Universidad de Oviedo

Programa de Doctorado en Ingeniería Química, Ambiental y Bioalimentaria

ESTRATEGIAS DIETÉTICAS PARA CONTRARRESTAR EL IMPACTO DE LA INGESTA DE XENOBIÓTICOS SOBRE LA MICROBIOTA INTESTINAL Y EL SISTEMA INMUNE

Doctorando:

Aida Zapico Linares

Oviedo, Enero 2025

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Directores:

Dra. Sonia González Solares

Dr. Celestino González González

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RESUMEN DEL CONTENIDO DE TESIS DOCTORAL

1.- Título de la Tesis	
Español/Otro Idioma: Estrategias dietéticas para contrarrestar el impacto de la ingesta de xenobióticos sobre la microbiota intestinal y el sistema inmune	Inglés: Dietary strategies to counteract the impact of xenobiotic intake on the intestinal microbiota and the immune system.

2.- Autor	
Nombre: Aida Zapico Linares	
Programa de Doctorado: Programa Oficial de Doctorado en Ingeniería Química, Ambiental y Bioalimentaria	
Órgano responsable: Centro Internacional de Postgrado	

RESUMEN (en español)

En paralelo con el aumento de enfermedades crónicas, se ha producido un alejamiento progresivo de la Dieta Mediterránea (DM) que ha sido reemplazada por un patrón occidentalizado. La incorporación de nuevas técnicas de cocinado y el incremento en el consumo de carne y de alimentos ultra-procesados, promueve la ingesta de xenobióticos como aminas heterocíclicas (AH), hidrocarburos aromáticos policíclicos (HAP), nitrosaminas y acrilamida, clasificados por la Agencia Internacional de Investigación en Cáncer como “carcinógenos” y “probables carcinógenos”. El nivel de ingesta de estos compuestos se ha asociado con un incremento del daño sobre la mucosa intestinal. Sin embargo, su potencial carcinogénico en el colón está influenciado por la microbiota intestinal, el sistema inmune y la dieta. Por el momento, existen escasas evidencias del potencial que algunos patrones dietéticos o componentes alimentarios poseen para actuar como moduladores del daño provocado por la ingesta de xenobióticos. Por ello, el objetivo general de la presente Tesis Doctoral ha sido identificar patrones dietéticos o componentes alimentarios, con potencial para contrarrestar el efecto de la ingesta de xenobióticos, sobre el mantenimiento de la homeostasis intestinal a través de la modulación de la microbiota intestinal y de los parámetros inmunes. Para la consecución de este objetivo, se reclutó una muestra de adultos sin patologías gastrointestinales diagnosticadas para la validación de una herramienta dietética, específicamente diseñada para la cuantificación de la ingesta de xenobióticos. Además, se evaluó el impacto de una intervención dietética basada en la DM sobre la modulación de la microbiota intestinal, marcadores sanguíneos y otros parámetros relacionados con el estado de salud. Finalmente, se evaluó el potencial de la fibra y de un probiótico, compuesto por cepas pertenecientes a *Lactobacillus* y *Bifidobacterium*, para revertir el daño producido sobre la mucosa del colon tras el consumo de xenobióticos en un modelo animal PhIP+DSS. La ingesta dietética, se analizó mediante las Tablas del Centro de Enseñanza Superior de Nutrición y Dietética, Marlett & Cheung, del *Phenol Explorer* o del Estudio Prospectivo Europeo Sobre Cáncer y Nutrición entre otras. El análisis de la composición y actividad microbiana fecal se realizó mediante la secuenciación del gen ARN ribosómico 16S y el análisis de metabolitos por cromatografía de gases. Asimismo, se determinaron los parámetros inmunes en plasma mediante citometría de flujo y se evaluó el daño sobre la mucosa del colon en animales, a través del análisis histológico de secciones incluidas en parafina con tinción hematoxilina-eosina. El cuestionario diseñado mostró precisión para la cuantificación de las AH (MeIQx, DiMeIQx, PhIP), y de las nitrosaminas (NDMA, NPIP, NPYR), mostrando niveles de ingesta en el rango descrito en población europea y americana. Del conjunto de compuestos evaluados, el PhIP presentó los niveles de ingesta más elevados, superando los umbrales asociados con un incremento del riesgo de alteración intestinal. Asimismo, el consumo de DiMeIQx, IQ, nitritos y nitrosaminas fue superior en aquellos individuos que presentaban una mayor alteración de la funcionalidad gastrointestinal, hemorroides o sangrados en heces. Por otro lado, la DM redujo la ingesta de xenobióticos, tales como DiB(a)A, HAP totales, NPIP y acrilamida, en paralelo con un descenso de la abundancia relativa de *Clostridia* UCG014, la reducción de parámetros pro-inflamatorios y el incremento de parámetros anti-inflamatorios. Finalmente, la suplementación con fibra redujo el daño en la mucosa del colon provocado por PhIP+DSS, y el probiótico redujo la longitud del colon. Además, ambas suplementaciones contrarrestaron el efecto de la exposición a PhIP+DSS, mediante la reducción de la abundancia relativa de *Clostridia* UCG014 y la modulación de marcadores inmunológicos de la vía Th17. La presente Tesis Doctoral proporciona una herramienta dietética válida para la recogida y cuantificación de la ingesta de xenobióticos. La DM mejoró el perfil inflamatorio y moduló la composición microbiana intestinal. Asimismo, la suplementación de animales expuestos a PhIP+DSS

con fibra, contrarrestó el daño provocado sobre la mucosa del colon y moduló la microbiota intestinal y del sistema inmune. Los hallazgos presentados podrían servir para la identificación de patrones dietéticos y/o componentes derivados de la dieta que serán de interés para el desarrollo de estrategias dietéticas personalizadas dirigidas a la prevención del cáncer colorrectal en la población.

RESUMEN (en Inglés)

A decline of the Mediterranean Diet (MD) in substitution of the Westernized pattern has been observed, in parallel with an increase incidence of chronic diseases. The use of new cooking techniques and increased consumption of meats and ultra-processed foods promotes the intake of xenobiotics, such as heterocyclic amines (HA), polycyclic aromatic hydrocarbons (PAH), nitrosamines and acrylamide. These compounds have been classified by the IARC as “carcinogenic” or “probably carcinogenic”, with their level of intake associated with an increased damage of the intestinal mucosa. However, the carcinogenic potential of these xenobiotics in the colon is influenced by the gut microbiota, the immune system and the diet. To date, little information is available on the potential of dietary patterns or food components to act as modulators of the damage provoked by the intake of xenobiotics. Therefore, the Main Objective of this Doctoral Thesis is to identify dietary patterns or diet-derived components with the potential to counteract the effect of xenobiotic consumption on the maintenance of intestinal homeostasis by the modulation of the intestinal microbiota and the immunological parameters. To achieve this Objective, adults without diagnosis of any gastrointestinal condition were recruited for the validation of a dietary instrument specifically developed for the quantification of xenobiotic intake. In addition, the impact of the MD on the modulation of gut microbiota, blood markers and parameters associated with the health status were evaluated. Finally, the potential of fibre and a probiotic, composed of *Lactobacillus* and *Bifidobacterium* to reverse the colon mucosal damage produced by the consumption of xenobiotics, was assessed in a PhIP+DSS animal model. Dietary intake was analysed by the Food Composition Tables of Centre for Higher Education in Nutrition and Dietetics, Marlett and Cheung, Phenol Explorer and the European Prospective Investigation into Cancer and Nutrition, among others. The analysis of composition and activity of faecal microbiota was performed through ribosomal RNA-gene sequencing and excreted metabolites were analysed by flow cytometer. The histological assessment of colon mucosa in animals was performed after embedding histologic sections in paraffin and staining with haematoxylin-eosin. The questionnaire designed for evaluating the intake of xenobiotics was particularly accurate for the quantification of HA (MeIQx, DiMeIQx, PhIP), nitrosamines (NDMA, NPIP, NPYR), showing levels of intake in the range of those described in European and American populations. Among all components evaluated, PhIP presented the highest level of intake, being greater than the threshold levels associated with an increased risk of intestinal mucosa alteration. In consonance, the consumption of DiMeIQx, IQ, nitrites and nitrosamines was higher in those individuals who presented a greater alteration of gastrointestinal functionality, haemorrhoids or bleeding in faeces. On the other hand, the MD reduced the intake of xenobiotics, such as DiB(a)A, total PAH, NPIP and acrylamide, in parallel with a reduction of the relative abundance of *Clostridia* UCG014, the reduction of pro-inflammatory mediators and the increment of anti-inflammatory parameters. Finally, the supplementation with fibre reduced the histological damage in the colon mucosa caused by PhIP+DSS, and the probiotic increased colon length. In addition, both supplementations counteracted the effect of PhIP+DSS treatment through the reduction of the relative abundances of *Clostridia* UCG014, and the modulation of immunological markers of the Th17 pathway. This Doctoral Thesis provides a dietary instrument valid for the registration and quantification of xenobiotic intake. The MD ameliorated the inflammatory profile and modulated gut microbiota composition. In addition, the supplementation of the animal model PhIP+DSS with fibre counteracted the colonic mucosa damage and modulated the intestinal microbiota and the immune system. The finding presented could serve for the identification of dietary patterns and/or diet-derived components of interest for the development of personalized dietary strategies aimed at the prevention of colorectal cancer in the population.

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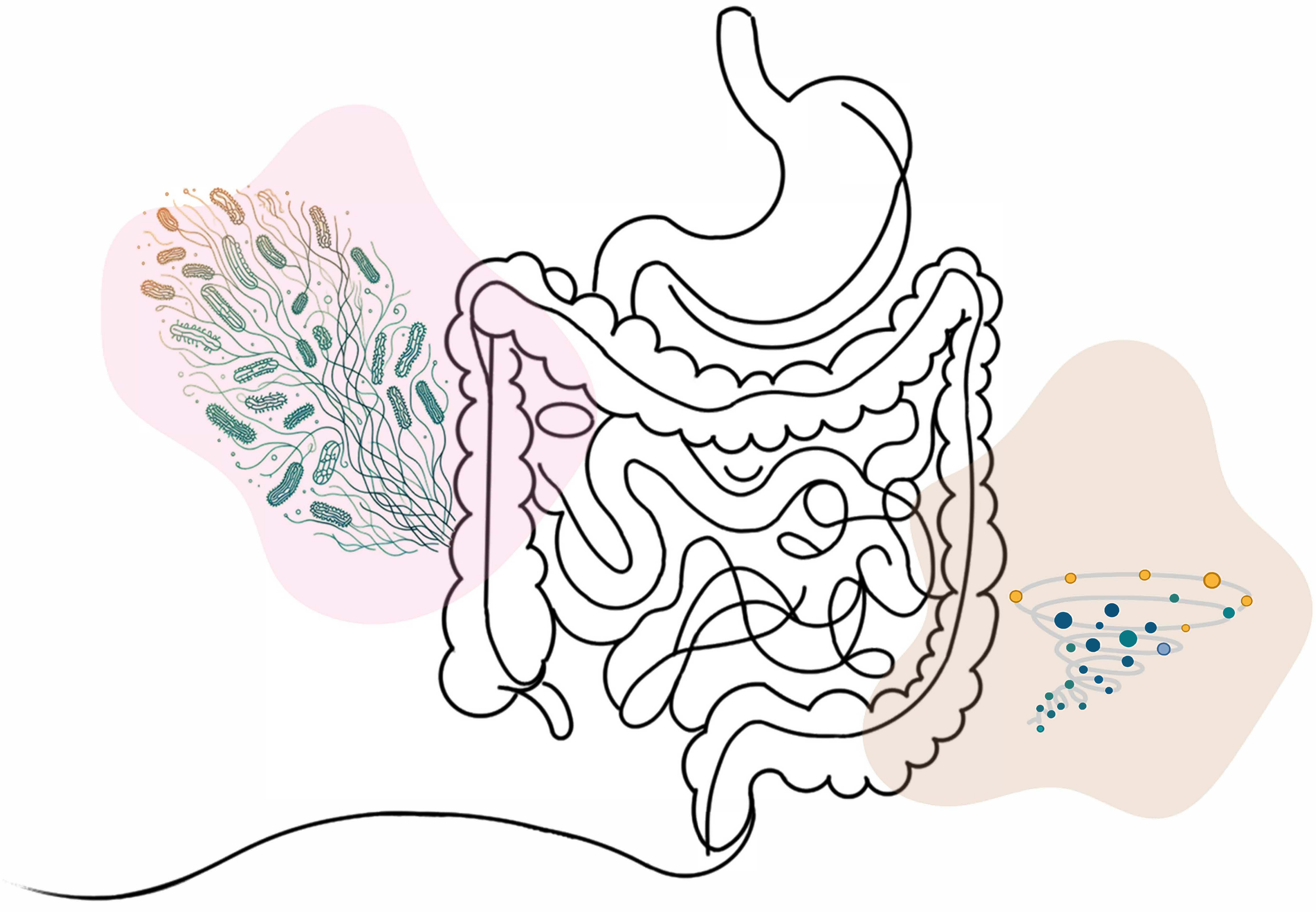

Aida Zapico Linares

Tesis Doctoral

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ISPA Instituto de Investigación Sanitaria del Principado de Asturias

INSTITUTO DE PRODUCTOS LÁCTEOS DE ASTURIAS IPLA CSIC Consejo Superior de Investigaciones Científicas

Agradecimientos

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A todos, gracias.

Nothing in biology makes sense except in the light of evolution.

(Dobzhansky, 1973)

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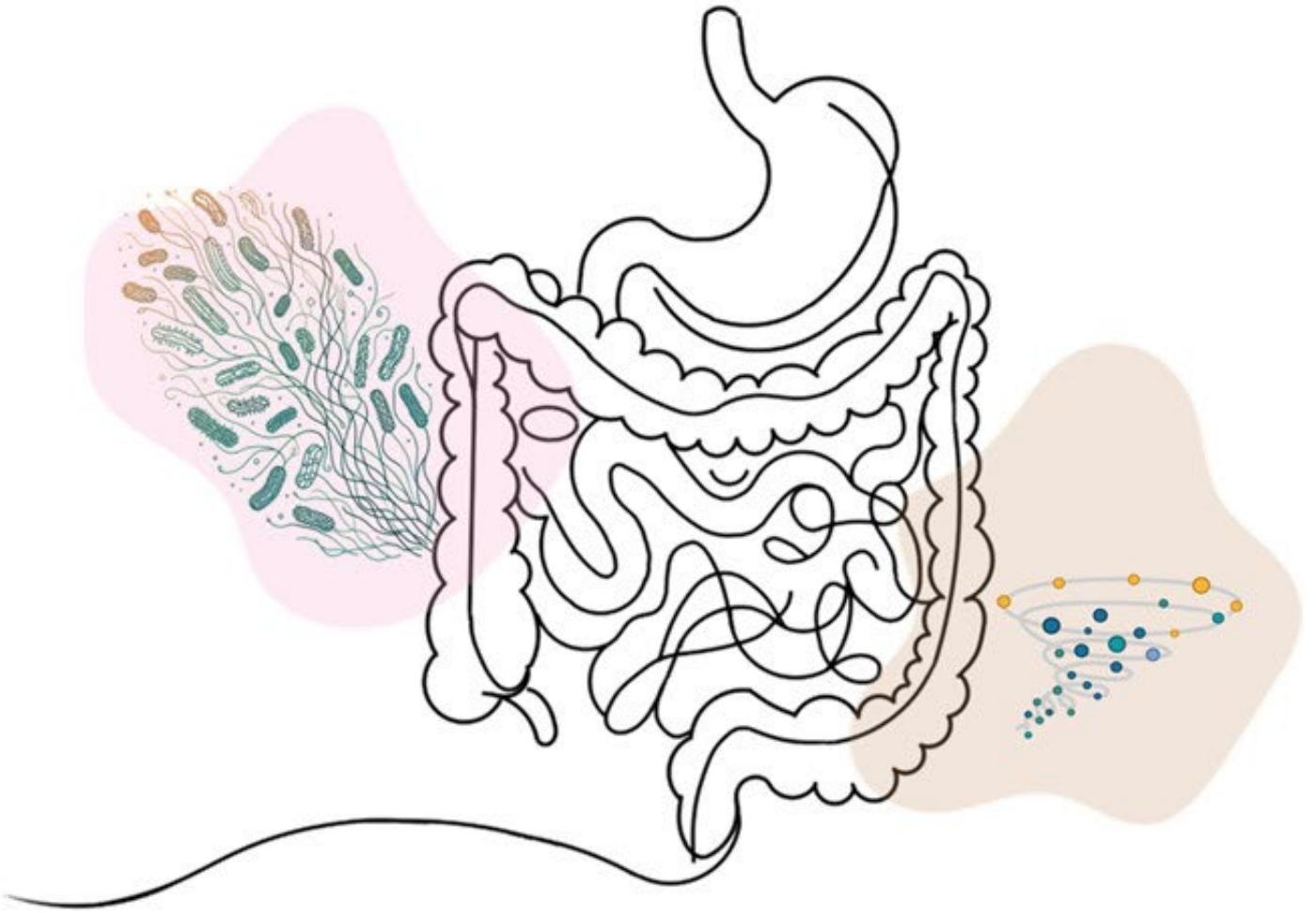

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A

AC	Aminocarbolinas
AGCC	Ácidos grasos de cadena corta
AH	Aminas heterocíclicas
AIA	Aminoimidazoazarenos
AKT	Proteína quinasa B
AOM	Azoxymetano
Apc	Poliposis adenomatosa familiar
ARNr	ARN ribosómico
AαC	Amino-α-carbolina

B

B(a)P	Benzo (a) pireno
BDI-II	Inventario de depresión de Beck-II

C

CAT	Capacidad antioxidante total
CCR	Cáncer colorrectal
CFCA	Cuestionario de frecuencia de consumo de alimentos
CG	Cromatografía de gases
CUPRAC	Capacidad antioxidante reductora del ion cúprico
CYP	Citocromo P450

D

DEBQ-C	Cuestionario Holandés de Comportamiento Alimentario Infantil
DiMeIQx	2-amino-3,4,8 trimetilimidazo (4,5-f) quinoxilina
DM	Dieta mediterránea
DSS	Sal sódica de sulfato de dextrano

E

EFSA	Autoridad Europea de Seguridad Alimentaria
EPIC	Estudio prospectivo europeo sobre cáncer y nutrición
ERO	Especies reactivas de oxígeno

F

FAO	Organización de las Naciones Unidas para la Alimentación y la Agricultura
FDA	Agencia de Alimentos y Medicamentos

G

GBD	Estudio sobre la carga global de las enfermedades
GPR	Receptores acoplados a proteína G

H

HAP	Hidrocarburos aromáticos policíclicos
HPLC	Cromatografía líquida de alta eficacia
HR	Cociente de riesgo

I

IARC	Agencia Internacional de Investigación en Cáncer
IDA	Ingesta diaria admisible
IC	Intervalo de confianza
IFN-γ	Interferón-γ
Ig	Inmunoglobulina
IL	Interleucina
IP-10	Proteína 10 inducida por interferón γ
IQ	2-amino-3-metilimidazol (4,5-f) quinolina
ISAPP	Asociación Científica Internacional para Probióticos y Prebióticos

M

MCP-1	Proteína quimioatrayente de monocitos-1
MDA	Malondialdehido
MeIQ	2-amino-3,4 dimetilimidazo (4,5-f) quinolina
MeIQx	2-amino-3,8 dimetilimidazo (4,5-f) quinoxilina
MNNG	N-metil-n′-nitro-n-nitrosoguanidina
MS	Espectrofotometría de masas

N

NDMA	N-nitrosodimetilamina
NF-κB	Factor nuclear-κβ
NIH	Institutos Nacionales de la Salud
NOC	Compuestos nitrosos
NPIP	N-nitrosopiperidina
NPYR	N-nitrosopirrolidina

P

p38	Proteína quinasa activada por mitógeno
PhIP	2-amino-1 metil-6-fenilimidazo (4,5,b) piridona
PREDIMED	Prevención con Dieta Mediterránea

R

R24h	Recordatorio dietético de 24 horas
RR	Riesgo relativo

S

SOD	Superóxido dismutasa
STAT3	Transductor de señal y activador de la transcripción 3

T

TGF-β	Factor de crecimiento transformante-β
RTT	Receptores de Tipo Toll
TNF-α	Factor de necrosis tumoral-α

W

WCRF/AICR	Red Global del Fondo Mundial para la Investigación del Cáncer/Instituto Americano de Investigación en Cáncer

Z

ZO	Zonula occludens

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Resumen

En los últimos años, en paralelo con el aumento de enfermedades crónicas, se ha producido un alejamiento progresivo de la Dieta Mediterránea (DM) que ha sido reemplazada por un patrón occidentalizado. La incorporación de nuevas técnicas de cocinado y el incremento en el consumo de carne y de alimentos ultra-procesados, promueve la ingesta de xenobióticos como aminas heterocíclicas (AH), hidrocarburos aromáticos policíclicos (HAP), nitrosaminas y acrilamida. Estos compuestos han sido clasificados por la Agencia Internacional de Investigación en Cáncer como "carcinógenos" y "probables carcinógenos", siendo sus niveles de ingesta asociados con un incremento del daño sobre la mucosa intestinal. Sin embargo, el potencial carcinogénico de estos xenobióticos en el colón está influenciado por la microbiota intestinal, el sistema inmune y la dieta. Por el momento, existen escasas evidencias del potencial que algunos patrones dietéticos o componentes alimentarios poseen para actuar como moduladores del daño provocado por la ingesta de xenobióticos. Por ello, el objetivo general de la presente Tesis Doctoral ha sido identificar patrones dietéticos o componentes alimentarios, con potencial para contrarrestar el efecto de la ingesta de xenobióticos, sobre el mantenimiento de la homeostasis intestinal a través de la modulación de la microbiota intestinal y de los parámetros inmunes.

Para la consecución de este objetivo, se reclutó una muestra de adultos sin patologías gastrointestinales diagnosticadas para la validación de una herramienta dietética, específicamente diseñada para la cuantificación de la ingesta de xenobióticos. Además, se evaluó el impacto de una intervención dietética basada en la DM sobre la modulación de la microbiota intestinal, marcadores sanguíneos y otros parámetros relacionados con el estado de salud. Finalmente, se evaluó el potencial de la fibra y de un probiótico, compuesto por cepas pertenecientes a *Lactobacillus* y *Bifidobacterium*, para revertir el daño producido sobre la mucosa del colon tras el consumo de xenobióticos en un modelo animal PhIP+DSS. La ingesta dietética, se analizó mediante las Tablas del Centro de Enseñanza Superior de Nutrición y Dietética, Marlett *&* Cheung, del *Phenol Explorer* o del Estudio Prospectivo Europeo Sobre Cáncer y Nutrición entre otras. El análisis de la composición y actividad microbiana fecal se

realizó mediante la secuenciación del gen ARN ribosómico 16S y el análisis de metabolitos por cromatografía de gases. Asimismo, se determinaron los parámetros inmunes en plasma mediante citometría de flujo y se evaluó el daño sobre la mucosa del colon en animales, a través del análisis histológico de secciones incluidas en parafina con tinción hematoxilina-eosina.

El cuestionario diseñado mostró precisión para la cuantificación de las AH (MeIQx, DiMeIQx, PhIP), y de las nitrosaminas (NDMA, NPIP, NPYR), mostrando niveles de ingesta en el rango descrito en población europea y americana. Del conjunto de compuestos evaluados, el PhIP presentó los niveles de ingesta más elevados, superando los umbrales asociados con un incremento del riesgo de alteración intestinal. Asimismo, el consumo de DiMeIQx, IQ, nitritos y nitrosaminas fue superior en aquellos individuos que presentaban una mayor alteración de la funcionalidad gastrointestinal, hemorroides o sangrados en heces. Por otro lado, la DM redujo la ingesta de xenobióticos, tales como DiB(a)A, HAP, NPIP y acrilamida, en paralelo con un descenso de la abundancia relativa de *Clostridia* UCG014, la reducción de parámetros pro-inflamatorios y el incremento de parámetros anti-inflamatorios. Finalmente, la suplementación con fibra redujo el daño en la mucosa del colon provocado por PhIP+DSS, y el probiótico redujo la longitud del colon. Además, ambas suplementaciones modularon marcadores inmunológicos de la vía Th17 y contrarrestaron el efecto de la exposición a PhIP+DSS sobre la microbiota intestinal, mediante la reducción de la abundancia relativa de *Clostridia* UCG014.

La presente Tesis Doctoral proporciona una herramienta dietética para la cuantificación de la ingesta de xenobióticos. La DM mejoró parámetros inflamatorios y moduló la composición microbiana intestinal. Asimismo, la suplementación de con fibra contrarrestó los efectos perjudiciales de la ingesta de PhIP+DSS. Los hallazgos presentados podrían servir para la identificación de patrones dietéticos y/o componentes derivados de la dieta que serán de interés para el desarrollo de estrategias dietéticas personalizadas dirigidas a la prevención del cáncer colorrectal en la población.

Summary

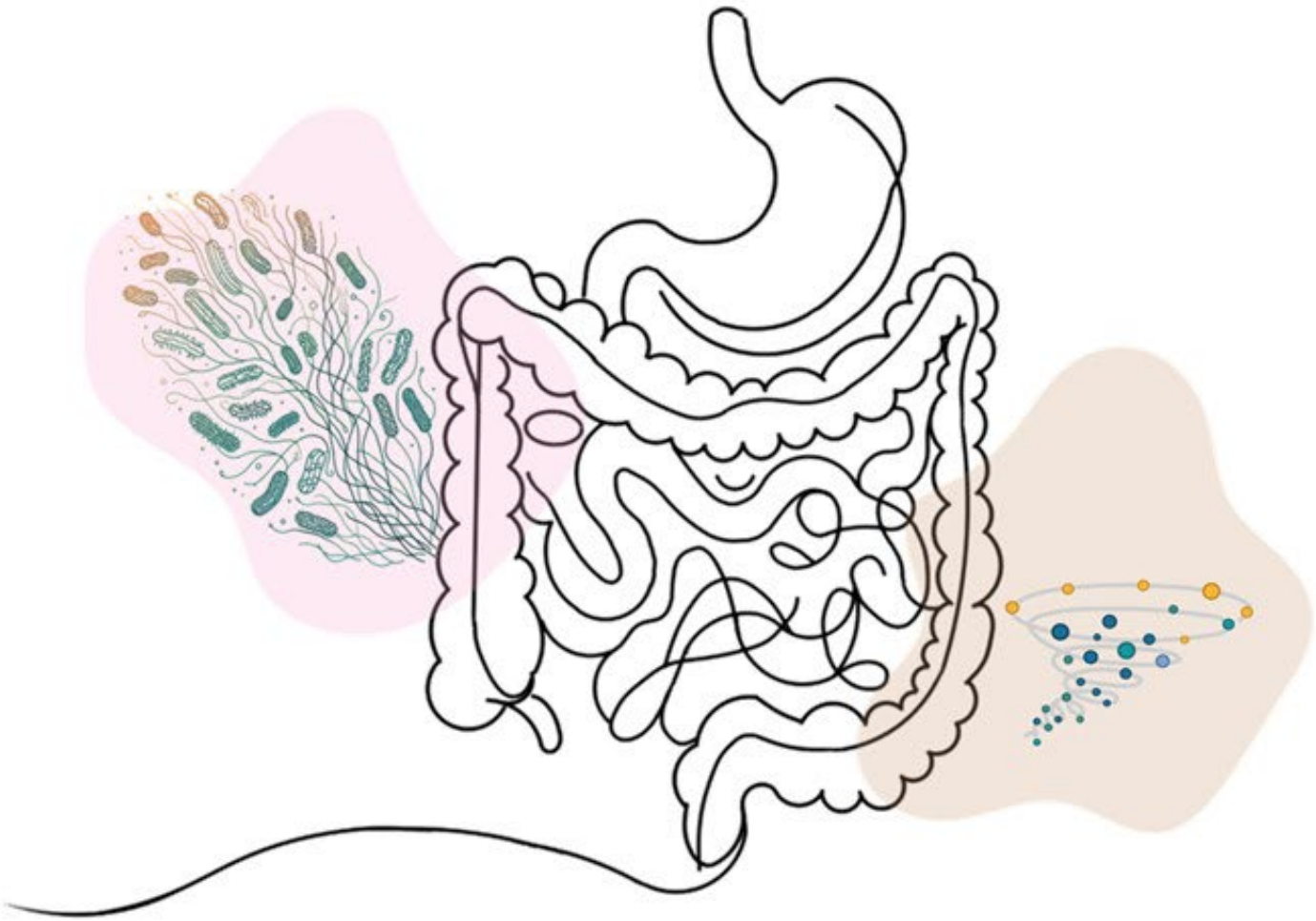
Recently, a decline of the Mediterranean Diet (MD) in substitution of the Westernized pattern has been observed, in parallel with an increase incidence of chronic diseases. The use of new cooking techniques and increased consumption of meats and ultra-processed foods promotes the intake of xenobiotics, such as heterocyclic amines (HA), polycyclic aromatic hydrocarbons (PAH), nitrosamines and acrylamide. These compounds have been classified by the IARC as "carcinogenic" or "probably carcinogenic", with their level of intake associated with an increased damage of the intestinal mucosa. However, the carcinogenic potential of these xenobiotics in the colon is influenced by the gut microbiota, the immune system and the diet. To date, little information is available on the potential of dietary patterns or food components to act as modulators of the damage provoked by the intake of xenobiotics. Therefore, the Main Objective of this Doctoral Thesis is to identify dietary patterns or diet-derived components with the potential to counteract the effect of xenobiotic consumption on the maintenance of intestinal homeostasis by the modulation of the intestinal microbiota and the immunological parameters.

To achieve this Objective, adults without diagnosis of any gastrointestinal condition were recruited for the validation of a dietary instrument specifically developed for the quantification of xenobiotic intake. In addition, the impact of the MD on the modulation of gut microbiota, blood markers and parameters associated with the health status were evaluated. Finally, the potential of fibre and a probiotic, composed of *Lactobacillus* and *Bifidobacterium* to reverse the colon mucosal damage produced by the consumption of xenobiotics, was assessed in a PhIP+DSS animal model. Dietary intake was analysed by the Food Composition Tables of Centre for Higher Education in Nutrition and Dietetics, Marlett and Cheung, Phenol Explorer and the European Prospective Investigation into Cancer and Nutrition, among others. The analysis of composition and activity of faecal microbiota was performed through ribosomal RNA-gene sequencing and excreted metabolites were analysed by flow cytometer. The histological assessment of colon mucosa in animals was performed after embedding histologic sections in paraffin and staining with haematoxylin-eosin.

The questionnaire designed for evaluating the intake of xenobiotics was particularly accurate for the quantification of HA (MeIQx, DiMeIQx, PhIP), nitrosamines (NDMA, NPIP, NPYR), showing levels of intake in the range of those described in European and American populations. Among all components evaluated, PhIP presented the highest level of intake, being greater than the threshold levels associated with an increased risk of intestinal mucosa alteration. In consonance, the consumption of DiMeIQx, IQ, nitrites and nitrosamines was higher in those individuals who presented a greater alteration of gastrointestinal functionality, hemorrhoids or bleeding in feces. On the other hand, the MD reduced the intake of xenobiotics, such as DiB(a)A, PAH, NPIP and acrylamide, in parallel with a reduction of the relative abundance of *Clostridia* UCG014, the reduction of pro-inflammatory mediators and the increment of anti-inflammatory parameters. Finally, the supplementation with fibre reduced the histological damage in the colon mucosa caused by PhIP+DSS, and the probiotic increased colon length. In addition, both supplementations modulated immunological markers of the Th17 pathway and counteracted the effect of PhIP+DSS treatment on gut microbiota through the reduction of the relative abundances of *Clostridia* UCG014.

This Doctoral Thesis provides a dietary instrument for the quantification of xenobiotic intake. The MD ameliorated inflammatory parameters and modulated gut microbiota composition. In addition, the supplementation with fibre counteracted the detrimental effects of PhIP+DSS intake. The findings presented could serve for the identification of dietary patterns and/or diet-derived components of interest for the development of personalized dietary strategies aimed at the prevention of colorectal cancer in the population.

INTRODUCCIÓN
INTRODUCTION



Con una gran influencia cultural, la alimentación ha supuesto a lo largo de la historia un importante factor selectivo para el desarrollo de la evolución humana (Arroyo P, 2008). Se entiende como alimentación el conjunto de actividades y procesos por los cuales, de manera consciente y voluntaria, se consumen alimentos que aportan las sustancias necesarias para la vida (Rivas-Gonzalo & Santos-Buelga, 1985). Mientras que los primeros homínidos, presentaban un patrón alimenticio basado en el consumo de productos vegetales, ricos en fibra alimentaria, el desarrollo de la postura erecta permitió el comienzo de la cacería y dio lugar a la diversificación de la dieta, al incorporarse alimentos de origen animal como las carnes (Arroyo P, 2008). Además, tuvo lugar el desarrollo de las primeras herramientas, como piedras o lascas, que permitían romper los huesos y aumentar la densidad energética de la dieta (Arroyo P, 2008). En el Paleolítico, a través del control del fuego, el hombre comenzó a cocinar los alimentos, lo que modificó la textura, inactivó la acción de algunos antinutrientes

naturalmente presentes en el alimento, redujo el riesgo de intoxicaciones por alimentos y mejoró la palatabilidad de estos, así como su masticación y digestión (Andrews & Johnson, 2020). Posteriormente, en el Neolítico se estableció la agricultura y la ganadería, lo cual aumentó la disponibilidad de provisiones y permitió el desarrollo de sociedades más complejas (Arroyo P, 2008; Ye & Gu, 2011). Sin embargo, no fue hasta la Edad Media, con el inicio del comercio y la importación de nuevos alimentos, como la patata o el tomate, cuando se diversificó la oferta de alimentos en la población. El tipo de alimentos, a los que se tenía acceso en esta etapa, comenzó a ser dependiente del estrato social (Alt *et al*., 2022). Mientras que el consumo de carne o pescados estaban reservados para la nobleza, los campesinos (el 90% de la población) presentaban un consumo basado en cereales, coles y carne de cerdo (Alt *et al*., 2022). Posteriormente, con la Revolución Industrial tuvo lugar un cambio en el sistema de producción de los alimentos, aumentando la eficiencia y reduciendo los costes (Arroyo P, 2008). En paralelo, la población pasó a mostrar un estilo de vida más sedentario, junto con un mayor consumo energético, de grasas saturadas y ácidos grasos trans y una menor ingesta de carbohidratos complejos y de fibra (Alt *et al*., 2022; Arroyo P, 2008). Además, durante esta etapa, estos cambios en el estilo de vida y hábitos alimentarios fueron acompañados de un incremento en la incidencia de enfermedades no transmisibles.

Las primeras civilizaciones fueron expuestas a cambios en el estilo de vida y en los hábitos alimentarios que se producían a lo largo de millones de años, permitiendo un proceso de adaptación biológica (Ye & Gu, 2011). Sin embargo, en las últimas etapas de la historia, el ambiente al que está expuesto el ser humano, ha cambiado demasiado rápido para permitir este proceso (Eaton *et al*., 1997). En base a esto, algunos autores proponen la existencia de un desfase biológico o discordancia evolutiva entre nuestro genoma actual y los cambios en el entorno (Arroyo P, 2008). En concreto, se calcula que hay un desfase de 10.000 años, indicando que el genoma actual de la población se encontraría adaptado a las condiciones del Pleistoceno y no ha tenido suficiente tiempo para adaptarse al nuevo ambiente (Eaton *et al*., 1988). Este desfase contribuiría a explicar el aumento de enfermedades crónicas de los últimos años asociadas, especialmente, con los hábitos alimenticios (Simopoulos, 1999; Konner & Eaton, 2010; Ye & Gu, 2011).

1. Pérdida del Patrón Mediterráneo

La Dieta Mediterránea (DM) se basa en el consumo de frutas, verduras, cereales mínimamente refinados, patatas, legumbres, frutos secos y semillas, junto con el uso de aceite de oliva, virgen o virgen extra, como principal fuente de grasa. Este patrón alimenticio también establece una ingesta moderada de lácteos fermentados, principalmente queso y yogur, el consumo de huevos (0 a 4 por semana), la ingesta de pescados y pollo en cantidades reducidas o moderadas, siendo preferible el consumo de carnes magras en detrimento de carnes rojas, junto con un consumo de vino moderado (Guasch-Ferré *&* Willett, 2021; Merra *et al*., 2021).

En las últimas décadas se ha observado un alejamiento del patrón dietético mediterráneo en los países desarrollados, que ha sido desplazado por un patrón occidentalizado, debido, entre otras cuestiones, a la globalización, a los sistemas de producción y consumo de alimentos, así como al turismo, la urbanización y la expansión generalizada de la cultura occidentalizada (Serra-Majem *&* Ortiz-Andrellucchi, 2018). En concreto, en los últimos 40 años, en Europa, se ha observado un aumento del 20% de la ingesta energética, principalmente derivado del incremento en la ingesta de lípidos (Balanza *et al*., 2007).

Este cambio en el patrón alimentario está fuertemente influenciado por la capacidad económica de los individuos. Aquellos con unos ingresos más elevados presentan patrones de consumo con una mayor presencia de productos frescos como frutas, verduras y pescados (Zujko *et al*., 2020), mientras que los sujetos con ingresos reducidos muestran ingestas más elevadas de productos ultra-procesados, cereales refinados y azúcares (da Costa *et al*., 2022; Rberg-Kjøllesdal *et al*., 2010).

La dificultad para acceder al consumo de alimentos seguros y nutritivos, necesarios para el adecuado desarrollo de un individuo, se conoce como inseguridad alimentaria (Kent *et al*., 2024). De acuerdo con la última publicación de la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), en España el coste de una dieta saludable ha aumentado un 7% entre 2017 y 2021 (**Figura 1**), en paralelo

con un aumento en el número de individuos sin acceso a alimentos adecuados, que ha pasado de 3,3 a 3,8 millones de personas (FAO *et al*., 2023).

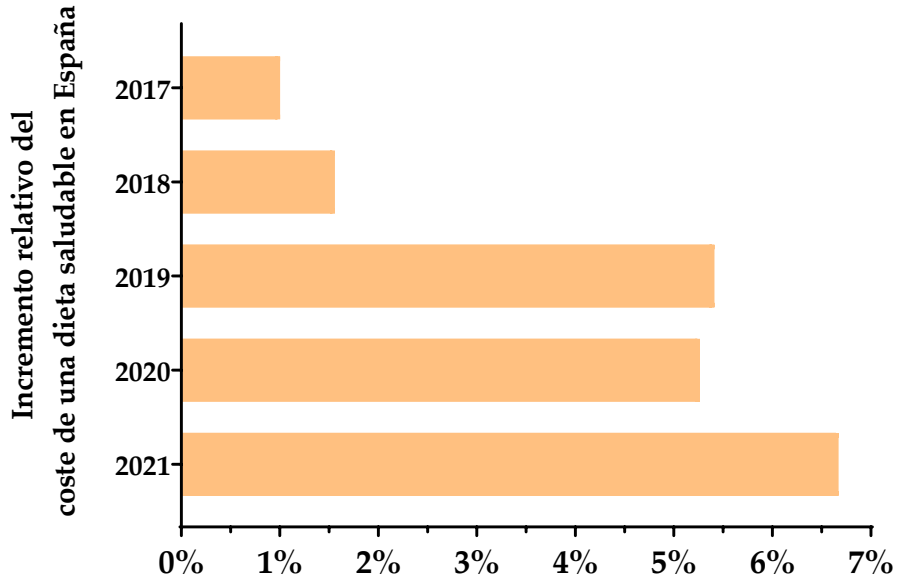


Figura 1. Incremento relativo del coste de una dieta saludable en España desde 2017 a 2021. Figura elaborada a partir de los datos de FAO *et al.*, 2023.

Estos resultados ponen de manifiesto la necesidad de llevar a cabo intervenciones dietéticas en colectivos en riesgo de inseguridad alimentaria. Asimismo, el bajo nivel económico se asocia directamente con el nivel educativo y contribuye a las malas elecciones alimentarias (Murakami *et al*., 2009). De esta forma, la educación alimentaria y nutricional resulta clave para promover el éxito de las intervenciones dietéticas en estos colectivos a corto y a largo plazo para, posteriormente, desarrollar políticas alimentarias que aseguren el acceso a los productos básicos de una dieta saludable (Han *et al*., 2021; Serra-Majem & Ortiz-Andrellucchi, 2018).

2. Dieta Occidentalizada

El patrón occidentalizado está basado en el consumo de productos ultra-procesados, como dulces, caramelos y bebidas azucaradas, de carne roja y procesada y de cereales refinados, en contraposición con un consumo reducido de frutas y vegetales, pescados, frutos secos y semillas. Esto, repercute en un consumo elevado de azúcares refinados, grasas saturadas y ácidos grasos omega-6, y reducidos de fibra alimentaria, así como de vitaminas y minerales y otros compuestos con acción

antioxidante (Christ *et al*., 2019; Malesza *et al*., 2021). El contenido energético de esta dieta es muy elevado y proviene en un 70% del consumo de alimentos de origen animal, aceites, grasas y azúcares (Clemente-Suárez *et al*., 2023).

2.1. Factor de riesgo de la salud gastrointestinal

La dieta occidentalizada se ha asociado con el empeoramiento de la salud gastrointestinal y un mayor riesgo de mortalidad por todas las causas en humanos (Entwistle *et al*., 2021; Malesza *et al*., 2021). Asimismo, la ingesta energética excesiva conlleva un mayor riesgo de obesidad y enfermedades no trasmisibles (Buscail *et al*., 2017; Clemente-Suárez *et al*., 2023; Di Polito *et al*., 2023; Malesza *et al*., 2021).

El consumo de alimentos ultra-procesados, se ha asociado con un aumento del riesgo de procesos inflamatorios a nivel intestinal (Krela-Kaźmierczak *et al*., 2022), similar al observado con la ingesta de carne roja (Jantchou *et al*., 2010). Más específicamente, el consumo de al menos 50 g/d de carne roja y 25 g/d de carne procesada produce un aumento del riesgo de cáncer colorrectal (CCR) (riesgo relativo (RR): 1,08; 95% intervalo de confianza (IC)=1,02-1,16 y RR: 1,06; 95% IC=1,02-1,10; respectivamente), tal y como se muestra a través de grandes estudios epidemiológicos como el Estudio sobre la Carga Global de las Enfermedades (GBD) (GBD *et al*., 2022). En base a estas evidencias, la Red Global del Fondo Mundial para la Investigación del Cáncer/Instituto Americano de Investigación en Cáncer (WCRF/ AICR) recomienda limitar la ingesta de carne roja a menos de 3 raciones a la semana (equivalentes a 64 g/d) y reducir, al mínimo posible, el consumo de carne procesada (Barrubés *et al*., 2020).

Entre los diferentes componentes de la carne que podrían explicar este potencial carcinogénico se encuentra su contenido en xenobióticos, derivados del cocinado y procesamiento de estos alimentos (Nogacka *et al*., 2019). De este modo, las técnicas de cocinado en el contexto del patrón occidentalizado son clave para explicar su efecto perjudicial sobre la salud intestinal.

2.2. Influencia del cocinado y procesado de los alimentos sobre la formación de potenciales carcinógenos

El procesado y cocinado de los alimentos altera su composición nutricional (Rodríguez-Ayala *et al*., 2022) y la biodisponibilidad de sus componentes (Lipski, 2010; Ozbek *et al*., 2024; Rodríguez-Ayala *et al*., 2022). Durante el procesado de alimentos como la carne, la industria alimentaria utiliza aditivos de tipo conservantes (como los nitritos), saborizantes (como el sabor ahumado) o edulcorantes (como la sacarina o el aspartamo) que alteran la homeostasis intestinal (Malesza *et al*., 2021; Roca-Saavedra *et al*., 2018), inducen cambios en el perfil microbiano, en la actividad inflamatoria, y pueden promover procesos carcinogénicos (Jarmakiewicz-Czaja *et al*., 2022; Malesza *et al*., 2021). El abandono de las técnicas de cocinado tradicionales, como el hervido o el asado (Rodríguez-Ayala *et al*., 2022), en pro de técnicas de cocinado más rápidas y palatables, como la fritura (Jacka *et al*., 2010), el ahumado (Rohrmann *&* Linseisen, 2016), la barbacoa (Stefani *et al*., 2009) o el consumo de alimentos en conserva (Madruga *et al*., 2023), ha contribuido a reducir la biodisponibilidad de nutrientes y promover en su lugar la formación de xenobióticos y productos de peroxidación lipídica entre otros (Vieira *et al*., 2017). Las nuevas técnicas de procesado y cocinado de los alimentos promueven, en consecuencia, un efecto perjudicial sobre la salud (Cahill *et al*., 2014; Sun *et al*., 2022).

Cambios en los hábitos y métodos de cocinado de la carne pueden tener una gran influencia sobre el nivel de ingesta de xenobióticos. Por ejemplo, el uso de marinados, durante el cocinado, a base de té verde o de zumo de limón, disminuyó la formación de hidrocarburos aromáticos policíclicos (HAP) hasta en un 70% (Bulanda *&* Janoszka, 2022). Del mismo modo, el cocinado de diferentes tipos de carne, con cebolla y ajo, o especias, con alta capacidad antioxidante, como pimentón, jengibre, pimienta negra, junto con cúrcuma o curri, redujo la formación de HAP y aminas heterocíclicas (AH) (Bulanda *&* Janoszka, 2022).

Por otro lado, las diferentes técnicas de cocinado desempeñan un papel clave en la formación de estos compuestos, tal y como se muestra en la **Figura 2**. En general, los métodos como la fritura, en el que el alimento se encuentra sumergido en aceite o el

cocinado a la plancha, al horno o a la barbacoa promueven la formación de estos compuestos, mientras que los cocinados al vapor, al microondas o estofados presentan el efecto contrario (Miller *et al.*, 2013; EFSA ANS Panel *et al.*, 2017a; Zimmerli *et al.*, 2001).

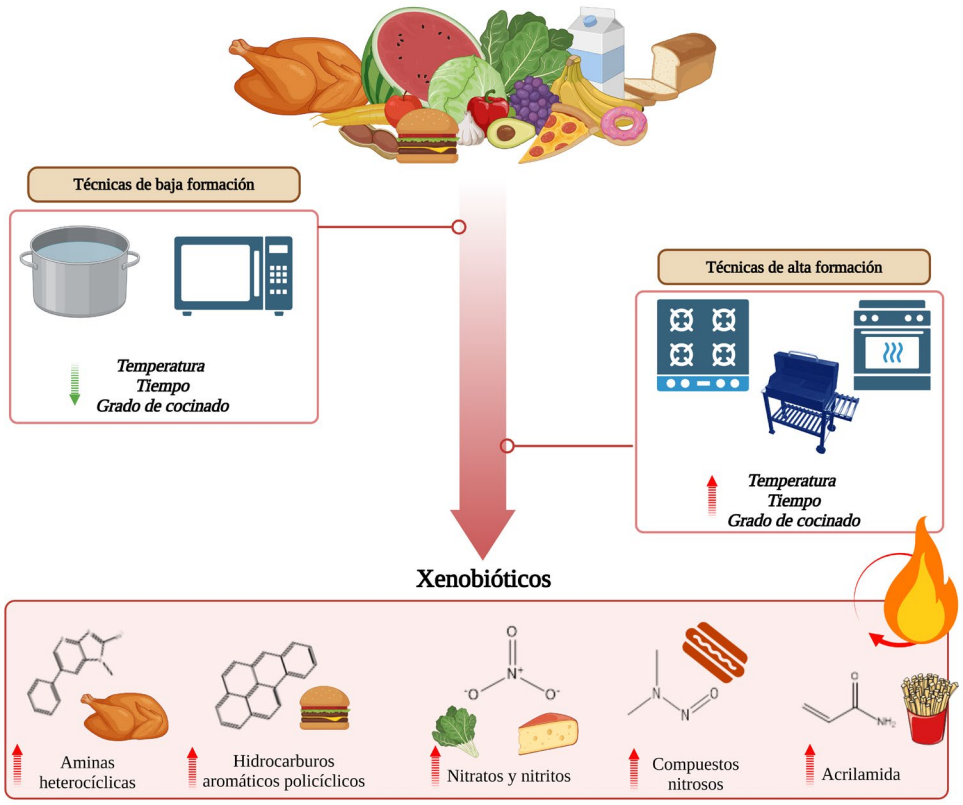


Figura 2. Impacto de diferentes técnicas y métodos de cocinado sobre la formación de xenobióticos. Creada con BioRender.com.

Por ejemplo, en el caso de 150 g de pollo, el contenido de 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona (PhIP) puede elevarse 3,5 veces si se ha cocinado a la plancha en lugar de al horno (1.575 ng *vs.* 450 ng). Además, en función del grado de dorado, el mismo tamaño de ración cocinado "bien hecho" en lugar de "medio hecho" puede suponer un incremento de 15 veces en la concentración final del potencial carcinogeno (1.575 ng *vs.* 105 ng) (Solyakov & Skog, 2002). Otros factores, como el cocinado con o sin piel y si esta se consume o no, o la presencia de otros elementos en el alimento que aumenten o disminuyan el efecto tóxico de estos compuestos, son cuestiones relevantes en la determinación del nivel de xenobióticos ingeridos y, en consecuencia, en el estudio de su impacto a largo plazo sobre la homeostasis intestinal (Nogacka *et al.*, 2019).

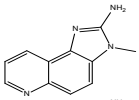
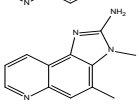
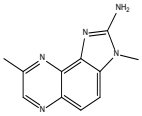
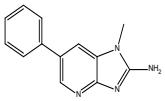
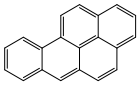
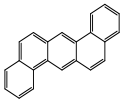
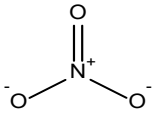
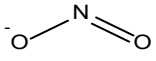
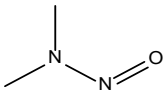
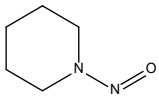
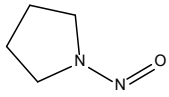
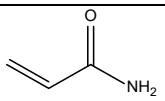
2.3. Xenobióticos: compuestos con potencial inflamatorio y carcinogénico

Los xenobióticos son sustancias extrañas al organismo, es decir, que no son producidas de forma endógena o no se encuentran originalmente en la composición del organismo dónde se han detectado (Croom, 2012). Tal y como se muestra en la **Tabla 1**, algunos de estos compuestos han sido clasificados por la IARC como carcinogénicos, probablemente carcinogénicos y posiblemente carcinogénicos para humanos.

2.3.1. Aminas heterocíclicas

Las AH presentan, generalmente, una estructura formada por 3 anillos aromáticos condesados con uno o más átomos de nitrógeno y un grupo amino exocíclico (Turesky *&* Le Marchand, 2011). Estos potenciales carcinógenos se forman sobre la superficie de alimentos ricos en proteína, como carnes y pescados, y los niveles formados, así como el grado de mutagenicidad, aumenta, con el tiempo y la temperatura del cocinado y, con el grado de dorado de los alimentos (Kondjoyan *et al.*, 2016; Nadeem *et al.*, 2021; Nogacka *et al.*, 2019). En función de estas condiciones, se pueden formar dos tipos de AH: aminocarbolinas (AC) y las aminoimidazoazarenos (AIA) (Nogacka *et al.*, 2019). Las AC se forman a temperaturas superiores a 300 ºC, mediante procesos de pirólisis, y presentan en su estructura un grupo piroindol, como es el caso de la amino-α-carbolina (AαC). Por otro lado, las AIA se forman a temperaturas inferiores (100 ºC - 300 ºC) a partir de la creatina o creatinina del músculo, procedente de carne y pescado, hexosa, aminoácidos y algunos dipéptidos mediante la reacción de Maillard (Chiavarini *et al.*, 2017; Miller *et al.*, 2013; Nogacka *et al.*, 2019). Estas AH se caracterizan por presentar el grupo 2-aminoimidazo junto con una quinolina, como 2-amino-3-metilimidazol (4,5-f) quinolina (IQ) o 2-amino-3,4 dimetilimidazo (4,5-f) quinolina (MeIQ), con una quinoxalina, como 2-amino-3,8 dimetilimidazo (4,5-f) quinoxilina (MeIQx) o 2-amino-3,4,8 trimetilimidazo (4,5-f) quinoxilina (DiMeIQx), o con un anillo de piridina, como PhIP (Galceran, 2001).

Tabla 1. Clasificación de los principales compuestos xenobióticos por la IARC.

	Estructura	Grupo carcinogénico	Ref
Aminas heterocíclicas			
IQ		Grupo 2A	(IARC, 2018)
MeIQ		Grupo 2B	(IARC, 2018)
MeIQx		Grupo 2B	(IARC, 2018)
PhIP		Grupo 2B	(IARC, 2018)
Hidrocarburos aromáticos policíclicos			
B(a)P		Grupo 1	(IARC, 2012)
DiB(a)A		Grupo 2A	(IARC, 2010a)
Nitratos, nitritos y NOC			
Nitratos ([a])		Grupo 2A	(IARC, 2010b)
Nitritos ([a])		Grupo 2A	(IARC, 2010b)
NDMA		Grupo 2A	(IARC, 1978)
NPIP		Grupo 2B	(IARC, 1978)
NPYR		Grupo 2B	(IARC, 1978)
Acrilamida		Grupo 2A	(IARC, 1994)

([a])Compuesto consumido bajo condiciones que conllevan la nitrosación endógena. B(a)P, benzo (a) pireno; DiB(a)A, dibenzo (a,h) antraceno; DiMeIQx, 2-amino-3,4,8 trimetilimidazo (4,5-f) quinoxilina; Grupo 1, carcinogénico para humanos; Grupo 2A, probablemente carcinogénico para humanos; Grupo 2B, posiblemente carcinogénico para humanos; IARC, Agencia Internacional de Investigación en Cáncer; IQ, 2-amino-3-metilimidazol (4,5-f) quinolina; MeIQ, 2-amino-3,4 dimetilimidazo (4,5-f) quinolina; MeIQx, 2-amino-3,8 dimetilimidazo (4,5-f) quinoxilina; NDMA, N-nitrosodimetilamina; NOC, compuestos nitrosos; NPIP, N-nitrosopiperidina; NPYR, N-nitrosopirrolidina; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona. Estructuras químicas creadas con ChemDraw 20.1.

Se han identificado más de 25 tipos de AH procedentes, principalmente, de la ingesta de carnes rojas y procesadas, pero también de carnes blancas y pescados (Chiavarini *et al*., 2017).

Entre las diferentes AH, las AC presentan un mayor nivel de mutagenicidad, seguido de las AIA como MeIQ, IQ, DiMeIQx, MeIQx y PhIP (de Kok & van Maanen, 2000; Le *et al*., 2016; Miller *et al*., 2013). Este último, a pesar de ser el menos carcinogénico, generalmente presenta los niveles de ingesta más elevados en la población (hasta 300 ng/d) (Carvalho *et al*., 2015; Le *et al*., 2016) y, junto con otras AH representadas en la dieta como MeIQx (en torno a 53 ng/d) y DiMeIQx (en torno a 5 ng/d) (Busquets *et al*., 2004; Le *et al*., 2016), se asocian con un aumento del riesgo de adenoma colorrectal (Chiavarini *et al*., 2017), tal y como se muestra en la **Tabla 2**. Entre estas, el PhIP es una AH que parece tener relación con la incidencia de CCR (Le *et al*., 2016; Rohrmann *et al*., 2009) y algunos autores han mostrado esta asociación en el caso de la ingesta de PhIP derivada de carne roja (cociente de riesgo (HR): 1,39; 95% IC=1,07-1,79), pero no de carne blanca (HR: 1,09; 95% IC=0,74-1,62) (Le *et al*., 2016). En base a estas evidencias, el PhIP se ha empleado en modelos animales para simular el dao intestinal provocado tras la exposición dietética a carcinógenos que puede derivar en la formación de CCR (Rosenberg *et al*., 2009; Yang *et al*., 2021). De esta forma, estos modelos difieren de los modelos de CCR tradicionales los cuales tienen como objetivo la simulación de un proceso oncológico generado de forma esporádica en humanos (Rosenberg *et al*., 2009; Snider *et al*., 2016). Estos pueden ser generados mediante la manipulación genética, como el modelo de poliposis adenomatosa familiar $(Apc)^{Min/+}$, o mediante el uso de promotores químicos de neoplasia, como azoxymetano (AOM)/ sal sódica de sulfato de dextrano (DSS) (Rosenberg *et al*., 2009; Snider *et al*., 2016).

2.3.2.Hidrocarburos aromáticos policíclicos

Los HAP se caracterizan por presentar dos o más anillos aromáticos unidos y se forman por la combustión incompleta de material orgánico (Yu, 2002). En función de su estructura química, se clasifican en HAP de bajo peso molecular con distribución gaseosa (formados por 2-3 anillos aromáticos) o de alto peso molecular en forma de partículas (4 o más anillos aromáticos) (Romo *et al* 2019). Entre estos últimos destaca el

benzo (a) pireno (B(a)P), clasificado por la IARC como Grupo 1 por su mayor potencial carcinogénico (**Tabla 1**) (Romo *et al*., 2019).

La exposición a HAP puede ocurrir a partir de una gran variedad de fuentes como la contaminación ambiental, el tabaco o la exposición ocupacional en la industria (Patel *et al*., 2020). Además, la dieta es una fuente de exposición relevante y la ingesta de estos compuestos se ha asociado con la promoción de tumores (Cheng *et al*., 2021). En el caso de individuos no fumadores, la dieta representa hasta un 70% del total de exposición a HAP (Patel *et al*., 2020).

Los HAP procedentes de la dieta se forman principalmente por procesos de cocinado en los que alimentos ricos en grasas y proteínas, como las carnes, son expuestos a temperaturas elevadas. La exposición del alimento a una fuente directa de calor, durante técnicas como la barbacoa o el grill, resulta en la formación de jugos y grasas que, al caer en el fuego, forman una llama con contenido en HAP y estos compuestos formados se adhieren a la superficie del alimento durante el cocinado (Miller *et al*., 2013; Nogacka *et al*., 2019). Se encuentran niveles más elevados de HAP en alimentos expuestos a un mayor tiempo o temperatura de cocinado y con un mayor contenido en grasa y proteínas (Zhang *et al*., 2021). La formación de estos compuestos es característica, principalmente, de productos como ahumados y carnes al grill (Pérez-Morales *et al*., 2016), aunque también se pueden encontrar en otros productos sometidos a altas temperaturas como los aceites, los cereales y las verduras (Nogacka *et al*., 2019; Scientific Committee on Food, 2002). De los más de 30 HAP identificados (IARC, 2018), la ingesta de B(a)P se ha asociado con un aumento del riesgo de adenoma colorrectal (Chiavarini *et al*., 2017) y, en base a la información disponible en la literatura, se han propuesto niveles de consumo asociados con potenciales efectos adversos en el caso de este xenobiótico (**Tabla 2**).

Tabla 2. Valores de ingesta de xenobióticos asociados con efectos adversos.

	Ingesta diaria	Parámetro de evaluación	Ref
Aminas heterocíclicas			
MeIQx	≥50 ng	↑ Riesgo de adenoma colorrectal	(Martínez Gongora *et al*., 2019)
PhIP	≥40 ng	↑ Riesgo de adenoma colorrectal	(Martínez Gongora *et al*., 2019)
Hidrocarburos aromáticos policíclicos			
B(a)P	100 mg/kg pc	BMDL10	(Joint FAO/WHO Expert Committee on Food Additives, 2005)
Nitratos, nitritos y NOC			
Nitratos	3,70 mg/kg pc	IDA	(EFSA ANS Panel *et al*., 2017a)
Nitritos	0,07 mg/kg pc	IDA	(EFSA ANS Panel *et al*., 2017b)
NDMA	0,05 µg	↑ Riesgo de cáncer de recto	(Loh et al 2011)
Acrilamida	0,17 mg/kg pc	BMDL10	(EFSA *et al*., 2022)

Se muestran aquellos xenobióticos para los cuales se ha encontrado información en la literatura. B(a)P, benzo (a) pireno; BMDL10, dosis mínima a partir de la cual se incrementa un 10% el riesgo de potenciales efectos adversos; IDA, ingesta diaria admisible; MeIQx, 2-amino-3,8 dimetilimidazo (4,5-f) quinoxilina; NDMA, N-nitrosodimetilamina; NOC, compuestos nitrosos; pc; peso corporal; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona.

2.3.3.Nitratos, nitritos y compuestos nitrosos

La exposición a los nitratos y nitritos proviene principalmente de la ingesta de alimentos, mientras que la contaminación externa contribuye de forma minoritaria (Jakszyn *et al*., 2006a). En concreto, los nitratos y nitritos son compuestos utilizados como aditivos en la industria alimentaria (Etemadi *et al*., 2018; Irigaray *et al*., 2007; Karwowska *&* Kononiuk, 2020). Durante el procesamiento térmico, los nitritos pueden reaccionar con aminas y dar lugar a la formación de compuestos nitrosos (NOC) con potencial carcinogénico (Bondonno *et al*., 2023; Pinaffi-Langley *et al*., 2024; Xie *et al*., 2023). De esta forma, la carne curada y preservada constituye la principal fuente de ingesta de los nitritos (Bondonno *et al*., 2023). Por otro lado, los nitratos proceden mayoritariamente de vegetales y, en este contexto, presentan un efecto protector para la salud promoviendo la salud cardiovascular (Bondonno *et al*., 2023).

