IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS

Iván Escobar Martínez

Impact of External Factors on the Bioavailability of (Poly)phenols: *Focus on Biological Rhythms and Proximity Consumption in Healthy and Obese Rats*

Iván Escobar Martínez

DOCTORAL THESIS
TARRAGONA 2024

UNIVERSITAT ROVIRA i VIRGILI

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Iván Escobar Martínez

Impact of External Factors on the Bioavailability of (Poly)phenols: Focus on Biological Rhythms and Proximity Consumption in Healthy and Obese Rats

DOCTORAL THESIS

Supervised by

Dr. Manuel Suárez Recio

Dr. Cristina Torres Fuentes

Nutrigenomics Research Group

Department of Biochemistry and Biotechnology

UNIVERSITAT ROVIRA i VIRGILI

Tarragona 2024

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA i VIRGILI

DEPARTAMENT DE BIOQUÍMICA I BIOTECNOLOGIA
Campus Sescelades
C/ Marcel·lí Domingo, 1
43007 Tarragona
Tel. +34 977 55 95 21
Fax +34 977 55 82 32

FEM CONSTAR que aquest treball, titulat **"Impact of External Factors on the Bioavailability of (Poly)phenols: Focus on Biological Rhythms and Proximity Consumption in Healthy and Obese Rats",** que presenta **Iván Escobar Martínez** per a l'obtenció del títol de Doctor, ha estat realitzat sota la nostra direcció al Departament Bioquímica i Biotecnologia de la Universitat Rovira i Virgili i que compleix els requisits per a l'obtenció de la Menció Internacional de Doctorat.

HACEMOS CONSTAR que el presente trabajo, titulado **"Impact of External Factors on the Bioavailability of (Poly)phenols: Focus on Biological Rhythms and Proximity Consumption in Healthy and Obese Rats"**, que presenta **Iván Escobar Martínez** para la obtención del título de Doctor, ha sido realizado bajo nuestra dirección en el Departamento Bioquímica y Biotecnología de la Universitat Rovira i Virgili y que cumple con los requisitos para la obtención de la Mención Internacional de Doctorado.

WE STATE that the present study, entitled **"Impact of External Factors on the Bioavailability of (Poly)phenols: Focus on Biological Rhythms and Proximity Consumption in Healthy and Obese Rats"**, presented by **Iván Escobar Martínez** for the award of the degree of Doctor, has been carried out under our supervision at the Department Biochemistry and Biotechnology from the Universitat Rovira i Virgili and that is eligible to apply for the International Doctoral Mention.

Tarragona, 8 de abril del 2024

Les directores de la tesi doctoral
Los directores de la tesis doctoral
Doctoral thesis supervisors

Dr. Manuel Suárez Recio

Dr. Cristina Torres Fuentes

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

This thesis is framed within the CIRCAFENOL (PID2021-128813OB-I00) and SEASONAL (PID2020-113739RB-I00) projects, aimed at studying the effects of (poly)phenol consumption and the influence of various factors such as rhythms, health status, and sex. Iván Escobar Martínez was supported by the Youth Employment Initiative from the European Social Fund, the Ministry of Science, the State Research Agency, and Universitat Rovira i Virgili (PEJ2018-002778-A) for the completion of this doctoral thesis. An international stay was carried out at the Department of Nutritional Sciences of King's College London, under the supervision of Dr. Ana Rodríguez Mateos. This international stay was supported by the Erasmus+ International Mobility Grant for Internships (2021 Call), as well as by the Spanish Society of Genetics (Travel Grant for 2021).

Aquesta tesi s'emmarca dins dels projectes CIRCAFENOL (PID2021-128813OB-I00) i SEASONAL (PID2020-113739RB-I00), amb l'objectiu d'estudiar els efectes del consum de polifenols i la influència de diversos factors com ara els ritmes, l'estat de salut i el sexe. Iván Escobar Martínez va rebre el suport de la Iniciativa d'Ocupació Juvenil del Fons Social Europeu, el Ministeri de Ciència, l'Agència Estatal de Recerca i la Universitat Rovira i Virgili (PEJ2018-002778-A) per a la realització d'aquesta tesi doctoral. A més, es va dur a terme una estada internacional al Departament de Ciències Nutricionals del King's College London, sota la supervisió de la Dr. Ana Rodríguez Mateos. Aquesta estada internacional va ser recolzada per la beca de mobilitat internacional Erasmus+ per a pràctiques (Convocatòria 2021), així com per la Societat Espanyola de Genètica (Borsa d'ajut de viatge de 2021).

Esta tesis se enmarca dentro de los proyectos CIRCAFENOL (PID2021-128813OB-I00) y SEASONAL (PID2020-113739RB-I00), cuyo objetivo es estudiar los efectos del consumo de polifenoles y la influencia de diversos factores como los ritmos, el estado de salud y el sexo. Iván Escobar Martínez recibió el apoyo de la Iniciativa de Empleo Juvenil del Fondo Social Europeo, el Ministerio de Ciencia, la Agencia Estatal de Investigación y la Universitat Rovira i Virgili (PEJ2018-002778-A) para la realización de esta tesis doctoral. Además, se llevó a cabo una estancia internacional en el Departamento de Ciencias Nutricionales del King's College London, bajo la supervisión de la Dr. Ana Rodríguez Mateos. Esta estancia internacional fue respaldada por la beca de movilidad internacional Erasmus+ para prácticas (Convocatoria 2021), así como por la Sociedad Española de Genética (Bolsa de ayuda de viaje de 2021).

