ±	117.44^{a}
SF300/55EFC	28.21 ±	4.66^{b}	10.94 ±	3.80^{b}	11.94 ±	1.43^{b}	6.54 ±	1.68^{abc}	0.64 ±	0.17^{b}	417.32 ±	69.85^{f}	123.77 ±	3.13^{bc}	419.08 ±	42.44^{bc}
P Values																
Pp	0.010		<0.001		<0.001		0.678		<0.001		<0.001		<0.001		<0.001	
Pt	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
*Pp*t*	<0.001		<0.001		<0.001		0.328		<0.001		<0.001		<0.001		<0.001	

Ws: water solubility; Sw: swelling water capacity; WRC: water retention capacity; FAC: fat absorption capacity; GAC: glucose retention capacity, DPPH and ABTS: antioxidant capacity, EFCC: cellulase-enhanced dietary fibre; EFCV: dietary fibre enhanced with Viscozyme; AUEFC: improved dietary fibre with ultrasound treatment and autoclave; SF150/45EFC: dietary fibre enhanced with supercritical fluids (150/45/2); SF300/55EFC: dietary fibre enhanced with supercritical fluids (300/55/2). [1]SD: standard deviation. [abcd] Values with different superscripts are significantly different ($p \leq 0.05$) between parts of the plant or treatments.

authors for broccoli by-products are significantly lower than those presented in this article. However, similar to the results obtained in this study, the results showed that the highest values for TDF and IDF coincided with the stem samples. In contrast, SDF results in previous studies were found to be higher in inflorescence samples (Li et al., 2021).

3.2. Effect of the different treatments on neutral sugar and pectins

Table 2 shows the results of the uronic acid profile and neutral sugars of the dietary fibre concentrates (free of reducing sugars, proteins and fats) after being treated with different treatments used to modify these compounds. Neutral sugar profile results revealed significant differences in the two study factors (plant parts and treatment). Regarding plant parts, stem samples showed higher values of glucose, xylose and fucose than leaf and inflorescence samples. However, they showed the lowest values of arabinose and rhamnose and average values of mannose and galactose. With respect to the different treatments applied, it can be observed that the enzymatic treatments (EFCC and EFCV) decreased the content of all the neutral sugars studied, but both treatments increased the content of uronic acids with respect to the control. In general terms, it can be observed that the treatments with supercritical fluids (SF150/45EFC and SF300/55EFC) and by autoclave and ultrasound (AUEFC) did not manage to modify the neutral sugar profile with respect to the control. However, the treatments with supercritical fluids (SF150/45EFC and SF300/55EFC) increased the uronic acid content. Supercritical fluids can be applied for biomass valorization as a pretreatment technique that aims to improve substrate accessibility by induced physical and/or chemical alteration of the lignocellulosic matrix. In general, increased temperatures and pressures improve reaction performance by enhancing solvent penetration through enlarged pores and fibre defects (Escobar et al., 2020). Previous work indicated that SFE conditions applied to pomegranate peel affected pectin content. It was found that temperatures of 45 °C and pressures of 300 bar combined with an extraction time of 2 h provided a higher percentage of galacturonic acid (Rivas, Casquete, de Guía Córdoba, et al., 2021).

Ma et al. (2022) modified potato dietary fibre with cellulase and xylanase enzymes and were able to increase uronic acid content after treatment and reduce glucose content, as shown in our results, and concluded that cellulase/xylanase treatment resulted in higher dietary fibre content. This modification improved the functional properties of dietary fibre from potato waste.

3.3. Functional properties

Table 3 shows the functional properties studies of dietary fibre from leaves, stems and inflorescences with the different treatments.

Table 3 presents the Ws results of dietary fibre concentrates from broccoli by-products. Regarding the part of the plant factor, the highest values of Ws corresponded to stems and inflorescences (29.75% and 29.29%, respectively), while the leaves obtained the lowest value (27.3%). Regarding the treatment applied, it was observed that the enzymatic treatments achieved an improvement in solubility with respect to the control, modifying the initial content of SDF of the three by-products previously presented in Table 1, with values of 56.2% with the enzyme cellulase and 53.7% with the viscoenzymatic multienzyme complex. It was also observed that the treatments with supercritical fluids improved the solubility of the fibre concentrates in both conditions studied (SF300/55EFC and SF150/45EFC) and achieved statistically higher solubility values than the control (25.3% and 28.2%, respectively). In contrast, the treatment applied by autoclaving and ultrasound proved to be ineffective for improving the Ws of the DF concentrates. The combined cellulase and xylanase enzymes on rice bran dietary fibre showed an effect on the enzymatic degradation of IDF which resulted in an increased solubility of the dietary fibre (Wen, Niu, Zhang, Zhao, & Xiong, 2017). In addition, other authors also verified that high temperatures and pressures were able to increase the solubility

Table 4
Growth percentage with respect to lactic acid bacteria control in the presence of fibre extracts.

	L. sakei			*E. faecium*			*L. plantarum*			*L. brevis*			*L. casei*		
	Mean		SD[1]	Mean		SD	Mean		SD	Mean		SD	Mean		SD
Parts of the plant (P)															
Leaves	38.86	±	21.53^{b}	31.61	±	14.60^{b}	39.55	±	24.19	23.48	±	9.88	50.67	±	27.00^{b}
Stems	38.70	±	20.12^{b}	33.38	±	12.59^{b}	23.44	±	10.42	22.36	±	25.28	20.37	±	13.44^{a}
Inflorescences	29.66	±	12.01^{a}	20.81	±	14.97^{a}	30.50	±	30.42	22.35	±	8.05	18.06	±	6.82^{a}
Treatments (T)															
EFCC	22.69	±	10.25^{a}	19.84	±	10.16^{ab}	36.47	±	26.30^{ab}	13.02		9.25^{a}	29.48	±	7.48
EFCV	56.82	±	16.85^{c}	41.75	±	19.77^{c}	49.12	±	29.69^{b}	29.58	±	15.60^{b}	33.18	±	25.46
AUEFC	17.12	±	9.58^{a}	14.88	±	7.71^{a}	10.83	±	2.97^{a}	16.04	±	13.59^{a}	16.68	±	19.68
SF150/45EFC	40.57	±	15.89^{b}	34.14	±	6.13^{bc}	27.59	±	8.33^{ab}	35.94	±	16.14^{b}	42.92	±	38.17
SF300/55EFC	35.46	±	1.76^{b}	32.38	±	9.32^{bc}	31.80	±	24.34^{ab}	18.85	±	14.84^{a}	26.24	±	7.34
Values P															
Pp	<0.001			0.012			0.177			0.837			0.001		
Pt	<0.001			0.010			0.033			<0.001			0.127		
*Pp*t*	<0.001			0.099			0.148			<0.001			0.356		

EFCC: cellulase-enhanced dietary fibre; EFCV: dietary fibre enhanced with Viscozyme; AUEFC: improved dietary fibre with ultrasound treatment and autoclave; SF150/45EFC: dietary fibre enhanced with supercritical fluids (150/45/2); SF300/55EFC: dietary fibre enhanced with supercritical fluids (300/55/2). [1]SD: standard deviation. [abc] Values with different superscripts are significantly different ($p \leq 0.05$) between parts of the plant or treatments.

of the compounds (Xu et al., 2016).

In the swelling study (Sw), the stem and inflorescence samples showed the highest mean values (10.48 and 11.66 mL/g, respectively), showing significant differences ($p \leq 0.05$) with respect to the leaf (8.50 mL/g). In terms of the different treatments studied, the highest swelling value was found in treatment SF150/45EFC, supercritical fluids, with significant differences to the rest of the treatments. The physical structure of the biomass changes when it is treated with supercritical fluids, since this technology uses solvents under high pressure conditions, changing the physical structure of the biomass by increasing the porosity of its fibers and its surface area (Escobar et al., 2020).The lowest values were for treatments EFCC (4.71 mL/g) and EFCV (5.42 mL/g) (enzymatic treatments). On the other hand, treatments AUEFC and SF300/55EFC obtained mean values (11.50 and 10.94 mg/L) with no significant differences with respect to the control. Similarly, the study of water retention capacity (WRC) showed higher values (11.50 and 10.67 g/g) in the stem and inflorescence samples, and treatment SF150/45EFC with supercritical fluids was the best performer with a mean value of 14.24 g/g. Regarding the FAC (lipid absorption capacity), no significant differences were observed between the different parts of the plant and the best treatment value was found in treatment SF150/45EFC (8.26 g/g), similar to that of Sw and WRC. Other authors have found that the enzymatic treatment conferred the highest water and lipid absorption capacity to the SDF obtained from coffee husk (Dong, Wang, Hu, Long, & Lv, 2020). It is true that higher values obtained in the hydration properties of dietary fibre (WRC and Sw) are associated with an increase in the solubility of the fibre, which leads to an increase in the contact area and lower particle sizes (Namir, Siliha, & Ramadan, 2015; Wang, Xu, Yuan, Fan, & Gao, 2015). However, it is also known that excessively small particle sizes can cause a decrease in the hydration properties of dietary fibre due to the denaturing of the bonds between polysaccharides in the dietary fibre matrix (Ma & Mu, 2016a, 2016b).

The glucose adsorption capacity GAC showed significant differences; the highest GAC value was shown by the stem samples (0.81 g/g), and all treatments improved this property, with the enzymatic treatments (EFCC and EFCV) showing the greatest improvement in glucose adsorption capacity (0.88 and 0.87 g/g). Huang et al. (2021a) in their study applied enzymatic treatments and high temperatures to a dietary fibre extract of tea leaves and their results showed a higher glucose adsorption capacity compared to untreated dietary fibre extracts. The improvement can be attributed to the change in the structure of the dietary fibre after treatment, which could suggest an increase in the specific surface area that would allow a greater capacity to absorb glucose (Zheng et al., 2019).

The functional properties of dietary fibre such as WRC and FAC, swelling capacity and prebiotic activity are associated with the physicochemical characteristics of cell wall polysaccharides (Rivas, Casquete, de Guía Córdoba, et al., 2021). Dietary fibre with high water-holding capacity can be used as a functional ingredient to modify the viscosity and texture of some formulated foods, while dietary fibre with high FAC allows fat stabilisation in emulsion-based products (Grigelmo-Miguel & Martín-Belloso, 1998). On the other hand, stomach swelling capacity and increased viscosity are associated with SDF, which slows nutrient absorption from the intestinal mucosa and reduces postprandial blood glucose and insulin responses (Mehta et al., 2015).

The analysis of non-extractable phenolic compounds associated with dietary fibre showed that inflorescences presented the highest values (341.23 mg/100 g of fibre concentrate), followed by leaf and stem samples (291.55 and 192.24 mg/100 g, respectively). In addition, the Viscozyme and supercritical fluids treatments improved the values obtained by the control (230.5 mg/100 g).

Finally, the antioxidant capacity of the fibre was determined by DPPH and ABTS methods. Regarding the plant part factor, the inflorescences showed the highest values and treatment with Viscozyme and supercritical fluids improved the values obtained with the DPPH method, although the results obtained with the ABTS method did not show this effect. Non-extractable phenolic compounds are strongly retained within the dietary fibre matrix which allows them to reach the colon where they are released by the bacterial microbiota showing significant biological activity (Dufour et al., 2018; Han et al., 2019; Huang et al., 2021b). By modifying insoluble dietary fibre from wheat bran through carboxymethylation, complex enzymatic hydrolysis and ultrafine grinding, Zhang et al. (2019) were able to improve its antioxidant capacity, with the ultrafine grinding method achieving the best results.

3.4. Growth of LAB and short-chain fatty acids production

Table 4 shows the growth percentages with respect to the control of the five lactic acid bacteria studied in the presence of dietary fibre extracts. In terms of bacterial growth, *L. sakei, E. faecium and* L. *casei* showed significant differences in growth rates in the presence of the different extracts. DF extracts from leaf and stem samples showed the highest growth values, while dietary fibre extracts from the inflorescences showed the lowest values. In contrast, no significant

Table 5
Production of short-chain fatty acids in mM of lactic acid bacteria (LAB) in the presence of the different soluble fibre extracts.

	Acetic acid			Propionic acid			Butyric acid			Isovaleric acid			Isobutyric acid			Isocaproic acid			Caproic acid			Valeric acid		
	Mean		SD[1]	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD	Mean		SD
Parts of the plant (P)																								
Leaves	177.83	±	150.76^{b}	1.49	±	1.94^{b}	0.08	±	0.10^{b}	0.44	±	0.04^{b}	0.14	±	0.17^{b}	0.66	±	0.94^{c}	0.10	±	0.20^{b}	0.29	±	0.53^{b}
Stems	134.24	±	109.65^{a}	0.20	±	0.16^{a}	0.02	±	0.02^{a}	0.25	±	0.16^{a}	0.05	±	0.03^{a}	0.02	±	0.14^{a}	0.03	±	0.04^{a}	0.09	±	0.12^{a}
Inflorescences	117.67	±	92.04^{a}	0.23	±	0.22^{a}	0.03	±	0.02^{a}	0.23	±	0.15^{a}	0.05	±	0.03^{a}	0.47	±	1.34^{b}	0.04	±	0.07^{a}	0.15	±	0.22^{a}
Treatments (T)																								
EFCC	258.19	±	81.69^{c}	1.84	±	2.07^{c}	0.11	±	0.09^{c}	0.58	±	0.43^{c}	0.21	±	0.16^{c}	1.39	±	1.68^{c}	0.16	±	0.19^{c}	0.42	±	0.59^{c}
EFCV	256.32	±	140.73^{c}	1.04	±	1.16^{b}	0.08	±	0.06^{b}	0.50	±	0.37^{b}	0.12	±	0.08^{b}	0.51	±	0.78^{b}	0.07	±	0.15^{b}	0.24	±	0.24^{b}
AUEFC	42.75	±	18.36^{a}	0.07	±	0.05^{a}	0.00	±	0.00^{a}	0.13	±	0.04^{a}	0.03	±	0.05^{a}	0.00	±	0.01^{a}	0.03	±	0.02^{a}	0.03	±	0.11^{a}
SF150/45EFC	73.22	±	34.38^{b}	0.09	±	0.03^{a}	0.01	±	0.11^{a}	0.15	±	0.04^{a}	0.02	±	0.01^{a}	0.00	±	0.02^{a}	0.01	±	0.01^{a}	0.08	±	0.21^{a}
SF300/55EFC	81.36	±	36.67^{b}	0.1	±	0.04^{a}	0.02	±	0.01^{a}	0.17	±	0.11^{a}	0.03	±	0.01^{a}	0.00	±	0.01^{a}	0.00	±	0.00^{a}	0.11	±	0.22a^{b}
Strains (S)																								
Lactobacillus sakei	156.04	±	136.05	0.63	±	1.26	0.04	±	0.08	0.46	±	0.54^{b}	0.11	±	0.16^{c}	0.27	±	0.58	0.02	±	0.03^{a}	0.36	±	0.69^{b}
Enterococcus faecium	156.73	±	156.99	0.77	±	1.55	0.05	±	0.08	0.32	±	0.29^{a}	0.09	±	0.14^{b}	0.25	±	0.56	0.10	±	0.19^{c}	0.16	±	0.23^{a}
Lactobacillus plantarum	160.68	±	117.02	0.66	±	1.32	0.05	±	0.07	0.29	±	0.28^{a}	0.08	±	0.10^{ab}	0.32	±	0.71	0.03	±	0.04^{a}	0.15	±	0.26^{a}
Lactobacillus brevis	145.14	±	126.60	0.63	±	1.26	0.05	±	0.07	0.26	±	0.26^{a}	0.07	±	0.08^{a}	0.41	±	0.89	0.06	±	0.11^{ab}	0.15	±	0.19^{a}
Lactobacillus casei	143.29	±	103.22	0.63	±	1.25	0.05	±	0.07	0.29	±	0.26^{a}	0.07	±	0.09^{a}	0.34	±	0.76	0.06	±	0.14^{ab}	0.15	±	0.20^{a}
P Values																								
Pp	<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001		
Pt	0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001		
Ps	0.061			0.193			0.792			<0.001			<0.001			0.058			<0.001			0.001		
*Pp*t*	<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001			<0.001		
*Pp*s*	0.983			0.357			0.996			0.050			<0.001			0.120			<0.001			0.150		
*Pt*s*	<0.001			0.572			0.999			<0.001			<0.001			<0.001			<0.001			0.174		
*Pp*t*s*	<0.001			0.454			0.995			0.001			0.003			<0.001			<0.001			0.031		

EFCC: cellulase-enhanced dietary fibre; EFCV: dietary fibre enhanced with Viscozyme; AUEFC: improved dietary fibre with ultrasound treatment and autoclave; SF150/45EFC: dietary fibre enhanced with supercritical fluids (150/45/2); SF300/55EFC: dietary fibre enhanced with supercritical fluids (300/55/2). [1]SD: standard deviation. [abc] Values with different superscripts are significantly different ($p \leq 0.05$) between parts of the plant, treatments or strains.

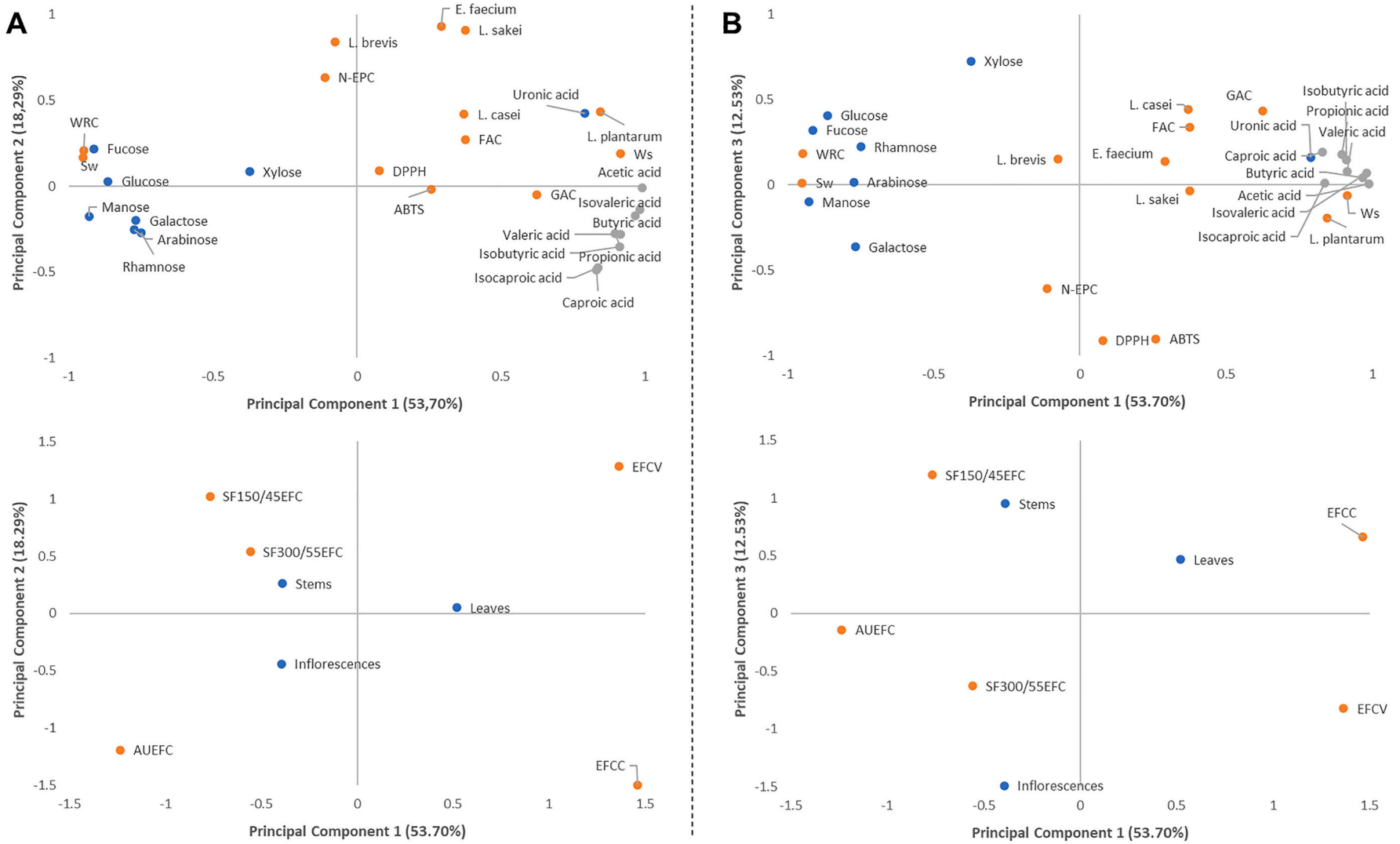

Fig. 1. Principal component analysis of the analytical results of the dietary fibre extracts studied. A: PC1 vs PC2; B: PC1 vs PC3. Loading plots: Ws: water solubility; Sw: swelling water capacity; WRC: water retention capacity; FAC: fat absorption capacity; GAC: glucose retention capacity, N-EPC: non-extractable phenolic compounds; DPPH and ABTS: antioxidant capacity; EFCC: cellulase-enhanced dietary fibre; EFCV: dietary fibre enhanced with Viscozyme; AUEFC: improved dietary fibre with ultrasound treatment and autoclave; SF150/45EFC: dietary fibre enhanced with supercritical fluids (150/45/2); SF300/55EFC: dietary fibre enhanced with supercritical fluids (300/55/2).

differences were observed in the growth of *L. plantarum* and *L. brevis* in the presence of the different extracts.

Regarding the treatment factor, all bacteria showed significant differences in their growth with the different treatments except L. *casei*. In general, it can be observed that the treatment that obtained the best results was treatment EFCV, Viscozyme, which achieved the highest values with respect to the other treatments in L. *sakei* (56.82%), *E. faecium* (41.75%) and L. *plantarum* (49.12%). Supercritical fluid 150/45/2, treatment SF150/45EFC, was found to be the second most effective treatment. Finally, none of the bacteria analysed exceeded 20% growth with respect to the control, and the worst results were obtained with treatment AUEFC, ultrasound treatment and autoclave. In addition, interactions between the different parts of the plant and the treatments studied were observed in the growth of L. *sakei* and *L. brevis*.

Rivas, Benito, Ruíz-Moyano, et al. (2021) evaluated the effect of dietary fibre obtained from pomegranate, tomato, grape and broccoli by-products on gastrointestinal transit survival, growth, and metabolism of LAB strains. The results showed that the by-products studied contained varying amounts of polysaccharides that affected the LAB microorganisms. Extracts with higher amounts of uronic acids favoured the growth of LAB in the study by Rivas, Casquete, Córdoba, et al. (2021), Rivas, Benito, Ruíz-Moyano, et al., 2021. Previously, Karam and Belarbi (1995) studied four strains of lactic acid bacteria that possessed pectinolytic activities, which allowed them to grow with polygalacturonic acid as their unique energy source by hydrolysing it and using galacturonic acid as a carbon source. Hence, the increase of these compounds with enzymatic treatment in our study therefore has a positive influence on improving the growth of the LAB.

Table 5 shows the production of SCFA from lactic acid bacteria grown in the presence of the different DF extracts studied. It can be noted that significant differences were found in the production of the different fatty acids in the factors studied. Regarding the plant part factor, it was observed that the leaf DF extracts showed the highest production of all the fatty acids analysed, showing statistically significant differences to the rest of the samples. As for the treatment, it was observed that the enzymatic treatments (EFCC and EFCV) showed significant differences to the rest of the treatments, and these also achieved the highest values in the production of all the SCFA analysed. Considering the different bacteria, *L. sakei* produced the highest values of isovaleric, valeric and isobutyric acid growing in the presence of the dietary fibre extracts. Furthermore, there was interaction between the different plant parts and the treatment applied. Ma et al., 2021 modified potato waste dietary fibre enzymatically with cellulase and xylanase in their study and their results showed significant effects on SCFA and gut microbiota compared to unmodified dietary fibre, mainly due to the increased soluble components and stronger physicochemical and functional characteristics of the enzymatically modified dietary fibre, such as water holding capacity, swelling capacity and glucose absorption capacity.

Fibre obtained from broccoli stems has been shown to produce an increased stimulation of SCFA production during fermentation with lactic accid bacteria, and furthermore an increased production of acetic acid was also observed in the presence of broccoli stem extract (Rivas, Benito, Ruíz-Moyano, et al., 2021. The increased amount and relative abundance of SCFA is considered a biomarker of a healthy state as they provide energy to colonocytes and resident bacteria, reduce gastrointestinal luminal pH to directly limit pathogen growth, enhance mineral absorption and promote bile acid excretion (Alexander, Swanson, Fahey Jr, & Garleb, 2019).

3.5. Multivariate analysis of the parameters related to DF extract studied from different subproducts

Principal component analysis (PCA) was carried out for the whole set of data to obtain an interpretable overview of the main information (Fig. 1). Principal components 1 (PC1), 2 (PC2) and 3 (PC3) explained a variability of 53.70%, 18.29% and 12.53%, respectively. High values for most short-chain fatty acids, *Lb. plantarum* growth and Ws were clearly explained by the positive axis of PC1 and were related to samples of leaves treated with enzymes (Fig. 1A). On the contrary, autoclave- and ultrasound-treated fibre (AUEFC) were associated with the negative axis of PC1 showing higher values for neutral sugars, WRC, and Sw. The second PC was mainly explained by the growth of most LAB located in the extreme of the positive axis, relating to high values of those in samples enhanced with Viscozyme (Fig. 1A). For the third PC, the values N-EPC were strongly associated with antioxidant activities (DPPH and ABTS), showing the highest levels in the inflorescence samples (Fig. 1B).

4. Conclusions

The results showed that there are significant differences in the composition and functional properties of the different parts of broccoli by-products and that supercritical fluid and enzyme treatments mainly affect their composition and properties. The value of non-extractable phenolic compounds was higher in the inflorescences and increased with supercritical fluid and enzyme treatments, as well as the antioxidant capacity. Treatment with enzymes improved especially the growth of LAB and the production of all the short-chain fatty acids analysed.

Therefore, the use of enzymatic treatments on DF obtained from broccoli by-products may improve the functional properties of broccoli fibre that could be applied to enhance human health benefits.

CRediT authorship contribution statement

María Ángeles Rivas: Methodology, Formal analysis, Investigation, Resources, Writing – original draft. **María J. Benito:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision. **Alberto Martín:** Methodology, Visualization. **María de Guía Córdoba:** Conceptualization, Funding acquisition. **Santiago Ruíz-Moyano:** Methodology, Formal analysis, Resources, Writing – review & editing. **Rocío Casquete:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

None.

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CAPÍTULO 4

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Functional properties of extracts and residual dietary fibre from pomegranate (*Punica granatum* L.) peel obtained with different supercritical fluid conditions

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ABSTRACT

The objective of this study was the optimization of supercritical fluid extraction (SFE) using CO_2 for extraction of bioactive compounds from pomegranate peel, evaluating the functional properties of different extracts (SFE extracts and alcohol-insoluble residues (AIR)). A Box–Behnken design combined with response surface methodology (RSM) was used to optimize extraction pressure (250–300 bar), temperature (45–55 °C) and time (2–4 h). The results showed that these SFE conditions have a significant effect on the responses studied. Antioxidant activities of the extracts, residual dietary fibre and pectin were the parameters that best fitted the quadratic model developed in this study. RSM optimization of these parameters was performed using Derringer's desired function methodology, estimating optimal conditions for extraction pressure (291 bar), temperature (46.5 °C) and time (2.5 h). The results reveal that supercritical technology is an interesting option for fractionating and recovering high-value compounds from pomegranate by-products.

1. Introduction

Pomegranate (*Punica granatum* L.) is widely grown in parts of Central Asia and North Africa (Holland, Hatib, & Bar-Ya'akov, 2009). It is also popularly cultivated in other subtropical and tropical regions such as Spain, Turkey and Egypt due to its high nutritional value and beneficial properties for health (Ercisli et al., 2011). However, the peel and internal membranes represent approximately 50% of the weight of this fruit, which generates a serious problem of by-products (Akhtar, Ismail, Fraternale, & Sestili, 2015). Therefore, the revaluation of bioactive compounds from the residues of agri-food industries has become a current topic in research since it brings added value to the main activity (Vardanega, Prado, & Meireles, 2015).

Pomegranate peel contains a high concentration of both extractable and non-extractable bioactive compounds such as phenolic compounds (Derakhshan et al., 2018), including flavonoids, anthocyanins and tannins, and terpenoids (Nile & Park, 2014; Pérez-Jiménez, Arranz, & Saura-Calixto, 2018; Ventura, 2012). Functional properties such as antioxidant activity (Singh, Singh, Kaur, & Singh, 2018), anti-cancer effect (Yusefi, Shameli, Ali, Pang, & Teow, 2020) and a beneficial effect in treating digestive tract disorders (Sun, Ma, Pan, Du, & Sun, 2019) have been associated with these phytochemicals. On the other hand, the functional properties of dietary fibre such as antioxidant activity, fat adsorption capacity (FAC), and prebiotic activity is associated to physicochemical characteristics of cell-wall polysaccharides. Furthermore, the hydration properties of fibre may affect the aspect and texture of food products (Colantuono, Vitaglione, Ferracane, Campanella, & Hamaker, 2017; Mantzourani et al., 2019; Wennberg & Nyman, 2004).

The conditions to which the by-product is subjected during extraction can cause important changes in its composition and structure and, therefore, in its functionality. Therefore, the recovery and purity of bioactive compounds depend on the extraction method. Conventional extraction methods such as Soxhlet and maceration using liquid solvents often present several disadvantages, e.g. thermal degradation of molecules due to the high temperature of extraction, or solvent residues in the extracts that can compromise their end use, as well as exerting a negative impact on the environment (Poojary et al., 2016). Supercritical fluid extraction (SFE) allows preservation of the natural qualities of

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bioactive compounds, guarantees food safety, reduces the environmental impact and minimizing energy costs at the same time (Cardoso, Serrano, Rodríguez, de la Ossa, & Lubián, 2012; da Silva, Santos, & Duarte, 2016). This technique lies in the recovery of relatively pure and clean extracts especially useful for functional food (Mushtaq, Sultana, Anwar, Adnan, & Rizvi, 2017). It is currently used for the recovery of phytochemicals from vegetable by-products such as citrus fruits (Ndayishimiye, Lim, & Chun, 2018), pomegranate peel (Mushtaq et al., 2017) and grape pomace (Oliveira et al., 2013). The impact of temperature and pressure applied in the supercritical CO_2 extractions on the recovery of bioactive compounds and antioxidant activity are well described in literature, being extremely important to optimize these extraction conditions for each sample (Ferrentino, Morozova, Mosibo, Ramezani, & Scampicchio, 2018; Pimentel-Moral et al., 2019). Pinto et al. (2020) demonstrated that high temperature (60 °C) and pressure (350 bar) provided a high antioxidant activity in *Castanea sativa* shells bioactive compounds. However, the impact of SFE conditions on extract characteristics of pomegranate peel has not been thoroughly studied and the information available on their effect on residual dietary fibre and its properties is limited.

Therefore, the aim of this study was to evaluate the functional properties of the extracts and residual dietary fibre from pomegranate peel obtained with different supercritical fluid conditions for the optimization of the SFE process.

2. Materials and methods

2.1. Plant material

The pomegranate (*Punica granatum* L.) samples used in this work were obtained from a local supermarket in the region of Extremadura, Spain. The fruits were peeled manually, and the peel dried in a forced-air oven at 45 °C for 48 h before being ground and screened to 1 mm particle size. The by-product was vacuum-packed in bags and stored at −20 °C until use.

2.2. Functional extracts

2.2.1. Extraction

Phytochemicals of pomegranate peel were extracted by SFE using CO_2. The assays were performed in a dynamic extraction mode unit in a Spe-ed SFE system (HELIX Applied Separations). Forty grams of sample was used, forming a fixed bed inside a 100 mL stainless steel column. The solvent flow rate was fixed at 2 L/min. A Box–Behnken experimental design (BBD) with three factors and two blocks was applied for modelling the influence of the variables pressure, temperature and extraction time on the performance of phenolic extraction (Table 1). The ranges of the parameters were defined after preliminary tests according to the conditions used by Mushtaq et al. (2017). Pressure ranges were determined taking into account that pressures lower than 150–200 bar can lead to low extraction rates, whereas over 300 bar compression costs can become uneconomical. Temperature levels were selected above the critical temperature of CO_2 (31.1 °C), and the upper limit was 60 °C to prevent the occurrence of thermal degradation in the extract (de Aguiar et al., 2013). The extracts were collected in 20 mL glass tubes and stored at −80 °C until analysis. The compositional analysis of Supercritical fluid extraction-Central points (SFE-CP) extracts was performed by HPLC-ES-QTOF (Supplemental material).

Table 1
Experimental runs of the Box–Behnken design (BBD) per block.

Pressure	Temperature	Time
Factorial points		
275	45	4
300	55	3
300	45	3
275	45	2
250	50	2
300	50	4
250	45	3
250	55	3
300	50	2
275	55	2
275	55	4
250	50	4
Supercritical fluid extraction-Central points (SFE-CP)		
275	50	3
275	50	3
275	50	3

For functional comparison, phenolic compounds were extracted by a conventional extraction method (CEM) as described by Casquete et al. (2015). Briefly, the dry samples (10 g) were mixed with 60 mL of ethanol/water (80: 20, v/v) and then placed on a magnetic mixer for 1 h in the absence of light at room temperature (25 °C) and filtered. This process was repeated twice. Excess ethanol was removed by heating at 37 °C in a rotary evaporator under vacuum. The resultant aqueous extracts were combined and lyophilized (Telstar, LyoBeta).

Once the phytochemicals were extracted by SFE, the pomegranate peel residue was vacuum-packed in bags and stored at ambient temperature until use for dietary fibre extraction.

2.2.2. Total phenolic content (TPC)

The TPC of the extracts, (10 mg extract/mL ethanol) was determined using Folin–Ciocalteu reagent (Wettasinghe & Shahidi, 1999) in a UV-1800 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA). Gallic acid was used as standard. Results are expressed as mg of gallic acid equivalents (GAE)/100 g extract. All experiments were conducted in triplicates.

2.2.3. Antioxidant activity

The antioxidant activity of the extracts (10 mg extract/mL ethanol) was determined by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical "DPPH•" according to the method of Teixeira, Canelas, Martins do Canto, Teixeira, and Dias (2009). The total antioxidant activity was expressed as mg of Trolox/100 g extract. All experiments were conducted in triplicates.

2.3. Dietary fibre residue

2.3.1. Extraction

The dietary fibre was extracted by the method of alcohol-insoluble residues (AIR) from pomegranate peel residues obtained after extraction of phenolic compounds. The AIR content was determined using the protocol described by Femenia, García-Pascual, Simal, and Rosselló (2003) with slight modifications.

Briefly, 5 g of each sample was homogenized with ethanol 85% (v/v) and boiled for 10 min, then filtered in vacuo with cellulose-free filters (Whatman, glass microfiber filters 934-AH™). This process was repeated with absolute ethanol. The solid residue was washed with acetone and excess solvent was removed after 24 h at a temperature of 23 ± 1 °C. Results were expressed in g/100 g peel residues. All experiments were conducted in triplicates.

2.3.2. Functional properties of alcohol insoluble residue

Different functional properties were determined as described below.

2.3.2.1. Swelling (Sw), water retention capacity (WRC) and lipid adsorption capacity (FAC). The determinations were carried out following the methodology described by Garau, Simal, Rosselló, and Femenia (2007). For Sw, 0.1 g of AIR was mix with 10 mL of distilled water. After 24 h, the volume acquired by the fibre was measured in a test tube. The results were expressed in mL water/g AIR. In the case of WRC, 0.2 g of AIR was

mix with 10 mL of distilled water. The samples were allowed to stand for 24 h and were centrifuged at 2000 g for 25 min. The solid residue obtained was weighed. The results were expressed in g water/g AIR. For FAC, the process was as in the previous case, replacing the 10 mL of water added with 5 mL of sunflower oil. The results were expressed in g oil/g AIR. All experiments were conducted in triplicate.

2.3.2.2. Antioxidant activity (non-extractable polyphenols). The determination of the antioxidant capacity of non-extractable polyphenols bound to AIR was achieved according to the method described by Arranz, Saura-Calixto, Shaha, and Kroon (2009) with some modifications. A total of 0.5 g was mixed with 20 mL of a methanol/water solution (50: 50) acidified with hydrochloric acid to pH 2. The mixture was incubated under stirring for 1 h and subsequently centrifuged for 20 min at 2500 g. This process was repeated with an acetone/water solution (70: 30). Excess solution was removed by heating at 37 °C in a rotary evaporator under vacuum. The resultant residue was resuspended in 30 mL of distilled water and the antioxidant capacity was determined by DPPH method. Results were expressed as mg Trolox/100 g AIR. All experiments were conducted in triplicate.

2.3.3. Pectin extraction

The pectin was extracted from the AIR and determined by spectrophotometric evaluation by the method of Blumenkrantz and Asboe-Hansen (1973). Firstly, acidic hydrolysis of AIR was carried out with 12 mol/L sulphuric acid (3 h at room temperature and 100 °C for 1 h); 0.5 mL of the hydrolysed sample was mixed with a solution of 50 mmol/L sodium borate in concentrated sulphuric acid, allowed to react for 10 min at 100 °C. The mixture was put into in ice water for 2 min. A volume of 100 μL of a solution of 0.15% *m*-phenylphenol in 0.5% NaOH was added into the tubes and vortexed. Samples were left for 30 min to react. Finally, the samples were measured at 524 nm in a UV-1800 spectrophotometer. Galacturonic acid was used as standard; the results were expressed as mg galacturonic acid/g AIR. All experiments were conducted in triplicate.

2.4. Statistical analysis

As shown in Table 1, a three-factor, three-level, two-block BBD with 15 experimental runs per block (12 at factorial points and 3 at the centre) combined with response surface methodology (RSM) was applied to determine the effects of SFE conditions on the characteristics of both extract and residual dietary fibre from pomegranate peel. RSM was performed employing StatGraphics Centurion XVI Version 8.0 software. The quadratic model was as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \varepsilon$$

where Y is the response variable predicted by the model; β_0 is an offset value; β_1, β_2 and β_3 are the regression coefficients for the main (linear) terms; β_{11}, β_{22} and β_{33} are quadratic effects; β_{12}, β_{13} and β_{23} are interaction effects; X_1, X_2 and X_3 are the independent variables; and ε is the experimental error. For each experimental factor, the software generated an analysis of variance (ANOVA), establishing the statistical significance at the 95% confidence level. Response surface plots, the optimal level for each variable analysed, and optimization of multiple responses by Derringer's desirability function were also obtained with the same statistical program.

In addition, one-way ANOVA for comparison of CEM and SFE central point (SFE-CP) samples of the BBD and principal component analysis (PCA) on the correlation matrix of the variables were performed using SPSS for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Effect of SFE conditions on TPC of extracts

Table 2 presents the descriptive statistics for each determination in the samples treated under the SFE conditions established in the BBD, and the differences found between CEM and SFE-CP samples.

SFE-CP samples showed higher values ($p < 0.05$) than CEM samples, reaching 4362 and 2554 mg GAE/100 g, respectively. In the SFE conditions included in the BBD, TPC values ranged from 2497 to 6326 mg GAE/100 g extract suggesting that, in optimal conditions, SFE extract present more concentration of phenolic compounds than CEM extract. The relative efficiency of SFE with respect to CEM is in agreement with other studies (Fabiani et al., 2014; Mushtaq et al., 2017). However, taking account the yields (1–1.5% for SFE and 26.9% for CEM) the absolute efficiency extraction of phenolic compounds was clearly higher for CEM.

Table 3 shows the results of ANOVA applied to assess the effects of the SFE parameters studied and the statistical significance of the model for each determination achieved in both SFE extracts and SFE residual dietary fibre extracts. It can be seen that the variables with the most significant effect on the TPC were the quadratic term of temperature and interactions between temperature and time. The TPC in pomegranate extract increased with an increase in pressure up to 270–280 bar, whereas at pressures above 280 bar the TPC decreased (Fig. 1A). The increase in TPC may be related to the density of the supercritical solvent (CO_2), which increases with pressure at constant temperature (Oliveira et al., 2013). On the other hand, at the lowest temperature and longest time, the TPC values of pomegranate peel extract increased, whereas they declined with a rise in temperature (Fig. 1A), most possibly due to the reduced density of carbon dioxide (Zahedi & Azarpour, 2011). A longer extraction time can contribute to additional disruption of cell walls and better penetration of solvent into cells (Kazemi, Karim, Mirhosseini, & Hamid, 2016).

The values of R^2 and adjusted R^2 statistics obtained for the model represented a good approximation for TPC (Table 3). The optimal SFE conditions for TPC extraction were a pressure of 270 bar, temperature of 45 °C and extraction time of 4 h, with an estimated value of 5407 mg GAE/100 g (Table 3). Mushtaq et al. (2017) obtained higher concentrations of phenolic compounds for pomegranate SFE extract. The divergence between the data from these authors and the present work was probably caused by the enzymatic pre-treatment. Enzymatic pre-treatment improves solvent distribution, reduces particle size and improves the mass transfer rate, leading to improved polyphenol extraction (da Silva, Rocha-Santos, & Duarte, 2016; Soylu, 2006).