En función de la presencia del grupo amida o amina, los NOC se clasifican en nitrosamidas, compuestos inestables que se descomponen de forma espontánea formando agentes alcalinizantes que reaccionan con el ADN, y nitrosaminas, compuestos que requieren una activación previa para ejercer una actividad genotóxica. Estos últimos son los únicos que se han detectado en alimentos (Bondonno *et al*., 2023) y, en concreto, N-nitrosodimetilamina (NDMA), N-nitrosopiperidina (NPIP) y N-nitrosopirrolidina (NPYR), son algunas de las nitrosaminas más abundantes en carnes procesadas (Bondonno *et al*., 2023).

A pesar de que las nitrosaminas pueden ingerirse con los alimentos, el 97% de la exposición procede de la formación endógena (Gushgari & Halden, 2018; Xie *et al*., 2023). Este proceso puede ocurrir por dos vías a nivel gástrico. En la primera de ellas, en condiciones de pH ácido, estos compuestos se forman tras la reacción de las aminas con el nitrito procedente de la ingesta previa de alimentos o de la actividad nitrato reductasa microbiana en la cavidad oral (de Kok & van Maanen, 2000). El segundo mecanismo, tiene lugar en condiciones de pH más elevado, tal y como como ocurre, por ejemplo, tras la infección por *Helicobacter pylori*, y se basa en el proceso de nitrosación por parte de la microbiota denitrificadora (de Kok & van Maanen, 2000). En este caso, el nitrito se oxida a óxido nítrico y, posteriormente, a agentes nitrosantes (tri- o tetróxido de nitrógeno), elementos que pueden reaccionar con aminas secundarias y nutrientes esenciales, como proteínas entre otros, produciendo NOC (de Kok & van Maanen, 2000). Por último, bajo condiciones de inflamación o infección crónica, la microbiota puede mediar la nitrosación endógena en otros órganos (de Kok & van Maanen, 2000).

Los niveles de NOC formados endógenamente, así como los niveles excretados en heces, los cuales se asocian con mayor mutagenicidad (Ruiz-Saavedra *et al*., 2024), están influenciados por la ingesta de nitritos y nitratos (de Kok & van Maanen, 2000). En consonancia con estas evidencias, algunos trabajos han observado que ingestas superiores de nitratos y nitritos se han asociado con un mayor riesgo de CCR (HR: 1,18; 95% IC=1,08–1,28, y, HR: 1,11; 95% IC=1,02–1,20, respectivamente) (Etemadi *et al*., 2018). De esta forma, se ha evaluado el potencial riesgo derivado de su ingesta (EFSA

CONTAM Panel *et al.*, 2023) y se han establecido valores de ingesta diaria admisible (IDA) (**Tabla 2**) (EFSA ANS Panel *et al.*, 2017a, 2017b).

2.3.4. Acrilamida

La acrilamida es un compuesto orgánico de tipo amida, formado a través de la reacción de Maillard mediante la condensación entre azúcares reductores, como glucosa o fructosa, y aminoácidos libres, como la asparagina (Dybing *et al*., 2005; Mucci *et al*., 2006).

En individuos no fumadores, la dieta representa la principal fuente de exposición a acrilamida (EFSA, 2015). Este compuesto, se forma durante el cocinado de los alimentos a altas temperaturas (superiores a 120 ºC), como ocurre durante la fritura de las patatas fritas caseras o de tipo chips comerciales, o durante el horneado o tostado de los cereales o del café (Dybing *et al*., 2005). Estos alimentos constituyen algunas de las principales fuentes dietéticas de este xenobiótico (Marques *et al*., 2024).

La ingesta de acrilamida se ha asociado con una mayor incidencia tumoral (Hirvonen *et al*., 2010; Hogervorst *et al*., 2009), posiblemente mediante la alteración de la expresión de genes involucrados en el desarrollo del proceso oncológico (Hogervorst *et al*., 2014; Hughes *et al*., 2017; Neophytou *et al*., 2023). Sin embargo, en el caso del CCR, existen discrepancias en la literatura sobre el posible riesgo derivado del consumo de acrilamida en humanos. Algunos autores han evaluado el consumo de acrilamida considerando exclusivamente alimentos con alto contenido en este compuesto y no han encontrado un incremento significativo del riesgo de CCR (Pelucchi *et al*., 2006). En contraposición, la ingesta de este potencial carcinógeno, derivado del conjunto de alimentos, se ha asociado con un mayor riesgo de CCR, al considerar la presencia de mutaciones en genes claves del inicio y la progresión tumoral (Hogervorst *et al*., 2014). De esta forma, además de un componente genético, la falta de unificación de los métodos de recogida de la información dietética y de las bases de datos utilizadas en cada caso (Pelucchi *et al*., 2006), podría contribuir a explicar estas diferencias.

2.4. Metabolismo y mecanismo de daño de los xenobióticos

Las AH y los HAP requieren una bioactivación previa para la producción de metabolitos carcinogénicos (Barnes *et al*., 2018). Este proceso de activación tiene lugar a través de reacciones de Fase I y Fase II a nivel hepático (**Figura 3**).

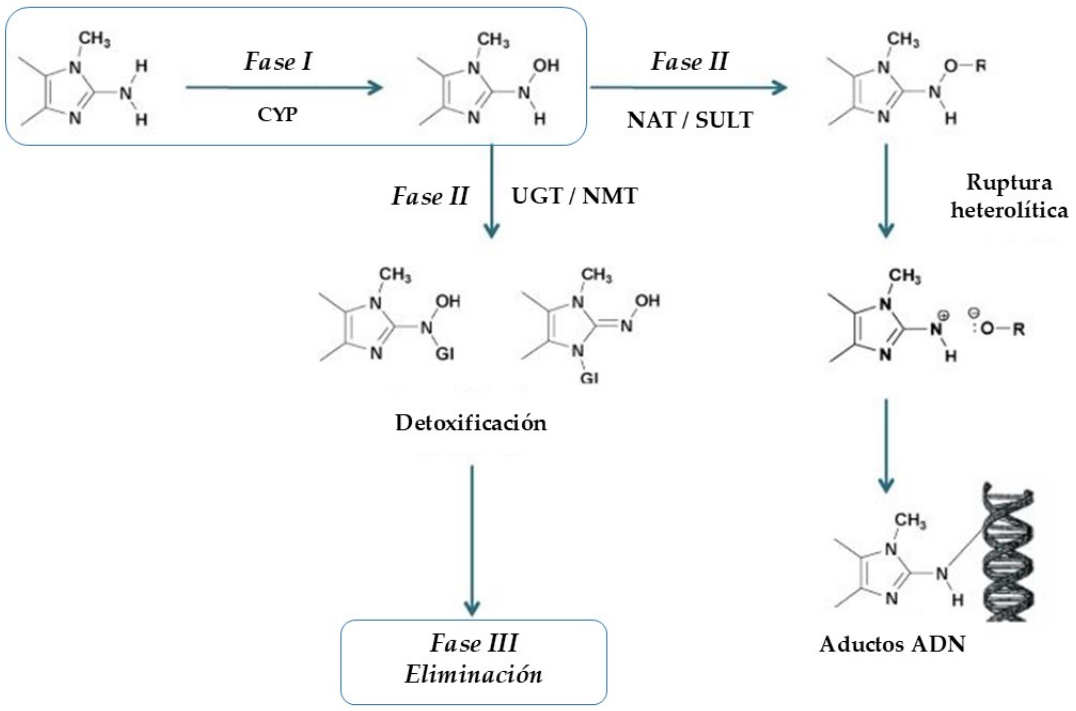


Figura 3. Ruta de bioactivación de aminas heterocíclicas. CYP, citocromo P450; NAT, N-acetiltransferasa; NMT, N-metiltransferasa; SULT, sulfotransferasa; UGT, UDP-glucoronosiltransferasa. Adaptado de Delannée *et al*., 2019 y Kim *et al*., 2015.

En la Fase I, el citocromo P450 (CYP) activa los carcinógenos mediante la oxidación, reducción o hidrólisis, mientras que en la Fase II se producen procesos de conjugación por los enzimas sulfotransferasas y las N-acetiltransferasas, dando lugar a la formación de metabolitos intermediaros activados (Barnes *et al*., 2018). Estos, compuestos son polares, presentan mayor solubilidad (Shimada, 2006) y pueden dar lugar a la formación de aductos de ADN, asociados con el desarrollo de procesos tumorales (La & Swenberg, 1996). En el caso del PhIP, a partir del metabolito inerte se producen compuestos metabólicamente activados como 4-OH-PhIP o N-acetil-PhIP (Kim *et al*., 2015). Del mismo modo, en la Fase II puede producirse la detoxificación de estos compuestos mediante enzimas como las UDP-glucuronosiltransferasas (Jarrar &

Lee, 2021). El conjunto de compuestos generados a nivel hepático puede ser transportado a la circulación sistémica o al intestino para su excreción, lo cual se conoce como Fase III (Abdelsalam *et al*., 2020).

Asimismo, el complejo CYP media la bioactivación de nitrosaminas como NDMA, dando lugar a la formación de intermediarios y aductos de ADN (Sen *et al*., 2012), y a partir de la acrilamida, cataliza la formación de glicidamida (Sen *et al*., 2012), metabolito considerado principal responsable de los efectos carcinogénicos y mutagénicos de la acrilamida (Sen *et al*., 2012).

2.5. Métodos de cuantificación de la ingesta de xenobióticos

A pesar de la evidencia obtenida hasta el momento sobre la asociación entre la ingesta de xenobióticos y el CCR, existen discrepancias en la literatura. Estas inconsistencias podrían deberse, entre otros factores, a los diferentes métodos empleados para su determinación en alimentos o a las bases de datos empleadas para la estimación de su ingesta.

Las técnicas analíticas más frecuentemente utilizadas son la cromatografía líquida de alta eficacia (HPLC), la cromatografía de gases (CG), así como su combinación con espectrofotometría de masas (MS), empleando los métodos HPLC-MS o CG-MS (Jakszyn *et al*., 2004). En el contexto de los xenobióticos, generados por el cocinado y procesado de los alimentos, resulta imprescindible realizar determinaciones del mismo alimento, o de diferentes partes del mismo, expuesto a diferentes técnicas y métodos de cocinado o de preparación.

La mejora en las técnicas analíticas en las últimas décadas ha permitido el desarrollo de bases de datos detalladas. Un ejemplo son las tablas con el contenido en sustancias potencialmente carcinógenas en alimentos desarrolladas por el Estudio Prospectivo Europeo Sobre Cáncer y Nutrición (EPIC) (Jakszyn *et al*., 2004). En ellas, se proporciona información sobre el contenido en AH, HAP, nitratos, nitritos y NOC en función de la técnica de cocinado o la temperatura y el tiempo de cocinado del alimento (Jakszyn *et al*., 2004). Del mismo modo, instituciones en Estados Unidos han proporcionado información sobre el contenido en AH y HAP en carnes (NIH, 2006) y,

de forma similar, la base de datos de la Agencia de Alimentos y Medicamentos (FDA) y la base de la Autoridad Europea de Seguridad Alimentaria (EFSA) disponen de información respecto al contenido en acrilamida y nitratos, respectivamente (EFSA, 2008; FDA, 2015). Hasta el momento, no se encuentra disponible en la literatura una base de datos harmonizada que considere la ingesta de los diferentes xenobióticos derivados de un mismo alimento con diferentes técnicas de cocinado. Este análisis permitiría estimar la ingesta del conjunto de xenobióticos en diferentes países.

La recogida de la información dietética se puede llevar a cabo por diferentes métodos. Entre ellos destaca el cuestionario de frecuencia de consumo de alimentos (CFCA) (Gunnar, 2014), que permite analizar los hábitos dietéticos a largo plazo (Zazpe *et al*., 2020). Esta herramienta se puede complementar con el registro del tamaño de las porciones de ingesta, lo cual aumenta la precisión de la misma (Sierra-Ruelas *et al*., 2020). A pesar de los errores intrínsecos del instrumento, tales como la precisión influenciada por capacidad memorística del individuo y una tendencia a la sobre-estimación de la ingesta (Sierra-Ruelas *et al*., 2020), este y otros métodos, como el recordatorio dietético de 24 horas (R24h), ya han sido utilizados en estudios oncológicos (Conway *et al*., 2022). En el contexto del CCR, los hábitos dietéticos están íntimamente ligados al riesgo de desarrollar este proceso tumoral (Feng *et al*., 2017; Tabung *et al*., 2017), por lo que el CFCA podría ser el método más recomendable. El mayor grado de precisión que proporciona aumenta la extensión y duración del cuestionario, por lo que, en su lugar, algunos autores se han limitado a analizar la ingesta de xenobióticos en un grupo reducido de alimentos (Sander *et al*., 2011). Por otro lado, el R24h es utilizado, frecuentemente, como método de validación, siendo esencial el registro de un número suficiente de días para recoger variabilidad y que la información obtenida sea representativa de la dieta habitual del encuestado (Murai *et al*., 2023; Ortega *et al*., 2015).

Hasta el momento, no hay ningún instrumento de recogida de la información dietética disponible en la literatura desarrollado y validado para la estimación de la ingesta de diferentes xenobióticos derivados del total de alimentos que componen la dieta. En este contexto y para llevar a cabo una cuantificación más precisa de la ingesta

de xenobióticos, el método de recogida de la información dietética debería, además, registrar los métodos y hábitos de cocinado de cada individuo.

2.6. Factores moduladores del efecto tóxico de los xenobióticos

Además de la cantidad de xenobióticos ingerida a lo largo de la vida, diferentes factores afectan a la modulación del potencial tóxico de estos compuestos, tal y como se ilustra en la **Figura 4**.

El nivel de activación o detoxificación de estos carcinógenos puede estar modulado por la presencia previa de otros xenobióticos. *In vitro*, el B(a)P promueve la expresión de genes codificantes de enzimas bioactivadores de AH, promoviendo el potencial mutagénico (Barnes *et al*., 2018). Además, cada individuo presenta diferente susceptibilidad debido a la presencia de polimorfismos en genes codificantes de enzimas de transporte y de la metabolización de compuestos xenobióticos como N-acetiltransferasa 1 y N-acetiltransferasa 2 entre otros (Chiavarini *et al*., 2017; de Castro *et al*., 2020).

A nivel intestinal, el perfil microbiano desempea un papel clave en la detoxificación o el incremento de la toxicidad de estos compuestos. Algunas bacterias con potencial probiótico, como algunos miembros de las especies *Lactobacillus acidophilus* o *Bifidobacterium longum* (Dominici *et al*., 2014), han mostrado capacidad de unir PhIP directamente a sus paredes celulares, reduciendo la biodisponibilidad, la absorción de este carcinógeno, así como su exposición al epitelio intestinal y promoviendo su eliminación (Dominici *et al*., 2014). Además, la unión de probióticos a los colonocitos limita el paso de xenobióticos a través de la barrera intestinal (Liu *et al*., 2010) y promueve la integridad del epitelio mediante el incremento de la expresión de proteínas de unión (Al-Sadi *et al*., 2021).

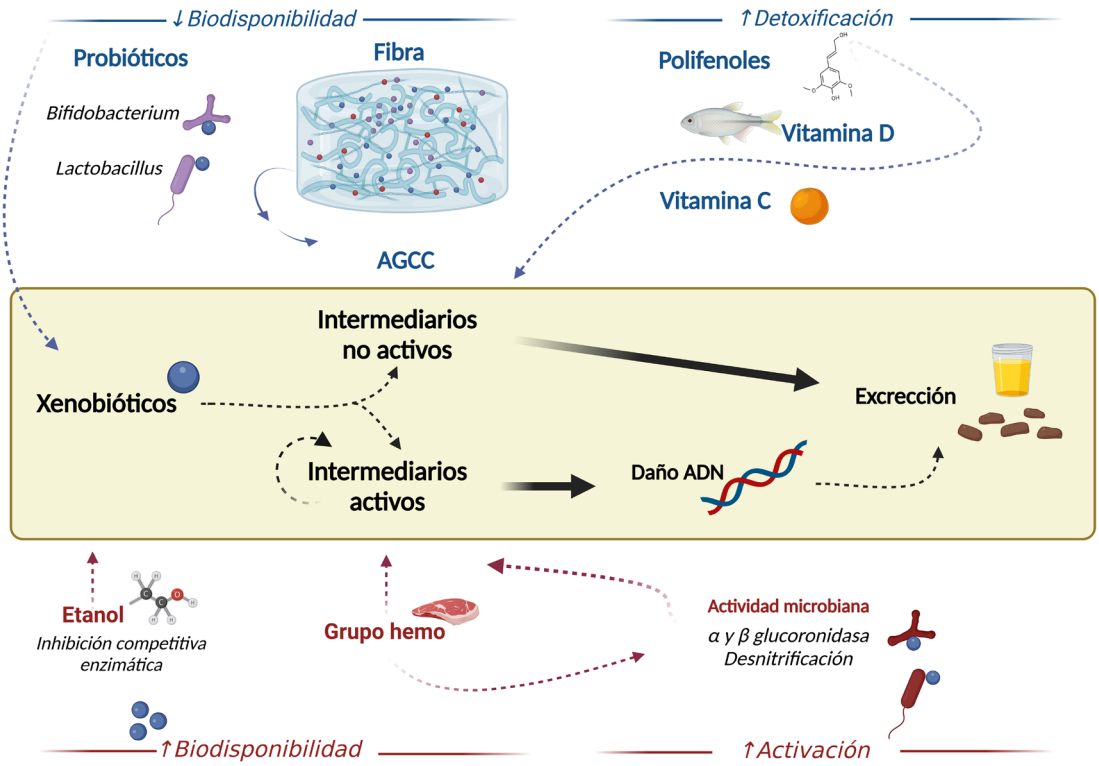


Figura 4. La microbiota y la dieta como factores modulantes del potencial carcinogénico de los xenobióticos. Creada con BioRender.com

Por otro lado, algunos microorganismos pueden reactivar los metabolitos previamente generados a través de la actividad β-glucuronidasa o nitrorreductasa, liberando intermediarios con potencial tóxico (Collins & Patterson, 2020). En este contexto, algunos autores han desarrollado herramientas bioinformáticas para el análisis del riesgo de desarrollar CCR en función del perfil microbiano y de la ingesta de carcinógenos (Ruiz-Saavedra *et al*., 2021).

Finalmente, el resto de los componentes dietéticos ingeridos a través de la dieta pueden influir en el potencial tóxico de estos metabolitos. Por un lado, la presencia del grupo hemo promueve la nitrosación endógena (de Kok & van Maanen, 2000), la generación de aldehídos citotóxicos y genotóxicos (Nogacka *et al*., 2019), la actividad de agentes nitrosantes como las bacterias (de Kok & van Maanen, 2000) y la peroxidación lipídica (Etemadi *et al*., 2018), dando lugar a la formación de aductos de ADN (Manson & Benford, 1999). Asimismo, el etanol actúa como inhibidor competitivo de enzimas metabolizadores de NDMA o acrilamida, dificultando su metabolismo y posterior degradación (ATSDR, 2023). Por otro lado, algunos autores han descrito una reducción

del efecto tóxico de estos compuestos cuando son consumidos en combinación con determinados componentes dietéticos. *In vitro*, la ingesta de vitamina C presenta un efecto reductor de la nitrosación endógena (Lundberg *et al*., 2008) y la fibra alimentaria soluble promueve la formación de geles que disminuyen la exposición de estos xenobióticos a la mucosa del epitelio (Nogacka *et al*., 2019). Además, metabolitos derivados de la fermentación de la fibra (van de Wouw *et al*., 2018), como los ácidos grasos de cadena corta (AGCC), junto con los polifenoles o la vitamina D, inducen la expresión de enzimas detoxificadores de xenobióticos como CYP3A4, reduciendo el potencial tóxico de estos compuestos (Gupta *et al*., 2004; Kimura *et al*., 2010; Mun *et al*., 2021).

En conjunto, el estado inflamatorio y/o carcinógeno del ambiente intestinal se encuentra determinado por el efecto global de los compuestos ingeridos sobre la actividad de la microbiota intestinal y el sistema inmune, influyendo, en última instancia, en el estado de homeostasis intestinal (Nogacka *et al*., 2019).

3. Microbiota Intestinal

La microbiota humana es el conjunto de microorganismos, incluyendo bacterias, arqueas, virus y algunos eucariotas unicelulares, que habitan el cuerpo humano y que han coevolucionado con nuestra fisiología hasta crear la relación de interdependencia que tenemos hoy en día (Barber *et al*., 2023). La composición microbiana está determinada por las condiciones de nicho ecológico y varía en las diferentes regiones del hospedador (Milani *et al*., 2017). En el tracto digestivo, la densidad microbiana es menor a nivel del estómago (10^1-10^3 UFC/ml) y del intestino delgado (10^3-10^8 UFC/ml), como resultado de las condiciones acidificantes derivadas del ácido, la bilis y las secreciones pancreáticas que dificultan la colonización por parte de los microorganismos (O'Hara & Shanahan, 2006). Esta densidad bacteriana se incrementa posteriormente a nivel del intestino grueso, siendo el colon la región más densamente poblada (10^{10}-10^{12} UFC/ml) (Eslami *et al*., 2020; O'Hara & Shanahan, 2006).

Durante aos, ha habido un gran interés en la comunidad científica por el estudio de estos microorganismos. Las técnicas dependientes de cultivo de microorganismos limitaban al principio este estudio, ya que no permiten el crecimiento

de todos los integrantes de la microbiota intestinal (Santos *et al*., 2020) ni el análisis de las dinámicas encontradas en comunidades bacterianas complejas (Rinninella *et al*., 2019). Actualmente, el desarrollo de las nuevas tecnologías ha hecho posible el uso de técnicas independientes del cultivo de microorganismos que permiten la identificación filogenética y la cuantificación de los diferentes componentes de la microbiota intestinal mediante el análisis de ácidos nucleicos (ADN y ARN) directamente a partir de muestras de heces (O'Hara & Shanahan, 2006).

La mayoría de estas técnicas se basan en la extracción, el aislamiento de ADN y la posterior secuenciación del genoma completo, o de una región especifica, a través de técnicas de secuenciación masiva. Para la secuenciación de una región específica se realiza, en primer lugar, un proceso de amplificación de genes seleccionados para el análisis de las regiones hipervariables del gen ARN ribosómico (ARNr) 16S mediante una reacción en cadena de la polimerasa (O'Hara & Shanahan, 2006). A continuación, la secuenciación de este producto de amplificación permite obtener gran cantidad de información relativa a la composición del perfil microbiano de forma eficiente y a bajo coste (Santos *et al*., 2020). Esta técnica no proporciona información sobre las propiedades funcionales de los microorganismos y, a pesar de que depende del desarrollo y mantenimiento de extensas bases de datos (Dekaboruah *et al*., 2020), es una de las más empleadas actualmente para el análisis de la diversidad y abundancia del ecosistema microbiano (Rinninella *et al*., 2019). Por otro lado, la secuenciación del genoma completo se lleva a cabo mediante las técnicas metagenómicas o de secuenciación *shotgun*. Estas son técnicas más avanzadas que, aunque resultan más costosas, permiten una mayor resolución taxonómica y el análisis de las dinámicas y la funcionalidad de las comunidades microbianas (Quince *et al*., 2017).

El desarrollo de la tecnología, en los últimos años, ha incrementado la evidencia disponible sobre la estrecha relación entre la microbiota y la salud. Entre los principales estudios realizados hasta el momento destaca el Proyecto del Microbioma Humano que, en 2007 y a partir del análisis de 15-18 muestras por individuo en 300 adultos sanos de Estados Unidos, caracterizó las comunidades microbianas presentes en las distintas regiones del cuerpo humano (Proctor *et al*., 2019) para analizar su posible asociación con diferentes patologías (Cho & Blaser, 2012). A partir de los

resultados obtenidos, algunos autores destacaron la complejidad de definir la composición microbiana "normal" de referencia, ya que esta se encuentra influenciada por múltiples factores que van desde el tipo de parto o lactancia, hasta el estilo de vida, la contaminación ambiental o la toma de antibióticos entre otros (Yatsunenko *et al*., 2012). Por ello, el uso de este término resulta controvertido en la literatura, lo que hace necesario caracterizar el perfil microbiano en grandes grupos poblacionales (al menos 500 individuos) pertenecientes a una misma región y con un estilo de vida y de alimentación similares (Latorre-Pérez *et al*., 2021).

En la población de los países desarrollados, la microbiota se caracteriza por presentar en mayor abundancia de microorganismos pertenecientes a los filos Bacteroidota (anteriormente Bacteroidetes), Bacillota (anteriormente Firmicutes) y Actinomycetota (anteriormente Actinobacteriota), seguido de Pseudomonadota (anteriormente Proteobacteria) y Verrucomicrobiota (anteriormente Verrucomicrobia) en menor proporción (Huttenhower *et al*., 2012; Latorre-Pérez *et al*., 2021; Singh *et al*., 2019). Estos resultados fueron similares a los obtenidos tras la caracterización de la microbiota intestinal en una muestra de 530 adultos sanos espaoles, excepto por la menor abundancia de Actinomycetota la cual es, en este caso, inferior a Pseudomonadota y Verrucomicrobiota (Latorre-Pérez *et al*., 2021). Valores reducidos de la ratio Bacillota/Bacteroidota (anteriormente Firmicutes/Bacteroidetes) se han asociado consistentemente con estados saludables, mientras que ratios elevadas se asocian con desordenes metabólicos (Ley *et al*., 2006).

De forma similar, la alteración de la diversidad taxonómica, de la composición microbiana y de la producción de metabolitos se ha asociado con un impacto sobre el estado de salud. Una menor consistencia de las heces refleja un menor tiempo de tránsito gastrointestinal y la menor reabsorción de agua a nivel del colon, lo cual, en última instancia, se ha asociado con cambios en la diversidad microbiana en humanos (Yap *et al*., 2021). El análisis de la diversidad α es un índice de la estructura de la comunidad bacteriana que considera la riqueza (número de grupos taxonómicos) y la uniformidad (distribución de abundancias entre grupos) (Willis, 2019) y se asocia habitualmente con estados de promoción de la salud (Merra *et al*., 2021). Asimismo, abundancias relativas superiores de familias como *Lachnospiraceae* y *Ruminococcaceae se*

han considerado como un marcador de la salud intestinal y una menor abundancia de estos microorganismos (Milani *et al*., 2017), y de otros como *Clostridium leptum* y *Faecalibacterium prausnitzii,* se han observado en patologías con inflamación intestinal (Wang *et al*., 2014). Asimismo, *Fusobacterium nucleatum*, *Bacteroides fragilis* y *Peptostreptococcus anaerobius* presentan un papel destacado en el desarrollo del CCR (Afzaal *et al*., 2022; Merra *et al*., 2021). En el caso de *F. nucleatum*, este microorganismo favorece la proliferación celular y suprime la respuesta inmune en humanos (Mehta *et al*., 2017).

Entre las principales funciones de la microbiota intestinal destacan acciones estructurales, mediante la regulación de la permeabilidad y la función de la barrera intestinal, o protectoras, a través de la prevención y resistencia a la colonización por patógenos (Singh *et al*., 2019). La microbiota intestinal presenta una actividad metabólica relevante, ya que interviene en la síntesis de vitaminas, la regulación del metabolismo lipídico y energético, y, la producción de AGCC entre otros (Singh *et al*., 2019). Los AGCC son de interés por sus efectos beneficiosos en el hospedador (Cronin *et al*., 2021). Además de la fibra, estos metabolitos bacterianos se producen a partir de almidón resistente y, en menor medida, de proteínas no digeridas y de un pequeo número de péptidos (Morrison & Preston, 2016). La concentración de estos metabolitos disminuye desde el ciego hasta el colon descendente y los AGCC excretados en mayor proporción son el ácido acético, el ácido propiónico y el ácido butírico (Hou *et al*., 2022; Zeng *et al*., 2014).

De los principales metabolitos derivados del metabolismo bacteriano, el ácido butírico es la principal fuente de energía de los colonocitos (Hou *et al*., 2022). Además, el ácido acético promueve la integridad del epitelio intestinal (Macia *et al*., 2015) y el ácido propiónico es un precursor de la gluconeogénesis en el hígado (Schnorr *et al*., 2014). En el intestino, estos AGCC son rápidamente absorbidos en la membrana apical de los colonocitos y, participan en la regulación de la homeostasis intestinal mediante la modulación de la acetilación y metilación de histonas (van de Wouw *et al*., 2018). Asimismo, se han observado niveles reducidos de ácido acético y ácido propiónico en pacientes con enfermedades inflamatorias (Machiels *et al*., 2014), poniendo de

manifiesto el papel potencialmente relevante de estos metabolitos en la promoción de la homeostasis intestinal.

4. Mediadores Inmunológicos

El análisis de los niveles circulantes de mediadores inmunológicos como las citocinas proporciona información respecto a un proceso influenciado por múltiples factores como la severidad, la cronicidad, la capacidad de respuesta del organismo ante un estímulo (Zhang & An, 2007), y la microbiota intestinal (Singh *et al*., 2019).

Las citocinas son proteínas de bajo peso molecular secretadas por células (Zhang & An, 2007) que presentan una actividad redundante, ya que la misma acción puede estar estimulada por diferentes tipos.

Algunas son pro-inflamatorias y presentan una función estimuladora y perpetuadora de la inflamación (interleucinas (IL) como IL-1, IL-2, IL-1β, o quimiocinas como la proteína quimioatrayente de monocitos-1 (MCP-1) y la proteína 10 inducida por interferón γ (IP-10)) (Borish & Steinke, 2003; Zhang & An, 2007). Otras son anti-inflamatorias y presentan acción inmunorreguladora, mediante el control de la respuesta pro-inflamatoria (IL-10, IL-4 o la proteína quimioatrayente de células B-1) (Borish & Steinke, 2003; Zhang & An, 2007). Asimismo, algunas citocinas, como IL-6 o el factor de crecimiento transformante-β (TGF-β), pueden actuar como pro o anti-inflamatorias (Borish & Steinke, 2003). Por otro lado, aunque algunos mediadores como el interferón γ (IFN-γ), el factor de necrosis tumoral-α (TNF-α), IL-2 o IL-22 se asocian, predominantemente, con la fase aguda (Arshad *et al*., 2020; Ribeiro *et al*., 2022), otros como IL-1, IL-6 o IL-17 pueden mediar tanto la fase aguda como crónica de la inflamación (Germolec *et al*., 2018; Huangfu *et al*., 2023).

La inflamación es la primera respuesta de la inmunidad innata ante la detección de un agente externo y se caracteriza por presentar enrojecimiento, calor, hinchazón y dolor en el sitio de infección (Sherwood & Toliver-Kinsky, 2004) y puede ser de fase aguda o crónica (Germolec *et al*., 2018). En la fase aguda tiene lugar un aumento del flujo vascular, como resultado de la entrada de leucocitos al foco de infección, la activación de células endoteliales y la expresión de moléculas de adhesión que facilitan

la unión de los linfocitos y monocitos circulantes, así como la acumulación de fagocitos y macrófagos en el sitio de infección (Germolec *et al*., 2018). Finalmente, la liberación de moléculas como IL-1, IL-6 y TNF-α permite la reacción global del organismo contra el agente estimulante, junto con la activación de otras células inmunitarias, como basófilos y mastocitos, y el desencadenamiento de la reacción de coagulación de la sangre en el sitio de infección (Germolec *et al*., 2018; Sherwood *&* Toliver-Kinsky, 2004). En su conjunto, este proceso tiene como objetivo la erradicación del estímulo que ha desencadenado el proceso de inflamación y/o la reparación del dao que ha provocado el estímulo (Germolec *et al*., 2018; Sherwood *&* Toliver-Kinsky, 2004).

En función de la severidad del dao estos mecanismos pueden ser suficientes (Germolec *et al*., 2018). Sin embargo, una inflamación continuada, debido a la persistencia de la exposición al estimulo o a la reacción inapropiada contra moléculas propias, puede desembocar en un proceso de inflamación crónica (Germolec *et al*., 2018). Durante esta fase, la respuesta inmune se perpetua a través de la liberación de moléculas estimuladoras de linfocitos como las citocinas (Hanada *&* Yoshimura, 2002). Estas tienen un papel fundamental en la regulación y resolución de la respuesta inflamatoria a nivel local y pueden dar lugar al desarrollo de inflamación sistémica (Hanada *&* Yoshimura, 2002).

Asimismo, en pacientes con inflamación crónica, se han detectado niveles alterados de marcadores de desorden metabólico como leptina, adiponectina o resistina (Karmiris *et al*., 2006). En concreto, mientras que los niveles de adiponectina y resistina se han encontrado elevados en estadios de inflamación, la leptina muestra niveles reducidos (Karmiris *et al*., 2006). En modelos animales, la dieta y los AGCC se ha destacado como factores moduladores de los niveles circulantes de estas adipocinas (Turpin *et al*., 2023).

4.1. Interacción del sistema inmunitario con la microbiota intestinal

El sistema inmunitario está sometido a una constante interacción con la microbiota intestinal (Malesza *et al*., 2021). A nivel intestinal, en esta interacción interviene el tejido linfoide asociado al intestino, el cual es la sección del sistema inmunitario más extensa del cuerpo humano pues contiene el 65% del tejido inmune y el 80% de los tejidos productores de inmunoglobulinas (Ig) (Hord, 2008). Entre sus principales funciones se encuentra la protección del hospedador frente a la exposición a compuestos nocivos o a microorganismos presentes en el lumen intestinal (Hord, 2008; Martel *et al*., 2022). De esta forma, el reconocimiento de antígenos puede activar la respuesta inmune a nivel local (intestinal) o en otras regiones (extra-intestinal) (Yoo *et al*., 2020).

En condiciones de homeostasis intestinal, la interacción entre el sistema inmune y la microbiota intestinal no desencadena una respuesta pro-inflamatoria (Martel *et al*., 2022). En su lugar, se inicia un proceso inflamatorio que conlleva la desregulación inmunitaria, como resultado del incremento de la permeabilidad del epitelio intestinal, de la alteración en la producción de mucus o de defectos en el sistema de reparación del epitelio intestinal, tal y como se muestra en la **Figura 5** (Martel *et al*., 2022).

En este caso, la ejecución de una respuesta inmunitaria tiene lugar a través de la infiltración de linfocitos T y B en la lámina propia junto con otras células del sistema inmune como macrófagos, células dendríticas y neutrófilos (Guan, 2019). Ante la falta de control de la respuesta inflamatoria, mediante experimentos *in vitro* se ha observado que células de la lámina propia se activan y producen niveles elevados de citocinas pro-inflamatorias como TNF, IL-1β, IFN-γ, IL-2 o IL-12 en el tejido local (Aggeletopoulou et al., 2024; Guan, 2019). Además, citocinas como TNF-α, IFN-γ y otras como IL-13, características de una respuesta Th2, pueden aumentar la permeabilidad intestinal y promover el deterioro de la función de la barrera intestinal (Guan, 2019).

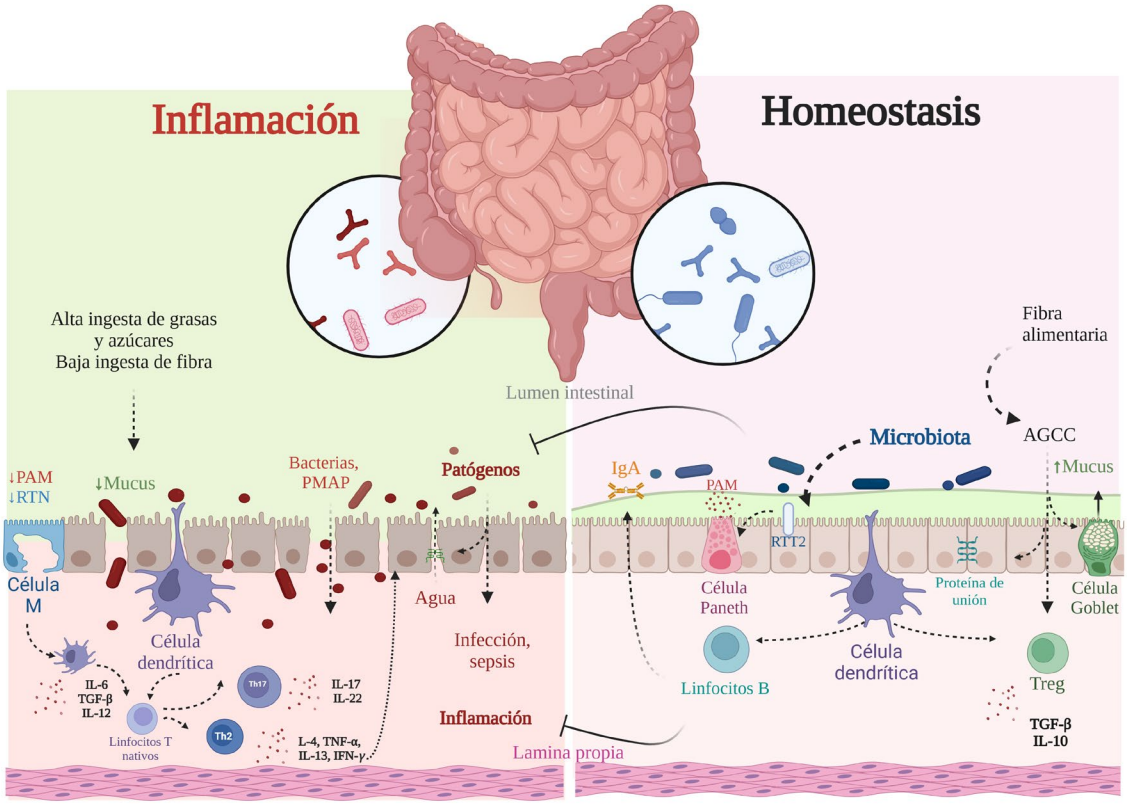


Figura 5. Mecanismos por los que la microbiota intestinal puede interaccionar con el sistema inmune y promover la homeostasis intestinal. AGCC, ácidos grasos de cadena corta; IFN-γ, interferón-γ; IgA, inmunoglobulina A; IL, interleucina; PMAP, patrones moleculares asociados a patógenos; RTN, receptores de tipo NOD; PAM, péptidos antimicrobianos.; RTT2, receptor de tipo Toll 2; TGF- β, factor de crecimiento transformante-β; TNF-α, factor de necrosis tumoral-α. Adaptado de Martel *et al*., 2022. Creada con BioRender.com.

La dieta y la microbiota tienen capacidad para modular la respuesta del sistema inmunitario (Christ *et al*., 2019) (**Figura 5**). Por tanto, todos los factores, en su conjunto, deberían ser analizados para descifrar las vías de promoción de la homeostasis intestinal.

5. Impacto de la Dieta sobre la modulación de la Microbiota Intestinal y los Mediadores Inmunológicos

La ingesta dietética tiene un gran impacto sobre la funcionalidad y estructura de la microbiota intestinal (Nagpal *et al*., 2019). De hecho, se ha observado que la diversidad dietética contribuye a mejorar la estabilidad de la composición microbiana (Johnson *et al*., 2019) y algunas intervenciones alimentarias en humanos han sido capaces de producir cambios en la composición microbiana en tan solo 24 horas (Wu *et al*., 2011). Sin embargo, se ha visto que estas variaciones producidas son temporales (David *et al*., 2014) y, en su lugar, son los hábitos dietéticos a largo plazo los que contribuyen a determinar el perfil microbiano individual (Leeming *et al*., 2019). En este sentido, de acuerdo con el método de clasificación de la microbiota intestinal en enterotipos (Arumugam *et al*., 2011), dietas ricas en proteína y grasa animal se relacionan con el enterotipo dominado por *Bacteroides*, mientras que una dieta rica en carbohidratos se asocia con el enterotipo dominado por *Prevotella* (Fu *et al*., 2022; Merra *et al*., 2021; Schnorr *et al*., 2014).

Algunos trabajos muestran que la DM se asocia con una mayor diversidad α (Willis, 2019) y una menor ratio Bacillota/Bacteroidota (Garcia-Mantrana *et al*., 2018). Además, en humanos, la DM promueve el crecimiento de géneros productores de ácido butírico con mayor afinidad a polisacáridos como *Bifidobacterium*, *Bacteroides*, *Parabacteroides*, *Prevotella* y *Faecalibacterium* (Barber *et al*., 2023; Khavandegar *et al*., 2024). Asimismo, se encontraron niveles reducidos de otras especies como *Collinsella aerofaciens* o *Flavonifractor plautii* (Barber *et al*., 2023; Khavandegar *et al*., 2024) y de forma similar, el género *Flavonifractor*, se asoció de forma directa con la ingesta de alimentos característicos del patrón occidentalizado (Latorre-Pérez *et al*., 2021). Estos hallazgos se encuentran en consonancia con los mostrados tras el análisis del efecto de la transición de una dieta de tipo occidentalizada a una de tipo mediterráneo isocalórica en pacientes con sobrepeso u obesidad durante 8 semanas (Meslier et al., 2020). En este caso, se incrementaron las abundancias relativas de bacterias degradadoras de fibra, como *F. prausnitzii*, y se redujo la abundancia de otra especie con potencial pro-inflamatorio como *Mediterraneibacter gnavus* (anteriormente

Ruminococcus gnavus) (Meslier *et al*., 2020). Además, en humanos *F. praustnitzii* se ha correlacionado positivamente con la adherencia a la DM (Khavandegar *et al*., 2024) y *Ruminococcus* negativamente (Gutiérrez-Díaz *et al*., 2016). Por otra parte, en diferentes grupos poblacionales, el consumo de una dieta rica en grasa y baja en fibra durante 2 semanas produjo un incremento de especies asociadas a un mayor riesgo de desarrollo de CCR como *F. nucleatum* (Afzaal *et al*., 2022; O'Keefe *et al*., 2015) y a nivel de familia, se ha encontrado una asociación inversa entre las abundancias relativas de este microorganismo y el consumo de carne (Gutiérrez-Díaz *et al*., 2016).

La DM se asocia con una mayor excreción fecal de AGCC (Bailey *&* Holscher, 2018; O'Keefe *et al*., 2015), los cuales presentan un papel anti-inflamatorio e inmunomodulador (Bander *et al*., 2020). En humanos, incrementos en la excreción del ácido butírico se han correlacionado con la disminución de los niveles circulatorios de citocinas como IL-12 e IL-17, mientras que incrementos en las concentraciones fecales de los ácidos acético y propiónico se asocian con una disminución de IL-12 y IP-10 (Pagliai *et al*., 2020). Del mismo modo, se han encontrado diferentes asociaciones en la literatura entre la ingesta de alimentos y los niveles circulantes de citocinas. En adolescentes, el consumo de vegetales se asoció con un aumento de IL-10, mientras que la ingesta de carne se asoció con mayores niveles de IL-2 (Arouca *et al*., 2018). En línea con estos resultados, se ha demostrado que la intervención con DM durante al menos 12 semanas en adultos redujo marcadores pro-inflamatorios como la proteína C reactiva (-0,98 mg/ml), la molécula de adhesión intercelular 1 (-23,73 ng/ml) e IL-6 (-0,42 pg/ml) y aumentó los niveles de adiponectina (1,69 μg/ml) (Schwingshackl *&* Hoffmann, 2014).

A su vez, la dieta puede modular la respuesta inmune a través del mecanismo del estrés oxidativo. El metabolismo normal de los seres vivos conlleva la producción de especies reactivas de oxígeno (ERO) como peróxido de hidrógeno, el radical hidroxilo o el anión superóxido, los cuales contribuyen a aumentar el estrés oxidativo por oxidación directa o mediante la formación de más especies reactivas (Birben *et al*., 2012; Gupta *et al*., 2021). Sin embargo, el organismo presenta actividad enzimática con acción antioxidante como la superóxido dismutasa (SOD), la catalasa o glutatión peroxidasa (Birben *et al*., 2012). Asimismo, la ingesta de componentes dietéticos como

la vitamina C y E o los carotenoides entre otros, actúan como antioxidantes no enzimáticos y disminuyen el estrés oxidativo (*Gupta et al.*, 2021), tal y como se muestra en la **Figura 6**.

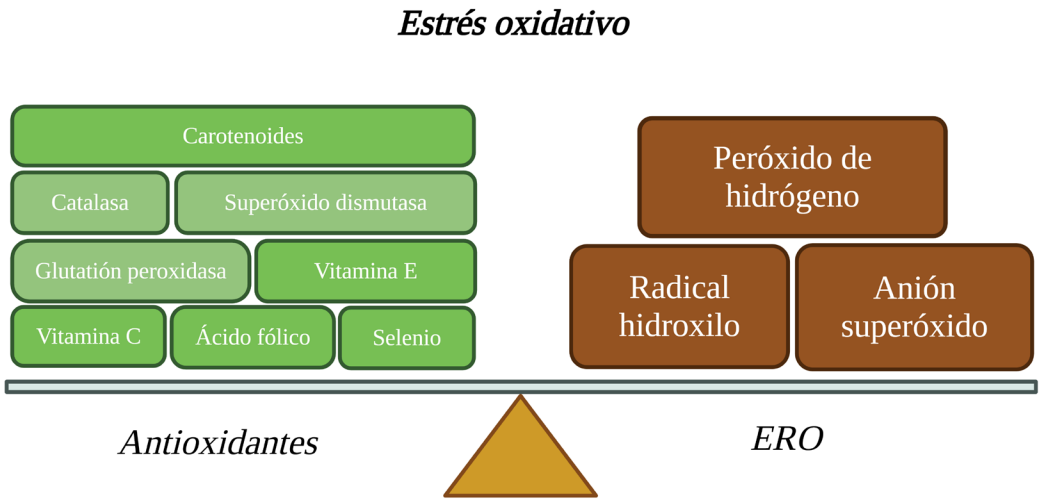


Figura 6. Compuestos que modulan el balance del estrés oxidativo. EDTA, ácido etilendiaminotetraacético; ERO, especies reactivas de oxígeno**.** Adaptado de Gupta *et al.*, 2021. Creada con BioRender.com.

Por el contrario, dietas ricas en ácidos grasos trans y/o azúcares promueven la generación de ERO e interactúan con carbohidratos, lípidos, proteínas y ácidos nucleicos (Birben *et al*., 2012). Como resultado, se altera la expresión de genes a través de factores de transcripción y cambios en la conformación de la cromatina, mediante la alteración del patrón de acetilación y desacetilación de histonas (Birben *et al*., 2012). Además, se generan productos de oxidación que presentan efectos mutagénicos como el MDA o el 8-hidroxideoxiguanosina (Cordiano *et al*., 2023; Tatsch et al., 2015). Asimismo, estos indicadores del daño oxidativo se han asociado directamente con los niveles de parámetros pro-inflamatorios en humanos (Tatsch *et al*., 2015).

La ingesta dietética modula la composición microbiana y los metabolitos derivados presentan un efecto inmunomodulador e influyen en el potencial antioxidante (González-Bosch *et al*., 2021, 2023; Kunst *et al*., 2023; Morris *et al*., 2022; Zhao *et al*., 2023). En base a estas evidencias, entre los diferentes componentes dietéticos, la fibra dietética, los polifenoles o los probióticos se han establecido como dianas de interés para la elaboración de estrategias dietéticas enfocadas al mantenimiento de la homeostasis intestinal.

5.1. Fibra dietética

La ingesta de prebióticos, como la fibra, se ha propuesto como mecanismo de modulación de la microbiota intestinal (Rose *et al*., 2021). De acuerdo con la definición de la Asociación Científica Internacional para Probióticos y Prebióticos (ISAPP), los prebióticos son "sustratos selectivamente utilizados por los microorganismos del hospedador y que confieren efectos beneficiosos en la salud" (G. R. Gibson *et al*., 2017). Estudios *in vitro* e *in vivo* han mostrado que los prebióticos promueven la mejora de la permeabilidad intestinal (Rose *et al*., 2021). Además, en humanos, producen alteraciones del perfil microbiano a través de incrementos en los niveles de bifidobacterias (Dou *et al*., 2022), incrementos en la excreción de AGCC (Baxter *et al*., 2019), reducción de la severidad de algunos síntomas intestinales como las flatulencias (Wilson *et al*., 2018) y un efecto protector frente al desarrollo de cáncer de colon (Zeng *et al*., 2014).

La fibra alimentaria reduce el contacto de los carcinógenos con el lumen intestinal a través de una disminución del tiempo de tránsito intestinal (fibra insoluble) y la formación de un gel viscoso (fibra soluble) (Timm *et al*., 2023). Además, la fibra promueve la reducción del pH, la modulación de la actividad microbiana y los parámetros inmunológicos (Zeng *et al*., 2014).

En el caso de la pectina, *in vitro,* ha mostrado promover el incremento de las abundancias relativas de *Ruminococaceae*, *Bacteroides* y *Lachnospira* (Pascale *et al*., 2022), junto con una reducción de la expresión del factor nuclear-κβ (NF-κβ), IL-1β, IL-6 (Donadio *et al*., 2023). Por otro lado, *in vivo*, la inulina estimula el crecimiento de bifidobacterias, inhibe el crecimiento de bacterias con potencial patogénico (Zeng *et al*., 2014) y reduce la expresión de TNF-α y IL-6 (Meng *et al*., 2020). Asimismo, en humanos, el consumo de almidón resistente altera las abundancias relativas de *Ruminococcus bromii* y promueve la integridad del epitelio intestinal mediante la producción de AGCC (Rosés *et al*., 2021; Zeng *et al*., 2014).

Los AGCC contribuyen a la homeostasis intestinal, ya que promueve la función de la barrera intestinal mediante la estimulación de la producción y secreción de moco, lo cual, regula y mantiene la función del sistema inmune innato y adaptativo (Hou *et al*., 2022; Makki *et al*., 2018). En este contexto, intervenciones en humanos con dietas altas

en proteínas y bajas en carbohidratos no solo redujeron la producción del total de AGCC y de ácido butírico, sino que también aumentaron la producción de metabolitos perjudiciales derivados de la fermentación de aminoácidos (Duncan *et al*., 2007). Por el contrario, los AGCC derivados de la ingesta de fibra se han mostrado protectores frente a dietas ricas en grasas en ratones (den Besten *et al*., 2015), posiblemente debido a la promoción de la expresión de leptina, la oxidación de ácidos grasos libres en el tejido adiposo de estos animales (Hou *et al*., 2022) y/o la reducción en los niveles de mediadores pro-inflamatorios TNF-α o NF-κβ, observada *in vitro* (Zeng *et al*., 2014).

5.2. Polifenoles

Diferentes estudios de intervención han mostrado el efecto de la ingesta de polifenoles sobre la modulación del perfil microbiano intestinal y parámetros inmunológicos (Plamada & Vodnar, 2022; Wan *et al*., 2021).

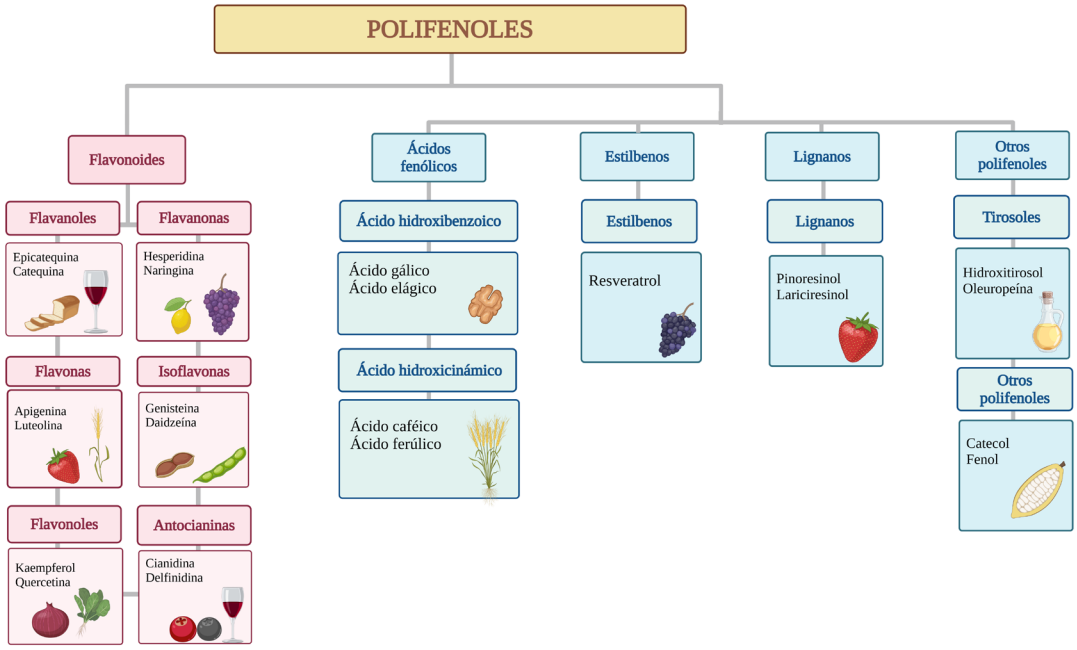


Figura 7. Clases, subclases y algunos tipos de polifenoles de acuerdo con la clasificación del *Phenol Explorer* (Neveu *et al*., 2010). Adaptado de Woodward *et al*., 2018. Creada con BioRender.com.

Existen más de 8.000 tipos de polifenoles (Nani *et al*., 2021), clasificados en clases y subclases, tal y como se muestra en la **Figura 7**. La biodisponibilidad de estos compuestos en el tracto intestinal esta influenciada por la abundancia de determinadas

especies bacterianas que presentan capacidad de metabolizarlos como *F. plautii*, degradadora de flavonoides (Latorre-Pérez *et al*., 2021). Asimismo, a través de su unión a la membrana celular, los polifenoles pueden producir un aumento de la permeabilidad de la membrana y dificultar el crecimiento bacteriano (Stapleton *et al*., 2007).

En el caso de flavonoides, como la quercetina, su ingesta tiene un impacto sobre el perfil microbiano fecal de roedores a través de la reducción de la ratio Bacillota/ Bacteroidota y de la abundancia relativa de algunos taxones, como *Erysipelotrichaceae* y *Bacillus*, incrementados en obesidad (Etxeberria *et al*., 2015). Además, se ha demostrado que la quercetina modula la respuesta inmune al inducir la producción de citocinas de la vía Th1, como INF-γ, e inhibir citocinas de la vía Th2, como IL-4 en líneas celulares (Li *et al*., 2016). De forma similar, la ingesta de resveratrol revirtió la ratio Bacillota/ Bacteroidota y estimuló el crecimiento de *Lactobacillus* y *Bifidobacterium* en diferentes modelos animales de colitis (Singh *et al*., 2019). Asimismo, *in vitro*, el resveratrol mejoró la barrera intestinal a través de una mayor expresión de proteínas de unión como ZO-1 (Zheng *et al*., 2024) y, promovió un efecto anti-inflamatorio reduciendo la producción de IL-6, TNF-α e IL-17 (Prakash *et al*., 2024). Finalmente, en animales se ha observado que el resveratrol afecta negativamente a la progresión del cáncer de colon (Singh *et al*., 2019) posiblemente como resultado de una reducción de la actividad enzimática bacteriana α-glucuronidasa o nitrorreductasa (Sengottuvelan *&* Nalini, 2006).

5.3. Probióticos

El consumo de probióticos ha despertado un gran interés en la comunidad científica por su potencial efecto protector sobre el desarrollo de patologías (Gul *&* Durante-Mangoni, 2024) entre las que se incluyen procesos inflamatorios intestinales (Nowak *et al*., 2015; Kumar, 2015). De acuerdo con la definición de la ISAPP, los probióticos se definen como "organismos vivos que, administrados en cantidades adecuadas, confieren un efecto beneficioso en el hospedador" (Hill *et al*., 2014). Estos microorganismos actúan a través de la disminución del tiempo de tránsito intestinal, la modulación de la composición microbiana o la unión directa a componentes derivados de la dieta como nutrientes o toxinas (Hill *et al*., 2014).

Además, algunos miembros de *Bacteroides* o *Clostridium* pueden modular la activación de compuestos tóxicos (Nowak *et al*., 2015) y la exposición de estos compuestos nocivos a la mucosa del colon, disminuyendo la potencial interacción de intermediarios tóxicos con el ADN, y, por tanto, reduciendo la formación de aductos de ADN en los colonocitos (Kumar *et al*., 2013; Nowak *et al*., 2015). Algunas cepas bacterianas con mayor evidencia de su potencial probiótico pertenecen a los grupos de los lactobacilos y las bifidobacterias (Aleksandrova *et al*., 2017). Sin embargo, en lugar de una sola cepa bacteriana, se ha propuesto que el uso de una mezcla de cepas probióticas podría tener un efecto sinérgico y, en consecuencia, presentar un mayor impacto sobre la salud del individuo (Tejero-Sariñena *et al*., 2013; Wei *et al*., 2022). En este contexto, el uso de probióticos mixtos ya ha mostrado mejorar la severidad de los síntomas gastrointestinales en humanos (Aleksandrova *et al*., 2017) y prevenir la aparición del CCR en roedores (Appleyard *et al*., 2011; Wei *et al*., 2022).

5.4. Impacto del consumo de xenobióticos sobre la microbiota intestinal y mediadores inmunológicos

Compuestos como las AH, los HAP o las nitrosaminas son potenciales carcinógenos que pueden alterar la homeostasis intestinal (Nogacka *et al*., 2019). A pesar de que hay escasas evidencias respecto a su potencial efecto modulador sobre la microbiota, algunos autores han observado cambios en el perfil microbiano en función del nivel de consumo de estos compuestos (Ruiz-Saavedra *et al*., 2023).

Individuos con niveles de consumo más elevados de AH, como PhIP y MeIQx (≥40 ng/d y ≥50 ng/d, respectivamente), presentaron abundancias relativas reducidas de *Akkermansiaceae* en heces (Ruiz-Saavedra *et al*., 2023). La especie *Akkermansia muciniphila* se considera esencial para el mantenimiento de la función de la barrera intestinal, ya que mejora la integridad y la regeneración del epitelio *in vivo* (Wade *et al*., 2023). En consonancia, abundancias relativas reducidas de esta especie bacteriana se han asociado con un mayor estado inflamatorio (Earley *et al*., 2019) y su administración oral en un modelo animal, redujo el estado inflamatorio mediante la disminución de IL-6, incrementada tras la ingesta de PhIP (Sfanos *et al*., 2015), junto con TNF-α, y el aumento de IL-10 (Raftar *et al*., 2022). Por otro lado, individuos con ingestas de HAP elevadas (≥0,75 μg/d), presentaron abundancias relativas superiores de *Coriobacteriaceae* e inferiores de

Bacteroidaceae. Del mismo modo, se han encontrado abundancias más elevadas de *Coriobacteriaceae* en individuos con bajas ingestas de fibra y enfermedades inflamatorias (Ruiz-Saavedra *et al*., 2023), así como en ratones alimentados con dietas altas en grasa (Tang *et al*., 2024). En consonancia, en humanos, esta familia se ha asociado inversamente con los niveles circulantes de mediadores pro-inflamatorios como IL-6 (Martínez *et al*., 2013), aumentado junto con TNF-α o IL-8 en ratones tras la exposición a HAP (Rojas *et al*., 2022). En el caso de individuos con niveles de ingesta más elevados de nitritos y de NDMA (≥1,69 mg/d y ≥0,124 μg/d, respectivamente), presentaron una menor abundancia relativa de *Bifidobacteriaceae* (Ruiz-Saavedra *et al*., 2023). *In vitro,* miembros pertenecientes a *Bacteroides faecis* y *Bifidobacterium bifidum*, han mostrado mejorar la función de la barrera intestinal promoviendo su integridad, mediante la restauración de la expresión de las proteínas de unión, e *in vivo*, mejoran la respuesta anti-inflamatoria en el colon, mediante el incremento de IL-10 y la reducción de IL-17, TNF-α, IFN-γ o IL-22 entre otras (Al-Sadi *et al*., 2021; Mohebali *et al*., 2020, 2023). Por otro lado, la ingesta de nitratos y nitritos a partir de alimentos de origen animal en adultos se ha asociado con un mayor riesgo de presentar niveles elevados de metabolitos bacterianos como N-óxido de trimetilamina, compuestos con potencial pro-inflamatorio que inducen la producción de IL-1 e IL-18 (Mirzababaei *et al*., 2024).

A pesar de estas evidencias, se ha observado que la ingesta de xenobióticos en paralelo con otros compuestos con potencial protector, como la fibra, los polifenoles o los micronutrientes como la vitamina C o la vitamina D, pueden reducir el potencial carcinogénico de los mismos y ejercer un efecto protector sobre la mucosa del colon promoviendo la homeostasis intestinal, posiblemente a través de la modulación en la microbiota intestinal y parámetros inflamatorios, tal y como se muestra en la **Figura 8**. Por lo tanto, resultaría necesario analizar las posibles vías de modulación de la microbiota intestinal y de mediadores inmunológicos en este contexto para el diseño de estrategias dietéticas que contrarresten el efecto de los xenobióticos y promuevan la salud del individuo.