UNIVERSITAT ROVIRA i VIRGILI

Grup de Recerca
NUTRIGENÒMICA URV

KING'S College LONDON

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
Unión Europea
Fondo Europeo de Desarrollo Regional

Erasmus+

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

ACKNOWLEDGMENTS

Parece que no llegaría este momento, pero al fin está aquí. Lo conseguimos, sí, en plural, porque esta tesis no es solo mi logro, sino el resultado del apoyo incondicional de muchas personas que me han acompañado en este largo camino.

En primer lugar, quiero dar las gracias a mis directores de tesis, Manuel y Cristina. Muchas gracias, por confiar en mí desde el primer momento y por todos los valiosos consejos que siempre me habéis trasmitido. Gracias también por el apoyo constante, porque, aunque sabemos que han sido años difíciles, siempre habéis estado ahí. También quería agradecer al Grup de Nutrigenòmica de la URV, por la gran acogida en el grupo, y por todo lo que me habéis enseñado. No podría olvidarme de las personas que han compartido tantos momentos conmigo como son la gente de nuestro despacho, y del despacho de al lado. Alba, Carme, Èlia, Florijan, Esther, Francesca, Helena, Josefina, Marina, Marta, Rafa, Romi, Álvaro, Vero, Niurka y Rosa. Muchas gracias por todos estos años de compañía y amistad. Néstor, Raúl, Jorge, Pauli, mis adorables perros salchichas. Habéis sido un pilar fundamental durante mi estancia en Tarragona. Gracias por todas las risas y por vuestro constante apoyo. Estoy seguro de que nos esperan muchos momentos de "CVA" juntos.

I am truly grateful to Dr Ana Rodríguez-Mateos for welcoming me into her amazing team at King's College London. This experience has been one of the most rewarding and enlightening stages of my life. I would also like to express my gratitude to Tracy. It has been a privilege to work alongside such a talented professional like you. Thank you for everything you have taught me and for your friendship. I am sure we will meet again soon!

A Xavi, Edu, María, mis compañeros de la "Resi", os llevo en el corazón, junto a todas las anécdotas que hemos vivido (que no son precisamente pocas…). Os considero como hermanos, y me tendréis para siempre.

Llegó el momento, de agradecer a los míos, a los de siempre, a los "Fanáticos y un Dino". Luis, Antoñoko, Luis primo y Nano, muchas gracias por ser tan especiales y auténticos. Este

trabajo también es vuestro. Me siento muy afortunado de tener esta pequeña y peculiar familia, que pase lo que pase siempre estarán presentes. Vuestro constante apoyo traducido en risas, lloros, fiestas, comidas, pádel, y más fiestas… hacen que las pequeñas cosas como una cerveza en una terraza al sol en vuestra compañía, sean las que le den color a la vida.

A mi familia, papá, mamá, Sandra, Silvia, Coco… simplemente gracias, no sería la persona que soy hoy en día si no fuera por vosotros. Vuestro apoyo y cariño ha sido el motor principal que me ha impulsado a seguir y nunca abandonar. Aunque os ha tocado un "Heredero" un poco cabezón que está constantemente haciendo cosas y no para de meterse en un "fregao" tras otro, siempre estáis dispuestos a sacrificaros para apoyarme en absolutamente todo. Gracias por transmitirme estos valores, sois mi ejemplo a seguir. Por otro lado, es imposible hablar de ejemplo y valores sin mencionar a toda la familia Escobar y Martínez, no puedo estar más orgulloso de tener la gran familia que tengo. Por último, quisiera hacer una mención especial, Abuelos, David y Tata, gracias por todo el cariño y sobre todo por inculcarme los valores de esfuerzo, constancia y dar siempre el 100% sin nunca esperar nada a cambio. Os quiero mucho familia, este logro es vuestro. ***"Vista larga, paso corto y …"***

Dejo para el final a la persona que realmente ha conseguido que este trabajo se haga realidad. Esa persona, es la más especial y única de todas. Gracias Ana, por apoyarme día a día y hacer que nunca me rinda. Has vivido conmigo toda esta montaña rusa de emociones durante todos estos años, y siempre has estado ahí para darme ese chute de motivación diaria que ha desembocado en este trabajo. Muchas gracias cariño, este logro no habría sido posible sin ti. Eres el verdadero corazón de esta tesis.

En definitiva, gracias a todos, habéis hecho que este camino haya merecido la pena.

Tarragona, Abril de 2024

A mi familia,

a mis amigos

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

El que jizo con el barro remojao,
en la ruea, sin más chismes que sus deos,
los pucheros, las botijas, los barriles,
los cacharros, las cazuelas, los barreños;
el que jizo la tinajas barrigúas
y endispués de cavilá tuvo el acuerdo
de los conos y los jornos encuadraos
y los chismes pa sacalos y metelos;
el que jizo que su nombre resonara
por la gran revolución de sus inventos
ondiquiera que las cepas dieran uvas,
muchas leguas en reondo de su pueblo,
no podía consentí que trompezara
su tesón, qu'era más juerte que los jierros,
en los riscos, chaparreras y coscojas
de la joya de los cuervos.

Era sangre d'otras épocas su sangre;
sus agallas parecían d'otros tiempos;
era un hijo d'estas tierras, de la raza
de castúos veteranos extremeños.

Y trunfó de los que tanto se bulraron,
y trunfó de los que tanto se riyeron,
y las cepas dieron uvas
remojás con el süor del tinajero.