3.2. Effect of SFE conditions on antioxidant activity of extracts

The SFE-CP extracts showed similar values for DPPH assay ($p > 0.05$) to the CEM extracts in terms of relative efficiency (Table 2), even though in the case of SFE-CP extract this activity is associated to both phenolic compounds as Trimethoxy flavone (Nevadensin), Lariciresinol and Methoxy flavone (Tricin) and terpenoids as Ursolic acid and Corosolic acid (Table 1S). Similar observations have been reported by other authors who noted that in vitro antioxidant capacity obtained from pomace residue remained after SFE-CO_2 (Bobinaitė et al., 2020). Some authors have reported that supercritical CO_2 and thermal treatments do not affect the antioxidant activity measured by the DPPH assay in prebiotic-enriched apple juice (Silva, Arruda, Eberlin, Pastore, & Meireles, 2019). However, SFE conditions had a great impact on the antioxidant activity of extracts, given that the values for DPPH assay ranged from 1959 to 842 mg Trolox/100 g for the extracts obtained under experimental runs established in the BBD (Table 1). In fact, ANOVA showed that the significant effects ($p < 0.01$) influencing the antioxidant capacity of extracts were all the quadratic terms of the studied parameters, the linear term of temperature and interactions between pressure

Table 2
Comparison of conventional extraction method (CEM) and supercritical fluid extraction-central points (SFE-CP) samples and descriptive statistics of runs included in Box–Behnken design (BBD) for all parameters studied.

	One-way ANOVA					BBD runs (descriptive statistics)			
	CEM		SFE-CP		p^*	Mean	SD	Max	Min
	Mean	SD[a]	Mean	SD					
Phenolic extracts									
TPC (mg GAE/100 g extract)	2554 ± 63		4362 ± 414		*0.002*	3590 ± 908		6326	2497
DPPH_E (mg Trolox/100 g extract)	1612 ± 376		1932 ± 39		*0.354*	1441 ± 271		1959	842
Dietary fibre from residues									
AIR (g/100 g peel)	45.46 ± 0.75		40.38 ± 1.07		*0.001*	40.90 ± 2.87		47.88	38.14
Sw (mL water/g AIR)	12.67 ± 0.47		7.85 ± 2.14		*0.024*	10.20 ± 3.61		19.37	5.03
WRC (g water/g AIR)	6.56 ± 0.33		12.04 ± 1.79		*0.006*	10.38 ± 2.11		14.50	6.13
FAC (g oil/g AIR)	7.76 ± 0.75		5.73 ± 0.59		*0.007*	6.19 ± 1.46		11.31	4.59
DPPH_R (mg Trolox/100 g AIR)	6160 ± 129		6775 ± 125		*0.040*	5315 ± 1326		7773	3393
Pectin (mg galacturonic/g AIR)	64.79 ± 9.07		30.38 ± 3.72		*0.001*	36.17 ± 8.37		66.00	24.05

TPC: total phenolic content; DPPH_E: antioxidant activity of extracts; AIR: alcohol-insoluble residue; Sw: swelling; WRC: water retention capacity; FAC: fat adsorption capacity; DPPH_R: antioxidant activity of dietary fibre from residues.
* p-Values lower than 0.05 are statistically significant.
[a] Values are represented as a mean ± standard deviation (n = 3).

Table 3
ANOVA results for response surface quadratic model and optimal supercritical fluid extraction (SFE) conditions of each determination studied.

Source	SFE extract		SFE residual dietary fibre					
	TPC	DPPH	AIR	Sw	WRC	FAC	DPPH	Pectin
ANOVA (p-values)								
A: Pressure	0.055	0.545	**0.007**	0.049	0.439	0.983	**0.001**	**0.000**
B: Temperature	0.264	**0.002**	**0.001**	0.301	**0.038**	0.201	**0.001**	0.845
C: Time	0.786	0.697	**0.005**	**0.040**	0.731	0.893	0.492	0.498
AA	**0.001***	**0.002**	0.546	0.482	0.116	0.749	**0.017**	0.126
AB	0.874	**0.001**	**0.001**	0.985	0.449	**0.000**	0.312	**0.001**
AC	0.502	0.367	0.931	0.666	0.446	0.829	0.155	0.941
BB	0.190	**0.001**	**0.001**	0.064	0.937	**0.039**	**0.001**	**0.003**
BC	**0.001**	**0.043**	**0.001**	0.389	0.744	0.816	**0.010**	0.487
CC	0.436	**0.001**	**0.001**	0.343	**0.012**	0.420	**0.030**	0.614
ANOVA (R-squared statistics)								
R^2	0.74	0.85	0.94	0.46	0.49	0.59	0.86	0.74
R^2 (adjusted by g.l.)	0.61	0.77	0.91	0.18	0.22	0.37	0.79	0.60
Optimal SFE conditions								
Pressure (bar)	270	267	300	–	–	–	250	300
Temperature (°C)	45	48	45	–	–	–	54	45
Time (h)	4	2.9	2.2	–	–	–	3.8	2
Estimated value	5407	1979	49.37				7579	56.70

TPC: total phenolic content (mg GAE/100 g extract); DPPH_E: antioxidant activity of extract (mg Trolox/100 g extract); AIR: alcohol-insoluble residue (g/100 g peel); Sw: swelling; WRC: water retention capacity; FAC: fat adsorption capacity; DPPH_R: antioxidant activity of dietary fibre from residues (mg GAE/100 g AIR); pectin (mg galacturonic/g AIR).
* p-Values lower than 0.05 are statistically significant (bold font).

and temperature, with an adjusted R^2 of 0.77 (Table 3). Fig. 1B shows the clear effect of SFE conditions (pressure, temperature and time) on DPPH_E. The optimum SFE conditions for obtaining extracts with a maximum antioxidant activity estimated as 1979 mg Trolox/100 g were pressure of 267 bar, temperature of 48 °C and extraction time of 2.3 h (Table 3), differing from the optimum SFE conditions for TPC mainly in a shorter extraction time. Hasnaoui, Wathelet, and Jiménez-Araujo (2014) found that the TPC is mainly responsible for the antioxidant activity of pomegranate peel from 12 cultivars. However, a longer extraction time can contribute to additional extraction of others antioxidant compounds such as terpenoids (Ursolic acid and Corosolic acid) (Table 1S). Other authors have reported that the total amount of TPC is not directly related to this antioxidant activity in prebiotic-enriched apple juice (Silva et al., 2019).

3.3. Effect of SFE conditions on AIR

The residue from SFE extraction is a good source of dietary fibre, containing more than the initial material (Bobinaitė et al., 2020). In our study, the AIR presented differences between CEM and SFE-CP, showing concentrations of 45.46 and 40.38 g AIR/100 g peel, respectively. However, for samples extracted by optimized SFE, a maximum value of 47.88 g AIR/100 g peel was reached (Table 2). The results obtained in the study are in concordance with those obtained by other authors for dietary fibre content in pomegranate peel (Hasnaoui et al., 2014). Likewise, the impact of SFE conditions on AIR is in agreement with previously reported data for SFE-CO_2 of palm kernel (Nik Norulaini, Moftah, Sawsan, Ahmad, & Mohd, 2011). The low p-values found in our study for ANOVA of most of the effects indicate that the regression model is highly significant and that the quadratic model represents a good approximation for this investigated response (Table 3). This is confirmed by the high coefficients of multiple determinations (R^2 0.94, adjusted R^2 0.91). Fig. 2A shows the effect of the different extraction parameters (temperature, pressure and time) and their interactions on the AIR content in pomegranate peel. At low temperatures and short extraction times, the AIR content approached a maximum value estimated as 49.37 g AIR/100 g peel at high pressure, reaching the optimal condition at a temperature of 45 °C, pressure of 300 bar and time of 2.2 h (Table 3). Limited information is available on the influence of the SFE parameters studied on the residual dietary fibre of vegetable

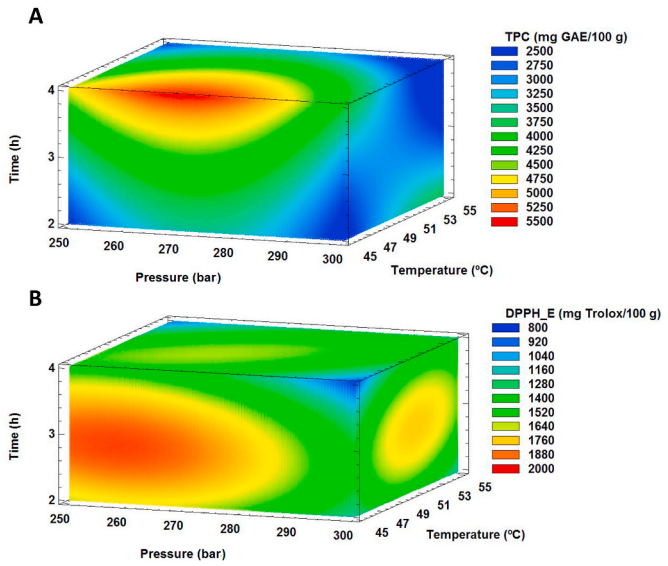

Fig. 1. Response surface mesh for total phenolic compounds (TPC) (A) and antioxidant activity of extracts (DPPH_E) (B) with respect to supercritical fluid extraction conditions.

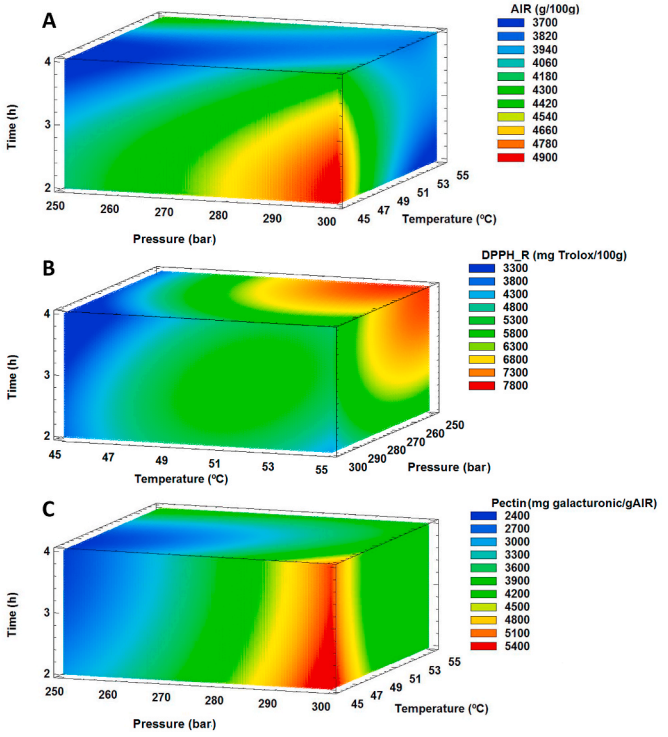

Fig. 2. Response surface mesh for alcohol-insoluble residues (AIR) (A), antioxidant activity of residue (DPPH_R) (B) and pectin (C) with respect to supercritical fluid extraction conditions.

by-products. Hincapié, Omaña, Hincapié, Arias, and Vélez (2010) observed that the drying temperature of citric by-products affects the AIR content in an inversely proportional way, the highest values of AIR in the citric by-product extract being obtained at a temperature of 45 °C, decreasing as the temperature increases. Other authors have observed a positive effect of high pressure on the extraction of both total dietary fibre and its soluble fraction (Xie et al., 2017; Yan, Liu, & Zheng, 2019).

3.4. Effect of SFE conditions on functional properties of AIR

The values of the functional properties of AIR (Sw, WRC, FAC and antioxidant activity) from pomegranate peel are shown in Table 2. The Sw and FAC values of the CEM samples were significantly higher than those found for SFE-CP samples, whereas the CEM samples showed lower values for WRC and DPPH determinations (Table 2). These values are similar to those published by other authors for pomegranate by-products (Viuda-Martos et al., 2012). However, according to the maximum values found in experimental runs of the BBD (Table 1), the efficiency of SFE under optimal conditions was clearly superior to that of CEM for all functional properties. The linear and quadratic effects of the variables, as well as the low value for the coefficient R^2 indicate that the influence of SFE conditions on Sw, WRC and FAC is not well fitted to the quadratic model designed (Table 3). However, pressure and time showed a significant linear effect ($p < 0.05$) on Sw, the dietary fibre obtained after extraction at the shortest time and highest pressure values being that with the highest values for Sw (Fig. 3A). This could be due to the high degradation suffered by the fibre under these conditions, which causes a smaller particle size and, therefore, a greater Sw (Román, Flórez, Gutiérrez, Martínez, & Medina, 2004). Putrino, Tedesco, Bodini, & de Oliveira (2020) indicated that the pre-treatment with supercritical CO_2 cause changes in the structure of the fibre, increasing the porosity, affecting the non-extractable phenolic compound contents and loosening the hydrogen bonds causing delignification. For the WRC of the residual dietary fibre, the SFE conditions that showed significant effects were temperature (linear effect) and time (quadratic effect) (Table 3). Maximum WRC values coincided with lower extraction temperatures (Fig. 3B), possibly due to higher temperature degrading the fibre structure and causing a lower WRC (Hincapié et al., 2010). FAC was affected by temperature (quadratic effect) and the interaction between

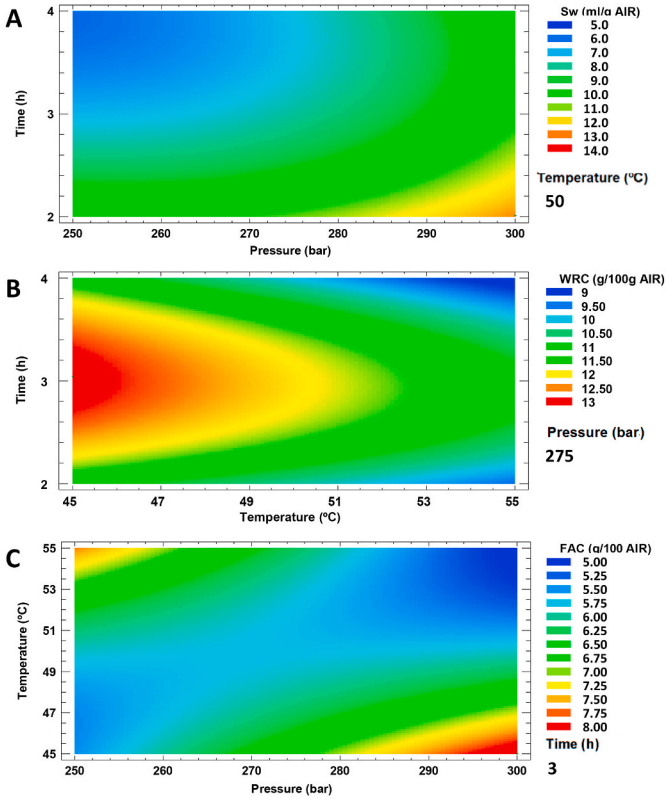

Fig. 3. Contour plots for swelling capacity (Sw) with respect to extraction pressure and time (A); water retention capacity (WRC) with respect to temperature and extraction time (B); fat adsorption capacity (FAC) with respect to extraction pressure and temperature (C).

temperature and pressure (Table 3), showing higher values for residual fibre extracted at the highest pressure and lowest temperature (Fig. 3C). For antioxidant activity, the quadratic model represented a good approximation, with R^2 and adjusted R^2 coefficients of 0.86 and 0.79, respectively (Table 3). Based on Fig. 2B, it can be observed that values for DPPH assay were higher in SFE residues obtained at the lowest pressures, highest temperatures and longest times, the estimated optimal value being 7579 mg Trolox/100 g AIR at temperature of 55 °C, pressure of 250 bar and time of 3.8 h (Table 3). The compounds responsible for the insoluble antioxidant activity could be phenolic compounds structurally linked to fibre (Hasnaoui et al., 2014) which are commonly ignored.

3.5. Effect of extraction parameters on pectin

The results for residual pectin, expressed in mg galacturonic acid/g AIR, showed significant differences between CEM and SFE-CP, with values of 64.79 and 30.38 mg/g AIR, respectively. However, according to the maximum values reached in the runs of the BBD (66 mg/g AIR), a similar amount of galacturonic acid was retained in determinate SFE conditions (Table 2).

It can be seen that the most significant effect on the residual pectin in the extracts treated by SFE was the linear term of pressure, the quadratic effect of temperature and the interaction of pressure and temperature. The quadratic model showed a good approximation for residual pectin with an adjusted R^2 statistic of 0.74 (Table 3). The lowest temperature (45 °C) and highest pressure (300 bar) combined with the shortest extraction time (2 h) were found to provide a higher percentage of galacturonic acid (Table 2; Fig. 2C). Limited information is available on the influence of SFE conditions on pectin. However, the effect of temperature, pressure and time in other extraction methods has been studied. Regarding the temperature and extraction time, other authors have observed high levels of residual pectin with short extractions at 45 °C. For grapefruit peel, the extraction yield using distilled water and microwave-assisted extraction was reduced at high temperature during long extraction times due to degradation of the pectin extracted (Bagherian, Zokaee Ashtiani, Fouladitajar, & Mohtashamy, 2011). Other authors have demonstrated that the microwave-assisted extraction of pectin is favoured by high pressures in combination with short times (Fishman, Chau, Hoagland, & Hotchkiss, 2006; Fishman, Coffin, Konstance, & Onwulata, 2000).

3.6. Multivariate analysis of parameters related to SFE extracts and residual dietary fibre under different SFE conditions

The optimization of the parameters that best fitted the quadratic model proposed in this study, the two fractions (extract and AIR), AIR and pectin content (Table 3), was performed using Derringer's desirability function methodology (Fig. 4). Estimated optimal SFE conditions (extraction pressure of 291 bar, temperature of 46.5 °C and time of 2.5 h) were determined, with a maximum DPPH_E of 1673 mg Trolox/100 g, DPPH_R of 4965 mg Trolox/100 g, AIR of 45.11 g/100 g, and pectin of 43.44 mg of galacturonic acid/g AIR, and an overall desirability value for this solution of 0.542.

4. Conclusions

The results of this study indicate that the SFE conditions applied to pomegranate peel affect the concentration and functional properties of extracts and residual dietary fibre. The introduction of Derringer's desirability function to the optimization of supercritical fluid conditions in the extraction, resulted in the selection of acceptable experimental conditions for an optimal SFE extracts and residual dietary fibre. Supercritical technology may be an interesting option for revaluating vegetable by-products such as pomegranate peel, and for optimizing the functional properties of extracts and residues obtained.

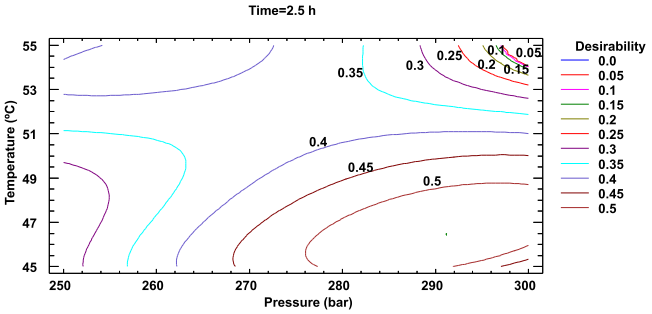

Fig. 4. Contours of estimated response surface of multiple response optimizations for maximum values of antioxidant activity of extracts (DPPH_E), antioxidant activity of residue (DPPH_R), alcohol-insoluble residues (AIR) and pectin.

CRediT authorship contribution statement

María Ángeles Rivas: Formal analysis, Investigation, Resources, Writing – review & editing. **Rocío Casquete:** Conceptualization, Methodology, Data curation, Data management, Writing – original draft, preparation, Writing – review & editing. **María de Guía Córdoba:** Conceptualization, Supervision, Funding acquisition. **María José Benito:** Writing – original draft, preparation, Visualization. **Alejandro Hernández:** Resources. **Santiago Ruiz-Moyano:** Methodology. **Alberto Martín:** Conceptualization, Methodology, Data curation, Data management, Writing – original draft, preparation, Supervision.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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CAPÍTULO 5

Broccoli leaf dietary fibre modification by supercritical technology for improved functional properties.

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Abstract

The response surface methodology was used to optimise the parameters of pressure, temperature and time for dietary fibre modification from broccoli leaves using supercritical technology. A model to predict the chemical composition and functional properties of the modified dietary fibre from broccoli leaves under specific supercritical treatment conditions was developed. The results showed that the treatment of the dietary fibre matrix with appropriate temperatures and pressures, 55 °C, 150 bar, 1 hour during the supercritical process causes the molecular chain breakdown of the polysaccharides and reduction of the molecular weight. In terms of composition, the dietary fibre analysed was composed almost entirely of galacturonic acid and the highest purification values were achieved at 55 °C, 300 bar during 1 hour, and the highest content of neutral sugars was found in glucose and fucose. In both cases, the results showed an increase of these compounds after supercritical fluids treatment (SFT) compared to the control. Regarding the effect of SFT conditions on the properties of dietary fibre, the results indicated that the application of temperature and pressure by supercritical fluid improved the water retention capacity, swelling and glucose absorption capacity properties of dietary fibre although excessive temperature and pressure damaged the internal structure, reducing these properties. As for the effect on the antioxidant activity property of dietary fibre, the application by supercritical fluid of temperature and pressure using prolonged times, resulted in the improvement of this property.

The use of the response surface methodology for the optimisation of the supercritical fluid conditions in the modification allowed the selection of acceptable experimental conditions for obtaining dietary fibre with optimal composition and properties, so that these conditions can be an interesting option to revalorise this vegetable by-product.

Keywords: Response surface methodology (RSM); broccoli leaves; dietary fibre; functional properties; supercritical fluid treatment (SFT)

1. Introduction

Response surface methodology (RSM) is a statistical and mathematical tool to explore and understand the complex interactions between multiple variables and their effects on a specific response.[1,2] RSM has been effective in the development, improvement and optimization of complex processes, as recent studies support.[3,4] This methodology highlights by its economic efficiency, as it reduces significantly the amount of experiments needed while providing a large amount of data and information.[5] Additionally, RSM allows analyzing the simultaneous influence of multiple factors and anticipating the response of the system to new conditions, which facilitates determining the optimal conditions to obtain the desired response, as previous research has indicated. Imtiaz et al.[6] reported that RSM was essential for optimizing the conditions of two extraction methods of bioactive compounds, microwave-assisted extraction and ultrasound-assisted extraction, designed to extract bioactive compounds from the leaves of *Thuja orientalis* tree. These models not only provided optimal conditions for the extraction of bioactive compounds and their functional activity, such as total phenolic content and antioxidant activity, but also allowed a deep understanding of the complex interactions between the factors involved.

Supercritical technology, particularly the use of supercritical carbon dioxide (CO_2) as a solvent, has emerged as a promising tool to purify bioactive compounds.[7] RSM promises to be useful in this context, allowing to adjust critical parameters such as pressure, temperature and time to optimize the extraction and purification of specific compounds as demonstrated in different works.[8-10] Until now, no research employing this methodology to set the conditions of supercritical equipment for modification of dietary fibre had been found. However, there are studies that have applied RSM to optimize dietary fibre extraction conditions by other methods. Behrouzian et al.[11] used RSM to optimize the extraction of dietary fibre from coffee bean skin using alkaline hydrogen peroxide. In this work, the different factors such as temperature, hydrogen peroxide concentration, pH and time were evaluated. The results demonstrated the effectiveness of this methodology to optimize the conditions for the extraction of dietary fibre.

Dietary fibre is an essential component of a healthy diet and plays a critical role in the prevention of several chronic diseases such as obesity, type 2 diabetes and cardiovascular

diseases.[12-17] In addition, several studies have suggested that dietary fibre may also play an important role in cancer prevention.[18] Ren et al.[19] characterized soluble dietary fibre from millet and reported its antitumor effect on colon cancer. In their study, they found that glucan inhibited cancer cell colony development and induced apoptosis. Therefore, its inclusion in the daily diet is recommended due to its great health benefits.

Broccoli (*Brassica oleracea* L. var. Italica) is a widely consumed food worldwide,[20] with increasing economic importance[21,22] due to the health benefits because of its rich chemical composition.[23-25] The edible part of broccoli has been the main focus of research; however, it only represents approximately 10-15% of the plant weight.[26] Therefore, it is necessary to investigate broccoli by-products to find new alternatives and uses. Broccoli by-products include non-commercial leaves, stems, and inflorescences, and are widely recognized for their high content of dietary fibre and bioactive compounds.[27] Consequently, they are valuable candidates for use as functional ingredients with health benefits.[28-30] In particular, broccoli plant leaves are an important source of vitamins, polyphenols, glucosinolates and dietary fibre.[31-36]

The bioactive activities of dietary fibre are closely related to its composition and solubility.[37] It has been reported in several studies that soluble dietary fibre shows superior bioactive properties compared to insoluble dietary fibre.[38] However, in plant by-products, the presence of insoluble dietary fibre is predominant,[39] which requires purification processes to improve its properties and release its potential. In recent research, several strategies have been explored to improve the functional properties of insoluble dietary fibre and adapt it to potential industrial applications in food. Rivas et al.[36] demonstrated that enzymatic modification of dietary fibre increased the soluble dietary fibre content in broccoli by-products, thus improving their oil-holding capacity, solubility, and glucose absorption capacity; on the contrary, water-holding capacity and swelling capacity were reduced. Xie et al.[40] in their study reported significant improvements in the antioxidant activity of dietary fibre from purple-fleshed potatoes when these were treated with high pressures. Purification of bioactive compounds yields a more concentrated and uniform product since it removes undesirable components, such as impurities and contaminants.[41] In addition to these advantages, purified dietary fibre is usually more accessible and its functional properties can be more clearly focused.[42] Thus, purification of

dietary fibre offers an innovative approach that increases its potential applications both in the food industry and in promoting human health.

Therefore, the aim of this research was to modify the dietary fibre obtained from broccoli leaves through supercritical technology. In this process, the pressure, temperature and time parameters were optimized using the response surface methodology. Furthermore, a model that allows the prediction of the chemical composition and functional properties of the modified dietary fibre from broccoli leaves under specific treatment conditions was developed.

2. Materials and Methods

2.1. Plant material

The broccoli by-products used in this work were derived from cultivar Parthenon in the region of Extremadura, Spain. The broccoli leaves by-products were used. The samples were dried in a forced air oven at 45°C for 24-48 h, ground with a mincer, packed in vacuum bags and stored at room temperature until use. All the experiments were performed in triplicate.

2.2. Total dietary fibre extraction

Total dietary fibre from broccoli leaves was extracted by the alcohol insoluble residue (AIR) method, a suitable method for plant matrices with low starch content. Cell wall polysaccharides were extracted using the protocol described by Femenia et al.[43] and modified by Rivas et al.'[44] Briefly, the dried samples were homogenized with 85 % (v /v) ethanol and brought to boiling for 10 min, then filtered under vacuum through non-cellulose filters (Whatman glass microfiber filters, 934-AH™). This process was repeated twice more, the last time with absolute ethanol. The solid residue was washed with acetone and the excess solvent was removed after 24 h at a temperature of 23 ± 1 °C. This process was considered as a control.

2.3. Dietary fibre treatment with supercritical fluids.

Treatment of dietary fibre extracted from broccoli leaves was carried out by supercritical fluid (SF) technology using CO_2. The process was carried out in a static unit in a Speed SF System 96 (HELIX applied separations). Twenty g of dietary fibre was used, within a 100 mL stainless steel column. The solvent flow rate was set at 2 l/min. A Box-Behnken experimental

design (BBD) with three factors and two blocks was applied to model the influence of the variables pressure, temperature and time on the performance of dietary fibre modification (Table 1). The parameter ranges were defined after preliminary tests according to the conditions in previous studies by Rivas et al.[36] After the process the modified dietary fibre was stored under vacuum until analysis.

2.4. Characterization of dietary fibre

The characterisation of dietary fibre was performed using the soluble part of the fibre. For this purpose, the samples were solubilised by mixing with water in a 1:10 ratio. The mixture was stirred for 30 minutes in a bath at a controlled temperature of 90°C. Subsequently, the supernatant was precipitated by the addition of absolute ethanol at 60°C. After centrifugation, the supernatant was removed and the soluble solid residue was dried at 45°C.

2.4.1. Neutral sugars and galacturonic acid profile

The soluble residue was hydrolysed using 12 M sulphuric acid. This process was carried out for 3 hours at room temperature, followed by an additional step at 100°C for 1 hour.

The neutral sugars and galacturonic acid profile of the soluble dietary fibre was determined by high performance liquid chromatography (HPLC) after hydrolysed using 12 M sulphuric acid. This hydrolysis was carried out for 3 hours at room temperature, followed by an additional step at 100°C for 1 hour.

HPLC analyses were performed using an Agilent LC 1260 Infinity II system (Waters, Milford, MA, USA) consisting of a separation module and a refractive index detector. A Rezex-ROA column (7.8 mm ID x 150 mm; Phenomenex, Torrance, CA, USA) was used in an isocratic mode, with water as the mobile phase at a flow rate of 0.6 ml/min. During elution, 10 µL of sample was injected, the column was maintained at a temperature of 80 °C, and the detector temperature was set at 40 °C.

2.4.2. Oligosaccharides

Soluble dietary fibre oligosaccharides in broccoli leaves were determined by HPLC. HPLC analyses were performed using an Agilent LC 1260 Infinity II system (Waters, Milford, MA, USA) consisting of a separation module and a refractive index detector. A Rezex-RNO column (10 mm inner diameter x 200 mm; Phenomenex, Torrance, CA, USA) was used in an

isocratic mode, with water as the mobile phase at a flow rate of 0.6 ml/min. During elution, 10 µL of sample was injected, the column was maintained at a temperature of 75 °C, and the detector temperature was set at 40 °C.

2.5. Functional properties of dietary fibre

2.5.1. Water solubility (WS)

The water solubility of the samples was determined following the method described by Wan et al.[45] with slight modifications. Sample was weighed 0.5 g and mixed with 10 mL of distilled water. Then it was incubated at 90 °C for 30 min in a thermostatically controlled water bath, followed by centrifugation at 2000 g for 15 min. The supernatant was collected, dried and weighed. WS was expressed as a percentage and was performed in triplicate.

2.5.2. Swelling (Sw), water holding capacity (WRC) and fat absorption capacity (FAC)

Sw, WRC and FAC of broccoli leaf dietary fibre were determined following the methodology described by Garau et al.[46] Sw results were expressed in ml water/g dietary fibre, WRC were expressed in g water/g dietary fibre and FAC results were expressed in g oil/g dietary fibre.

2.5.3. Glucose absorption capacity (GAC).

The GAC of dietary fibre was analyzed according to the method described by Niu et al.[47] with some modifications. Briefly, 0.1 g of sample was mixed with 25 ml of a 50 mM glucose solution. This was incubated at 37 °C for 6 h with constant shaking and then centrifuged at 4500 g for 10 min. The glucose content of the supernatant was analyzed using HPLC equipment. GAC was expressed as mg glucose retained/mg dietary fibre.

2.5.4. Antioxidant activity (non-extractable polyphenol N-EPC)

N-EPCs bound to dietary fibre from broccoli leaves were extracted according to the method described by Arranz et al.[48] and determined using the Folin-Ciocalteu reagent.[49] The antioxidant capacity of the samples was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrogen oxide depletion) method and the ability to scavenge the ABTS radical (2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid).[50,51]

2.6. Statistical analysis

As shown in Table 1, a two-block, three-level, three-factor Box-Behnken desing (BBD) with 15 experimental runs per block (12 at factor points and 3 in the middle) combined with response surface methodology (RSM) was applied to determine the effects of SFT conditions on the composition and functional properties of dietary fibre modified from broccoli leaf. RSM was performed using StatGraphics Centurion XVI Version 8.0 software. The quadratic model was as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \varepsilon$$

where Y is the response variable predicted by the model; β_0 is an offset value; β_1, β_2 and β_3 are the regression coefficients for the main (linear) terms; β_{11}, β_{22} and β_{33} are quadratic effects; β_{12}, β_{13} and β_{23} are interaction effects; X_1, X_2 and X_3 are the independent variables; and ε is the experimental error. For each experimental factor, the software generated an analysis of variance (ANOVA), establishing statistical significance at the 95% confidence level. The same statistical program was also used to obtain response surface graphs, the optimum level for each variable analyzed and the optimization of multiple responses using Derringer's desirability function.

In addition, one-way ANOVA was performed for the comparison of control and supercritical fluid treatment central point (SFT-CP).

3. Results and Discussion

3.1. Effect of SFT conditions on the composition and structure of broccoli leaf dietary fibre.

3.1.1. Dietary fibre structure

According to Naveed et al.[52] oligomers consisting of 2 to 20 monosaccharide units are known as oligosaccharides. The detected peak has a degree of polymerisation (DP) between 7 and 12, suggesting that the analysed samples can be classified as oligosaccharides. Table 2 shows the results of the structural analysis of broccoli leaf dietary fibre. The modified samples and the control sample showed the same oligosaccharide or group of oligosaccharides but at different concentrations. The control sample showed the lowest value (592.94 g/g) in the DP concentration (7-12), compared to the SFT-CP samples (899.49 mg/g) and even the lowest value (769.35 mg/g) when the SFT conditions included in the BBD were applied (Table 2).

Table 3 shows the results of ANOVA for quadratic response surface model and optimal supercritical fluid conditions for each parameter of treated dietary fibre. The effects of SFT conditions on DP concentration (7-12) were adjusted to the designed quadratic model with an R^2 value of 0.70 (Table 3). Significant linear effects were observed with temperature, pressure and time, as well as quadratic effect with temperature. In addition, a significant interaction between pressure and temperature was identified. Figure 1 shows that at lower pressures and shorter times, the DP values increased, but decreased with lower temperatures, being the optimal conditions for maximized DP concentration (7-12) at 55 °C, 150 bar and 1 hour treatment. As shown in table 3, this optimal condition achieved values near to 100 % DP (7-12), so it could be affirmed that the SFT with the appropriate conditions was able to modify the dietary fibre efficiently and produce a dietary fibre concentrate composed only by oligosaccharides. The results suggest that the treatment of the dietary fibre matrix with appropriate temperatures and pressures during the supercritical process causes the breakdown of polysaccharides molecular chain and the reduction of the molecular weight.[53,54]

Dietary fibre modification by supercritical fluids to obtain oligosaccharides is a topic for research. However, there are some studies investigating the use of supercritical fluids in the extraction of polysaccharides. Gong et al.[55] used supercritical fluid extraction (SFE-CO_2) with ethanol as co-solvent to obtain a polysaccharide from Ginkgo leaves, the results showed that the polysaccharides obtained had a lower molecular weight than the polysaccharides derived from Ginkgo leaves obtained by hot water extraction. In a more recent study, Manthei et al.[56] investigated the use of apple bagasse and orange peel by subjecting them to high-pressure homogenisation, enzymatic hydrolysis and a combination of both to evaluate the production of oligosaccharides. The results showed that pretreatment of the substrates with high-pressure homogenisation enhanced the release of pectic oligosaccharides in orange peel, reaching a content of 44.51 g/100 g peel.

3.1.2. Dietary fibre composition

Table 2 shows that the results of galacturonic acid showed differences between the control and SFT-CP, showing concentrations of 98.54 and 98.51 %, respectively. However, analysis of the experimental runs using the response surface design (BBD) shows a maximum

value of 98.91(p/p%). Due to the high content of galacturonic acid, it can be stated that the pectin concentration is high. The typical structure of pectin is composed of a backbone consisting of galacturonic acid linked by α-D-1,4-glycosidic bonds, with the backbone connected to side chains containing neutral sugars through various glycosidic bonds.[57,58] This result seems to indicate that the dietary fibre analysed is composed almost entirely of homogalacturonan domain as a very high percentage of its composition is galacturonic acid, and to a lesser extent of ramnogalacturonan domains I and II which have complex side chains containing neutral monosaccharides such as Ara, Gal and others.[59] Furthermore, galacturonic acid content adjusted adequately to the designed quadratic model with a high R^2 coefficient value of 0.91 (Table 3). As the pressure and temperature increased, higher values of galacturonic acid were purified obtaining the maximum estimated values (98.97%) at 300 bar pressure, 55 ◦C temperature and 1 h extraction time (Figure 2A and Table 3).

The results of the neutral sugars as constituents of broccoli leaf dietary fibre are also presented in Table 2. Glucose and fucose were the major neutral sugars, both showing lower values in the control samples (0.73 and 0.56 (w/w%), respectively) compared to the SFT-CP samples (0.86 and 0.79 (w/w%), respectively) ($p<0.05$). On the other hand, xylose had a significantly lower value in the SFT-CP samples compared to the control sample. Finally, arabinose was not found in either the control or the SFT-CP samples. However, it should be noted that the maximum values of all monosaccharides were higher when the SFT conditions included in the BBD were applied (Table 2). The concentration of all monosaccharides adjusted the designed quadratic model with R^2 values higher than 0.8 in all cases except for xylose (Table 3). It is also worth mentioning that all monosaccharides presented maximum conditions in terms of pressure, although all of them coincided in similar temperatures between 44º and 40 ºC and treatment times between 2 and 3 hours. Figures 2B and 2D shows that glucose and fucose values decreased at higher temperatures and in the case of arabinose (Figure 2C) the values increased at the extreme points of pressure during long times and lower temperatures.

Neutral sugars, together with galacturonic acid, show an increase after SFE treatment compared to the control (Table 2). This increase could be attributed to a higher pectin release as a consequence of cell wall degradation accompanied by the breaking of glycosidic bonds, as

observed by other researchers when treating and modifying dietary fibre fractions by different methods.[60,61]

Although there are no specific studies on the dietary fibre purification using supercritical fluids, several investigations have explored the effects of temperature and pressure on dietary fibre pectin as well as galacturonic acid content. In addition, some studies support the use of supercritical CO_2 to improve dietary fibre pectin. In a previous study, the impact of temperature, pressure and extraction time on pectin from pomegranate peel dietary fibre treated with supercritical fluids was evaluated. Specific conditions, such as a temperature of 45 °C, a pressure of 300 bar and an extraction time of 2 hours, were found to maximise the galacturonic acid content.[44] The optimal time value is higher than the one achieved in this work (Table 3), however, the plant material is different and in this work the supercritical treatment was applied directly on the dietary fibre fraction which would leave more exposed the pectin present and the rest of its components. Pedraza-Guevara et al.[62] focused their research on the development of environmentally friendly and sustainable processes using compressed fluids to obtain high quality pectin from green papaya flour. Their research highlighted that the introduction of CO_2 into the matrix caused the breaking of hydrogen bonds, increasing the porosity of the cell membrane and facilitating the recovery of pectin, without affecting the monosaccharides and promoting a high content of galacturonic acid. In another study, Xie et al.[63] explored the effects of high-pressure processing on waste potato peel pectins, finding an increase in galacturonic acid content and a decrease in the degree of esterification. This work underlines the ability of high pressure to modify pectin characteristics. Benvenutti et al.[64] focused on the recovery of pectin from jaboticaba by-product by subcritical water extraction modified by deep eutectic solvent. They identified that the maximum pectin yield was reached at 122 °C, higher temperatures caused a decrease in yield, possibly due to damage to the pectin structure.

3.2. Effect of SFT conditions on the functional properties of broccoli leaf dietary fibre

Table 4 presents the descriptive statistics of the functional properties of the treatment dietary fibre under the SFT conditions set out in the BBD, and the differences found between the control and SFT-CP samples. In general, SFT-CP samples showed significantly higher values ($p<0.05$) than the control samples for all functional properties except FAC.

312 *3.2.1. Effect of SFT conditions on the Ws of dietary fibre*

313 The Ws values ranged from 17.98 to 9.02 % when the SFT conditions included in the
314 BBD were applied (Table 4). Significant effects ($p<0.05$) were observed for pressure (quadratic),
315 temperature (linear) and the interaction of pressure and time on Ws (Table 5).

316 With regard to dietary fibre modification, the results obtained indicate a linear increase
317 in fibre solubility with increasing temperature, as revealed by several studies. Ullah et al.[65]
318 examined the properties of insoluble dietary fibre from okara and showed that the solubility of
319 the insoluble dietary fibre increased steadily with increasing pretreatment temperature. On the
320 other hand, the influence of pressure on solubility is more complex and less predictable, as
321 evidenced in the study by Ouyang et al.[66] on grapefruit dietary fibre. Ultra-high pressures above
322 500 MPa resulted in total degradation of dietary fibre, while pressures between 100 to 400 MPa
323 increased solubility by converting insoluble fractions into soluble ones. In addition, previous
324 studies, such as the one by Xie et al.[40] indicate that high pressure can convert insoluble
325 cellulose and hemicellulose fractions into soluble ones, thus improving the solubility of dietary
326 fibre.

327 *3.2.2. Effect of SFE conditions on the functional properties of dietary fibre*

328 Sw, WRC and FAC values of dietary fibre treated with SF from broccoli leaves are
329 shown in Table 4. The maximum values found in the BBD experimental runs (Table 4) show
330 that the efficiency of SFT under optimal conditions was clearly higher than that of the control
331 samples for all technological properties. Sw and WRC did fit the model with an R^2 coefficient
332 value of 0.55 in both cases. For both properties, the SFE conditions that showed significant
333 effects were temperature (linear and quadratic effect) and the interaction between temperature
334 and time (Table 5). The maximum values of Sw and WRC coincided with low temperatures
335 (40.0 ºC) and pressures (150 bar) and shorter purification times (1 hour) (Figure 3). In the case
336 of FAC, the variability of the parameter was mainly associated to the interaction between
337 pressure and temperature factors (Table 5).

338 The results suggested that the application of temperature and pressure by supercritical
339 fluid improved these properties of the dietary fibre. However, the results also showed that too
340 high temperatures and pressures degrade the dietary fibre resulting in lower Sw and WRC
341 (Figure 3). The results obtained from different researches support our findings and reveal the

relevance of temperature and pressure in the modification of dietary fibre to improve its properties. Wang et al.[67] showed that modifications on dietary fibre from lychee pomace by techniques such as high-pressure homogenisation and high hydrostatic pressure created more porous structures, improving its WRC, Sw and FAC.[67] Ouyang et al.[66] also confirmed that Ultra High Pressure treatment significantly increased WRC and FAC of grapefruit dietary fibre, although excessive pressure damaged the internal structure, reducing these properties. On the other hand, Dong et al.[68] used a combination of ultrasound and high-temperature cooking to improve these properties of bamboo dietary fibre, increasing its WRC and FAC due to smaller particle size and looser structure. Similarly, Su et al.[69] applied a lactic acid-assisted subcritical water treatment to dietary fibre from beer bagasse, where high temperature (180 °C) during the process resulted in a more porous structure and smaller particles, significantly improving the WRC and FAC of dietary fibre. These studies underline the positive influence of temperature and pressure conditions on dietary fibre properties, showing how appropriate modification can lead to significant improvements in its functionality, which is crucial for its application in food products.