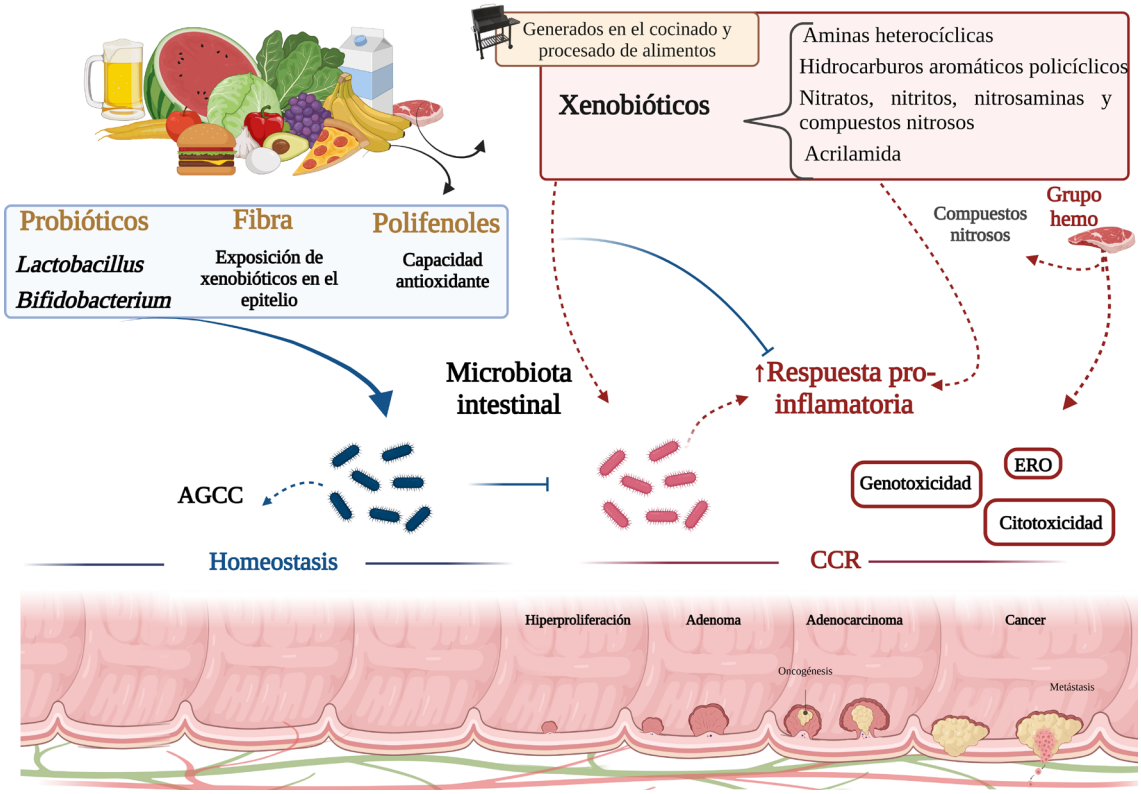


Figura 8. Posibles mecanismos de promoción e inhibición de la homeostasis intestinal mediados por la microbiota intestinal y mediadores inflamatorios, tras la ingesta de xenobióticos, probióticos, fibra y/o polifenoles. ERO, especies reactivas de oxígeno. Adaptado de Nogacka *et al.*, 2019. Creada con BioRender.com.

5.5. Evidencias del efecto protector de la dieta frente al daño en la mucosa del colon provocado por la ingesta de xenobióticos.

El epitelio colónico está expuesto a una mezcla compleja de compuestos dietéticos, entre los cuales algunos presentan un papel promotor y otros presentan un papel disruptor de la homeostasis intestinal (Nogacka *et al.*, 2019). A lo largo de esta introducción se han descrito los posibles mecanismos por los que algunos componentes de la dieta podrían desencadenar un efecto protector frente a la ingesta de xenobióticos a través de la modulación de la microbiota y mediadores inmunológicos. Sin embargo, el número de estudios de intervención en animales de experimentación realizado hasta la fecha es escaso. Estas intervenciones permitirían en primer lugar, mimetizar las condiciones carcinogénicas y, en segundo lugar, analizar el conjunto de interacciones y vías a través de las cuales los componentes dietéticos

pueden ejercer un efecto protector reduciendo el daño sobre la mucosa del colon provocado por la ingesta de xenobióticos. La información disponible hasta el momento en este contexto se muestra en la **Tabla 3**.

Entre los xenobióticos formados por el cocinado y procesado de los alimentos el B(a)P ha sido el más estudiado hasta el momento (Attia *et al*., 2023; He *et al*., 2019; Z. Wang *et al*., 2022). En dos intervenciones se mostró el efecto protector de la ingesta de polifenoles (Attia *et al*., 2023; He *et al*., 2019) a través de una reducción del daño sobre la mucosa del colon, en paralelo con cambios en la composición microbiana a través del incremento en la abundancia de géneros pertenecientes a la familia *Erysipelotrichaceae* (Attia *et al*., 2023; He *et al*., 2019), y la mejora del estado oxidativo a nivel del colon (He *et al*., 2019). De forma similar, un estudio mostró un efecto protector del selenio sobre el daño en el colon inducido por B(a)P (Z. Wang *et al*., 2022). En este caso, se observaron cambios en el perfil microbiano intestinal caracterizados por un aumento de bacterias pertenecientes a *Lachnospiraceae*, *Bacteroides* y *Prevotella*, junto con una diminución de la expresión de mediadores pro-inflamatorios como TNF-α, IL-1β y IL-18 (Z. Wang *et al*., 2022). Además, se observaron mejoras en el estado oxidativo y una menor activación de xenobióticos, en paralelo con una menor formación de aductos de ADN (Z. Wang *et al*., 2022).

A pesar de que PhIP es la AH que presenta los niveles de ingesta más elevados en la población (Carvalho *et al*., 2015; Le *et al*., 2016), no se han encontrado estudios de experimentación animal que evalúen el efecto protector de componentes dietéticos sobre el daño en el colon causado por este xenobiótico, la modulación de la microbiota intestinal y otros marcadores inflamatorios. No obstante, se evaluó el efecto protector de la ingesta de triterpenos, procedentes del hongo *Ganoderma lucidum*, en un modelo animal PhIP+DSS (100 ppm) (Sliva *et al*., 2012). Los autores observaron una reducción del acortamiento del colon junto con una menor hiperplasia y formación de criptas aberrantes y de tumores, una disminución de la infiltración de macrófagos y una menor expresión de genes metabolizadores de xenobióticos como CYP1A2 o CYP3A4 (Sliva *et al*., 2012). Finalmente, tampoco se han encontrado estudios en este contexto en el caso de las nitrosaminas que presentan un consumo más frecuente en la población. Sin embargo, el daño provocado por la ingesta de nitrosaminas minoritarias como N-

metil-n′-nitro-n-nitrosoguanidina (MNNG) se ha mostrado aminorado por el consumo de *Lacticaseibacillus rhamnosus* (anteriormente *Lactobacillus rhamnosus*) (10^{10} UFC/kg/d) (Gosai *et al*., 2011). En concreto, el probiótico produjo una mejora del epitelio intestinal con indicios de inflamación leve en la mucosa del colon, en paralelo con una mayor actividad antioxidante y una menor actividad de genes involucrados en la conversión de pro-carcinógenos en heces (Gosai *et al*., 2011).

A lo largo de la presente Tesis Doctoral se han recogido evidencias acerca de la influencia de la dieta sobre la modulación de la microbiota intestinal y marcadores sanguíneos asociados con la inflamación. El análisis de estos factores contribuiría a la elaboración de estrategias dietéticas personalizadas dirigidas a reducir el impacto del consumo de xenobióticos.

Tabla 3. Sinopsis de los ensayos de experimentación animal realizados hasta el momento, evaluando el efecto protector de la ingesta de componentes dietéticos sobre el daño provocado en la mucosa colónica tras la ingesta de xenobióticos, a través del análisis de las alteraciones de la microbiota intestinal y/o parámetros inflamatorios.

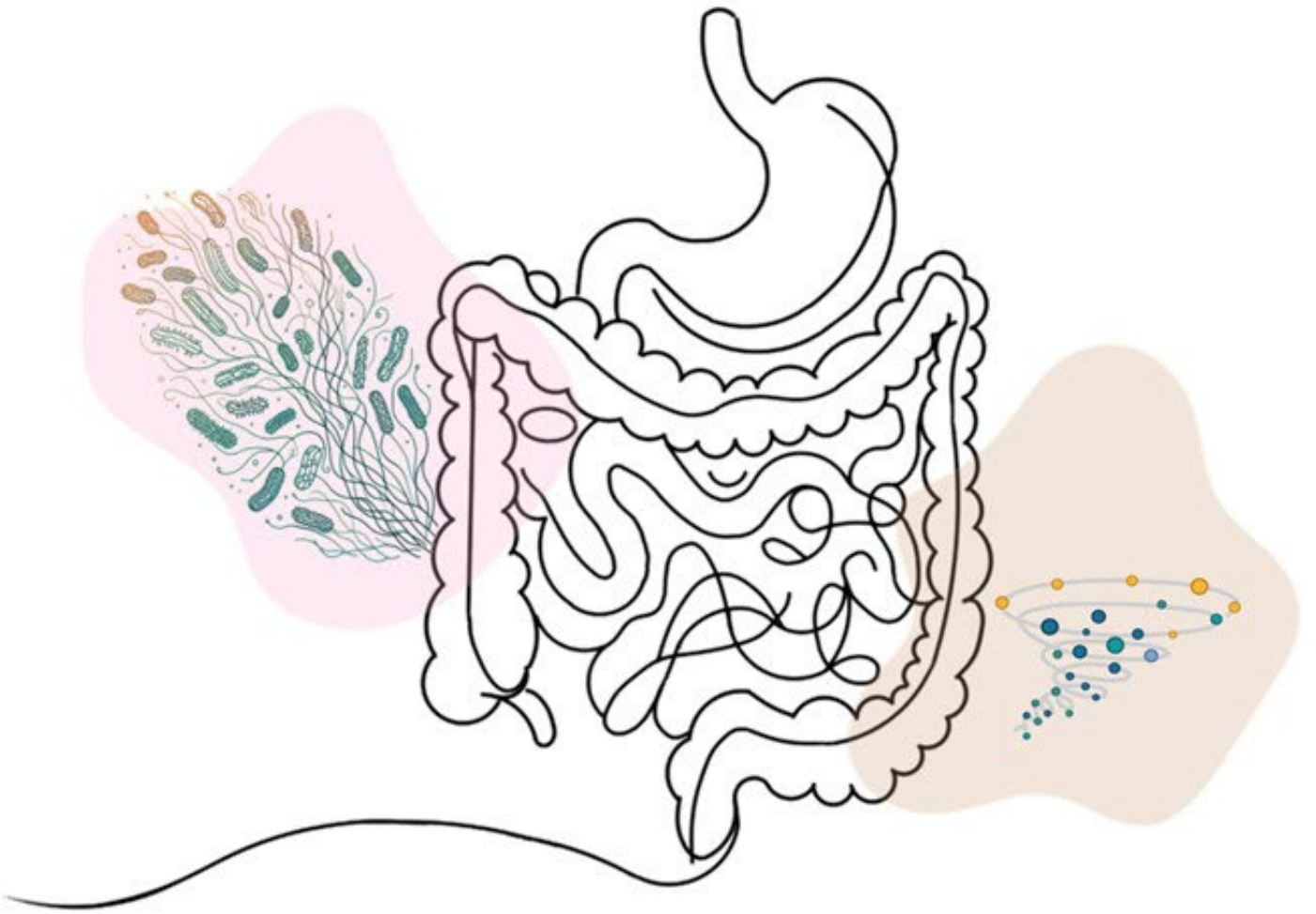
Xenobiótico	Potencial compuesto protector	Modelo animal (n) / Duración	Impacto del compuesto protector				Conclusión	Ref
			Histología/ Longitud del colon	Microbiota intestinal *(Abundancia relativa / Metabolitos en heces)*	Inflamación (colon)	Otros		
B(a)P								
150 µg/Kg/d	Tanino ácido gálico (50 mg ácido gálico equivalente /kg/d)	Rata ♂ Sprague–Dawley (n=12/grupo) 1 mes	↓Displasia en el epitelio de las criptas intestinales	↑*Erysipelotrichaceae* UCG003, *Acidaminococcaceae* y *Phascolartobacterium* ↓*Prevotellaceae* UCG003 ↑*Lactobacillaceae* y *Allobaculum* ↓Ácidos grasos y etilenglicol	↓Parcialmente la infiltración de células inflamatorias y congestión leve de vasos sanguíneos de la mucosa		Restauración parcial del daño en el colon	(Attia *et al.*, 2023)
50 mg/kg/d	Flavona isoorientina (20 mg/kg/d)	Ratón ♂ BALB/c (n=9/grupo) 1 mes y 12 días		↑*Faecalibaculum* ([a]), *Lactobacillus*, *Enterococcus*, *Blautia* ↓*Alloprevotella*, *Acinetobacter* ([a]), *Veillonella* ([a])		<u>Estrés oxidativo:</u> ↑Actividad antioxidante (T-SOD y GSH-PX). ↓Productos de oxidación (MDA y H_2O_2)	Atenuación del daño colónico y de las alteraciones en la composición microbiana	(He *et al.*, 2019)
50 mg/kg/d	Proteína de soja negra enriquecida en selenio (84 mg selenio /kg/d)	Ratón ♂ BALB/c (n=6/grupo) 1 mes	Mejora en la organización celular	↑*Lachnospiraceae bacterium*, *L. reuteri*, *Bacteroides thetaiotaomicron*, *Bacteroides caecimuris*, *Roseburia*, *Parabacteroides*, *Romboutsia* y *Prevotella* ↓*Akkermansia* ↓Ácido acético y etilenglicol	↓Infiltración de células inflamatorias en la mucosa ↓Expresión del inflamasoma NLRP3, TNF-α, IL-1β, IL-18, iNOS, COX-2	<u>Estrés oxidativo:</u> ↑Actividad antioxidante (GSH-Px y SOD) ↓Productos de oxidación (MDA y H_2O_2)	Atenuación del daño colónico y modulación de la composición microbiana	(Z. Wang *et al.*, 2022)

(Continuación Tabla 3)

					Metabolismo xenobióticos: ↓Expresión de AhR, CYP1A1, CYP1B1 y GST-P1 ↓Niveles de BPDE-ADN		
PhIP							
100 mg/kg/d + DSS (2%)	Triterpeno de hongo *Ganoderma lucidum* (0-500 mg de *G. lucidum* /kg/d)	Ratón ♂ ICR (CD-1) (n=10/grupo) — 4 meses	↓Hiperplasia focal, formación de criptas aberrantes y tumores ↓Acortamiento del colon	↓Infiltración de macrófagos	Metabolismo xenobióticos: ↓Expresión de CYP1A2, CYP3A4, ciclina D1 y COX-2	Alivia daño colónico a través de la modulación de genes asociados al metabolismo de xenobióticos	(Sliva *et al.*, 2012)
MNNG							
15 mg/kg/d	Probiótico *Lacticaseibacillus rhamnosus* 231 (10^{10} UFC/kg/d)	Rata ♂ Wistar (n=6/grupo) — 1 mes	Epitelio intestinal normal con inflamación muy leve	↓Inflamación (puntuación histológica de inflamación reducida a muy leve)	Estrés oxidativo: ↓Actividad GST y ↑GSH Metabolismo xenobióticos: ↓Actividad azo- y nitrorreductasa	Reduce la inflamación en paralelo con una mayor actividad antioxidante y menor oxidante	(Gosai *et al.*, 2011)

Se han seleccionado intervenciones en animales de experimentación en las que se considere: 1) aminas heterocíclicas, hidrocarburos aromáticos policíclicos, nitratos, nitritos, nitrosaminas o acrilamida; 2) el daño provocado sobre la mucosa del colon y, 3) las alteraciones de la microbiota intestinal (composición y/o metabolitos derivados) y/o parámetros inflamatorios. En la Tabla se muestra el efecto del factor dietético protector (en animales expuestos a xenobiótico) en comparación con el grupo control (sólo xenobiótico). ([a])Microorganismos significativamente modificados por el carcinógeno en los que el compuesto protector atenúa las alteraciones. Ahr, receptor de hidrocarburos de arilos; B(a)P, benzo(a)pireno; BPDE, benzo(a)pireno diol epóxido; COX-2, ciclooxigenasa-2; CYP, citocromo P450; DSS, sal sódica de sulfato de dextrano; GSH, glutatión reductasa; GSH-Px, glutatión peroxidasa; GST, glutatión S-transferasa; GST-P1, glutatión S-transferasa P; iNOS, óxido nítrico sintasa; MDA, malondialdehido; MNNG, N-metil-n'-nitro-n-nitrosoguanidina; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona; SOD, superóxido dismutasa; T-SOD, superóxido dismutasa total; TNF-α, factor de necrosis tumoral-α.

HIPÓTESIS Y OBJETIVOS
HYPOTHESIS & OBJETIVES



En este contexto, la **HIPÓTESIS** de esta Tesis Doctoral ha sido:

Algunos componentes dietéticos tienen capacidad para contrarrestar el daño provocado por la ingesta de xenobióticos, formados con el cocinado y procesado de los alimentos, en la mucosa del colon mediante la modulación de la composición microbiana y del sistema inmunitario.

A partir de esta hipótesis, el **OBJETIVO GENERAL** propuesto en esta Tesis Doctoral ha sido:

Identificar patrones dietéticos o componentes alimentarios con potencial para contrarrestar el efecto de la ingesta de compuestos dietéticos con potencial carcinogénico sobre el mantenimiento de la homeostasis intestinal a través de la modulación de la microbiota intestinal y el sistema inmune.

Para la consecución de este objetivo general, se han planteado los siguientes objetivos parciales:

Objetivo 1. Identificar los carcinógenos derivados del cocinado y procesado de los alimentos con un nivel de consumo superior a los umbrales descritos en la literatura.

1.1. Desarrollar y validar una herramienta dietética para la estimación de la ingesta de xenobióticos con potencial carcinogénico.

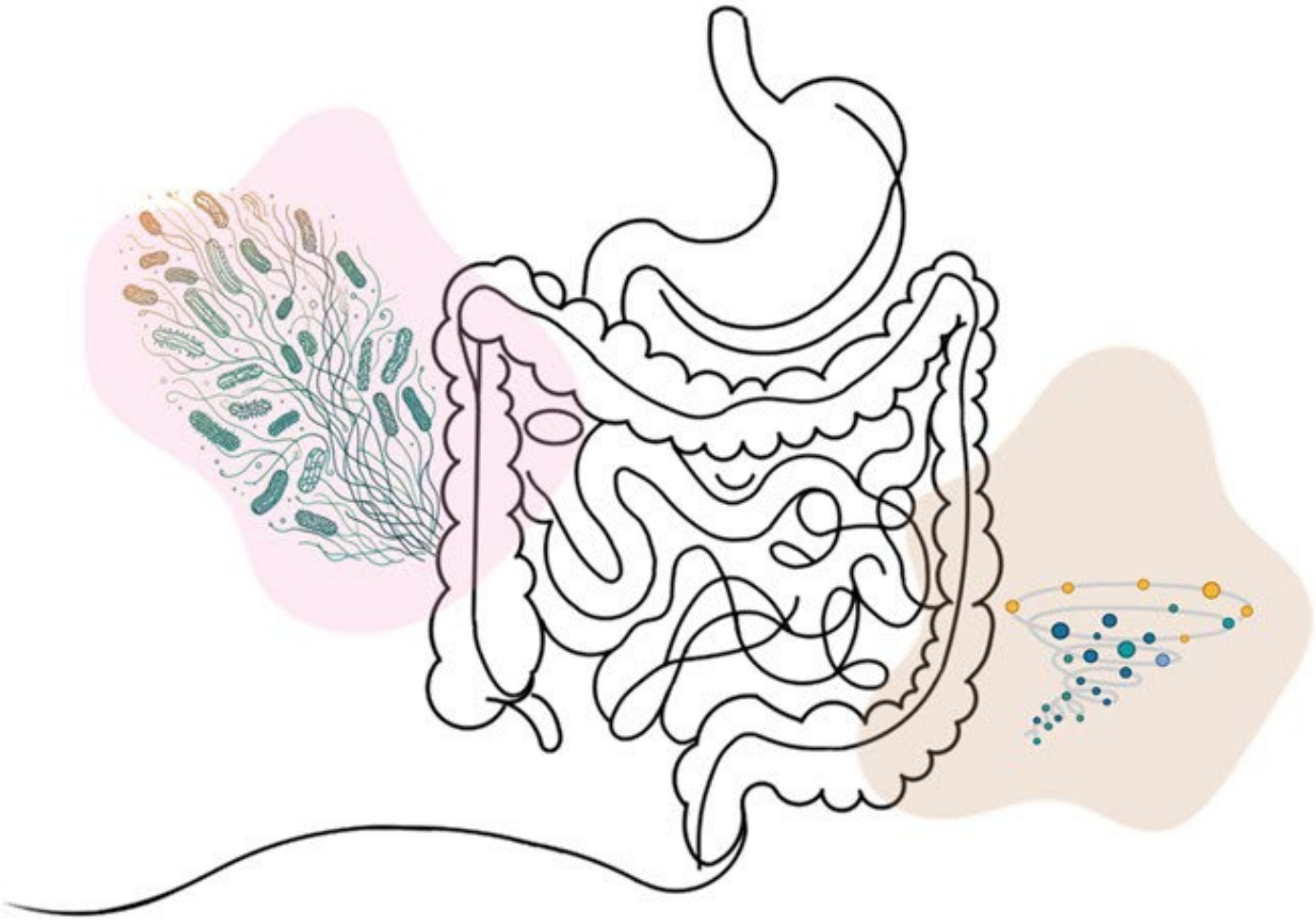
1.2. Analizar el nivel de consumo de xenobióticos en dos muestras poblacionales de la región.

Objetivo 2. Describir las asociaciones entre el consumo de componentes con potencial carcinogénico o bioactivo y la microbiota intestinal o el sistema inmune.

Objetivo 3. Evaluar el impacto de la Dieta Mediterránea sobre la microbiota intestinal, el sistema inmune y marcadores biológicos relacionados con el estado de salud.

Objetivo 4. Evaluar el potencial de la ingesta de prebióticos y probióticos como agentes protectores del daño en la mucosa del colon producido por el consumo de xenobióticos a través de la modulación de la microbiota y el sistema inmune.

RESULTADOS
RESULTS



En base al objetivo general de la presente Tesis Doctoral, se ha seguido la metodología ilustrada brevemente en la **Figura 9**.

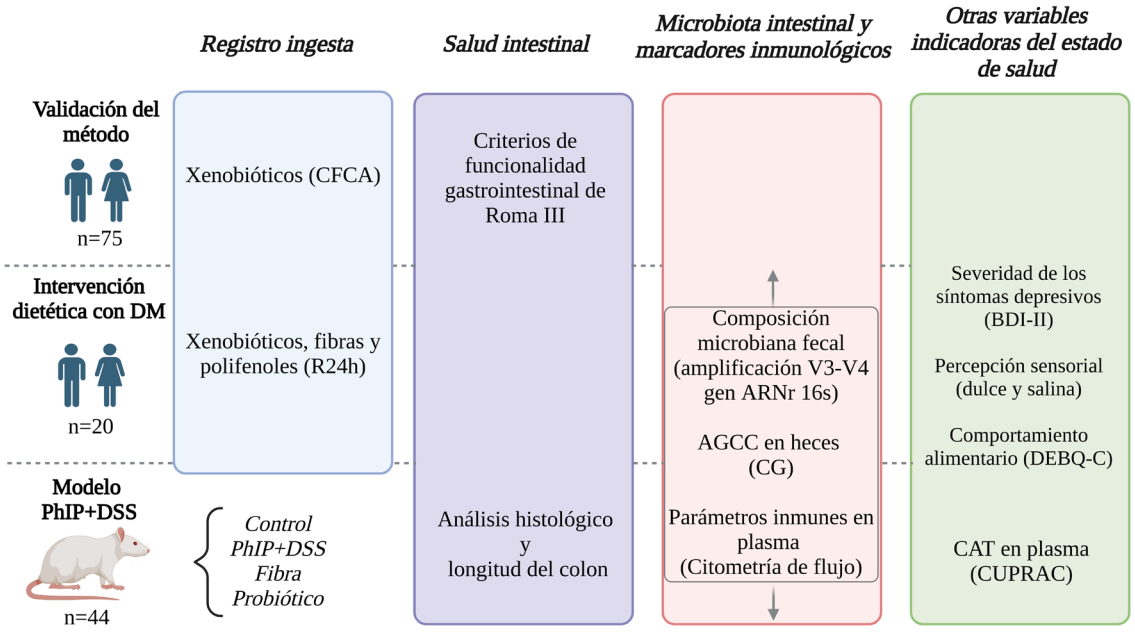


Figura 9. Breve descripción metodológica de cada muestra de estudio. AGCC, ácidos grasos de cadena corta; ARNr, ARN ribosómico; BDI-II, inventario de depresión de Beck-II; CAT, capacidad antioxidante total; CFCA, cuestionario de frecuencia de consumo de alimentos; CG, cromatografía de gases; CUPRAC, capacidad antioxidante reductora del ion cúprico; DEBQ-C, cuestionario holandés de comportamiento alimentario infantil; DM, dieta mediterránea; DSS, sal sódica de sulfato de dextrano; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona; R24h, recordatorio dietético de 24 horas.

Para la consecución de cada objetivo parcial, a continuación, se detalla brevemente la metodología empleada y los principales resultados obtenidos.

Objetivo 1

Identificar los carcinógenos derivados del cocinado y procesado de los alimentos con un nivel de consumo superior a los umbrales descritos en la literatura.

La dieta es una fuente de exposición a compuestos potencialmente perjudiciales para la salud intestinal. Existen evidencias de que los xenobióticos, formados durante el cocinado y el procesado de algunos alimentos como las carnes, presentan potencial carcinogénico y alteran la homeostasis intestinal. Para mejorar el conocimiento de su efecto sobre la salud, resulta necesario el desarrollo y la validación de herramientas harmonizadas que consideren, de forma detallada, los hábitos de cocinado y que estén específicamente diseñadas para la estimación del consumo de estos compuestos. Esto permitiría cuantificar con mayor precisión los niveles de ingesta, analizar su impacto sobre la salud y descifrar los mecanismos de modulación de la microbiota intestinal, con el fin último de establecer recomendaciones nutricionales específicas. El trabajo desarrollado en relación con los Objetivos 1.1 y 1.2 se recoge en los siguientes Artículos científicos:

Artículo 1: Zapico A., Ruiz-Saavedra S., Gómez-Martín M., de Los Reyes-Gavilán C.G., González S. (2002). Pilot study for the dietary assessment of xenobiotics derived from food processing in an adult Spanish sample. *Foods*, *11*(3), 470–489. doi: 10.3390/foods11030470.

Artículo 2: Zapico A., Arboleya S., Ruiz-Saavedra S., Gómez-Martín M., Salazar N., Nogacka A.M., Gueimonde M., de Los Reyes-Gavilán C.G., González S. (2022). Dietary xenobiotics, (poly)phenols and fibers: exploring associations with gut microbiota in socially vulnerable individuals. *Frontiers in Nutrition*, *9*, 1000829–1000835. https://doi.org/10.3389/fnut.2022.1000829

En el **Artículo 1** se describe el desarrollo y la validación de un instrumento de recogida de la información dietética específico para la estimación de la ingesta de xenobióticos en una muestra poblacional. El CFCA desarrollado incorporó imágenes para el registro del tamaño de ración y la estandarización del grado de cocinado (muy hecho, bien hecho o poco hecho), además de información relativa a los hábitos de

cocinado (por ejemplo, el consumo de alimentos con o sin piel o su cocinado con o sin piel) y las técnicas utilizadas (cocido, plancha, frito, horno o barbacoa). Este cuestionario fue cumplimentado por 70 adultos sin patologías digestivas declaradas y validado posteriormente a través de un R24h. Adicionalmente, en los **Artículo 1** y **Artículo 2**, se describió, en diferentes muestras de estudio, la ingesta de xenobióticos tales como AH, HAP, nitratos, nitritos, NOC y acrilamida, mediante la recopilación de referencias europeas y americanas como las del EPIC (Jakszyn *et al*., 2004), la EFSA (EFSA, 2008, 2012) y, la FDA (FDA, 2015) entre otras (Campillo *et al*., 2011; De Mey *et al*., 2014; Falcó *et al*., 2003; Hellenäs *et al*., 2013; Konings *et al*., 2003; Lee, 2019; NIH, 2006; Palacios-Colón *et al*., 2022; Park *et al*., 2015; Svensson *et al*., 2003).

En el **Artículo 1** y la **Tabla Suplementaria 1.1** se muestran los resultados obtenidos en la validación del método en el que participó el 56% de la muestra inicial. Por primera vez, se han obtenido correlaciones de Spearman significativas en el 64% de los xenobióticos evaluados con índices de correlación entre 0,2 y 0,8 (**Tabla Suplementaria 1.1**). En relación con los niveles de consumo y las fuentes dietéticas, en adultos sin patologías conocidas (**Artículo 1)**, las AH procedían principalmente del consumo de carne "a la plancha" y el PhIP fue el xenobiótico que presentaba los niveles de ingesta más elevados en la muestra de estudio (188 ng/d). En el caso de los HAP, el B(a)P (0,03 μg/d) provenía de la ingesta de diversos alimentos, como aceites y grasas, los nitritos (3,14 mg/d) y los NOC, como NDMA (0,17 μg/d), NPIP (0,09 μg/d) o NPYR (0,15 μg/d), procedían del consumo de jamón cocido y jamón serrano; y la acrilamida (15,12 μg/d) de patata.

Por otro lado, en individuos en riesgo de inseguridad alimentaria (**Artículo 2**), las AH procedían, principalmente, del consumo de pollo y PhIP presentó los niveles de ingesta más elevados (0-299 ng/d). El consumo de HAP, como el B(a)P (0,02-0,06 μg/d), provenía de aceite de girasol, mientras que la ingesta de nitritos (0-1 mg/d) y de NOC, como NDMA (0,02-0,09 μg/d), NPIP (0,01-0,05 μg/d) o NPYR (0,01-0,06 μg/d), derivaban del consumo de carnes procesadas como jamón cocido y chorizo. Por último, la ingesta de acrilamida (6-12 μg/d) procedía principalmente de galletas.

ARTÍCULO 1

foods

MDPI

Article

Pilot Study for the Dietary Assessment of Xenobiotics Derived from Food Processing in an Adult Spanish Sample

Aida Zapico [1,2], Sergio Ruiz-Saavedra [1,2], María Gómez-Martín [1,2], Clara G. de los Reyes-Gavilán [2,3] and Sonia González [1,2,*]

1 Department of Functional Biology, University of Oviedo, 33006 Oviedo, Spain; aiida.zapiico@gmail.com (A.Z.); ruizssergio@uniovi.es (S.R.-S.); gomezmarmaria@uniovi.es (M.G.-M.)
2 Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), 33011 Oviedo, Spain; greyes_gavilan@ipla.csic.es
3 Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), 33300 Villaviciosa, Spain
* Correspondence: soniagsolares@uniovi.es; Tel.: +34-985-104-209

Abstract: Background: Although xenobiotics from food processing have gained support as possible drivers of the relationship between diet and some types of cancer, there are still few studies characterizing the intake of these compounds among different populations. Aim: To describe the intake of heterocyclic amines (HAs), polycyclic aromatic hydrocarbons (PAHs), nitrates, nitrites, nitrosamines, and acrylamide; and to identify dietary and lifestyle related factors.Methods: This was a descriptive cross-sectional study in 70 adult volunteers. Intake was registered by means of a food frequency questionnaire, including cooking methods, temperature, and degree of browning. The European Prospective Investigation into Cancer (EPIC) and the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) databases were used for xenobiotic estimation in conjunction with data from the European Food Safety Authority (EFSA) and U.S. Food and Drug Administration (FDA). Results: Dietary HAs (amino-alpha-carboline (AαC), 2-amino-3-methylimidazo (4,5,f) quinoline (IQ), 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline (MeIQx), 2-amino-3,4,8 trime-thylimidazo (4,5,f) quinoxaline (DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine (PhIP)) were mainly derived from meat and meat products, while benzo (a) pyrene (B(a)P), dibenzo (a) anthracene (DiB(a)A), and total PAHs were explained by oils and fats, alcoholic beverages, and milk, respectively. Microwaved, fried, grilled, broiled, barbecued, and braised cooking methods were mainly responsible for HAs and PAHs consumption. Conclusion: Based on the wide presence and levels of intake of these compounds in different sources, more efforts should be made to adjust their intake to the levels recommended by health agencies.

Keywords: diet; xenobiotics; heterocyclic amines; polycyclic aromatic hydrocarbons; nitrosamines; acrylamide; gastrointestinal health

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1. Introduction

Solid evidence supports the importance of environmental factors, with special focus on diet, in the development of different types of cancer [1,2]. Several hypotheses have been postulated to elucidate the possible mechanisms of this association. In general terms, red and processed meats have been identified as risk factors for cancer, contrary to what has been considered for plant-based foods, which seem to exhibit a protective effect [1–3]. Based on existing scientific evidence, the International Agency for Research on Cancer (IARC) evaluated red meat as "probably carcinogenic to humans" (Group 2A) and processed meat as "carcinogenic to humans" (Group 1) in 2015 [4]. However, in addition to the nutritional and bioactive compounds contained in these foods, different chemical substances could be incorporated as a consequence of the cooking, preservation, and processing performed to improve their digestibility, palatability, and safety [2]. From them, heterocyclic amines

(HAs) and polycyclic aromatic hydrocarbons (PAHs), which are not naturally present in foodstuffs, but are formed during high-temperature cooking of foods, have been targeted as mediators of this relationship along with nitrites and nitrates, which are commonly used as food additives [1,5–9]. HAs are formed from muscle creatine and/or creatinine, sugars, and amino acids by the Maillard reaction [5]. Even though these elements are present at elevated levels in meat and fish muscle, the major dietary source is usually meat and meat products [10]. Another targeted compound, classified as a probable carcinogen to humans (Group 2A) by the IARC, is the acrylamide generated by the Maillard reaction in baked or fried carbohydrate-rich food sources, such as potatoes or cereals, by the condensation reaction between reducing sugars (glucose or fructose) and free amino acids (i.e., asparagine) [11,12].

Finally, PAHs are not usually present in raw foods, but have been reported in foods from industrialized areas as a result of the atmospheric contamination, exposure to which these products are subjected [13]. High levels of PAHs have been found in smoked products and grilled meats [5,7,14], formed by pyrolysis processes of organic matter at high temperatures; i.e., by direct contact of lipids with a flame or heat source, from smoke produced in cooking, or by incomplete combustion of wood or charcoal during the cooking process [14]. Once formed, these compounds are deposited on the meat being cooked [7].

All these chemical compounds with possible carcinogenic activity for humans are grouped under the denomination of xenobiotics; i.e., foreign substances that are not produced or are not found in the composition of the living organism [4]. In interpreting the scientific evidence between xenobiotics resulting from food processing and gastrointestinal health, several aspects should be considered; the risk of chronic dietary exposure to potentially carcinogenic compounds depends on the dose, frequencies, and combinations of xenobiotics being taken; the times of exposure to each compound, and the individual genetic susceptibilities. Then, differences in the way of recovering information, such as those regarding cooking questionnaires, the reference period of reported intake, and the use of different food composition databases, may at least partly explain the lack of consensus among studies [15,16]. Although no harmonized methods have been found in the literature, at the European level [17], when evaluating the dietary exposure to xenobiotics, the accurate assessment of individual food consumption is essential. The development of improved Food Frequency Questionnaires (FFQs) including cooking method, degree of doneness, and browning, apart from the traditional questions about food type, amount, and frequency of consumption, is necessary for nutritional assessment. Given that the intake of dietary xenobiotics may have an important impact on human health, our aim in the present work was to quantify their uptake in the population in order to define potential therapeutic targets, as well as to identify associations with other dietary components with which they may interact, increasing or reducing their genotoxic potential. This information could be useful to provide the basis for a more holistic view on the relationship between diet and health in the future.

2. Subjects and Methods

2.1. Sample Recruitment and Study Design

Recruitment of the sample was carried out by the nutrition group at the University of Oviedo by contacting individuals enrolled in the first semester of 2020/2021 at the University Program for Older Adults of the University of Oviedo (PUMUO) (n = 75). Eligibility criteria were to be over 50 years of age and not having been diagnosed with any digestive disorders. Those individuals interested in participating were informed of the objectives of the study and signed an informed consent form. Once the data were analyzed, all those who reported the existence of a major health condition (with the most frequent being cancer, Parkinson's disease, or irritable bowel disease) or outlier daily intakes (energy intake below 1000 kcal/day or above 4000 kcal/day) were excluded.

This project was evaluated and approved by the Regional Ethics Committee of Clinical Research of Asturias (Ref. 163/19) and by the Committee on Bioethics of CSIC (Ref. 174/2020).

The procedures were performed in accordance with the fundamental principles set out in the Declaration of Helsinki, the Oviedo Bioethics Convention, and the Council of Europe Convention on Human Rights and Biomedicine, as well as in Spanish legislation on bioethics. Directive 95/46/EC of the European Parliament and the Council of 24 October 1995, on the protection of individuals regarding the processing of personal data and on the free movement of such data, was strictly followed.

2.2. General Characteristics and Food Frequency Questionnaire (FFQ)

General characteristics of the questionnaire included information on age, nationality, gender, weight, height, educational level, and economic income, as well as questions related to lifestyle, physical activity, and gastrointestinal health, among others.

The auto-administered FFQ was constructed with 155 items and required an estimated duration of 30–45 min to be filled out. In addition to food and culinary preparations, the specific type of food was recorded, as well as cooking methods and other related questions, when necessary. For each food, the frequency of intake and portion size were registered by means of a validated photograph album adapted from the Pilot Study for Assessment of Nutrient intake and Food Consumption Among Kids in Europe (PANCAKE) study [18]. A specific section about cooking habits (boiled, fried, grilled, baked/broiled, or barbecued) and the degree of cooking or toasting in the case of meats, fried potatoes, or toasted bread (undercooked, medium, well-done, very well-done) were included in the FFQ. To standardize this point, photographs of the different temperatures, in which the degree of browning increased progressively, were developed specifically for this study: low, medium, well-done or very well-done were incorporated. Additionally, complementary questions such as which part of the food was consumed (breast or thigh in the case of chicken) or the possible consumption and/or cooking of the skin (cooking with skin and eating the skin; cooking with skin, but not consuming it; and cooking without skin) were incorporated in order to improve the quality of the information.

A 24 h dietary recall (R24h) was used to record the intake of each individual over the course of a day, as a method of validation of the FFQ, in a total of 39 participants. For this purpose, a survey was designed consisting of 14 questions in which the participant was asked to record in as much detail as possible everything consumed for breakfast, lunch, afternoon snack, and dinner. They were asked to specify the ingredients used in each preparation; the size of the portion; the type of food, if applicable; the possible accompaniment with drink or bread; the way the food was cooked; the cooking of the meats with or without skin; and the possible subsequent intake of the skin. Finally, the degree of toastiness of bread, French fries, and meat was collected by means of visual images. Spearman correlation analyses were conducted between the information obtained throughout the FFQ and R24h for the intake of the main xenobiotic compound; 71% of the xenobiotics studied showed significant Spearman correlations, ranging from r = 0.20 (2-amino-3,4 dimethylimidazo (4,5,f) quinoline ((MeIQ)) to r = 0.75 (combined nitroso compounds (Comb.)) ($p < 0.05$; data not shown). These values have been previously considered acceptable in the literature [19,20].

Body mass index (BMI) was calculated using the formula: weight (kg)/height $(m)^2$. Subjects were classified in normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese ($\geq$30.0 kg/m^2), based on the Spanish Society for the Study of Obesity (SEEDO) criteria [21].

2.3. Xenobiotic Estimation and Nutritional Analyses

Based on food consumption per individual, cooking method, cooking time, and degree of browning, the nutritional analysis of the sample was carried out. For this purpose, information on the consumption of HAs, PAHs, nitrates, and nitrites was obtained mainly from the European Prospective Investigation into Cancer and Nutrition (EPIC) Carcinogen Database [22]. The EPIC database compiles information obtained from 139 references regarding the content per 100 g of food in nitrosamines, HAs, PAHs, nitrites, and nitrates

in more than 200 food items. The food composition table is classified according to the preservation method, cooking method, degree of browning, and temperature [22]. This information was provided by the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) database for those foods or culinary preparations not included in the EPIC database [23]. Acrylamide content was provided by the European Food Safety Authority (EFSA) categorization of European food products for monitoring purposes [24] and U.S. Food and Drug Administration (FDA) composition tables [25]. For each compound, the foods that accounted for at least 80% of its total intake were identified. The classification of the food groups was carried out according to the classification into 18 food groups of the Centre for Higher Education in Nutrition and Dietetics (CESNID) food composition tables [26]. For the meat and meat derivatives group, the IARC definition was used to break down the red meat and processed meat groups [4].

The analysis was completed with the CESNID [26] and the United States Department of Agriculture (USDA) food composition tables [27]. The polyphenol content of the foods was extracted from the Phenol Explorer (PHEX) database [28], and fiber content from the tables of Marlett and Cheung [29].

2.4. Digestive Function Self-Assessment Questionnaire

This questionnaire included some of the broader Rome III Criteria gastrointestinal functionality symptoms [30]. The 12 variables selected were: stomach pain, belching or reflux, heartburn, bloating, flatulencies, unpleasant taste in the mouth, nausea, bad breath, loss of appetite, abdominal pain, chest discomfort at night, and abdominal distention. Both the presence and intensity (from never to mild, moderate, severe, or very severe) of each of these symptoms were evaluated by the participant. The results obtained for each individual were represented by the percentage of symptoms presenting each of the intensities.

2.5. Statistical Analyses

Results were analyzed using the IBM SPSS software version 25.0 (IBM SPSS, Inc., Chicago, IL, USA) and RStudio software version 1.4.1103. Goodness of fit to the normal distribution was checked by means of the Kolmogorov–Smirnov test. As normality of the variables was not achieved, nonparametric tests were used. Overall, categorical variables were summarized as percentages and continuous ones using mean and standard deviations. *T*-test and Chi-squared analyses were performed for continuous and categorical variables, respectively (p-value < 0.05) with a Bonferroni correction. To deeper explore the associations between xenobiotics and dietary components, Spearman correlation analyses were conducted. A heatmap was generated using the RStudio software version 1.4.1103 package corrplot. GraphPad Prism 8 was used for graphical representations.

3. Results

3.1. Description of the Sample

A general description of general and health-related parameters is shown in Table 1. The sample had a mean age of 59 years with a BMI of 27 kg/m^2, indicative of overweight. Concerning health-related parameters, most of the sample did not have a previous history of first- or second-degree colorectal cancer (CRC), and only around a 17% had asthma and/or allergies or hypertension, and 9% had diabetes. In relation to intestinal disorders (diarrhea, constipation, hemorrhoids, fissures, and fistulas or abscesses), statistically significant differences were detected according to gender in the percentage of hemorrhoids (higher in women) and in the absence of intestinal pathology (higher in men). The average stool frequency was once a day, and stool consistency was normal in most cases (60%). In addition, the proportion of individuals reporting the presence of bleeding (36%) was notable, albeit occasional (96%). The self-assessment of gastrointestinal functionality, adapted from the Rome III Criteria, showed that most subjects presented a moderate level of symptoms and an acceptable gastrointestinal health status.

Table 1. General characteristics and description of gastrointestinal functionality, adapted from the Rome III Questionnaire, by gender.

Characteristics	Total (*N* = 70)	Gender	
		Male (*N* = 25)	Female (*N* = 45)
Age (years)	59 ± 12	62 ± 7	57 ± 14
<57	24 (34%)	5 (20%)	19 (42%)
57–65	18 (26%)	9 (36%)	9 (20%)
>66	28 (40%)	11 (44%)	17 (38%)
Energy intake (kcal/day)	1885.87 ± 581.71	1935.28 ± 569.40	1858.42 ± 593.01
Weight (kg)	74.70 ± 16.02	84.48 ± 16.84	69.15 ± 12.67 *
Height (m)	1.66 ± 0.08	1.74 ± 0.07	1.62 ± 0.05 *
BMI (kg/m^2)	26.90 ± 4.64	27.77 ± 4.67	26.41 ± 4.60
Normal weight (18.5–24.9)	25 (36%)	7 (28%)	18 (40%)
Overweight (25.0–29.9)	32 (46%)	13 (52%)	19 (42%)
Obese (≥30.0)	12 (17%)	5 (20%)	7 (16%)
Na	1 (1%)	0 (0%)	1 (2%)
Smoking status			
Current smoker	7 (10%)	3 (12%)	4 (9%)
Former smoker	27 (39%)	14 (56%)	13 (29%) *
Never smoker	36 (51%)	8 (32%)	28 (62%) *
Exercise (hours/week)	1.13 ± 1.93	1.80 ± 2.20	0.76 ± 1.68 *
Sleeping (hours/day)	6.93 ± 1.11	6.80 ± 1.08	7.00 ± 1.13
Family CRC history			
Presence	11 (16%)	5 (20%)	6 (13%)
Absence	52 (74%)	18 (72%)	34 (76%)
Na	6 (9%)	2 (8%)	4 (9%)
Previous pathologies			
Hypertension	11 (16%)	7 (28%)	4 (9%) *
Diabetes	6 (9%)	3 (12%)	3 (7%)
Obesity	28 (40%)	12 (48%)	16 (36%)
Asthma and/or allergies	12 (17%)	4 (16%)	8 (18%)
None	14 (20%)	4 (16%)	10 (22%)
Intestinal pathologies			
Diarrhea	1 (1%)	0 (0%)	1 (2%)
Constipation	9 (13%)	1 (4%)	8 (18%)
Hemorrhoids	29 (41%)	6 (24%)	23 (51%) *
Fissures	2 (3%)	1 (4%)	1 (2%)
None	30 (43%)	17 (68%)	13 (29%) *
Bleeding frequency			
Daily	1 (1%)	1 (4%)	0 (0%)
At least once a week	0 (0%)	0 (0%)	0 (0%)
Occasionally	24 (34%)	8 (32%)	16 (36%)
Never	45 (64%)	16 (64%)	29 (64%)
Rome III Criteria			
No discomfort	49 ± 28	50 ± 24	49 ± 30
Mild discomfort	31 ± 21	33 ± 19	29 ± 22
Moderate discomfort	11 ± 12	8 ± 10	12 ± 14
Severe discomfort	1 ± 7	1 ± 2	2 ± 9
Very severe discomfort	1 ± 8	0 ± 2	2 ± 10
Na	7 ± 20	8 ± 23	7 ± 19
Stool frequency [a]	7 ± 2	7 ± 2	7 ± 3
Stool consistency			
Liquid	0 (0%)	0 (0%)	0 (0%)
Soft	42 (60%)	15 (60%)	27 (60%)
Hard	27 (39%)	10 (40%)	17 (38%)

Values are presented as mean ± standard deviation or number of the subjects and percentage (%). CRC, colorectal cancer; Na, not available. ([a]) Number of depositions per week; (*) significant differences were found according to gender (p-value < 0.05).

The variation in the average daily intake of the different food groups according to gender is presented in Table 2. A higher intake of potatoes and tubers, alcoholic beverages, and other foods was observed in men.

Table 2. Differences in the intake of the major food groups in the study sample, by gender.

Food Groups Intake (g/Day)	Total (*N* = 70)	Gender	
		Male (*N* = 25)	Female (*N* = 45)
Cereals and cereals products	195.09 ± 138.37	185.08 ± 106.56	200.66 ± 154.09
Whole grain cereals	57.69 ± 118.62	23.31 ± 41.01	76.78 ± 141.78
Milk and dairy products	392.43 ± 236.26	323.14 ± 216.16	425.92 ± 242.56
Meat and meat products	147.47 ± 89.62	146.89 ± 72.32	147.79 ± 98.70
White meat	48.77 ± 37.88	48.05 ± 39.05	49.16 ± 37.66
Red meat	42.17 ± 30.04	47.13 ± 33.94	39.42 ± 27.66
Processed meat	58.90 ± 52.99	54.00 ± 28.47	61.62 ± 62.77
Eggs	43.51 ± 29.53	49.23 ± 33.74	40.33 ± 26.79
Fish	61.83 ± 36.99	63.46 ± 30.00	60.93 ± 40.66
Seafood	22.82 ± 19.64	22.92 ± 19.16	22.77 ± 20.12
Oils and fats	16.18 ± 8.57	18.05 ± 9.09	15.15 ± 8.19
Vegetables	308.53 ± 179.13	262.94 ± 153.23	333.86 ± 188.88
Legumes	42.61 ± 76.11	49.79 ± 77.89	38.62 ± 75.70
Potatoes and tubers	50.38 ± 31.75	60.50 ± 32.11	44.76 ± 30.46 *
Fruits	130.68 ± 90.87	156.27 ± 126.20	116.47 ± 60.69
Nuts and seeds	13.29 ± 17.60	9.12 ± 9.00	15.61 ± 20.65
Sugar and sweets	7.45 ± 10.11	9.93 ± 12.44	6.07 ± 8.39
Snacks	2.09 ± 4.45	3.16 ± 4.55	1.49 ± 4.32
Sauces and condiments	8.17 ± 7.17	8.04 ± 5.25	8.24 ± 8.10
Other foods	10.20 ± 14.37	14.84 ± 19.64	7.62 ± 9.72 *
Nonalcoholic beverages (mL/day)	225.86 ± 231.79	283.30 ± 325.24	193.96 ± 153.74
Alcoholic beverages (mL/day)	133.42 ± 171.11	191.02 ± 175.93	101.42 ± 161.55 *

Values are presented as mean ± standard deviation. (*) Significant differences were found between genders (p-value < 0.05).

3.2. Xenobiotics: Doses and Dietary Origin

With respect to the consumption of xenobiotics in the sample, no gender-specific statistically significant differences were found for any of the xenobiotic compounds considered (Supplementary Table S1). The average intake values for HAs, hydrocarbons, and acrylamide were within the range reported for the main sources of carcinogens, as can be seen in Table 3 [31–37].

Table 3. Comparison between mean xenobiotic intake in the study sample with other studies using EPIC and CHARRED databases.

Xenobiotics	Value (*N* = 70)	Type of Study					
		Reference Value	Sample Size (Gender)	Age (Years)	Health Status	Country	Reference
Heterocyclic amines (ng/day)							
MeIQx	29.48 ± 27.85	16.8 (±29.7)	*n* = 3.699 (MF)	35–65	Healthy	DE	[31] [a]
		102.7	*n* = 561 (MF)	>20	Na	BR	[32] [b]
DiMeIQx	8.18 ± 7.96	3.0 (±4.5)	*n* = 3.699 (MF)	35–65	Healthy	DE	[31] [a]
		9.8	*n* = 561 (MF)	>20	Na	BR	[32] [b]
PhIP	187.59 ± 257.04	41.0 (±117.5)	*n* = 3.699 (MF)	35–65	Healthy	DE	[31] [a]
		324.3	*n* = 561 (MF)	>20	Na	BR	[32] [b]

Table 3. *Cont.*

Xenobiotics	Value (N = 70)	Type of Study					
		Reference Value	Sample Size (Gender)	Age (Years)	Health Status	Country	Reference
Total HAs	226.99 ± 285.50	69.4 436.8	n = 21.462 (MF) n = 561 (MF)	35–65 >20	Na Na	DE BR	[33] [a] [32] [b]
Polycyclic aromatic hydrocarbons (µg/day)							
B(a)P	0.03 ± 0.03	0.14 (±0.07)	n = 40.690 (MF)	35–64	Na	SP	[34] [a]
DiB(a)A	0.07 ± 0.10	0.06	n = 3.890.240 (M)	20–65	Na	SP	[35] [c]
Total PAHs	5.04 ± 3.84	8.57 (±2.69)	n = 40.690 (MF)	35–64	Na	SP	[34] [a]
Nitrates, nitrites, and nitroso compounds							
Nitrites (mg/day)	3.14 ± 2.90	1.48 (±0.51)	n = 20.095 (MF)	40–79	Healthy	UK	[36] [a]
NDMA (µg/day)	0.17 ± 0.14	0.06 (±0.05)	n = 20.095 (MF)	40–79	Healthy	UK	[36] [a]
NPIP (µg/day)	0.09 ± 0.09						
NPYR (µg/day)	0.15 ± 0.16	72.3 (±19.2) [d]	n = 20.095 (MF)	40–79	Healthy	UK	[36] [a]
Comb. (ng/day)	1.71 ± 5.10						
Acrylamide (µg/day)	15.12 ± 11.60	20.6 (±12.1)	n = 22.783 (F)	29–69	Cases & healthy	SP	[37] [a]

Values are presented as mean ± standard deviation. ([a]) Study/data from the European Prospective Investigation on Cancer (EPIC); ([b]) study from the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED); ([c]) study not belonging to either EPIC nor CHARRED, for which sample size was calculated using the National Statistics Institute (Spanish Statistics Office, available at: https://www.ine.es/en) to date (12 January 2021); ([d]) sum of all nitrosamines formed endogenously such as NPIP, NPYR, and Comb. MF, male and female; M, male; F, female; MeIQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; DiMeIQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine; Total HAs, total heterocyclic amines; B(a)P, benzo (a) pyrene; DiB(a)A, dibenzo (a) anthracene; Total PAHs, total polycyclic aromatic hydrocarbons; NDMA, *N*-nitrosodimethylamine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; Comb., combined nitroso compounds.

According to our results, in the studied sample, dietary HAs (amino-alpha-carboline (AαC), 2-amino-3-methylimidazo (4,5,f) quinoline (IQ), 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline (MeIQx), 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline (DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine (PhIP)) were mainly derived from meat and meat products, with the exception of MeIQ, which was provided by fish (Figure 1). On the other hand, benzo (a) pyrene (B(a)P), dibenzo (a) anthracene (DiB(a)A), and total PAHs were more diversified in terms of dietary origin, being the main dietary sources oils and fats, alcoholic beverages, and milk, respectively. Nitrates derived predominantly from vegetables, while nitrites and *N*-nitroso compounds (NOCs) were mainly found in meat and meat products, and oils and fats. Acrylamide was provided at 64% by the group of cereals and derivatives. According to Figure 1, the main contributor to acrylamide intake was potato (33%) (fried potato and potato chips), followed by cookies (26%) (Maria-type cookies and whole meal cookies), and bread (22%) (loaf white bread and sliced white bread).

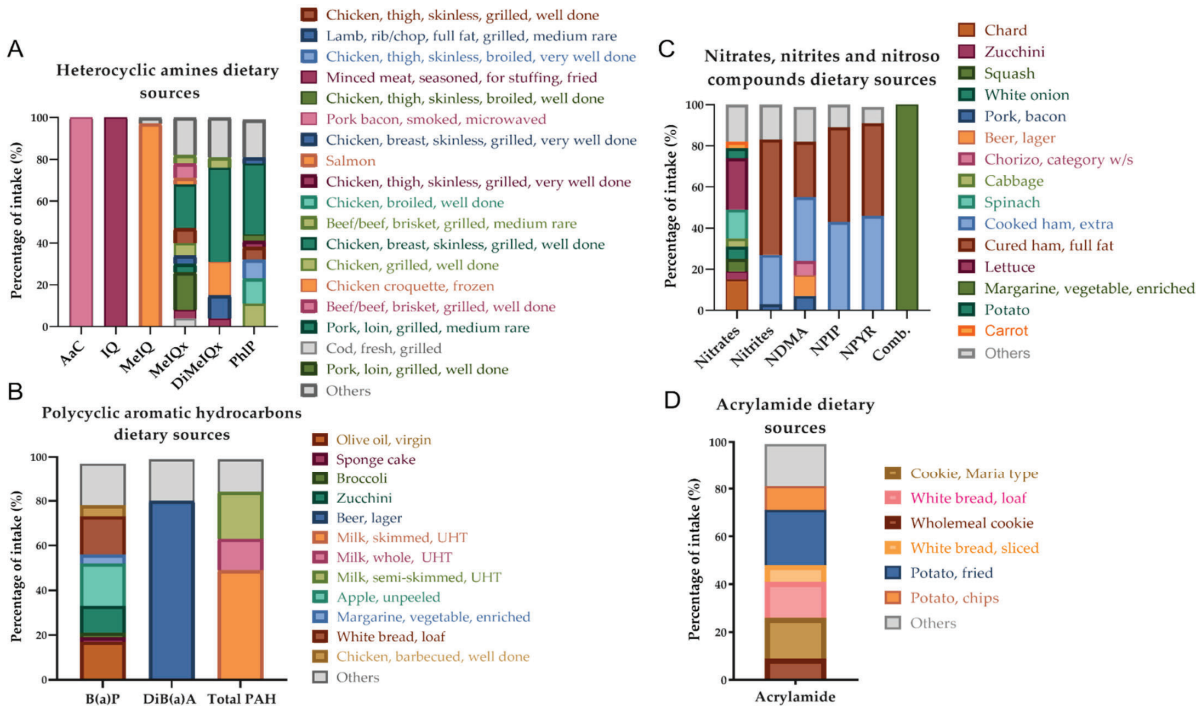

Figure 1. Main dietary sources of xenobiotics in the study sample. (**A**) Heterocyclic amine dietary sources. AαC, amino-alpha-carboline; IQ, 2-amino-3-methylimidazo (4,5,f) quinoline; MeIQ, 2-amino-3,4 dimethylimidazo (4,5,f) quinoline; MeIQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; DiMeIQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine. (**B**) Polycyclic aromatic hydrocarbon dietary sources. B(a)P, benzo (a) pyrene; DiB(a)A, dibenzo (a) anthracene; Total PAHs, total polycyclic aromatic hydrocarbons. (**C**) Nitrate, nitrite, and nitroso compound dietary sources. NDMA, *N*-nitrosodimethylamine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; Comb., combined nitroso compounds. (**D**) Acrylamide dietary sources.

As depicted in Figure 2, microwaved, fried, grilled, broiled, barbecued, and braised cooking methods were mainly responsible for the intake of HAs and PAHs through the cooking of meat in the sample, whereas nitrates, nitrites, and nitrosamines (*N*-nitrosodimethylamine (NDMA), *N*-Nitrosopiperidine (NPIP), and *N*-Nitrosopyrrolidine (NPYR)) derived from grilled and other nonspecified methods. Processed meats were the main dietary source of these compounds (Figure 1), and they also contributed to the intake of hydrocarbons (B(a)P, not available) and amines (AαC and MeIQx, microwaved).

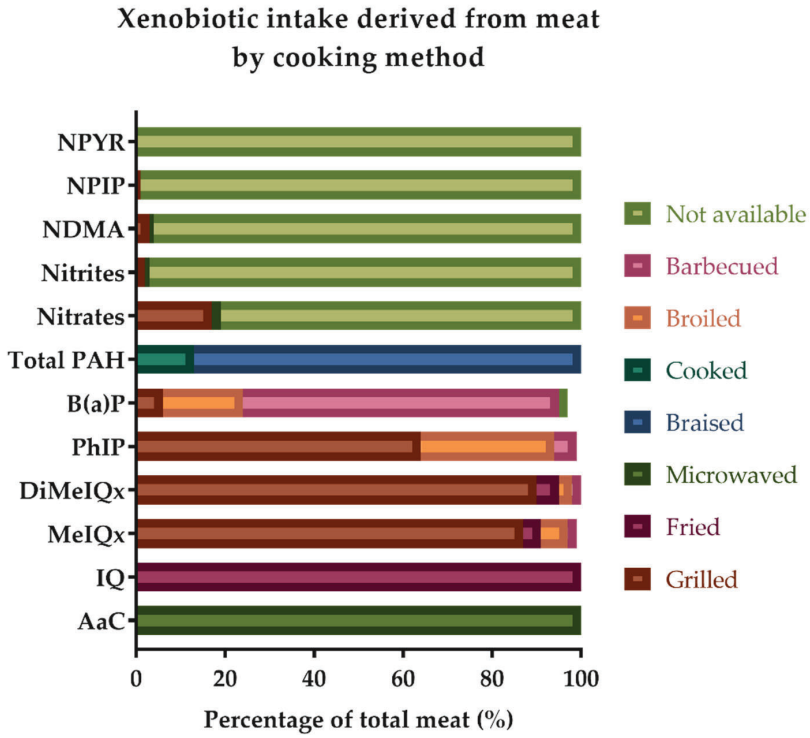

Figure 2. Impact of meat cooking method over the xenobiotic intake in the study sample (percentages may not sum to 100% because of rounding). The label "Cooked" was used by the authors Jakszyn, P. et al. as a general cooking method descriptor. AαC, amino-alpha-carboline; IQ, 2-amino-3-methylimidazo (4,5,f) quinoline; MeIQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; DiMeIQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine; B(a)P, benzo (a) pyrene; DiB(a)A, dibenzo (a) anthracene; Total PAHs, total polycyclic aromatic hydrocarbons; NDMA, *N*-nitrosodimethylamine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine.