"La viña del tinajero" (*El Miajón de los castúos*), Luís Chamizo.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

SUMMARY

Phenolic compounds are substances produced by plants in response to stress. These compounds possess antioxidant and anti-inflammatory properties, and their consumption has been associated with various health benefits. However, a comprehensive understanding of these benefits requires an investigation into their bioavailability. Several factors, such as compound structure, environmental conditions, sex, gut microbiota, and diet, have been identified as influencing their absorption and metabolism. In addition to these factors, biological rhythms, including circadian and seasonal patterns, are emerging as crucial determinants in their effects. Disruptions in these rhythms associated with contemporary lifestyle factors have been shown to impact metabolic status, potentially influencing (poly)phenol bioavailability. Considering all of this, the main objective of this thesis is to evaluate whether the metabolism and bioavailability of (poly)phenols can be influenced by different biological rhythm patterns and proximity consumption in the context of healthy and altered dietary habits. To achieve this, we first investigated the influence of circadian and seasonal rhythms on the bioavailability of phenolic compounds derived from grape seed proanthocyanidins extract (GSPE) in healthy and diet-induced obese rats. Subsequently, we characterized the phenolic profile of sweet cherry cultivars from two geographical origins and evaluated their bioavailability after acute consumption. The results revealed significant effects of administration time and photoperiod on phenolic compound bioavailability, highlighting the interplay between dietary habits and time of administration. Moreover, geographical origin significantly influenced the phenolic profile of sweet cherries, exhibiting the local cherries higher phenolic content. These findings underscore the importance of considering biological rhythms and environmental factors in the bioavailability of (poly)phenols, which may contribute to enhancing food consumption strategies and fostering a deeper understanding of the relationship between diet and health.

RESUMEN

Los compuestos fenólicos son sustancias producidas por las plantas en respuesta al estrés. Estos compuestos poseen propiedades antioxidantes y antiinflamatorias, y su consumo se ha asociado con beneficios para la salud. Se han identificado varios factores, como la estructura de los compuestos, las condiciones ambientales del cultivo, el sexo, la microbiota intestinal y la dieta, como determinantes en su absorción y metabolización. Además, los ritmos biológicos, tanto circadianos como estacionales, están emergiendo como cruciales en sus efectos. Las alteraciones en estos ritmos asociados a factores del estilo de vida moderno han demostrado impactar el estado metabólico, potencialmente influenciando la biodisponibilidad de los (poli)fenoles. Con todo ello, el objetivo principal de esta tesis es evaluar si el metabolismo y la biodisponibilidad de los (poli)fenoles pueden ser influenciados por diferentes patrones de ritmo biológico y consumo de proximidad en el contexto de hábitos dietéticos saludables y alterados. Para ello, primero investigamos la influencia de los ritmos circadianos y estacionales en la biodisponibilidad de los compuestos fenólicos derivados del extracto de proantocianidinas de semilla de uva (GSPE) en ratas sanas y obesas. Posteriormente, caracterizamos el perfil fenólico de dos cultivos de cereza dulce de diferentes orígenes geográficos y evaluamos su biodisponibilidad después del consumo agudo. Los resultados revelaron efectos significativos del momento de administración y el fotoperíodo en la biodisponibilidad, destacando la interacción entre los hábitos dietéticos y el momento de administración. Además, el origen geográfico influyó significativamente en el perfil fenólico de las cerezas, exhibiendo las locales un mayor contenido. Estos hallazgos subrayan la importancia de considerar los ritmos biológicos y los factores ambientales de producción en la biodisponibilidad lo que puede contribuir a mejorar las estrategias de consumo de alimentos y fomentar una comprensión más profunda de la relación entre la dieta y la salud.

RESUM

Els compostos fenòlics són substàncies produïdes per les plantes en resposta a l'estrès. Aquests compostos posseeixen propietats antioxidants i antiinflamatòries, i el seu consum s'ha associat amb beneficis per a la salut. S'han identificat diversos factors, com ara l'estructura dels compostos, les condicions ambientals, el sexe, la microbiota intestinal i la dieta, com a determinants en la seva absorció i metabolització. A més d'aquests factors, els ritmes biològics, incloent-hi els circadians i estacionals, estan emergint com a crucials en els seus efectes. Les alteracions en aquests ritmes associades amb factors de l'estil de vida han demostrat impactar l'estat metabòlic, potencialment influenciant la biodisponibilitat dels (poli)fenols. Tenint en compte tot això, l'objectiu principal d'aquesta tesi és avaluar si el metabolisme i la biodisponibilitat dels (poli)fenols poden ser influenciats per diferents patrons de ritme biològic i consum de proximitat en el context d'hàbits dietètics saludables i alterats. Per aconseguir això, primer vam investigar la influència dels ritmes circadians i estacionals en la biodisponibilitat dels compostos fenòlics derivats de l'extracte de proantocianidines de llavor de raïm (GSPE) en rates sanes i obeses induïdes per dieta. Posteriorment, vam caracteritzar el perfil fenòlic de cultivars de cirera dolça de dos orígens geogràfics i vam avaluar-ne la biodisponibilitat després del consum agut. Els resultats van revelar efectes significatius del moment d'administració i el fotoperíode en la biodisponibilitat de compostos fenòlics, destacant la interacció entre els hàbits dietètics i el moment d'administració. A més, l'origen geogràfic va influir significativament en el perfil fenòlic de les cireres, exhibint les locals un major contingut fenòlic. Aquests resultats subratllen la importància de considerar els ritmes biològics i els factors ambientals en la biodisponibilitat, la qual cosa pot contribuir a millorar les estratègies de consum d'aliments i fomentar una comprensió més profunda de la relació entre la dieta i la salut.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