3.2.3. Effect of SFT conditions on GAC of dietary fibre

Table 4 presents the descriptive statistics of GAC under the SFT conditions set out in the BBD, and the differences found between control and SFT-CP samples. The SFE-CP samples showed higher values (0.92 g/g) than the control samples when GAC was measured ($p<0.05$). GAC values ranged from 1.25 to 0.55 g/g when applying the SFT conditions included in the BBD showing lower values in the control than SFT-CP samples (Table 4). This parameter adjusted adequately to the designed quadratic model with an R^2 coefficient value of 0.67 (Table 5). In addition, Figure 4 shows that the GAC values increase at pressures between 150 and 240 at short times and low temperatures, estimating the maximum values (1.22 g/g) at 191 bar pressure, 40ºC temperature and 1 hour (Table 5).

The GAC values obtained for the dietary fibre of the control sample are similar to those published in a previous work, although they are higher than those published by other authors.[44,70] As for the modification and improvement after treatment with supercritical fluid, the effects of time and pressure are evident. The differences can be attributed to the change in the

structure of the dietary fibre after temperature and pressure condition impact on the matrix, which could suggest an increase in the specific surface area that would allow a higher glucose absorption capacity.[71]

3.2.4. Effect of SFT conditions on FNE content and antioxidant capacity of dietary fibre

Table 4 presents the descriptive statistics of the phenol non-extractable (FNE) content and antioxidant activity of the treated dietary fibre under the SFT conditions established in the BBD, and the differences found between the control samples and the supercritical centre point conditions (SFT-CP). The SFT-CP samples showed higher values ($p<0.05$) with respect to the control in both FNE content and antioxidant activity. In the BBD experimental runs (Table 4), it was observed that the maximum values of FNE (509.19 mg GAE/100g) and antioxidant activity measured by the DPPH (176.33 mg Trolox/100g) and ABTS (545. 15 mg Trolox/100g) indicated a clear increase with SFT compared to control samples (251.25 mg GAE/100g, 84.57 mg Trolox/100g and 399.40 mg Trolox/100g, respectively). Both FNE content and antioxidant activity by the DPPH method showed high R^2 coefficients of 0.83 and 0.78, respectively.

Figure 5 shows that under the conditions of low pressure (150 bar), low temperature (40 °C) and extended times (3 h), the FNE content and antioxidant activity (DPPH) approached a maximum estimated value of 541.62 mg GAE/100g and 188.63 mg Trolox/100g of dietary fibre, respectively (Table 5). These results highlight the positive influence of these factors on FNE content and antioxidant activity and justify their use to purify and improve dietary fibre from broccoli leaves.

In previous research, various improvement strategies, including supercritical fluid optimization, were employed to study the effect of temperature, pressure and time parameters on dietary fibre in broccoli by-products.[36] Two specific supercritical conditions, 300 bar at 55 °C and 150 bar at 45 °C, both for 2 hours, were explored to assess their impact on the functional properties of dietary fibre, including FNE content and antioxidant activity. The 300 bar and 55 °C condition was able to improve the levels of FNE content and antioxidant activity, as measured by the DPPH method, compared to the control group. It is important to mention that the pressure and temperature conditions in these previous studies exceed the optimum conditions obtained in the present work. However, these differences could be attributed to the treatment

time. In another previous study, pomegranate peel after supercritical extraction of phenolic compounds was investigated, and it was determined that 3.8 hours at a temperature of 55 °C and a pressure of 250 bar were needed to reach the maximum antioxidant activity in the dietary fibre extracted from the residue resulting from supercritical extraction.[44] These findings underline the relevance of extraction time, as well as temperature and pressure, in improving the functional properties of dietary fibre in plant by-products.

3.3. Multivariate analysis of parameters related to SFT extracts and residual dietary fibre under different SFT conditions

Based on the goodness of fit models, the functional properties swelling (Sw), water retention capacity (WRC), glucose absorption capacity (GAC), total non-extractable phenolic compounds (FNE) and antioxidant activity of extract (DPPH) were used for overall optimisation of the SFT conditions (Table 5). Figure 6 shows the Derringer desirability function methodology for estimating optimal SFT conditions (191 bar pressure, 40°C temperature and 1 h time), with maximum values of Sw, WRC, GAC, FNE and DPPH (11.53 mL/g, 12.53 g/g, 1.22, 467.60 mg GAE/100 g and 134.55 mg Trolox/100 g, respectively) and a global desirability value for this solution of 0.805.

4. Conclusions

The results of this study indicate that the SFT conditions applied to broccoli leaves affect the composition and functional properties of the obtained dietary fibre. The use of the response surface methodology for the optimisation of the supercritical fluid conditions resulted in the selection of acceptable experimental conditions to obtain dietary fibre with optimal composition and properties. Supercritical technology can be an interesting option to revalorise this vegetable by-product in order to optimise their properties.

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Figure 1. Response surface mesh for oligosaccharide degree of polymerization between 7-12 (DP (7-12)) with respect to SFT conditions.

Figure 2. Response surface mesh for galacturonic acid (A), glucose (B), arabinose (C) and fucose (D) with respect to SFT conditions.

Figure 3. Contour plots for swelling capacity (Sw) with respect to temperature and pressure (A); water retention capacity (WRC) with respect to temperature and pressure (B).

Figure 4. Response surface mesh for glucose absorption capacity (GAC) with respect to SFT conditions.

Figure 5. Response surface mesh for total non-extractable phenolic compounds (FNE) (A) and antioxidant activity of extract (DPPH) (B) with respect to SFT conditions.

Figure 6. Contours of estimated response surface of multiple response optimizations for maximum values of swelling (Sw), water retention capacity (WRC), glucose absorption capacity (GAC), total non-extractable phenolic compounds (FNE) and antioxidant activity of extract (DPPH).

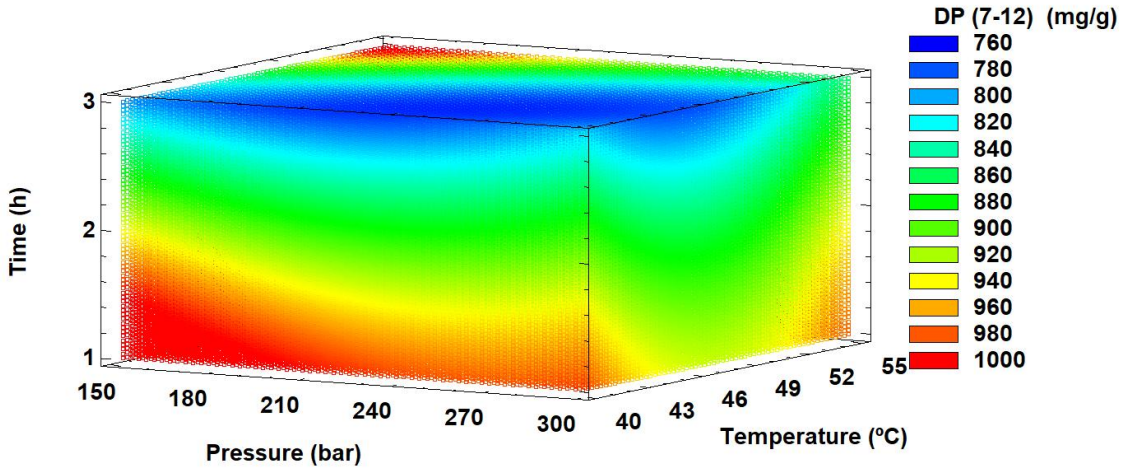
DP (7-12) (mg/g)
760
780
800
820
840
860
880
900
920
940
960
980
1000
Time (h)
3
2
1
150
180
210
240
270
300
Pressure (bar)
40
43
46
49
52
55
Temperature (ºC)

Fig. 1

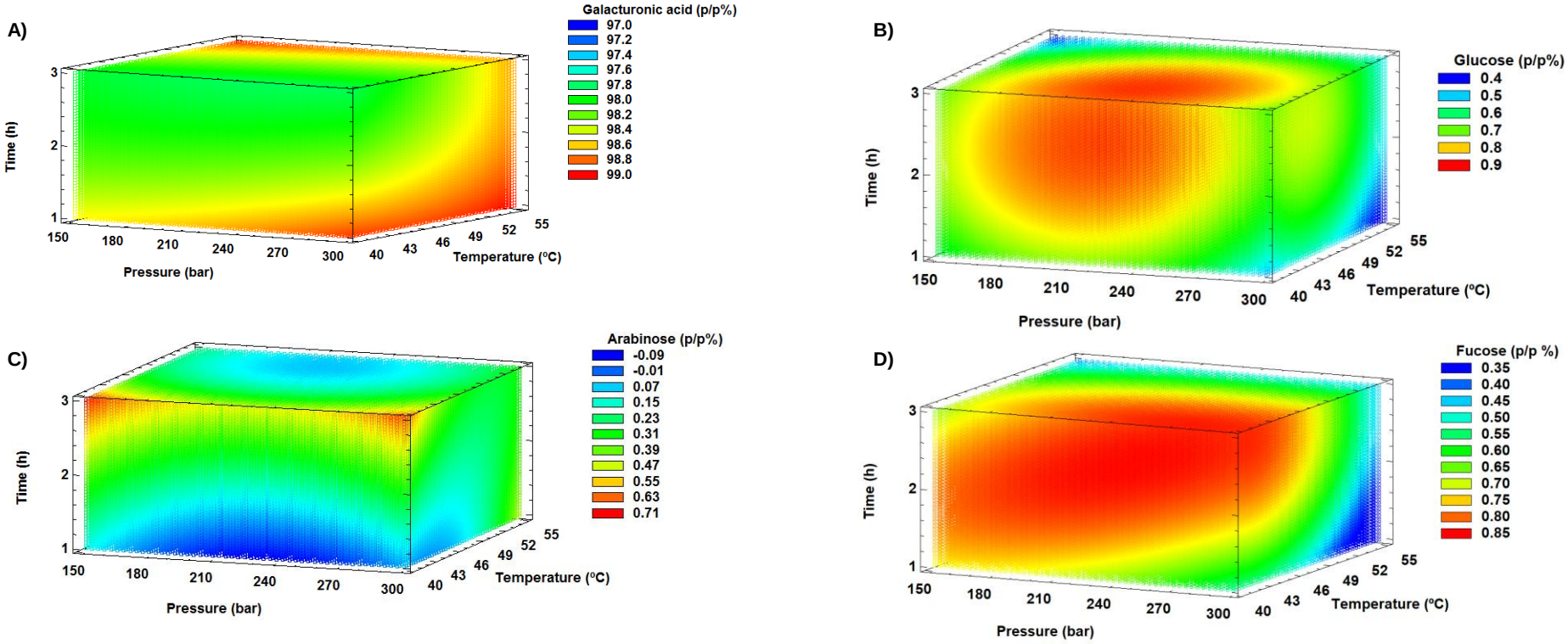
A)
Galacturonic acid (p/p%)
97.0
97.2
97.4
97.6
97.8
98.0
98.2
98.4
98.6
98.8
99.0
Time (h)
Pressure (bar)
Temperature (°C)
B)
Glucose (p/p%)
0.4
0.5
0.6
0.7
0.8
0.9
C)
Arabinose (p/p%)
-0.09
-0.01
0.07
0.15
0.23
0.31
0.39
0.47
0.55
0.63
0.71
D)
Fucose (p/p %)
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85

Fig 2.

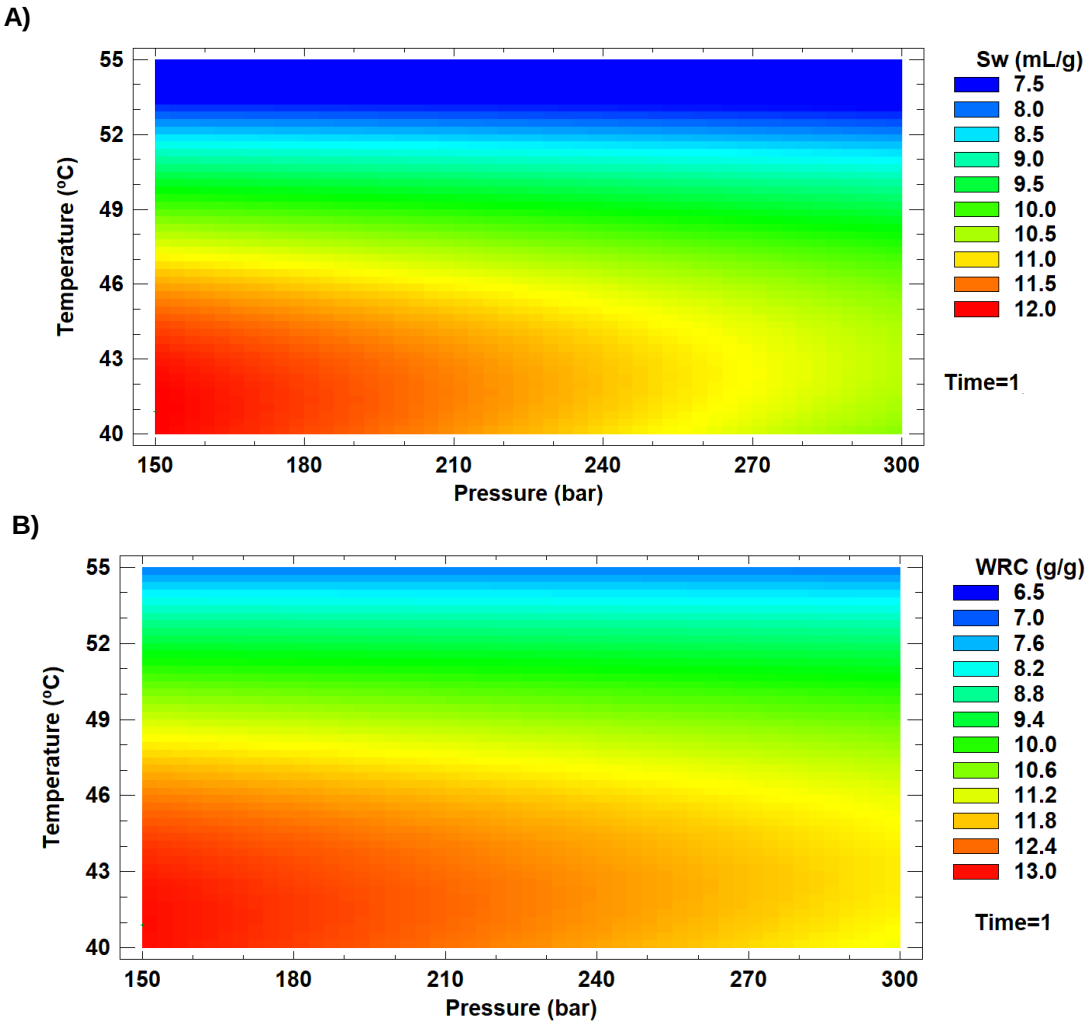
A)
Temperature (°C)
55
52
49
46
43
40
150
180
210
240
270
300
Pressure (bar)
Sw (mL/g)
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
11.5
12.0
Time=1
B)
Temperature (°C)
55
52
49
46
43
40
150
180
210
240
270
300
Pressure (bar)
WRC (g/g)
6.5
7.0
7.6
8.2
8.8
9.4
10.0
10.6
11.2
11.8
12.4
13.0
Time=1

Fig. 3

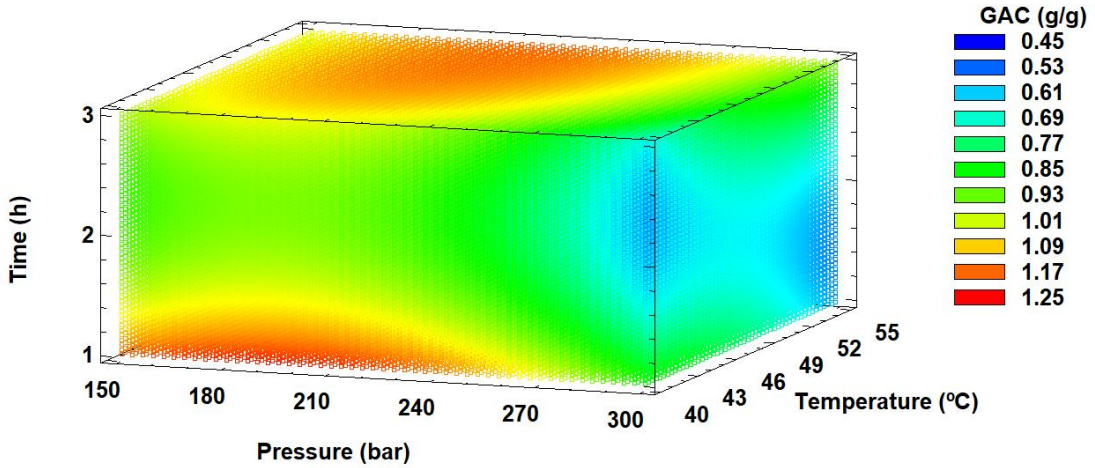
GAC (g/g)
0.45
0.53
0.61
0.69
0.77
0.85
0.93
1.01
1.09
1.17
1.25
Time (h)
3
2
1
150
180
210
240
270
300
Pressure (bar)
40
43
46
49
52
55
Temperature (°C)

Fig. 4

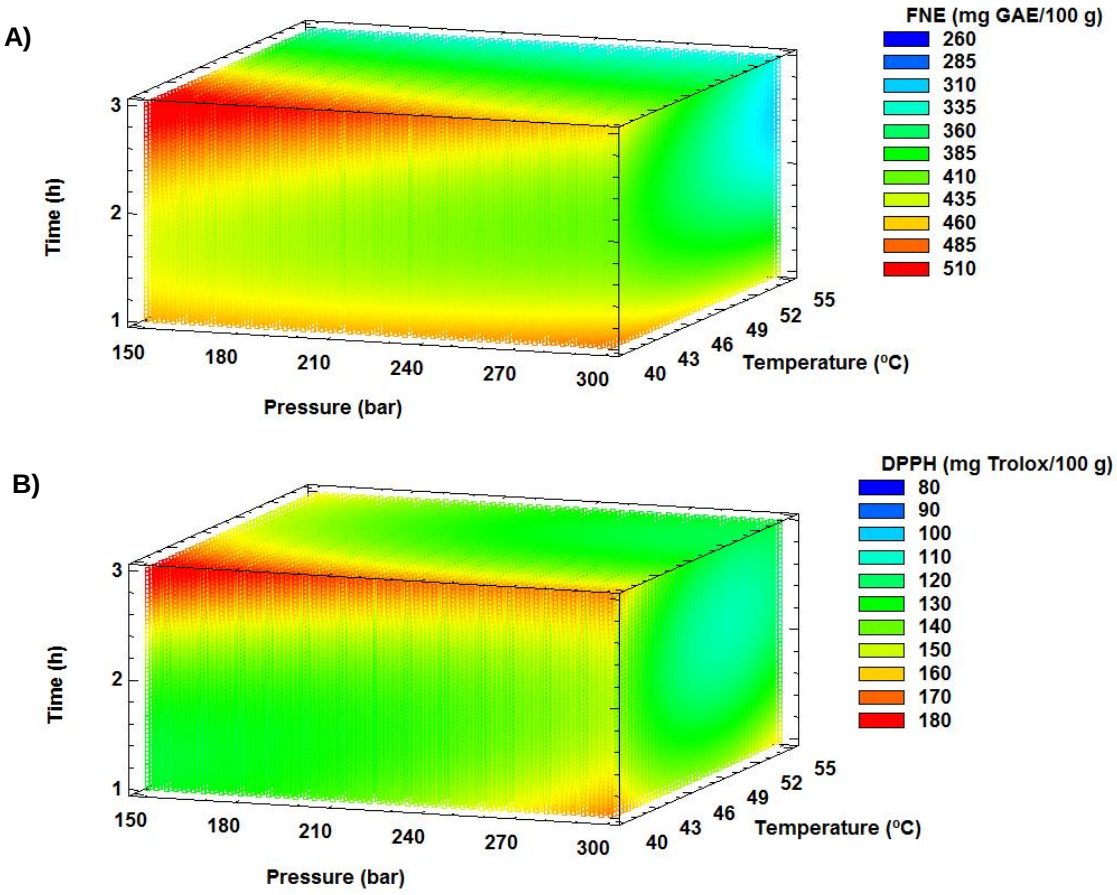
A)
FNE (mg GAE/100 g)
260
285
310
335
360
385
410
435
460
485
510
Time (h)
1
2
3
Pressure (bar)
150
180
210
240
270
300
Temperature (°C)
40
43
46
49
52
55
B)
DPPH (mg Trolox/100 g)
80
90
100
110
120
130
140
150
160
170
180
Time (h)
1
2
3
Pressure (bar)
150
180
210
240
270
300
Temperature (°C)
40
43
46
49
52
55

Fig. 5

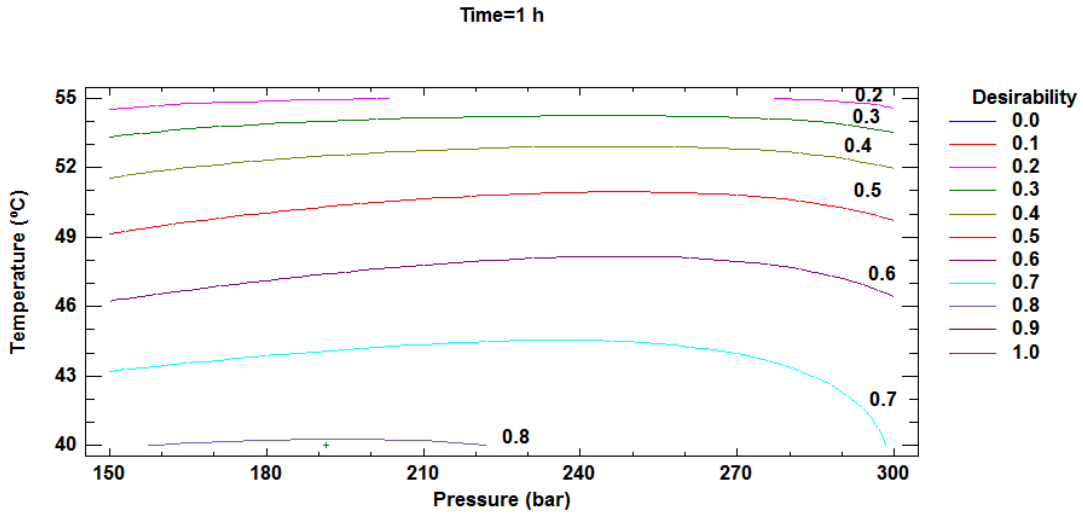
Time=1 h
Temperature (°C)
55
52
49
46
43
40
150
180
210
240
270
300
Pressure (bar)
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Desirability
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Fig. 6

1 **Table 1** Experimental runs of the Box–Behnken design (BBD) per block for

2 supercritical fluid treatment.

Pressure (bar)	Temperature (ºC)	Time (h)
Factorial points		
225	55.0	3.00
300	47.5	1.00
225	55.0	1.00
300	55.0	2.00
150	47.5	3.00
300	40.0	2.00
150	55.0	2.00
225	40.0	3.00
300	47.5	3.00
150	40.0	2.00
150	47.5	1.00
225	40.0	1.00
Central points (SFT-CP)		
225	47.5	2.00
225	47.5	2.00
225	47.5	2.00

Table 2 Comparison of control and supercritical fluid treatment-central points (SFT-CP) samples and descriptive statistics of runs included in BBD for oligosaccharide degree of polymerization (DP) and soluble fiber constituents.

	One-way ANOVA					BBD runs (descriptive statistics)			
	CONTROL		SFT-CP						
	Mean	SD[a]	Mean	SD	*p**	Mean	SD	Max	Min
DP (7-12)	592.94 ±	30.87	899.49 ±	53.33	*0.000*	947.46 ±	127.26	998.91	769.35
Soluble fiber constituents									
Galacturonic acid	98.54 ±	0.02	98.31 ±	0.09	*0.020*	98.46 ±	0.34	98.91	97.77
Glucose	0.73 ±	0.01	0.86 ±	0.05	*0.027*	0.62 ±	0.11	0.87	0.44
Xylose	0.16 ±	0.05	0.03 ±	0.03	*0.008*	0.04 ±	0.04	0.16	0.01
Arabinose	0.00 ±	0.00	0.00 ±	0.00	-	0.24 ±	0.17	0.42	0.00
Fucose	0.56 ±	0.02	0.79 ±	0.04	*0.001*	0.62 ±	0.16	0.89	0.38

DP (7-12): oligosaccharide degree of polymerization between 7-12. DP (7-12) (mg/g fibre dietary); Fiber constituents (p/p%)
* p-Values lower than 0.05 are statistically significant.
[a] Values are represented as a mean ± standard deviation (n = 3).

Table 3. ANOVA of quadratic response surface model and optimal supercritical fluid treatment (SFT) for oligosaccharide degree of polymerization (DP) and soluble fiber constituents.

		Soluble fiber constituents				
Source	**DP (7-12)**	**Galacturonic acid**	**Glucose**	**Xylose**	**Arabinose**	**Fucose**
ANOVA (p-values)						
A: Pressure	**0.000***	0.173	0.667	0.662	0.810	**0.024**
B: Temperature	**0.001**	**0.000**	**0.000**	0.950	0.238	**0.000**
C: Time	**0.000**	**0.000**	**0.000**	0.387	**0.000**	**0.006**
AA	0.223	0.246	**0.000**	0.302	**0.000**	**0.044**
AB	**0.029**	0.329	0.099	0.482	0.3349	0.203
AC	0.364	**0.011**	0.154	0.105	0.641	**0.002**
BB	**0.023**	0.093	**0.000**	0.517	**0.000**	**0.000**
BC	0.541	**0.000**	0.807	0.430	**0.000**	0.802
CC	0.530	**0.017**	**0.003**	0.091	0.430	**0.003**
ANOVA (R-squared statistics)						
R^2	0.80	0.91	0.89	0.34	0.87	0.88
R^2 (adjusted by g.l.)	0.70	0.86	0.83	0.02	0.80	0.81
Optimal SFT conditions						
Pressure (bar)	150	300	223	-	150	233
Temperature (°C)	54.8	55.0	44.4	-	40.0	42.7
Time (h)	1.00	1.00	2.40	-	3.00	2.30
Estimated value	999	98.9	0.89	-	0.69	0.85

DP (7-12): oligosaccharide degree of polymerization between 7-12. DP (7-12) (mg/g fibre dietary); Fiber constituents (p/p%)

* p-Values lower than 0.05 are statistically significant. [a] Values are represented as a mean ± standard deviation (n = 3).

Table 4 Comparison of control and supercritical fluid treatment-central points (SFT-CP) samples and descriptive statistics of runs included in BBD for all properties studied.

	One-way ANOVA				BBD runs (descriptive statistics)			
	CONTROL		SFT-CP					
	Mean	SD[a]	Mean	SD	*p**	Mean	SD	Max	Min
Ws	11.98	± 0.94	15.00	± 0.94	*0.001*	14.34	± 2.35	17.98	9.02
Sw	8.84	± 0.62	10.49	± 0.64	*0.003*	9.14	± 1.78	12.38	5.82
WRC	9.84	± 0.62	11.49	± 0.64	*0.003*	10.14	± 1.78	13.38	6.82
FAC	7.36	± 0.53	7.90	± 0.73	*0.267*	8.03	± 1.33	10.95	5.63
GAC	0.72	± 0.01	0.92	± 0.12	*0.023*	0.87	± 0.22	1.25	0.55
FNE	215.28	± 15.91	355.95	± 26.68	*0.000*	403.77	± 64.41	509.19	266.84
DPPH	84.57	± 3.14	108.07	± 15.66	*0.031*	137.65	± 17.72	176.33	100.65
ABTS	399.40	± 8.34	459.84	± 36.99	*0.021*	488.39	± 27.28	545.15	440.47

Ws: solubility (%); Sw: swelling (mL/g); WRC: water retention capacity (g/g); FAC: fat adsorption capacity (g/g); GAC: glucose absorption capacity (g/g); FNE: total non-extractable phenolic compounds (mg GAE/100 g); DPPH: antioxidant activity of extract (mg Trolox/100 g); ABTS: antioxidant activity of extract (mg Trolox/100 g)

* p-Values lower than 0.05 are statistically significant.

[a] Values are represented as a mean ± standard deviation (n = 3).

Table 5 ANOVA of quadratic response surface model and optimal supercritical fluid treatment (SFT) conditions for the functional properties studied.

Source	Ws	Sw	WRC	FAC	GAC	FNE	DPPH	ABTS
ANOVA (p-values)								
A: Pressure	0.135	0.352	0.352	**0.045**	**0.000**	0.750	0.199	0.759
B: Temperature	**0.022**	**0.001**	**0.001**	0.393	**0.030**	**0.000**	**0.000**	**0.041**
C: Time	0.131	0.355	0.355	0.836	0.073	0.075	**0.001**	0.783
AA	**0.013**	0.945	0.945	0.420	**0.000**	0.376	0.053	0.603
AB	0.060	0.213	0.213	**0.000**	**0.048**	**0.027**	0.354	0.753
AC	**0.000**	0.554	0.554	0.233	0.825	**0.002**	**0.000**	0.092
BB	0.168	**0.000**	**0.000**	0.568	0.122	0.398	**0.000**	0.072
BC	0.062	**0.000**	**0.000**	**0.023**	**0.000**	**0.004**	**0.008**	0.493
CC	0.382	0.291	0.291	**0.049**	**0.000**	**0.000**	**0.000**	**0.016**
ANOVA (R-squared statistics)								
R^2	0.59	0.66	0.66	0.50	0.75	0.87	0.83	0.35
R^2 (adjusted by g.l.)	0.46	0.55	0.55	0.34	0.67	0.83	0.78	0.14
Optimal SFT conditions								
Pressure (bar)	-	150	150	-	191	150	150	-
Temperature (°C)	-	40.9	40.9	-	40.0	40.0	40.0	-
Time (h)	-	1.00	1.00	-	1.00	3.00	3.00	-
Estimated value	-	12.0	13.0	-	1.20	541	188	-

Ws: solubility (%); Sw: swelling (mL/g); WRC: water retention capacity (g/g); FAC: fat adsorption capacity (g/g); GAC: glucose absorption capacity (g/g); FNE: total non-extractable phenolic compounds (mg GAE/100 g); DPPH: antioxidant activity of extract (mg Trolox/100 g); ABTS: antioxidant activity of extract (mg Trolox/100 g). * p-Values lower than 0.05 are statistically significant (bold font).

CAPÍTULO 6

Impact of simulated human gastrointestinal digestion of dietary fiber obtained from different vegetable by-products on functional properties.

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Abstract

The aim of this study was to analyze the impact of simulated human digestion process on the composition and functional properties of dietary fiber derived from pomegranate peel, tomato peel, broccoli stems, and grape stems by-products. For this purpose, a computer-controlled simulated digestion system consisting of three bioreactors (simulating the stomach, small intestine, and colon) was utilized. Non-extractable phenols associated with dietary fiber and their influence on antioxidant capacity and antiproliferative activity were investigated throughout the simulated digestive phases. Additionally, modifications in oligosaccharide composition, microbiological population, and short-chain fatty acid produced within the digestion media were examined. The type and composition of each dietary fiber significantly influenced its functional properties and behaviour during intestinal transit. Notably, dietary fiber from pomegranate peel kept its high phenol content throughout colon digestion, potentially enhancing intestinal health due to its strong antioxidant activity. Similarly, dietary fiber from broccoli and pomegranate peel demonstrated anti-proliferative effects in both small and large intestines, prompting significant modifications in colonic microbiology. Moreover, these fiber types promoted the growth of bifidobacteria over lactic acid bacteria Thus, these results suggests that dietary fiber from pomegranate peel seems to be a promising functional food ingredient for improving human health.

Keywords: Digestion process, Dietary fiber, By-products, Antioxidant capacity, Antiproliferative activity

1. Introduction

Today, agricultural industries generate large amounts of agricultural by-products throughout the world. Agricultural by-products come from wastes, from inedible parts derived from the cultivation and processing of food products (Pagano et al., 2021). The agricultural residues of crops are composed of leaves, flowers, stems and roots, while the by-products of the food industry are represented by fruits, peels and discarded seeds (Chamorro et al., 2022). The generation of agricultural residues and by-products causes a serious economic and environmental problem (Lin et al., 2021). However, they can contain a high concentration of valuable bioactive compounds such as phenolic compounds (Abbasi-Parizad et al., 2021; Alañón et al., 2021; Maatallah et al., 2020), terpenes (Gómez-García et al., 2021), fatty acids (Petropoulos et al., 2020; Roselló-Soto et al., 2019), polysaccharides (Shehata et al., 2020; Rivas et al., 2021a) and proteins (De Iseppi et al., 2021). In addition, it should be noted that plant by-products are a powerful source of dietary fibers (Chitrakar et al., 2020; Pathania & Kaur, 2021; Rivas et al., 2021b) that have various functional properties and are associated with good applications in food and many benefits for people's health (Fabek et al., 2014). Therefore, their extraction and application for different purposes in various fields, such as the food, pharmaceutical, cosmetic, textile and biofuel industries, among others, can result in new value-added products (Donner et al., 2021; Panwar et al., 2021).

The physiological effects of the by-products of dietary fiber depend on the composition and structural characteristics of the plant cell wall, as well as on the techno-functional properties (Benitez et al., 2019). Previous studies reported that tomato peel is an important source of high-quality dietary fiber (Gu et al., 2020), with

a content of approximately 48% insoluble dietary fiber and 9% soluble dietary fiber (Shao et al., 2013) and with excellent functional properties, in particular great gelling capacity (Li et al., 2018) and high capacity for glucose retention, which could have a positive effect in protecting against postprandial hyperglycemia (Li et al., 2022). In addition, other vegetable by-products such as grape stem, pomegranate peel, and broccoli leaf, also showed good functional and technological properties. The peel of the pomegranate was found to be rich in antioxidant dietary fiber (Rivas et al., 2021c), while the fiber from grape stem (Rivas et al., 2021b; Luo et al., 2023) and broccoli leaf (Rivas et al.,2021d; Shang et al., 2023) were shown to have prebiotic capacity and capacity to stimulate the increase the short-chain fatty acids (SCFAs) amount in *in vitro* test, respectively.

Most previous works on isolated fractions or extracts of dietary fiber of various by-products have been focused on the evaluation of their composition, structure and functional properties, but there are hardly any studies that consider the analysis of the functional properties throughout the simulated digestion process. Ribeiro et al. (2021) evaluated the impact of *in vitro* simulation of gastrointestinal (GI) digestion on antioxidant dietary fiber powder from olive pomace. They concluded that dietary fiber, containing free and bound phenolics, can reach the colon and potentially offer health benefits such as antioxidant, antimicrobial and anti-inflammatory activity. Additionally, dietary fiber may positively interact with lipids by decreasing the bioaccessibility of saturated fatty acids and facilitating the absorption of unsaturated fatty acids. Studying the effects of GI stresses on the structure and functional properties of dietary fiber is crucial to understanding their potential impact on human health when incorporated as food ingredients. Thus, the objective of this study was through the use of a computer-controlled simulated GI digestion equipment, to

evaluate *in vitro* digestibility, focusing on knowing the composition, structure and functional properties of extracts of dietary fiber from pomegranate peel, tomato peel, broccoli stems and grape stems. This research can serve as a scientific basis to promote the use as functional food and / or additive in the food industry of agricultural by-products undervalued until now.

2. Material and methods

2.1. Plant material and dietary fiber extraction

The by-products used in this study (pomegranate peel, tomato peel, broccoli leaf and grape stems) were provided by industries from Autonomous Community of Extremadura, Spain. They were dried, until around 6% moisture content, in a forced air oven (Model Digitronic-TFT, SELECTA, Barcelona, Spain) with a flow rate of 2 m^3/min and an air temperature of 45 °C, followed by a vacuum packaging individually in a plastic bag using a vacuum packaging machine (Model SV-420S, Sammic, Azkoitia, Spain). The vacuum bags were stored at room temperature until use.

The extraction of dietary fiber from the by-products was carried out following the alcohol-insoluble residues (AIR) method described by Rivas et al. (2021b). Once extracted, the extracts were ground and passed through a 1 mm sieve.

2.2.- Preparation of a base feed supplemented with different types of dietary fiber.

Chickpea paste, obtained with washed cooked chickpeas and 400 ml of sterile distilled water followed by grinding in a Thermomix (Wuppertal, Vorwerk, Germany) at high speed for 5 minutes was used as dietary fiber control. Dietary fiber extracts (50 g) from each by-products were individually added to chickpea paste by

normalizing the amount of fiber (20% of dried extract approx..) through the addition of water.

2.3.-Fecal inoculum

Fecal sample was collected from a 45 aged healthy human volunteer, who was on a non-specific Mediterranean diet, had no metabolic and GI diseases, non-smokers and had not received any antibiotics, pre- or probiotic supplements for at least 6 months before the fecal donation. A voluntary informed consent was obtained from the donors prior to this study. Twenty g of fecal sample were diluted in 80 mL solution of 58% glicerol in phosphate-buffered saline (PBS) 0.1 M at pH 7.0. Immediately afterwards, the fecal inoculum was stored at -80º °C until used. Only one donor was used for the experiment to prevent diversity associated to different fecal samples.

2.4. Human simulated digestion: experimental design

For simulated digestion of the different dietary fibers and control a dynamic *in vitro* simulator model of the human digestion system, consisting in three bioreactor BIOSTAT A sytems (Sartorius Stedim Biotech, Göttingen, Germany) connected in series by peristaltic pumps, was used. These three glass vessels simulated the conditions of stomach, small intestine, and colon (ascending, transverse, and descending colon) respectively. Parameters of simulated digestion in each section of the system were entirely computer-controlled and showed in Table 1. Initial feed consisted of 750 mL of sterile PBS for each bioreactor, which were equilibrate at specific initial conditions of temperature, pH and O_2 pressure for 2 h. Before starting the digestion process, the 3^{th} bioreactor (colon compartment) were inoculated with

25 mL of De Man Rogosa Sharp broth (MRS; Condalab, Madrid, Spain) medium and 10 mL of fecal inoculum. So, the stabilization phase for the colon microbiome was for 12 h before effective use of this bioreactor. During digestion process, peristaltic pumps add a specific amount of food supplement, HCl, pepsin, $NaHCO_3$, pancreatic and biliary fluids (Sigma-Aldrich Chemistry, St. Louis, MO, USA) in simulate stomach and small intestine (Table 1). The digestion process was repeated twice for each dietary fiber and control, and samples were taken for analysis in triplicate at different times during the process. Samples were collected at the initial phase, end of stomach stage (sampling 1.1 and 1.2), end of small intestine stage (sampling 2.1 and 2.2), and in colon stage: 8 h (ascending: sampling 3.2), 20 h (transverse: sampling 3.3) and 36 h (descending: sampling 3.4).

2.5. Characterization of digestion extracts

2.5.1. Dietary fiber content

The dietary fiber content of the different by-products was calculated before and during the different stages of the digestion process by the AIR method described in section 2.1. The results were expressed in g of AIR / 100 g of solid residue.

2.5.2. Dietary fiber composition

To determine the content of galacturonic acid and the profile of neutral sugars, the dietary fiber extract underwent hydrolysis with 12 M sulfuric acid (3 hours at room temperature and 1 hour at 100 °C). Subsequently, the released monosaccharides and galacturonic acids were determined using HPLC. The HPLC analyses in this study were conducted using an Agilent LC 1260 Infinity II HPLC system (Waters, Milford, MA, USA), comprising a separation module and RI detector. The HPLC

system was equipped with a Rezex ROA-Organic Acid H+ (8%) column (7.8 mm ID x 150 mm; Phenomenex, Torrance, CA, USA). In isocratic mode, the mobile phase consisted of water, with a flow rate of 0.6 mL/min. In elution mode, the sample injection volume was 10 µL, the column temperature was 80 °C, and the detector temperature was 40 °C.

2.5.3. Non-extractable phenolic compounds and their antioxidant capacity

The determination of non-extractable phenolic to dietary fiber and their antioxidant capacity involved an initial extraction of phenolic compounds from the AIR, following the method outlined by Rivas et al. (2021b).

Phenolic content in the extracts was quantified using Folin-Ciocalteu reagent (Wettasinghe & Shahidi, 1999) via a UV-1800 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA), with gallic acid as the standard. Results are expressed as mg gallic acid equivalents (GAE) per 100 g of extract.

Antioxidant activity of the extract solutions (10 mg of extract per mL of ethanol) was assessed by bleaching the violet solution of the 1,1-diphenyl-2-picrylhydrazyl radical using the DPPH method as described by Teixeira et al. (2009), and by measuring the ability to eliminate the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical (ABTS) following the method of Re et al. (1999). Total antioxidant activity was expressed as mg of Trolox per 100 g of extract.