To further study the interaction between the consumption of xenobiotics and other dietary components, a correlation analysis was carried out (Figure 3). It was noteworthy that MeIQ was inversely related to elements of vegetable origin such as fiber (total and insoluble and soluble, insoluble cellulose, insoluble hemicellulose, soluble hemicellulose, and Klason lignin), other polysaccharides (starch and digestible polysaccharides), calcium, manganese, or sodium, among others; while other compounds such as IQ, from minced seasoned meat, were inversely related to the dietary total oxygen radical absorbance capacity (ORAC), as well as with compounds with high ORAC such as flavonoids, total phenolics, and insoluble and soluble pectin. Most of the xenobiotics quantified in the sample correlated significantly with the intake of cholesterol, total lipids, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), animal protein, iron, and sodium. When exploring the difference in the consumption of xenobiotics according to lifestyle and health-related gastrointestinal variables (Table 4), a higher mean consumption of nitrites, NDMA, NPIP, and NPYR in individuals who slept less than 7 h/day and in those who reported some occasional intestinal discomfort (such as hemorrhoids or fissures) was found. No significant differences were found according to smoking.

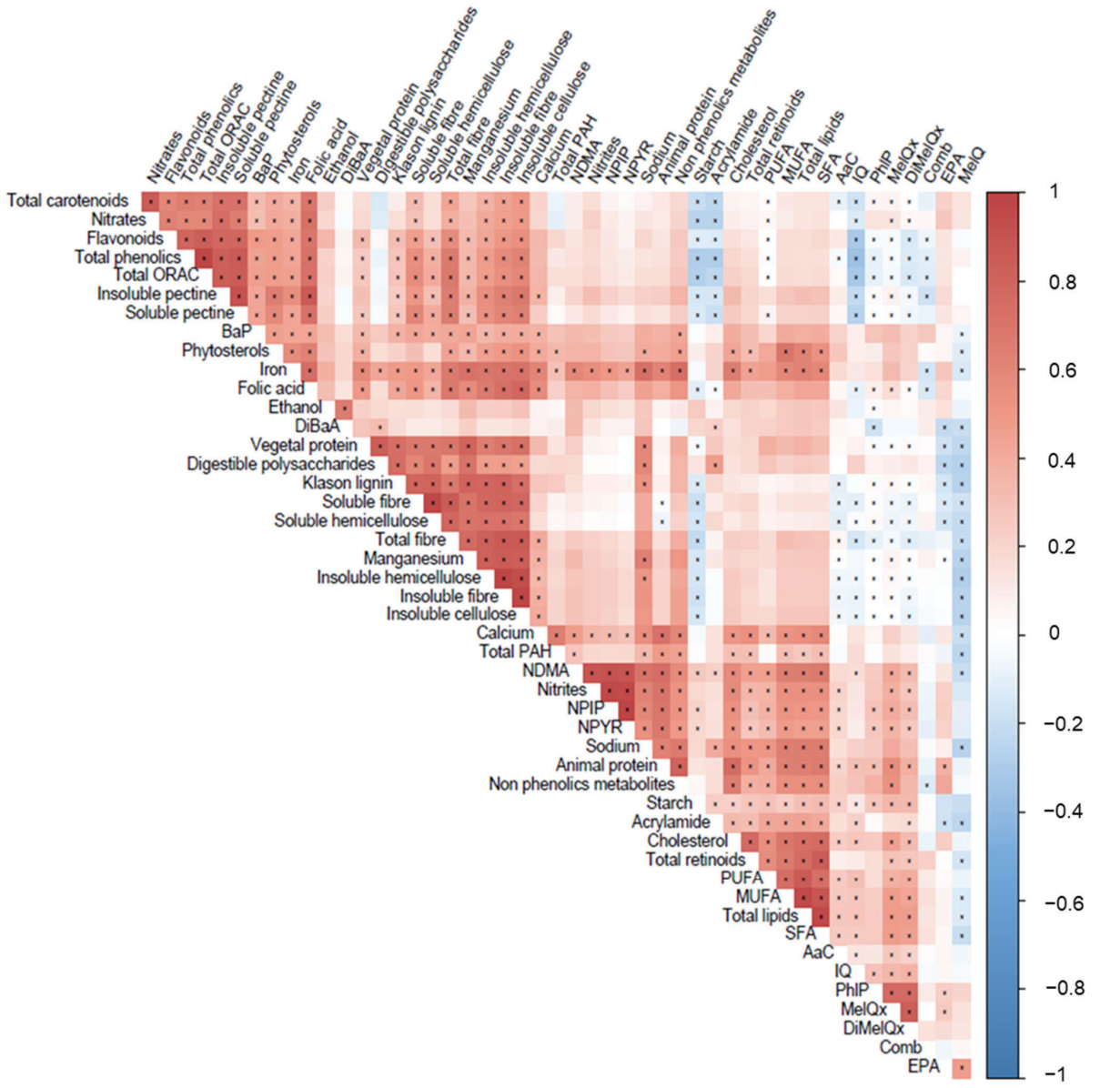

Figure 3. Graphical representation of correlations found between xenobiotic intake and other components derived from the diet. Only components showing significant correlations are represented. (*) *p*-value < 0.01. Total ORAC, total oxygen radical absorbance capacity; B(a)P, benzo (a) pyrene; DiB(a)A, dibenzo (a) anthracene; total PAHs, total polycyclic aromatic hydrocarbons; NDMA, *N*-nitrosodimethylamine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; AαC, amino-alpha-carboline; IQ, 2-amino-3-methylimidazo (4,5,f) quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine; MeIQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; DiMeIQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; Comb., combined nitroso compounds; EPA, eicosapentaenoic acid; MeIQ, 2-amino-3,4 dimethylimidazo (4,5,f) quinoline.

Table 4. Mean xenobiotic intake according to health characteristics.

Mean Daily Intake	Heterocyclic Amines (ng/Day)					Polycyclic Aromatic Hydrocarbons (μg/Day)				Nitroso Compounds (μg/Day)						Acrylamide (μg/Day)
	AαC	IQ	MeIQ	MeIQx	DiMeIQx	PhIP	B(a)P	DiB(a)A	Total PAHs	Nitrates (mg/Day)	Nitrites (mg/Day)	NDMA	NPIP	NPYR	Comb (ng/Day)	Acrylamide
BMI (kg/m^2)																
Normal weight	0.01	0.15	1.79	27.92	7.88	159.69	0.03	0.06	3.89	118.39	2.85	0.16	0.08	0.14	2.80	11.96
Overweight	0.03	0.11	1.48	27.24	7.26	152.55	0.03	0.07	4.88	123.03	3.43	0.18	0.10	0.16	1.25	16.36
Obese	0.00	0.18	1.45	37.48	9.97	330.26	0.04	0.07	7.95 *	153.24	2.91	0.16	0.08	0.13	0.83	16.17
Smoking status																
Current smoker	0.01	0.15	1.95	35.17	10.32	177.52	0.04	0.04	4.35	110.49	2.41	0.13	0.07	0.12	1.43	21.37
Former smoker	0.01	0.14	1.46	25.88	7.57	226.07	0.03	0.09	4.82	130.36	2.73	0.14	0.07	0.11	1.11	12.16
Never smoker	0.03	0.14	1.60	31.09	8.21	160.69	0.03	0.05	5.35	125.77	3.58	0.19	0.11	0.18	2.22	16.13
Exercise																
Active	0.01	0.13	1.54	29.17	9.05	210.78	0.03	0.06	3.65	110.16	2.31	0.14	0.06	0.09	1.20	15.09
Sedentary	0.02	0.14	1.60	29.66	7.69	174.71	0.03	0.07	5.82 *	134.82	3.59	0.19	0.10 *	0.18 *	2.00	15.15
Sleeping																
≥7 h/day	0.02	0.13	1.47	27.88	7.24	185.21	0.03	0.06	4.61	130.64	2.65	0.14	0.07	0.12	1.20	13.38
<7 h/day	0.02	0.15	1.86	33.51	10.51	193.55	0.03	0.09	6.14	114.43	4.35 *	0.23 *	0.13 *	0.21 *	3.00	19.50 *
Intestinal pathologies																
Constipation	0.00	0.19	2.17	38.64	11.05	261.38	0.05	0.02	8.33	94.33	3.24	0.14	0.09	0.15	1.11	9.83
Regular transit	0.02	0.13	1.49	28.13	7.75	176.71	0.03	0.07	4.56 *	130.68	3.12	0.17	0.09	0.15	1.80	15.91
Hemorrhoids	0.03	0.15	1.47	33.13	9.70	195.17	0.03	0.07	4.83	126.60	4.01	0.22	0.12	0.20	0.34	16.67
No hemorrhoids	0.01	0.13	1.66	26.90	7.10	182.23	0.03	0.06	5.19	125.59	2.52 *	0.13 *	0.06 *	0.11 *	2.68	14.03
Fissures	0.05	0.40	2.73	36.88	10.96	469.58	0.02	0.04	2.72	120.76	7.87	0.37	0.24	0.39	5.00	16.54
No fissures	0.02	0.13 *	1.55	29.27	8.09	179.30	0.03	0.07	5.11	126.17	3.00 *	0.16 *	0.08 *	0.14 *	1.62	15.08
Bleeding																
Ever	0.03	0.14	1.43	28.74	8.94	180.16	0.03	0.09	4.76	124.45	3.96	0.22	0.12	0.20	0.80	15.21
Never	0.01	0.14	1.66	29.90	7.75	191.72	0.03	0.05	5.20	126.88	2.67	0.14 *	0.07	0.12	2.22	15.08

Table 4. *Cont.*

Mean Daily Intake	Heterocyclic Amines (ng/Day)					Polycyclic Aromatic Hydrocarbons (μg/Day)				Nitroso Compounds (μg/Day)						Acrylamide (μg/Day)
	AαC	IQ	MeIQ	MeIQx	DiMeIQx	PhIP	B(a)P	DiB(a)A	Total PAHs	Nitrates (mg/Day)	Nitrites (mg/Day)	NDMA	NPIP	NPYR	Comb (ng/Day)	Acrylamide
Rome III Criteria																
Moderate or greater [a]	0.00	0.20	1.89	36.92	19.70	258.23	0.03	0.01	10.56	82.01	0.54	0.03	0.01	0.02	5.00	10.56
Never or mild [b]	0.02	0.14	1.57	29.27	7.84 *	185.51	0.03	0.07	4.88 *	127.31	3.21	0.17	0.09	0.15	1.62	15.26

([a]) Moderate or greater discomfort for more than 50% of the symptoms; ([b]) no discomfort or mild for a maximum of 50% of the symptoms; (*) significant differences were found between values belonging to the same category (p-value < 0.05). AαC, amino-alpha-carboline; IQ, 2-amino-3-methylimidazo (4,5,f) quinoline; MeIQ, 2-amino-3.4 dimethylimidazo (4,5,f) quinoline; MeIQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; DiMeIQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine; B(a)P, benzo (a) pyrene; DiB(a)A, dibenzo (a) anthracene; Total PAHs, total polycyclic aromatic hydrocarbons; NDMA, *N*-nitrosodimethylamine; NPIP, *N*-nitrosopiperidine; NPYR, *N*-nitrosopyrrolidine; Comb., combined nitroso compounds.

4. Discussion

The increasing and progressive incidence of some diseases such as cancer makes it urgent to develop adequate instruments for improving our understanding of the disease in order to increase the efficacy of medical treatments, but also for contributing to developing social guidelines to prevent the onset of the pathology. Diet is one of the modifiable lifestyle factors mainly contributing to the incidence and severity of some human pathologies [14]. As all dietary components and their interactions are important in the risk assessment, xenobiotic compounds formed during food cooking and processing have been targeted as mediators of the relationship between diet and cancer [1,3–5]. Overall, the comprehensive analyses carried out in this dietary study on an adult sample population enabled us to compare the intake of the main xenobiotics in our sample with that reported by other reference authors, and to specify their major dietary sources according to the cooking method. The identification of other dietary and lifestyle factors associated with the consumption of these compounds may be useful for the design of future studies attempting to understand their impact on health in more detail.

The HAs levels reported here were similar to those observed by other authors in different population groups with equivalent consumption of meat and meat products, vegetables, and fruits [32,38]. It should be noted that the dietary sources of some amines were less varied than those of other compounds in the same category. For example, 80% of the intake of MeIQx in the study sample was explained by 11 foods, followed by PhIP with eight and DiMeIQx with five. The best contributors to the intake of MeIQx, DiMeIQx, and PhIP were poultry meat (chicken, thigh, skinless, grilled, well done and very well-done; chicken, thigh, skinless, well-done; chicken, well-done; chicken, grilled, well-done), other animal meat sources (pork, grilled loin, well-done; beef/beef, brisket, grilled, medium-rare and well-done), or meat preparations (minced, seasoned, stuffing, fried, and chicken croquettes). In addition, MeIQx intake was also derived from the consumption of fish such as cod, fresh, grilled, and salmon. AαC and IQ amines were mainly supplied by animal foods, such as pork bacon, smoked, microwaved; and minced meat, seasoned for stuffing, fried, respectively. The 80% of DiB(a)A intake in the sample derived from lager beer, while milk, skimmed, UHT; milk, whole, UHT; milk, semi skimmed, UHT were the main dietary sources of total PAHs. The Comb. component was exclusively provided by vegetable, enriched margarine, while the nitrites NPIP and NPYR came mainly from processed meat products such as fatty cured ham and extra cooked ham. NDMA also came from other meats (chorizo, category w/s; and pork, bacon) (31%) and alcoholic beverages (beer, lager) (10%). Finally, nitrates were the compounds with the greatest variety of dietary sources. They were provided by vegetables, mainly lettuce, chard, and spinach.

The cooking methods of frying, grilling, barbecuing, microwaving, and stewing were mainly responsible for the consumption of HAs and PAHs from meats in the sample study, while some of these techniques, such as microwaving, are recognized as the lowest-driving xenobiotic-formation methods [7,39]. Nitrates were generated after grilling of meats and by other cooking methods that were not available in the database. These results were similar to the ones obtained by other authors [32,34,40]. Since for some references, the food composition table used had no information on the type of cooking, it was assumed that the resulting outcomes were dependent on the information available in the literature. For example, AαC was derived from a single food item (pork bacon, smoked, microwaved) that was always microwaved [41], while other compounds, such as DiMeIQx, appeared in the information for several food items, including chicken, breast, skinless with different cooking methods available (grilled, fried, broiled, and barbecued) [41,42].

In general, in our sample population, processed meats contributed mainly to the intake of nitrates, nitrites, and nitrosamines (NDMA, NPIP, and NPYR), although they also contributed to the intake of other compounds such as hydrocarbons (B(a)P) and HAs (AαC and MeIQx). Regarding meats, white meat was mainly consumed grilled (in Spain, this method implies the use of low amount of oil in a pan), while red meat was mostly cooked through frying (which implied food submerged in oil). As other authors have already

pointed out, the cultural differences in the cooking methods employed are some of the main causes of variations in xenobiotic intake between populations [33]. On the other hand, the proportional contribution of white meat and red meat to the total intake of xenobiotics was similar, since, although the content of xenobiotics was lower in white meat, it was consumed more frequently and in greater daily quantities than red meat. These results may appear contradictory to current recommendations. However, it should be noted that there is scientific evidence showing that the potential carcinogenicity of red meat could be greater for the same intake of these xenobiotics, since another series of components such as heme groups or iron, which are found in higher levels in red meat, can promote endogenous nitrosation, which can contribute to an increased intake of xenobiotics by consumption of red meat [43]. Furthermore, other studies have found a link between proximal CRC and PhIP intake only when it came from red meat and not from white meat [6]. NDMA presented a higher intake level than the one reported in the literature [36], whereas no work estimating the intake of the rest of nitrosamines (NPIP, NPYR, and Comb) was available for comparison. The daily intake for nitrites (3 mg/day), although higher than the one reported by other authors (1 mg/day [36]), remained below the maximum intake recommended for an average weight of 75 kg (0.07 mg/kg body per day; 5 mg/day) [40], and the same applied to nitrates and acrylamide. These compounds showed mean daily intakes of 126 mg/day and 15 μg/day, respectively, which were lower than the maximum recommended intakes of 3.7 mg/kg body weight per day (278 mg/day) [44] and 0.17 mg/kg body weight per day (13 mg/day) [45] in each case. The main sources of acrylamide intake in our human sample were potato with 33% and bread with 22%, similar to previous studies in France and other European populations [11].

On the other hand, it was noteworthy that the direct associations reported between most of the main xenobiotic compounds and dietary compounds were from an animal origin, such as cholesterol, total lipids, MUFAs, PUFAs, SFAs, animal protein, iron, and sodium. These components were positively, and in most cases significantly, related to compounds belonging to the group of HAs. Nitrates, which mainly come from plant-based foods, have been directly related to compounds such as fiber and its subtypes (insoluble fiber, soluble fiber, insoluble cellulose, insoluble hemicellulose, insoluble pectin, soluble pectin), total carotenoids, total phenolics, flavonoids, or total ORAC, all of which have a proven beneficial impact on intestinal homeostasis preservation [14]. Thus, the upper limit of safety for the intake of xenobiotics may be conditioned by the subject's antioxidant intake. In this regard, it has been shown that the intake of nitrates over 142.5 mg/day increases the risk of colon cancer only in those cases with a daily intake of vitamin C under 83.9 mg/day [46], and the intake of NDMA $\geq$ 0.07 μg/day was associated with an increased risk of this pathology with levels of vitamin E under the recommended amounts [47].

When comparing the HA intakes in our sample with the ones from other populations, it was noticeable that those studies from other countries in Europe showed lower amounts of MeIQx, DiMeIQx, PhIP, and total HAs consumption [31,33]. In Sweden, the calculation of individual mean PhIP intake still was maintained lower compared with the value in our sample (188 ng/day vs. 72 ng/day), but MeIQx and DiMeIQx intakes were higher (29 ng/day vs. 72 ng/day and 8 ng/day vs. 16 ng/day, respectively) [48]. Most of these European studies were related to the EPIC database, while studies from other continents were mainly based on the CHARRED database. In our study, we combined references from both databases. Indeed, when comparing with studies performed in the USA as a multiethnic cohort (MEC) study or from other countries such as Brazil, the value of total HAs and the quantified subclasses in those studies were higher than ours, except for DiMeIQx in MEC [15]. This could explain why we found values in between those of the European and non-European countries in our sample. The combination of both databases in order to obtain more standardized quantifications would be interesting in a more globalized and "diet-westernized" world, although xenobiotic formation is finally highly dependent on the culinary methods applied. As HAs have been highlighted as one

of the responsible actors in the increasing CRC incidence, it is crucial to further elucidate how the quantities and the combinations of different HAs would impact on our health. For example, a meta-analysis performed in 2017 revealed an increase in the odds ratio (OR) for colorectal adenoma (CRA) risk of 1.26 for a 50 ng/day increment in MeIQx intake, but just an increase of 1.01 for a 100 ng/day increment in PhIP intake [5].

Regarding PAHs, the total amount recommended by the World Health Organization (WHO) ranges from less than 1 μg/day to 2 μg/day [49], and the Spanish Agency for Food Safety and Nutrition (AESAN) established the "No Observed Adverse Effect Level" (NOAEL) at 6.5 ng/kg/day per person [50], which for a 75 kg person would mean a maximum intake of 0.49 μg/day. In our sample, we found higher levels of total PAHs, although these values were in accordance with other studies performed previously in Spain [34,35].

The validation performed by an R24h showed an acceptable degree of accuracy in quantifying most of the xenobiotics in the diet; however, this observational study showed a few limitations. First, due to the high precision required for data collection, some differences between volunteers who regularly cooked and those who ate away from home might have occurred. Second, despite that the main strength of the study was the degree of detail in the questions and the use of photographic models, for the dietary collection of information, it represented an indirect estimation that was subjected to the systematic error inherent to this methodology. Third, quantification of the levels of these compounds in the organism would be desirable in the future as an additional validation step of the methodology applied. Finally, some recent publications demonstrated that some cooking methods such as air frying could reduce the formation of acrylamide and PAHs in comparison with deep-fat frying [51]. However, since this information was not available in the xenobiotics database, it was not considered.

5. Conclusions

Due to the wide presence of these compounds and their different sources, it is difficult to assess the impact of these dietary compounds on our health, but efforts should be made to adjust their intake to the levels recommended by health agencies.

In short, this preliminary exploratory study of the intake of dietary xenobiotics as potential carcinogens in a Spanish sample population can lay the foundation for short- and long-term broader and deeper multidisciplinary studies for the risk assessment of dietary exposure to these compounds and the onset of precancerous states.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/foods11030470/s1, Table S1: Description of mean daily xenobiotic intake in the study sample, by gender.

Author Contributions: S.G. and C.G.d.l.R.-G. designed the study; S.G. and A.Z. recruited participants; A.Z. performed the nutritional assessment and statistical analyses; S.G., A.Z. and S.R.-S. drafted the manuscript; M.G.-M. designed the graphical abstract. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This project was evaluated and approved by the Regional Ethics Committee of Clinical Research of Asturias (Ref. 163/19) and by the Committee on Bioethics of CSIC (Ref. 174/2020). The procedures were performed in accordance with the fundamental principles set out in the Declaration of Helsinki, the Oviedo Bioethics Convention, and the Council of Europe Convention on Human Rights and Biomedicine, as well as in Spanish legislation on bioethics. Directive 95/46/EC of the European Parliament and the Council of 24 October 1995, on the protection of individuals regarding the processing of personal data and on the free movement of such data, was strictly followed.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: We thank all the participants involved in the study.

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

Abbreviations

AESAN	Spanish Agency for Food Safety and Nutrition
AαC	Amino-alpha-carboline
B(a)P	Benzo (a) pyrene
BMI	Body mass index
CESNID	Centre for Higher Education in Nutrition and Dietetics
CHARRED	Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease
Comb.	Combined nitroso compounds
CRA	Colorectal adenoma
CRC	Colorectal cancer
DiB(a)A	Dibenzo (a) anthracene
DiMeIQx	2-Amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline
EFSA	European Food Safety Authority
EPIC European	Prospective Investigation into Cancer and Nutrition
FDA	U.S. Food and Drug Administration
FFQ	Food Frequency Questionnaire
HAs	Heterocyclic amines
IARC	International Agency for Research on Cancer
IQ	2-Amino-3-methylimidazo (4,5,f) quinoline
MEC	Multiethnic cohort
MeIQ	2-Amino-3.4 dimethylimidazo (4,5,f) quinoline
MeIQx	2-Amino-3,8 dimethylimidazo (4,5,f) quinoxaline
MUFA	Monounsaturated fatty acid
NDMA	*N*-nitrosodimethylamine
NOAEL	No observed adverse effect level
NOCs	*N*-Nitroso compounds
NPIP	*N*-Nitrosopiperidine
NPYR	*N*-Nitrosopyrrolidine
OR	Odds ratio
ORAC	Oxygen radical absorbance capacity
PAHs	Polycyclic aromatic hydrocarbons
PANCAKE	Assessment of Nutrient Intake and Food Consumption Among Kids in Europe
PHEX	Phenol Explorer
PhIP	2-Amino-1-methyl-6-phenylimidazo (4,5,b) pyridine
PUFA	Polyunsaturated fatty acid
PUMUO	University Program for Older Adults of the University of Oviedo
R24h	24-h dietary recall
SEEDO	Spanish Society for the Study of Obesity
SFA	Saturated fatty acid
USDA	United States Department of Agriculture
WHO	World Health Organization

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EDITED BY
Kai Wang,
Chinese Academy of Agricultural Sciences (CAAS), China

REVIEWED BY
Maria Carolina Rodriguez Daza,
Wageningen University and Research, Netherlands
Rupesh Kumar Basniwal,
Amity University, India
Iftikhar Ali Khan,
Shenzhen University, China

*CORRESPONDENCE
Clara G. de los Reyes-Gavilán
greyes_gavilan@ipla.csic.es
Sonia González
soniagsolares@uniovi.es

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Dietary xenobiotics, (poly)phenols and fibers: Exploring associations with gut microbiota in socially vulnerable individuals

Aida Zapico[1,2], Silvia Arboleya[2,3], Sergio Ruiz-Saavedra[1,2], María Gómez-Martín[1,2], Nuria Salazar[2,3], Alicja M. Nogacka[2,3], Miguel Gueimonde[2,3], Clara G. de los Reyes-Gavilán[2,3]* and Sonia González[1,2]*

[1]Department of Functional Biology, University of Oviedo, Oviedo, Spain, [2]Diet Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain, [3]Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa, Spain

Objectives: Although xenobiotics derived from food processing may cause modifications in the composition of the gut microbiota (GM) evidence is scarce. The aim of this study is to evaluate the impact of potential dietary carcinogens as heterocyclic amines (HAs), polycyclic aromatic hydrocarbons (PAHs), nitrates, nitrites, nitroso compounds and acrylamide, in combination to fibers (poly)phenols on the GM composition in a group of materially deprived subjects.

Study design: Transversal observational study in a sample of 19 subjects recipients of Red Cross food aid. Dietary information was recorded by means of 3 non-consecutive 24 h recalls. Questions focused on the type of cooking and the extent of cooking and roasting were included. Information on potential carcinogens was mainly obtained from the European Prospective Investigation into Cancer and Nutrition (EPIC) and Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) Carcinogen Databases. Microbial composition was determined by 16S ribosomal RNA gene sequencing in fecal samples.

Results: Higher levels of Lachnospiraceae and Eggerthellaceae families were found in individuals consuming less than 50 ng/day of 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline (MeIQx) (considered as lower risk dose for colorectal adenoma) while those consuming more than 40 ng/day of 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine (PhIP) (higher risk for colorectal adenoma) showed lower relative abundance of

Muribaculaceae and greater presence of Streptococcaceae and *Eubacterium coprostanoligenes* group.

Conclusion: The associations identified between diet and processing by-products on GM in this study could be used as potential targets for the designing of dietary interventions tailored to this collective.

KEYWORDS

xenobiotic, microbiota, sustainable diet, fiber, Mediterranean diet, meat

Introduction

Gut microbiota (GM) is the set of microorganisms, including bacteria, archaea, viruses, and some unicellular eukaryotes, which inhabit the digestive tract, the colon being the area most densely populated (1). In adults living in developed countries, the most abundant intestinal microorganisms are bacteria belonging to the phyla Bacteroidota, Bacillota (former Firmicutes), Actinobacteriota, Pseudomonadota (former Proteobacteria), and Verrucomicrobiota in a lesser proportion (2–4). In the last years, there was increasing scientific evidence supporting the critical role of the GM in the maintenance of gut homeostasis and in the prevention of different non-communicable diseases, particularly metabolism-related conditions, and several types of cancers (4). From environmental factors, diet plays a fundamental role in shaping the composition and activity of the GM and, thus, determines the inter-relationship between the gut microbiome and the host (5). In humans, the transition from the ancestral diet to the current westernized pattern, represented by a high presence of fats, sugars, animal proteins and processed foods, has shown to impact on GM composition and activity, by means of the reduction in the abundance of certain microorganisms such as *Prevotella* or *Xylanobacter* with capacity to degrade cellular wall components from plants as cellulose and xylan (6) and by a drastic decrease in microbial diversity (6–8). This dietary change may be particularly striking in socially disadvantaged groups where an increase in consumption of processed foods high in fat, sugar or salt has been detected (9), as well as a decrease in the intake of fresh products, such as fruit and vegetables or fish (10). Apart from the nutritional aspects, modern diets have led to an increased intake of processed food. Xenobiotic is a general term used to define "*chemical substances that are foreign to animal life*" including vegetable derived compounds, drugs or additives (11). Within this broad term, the International Agency of Research in Cancer (IARC) provides a particular category for those substances which exhibit demonstrated cytotoxic and genotoxic character (12). In this sense, heterocyclic amines (HAs) and polycyclic aromatic hydrocarbons (PAHs) are generated during high-temperature cooking by grilling, barbecuing, or frying processes, being their concentration in the food directly proportional to the exposure time and temperature (13, 14). Some authors have proposed that these substances may lead to modifications of the GM composition and functions, with influence in host homeostasis (15, 16), although scientific evidence in this field is still scarce (17). The GM also plays a pivotal role as producer and transformer of xenobiotics to activated derivatives, and/or as attenuator of the toxic action of these compounds by diverse mechanisms (18). Some lactic acid bacteria and other microorganisms can bind or metabolize xenobiotics, contributing either to their sequestration and excretion in feces, or to their transformation into less toxic compounds (19, 20) whereas sometimes the GM can metabolize xenobiotics and transform them into derived molecules with enhanced toxicity (17). Some gastrointestinal microbes can generate toxic compounds themselves, as is the case of the colibactin produced by *Escherichia coli* group B (21) or enterotoxins formed by *Bacteroides fragilis,* among others (22). The modification of the toxicity of some dietary xenobiotics can also occur through interactions between the GM and host-detoxification mechanisms. This mainly involves impairment of the activity of hepatic enzymes from the cytochrome P450 complex that participates in the hepatic phase I of detoxification (23) and the reactivation of deactivated glucuronic acid-conjugated compounds during the phase II of detoxification in the liver, by the activity of microbial β-glucuronidases harbored by some intestinal enterobacteria and by members of *Clostridium* and *Bacteroides* genera (24). It is also worth mentioning that some members of the GM can metabolize dietary (poly)phenols, promoting the increase of their biological health benefits, as it is the case of the transformation of ellagitannins into urolithins by members of *Clostridium leptum* group and *Bacteroides/Prevotella* (25) and the conversion of lignans into enterolignans, process in which can participate some *Clostridium*, *Bacteroides*, *Peptostreptococcus* and *Eggerthella* strains, among others (26). Dietary fiber can also act as a sequestrating agent of some toxic dietary compounds, decreasing intestinal toxicity (27). Based on this evidence, the main objective of this pilot study was to analyze the associations between diet and GM, with special emphasis on bioactive compounds and xenobiotics derived from food processing,

in a group of materially deprived subjects with a diet rich in processed foods.

Materials and methods

Participants and study design

The MESAS (Economic, Healthy, and Sustainable Menus) pilot project corresponds to an educational and dietary program in deprived human groups addressed to recipients of food aid from the Red Cross of Asturias (North of Spain). The aid received usually consists of basic non-perishable foodstuffs packs. Thus, in order to offer didactic and dietary tools adapted to these individuals and to facilitate the acquisition of fresh food for the achievement of a balanced and healthy diet, the aim of the MESAS project is to identify the main dietary targets in this group. The data presented in this work are relative to the basal status of the sample studied in which twenty adult subjects with non- declared pathologies were randomly selected among those receiving food aid from Alimerka Foundation provided by the non-profit organization Spanish Red Cross. Inclusion criteria were not to be diagnosed with any chronic condition and not to have consumed antibiotics in the last month. The subjects participating in the aid program were randomly recruited and informed of the objectives of the study, being those interested in participating invited to a personal interview in which the purpose of the project and the required involvement were explained. The volunteers who agreed to participate signed an informed consent form. From the population sample, only those for which both dietary and GM information was available were included in the present study ($n = 19$).

The whole procedure and methodology of this project was approved by the Ethics Committee of the Hospital Universitario Central de Asturias (CEImPA2021.307). The procedures were performed in accordance with the fundamental principles set out in the Declaration of Helsinki, the Oviedo Bioethics Convention, and the Council of Europe Convention on Human Rights and Biomedicine, as well as in Spanish legislation on Bioethics. Directive 95/46/EC of the European Parliament and the Council of October 1995, on the protection of individuals regarding the processing of personal data and on the free movement of such data was strictly followed.

Nutritional assessment

Information regarding the dietary intake of the participants was collected by means of a one unique personalized interview, of no more than 30 min of duration, through three non-consecutive 24 h recalls. At the same interview, participants were scheduled for blood collection and were given the fecal sample collection bottle. Specific questions about cooking habits (boiled, fried, grilled, baked/broiled, or barbecued) and the degree of doneness or toasting in the case of meats, fried potatoes, or toasted bread (undercooked, medium, well-done, very well-done) were included. In order to standardize this point, photographs of the different cooking temperatures, in which the degree of browning increased progressively, were developed specifically for this study. Additionally, complementary questions such as which part of the food was consumed (breast or thigh in the case of chicken) or the possible consumption and/or cooking of the skin (cooking with skin and eating the skin; cooking with skin, but not consuming it; and cooking without skin) were incorporated to improve the quality of the information. The classification of the food into groups was carried out according to the Centre for Higher Education in Nutrition and Dietetics (CESNID) criteria. Food composition tables of CESNID (28) and the United States Department of Agriculture (USDA) (29) were used to transform food consumption into energy and macronutrients intake. The fiber and phenolic content of the foods was extracted from Marlett and Cheung tables (30) and the Phenol Explorer 3.6 (31). For each dietary compound, the five major food sources were identified.

Nutritional assessment of xenobiotics derived from food processing

The nutritional analysis of the sample was carried out based on food consumption per individual. Information on HAs, PAHs, nitrates, and nitrites was mainly obtained from the European Prospective Investigation into Cancer and Nutrition (EPIC) Carcinogen Database (32). The EPIC database compiles information from 139 references regarding the content of these compounds per 100 g of food in more than 200 food items. The food composition table is classified according to the preservation method, cooking method, degree of browning, and temperature (32). For those foods or culinary preparations not included in the EPIC database, information was completed with the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) database (33) in the case of HAs and benzo (a) pyrene (B(a)P), and the European Food Safety Authority (EFSA) data in the case of nitrates (34). Acrylamide content was provided by the U.S. Food and Drug Administration (FDA) composition tables (35) and other external reference sources have been used when necessary for acrylamide (36–38), HAs (39), total PAHs (40) and nitrosamines (41–44).

Anthropometrical and biochemical determinations

Height (m) and weight (kg) were taken by standardized protocols (45) and Body Mass Index (BMI) was calculated using the formula: weight/(height)^2. Subjects were classified as normal weighted (18.5–24.9 kg/m^2), overweighted (25.0–29.9 kg/m^2), and obese ($\geq$ 30.0 kg/m^2), based on the Spanish Society for

the Study of Obesity (SEEDO) criteria (46). The percentage of body fat was estimated through bioelectrical impedance in a calibrated TANITA equipment (Tanita Corporation of America, Inc., Arlington Heights, IL, USA). Waist and hip circumferences were measured with an inelastic and extensible tape according to standard criteria (47) and waist-hip ratio was calculated as the ratio of waist circumference over the hip circumference.

Fasting blood samples were drawn by venipuncture and collected in separate tubes for serum and plasma. Samples were kept on ice and centrifuged (1,000 x g, 15 min) within 2–4 h after collection. Plasma and serum aliquots were stored at −20°C until analysis were performed. From blood samples, biochemical analysis of fasting plasma glucose, cholesterol, high- and low-density lipoproteins (HDL and LDL) and triglycerides were determined by using an automated biochemical autoanalyzer in external laboratories.

Fecal microbiota analysis

Fecal samples were collected within ± 24 h of blood collection in sterile containers supplied to each volunteer along with the instructions for sample collection. Samples were frozen within a period no longer than two hours from deposition and stored at −20°C until analysis. At lab, fecal samples were weighted, diluted 1/10 (p/v) in sterile PBS solution and homogenized at full speed (260 rpm) in a LabBlender 400 stomacher (Seward Medical, London, UK) for 3 min. Samples were centrifuged (13 000 rpm, 15 min, 4°C) and then, supernatant, and bacterial pellet were separated. From the pellet obtained, DNA was extracted in accordance with the Q Protocol for DNA extraction defined by the International Human Microbiome Standards Consortium (48) using QIAamp Fast DNA Stool Mini Kit (Qiagen, Sussex, UK). Quantification of extracted/purified DNA and 260/280 ratio was performed using Take3 Micro-Volume plate and Gen5 microplate reader (BioTek Instrument Inc., Winooski, VT, USA). DNA was finally kept frozen at −80°C until analysis.

Variable region V3-V4 of bacterial 16S rRNA genes present in each fecal community was amplified by PCR and the resulting amplicons were sequenced on an Illumina NovaSeq 6000 platform instrument. Following sequencing of the library, the obtained individual sequence reads were filtered to remove low quality sequences. All Illumina quality-approved, trimmed, and filtered data were exported, and the information was integrated in order to generate *de novo* 16S rRNA Operational Taxonomic Units (OTUs) with ≥97% sequence homology using Uparse software (Uparse v7.0.1090) (49). All reads were classified to the lowest possible taxonomic rank using Quantitative Insights Into Microbial Ecology (QIIME) and a reference dataset from the SILVA 138 database (50). The whole procedure of sequencing and annotation was undergone at Novogene Bioinformatics Technology Co., Ltd.

Statistical analysis

Results were analyzed using the IBM SPSS software version 25.0 (IBM SPSS, Inc., Chicago, IL, USA). Goodness of fit to the normal distribution was checked by means of the Kolmogorov-Smirnov test. As normality of the variables was not achieved, non-parametric tests were used. Overall, categorical variables were summarized as number and percentage (n (%)) and continuous variables as median and percentiles 25 and 75 (P_{25} – P_{75}). Spearman correlation and stepwise regression analysis (adjusted for BMI, age and energy intake) were conducted. Heatmaps were generated using ClustVis web tool (51) and logarithmic Linear Discriminant Analysis (LDA) Effect Size (LEfSe) within the Galaxy web application (52). LefSe graphs were created for the xenobiotic compounds, derived from food processing, showing statistically significant results in Spearman correlations and stepwise regression analysis conducted and for which a risk threshold was available in the literature.

Results

General characteristics of the sample

A general description of the main general lifestyle, anthropometric and clinical history characteristics of the population under study is shown in **Table 1**. The volunteers were mostly women under 50 years living in a family unit of 3 or 4 members. Regarding lifestyle and anthropometric characteristics, about half of the sample reported less than 6 hours of sleep per day and a BMI ≥ 30 kg/m^2.

Dietary pattern: Food groups, xenobiotics, (poly)phenols, and fibers

A brief description of the food intake in the sample is presented in **Table 2**. The median energy daily intake is approximately 1,500 kcal, being meat and derivates above the maximum recommended level of 100 g/day. Also, the daily intake of red meat and processed meats were below the upper limits of 100 g/day and 50 g/day recommended, respectively, by the World Cancer Research Fund International (WCRF) (53). No ethanol consumption has been reported in the sample.

The major food sources of xenobiotics, fibers and (poly)phenols in the sample are shown in **Table 3**. The HA 2-amino-3,8 dimethylimidazol (4,5, f) quinoxaline (MeIQx) is provided by chicken, breast, pork and minced meat. Insoluble dietary fiber derives from white bread, potato, and pasta, among others. In addition, coffee has been identified as one of the major contributors to (poly)phenol and phenolic acids consumption (**Table 3**).

TABLE 1 General characteristics and clinical history of the sample population.

Characteristics	*N* = 19
General	
Age (years)	41 (32 – 51)
Gender	
Female	16 (84)
Educational level	
Primary	4 (21)
Secondary	5 (26)
Technical	7 (37)
University	3 (16)
Family members (n)	
1 – 2	7 (37)
3 – 4	10 (53)
≥ 5	2 (11)
Lifestyle	
Sleep (hours/day)	5.5 (5.0 – 7.5)
Physical activity (walking min/day)	60.0 (21.4 – 90.0)
Smoking status	
Current smoker	5 (26)
Occasional alcohol consumption	6 (32)
Anthropometric	
BMI (kg/m^2)	27.85 (22.39 – 36.00)
Underweight (≤18.5)	1 (5)
Normal weight (18.5–24.9)	6 (32)
Overweight (25.0–29.9)	3 (16)
Obese (≥30.0)	9 (47)
Total body fat (%)	45 (29 - 53)
Waist-hip ratio	0.85 (0.80 – 0.91)
Clinical history	
Chronic conditions	
Respiratory diseases	9 (47)
Stool frequency (times/week)	7.0 (5.0 – 10.0)
Stool consistency	
Liquid	1 (5)
Soft	13 (68)
Hard	5 (26)
Presence of occasional bleeding	2 (11)
Biochemical parameters	
Glucose (mg/dL)	90.0 (83.0 – 93.0)
Triglycerides (mg/dL)	130.0 (69.0 – 162.0)
Total cholesterol (mg/dL)	205.0 (193.0 – 226.0)
LDL (mg/dL)	123.0 (108.0 – 137.0)
HDL (mg/dL)	54.0 (46.0 – 67.0)
Total cholesterol/HDL ratio	3.7 (2.9 – 4.5)
LDL/HDL ratio	2.2 (1.8 – 3.0)

Data is expressed as median (P_{25} – P_{75}) or as number of participants (*n* (%)) for continuous and categorical variables, respectively.
BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

TABLE 2 Description of the energy and food groups intake in the sample of study.

Characteristics	*N* = 19
Energy (kcal/day)	1510.35 (1201.45 - 1667.14)
Food groups (g/day)	
Cereals and cereals products	121.53 (60.70 - 151.63)
Whole grain cereals	0.00 (0.00 - 5.90)
Milk and dairy products	187.50 (92.00 – 300.00)
Meat and meat products	110.00 (93.99 - 177.04)
White meat	53.58 (33.30 - 100.38)
Red meat	27.08 (5.90 - 63.33)
Processed meat	13.33 (6.67 - 31.33)
Eggs	52.67 (24.43 - 69.93)
Fish	25.33 (0.00 – 53.00)
Seafood	0.00 (0.00 – 20.00)
Oils and fats	16.73 (11.00 - 23.99)
Vegetables	104.96 (43.37 – 190.00)
Legumes	11.67 (2.50 - 33.33)
Potatoes and tubers	43.18 (8.33 – 59.00)
Fruits	116.56 (59.10 - 170.35)
Nuts and seeds	0.00 (0.00 – 10.00)
Sugar and sweets	12.33 (7.00 - 23.33)
Snacks	0.00 (0.00 – 0.00)
Sauces and condiments	18.50 (2.50 - 30.83)
Other foods	0.00 (0.00 - 113.33)
Non-alcoholic beverages (mL/day)	300.00 (143.33 – 400.00)
Alcoholic beverages (mL/day)	(0.00 – 0.00)

Data is expressed as median (P_{25} – P_{75}).

Gut microbiota profile

The GM diversity determined by the Shannon index and richness measured as Observed species were 6.13 and 706, respectively (Figures 1A,B). At the phylum level, Bacillota was the most abundant, followed by Actinobacteriota, Bacteroidota, and Pseudomonadota (Figure 1C). At the family level, the most abundant was Lachnospiraceae, followed by Bifidobacteriaceae, Ruminococcaceae, Prevotellaceae and then Coriobacteriaceae (Figure 1D). GM relative abundances at the phylum level, disaggregated by individual evidenced a global increase of Bacteroidota at the expenses of the decrease of Bacillota from individuals MESAS11 to MESAS19, (with the exception of individual MESAS12) in contrast to an increase of Actinobacteriota at the expenses of the reduction of Bacillota in the remaining individuals (Figure 1E).

Dietary patterns and gut microbiota

In order to look for dietary, anthropometric, biochemical or lifestyle factors that could be related with variations in the two most abundant intestinal microbial phyla (Bacillota and Actinobacteriota) in the sample, individuals were clustered in two groups according to the Bacillota/Actinobacteriota abundance ratio: group 1 (ratio ≥2) and group 2 (ratio <2). Individuals in the group 2 presented a significantly higher consumption of red meat and significantly higher fasting glucose levels as well as a total/HDL-cholesterol atherogenic

TABLE 3 Top dietary sources of xenobiotics (poly)phenols and fibers in the sample of study.

Compound	Intake	Dietary sources	(%)
Xenobiotic compounds			
Heterocyclic amines (ng/day)			
MeIQx	40.62 (3.54 – 65.45)	Chicken, breast, skinless; pork loin; minced meat, seasoned, for stuffing; beef, topside; cod, fresh	74
DiMeIQx	1.60 (0.00 – 23.35)	Chicken, breast, skinless; minced meat, seasoned, for stuffing; beef, topside; lamb, shoulder, lean and fat; pork, chops, lean and fat	58
PhIP	2.95 (0.00 – 298.97)	Chicken, breast, skinless; minced meat, seasoned, for stuffing; beef, topside; chicken, thigh, skinless; lamb, rib/chop, full fat	62
Polycyclic aromatic hydrocarbons (μg/day)			
B(a)P	0.04 (0.02 – 0.06)	Sunflower oil; yogurt, whole; olive oil, virgin; banana; apple, unpeeled	42
DiB(a)A	0.00 (0.00 – 0.01)	Chicken egg, whole; tea, infusion; chocolate, with milk; white bread, stick; coffee, infusion	86
Total PAH	0.70 (0.50 – 1.35)	Pizza, tomato and cheese, baked; wheat flour; pasta; white bread, stick; potato	64
Nitrates, nitrites and nitroso compounds			
Nitrates (mg/day)	38.19 (20.29 – 90.45)	Lettuce; potato; onion; green bean; carrot	79
Nitrites (mg/day)	0.63 (0.31 – 1.06)	Cooked ham, extra; chicken egg, whole; potato; sausage, Frankfurt type; cured ham, lean and fat	76
NDMA (μg/day)	0.03 (0.02 – 0.09)	Cooked ham, extra; chorizo, category w/s; Manchego cheese, semi-matured; black pudding; cured ham, lean and fat	79
NPIP (μg/day)	0.02 (0.01 – 0.05)	Cooked ham, extra; chorizo, category w/s; black pudding; cured ham, lean and fat; sausage, Frankfurt type	79
NPYR (μg/day)	0.03 (0.01 – 0.06)	Cooked ham, extra; chorizo, category w/s; black pudding; cured ham, lean and fat; sausage, Frankfurt type	79
Acrylamide (μ g/day)	8.73 (6.44 – 11.62)	Cookie; potato; white bread, loaf; wholemale bread, loaf; white bread, stick	91
(Poly)phenols (mg/day)			
Total (poly)phenols	683.17 (345.80 – 1208.90)	Coffee, infusion; potato; lentils; soluble cocoa powder; banana	62
Flavonoids	62.37 (10.71 – 183.03)	Onion; orange juice, commercial; orange juice, fresh; cherry; orange	52
Phenolic acids	226.37 (101.87– 605.21)	Coffee, infusion; potato; green olive, in brine; cherry; carrot	93
Lignans	12.98 (8.73 – 27.08)	Potato; green bean; tomato; melon; carrot	59
Other (poly)phenols	13.74 (4.84 – 23.24)	Coffee, infusion; olive oil, virgin; green olive, in brine; pasta; olive oil	88
Stilbenes	0.00 (00.00 – 0.02)	Lentil; vinegar; green grape; peanut butter; red wine	48
Dietary fiber (g/day)			
Total	11.86 (8.29 – 15.06)	White bread, stick; potato; pasta; chick peas; white bread, loaf	35
Soluble	1.46 (1.05 – 2.07)	White bread, stick; potato; pasta; white bread, loaf; tomato	50
Insoluble	5.97 (4.56 – 10.25)	White bread, stick; potato; pasta; wholemale bread, loaf; onion	43
Starch	23.31 (9.07 – 42.44)	Pasta; breadcrumbs; pizza, tomato and cheese, baked; white bread, loaf; wheat flour	87
Celulose	2.15 (1.74 – 3.37)	Potato; white bread, stick; pasta; lentils; onion	43
Klason Lignine	1.13 (0.71 – 1.49)	White bread, stick; pasta; white bread, loaf; banana; wholemale bread, loaf	56
Hemicelulose			
Soluble	1.14 (0.48 – 1.36)	White bread, stick; pasta; potato; white bread, loaf; cookie	63
Insoluble	2.12 (1.51 – 3.62)	Pasta; potato; white bread, stick; wholemale bread, loaf; onion	49
Pectin		White bread, stick; potato; pasta; chick peas; white bread, loaf	
Soluble	0.45 (0.26 – 0.62)	Potato; banana; tomato; onion; carrot	58
Insoluble	0.71 (0.45 – 1.18)	Potato; onion; lettuce; green bean; carrot	50

Data is expressed as median (P_{25} – P_{75}). For each dietary compound five major food dietary sources and mean percentage of contribution (%) to the total intake in the sample are shown. MeIQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; DiMeIQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine; B(a)P, benzo (a) pyrene; DiB(a)A, dibenzo (a) anthracene; Total PAH, total polycyclic aromatic hydrocarbons; NDMA, N-nitrosodimethylamine; NPIP, N-Nitrosopiperidine; NPYR, N-Nitrosopyrrolidine.

index higher than individuals from the group 1, without reaching risk threshold values of 110 mg/dL and 4.5 defined for these blood parameters, respectively (Table 4). When comparing the GM between both groups of individuals, several taxa displayed significant differences (Table 4). Remarkably, the only significant variation within the Actinobacteriota phylum was found for the family Bifidobacteriaceae and the genus *Bifidobacterium*, which had twice the relative abundances in the group that consumed more red meat than individuals who consumed less red meat. No other anthropometric,

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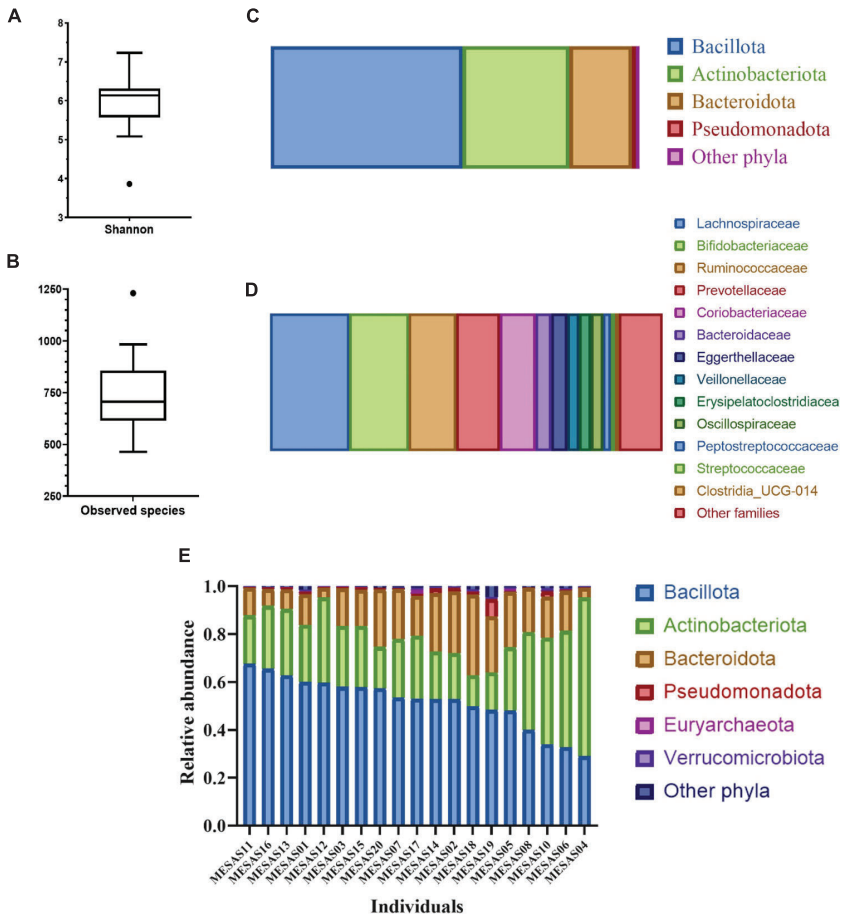

FIGURE 1
Diversity indices and GM relative abundance composition. Box plot of Shannon index **(A)** and Observed species index **(B)**. GM profile at phylum **(C)** and family level **(D)**; only taxa showing mean relative abundances higher than 1% are shown. GM main phyla relative abundance distribution in the sample of study disaggregated by individual **(E)**.

biochemical, lifestyle or GM parameters displayed significant differences between these two subgroups in the sample (data not shown).

Xenobiotics, (poly)phenols, dietary fibers, and gut microbiota

Associations of dietary components with GM are shown at the phylum (Figure 2A) and family level (Figure 2B). Pseudomonadota and Verrucomicrobiota are the phyla showing the most significant direct correlations with xenobiotics (HAs, total PAHs, and nitrates). Verrucomicrobiota is also significantly associated with some fibers and (poly)phenols, as well as Euryarchaeota and Bacteroidota. These phyla are inversely correlated with starch, (poly)phenols and phenolic acids and lignans, respectively. At the family level (Figure 2B), 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline (DiMeIQx) and 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine (PhIP) are directly associated with Streptococcaceae, this family being inversely correlated with the nitrosamine N-Nitrosopiperidine (NPIP). From (poly)phenols, inverse associations were detected for some of them with Clostridia UCG-014, Prevotellaceae and Ruminococcaceae while starch is directly associated with Lachnospiraceae and Eggerthellaceae. Based on these results, a stepwise regression analysis was conducted for the major microbial phyla and dietary sources, potential carcinogens, and (poly)phenol, controlled by age, BMI and energy intake (Table 5). The results obtained revealed that the associations represented in Figure 2 remained after adjusting for energy intake and BMI. It is also remarkable that in most cases dietary sources are not the main variables explaining the correlations

TABLE 4 Dietary and microbiota differences according to microbiota profile distribution of main phyla in the sample of study.

Characteristics	Group 1 ($N = 13$)	Group 2 ($N = 6$)
Dietary		
Red meat (g/day)	21.00 ± 21.89	70.20 ± 36.65*
Biochemical parameters		
Fasting glucose (mg/dL)	85.31 ± 7.84	95.50 ± 5.32*
Total cholesterol/HDL ratio	3.49 ± 0.73	4.78 ± 1.31*
Microbiota composition		
Bacillota	56.85 ± 5.91	40.57 ± 11.51*
Lachnospiraceae	22.42 ± 4.66	14.58 ± 3.66*
Agathobacter	6.39 ± 1.61	3.41 ± 1.96*
Blautia	3.77 ± 1.40	2.75 ± 1.24*
Roseburia	1.21 ± 0.90	0.49 ± 0.04*
Ruminococcaceae	13.27 ± 2.99	9.01 ± 0.98*
Faecalibacterium	5.54 ± 1.44	3.38 ± 0.89*
Ruminococcus torques group	1.33 ± 0.81	0.63 ± 0.17*
Peptostreptococcaceae	2.84 ± 1.70	1.46 ± 0.51*
Romboutsia	2.26 ± 1.30	1.16 ± 0.41*
Actinobacteriota	21.88 ± 4.67	43.67 ± 13.49*
Bifidobacteriaceae	9.41 ± 4.02	26.95 ± 19.31*
Bifidobacterium	9.40 ± 4.02	26.94 ± 19.31*
Pseudomonadota	1.44 ± 1.82	0.60 ± 0.81*

Data is expressed as mean ± standard deviation. For microbiota composition relative abundance (%) for those taxa >1% are shown.
(*) Only significant results found by the U-Mann Whitney test *p*-value <0.05 are displayed on the table.
HDL, high density lipoprotein.

found but the specific dietary component instead is sufficient by itself to explain at least for the minimum coefficient of the association.

Analysis of the gut microbiota according to the risk for xenobiotic intake levels

Since xenobiotic intake can vary widely between individuals, a LEfSe analysis was conducted to detect differences in GM profiles between individuals consuming xenobiotics below or over the risk doses described in the literature. PhIP and MeIQx were the only carcinogenic compounds showing significant associations with specific taxa of the GM both by Spearman correlation and by stepwise regression analysis for which risk daily consumption doses were reached in the sample. In the case of MeIQx (**Figure 3A**), higher levels of Lachnospiraceae and Eggerthellaceae families were found in individuals consuming less than 50 ng/day (lower risk for colorectal adenoma) (54) while those consuming more than 40 ng/day of PhIP (higher risk for colorectal adenoma) (54) showed lower relative abundance of Muribaculaceae and greater presence of Streptococcaceae and *Eubacterium coprostanoligenes* group (**Figure 3B**).

Discussion

In developed countries, foods with solid science-based evidence on their benefits for health, such as fruit, vegetables, whole grains, and fish, usually have a relatively high cost. As a result, vulnerable at-risk groups often adopt diets that are far from the Mediterranean dietary standard (9), increasing the risk long-term to suffer non-communicable diseases, such as diabetes, hypertension, hyperlipidemia and obesity (55, 56). To the best of our knowledge, this is the first study examining the potential impact of the diet of a socially vulnerable population on the composition of the GM, providing a more in-depth analysis into the HAs, PAHs, nitrates, nitrites, nitroso compounds and acrylamide resulting from food processing. In spite of this, it is interesting to note that in our sample those individuals consuming significantly higher amounts of red meat also displayed significantly higher levels of microorganisms from the genus *Bifidobacterium* than individuals with lower intake of meat. Members of the genus *Bifidobacterium* are considered beneficial for health (57). Lower intestinal *Bifidobacterium* levels have been generally associated with higher consumption of red meat and animal meat (58) in the context of high fat and high calorie westernized diets, with high consumption of meat. However, the population analyzed in the present study are socially disadvantaged individuals whose daily intake of red meat did not exceeded the maximum recommended (53). In this regard, lower levels of fecal *Bifidobacterium* have been also reported in vegetarian individuals with respect to omnivores (59).

Our results evidence that the intake of some (poly)phenol, fibers and xenobiotics derived from food processing were associated with the GM composition, with a differential impact as depending on the microbial groups. It seems plausible that the consumption of processed foods, as well as fast cooking techniques, may lead to a higher intake of carcinogens in this sample. In this regard, only 42% and 37% of the sample had a MeIQx or PhIP intake above the recommended values (50 ng/day and 40 ng/day, respectively) (54). The average daily intakes of nitrate (54 mg) and acrylamide (10 μg) were within the normal limits, with no volunteers exceeding threshold levels (3.70 and 0.17 mg/kg/day, respectively) (60, 61). When comparing the levels of xenobiotics intake obtained in the present work with those of other studies in the general population at the same geographical location, a higher consumption of MeIQx and lower of phenolic compounds and fibers was observed in our sample (62). This is consistent with the lower consumption of fruit, vegetables, and plant-based foods in this human group.

Net effects exerted by dietary xenobiotics on the GM are dependent on their intake levels, the diet considered globally, and the interactions between these compounds and the GM (18). Indeed, microorganisms from the GM can present different degrees of sensitivity/resistance to dietary xenobiotics. Some

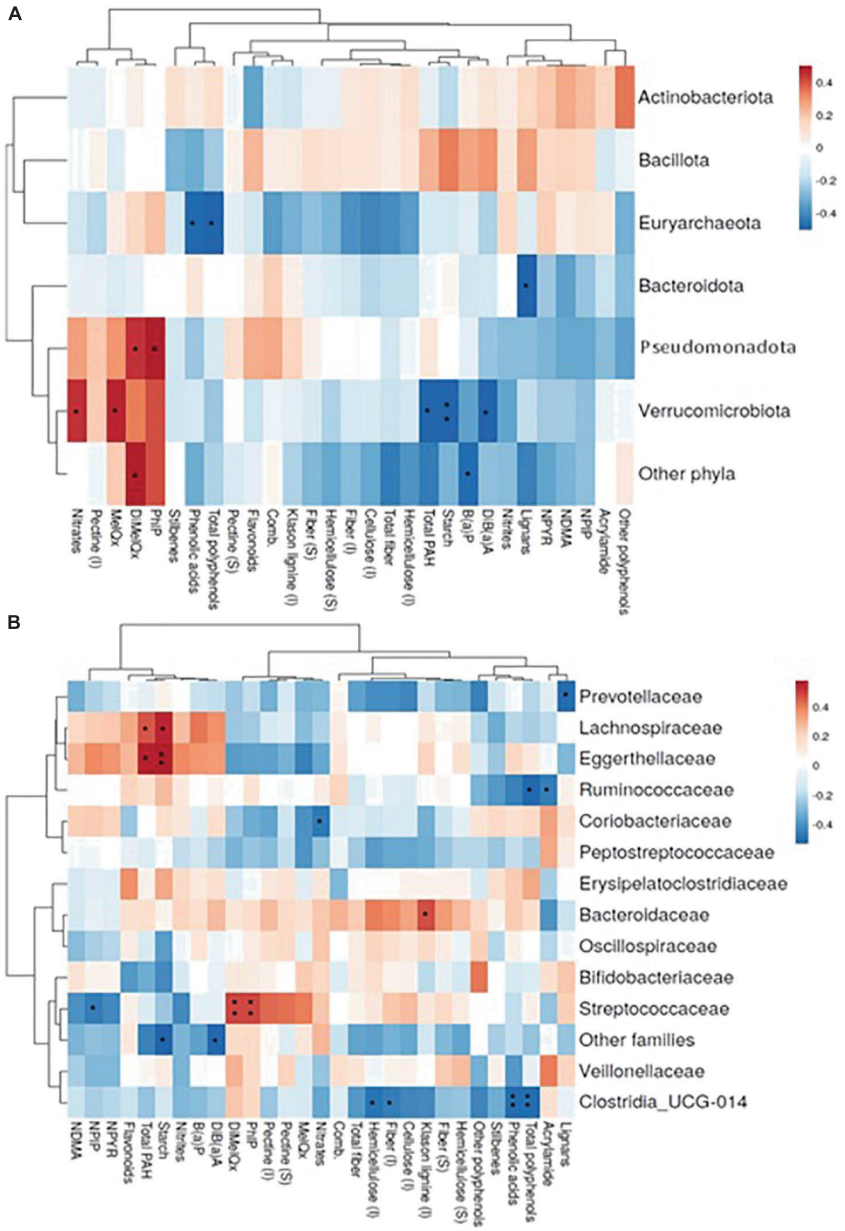

FIGURE 2
Spearman correlations representation between the most abundant bacterial phyla **(A)** or families **(B)** (rows) and consumption of xenobiotics and bioactive compounds derived from the diet in the sample (columns). (∗), (∗∗) p − value $<$ 0.05 and 0.01, respectively. Only taxa showing mean relative abundances higher than 1% are shown. MeIQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; DiMeIQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine; B(a)P, benzo (a) pyrene; DiB(a)A, dibenzo (a) anthracene; Total PAH, total polycyclic aromatic hydrocarbons; NDMA, N-nitrosodimethylamine; NPIP, N-Nitrosopiperidine; NPYR, N-Nitrosopyrrolidine; (I), insoluble; (S), soluble.

members of the GM can bind toxics, contributing to their elimination with feces, and others can metabolize toxics, directly or through microbial metabolic interactions, resulting in new derived compounds with higher or lower toxicity. In this way, some of the variations in relative abundances of gut microbial taxa studied in the present work may be related with

TABLE 5 Analysis of variables accounting for significant correlations found between microbiota and the intake of xenobiotics, fibers and (poly)phenols, at the phylum level.