ABBREVIATIONS

ABTS	2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonate)
AOAC	Association of Official Analytical Chemists
BMAL1	Brain and Muscle ARNT-like protein 1
BW	Body weight
CAD	Charged aerosol detection
CAF	Cafeteria diet
CBG	Cyanogenic β-Glucosidase
CE	Capillary electrophoresis
CLOCK	Circadian locomotor output cycles kaput
COMT	Catechol *O*-methyltransferase
COX	Cyclooxygenases
CRY1	*Cryptochrome*
CXLE	Carbon dioxide-expanded liquid extraction
DAD	Diode array detector
DMAC	Dimethylaminocinnamaldehyde
DPPH	2,2-diphenyl-picrylhydrazyl
ECD	Electrochemical detector
ESI	Electrospray ionization
FID	Flame ionization detector
FLD	Fluorescence detector
GAE	Gallic acid equivalents
GC	Gas chromatography

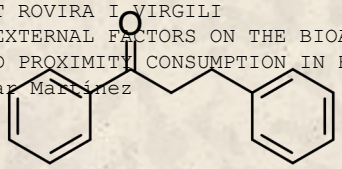
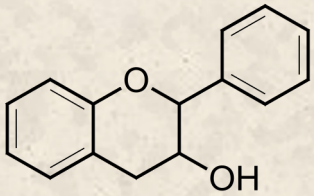
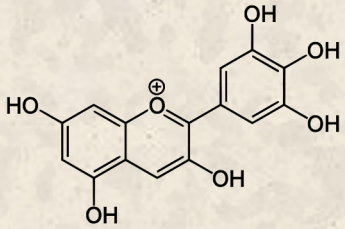
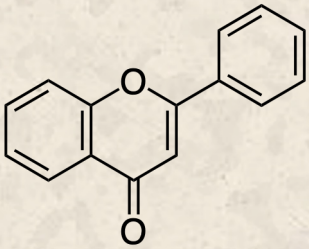
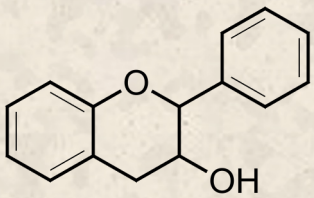
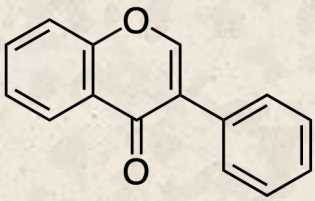
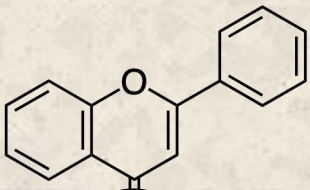
HBA	Hydroxybenzoic acids
HCA	Hydroxycinnamic acids
HDL	High-density lipoprotein
HHPE	High hydrostatic pressure extraction
HPLC	High-performance liquid chromatography
HSCCC	High-speed countercurrent chromatography
HVED	High voltage electrical discharge
IR	Insulin resistance
LC	Local sweet cherries
LDL	Low-density lipoprotein
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LOX	Lipoxygenase
LPH	Lactase-phlorizin-hydrolase
LSE	Liquid-solid extraction
LSR	Liquid-solid ratio
m/z	Mass-to-charge ratio
MAE	Microwave-assisted extraction
MS	Mass spectrometry
MS/MS	Mass spectrometry in tandem mode
NLC	Non-local sweet cherries
ORAC	Oxygen radical absorbance capacity
PAL	Phenylalanine ammonia-lyase

PEF	Pulse electric field
PER	*Period*
PHWE	Pressurized hot water extraction
PLA2	Phospholipase A2
PLE	Pressurized liquid extraction
PME	Pectin methylesterase
Q-TOF	Quadrupole-time-of-fligth
Q-trap	Quadrupole ion trap
QqQ	Triple quadrupole
RE	Relative Error
ROR	RAR-related orphan receptor
RSM	Response Surface Methodology
RT	Retention Time
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SGLT1	Sodium-glucose-linked transporter 1
SLE	Solid-liquid extraction
SPE	Solid phase extraction
ST	Standard diet
SULT	Sulfotransferases
TDF	Total dietary fiber
TOF	Time-of-flight
UAE	Ultrasound assisted extraction
UGT	UDP-glucuronosyltransferases

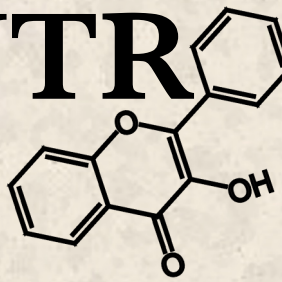
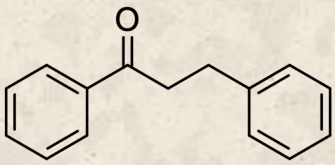
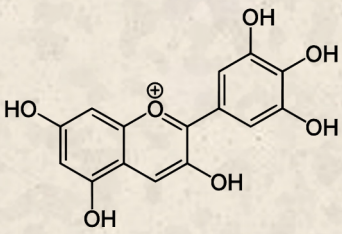
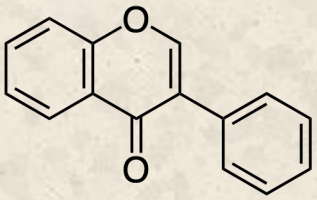
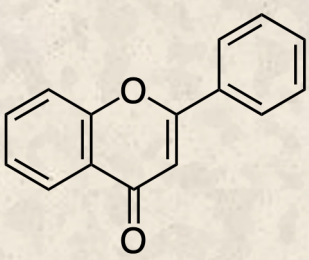
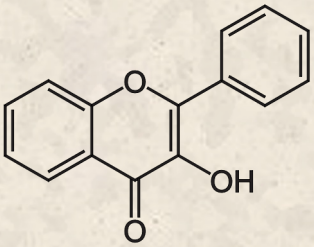
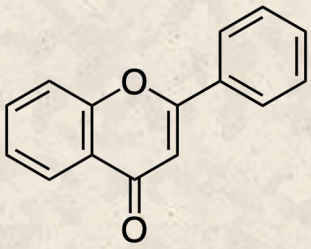
UHPLC	Ultra high-performance liquid chromatography
UV	Ultraviolet
ZT	Zeitgeber time

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

INTRODUCTION

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

1. Phenolic compounds

Phenolic compounds, commonly known as (poly)phenols, are a group of natural chemical compounds found in vegetables, fruits, grains and derived beverages. These molecules form a heterogeneous class of bioactive compounds that are synthesised by most plants as a defence mechanism against various internal threats including free radicals, as well as external agents such as pathogens, UV radiation, fungi, insects and animals [1,2].