2.5.4. Microbial population

For microbial counts, each sample from each stage of digestion, 10 mL was placed aseptically in a sterile plastic bag with 90 mL of 1% peptone water (Condalab)

and homogenized for 120 s in a stomacher instrument (Lab-Blender 400 Seward Lab., London, England). Serial 10-fold dilutions were made with peptone water and 0.1 mL aliquots of each dilution were inoculated into agar plates. Total viable counts were counted on plate count agar (PCA; Condalab) after incubation at 30°C for 48 hours. Lactic acid bacteria (LAB) were enumerated on MRS agar (Condalab) acidified to pH 5.6 with acetic acid (10%) at 30 °C after 48 h. Bifidobacteria were enumerated on MRS agar (Condalab) supplemented after sterilization with L-cysteine-HCl 500 mg/L, Mupirocin 100 mg/L, Kanamycin 25 mg/L, 2,3,5-triphenyltetrazolium chloride 25 mg/L, Polymyxin B 4.28 mg/L(Ruiz-Moyano et al., 2013) under anaerobic conditions using an Oxoid AnaeroGen 3.5 L sachet (Thermo Scientific, Massachusetts, USA) and an anaerobic jar for 72 h at 37 °C. Enterococci were counted on Slanetz and Bartley agar (SB; Condalab) at 37°C for 48 h. The staphylococci were counted in Baird-Parker agar (BP; Condalab) supplemented with potassium tellurite and egg yolk the emulsion was evaluated after incubation at 37 °C for 48 h. Enterobacteria were counted on violet red bile glucose agar (VRBG; Condalab), after incubation at 30 °C for 24 h. Yeast were counted on potato dextrose agar (PDA; Condalab) acidified to pH 3.5 with a sterilized solution of tartaric acid (10%) after incubation at 25°C for 72 h. Microbial counts were expressed in log CFU/mL.

2.5.5. SCFAs

To assess the impact of each dietary fiber on microbial activity the level of SCFAs across various stages of digestion was measured. Five hundred µL of the digested extract was combined with 500 µL of ultrapure water and 100 µL of internal standard (2-ethylbutyric acid). Subsequently, 0.5 µL of this mixture was injected into

a gas chromatograph equipped with a split/split-less injector and a flame ionisation detector (Shimadzu 2010 Plus). SCFAs were separated using a DB-FFAP capillary column (30 m x 0.25 mm id; 0.25 µm) using the chromatography conditions described by Rivas et al. (202d). Identification of individual SCFAs was achieved by comparing their retention times with those of the reference standard mixtures (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and hexanoic; Sigma-Aldrich). SCFA concentrations were determined as the ratio of the peak area of the analyte to the internal standard (2-ethylbutyric acid), according to Brighenti (1997).

2.5.6. Soluble oligosaccharides

An HPLC analysis was performed using an Agilent 1260 Infinity LC system (Agilent Technologies) equipped with a refractive index detector (RID). Data acquisition was controlled by OpenLAB CDS ChemStation Edition™ software (Agilent Technologies). Prior to injection, a 500 µL aliquot of the digestion medium was diluted in 1.0 mL of HPLC water and filtered through a 0.45 µm nylon membrane. An injection volume of 10 µL was used. Chromatographic separation was carried on using a Phenomenex Rezex RNO-Oligosaccharide Na+ (4%) column (200 x 10 mm ID; Phenomenex)) with a particle size of 12 µm and a Rezex RNO-Oligosaccharide Na+ (4%) guard cartridge (60 x 10 mm ID; Phenomenex) 8.0 µm internal particles protected by a PL Hi-Plex H guard column (60 x 10 mm rezex TM RNO-oligosaccharide Na+. The column temperature was maintained at 80°C, while the RID flow cell temperature was set to 40°C. The mobile phase consisted of HPLC water with a flow rate of 0.5 mL/min and a run time of 20 minutes. Detection employed the RID for changes in refractive index.

2.5.7 Antiproliferative activity

The antiproliferative test was carried out using the HT-29 ATCC HTB-38 human colorectal adenocarcinoma cell line. The cells were seeded at a density of 10^4 cells per well in 96-well flat plates in high glucose, pyruvate, no glutamine Dulbecco's Modified Eagle's Medium (DMEM; Gibco-Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco-Thermo Fisher Scientific), 1% L-glutamine (200 mM; Gibco-Thermo Fisher Scientific) and antibiotics including 100 IU/mL penicillin and 100 µg/mL streptomycin (Gibco-Thermo Fisher Scientific). After incubation for 24 h at 37 °C in an atmosphere containing 5% CO_2, culture media were substituted with 200 µL of complete culture media supplement with 50 µL of digestion extracts sterilised by filtration through 0.22 µm filters (Thermo Fisher Scientific). The extracts corresponding to the initial phase were previously diluted 1/8, those from the small intestine were diluted 1/2 and those from the colon were added without dilution. After 24 h treatment, the culture medium was removed and cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium solution (MTT, 0.5 mg/mL; Sigma-Aldrich) for 1 h. The formazan blue crystals were then dissolved in 200 µL of dimethyl sulfoxide (DMSO; Sigma-Aldrich) and the absorbance measured at 570 nm using a Fluostar Optima microplate reader (BMG LABTECH, Offenburg, Germany). The cells treated with hydrogen peroxide (2 mL/ 100 mL) were used as the positive control, while untreated cells were used as a negative control. The antiproliferative effect was calculated as the percentage of growth inhibition with respect to the negative control cells.

2.. Statistical Analysis

Data were analysed using SPSS for Windows (version 21.0, IBM Corp.) with descriptive statistics calculated. Differences within and between groups were assessed with one-way analysis of variance (ANOVA) followed by Tukey's HSD test ($p \leq 0.05$) for between-group comparisons. Additionally, principal component analysis (PCA) was performed on the correlation matrix of the variables.

3. Results and Discussion

3.1 Content and composition of dietary fiber of digestion extracts

Table 2 shows the initial values of dietary fiber parameters of the control (chickpea) and by-products analyzed. The content of dietary fiber ranges from 18.8 % for tomato peel to 22.7 % for chickpea (control), without significant differences between them. The dietary fiber values of the chickpea agree with those published by other authors (Khatoon and Prakash, 2004). With respect to dietary fiber constituents, statistically significant differences were found in all reducing sugars analyzed. Specifically, the broccoli leaf and grape stem presented lower values of glucose than the control while these by-products and the pomegranate peel presented the highest values of xylose, galactose and mannose compared to the control and the tomato peel. Rivas et al. (2021d) characterized dietary fiber obtained from several vegetable by-products to study the improvement of the viability and metabolism of intestinal probiotic bacteria, obtaining similar results to ours for the composition of neutral sugars for winemaking by-products, broccoli, tomato, and pomegranate peel. The higher glucose values for the tomato and pomegranate peel can be explained by the high cellulose content present in fruit and vegetable skins and peels (Wenjuan et al., 2022). No significant differences were found in the

galacturonic acid and rhamnose/arabinose content of the dietary fibers from the by-products under study (Table 2).

During the simulated digestion process, after passing through the stomach, the dilution effect in the fiber content for all dietary fibers was observed. It should be noted that in the case of broccoli leaf and pomegranate digestions this reduction was significantly higher than the others (Table 2 and 3). Although most dietary fibers cannot be digested under simulated saliva and stomach conditions (Su et al., 2019), part of the soluble dietary fiber disappeared in the stomach by the solubilization of non-cellulosic and non-starch polysaccharides (Abelilla and Stein, 2019). Regarding the composition profiles of dietary fiber, in general were not affected after passing through the stomach, as expected from what was published by other authors (Ding et al., 2019; Gao et al., 2019).

In the case of the small intestine, the dietary fiber values decreased by dilution effect associated with the change of bioreactor, pH adjustment and the addition of pancreatin and bile salts solution (Table 1). The differences between the dietary fibers digestions found in the stomach stage for both total dietary fiber and their constituents were overall maintained in the small intestine simulation (Table 4). However, lower values of glucose and higher of xylose, galactose and mannose were observed in dietary fiber constituents with respect to the previous digestion stage. The higher values of neutral sugars (mainly xylose, galactose, mannose) with respect to the stomach stage, associated with a high content of galacturonic acid in the samples, seem to indicate a greater degradation of the pectin. Dietary fiber with shorter chains and branches as well as a loosening of the structure allow a better

hydrolysis of the polysaccharides studied during the analysis and, finally, a greater detection of these monosaccharides (Garna et al., 2006).

Table 5 shows the content of dietary fiber and its components after passing through the colon. The dietary fiber values in the stages of colon digestion showed low fermentation, which is positively associated with an impact on gut health (Wang et al., 2019). At the end, no differences were observed between dietary fiber from control and by-products. The most easily fermentable substrates are characterized not only by their chemical composition but also by their ease of access for the microbiota (Flanagan et al., 2020). In terms of dietary fiber composition, no significant differences were noted between digestions in the galacturonic acid content. Regarding monosaccharide content, there appears to be a slight reduction compared to the previous stage. Additionally, when comparing across colon stages, values consistently show lower levels in samples from the distal colon. These findings are anticipated, as microorganisms tend to metabolize monosaccharides more rapidly than carbohydrates with longer chains, making them the preferred fermentation substrates. This observation aligns with the research by Stewart et al. (2008), which emphasized the significance of carbohydrate chain length in fermentation. They highlighted the rapid fermentation of short-chain fructans, primarily occurring in the proximal colon.

3.2. Functional properties of dietary fiber

Non-extractable phenols associated with dietary fiber is showed in the Table 2. The initial highest value was found in pomegranate peel, followed by the grape stem, whereas broccoli leaf, tomato peel and control presented the lowest values. Previous studies corroborate the high values of non-extractable phenolic compounds found in

these by-products (Rivas et al., 2021b; Rivas et al., 2021c). The high initial antioxidant capacity of the pomegranate peel and grape stem digestions, evaluated by both, ABTS and DPPH methods, evidenced the positive relationship between the content of non-extractable phenols and the antioxidant capacity of these dietary fibers (Ferri et al., 2016; Granato et al., 2018). However, antioxidant capacity has been also associated to other residual compounds linked to dietary fiber such as some soluble polysaccharides (Li et al., 2020), terpenoids (Cutillas et al., 2018), fatty acids (Morshedloo et al., 2022), among others.

During the simulated digestion process, after passing through the stomach, the losses of phenolic compounds associated with dietary fiber are relatively small since dietary fiber protects them from GI conditions (Tang et al., 2022; Tomas, 2022). However, the reduction tendence in the values of non-extractable phenols may be due to degradation or loosening of the dietary fiber structures under digestion conditions (Liu et al., 2021), that can favour antioxidant activity due to a greater accessibility to the linked compounds that support this activity (Table 3). In fact, the antioxidant capacity of polyphenols depends not only on the quantity but also on the percentage that can be released under intestinal tract conditions and therefore remains available for absorption (Garzón et al., 2020). Like as the initial, the fiber-added from pomegranate peel and grape stems showed the highest antioxidant activity in the simulated stomach stage.

At the end of small intestine stage, the content of non-extractable phenolic compounds associated with dietary fiber tended to decrease in the broccoli leaves and grape stems digestions, while it increased in the rest of dietary fibers tested, especially in the pomegranate peel (Table 4). This result agrees with the DPPH

values observed in pomegranate dietary fiber digestion. The increase in non-extractable phenolic compounds may be since phenolic acids released during gastric processing are reabsorbed into the cell wall under small intestine conditions (Padayachee et al., 2013). In any case, for all dietary fibers evaluated, the antioxidant activity values were higher than those obtained at the beginning of digestion, demonstrating that dietary fiber protects the compounds responsible for antioxidant activity in its passage through the GI tract (Bermúdez-Oria et al., 2020). Depolymerization of polysaccharides and changes in reducing sugars after simulated digestion may influence antioxidant activity (Shao et al., 2022; Wang et al., 2018).

Finally, approximately 50% of the initial phenolic content found in the broccoli leaves, pomegranate peel, and grape stem dietary fibers reached the colon (Table 5). For control and tomato peel digestions, the content of polyphenols associated with the fiber was similar or higher in the colon stages compared to the initial contents. In general, the results of antioxidant capacity, ABTS and DDPH data, suggest that there was no substantial loss of this capacity after digestion, increasing even in some cases. These data suggest that the phenolic compounds associated with dietary fiber are not completely bioaccessible in the small intestine, so a significant fraction moves to the colon where they could act as a substrate for microbiota modulation and may have a positive impact on health. In this context, dietary fiber extracted from pomegranate peel stood out from the rest of the fibers studied.

Regarding the results of antiproliferative activity, no significant differences in the initial stage were found between the different dietary fibers, oscillating the values between 80.4 and 103.2% of cell survival compared to the positive control. The values of the antiproliferative capacity of dietary fiber also did not show significant

differences after passing through the stomach (Table 3). However, as can be seen in Table 4 and Table 5, overall, the digestion of dietary fiber from broccoli leaves, pomegranate peel and grape stem showed greater antiproliferative capacity of HT-29 cells compared to the control after passing through the small intestine and colon. These results agree with those published by other authors Sharma et al. (2020) demonstrated in their study that xyloligosaccharide-rich dietary fiber from Azadirachta sawdust inhibits the growth of human colorectal cancer (HT-29) cells.

3.3. Oligosaccharides

The amount of oligosaccharides (degree of polymerization: 2-7) present in the the dietary fibers studied at simulated stomach is showed in Table 3. Pomegranate peel presented the highest value of total oligosaccharides (38.71 g/L) in simulated stomach followed by tomato peel and grape stem with 29.87 and 17 .58 g/L, respectively. These differences are mainly associated to DP7 that was the predominant oligosaccharide found in all dietary fibers tested. In fact, no statistically significant differences were found between the dietary fibers in the content of oligosaccharides with degree of polymerization 5 and 6 (DP 5-6). Regarding the oligosaccharides with DP 2-4, the highest contents were found in pomegranate peel and tomato peel dietary fibers digestions, followed by the control.

The differences in DP7 content between the different dietary fibers are clear, pointing to the differences between the dietary fibers evaluated and how stomach acid modified them. Although there are currently no references on the production of oligosaccharides from dietary fiber after passing through the stomach, there are numerous works in which oligosaccharides are chemically and enzymatically produced from dietary fiber. Zhang et al. (2018) treated citrus peel pectin and by

chemical degradation with trifluoroacetic acid acquired three pectic oligosaccharides, with a molecular weight range (Mw) of 3000–4000 Da, 2000–3000 Da and less than 2000 Da. Additionally, Yang et al. (2022) demonstrated that acid hydrolysis with lactic acid combined with an enzymatic treatment with xylanases was an effective way to produce high purity xyloligosaccharides from poplar wood.

Table 4 shows the oligosaccharides present after passing through the small intestine. Overall, an increase in oligosaccharide values was observed, except in the broccoli leaf dietary fiber digestion, corroborating the variability of the fiber degradation depending on its mixture composition (Capuano, 2017). Therefore, the impact of digestion on fiber degradation and oligosaccharide production can be highly variable since there are several factors that can influence such as the different types of fiber, the food matrix, and the microbiological profile, among others (Luzardo-Ocampo et al., 2017). Regarding the higher content of DP2 with respect to the previous stage, it may be due to the exposure of the substrate with mild alkali combined with enzymatic action, which resulted in a higher yield of oligosaccharides with a lower degree of polymerization. (Aachary and Prapulla, 2009). It should also be noted that the oligosaccharides studied are resistant to the conditions of the small intestine and reach the colon available for use by the microbiota (Ferreira-Lazarte et al., 2017; Intaratrakul et al., 2022). At colon phase, after 8 h the level of oligosaccharides decreased sharply to level below 0.1 g/L in all dietary fiber digestions evaluated. This result could be related with the metabolic activity of the colon microbiota, which in general is prone to consume oligosaccharides with lower DP, as we have analysed (Steawrt et al., 2008; Singh et al., 2021). Previous studies on simulated colon conditions have shown similar trend (Yang et al., 2013; van Trijp et al., 2020; Zhang et al., 2020).

3.4. Microbial population dynamic

Table 4 and 5 shows the microbiological population dynamic during the digestion process in small intestines and colon stages, respectively. Regarding the results of total viable bacteria, the counts increase from 2.8 Log CFU/mLin stomach to values higher than 8 Log CFU/mLin small intestine and colon. The level of total viable bacteria throughout digestion was clearly associated to the enterobacteria counts, which was the predominant microbial group, not showing significant differences between the dietary fibers studied at the colon stages (Table 5). However, in the case of enterobacteria, all dietary fibers digestion presented higher counts than the control in the colon, with broccoli leaf dietary fiber accounting for the lowest values among them. The differences in dietary fiber composition could explain these results. In fact, dietary fiber constitutes such as cellulose have demonstrated a positive effect on the *Enterobacteriaceae* populations in the ileum of growing pigs (Owusu-Asiedu et al., 2006). Regarding colon, *Enterobacteriaceae* counts increased with the addition of dietary fiber, which is corroborated by other authors who stated that a diet rich in dietary fiber mainly included changes in Firmicutes, Verrucomicrobia, *Enterobacteriacea*, *Prevotella*, and Bacteroides (Yao et al., 2022).

Respect to the counts of *Enterococcus* and *Staphylococcus*, both microbial groups increased from values lower than 2 log CFU/mL at stomach to values higher than 6 log CFU/mL during the colon stages, except for *Staphylococcus* that decreasing to 3.9 log CFU/mL at distal colon stage (Tables 4 and 5). On the other hand, significant differences were found between the dietary fibers estudied, with values ranging between 5.82-7.47 log CFU/mL and 3.62-7.25 log CFU/mL, for of *Enterococcus* and *Staphylococcus*, respectively. For both microbial groups, newly the tomato and

pomegranate peel fibers showed the highest mean counts in colon. In previous works, the growth capacity of several *Enterococcus faecium* strains were tested in the presence of dietary fiber from grape stems, proving to be an effective substrate for promoting their growth (Rivas et al., 2021b).

Dietary fiber is also the main energy substrate for LAB and bifidobacteria in colon, which have specific enzymes that break down these complex carbohydrates. In general, bifidobacteria counts on the contrary of LAB, were stimulate in proximal colon (Table 5). Several studies have shown the capacity of of bifidobacteria to metabolize dietary fiber from different source (Gómez et al., 2016; Ruiz et al., 2017; Van den Abbeele et al., 2021), although this ability is variable among bifidobacteria strains and species. Strains of *Bifidobacterium longum*, *Bifidobacterium breve* *Bifidobacterium adolescentis* and *Bifidobacterium animalis* subsp. *lactis* harbour different types of enzymes (glycosyl hydrolase) and transporters involved in the degradation of vegetable oligosaccharides and polysaccharides (Wang et al., 2022). Thus, dietary fibers with different structures can modulate bifidobacteria growth in different ways. In our study, this fact is observed in the mean counts of bifidobacteria for the most dietary fiber studied. Concretely, dietary fibers from grape stem broccoli leaf and tomato peel presented mean values over 5 log CFU/mL in colon, whereas the control showed the lowest counts (Table 5). This result agrees with previous works that evaluate the bifidogenic effect of different type of fibers (de Oliveira et al., 2023; Luo et al., 2023; Goñi et al., 2024).

Regarding to yeast population, the mean counts ranged from 3.2 log CFU/mL in the proximal colon to 1.1 log CFU/mL in distal colon, according to the results obtained on fungi in GI tracts of human by other authors (Li et al.,2018; Borges et al. 2018). The

positive effect of diary fiber on this population was evident with higher counts in all dietary fibers with respect to the control, especially in the case of pomegranate peel (Table 5).

3.5. SCFAs level during simulated digestion

The variation of the main SCFAs found during the simulated digestion, stomach, small intestine, and colon, is presented in Table 3, Table 4 and Table 5, respectively. After stomach digestion, the mean values of acetic, propionic and butyric acids were 77.9, 23.5, and 3.9 mM, respectively. The rest of SCFAs studied showed values lower than 0.7 mM at each stage of digestion for all fibers evaluated. At small intestine stage, taking into account that in our experiment the transfer of the digestion contents from the stomach to the small intestine involves a dilution by half, in general the levels of SFCAs experienced a notably increase for broccoli leaf, pomegranate peeland grape stem fiber, whereas in the case of fiber from control and tomato peel were similar for acetic and butyric acid (Table 4). Finally, considering again the dilution of the transfer of the contents from the small intestine to the colon, in all dietary fibers assayed acetic acid and butyric acid increased slightly, whereas the propionic acid increased notably up to around 10 times. These values of SFCAs were stable throughout colon digestion with mean values of 45.9, 1033 and 7.9 mM for acetic acid, propionic acid and butyric acid, respectively. Among dietary fibers, pomegranate peel fiber presented the highest average acetic and butyric acid values, without significant different respect to the control (Table 5). As other authors have pointed out, acetic, propionic and butyric acid are the main metabolites released during the fermentation process of prebiotic compounds (Koh et al., 2016). The sum of these metabolites is used as an indicator of fiber fermentability

(Jonathan et al., 2012). Based on our data, a selective growth stimulation of SFCAs producing bacteria was observed at small intestine and colon stages, especially for propionic acid-producing bacteria and at lower extend for butyric acid producing bacteria. The increase of SFCAs level in the intestine has been reported as playing an important role on human health. In particularly, Butyric acid has been the most widely studied since its prominent effects on health, it protects against colitis and colonic cancer and displays anti-inflammatory and immunomodulatory (Russel et al., 2013; Russo et al., 2019). Acetic acid is a precursor for butyric acid production and impact on lipid metabolism (Morrisson et al., 2016), whereas propionic acts as inhibitor of gluconeogenesis and cholesterol synthesis in the liver and exerts protection of human intestines against pathogens throughout its antibacterial and anti-inflamatory capacity (Markowiak-Kopeć, et al., 2020).

3.6.-Multivariate analysis

Principal component analysis (PCA) was carried out for stomach-small intestine (Figures 1 and 2) and colon stages (Figure 3 and 4) data to obtain an interpretable overview of the main information. In the case of PCA for stomach and small intestine digestion, principal components 1 (PC1), 2 (PC2), and 3 (PC3) explained a variability of 34.58, 17.24 and 14.00 %, respectively (Figures 1 and 2). PC1 was clearly related to digestion stage, being the highest values for microbial counts explained by the positive axis of PC1 and associated to samples of small intestine (Figure 1). On the contrary, samples of stomach were in negative axis of PC1 and associated to higher values of fiber, glucose, and antiproliferative activity. The variability associated to each dietary fiber were mainly explained by the PC2 and PC3 (Figure 2). High values of non-extractable phenols, antioxidant capacity, and DP3_1 were associated

to pomegranate peel fiber as opposed to the control, which is linked to production of some SCFAs such as acetic, propanoic and isobutyric acids. On the other hand, the tomato peel fiber was related to high values of Ram/Arb, DP2_2 and SCFAs such as acetic and isobutyric acids, in contrast to broccoli and grape stem fibers (Figure 1 and 2).

With respect to PCA for colon stages, the study of PC_1 (31.57% of variability) and PC_2 (24.00% of variability) corroborated the association of high values of non-extractable phenols and antioxidant capacity of the fiber obtained from the pomegranate peel, and the presence of higher amount of most of SCFAs in the control, together high counts in MRS media (Figure 3 and 4). In opposition to the control, broccoli and grape stem fibers were related to low values of SCFAs and high count of bifidobacteria, locating in the negative axis of PC_1. In this case, the factor “stage” was low influence in the global variability. In fact, PC_3 (14.02% of variability) was mainly associated to variability of tomato peel fiber, which was related to the highest values of fiber (Figure 3 and 4).

In conclusion, the source and composition of dietary fiber play a crucial role in determining its functional properties and behavior during intestinal transit. Notably, dietary fiber derived from pomegranate peel maintains its high phenol content throughout the entire process of digestion simulation in the colon. This persistence of phenols may enhance intestinal health owing to its heightened antioxidant activity. Similarly, dietary fiber sourced from broccoli and grape stem exhibits anti-proliferative activity within both the small intestine and colon, while also leading to significant modification in colonic microbiology. This type of dietary fiber favors the proliferation of bifidobacteria as opposed to LAB and high amount of SCFAs.

However, for a more comprehensive understanding of the impact of various types of dietary fiber on the microbial community comprising colonic microbiota, it is imperative to identify, quantify, and monitor them using High Throughput Sequencing techniques in future research endeavors.

Author Contributions

Conceptualization, A.M., M.G.C. and S.R.M; methodology, M.A.R., S.R.M. and M.V.H. formal analysis, M.A.R. and M.V.H.; investigation, M.A.R., R.C. and M.V.H.; resources, R.C., M.G.C. and A.M.; data management, R.C. and A.M.; writing—original draft preparation, M.A.R.; writing—review and editing, M.A.R., M.J.B., S.R.M. and A.M.; visualization, S.R.M. and A.M.; supervision, M.J.B., R.C., S.R.M. and A.M.; funding acquisition, M.G.C., A.M. and R.C.. All authors have read and agreed to the published version of the manuscript.

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Figure 1. Loading plot (A) and score plot (B) after principal component analysis of dietary fiber, fiber constituents (Cons), fiber properties (Prop), oligosaccharides (Oligos), SCFAs, microbial population (microb.), and antiproliferative activities (antip.) of the stomach (1_2) and small gut (2_2) samples in the planes defined by the two first principal components (PC1 and PC2). The variable codes are those defined in tables 3, 4 and 5.

Figure 2. Loading plot (A) and score plot (B) after principal component analysis of dietary fiber, fiber constituents (Cons), fiber properties (Prop), oligosaccharides (Oligos), SCFAs, microbial population (microb.), and antiproliferative activities (antip.) of the stomach (1_2) and small gut (2_2) samples in the planes defined by the first and third principal components (PC1 and PC3). The variable codes are those defined in tables 3, 4 and 5.

Figure 1. Loading plot (A) and score plot (B) after principal component analysis of dietary fiber, fiber constituents (Cons), fiber properties (Prop), oligosaccharides (Oligos), SCFAs, microbial population (microb.), and antiproliferative activities (antip.) of the colon samples (proximal 3_2, transverse 3_3, and distal 3_4) in the planes defined by the two first principal components (PC1 and PC2). The variable codes are those defined in tables 3, 4 and 5.

Figure 2. Loading plot (A) and score plot (B) after principal component analysis of dietary fiber, fiber constituents (Cons), fiber properties (Prop), oligosaccharides (Oligos), SCFAs, microbial population (microb.), and antiproliferative activities (antip.) of the colon samples (proximal 3_2, transverse 3_3, and distal 3_4) in the planes defined by the first and third principal components (PC1 and PC3). The variable codes are those defined in tables 3, 4 and 5.

Table 1. Simulate digestion program in in three bioreactor BIOSTAT A sytem: experimental conditions, reactive concentrations, and sampling description.

Stage	Conditions					Operation		
Time	Vol (mL)	Temp (ºC)	pH[1]	pO[2]	Stirr (rpm)	Type	Vol (mL)	Description
Digestion								
0:00:00	600	20	6-6.5	21	---	Sampling	-100	Initial sample
0:10:00	0					Transfer to	500	Stomach
Stomach (biorreactor 1)								
0:00:00	750	37	2.5	<0.5	150	Initial content	750	PBS
0:10:00	1350					Transfer from	500	Batch
						Supplementation	100	Pepsin 4.5%
						Sampling 1_1	-25	Microbial control
3:00:00	1225					***Sampling 1_2***	-100	Stomach sample
3:15:00	500					Transfer to	725	Small instestine
Small instestine (biorreactor 2)								
0:00:00	750	37	6.5	<0.5	150	Initial content	750	PBS
3:15:00	1475					Transfer from	750	Stomach
						Supplementation	100	Pancreatin (3%); Bilis (7.5%)
						Sampling 2_1	-25	Microbial control
7:00:00			6.5			Start pH grad.[3]		
11:00:00	1450		7			End pH grad.		
						Sampling 2_2	-100	Small instestine sample
11:15:00	500					Transfer to	950	Large gut
Colon (biorreactor 3)								
0:00:00	750	37	5.7	<0.5	150	Initial content	750	PBS
0:05:00	770					Supplementation	25	MRS
0:10:00	790					Supplementation	15	Feacal inoculum
11:00:00	750					Sampling 3_1	-40	Microbial control
11:15:00	1700					Transfer from	1000	Small intestine
			5.7			Start pH grad.		
24:00:00	1600					***Sampling 3_2***	-100	Ascending sample
31:00:00			6			pH grad. point		
48:00:00	1500					***Sampling 3_3***	-100	Transverse sample
50:00:00			6.4			pH grad. point		
70:00:00			6.8			End pH grad.		
72:00:00	1400					***Sampling 3_4***	-100	Descending sample

[1]Manual adjustment of the initial pH with HCl 5M and Na_2CO_3 2M; Automatic pH adjustment during the digestion process with HCl 0.5 M and Na_2CO_3 0.2M.
[2]Oxygen pressure adjusted with Nitrogen: CO_2 (99.5:0.5).
[3]Grad.: gradient

Table 2. Constituents and properties of feed supplemented with different types of dietary fiber prior to digestion.

	Control (Chickpea pasta)		Dietary fibers								p-Values
			Broccoli leaf		Pomegranate peel		grape stem		Tomato peel		
	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Dietary fiber (g/100g)	22.7	0.1	22.6	0.0	20.2	2.9	22.3	0.1	18.8	0.2	0.703
*Fiber constituents (*mg/g dietary fiber)											
GalA	578	9	588	10	686	46	669	109	526	8	0.121
Glc	34.3	0.2^{b}	27.2	0.5^{ab}	35.5	2.6^{b}	25.6	5.1^{a}	34.2	0.8^{b}	0.035
Xyl/Gal/Man	<0.5	0.00^{a}	2.12	0.09^{b}	2.43	0.13^{b}	2.21	0.28^{b}	<0.5	0.00^{a}	0.000
Rha/Ara	3.3	0.6	2.3	0.4	2.4	0.2	1.9	0.2	2.8	1.0	0.310
Fiber properties											
N-EPC (mg GAE/100 g)	1.6	0.1^{a}	3.7	0.1^{a}	83.5	8.2^{c}	15.7	1.0^{b}	2.2	0.2^{a}	0.000
ABTS (mg Trolox/100 g)	229	34^{ac}	261	25^{ad}	175	1^{a}	266	5^{bcd}	216	2^{ab}	0.025
DPPH (mg Trolox/100 g)	70	1^{ac}	55	5^{ab}	621	58^{d}	95	17^{bc}	23	8^{a}	0.000
Antiproliferative (% growth inhibition)	100.0	3.9	80.4	3.5	103.2	21.1	85.3	9.4	93.1	4.1	0.120

GalA: galacturonic acid; Glc: glucose; Xyl/Gal/Man: xylose/galactose/mannose; Rha/Ara: rhamnose/arabinose; N-EPC: non-extractable phenolic compounds. DPPH and ABTS: antioxidant capacity. [1]SD: standard deviation. [abcd] Values with different superscripts are significantly different ($p \leq 0.05$) between samples.

Table 3. Characterization of dietary fiber, oligosaccharides and SCFAs in digestion samples after stomach simulation (Sampling 2_1).

	Control (Chickpea pasta)		Dietary fibers								p-Values
			Broccoli leaf		Pomegranate peel		Grape stem		Tomato peel		
	Mean	SD[1]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Dietary Fiber (g/100g)	17.27	0.41^{b}	12.76	0.06^{a}	12.37	1.05^{a}	16.39	0.16^{b}	15.53	0.08^{b}	0.001
Fiber constituents (mg/g dietary fiber)											
GalA	577.9	9.5	587.9	10.2	686.1	45.8	669.3	108.8	526.2	7.8	0.568
Glc	34.3	0.2^{ab}	27.2	0.5^{a}	35.5	2.6^{b}	25.6	5.1^{ab}	34.2	0.8^{c}	0.001
Xyl/Gal/Man	<0.5	0.0^{a}	2.1	0.1^{b}	2.4	0.1^{b}	2.2	0.3^{b}	<0.5	0.0^{a}	0.000
Rha/Ara	3.3	0.6^{a}	2.3	0.4^{a}	2.4	0.2^{a}	1.9	0.2^{a}	2.8	1.0^{b}	0.335
Fiber properties											
N-EPC (mg GAE/100 g)	0.79	0.09^{a}	1.99	1.29^{a}	62.31	2.97^{c}	12.89	0.59^{b}	1.68	0.21^{a}	0.000
ABTS (mg Trolox/100 g)	216	6^{ab}	387	13^{c}	933	31^{d}	387	43^{ac}	291	5^{b}	0.000
DPPH (mg Trolox/100 g)	111.1	3.7^{a}	62.7	9.9^{a}	636.4	23.5^{c}	235.1	15.1^{b}	90.7	1.7^{a}	0.000
Antiproliferative[2]	105.1	4.5	80.0	3.3	105.0	33.7	87.6	19.3	92.2	8.6	0.291
Oligosaccharides (g/L)											
DP7	6.42	0.43^{b}	2.29	0.28^{a}	23.60	0.04^{e}	11.73	0.34^{c}	19.99	0.66^{d}	0.000
DP6	0.04	0.02	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.006
$DP5_1$	0.00	0.00	0.04	0.01	0.02	0.00	0.08	0.00	0.05	0.01	0.000
$DP5_2$	0.10	0.00	0.04	0.00	0.08	0.01	0.00	0.00	0.06	0.00	0.000
$DP4_1$	0.95	0.01^{b}	0.56	0.01^{a}	0.20	0.15^{a}	0.92	0.02^{b}	0.86	0.01^{b}	0.000
$DP4_2$	0.00	0.00^{a}	0.67	0.04^{b}	0.66	0.09^{b}	1.16	0.07^{d}	1.02	0.01^{c}	0.000
$DP3_1$	1.72	0.06^{b}	1.01	0.02^{a}	2.78	0.05^{c}	1.05	0.04^{a}	1.08	0.01^{a}	0.000
$DP3_2$	0.93	0.02^{c}	0.00	0.00^{a}	0.00	0.00^{a}	0.85	0.00^{b}	0.00	0.00^{a}	0.000
$DP2_1$	1.14	0.00^{b}	1.07	0.00^{a}	4.54	0.04^{d}	1.76	0.01^{c}	1.10	0.00^{ab}	0.000
$DP2_2$	1.60	0.02^{b}	2.57	0.01^{b}	6.83	0.04^{b}	0.00	0.00^{a}	2.07	0.00^{b}	0.000
$DP2_3$	0.00	0.00^{a}	0.00	0.00^{a}	0.00	0.00^{a}	0.00	0.00^{a}	4.64	0.00^{b}	0.000
SCFAs (mM)											
Acetic acid (Ac_A)	191.08	18.22^{c}	20.44	0.33^{a}	60.17	0.64^{ab}	18.51	0.30^{a}	99.36	18.24^{b}	0.000
Propionic acid (Pro_A)	34	9^{b}	32	9^{a}	33	9^{c}	33	9^{d}	24	9^{e}	0.000
Butiric acid (Bu_A)	5.49	1.20	2.09	0.13	3.74	0.59	1.77	0.27	6.42	1.45	0.012
Isovaleric acid (Isov_A)	0.63	0.12^{b}	0.08	0.00^{a}	0.19	0.02^{a}	0.06	0.01^{a}	0.05	0.01^{a}	0.001
Isobutiric acid (Isob_A)	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.11	0.502
Isocaproic acid (Isoc_A)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.22	1.72	0.486

GalA: galacturonic acid; Glc: glucose; Xyl/Gal/Man: xylose/galactose/mannose; Rha/Ara: rhamnose/arabinose; N-EPC: non-extractable phenolic compounds. DPPH and ABTS: antioxidant capacity; DP: polymerization degree. [1]SD: standard deviation. [abcde] Values with different superscripts are significantly different ($p \leq 0.05$) between samples.[2] % growth inhibition.

Table 4. Characterization of dietary fiber, oligosaccharides and SCFAs in digestion samples after small intestine simulation (Samplig 2_2).

	Control (Chickpea pasta)		By-products								*p-Values*
			Broccoli leaf		Pomegranate peel		Grape stem		Tomato peel		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Fiber (g/100 g)	7.03	± 0.02^{b}	5.88	± 0.03^{a}	6.53	± 0.28^{b}	8.56	± 0.04^{c}	8.97	± 0.07^{c}	0.000
Fiber constituents (mg/g dietary fiber)											
GalA	626.8	± 59.4	613.1	± 0.2	699.3	± 56.3	565.0	± 7.0	708.4	± 161.7	0.442
Glc	22.6	± 2.4	18.8	± 0.0	21.9	± 2.1	18.1	± 0.3	23.6	± 5.9	0.384
Xyl/Gal/Man	2.6	± 0.2^{a}	3.9	± 0.0^{ab}	4.0	± 0.6^{b}	2.9	± 0.0^{ab}	2.8	± 0.5^{ab}	0.023
Rha/Ara	8.9	± 1.1^{b}	4.9	± 0.1^{a}	6.5	± 1.1^{ab}	3.9	± 0.2^{a}	5.5	± 1.3^{ab}	0.018
Fiber properties											
N-EPC (mg GAE/100 g)	1.24	± 0.13^{a}	1.23	± 0.21^{a}	75.81	± 2.86^{b}	6.25	± 0.16^{a}	4.25	± 0.48^{a}	0.000
ABTS (mg Trolox/100 g)	356	± 18^{a}	310	± 3^{a}	708	± 55^{b}	351	± 51^{a}	246	± 11^{a}	0.000
DPPH (mg Trolox/100 g)	27.0	± 16.7^{a}	3.2	± 1.7^{a}	860.6	± 63.9^{c}	149.0	± 9.8^{b}	2.8	± 1.5^{a}	0.000
Antiproliferative (% growth inhibition)	99.0	± 1.5^{b}	76.5	± 3.2^{a}	55.6	± 6.1^{a}	56.9	± 10.5^{a}	82.2	± 13.7^{ab}	0.002
Oligosaccharides (g/L)											
DP7	18.59	± 0.00^{b}	1.74	± 0.25^{a}	25.50	± 0.02^{d}	22.68	± 0.01^{c}	22.14	± 0.04^{e}	0.000
DP6	0.28	± 0.00^{b}	0.00	± 0.00^{a}	0.00	± 0.00^{a}	1.05	± 0.14^{c}	0.00	± 0.00^{a}	0.000
$DP5_1$	0.00	± 0.00^{a}	0.00	± 0.00^{a}	0.59	± 0.01^{a}	0.47	± 0.11^{b}	1.18	± 0.17^{c}	0.000
$DP5_2$	0.79	± 0.05^{d}	0.26	± 0.00^{b}	0.00	± 0.00^{a}	0.00	± 0.00^{a}	0.67	± 0.13^{c}	0.000
$DP4_1$	0.52	± 0.17^{c}	0.00	± 0.00^{a}	0.26	± 0.01^{d}	0.11	± 0.01^{b}	0.22	± 0.03^{c}	0.008
$DP4_2$	0.00	± 0.00^{a}	0.44	± 0.00^{b}	0.54	± 0.00^{b}	0.43	± 0.01^{b}	0.69	± 0.01^{c}	0.000
$DP3_1$	1.17	± 0.00^{c}	0.90	± 0.02^{a}	2.73	± 0.00^{d}	0.88	± 0.01^{a}	1.07	± 0.01^{b}	0.000
$DP3_2$	0.90	± 0.00^{b}	0.00	± 0.00^{a}	0.00	± 0.00^{a}	1.23	± 0.01^{c}	0.00	± 0.00^{a}	0.000
$DP2_1$	1.84	± 0.01^{c}	0.00	± 0.00^{a}	3.37	± 0.00^{d}	12.36	± 0.10^{e}	1.07	± 0.00^{b}	0.000
$DP2_2$	13.06	± 0.11^{b}	0.00	± 0.00^{a}	19.28	± 0.10^{d}	0.00	± 0.00^{a}	17.37	± 0.05^{c}	0.000
SCFAs (mM)											

Acetic acid (Ac_A)	104.62 ± 0.15[b]	36.83 ± 12.5[a]	65.31 ± 8.37[ab]	24.03 ± 7.90[a]	50.10 ± 16.9[a]	0.004
Propionic acid (Pro_A)	60 ± 9	59 ± 9	60 ± 9	60 ± 9	51 ± 9	0.000
Butiric acid (Bu_A)	2.34 ± 0.83	5.33 ± 1.19	3.30 ± 0.02	1.80 ± 0.23	3.80 ± 1.24	0.051
Isovaleric acid (Isov_A)	0.31 ± 0.13[b]	0.02 ± 0.01[a]	0.13 ± 0.01[a]	0.14 ± 0.08[d]	0.07 ± 0.09[a]	0.084
Isobutiric acid (Isob_A)	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.065
Isocaproic acid (Isoc_A)	0.06 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.03	0.550
Valeric acid (Val_A)	0.17 ± 0.10	0.01 ± 0.01	0.11 ± 0.01	0.03 ± 0.04	0.11 ± 0.05	0.124
Microbial population (Log CFU/mL)						
Total viable counts (TVC)	8.91 ± 0.04[b]	9.18 ± 0.04[c]	7.81 ± 0.05[a]	9.42 ± 0.01[d]	8.77 ± 0.00[b]	0.000
Enterobacteria (Ent_B)	8.54 ± 0.31[b]	9.35 ± 0.07[c]	7.81 ± 0.05[a]	9.22 ± 0.06[c]	9.00 ± 0.00[a]	0.001
Lactic acid bacteria (LAB)	5.18 ± 0.02[b]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.000
Enterococci (Ent_c)	4.19 ± 1.26[ab]	5.93 ± 0.01[bc]	3.00 ± 0.00[a]	7.39 ± 0.08[c]	4.94 ± 0.19[ab]	0.004
Staphylococci (Sta)	4.95 ± 0.14[a]	6.11 ± 0.01[b]	5.76 ± 0.14[b]	7.47 ± 0.15[c]	7.14 ± 0.03c	0.000

GalA: galacturonic acid; Glc: glucose; Xyl/Gal/Man: xylose/galactose/mannose; Rha/Ara: rhamnose/arabinose; N-EPC: non-extractable phenolic compounds. DPPH and ABTS: antioxidant capacity; DP: polymerization degree. [1]SD: standard deviation. [abcde] Values with different superscripts are significantly different ($p \leq 0.05$) between samples.

Table 5. Characterization of dietary fiber, oligosaccharides and SCFAs in digestion samples throughout colon stage.