		Variables	R^2	β	*p*
Bacteroidota					
	Model 1	Lignans	0.302	−0.584	0.009
	Model 2	Lignans	0.428	−0.754	0.001
		Apricot		0.425	0.044
	Model 3	Lignans	0.626	−1.054	0.000
		Apricot		0.508	0.006
		Age		0.523	0.008
	Model 4	Lignans	0.778	−0.915	0.000
		Apricot		1.253	0.000
		Age		0.635	0.000
		Pear		−0.915	0.005
	Model 5	Lignans	0.829	−0.893	0.000
		Apricot		1.225	0.000
		Age		0.564	0.001
		Pear		−0.920	0.002
		Melon		−0.232	0.041
Pseudomonadota					
	Model 1	Chicken, thigh, skinless	0.238	0.529	0.020
	Model 2	Chicken, thigh, skinless	0.423	0.462	0.021
		PhIP		0.459	0.022
Verrucomicrobiota					
	Model 1	MeIQx	0.349	0.621	0.005
	Model 2	MeIQx	0.541	0.591	0.002
		Pasta		−0.456	0.012
Other phyla					
	Model 1	DiMeIQx	0.191	0.486	0.035

Results from stepwise regression analysis between significant correlated associations of main phyla relative abundances and the intake of dietary compounds (xenobiotics, fibers and (poly)phenols). The variables considered for each analysis are BMI, age, energy intake and ALL dietary sources involved in each case. Only significant results are shown. β, standarized regression coefficient; R^2, adjusted coefficient of multiple determination; *p*; *p* value. MeIQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; DiMeIQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine.

the microbial fitness exhibited against some of the potential carcinogens, fibers and (poly)phenols. The effect of each toxic compound could be enhanced or attenuated by other dietary components, which can result in associations of xenobiotics and/or bioactive compounds with the GM that vary depending on the characteristics of the GM itself, dietary habits and lifestyle. Therefore, although causality cannot be established from this type of research, it is necessary to highlight the difficulty in evaluating the impact of dietary components in human populations. In this sense, food groups, such as vegetables, which are sources of nitrites but also of (poly)phenols and fiber may have a different association with the GM than those compounds derived from cooked meat. For some dietary phenolics and fibers it has been demonstrated experimentally that they can counteract, total or partially, the effects of potentially harmful xenobiotics, even avoiding their formation during cooking, thus decreasing the potential toxicity of foreign chemicals to the organism (63–65). These associations between the GM and xenobiotics have been demonstrated by *in vitro* and *in vivo* systems. For instance, the formation of quinone-derived compounds was prevented from (poly)phenols of green tea in the presence of N-nitrosamine by the action of gut microbiome (66). Likewise, dietary wheat bran was shown to attenuate chronic cadmium toxicity in rats (67) and mulberry and dandelion water extracts were shown to prevent alcohol-induced steatosis and alleviate gut microbiome dysbiosis in rats (68). However, as our sample presented an intake of the HA MeIQx higher than the recommended levels and a low intake of phenolic compounds, the potential protective effect of dietary (poly)phenols would be presumably lower than in a population with higher intake of fruits and vegetables. Nevertheless, the long-term effect of the interaction between dietary bioactive compounds such as (poly)phenols and fibers from vegetables and xenobiotics derived from food processing, as HAs and PAHs, at the intestinal level and their effect on the GM remains largely unknown.

At the phylum taxonomic level, associations of several xenobiotics (mainly HAs) with the GM seem to have an opposite direction to that of several (poly)phenols whereas other potentially harmful xenobiotics display associations similar to dietary compounds derived from plants. This is the case of total PAH, dibenzo (a) anthracene (DiB(a)A) and starch, all of them showing negative association with Verrucomicrobiota and Euryarchaeota and positive correlation with the phylum Bacillota. In addition, both Pseudomonadota and Verrucomicrobiota are directly correlated with some HAs (MeIQx, DiMeIQx, and PhIP) and nitrates. Verrucomicrobiota is also significantly associated with some bioactive dietary components, as well as Euryarchaeota and Bacteroidota. Thus, these three phyla (and the family Prevotellaceae within the phylum Bacteroidota) are inversely correlated with starch, (poly)phenols and phenolic acids, and lignans, respectively. It has been generally reported that the intake of dietary sources rich in (poly)phenols and/or fiber can shape the GM by promoting the abundance of beneficial bacteria and inhibiting some pathogenic microbial groups (69–71) whereas food chemicals can disrupt human GM and impact negatively the intestinal homeostasis (72). Studies focusing on the association between xenobiotics and GM are still scarce and data available are mainly from *in vitro* models and *in vivo* murine models. In this regard, Kim and Hur (73) found during *in vitro* simulated human digestion, that the mutagenicity of HAs was drastically reduced in the presence of enterobacteria, *Escherichia coli* and *Lactobacillus sakei*. Ribière et al. (74) evidenced in a murine model that oral exposure to B(a)P induced an increase in the relative abundance of Bacteroidaceae, Porphyromonadaceae and Paraprevotellaceae and decreased Lactobacillaceae and

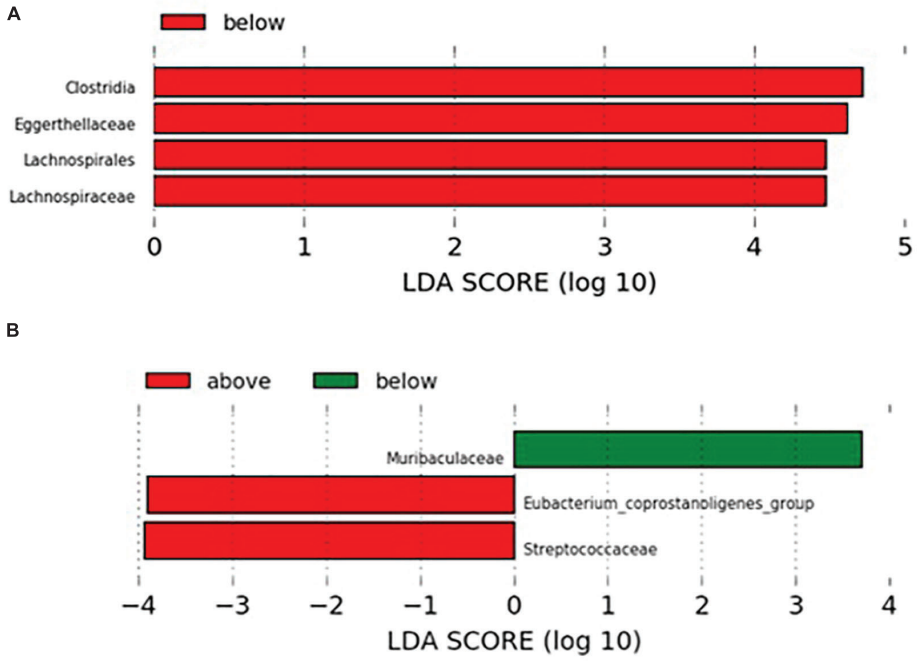

FIGURE 3
LEfSe analysis of microbial taxa differentially altered as a function of safety intake thresholds for some xenobiotics. **(A)** MeIQx at levels below (n = 11) or over (n = 8) the risk dose (50 ng/day) (54) and **(B)** PhIP at levels below (n = 12) or over (n = 7) the risk dose (40 ng/day) (54). Only families with relative abundance of at least 1% in at least two samples were considered in the analysis.

Verrucomicrobioaceae families. Furthermore, the genus *Bifidobacterium* and families Coriobacteriaceae, Rikenellaceae and Desulfovibrionaceae increased in the presence of this xenobiotic derived from food processing.

Consistent with some of the associations found for HAs and the most abundant intestinal microbial taxa, we evidenced differentially higher abundance of the genus *Streptococcus* and members of the *Eubacterium coprostanoligenes* group in the GM of individuals with daily intake of PhIP in doses considered at higher risk. In contrast, individuals with lower intake of this xenobiotic displayed differentially higher abundance of the family Muribaculaceae. Differentially higher abundance of Eggerthellaceae and Lachnospiraceae was found in those individuals with daily intake of MeIQx below the doses considered at risk. *Eubacterium coprostanoligenes* has been related with the maintenance of intestinal mucosal barrier function (75), so that their differentially higher levels in individuals with higher intake of PhIP may be interpreted as a reinforcement of the protection of the intestinal mucosa against moderately high intake of this xenobiotic. In contrast, Lachnospiraceae tends to be differentially reduced in pathological states (76–78) whereas Muribaculaceae, a recently described family, has been related with long-term health effects (79, 80).

Interpreting the findings on the relationship between xenobiotics and GM obtained in our human sample is challenging. The scientific literature currently available generally describes positive association of the genus *Akkermansia* (phylum Verrucomicrobiota) with dietary resistant starch (81), which is apparently contradictory with our results. These differences could be partially related to the inverse relationship between the levels of Clostridia and Lachnospirales (Bacillota phylum) and Eggerthellaceae (Actinobacteriota phylum) and MeIQx intake, as well as to the existence of some key species in the degradation of resistant starch, such as *Ruminococcus bromii* (82). The previously reported negative association between resveratrol with the intestinal microbial genus *Methanobrevibacter* (Euryarchaeota phylum) in humans is according to our results and could be related with the complex crosstalk among the (poly)phenols consumption, intestinal permeability and GM composition (83). The inverse association between the relative abundance of Prevotellaceae (phylum Bacteroidota) and lignans could be related with some other positive association found by us at the family level for other members of the GM with lignans, as it is the case of Peptostreptococcaceae and Coriobacteriaceae (genus *Eggerthella*), two groups of microorganisms with strains able to participate in the metabolization of lignans (26). Other

positive and negative associations found in the present work between xenobiotics (toxic and bioactive compounds) and GM may be due to changes in the relative abundance of the different microbial taxa at a variable extent, depending on their interaction with dietary xenobiotics. Our work suggests a comparatively higher potential carcinogens exposure and a lower consumption of protective bioactive compounds in the healthy vulnerable population under study with respect to other groups at the same geographical location (62). This was accompanied by differential intestinal microbial altered profiles in those individuals with intake of certain xenobiotics over the risk threshold, which can potentially increase the risk of long-term non-communicable diseases. Comparing dietary habits of African American volunteers (a population presenting increased incidence and mortality by colorectal cancer) and Caucasian Americans evidenced higher intake of HAs and decreased intake of vitamins, including vitamin D in the first group, which was correlated with differences in GM composition (84). Groups of economically and socially vulnerable individuals may be susceptible for early basic nutritional interventions to improve their nutritional and GM profile if these results will be confirmed in future studies.

Conclusion

The results obtained point to a possible association between potential carcinogens in the diet and the composition of the GM in subjects with a low socioeconomic level, despite the limited sample size of this work. However, when extrapolating the results, it should be taken into account the proportion of gender in the sample and the high BMI, both factors that could influence the composition of the GM. If confirmed in future studies, these data would serve to evidence the need for strategies aimed at nutritional intervention in these groups for the promotion of health.

Data availability statement

The raw data presented in the study are deposited in the NCBI BioProject repository, accession number: PRJNA870886.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Hospital Universitario Central de Asturias (CEImPA2021.307). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SG and CR-G designed the study. SG and AZ recruited the participants. AZ performed the nutritional assessment and statistical analysis. SA, SR-S, MG-M, NS, AN, and MG contributed and assisted to methodology and analytical tools. SG, CR-G, and AZ drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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MATERIAL SUPLEMENTARIO

Tabla Suplementaria 1.1. Correlación en los niveles de ingesta de xenobióticos estimados a través de los métodos CFCA y R24h.

Xenobiótico	(n=39)
Aminas heterocíclicas	
MeIQ	0,20
MeIQx	0,45**
DiMeIQx	0,37*
PhIP	0,39*
Hidrocarburos aromáticos policíclicos	
B(a)P	0,10
DiB(a)A	0,04
HAP totales	0,68**
Nitratos	0,29
Nitritos	0,31
Compuestos nitrosos	
NDMA	0,35*
NPIP	0,38*
NPYR	0,38*
Comb.	0,75**
Acrilamida	0,24

Los datos se presentan como índices de correlación de Spearman. (*) (**) *p* valor <0,05 y 0,01, respectivamente. B(a)P, benzo (a) pireno; CFCA, cuestionario de frecuencia de consumo de alimentos; Comb., combinación de compuestos nitrosos; DiB(a)A, dibenzo (a,h) antraceno; DiMeIQx, 2-amino-3,4,8 trimetilimidazo (4,5-f) quinoxilina; HAP, hidrocarburos aromáticos policíclicos; MeIQ, 2-amino-3,4 dimetilimidazo (4,5-f) quinolina; MeIQx, 2-amino-3,8 dimetilimidazo (4,5-f) quinoxilina; NDMA, N-nitrosodimetilamina; NPIP, N-nitrosopiperidina; NPYR, N-nitrosopirrolidina; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona; R24h, recordatorio de 24 horas.

Objetivo 2

Describir las asociaciones entre el consumo de componentes con potencial carcinogénico o bioactivo y la microbiota intestinal o el sistema inmune.

Algunos grupos microbianos han demostrado, mediante experimentos *in vitro*, su capacidad para modular el efecto carcinogénico de los xenobióticos. Sin embargo, la evidencia disponible hasta el momento es escasa. Por el contrario, existe mayor evidencia del potencial de componentes bioactivos, como las fibras o los polifenoles, para modular la composición microbiana y la inflamación. Por ello, resulta necesario el análisis de las asociaciones entre el consumo de compuestos con potencial carcinogénico o bioactivo, la microbiota intestinal y los parámetros inmunes en la población. Estos resultados contribuirían a ampliar la información disponible en la literatura para la identificación de las posibles vías, por las que a través de la modulación microbiana y de la respuesta inmune, los diferentes componentes dietéticos podrían alterar o promover la homeostasis intestinal. El trabajo desarrollado en relación con el con el Objetivo 2 se recoge en el siguiente Artículo científico:

> **Artículo 2**: Zapico A., Arboleya S., Ruiz-Saavedra S., Gómez-Martín M., Salazar N., Nogacka A.M., Gueimonde M., de Los Reyes-Gavilán C.G., González S. (2022). Dietary xenobiotics, (poly)phenols and fibers: exploring associations with gut microbiota in socially vulnerable individuals. *Frontiers in Nutrition*, *9*, 1000829–1000835. https://doi.org/10.3389/fnut.2022.1000829.

En el **Artículo 2**, en la **Figura Suplementaria 2.1** y en la **Tabla Suplementaria 2.1**, se describen las asociaciones entre la ingesta de xenobióticos, fibras o polifenoles y los marcadores microbiológicos o sanguíneos. Para ello, se analizó la ingesta dietética en individuos en riesgo de inseguridad alimentaria a través de tres R24h, y el consumo de la fibra, los polifenoles y los xenobióticos se cuantificó utilizando la información de Marlett *&* Cheung (Marlett *&* Cheung, 1997), del *Phenol Explorer* (Neveu *et al*., 2010) o del EPIC (Jakszyn *et al*., 2004) entre otros (Campillo *et al*., 2011; De Mey *et al*., 2014; European Food Safety Authority (EFSA), 2008; Falcó *et al*., 2003; Food *&* Drug Administration (FDA), 2015; Hellenäs *et al*., 2013; Konings *et al*., 2003; Lee, 2019; NIH,

2006; Palacios-Colón *et al*., 2022; Park *et al*., 2015; Svensson *et al*., 2003). En estos individuos se recogieron muestras de heces para la determinación de la composición microbiana mediante la amplificación y secuenciación de la región V3-V4 del gen ARNr 16S y la determinación de AGCC mediante CG. Asimismo, se recogieron muestras de sangre para la determinación de los parámetros inmunes en plasma mediante citometría de flujo.

Los resultados obtenidos muestran que, en los individuos en riesgo de inseguridad alimentaria, el consumo de HAP se asoció directamente con los niveles de TNF-α, mientras el consumo de nitritos se asoció con los niveles de MCP-1 (**Figura Suplementaria 2.1A**) y la ingesta de acrilamida se asoció inversamente con *Ruminococcaceae* (**Artículo 2**). Asimismo, en el caso de las AH, se describieron diferencias en la composición microbiana y en parámetros inmunológicos en función del consumo superior o inferior a las dosis de riesgo de adenoma colorrectal descritas (**Tabla Suplementaria 2.1**). En concreto, aquellos con un consumo de MeIQx inferior (<50 ng/d) mostraron niveles superiores de MCP-1, *Eggerthellaceae* y *Lachnospiraceae*. Por otro lado, individuos con ingestas de PhIP superiores (≥40 ng/d) mostraron niveles más elevados de IL-17 y de las abundancias relativas de *Eubacterium coprostanoligenes group* y *Streptococcaceae*, mientras que individuos con ingestas de PhIP inferiores presentaron una mayor abundancia de *Muribaculaceae*.

Por otro lado, el consumo de polifenoles se asoció directamente con los niveles circulantes de IL-2, IL-12 y TGF-β/IL-2 (**Figura Suplementaria 2.1B**) y se correlacionó inversamente con las abundancias relativas de *Clostridia* UCG014 y *Ruminococcaceae* (**Artículo 2**). En el caso de los lignanos, su ingesta se asoció directamente con los niveles circulantes de IL-14 e IL-4 y asociaciones similares se encontraron con subtipos de la fibra solubles e insolubles. En concreto, la ingesta de fibra insoluble se asoció directamente con los niveles de IL-17 e IL-4 e indirectamente con *Clostridia* UCG014; la ingesta de lignina de Klason se asoció directamente con las abundancias relativas de *Bacteroidaceae* y, el consumo de almidón se asoció positivamente con las abundancias de *Eggerthellaceae* y *Lachnospiraceae.*

MATERIAL SUPLEMENTARIO

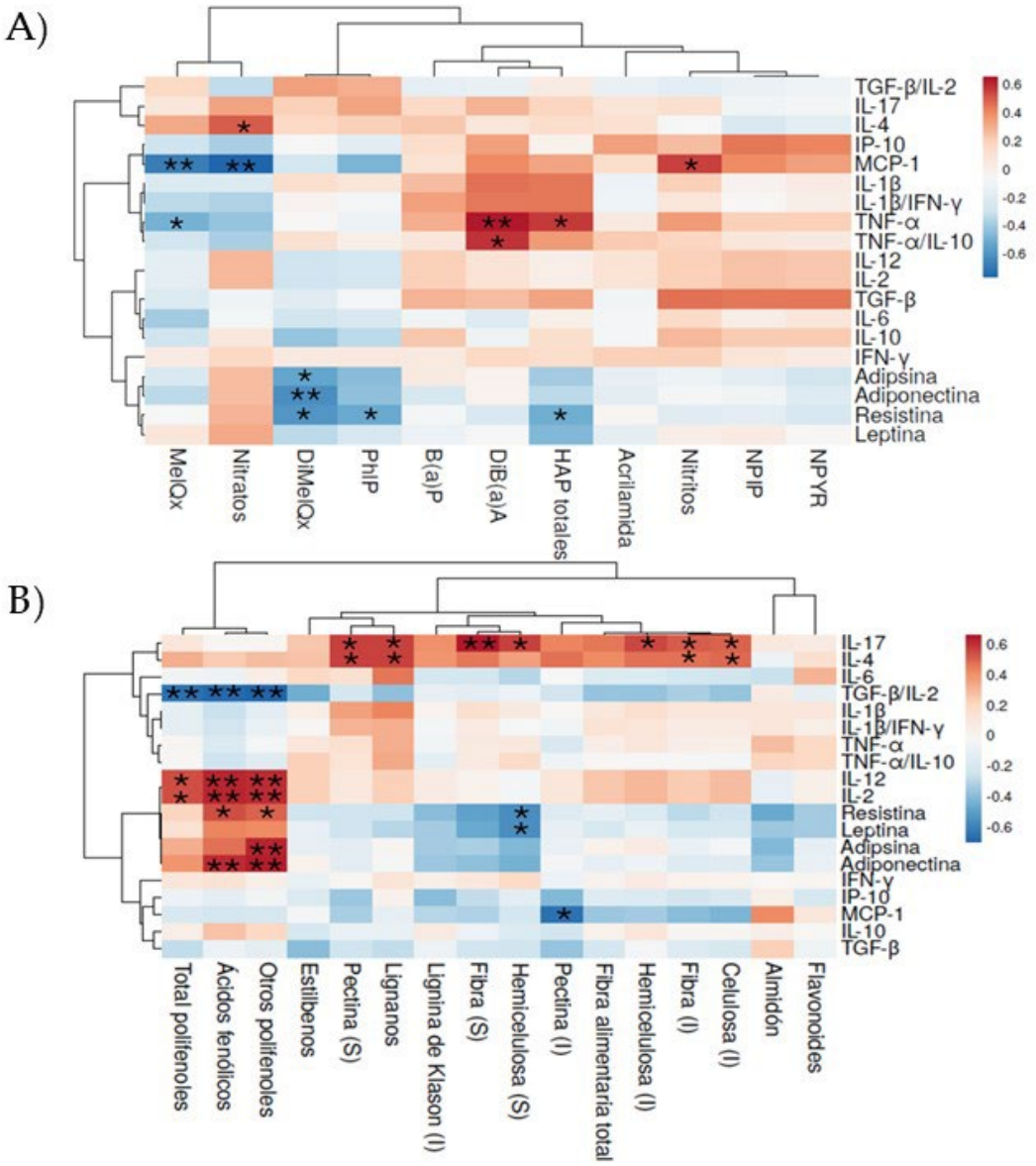

Figura Suplementaria 2.1. **Representación de las correlaciones de Spearman obtenidas en los individuos en riesgo de inseguridad alimentaria entre los niveles circulantes de parámetros inflamatorios (filas) y el consumo de xenobióticos (A), fibras y polifenoles (B) derivados de la dieta (columnas).** (*) (**) *p* valor <0,05 y 0,01, respectivamente**.** B(a)P, benzo (a) pireno; DiB(a)A, dibenzo (a,h) antraceno; DiMeIQx, 2-amino-3,4,8 trimetilimidazo (4,5-f) quinoxilina; HAP, hidrocarburos aromáticos policíclicos; IFN-γ, interferón-γ; IL, interleucina; IP-10; proteína 10 inducida por interferón γ; MCP-1, proteína quimioatrayente de monocitos-1; MeIQx, 2-amino-3,8 dimetilimidazo (4,5-f) quinoxilina; NDMA, N-nitrosodimetilamina; NPIP, N-nitrosopiperidina; NPYR, N-nitrosopirrolidina; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona; TGF-β, factor de crecimiento transformante-β; TNF-*α*, factor de necrosis tumoral-*α*.

Tabla Suplementaria 2.1. Concentración de marcadores sanguíneos relacionados con el estado inflamatorio y metabólico de la muestra en función del nivel de consumo de PhIP y MeIQx ([a]).

(pg/ml)	PhIP		MeIQx	
	<40 ng/d	≥40 ng/d	<50 ng/d	≥50 ng/d
IL-1β	90,34 (67,75-97,86)	94,85 (72,27-152,07)	93,35 (79,80-126,47)	87,33 (65,50-106,90)
IL-2	87,03 (26,73-127,74)	30,57 (19,84-208,09)	54,43 (28,27-76,12)	112,84 (19,84-184,13)
IL-4	131,58 (104,77-205,86)	203,76 (169,94-320,79)	134,20 (123,69-175,02)	249,57 (116,07-319,3)
IL-6	24,29 (20,52-30,66)	21,04 (20,78-24,68)	25,72 (20,52-30,66)	21,43 (20,78-23,51)
IL-10	101,01 (99,09-110,63)	98,12 (87,54-107,74)	101,01 (99,09-107,74)	99,57 (87,06-110,63)
IL-12	66,87 (20,71-97,63)	23,67 (15,38-157,39)	42,01 (21,89-58,58)	86,39 (15,38-139,64)
IL-17	78,03 (68,05-87,28)	97,67 (93,22-123,76)*	87,28 (69,52-122,26)	88,77 (70,26-96,56)
IFN-γ	13,20 (12,02-16,20)	13,69 (13,36-15,36)	13,36 (12,02-16,20)	13,53 (13,20-14,53)
TGF-β	20,37 (16,17-22,78)	17,97 (16,77-25,79)	20,37 (16,77-25,79)	17,97 (15,87-21,58)
TNF-α	32,64 (30,68-37,21)	32,64 (30,68-48,96)	34,60 (31,33-38,52)	31,01 (26,44-33,30)
IP-10 (ng/ml)	83,39 (76,00-113,99)	88,66 (67,55-120,33)	92,88 (76-113,99)	81,28 (71,78-102,39)
MCP-1	45,78 (35,94-59,55)	37,25 (30,70-43,81)	45,12 (43,81-59,55)	35,94 (33,32-39,88)*
Resistina (ng/ml)	15,52 (7,21-48,09)	3,85 (2,74-15,20)	7,87 (3,23-15,20)	14,76 (4,77-50,30)
Leptina (ng/ml)	78,54 (66,92-119,46)	29,82 (11,27-87,21)	66,92 (11,27-80,45)	94,49 (30,78-169,92)
Adipsina (μg/ml)	154,35 (33,41-178,95)	91,59 (0,02-149,32)	132,45 (0,02-170,05)	120,05 (45,80-164,14)
Adiponectina (μg/ml)	7,19 (4,31-9,47)	3,80 (0,03-8,96)	4,63 (0,06-9,60)	5,66 (1,92-8,30)
TGF-β/IL-2	0,32 (0,14-0,77)	0,59 (0,20-0,78)	0,59 (0,37-0,76)	0,20 (0,13-0,83)
TNF-α/IL-10	0,32 (0,29-0,36)	0,31 (0,28-0,50)	0,34 (0,31-0,37)	0,31 (0,28-0,34)
IL-1β/IFN-γ	6,97 (5,54-7,73)	6,18 (4,28-11,11)	7,39 (5,54-7,87)	6,09 (4,51-7,61)

Los datos se presentan como mediana (P_{25} – P_{75}). ([a])Dosis a partir de la cual se incrementa el riesgo de adenoma colorrectal (Martínez Gongora *et al*., 2019). (*) *p* valor <0.05 por prueba U de Mann-Whitney. IFN-γ, interferón-γ; IL, interleucina; IP-10; proteína 10 inducida por interferón γ; MCP-1, proteína quimioatrayente de monocitos-1; MeIQx, 2-amino-3,8 dimetilimidazo (4,5-f) quinoxilina; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona; TGF-β, factor de crecimiento transformant-β; TNF-α, factor de necrosis tumoral-α.

Objetivo 3

Evaluar el impacto de la Dieta Mediterránea sobre la microbiota intestinal, el sistema inmune y marcadores biológicos relacionados con el estado de salud.

Los colectivos vulnerables tienen un menor acceso a la compra de productos frescos y muestran en su lugar, una tendencia hacia el consumo de productos ultra-procesados, azúcares y grasas. Este patrón alimentario se asocia con un mayor riesgo de enfermedades no transmisibles como obesidad, depresión o CCR, caracterizadas por alteraciones de la composición microbiana y la promoción de un estado pro-inflamatorio. En contraposición, el patrón mediterráneo promueve la salud intestinal a través de la interacción de componentes como la fibra o los polifenoles con la microbiota intestinal y el sistema inmune. Sin embargo, hasta el momento no se ha evaluado el potencial de la DM para reducir la ingesta de compuestos con potencial carcinogénico y mejorar el estado de salud de individuos en riesgo de inseguridad alimentaria a través de la modulación de la microbiota y de los parámetros inflamatorios. El trabajo desarrollado en relación con el Objetivo 3 se recoge en el siguiente Artículo científico:

> **Artículo 3**: Zapico A., Arboleya S., Salazar N., Perillán C., Ruiz-Saavedra S., de Los Reyes-Gavilán C.G., Gueimonde M., González S. (2023). Impact on fecal microbiota and health-related markers of an intervention focused on improving eating behavior in people at risk of food insecurity. *Nutrients*, *15*(16). https://doi.org/10.3390/nu15163537.

En el **Artículo 3** se describe el impacto de una intervención educacional y dietética de 4 semanas de duración sobre 20 individuos receptores de ayudas alimentarias de la Cruz Roja. La intervención consistió en un taller educacional, en el que se expusieron las bases de la DM de acuerdo con los criterios del proyecto Prevención con Dieta Mediterránea (PREDIMED). Además, se proporcionó a cada voluntario una ayuda económica para la compra de productos frescos junto con material educativo específicamente desarrollado para el estudio: un menú mensual, recetas y una lista de la compra. Antes y después de la intervención se analizó el nivel

de adherencia a las recomendaciones mediante el cuestionario PREDIMED. Además, se registró la ingesta dietética mediante tres R24h y se cuantificó el consumo de macronutrientes, micronutrientes y otros componentes como la fibra, los polifenoles y los xenobióticos utilizando la información del Centro de Enseñanza Superior de Nutrición y Dietética (Farran *et al*., 2004), Marlett *&* Cheung (Marlett *&* Cheung, 1997), del *Phenol Explorer* (Neveu *et al*., 2010) o del EPIC (Jakszyn *et al*., 2004) entre otros (Campillo *et al*., 2011; De Mey *et al*., 2014; European Food Safety Authority (EFSA), 2008; Falcó *et al*., 2003; Food *&* Drug Administration (FDA), 2015; Hellenäs *et al*., 2013; Konings *et al*., 2003; Lee, 2019; NIH, 2006; Palacios-Colón *et al*., 2022; Park *et al*., 2015; Svensson *et al*., 2003). Asimismo, antes y después de la intervención, se recogieron muestras de heces para el análisis del perfil microbiano fecal, mediante la amplificación y secuenciación de la región V3-V4 del gen ARNr 16S, y para la determinación de AGCC, a través de CG. Además, se recogieron muestras sanguíneas para el análisis de los niveles circulantes de parámetros inmunitarios, mediante citometría de flujo. Adicionalmente, se analizaron otras variables indicadoras del estado de salud, tales como la severidad de los síntomas depresivos, mediante el Inventario de Depresión de Beck-II (BDI-II) (Sanz *et al*., 2014); el comportamiento alimentario, a través del Cuestionario Holandés de Comportamiento Alimentario Infantil (DEBQ-C) (Bailly *et al*., 2012) y la percepción sensorial (sensibilidad y discriminación dulce y salina), utilizando el método y concentraciones similares a las de otros autores (Arguelles *et al*., 2007; Málaga *et al*., 2005; Webb *et al*., 2015).

La descripción del impacto de la intervención educacional y dietética sobre la microbiota intestinal, los parámetros inmunes y otros marcadores biológicos relacionados con el estado de salud se muestran en el **Artículo 3** y las **Tabla Suplementaria 3.1-3.3**. Tras la intervención, el 35% de la muestra mejoró al menos 3 variables del cuestionario PREDIMED y estos individuos mostraron un aumento del consumo de verduras (75%) y polifenoles como los lignanos (236%) junto con una disminución en la ingesta de cereales (48%), carnes procesadas (100%) y xenobióticos como DiB(a)A (56%), total de HAP (34%), NPIP (100%) y acrilamida (66%). Además, este grupo mostró una menor abundancia relativa de Actinomycetota tras la intervención (27 a 17%), probablemente debido a las menores abundancias de

Bifidobacteriaceae (12 a 7%), *Coriobacteriaceae* (9 a 5%) y *Eggerthellaceae* (5 a 4%), en paralelo con un incremento de *Ruminococcaceae* (filo Bacillota) y un aumento de los niveles circulatorios de TGF-β/IL-2 (51%). El resto de la muestra mejoró a lo sumo 2 criterios del cuestionario PREDIMED, en paralelo con un aumento del Bacillota (51 a 61%) y una reducción de *Prevotellaceae* (11 a 5%) y *Clostridia* UCG014 (2 a 1%), un incremento de IL-10 y adiponectina (14 y 43%, en cada caso) y una reducción de IL-2, IL-6 e IL-12 (54, 23 y 52%, respectivamente). Asimismo, este grupo mostró una reducción de la severidad de los síntomas depresivos (36%), un incremento de la capacidad de discriminación dulce (1 punto) y una reducción de la alteración del comportamiento alimentario, en concreto, en la conducta emocional (20%) y externa (17%). Los individuos que presentaron mejoras en el índice de severidad de síntomas depresivos en este grupo, mostraron ingestas más elevadas de aceites y grasas (17 *vs.* 12 g/d), junto con patatas y tubérculos (96 *vs.* 25 g/d) tras la intervención, en paralelo con un incremento en la abundancia relativa de *Oscillospiraceae* (2 a 3%) y de TGF-β/IL-2 (0,6 a 1).

ARTÍCULO 3

nutrients

MDPI

Article

Impact on Fecal Microbiota and Health-Related Markers of an Intervention Focused on Improving Eating Behavior in People at Risk of Food Insecurity

Aida Zapico [1,2], Silvia Arboleya [2,3], Nuria Salazar [2,3], Carmen Perillán [1], Sergio Ruiz-Saavedra [2,3], Clara G. de los Reyes-Gavilán [2,3], Miguel Gueimonde [2,3] and Sonia González [1,2,*]

1 Department of Functional Biology, University of Oviedo, 33006 Oviedo, Spain; aida.zapico@ispasturias.es (A.Z.); perillanmaria@uniovi.es (C.P.)
2 Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), 33011 Oviedo, Spain; silvia.arboleya@ipla.csic.es (S.A.); nuriasg@ipla.csic.es (N.S.); sergio.ruiz@ipla.csic.es (S.R.-S.); greyes_gavilan@ipla.csic.es (C.G.d.l.R.-G.); mgueimonde@ipla.csic.es (M.G.)
3 Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), 33300 Villaviciosa, Spain
* Correspondence: soniagsolares@uniovi.es; Tel.: +34-985-104-209

Abstract: Non-communicable diseases are particularly prevalent among low-income individuals and are associated with the consumption of processed foods, fat, and sugars. This work aims to evaluate the impacts of a nutrition education intervention for low socio-economic individuals on sensory perception, health-related parameters and gut microbiota. Twenty low-income adults underwent a 4-week intervention. Dietary information (three 24 h recalls), detection thresholds and discrimination scores (salty and sweet), and severity of depressive symptoms (Beck Depression Inventory-II (BDI-II)) were collected. Fecal microbial composition and short chain fatty acids were determined by 16S ribosomal RNA-gene sequencing and gas chromatography, respectively. After the intervention, 35% of subjects presented higher compliance with dietary recommendations, increased consumption of vegetables and lignans and reduced consumption of processed meats and nitrosamines, together with depleted levels of Actinomycetota. Higher discrimination for salty and sweet and lower BDI-II scores were also obtained. This nutrition education intervention entailed changes in dietary intake towards healthier food options, reduced potentially carcinogenic compounds and improved scores for discrimination and severity of depressive symptoms. The confirmation of these results in future studies would enable the design of strategic policies contributing to the optimal nutrition of materially deprived families through affordable healthy plant-based interventions.

Keywords: vulnerable subjects; sensorial perception; gut microbiota; processed food; depression; sustainable diet

Citation: Zapico, A.; Arboleya, S.; Salazar, N.; Perillán, C.; Ruiz-Saavedra, S.; de los Reyes-Gavilán, C.G.; Gueimonde, M.; González, S. Impact on Fecal Microbiota and Health-Related Markers of an Intervention Focused on Improving Eating Behavior in People at Risk of Food Insecurity. *Nutrients* **2023**, *15*, 3537. https://doi.org/10.3390/nu15163537

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1. Introduction

Solid evidence demonstrates the importance of a healthy lifelong dietary pattern for maintaining overall health status. The growing prevalence of non-communicable diseases worldwide has been associated with the loss of traditional eating patterns and the increased consumption of ultra-processed foods, fat, salt, and refined sugar in the diet [1]. While this situation is widespread among the population in developed countries, it is particularly striking in some groups, such as those at risk of vulnerability who receive food aid [2]. Socio-economically deprived groups exhibit a suboptimal eating behavior, associated with the higher cost of healthy foods and inadequate knowledge and skills in relation to healthy food choice and food preparation [3–8]. In addition, poor lifestyle habits, such as sedentary behavior or smoking, and stress have frequently been reported in these subjects [9–13].

Recent literature has shown that processed foods have a negative impact on dietary quality and health due to the poor nutrient density, their high additive content, and

association with higher intakes of sugars and trans-fatty acids [14]. Sugar-sweetened beverages and processed meats [14,15] have been associated with an increased risk of non-communicable diseases including colorectal cancer [16–18]. Along with that, the scarce consumption of fruits, vegetables and whole grains may lead to a reduced intake of fiber or (poly)phenols, which have been identified as key players in microbiota modulation in addition to their anti-inflammatory and antioxidant properties [17,19,20].

Over the last decades, a consensus has been reached on the impact of microbiota alteration in host health [21]. The transition from a Westernized diet to a Mediterranean-type pattern, for 4 weeks, with constant energy intake and physical activity has shown noticeable changes in the intestinal microbiome in people with cardiometabolic risk, with these changes being proportional to the degree of adherence [22]. Regarding individual dietary components, a higher intake of whole grain cereals was associated with an increase in Bifidobacteria in healthy humans [23], whereas a high salt ingestion in experimental animals was linked to a decrease in *Lactobacillus* and Prevotellaceae and to an enrichment in Erysipelotrichaceae and Oscillospiraceae [24,25]. While Erysipelotrichaceae has been associated with inflammation [26], higher levels of bifidobacteria and lactobacilli are recognized as beneficial for human health, improving outcomes such as obesity and depression [27–29]. Some authors have shown an increase in the microbial diversity index with Mediterranean diet (MD) interventions, although others have found the opposite or no association [21]. Throughout the gut–brain axis, microbial metabolites, and immune, neuronal, and metabolic pathways could drive dietary modulation [30]. It is possible that the high prevalence rates of stress, anxiety, and depression, described in people at risk of socio-economic vulnerability, may be associated with unhealthy food preferences. Foods with a high content in sugars and fats have been associated with a self-rewarding effect in response to the increase in cortisol levels [31–33]. In this regard, dietary interventions with a high fiber diet in obese females had a positive impact on stress [34] and depression [35] linked to changes in the abundance of some beneficial species from the intestinal microbiota such as *Bifidobacterium longum* [36].

Based on this evidence, the objective of this study was to evaluate the impact of an educational and dietary intervention on sensorial perception, health-related parameters and the composition and activity of the intestinal microbiota in a group of subjects in a socio-economically vulnerable situation.

2. Materials and Methods

2.1. Participants and Recruitment

The MESAS (Economic, Healthy, and Sustainable Menus) project consists of an educational and dietary program promoted by the Alimerka Foundation for low-income individuals belonging to local assemblies of the Red Cross of Asturias (northern Spain). Volunteers were recruited from the Red Cross of Asturias and informed about the objectives of the study. An informed written consent was obtained before enrolment. Exclusion criteria were to be diagnosed with any gastrointestinal chronic condition or to have consumed antibiotics in the last month.

The Ethical Committee of the Hospital Universitario Central de Asturias (CEImPA2021.307) approved the whole procedure and methodology of this project. The procedures were performed in accordance with the fundamental principles set out in the Declaration of Helsinki, the Oviedo Bioethics Convention, and the Council of Europe Convention on Human Rights and Biomedicine, as well as in Spanish legislation on Bioethics. Directive 95/46/EC of the European Parliament and the Council of October 1995 on the protection of individuals regarding the processing of personal data was strictly followed.

2.2. Study Design

Twenty volunteers were recruited and scheduled for a baseline face-to-face interview conducted by trained interviewers and for blood collection in the week before the dietary and educational intervention. For the collection of fecal samples, participants were provided

with sterile containers and detailed instructions (Figure 1). For the initial sample size and considering microbial relative abundances, the statistical power of our results with a type I error probability of 0.05 was 95–98% (Power and Sample Size Calculation version 3.0.43 (Vanderlbilt University, Nashville, TN, USA)).

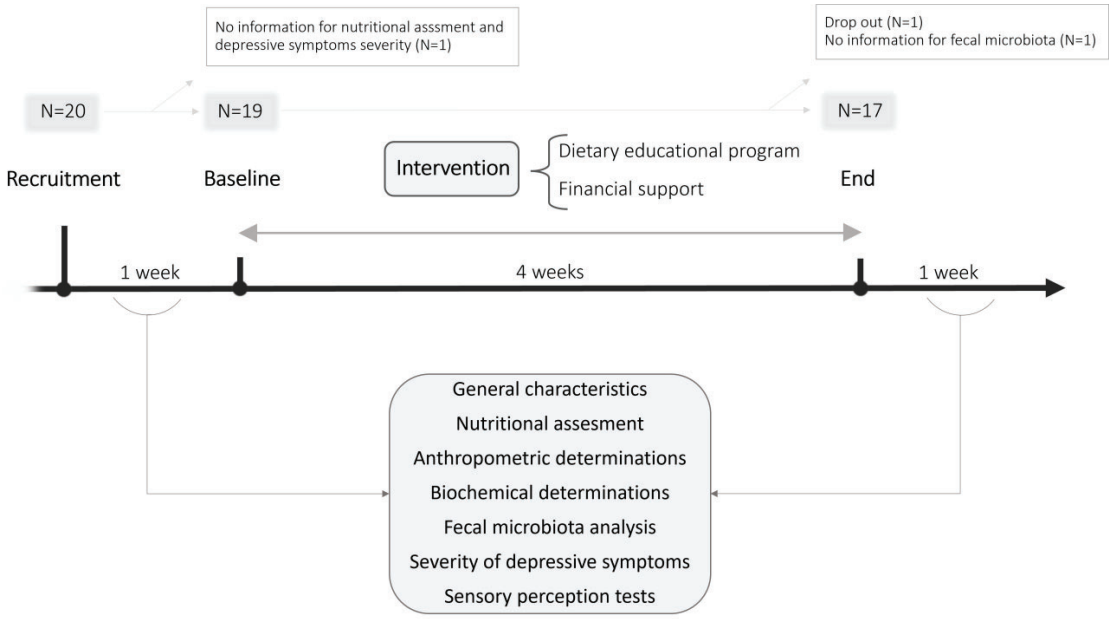

Figure 1. Scheme of study design and timeline.

The intervention consisted of a 1 h educational intervention explaining the basis of a healthy and affordable diet based on the consumption of locally produced fresh products. This activity was complemented with financial support for the acquisition of fresh products provided by Alimerka Foundation, along with dietary education materials (general dietary recommendations, a monthly meal plan, recipes and a shopping list adjusted to the budget). This allowed them to complement the assistance that they receive regularly with non-perishable foodstuffs through the Asturias Red Cross. The material used in the intervention was developed specifically for the purpose of this project and is available at: https://www.fundacionalimerka.es/wp-content/uploads/2022/01/MESAS_guia-completa.pdf (accessed on 18 May 2022) in Spanish language.

At baseline and at the end of the study, general characteristics of the sample population, nutritional assessment, anthropometric determinations, blood analyses and fecal sample collection were assessed, together with a self-completed depression test and sensory perception assays. Participants were encouraged to contact the interviewer if they had any questions or concerns during the study. Seventeen subjects completed the intervention.

2.3. General Characteristics

Information about age, gender, educational level, family size, as well as questions related to energy expenditure, smoking habit, alcohol consumption and the presence of chronic conditions were included in the questionnaire.

The highest educational level attained by each volunteer was registered and classified into primary, secondary, technical or university higher education. The physical activity of the previous week was quantified by using the International Physical Activity Questionnaire (IPAQ) [37,38]. The total metabolic equivalent of task (MET) and the IPAQ classification into three categories of physical exercise were obtained [38].

2.4. Nutritional Assessment

Information regarding the dietary intake of the participants was collected during the face-to-face interviews of no more than 30 min of duration through three nonconsecutive 24 h recalls. In addition, information on cooking habits (boiled, fried, grilled, baked/broiled, or barbecued) and the part of the food that was finally consumed (breast or thigh in the case of chicken) as well as the possible consumption and/or cooking of the skin (cooking with skin and eating the skin; cooking with skin, but not consuming it; and cooking without skin) were included. Standardization of the information reported was achieved by using photographs of different serving sizes and others in which the degree of browning increased progressively, as has been previously reported [18,39].

Food consumption was classified into food groups according to the Centre for Higher Education in Nutrition and Dietetics (CESNID) criteria. CESNID food composition tables were used to transform food consumption into energy and macronutrients intake [40]. United States Department of Agriculture (USDA) and Marlett and Cheung tables were used to calculate the starch and fiber content, respectively [41,42]. (Poly)phenols were extracted from Phenol Explorer version 3.6. [43]. Regarding the consumption of food-derived xenobiotics, the European Prospective Investigation into Cancer and Nutrition (EPIC) Potential Carcinogen Database was the main data source for the content of heterocyclic amines, polycyclic aromatic hydrocarbons (PAHs), nitrates, and nitrites per g of food [44]. When necessary, missing information was completed by additional sources such as the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) database [45], the European Food Safety Authority (EFSA) data [46], the U.S. Food and Drug Administration (FDA) composition tables [47] and others [48–56].

The validated 14-point MD screener "PREvención con Dieta MEDiterránea" (PREDIMED) was used to assess adherence to a Mediterranean pattern [57,58]. The degree of compliance with the dietary intervention was evaluated based on the number of PREDIMED criteria that improved with the intervention. Those subjects that improved 2 or fewer criteria were classified as lower compliance (LC) while those who improved 3 or more items were considered as higher compliance (HC).

2.5. Severity of Depressive Symptoms

Depressive symptoms severity was estimated using the validated 21-item Spanish Beck Depression Inventory-II (BDI-II) [59]. Volunteers rated each item on an intensity scale ranging from 0 to 3, with a maximum possible score of 63 points. The severity of depressive symptoms was categorized as minimal (0–13 points), mild (14–19), moderate (20–28), or severe (29–63) using previously established references [59,60].

2.6. Anthropometric Determinations

Height (m) and weight (kg) were assessed by standardized protocols [61] and body mass index (BMI) was calculated using the formula: weight/(height)^2. Spanish Society for the Study of Obesity (SEEDO) criteria [62] were used to classify subjects as normal weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese ($\geq$30.0 kg/m^2). For body fat percentage, bioelectrical impedance in a calibrated TANITA device (Tanita Corporation of America, Inc., Arlington Heights, IL, USA) was employed, and waist and hip circumferences were measured using an inelastic and extensible tape, as indicated by standard criteria [63].

2.7. Biochemical and Microbiological Analysis

Twelve-hour fasting blood samples were drawn by venipuncture and collected in separate tubes for serum and plasma. The samples were kept on ice and centrifuged (1000$\times$ *g*, 15 min) within 2–4 h after collection. Plasma and serum aliquots were stored at $-$20 °C until analyses. From the blood samples, the biochemical parameters fasting plasma glucose, cholesterol, high- and low-density lipoproteins (HDL and LDL), triglycerides, uric acid, creatinine and iron were determined by standard methods in external laboratories.

Fecal samples were collected within ±24 h of blood collection in sterile containers supplied to each volunteer along with the instructions for sample collection. The samples were frozen after deposition within a period not exceeding two hours and transported to the laboratory. Fecal sample specimens were diluted 1/10 (w/v) in sterile PBS solution and homogenized at full speed in a LabBlender 400 stomacher (Seward Medical, London, UK) for 3 min. The samples were centrifuged for 15 min at 4 °C and 17,530× g and the obtained supernatants were separated from the pellets and kept frozen at −20 °C until use. From the pellet obtained, DNA was extracted in accordance with the Q Protocol for DNA extraction defined by the International Human Microbiome Standards Consortium [64] using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Sussex, UK). The quantification of extracted/purified DNA and the 260/280 ratio were performed using the Take3 Micro-Volume plate and Gen5 microplate reader (BioTek Instrument Inc., Winooski, VT, USA). The DNA obtained was kept frozen at −80 °C until analysis.

The variable region V3–V4 of the bacterial 16S rRNA genes present in each fecal community was amplified by PCR and the resulting amplicons were sequenced on an Illumina NovaSeq 6000 platform instrument (San Diego, CA, USA). The obtained individual sequence reads were filtered to remove low quality sequences. All Illumina quality-approved, trimmed, and filtered data were integrated to generate de novo 16S rRNA Operational Taxonomic Units (OTUs) with ≥97% sequence homology using Uparse software (Uparse v7.0.1090). A classification of all reads to the lowest possible taxonomic rank was performed using Quantitative Insights Into Microbial Ecology (QIIME) and a reference dataset from the SILVA 138 database. The whole procedure of sequencing and annotation was performed at Novogene Bioinformatics Technology Co., Ltd, Cambridge, UK.

Short chain fatty acids (SCFA) were analyzed by gas chromatography from the supernatants of 1 mL of the homogenized feces [65]. A chromatograph 6890N (Agilent Technologies Inc., Palo Alto, CA, USA) connected to a mass spectrometry detector (MS) 5973N (Agilent Technologies) and a flame ionization detector (FID) was used for SCFA identification and quantification, as described in previous works [66].

2.8. Salt and Sweet Sensitivity and Discrimination

Our survey was conducted in early summer in Asturias (Spain) with an average room temperature of 20 °C. The sensitivity and discrimination tests were specifically designed for this research, using concentrations already validated in the literature [67–69].

For the sensory perception tests, NaCl and sucrose were dissolved in mineral water (low mineral content) to create salty and sweet tastes, respectively. Throughout the assessment, plain water was offered for mouth rinsing and the order in which the solutions were tasted was freely chosen. Taste solutions were kept in the dark at 5 °C when not in use.

A test based on exposure to 5 different concentrations of sucrose was designed, including the detection and recognition thresholds developed by Webb et al., (0, 5, 15, 30 and 21,950 mM) [69]. Each concentration was anonymized with a random number, with volunteers having to report detection or not of sweet taste for the sensitivity tests and ordering the 5 concentrations from lowest to highest in order to determine the discrimination of each taste. For each correct answer, 1 score was given. The same procedure was carried out with NaCl for salty taste with 5 different concentrations, including the detection and recognition thresholds developed by Malaga et al., (0, 5, 10, 15 and 50 mM) [68].

Sensory perception tests at baseline and after the intervention were completed by 80% of the individuals selected for this study (n = 14).

2.9. Statistical Analysis

IBM SPSS software version 25.0 (IBM SPSS, Inc., Chicago, IL, USA) was used to analyze all the collected data. Goodness of fit to the normal distribution was checked by means of the Kolmogorov–Smirnov test, and as normality of the variables was not achieved, non-parametric tests were used. Categorical variables were presented as number and percentage (n (%)) and continuous variables as the median and 25th and 75th percentiles (P_{25}–P_{75})

or mean ± SD. For categorical variables Mc Nemar and Fisher tests were performed, and continuous variables were analyzed using the Wilcoxon and Mann–Whitney U tests with Bonferroni correction, for comparisons within each group (T0 vs. T1) and between groups (T0 vs. T0 and T1 vs. T1), respectively (p value < 0.05). GraphPad Prism 8 (La Jolla, CA, USA) was used for graphical representations.

3. Results

3.1. Characteristics of the Study Sample

The general baseline characteristics of the sample are shown in Table 1. The sample comprised 82% adult women (median age 40 years old), and 35% and 24% of the study sample presented occasional alcohol consumption and smoking habit, respectively.

Table 1. General characteristics of the study sample.

	Total Sample n = 17
Age (years)	41 (34–50)
Gender	
Female	14 (82)
Educational level	
Primary	4 (24)
Secondary	4 (24)
Technical	7 (41)
University	2 (12)
Family size(n)	
1–2	6 (35)
3–4	9 (53)
≥5	2 (12)
Lifestyle	
Sleep (hours/day)	6 (5–7)
Physical activity (walking min/day)	60 (21–90)
IPAQ classification	
Low/inactive	3 (18)
Moderate	8 (47)
Vigorous	6 (35)
Total METs	2010 (1315–2772)
Smoking status	
Current smoker	4 (24)
Former smoker	2 (12)
Never smoker	11 (65)
Occasional alcohol consumption [(a)]	6 (35)
Chronic conditions	
Respiratory diseases	9 (53)

Data are expressed as median (P_{25}–P_{75}) and n (%) at baseline. IPAQ, International Physical Activity Questionnaire; METs, metabolic equivalents of task. [(a)] Frequency of consumption was lower than twice a week.

3.2. Effect of the Intervention on Dietary and Nutritional Intake

Differences in the adherence to MD during the study are shown in Figure 2. Higher scores in PREDIMED were observed with intervention. The impact of the intervention on the daily intake of energy and major food groups for both LC and HC groups is shown in Table 2. The HC group significantly increased the daily intake of vegetables (144 to 251 g/day) and decreased the consumption of cereal products (118 to 57 g/day) and processed meats (12 to 0 g/day). Regarding bioactive compounds, subjects with HC showed lower consumption of starch (35 to 2 g/day) and higher consumption of lignans (10 to 32 g/day) after the intervention (Table 3). In addition, the intervention resulted in a reduced level of food-derived xenobiotic compounds in the HC group (Table 4), specifically dibenzo (a) anthracene (4 to 2 ng/day), acrylamide (8 to 3 µg/day), total PAHs (1108 to 373 ng/day) and n-nitrosopiperidine (28 to 0 ng/day). On the other hand, the consumption

of nitrates was increased in both groups after the intervention (30 to 67 mg/day and 101 to 153 mg/day, in the LC and HC, respectively).

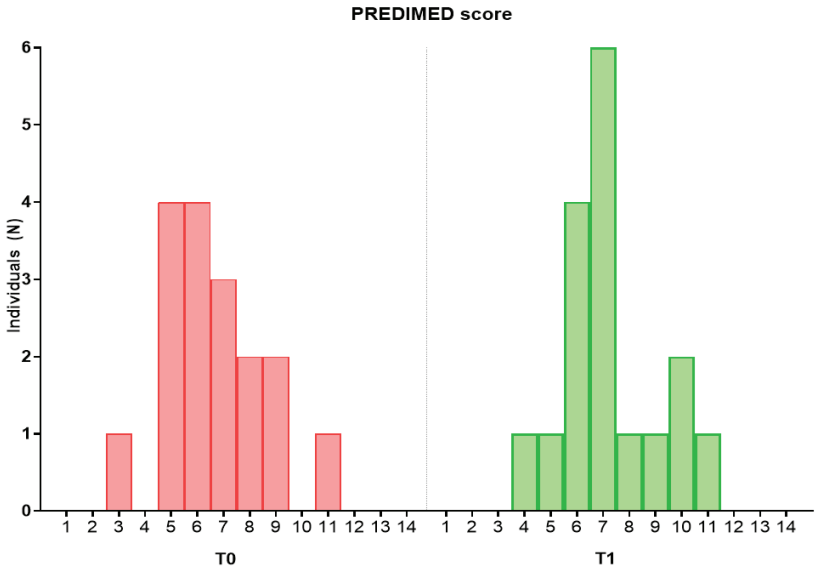

Figure 2. "PREvención con DIeta MEDiterránea" (PREDIMED) score of the sample across the study. T0, baseline; T1, end.

Table 2. Daily intake of energy and main food groups at baseline and after the intervention according to the degree of compliance with the dietary intervention.

	LC n = 11		HC n = 6	
	T0	T1	T0	T1
Energy (Kcal/day)	1471.98 (999.59–1641.27)	1437.07 (1197.17–1576.69)	1524.55 (1262.86–1849.45)	1376.52 (1123.48–1621.64)
Food groups intake (g/day)				
Cereals and cereals products	123.30 (58.33–148.07)	119.97 (94.44–146.20)	117.87 (113.29–174.66)	56.73 (41.20–92.75) * †
Whole grain cereals	0.00 (0.00–0.00)	10.00 (0.00–23.33)	0.00 (0.00–32.50)	4.33 (0.00–30.00)
Milk and dairy products	293.53 (148.23–451.66)	245.90 (179.33–530.68)	123.67 (31.67–187.50)	116.48 (84.17–204.73) †
Meat and meat products	110.00 (93.99–172.48)	94.83 (69.17–175.16)	156.58 (108.83–206.13)	102.98 (76.67–156.83)
White meat	53.58 (33.30–107.95)	55.33 (5.58–108.91)	53.58 (53.33–80.09)	79.02 (0.00–108.91)
Red meat	27.08 (5.90–63.33)	31.25 (0.00–50.00)	38.96 (5.90–110.16)	43.96 (0.00–47.92)
Proccesed meat	13.33 (0.00–31.33)	23.75 (13.33–40.00)	11.67 (6.67–23.33)	0.00 (0.00–0.00) * †
Eggs	53.33 (21.33–69.93)	42.67 (26.67–76.00)	58.30 (45.43–72.00)	34.28 (20.67–42.67)
Fish	21.67 (0.00–33.75)	59.67 (43.33–84.00)	27.97 (0.00–75.00)	61.25 (13.33–94.62)
Seafood	0.00 (0.00–29.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–26.66)
Oils and fats	14.09 (10.50–23.99)	15.53 (11.14–19.11)	21.00 (12.33–25.57)	24.01 (17.00–25.28)
Vegetables	67.71 (34.73–190.00)	98.80 (56.83–111.73)	144.48 (43.37–207.33)	251.35 (169.67–357.00) * †
Legumes	11.67 (0.00–33.33)	12.77 (0.00–46.67)	16.94 (7.77–150.00)	35.00 (0.00–200.00)
Potatoes and tubers	46.14 (8.33–88.83)	62.86 (33.33–123.00)	37.97 (23.00–59.00)	79.42 (24.60–101.00)
Fruits	96.17 (62.50–170.35)	139.35 (42.34–164.58)	151.00 (39.60–336.40)	262.08 (114.76–383.94)
Nuts and seeds	0.00 (0.00–0.00)	0.00 (0.00–0.00)	5.00 (0.00–16.67)	8.34 (0.00–16.67) †
Sugar and sweets	10.00 (0.00–20.80)	11.67 (4.17–18.67)	11.42 (7.00–64.59)	4.67 (0.00–14.00)
Snacks	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–11.67) †
Sauces and condiments	18.50 (1.70–24.30)	13.83 (2.75–20.46)	21.37 (5.37–31.13)	10.14 (1.67–21.26)
Other foods	0.00 (0.00–125.00)	0.00 (0.00–75.00)	5.00 (0.00–83.33)	15.00 (0.00–40.00)
Non alcoholic beverages [(a)]	268.33 (126.67–333.33)	250.00 (183.33–517.67)	345.84 (187.50–403.33)	225.00 (66.67–366.67)
Soft drinks [(a)]	0.00 (0.00–0.00)	0.00 (0.00–83.33)	0.00 (0.00–166.67)	0.00 (0.00–83.33)
Alcoholic beverages [(a)]	0.00 (0.00–0.00)	0.00 (0.00–33.33)	0.00 (0.00–0.00)	0.00 (0.00–0.00)

Data are expressed as median (P_{25}–P_{75}). Statistical differences were found by Wilcoxon (*) and Mann–Whitney U tests (†) for comparisons within each group (T0 vs. T1) and between groups (T0 vs. T0 and T1 vs. T1), respectively (p value < 0. 05). [(a)] (mL/day). LC, lower compliance; HC, higher compliance; T0, baseline; T1, end.

Table 3. Daily intake of bioactive compounds at baseline and after the intervention according to the degree of compliance with the dietary intervention.