In the past few years, many studies have revealed that (poly)phenols possess distinctive chemical structures that enable them to engage with biomolecules and exhibit diverse biological activities, such as antioxidant and anti-inflammatory properties [3–6]. These activities are mediated by the ability of (poly)phenols to affect, regulate or inhibit different cell signalling pathways involved in inflammation, oxidative stress and cancer [7]. (Poly)phenols have been shown to interact with several enzymes, such as phospholipase A2 (PLA2), lipoxygenase (LOX) and cyclooxygenases (COX-1 and COX-2). (Poly)phenols also have antioxidant properties that allow them to scavenge free radicals and chelate metal ions, thus reducing the damage caused by oxidative stress [2]. Moreover, (poly)phenols can influence the composition and function of the gut microbiota, which has significant implications in health. These complex biological functions play a pivotal role in preventing chronic diseases, including cardiovascular disorders [5,8] and type-II diabetes [9,10]. Moreover, (poly)phenols act as bioactive agents by boosting the body's immune system [11,12], leading to the suppression of cellular inflammation and tumour angiogenesis [13]. **Table 1** shows an overview of the different potential attributes of (poly)phenols against health disorders.

Table 1. Biological effects of phenolic compounds.

Diseases	Effects of phenolic compounds	References
Cardiovascular Disease	- Foods rich in flavonoids have been linked to improving overall vascular health through decreasing platelet activation, improving ventricular health, exerting anti-inflammatory effects, modulating enzymes and lowering blood pressure, both systolic and diastolic. - Cardiovascular disease incidence can be reduced by increasing HDL and reducing total cholesterol/HDL and LDL/HDL ratios. Cholesterol oxidation can be inhibited by flavonoids and resveratrol.	8,14–23
Obesity	- (Poly)phenols, such as curcumin, catechins and resveratrol have anti-obesogenic properties that can help with weight loss and weight maintenance. They affect different aspects of fat metabolism, such as oxidation, lipogenesis, inflammation and energy expenditure. They also inhibit the digestion and absorption of fats, carbohydrates and proteins in the digestive tract by binding to proteins and blocking digestive enzymes. - They prevent the differentiation of pre-adipocytes into mature adipose cells and protect against obesity-induced metabolic disorders such as hyperglycaemia, insulin resistance, leptin resistance and dyslipidaemia.	24–32
Type-2-diabetes	- Some (poly)phenols such as anthocyanins, ferulic acid and chlorogenic acid have anti-inflammatory and antioxidant effects related to type-2 diabetes. They slow starch digestion, modify glucose transport and protect insulin-producing cells. These actions help control blood sugar levels. - They also improve insulin sensitivity and glucose uptake, enhance the effects of anti-diabetic drugs and reduce blood lipids and body weight in diabetic rats.	31–37

Cancer	- Flavonoids can reduce the risk of cancer by neutralizing free radicals and stopping cell growth in tumours. Flavanols, catechins, anthocyanins, isoflavones, flavanones, and flavones are some examples of flavonoids that have shown positive effects on colon, prostate, epithelial, endometrial, and breast cancers.	38–45
Inflammation	- (Poly)phenols have the potential to attenuate both systemic and localized inflammation by rebalancing redox levels, thereby reducing oxidative stress. Additionally, they may modify inflammatory reactions by mitigating cytokine pathways.	46–53
Neurodegenerative diseases	- Some (poly)phenols, such as curcumin, catechin, resveratrol, epigallocatechin gallate, myricetin and ginsenosides, can have neuroprotective effects against neurodegenerative diseases by modulating the immune system and scavenging the oxidative stress that damages the neurons. They can also inhibit the neurotoxic effects of the β-amyloid proteins, which is the main cause of Alzheimer's disease. - Additionally, they can chelate iron, which contributes to reduce neurotoxicity and prevents neuronal death.	54–59
Visual function	- Anthocyanin-enriched bilberry extract can improve visual function by protecting the retinal pigment epithelium from oxidative damage. Green-tea (poly)phenols can also protect the retinal pigment epithelium from UVB damage.	60,61
Cognitive function	- (Poly)phenol-rich blueberry extract can enhance cognitive function in adult mice by improving learning and memory and increasing brain antioxidant properties. Blueberry juice can also improve memory performance in older adults.	62,63
Allergy	- Grape seed extract can have anti-allergic activity by inhibiting the release of inflammatory mediators, reducing the expression of ImmunoglobulinE receptors, and lowering calcium influx in mast cells.	64

1.1. Classification and structure

(Poly)phenols represent a heterogeneous group of compounds with an aromatic (benzene or phenol) ring containing at least one hydroxyl group. Their structures can range from simple molecules to high molecular weight polymers [1,2]. Classification of these compounds has caused some debate, but the most widely accepted approach divides phenolics into two main groups: flavonoids and non-flavonoids [6] (**Figure 1**).