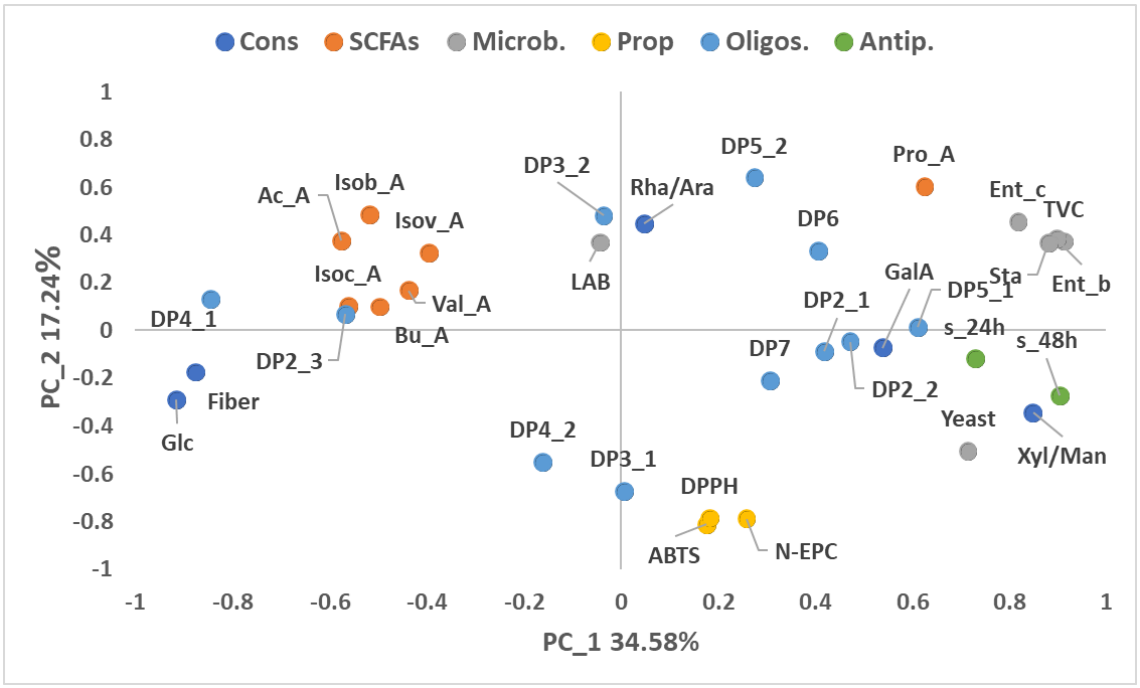
	Dietary fibers					Colon stages			Total			
	Control	Broccoli leaf	Pomegranate peel	Grape stem	Tomato peel	proximal	transverse	Distal	Mean	SD[1]	Min	Max
Fiber (g/100g)	5.9[b]	4.6[a]	5.9[b]	6.6[b]	7.6[c]	6.9[b]	5.9[a]	5.6[a]	6.1	1.2	3.7	8.5
Fiber constituents (mg/g dietary fiber)												
GalA	600.3	593.0	659.8	589.4	690.6	626.8	625.4	627.7	626.6	75.1	560.6	802.9
Glc	20.8[c]	13.2[ab]	17.8[bc]	11.8[a]	26.6[d]	19.3[b]	19.1[ab]	15.8[a]	18.1	6.5	8.2	32.8
Xyl/Gal/Man	2.4[a]	3.0[ab]	3.9[c]	2.5[ab]	3.2[b]	3.1	3.1	2.8	3.0	0.8	1.9	4.7
Rha/Ara	8.5[d]	3.0[b]	5.7[c]	1.3[a]	7.0[cd]	5.4[b]	5.8[b]	4.0[a]	5.1	3.0	0.7	10.4
Fiber properties												
N-EPC (mg GAE/100 g)	3.7[a]	2.3[a]	39.3[c]	6.3[b]	3.2[a]	11.1[b]	13.0[c]	8.8[a]	10.9	14.8	1.3	45.7
ABTS (mg Trolox/100 g)	302[ab]	345[b]	732[d]	436[c]	274[a]	405[a]	447[b]	402[a]	418	186	246	806
DPPH (mg Trolox/100 g)	20[a]	22[a]	534[c]	99[b]	16[a]	141[b]	160[b]	115[a]	138	208	4	657
Antiproliferative (% growth inhibition)	103.14[c]	80.012a[b]	89.475[abc]	77.328[a]	96.861[bc]	95.653[b]	80.337[a]	91.602[ab]	89.32	15.10	52.75	112.44
SCFAs (mM)												
Acetic acid (Ac_A)	82.1[b]	27.6[a]	70.8[b]	17.6[a]	31.6[a]	47.7	44.7	45.4	45.9	28.5	12.1	100.5
Propionic acid (Pro_A)	873	712	1033	1194	1354	1006	1033	1060	1033	232	679	1387
Butiric acid (Bu_A)	13.0[c]	5.4[a]	10.4[bc]	3.8[a]	7.1[ab]	7.8	7.7	8.4	7.9	4.2	2.8	17.1
Isovaleric acid (Isov_A)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Isobutiric acid (Isob_A)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Isocaproic acid (Isoc_A)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Caproic acid (Cap_A)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Valeric acid (Val_A)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Microbial population (Log CFU/mL)												
Total viable counts (TVC)	8.9	8.6	9.0	7.9	8.7	8.4	8.8	8.7	8.6	1.1	3.0	9.7
Enterobacteria (Ent_b)	8.0[a]	8.4[b]	8.8[c]	8.7[c]	8.8[c]	8.6[b]	8.6[b]	8.4[a]	8.5	0.4	7.5	9.1

	Dietary fibers					Colon stages			Total			
	Control	Broccoli leaf	Pomegranate peel	Grape stem	Tomato peel	proximal	transverse	Distal	Mean	SD[1]	Min	Max
Lactic acid bacteria (LAB)	5.4^{d}	0.0^{a}	2.4^{b}	0.0^{a}	2.9^{c}	3.2^{c}	2.2^{b}	1.1^{a}	2.1	2.8	0.0	7.8
Enterococci (Ent_c)	5.8^{a}	6.4^{b}	7.5^{c}	5.8^{a}	7.4^{d}	6.7	6.5	6.5	6.6	0.9	4.3	8.0
Staphylococci (Sta)	5.3^{c}	3.9^{b}	7.3^{d}	3.6^{a}	7.0^{d}	6.0^{b}	6.4^{c}	3.9^{a}	5.4	2.3	0.0	7.3
Bifidobacteria (Bf)	1.6^{a}	5.1^{c}	2.8^{b}	5.4^{d}	5.2^{cd}	3.2^{a}	4.9^{c}	3.9^{b}	4.0	2.1	0.0	5.7

GalA: galacturonic acid; Glc: glucose; Xyl/Gal/Man: xylose/galactose/mannose; Rha/Ara: rhamnose/arabinose; N-EPC: non-extractable phenolic compounds. DPPH and ABTS: antioxidant capacity; DP: polymerization degree. [1]SD: standard deviation. ^{abcde} Values with different superscripts are significantly different ($p \leq 0.05$) between samples.

Figure 1

A


Cons
SCFAs
Microb.
Prop
Oligos.
Antip.
PC_2 17.24%
PC_1 34.58%
Ac_A
Isob_A
Isov_A
Isoc_A
Val_A
Bu_A
DP2_3
DP4_1
Fiber
Glc
DP3_2
LAB
Rha/Ara
DP5_2
DP6
Pro_A
Ent_c
TVC
Sta
Ent_b
GalA
DP5_1
DP2_1
s_24h
s_48h
DP7
DP2_2
Yeast
Xyl/Man
DP4_2
DP3_1
DPPH
ABTS
N-EPC

B

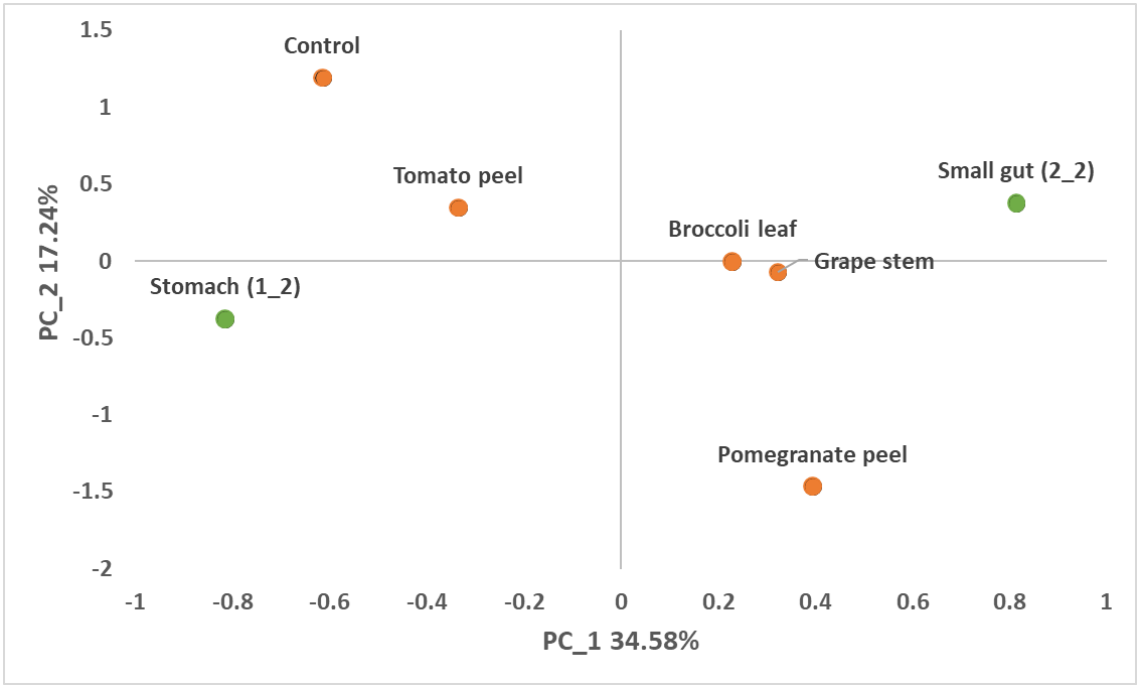
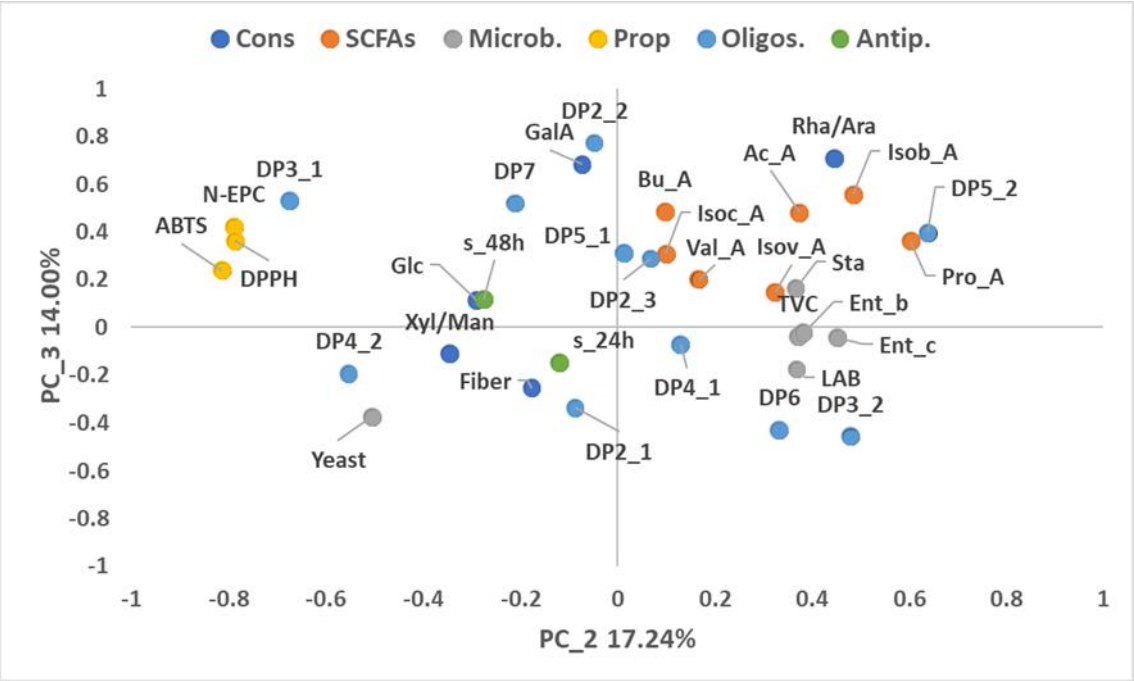

PC_2 17.24%
PC_1 34.58%
Control
Tomato peel
Small gut (2_2)
Broccoli leaf
Grape stem
Stomach (1_2)
Pomegranate peel

Figure 2

A


Cons
SCFAs
Microb.
Prop
Oligos.
Antip.
DP2_2
GalA
Rha/Ara
Ac_A
Isob_A
DP3_1
DP7
Bu_A
DP5_2
N-EPC
Isoc_A
ABTS
DP5_1
Val_A
Isov_A
Sta
s_48h
Glc
Pro_A
DPPH
DP2_3
TVC
Ent_b
Xyl/Man
DP4_2
s_24h
Ent_c
DP4_1
LAB
Fiber
DP6
DP3_2
Yeast
DP2_1
PC_3 14.00%
PC_2 17.24%

B

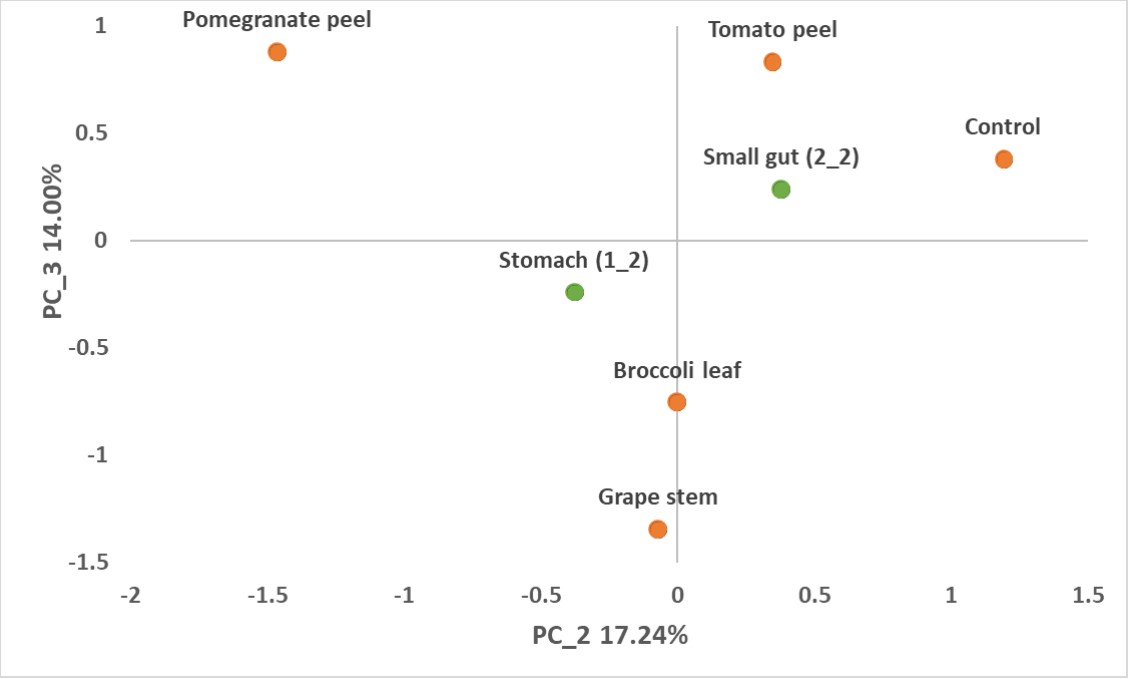
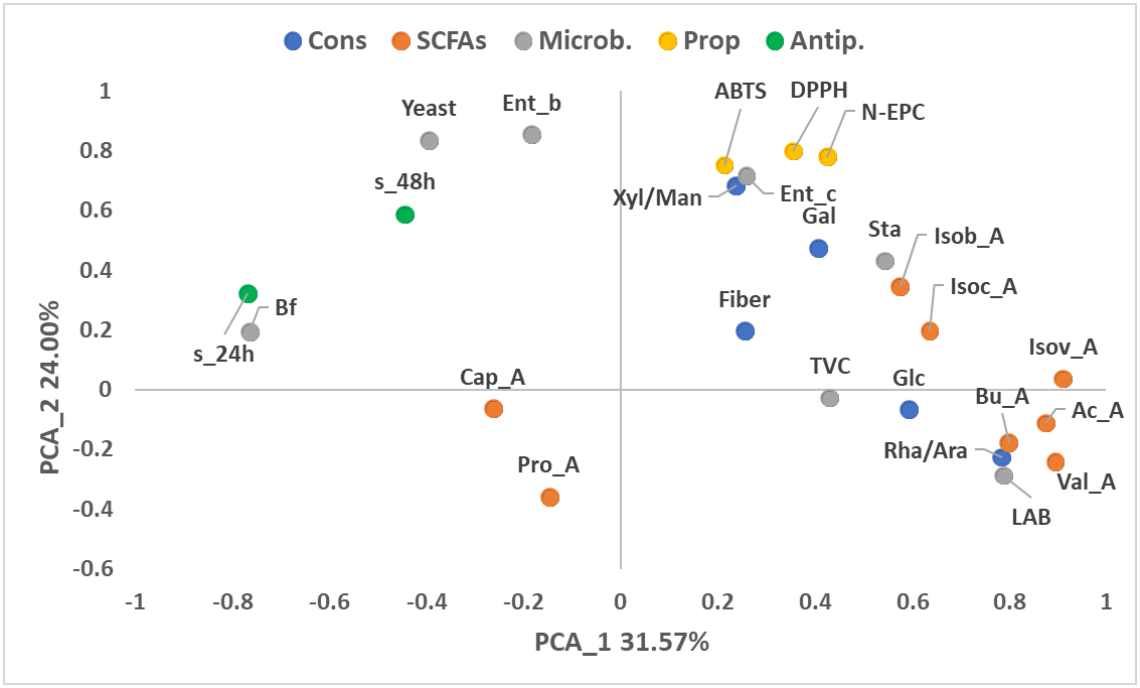

Pomegranate peel
Tomato peel
Control
Small gut (2_2)
Stomach (1_2)
Broccoli leaf
Grape stem
PC_3 14.00%
PC_2 17.24%

Figure 3

A


Cons
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Microb.
Prop
Antip.
ABTS
DPPH
N-EPC
Yeast
Ent_b
s_48h
Xyl/Man
Ent_c
Gal
Sta
Isob_A
Isoc_A
Bf
s_24h
Fiber
Isov_A
Cap_A
TVC
Glc
Bu_A
Ac_A
Rha/Ara
Pro_A
Val_A
LAB
PCA_2 24.00%
PCA_1 31.57%

B

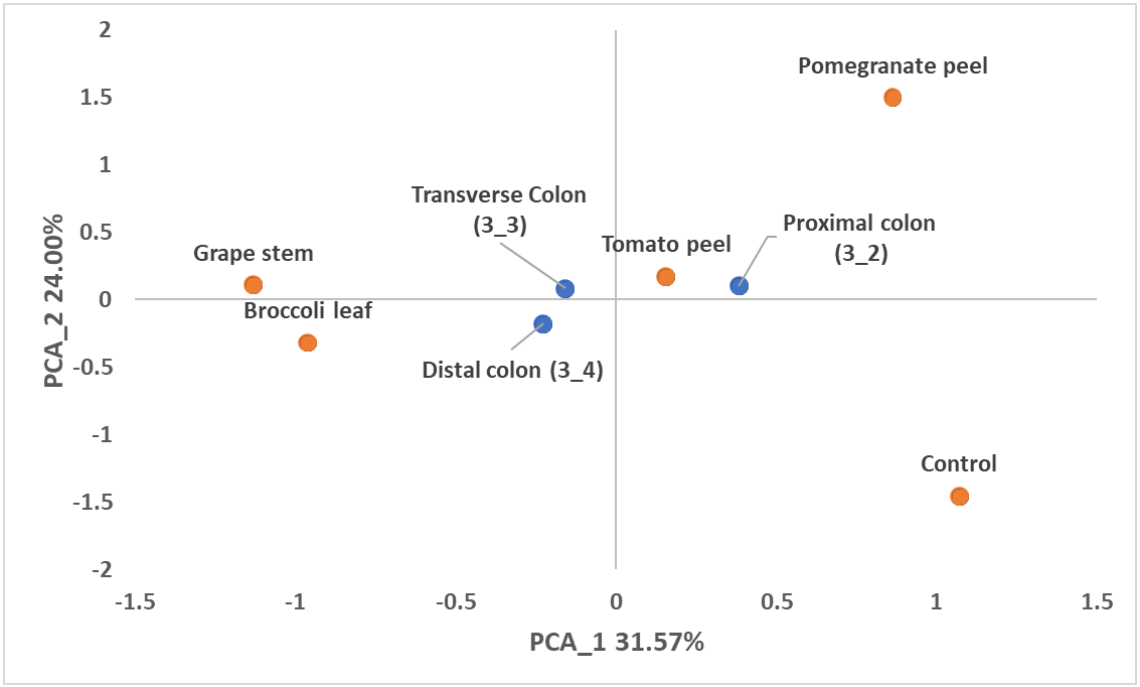
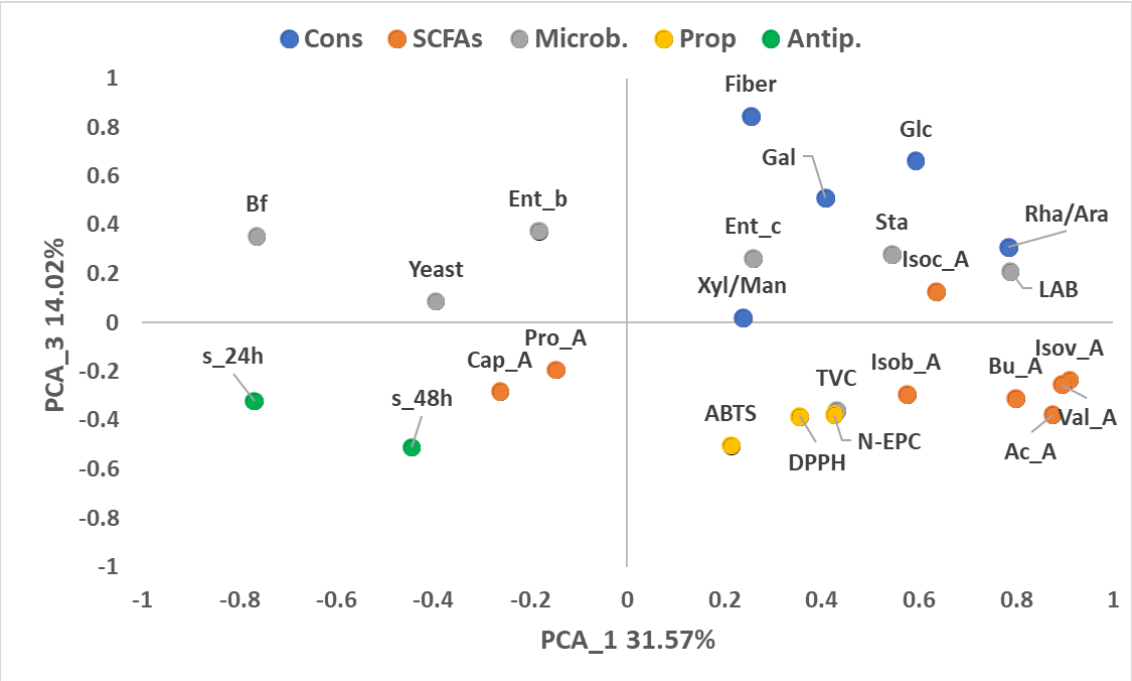

Pomegranate peel
Transverse Colon (3_3)
Proximal colon (3_2)
Grape stem
Tomato peel
Broccoli leaf
Distal colon (3_4)
Control
PCA_2 24.00%
PCA_1 31.57%

Figure 4

A


Cons
SCFAs
Microb.
Prop
Antip.
Fiber
Glc
Gal
Bf
Ent_b
Ent_c
Sta
Rha/Ara
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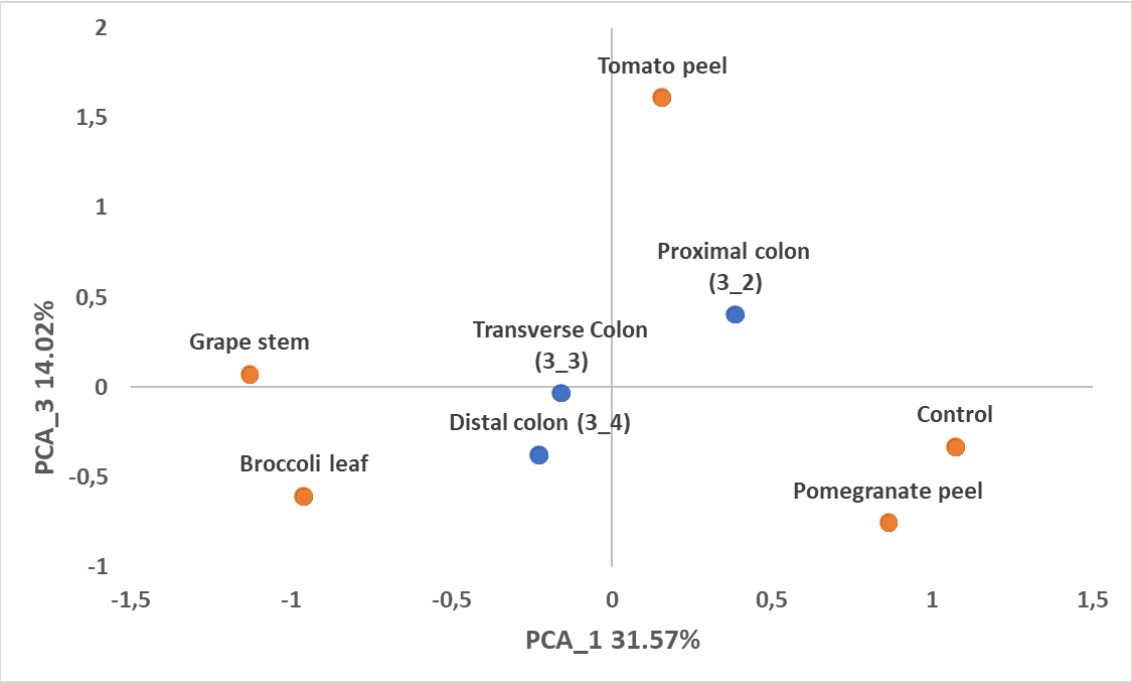
Yeast
Xyl/Man
LAB
Pro_A
Isov_A
s_24h
Cap_A
TVC
Isob_A
Bu_A
s_48h
ABTS
Val_A
N-EPC
DPPH
Ac_A
PCA_3 14.02%
PCA_1 31.57%

B


Tomato peel
Proximal colon
(3_2)
Grape stem
Transverse Colon
(3_3)
Distal colon (3_4)
Control
Broccoli leaf
Pomegranate peel
PCA_3 14.02%
PCA_1 31.57%

CAPÍTULO 7

Effect of applying wine lees dietary fiber as sugar replacer on the quality of tomato sauces

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Abstract

This study evaluated the use of dietary fiber from wine lees as a sugar replacement in tomato sauces, analyzing their physicochemical, nutritional, functional, and sensory characteristics. Nine tomato sauces were prepared: one full sugar (FS) and eight with reduced sugar (SR). Four SR sauces were formulated by replacing sugar with directly solubilized fiber, and the other four used solubilized and lyophilized fiber at varying levels to achieve 25% (SR25), 50% (SR50), 75% (SR75), and 100% (SR100) sugar reduction. All sauces were stored at 4°C for 12 days. Physicochemical (moisture, ash, pH, acidity, soluble solids), nutritional (total sugars, dietary fiber), and functional properties (swelling capacity, water retention capacity, fat absorption capacity, glucose absorption capacity) were analyzed every 3 days during storage. Finally, sensory evaluation assessed the visual appearance, taste, aroma, and overall acceptability of the sauces at day 0 and day 12. The results indicated that dietary fiber addition significantly impacted several characteristics, including pH, acidity, soluble solids, sugar content, and total dietary fiber. Additionally, changes were observed in antioxidant capacity and the polyphenol profile. The sauces exhibited promising functionalities, such as improved water holding capacity and fat binding ability (swelling and water retention capacity, fat absorption capacity). The sensory evaluation revealed good consumer acceptance for sauces modified with dietary fiber, particularly those with 25% sugar reduction (SR25).

These findings highlight the potential of utilizing by-products like wine lees to enhance the nutritional and functional quality of food products, while promoting sustainability in the food industry.

Keywords: Sugar reduction, by-products, dietary fibre, functional properties, tomato sauces

1. Introduction

The tomato industry plays a crucial role in vegetable processing at both the European and global levels (World Processing Tomato Council, 2016). Tomato-based products are widely used as ingredients in a variety of manufactured foods, including soups, ketchup, and sauces (Vallverdú-Queralt et al., 2014; Bureau et al., 2020; Yu et al., 2023). Tomatoes and their derivatives are rich in bioactive compounds such as vitamins (Hernández et al., 2007), carotenoids (Knockaert et al., 2012), flavonoids (Briones-Labarca et al., 2019), and minerals (Fragni et al., 2018). Previous studies have demonstrated a significant correlation between the consumption of tomatoes and their derivatives, and a decreased risk of developing cardiovascular diseases and certain types of cancer (Tomas et al., 2017; Tomas et al., 2018). However, it is worth noting that commercial tomato sauces often contain high concentrations of fructose or glucose (El Haggar et al., 2023). Additionally, commercial tomato sauces often lack significant amounts of protein and dietary fiber (Rani and Khetarpaul, 2009).

The current public health framework emphasizes the importance of increasing dietary fiber intake and reducing simple sugar consumption as part of a balanced diet (World Health Organization, 2015; Bates et al., 2020). Dietary fiber is composed of a mixture of plant carbohydrate polymers, both oligosaccharides and polysaccharides, and is classified as either water-soluble or insoluble (Johansson et al., 2000; Chen et al., 2021). Today, the health benefits of dietary fiber are widely known. Increased dietary fiber intake has been reported to reduce the risk of chronic diseases, including obesity (Moyano et al., 2016; Lambert et al., 2017; Delzenne et al., 2020; Ni et al., 2023), cardiovascular diseases (McRae, 2007; Satija and Hu, 2012), type 2 diabetes (Mao et al., 2021), gastrointestinal diseases (Ribeiro et al., 2021; Day et al., 2021), and certain types of cancer (Mao et al., 2017; Zheng et al., 2018).

Replacing simple sugars with dietary fiber in food is considered an effective strategy to address this recommendation. Polysaccharides from dietary fiber have been shown to be effective substitutes for simple sugars due to their properties as sweetening (Gerschenson et al., 2017). Rodriguez-Rodriguez-Garcia et al. (2022) compared the effectiveness of four types of soluble fibers, two inulin and two dextrose fibers, as potential sucrose substitutes in short-dough biscuits. They evaluated how these fibers affected dough rheology, biscuit dimensions, texture, color, and sensory profile. The results revealed that soluble fibers could successfully replace sugar in these biscuits, significantly reducing sucrose content compared to the control. Although minor changes in organoleptic properties were observed, all sugar-reduced biscuits were found to be noticeably firmer and crispier in their sensory profile. Carcelli et al. (2021) developed and

characterized three sugar-reduced strawberry sauce recipes (30 %, 50 %, and 70 %), replacing sugar with a semi-solid fiber syrup. The results showed that in addition to reducing sugar content, dietary fiber content increased from 0 g/100 g of sauce to 31.5 g/100 g of sauce, allowing them to claim, "reduced sugar" and "high in dietary fiber" on the product label.

According to the 2030 Agenda for Sustainable Development, the inclusion of by-products in the human diet can be a valid option for finding a balance between human health and environmental impact (United Nations Resolution, 2022). Rivas et al, (2021a) observed that winemaking by-products, such as lees, stand out for their high concentration of dietary fiber, neutral sugars and antioxidant compounds. They represent approximately 25% of the waste generated in the winemaking process (De Iseppi et al., 2020). In addition, they present good functional properties that make them suitable for use in the production of ingredients for the development of new food products.

Therefore, the aim of this study was to evaluate the use of dietary fiber obtained from wine lees to replace or decrease the use of reducing sugars in the elaboration of tomato sauces, evaluating their physicochemical, nutritional, functional and sensory characteristics.

2. Materials and Methods

2.1. Plant material

For the preparation of the sauces, tomato concentrates, sucrose and fiber obtained from lees from the first fermentation of white wine as sugar replace were used were used. These samples were supplied by industries in the Extremadura region, Spain. The wine lees were lyophilized (LyoBeta, Telstar, Barcelona, Spain) and vacuum-packed at room temperature for later use. Meanwhile, the tomato concentrates were stored under refrigeration until their subsequent use for sauce preparation.

2.2. Sauces manufacturing

The fiber used as sugar replacers was extracted from the vinification lees samples was carried out using the Alcohol Insoluble Residue (AIR) method as described by Femenia et al. (2003) and modified by Rivas et al. (2021a). Once the AIR was extracted from the vinification lees, it was solubilized (autoclaved at 121°C for 16 minutes, followed by ultrasound treatment for one hour). Solubilized fiber was added to the sauces in two ways: directly solubilized and lyophilized. The absence of reducing sugars was verified in the composition of the solubilized fiber before use.

Nine tomato sauces were prepared, one with full sugar (FS) and eight with reduced sugar (SR), following an industrial recipe. Four of the SR tomato sauces were obtained by replacing sugar with directly solubilized fiber and another 4 with solubilized and lyophilized fiber at different percentages to reach the 25 % (SR25), 50 % (SR50), 75 % (SR75) and 100 % (SR100) sugar reduction.

All produced tomato sauces were stored at 4°C for 12 days. Physicochemical, nutritional, and functional, were performed in triplicate every 3 days during storage period. The sensory analysis was performed at 0 any 12 days.

2.3. Characterisation of tomato sauces

2.3.1. Physicochemical analyses

Determinations of moisture and ash content were carried out following the methods of AOAC International (2005), where the samples were dried at 105°C and 500°C, respectively, until a constant weight was achieved. pH and total acidity were determined according to the ISO 2917:1999 standard using an automatic titrator (METROHM mod. 855 Robotic Titrosampler), expressing the results of total acidity in g/100 g of citric acid. Soluble solids content was measured using a refractometer (Mettler Toledo), and the results were expressed in °Brix.

2.3.2. Determination of soluble sugars

The soluble sugars in the sauces were extracted with distilled water and then the total soluble sugars (STT) and reducing sugars (RS) were determined. The determination of TTS was carried out using the sulfuric acid-UV method proposed by Albalasmeh et al. (2013), while reducing sugars were determined using the dinitrosalicylic acid (DNS) method (Miller, 1959), calibrated with standard glucose solutions. All results are expressed in g/100 g of dry sample.

2.3.3. Total phenolic compound content

Total phenolic compounds (TPC) from the sauces were extracted using the method outlined by Casquete et al. (2015). In summary, 5 g of sauce were mixed with 30 mL of ethanol/water (80:20, v/v) and stirred on a magnetic mixer for 1 hour at room temperature (25°C) in the dark. This process was repeated twice. Excess ethanol was removed by vacuum evaporation at 37°C using a rotary evaporator.

Total phenolic content was determined using Folin-Ciocalteu reagent (Wettasinghe and Shahidi, 1999) on a UV-1800 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA), with gallic acid as the standard. Results are expressed in mg gallic acid equivalents (GAE) per 100 g of extract.

123 *2.3.4. Antioxidant activity*

124 The antioxidant activity of the sauce extracts (10 mg of extract/mL ethanol) was assessed using
125 the DPPH (2,2-diphenyl-1-picrylhydrogen oxide depletion) method (Teixeira et al., 2009) and the ABTS
126 radical (2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) scavenging method (Re et al., 1999).
127 Antioxidant activity was expressed in mg of Trolox/ 100 g of extract.

128 *2.3.5. Total dietary fibre content and neutral sugar and galacturonic acid*

129 Total dietary fibre from sauces was extracted by the alcohol insoluble residue (AIR) metho
130 described above in section 2.2. The results were expressed in g/100 g of sauce.

131 At 0 and 12 days storage, the AIR of the sauces underwent hydrolysis using 12 M sulfuric acid (3
132 hours at room temperature followed by 1 hour at 100°C). After hydrolysis, the hydrolysed monosaccharides
133 and galacturonic acids were quantified using HPLC.

134 HPLC analysis was conducted using an Agilent LC 1260 Infinity II HPLC system (Waters,
135 Milford, MA, USA), including a separation module and a refractive index detector. A Rezex-ROA column
136 (7.8mm ID x 150mm; Phenomenex, Torrance, CA, USA) was used. In isocratic mode, water was used as
137 the mobile phase at a flow rate of 0.6 mL/min. Sample injection volume was 10 µL, with the column and
138 detector temperatures set to 80°C and 40°C, respectively.

139 *2.5. Functional properties of fiber from tomato sauces*

140 *2.5.1. Swelling, water holding capacity and fat absorption capacity*

141 The swelling capacity (Sw), water retention capacity (WRC), and fat absorption capacity (FAC)
142 of the extracted dietary fiber (AIR) were determined following the method by Garau et al. (2007).

143 For Sw determination, 0.1 g of AIR was placed in a calibrated cylinder, and 10 mL of distilled
144 water was added. After 24 hours of incubation at room temperature (20-25°C), the increase in volume was
145 measured and expressed in mL water/g AIR.

146 To determine WRC, 0.2 g of AIR was hydrated in 10 mL of distilled water and left for 24 hours
147 at room temperature. Subsequently, the sample was centrifuged at 2000 g for 25 minutes, and the weight
148 of the resulting solid residue was measured. WRC is expressed in g water /g AIR.

149 For FAC determination, the process was similar to WRC determination, but using 5 mL of
150 sunflower oil instead of water. The resulting weight of oil absorbed by the AIR was measured and expressed
151 in g oil/g AIR.

152

153 *2.5.2. Glucose absorption capacity*

154 The glucose absorption capacity (GAC) of dietary fiber was estimated according to the method
155 described by Niu et al., (2018) with some modifications. Specifically, 0.1 g of AIR from the sauces was
156 mixed with 25 mL of a 50 mM sample glucose solution, which incubated at 37° C for 6 h with constant
157 shaking and then centrifuged at 4500 g for 10 min. The glucose content of the supernatant was analyzed
158 using HPLC equipment. GAC is expressed in terms of mg retained glucose/mg dietary fibre.

159 *2.5.3. Antioxidant activity (non-extractable polyphenol N-EPC)*

160 The antioxidant capacity of the non-extractable polyphenols bound to AIR was carried out by
161 means of a previous extraction of the phenolic compounds from the cell walls, this extraction was carried
162 out following the method described by Arranz et al. (2009) with some modifications. 0.5 g of AIR was
163 mixed with 20 mL of a methanol / water solution (50:50) acidified with hydrochloric acid to pH 2. The
164 mixture was incubated with shaking for 1 hour and subsequently centrifuged for 20 minutes at 2500 g. This
165 process was repeated with an acetone / water solution (70:30). Excess solution was removed by heating to
166 37 ºC in a vacuum rotary evaporator (Hei-VAP Precision, Heidolph, Germany). The resulting residue was
167 resuspended in 30 mL of distilled water. The determinations of content of non-extractable phenolic
168 compounds and their antioxidant capacity were previously described in the section 2.3.4 y 2.3.3.

169 *2.6. Colour*

170 The color of the sauces was measured at 0 and 12 days with a colorimeter (Konica Minolta model
171 CM-600d) using the standard D65 illuminator. The results were expressed in terms of the CIELab scale
172 parameters: (L *: luminosity; a *: red-green coordinate; b *: yellow-blue coordinate) calculating the global
173 chromaticity of the sample, saturation or color intensity (C *) and Hue angle or pitch (H *).

174 *2.7. Sensory analysis*

175 Sensory properties and acceptability of tomato sauces were studied using a quantitative descriptive
176 analysis and hedonic test on both fresh and stored sample (time 0, 60 days). Tomato sauce samples were
177 identified with a random number and introduced to 15 trained judges to evaluate differences in visual
178 appearance (color intensity and appearance), flavor (sweetness, and sourness) and aroma (sweet aroma acid
179 aroma, unpleasant smell) parameters. The attributes were scored according to a scale from 0 to 10. Judges
180 were allowed to drink water between the samples to cleanse the palate. In addition, a hedonic test was
181 carried out where the samples were compared randomly, evaluating their acceptability in groups of two so
182 that all the lots were compared to each other. The rating scale used was 0 to 10.

2.8. Statistical analysis

Statistical analysis of the data was carried out using SPSS for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics of the data were determined, and the differences within and between groups were studied by three-way analysis of variance (ANOVA) and separated by Tukey's honest significant difference test ($p \leq 0.05$).

3. Results and Discussion

3.1. Characterisation of tomato sauces

3.1.1. Physicochemical analyses

Table 1 shows the results obtained from the analysis of various physicochemical parameters. Moisture and ash values were not significantly affected by the amount of added fiber, the application way, or storage time. This finding aligns with previous reports where adding dietary fiber from by-products did not impact product moisture (Kumar and Ray, 2016; Torbica et al., 2016). However, significant differences were observed in pH values between the different sauces and storage days. As the added fiber content increased, the pH decreased. Regarding storage time, the pH values remained stable for the first 9 days, with a statistically significant decrease observed on day 12. It's worth noting that pH is closely related to acidity. Total acidity also showed significant differences between the sauces (Table 1). Full sugar sauces (FS) had the lowest acidity (1.15%), while those with 100% sugar reduction (SR100) had the highest (1.54%). However, storage time did not significantly impact acidity. These results suggest that adding dietary fiber to the sauces reduced their pH and increased their acidity by lowering the content of reducing sugars. Similar findings were reported by Simanca et al. (2013), who observed a decrease in pH and an increase in acidity when fortifying yogurt with wheat bran dietary fiber.