Bioactive Compounds	LC n = 11		HC n = 6	
	T0	T1	T0	T1
Total dietary fiber	9.06 (6.77–14.45)	11.37 (9.01–15.73)	13.70 (12.44–15.06)	15.25 (12.37–19.66)
Soluble dietary fiber	1.34 (0.80–1.78)	1.25 (1.01–1.90)	2.00 (1.44–2.29)	1.51 (1.25–2.94)
Insoluble dietary fiber	5.04 (3.72–8.09)	5.85 (4.30–7.63)	9.25 (7.11–11.97) †	7.40 (5.64–12.78)
Starch	17.46 (9.07–35.87)	9.70 (7.44–18.85)	35.19 (10.07–49.84)	1.83 (0.11–14.04) *
Celulose	1.92 (1.66–3.31)	2.25 (1.70–3.30)	3.19 (2.31–3.80) †	2.93 (2.36–4.30)
Klason lignine	0.87 (0.43–1.18)	0.83 (0.73–1.21)	1.47 (1.27–2.04) †	1.15 (0.74–1.54)
Hemicellulose				
Soluble hemicellulose	1.05 (0.47–1.26)	0.85 (0.65–1.31)	1.27 (1.09–1.53) †	0.62 (0.60–1.60)
Insoluble hemicellulose	1.63 (1.18–2.71)	1.87 (1.47–2.40)	2.89 (2.51–3.63)	2.09 (1.88–4.32)
Pectin				
Soluble pectin	0.36 (0.26–0.47)	0.50 (0.30–0.57)	0.56 (0.32–0.65)	0.79 (0.45–1.23)
Insoluble pectin	0.61 (0.39–0.94)	0.74 (0.44–0.92)	1.05 (0.59–1.28)	1.26 (1.05–1.72)
Total (poly)phenols	346.16 (206.13–1208.90)	907.60 (552.78–1082.57)	869.88 (738.35–1211.61)	841.02 (795.02–913.25)
Flavonoids	14.70 (6.30–117.65)	61.82 (45.13–110.77)	105.13 (16.54–183.03)	57.23 (18.16–142.95)
Phenolic acids	186.24 (80.77–361.26)	271.21 (177.11–573.50)	589.79 (226.37–888.03)	324.55 (272.85–643.02)
Lignans	13.33 (4.32–27.69)	8.45 (4.79–16.00)	9.51 (8.73–14.92)	31.96 (18.06–51.23) * †
Other (poly)phenols	7.48 (2.12–16.29)	7.52 (6.05–11.87)	12.49 (9.79–22.45)	9.93 (6.85–36.28)
Stilbenes	0.00 (0.00–0.03)	0.01 (0.00–0.07)	0.01 (0.00–0.02)	0.00 (0.00–0.02)

Data are expressed as median (P_{25}–P_{75}). Statistical differences were found by Wilcoxon (*) and Mann–Whitney U tests (†) for comparisons within each group (T0 vs. T1) and between groups (T0 vs. T0 and T1 vs. T1), respectively (p value < 0.05). LC, lower compliance; HC, higher compliance; T0, baseline; T1, end.

Table 4. Daily intake of food-derived xenobiotic compounds at baseline and after the intervention according to the degree of compliance with the dietary intervention.

Xenobiotics	LC n = 11		HC n = 6	
	T0	T1	T0	T1
Heterocyclic amines (ng/day)				
IQ	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)
MeIQ	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–6.84)
MeIQx	47.91 (0.00–65.45)	81.47 (10.66–112.83)	36.73 (7.08–154.13)	54.63 (8.00–84.08)
DiMeIQx	2.33 (0.00–23.35)	33.20 (0.00–66.40)	1.18 (0.00–70.46)	25.00 (16.00–37.99)
PhlP	7.00 (0.00–298.97)	580.97 (10.94–1162.04)	66.50 (0.00–1743.00)	397.55 (4.00–603.31)
Polycyclic aromatic hydrocarbons (ng/day)				
B(a)P	36.30 (21.80–50.40)	46.10 (24.40–63.80)	52.20 (33.20–68.90)	36.40 (35.20–41.40)
DiB(a)A	3.20 (2.00–5.10)	4.30 (2.00–6.60)	4.30 (2.50–22.60)	2.40 (1.20–3.50) *
Total PAHs	616.10 (266.10–1190.70)	623.50 (368.60–1092.80)	1108.20 (660.70–1347.00)	372.80 (134.80–727.80) *
Nitrates. nitrites and nitroso compounds (ng/day)				
Nitrates (mg/day)	30.62 (17.77–48.18)	67.45 (24.00–100.83) *	100.77 (27.73–119.99)	153.26 (126.99–182.58) * †
Nitrites (mg/day)	0.63 (0.31–1.06)	1.24 (0.65–1.81)	0.65 (0.43–0.78)	0.36 (0.30–0.83) †
NDMA	34.70 (3.20–85.30)	86.70 (44.20–112.70)	24.90 (19.80–34.70)	0.00 (0.00–90.50)
NPIP	25.30 (0.00–45.50)	36.10 (24.00–63.30)	15.80 (8.00–25.30)	0.00 (0.00–0.00) * †
NPYR	45.30 (0.00–68.00)	64.60 (29.00–105.30)	28.30 (11.50–45.30)	0.00 (0.00–0.00) †
Comb	0.00 (0.00–0.00)	0.00 (0.00–4.70)	0.70 (0.00–1.90)	0.00 (0.00–0.00)
Acrylamide (µg/day)	8.73 (6.44–11.62)	12.24 (7.70–20.21)	8.44 (7.22–10.44)	2.83 (1.51-6.13) * †

Data are expressed as median (P_{25}–P_{75}). Statistical differences were found by Wilcoxon (*) and Mann–Whitney U tests (†) for comparisons within each group (T0 vs. T1) and between groups (T0 vs. T0 and T1 vs. T1), respectively (p value < 0.05). B(a)P, benzo (a) pyrene; Comb, Combined nitroso compounds; DiB(a)A, dibenzo (a) anthracene; DiMelQx, 2-amino-3,4,8 trimethylimidazo (4,5,f) quinoxaline; HC, higher compliance; IQ, 2-amino-3-methylimidazo (4,5,f) quinoline; LC, lower compliance; MelQ, 2-amino-3,4 dimethylimidazo (4,5,f) quinoline; MelQx, 2-amino-3,8 dimethylimidazo (4,5,f) quinoxaline; NDMA, N-nitrosodimethylamine; NPIP, n-nitrosopiperidine; NPYR, n-nitrosopyrrolidine; PAH, polycyclic aromatic hydrocarbons; PhlP, 2-amino-1-methyl-6-phenylimidazo (4,5,b) pyridine; T0, baseline; T1, end.

3.3. Effect of the Intervention on Anthropometric and Biochemical Parameters, Severity of Depressive Symptoms and Sensitivity and Discrimination for Salt and Sweet Taste

No significant changes were found in anthropometric values (Table S1) or biochemical parameters (Table S2) after the intervention except for total body fat, which increased in volunteers with HC (32 to 35%). The severity of depressive symptoms was improved in both the LC and HC groups. The proportion of individuals with an ameliorated BDI-II total score for depressive symptoms after the intervention was higher in the HC group (83 vs. 64%), whereas a significant lower BDI-II score after the intervention was only found in the LC group (score change from 14 to 9) (Figure 3, Table S3). Regarding the sensory tests, no significant differences were observed in salt and sweet detection thresholds after the intervention, whereas discrimination scores were increased in both LC and HC groups (Figure 4, Table S4). The proportion of individuals with a higher discrimination score after the intervention was higher in the HC group (67 vs. 50%), although the observed changes reached statistical significance only for sweet taste in the LC (score change from 3 to 4) (Figure 4, Table S4).

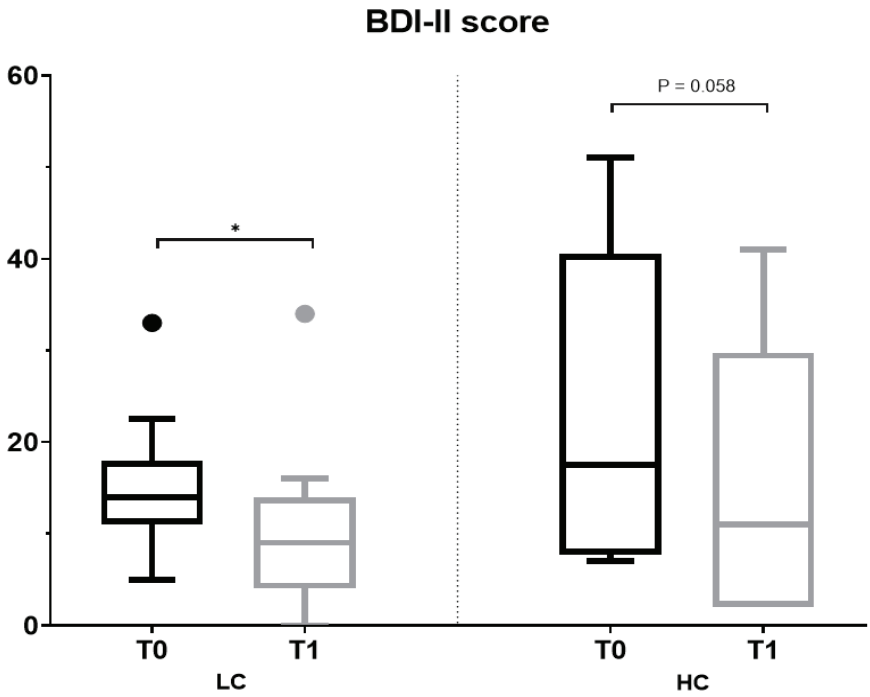

Figure 3. Analysis of Beck Depression Inventory-II (BDI-II) total score at baseline and after the intervention by the degree of compliance with the dietary intervention. (*) Statistical differences were found by Wilcoxon test for comparisons within each group (T0 vs. T1) (p value < 0.05). HC, higher compliance; LC, lower compliance; T0, baseline; T1, end.

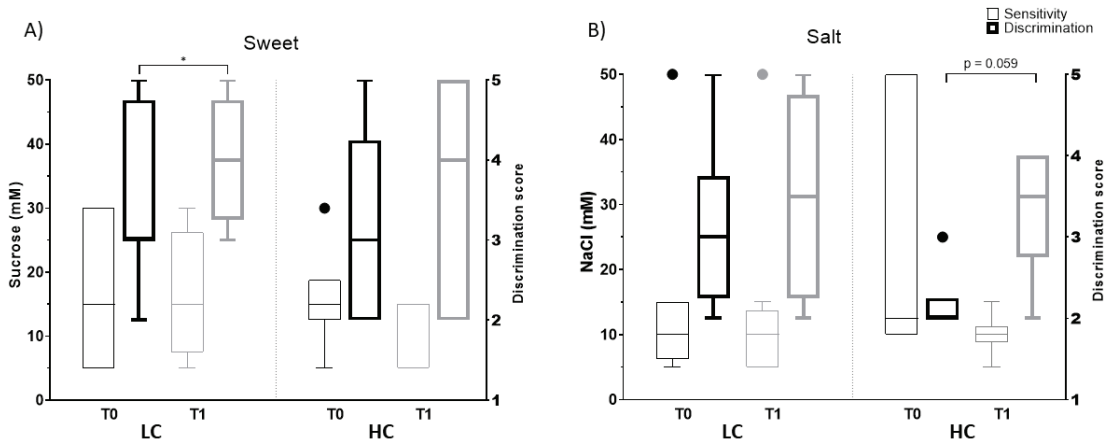

Figure 4. Analysis of sensitivity thresholds (left Y axis) and discrimination scores (right Y axis) for sweet (**A**) and salt (**B**) at baseline and after the intervention by the degree of compliance with the dietary intervention. Statistical differences were found by Wilcoxon (*) for comparisons within each group (T0 vs. T1) (*) (p value < 0.05). HC, higher compliance; LC, lower compliance; T0, baseline; T1, end.

3.4. Effect of the Intervention on the Fecal Profile of Microbiota and SCFA

No differences were observed in the Shannon index or the observed species across the study (Figure 5A,B). The distribution of microbiota relative abundances is shown in Figure 5C,D) and Table S5. Those with LC presented an increase in Bacillota (51 to 61%), and the Oscillospiraceae family in this phylum increased from 2 to 3%. This was accompanied by a reduction in Prevotellaceae (Bacteroidota phylum) from 11 to 5%. Individuals with HC were characterized by a depletion of Actinomycetota (27 to 17%) and, within this phylum, of the families Bifidobacteriaceae, Coriobacteriaceae and Eggerthellaceae (12 to 7%, 9 to 5% and 5 to 4%, respectively) after the intervention. Regarding SCFA, only caproic acid was found to be 15% lower after the intervention in the LC group (Figure 6).

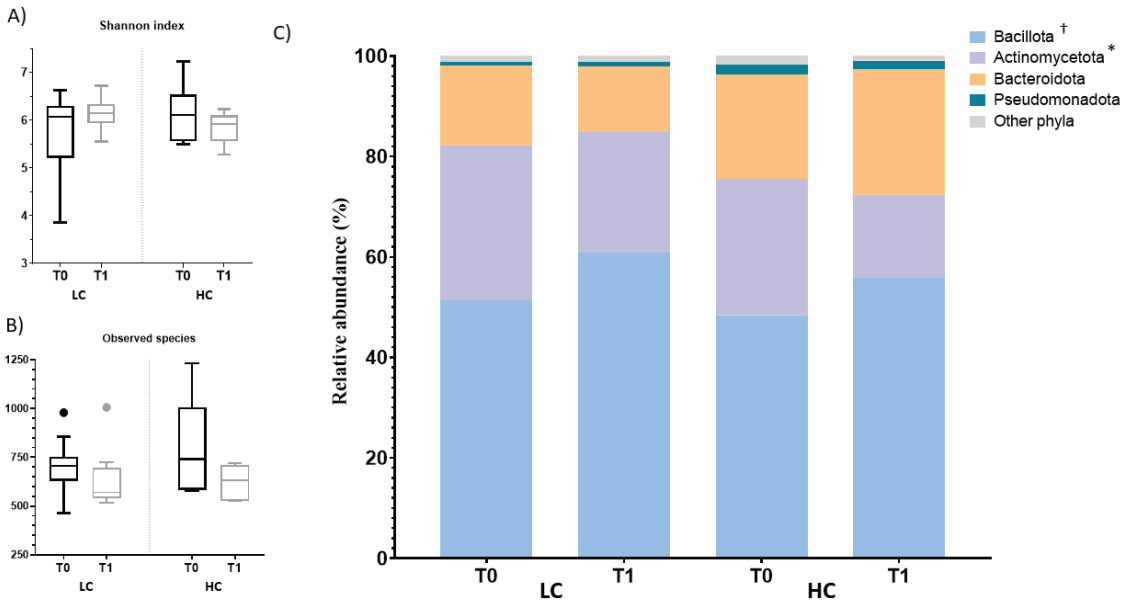

Figure 5. *Cont.*

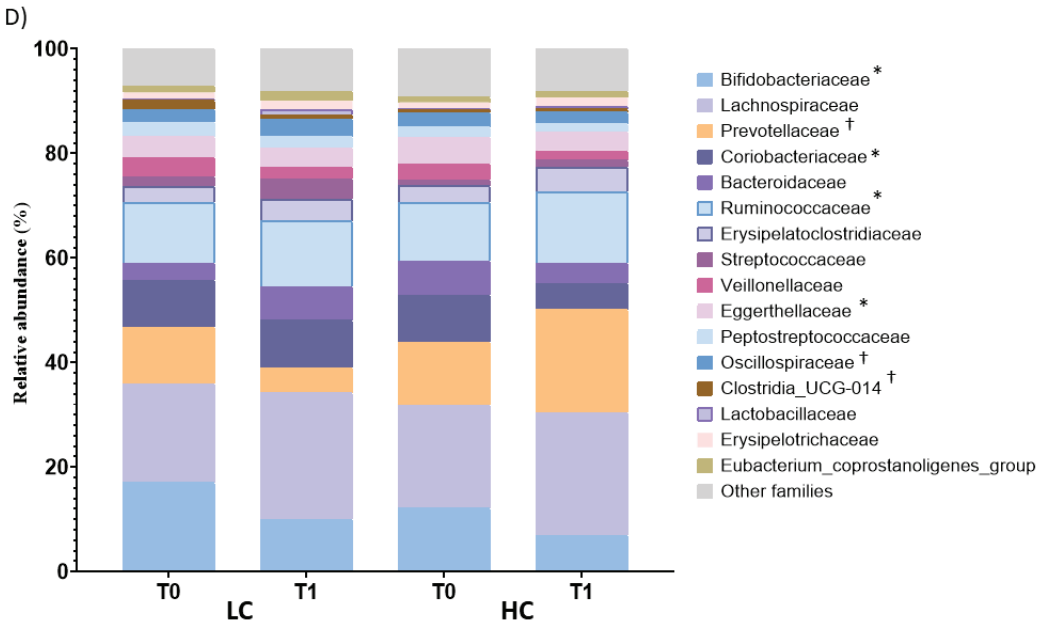

Figure 5. Analysis of microbiota diversity indexes and microbiota relative abundance profile composition at baseline and after the intervention by the degree of compliance with the dietary intervention. Box plot of (**A**) Shannon and (**B**) Observed species. No significant differences were found by Wilcoxon test (p value < 0.05). Microbiota relative abundance distribution to (**C**) phylum and (**D**) family level. Statistical differences were found by Wilcoxon for comparisons within each group (T0 vs. T1) in the LC (†) and HC (*) (p value < 0.05). Only taxa with relative abundance greater than 1% in mean values and in at least two samples are presented. HC, higher compliance; LC, lower compliance; T0, baseline; T1, end.

3.5. Dietary Intake and Shifts of Microbiota Profile Composition among Individuals with Reduced Severity of Depressive Symptoms

Both groups had improved BDI-II scores after the intervention (Figure 3). Subjects from the LC group with better BDI-II scores presented higher consumption of oils and fats (17 vs. 12 g/day), potatoes and tubers (96 vs. 25 g/day) (Table 5), together with a higher relative abundance of Oscillospiraceae (2.31 to 3.47%) (Figure 7, Table S6). In the case of the HC group, a reduced consumption of snacks (22 vs. 0 g/day) and soft drinks (317 vs. 0 g/day) was observed for those individuals with better BDI-II scores (p value 0.083) (Table 5), together with shifts in the microbiota profile. These modifications were in line with the general profile change observed in the whole HC group with the intervention (Figure 5C,D): reduced abundances of Actinomycetota and the families Coriobacteriaceae and Eggerthellaceae, and higher abundances of Ruminococcaceae (p value 0.043).

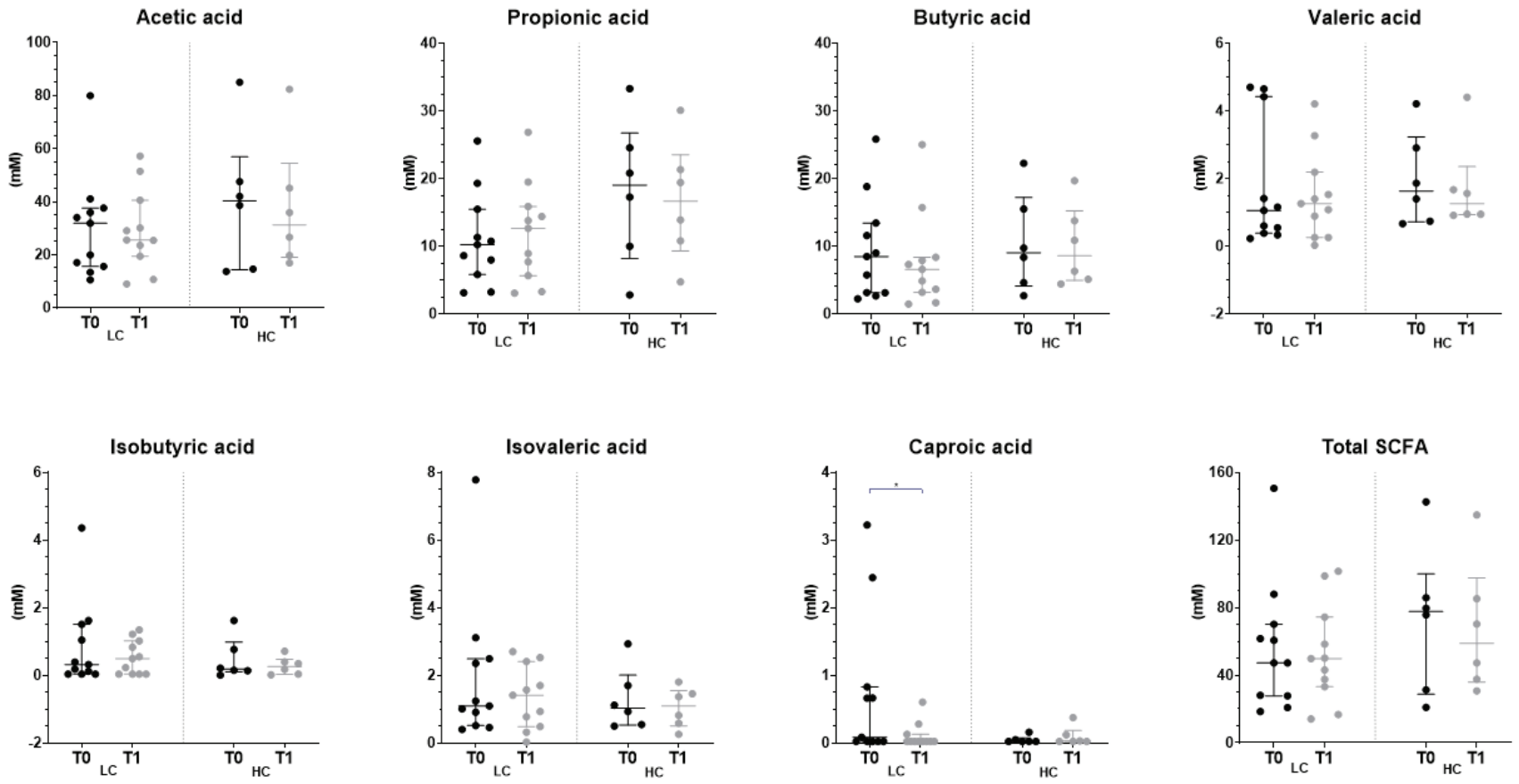

Figure 6. Analysis of levels of fecal SCFA at baseline and after the intervention according to the degree of compliance with the dietary intervention. Box plot data are represented as median and interquartile range. Statistical differences were found by Wilcoxon (*) for comparisons within each group (T0 vs. T1) (p value < 0.05). HC, higher compliance; LC, lower compliance; T0, baseline; T1, end; Total SCFA, sum of acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid, isovaleric acid and caproic acid; SCFA, short-chain fatty acid.

Table 5. Daily intake of energy and food groups at the end of the study according to the degree of compliance with the dietary intervention and the improvement of depressive symptoms.

	LC		HC	
	Improved BDI-II Score n = 7	**No Improvement of BDI-II Score n = 4**	**Improved BDI-II Score n = 5**	**No Improvement of BDI-II Score n = 1**
Energy (kcal/day)	1437.07 (1977.06–1250.26)	1317.42 (1467.85–1196.86)	1255.91 (1497.14–1123.48)	2101.11 (2101.11–2101.11)
Food groups intake (g/day)				
Oils and fats	17.00 (15.53–21.16)	11.46 (6.41–13.01) *	24.15 (17.00–25.28)	23.86 (23.86–23.86)
Olive oil	13.33 (11.00–17.00)	6.89 (4.06–9.83)	16.33 (11.00–17.48)	15.45 (15.45–15.45)
Cereals and cereals products	119.97 (94.44–147.27)	117.06 (67.89–139.27)	46.27 (41.20–67.19)	136.44 (136.44–136.44)
Whole grain cereals	10.00 (0.00–24.17)	12.27 (4.44–19.50)	0.00 (0.00–30.00)	8.67 (8.67–8.67)
Milk and dairy products	398.34 (215.00–545.84)	176.75 (137.84–355.01)	99.63 (84.17–133.33)	204.73 (204.73–204.73)
Meat and meat products	94.83 (69.17–175.16)	125.87 (60.53–194.29)	128.2 (77.76–156.83)	0.00 (0.00–0.00)
White meat	55.33 (5.58–69.17)	82.12 (26.79–127.42)	80.28 (77.76–108.91)	0.00 (0.00–0.00)
Red meat	31.25 (0.00–58.33)	33.33 (13.33–45.00)	47.92 (40.00–47.92)	0.00 (0.00–0.00)
Proccesed meat	33.33 (10.00–41.33)	21.88 (16.67–25.63)	0.00 (0.00–0.00)	0.00 (0.00–0.00)
Eggs	64.00 (32.00–84.67)	34.67 (13.34–46.84)	25.88 (20.67–42.67)	106.00 (106.00–106.00)
Fish	45.83 (3.00–107.33)	59.67 (53.62–71.84)	90.00 (32.50–94.62)	13.33 (13.33–13.33)
Seafood	0.00 (0.00–19.33)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	26.66 (26.66–26.66)
Vegetables	90.11 (48.83–107.67)	105.27 (77.82–112.70)	193.00 (169.67–309.70)	372.27 (372.27–372.27)
Legumes	12.77 (0.00–53.00)	11.67 (0.00–35.00)	23.33 (0.00–46.67)	200.00 (200.00–200.00)
Potatoes and tubers	95.67 (47.83–150.67)	25.00 (16.52–64.19) *	66.67 (24.60–92.17)	241.00 (241.00–241.00)
Fruits	118.68 (42.34–164.58)	144.68 (69.68–168.9)	372.20 (114.76–383.94)	151.96 (151.96–151.96)
Nuts and seeds	0.00 (0.00–0.00)	0.00 (0.00–0.00)	16.67 (0.00–16.67)	0.00 (0.00–0.00)
Sugar and sweets	11.67 (4.17–18.67)	7.00 (0.00–19.50)	9.33 (0.00–14.00)	0.00 (0.00–0.00)
Snacks	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	21.67 (21.67–21.67)
Sauces and condiments	13.83 (0.67–17.33)	22.28 (6.96–56.22)	5.10 (1.67–15.17)	27.56 (27.56–27.56)
Other foods	0.00 (0.00–83.33)	0.00 (0.00–37.50)	30.00 (0.00–40.00)	0.00 (0.00–0.00)
Non alcoholic beverages (mL/day)	250.00 (183.33–517.67)	276.67 (135.00–462.50)	200.00 (66.67–250.00)	366.67 (366.67–366.67)
Soft drinks (mL/day)	0.00 (0.00–0.00)	41.67 (0.00–151.67)	0.00 (0.00–0.00)	316.67 (316.67–316.67)
Alcoholic beverages (mL/day)	0.00 (0.00–66.67)	2.00 (0.00–18.67)	0.00 (0.00–0.00)	0.00 (0.00–0.00)

Data are expressed as median (P_{25}–P_{75}). Statistical differences were found by Mann–Whitney U tests (*) for comparisons within each (p value < 0.05). LC, lower compliance; HC, higher compliance.

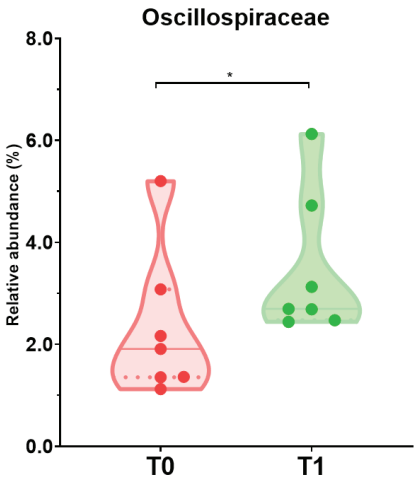

Figure 7. Relative abundance of Oscillospiraceae at baseline and after the intervention in individuals with lower compliance (LC) who presented successful amelioration of severity of depressive symptoms (n = 7). (*) Statistical differences were found by Wilcoxon test for comparisons within each group (T0 vs. T1) (p value < 0.05). T0, baseline; T1, end.

4. Discussion

The present work highlights the impact of a nutrition education intervention in a low-income group of adults on different health-related parameters and gut microbiota profiles.

The starting point was marked by a dietary pattern with a low consumption of vegetables, fruits, and legumes in comparison with the recommendations (at least 400 g/day of vegetables and fruits and 50 g/day of legumes) [70,71]. Alcohol consumption was rarely present in the studied population (12% of the sample), in contrast to previous studies, analyzing the consumption of this toxic as related to the socio-economic status, although a similar proportion of regular smokers was found (24%) [9].

After the intervention, while 64% of the volunteers made between zero and two modifications in the PREDIMED items, 35% increased at least three criteria. This change was characterized by a greater intake of vegetables together with a reduction in the consumption of cereals and processed meats. In consequence, higher levels of some food-derived bioactive dietary components were achieved. Whereas the level of intake of starch was depleted, the ingestion of (poly)phenols, such as lignans, was tripled. High intakes of bioactive dietary components, such as fiber and (poly)phenols, are considered as possible protective factors against non-communicable diseases [17,34], this effect being partially linked to microbiota modulation [72]. In this regard, increased abundance of *Ruminococcus* (Bacillota phylum) and a reduced proportion of Actinomycetota, and the families Bifidobacteriaceae, Coriobacteriaceae and Eggerthellaceae, which belong to this phylum, were observed in the group of volunteers showing HC to the intervention. These results are in agreement with previous studies reporting a lower abundance of Actinomycetota with low-fat and high-fiber diets [73]. Also, fructo-oligosaccharides, fiber and inulin derived from flour-based products, such as cereals, have been shown to increase the relative abundance of *Bifidobacterium* [74]. Therefore, it is plausible that the reduction in the consumption of cereals and processed meat, together with the increase in lignans, could be associated with the shifts in this microbial genus. (Poly)phenols have been shown to inhibit the growth of this genus [75], and lower abundances have already been associated with a higher consumption of (poly)phenols and processed meats in previous works [76].

Depleted abundances of Coriobacteriaceae could be due to the reduced consumption of processed meats. Previous studies have also shown an association between this family, particularly the genus *Senegalimassilia* (also depleted), with starch dietary consumption, whereas starch and *Slackia* (Eggerthellaceae family) have also been associated in previous works [39]. Also, the increased levels of Ruminococcaceae, among other taxa with an affinity for polysaccharides, have been associated with an MD pattern [21]. The intervention did not significantly affect the alpha diversity of the microbiota, which is consistent with the results of a recent review on MD and microbiota [21].

To our knowledge, this is the first study analyzing the impact of a nutrition education intervention on xenobiotics consumption in a sample of low-income individuals. The intervention was effective in decreasing PAHs and nitrosamines. On the contrary, and probably resulting from the higher intake of vegetables during the intervention, an increased intake of nitrates was observed in the entire sample. These results are of great interest in order to strengthen healthy dietary interventions in this population group. According to our initial hypotheses, dietary intervention affects the BDI-II score and gut microbiota composition [77–80]. The depressive severity score was ameliorated in both the LC and HC groups. Although the proportion of subjects showing improvement in the HC group was higher, a significantly improved BDI-II score was only obtained in the LC group. Among these participants, a higher intake of oils and fats, potatoes and tubers and higher relative abundance of Oscillospiraceae were found. Although some authors have found an association between increased relative abundances of this microbial family and depression, the evidence in the literature is still inconsistent [81–84], and depression may remain constant after dietary interventions, according to previous research [85]. In all groups of individuals (LC and HC) an improvement in taste discrimination, both sweet and salty, was observed.

The LC group showed higher discrimination for sweet after the intervention without associated changes in microbial profile. Although it has been reported that sensitivity and discrimination tests can be affected by alcohol consumption, smoking, age, gender, or BMI [86–92], these variables remained constant across the intervention.

For the interpretation of our data, it is important to keep in mind that this segment of the population is often unable to freely choose their dietary pattern. The financial budget provided to each volunteer along with the complex low socio-economic environment in which the people involved live, the limited sample size, and the short duration of the dietary intervention limit the strength and the potential impact of results obtained.

5. Conclusions

In conclusion, this nutrition education intervention for materially deprived subjects demonstrated changes in dietary intake towards healthier food options and lowered the consumption of potentially carcinogenic compounds. Accompanied by shifts in fecal microbiota, this work has shown the potential of a nutrition education intervention to improve the sensitivity and the severity of depressive symptoms. Further research is required to confirm the obtained results in this study. This would allow for the design of future strategic policies that would contribute to the optimal nutrition of materially deprived families through healthy, plant-based affordable interventions. Furthermore, despite the small sample size, to our knowledge, this is the first study analyzing the whole picture of the impact of a nutrition education intervention for materially deprived subjects on diet, bioactive and xenobiotics consumption, fecal microbiota, sensitivity and discrimination of flavors and mood depression.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu15163537/s1. Table S1: Anthropometric parameters at baseline and after the intervention according to the degree of compliance with the dietary intervention; Table S2: Biochemical parameters at baseline and after the intervention according to the degree of compliance with the dietary intervention; Table S3: Beck Depression Inventory-II (BDI-II) categories of depressive symptoms and total score at baseline and after the intervention according to the degree of compliance with the dietary intervention; Table S4: Sensitivity thresholds and discrimination scores for salt and sweet at baseline and after the intervention according to the degree of compliance with the dietary intervention; Table S5: Microbiota diversity indexes and relative abundance profile composition at baseline and after the intervention according to the degree of compliance with the dietary intervention.; Table S6: Microbiota diversity indexes and relative abundance profile composition at baseline and after the intervention in those individuals with LC showing amelioration of depressive symptoms after the intervention.

Author Contributions: S.G. and C.G.d.l.R.-G. designed the study. S.G. and A.Z. recruited the participants. A.Z. performed the nutritional assessment and statistical analysis. C.P. performed the sensory perception tests. S.A., S.R.-S., N.S. and M.G. contributed to and assisted with the methodology and analytical tools. S.G., C.G.d.l.R.-G. and A.Z. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The studies involving human participants were reviewed and approved by the Ethics Committee of the Hospital Universitario Central de Asturias (CEImPA2021.307). The patients/participants provided their written informed consent to participate in this study. The procedures were performed in accordance with the fundamental principles set out in the Declaration of Helsinki, the Oviedo Bioethics Convention, and the Council of Europe Convention on Human Rights and Biomedicine, as well as in Spanish legislation on Bioethics. Directive 95/46/EC of the

European Parliament and the Council of October 1995 on the protection of individuals regarding the processing of personal data was strictly followed.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

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

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Tabla Suplementaria 3.1. Concentración de marcadores sanguíneos, antes y después de la intervención, en función del grado de cumplimiento de las recomendaciones dietéticas.

(pg/ml)	Mejora ≤2 criterios PREDIMED (n=11)		Mejora ≥3 criterios PREDIMED (n=6)	
	T0	T1	T0	T1
IL-1β	94,85 (67,75-152,07)	108,40 (79,80-156,58)	81,31 (72,27-93,35)	73,02 (64,74-82,81)
IL-2	54,43 (26,73-100,28)	25,20 (22,14-27,50)*	115,04 (21,37-187,25)	23,67 (18,31-26,73)
IL-4	155,23 (76,91-205,86)	198,15 (96,18-265,95)	231,61 (134,20-317,81)	168,81 (96,36-268,41)
IL-6	25,72 (20,78-65,99)	19,49 (18,97-20,78)*	20,91 (20,52-21,82)	22,99 (21,56-25,72)
IL-10	101,01 (92,35-106,78)	114,48 (105,82-117,36)*	104,38 (96,20-116,40)	110,63 (92,35-117,36)
IL-12	42,01 (20,71-76,92)	19,53 (17,16-21,3)*	87,87 (16,57-142,01)	18,34 (14,20-20,71)
IL-17	70,26 (68,05-123,76)	95,45 (84,32-117,78)	91,37 (86,54-97,67)	83,58 (82,10-109,58)
IFN-γ	13,36 (12,02-15,36)	15,36 (14,03-16,03)*	14,20 (13,36-16,37)	13,78 (13,53-14,86)
TGF-β	17,97 (16,17-20,98)	25,19 (17,97-35,44)	21,58 (16,17-25,79)	20,68 (17,97-23,98)
TNF-α	33,29 (30,68-48,96)	31,99 (30,03-41,13)	31,66 (30,68-34,60)	31,99 (31,33-36,56)
IP-10 (ng/ml)	92,88 (80,22-113,99)	95,00 (82,33-103,44)	74,95 (67,55-82,33)	80,22 (67,55-90,77)
MCP-1	43,81 (35,94-46,43)	45,12 (33,32-52,99)	36,60 (30,70-46,43)	44,47 (43,81-52,99)
Resistina (ng/ml)	7,21 (3,23-34,68)	12,87 (9,38-34,27)	18,75 (2,90-22,30)	19,25 (7,17-34,76)
Leptina (ng/ml)	66,92 (11,27-80,45)	78,37 (52,88-100,56)	103,34 (13,52-170,61)	69,79 (52,71-105,24)
Adipsina (μg/ml)	96,41 (0,02-170,05)	156,75 (123,84-194,24)	140,89 (0,019,79-178,95)	159,29 (120,10-173,98)
Adiponectina (μg/ml)	4,63 (0,06-8,59)	6,64 (5,11-8,29)*	8,49 (0,03-11,37)	5,90 (5,27-6,62)
TGF-β/IL-2	0,59 (0,15-0,78)	0,89 (0,81-1,13)*	0,28 (0,14-0,76)	0,85 (0,64-0,98)*

Los datos se presentan como mediana (P_{25} – P_{75}). (*) *p* valor <0,05 por prueba de Wilcoxon (T0 *vs*. T1) dentro de cada grupo. IFN-γ, interferón-γ; IL, interleucina; IP-10; proteína 10 inducida por interferón γ; MCP-1, proteína quimioatrayente de monocitos-1; PREDIMED, Prevención con Dieta Mediterránea; T0, basal; T1, tras la intervención; TGF-β, factor de crecimiento transformante-β; TNF-α, factor de necrosis tumoral-α.

Tabla Suplementaria 3.2. Análisis de la conducta alimentaria, antes y después de la intervención, en función del grado de cumplimiento de las recomendaciones dietéticas.

Comportamiento	Mejora ≤2 criterios PREDIMED (n=11)		Mejora ≥3 criterios PREDIMED (n=6)	
	T0	T1	T0	T1
Externo (%)				
No	40 (20-80)	60 (20-80)*	40 (40-60)	40 (20-60)
A veces	0 (0-20)	0 (0-0)	0 (0-20)	20 (0-20)
Sí	40 (20-80)	40 (20-60)	50 (20-60)	30 (20-60)
Emocional (%)				
No	57 (29-71)	71 (57-86)*	57 (29-71)	43 (43-43)
A veces	0 (0-14)	0 (0-0)	7 (0-14)	0 (0-0)
Sí	43 (14-71)	29 (14-43)*	29 (29-57)	57 (43-57)
Restrictivo (%)				
No	71 (71-100)	86 (71-100)	57 (57-71)	64 (43-86)
A veces	0 (0-0)	0 (0-0)	7 (0-29)	0 (0-14)
Sí	14 (0-29)	14 (0-29)	14 (14-43)	21,5 (0-57)

Los datos se presentan como mediana (P_{25} – P_{75}). Basado en cuestionario de DEBQ-C. (Baños *et al* 2011). (*) *p* valor <0,05 por prueba de Wilcoxon (T0 *vs.* T1) dentro de cada grupo. DEBQ-C, Cuestionario Holandés de Comportamiento Alimentario Infantil; PREDIMED, Prevención con Dieta Mediterránea; T0, basal; T1, tras la intervención.

Tabla Suplementaria 3.3. Concentración de marcadores sanguíneos, antes y después de la intervención, en individuos con mejora de la severidad de los síntomas depresivos, en función del grado de cumplimiento de las recomendaciones dietéticas.

(pg/ml)	Mejora ≤2 criterios PREDIMED (n=7)		Mejora ≥3 criterios PREDIMED (n=5)	
	T0	T1	T0	T1
IL-1β	126,47 (88,83-197,23)	124,96 (91,84-269,5)	79,80 (72,27-82,81)	64,74 (64,74-81,30)
IL-2	54,43 (26,73-127,74)	25,20 (22,90-27,50)	160,16 (69,91-187,25)	24,43 (18,31-26,73)
IL-4	203,76 (76,91-267,88)	205,33 (82,17-305,02)	293,28 (169,94-317,81)	209,71 (96,36-268,41)
IL-6	25,72 (22,86-30,66)	19,23 (18,45-21,82)	21,04 (20,78-21,82)	22,34 (21,56-23,64)
IL-10	101,01 (92,35-110,63)	114,48 (107,74-117,36)	107,74 (101,01-116,40)	113,52 (92,35-117,36)
IL-12	42,01 (20,71-97,63)	19,53 (17,75-21,30)	121,89 (53,84-142,01)	18,93 (14,20-20,71)
IL-17	91,73 (57,72-123,76)	106,60 (88,77-127,49)	95,45 (87,28-97,67)	85,06 (82,10-109,58)
IFN-γ	13,69 (12,02-16,20)	15,36 (14,03-18,87)*	15,03 (13,36-16,37)	13,86 (13,69-14,86)
TGF-β	17,97 (16,17-32,42)	27,00 (17,97-37,26)	22,78 (20,37-25,79)	22,78 (17,97-23,98)
TNF-α	38,52 (31,33-50,92)	31,99 (29,38-41,13)	30,68 (30,68-32,64)	31,99 (31,33-31,99)
IP-10 (ng/ml)	109,77 (76,00-113,99)	84,44 (82,33-97,11)	73,89 (67,55-82,33)	84,44 (67,55-90,77)
MCP-1	43,81 (35,94-59,55)	39,88 (33,32-46,43)	35,94 (30,70-37,25)	45,12 (43,81-52,99)
Resistina (ng/ml)	7,87 (3,23-48,09)	17,87 (10,74-51,49)	22,30 (15,20-22,30)	30,09 (7,17-34,76)
Leptina (ng/ml)	75,77 (9,54-80,45)	68,31 (41,26-108,02)	119,46 (87,21-170,61)	67,79 (52,71-71,78)
Adipsina (μg/ml)	143,69 (0,02-179,19)	167,85 (128,92-194,24)	149,32 (132,45-178,95)	155,24 (120,10-163,34)
Adiponectina (μg/ml)	4,63 (0,046-8,59)	6,64 (6,07-7,31)	8,96 (8,01-11,37)	6,28 (5,53-6,62)
TGF-β/IL-2	0,59 (0,12-0,78)	0,98 (0,76-1,43)*	0,20 (0,14-0,37)	0,90 (0,64-0,98)

Los datos se presentan como mediana (P_{25} – P_{75}). (*) p valor <0,05 por prueba U de Mann-Whitney. IFN-γ, interferón-γ; IL, interleucina; IP-10; proteína 10 inducida por interferón γ; MCP-1, proteína quimioatrayente de monocitos-1; PREDIMED, Prevención con Dieta Mediterránea; T0, basal; T1, tras la intervención; TGF-β, factor de crecimiento transformante-β; TNF-α, factor de necrosis tumoral-α.

Objetivo 4

Evaluar el potencial de la ingesta de prebióticos y probióticos como agentes protectores del daño en la mucosa del colon producido por el consumo de xenobióticos a través de la modulación de la microbiota y el sistema inmune.

La fibra alimentaria y los probióticos se han asociado con modulaciones en la composición microbiana y mejoras del estado inflamatorio, promoviendo la homeostasis intestinal y reduciendo el riesgo de CCR. En contraposición, la AH PhIP ha sido clasificada como posible carcinógeno por la IARC y, entre los xenobióticos evaluados, es el que presenta los niveles de ingesta más elevados. Sin embargo, no existe información disponible en la literatura que evalúe el potencial de la fibra o de los probióticos como agentes protectores del daño en la mucosa del colon producido por el consumo de PhIP. Con este fin, el uso del modelo animal PhIP+DSS con dosis de consumo extrapolables a las observadas en humanos, permitiría mimetizar el ambiente colónico derivado de la ingesta de AH durante largos periodos de tiempo. Asimismo, mediante la suplementación con fibra o probiótico sería posible evaluar el potencial protector de estos compuestos junto con los cambios en la microbiota intestinal y los marcadores sanguíneos relacionados con el estado inflamatorio. El trabajo desarrollado en relación con el Objetivo 4, se recoge en el siguiente Artículo científico:

> **Artículo 4**: Zapico, A., Salazar, N., Arboleya, S., González del Rey, C., Diaz, E., Alonso, A., Gueimonde, M., de los Reyes-Gavilán, C.G., Gonzalez, C., González, S. (2024). Potential of fiber and probiotics to fight against the effects of PhIP + DSS-induced carcinogenic process of the large intestine. *Journal of Agricultural and Food Chemistry*, *72*(45), 25161–25172. https://doi.org/10.1021/acs.jafc.4c07366.

En el **Artículo 4** se describe la intervención realizada en ratas macho Fischer 344. Estas se distribuyeron aleatoriamente en 4 grupos (n=11/grupo): control (dieta estándar), grupo PhIP+DSS (dieta estándar + PhIP (1 ppm) y DSS (1,5% p/v)), grupo Probiótico (dieta con probiótico compuesto principalmente por cepas de *Lactobacillus* y *Bifidobacterium* (2,2 x 10^9 UFC/d) + PhIP+DSS) y grupo Fibra (dieta enriquecida en fibra (5,9%) + PhIP+DSS). Tras una semana de aclimatación con dieta estándar, los animales

fueron expuestos durante 3 semanas a PhIP+DSS junto con la dieta correspondiente y, posteriormente, se realizó una semana de descanso sin PhIP+DSS. Diariamente se registró el peso, junto con la ingesta de bebida y comida de los animales por el método de doble pesada. Los parámetros inmunitarios (citometría de flujo) y la capacidad antioxidante total (CAT) (método CUPRAC), se determinaron en muestras de plasma obtenidas al final del estudio. Tras el sacrificio, el colon fue extraído y fijado con formaldehido (10%) para el análisis histológico de secciones representativas que se incluyeron en parafina y se tiñeron con hematoxilina-eosina. La composición microbiana y la determinación de AGCC se realizó en muestras fecales recogidas en cuatro tiempos a lo largo del estudio (inicio, antes y después de la administración de PhIP+DSS y al final de la intervención) mediante la amplificación y secuenciación de la región V3-V4 del gen del ARNr 16S y mediante CG, respectivamente.

Los resultados obtenidos en este trabajo se describen en el **Artículo 4**, la **Tabla Suplementaria 4.1** y la **Figura Suplementaria 4.1**. En comparación con el grupo PhIP+DSS, la ingesta de fibra redujo parcialmente la presencia de inflamación difusa (54%) y la pérdida de criptas (60%), mientras que el probiótico mostró un incremento de la longitud del colon (24%), en paralelo con una tendencia no significativa de reducción del daño histológico. Además, la abundancia relativa de *Clostridiaceae* y los niveles circulantes de IL-6 (**Figura Suplementaria 4.1A**) se asociaron con un mayor daño en la mucosa en el grupo PhIP+DSS y, tras la intervención, estos animales mostraron un incremento en las abundancias relativas de *Clostridia* UCG014, la cual se redujo tras la suplementación con fibra (4,0 *vs*. 9,0%) y con probiótico (2,5 *vs*. 9,0%). Adicionalmente, la ingesta de fibra incrementó los niveles de IL-17F (5 *vs.* 4 pg/ml) (**Tabla Suplementaria 4.1**), junto con la abundancia de *Bacteroidaceae* y *Bacteroides* (6,1 *vs*. 1,9%) y una disminución en los niveles de *Monoglobaceae* y *Monoglobus* (0,4 *vs*. 1,2%). Asimismo, la ingesta de probiótico aumentó la excreción del ácido valérico (0,54 *vs*. 0,32 μmol/g) y redujo las abundancias relativas de *Eubacterium coprostanoligenes group* (familia y género) (0,4 *vs.* 1,2%) y de *Ruminococcus* (1,8 *vs.* 2,7%). Finalmente, la suplementación con probiótico redujo la concentración de IL-22 (11 *vs.* 8 pg/ml) (**Tabla Suplementaria 4.1**) y este grupo mostró asociaciones inversas entre los niveles circulantes de IL-6, TNF-α y la CAT (**Figura Suplementaria 4.1B**).

ARTÍCULO 4

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Potential of Fiber and Probiotics to Fight Against the Effects of PhIP + DSS-Induced Carcinogenic Process of the Large Intestine

Aida Zapico, Nuria Salazar, Silvia Arboleya, Carmen González del Rey, Elena Diaz, Ana Alonso, Miguel Gueimonde, Clara G. de los Reyes-Gavilán, Celestino Gonzalez, and Sonia González*

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ABSTRACT: We determined the in vivo counteracting effect of fiber and probiotic supplementation on colonic mucosal damage and alterations in gut microbiota caused by 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP) and sodium dextran sulfate (DSS). Male Fischer-344 rats were randomly divided into 4 groups: control (standard diet), PhIP + DSS group (standard diet + PhIP + DSS), fiber (fiber diet + PhIP + DSS), and probiotic (probiotic diet + PhIP + DSS). The intake of PhIP + DSS for 3 weeks induced colonic mucosal erosion, crypt loss, and inflammation, and the distal colon was more severely damaged. Fiber alleviated colonic mucosal damage by reducing crypt loss and inflammation, while the probiotic increased colon length. The intake of PhIP + DSS increased the fecal relative abundance of *Clostridia* UCG014 along the intervention, in contrast to the lower abundances of these taxa found after PhIP + DSS administration in the rats supplemented with probiotics or fiber. Fiber supplementation mitigated the histological damage caused by PhIP + DSS shifting the gut microbiota toward a reduction of pro-inflammatory taxa.

KEYWORDS: *fiber, PhIP, probiotic, microbiota, colorectal cancer, treatment strategies, heterocyclic amines, xenobiotics*

INTRODUCTION

Epidemiologic studies have shown that diet is among the most important environmental factors contributing to the development of cancer in humans. Dietary exposure to carcinogens depends on the type of food, its preparation, and nutrient composition.[1] In particular, heterocyclic aromatic amines (HCAs) are formed mainly by the pyrolysis of aromatic amino acids and creatine from protein-containing foods during cooking at high temperatures and have shown a high mutagenic potential.[2] 2-Amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP) was the HCA with the highest level of consumption in recent studies carried out by our team in a sample of adults from Asturias (northern Spain)[1,3] due to its wide presence in many types of meat.[4] PhIP has been classified by the International Agency for Research on Cancer (IARC) as "possibly carcinogenic to humans"[5] and has been extensively studied in animal models of colorectal cancer (CRC) in combination with the compound sodium dextran sulfate (DSS), which increases the susceptibility to PhIP-induced carcinogenesis of the large bowel.[6,7] Within the proposed mechanisms for this association, PhIP contributes to the formation of DNA adducts[6] and preneoplastic lesions as aberrant crypt foci (ACF) in the colonic mucosa[8] and to a higher incidence and multiplicity of intestinal mucosal lesions and adenocarcinomas in comparison to other HCAs.[9] However, most studies have used pharmacological doses rather than amounts that could be provided by the usual diet, which range from 80 to 190 ng/d.[6,10]

While evidence is still limited, the consumption of PhIP led to an enrichment of *Lactobacillus* and a reduction of *Prevotellaceae* UCG-001 and *Clostridiaceae* in the gut microbiota of mice.[10] In addition, while specific gut microbes such as *Bacteroides fragilis*, *Escherichia coli*, and *Enterococcus faecalis* have been linked to CRC,[11−15] several probiotic strains, including members of the species *Bifidobacterium longum*,[16] *Lactobacillus acidophilus*,[17] and *Lacticaseibacillus rhamnosus*,[18] have shown beneficial effects in various murine models of colon cancer. Although the protective mechanisms are unknown, *L. rhamnosus* strains have been specifically associated with induction of epithelial cell apoptosis, and suppression of the nuclear factor kappa B (NFκB) signaling pathway associated with inflammation.[18]

Rebalancing the gut microbiome, enhancing the intestinal mucosal barrier function, and modulating the immune response are among the potential mechanisms that contribute to explaining the beneficial actions of probiotic mixtures.[19] Also, to act as a carcinogen, PhIP needs to be enzymatically activated in the body, and the colon microbiota has shown to mediate its activation.[20] PhIP has been extensively studied in animal models in combination with the colitis-promoting compound DSS.[7] Dietary fiber can act as a sequestering agent for some toxic compounds, contributing to decreasing the intestinal toxicity,[21] and to modulate the microbiota by promoting the growth of beneficial bacteria and inhibiting some pathogenic groups.[22] Dietary fiber has evidenced an amelioration of colitis symptoms,[23] with higher intakes being associated with a lower risk of CRC.[24] In addition to

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maintaining gut homeostasis, the production of metabolites such as short-chain fatty acids (SCFA) as end microbial fermentation products of dietary fiber in the intestine may underlie its protective effect.[25] There is strong evidence suggesting the modulation of gut microbiota by diet and dietary compounds, so further research was performed to elucidate the possible role of HCAs. Previous results pointed to PhIP and dietary-derived bioactive compounds, such as fibers, as potential drivers of gut microbiota.[26] In addition, significant associations were found between the level of intake of these compounds and shifts of microbiota composition according to the severity of the damage to the colon mucosa.[27] Based on this evidence, the aim of the present study was to mimic the human context to explore the potential of fiber and probiotics to counteract the impact of the intake of PhIP + DSS on mucosal damage and fecal microbiota in a rat model. For this purpose, we have used a PhIP dose extrapolated from daily dietary intake in humans and DSS to promote inflammation of the intestinal mucosa similar to that produced by long-term consumption of a pro-inflammatory diet.

MATERIALS AND METHODS

This animal intervention has been carried out as a part of the broader project "Effect of diet and exposure to xenobiotics generated in food processing on the genotoxic/cytotoxic capacity of the intestinal microbiota" (reference: RTI2018−098 288−B-I00), financed by the Spanish Agency of Research (AEI), that aimed to analyze the impact of the intake of HCAs over damage of colon mucosa and the possible counteracting effect of the intake of probiotics and prebiotics.

Chemicals. PhIP (Catalogue number: 105650−23−5; Lot number: 134288) was obtained from MedChemExpress (Sollentuna, Sweden) and DSS (M.W. 36−50 kDa; Catalogue number: 160110; Lot number: S7980) was obtained from MP Biomedicals (Loughborough, UK). All animal diets: standard (Standard Rodent Diet A40) and supplemented with probiotics (Standard Rodent Diet A04+MP36) and fiber (Standard Rodent Diet A05) were obtained from SAFE (Augy, France). The probiotic Lactiplus VSL#3 was obtained from PiLeJe (Paris, France). QIAamp Fast DNA Stool Mini Kits were purchased from Qiagen (Sussex, UK).

Animals. The animal experiment conducted was performed in accordance with the protocols and procedures approved by the Ethics and Animal Experimentation Committee at the University of Oviedo, Spain (PROAE 48/2019). 44 male Fischer 344/NHsd rats (200 g of body weight; 7 weeks old) were purchased from Charles River Laboratories (Les Oncins, France) and maintained at the Bioterium of the University of Oviedo (N° REGISTER: ES 33044 0003591) under controlled 12 h light−dark cycle and at a constant temperature of 22 ± 2 °C and relative humidity of 55 ± 15%. Animals were randomly placed in polypropylene cages (2 to 3 rats per cage) and acclimatized for 1 week with free access to tap water and pelleted food. After acclimatization, each cage was randomly assigned to one of the four groups. Considering the sample size in each group (n = 11) and microbial relative abundances in rat feces, the statistical power of our results with a type I error probability of 0.05 is 95−98% (G*Power version 3.1.9.6 Franz Faul, Universität Kiel, Germany).

Treatments and Experimental Design. All animals were fed a standard diet during acclimatization, and those in the control and the PhIP + DSS groups were maintained on the standard diet for the entire duration of the intervention (5 weeks). The probiotic group received a customized diet with the probiotic Lactiplus VSL#3 composed of four *Lactobacillus* strains (*L. acidophilus BA05*, *Lactobacillus plantarum BP06*, *Lactobacillus paracasei BP07*, and *Lactobacillus helveticus BD08z*), three *Bifidobacterium* strains (*Bifidobacterium breve BB02*, *Bifidobacterium animalis* subsp. *lactis BL03x*, and *B. animalis* subsp. *lactis BL04y*), and one *Streptococcus* strain (*Streptococcus thermophilus BT01*). The probiotic was incorporated into the diet as a lyophilized powder and consumed by animals at 2.2 × 10^9 CFU/d. The fiber group received a prebiotic diet containing 5.9% (w/w) of fiber. All customized diets were provided by the company SAFE (Augy, France) in pellet form and were available *ad libitum*. Their compositions are detailed in Table 1. Probiotic and fiber diets were administered from baseline (T0) to the end of the study (T3) (5 weeks), as described in Figure 1.

Table 1. Nutritional Composition of the Commercial Diets Administered in the Study

	standard[c]	probiotic[d]	fiber[e]
energy content (kcal/g)[a]	3.34	3.21	3.22
macronutrients			
carbohydrates[b]			
g/kg	610	580	617
kcal (% of total)	73.05	72.27	76.65
protein			
g/kg	152	154	118
kcal (% of total)	18.20	19.19	14.66
fats			
g/kg	32	30	31
kcal (% of total)	8.62	8.33	8.66
supplementation			
probiotic (mg/kg)	0	1300	0
fiber (%)	4.10	3.74	5.90

[a]Metabolizable energy in the standard, probiotic, and fiber diet was 3.10, 3.15, and 2.83 kcal/g, in each case. [b]Nitrogen-free extract representing sugars and starches. [c]Standard Rodent Diet A40, SAFE, Augy, France. [d]Standard Rodent Diet A04+MP36, SAFE, Augy, France. [e]Standard Rodent Diet A05, SAFE, Augy, France.

The experimental groups (n = 11 animals/group) were the negative control (standard diet), the positive control, or PhIP + DSS (standard diet + PhIP + DSS), and the intervention groups: probiotic (probiotic diet + PhIP + DSS) and fiber (fiber-enriched diet + PhIP + DSS) (Figure 1). After 1 week of customized diet supplementation (T1), animals in the PhIP + DSS, probiotic, and fiber groups were administered the dietary carcinogen PhIP and DSS. PhIP was administered after anesthesia with isoflurane by oral gavage at 1 mg/kg of body weight (1 ppm) in sterile water. DSS (1.5%, w/v) was dissolved in tap water at room temperature and available *ad libitum* in substitution of normal drinking water. Animals in the PhIP + DSS, probiotic, and fiber groups were exposed to 3 weeks of PhIP + DSS treatment (5 days of treatment per week) (Figure 1). The control group was anesthetized for the administration of sterile water by oral gavage in substitution of PhIP. PhIP was effective in inducing carcinogenesis in previous studies combined with 1.5% DSS,[6] and the selected concentration of PhIP replicates reported human consumption levels in a previous study (188 ng/day).[1] After PhIP + DSS administration (T2), rats underwent 1 week of washout without PhIP + DSS and were sacrificed by CO_2 asphyxiation (T3). All animals survived the intervention (n = 44). Cecum and colon were then excised. Body weight and water and food intake were recorded daily throughout the study, and mean values obtained are presented in Table S1.

Colon Length and Histologic Assessment. Colon length was measured in a relaxed position without stretching from the ileocecal junction by using a 1 cm square grid. This information is available for only 21 animals. After measurement, feces were removed, and the colon was flushed with phosphate-buffered saline (PBS) to remove residual bowel content and divided into proximal, mild, and distal colon. The 3 segments from a total of 44 rats were then stored in formaldehyde. Swiss rolls were prepared by fixing each colon segment in 10% formaldehyde, embedding them in paraffin, and then sectioning into 3 μm thick slices for hematoxylin−eosin staining. A total of 528 histological sections (4 sections per colon segment within each animal) were examined with NanoZoomer S20MD (Hamamatsu

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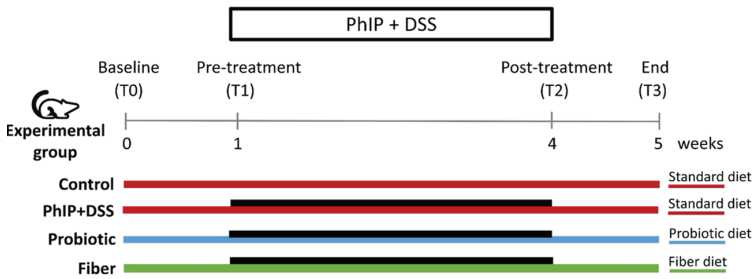

Figure 1. Experimental design. Different colored lines indicate the period of diet administration, and the black line indicates the period of PhIP and DSS administration. Fecal samples were collected at each time interval (T0, T1, T2, and T3). DSS, sodium dextran sulfate, and PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine.