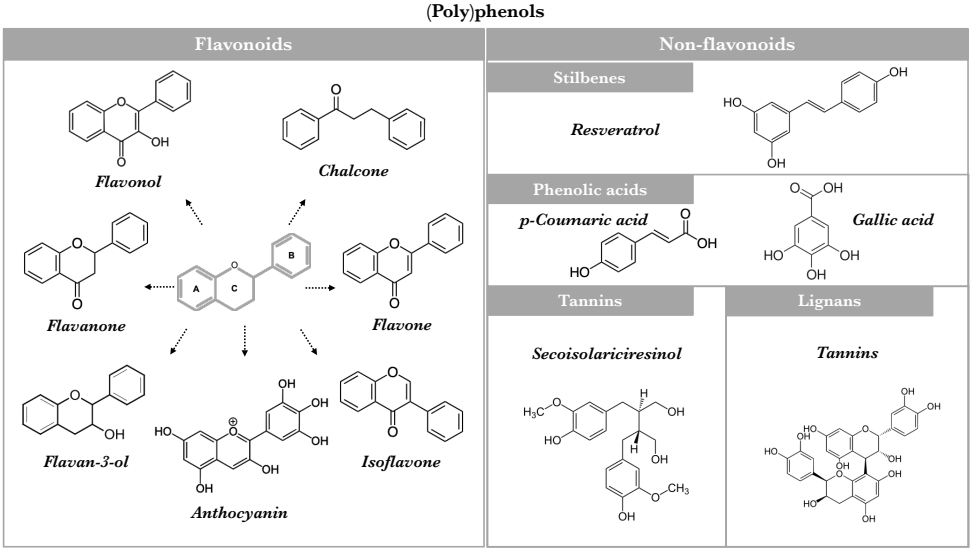


Figure 1. Main (poly)phenols classes and subclasses with their chemical structures.

1.2. Flavonoids

Flavonoids are generally the most widely described class of phenolic compounds in the scientific literature, constituting one of the most characteristic classes of compounds in higher plants [65]. Their chemical structure is composed of 15 carbon atoms arranged as C6-C3-C6, forming two aromatic rings, A and B, linked by a C ring with three carbon atoms in their backbone [1,6,65]. Flavonoids can be classified into different subclasses based on the degree of oxidation in the heterocyclic ring,

including flavan-3-ols, flavonols, flavones, isoflavones, flavanones, anthocyanidins, and chalcones [3,66]. **Figure 2** shows a comprehensive overview of the main phenolic compounds in each flavonoid subclass and their food sources.

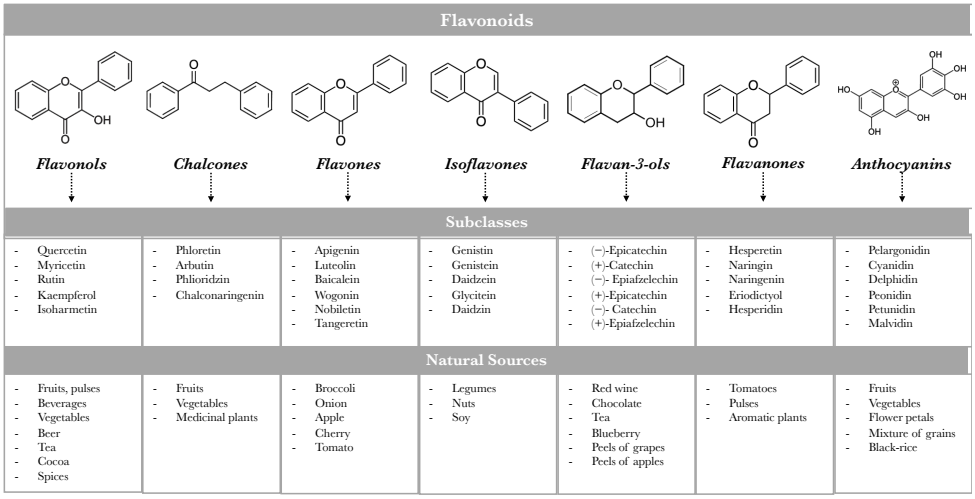


Figure 2: Main flavonoid subclasses. Their basic chemical structure and natural source.

1.2.1. Flavan-3-ols

The most complex subclass of flavonoids is flavan-3-ols, which are characterized by the hydroxyl group bound to position 3 of the C ring [3]. They can be found both as monomers (catechins) and as oligomers and polymers (proanthocyanidins). These are also called condensed tannins and can have up to 50 units in their structure. Proanthocyanidins that are composed only of (epi)catechin units are named procyanidins, and they are the most common type of proanthocyanidins in plants [1,67]. The most prevalent flavan-3-ol compounds in nature are (+)-catechin and (-)-epicatechin [1]. Flavan-3-ols differ from other classes of flavonoids in that they do not have glycosylated forms in foods [67]. However, they can be

conjugated with gallic acid, forming gallate flavan-3-ols, such as epicatechin gallate, catechin gallate, epigallocatechin gallate and gallocatechin gallate [1,6]. Flavan-3-ols are abundant in many fruits, such as apricots, apples and grapes; they also occur in red wine, but the richest sources are green tea, chocolate and fruit skins [3,67].

1.2.2. Flavanones

Flavanones comprises a large range of compounds with O and/or C substitutions on the A or B ring [68]. Various glycosides can combine with the same aglycone to form different flavanones. This characteristic is typical of flavanones found in citrus. These compounds, including naringin and rutin, are responsible for the bitter taste found in the juice and peel of citrus fruits [3,67,68]. Flavanones are widely distributed in about 42 higher plant families. Among flavanones, naringenin and hesperidin, both aglycones and their glycosides, are very common in foods [68]. In fact, in human food, flavanones are found in tomatoes and some aromatic plants such as mint, and in citrus fruits such as orange and lemons they can be found in high concentrations [3,67].