The results of soluble solids content analysis showed significant differences between the types of sauces and the storage days. When comparing the sauces, the full sugar sauces (FS) had the lowest value (13.72), while the 50% sugar-reduced sauces (SR50) showed the highest values (Table 1). On the other hand, regarding the storage period, day 0 had the highest value (14.96), which decreased to 13.81 on day 60.

3.1.2. Soluble sugars content

The concentration of soluble sugars and total reducing sugars in the different analyzed sauces values are reported in table 2. Significant differences were found between the different sauces and storage

time for both total and reducing sugar values. These values ranged from 10.12 to 12.67 g/100g for total sugars and 3.08 to 4.51 mg/g for reducing sugars. Furthermore, both total and reducing sugars behaved similarly across the different sauces. The concentration of both decreased as the content of dietary fiber increased, with the 100% fiber sauce (SR100) showing the lowest values in both cases. These results agree with the work of Carcelli et al. (2021). In their study, they developed and characterized three recipes for strawberry sauces with reduced sugar content (30 %, 50 %, and 70 %) by replacing sugar with a semi-solid fiber syrup. They observed not only a reduction in sugar content but also an increase in dietary fiber content, from 0 g/100g to 31.5 g/100g.

3.1.3. Total phenolic compound content

The content of phenolic compounds in the sauces showed significant differences depending on the amount of added fiber and storage time, but not on the application method (lyophilized or solubilized) (Table 2).

Full sugar sauce (FS) contained 596.31 mg EAG/100 g of total phenols, similar to the 25% sugar-reduced sauce (584.88 mg EAG/100 g). Highlight that the amount of total phenols increased with increasing fiber content in the sauces with a 50% sugar reduction, reaching a maximum value of 829.95 mg EAG/100 g in the SR100 sauce. This relationship between fiber application and increased phenolic compounds was observed in other studies. Guzmán (2015) found that adding by-products of tomato, apple, rice, and Nopal flour as dietary fiber sources to sliced bread increased the total phenolic content. Additionally, a positive correlation exists between dietary fiber and phenolic compound content. These compounds interact, offering advantages such as the encapsulation of polyphenols in dietary fiber and potential prebiotic effects due to synergies that may lead to health benefits (Tang et al., 2020).

3.1.4. Antioxidant activity

The antioxidant capacity of the sauces, measured by both the DPPH and ABTS methods, revealed that the FS sauce had the lowest activity (Table 2). Conversely, sauces with the most added fiber (SR75 and SR100) exhibited the highest antioxidant activity (Table 2). These results are agreed with previous studies by Sáyago-Ayerdi et al. (2009), who reported increased antioxidant capacity in chicken burgers with the addition of grape dietary fiber.

The increase in antioxidant capacity for the sauces may be due to the positive correlation observed between phenolic compound content and antioxidant activity, as verified by other authors (Solari-Godiño et al., 2017).

When considering the storage period, a contrasting effect was observed. The highest values were found at the end of the shelf life, on day 12. Other authors observed a decrease in antioxidant activity during storage of food products (Sen et al., 2015 and Deng et al., 2022). This may be explained by the degradation of dietary fiber in the sauces during storage, releasing compounds with antioxidant activity. This phenomenon has also been reported by Bermúdez-Oria et al. (2020).

It's important to consider that the bioavailability and bioaccessibility of phenolic compounds, which are often responsible for antioxidant activity, can be affected by interactions between dietary fiber and the food matrix (Jakobek and Matić, 2019).

3.1.5. Total dietary fibre content and neutral sugar and galacturonic acid

Table 2 presents the descriptive statistics for each analyzed sauce. It reveals significant differences in dietary fiber content between sauces formulated with different fiber concentrations. However, no significant differences were observed based on the application method (lyophilized or solubilized) or throughout the storage period.

Dietary fiber values ranged from 33.84% to 38.24%. As expected, the FS sauce (with no added fiber) had the lowest percentage. The fiber content increased progressively with increasing dietary fiber addition in the sauce, which aligns with previous research (Cedeño and Zambrano, 2014; Kumar and Ray, 2016; Torbica et al., 2016;).

Table 3 shows the results of the neutral sugar profiles of the dietary fiber in the sauces analyzed in this study. Galacturonic acid was the main monosaccharide in all the formulated sauces, followed by glucose, fucose, and arabinose. Small concentrations of mannose, galactose, and rhamnose were also identified as minor sugars.

Significant differences were observed in mannose and galactose content between full sugar (FS) sauce and with added dietary fiber sauce. The FS sauce had the lowest values of both monosaccharides, while the SR75 sauce had the highest. The increase in mannose and glucose content due to the application of dietary fiber from winemaking lees is a positive aspect, considering the health benefits offered by these monosaccharides. Recent studies highlight the direct anticancer effects of mannose, observed both *in vitro* and *in vivo* through simple supplementation. Mannose ingestion can be achieved through cell culture media, drinking water, or incorporation into food products. Additionally, mannose has been shown to improve the effectiveness of various cancer treatments, including chemotherapy, radiotherapy, targeted therapy, and immunotherapy (Jin et al., 2023). Similarly, preclinical research in mice suggests that galactose, particularly

when replacing a portion of dietary glucose post-weaning, contributes positively to liver health. This is achieved by reducing triglyceride accumulation and liver inflammation. Additionally, an improvement in insulin sensitivity and a decrease in adipose tissue mass have been observed, indicating long-term benefits for body weight regulation (Bouwman et al., 2019).

Regarding storage time, all monosaccharide concentrations were lower at the end of the storage period (Day 12). Interestingly, sauces formulated with solubilized fiber exhibited higher monosaccharide content compared to those with solubilized and lyophilized fiber.

Although there are currently no studies on the neutral sugar profile of tomato sauces with different forms of fiber, previous research has analyzed the sugar profile of tomato byproducts, particularly the skin. The profile obtained in this study is similar, with galacturonic acid, glucose, and fucose as the major sugars, and rhamnose and mannose among the minor ones (Rivas et al., 2021b).

3.2. Functional properties of fiber from tomato sauces

3.2.1. Swelling, water holding capacity and fat absorption capacity

Sw, WRC and FAC values of dietary fibre from sauce are shown in Table 4. The results of Sw show a decrease in values as more fiber is added to the sauce. The SR25 sauce had the highest Sw value (17.17 mL of water/g of fiber), which decreased to 14.55 mL of water/g of fiber in the SR100 sauce. Swelling capacity increased throughout the storage days, reaching a maximum on day 6 of 17.79 mL water/g fiber.

The results of the water retention capacity (WRC) showed that the SR100 sauce had the highest value, reaching 18.85 g/g of fiber. This value decreased with decreasing fiber content, with the full sugar (FS) sauce having the lowest WRC (16.37 g/g). These findings align with observations by Bakirci et al. (2016) who reported increased WRC in yogurt with added pumpkin dietary fiber. Similarly, other studies have shown that dietary fiber, particularly soluble fiber, improves water retention capacity in various food products (Baenas et al., 2020; Popoola-Akinola et al., 2022).

Additionally, WRC increased during storage, peaking on day 6. This value was 4 g/g of fiber higher compared to day 0.

The results for free water capacity (FAC) in Table 4 did not show significant differences between the sauces. However, significant differences were observed based on storage time, although the magnitude of these differences was minimal.

Changes in the technological properties of dietary fiber are associated with alterations in the chemical structure of plant polysaccharides. These structural changes can affect various factors such as surface properties, charge density, particle size, insoluble fiber content, and the hydrophobic nature of the fiber particle (Abirami et al., 2014)

3.2.2. Glucose absorption capacity

Table 4 presents the glucose absorption capacity of the dietary fiber in the sauces. While no statistically significant differences were observed between the different sauces, the SR75 sauce exhibited the highest value (0.88 mg/g) and the full sugar sauce (FS) the lowest (0.81 mg/g).

During storage, glucose absorption capacity showed significant reductions. The value decreased to 0.77 mg/g on the last day compared to 0.92 mg/g on day 0. This coincides with the higher galacturonic acid content observed at the beginning of storage. Higher galacturonic acid suggests a greater content of soluble dietary fiber, which translates to larger porosity and consequently, a greater capacity to absorb glucose. This finding aligns with observations by Zheng et al. (2022) who reported a close link between porosity and glucose absorption by dietary fiber.

3.2.3. Non-extractable phenol contents

Table 4 shows the content of non-extractable phenolics. The SR50, SR75, and SR100 sauces exhibited the highest values (with a maximum of 416.95 mg EAG/100 g), with no significant differences observed among them. The storage period also significantly impacted the content of non-extractable phenolics. Values ranged from 251.94 to 506.04 mg EAG/100 g, with the lowest value on day 0 and the highest value after 12 days of storage. Other studies support our findings, highlighting insoluble dietary fiber as a good source of non-extractable phenolics (Acosta-Estrada et al., 2014; Xu et al., 2020). Previous research indicates that most free and bound phenolics typically decrease during storage (Liu et al., 2021; Galani et al., 2017). However, some studies report an increase in phenolic compounds after storage. This might be due to enzyme activity that breaks down cellular components, releasing previously bound phenolics into the free fraction or making them more easily extractable (Ziegler et al., 2020).

3.2.4. Antioxidant capacity from non-extractable phenol contents

Table 4 shows the antioxidant activity associated with non-extractable phenolics. Significant differences were observed between the sauces and storage days using both ABTS and DPPH methods. Antioxidant activity decreased with increasing fiber content in the sauces. The SR100 sauce, with the highest fiber content, exhibited the lowest antioxidant capacity (41.41 mg Trolox/100 g by ABTS and 36.64

mg Trolox/100 g by DPPH). Antioxidant activity also decreased over storage time, with the lowest values observed on the last day. This may be due to the previously observed degradation of dietary fiber during storage, potentially releasing compounds with antioxidant activity into the sauces.

The formt application of dietary fiber also influenced antioxidant activity (Table 4). Soluble fiber application resulted in superior results compared to lyophilized fiber. This suggests an interaction between the application method and the food matrix, potentially leading to synergistic or antagonistic effects on the bioactivity of the compounds (Suharoschi et al., 2019).

3.3. Colour

The addition of dietary fiber significantly impacted the color of the sauces, particularly reflected in the a* coordinate and C* value. However, no relationship was found between fiber addition and the b* coordinate, L* parameter (brightness), or H* value (hue angle) (Table 5).

The a* coordinate, which represents greenness-redness, decreased with increasing fiber content. The full sugar (FS) sauce had the highest value (12.93), while SR75 and SR100 sauces had lower values (around 9.04). Similarly, the C* value, indicating color saturation, decreased with increasing fiber content, with a more pronounced effect in sauces with higher fiber content (SR75 and SR100). On the contrary, the H* value, representing hue angle, significantly increased with fiber addition. The FS and SR25 sauces had the lowest H* values, while it progressively increased with fiber content, reaching a maximum of 27.38 in the SR100 sauce. The b* coordinate (yellowness-blueness) and L* parameter remained relatively constant across all analyzed sauces compared to the FS. These changes in color coordinates are likely due to the presence of pigments in the dietary fiber derived from the byproducts (Pathania and Kaur, 2022).

3.4. Sensory analysis

Table 5 shows the scores assigned by tasters for the the appearance and color intensity of the sauces. Significant differences were observed for both variables across the different sauces and storage time. The full sugar (FS) sauce and the SR25 sauce received the highest scores for appearance (7.56 and 8.21, respectively) and color intensity (7.94 and 7.56, respectively), with no significant difference between them. Conversely, the SR100 sauce received the lowest scores for both appearance and color intensity. A similar trend was observed for taste and aroma ratings. The FS and SR25 sauces received the highest scores, with no significant difference between them, while the SR100 sauce received the lowest scores.

Regarding the acceptability of the sauces, the SR25 sauce, with a 25% sugar reduction, received the highest acceptability score (7.56) and showed no significant difference compared to the full sugar (FS) sauce (7.31). Conversely, the SR100 sauce received the lowest score (5.23) for acceptability. Other studies observed similar results (Devi et al., 2023). These authors added dietary fiber and phytochemicals from pumpkin seeds to cookies. Their study showed that cookies with 3% added fiber received higher overall acceptability ratings compared to the control group, but no significant differences were observed.

On the other hand, acceptability assessments on day 0 and day 12 showed no significant differences, indicating good sensory stability of the sauces during storage. These results are consistent with those reported by other researchers (Paglarini et al., 2022).

4. Conclusions

This study highlights the promising benefits of incorporating dietary fiber from wine lees into tomato sauces as a replacement or reduction for simple sugars. Sauces formulated with a 25% sugar reduction (SR25) showed positive correlations with the color coordinate (a*), sugar concentration, pH, and sensory parameters like color intensity, sweetness, and overall acceptability. These characteristics were similar to full sugar (FS) sauces. These results suggest that SR25 sauces have similar consumer appeal to full sugar (FS) sauces. However, further research is necessary to understand the long-term effects of dietary fiber addition and optimize the formulation for practical applications in the food industry.

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Table 1. Moisture (%), ash (%), pH, total acidity (%) and ºBrix of tomato sauces: FS: full sugar; SR25: sugar reduced of 25 %; SR50: sugar reduced of 50 %; SR75: sugar reduced of 75 %; SR100: sugar reduced of 100 %, application form (directly solubilized fiber and solubilized and lyophilized fiber) and days of storage at 4ºC.

Factors	**Moisture**		**Ash**		**pH**		**Total acidity**		**º Brix**	
Levels	Mean	SD*	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sauces (S)										
FS	83.51	0.41	14.03	0.61	4.47	0.07^{e}	1.15	0.06^{a}	13.72	1.04^{a}
SR25	83.24	0.65	14.51	0.77	4.41	0.07^{d}	1.24	0.06^{b}	14.17	0.74^{b}
SR50	82.96	0.38	14.18	1.24	4.31	0.04^{c}	1.35	0.07^{c}	14.55	0.62^{c}
SR75	81.20	8.23	14.07	1.44	4.25	0.02^{b}	1.46	0.06^{d}	14.43	0.57^{c}
SR100	82.54	13.17	14.09	0.34	4.22	0.02^{a}	1.54	0.05^{e}	14.12	0.62^{b}
Application (A)										
Lyophilized	82.98	0.65	14.47	0.69	4.32	0.10	1.37	0.12	14.36	0.70
Solubilized	82.29	9.72	13.98	1.15	4.31	0.10	1.37	0.16	14.16	0.74
Days (D)										
0	83.12	0.35	14.31	0.39	4.33	0.10^{b}	1.40	0.16	14.96	0.64^{c}
3	82.96	0.75	14.48	0.46	4.34	0.10^{b}	1.37	0.16	14.33	0.21^{b}
6	83.41	0.50	13.62	1.41	4.32	0.08^{b}	1.37	0.12	13.81	0.99^{a}
9	80.48	16.33	14.47	0.72	4.32	0.11^{b}	1.35	0.14	14.26	0.44^{b}
12	83.01	0.54	14.09	1.35	4.27	0.08^{a}	1.36	0.14	13.88	0.47^{a}
P-values										
P_S	0.94		0.58		**<0.001**		**<0.001**		**<0.001**	
P_A	0.64		**0.02**		**<0.001**		**<0.001**		0.06	
P_D	0.94		0.08		**<0.001**		0.19		**<0.001**	
P_{D*A}	0.80		0.15		**<0.001**		0.35		**<0.001**	
P_{D*S}	1.00		0.42		**<0.001**		0.16		**<0.001**	
P_{A*S}	0.87		0.47		**<0.001**		.035		**<0.001**	

** SD: standard deviation.* ^{abcd} *Values with different superscripts are significantly different between each of the factors (Tukey's test; $p < 0.05$).*

Table 2. Total sugar and reducing sugars (g/100 g), total phenolic compounds (TPC) (mg GAE/ 100 g dry extract), antioxidant activity (mg Trolox/100 g dry extract) by two methods (DPPH and ABTS) and dietary fiber (%) of tomato sauces: FS: full sugar; SR25: sugar reduced of 25 %; SR50: sugar reduced of 50 %; SR75: sugar reduced of 75 %; SR100: sugar reduced of 100 %, application form (directly solubilized fiber and solubilized and lyophilized fiber) and days of storage at 4ºC.

Factors	**Total sugars**		**Reducing sugars**		**TPC**		**DPPH**		**ABTS**		**Dietary fiber**	
Levels	Mean	SD*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sauces (S)												
FS	11.24	1.82^{ab}	4.36	0.28^{c}	596.31	39.01^{a}	35.27	5.76^{a}	57.38	14.74	33.84	1.48^{a}
SR25	11.89	1.76^{b}	3.81	1.14^{b}	584.88	99.34a	39.68	7.82^{ab}	62.70	11.80	34.14	1.68^{a}
SR50	11.43	1.97^{ab}	3.79	1.19^{b}	626.9	116.54^{a}	41.44	9.83^{bc}	58.33	16.93	36.03	1.371^{b}
SR75	11.32	2.27^{ab}	3.73	1.16^{b}	713.89	210.31^{b}	46.43	13.58c	60.01	11.05	38.24	1.68^{c}
SR100	10.12	2.99^{a}	3.49	1.20^{a}	829.95	405.85^{b}	43.37	13.29^{bc}	64.36	10.85	38.17	1.46^{c}
Application (A)												
Lyophilized	11.97	2.48	3.08	1.30	709.83	210.07	40.32	11.85	62.35	11.32	36.4	2.69
Solubilized	10.58	1.94	4.34	0.42	639.97	258.08	43.66	10.20	59.3	14.61	36.28	2.12
Days (D)												
0	12.67	2.78^{b}	3.89	0.35^{c}	604.53	80.28^{a}	40.52	9.04^{ab}	64.16	12.43^{b}	35.88	2.42
3	11.11	1.73^{ab}	4.13	0.82^{d}	718.76	351.20^{a}	35.63	7.52^{a}	61.69	12.14^{a}	35.87	1.81
6	11.25	2.41^{ab}	4.50	0.31^{e}	618.75	112.58^{a}	40.9	14.14^{ab}	62.05	16.69^{b}	36.74	2.60
9	10.61	1.34^{a}	3.31	1.22^{b}	65.74	74.17^{a}	44.46	6.19^{b}	52.7	9.56^{a}	36.74	2.27
12	10.34	2.37^{a}	4.51	0.12^{a}	987.91	328.65^{b}	56.67	9.36^{c}	65.3	7.41^{b}	36.43	2.79
P-values												
P_S	**0.02**		**<0.001**		**<0.001**		**<0.001**		0.5		**<0.001**	
P_A	**<0.001**		**<0.001**		0.05		0.62		0.28		**<0.001**	
P_D	**0.003**		**<0.001**		**0.04**		**0.001**		**<0.000**		**<0.001**	
P_{D*A}	**0.03**		**<0.001**		**0.02**		**0.2**		**<0.001**		**<0.001**	
P_{D*S}	0.07		**<0.001**		**0.004**		0.18		**<0.001**		0.05	
P_{A*S}	0.42		**<0.001**		**<0.001**		0.37		**0.01**		**<0.001**	

** SD: standard deviation. ^{abcd} Values with different superscripts are significantly different between each of the factors (Tukey's test; $p < 0.05$).*

Table 3. Galacturonic acid content and neutral sugar profile (mg/g of fiber) of tomato sauces: FS: full sugar; SR25: sugar reduced of 25 %; SR50: sugar reduced of 50 %; SR75: sugar reduced of 75 %; SR100: sugar reduced of 100 %, application form (directly solubilized fiber and solubilized and lyophilized fiber) and at 0 and 12 days of storage at 4ºC.

Factors	**Galacturonic acid**		**Glucose**		**Arabinose**		**Fucose**		**Rhamnose**		**Manose**		**Galactose**	
Levels	Mean	DT*	Mean	DT	Mean	DT	Mean	DT	Mean	DT	Mean	DT	Mean	DT
Sauces (S)														
FS	818.40	113.88	23.66	3.42	1.03	0.01	2.31	0.25	0.06	0.00	0.36	0.05^{a}	0.75	0.12^{a}
SR25	923.03	77.42	24.59	1.75	1.05	0.02	2.41	0.13	0.06	0.01	0.45	0.05^{ab}	0.94	0.11^{ab}
SR50	925.46	126.47	23.92	4.24	1.04	0.04	2.31	0.15	0.06	0.01	0.50	0.10^{b}	1.05	0.22^{b}
SR75	935.44	105.21	22.86	2.44	1.05	0.03	2.34	0.22	0.06	0.01	0.54	0.08^{b}	1.14	0.16^{b}
SR100	850.85	163.38	21.46	4.38	1.03	0.05	2.28	0.16	0.06	0.02	0.49	0.10^{ab}	1.03	0.22^{ab}
Application (A)														
Lyophilized	867.73	120.08	22.22	3.14	1.03	0.02	2.34	0.14	0.05	0.01	0.46	0.07	0.96	0.15
Solubilized	949.67	110.94	24.20	3.54	1.06	0.04	2.33	0.20	0.07	0.01	0.54	0.09	1.12	0.19
Days (D)														
0	942.36	57.40	24.07	2.22	1.05	0.02	2.40	0.18	0.06	0.01	0.50	0.06	1.04	0.13
12	854.96	152.97	22.45	4.18	1.04	0.04	2.27	0.15	0.06	0.02	0.47	0.12	0.97	0.25
P-values														
P_S	0.409		0.290		0.367		0.527		0.778		0.213		0.213	
P_A	**0.048**		0.109		**0.022**		0.836		**0.022**		**0.021**		**0.021**	
P_D	**0.019**		0.082		0.635		**0.035**		0.635		0.178		0.178	
P_{D*A}	**0.043**		0.095		0.278		0.451		0.278		0.159		0.159	
P_{D*S}	0.541		0.490		0.704		0.781		0.704		0.466		0.466	
P_{A*S}	0.868		0.981		0.999		0.700		0.999		0.869		0.869	

** SD: standard deviation. [abcd] Values with different superscripts are significantly different between each of the factors (Tukey's test; $p < 0.05$).*

Table 4. Swelling capacity (Sw) (ml water /g fiber), water retention capacity (WRC) (g water/g fiber), fat absorption capacity (FAC) (g oil/g fiber), glucose adsorption capacity (GAC) (mg/g), non-extractable phenols (NE-F) (mg EAG/100 g AIR), antioxidant activity (mg Trolox/100 g dry extract) by two methods (DPPH and ABTS) of fiber from tomato sauces: FS: full sugar; SR25: sugar reduced of 25 %; SR50: sugar reduced of 50 %; SR75: sugar reduced of 75 %; SR100: sugar reduced of 100 %, application form (directly solubilized fiber and solubilized and lyophilized fiber) and days of storage at 4ºC.

Factors	**Sw**		**WRC**		**FAC**		**GAC**		**NE-F**		**DPPH-NEF**		**ABTS-NEF**	
Levels	Mean	SD*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sauces (S)														
FS	16.01	2.838^{b}	16.37	2.81^{ab}	13.88	3.84	0.81	0.14	306.21	40.88^{a}	50.52	16.20^{c}	60.69	17.42^{c}
SR25	17.17	1.931^{b}	18.1	2.02^{c}	14.92	4.62	0.85	0.21	365.71	130.12^{b}	25.60	9.24^{a}	59.63	39.05^{bc}
SR50	16.65	2.003^{b}	17.56	2.09^{bc}	14.99	3.43	0.82	0.15	416.95	156.72^{c}	29.68	11.55^{ab}	47.91	26.50^{ab}
SR75	16.03	2.843^{b}	16.98	2.99^{abc}	14.6	2.83	0.88	0.12	391.26	128.96^{bc}	34.47	11.65^{b}	47.24	32.17^{a}
SR100	14.55	3.214^{a}	18.85	3.49^{c}	16.27	3.42	0.82	0.14	395.95	143.48^{bc}	36.64	18.54^{b}	41.41	23.13^{a}
Application (A)														
Lyophilized	17.28	1.59	18.28	1.59	4.96	3.26	0.89	0.16	353.29	152.6	25.77	11.95	29.36	13.56
Solubilized	15.14	3.02	15.82	3.16	15.1	4.03	0.8	0.13	415.36	104.31	41.45	13.29	72.63	26.47
Days (D)														
0	13.78	3.770^{a}	14.78	3.77^{a}	16.44	5.76^{b}	0.92	0.20^{b}	251.94	173.9^{a}	28.99	10.68^{b}	55.38	25.9^{c}
3	16.07	1.983^{b}	17.07	1.98^{b}	15.6	2.84^{ab}	0.83	0.17^{ab}	423.07	59.59^{c}	38.92	13.9^{c}	60.12	28.87^{c}
6	17.79	1.843^{c}	18.79	1.84^{c}	14.56	2.94^{ab}	0.8	0.12^{ab}	362.36	42.97^{b}	36.34	18.71^{bc}	61.1	36.25^{c}
9	16.15	1.795^{b}	17.39	1.73^{bc}	13.21	1.85^{a}	0.87	0.01^{ab}	449.64	116.52^{c}	36.42	11.77^{c}	35.89	22.6^{b}
12	16.67	2.043^{bc}	17.81	1.78^{bc}	15.25	1.61^{ab}	0.77	0.14^{a}	506.04	74.13^{d}	18.36	9.53^{a}	22.66	5.96^{a}
P-values														
P_S	**<0.001**		**<0.001**		**<0.001**		**<0.001**		**0.045**		**<0.001**		0.538	
P_A	**<0.001**		**<0.001**		**<0.001**		**<0.001**		0.510		**<0.001**		**0.002**	
P_D	**<0.001**		**<0.001**		**<0.001**		**<0.001**		**0.040**		**<0.001**		**0.020**	
P_{D*A}	**<0.001**		0.050		**0.040**		**<0.001**		**0.010**		**<0.001**		0.279	
P_{D*S}	**<0.001**		**<0.001**		**<0.001**		0.240		**0.040**		0.150		**0.026**	
P_{A*S}	**<0.001**		0.530		0.160		**0.050**		0.470		0.080		0.605	

** SD: standard deviation. [abcd] Values with different superscripts are significantly different between each of the factors (Tukey's test; p < 0.05).*

Table 5. CIELab scale parameters (L *: luminosity; a *: red-green coordinate; b *: yellow-blue coordinate; C *: saturation or color intensity; Hue angle or pitch H *) and sensory analysis of tomato sauces: FS: full sugar; SR25: sugar reduced of 25 %; SR50: sugar reduced of 50 %; SR75: sugar reduced of 75 %; SR100: sugar reduced of 100 %, application form (directly solubilized fiber and solubilized and lyophilized fiber) and at 0 and 12 days of storage at 4ºC.

	Sauces (S)						*Application (A)*				*Days (D)*				
	FS	RS25	RS50	RS75	RS100	P_S	Lyophilized	Soluble	P_A	P_{SA}	0	12	P_D	P_{SD}	P_{AD}
Color coordinates															
a*	12,93	10,18	10,99	9,04	9,04	***	8,13	11,78	***	**	11,84	9,3	***	***	***
b*	5,59	3,93	5,54	3,95	5,34	***	3,7	5,66	***	***	6,9	4,88	***	***	***
L*	36,32	36,17	36,65	36,28	36,75	-	36,52	36,39	-	-	36,11	37,58	***	***	**
C*	14,22	10,96	12,41	9,93	10,65	***	8,99	13,23	***	*	13,94	10,53	***	***	***
H*	2,51	20,62	24,47	22,21	27,38	***	23,09	23,7	-	**	25,92	27,65	***	***	***
Sensory analysis															
Color intensity	7,94	7,17	6,38	5,14	3,98	***	6,05	5,89	**	-	6,38	5,47	*	-	-
Appearance	7,56	8,21	7,32	6,36	5,41	***	7,25	6,71	**	*	6,25	7,25	*	*	-
Sweet taste	6,38	6,77	6,05	5,32	4,54	***	6,04	5,59	*	-	5,81	5,54	-	-	*
Acid taste	3,56	3,15	3,37	4,27	5,04	**	3,85	3,94	-	-	4,43	3,61	-	-	-
Sweet scent	6,35	6,22	5,89	5,66	5,17	**	6,2	5,57	**	-	5,29	5,69	**	-	**
Acid scent	4,13	3,4	3,68	4,52	5,2	**	3,73	4,48	**	-	4,22	4,05	-	-	-
Unpleasant smell	1,02	1,05	1,19	1,6	0,69	-	1,13	1,47	*	-	1,72	1,23	*	-	-
Scent others	0,74	0,7	0,15	0,16	0,56	-	0,14	0,39	-	-	0,37	0,72	-	-	-
Acceptability	7,31	7,56	7,27	5,93	5,23	***	6,83	6,45	-	-	6,94	6,3	-	-	-

*Values de P *** < 0.001; P **<0.05; P *<0.1*

DISCUSIÓN GENERAL

Subproductos como fuente de fibra dietética

El consumo de **fibra dietética** es esencial para una alimentación saludable y está asociado con múltiples beneficios para la salud (Thomas, 2023). La fibra dietética ayuda a prevenir enfermedades digestivas y cardíacas y se considera uno de los cinco "nutrientes de interés" (McGuire, 2011). Las recomendaciones de ingesta varían según la edad, pero una dieta rica en fibra dietética es esencial para todas las etapas de la vida (OMS, 2003). Además, la fibra dietética tiene efectos fisiológicos importantes en el proceso de digestión, mejorando la salud gastrointestinal, regulando la saciedad y la absorción de nutrientes, y promoviendo un microbioma intestinal saludable (Johnson, 2016; Li y Uppal, 2010; Mudgil y Barak, 2013; Lattimer y Haub, 2010; Hua y col., 2021; Rastall y Gibson, 2015).

Actualmente se generan a nivel mundial 37 millones de toneladas de **residuos agrícolas** (Sarfraz y col., 2023) ricos en fibra dietética, además de en otras sustancias como polisacáridos solubles, compuestos fenólicos (Bondam y col., 2022) y ácidos grasos (Gottardo y col., 2022), lo que los hace valiosos para la obtención de aditivos e ingredientes funcionales (Perez-Pirotto y col., 2022; Yin y col., 2022; Chamorro y col., 2022; de Oliveira y col., 2022; Nakamura y col., 2023). Industrias como la viticultura generan subproductos como el orujo de uva, que posee una alta concentración de fibra dietética, polisacáridos y compuestos fenólicos (González-Ballesteros y col., 2018; Antonić y col., 2020). Además, el procesamiento de la granada y del tomate también genera subproductos con un alto contenido de fibra dietética y compuestos bioactivos (Hasnaoui y col., 2014; Abbasi-Parizad y col., 2020). Por otro lado, los subproductos del brócoli, como sus tallos, hojas e inflorescencias no comerciales, son especialmente ricos en fibra dietética, además de contener otros compuestos bioactivos como fenoles, vitamina C y glucosinolatos (Sánchez-Pujante y col., 2020; Salas-Millán y col., 2022). Estos subproductos agrícolas presentan oportunidades prometedoras para su aplicación en diversas industrias, incluida la alimentaria, donde pueden ser utilizados como aditivos e ingredientes funcionales.

En este contexto, la presente Tesis Doctoral investigó **una variedad de subproductos** generados por cultivos e industrias importantes en la región de Extremadura, como los del **cultivo del brócoli y la granada,** así como los subproductos

procedentes del procesamiento del **tomate** y los generados por las **industrias vitivinícolas** durante su actividad. Los resultados obtenidos de los distintos ensayos realizados muestran que todos los subproductos elegidos contienen cantidades significativas de fibra dietética, con valores que oscilan entre aproximadamente 45 g/100 g de subproducto seco en la cáscara de granada y 82 g/100 g de subproducto seco en el **hollejo y las lías de vinificación** (Rivas y col., 2021a: Rivas y col., 2021b). Además, la presencia significativa de fibra dietética en estos subproductos vegetales se sustenta en la evidencia respaldada por otros estudios (Navarro-González y col., 2011; Zhu y col., 2015; Siddiqui y col., 2024; Shang y col., 2023).

Composición de la fibra dietética

Como ya se ha comentado, la fibra dietética, compuesta principalmente por polisacáridos, desempeña un papel crucial en la salud humana (Chebli y Geitmann, 2017). Estos **polisacáridos incluyen celulosa, hemicelulosas y polisacáridos pécticos**, que forman parte de la estructura de la pared celular (Li y col., 2021b). La funcionalidad de la fibra dietética depende de su composición y de cómo interactúa con el cuerpo humano. Es por ello de vital importancia conocer la composición de la fibra dietética procedente de los distintos subproductos. Así, se analizó la composición de la fibra dietética del hollejo, raspón y lías de vinificación observándose que el compuesto más abundante en el hollejo y el raspón fue el **ácido urónico**, seguidos de los **azúcares neutros** glucosa y fucosa en el hollejo y manosa en el raspón. Otros azúcares neutros como arabinosa, xilosa, galactosa y ramnosa estaban presentes en menor cantidad. En las lías de vinificación, la glucosa fue el azúcar mayoritario, seguida de ramnosa y manosa. Ortega-Regules y col. (2008) mostraron que el perfil de azúcar neutro de los subproductos de la vinificación era muy variable, dependiendo del cultivar utilizado. En el caso de los otros subproductos estudiados, la investigación reveló una gran diversidad en el perfil de azúcares neutros en la fibra dietética del brócoli. Las muestras de tallo mostraron valores mayores de glucosa, xilosa y fucosa que las muestras de hojas e inflorescencias. Sin embargo, no se encontraron diferencias significativas en el contenido de ácidos urónicos entre la fibra dietética procedente de las distintas partes de la planta de brócoli. Por otro lado, la fibra dietética de piel de tomate mostró los valores más altos de ácidos urónicos con un valor medio de 934,72 mg/g. En cuanto al perfil de azúcares neutros, se observó que los principales monosacáridos fueron glucosa y fucosa

en la piel de tomate y cáscara de granada. Estos hallazgos sugieren una diversidad en la composición de la fibra dietética entre los subproductos estudiados lo que puede tener implicaciones importantes en sus propiedades nutricionales y funcionales.

En conclusión, la composición de azúcares neutros y ácidos urónicos de la fibra dietética de los subproductos estudiados está influenciada por la naturaleza de los subproductos y una gran variedad de factores. Los resultados obtenidos de los diferentes estudios realizados en la presente Tesis Doctoral subrayan la importancia de comprender la composición química de la fibra dietética de los subproductos para su valorización en diversas aplicaciones industriales y nutricionales.

Propiedades funcionales

Entre las propiedades funcionales de la fibra dietética, destaca su **capacidad prebiótica**. La fermentación de ciertos polisacáridos presentes en la fibra dietética por bacterias gastrointestinales potencialmente beneficiosas puede tener efectos positivos para la salud, al modificar la composición de las comunidades bacterianas, convirtiendo así a la fibra dietética en un agente prebiótico. Importantes investigaciones han demostrado que los polisacáridos derivados de las paredes celulares de las plantas tienen una alta actividad prebiótica y pueden estimular el crecimiento de ciertas bacterias beneficiosas (Lordan y col., 2020; Baenas y col., 2020; Redondo-Cuenca y col., 2023; Khodaei y col., 2016; Prandi y col., 2018). Así, en la evaluación del efecto de la fibra dietética obtenida de subproductos de granada, tomate, uva y brócoli sobre la supervivencia, el crecimiento y el metabolismo del tracto gastrointestinal de cepas de bacterias ácido-lácticas (LAB) se observaron diferencias significativas en función de la fibra dietética utilizada. Aunque se esperaba un mayor crecimiento bacteriano en presencia de la fibra dietética de piel de tomate debido a la presencia de componentes fermentables como pectinas y hemicelulosas, solo se observó un crecimiento modesto, similar al observado cuando crecieron en presencia de fibra dietética de las muestras de lías vinificación como única fuente de carbono. Por el contrario, cuando las cepas de LAB crecieron en presencia de fibra dietética extraída de muestras de raspón se observó el mayor crecimiento de estos microorganismos (Rivas y col., 2021c; Rivas y col., 2021b). Este fenómeno podría estar relacionado con la presencia de polifenoles no extraíbles en el raspón de la uva, según se observó en los datos. Investigaciones previas, como la de Landete y col. (2007), han demostrado que bacterias como *Lactobacillus*

spp. tienen la capacidad de degradar compuestos fenólicos a otras moléculas con alto valor añadido, lo que sugiere que las cepas estudiadas podrían utilizar algunos fenoles para su metabolismo. Por otro lado, se observaron diferencias significativas en las tasas de crecimiento de ciertas cepas de LAB en presencia de diferentes extractos de fibra dietética procedente de los subproductos del cultivo del brócoli, destacando los valores más altos de crecimiento con extractos de fibra dietética de hojas y tallos y los valores más bajos con extractos de fibra dietética de inflorescencias (Rivas y col., 2022).

La capacidad de las bacterias para utilizar la fibra como fuente de energía se ve influida por diversos factores, como el grado de polimerización, el peso molecular, el tamaño de la cadena y la presencia de ramificaciones en la molécula. Por ejemplo, los polisacáridos, como la pectina, tienen estructuras complejas que se metabolizan de manera más lenta (Hamaker y Tuncil, 2014). Además, la velocidad y la eficacia de la fermentación prebiótica de la fibra dietética por parte de la microbiota intestinal dependen también de aspectos como la solubilidad, la porosidad y la estructura y organización de la pared celular de la fibra (Capuano, 2017). También, es importante tener en cuenta que otros componentes de fibra dietética, como las proteínas y los compuestos fenólicos, pueden influir en la fermentabilidad y en el efecto prebiótico (Aprikian y col., 2003).

Debido a la fermentación de la fibra dietética en el colon por la microbiota intestinal. Se producen **ácidos grasos de cadena corta (SFCA)**, como butírico, propiónico y acético (Baenas y col., 2020), que se asocian con una amplia gama de propiedades fisiológicas locales y sistémicas beneficiosas para la salud (Rastall y Gibson, 2015). Así, se examinó la producción de SFCA de ciertos microorganismos cuando crecieron en presencia de fibra dietética extraída de los distintos subproductos vegetales estudiados. Los resultados revelaron que bacterias probióticas cultivadas en distintos extractos de fibra dietética demostraron un potencial interesante para influir en la salud gastrointestinal y general. Específicamente, se encontró que la fibra extraída de los tallos de brócoli estimuló una mayor producción de ácidos grasos de cadena corta, especialmente ácido acético, durante la fermentación por LAB. Este aumento en la producción de SFCA se considera un indicador positivo de la salud intestinal, ya que estas sustancias pueden proporcionar energía a las células del colon, mantener un pH gastrointestinal adecuado y facilitar la absorción de minerales, entre otros beneficios

(Alexander y col., 2019). Comparativamente, los otros subproductos vegetales analizados, como la cáscara de granada, la piel de tomate y los subproductos de vinificación, también mostraron efectos sobre la producción de SFCA, aunque en diferentes magnitudes y perfiles. Esto sugiere que, si bien estos subproductos también pueden tener efectos beneficiosos sobre la microbiota intestinal, su capacidad para promover la producción de SFCA puede ser relativamente menor en comparación con la fibra dietética del brócoli. Estos hallazgos resaltan la importancia de considerar la composición específica de la fibra dietética en diferentes subproductos vegetales y su impacto potencial en la salud intestinal. Aunque la cáscara de granada, la piel de tomate y los subproductos de vinificación también pueden proporcionar beneficios para la salud gastrointestinal debido a su capacidad antioxidante y otras propiedades, los resultados sugieren que la fibra dietética de los tallos de brócoli puede tener un potencial particularmente alto para promover una microbiota intestinal saludable y la producción de metabolitos beneficiosos como los SFCA. En conjunto, estos hallazgos respaldan la posibilidad de utilizar fibra dietética procedente de subproductos vegetales para desarrollar alimentos funcionales con el fin de fomentar una microbiota intestinal diversa y funcionalmente beneficiosa, lo que puede tener efectos positivos en la salud gastrointestinal y general.

Otra función destacada de la fibra dietética es su **capacidad antioxidante**, la cual puede ayudar a combatir el estrés oxidativo asociado con diversas enfermedades. La terapia antioxidante podría ser beneficiosa en el tratamiento y prevención de afecciones como la diabetes mellitus, las lesiones por reperfusión, enfermedades inflamatorias, así como en la prevención de procesos crónicos como la aterosclerosis y la carcinogénesis (Zeng y col., 2020; Li y col., 2020a; Hamed y col., 2020; Zhang y col., 2021). Los resultados obtenidos de la actividad antioxidante de la fibra dietética de distintos subproductos vegetales estudiados, como la cáscara de granada, los raspones, hollejos y lías de la vinificación, así como los subproductos del brócoli (hojas, tallos e inflorescencias), revelan diferencias entre las muestras analizadas. De manera general, los ensayos realizados en el transcurso de la presente Tesis Doctoral han mostrado los siguientes resultados sobre la actividad antioxidante de la fibra dietética de los subproductos vegetales. Se observó que la fibra dietética procedente de los subproductos de vinificación exhibieron la mayor actividad antioxidante, en concreto, la fibra dietética de raspón seguido por la fibra dietética procedente del hollejo y de las lías

de vinificación, lo cual está en consonancia con el contenido fenólico total presente en estas muestras. En el caso de la cáscara de granada, los extractos de fibra dietética también exhibieron una actividad antioxidante elevada determinada mediante el método DPPH (1612 mg Trolox/100 g), lo que indica la presencia de compuestos fenólicos y otros antioxidantes asociados con la fibra dietética. Esta **relación entre el contenido de compuestos fenólicos y la actividad antioxidante** ha sido consistentemente observada en estudios previos, donde se ha demostrado que el aumento de la actividad antioxidante está relacionado con el contenido de compuestos fenólicos en la fibra dietética (Hasnaoui y col., 2014; Peixoto y col., 2018). Ferri y col. (2016) informaron que las semillas y los extractos de piel cruda de varios cultivares de uva exhibieron una alta capacidad antioxidante medida por el método ABTS, que se correlacionó positivamente con el contenido total de polifenoles. Por otro lado, en el caso de los subproductos del brócoli, se observa una mayor actividad antioxidante en las inflorescencias en comparación con las hojas y los tallos. Sin embargo, es importante mencionar que, aunque mostraron actividad antioxidante, esta fue menor en comparación con los subproductos de vinificación y de la granada.