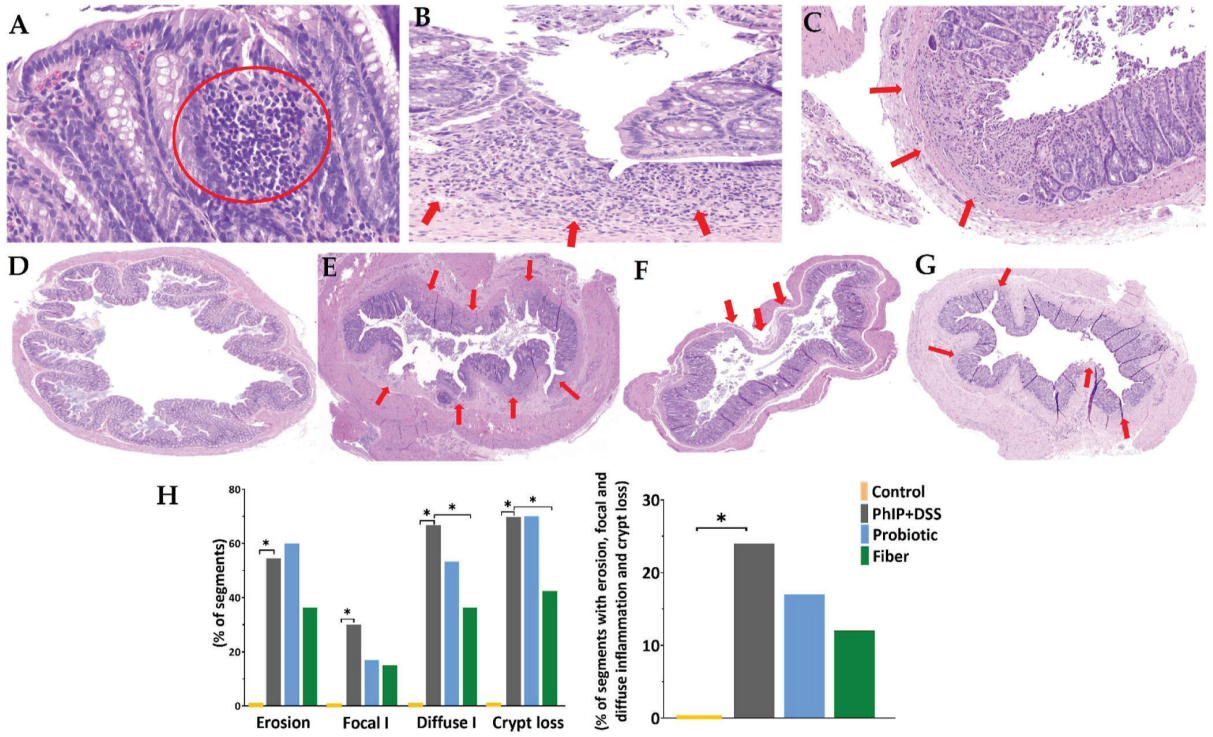

Figure 2. Histologic colonic mucosal damage induced by PhIP + DSS treatment and counteracting effect by probiotic or fiber supplementation. Histologic sections stained with hematoxylin−eosin showing (A) focal I through lymphoid infiltration, ×30; (B) diffuse I, ×20; and (C) erosion and crypt loss, ×10. Histologic sections of distal colon of (D) control group ×2; (E) PhIP + DSS group ×2.5; (F) probiotic group ×2.5; and (G) fiber group ×2.5. Red arrows indicate erosion, diffuse I, and crypt loss. (H) Percentage of colon segments presenting each histologic feature: erosion, focal and diffuse I, and crypt loss; and percentage of colon segments presenting all features within each group. Statistical analysis for (H) pairwise comparisons of the control vs PhIP + DSS group, the PhIP + DSS vs probiotic group, and the PhIP + DSS vs fiber group was performed with the X^2 test (n = 33 for each group) (*$p < 0.05$). DSS, sodium dextran sulfate; I, inflammation; PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine.

Photonics, France) for the presence of the following features: erosion, crypt loss, diffuse, and focal inflammation, and ACF without cell alterations, hyperplasia, or dysplasia. The results obtained are presented as the proportion of colon segments affected by each or all histologic features. No ACF was observed in the samples of study.

Feces Collection, DNA Extraction, Analysis of Fecal Microbiota Using High Throughput Sequencing, and SCFA Determinations. Fecal samples were collected in sterile containers at baseline (T0), before PhIP + DSS administration or pretreatment (T1), after PhIP + DSS administration or post-treatment (T2), and at the end of the study (T3) (Figure 1). Fecal samples were obtained in the morning, directly from each animal, to avoid contamination. However, at given intervals, some animals did not excrete fresh feces at any of the intervals of study (n = 8). Samples were stored at −80 °C for further analyses. The stool microbiota composition was determined by 16S rRNA gene sequencing for 36 animals. Fecal samples were diluted 1/7 (w/v) in sterile PBS solution and homogenized for 3 min at full speed in a LabBlender 400 stomacher (Seward Medical, London, UK). They were centrifuged for 15 min at 4 °C and 14,000 rpm, and the supernatants obtained were separated from the pellets and kept frozen at −20 °C until use. DNA was extracted by using the QIAamp Fast DNA Stool Mini Kit with an additional bead-beating step. The quantification of DNA and the determination of the 260/280 ratio were performed using the Take3Micro-Volume plate and Gen5 microplate reader (BioTek Instrument Inc., Winooski, VT, USA). The DNA obtained was kept

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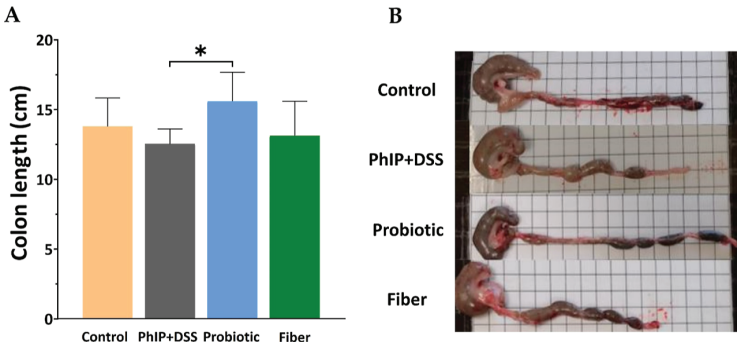

Figure 3. Impact of PhIP + DSS on colon length and counteracting effect by probiotic or fiber supplementation. (A) Colon length of each group. Bar plots represent the mean ± SD values obtained. Statistical analysis for pairwise comparisons of the control vs PhIP + DSS group, the PhIP + DSS vs probiotic group, and the PhIP + DSS vs fiber group was performed through a *T*-test (Control $n = 6$, PhIP + DSS $n = 5$, Probiotic $n = 5$, Fiber $n = 5$) (*$p < 0.05$). (B) Macroscopic appearance of the colon after excision. PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine.

frozen at −80 °C until analysis. Variable region V3−V4 of bacterial 16S rRNA genes present in each fecal community was amplified by PCR, and the resulting amplicons were sequenced on an Illumina NovaSeq 6000 platform instrument. The obtained individual sequence reads were filtered to remove low-quality sequences. All Illumina quality-approved, trimmed, and filtered data were integrated to generate de novo 16S rRNA Amplicon Sequence Variants with ≥97% sequence homology using Uparse software (Uparse v7.0.1090). A classification of all reads to the lowest possible taxonomic rank was performed using Quantitative Insights Into Microbial Ecology (QIIME2) and a reference data set from the SILVA 138 database. The 121 fecal samples analyzed yielded an average of ∼90,000 filtered partial sequences per sample and a final number of 3826 total ASVs. The whole procedure of sequencing and annotation was undergone at Novogene Bioinformatics Technology Co., Ltd. For the representation of the obtained results, only taxa with relative abundance greater than 1% in at least two samples and obtained mean values were considered. SCFA were analyzed by gas chromatography from the supernatants of 1 mL of the homogenized feces.[28] A chromatograph 6890N (Agilent Technologies Inc., Palo Alto, CA, USA) connected to a mass spectrometry detector 5973N (Agilent Technologies) and a flame ionization detector was used for the identification and quantification of SCFA, as described in previous works.[29]

Statistical Analysis. Results were analyzed using IBM SPSS software version 27.0 (IBM SPSS, Inc., Chicago, IL, USA). The goodness of fit to the normal distribution was checked by means of the Kolmogorov−Smirnov test. The analysis of categorical variables was performed with the X^2 test. Continuous variables were analyzed through analysis of the variance (ANOVA) and Tukey's posthoc HSD tests for intragroup comparisons to detect differences along the study within the same group and through *T*-test for pairwise comparisons at a specific time interval to detect differences between the control vs PhIP + DSS group, the PhIP + DSS vs probiotic group, and the PhIP + DSS vs fiber group. Spearman correlation analyses were conducted, and heatmaps were generated using the ClustVis web tool.[30] Graphical representations were obtained using GraphPad Prism 8 (La Jolla, CA, USA), and the Table of Contents was created using BioRender software.

RESULTS

Food Intake and Body Weights. No differences in body weight between the four groups were found at the beginning or end of intervention, but the fiber group presented a lower body weight gain in comparison to the PhIP + DSS group (54 vs 69 g; $p = 0.003$) (Table S1), probably due to the lower metabolizable energy (2.83 vs 3.44 kcal/g, respectively) (Table 1). The daily consumption of food and water was monitored daily, and no significant differences were found between groups.

Impact of PhIP + DSS on Colon Length and Mucosal Damage and the Counteracting Effect by Probiotic or Fiber Supplementation. The administration of 1 ppm of PhIP and 1.5% (w/v) of DSS for 3 weeks damaged the colonic mucosa of rats in the PhIP + DSS group (Figure 2). The histologic features assessed by hematoxylin−eosin stain are depicted in Figure 2A−C and include focal (Figure 2A) and diffuse (Figure 2B) inflammation and erosion and crypt loss (Figure 2C). In Figure 2D, the colonic mucosa of the control group receiving a standard diet is presented without lesions. In comparison to controls, the PhIP + DSS treatment group (Figure 2E) presented colon segments with erosion (55 vs 0%; $p < 0.001$), crypts loss (70 vs 0%; $p < 0.001$), focal (30 vs 0%; $p < 0.001$), and diffuse (67 vs 0%; $p < 0.001$) inflammation (Figure 2H). Similar results were obtained for the proportion of colon segments presenting all histologic findings simultaneously in the PhIP + DSS treatment group vs controls (24 vs 0%; $p = 0.003$) (Figure 2H). No ACF was found in this work. Considering the three colonic segments, the distal colon presented a higher grade of histologic alteration (Figure S1). In the PhIP + DSS group, crypt loss and diffuse inflammation were present in 91% of the distal segments and in 45% of the proximal segments, while focal inflammation was more common in proximal segments (64%) compared to mild (9%) and distal (18%) segments (Figure S1).

The administration of 5.9% (w/w) fiber counterbalanced colonic mucosal damage (Figure 2G). In comparison to the PhIP + DSS group, the fiber group presented a reduced proportion of segments with diffuse inflammation (36 vs 67%; $p = 0.014$) and crypts loss (42 vs 70%; $p = 0.026$) (Figure 2H). The administration of the probiotic (Figure 2F) evidenced a similar tendency toward alleviation of histologic damage compared to the PhIP + DSS group through nonstatistically significant reductions in the proportion of segments with focal (17 vs 30%; $p = 0.204$) and diffuse inflammation (53 vs 67%; $p = 0.280$) (Figure 2H).

In addition to histologic assessment, the colon length was measured (Figure 3A−B). Whereas a nonsignificant 10% reduction in colon length was found in the PhIP + DSS group compared to controls (12.54 vs 13.80 cm; $p = 0.695$), the colon was 24% longer in the probiotic group when compared to the PhIP + DSS treatment group (15.60 vs 12.54 cm; $p =$

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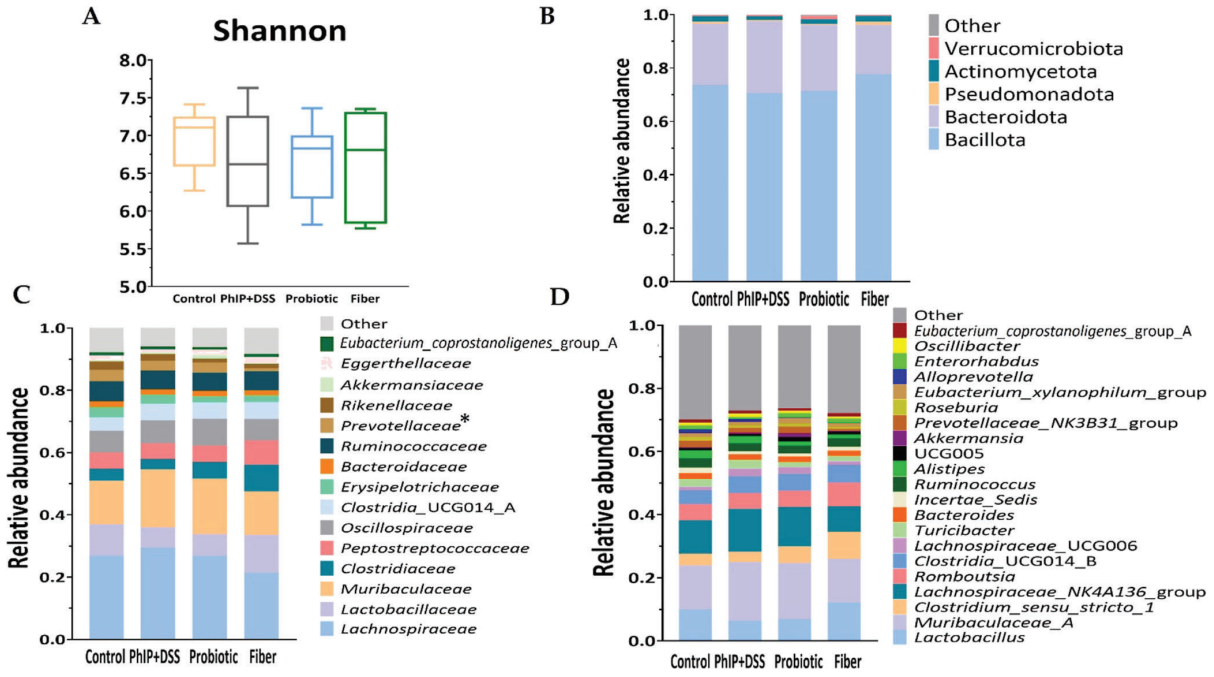

Figure 4. Baseline diversity and profile composition of the gut microbiota in each group. (A) Shannon diversity index. The lines within the boxes represent the median, and the bounds of boxes represent the first and third quartiles (25th and 75th percentiles, respectively). The whiskers denote the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively. Microbiota profile composition at (B) phylum, (C) family, and (D) genus level. Bar plots represent the mean values obtained for each taxa. Only those with a mean relative abundance greater than 1% are shown. Statistical analysis for (A–D) pairwise comparisons of the control vs PhIP + DSS group, the PhIP + DSS vs probiotic group, and the PhIP + DSS vs fiber group at the baseline was performed through a *T*-test, and results were found only for the PhIP + DSS vs fiber groups (Control n = 8, PhIP + DSS n = 7, Probiotic n = 8, Fiber n = 7) (*p < 0.05). PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine.

0.019). No significant differences in colon length were found between PhIP + DSS and fiber groups.

Impact of PhIP + DSS Treatment on Gut Microbiota Composition and Counteracting Effect by Probiotic or Fiber Supplementation. The metataxonomic analyses based on sequencing the V3–V4 region of the 16S rRNA gene revealed substantial differences in the composition of the fecal microbiota between the different groups of treatment. Globally, the microbial communities of all samples from the four groups of animals were assigned to 5 phyla, 28 families, and 52 genera. At baseline, no differences were found in alpha diversity for the Shannon index (Figure 4A) and in microbiota composition at the taxonomic levels of phylum, family, and genus among the four experimental groups (Figure 4B–D), except for the family *Prevotellaceae*, which presented a reduced relative abundance in the fiber group compared to the PhIP + DSS group (1.0 vs 3.1%; p = 0.027). At the phylum level, Bacillota was the most abundant, followed by Bacteroidota and Actinomycetota. At the family level, the most abundant was *Lachnospiraceae*, followed by *Muribaculaceae*, *Oscillospiraceae* and *Lactobacillaceae*.

Longitudinal Analysis. The longitudinal effect of the intervention on the Shannon index and microbiota composition within each group of animals is shown in Figure 5A–D and Tables S2–S5. Significant variations in the Shannon diversity index along the intervention were detected only in the fiber group, for which the highest diversity index was obtained after PhIP + DSS administration (T2) (Figure 5A).

Regarding the gut microbiota composition, the PhIP + DSS group of treatment presented variations along the intervention in the relative abundance of the family *Clostridia* UCG014_A and the genus *Clostridia* UCG014_B, the families *Oscillospiraceae* and *Monoglobaceae* and the genus *Monoglobus*, and the family *Rikenellaceae* and the genus *Alistipes* (Figure 5B–D). Specifically, in the case of *Monoglobaceae* and the genus *Monoglobus* as well as for *Clostridia* UCG014 (family and genus) (Figure S2), the PhIP + DSS treatment (T2) promoted the increase of these microbial groups, followed by a significant reduction in their relative abundance 1 week after the cessation of PhIP + DSS administration (T3) from 1.2 to 0.4% (p = 0.040) (*Monoglobaceae* and *Monoglobus*) and from 9.0 to 3.9% (p = 0.013) (*Clostridia* UCG014, family and genus). These results are in contrast to the increased relative abundance of *Oscillospiraceae* from pretreatment (T1) to 1 week after cessation of the administration of PhIP + DSS (T3) (from 6.3 to 9.3%; p = 0.031) in the same group. In Figure 5B–D, it can be observed that the probiotic group also presented variations along the study in the relative abundances of *Clostridia* UCG014 (at the family and genus level) and *Lachnospiraceae* UCG006. Among them, an increment in the relative abundance of *Lachnospiraceae* UCG006 was detected from pretreatment (T1) to 1 week after cessation of PhIP + DSS administration (T3), (from 0.8 to 3.0%; p = 0.035) (Figure S2), and the opposite nonsignificant trend was observed for the relative abundance of *Clostridia* UCG014 (family and genus) in comparison to the PhIP + DSS group: from 5.3% at baseline (T0) to 2.5% after PhIP + DSS

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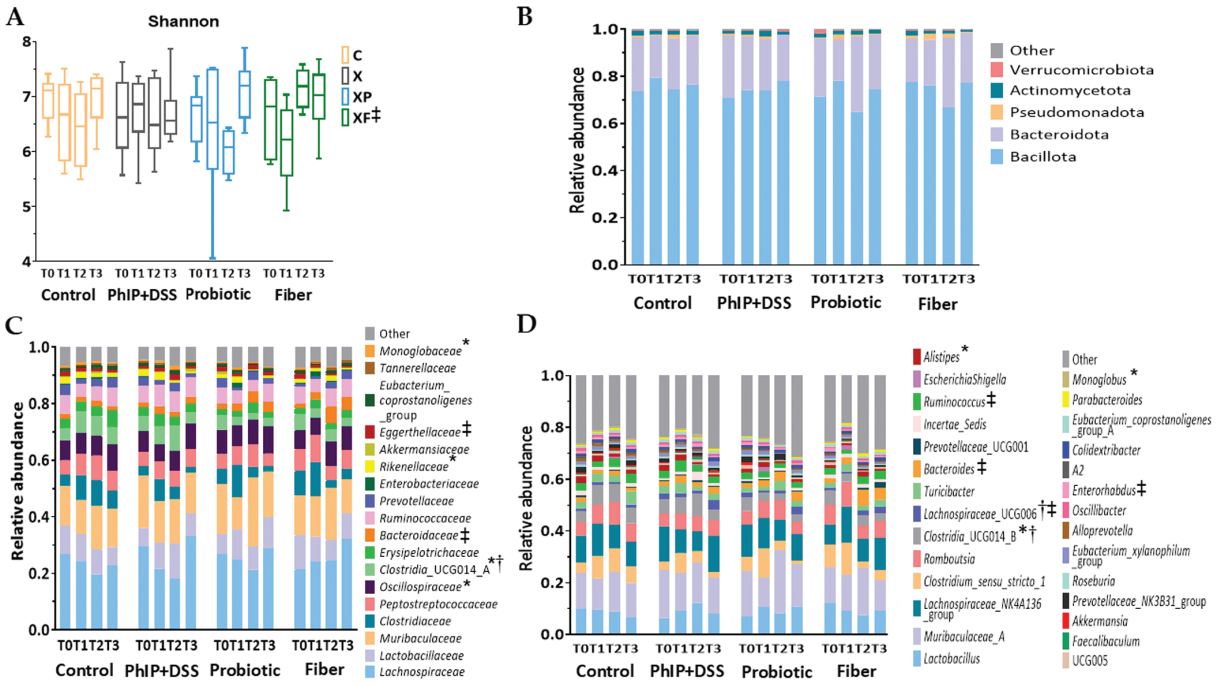

Figure 5. Gut microbiota diversity and profile composition across the study for each group. (A) Shannon diversity index. The lines within the boxes represent the median, and the bounds of boxes represent the first and third quartiles (25th and 75th percentiles, respectively). The whiskers denote the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively. Microbiota profile composition at (B) phylum, (C) family, and (D) genus level. Bar plots represent the mean values obtained for each taxa, and only those with a mean relative abundance greater than 1% are shown. Statistical analysis (A–D) for differences across the study (T0, T1, T2, and T3) was performed by one-way ANOVA for each group (Control $n = 8$, $n = 8$, $n = 9$, and $n = 9$; PhIP + DSS $n = 7$, $n = 8$, $n = 5$, and $n = 9$; Probiotic $n = 8$, $n = 7$, $n = 4$, and $n = 7$; and Fiber $n = 7$, $n = 8$, $n = 8$, and $n = 9$ at T0, T1, T2, and T3, respectively) ($p < 0.05$ for PhIP + DSS (*), Probiotic (†), and Fiber (‡) groups). No significant differences were found in the control group across the study. p-values are provided in Tables S2–S5. PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine; T0, baseline; T1, pretreatment; T2, post-treatment; and T3, end of the study.

administration (T2) ($p = 0.088$) (Figure S2). The fiber group presented variations throughout the intervention in the relative abundance of the phylum Actinomycetota and related taxa *Eggerthellaceae* and *Enterorhabdus*, as well as *Bacteroidaceae* and *Bacteroides*, and genera *Lachsnopiraceae* UCG006 and *Ruminococcus* (Figure 5B–D). Whereas in comparison to baseline (T0) reduced relative abundance at the end of the study (T3) was noted in the case of Actinomycetota (from 2.1 to 0.7%; $p = 0.016$), *Eggerthellaceae* (from 1.9 to 0.5%; $p = 0.008$), and *Enterorhabdus* (from 1.2 to 0.4%; $p = 0.009$), an increasing trend from pretreatment (T1) was presented by *Ruminoccus* (from 1.3 to 3.2%; $p = 0.033$) (Figure S2).

Cross-Sectional Analysis. The effect of the different diets was determined at the end of the intervention. Compared to the PhIP + DSS group, both the probiotic and fiber supplementations reduced the relative abundance of *Clostridia* UCG014 (family and genus) (2.5 and 4.0% vs 9.0%; $p = 0.010$, both cases) (Figure 6). In comparison to the PhIP + DSS group, supplementation with the probiotic led to reduced relative abundance of *Eubacterium coprostanoligenes* group (family and genus) (0.4 vs 1.2%; $p = 0.032$) and *Ruminococcus* (1.8 vs 2.7%; $p = 0.028$) (Figure S3), whereas supplementation with fiber reduced the relative abundance of *Monoglobaceae* and *Monoglobus* (0.4 vs 1.2%; $p = 0.021$) and UCG005 (0.6 vs 1.2%: $p = 0.030$) and increased *Bacteroidaceae* and *Bacteroides* (6.1 vs 1.9%; $p = 0.040$) and *Alistipes* (1.6 vs. 0.7%; $p = 0.041$) (Figure S3).

The level of fecal SCFA after the administration of PhIP + DSS for each group of animals is displayed in Figure 7. The PhIP + DSS group showed significantly higher fecal levels of propionic acid (9.53 vs 5.90 μmol/g; $p = 0.015$) and lower levels of isobutyric acid (0.15 vs 0.33 μmol/g; $p = 0.001$), isovaleric acid (0.17 vs 0.57 μmol/g; $p = 0.001$), branched SCFA (BCFA) (0.33 vs 0.77 μmol/g; $p = 0.001$), and valeric acid (0.28 vs 0.52 μmol/g: $p = 0.014$) as compared to controls. The administration of the probiotic led to a partial restoration of valeric acid excretion levels, and increased concentrations were observed in comparison to the PhIP + DSS group (0.54 vs 0.32 μmol/g; $p = 0.042$). No significant differences in fecal SCFA levels were found in the fiber group compared to the PhIP + DSS group after PhIP + DSS administration, according to the *T*-test.

The associations found between the relative abundance of gut microbiota families and the concentration of SCFA excreted in feces for each experimental group after PhIP + DSS administration are shown in Figure 8. The control group presented a direct association between *Clostridia* UCG014_A and isovaleric acid, and the opposite direction was found for this association in the case of animals from the PhIP + DSS group of treatment and the probiotic group. The group supplemented with the probiotic presented the greatest number of significant associations and is the only one noting significant associations with valeric acid through direct correlations with the *Ruminococcaceae* and *Lachnospiraceae*

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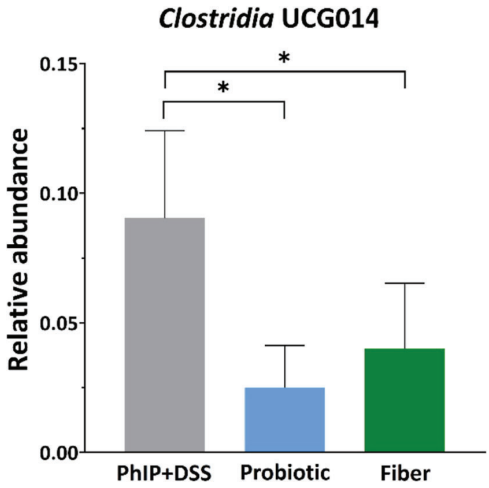

Figure 6. Effect of probiotic or fiber supplementation on the relative abundance of *Clostridia* UCG014 after PhIP + DSS administration. Bar plots represent mean relative abundance ±SD. Statistical analysis for pairwise comparisons of the PhIP + DSS vs probiotic group and the PhIP + DSS vs fiber group at post-treatment was performed by a *T*-test (PhIP + DSS $n = 5$, Probiotic $n = 4$, Fiber $n = 8$) (*$p < 0.05$). Only microbial groups with significant differences for both comparisons are shown. DSS, sodium dextran sulfate; PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine.

families and inversely with the family *Erysipelotrichaceae*. In addition, the relative abundances of gut microbiota families after PhIP + DSS administration were correlated with the occurrence of histological alterations according to the experimental group (Figure 9). The PhIP + DSS group presented direct correlations between the occurrence of erosion and crypt loss and the relative abundance of *Clostridiaceae*, and inverse associations were found in the fiber group between focal and diffuse inflammation and the relative abundance of *Monoglobaceae*. In the probiotic group, inverse associations were found between erosion and the levels of *Bacteroidaceae* and *Lactobacillaceae*.

DISCUSSION

In this work, an animal PhIP + DSS model was used to mimic the potential of dietary fiber and a mixed probiotic to counteract the impact of the intake of HCAs formed during the cooking of foods such as meat on colon damage and gut microbiota. PhIP has been shown to promote the formation of genotoxic metabolites and DNA adducts over time, and DSS increases PhIP susceptibility of colonic epithelial cells.[31] The coutilization of PhIP with DSS has been previously shown to shorten the time of progression of PhIP-induced tumors in murine models from 52 to 82 weeks (when PhIP is administered alone) to 6−24 weeks.[6] In our study, the administration of 1 ppm of PhIP concomitantly with DSS to Fischer 344 rats for 3 weeks resulted in an effective combination to provoke histologic damage in the colonic mucosa through erosion, focal and diffuse inflammation, and crypt loss. This supports the model of short-term induction of carcinogenesis used in the present work as suitable for investigating diet-related colon carcinogenesis[32] by mimicking the long-term exposure to PhIP. Also, the lower concentrations of PhIP (1 ppm) employed in this work as compared to

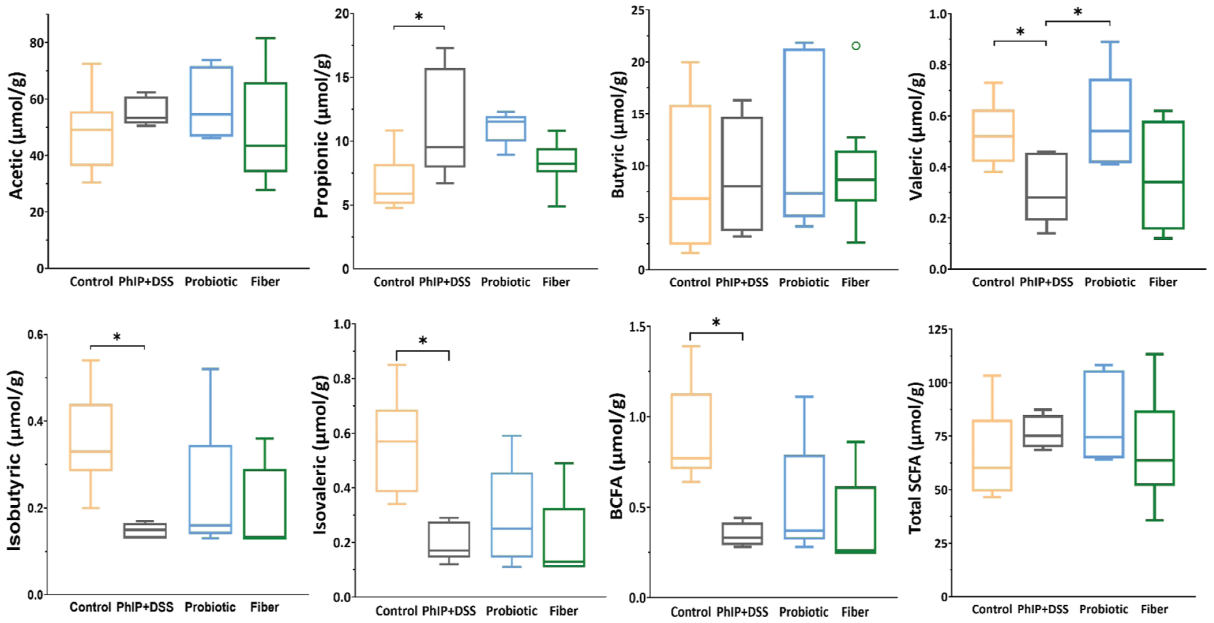

Figure 7. Analysis of fecal SCFA after the administration of PhIP + DSS. The lines within the boxes represent the median, and the bounds of the boxes represent the first and third quartiles (25th and 75th percentiles, respectively). The whiskers denote the lowest and highest values within 1.5 times the IQR from the first and third quartiles, respectively. Statistical analysis for pairwise comparisons of the control vs PhIP + DSS group, the PhIP + DSS vs probiotic group, and the PhIP + DSS vs fiber group at post-treatment was performed through *T*-test (Control $n = 9$, PhIP + DSS $n = 5$, Probiotic $n = 4$, and Fiber $n = 8$) (*$p < 0.05$). BCFA, branched chain fatty acids; DSS, sodium dextran sulfate; PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine; and SCFA, short chain fatty acids.

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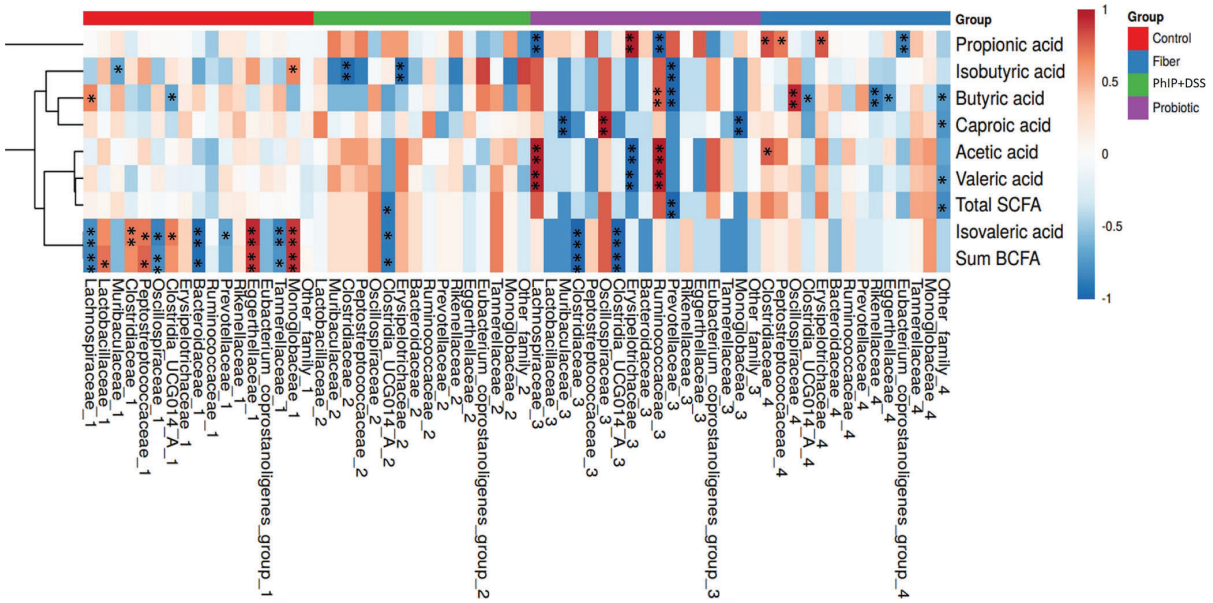

Figure 8. Spearman correlations representation between the level of fecal SCFA (rows) and most abundant bacterial families (columns) by group of study after the administration of PhIP + DSS. (*) and (**) $p < 0.05$ and 0.01, respectively. Only taxa showing mean relative abundances higher than 1% are shown. (Control $n = 9$, PhIP + DSS $n = 5$, Probiotic $n = 4$, and Fiber $n = 8$). BCFA, branched chain fatty acids; DSS, sodium dextran sulfate; PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine; and SCFA, short chain fatty acids.

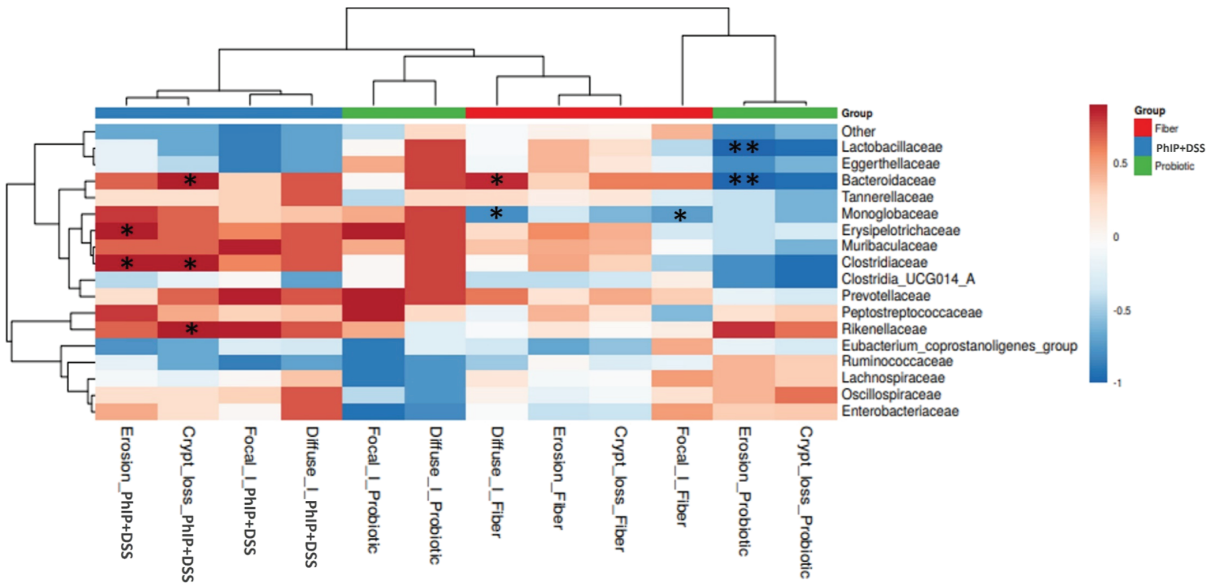

Figure 9. Spearman correlations representation between most abundant bacterial families (rows) and the histological alterations (erosion, diffuse and focal inflammation, and crypt loss) by group of study after the administration of PhIP + DSS. (*), (**) $p < 0.05$ and 0.01, respectively. Only taxa showing mean relative abundances higher than 1% are shown. No histological alterations were found in the control group. (PhIP + DSS $n = 5$, Probiotic $n = 4$, and Fiber $n = 8$). DSS, sodium dextran sulfate and PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine.

previous studies were used based on the level of consumption observed among humans in previous works from our team.[1,3]

The greater presence of histologic features in distal segments is similar to the distribution of colon tumors reported in human studies during the early onset of CRC[33] and is also in accordance with the absence of tumors found after a similar intervention study conducted by other authors (5 day treatment with PhIP at lower doses of administration than the used by us (0.1 mg/kg)).[6] In contrast to the higher concentrations of PhIP used in some previous studies, aiming to induce a tumor in the minimum time possible,[6,7,10] the dose administered in the present work is comparable to the estimated levels of the regular consumption of this compound in humans.[34] Colon length has been widely used as a

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morphologic marker of the degree of inflammation, as its shortening is associated with relevant histological changes[23] and carcinogenesis.[35] The nonsignificant trend toward colon shortening (10%) observed in our experimental model is consistent with these studies, considering that the doses employed were not sufficient to induce carcinogenicity.

There is a consensus regarding the involvement of gut microbiota in the development of inflammation processes in the colon mucosa. The histologic findings in the present study are parallel to the increased relative abundance of *Clostridia* UCG014, and in the case of erosion and crypt loss, these were directly associated with the relative abundance of *Clostridiaceae*. This lies in accordance with the results obtained by other authors, in which higher relative abundances of *Clostridiaceae_1* and *Clostridium_sensu_stricto_1* were detected after similar intervention periods and the administration of slightly higher doses of PhIP (10 ppm).[10] The family *Clostridiaceae* has already been identified as pro-inflammatory,[36] and the levels of *Clostridia* UCG014 are elevated in colitis[23] and CRC[37] models. The increase of Clostridia in the group of animals treated with PhIP + DSS was parallel to a greater excretion of propionic acid and reduced valeric and BCFA, as previously reported in a chemically induced CRC animal model (azoxymethane (AOM)/DSS).[36] Among these, inverse associations were found between *Clostridia* UCG014_A and BCFA in this work, which have previously been associated with the impairment of gut barrier integrity.[38]

To our knowledge, this is the first study analyzing the effect of the administration of a mixed probiotic on the gut microbiota composition to reduce the negative impact of dietary xenobiotics at the intestinal level. There is strong evidence suggesting the convenience of using a combination of probiotics rather than a single strain in the modulation of gut microbiota.[19] In this sense, the intake of the multistrain probiotic composed of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* strains in this work (2.2×10^9 CFU/d) were based on the recommended intake in humans (10^9 CFU/d), considering an adult with an average body weight of 70 kg, and in consonance with our results, it has shown beneficial effects in colitis and in the progression of colonic neoplastic lesions when used at doses around 2×10^9 CFU/d in previous animal studies.[39] It was also found in the present work that probiotic administration counteracted colon shortening in comparison to that of the PhIP + DSS group. These results are in consonance with the longer colon length reported by other authors after supplementation with the same combination of probiotics in experimental colitis models.[40] In addition, the previously reported increased colon length was not associated with variations in leukocyte infiltration in the lamina propria and submucosa.[41] This may indicate a colon elongation effect by probiotic supplementation independent of inflammation of the colonic mucosa, which would explain the nonsignificant variations in the proportions of colon segments with histological features observed after the probiotic supplementation in this work. In addition, erosion was found to be inversely associated with *Lactobacillaceae* and *Bacteroidaceae* in this group. In the present work, we have found that the longer colon length was parallel to an increased abundance of *Adlercreutzia* compared to the PhIP + DSS group (0.7 vs 0.3%, $p = 0.010$, data not shown); this microorganism was previously positively associated with colon length,[42] amelioration of colitis,[43] and excreted valeric acid.[44] The probiotic promoted an increase in the fecal concentration of SCFA valeric acid, restoring the levels obtained in the control group. This SCFA has been directly correlated with the circulating levels of anti-inflammatory cytokines in previous works,[43] which could be due to *B. animalis* subsp. *lactis BL03x*, one of the components of the probiotic mixture that has been shown to produce valerate in *in vitro* fermentations.[38] Our results revealed that the multistrain probiotic can be effective in reversing the increase of *Clostridia* UCG014 that occurred in the group of rats treated with PhIP + DSS, in agreement with the similar depletion observed by other authors for the genus *Clostridium* in the mucosal-adherent microbiota of AOM/IL10−/− mice after probiotic administration.[11] In the present work, the probiotic was administered before the onset of inflammation, which has been associated with a prophylactic effect.[45] This probiotic effect is time and dose dependent and may not be necessarily the same when it is administered after the onset of colonic inflammation.[19]

The microorganisms present in this commercial formula have been studied for their potential use as probiotics in the treatment and prevention of CRC.[19] Based on the evidence showing that some lactobacilli strains mediate the conversion of PhIP into intermediates with reduced mutagenic potential[46] and the role of *Bifidobacterium* in the maintenance of the intestinal barrier integrity;[47] the chosen probiotic may be useful to revert shifts of the microbiota resulting from the administration of PhIP + DSS, despite the absence of modifications of its bacterial groups in feces.[39] The authors suggest that this may be due to its effect on the regulation of the composition of beneficial and harmful bacteria[19] and differential ability to colonize feces and colonic mucosa.[39] In contrast to other studies, in our work, the mixed probiotic was administered with food instead of water. However, a reduction in markers related to inflammation has been observed in previous works independent of the administration method (orally gavage or dissolved in drinking water).[39,48]

There is strong evidence supporting the protective effect of dietary fiber consumption against CRC and their role as a modulator of the intestinal microbiota.[13] In spite of this and to the best of our knowledge, no previous studies have approached the consumption of fiber in the context of an animal model treated with PhIP + DSS. In our study, the administration of an isocaloric diet enriched in fiber (6% fiber) to rats treated with PhIP + DSS reduced damage to the colonic mucosa by means of reducing crypt loss and diffuse inflammation, and by a nonsignificant partial counteracting effect on decreased colon length. Consistent with our results, increased colon length and restoration of colonic mucosal damage have been reported in animals with reduced DSS colitis scores and a similar dietary fiber content (5%).[49] Dietary fiber promoted shifts in the gut microbiota by increasing the alpha diversity and the relative abundance of *Ruminococcus* (*Ruminococcaceae* family). In addition, a reduced relative abundance of *Clostridia* UCG014 and an increase of taxa belonging to the phylum Bacteroidota such as *Alistipes* (*Rikenellaceae* family) and *Bacteroidaceae* and genus *Bacteroides* were found in the present work as compared to rats treated with PhIP + DSS following a standard diet. Low relative proportions of *Rikenellaceae* and *Ruminococcus* have been found after DSS exposure in previous studies,[50] these microorganisms displaying negative associations with pro-inflammatory cytokine levels in colitis.[51] In contrast, higher relative abundances of Bacteroidota and *Rikenellaceae* were noted in colitis models fed with similar dietary fiber content as those used by us

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(5%),[23,49] whereas increased abundance of *Bacteoridaceae* and *Ruminocccaceae* were found in mice fed with a high-fiber diet (35%) or pectin supplementation for 1 week (10%).[52] Among these, the species *Ruminococcus bromii* seems to play a pivotal role in the degradation of a subtype of fiber.[53] The increased relative abundance of these families in animals fed with high-fiber diets has been associated with the remodeling of the gut microbiota and the restoration of the intestinal barrier[43] and thus may partially explain the mechanisms by which fiber supplementation reduced colonic mucosal damage in a PhIP animal model. In addition to shifts in gut microbiota composition, reduced absorption of HCAs in the presence of fiber has been previously described,[4] which may contribute to the protective effect of dietary fiber intake at the colonic level. Moreover, although the use of an animal model limits the extrapolation of the results obtained to humans, our findings contribute to supporting the benefits of probiotic supplementation on colon elongation and the beneficial effect of dietary fiber on reducing colonic mucosal damage, inflammation, and thus the risk of CRC, probably through the modulation of gut microbiota composition, among other mechanisms.

In the present work, the consumption of PhIP + DSS in a dose mimicking a high regular PhIP dietary intake in humans provoked damage to the colonic mucosa through erosion, inflammation, and crypt loss, accompanied by shifts in the composition of the gut microbiota profile toward higher abundances of pro-inflammatory taxa such as *Clostridia* UCG014. At the doses administered, fiber was more effective than the mixed probiotic in alleviating damage induced by PhIP + DSS to the intestinal mucosa, while the probiotic favored colon elongation compared to animals in the PhIP + DSS group without probiotic supplementation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.4c07366.

Body weights and food and water intake, histologic colonic mucosal damage induced by PhIP + DSS and counteracting effect by probiotic or fiber supplementation in each colon segment, diversity indices and microbial relative abundances along the study in the control group, PhIP + DSS group, probiotic group, and fiber group, posthoc analysis of the relative abundances of gut microbiota taxa that changed significantly across the study within each experimental group, and effect of probiotic or fiber supplementation on the relative abundance of gut microbiota after the administration of PhIP and DSS (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Sonia González − *Department of Functional Biology, University of Oviedo, Oviedo 33006, Spain; Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo 33011, Spain;* orcid.org/0000-0003-2602-7036; Phone: +34-958-104-209; Email: soniagsolares@uniovi.es

Authors

Aida Zapico − *Department of Functional Biology, University of Oviedo, Oviedo 33006, Spain; Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo 33011, Spain*

Nuria Salazar − *Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa 33300, Spain; Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo 33011, Spain*

Silvia Arboleya − *Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa 33300, Spain; Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo 33011, Spain*

Carmen González del Rey − *Anatomical Pathology Service, Central University Hospital of Asturias (HUCA), Oviedo 33011, Spain*

Elena Diaz − *Department of Functional Biology, University of Oviedo, Oviedo 33006, Spain*

Ana Alonso − *Department of Functional Biology, University of Oviedo, Oviedo 33006, Spain*

Miguel Gueimonde − *Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa 33300, Spain; Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo 33011, Spain;* orcid.org/0000-0002-0192-901X

Clara G. de los Reyes-Gavilán − *Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Villaviciosa 33300, Spain; Diet, Microbiota and Health Group, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo 33011, Spain*

Celestino Gonzalez − *Department of Functional Biology, University of Oviedo, Oviedo 33006, Spain*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.4c07366

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Notes

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■ ABBREVIATIONS USED

ACF, Aberrant crypt foci
AEI, Spanish Agency of Research
ANOVA, analysis of the variance
AOM, azoxymethane
ASV, amplicon sequence variants
BCFA, branched chain fatty acids
CRC, colorectal cancer
DSS, sodium dextran sulfate
HCAs, heterocyclic aromatic amines
IARC, International Agency for Research on Cancer

NFκB, nuclear factor kappa B
PBS, phosphate-buffered saline
PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine
QIIME2, Quantitative Insights Into Microbial Ecology
SCFA, short chain fatty acids

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Tabla Suplementaria 4.1. Concentración de marcadores sanguíneos en función del grupo experimental.

	Control (n=8)	PhIP+DSS (n=8)	Probiótico (n=8)	Fibra (n=8)
Parámetros inflamatorios (pg/ml)				
IL-6	17,19 (13,25-30,41)	21,06 (16,91-42,12)	14,79 (14,27-35,26)	25,70 (18,03-32,41)
IL-10	8,75 (8,39-11,04)	12,23 (8,40-15,84)	8,27 (7,81-9,47)	8,76 (7,81-15,68)
IL-17A	14,26 (8,31-23,97)	12,52 (8,85-20,04)	10,10 (8,31-14,23)	11,63 (8,85-18,68)
IL-17F	5,04 (4,77-5,45)	4,35 (4,08-4,81)	4,27 (4,12-4,47)	5,49 (5,11-5,91) ‡
IL-22	8,82 (7,89-11,00)	11,10 (9,61-11,83)	8,44 (7,80-9,11)†	9,69 (9,30-11,66)
IFN-γ	7,80 (7,23-9,63)	9,88 (8,40-17,98)	9,50 (8,04-10,52)	8,64 (7,80-11,45)
TNF-α	6,25 (5,69-6,83)	6,75 (5,93-7,08)	5,85 (5,69-16,72)	6,50 (6,17-7,09)
GM-CSF	25,38 (23,36-51,37)	26,71 (22,18-61,96)	33,14 (22,96-42,62)	48,19 (24,22-74,02)
TNF-α/IL-10	0,69 (0,61-0,73)	0,55 (0,45-0,66)	0,71 (0,60-0,82)	0,71 (0,45-0,79)
Capacidad antioxidante total				
(mM equivalente de Trolox)	11,53 (10,66-11,96)	9,49 (9,07-10,58)*	10,18 (9,26-11,12)	10,19 (9,57-10,82)

Los datos se presentan como mediana (P_{25} – P_{75}). *p* valor <0,05 por prueba T para comparaciones por pares: PhIP+DSS *vs*. Control (*), PhIP+DSS *vs*. Probiótico (†), y, PhIP+DSS *vs*. Fibra (‡). DSS, sal sódica de sulfato de dextrano; GM-CSF, factor estimulante de colonias de granulocitos y macrófagos; IFN-γ, interferón-γ; IL, interleucina; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona; TNF-α, factor de necrosis tumoral-α.

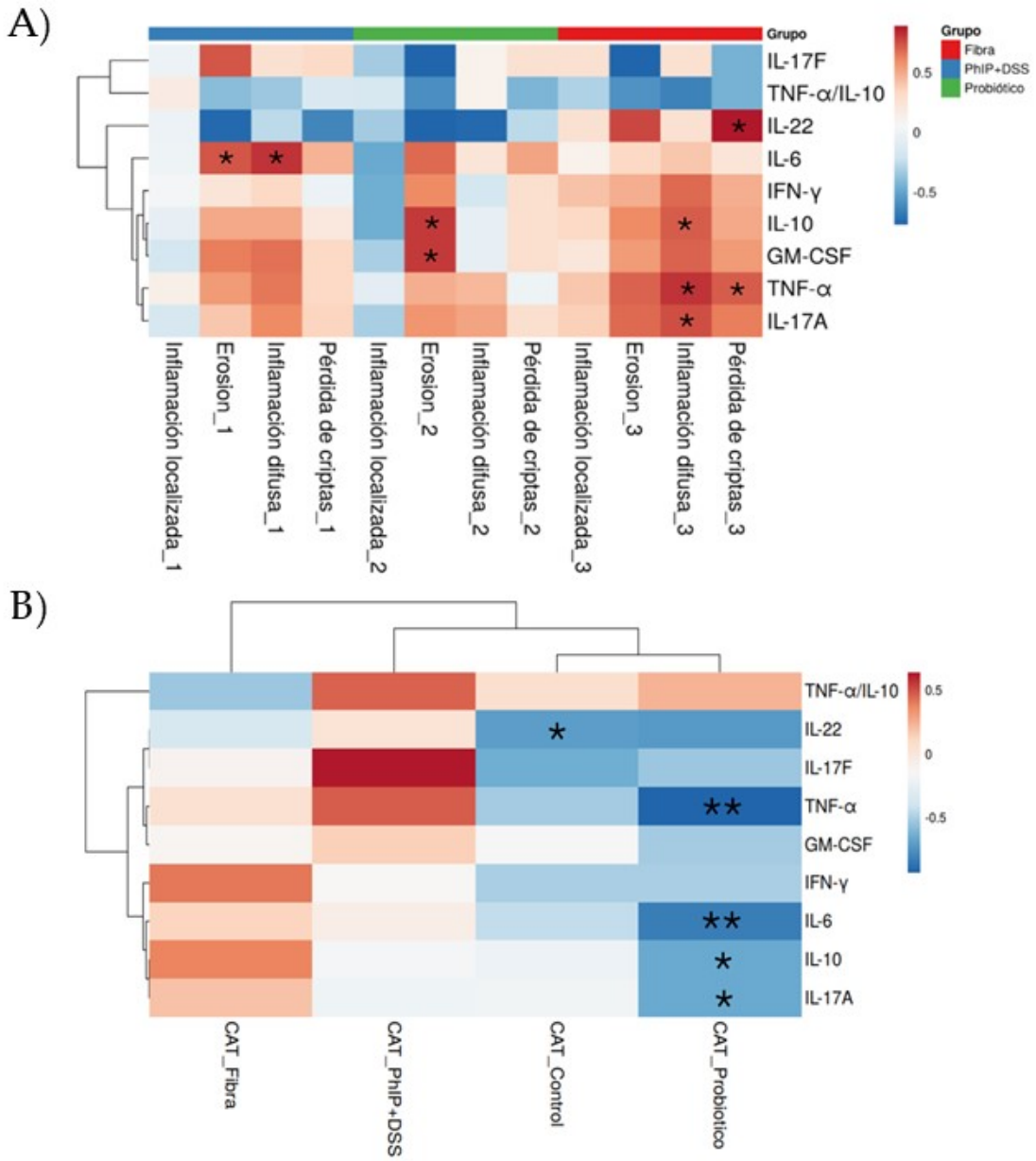
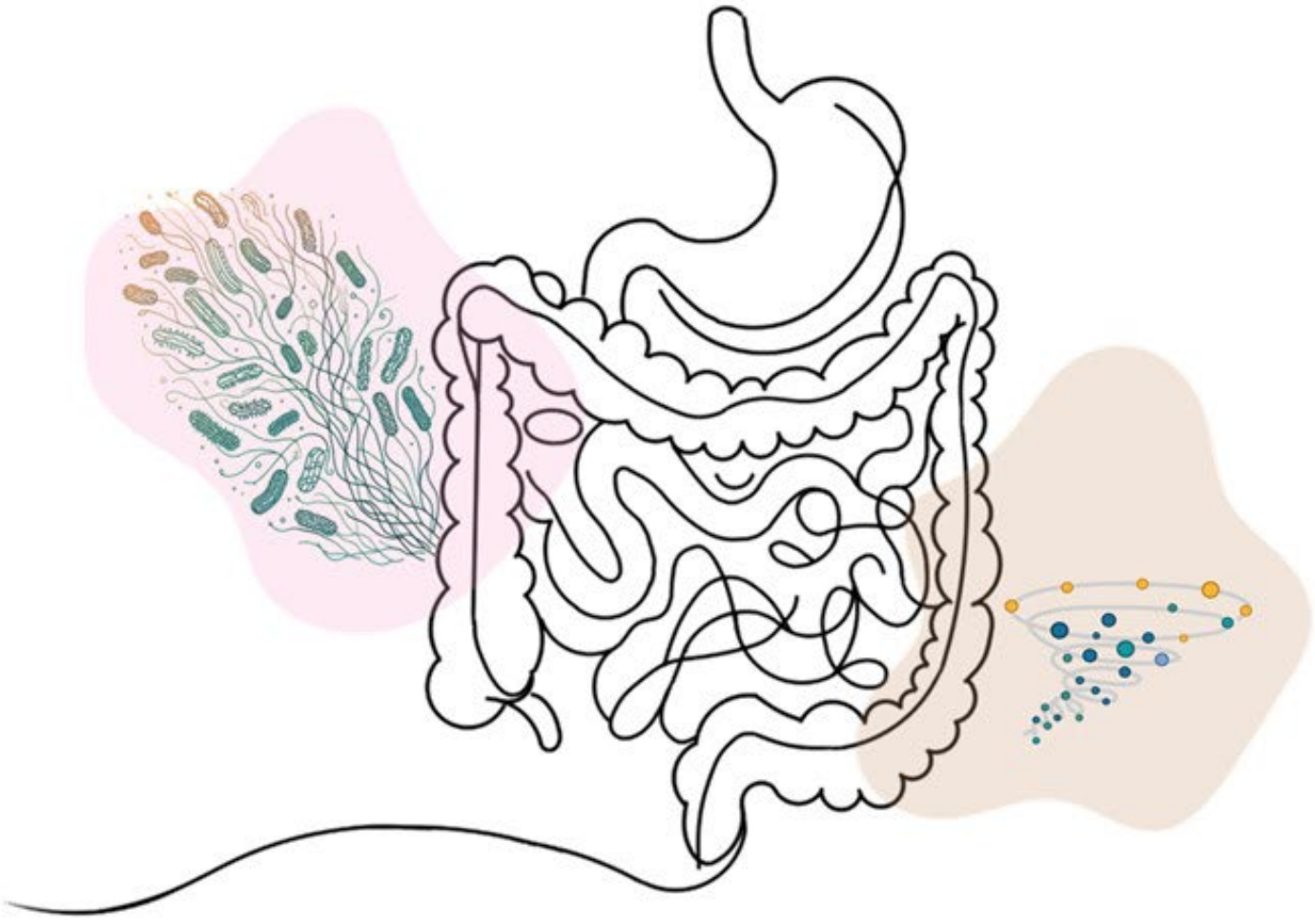


Figura Suplementaria 4.1. Representación de las correlaciones de Spearman obtenidas entre los niveles circulantes de parámetros inflamatorios (filas) y las alteraciones histológicas en la mucosa (A) o CAT (B) en función del grupo experimental (columnas). (*) (**) *p* valor <0,05 y 0,01, respectivamente. CAT, capacidad antioxidante total; DSS, sal sódica de sulfato de dextrano; GM-CSF, factor estimulante de colonias de granulocitos y macrófagos; IFN-γ, interferón-γ; IL, interleucina; PhIP, 2-amino-1 metil-6-fenilimidazo (4,5,b) piridona; TNF-α, factor de necrosis tumoral-α.

DISCUSIÓN
DISCUSSION



1. Cuantificación de la Ingesta de Compuestos con Potencial Carcinógeno

1.1. Desarrollo y validación de una herramienta para la estimación de la ingesta de xenobióticos

Como parte de la presente Tesis Doctoral se muestra por primera vez en la literatura el desarrollo y la validación de un instrumento dirigido específicamente a la estimación de la ingesta de xenobióticos. A través del trabajo llevado a cabo, el CFCA desarrollado se ha identificado como un instrumento útil de recogida de la información dietética (**Artículo 1**), permitiendo registrar los hábitos dietéticos a largo plazo y las asociaciones con el desarrollo de síntomas gastrointestinales vinculados a hábitos dietéticos poco saludables que se relacionan con un mayor riesgo de CCR.

Asimismo, se ha mostrado que el método desarrollado y validado para la estimación de la ingesta de xenobióticos resulta especialmente preciso en el 64% de los xenobióticos analizados. En concreto, presenta un índice de correlación "aceptable" (≥ 0,3) en el caso de DiMeIQx, PhIP, nitratos, nitritos y nitrosaminas, y esta precisión es superior y se considera "buena" (índice de correlación ≥ 0,5) en el caso de MeIQx, la categoría "HAP totales" y Comb (Sander *et al*., 2011; Watson *et al*., 2015; Zazpe *et al*., 2020) (**Tabla Suplementaria 1.1**). Teniendo en cuenta la validez del método desarrollado, en la presente Tesis Doctoral se ha llevado a cabo el análisis de la ingesta de los compuestos con potencial carcinógeno.

1.2. Niveles de consumo y fuentes dietéticas de xenobióticos en población española

Los datos obtenidos empleando este cuestionario han puesto de manifiesto que los valores de consumo de AH, HAP, nitratos, nitritos, NOC y acrilamida (**Artículo 1** y **Artículo 2**), son similares a los valores de ingesta mostrados en otros estudios disponibles en la literatura (Carvalho *et al*., 2015; Falcó *et al*., 2003; Ibáñez *et al*., 2005; Loh *et al*., 2011; Obón-Santacana *et al*., 2014; Rohrmann *et al*., 2007, 2009).

La ingesta de AH procedió principalmente de carnes y carnes procesadas, cocinadas mediante métodos "a la plancha" y "al horno", en consonancia con las descritas en la literatura (Iwasaki *et al*., 2010; Jahurul *et al*., 2010; Nguyen *et al*., 2022; Voskuil *et al*., 1999). Del mismo modo, estas fueron las técnicas de cocinado de las carnes que más contribuyeron a la ingesta de PhIP y MeIQx. PhIP destaca por presentar los niveles de ingesta más elevados entre el conjunto de los potenciales carcinógenos evaluados (**Artículo 1** y **Artículo 2**), tal y como se ha descrito en otros trabajos (Carvalho *et al*., 2015; Le *et al*., 2016). En concreto, el consumo de PhIP en individuos sin patologías conocidas fue 188 ng/d, mientras que individuos en riesgo de inseguridad alimentaria presentaron una ingesta de hasta 299 ng/d. Estos niveles de consumo son entre 5 y 8 veces superiores a la ingesta asociada con un incremento del riesgo de adenoma colorrectal (≥40 ng/d) (Martínez Gongora *et al*., 2019) y entre 2 y 3 veces superiores a la ingesta asociada con un incremento del riesgo de CCR (103 ng/d) (Dao *et al*., 2020). Asimismo, teniendo en cuenta que el consumo de PhIP se ha asociado directamente con un incremento de la mutagenicidad fecal (Ruiz-Saavedra *et al*., 2022), los resultados expuestos en esta Tesis resaltan el potencial papel del PhIP como factor de riesgo en la alteración de la homeostasis intestinal y ensalzan el uso de esta AH como una posible diana de interés en estrategias dietéticas dirigidas a reducir el consumo y/o el impacto de la ingesta de este xenobiótico. Por otra parte, tras el PhIP, el MeIQx fue la AH que presentaba los niveles de ingesta más elevados, con valores en el rango de los descritos por otros autores (Carvalho *et al*., 2015; Rohrmann *et al*., 2009). En concreto, se obtuvieron ingestas entre 4-65 ng/d (**Artículo 1** y **Artículo 2**), por lo que, considerando que en algunos casos los niveles de consumo fueron ligeramente superiores a los valores asociados con un incremento del riesgo de adenoma colorrectal (≥50 ng/d) (Martínez Gongora *et al*., 2019), la AH MeIQx podría considerarse, asimismo, una potencial diana de interés en el desarrollo de estrategias dietéticas dirigidas a la prevención de CCR.