1.2.3. Flavonols

Flavonols also known as 3-hydroxyflavone are the most common of the flavonoids, as they are found in all the plant kingdom, except for fungi and algae [1,69]. Flavanols have several specific substitutions in the A and B rings, which are linked with a three-carbon chain. Hydroxyl groups replace the 5 and 7 positions on the A ring of flavonols. Flavonols have more 3-OH groups than other flavonoids. They are abundant in the epidermal cells of plant tissues and filter some harmful solar wavelengths (such as UV) to protect DNA [3]. The main flavonols in the diet, kaempferol, quercetin,

isorhamnetin and myricetin are usually found as *O*-glycosides with conjugation at the 3-position [1,69]. There are more than 200 kaempferol sugar conjugates, though the aglycones are few [3,69]. The most abundant sources are onions, broccoli, curly kale, leeks, tomatoes, apricots, blueberries, and cherries. Red wine and tea also have significant amounts of these compounds [1,3,67,69].

1.2.4. Flavones

Flavones, such as apigenin, baicalein, luteolin and wogonin are similar to flavonols in structure, except that they have no oxygen at C-3 3. Most of flavones are 7-*O*-glycosides, which are present in celery, tea, red pepper, and oranges. While found in smaller amounts than flavonols, they remain one of the most prevalent groups of flavonoids [3]. Furthermore, polyethoxylated flavones, like nobiletin and tangerine are present in citrus species [1,67].

1.2.5. Isoflavones

Isoflavones are biologically active compounds with estrogenic properties and are often called phytoestrogen [6,67]. Isoflavones have the B-ring attached at C-3 instead of at the C-2 position [67]. They have hydroxyl groups at the 7′and 4′positions in a configuration similar to that of the hydroxyl groups of the oestradiol molecule [67]. The main components are genistein, daidzein, biochanin A, and glycitein [6]. They are commonly accumulated in leguminous plants with large amounts of daidzein and genistein in soybean [3,6]. Other sources such as apple, black currant, apricot, sweet potato, plum, cherry, cabbage, date, onion, nuts, wheat and pineapple have been reported [3,6].

1.2.6. Chalcones

Chalcones are natural open-chain flavonoids with prenyl moieties on both rings. They are found in several plant families and have various pharmacological effects. Chalcones are an important group of natural compounds that are found in large amounts in fruits (such as citruses, apples), vegetables (such as tomatoes, shallots, bean sprouts, potatoes) and various plants and spices (such as licorice) [70]. They are precursors for flavonoids and isoflavonoids and can be synthesized or modified from simple aromatic compounds [3,71]. Xanthohumol and isbavirachalone are two examples of bioactive chalcone derivatives [3,71].

1.2.7. Anthocyanidins

Anthocyanidins are pigments that are soluble in water [1,72]. The basic structural unit of anthocyanins is the flavylium ion (2-phenylchromenylium), their diversity is determined by the arrangement and number of hydroxyl and methoxyl groups as substituents within the flavin structure [3]. The most common anthocyanidin aglycones are cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin, which are attached to sugars and organic acids to form a variety of anthocyanins that are pigments dissolved in the vacuole sap of the epidermal tissues of the flowers and fruits, giving them a pink, red, blue or purple colour [1,67]. They have different chemical forms and, depending on the pH, they can be coloured or colourless. Also, these compounds are stabilized by making complexes with other flavonoids (co-pigmentation) [67]. In the human diet, anthocyanins are found plentifully in red grapes, red wine, cherries, berries and some leafy and root vegetables [3,6,67].

1.3. Non-Flavonoids

Non-flavonoids are a group of phenolic compounds that are widely distributed in nature and have various beneficial effects on health [1,6,73]. They differ from flavonoids in their chemical structure and biosynthesis pathways [74]. Non-flavonoids include phenolic acids, lignans, stilbenes and tannins. They are found in fruits, vegetables, cereals, legumes, nuts, spices, and beverages. They have antioxidant, anti-inflammatory, anticancer, antidiabetic, and cardioprotective properties, among others [1,73]. Non-flavonoids are promising natural products for the prevention and treatment of various human diseases [1,73].

1.3.1. Phenolic Acids

Phenolic acids are a major class of plant phenolics that exist in free and bound forms. They can be classified into two subgroups: benzoic acid derivatives and cinnamic acid derivatives, which have C6–C1 and C6–C3 structures, respectively [6,75]. They are present in various fruits like apricots, cherries, peaches, as well as vegetables like eggplant, cereals, and spinach [76].

- Benzoic acid derivatives include hydroxybenzoic acids (HBAs), such as gallic, *p*-hydroxybenzoic, protocatechuic, vanillic, and syringic acids. They are part of complex structures like hydrolysable tannins [6,75,76].

- Cinnamic acid derivatives include hydroxycinnamic acids (HCAs), like *p*-coumaric, caffeic, ferulic, and synapic acids. They are mainly found as conjugates called chlorogenic acids and are rich in fruits like apples, blueberries, and coffee [6,75,76].

1.3.2. Other non-flavonoids

Other non-flavonoid phenolic compounds include lignans, stilbenes, tannins, and xanthones.

- Lignans are natural products formed by the oxidative dimerization of 2 phenyl-propane units. They are found in various plant foods such as linseed (the richest source), vegetables, fruits, nuts, oilseeds, garlic, olive oil, wine, tea, beer, and coffee [1,75].