En resumen, los resultados obtenidos indican que la fibra dietética presente en los subproductos vegetales analizados posee una notable actividad antioxidante, la cual está asociada principalmente con el contenido de compuestos fenólicos. Estos hallazgos sugieren el potencial de estos subproductos como fuentes de fibra con propiedades antioxidantes, lo que podría tener implicaciones importantes para su uso en la industria alimentaria y en la promoción de la salud humana.

Propiedades tecnologías

Según un estudio llevado a cabo en la presente Tesis Doctoral, los resultados sobre la **capacidad de adsorción de glucosa (CAG)** en los subproductos del brócoli apuntan a un potencial significativo para influir en la absorción de dicho monosacárido. Es importante destacar que las muestras de tallo mostraron la mayor capacidad de adsorción de glucosa en comparación con el resto de los subproductos del brócoli analizados, lo que sugiere una composición específica de la fibra dietética en esta parte de la planta que favorece esta interacción. Trabajos anteriores han demostrado que la fibra dietética procedente de diferentes fuentes puede adsorber la glucosa de manera diferente (Peerajit y col., 2012).

Entre las propiedades tecnológicas y físico-químicas más importantes de la fibra dietética se incluyen, su **capacidad de hidratación** y la **capacidad para captar moléculas orgánicas**. Estas propiedades fisicoquímicas de los polisacáridos de la pared celular, varían según su composición. La **capacidad de retención de agua (WRC)**, relacionada principalmente con la fibra dietética insoluble, previene y trata diferentes trastornos intestinales aumentando el volumen fecal y reduciendo el tiempo de tránsito gastrointestinal. En términos tecnológicos alimentarios, la fibra dietética con alto WRC se puede utilizar como ingrediente funcional para evitar la sinéresis y modificar la viscosidad y textura de algunos alimentos formulados, mientras que la fibra dietética con alta **capacidad de retención de aceite (FAC)** permite la estabilización de la grasa en productos a base de emulsión (Grigelmo-Miguel y col., 1998). Otra propiedad tecnológica importante de la fibra dietética es la **capacidad de hinchamiento (Sw)** asociado a la sensación de saciedad en el estómago y un aumento en la viscosidad de la digesta y que está vinculado con fibra dietética soluble, la cual ralentiza la absorción de nutrientes de la mucosa intestinal y reduce las respuestas posprandiales de glucosa e insulina en sangre (Mehta y col., 2015).

La evaluación de las propiedades tecnológicas de las fibras dietéticas derivadas de subproductos vegetales es esencial para su potencial aplicación en la industria alimentaria. Por tanto, en esta Tesis Doctoral se exploraron diversas propiedades tecnológicas de las fibras dietéticas extraídas de distintos subproductos vegetales. Los resultados revelaron que, en términos de Sw, la fibra dietética de la cáscara de granada mostró el valor más alto, seguida por la fibra dietética de los subproductos del brócoli, mientras que los subproductos de vinificación presentaron el menor valor. En cuanto a la WRC los resultados de los trabajos mostraron los valores más altos en la fibra dietética proveniente de los subproductos de brócoli, especialmente en la fibra dietética de los tallos, seguidos por los subproductos de vinificación, particularmente las lías. Estos resultados sugieren que las fibras dietéticas de los diferentes subproductos exhiben variaciones importantes tanto en Sw como en WRC. Estas diferencias pueden atribuirse a las disparidades en la estructura y composición de la fibra, dado que estos dos aspectos son determinantes en la cinética de absorción de agua (Chau y col., 2007; Lattimer y Haub, 2010). Finalmente, en relación con la FAC, se encontró que la cáscara de granada mostró el valor más elevado, seguida de la fibra dietética de las inflorescencias del brócoli. Por otro lado, los subproductos de la vinificación exhibieron

los valores más bajos de FAC. Los altos valores de FAC son beneficiosos ya que están asociados con la retención de aceite durante la digestión de los alimentos y reducen los niveles de colesterol sérico (Navarro-González y col., 2011).

En resumen, tanto los subproductos de la granada y el brócoli como los subproductos de vinificación muestran propiedades tecnológicas prometedoras en términos de capacidad de Sw, WRC y FAC. Sin embargo, las diferencias específicas entre los distintos tipos de subproductos subrayan la importancia de seleccionar cuidadosamente los materiales vegetales para optimizar las propiedades funcionales de las fibras dietéticas en aplicaciones alimentarias.

Modificaciones de la fibra dietética

Las características bioactivas de la fibra dietética están estrechamente relacionadas con su composición y solubilidad (Huang y col., 2020). La estructura, unidades de monosacáridos, tipo de enlace, configuración espacial, distribución de cadenas ramificadas y peso molecular son factores determinantes que influyen en la bioactividad de la fibra dietética (Huang y col., 2020; Gao y col., 2017). Dado el alto peso molecular de los polisacáridos naturales que forman la fibra dietética, se requiere su modificación y degradación para mejorar su solubilidad y facilitar su acceso a las células (Liu y col., 2017; Tang y col., 2014). Dicho de otra manera, **la fibra dietética soluble posee generalmente propiedades bioactivas superiores** en comparación con la fibra dietética insoluble, gracias a su fermentabilidad y viscosidad (Ma y Mu, 2016). Considerando que la fibra dietética derivada de los subproductos vegetales consiste principalmente en fibra insoluble (Chitrakar y col., 2020; He y col., 2021), se hace necesario modificarla y degradarla para mejorar sus propiedades y funciones. Se emplean diversos métodos, incluyendo químicos, físicos y biológicos, para modificar los polisacáridos que conforman la fibra dietética, reduciendo su tamaño y peso molecular para potenciar su bioactividad (Zheng y col., 2019b; Hashemifesharaki y col., 2020; Li y col., 2020a; Liu y col., 2021).

Tratamientos enzimáticos

Los **tratamientos enzimáticos** demostraron ser efectivos para modificar la composición de la fibra dietética extraída de los subproductos del brócoli, aumentando

el contenido de ácidos urónicos y mejorando la actividad funcional específica. Ma y col. (2022) modificaron la fibra dietética de patata con las enzimas celulasa y xilanasa y sus resultados mostraron un aumento del contenido de ácido urónico después del tratamiento. También se observó que los tratamientos enzimáticos lograron una mejora en la solubilidad respecto al control, modificando el contenido inicial de fibra dietética soluble. Wen y col. (2017) en su estudio vieron mejorada la solubilidad de la fibra dietética del salvado de arroz cuando esta fue tratada conjuntamente con las enzimas celulasa y xilanasa. En cuanto a la CAG los tratamientos con enzimas mejoraron esta propiedad, mostrando los valores más elevados de CAG (0,88 y 0,87 g/g) con respecto al control. La mejora de la CAG tras el tratamiento enzimático es corroborada por otros autores (Huang y col., 2021a). Por otro lado, se observó que la mayoría de las LAB experimentaron un aumento significativo en su crecimiento cuando se utilizaron enzimas en la mejora de la fibra dietética, específicamente el complejo multienzimático Viscozyme. El mayor crecimiento puede atribuirse a un mayor contenido de pectina como se ha visto en trabajos previos (Rivas y col., 2021c). Además, los resultados revelaron que los microorganismos crecidos tras el tratamiento enzimático produjeron los valores más altos de SFCA en comparación al resto de los tratamientos. Ma y col., 2021 modificaron enzimáticamente la fibra dietética de desechos de patata con celulasa y xilanasa, los resultados mostraron efectos significativos sobre los SFCA y la microbiota intestinal en comparación con la fibra dietética no modificada.

Atendiendo a los compuestos fenólicos no extraíbles y la capacidad antioxidante, se observaron valores superiores en la fibra dietética procedente de las inflorescencias y aumentaron tras el tratamiento enzimático. Zhang y col. (2019) al modificar la fibra dietética insoluble del salvado de trigo mediante carboximetilación, hidrólisis enzimática compleja y molienda ultrafina, lograron mejorar su capacidad antioxidante. Esto sugiere que la aplicación de enzimas puede ser una estrategia prometedora para mejorar la fibra dietética de los subproductos del brócoli, mejorando algunas de sus propiedades funcionales.

Fluido supercrítico

En la presente Tesis Doctoral, se examinaron los efectos de la extracción previa de compuestos bioactivos de la cáscara de granada en el residuo resultante y en la fibra dietética extraída posteriormente, utilizando distintas condiciones de **fluido**

supercrítico para optimizar el proceso. Se identificaron varias repercusiones del tratamiento previo en el subproducto y en la fibra dietética extraída posteriormente. En primer lugar, se comprobó que las condiciones óptimas de extracción supercrítica conducían a un mayor rendimiento en la recuperación posterior de la fibra dietética de la cáscara de granada. Respecto al efecto de los parámetros de extracción sobre la pectina residual, se encontró que la presión, la temperatura y el tiempo de extracción tuvieron un impacto significativo en el contenido de ácido galacturónico Las condiciones óptimas de extracción supercrítica resultaron en un mayor contenido de ácido galacturónico, lo que sugiere una mayor retención de pectina en el residuo. Por otro lado, se detectaron cambios significativos en las propiedades tecnológicas y funcionales de la fibra dietética de la cáscara de granada sometida a extracción previa mediante fluidos supercríticos en comparación con el método convencional. La Sw alcanzó su valor máximo con las condiciones óptimas tras la extracción supercrítica. Los valores máximos de la WRC coincidieron con temperaturas de extracción más bajas, lo que sugiere que temperaturas más altas podrían degradar la estructura de la fibra y reducir la WRC (Hincapié y col., 2010). Además, la FAC se vio afectada por la temperatura y por la interacción entre temperatura y presión, mostrando valores más altos para la fibra residual extraída a presiones más altas y temperaturas más bajas. Las modificaciones en las propiedades tecnológicas, tras el tratamiento previo del subproducto con extracción supercrítica, podrían atribuirse a la degradación de la fibra dietética en tales condiciones. Esta degradación conlleva a una reducción en el tamaño de partícula, lo que a su vez modifica las propiedades tecnológicas (Román y col., 2004).

Los tratamientos con fluidos supercríticos también mostraron resultados positivos al mejorar la capacidad de Sw, WRC y FAC de la fibra dietética extraída de los subproductos del brócoli. La estructura física de la biomasa cambia cuando es tratada con fluidos supercríticos, ya que esta tecnología utiliza solventes en condiciones de alta presión, cambiando la estructura física de la biomasa al aumentar la porosidad de sus fibras y su área superficial (Escobar y col., 2020). Además, el tratamiento con fluidos supercríticos aumentó el contenido de compuestos fenólicos no extraíbles de esta matriz, lo que contribuye a una mayor capacidad antioxidante. Estos hallazgos sugieren que los fluidos supercríticos podrían ser una herramienta eficaz para mejorar la funcionalidad de la fibra dietética. En esta línea, el análisis de la actividad antioxidante de la fibra dietética de la cáscara de granada reveló que los valores del ensayo DPPH

fueron mayores tras someterse el subproducto a una extracción previa mediante fluidos supercríticos. El valor óptimo estimado fue de 7579 mg Trolox/100 g de materia seca a una temperatura de 55 °C, presión de 250 bar y tiempo de 3,8 horas. Estos compuestos antioxidantes podrían ser compuestos fenólicos estructuralmente unidos a la fibra, los cuales a menudo son pasados por alto en los análisis convencionales (Hasnaoui y col., 2014). Putrino y col. (2020) indicaron que el pretratamiento con CO_2 supercrítico provoca cambios en la estructura de la fibra, aumentando la porosidad, afectando el contenido de compuestos fenólicos no extraíbles y aflojando los enlaces de hidrógeno, lo que provoca la deslignificación.

En resumen, estos estudios destacan la importancia de investigar y desarrollar métodos de mejora y modificación de la fibra dietética procedente de los subproductos vegetales, ya que pueden ofrecer beneficios tanto para la industria alimentaria como para la salud humana. Los resultados de estos estudios ofrecen una perspectiva interesante sobre cómo diferentes tratamientos pueden influir en las propiedades de la fibra dietética de los subproductos del brócoli y la granada. Sin embargo, es importante considerar que cada tratamiento tiene sus propias ventajas y limitaciones. Por ejemplo, los tratamientos enzimáticos pueden ser más selectivos en la modificación de ciertos componentes de la fibra (Karaki y col., 2016), mientras que los fluidos supercríticos permiten recuperar compuestos bioactivos con un alto grado de pureza y extractos limpios especialmente útiles para alimentos funcionales (Mushtaq y col., 2015). Además, el pretratamiento con CO_2 supercrítico puede mejorar significativamente las propiedades tecnológicas y funcionales de la fibra dietética como se ha demostrado en la extraída del subproducto de cáscara de granada. Estos hallazgos tienen implicaciones importantes para el desarrollo de procesos de valorización de subproductos agrícolas y la producción de ingredientes funcionales con potenciales beneficios para la salud. Sin embargo, se necesitan más investigaciones para comprender completamente los mecanismos involucrados en estos procesos y optimizar las condiciones de extracción para maximizar el rendimiento y la calidad de los productos obtenidos.

Modelización de las condiciones de fluido supercrítico

Con base en los resultados y conclusiones obtenidos sobre el impacto de las condiciones de fluido supercrítico sobre la modificación de la fibra, se diseñó un **modelo para predecir** la **composición química** y las **propiedades funcionales** de la

fibra dietética de las hojas de brócoli modificada mediante tecnología supercrítica bajo condiciones de tratamiento específicas. Durante este proceso, se optimizaron los parámetros de presión, temperatura y tiempo mediante la **metodología de superficie de respuesta**.

Los resultados obtenidos de este estudio indican que las condiciones de tratamiento con fluidos supercríticos aplicadas a la fibra dietética extraída de las hojas de brócoli afectan significativamente a su composición y propiedades funcionales. La aplicación de la metodología de superficie de respuesta permitió seleccionar condiciones experimentales óptimas que dieron como resultado un extracto de fibra dietética mejorado. Concretamente, se observó que las condiciones óptimas de tratamiento supercrítico dieron como resultado un concentrado de fibra dietética compuesta principalmente por oligosacáridos con un grado de polimerización entre 7 y 12. Los resultados sugieren que el tratamiento de la matriz de fibra dietética con temperaturas y presiones apropiadas durante la extracción supercrítica provoca la ruptura de la cadena molecular de los polisacáridos y la reducción del peso molecular (Barbosa y col, 2020; Cai y col., 2021). La modificación de fibra dietética mediante fluidos supercríticos para la obtención de oligosacáridos es un tema de investigación. Sin embargo, existen algunos estudios que investigan el uso de fluidos supercríticos en la extracción de polisacáridos. Gong y col. (2021) utilizaron la extracción con fluidos supercríticos con etanol como cosolvente para obtener un polisacárido de hojas de Ginkgo, los resultados mostraron que los polisacáridos obtenidos tenían un peso molecular menor que los polisacáridos derivados de hojas de Ginkgo obtenidas mediante extracción con agua caliente.

Además, se observó que las condiciones óptimas también mejoraron la composición química de la fibra dietética aumentando el contenido de ácido galacturónico y los niveles de azúcares neutros. Este aumento podría deberse a una mayor liberación de pectina como consecuencia de la degradación de la pared celular acompañada de la rotura de enlaces glicosídicos, como observaron otros investigadores al tratar y modificar fracciones de fibra dietética mediante diferentes métodos (Gan y col., 2020; Yang y col., 2022). Xie y col. (2018) exploraron los efectos del procesamiento a alta presión sobre las pectinas de cáscara de patata, encontrando un

aumento en el contenido de ácido galacturónico y una disminución en el grado de esterificación.

En cuanto a las propiedades tecnológicas, se observó un aumento significativo en la solubilidad en agua (Ws), Sw, WRC y FAC de la fibra dietética modificada bajo las condiciones óptimas de tratamiento con fluidos supercríticos. Estos resultados sugieren que el tratamiento con fluidos supercríticos mejora la capacidad de hidratación de la fibra dietética, lo que podría tener implicaciones importantes para su aplicación en productos alimenticios. Xie y col. (2017) señalan que las altas presiones tienen el potencial de modificar las fracciones insolubles de celulosa y hemicelulosa, convirtiéndolas en solubles y, por lo tanto, mejorar la solubilidad de la fibra dietética. Wang y col. (2023) demostraron que las modificaciones en la fibra dietética del orujo de lichi mediante técnicas como la homogeneización a alta presión y la presión hidrostática crearon estructuras más porosas, mejorando su WRC, Sw y FAC. Ouyang y col. (2023) también confirmaron que el tratamiento con presión ultra alta aumentó significativamente la WRC y FAC de la fibra dietética de pomelo, aunque la presión excesiva dañó la estructura interna, reduciendo estas propiedades. De manera similar, Su y col. (2024) aplicaron un tratamiento con agua subcrítica asistido por ácido láctico a la fibra dietética del bagazo de cerveza, donde la alta temperatura (180 °C) durante el proceso dio como resultado una estructura más porosa y un tamaño más pequeño de partículas, mejorando significativamente la WRC y FAC de la fibra dietética. Estos estudios subrayan la influencia positiva de las condiciones de temperatura y presión sobre las propiedades tecnológicas de la fibra dietética, mostrando cómo una modificación adecuada puede conducir a mejoras significativas en su funcionalidad, lo cual es crucial para su aplicación en productos alimenticios.

En cuanto a la CAG los efectos de la temperatura y la presión son evidentes tras el tratamiento con fluidos supercríticos. Las diferencias se pueden atribuir al cambio en la estructura de la fibra dietética después del impacto de la temperatura y la presión en la matriz, lo que podría sugerir un aumento en la superficie específica que permitiría una mayor CAG (Zheng y col., 2019). Además, se encontró un aumento en el contenido de compuestos fenólicos no extraíbles y en la actividad antioxidante de la fibra dietética modificada con tratamiento supercrítico, resultados que concuerdan con trabajos previos (Rivas y col., 2021a; Rivas y col., 2022).

En resumen, este estudio demuestra que el tratamiento con fluidos supercríticos puede ser una estrategia efectiva para modificar y mejorar la composición y las propiedades tecnológicas y funcionales de la fibra dietética de las hojas de brócoli. Las condiciones óptimas de tratamiento supercrítico seleccionadas fueron capaces de producir una fibra dietética compuesta principalmente por oligosacáridos con un grado de polimerización adecuado, así como de mejorar propiedades tecnológicas y funcionales, lo que podría tener implicaciones importantes para el desarrollo de alimentos funcionales y saludables.

Impacto de la digestión en las propiedades de la fibra dietética

En el Capítulo 6, se propuso analizar el efecto del proceso de **simulación de la digestión humana** en la **composición** y las **propiedades funcionales** de la **fibra dietética** obtenida a partir de subproductos como la cáscara de granada, la piel de tomate, los tallos de brócoli y los tallos de uva. Se empleó un sistema de digestión simulada controlado por computadora que constaba de tres biorreactores diseñados para simular las condiciones del estómago, el intestino delgado y el colon. Comprender los mecanismos de digestión gastrointestinal es fundamental para el diseño de alimentos funcionales. Por esta razón, la digestión gastrointestinal ha sido objeto de numerosos estudios (Krul y col., 2000; Havenaar y col., 2000; McDougall y col., 2005; Delgado y col., 2011; Cian y col., 2015; Ketnawa y col., 2018; Spínola y col., 2019; Pimentel y col., 2020; Ye y col., 2022). Los métodos de digestión gastrointestinal simulados integran los principales parámetros de la digestión (duración, pH, temperatura, agitación y concentración de líquidos digestivos y enzimas) y abarcan las etapas oral, gástrica e intestinal, y en algunos casos, la fermentación colónica (Makran y col., 2020). La fibra dietética tiene un papel clave durante todas las etapas de la digestión, desde la masticación hasta la evacuación de las heces.

Durante la simulación de la digestión, se observó un efecto de dilución en el contenido de fibra dietética y su composición después de la fase estomacal, siendo más evidente en las hojas de brócoli y la cáscara de granada. Sin embargo, los perfiles de composición de la fibra dietética no se vieron afectados significativamente después de esta etapa. En el intestino delgado, se registraron valores más bajos de glucosa y más altos de xilosa, y manosa en los constituyentes de la fibra dietética, indicando una mayor degradación de la pectina. En el colon, la fermentación fue baja, sin diferencias

notables entre el control y los subproductos. Hubo una ligera disminución en el contenido de monosacáridos en comparación con la etapa anterior. Estos hallazgos sugieren que la composición y la degradación de la fibra dietética varían a lo largo del tracto digestivo, lo que puede tener implicaciones para la salud intestinal. Estos resultados son consistentes con investigaciones previas que destacan la importancia de la longitud de la cadena de carbohidratos en la fermentación (Stewart y col., 2008).

La elevada capacidad antioxidante de la fibra dietética de la cáscara de granada y el tallo de uva antes del proceso de digestión, evaluada tanto por los métodos ABTS como DPPH, evidenció la **relación positiva entre el contenido de fenoles no extraíbles y la capacidad antioxidante** de estas fibras dietéticas (Ferri y col., 2016; Granato y col., 2018). Durante el proceso de digestión simulada, después de pasar por el estómago, las pérdidas de compuestos fenólicos asociados con la fibra dietética son relativamente pequeñas, ya que la fibra dietética los protege de las condiciones del tracto gastrointestinal (Tomas, 2022; Tang y col., 2022). Sin embargo, la tendencia a la reducción en los valores de fenoles no extraíbles puede deberse a la degradación o aflojamiento de las estructuras de la fibra dietética bajo las condiciones de digestión (Liu y col., 2021), lo que puede favorecer la actividad antioxidante debido a una mayor accesibilidad a los compuestos vinculados que respaldan esta actividad. De hecho, la capacidad antioxidante de los polifenoles depende no solo de la cantidad, sino también del porcentaje que puede liberarse bajo las condiciones del tracto intestinal y, por lo tanto, permanece disponible para la absorción (Garzón y col., 2020). Los compuestos fenólicos no extraíbles asociados con la fibra dietética muestran una tendencia a disminuir durante la digestión en el intestino delgado para algunas fuentes de fibra, mientras que aumentan para otras, especialmente en la cáscara de granada. Este fenómeno puede estar relacionado con la liberación y reabsorción de ácidos fenólicos durante la digestión gástrica e intestinal (Padayachee y col., 2013). Sin embargo, la **actividad antioxidante** global se mantiene alta incluso después de la digestión, lo que sugiere que una **fracción significativa de estos compuestos permanece bioaccesible y se transfiere al colon**, donde puede influir en la salud intestinal a través de la modulación de la microbiota (Bermúdez-Oria y col., 2020).

Atendiendo a la **capacidad antiproliferativa** de las células HT-29, utilizadas como modelo de cáncer colorrectal, ésta aumenta después de la digestión para algunas

fuentes de fibra, especialmente la de hojas de brócoli, cáscaras de granada y tallos de uva. Estos resultados son consistentes con investigaciones anteriores que destacan el potencial de ciertas fibras dietéticas para inhibir el crecimiento de células cancerosas colorrectales (Sharma y col., 2020).

Durante la **simulación de la digestión** gástrica, se observó una variabilidad significativa en la cantidad y composición de **oligosacáridos** presentes en las fibras dietéticas estudiadas. La cáscara de granada mostró el mayor contenido de oligosacáridos, destacando el oligosacárido con un grado de polimerización de 7 como el más predominante. Aunque no hay referencias directas sobre la producción de oligosacáridos a partir de fibra dietética después de la digestión gástrica, estudios previos han demostrado la producción de oligosacáridos a partir de otras fuentes de fibra mediante métodos químicos y enzimáticos. Zhang y col. (2018) trataron pectina de cáscara de cítricos por degradación química con ácido trifluoroacético y adquirieron tres oligosacáridos pécticos, con un rango de peso molecular (Mw) de 3000-4000 Da, 2000-3000 Da y menos de 2000 Da. Además, Yang y col. (2022) en su estudio demostraron que la hidrólisis ácida con ácido láctico combinada con un tratamiento enzimático con xilanasa fue una forma efectiva de producir xiloligosacáridos de alta pureza a partir de madera de álamo. En el intestino delgado, se observó un aumento generalizado en los valores de oligosacáridos, con una disminución notable en la digestión de la fibra de hojas de brócoli. El impacto de la digestión en la degradación de la fibra y la producción de oligosacáridos puede ser muy variable ya que existen varios factores que pueden influir como los diferentes tipos de fibra, la matriz del alimento y el perfil microbiológico, entre otros (Luzardo-Ocampo y col., 2017). Cabe señalar también que los oligosacáridos estudiados son resistentes a las condiciones del intestino delgado y llegan al colon disponibles para ser utilizados por la microbiota (Ferreira-Lazarte; Intaratrakul y col., 2022). En la fase de colon, después de 8 h, el nivel de oligosacáridos disminuyó drásticamente en todas las digestiones de fibra dietética evaluadas. Este resultado podría estar relacionado con la actividad metabólica de la microbiota del colon, que en general es propensa a consumir oligosacáridos con menor grado de polimerización (Steawrt y col., 2008; Singh y col., 2021). Estudios anteriores sobre condiciones de colon simuladas han mostrado una tendencia similar (Yang y col., 2013; van Trijp y col., 2020; Zhang y col., 2020). Los oligosacáridos estudiados mostraron resistencia a las condiciones del intestino delgado y llegaron al colon disponibles para

su uso por parte de la microbiota. Sin embargo, después de un período en el colon, se observó una disminución en el nivel de oligosacáridos en todas las digestiones de fibra dietética evaluadas, posiblemente debido a la actividad metabólica de la microbiota del colon.

En cuanto a la dinámica de la **población microbiológica** durante el **proceso de digestión** en las etapas de intestino delgado y colon. El nivel de bacterias aerobias mesófilas a lo largo de la digestión estuvo claramente asociado al recuento de enterobacterias, que fue el grupo microbiano predominante. Respecto al colon, los recuentos de *Enterobacteriaceae* aumentaron con la adición de fibra dietética, lo que es corroborado por otros autores que afirman que una dieta rica en fibra dietética incluía principalmente cambios en *Firmicutes*, *Verrucomicrobia*, *Enterobacteriaceae*, *Prevotella* y *Bacteroides* (Yao y col., 2022). Respecto a los recuentos de *Enterococos* y *Estafilococos*, ambos grupos microbianos aumentaron desde valores inferiores a 2 log UFC mL^{-1} en el estómago hasta valores superiores a 6 log UFC mL^{-1} durante las etapas de colon, excepto *Staphylococcus* que disminuyó a 3,9 log UFC mL^{-1} en la etapa de colon distal. Para ambos grupos microbianos, las fibras de piel de tomate y cáscara de granada mostraron los recuentos medios más altos en el colon. En trabajos anteriores se probó la capacidad de crecimiento de varias cepas de *Enterococcus faecium* en presencia de fibra dietética procedente de tallos de uva, demostrando ser un sustrato eficaz para promover su crecimiento (Rivas y col., 2021b). La fibra dietética también es el principal sustrato energético de las LAB y las *bifidobacterias* del colon, que tienen enzimas específicas que descomponen estos carbohidratos complejos. En general, los recuentos de *bifidobacterias*, a diferencia de las LAB, se estimularon durante las primeras etapas en el colon. Varios estudios han demostrado la capacidad de las *bifidobacterias* para metabolizar la fibra dietética de diferentes fuentes (Gómez y col., 2016; Ruiz y col., 2017; Van den Abbeele y col., 2021), aunque esta capacidad es variable entre cepas de *bifidobacterias* y especies. Cepas de *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium adolescenteis* y *Bifidobacterium animalis subsp. lactis* albergan diferentes tipos de enzimas (glicosil hidrolasa) y transportadores involucrados en la degradación de oligosacáridos y polisacáridos vegetales (Wang y col., 2022).

Durante la simulación de la digestión, se observaron cambios en los niveles de los **SFCA** en diferentes etapas del proceso, incluyendo el estómago, el intestino delgado y el colon. Después de la digestión estomacal, se registraron niveles promedio de ácido acético, propiónico y butírico de 77,9 mM, 23,5 mM y 3,9 mM, respectivamente. Los demás SFCA analizados mostraron concentraciones por debajo de 0,7 mM en todas las etapas de digestión para todas las fibras dietéticas evaluadas. Como han señalado otros autores, el **ácido acético, propiónico y butírico son los principales metabolitos** liberados durante el proceso de fermentación de los compuestos prebióticos (Koh y col., 2016). La suma de estos metabolitos se utiliza como indicador de la fermentabilidad de la fibra (Jonathan y col., 2012). Según nuestros datos, se observó una estimulación selectiva del crecimiento de las bacterias productoras de SFCA en las etapas del intestino delgado y del colon, especialmente para las bacterias productoras de ácido propiónico y en menor medida para las bacterias productoras de ácido butírico. Se ha informado que el aumento del nivel de SFCA en el intestino desempeña un papel importante en la salud humana. En particular, el ácido butírico ha sido el más estudiado por sus destacados efectos sobre la salud, protege contra la colitis y el cáncer de colon y tiene propiedades antiinflamatorias e inmunomoduladoras (Russel y col., 2013; Russo y col., 2019). El ácido acético es un precursor de la producción de ácido butírico y tiene un impacto en el metabolismo de los lípidos (Morrisson y col., 2016), mientras que el propiónico actúa como inhibidor de la gluconeogénesis y la síntesis de colesterol en el hígado y ejerce protección en el intestino humano contra patógenos gracias a su acción antibacteriana y capacidad inflamatoria (Markowiak-Kopeć, y col., 2020).

En resumen, la fuente y composición de la fibra dietética son factores determinantes en sus propiedades funcionales y su comportamiento durante la digestión. Es evidente que diferentes tipos de fibra tienen efectos distintos en la microbiota intestinal y en la producción de metabolitos beneficiosos como los SFCA. Sin embargo, se necesitan investigaciones adicionales, posiblemente utilizando técnicas avanzadas de secuenciación, para comprender completamente cómo interactúan los distintos tipos de fibra con la microbiota intestinal y cómo estas interacciones afectan la salud intestinal en general.

La fibra dietética como sustituto en la industria alimentaria. Aplicación en salsa de tomate

Por último, en el capítulo 7 se evaluó el efecto de la **adición de fibra dietética** obtenida de lías de vinificación **como sustituto de los azúcares añadidos**, en las características fisicoquímicas, nutricionales, funcionales y sensoriales de las **salsas de tomate** elaboradas.

La **incorporación de fibra dietética** en determinados alimentos puede provocar cambios en la textura, en las características organolépticas y en la vida útil de los alimentos. Además, la aplicación de la fibra dietética en los alimentos puede atender a diversas razones, tales como, su uso como sustituto de otros compuestos menos saludables (grasa o azúcares, fundamentalmente), o simplemente para suplementar alimentos que contienen poca fibra dietética o que carecen de ella. Los polisacáridos procedentes de la fibra dietética pueden ser utilizados como sustitutos de azúcares simples por sus propiedades como edulcorantes (Gerschenson y col., 2017). Rodriguez-Garcia y col. (2022) en su estudio compararon la funcionalidad de cuatro fibras solubles, dos de inulina y dos de dextrosa como posibles sustitutos de la sacarosa en galletas de masa corta evaluando su efecto sobre la reología de la masa, las dimensiones de la galleta, la textura, el color y el perfil sensorial. Los resultados mostraron que las fibras solubles pueden reemplazar con éxito el azúcar en galletas de masa corta reduciendo de manera significativa el contenido de sacarosa con respecto al control. Sin embargo, se encontraron cambios poco importantes en las propiedades organolépticas, todas las galletas reducidas en azúcar fueron significativamente más firmes y crujientes en el perfil sensorial.

Los resultados obtenidos en este capítulo sugieren que la **adición de fibra dietética** a las salsas tiene un **impacto significativo en su composición, propiedades funcionales y características sensoriales**. En términos de contenido de azúcares solubles y reductores, se observó una disminución gradual a medida que aumentaba la cantidad de fibra dietética añadida a las salsas. Los resultados obtenidos concuerdan con los obtenidos por Carcelli y col. (2021), en su estudio desarrollaron y caracterizaron tres recetas de salsas de fresa reducidas en azúcar (30%, 50% y 70%), reemplazándola por un almíbar de fibra semisólida y lograron además de reducir el contenido de azúcar, aumentar el contenido de fibra dietética de 0 g/100 g de salsa a 31,5 g/100 g de salsa, lo

que les permitió informar en la etiqueta del producto "Reducido en azúcar" y "Alto en fibra".

En cuanto al estudio del contenido de compuestos fenólicos y capacidad antioxidante de las salsas y los resultados mostraron diferencias significativas cuando se les añadió diferentes cantidades de fibra dietética y en los diferentes días del periodo de almacenamiento. La relación que existe entre la aplicación de fibra y el aumento de compuestos fenólicos está respaldada por lo publicado por otros autores. Los resultados obtenidos por Guzmán (2015) en su estudio sugieren que la adición de subproductos de tomate, manzana, el arroz y la harina de Nopal como fuente de fibra dietética en el pan de molde aumentaron el contenido total de compuestos fenólicos. Lo que evidenció una correlación positiva en el contenido de fibra dietética y compuestos fenólicos. Estos tienen una clara interacción que ofrece varias ventajas como la encapsulación de polifenoles en la fibra dietética y posibles efectos prebióticos como consecuencia de sinergias entre ambos tipos de compuestos que pueden resultar beneficiosos para la salud (Tang y col., 2020). Otros trabajos previos también obtuvieron resultados similares, Sáyago-Ayerdi y col. (2009), agregaron fibra dietética de uvas a hamburguesas de pollo y concluyeron que agregar más cantidad de fibra aumentaba la capacidad antioxidante. El aumento de la capacidad antioxidante de las salsas puede deberse a la correlación positiva observada entre el contenido de compuestos fenólicos y la actividad antioxidante. Esta correlación también es verificada por otros autores (Solari-Godiño, y col., 2017). Durante el almacenamiento de diferentes productos alimenticios la actividad antioxidante suele verse reducida (Sen y col., 2015; Deng y col., 2022), el hecho de que los resultados de nuestro estudio muestren lo contrario puede deberse a que durante el almacenamiento la fibra dietética presente en las salsas se degrada liberando compuestos con actividad antioxidante. Este hecho también ha sido corroborado por otros autores (Bermúdez-Oria y col., 2020). En este sentido, la biodisponibilidad y bioaccesibilidad de los compuestos fenólicos responsables en muchos casos de la actividad antioxidante pueden verse afectadas por las interacciones moleculares entre la fibra dietética y la matriz alimentaria (Jakobek y Matić, 2019).

En cuanto a los resultados del perfil de azúcares neutros que forman parte de la fibra dietética de las salsas elaboradas en este estudio. Los principales monosacáridos en todas las salsas elaboradas fueron el ácido galacturónico, seguido de glucosa, fucosa y

arabinosa. También se encontraron pequeñas concentraciones de manosa, galactosa y ramnosa, que resultaron ser los azúcares menores. En el caso de manosa y galactosa se encontraron diferencias significativas entre el lote control y los lotes con fibra dietética añadida, siendo los valores más bajos de ambos monosacáridos los pertenecientes al control y los más elevados en el lote con 75% de fibra añadida. El aumento del contenido de manosa y glucosa mediante la aplicación de fibra dietética procedente de lías vinícolas es un aspecto positivo por los beneficios para la salud que ofrecen ambos monosacáridos. Estudios recientes destacan los efectos anticancerígenos directos de la manosa, tanto *in vitro* como *in vivo*, logrado mediante simple suplementación en medios de cultivo celular o agua potable. Además, se ha observado que la manosa mejora la eficacia terapéutica de tratamientos como la quimioterapia, la radioterapia, la terapia dirigida y la inmunoterapia (Jin y col., 2023). Asimismo, investigaciones preclínicas en ratones indican que la galactosa, especialmente cuando reemplaza parte de la glucosa en la dieta post-destete, contribuye positivamente a la salud del hígado al reducir la acumulación de triglicéridos y la inflamación hepática. También es evidente una mejora en la sensibilidad a la insulina y una disminución de la masa de tejido adiposo, lo que indica beneficios a largo plazo en la regulación del peso corporal (Bouwman y col., en 2019).

Los resultados de la WRC revelaron que el valor máximo se alcanzó cuando se aplicó el mayor contenido de fibra dietética y disminuyó con el contenido de fibra hasta mostrar un valor mínimo. Bakirci y col. (2016) observaron un aumento en la capacidad de retención de agua al agregar fibra dietética de calabaza al yogur. Además, otros autores también han informado que la fibra dietética, especialmente la fibra dietética soluble en diversos productos alimenticios mejoraba la WRC (Baenas y col., 2020; Popoola-Akinola y col., 2022). Los cambios en las propiedades tecnológicas de la fibra dietética están asociados con cambios en la estructura química de los polisacáridos vegetales que afectan factores como las propiedades de la superficie, la densidad de carga, el tamaño de las partículas, el contenido de fibra insoluble y la naturaleza hidrofóbica de la partícula de fibra (Abirami y col., 2014).

El contenido de fenoles no extraíbles estuvo relacionado con los lotes a los que se aplicó un 50%, 75% y 100% de fibra dietética. Otros autores también demostraron que la fibra dietética, especialmente la fibra insoluble, es una buena fuente de

compuestos fenólicos no extraíbles. (Acosta-Estrada y col., 2014; Xu y col., 2020). Según investigaciones anteriores, la mayoría de los compuestos fenólicos en la fracción libre y unida mostraron el mismo patrón de reducción durante diferentes tipos de almacenamiento (Liu y col., 2021; Galani y col., 2017). Sin embargo, se demostró que algunos compuestos fenólicos aumentan después del almacenamiento, lo que podría estar asociado con la acción de enzimas que actúan sobre la descomposición de los constituyentes celulares, provocando que compuestos previamente asociados con proteínas y carbohidratos se lixiven hacia la fracción libre o se extraigan más fácilmente (Ziegler y col., 2020). Lo cual concuerda con nuestros resultados, por lo que sería conveniente en futuros estudios analizar la composición de la fracción fenólica para determinar la causa del aumento de compuestos fenólicos durante el almacenamiento.

En cuanto al análisis sensorial la variable aceptabilidad, mostró que el lote con un 25% de adición de fibra dietética obtuvo el mayor puntaje (7,56) y no mostró diferencias significativas respecto al lote control (7,31). Por otro lado, el lote con una adición del 100% presentó el puntaje más bajo en el parámetro de aceptabilidad, alcanzando un valor de 5,23. Cabe señalar que estos hallazgos se alinean con los obtenidos por Devi y col. (2023) en su estudio, donde extrajeron y agregaron fibra dietética y fitoquímicos de semillas de calabaza maduras para usar en la preparación de galletas. Los resultados mostraron que las galletas suplementadas con un 3% de fibra dietética recibieron calificaciones más altas en cuanto a aceptabilidad general en comparación con el grupo de control. Sin embargo, al igual que en nuestro estudio, no se encontraron diferencias significativas en estas valoraciones. Las evaluaciones de aceptabilidad los días 0 y 12 no mostraron diferencias significativas. Por tanto, los lotes mostraron una buena estabilidad sensorial durante el almacenamiento, resultados que concuerdan con los publicados con otros autores (Paglarini y col., 2022).

En conclusión, la adición de fibra dietética a las salsas tiene un impacto significativo en su composición, propiedades funcionales y características sensoriales, con beneficios potenciales para la salud asociados con el aumento de compuestos bioactivos y actividad antioxidante. Sin embargo, es importante considerar los posibles efectos en el producto final y optimizar las formulaciones para maximizar los beneficios para la salud sin comprometer la calidad sensorial.

CONCLUSIONES

PRIMERA

Los subproductos de la vinificación son fuentes potenciales de fibra dietética de alta calidad con buenas propiedades funcionales para aplicaciones alimentarias. En concreto, la fibra dietética obtenida de hollejos y raspones presenta una alta concentración de polifenoles no extraíbles unidos a polisacáridos con una alta actividad antioxidante y potencial para su uso en el desarrollo de prebióticos. En el caso de la fibra obtenida de las lías, rica en azúcares neutros totales, destaca por sus buenas propiedades funcionales en cuanto a la capacidad de retención de agua y la capacidad de absorción de aceite.

Winemaking by-products are potential sources of high quality dietary fibre with good functional properties for food applications. In particular, dietary fibre obtained from skins and stems has a high concentration of non-extractable polyphenols linked to polysaccharides with high antioxidant activity and potential for use in the development of prebiotics. In the case of fibre obtained from lees, rich in total neutral sugars, it stands out for its good functional properties in terms of water retention capacity and oil absorption capacity.

SEGUNDA

La fibra dietética obtenida de subproductos de granada, tomate, uva y brócoli tuvo distinto efecto sobre la supervivencia, el crecimiento y el metabolismo de microorganismos probióticos en condiciones del tránsito gastrointestinal. El efecto protector de la fibra obtenida de la piel del tomate fue superior a la de los demás subproductos mientras que la fibra extraída de los tallos de uva favoreció el crecimiento de bacterias ácido lácticas. La fibra extraída de los tallos de brócoli y de la cáscara de granada estimula la producción de ácidos grasos de cadena corta. Estos subproductos pueden considerarse como fuentes de fibra dietética de alta calidad para aplicaciones conjuntas con microorganismos probióticos. Su uso como prebióticos en la industria alimentaria ayudaría a desarrollar nuevos Ingredientes de alto valor añadido.