Junto con las AH, los HAP son potenciales carcinogénicos asociados con el desarrollo de procesos neoplásicos en el colon. Estos compuestos proceden principalmente del consumo de aceites y grasas, cereales, bebidas alcohólicas y leche, en consonancia con lo referido por otros autores en población española (Falcó *et al*.,

2003; Ibáñez *et al*., 2005) y de Reino Unido (Scientific Committee on Food, 2002). En el caso de DiB(a)A, la cerveza fue la principal fuente de ingesta de este potencial carcinógeno, excepto en el caso del colectivo en riesgo de inseguridad alimentaria, quienes declararon no consumir alcohol en el 68% de los casos. Los niveles de consumo de DiB(a)A observados en esta Tesis (0,00-0,07 μg/d) se encuentran dentro del rango de valores descritos por otros autores en España (Falcó *et al*., 2003; Ibáñez *et al*., 2005) y resultados similares se obtienen en el caso del B(a)P. En esta Tesis, el consumo de B(a)P en las dos muestras poblacionales (0,02-0,06 μg/d) dista considerablemente de las ingestas asociadas con un incremento del potencial efecto perjudicial definido por la Organización Mundial de la Salud como potencial dosis de riesgo (100 mg/ kg de peso corporal). En concreto, considerando un peso corporal de 75 kg, las ingestas reflejan valores hasta 9 órdenes de magnitud inferiores a esta dosis (Joint FAO/WHO Expert Committee on Food Additives, 2005). A pesar de que los hábitos alimenticios mediterráneos y americanos difieren considerablemente, en la presente Tesis, la técnica de cocinado "a la barbacoa" fue la que más contribuyó a la ingesta de B(a)P derivada de la carne, al igual que ocurre en individuos estadounidenses (Kazerouni *et al*., 2001).

De forma similar a lo mostrado con los HAP, en el conjunto de sujetos evaluados el nivel de consumo de nitratos es entre 2 y 3 veces inferior a la IDA de la EFSA de 278 mg/d considerando un peso medio de 75 kg (EFSA ANS Panel *et al*., 2017a), dado que se han observado niveles de consumo entre 20 y 126 mg/d en la presente Tesis Doctoral. Mientras que los nitratos proceden principalmente de alimentos de origen vegetal como acelga, calabacín, lechuga o patata, los nitritos y las nitrosaminas derivaron del consumo de carnes procesadas, principalmente de jamón cocido, jamón serrano y chorizo. Estos resultados son similares a los hallados en la literatura (Griesenbeck *et al*., 2009; Jakszyn *et al*., 2006b; Loh *et al*., 2011). Además, la procedencia de estos potenciales carcinógenos de alimentos de origen vegetal o animal sugiere potenciales diferencias en el efecto de estos compuestos sobre la homeostasis intestinal, tal y como ya han identificado algunos autores (Bondonno *et al*., 2024).

En el caso de los nitritos, los niveles de consumo observados (entre 0 y 3 mg/d), se encuentran dentro del rango descrito en individuos de Reino Unido (1,5 mg/d) (Loh *et al*., 2011). Además, estos valores son entre 2 y 3 veces inferiores a la IDA de la EFSA

de 5 mg/d, considerando una persona con un peso medio de 75 kg (EFSA ANS Panel *et al*., 2017b). Por otro lado, recientemente, la EFSA realizó una evaluación del riesgo de las nitrosaminas en alimentos (EFSA CONTAM Panel *et al*., 2023) y en esta, se mostraron niveles de consumo de las nitrosaminas evaluadas en este trabajo, considerando una persona de 75 kg, entre 0,3 y 3,8 μg/d, lo cual se encuentra dentro del orden de ingesta estimado en esta Tesis (entre 0,01 y 0,17 μg/d). Sin embargo, cabe destacar que la ingesta de nitrosaminas cuantificada no representa el total de NOC en el organismo, ya que existe una producción endógena de estos potenciales carcinógenos la cual, influenciada por la ingesta de nitritos y nitratos, no ha podido ser evaluada en este trabajo (Gushgari & Halden, 2018; Xie *et al*., 2023).

Por último, el consumo de acrilamida procedió principalmente de galletas, patata y pan, alimentos que se han identificado entre las principales fuentes dietéticas en países europeos, asiáticos y americanos (Dybing *et al*., 2005; Marques *et al*., 2024). En esta línea, los niveles de consumo observados en los sujetos participantes (6-15 μg/d) se encuentran dentro del rango descrito tras considerar un total de 94 países de los cuales el 69% eran europeos (entre 2 y 115 μg/d) (Timmermann *et al*., 2021). Además, estos valores son 3 órdenes de magnitud inferiores a los establecidos por la EFSA como nivel de consumo asociado con un incremento del riesgo de potenciales efectos adversos (13 mg/d), considerando un peso medio de 75 kg (EFSA *et al*., 2022). En base a los resultados obtenidos y las discrepancias encontradas en la literatura entre la ingesta de acrilamida y el riesgo de CCR (Hogervorst *et al*., 2014; Pelucchi *et al*., 2006), este xenobiótico no parece ser una diana terapéutica de interés en los grupos poblacionales de estudio.

Tal y como hemos observado, entre el conjunto de xenobióticos cuya ingesta ha sido evaluada, el consumo de HAP, nitratos, nitritos, nitrosaminas o acrilamida es considerablemente inferior a los umbrales descritos por entidades internacionales. Sin embargo, en esta Tesis no ha sido posible evaluar la exposición total a estos compuestos, dado que, junto con la dieta, estos potenciales carcinógenos pueden derivar de fuentes como la contaminación ambiental o el tabaco (EFSA, 2015; Jakszyn *et al*., 2006a; Patel *et al*., 2020). No obstante, una de las fortalezas de la presente Tesis Doctoral es que la mayoría de los sujetos de la muestra de estudio no son fumadores,

por lo que no se espera que esto afecte de manera significativa a la exposición cuantificada para la extracción de conclusiones. En contraposición, las AH provienen exclusivamente de la dieta y, a pesar de que el PhIP es el xenobiótico que presenta los niveles de ingesta más elevados de forma consistente en la literatura, no se ha encontrado umbrales de consumo descritos para esta u otras AH por parte de las instituciones de referencia. Asimismo, los niveles de consumo de PhIP son próximos a los asociados con un mayor riesgo de alteración de la mucosa, por lo que, en base a las evidencias expuestas en la presente Tesis Doctoral, resulta evidente la necesidad de considerar el PhIP como una de las principales dianas de interés para la elaboración de estrategias dietéticas dirigidas a la prevención del CCR.

2. Impacto de la Ingesta de Xenobióticos sobre la Salud Intestinal

A continuación, se evaluó el impacto del consumo de los xenobióticos sobre la salud gastrointestinal. Para ello se utilizaron como indicadores los criterios de funcionalidad gastrointestinal de Roma III (**Artículo 1**) y el análisis histológico de la mucosa del colon (**Artículo 4**).

Por primera vez en la literatura, se ha identificado un consumo más elevado de xenobióticos asociado a la existencia y a la gravedad de alteraciones gastrointestinales en individuos sin patologías conocidas. Los criterios de Roma III se emplean para evaluar la funcionalidad gastrointestinal (Shih *&* Kwan, 2007). En esta Tesis, aquellos sujetos con molestias gastrointestinales de gravedad moderada o superior presentaron niveles de consumo más elevados de DiMeIQx y HAP (**Artículo 1**), indicando una asociación entre el consumo de xenobióticos y la alteración de la función intestinal. Esta asociación resulta especialmente relevante dado que se han descrito diferentes alteraciones de la funcionalidad gastrointestinal de forma previa al diagnóstico de CCR (Demb *et al*., 2024; Fritz *et al*., 2023). Asimismo, aquellos individuos que presentaban hemorroides y/o sangre en heces, indicadores del inicio del CCR (Fritz *et al*., 2023; González-Bernardo *et al*., 2019; Wu *et al*., 2021), mostraron niveles de consumos superiores de IQ, nitritos y nitrosaminas, poniendo de manifiesto la posible asociación entre el consumo de estos xenobióticos y la alteración de la homeostasis intestinal en individuos sin patologías digestivas declaradas.

Además, se ha analizado el impacto de la ingesta de xenobióticos sobre la mucosa del colon en estadios previos al desarrollo de CCR. Para ello, a través de un ensayo de experimentación animal, se seleccionó el PhIP por ser el xenobiótico que presentaba los niveles de ingesta más elevados en la población. Simulando las dosis de consumo observadas en el **Artículo 1** (188 ng/d), se empleó un modelo PhIP+DSS para reproducir, las condiciones inflamatorias que tienen lugar en individuos con hábitos dietéticos caracterizados por el consumo de una dieta rica en este xenobiótico (Cheung *et al*., 2011). La administración de DSS aumenta la susceptibilidad del xenobiótico (Chen *et al*., 2017; Yang *et al*., 2021) y acelera el proceso tumoral inducido por PhIP (Abdel Salam *et al*., 2014; Yang *et al*., 2021).

El tratamiento con PhIP+DSS produjo un daño histológico sobre la mucosa del colon caracterizado por la presencia de erosión, focos de inflamación, inflamación difusa, pérdida de criptas y la ausencia tumores, en consonancia con los resultados obtenidos con dosis y tiempos de tratamiento similares (Yang *et al*., 2021). Además, se observó una mayor afectación distal, lo cual es consistente con lo mostrado por otros autores tras la administración del mismo xenobiótico (Nakanishi *et al*., 2007) y podría reflejar la fase inicial del desarrollo de CCR. Esto es debido a que PhIP puede inducir el proceso oncogénico (Chen *et al*., 2017; Vanhaecke *et al*., 2008) y existe una mayor distribución de tumores en la zona distal durante las fases iniciales en humanos (Cheng *et al*., 2011; Nascimento-Gonçalves *et al*., 2021).

Los resultados obtenidos amplían las evidencias existentes sobre el impacto de la ingesta de potenciales carcinógenos en el desarrollo de alteraciones previas al CCR (Miller *et al*., 2013; Ruiz-Saavedra *et al*., 2022). En paralelo con la pérdida de la homeostasis intestinal, se han descrito cambios en el perfil microbiano y/o la respuesta inmune (Wu & Wu, 2012; Zheng *et al*., 2020). En base a esto, se ha analizado el impacto de la ingesta de xenobióticos sobre la composición y actividad de la microbiota intestinal y marcadores sanguíneos.

2.1. Modulación del potencial efecto carcinógeno de los xenobióticos por la microbiota intestinal y el sistema inmunitario

La microbiota intestinal y la respuesta inmune, además de desempeñar un papel clave en la regulación de la homeostasis intestinal (Wu & Wu, 2012; Zheng *et al*., 2020), podrían modular el potencial carcinogénico de estos xenobióticos (Al-Sadi *et al*., 2021; Dominici *et al*., 2014; Liu *et al*., 2010; Nowak & Libudzisz, 2009). De esta forma, se ha llevado a cabo un análisis de las asociaciones encontradas entre el consumo de estos potenciales carcinógenos y la microbiota intestinal (**Artículo 2**) o los marcadores sanguíneos (**Figura Suplementaria 2.1A** y **Tabla Suplementaria 2.1**) en individuos en riesgo de inseguridad alimentaria. Estos hallazgos podrían contribuir a identificar posibles vías de modulación del potencial efecto carcinogénico derivado de la ingesta de xenobióticos.

En concreto, este análisis se ha centrado en los xenobióticos que presentaron umbrales de ingesta asociados con un incremento del riesgo de alteración intestinal, el MeIQx y el PhIP. Por un lado, los individuos con una ingesta de MeIQx inferior al consumo asociado con un incremento de este riesgo presentaron niveles superiores de MCP-1. Dado que esta quimiocina atrae a los monocitos y promueve la infiltración de macrófagos a través de la vía Th17 (Hsieh *et al*., 2024), la elevación de los niveles de MCP-1 podrían reflejar un estado de inflamación de bajo grado asociado al consumo de MeIQx (Quetglas-Llabrés *et al*., 2024; Singh *et al*., 2021). Además, individuos con ingestas reducidas de esta AH mostraron un incremento de las abundancias relativas de *Eggerthellaceae* y *Lachnospiraceae* y, en base a las evidencias disponibles en la literatura, estas familias podrían ejercer diferentes efectos sobre la homeostasis intestinal. Por un lado, el aumento en las abundancias de *Eggerthellaceae* podría sugerir el desarrollo de fases iniciales de alteración de la salud gastrointestinal y del desarrollo neoplásico, dado que abundancias relativas superiores de esta familia se han encontrado en individuos con pólipos hiperplásicos (Ruiz-Saavedra *et al*., 2024). Además, estos microorganismos se han asociado con la ingesta de HAP en esta Tesis y en otros trabajos (Ruiz-Saavedra *et al*., 2023). Por otro lado, en el caso de *Lachnospiraceae*, los resultados obtenidos podrían indicar un potencial efecto reductor

del daño producido por la ingesta de potenciales carcinógenos. Considerada como marcador de la salud intestinal (Milani *et al*., 2017), *Lachnospiraceae* está involucrada en la respuesta inmune anti-tumoral del CCR (Almeida *et al*., 2021) y se ha visto aumentada con la mejora de la mucosa colónica (Q. Wang *et al*., 2020) y disminuida tras la ingesta de HAP y en alteraciones inflamatorias intestinales (Lobionda *et al*., 2019; Ribière *et al*., 2016). De esta forma, la ingesta de MeIQx en niveles reducidos, parece presentar asociaciones similares a las encontradas tras el consumo de HAP y podría asociarse con un estado de reparación y/o de inflamación de bajo grado mediado por parámetros de la vía Th17. No obstante, sería necesario llevar a cabo más estudios para confirmar los resultados obtenidos.

En el caso del PhIP, aquellos individuos que presentaban ingestas asociadas con un mayor riesgo de adenoma colorrectal mostraron abundancias relativas más elevadas de *Streptococcaceae* y *Eubacterium coprostanoligenes group*, en paralelo con un incremento en los niveles circulantes de IL-17. Y asociaciones similares se encontraron entre las abundancias relativas de *Streptococcaceae* y la ingesta de otros xenobióticos como DiMeIQx o NPIP. En consonancia, individuos con pólipos y CCR mostraron un incremento en las abundancias relativas de las familias bacterianas *Streptococcaceae* y de *Eubacterium coprostanoligenes group* (Qi *et al*., 2022; Senthakumaran *et al*., 2023). Asimismo, a través de la vía Th17, la IL-17 media la inflamación crónica y también está involucrada en el desarrollo del CCR (Marques *et al*., 2021). En consecuencia, teniendo en cuenta que el PhIP es el xenobiótico con los niveles de consumo más elevados, y en base a las asociaciones observadas, los resultados obtenidos podrían indicar una potencial asociación entre el consumo de PhIP, la modulación de la composición microbiana y, la respuesta inmune hacia un estado pro-inflamatorio. De esta forma, con el fin de confirmar las asociaciones observadas, se llevó a cabo un análisis del impacto del consumo de este xenobiótico sobre la microbiota intestinal (**Artículo 4)** y los parámetros sanguíneos mediante el ensayo de experimentación animal (**Tabla Suplementaria 4.1** y **Figura Suplementaria 4.1**).

El tratamiento con PhIP+DSS podría confirmar algunas de las asociaciones observadas en humanos ya que, en paralelo con un mayor daño histológico sobre la mucosa del colon, el tratamiento produjo un incremento de la abundancia relativa de

familias previamente elevadas en modelos de colitis y de CCR como *Clostridia* UCG014 (Lin *et al*., 2022; Qiao *et al*., 2022).

Los resultados obtenidos concuerdan con los mostrados por otros autores en los que dosis de PhIP ligeramente superiores (10 ppm) provocaron un incremento de *Clostridiaceae* (Zhao *et al*., 2021), descrita como una familia con efecto pro-inflamatorio (K. Wang *et al*., 2022). Por otro lado, los AGCC desempeñan un papel importante en la promoción de la homeostasis intestinal (Fan *et al*., 2023). De hecho, se ha observado que una menor producción de AGCC podría incrementar la severidad del daño (Fan *et al*., 2023) y niveles reducidos se han identificado en el CCR, tanto en modelos animales (Fan *et al*., 2023; K. Wang *et al*., 2022) como en humanos (Ziemons *et al*., 2023). En consonancia, la administración de PhIP+DSS en el ensayo animal redujo la excreción de los AGCC ramificados y del ácido valérico. Además, los niveles de estos metabolitos se han asociado directamente con la abundancia de *Clostridiaceae*, lo cual contribuye a aumentar las evidencias disponibles hasta el momento por las que esta familia podría mediar la alteración de la homeostasis intestinal. En consonancia, entre los principales productores de estos AGCC mediante la fermentación de aminoácidos ramificados, se encuentran géneros próximos como *Clostridium* (Rios-Covian *et al*., 2020) y este se ha destacado como predictor de estos metabolitos en omnívoros (Trefflich *et al*., 2021).

Por otro lado, existen evidencias de que la IL-17 podría actuar como mediadora del efecto del ácido valérico en la modulación del estado inflamatorio (Zeng *et al*., 2023). Esto resulta especialmente interesante considerando que, en paralelo con una menor excreción de ácido valérico, el PhIP+DSS redujo de forma no significativa los niveles circulantes de IL-17F e IFN-γ. Asimismo, el daño en la mucosa observado en el grupo PhIP+DSS se asoció directamente con los niveles del mediador pro-inflamatorio IL-6. De esta forma, los resultados obtenidos contribuyen a ampliar la información disponible en la literatura, mostrando vías por las que el PhIP+DSS podría alterar la homeostasis intestinal mediante la actividad microbiana y posiblemente, la respuesta inmunológica. Adicionalmente, la ingesta de xenobióticos se ha asociado con un incremento del estrés oxidativo durante la pérdida de la homeostasis intestinal (Lin *et al*., 2020; Y. Wang *et al*., 2020) y un efecto similar se ha observado en la presente Tesis tras la administración de PhIP+DSS. En paralelo con un incremento del daño sobre la

mucosa, la ingesta del potencial carcinógeno produjo una reducción de la CAT (**Figura Suplementaria 4.1**), lo cual es similar a lo descrito por otros autores con el mismo modelo animal (Lin *et al*., 2020; Sliva *et al*., 2012).

En la presente Tesis Doctoral se ha podido confirmar que el consumo de PhIP, con niveles de ingesta similares a las observados en humanos, produce un incremento del daño histológico en la mucosa del colon en paralelo con un aumento de la abundancia relativa de familias con potencial pro-inflamatorio como *Clostridia* UCG014, la reducción de AGCC como el ácido valérico, la alteración de los niveles de citocinas de la vía Th17 y el incremento del estrés oxidativo. En base a estos resultados, se evaluó el potencial de diferentes estrategias de intervención para reducir el impacto de la ingesta de estos potenciales carcinógenos.

3. Estrategias Dietéticas para Contrarrestar el Impacto de la Ingesta de Xenobióticos

La DM cuenta con evidencia sólida sobre su efecto protector de la homeostasis intestinal (Del Chierico *et al*., 2014). En concreto, la ingesta de frutas, verduras, legumbres y cereales integrales, las cuales aportan un alto contenido en fibras, polifenoles, vitaminas y minerales (Román *et al*., 2019), se han asociado con la reducción del riesgo de CCR (Aranda-Olmedo *&* Rubio, 2020; Duijnhoven *et al*., 2009; GBD *et al*., 2022; Kopf *et al*., 2018; Papandreou *et al*., 2019). Sin embargo, existe una pérdida progresiva de este patrón alimentario en los últimos años (Serra-Majem *&* Ortiz-Andrellucchi, 2018) que afecta especialmente a individuos en riesgo de inseguridad alimentaria (da Costa *et al*., 2022). De esta forma, resultaría interesante evaluar el impacto de una intervención educacional y dietética sobre el consumo de xenobióticos y la modulación de la composición microbiana y los parámetros inmunológicos en este colectivo.

En la presente Tesis Doctoral, la intervención de 4 semanas produjo una mejora del perfil alimentario (**Artículo 3**). En concreto, se produjo un incremento en el consumo de verduras junto con una reducción en el consumo de carnes procesadas y de cereales. Asimismo, se incrementó el consumo de lignanos y se redujo la ingesta de DiB(a)A, "HAP totales", NPIP y acrilamida mostrando, por primera vez en la literatura, el potencial de una intervención con DM para reducir la ingesta de

xenobióticos, y, en consecuencia, disminuir el potencial riesgo de CCR derivado de la dieta. En el caso de la fibra dietética, independientemente del grado de cumplimiento de las recomendaciones, se superó el umbral asociado con un mayor riesgo de CCR en algunos estudios (<10 g/d) (Park *et al*., 2005).

De esta forma, tras comprobar que la intervención mejoró el perfil alimentario de los sujetos en riesgo de inseguridad alimentaria, se evaluó el impacto de la intervención sobre la modulación de la composición microbiana y la respuesta inmunitaria, junto con el impacto sobre variables asociadas con el estado de salud.

3.1. Impacto de la intervención sobre la modulación de la microbiota intestinal, el perfil inflamatorio y variables indicadoras del estado de salud

Tras 4 semanas de intervención dietética, la DM produjo cambios en la composición microbiana de los individuos en riesgo de inseguridad alimentaria (**Artículo 3**). En concreto, se redujo la abundancia relativa de Actinomycetota y de familias pertenecientes como *Coriobacteriaceae* y *Eggerthellaceae* u otras como *Clostridia* UCG014 (filo Bacillota). Estas variaciones son similares a las observadas en el caso de intervenciones con dietas ricas en fibra y bajas en grasa (Fritsch *et al*., 2021) y, además, considerando las asociaciones encontradas entre la dieta y la microbiota en la literatura, estos cambios en el perfil microbiano podrían estar mediados por las variaciones en la ingesta de estos sujetos. Por un lado, se ha descrito que individuos con consumos más elevados de carne mostraron un incremento de las abundancias relativas de *Coriobacteriaceae* (Ruiz-Saavedra *et al*., 2023), por lo que la reducción observada de esta familia bacteriana podría estar asociada con la menor ingesta de carne procesada de la muestra de estudio. Asimismo, junto con *Eggerthellaceae*, ambas familias se han mostrado elevadas en el colon de ratones alimentados con una dieta rica en grasa promoviendo el desarrollo de CCR (Tang *et al*., 2024) y, además, *Clostridia* UCG014 se ha mostrado elevada en modelos de colitis (Lin *et al*., 2022). De esta forma, el potencial de la intervención dietética para reducir estos microorganismos podría ser considerado como uno de los mecanismos protectores. Finalmente, a lo largo de la presente Tesis Doctoral, *Clostridia* UCG014 se ha mostrado consistentemente vinculada con el consumo de xenobióticos, lo cual podría sugerir que las abundancias reducidas

tras la intervención podrían estar influenciadas por la menor ingesta de estos potenciales carcinógenos. Además, esta familia se ha mostrado inversamente asociada con el consumo de fibra y polifenoles en una de las muestras de estudio (**Artículo 2**). En base a la evidencia disponible en la literatura, los resultados obtenidos parecen indicar cambios en el perfil microbiano asociados con un potencial efecto protector sobre la mucosa del colon.

A continuación, en base a las asociaciones descritas entre la composición microbiana y los marcadores sanguíneos, se analizó el impacto de la intervención sobre los parámetros inflamatorios (**Tabla Suplementaria 3.1**). Conforme a lo esperado, se observó una mejora del perfil inflamatorio en los sujetos de estudio. En concreto, los individuos mostraron un incremento en los niveles de IL-10 y adiponectina, junto con una reducción de IL-2, IL-6 o IL-12. En consonancia, algunos autores han mostrado que aquellos individuos con una mayor adherencia a la DM tendían a presentar niveles elevados de IL-10 (Ghosh *et al*., 2020) y reducidos de IL-2 y IL-12 (Beam *et al*., 2021). Del mismo modo, revisiones sistemáticas ponen de manifiesto el potencial de intervenciones con DM para aumentar los niveles de adiponectina y reducir IL-6, sin aparente influencia del índice de masa corporal (Schwingshackl *&* Hoffmann, 2014). Estos resultados concuerdan con los obtenidos en la presente Tesis Doctoral, dado que la mejora del perfil microbiano ha sido independiente de cambios significativos en los niveles circulantes de parámetros bioquímicos o en las determinaciones antropométricas realizadas, excepto en el caso del porcentaje de grasa corporal, el cual se incrementó ligeramente tras la intervención (de 32 a 35%).

Finalmente, en paralelo con la mejora del perfil inflamatorio y de la composición microbiana, la intervención con DM tuvo un impacto positivo sobre otras variables asociadas con el estado de salud. En concreto, se ha mostrado que los individuos en riesgo de inseguridad alimentaria redujeron la severidad de los síntomas depresivos, aumentaron la percepción sensorial a través de una mayor discriminación al sabor dulce y salado, y mejoraron el comportamiento alimentario mostrando una ingesta menos influenciada por estímulos externos y emocionales (**Artículo 2** y **Tabla Suplementaria 3.2**). Estos resultados confirman los mostrados por otros autores, mostrando el potencial de la DM para mejorar estas variables, las cuales se utilizan

como indicadores de la salud en individuos (Calderón García *et al*., 2024; Cattaneo *et al*., 2023; Sánchez-Villegas *et al*., 2013). Además, se han detectado modulaciones específicas en la composición microbiana y el sistema inmune de aquellos sujetos que mostraron mejoras en la severidad de los síntomas depresivos. De hecho, tras la intervención, los individuos con mejoras mostraron un incremento en la abundancia relativa de *Oscillospiraceae* (**Artículo 2**) y en los niveles TGF-β/IL-2 (**Tabla Suplementaria 3.3**), parámetros que ya se han visto involucrados en la depresión (Beurel *et al*., 2022; McGuinness *et al*., 2022; Mennella *et al*., 2014; Petty *et al*., 2020). Estos resultados contribuyen a aumentar las evidencias acerca del papel de la DM sobre la promoción de la salud a través de la modulación de la composición microbiana y parámetros inmunológicos.

A pesar de las evidencias mostradas, el complejo contexto socioeconómico de estos individuos y la ayuda económica aportada ha limitado el tamaño muestral y la adherencia a las recomendaciones. Por ello, la obtención de mejoras significativas en los indicadores de salud de los individuos y la modulación descrita de la composición microbiana y de marcadores sanguíneos, a pesar de las dificultades encontradas, pone de manifiesto el potencial de este tipo de intervenciones para mejorar los hábitos alimentarios y, en consecuencia, la salud de un colectivo con recursos limitados que constituye una fracción cada vez mayor de la población.

3.2. Evaluación del potencial de los prebióticos para reducir el daño derivado de la ingesta de xenobióticos

Existe una amplia evidencia en la literatura acerca del papel de la fibra dietética frente al CCR y su efecto promotor de la homeostasis intestinal (Barrubés *et al*., 2020; Martel *et al*., 2022). Sin embargo, hasta el momento no se habían realizado ensayos en animales que evaluaran la capacidad de la fibra para contrarrestar el daño provocado por la ingesta de xenobióticos sobre la mucosa del colon.

En esta Tesis se ha mostrado por primera vez, que la suplementación de animales expuestos a PhIP+DSS, con una dieta isocalórica enriquecida en fibra (6%) redujo el daño observado sobre la mucosa del colon a través de una reducción de la

inflamación difusa y de la pérdida de criptas (**Artículo 4**). Estas alteraciones histológicas son características de la inflamación crónica (Yusuf *et al*., 2022), por lo que los resultados obtenidos se encuentran en consonancia con otros trabajos en los que se ha demostrado la capacidad de la fibra para reducir la inflamación crónica de bajo grado en modelos en colitis (Miles *et al*., 2017). Además, utilizando una dosis de suplementación dietética con fibra similar (5%), se ha observado una reducción de la severidad de la inflamación en paralelo con una mejora del daño histológico sobre la mucosa en modelos de colitis (Macia *et al*., 2015). Entre los diferentes mecanismos propuestos, por los que la fibra pudiera tener un efecto reductor del daño derivado de la ingesta de PhIP, se encuentra la formación de geles viscosos en el lumen del tracto intestinal, los cuales podrían retener los compuestos con potencial carcinogénico reduciendo su absorción intestinal y su contacto con la mucosa del colon (Nogacka *et al*., 2019). Asimismo, existen evidencias de la fibra como posible moduladora del potencial carcinogénico del PhIP a través de la modulación de la composición microbiana y de la respuesta del sistema inmune (Willenberg *et al*., 2015a).

A partir de los resultados obtenidos, podemos confirmar que la fibra ejerce un efecto reductor del daño provocado por la ingesta de xenobióticos y mejora el daño histológico en la mucosa. En base a estas evidencias, el siguiente paso en la presente Tesis Doctoral ha sido profundizar en los mecanismos por los que la fibra puede ejercer este efecto protector mediante la modulación de la microbiota intestinal y los marcadores sanguíneos.

3.2.1.Modulación del efecto protector mediante la microbiota intestinal y el sistema inmunitario

En la presente Tesis Doctoral, los individuos en riesgo de inseguridad alimentaria mostraron que el consumo de componentes con potencial prebiótico como la fibra, se asociaba inversamente con las abundancias relativas de familias con potencial pro-inflamatorio vinculadas con el consumo de xenobióticos, tales como *Eggerthellaceae* o *Clostridia* UCG014 (**Artículo 2**). Asimismo, la ingesta de fibra dietética en estos sujetos se asoció directamente con los niveles relativos de familias con potencial anti-inflamatorio como *Bacteroidaceae*, junto con los niveles circulantes de

parámetros como IL-17, integrantes de la vía Th17 (**Figura Suplementaria 2.1**). Asociaciones similares se encontraron en otros estudios, puesto que algunas de las principales fuentes de ingesta de fibra se asociaron con los niveles circulantes de IL-17 en individuos con pólipos (González *et al*., 2023). En la presente Tesis Doctoral, estas asociaciones observadas en humanos, a través de las cuales la ingesta de fibra dietética podría mediar un efecto protector de la homeostasis intestinal mediante la modulación de la composición microbiana y parámetros inmunológicos, fueron confirmadas en el estudio con animales de experimentación llevado a cabo.

En paralelo con un menor daño histológico sobre la mucosa, la administración de un 6% de fibra redujo las abundancias relativas de *Clostridia* UCG014, la cual se encontraba elevada tras el tratamiento con PhIP+DSS y está asociada con el desarrollo de pólipos (Lin *et al*., 2022). De esta forma, los resultados obtenidos parecen indicar que la reducción de este género bacteriano podría ser uno de los mecanismos por los que la ingesta de fibra reduce el riesgo de CCR. En consonancia, la suplementación con fibra produjo cambios en parámetros sanguíneos que ya se han visto asociados con variaciones en las abundancias de *Clostridia* UCG014 (Wang *et al*., 2024). Concretamente, el prebiótico produjo un incremento en los niveles circulantes de IL-17A, por lo que, dado que IL-17A participa en procesos de reparación y regeneración del epitelio a través de la inducción de la expresión de proteínas de unión (Lee *et al*., 2015), los niveles elevados de este parámetro inmunológico podrían estar involucrados en la mejora del epitelio intestinal. En conjunto, los resultados obtenidos amplían las evidencias disponibles en la literatura, mostrando posibles mecanismos por los que la fibra alimentaria alivia las alteraciones derivadas de la ingesta de PhIP+DSS a través de la reducción de *Clostridia* UCG014 y la modulación de parámetros de la vía Th17. Del mismo modo, la suplementación con fibra podría ejercer un efecto promotor de la homeostasis intestinal a través del incremento de la familia *Bacteroidaceae*, dentro de la cual especies como *B. faecis* han demostrado mejorar la función de la barrera epitelial a través de la restauración de la expresión de proteínas de unión y la promoción de la respuesta anti-inflamatoria en el colon (Mohebali *et al*., 2023).

En conjunto, la presente Tesis Doctoral ha puesto por primera vez de manifiesto, el potencial efecto protector que ejerce la fibra dietética sobre el daño

provocado en la mucosa del colon por el consumo de PhIP+DSS, mediante la modulación de la composición microbiana y la respuesta inmune. Estos resultados contribuyen a ampliar las evidencias existentes que respaldan el uso de prebióticos como la fibra, como una posible diana de acción en el diseño de estrategias dietéticas dirigidas a reducir el riesgo de CCR en la población.

3.3. Evaluación del potencial de un probiótico para reducir el daño derivado de la ingesta de PhIP

Además de la ingesta de prebióticos como la fibra, el consumo de probióticos ha sido considerado como una estrategia de interés para la promoción de la homeostasis intestinal dado que, promueve la modulación de la composición microbiana y de la respuesta inmune (Aleksandrova *et al*., 2017). En consecuencia, en esta Tesis se ha evaluado el potencial de probióticos para reducir el daño derivado de la ingesta de xenobióticos.

El probiótico mixto utilizado, compuesto por cepas pertenecientes a *Lactobacillus*, *Bifidobacterium* y *Streptococcus*, presenta evidencias en la literatura de su efecto protector frente al desarrollo de la inflamación y la progresión de neoplasias (Appleyard *et al*., 2011; Chung *et al*., 2017; Do *et al*., 2016; Friederich *et al*., 2011; Kumar *et al*., 2017; Tursi *et al*., 2010; Wang *et al*., 2018). Sin embargo, hasta el momento no se ha analizado el potencial de este probiótico para contrarrestar las alteraciones derivadas de la ingesta de estos potenciales carcinógenos. Algunos autores, mediante ensayos *in vitro,* han mostrado que cepas pertenecientes a los géneros integrantes del probiótico reducen el efecto carcinogénico del PhIP por su unión directa a las paredes celulares y su metabolización, lo cual contribuye a disminuir la biodisponibilidad y, en consecuencia, su potencial carcinógeno sobre la mucosa (Dominici *et al*., 2014; Nowak *et al*., 2014; Nowak *&* Libudzisz, 2009). En base a estas evidencias, en la presente Tesis Doctoral se ha evaluado el potencial de este probiótico para reducir el daño provocado por la ingesta de PhIP+DSS sobre la mucosa del colon.

En línea con los resultados observados en modelos animales de colitis (Fitzpatrick *et al*., 2007; Liu *et al*., 2019; Sang *et al*., 2014), la ingesta del probiótico en esta

Tesis (2,2 x 10^9 UFC/d) aumentó la longitud del colon. Este parámetro es habitualmente utilizado como marcador de la inflamación intestinal (Chou *et al*., 2021; Qiao *et al*., 2022; Wang *et al*., 2017) y los resultados obtenidos podrían indicar una mejora del ambiente inflamatorio dado que el acortamiento del colon se ha asociado con un mayor índice de la actividad inflamatoria (Kim *et al*., 2020) y con el desarrollo tumoral (Fan *et al*., 2023). De este modo, los resultados obtenidos confirman los mostrados en otros trabajos, en los que dosis similares de probiótico (1,8 x 10^9 UFC/d) produjeron una elongación del colon en paralelo con una menor afectación histológica de la mucosa (Fitzpatrick *et al*., 2007).

3.3.1.Modulación del efecto protector mediante la microbiota intestinal y el sistema inmunitario

En paralelo con una elongación del colon, la administración del probiótico contrarrestó las alteraciones en la composición microbiana intestinal asociadas a la administración de PhIP+DSS. Por un lado, el probiótico redujo la abundancia relativa de *Eubacterium coprostanoligenes group*, la cual se encontraba elevada con niveles de consumo de este xenobiótico de riesgo de adenoma colorrectal (**Artículo 2**). Asimismo, al igual que la fibra dietética, el probiótico contrarrestó el incremento en la abundancia relativa de *Clostridia* UCG014 tras la exposición a PhIP+DSS, la cual, junto con *Eubacterium coprostanoligenes group*, mostraba abundancias elevadas en individuos con pólipos (Lin *et al*., 2022; Senthakumaran *et al*., 2023). En base a los resultados obtenidos, el probiótico parece contrarrestar las alteraciones en la composición microbiana asociadas con el desarrollo de CCR.

En relación con los mecanismos de acción, en el grupo suplementado con probiótico la abundancia relativa de *Lactobacillaceae* se asoció de forma inversa con la erosión en la mucosa. *Lactobacillaceae* es una de las familias integrantes del probiótico, y dado que ha mostrado reducir la permeabilidad e inflamación en trabajos previos (Li *et al*., 2023), podría mediar el efecto protector del mismo. Asimismo, la suplementación contrarrestó el efecto de PhIP+DSS mediante la restauración de los niveles excretados del ácido valérico. En base al potencial efecto de los AGCC como promotores de la homeostasis intestinal (Hou *et al*., 2022; Makki *et al*., 2018), el incremento de la

excreción de este metabolito podría ser uno de los mecanismos de acción. Además, la mayor longitud del colon observada ha tenido lugar en paralelo con un aumento de la abundancia de *Adlercreutzia* (0,7 *vs.* 0,3%), asociada positivamente con la longitud del colon en otros estudios (Liu *et al*., 2019), con los niveles excretados de ácido valérico (Huang *et al*., 2022) y, con la mejora de estados de colitis (Yang *et al*., 2022). En conjunto, los resultados obtenidos parecen indicar un efecto reductor del impacto de la ingesta del xenobiótico a través de la elongación del colon y la modulación de la composición y actividad microbiana que, en base a la información disponible en la literatura, podría asociarse con una reducción de los parámetros inflamatorios.

En este sentido, la suplementación con probiótico modificó la respuesta inflamatoria mediante la reducción de los niveles de IL-22. Esta citocina es producida en el inicio de la respuesta inmune, se encuentra involucrada en la reparación de tejidos (Arshad *et al*., 2020) y la vía Th17 (Ivanov *et al*., 2009; Kim *et al*., 2022). De este modo, los niveles circulantes reducidos en esta Tesis concuerdan con la reducción observada en pacientes de CCR tras el consumo de cepas probióticas pertenecientes a los mismos géneros (*Lactobacillus* y *Bifidobacterium*), junto con IL-6 e IL-2 (Zaharuddin *et al*., 2019).

Por otro lado, el grupo Probiótico mostró asociaciones inversas entre la CAT y los niveles de mediadores pro-inflamatorios TNF-α o IL-6, los cuales se han visto significativamente reducidos tras el consumo del probiótico mixto (Wang *et al*., 2018). Por lo tanto, estas asociaciones podrían indicar la existencia de cambios en la respuesta inflamatoria asociados a la alteración del estado oxidativo en este grupo. En este sentido, se ha mostrado que el consumo de cepas probióticas pertenecientes a las mismas especies que las contenidas en el probiótico empleado, modula el estrés oxidativo contribuyendo a la promoción de la homeostasis intestinal (Zhao *et al*., 2023). Sin embargo, la CAT parece ser altamente dependiente del tejido utilizado (dos Santos Cruz *et al*., 2020), lo cual podría explicar la ausencia de cambios significativos en esta Tesis, al igual que ya se ha mostrado en humanos con dosis de probiótico similares (Valentini *et al*., 2015).

Al igual que la fibra, el probiótico contrarrestó los niveles elevados de familias bacterianas con potencial pro-inflamatorio como *Clostridia* UCG014 y, además, restauró la excreción del ácido valérico y moduló los niveles de IL-22.

4. Limitaciones del Estudio

Los resultados obtenidos en la presente Tesis Doctoral amplían la información disponible en la literatura sobre el potencial de la dieta para contrarrestar el impacto del consumo de xenobióticos, a través de la modulación de la composición microbiana y marcadores sanguíneos. Sin embargo, existen potenciales limitaciones del estudio que podrían afectar a la extrapolación de conclusiones y el establecimiento de perspectivas futuras.

4.1. Aplicabilidad y limitaciones del método desarrollado para la cuantificación de la ingesta de xenobióticos

En base al gran grado de detalle necesario para la estimación de la ingesta de xenobióticos (método y grado de cocinado), el análisis del consumo de estos potenciales carcinógenos resulta difícilmente aplicable a sujetos que habitualmente realizan comidas fuera de casa. Además, a pesar de que la principal fortaleza del estudio fue el grado de detalle en las preguntas y el uso de modelos fotográficos, el registro de la información dietética constituye una estimación indirecta de la exposición a xenobióticos que está limitada por el error sistemático inherente a esta metodología. En concreto, tal y como ya han señalado alguno autores, según el método de recogida de la información dietética puede existir una infraestimación o una supra-estimación de la misma (R.S. Gibson *et al*., 2017; Gunnar, 2014).

En este contexto, la determinación de biomarcadores de la ingesta mediante la cuantificación de los niveles de xenobióticos o de metabolitos derivados en muestras fecales, contribuiría a reducir los errores intrínsecos del método de registro de la información dietética y permitiría llevar a cabo una validación adicional de la herramienta desarrollada. En esta línea, el grupo de investigación ha validado recientemente la herramienta en el caso de las nitrosaminas (Ruiz-Saavedra *et al*., 2024), sentando las bases para el desarrollo de futuros trabajos, de especial interés en el caso de HAP o acrilamida por su posible exposición ambiental (EFSA, 2015; Jakszyn *et al*., 2006a; Patel *et al*., 2020).

4.2. Factores limitantes de la adherencia a las recomendaciones dietéticas en la intervención

Una de las fortalezas de la presente Tesis Doctoral es el impacto social de la intervención educacional y dietética realizada sobre individuos en riesgo de inseguridad alimentaria. A pesar del complejo contexto socioeconómico de estos sujetos, se han obtenido mejoras significativas en la ingesta de componentes con potencial efecto protector y en otras variables utilizadas como indicadores del estado de salud en la literatura. Además, la administración de la ayuda económica a cada voluntario ha sido un factor limitante del tamaño muestral, de la duración y, en consecuencia, del posible impacto de la intervención. Sin embargo, el poder estadístico de los resultados obtenidos, con una probabilidad de error de tipo I de 0,05, ha sido del 95-98%, considerando el tamaño muestral inicial y las abundancias relativas microbianas (Power and Sample Size Calculation version 3.0.43; Vanderlbilt University, Nashville, TN, USA). Estas evidencias ponen de manifiesto el potencial de intervenciones educacionales y dietéticas para promocionar el estado de salud e identificar dianas de acción para la prevención de patologías asociadas con los hábitos alimentarios, especialmente en los colectivos más desfavorecidos.

4.3. Heterogeneidad del tamaño muestral en el ensayo de experimentación animal

El estudio en roedores ha permitido analizar el impacto del tratamiento con PhIP+DSS con un poder estadístico de 95–98%, considerando las diferencias en la abundancia relativa de la composición microbiana con una probabilidad de error de tipo I de 0,05 (G*Power version 3.1.9.6 Franz Faul, Universität Kiel, Germany). Sin embargo, en algunos intervalos de tiempo, el tamaño muestral se ha visto afectado por la metodología utilizada. Por ejemplo, en el caso de la microbiota intestinal, las muestras de heces fueron recogidas cada mañana directamente de cada animal para evitar la posible contaminación y, consecuentemente, del total de la muestra, 36 animales dispusieron de información microbiana a lo largo del tiempo.

5. Perspectivas Futuras

En base a los resultados obtenidos en la presente Tesis Doctoral, a continuación, se detallan algunas posibles líneas de trabajo para corroborar y fortalecer los hallazgos encontrados.

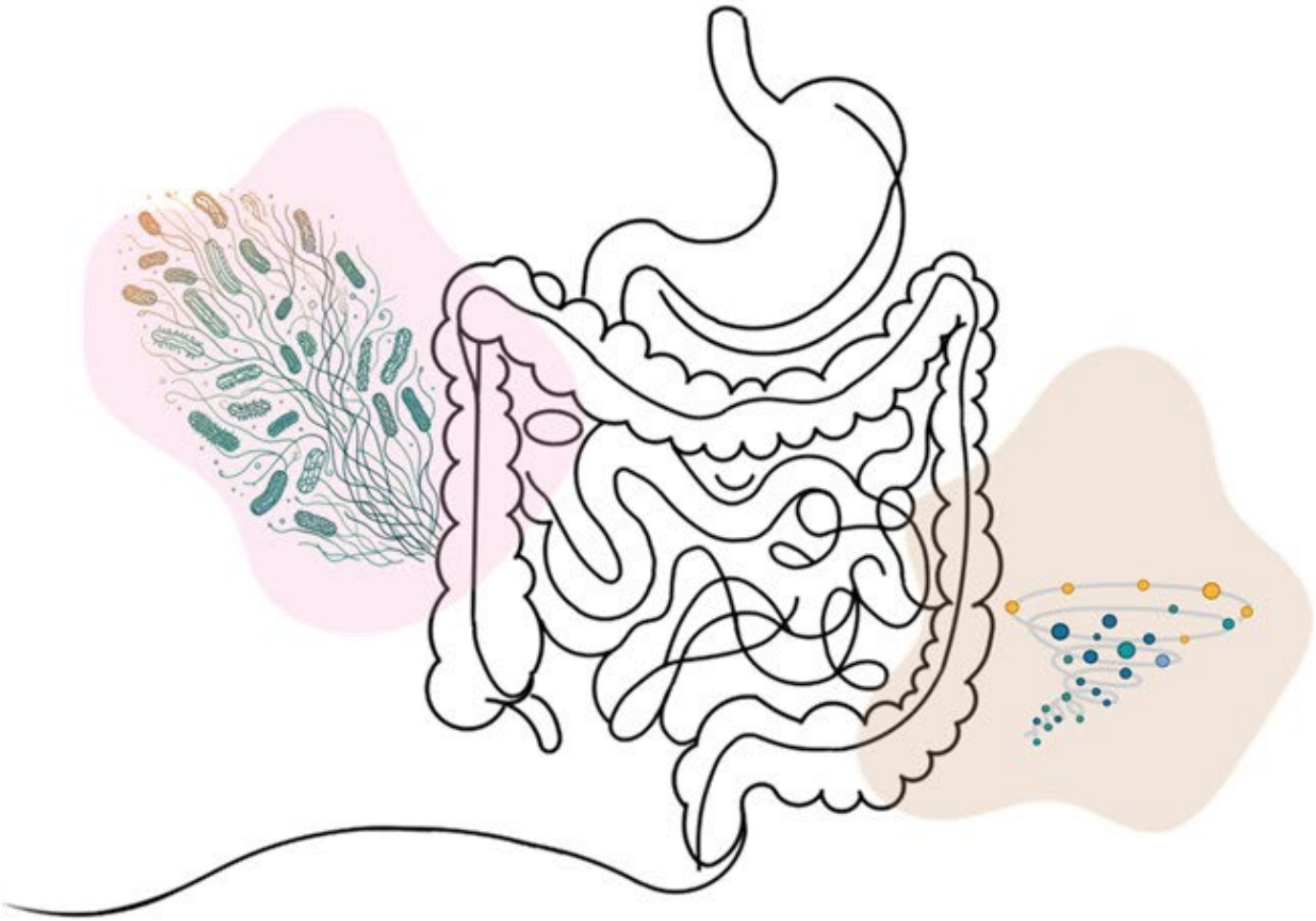
5.1. Ampliación de la validez experimental de método desarrollado

Al igual que ya se ha hecho en el caso de las nitrosaminas, en vista a los resultados obtenidos, sería de interés validar experimentalmente el método desarrollado para la cuantificación de la ingesta de xenobióticos como el PhIP. Para ello, se podría correlacionar la ingesta de este potencial carcinógeno con los niveles fecales de metabolitos bacterianos como PhIP-M1 (Willenberg *et al*., 2015b) o derivados de su metabolización por el CYP como 4'-OH-PhIP o N^2-OH-PhIP (Kim *et al*., 2015; Lu *et al*., 2007). Los resultados obtenidos podrían utilizarse como método de doble validez de la ingesta.

5.2. Intervención en colectivos socioeconómicamente vulnerables

En vista al impacto social de los hallazgos obtenidos, resulta evidente la necesidad de profundizar en posibles estrategias de intervención en los colectivos más desfavorecidos. Para ello, sería interesante llevar a cabo una intervención educacional y educativa de mayor duración y tamaño muestral, con contacto con los voluntarios en puntos intermedios del estudio. Además, se establecerían recomendaciones personalizadas en base a los hábitos de cocinado propios de cada individuo, para promover el uso de técnicas culinarias que conlleven una menor formación de xenobióticos. Finalmente, en base a los resultados obtenidos en el estudio de experimentación animal, podría ser de interés analizar el impacto de la suplementación dietética con fibra en un subgrupo de individuos, utilizando dosis similares a las empleadas en animales. De esta forma, los resultados obtenidos en esta intervención permitirían confirmar los hallazgos mostrados en la presente Tesis Doctoral y ampliar el conocimiento en la literatura sobre estrategias dietéticas que contrarresten el impacto de la ingesta de xenobióticos.

CONCLUSIONES
CONCLUSIONS



Primera.- El método desarrollado para la cuantificación de la ingesta de xenobióticos es especialmente preciso en la estimación de aminas heterocíclicas, nitratos, nitritos y nitrosaminas.

Segunda.- El PhIP se reveló como la principal diana terapéutica en la población, resaltando la necesidad de llevar a cabo estrategias dietéticas dirigidas a reducir sus niveles de consumo y/o el impacto de su ingesta en la población.

Tercera.- La intervención educacional y dietética, basada en los parámetros de la Dieta Mediterránea, es efectiva para la modulación de la composición microbiana fecal, así como, la mejora de marcadores de inflamación, contribuyendo a la comprensión del binomio dieta-microbiota en individuos en riesgo de inseguridad alimentaria.

Cuarta.- La suplementación con fibra dietética se identificó como una estrategia de interés para contrarrestar los efectos perjudiciales de la ingesta de PhIP+DSS.

Quinta.- A pesar de que la administración del probiótico parece modular la actividad y composición microbiana fecal, no es posible establecer su potencial para revertir, a nivel intestinal, el daño ocasionado por la ingesta de PhIP+DSS

First.- The developed method for the quantification of the intake of xenobiotics showed a higher degree of accuracy in the estimation of heterocyclic amines, nitrates, nitrites and nitrosamines.

Second.- PhIP emerged as the main therapeutic target in the population, highlighting the need to implement dietary strategies aimed at reducing its level of consumption and/or the impact of its intake in the population.

Third.- The educational and nutrition intervention, based on the Mediterranean Diet, proved to be effective in modulating the composition of the faecal microbiota, as well as improving markers of inflammation, contributing to the understanding of the interplay diet–microbiota in individuals at risk of food insecurity.

Fourth.- Dietary fibre supplementation was identified as a strategy of interest to counteract the detrimental effects of the intake of PhIP+DSS.

Fifth.- Although the intake of the probiotic seems to modulate the activity and composition of faecal microbiota, it is not possible to establish its potential to reverse, at the intestinal level, the damage provoked by PhIP+DSS intake.

BIBLIOGRAFÍA

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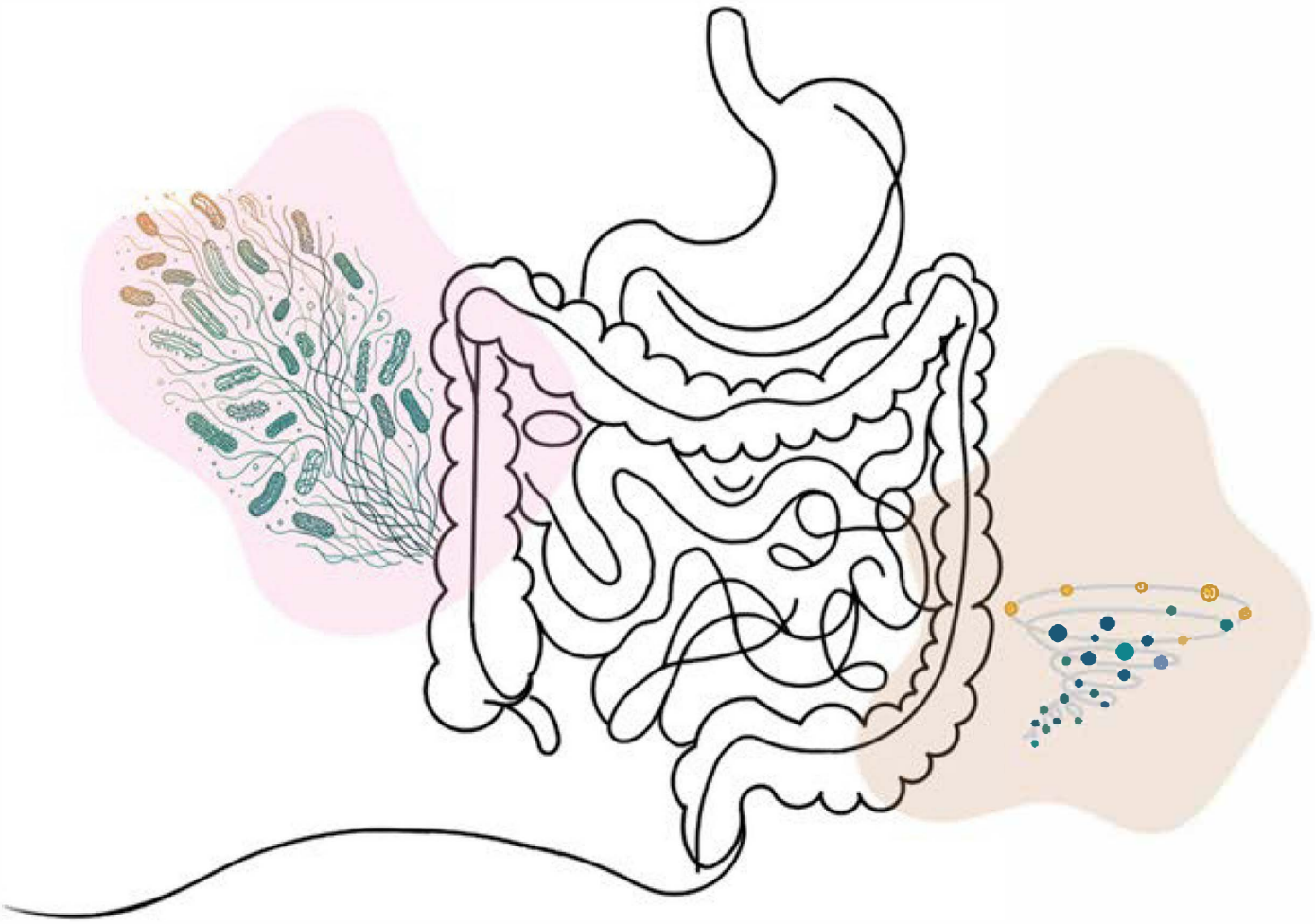

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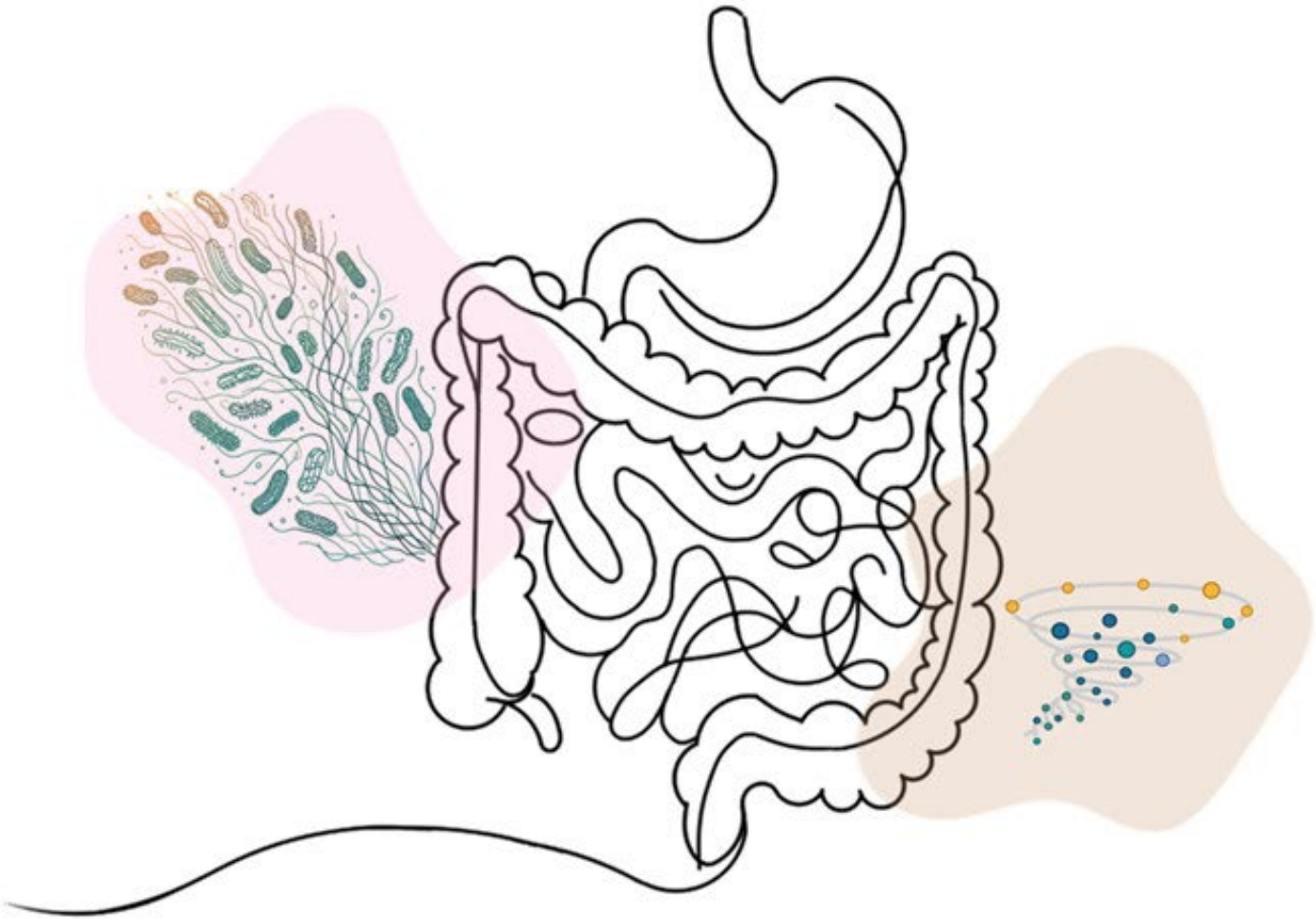
ANEXOS
ANNEXES



ANEXO I. Informe sobre la calidad de las publicaciones científicas recogidas en esta tesis.

La información sobre la calidad de los artículos que componen la presente Tesis Doctoral ha sido recogida a partir de "*Web of Science*" (https://www.recursoscientificos.fecyt.es/). Para cada artículo, se han recopilado los siguientes indicadores de la calidad: el **área Science Citation Index (SCI)** a la que se encuentra vinculada la revista de publicación; el **factor de impacto** de la revista correspondiente al "*Journal Impact Factor*" del año de publicación del artículo o en su lugar, al más próximo a su publicación (*); el **cuartil (Q)** de la revista dentro de cada área correspondiente; y, por último, las **citas**, es decir, el número de veces que se ha citado cada artículo, de acuerdo con "*Scopus*", hasta el momento de escritura de la Tesis (Noviembre del 2024).

Artículo 1: Zapico A., Ruiz-Saavedra S., Gómez-Martín M., de Los Reyes-Gavilán C.G., González S. (2002). Pilot study for the dietary assessment of xenobiotics derived from food processing in an adult Spanish sample. *Foods*, *11*(3), 470–489. doi: 10.3390/foods11030470.

Área SCI	Factor de impacto	Q	Citas
Food Science & Technology	5,2	Q1 (34/142)	9

Artículo 2: Zapico A., Arboleya S., Ruiz-Saavedra S., Gómez-Martín M., Salazar N., Nogacka A.M., Gueimonde M., de Los Reyes-Gavilán C.G., González S. (2022). Dietary xenobiotics, (poly)phenols and fibers: exploring associations with gut microbiota in socially vulnerable individuals. *Frontiers in Nutrition*, *9*, 1000829–1000835. https://doi.org/10.3389/fnut.2022.1000829.

Área SCI	Factor de impacto	Q	Citas
Nutrition & Dietetics	5,0	Q2 (28/88)	6

Artículo 3: Zapico A., Arboleya S., Salazar N., Perillán C., Ruiz-Saavedra S., de Los Reyes-Gavilán C.G., Gueimonde M., González S. (2023). Impact on fecal microbiota and health-related markers of an intervention focused on improving eating behavior in people at risk of food insecurity. *Nutrients*, *15*(16). https://doi.org/10.3390/nu15163537.

Área SCI	Factor de impacto	Q	Citas
Nutrition & Dietetics	4,8	Q1 (18/114)	1

Artículo 4: Zapico, A., Salazar, N., Arboleya, S., González del Rey, C., Diaz, E., Alonso, A., Gueimonde, M., de los Reyes-Gavilán, C.G., Gonzalez, C., González, S. (2024). Potential of fiber and probiotics to fight against the effects of PhIP + DSS-induced carcinogenic process of the large intestine. *Journal of Agricultural and Food Chemistry*, *72*(45), 25161–25172. https://doi.org/10.1021/acs.jafc.4c07366.

Área SCI	Factor de impacto (*)	Q	Citas
Chemistry, Applied	5,7	Q1 (12/74)	0

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