Dietary fibre obtained from pomegranate, tomato, grape and broccoli by-products had different effects on the survival, growth and metabolism of probiotic microorganisms under gastrointestinal transit conditions. The protective effect of the fibre obtained from tomato peel was superior to that of the other by-products while the fibre extracted from grape stems favoured the growth of lactic acid bacteria. Fibre extracted from broccoli

stems and pomegranate peel stimulates the production of short-chain fatty acids. These by-products can be considered as sources of high quality dietary fibre for combined applications with probiotic microorganisms. Their use as prebiotics in the food industry would help to develop new high value-added ingredients.

TERCERA

De las diferentes partes de los subproductos del brócoli estudiados (inflorescencias, tallos y hojas), la fibra dietética obtenida a partir de las inflorescencias presenta una mejor aptitud como ingrediente funcional por su mayor contenido de compuestos fenólicos no extraíbles y capacidad antioxidante. Los tratamientos con fluidos supercríticos y enzimas, especialmente el tratamiento con enzimas, mejoran las propiedades funcionales de los subproductos del brócoli en lo referente al crecimiento de bacterias ácido-lácticas y su producción de ácidos grasos de cadena corta, lo que los hace más atractivos para aplicaciones alimentarias y nutracéuticas.

Among the different parts of the broccoli by-products studied (inflorescences, stems and leaves), the dietary fibre obtained from the inflorescences presents a better suitability as a functional ingredient due to its higher content of non-extractable phenolic compounds and antioxidant capacity. Supercritical fluid and enzyme treatments, especially enzyme treatment, improve the functional properties of broccoli by-products in terms of lactic acid bacteria growth and their production of short-chain fatty acids, making them more attractive for food and nutraceutical applications.

CUARTA

Las condiciones de fluidos supercríticos aplicadas a la cáscara de granada afectan a la concentración y las propiedades funcionales tanto de los extractos como de la fibra dietética residual. La modelización de las condiciones del fluido supercrítico y el uso de herramientas como la función de deseabilidad de Derringer, podría ser una opción interesante para revalorizar subproductos vegetales y mejorar las propiedades funcionales tanto de los extractos obtenidos y como de la fibra residual.

Supercritical fluid conditions applied to pomegranate peel affect the concentration and functional properties of both extracts and residual dietary fibre. The modelling of supercritical fluid conditions and the use of tools such as the Derringer desirability

function could be an interesting option to revalorise plant by-products and improve the functional properties of both the extracts obtained and the residual fibre.

QUINTA

Las condiciones de fluidos supercríticos aplicadas a las hojas de brócoli afectan a la composición y propiedades funcionales de la fibra dietética obtenida. El uso de la metodología de superficie de respuesta para la optimización de las condiciones del fluido supercrítico permite la selección de condiciones experimentales específicas para obtener fibra dietética con una composición y propiedades óptimas. La tecnología supercrítica puede ser una opción interesante para revalorizar este subproducto vegetal y optimizar sus propiedades.

The supercritical fluid conditions applied to broccoli leaves affect the composition and functional properties of the dietary fibre obtained. The use of response surface methodology for the optimisation of supercritical fluid conditions allows the selection of specific experimental conditions to obtain dietary fibre with optimal composition and properties. Supercritical technology can be an interesting option to revalorise this vegetable by-product and optimise its properties.

SEXTA

La fuente y composición de la fibra dietética influyen en sus propiedades funcionales y su comportamiento durante el tránsito intestinal. La fibra dietética derivada de la cáscara de granada mantiene su alto contenido de compuestos fenólicos y actividad antioxidante durante la simulación de la digestión en el colon. La fibra dietética extraída de brócoli y raspón presenta actividad antiproliferativa tanto en el intestino delgado como en el colon, promoviendo la proliferación de bifidobacterias en lugar de lactobacilos y la producción de ácidos grasos de cadena corta.

The source and composition of dietary fibre influence its functional properties and behaviour during intestinal transit. Dietary fibre derived from pomegranate peel maintains its high content of phenolic compounds and antioxidant activity during simulated digestion in the colon. Dietary fibre extracted from broccoli and grape stems has anti-proliferative activity both in the small intestine and in the colon, promoting the

proliferation of bifidobacteria instead of lactobacilli and the production of short-chain fatty acids.

SEPTIMA

La fibra dietética obtenida de lías de vino tiene un alto potencial para mejorar la calidad nutricional y la sostenibilidad de los productos a base de tomate. Las propiedades fisicoquímicas y nutricionales del producto como pH, acidez, contenido de sólidos solubles y fibra dietética total presentan un comportamiento positivo con la adición de la fibra, al igual que las propiedades funcionales como capacidad de hinchamiento, capacidad de retención de agua y actividad antioxidante. Todo ello con una aceptación sensorial positiva. Aunque estos resultados son prometedores, se necesita más investigación para comprender completamente los efectos a largo plazo y optimizar la formulación para aplicaciones prácticas.

Dietary fibre obtained from wine lees has a high potential to improve the nutritional quality and sustainability of tomato-based products. The physicochemical and nutritional properties of the product such as pH, acidity, soluble solids content and total dietary fibre show a positive behaviour with the addition of the fibre, as well as the functional properties such as swelling capacity, water retention capacity and antioxidant activity. All with positive sensory acceptability. Although these results are promising, more research is needed to fully understand the long-term effects and to optimise the formulation for practical applications.

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ANEXO

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Review

Strategies to Increase the Biological and Biotechnological Value of Polysaccharides from Agricultural Waste for Application in Healthy Nutrition

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Abstract: Nowadays, there is a growing interest in the extraction and identification of new high added-value compounds from the agro-food industry that will valorize the great amount of by-products generated. Many of these bioactive compounds have shown beneficial effects for humans in terms of disease prevention, but they are also of great interest in the food industry due to their effect of extending the shelf life of foods by their well-known antioxidant and antimicrobial activity. For this reason, an additional research objective is to establish the best conditions for obtaining these compounds from complex by-product structures without altering their activity or even increasing it. This review highlights recent work on the identification and characterization of bioactive compounds from vegetable by-products, their functional activity, new methodologies for the extraction of bioactive compounds from vegetables, possibly increasing their biological activity, and the future of the global functional food and nutraceuticals market.

Keywords: agricultural waste; polysaccharides; bioactive function; extraction methods; nutraceuticals

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1. Polysaccharides from Agricultural Waste

Nowadays, 37 million tons of agricultural residues are generated worldwide [1] causing a serious economic and environmental problem [2]. These residues are made up of waste and plant by-products, such as skins and seeds. Plant by-products are rich sources of dietary fiber, soluble polysaccharides, phenolic compounds and fatty acids, making them particularly interesting for use as additives and functional ingredients.

Polysaccharides from agro-food industry waste constitute one of the most important renewable resources. The great variety of their chemical composition and structure, and their biodegradability and safety make them ideal for application in diverse fields, such as the food, pharmaceutical, cosmetics, tissue engineering and biofuels industries, among others [3,4].

1.1. Polysaccharide Classification

Natural polysaccharides from agricultural residues form part of the plant cell walls that are highly variable in terms of structure and composition [5]. In general, cell walls are composed of high molecular weight polysaccharides, which are mainly lignin, cellulose, hemicelluloses, pectins and other non-starch polysaccharides, such as inulin and oligosaccharides [6]. Polysaccharides that form cell walls are classified as insoluble and soluble based on their ability to be soluble in water. Insoluble polysaccharides are lignin, cellulose, hemicelluloses and pectins (insoluble); the soluble polysaccharide group consists of other pectins and hemicelluloses [7].

1.1.1. Cellulose

Cellulose is the most abundant biopolymer on Earth, with an annual production of about 50 thousand tons [8]. Composed exclusively of β-glucose molecules linked by β-1,4-glycosidic bonds, cellulose is characterized by its capacity for chemical modification and hydrophilicity [9]. Cellulose is found in different agricultural residues, such as garlic skin [10], corn [11], grape pomace [12] and carrot [13]. Cellulose has multiple applications in the food industry; among others, its application as a fat substitute [14] has been proven to improve food texture [15], and it has also been widely used as a film to protect food. Riaz et al. [16] manufactured cellulose-based coatings from Chinese chive root extract, and the results showed that the coatings possessed antioxidant and antimicrobial properties.

1.1.2. Hemicellulose

Hemicellulose is a heteropolysaccharide, composed of a heterogeneous set of polysaccharides, itself composed of two types of monosaccharides linked by β-bonds, which form a branched linear chain. It is the second most abundant component of agricultural residues, representing approximately 20–40% [17,18]. Hemicelluloses include glucans, xyloglucans, mannans, xylans and β-(1→3,1→4)-glucans [19]. However, the polysaccharides that constitute the majority of hemicelluloses are mannan and xylan [20].

Xylan is the most abundant hemicellulose found in nature [21]. Xylan from agricultural residues can be hydrolyzed and converted into xylose; furthermore, it can be turned into xylooligosaccharides (XOS) with different degrees of polymerization [22]. Aachary and Prapulla [23] reported that XOS with a degree of polymerization of 2–3 have maximum prebiotic potential. XOS are extracted industrially from corn and sugarcane [24,25], although they can also be obtained from agricultural by-products such as pineapple rind [26], straw rice [27] and quinoa stems [28].

Another polysaccharide that is part of the hemicelluloses is mannan, which is significantly present in agricultural residues. By enzymatic hydrolysis, mannan can be turned into mannooligosaccharides (MOS) [29]. MOS are considered as emerging prebiotics and can be obtained from different agricultural residues, such as copra flour [30].

1.1.3. Pectic Polysaccharides

Pectins, mostly considered soluble fiber, are part of the cell wall of plants and are heteropolysaccharide polymers rich in polygalacturonic acid that can be composed of up to 17 different monosaccharides [31], which is why it is characterized as one of the most structurally complex natural plant polysaccharides [32]. It is composed of three structurally distinct domains: homogalacturonan (HG), rhamnogalacturonan (RG-I) and rhamnogalacturonan (RG-II).

Pectins are traditionally obtained from agricultural by-products, such as citrus peels and apple pomace [33,34]. The increasing global demand for this heteropolysaccharide due to the numerous health benefits attributed to it [35] means that alternative sources of pectin are being sought in other vegetables and by-products, such as eggplant [36], tomato peel [37,38], broccoli stem [39] and pomegranate peel [40], among others.

The main component of pectins is HG, a polymer with a linear homogeneous chain of α-1,4-glycoside linked to D-galacturonic acid [41]. Methoxy esters, located in the C6 carboxyl groups of D-galacturonic acid, are substitutions generally found in the HG region and play an important role in the known functional properties and health benefits of these pectic polysaccharides [42]. RG-I is the second most abundant pectic polysaccharide in the cell wall of plants [43]. It is composed of a repeating backbone of galacturonic acid and rhamnose disaccharide, usually with neutral side chains [44]. RG-I polysaccharides have been demonstrated to have a number of promising bioactive properties [45]; their bioactivity is attributed to their composition and structure [46]. Although less common in the pectic fraction, RG-IIs are polysaccharides with abundant bioactive properties and many human health benefits. Their structure comprises a main chain linked to galacturonic acid and side chains of highly complex oligosaccharides and other unusual monosaccharides [47].

In addition, depolymerization of pectin releases pectic oligosaccharides (POS) [48]. POS are currently described as emerging prebiotics with numerous health benefits [49].

1.2. Bioactive Function

Table 1 summarizes the applications of polysaccharides obtained from by-products in the food industry. In addition to the prebiotic effect, polysaccharides have been studied for their antioxidant and antimicrobial activity; due to this activity, important effects have been reported: the prevention of diseases such as cancer, the regulation of chronic metabolic syndrome, application as immunomodulators and anti-inflammatories, and anti-diabetic, anticoagulant and antiviral activity. Additionally, technological properties are found, since they are used as a fat substitute, to improve the texture of food and as a meat preservative.

1.2.1. Prebiotic Activity

Significant research indicates that polysaccharides derived from plant cell walls have high prebiotic activity [50] and are able to stimulate the growth of certain bacteria and promote the production of certain short-chain fatty acids (SCFA) [51].

Many authors have demonstrated the prebiotic effect of different polysaccharides from agricultural waste; in vitro experiments performed with XOS extracted from barley straw demonstrated their suitability for use as a prebiotic ingredient [52]. Another type of hemicellulosic polysaccharide that demonstrates prebiotic character is MOS as they resist digestion in the upper gastrointestinal tract [53]. Pectic polysaccharides have also shown prebiotic activity. The prebiotic properties of RG-I have been tested; Khodaei et al. [54] extracted RG-I from potatoes and demonstrated the growth of beneficial bacteria (*Bifidobacterium* spp. and *Lactobacillus* spp.). POS were extracted from sugar beets, and selective growth was observed of bacteria of the genus *Lactobacillus* [55].

1.2.2. Antioxidant Activity

Nowadays, there is growing evidence that many types of polysaccharides possess important antioxidant activity. Zeng et al. [56] investigated the structural characteristics of two polysaccharides obtained from Chinese water chestnut shells and showed that both had potential antioxidant activity. The low molecular weight polysaccharide extracted from *Cucurbita moschata* also showed proven antioxidant activity, exhibiting significant scavenging ability against ABTS and DPPH radicals [57]. Heteropolysaccharides composed of rhamnose, glucose, galactose, mannose, xylose, arabinose and galacturonic acid extracted from pistachio husk showed significant antioxidant potential [58]. Four pectin-like polysaccharides (PKP-E-1− 1, -1− 2, -2− 1, and -2− 2) extracted from *Pinus koraiensis* pineapples showed promising potential antioxidant properties for food applications [59]. The antioxidant activity was measured by determining the elimination capacity of hydroxyl radicals of polysaccharides and comparing it with that of ascorbic acid.

Oxidative stress caused by free radicals has been involved in the pathogenesis of several human diseases, as natural antioxidant defenses have been found to be defective in some of the same diseases. This has led to the suggestion that disease progression may be slowed by supplementation with natural antioxidants. Antioxidant therapy may be beneficial in diseases including diabetes mellitus, reperfusion injury and inflammatory diseases, as well as in the prevention of chronic processes such as atherosclerosis and carcinogenesis.

Table 1. Summary of applications of polysaccharides from by-products in the food industry.

Compound	By-Products	Beneficial Effects	References
Cellulose	Garlic skin, corn, grape pomace, carrot, Chinese chive root extract	Technological processes: as a fat substitute, to improve the texture of food. Nutritional, antioxidant and antimicrobial activity.	[10–16]
Xylan/xylooligosaccharides, methylglucuronoxylan (hemicellulose)	Corn (corncobs), sugar cane, pineapple rind, straw rice, quinoa stems, soybean, mango seed, barley straw, neem plant, Spanish chestnut	Prebiotic activity Disease prevention: preventing cancer	[22–24,26–28,52,60,61]
Mannan/mannooligosaccharides (hemicellulose)	Copra flour/meal, palm kernel cake, guar gum, potato peel	Prebiotic activity	[29,30]
Pectins, pectic oligosaccharides (POS), homogalacturonan (HG), rhamnogalacturonan (RG-I), rhamnogalacturonan (RG-II)	Citrus peel, mandarin citrus peel, apple pomace, eggplant, tomato peel, broccoli stem, pomegranate peel, potato pulp	Prebiotic activity Disease prevention: preventing cancer, regulating chronic metabolic syndrome, immunomodulatory, anti-inflammatory and probiotic uses	[33,34,36–40,54,55,62–70]
Heteropolysaccharides, WVP-1 (mannose, glucose, galactose and arabinose) WVP-2 (mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose and arabinose)	Mango pomace, chestnut shells	Disease prevention: preventing cancer, immunomodulatory, anti-diabetic, anticoagulant, antiviral Biological activity: antioxidant activity	[56,71]
Natural low molecular weight polysaccharides (SLWPP-3)	Pumpkin by-products	Biological activity: antioxidant activity Disease prevention: anti-diabetic	[72,73]
Heteropolysaccharides (rhamnose, glucose, galactose, mannose, xylose, arabinose and galacturonic acid)	Pistachio external hull	Technological process: meat preservative Biological activity: antioxidant activity	[58]
Heteropolysaccharides: PKP-E	Pinecones	Biological activity: antioxidant activity	[59]
Glucan, inulin	Cereals	Biological activity: immunomodulatory, anti-inflammatory, antimicrobial activity	[74–76]

1.2.3. Anti-Diabetic Activity

Diabetes is a growing global problem and a heavy financial cost burden on health services. Millions of people suffer from the disease which causes many deaths each year, as well as being associated with an increased risk of other health problems. Polysaccharides have a significant hypoglycemic effect and therefore may be useful to prevent diabetes mellitus resulting from defects in insulin production or action that cause hyperglycemia. Li et al. [72] reported the hypoglycemic effects of polysaccharides extracted from pumpkin by-products. Different by-product polysaccharides have gained popularity among researchers due to their numerous bioactive properties, including inhibitory effects against starch hydrolyzing enzymes such as α-amylase and α-glucosidase, highlighting their potential as anti-diabetic agents in the treatment and prevention of diabetes mellitus [73].

1.2.4. Anticancer Activity

The anticancer effect of natural polysaccharides from plant residues is currently under study. Sharma et al. [61] demonstrated in their study that XOS extracted from sawdust of *Azadirachta* inhibits the growth of human colorectal cancer cells (HT-29). In another study, three major polysaccharides were isolated from mango pomace, composed of seven

monosaccharides (mannose, rhamnose, glucose, galactose, xylose, arabinose and fucose) and two uronic acids, and the isolated polysaccharides showed significant anticancer activity against HepG2, MCF-7, A549, HeLa, A2780, HCT-116 and BGC- cells [71]. HG from the extraction of *Hippophae rhamnoides* berries has also been shown to have an antitumor effect [64]. RG-I have been shown to be able to promote cell adhesion and migration [77] and immunomodulatory activity [62].

1.2.5. Anti-Inflammatory Activity

Natural polysaccharides such as glucan [74], inulin [75] and pectins [78] have been shown to have strong anti-inflammatory activity. As demonstrated by Bermudez-Brito et al. [63], pectic polysaccharides exhibit higher anti-inflammatory activity than inulin and glucan. Moreover, pectic polysaccharides show high anti-inflammatory activity in their three domains (HG, RG-I and RG-II) [70].

Hosary et al. [76] isolated glucan from the plant *Avena sativa* L. and demonstrated its anti-inflammatory capacity, showing its high potential for use as a wound-healing hydrogel; it also showed antimicrobial activity against *Staphylococcus aureus* and *Micrococcus luteus*.

1.2.6. Antimicrobial Activity

Several studies have shown the antimicrobial activity of natural polysaccharides as they have a strong ability to inhibit the growth of a wide range of infectious and spoilage micro-organisms. Rostami et al. [79] extracted polysaccharides from *Malva sylvestris* and showed that Gram-positive bacteria (*Bacillus cereus* PTCC 1015 and *Staphylococcus aureus* PTCC 1112) were less sensitive than Gram-negative bacteria (*Escherichia coli* PTCC 1763 and *Salmonella typhimurium* PTCC 1709) to the different polysaccharide extracts obtained. However, Hosary et al. [76] developed a hydrogel using polysaccharides derived from Egyptian *Avena sativa* L. The developed product showed slight activity against *Candida albicans* and high activity only against the Gram-positive bacterial strains *Staphylococcus aureus* ATCC 297373 and *Micrococcus luteus* ATCC 10240, and none against the two Gram-negative strains used in the study, *Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 25619. In the same way, Hashemifesharaki et al. [80] obtained polysaccharides by microwave-assisted extraction of marshmallow root and increased the antimicrobial activity of the polysaccharide extract mainly against Gram-positive bacteria, in particular, *Staphylococcus aureus* PTCC 1189 and *Bacillus circulans* ATCC 4516.

2. Extraction of Polysaccharides: Methods and Influence on the Bioactive Function

The different extraction methods, the extraction solvent, the pH, the ratio of raw material to solvent, the temperature and the time have a significant influence on the yield, technological properties and functions of bioactive polysaccharides [81,82]. Each extraction method has its advantages and disadvantages; therefore, the extraction method chosen should be adapted to the final purpose, the nature of the by-product and the cost of the procedure.

Table 2 shows an overview of the optimized extraction methods and their influence on the bioactive function of polysaccharides obtained from plant by-products.

2.1. Hot Water Extraction (HWE)

HWE is one of the most widely used methods to extract polysaccharides, being conventional, simple and cheap. However, the use of HWE is limited due to its low yield; only extracellular polysaccharides can be obtained since the cell wall is not degraded [83]. High temperatures and long extraction times are needed to achieve high yields [84], which results in degradation of the structure and a decrease in quality and bioactivity [85,86]. Therefore, there is a need to explore new methods of polysaccharide extraction that ensure a good yield besides maintaining the bioactive and functional characteristics of the polysaccharides.

Several authors have reported the optimization of HWE conditions and assurance of good yields. Romdhane et al. [87] have reported the effect of temperature, time and ratio of water to raw mate-rial on the extraction yield of WMRP using the classical "one factor at a time" methodology. These authors obtained a good yield of polysaccharides extracted from watermelon rind, and they showed good functional activity and significant antioxidant capacity. Khatib et al. [88] showed that the use of hot water maximized solubility and extractability of the crude polysaccharides from Laffan and Wonderful pomegranate mesocarp with prebiotic properties in vitro by serving as an excellent substrate for the growth of potentially probiotic bacteria, such as *Lactobacillus* and *Bifidobacterium* strains. Sharifian-Nejad et al. [89] optimized the extraction of polysaccharides from oleaster fruits depended on temperature, water/dry matter ratio, time, and alcohol ratio and the highest polysaccharide purity at a temperature of 60 °C; and obtained the best results at 53:1 water/dry matter ratio (*V*/*W*); time, 5 h; and alcohol ratio of 2.9 (*V*/*V*) with a solubility of 67.46%.

2.2. Ultrasound-Assisted Extraction (UAE)

UAE is based on a phenomenon called acoustic cavitation, which involves the generation and formation of gas vapor-filled bubbles in a liquid that expand and finally collapse. Cavitation generates circulating liquid currents and turbulence as well as an increase in temperature and pressure [90]. This leads to an increase in the overall extraction yield [91]. Among the advantages of UAE are that it is considered one of the most cost-effective techniques for the extraction of polysaccharides [90], apart from being efficient, fast and environmentally friendly.

UAE has been used to extract pectic polysaccharides from the peel of fruit and vegetables, such as eggplant [36], pomegranate [92], tomato [93], custard apple [94] and mango [95]. UAE of tomato peel was able to efficiently extract two valuable bioactive ingredients (pectin and polyphenols) simultaneously, in addition to shortening the extraction time with respect to conventional extraction techniques [37]. It has also been reported that high yields of hemicellulose polysaccharides can be obtained with short extraction times, especially xyloglycans by UAE in grape pomace [96]. UAE turns out to be efficient for extracting prebiotic sugars from industrial artichoke residues: 1-kestose, nystose, fructofuranosylnystose and raffinose were successfully extracted, obtaining an extract of approximately 9.6 mg of prebiotic saccharides/g of dry raw material [97].

2.3. Microwave-Assisted Extraction (MAE)

MAE involves the penetration of electromagnetic radiation into a solid matrix. The heating generated is due to the molecular friction caused by the ionic conduction of the dissolved ions and the rotation of the dipoles of the polar solvent, which favor the extraction of the bioactive compounds. Both the heating produced and the internal pressure originated cause rupture of the cell; as a consequence, the structure is altered, which facilitates the release into the solvent of the bioactive compounds, improving the transfer coefficient [98–100]. MAE is a promising technique for polysaccharide extraction; it has advantages such as high yields, less solvent used, shorter extraction times and being environmentally friendly [101–103].

Currently, MAE is widely used in the extraction of polysaccharides from various sources of by-products, such as banana peel [104], grapefruit peel [105], broccoli by-products [106] and cocoa shell [107], among others. Dao et al. [108] reported higher yields (18.73%) and higher purity of pectic polysaccharides extracted from fruit peels, such as dragon fruit and passion fruit, compared to conventional methods. In addition, extraction times were shortened, resulting in a reduction in energy consumption. High yields of hemicellulose polysaccharides such as xylan, glucuronoxylan and xyloxylan extracted by MAE from tobacco plant residues have also been reported [109].

Table 2. Review of optimized extraction methods and their influence on the bioactive function of polysaccharides obtained from plant by-products.

Optimized Extraction Method	Compounds	By-Products	Influence on Bioactive Function	Reference
Hot water extraction	Polysaccharides	White mulberry	Anti-diabetic, immunomodulatory, anti-inflammatory, antioxidant, hepatoprotective, renoprotective and anti-obesity activity; effect on gut microbiota	[84]
Hot water extraction	Polysaccharides	Watermelon rind	Antihypertensive and antioxidant activity	[87]
Hot water extraction	Polysaccharides	Pomegranate fruit	Prebiotic activity	[88]
Hot water extraction	Polysaccharides	Oleaster fruit	N.d. *	[89]
Ultrasound-assisted extraction	Polysaccharides/starch, pectin	Yam tubers, fruit peel, tomato processing, potato . . .	Antioxidant, anticoagulant, antitumor, anti-inflammatory and prebiotic activity	[90]
Ultrasound-assisted extraction	Polysaccharides/pectin	Fruit and vegetable peel: eggplant	Antioxidant activity	[36]
Ultrasound-assisted extraction	Polysaccharides/pectin	Pomegranate peel	N.d. *	[92]
Ultrasound-assisted microwave extraction	Polysaccharides/pectin	Tomato peel	N.d. *	[93]
Ultrasound-assisted extraction	Polysaccharides/pectin	Custard apple peel	N.d.*	[94]
Ultrasound-assisted extraction	Polysaccharides/pectin	Mango peel	N.d. *	[95]
High hydrostatic pressure and ultrasound-assisted extraction	Polysaccharides/pectin	Tomato peel	N.d. *	[37]
Ultrasound-assisted extraction	Hemicellulose polysaccharides/xyloglycans	Grape pomace	N.d. *	[96]
Ultrasound-assisted extraction	Fructooligosaccharides	Artichoke industrial waste	Prebiotic activity	[97]
Microwave-assisted extraction	Polysaccharides	Marshmallow roots	Antioxidant, antimicrobial and antitumor activity	[80]
Microwave-assisted extraction	Polysaccharides	*Chuanminshen violaceum* root	Increased antioxidant activity	[86]
Microwave-assisted extraction	Polysaccharides/pectin	*Carica papaya* L. peel	N.d. *	[98]
Microwave-assisted extraction	Polysaccharides	Kiwifruit	Antioxidant activity	[99]
Microwave-assisted extraction	Polysaccharides	*Camptotheca acuminata* fruits	Antioxidant and immunomodulatory activity	[100]
Surfactant and microwave-assisted extraction	Polysaccharides/pectin	Orange peel	N.d. *	[101]
Microwave-assisted extraction	Polysaccharides	Waste jamun fruit seeds	N.d. *	[102]
Microwave-assisted extraction	Polysaccharides	*Sargassum pallidum*	Hypoglycemic activity	[103]
Microwave-assisted extraction	Polysaccharides/pectin	Banana peel	N.d. *	[104]
Hot-solvent microwave extraction	Polysaccharides/pectin	Pomelo peel	N.d. *	[105]
Microwave hydrodiffusion and gravity	Polysaccharides	Broccoli	N.d. *	[106]
Microwave-assisted extraction	Polysaccharides	Cocoa bean shell	Antioxidant activity	[107]

Table 2. *Cont.*

Optimized Extraction Method	Compounds	By-Products	Influence on Bioactive Function	Reference
Microwave-assisted extraction	Polysaccharides/pectin	Fruit peels	N.d. *	[108]
Microwave-assisted extraction	Hemicellulose polysaccharides/xyloglycans	Tobacco plant residues	N.d. *	[109]
Enzyme-assisted extraction	Polysaccharides	*Fritillaria pallidiflora* Schrenk	Antioxidant, antimicrobial, anti-inflammatory, antitumor and antihypertensive activity	[110]
Enzyme-assisted extraction	Polysaccharides	*Malva sylvestris* plant	Increased antioxidant, antitumor and antimicrobial activity	[79]
Enzyme-assisted extraction	Polysaccharides	Cup plant (*Silphium perfoliatum* L.)	Antioxidant and hypoglycemic activity	[111]
Enzyme-assisted extraction	Polysaccharides/pectin	Kiwi pomace	N.d. *	[112]
Enzyme-assisted extraction	Polysaccharides/pectin	Apple pomace	Antioxidant and anticancer activity	[113]
Enzyme-assisted extraction	Polysaccharides/pectin	Pomegranate peel	Antioxidant activity	[114]
Enzyme-assisted extraction	Polysaccharides	*Dendrobium chrysotoxum*	Immunological activity	[115]
Enzyme-assisted supercritical fluid extraction	Polysaccharides	Pomegranate peel	Antioxidant activity	[116]
Supercritical fluid extraction	Polysaccharides	*Artemisia sphaerocephala* Krasch. seeds	N.d.*	[117, 118]
Supercritical fluid extraction	Polysaccharides	Pomegranate peel	Antioxidant activity	[118]
Deep extraction with eutectic solvent/microwave-assisted extraction	Polysaccharides	Bladder-wrack (*Fucus vesiculosus*)	Antioxidant activity, cell growth inhibition	[119]
Accelerated solvent extraction	Polysaccharides	Bamboo shoots	Antioxidant activity	[120]
Dynamic high-pressure microfluidization	Polysaccharides	*Nelumbo nucifera* leaves	Antioxidant activity	[121]
Ultrasonic-cellulase synergistic extraction	Polysaccharides	Pineapple pomace	Hypoglycemic and anticancer activity	[122]
Deep extraction with eutectic solvent	Polysaccharides/pectin	Pomelo peel	N.d. *	[123]

* Not determined.

2.4. Enzyme-Assisted Extraction (EAE)

While the UAE and MAE methods break the cell wall, EAE degrades the cell parts by enzymatic hydrolysis, which causes an improvement in the yield and biological activity of polysaccharides [110,124]. The method is selective for the extracted bioactive compounds and environmentally friendly [80]. The extraction yield depends on several factors, such as the liquid–solid ratio, pH, amount of enzyme, temperature and extraction time [111,125].

EAE has been used effectively for extraction of pectin from kiwifruit pomace, demonstrating a higher pectin yield by enzymatic extraction with Celluclast (cellulases, polygalacturonase, pectin lyase and rhamnogalacturonan lyase) than by acid extraction with citric acid [112]. Wikiera et al. [113] also reported higher extraction yields for pectin (15.3%) from apple pomace by EAE compared to acid extraction with sulfuric acid. EAE achieved higher polysaccharide extraction yields from pomegranate peel than those obtained with HWE and UAE, the extracted polysaccharides having strong antioxidant properties [114].

Extraction of polysaccharides from *Dendrobium chrysotoxum* by EAE provided 1.25-fold higher yields than with HWE [115].

2.5. Supercritical Fluid Extraction (SFE)

SFE is an emerging technology in the extraction of bioactive compounds that allows the natural qualities of the compounds to be preserved and ensures food safety [126]. The critical point of CO_2 (31 °C and 7.38 MPa) allows the recovery of bioactive compounds with a high degree of purity and especially useful clean extracts for functional foods [116]. SFE technology has, to a large extent, been used for apolar substances, although selective extraction of polar compounds is possible by using modifications. Therefore, the use of cosolvents, such as ethanol and methanol, can increase the efficiency of polysaccharide extraction [118]. However, SFE conditions and information available on polysaccharide extraction and its properties are limited. Chen et al. [117] reported a yield of 18.59% for SFE of polysaccharides from *Artemisia sphaerocephala* plants with extraction parameters of 45 MPa at 45 °C, with a CO_2 flow of 20 L/h for 2 h. Rivas et al. [118] optimized SFE using CO_2 to obtain high-value compounds from pomegranate peel by-products.

2.6. Other Extraction Methods

New polysaccharide extraction methods are being developed, such as deep extraction with eutectic solvents [119], accelerated extraction with solvents [120,123] and high-pressure dynamic microfluidization [121]. In addition, the previously mentioned methods are also combined to improve the yields and bioactive functions of polysaccharides.

The polysaccharides, mannose, rhamnose, glucose, galactose, xylose, arabinose, fucose, glucuronic acid and galacturonic acid, were extracted from pineapple pomace by combining UAE and EAE to provide ultrasonic-cellulase synergistic extraction; the extracted polysaccharides demonstrated the ability to inhibit development of HepG2 cells resistant to insulin and therefore can be considered potential ingredients to develop a new beneficial food [122]. In addition, Liew et al. [123] reported a 1.5 to 3.5 times higher yield for the extraction of pectic polysaccharides combining UAE and EAE compared to separate extraction methods.

3. Strategies to Increase the Biological Activity

The bioactivity of polysaccharides depends on their structure, monosaccharide units, bond type, spatial configuration, branched-chain distribution and molecular weight [127,128]. The high molecular weight of polysaccharides in nature makes it necessary to modify and degrade polysaccharides to improve their solubility and make them more accessible to cells [129,130]. Chemical [131], physical [80] and biological [57,132] methods are used to modify and degrade polysaccharides, thereby reducing their size and molecular weight to improve their bioactivity.

3.1. Enzymatic Modification

Enzymatic degradation improves the properties and bioactivity of polysaccharides by modifying their structure and reducing the molecular weight [133]. The main advantage is the high selectivity, which allows polysaccharides to be modified in a personalized way, obtaining well-defined structures [134]. Enzymatic modification, in addition to improving polysaccharide properties and oligosaccharide production [133], also increases the possibility of extracting non-extractable polyphenols associated with cell wall polysaccharides [135–137].

The acquisition of POS with a specific degree of polymerization (DP) by enzymatic treatment of pectic polysaccharides extracted from onion skins has been reported. Pectin treatment was carried out with three enzymes (EPG-M2, Viscozyme and pectinase), EPG-M2 being the one that showed the best results for the production of POS [138]. Mathew et al. [139] improved by means of enzymatic treatment the soluble polysaccharide arabinoxylan extracted from wheat bran through the action of enzymes (endoxy-

lanases GH10 and GH11), managing to produce XOS and arabino-xylooligosaccharides with prebiotic potential. Enzyme treatment with α-amylase and amyloglucosidase of the immunostimulatory polysaccharides isolated from red ginseng was proven to improve the modulating activity of the intestinal immune system compared to non-enzymatically treated polysaccharides [140].

3.2. Ultrasound Modification

Ultrasound degradation is a physical method that allows operation at high or low frequency depending on the intended purpose [90]. Degradation by ultrasound modifies the molecular weight and solubility and improves the properties and bioactivity of polysaccharides [141,142]. In addition, ultrasound treatment is an environmentally friendly, fast method that saves energy [143].

Chen et al. [144] demonstrated in their study the effect of ultrasound treatment on the digestibility of five fractions of polysaccharides extracted from bamboo shoots (*Chimonobambusa quadrangularis*). The results indicated that there was an improvement in the prebiotic potential of the polysaccharides. Similarly, Zeaiter et al. [145] reported higher prebiotic activity in artichoke polysaccharides extracted using the ultrasound technique compared to other treatments. In addition to improving prebiotic activity, modification by ultrasound has been shown to enhance other bioactive properties, such as antioxidant activity, ultrasonic treatment being reported to improve the antioxidant activity of pectic polysaccharides extracted from hawthorn [146]. Modification by ultrasound has also been reported to improve the bioactivity of polysaccharides, such as anti-inflammatory [147] and antitumor properties [148].

3.3. Microwave Modification

The treatment of polysaccharides by microwave technology is a promising way to obtain polysaccharides with suitable properties and bioactivity. The bioactivity of polysaccharides of natural origin is conditioned by their structure, composition and number of monosaccharides, molecular weight, type of bond and DP [149]. Therefore, despite being a promising approach, it is a novel technique for polysaccharide improvement, and the effect on bioactivity needs to be further investigated and studied. However, there is evidence that polysaccharides obtained and treated with microwave technology have antioxidant [150], antifungal [151], antiviral [152], antitumor [80] and antibacterial activity [153].

The minimum inhibitory concentrations of the pathogens *E. coli*, *B. subtilis* and *S. aureus* were determined in the presence of microwave-treated and conventionally treated ginseng polysaccharides, and higher antibacterial activity was reported for microwave-treated polysaccharides compared to those that were treated with conventional technology [6]. Hashemifesharaki et al. [80] demonstrated in their study that microwave treatment is able to purify the homogeneous fractions of polysaccharides extracted from marshmallow root, and these showed significant improvement in their antiradical, antioxidant, antimicrobial and antitumor activity.

3.4. Other Treatments

Other techniques are being investigated for the modification of polysaccharides, such as cold plasma processing [154,155], pulsed electric field processing [156], high pressure [83], high-pressure UAE [157,158], supercritical fluids [159,160] and degradation with ascorbic acid [161,162].

Cold plasma is an emerging technology in the processing of temperature-sensitive biological compounds [163], and it is also considered an environmentally friendly technology [164]. It has been used to modify the properties and bioactivity of polysaccharides [165]. The polysaccharide galactomannan was extracted from the seeds of the legume fenugreek and modified by cold plasma degradation; the results showed improved functional and rheological properties of the polysaccharide [166].

Pulsed electric field technology is an environmentally friendly non-thermal technology [167]. It is used for the extraction and modification of polysaccharides and oligosaccharides, such as pectin [168–170] and inulin [171], improving their properties.

High pressure is an increasingly popular non-thermal method in the processing of bioactive compounds. It has been used for the modification of macromolecules, significantly improving their functional properties and bioactivity. It acts on polysaccharides by causing changes in their structure, bonding, and monosaccharide composition and number [172]. Porfiri et al. [173] showed the high potential of high pressure to modify polysaccharides, achieving the solubilization of certain compounds in soybean regarded as insoluble polysaccharides.

4. Future Perspective: Nutritional Therapy

Polysaccharides obtained from agricultural waste by-products are heterogeneous and structurally different, which complicates the research. The beneficial effects of these polysaccharides have already been demonstrated, and they could be used as nutraceuticals. Nutraceuticals, according to the recognized definition, are foods or parts of foods that confer medical or health benefits, including the prevention and/or treatment of a disease. The term "nutraceutical" was introduced in 1989 by Stephen DeFelice and combines two words: "nutrient" (a nutritional food component) and "pharmaceutical" (a drug) [174]. Polysaccharides obtained from agricultural waste usually have limited aqueous solubility and bioavailability that can be improved by using the technologies mentioned earlier in this review. Figure 1 shows a summary of the different polysaccharides obtained from agricultural waste, their health benefit applications and methods for obtaining them.

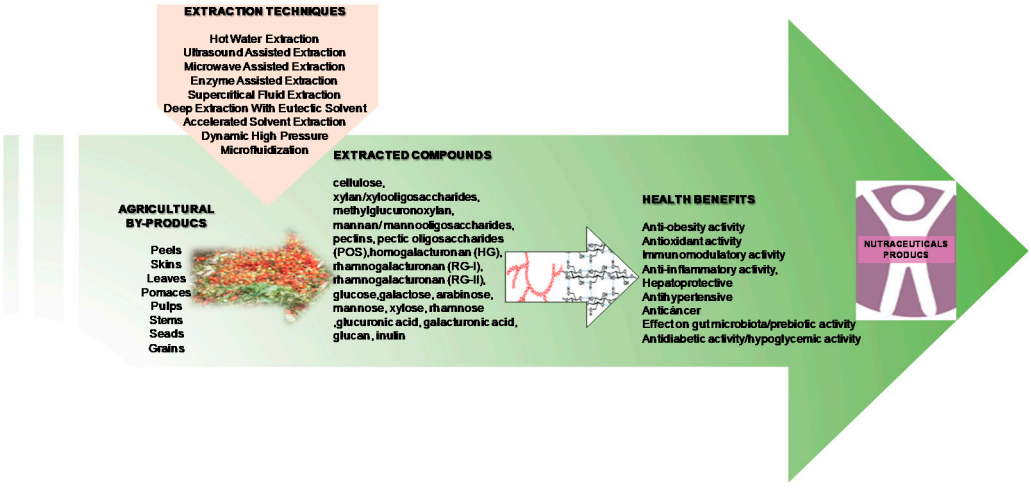

Figure 1. Overview of extraction of agricultural waste by-product polysaccharides with different health-beneficial activity for nutraceutical use.

It is of great interest to explore the therapeutic use of these polysaccharides in humans, considering their prebiotic, antioxidant, anticancer, hypoglycemic, anti-inflammatory and antimicrobial activity. Current therapeutic strategies are focused on the use of natural compounds to avoid the use of drugs that are often ineffective and have adverse health effects. Although more studies should be developed in this field, recently, several different non-digestible polysaccharides have been studied for their therapeutic use, most of them focused on intestinal health and intestinal microbiota [175]. Different seaweed polysaccharides, used for drug development, have been shown to be effective for the treatment of Inflammatory Diseases of the Intestine [176]. Saeed et al., [177] described the therapeutic benefits of a fucose-rich polysaccharide from brown algae, defining its importance for the treatment of ischemic diseases and as an anticancer agent to treat colon cancer. Other medicinal plants, which are potential sources of biologically active polysaccharides, showed immunological, antitumor, antihyperlipidemic, cardioprotective,

analgesic, anti-inflammatory, antidiabetic, antioxidant and hepatoprotective effects. Several polysaccharides isolated from different parts of *Lagenaria siceraria* medicinal plant [178] have been proposed for therapeutic use.

5. Conclusions

In order to obtain the best characteristics of the polysaccharides for therapeutic use, researchers have observed how the method used to acquire these compounds can be crucial for increasing the concentration of the polysaccharides extracted and significantly improving their biological activity. The methods reviewed here have been optimized to enhance the solubility rate and decrease the molecular size of polysaccharides, improving their bioavailability without modifying their healthy functional properties. According to the existing studies and literature resources, careful attention should be focused on the bioactive effect of polysaccharides from agricultural waste under in vivo clinical conditions. The use of these functional polysaccharides will be a promising approach in promoting public health. Further research is needed to elucidate the relationship between chemical structure, biological activity and behavior at the gastrointestinal tract level.

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