- Stilbenes are phenolic compounds with a 1,2-diphenylethylene structure that are present in various plant foods such as grapes, berries, peanuts, red wine, and some medicinal plants. The main dietary sources are wines, peanuts, grapes, and peanut products. Some examples of stilbenes are resveratrol, pterostilbene, and 3′-hydroxypterostilbene. Resveratrol is the most studied stilbene, which has antioxidant activity in the cardiovascular system [1,75].

- Tannins are phenolic compounds with various biological activities that are abundant in cereals, leguminous seeds, fruits, vegetables, and brown seaweeds. They can be classified into hydrolysable tannins (tannic acid) and condensed tannins (proanthocyanidins) based on their chemical structures [1,75].

2. Absorption and metabolism

(Poly)phenols are commonly found in dietary sources as esters, glycosides or polymeric forms that are not directly absorbed [77]. Upon ingestion, dietary (poly)phenols undergo extensive metabolism, with absorption occurring primarily in both the small and large intestines [78]. **Figure 3** schematically illustrates the complex process of digestion, absorption, distribution, metabolism and excretion (ADME) of phenolic compounds.

2.1. Oral and gastrointestinal digestion

In the oral cavity, (poly)phenols are partially released from the food matrix. Salivary *a-amylase* can hydrolyse glycosides into their aglycone counterparts, but the impact of this step is limited due to the short contact time [79]. However, the breakdown of the food matrix particles in the oral cavity facilitates better enzymatic accessibility in subsequent digestion steps [79].

Phenolic compounds are predominantly released from the food matrix during gastric digestion. Pepsin activity, in conjunction with peristaltic movements and the low pH of the stomach (usually between 2-4), further reduces the particle size of the food matrix [79]. The low pH also promotes the diffusion of phenolic compounds from the food matrix into the aqueous phase by reducing ionic interactions. The harsh chemical environment of the stomach is not conducive to most phenolic compounds, except for anthocyanins, which remain stable at acidic pH [80]. Although the intestine is the primary site of absorption of phenolic compounds, it has been described that certain phenolic families, such as anthocyanins and phenolic acids such as caffeic acid, can be absorbed at the gastric level [81–83].

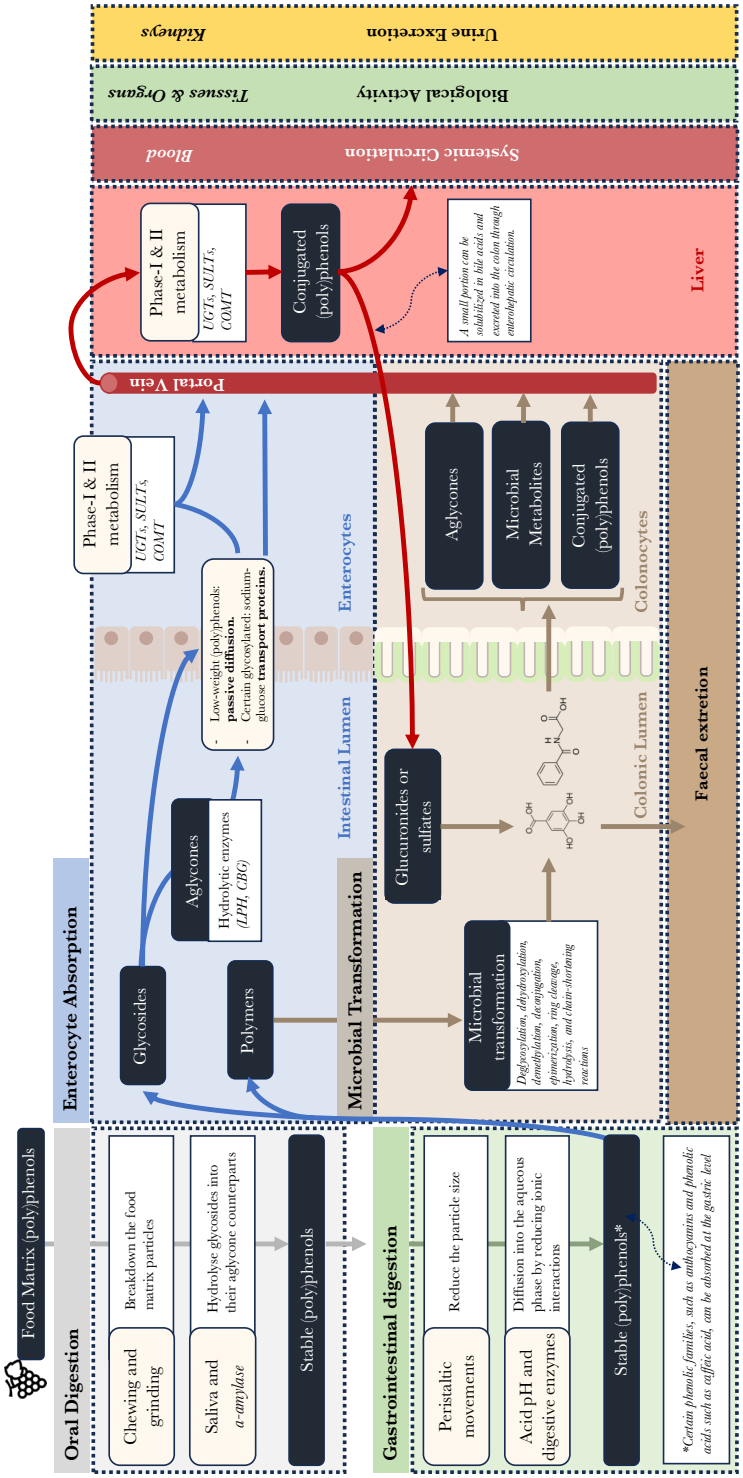


Figure 3: Schematic representation of the digestion, absorption, distribution, metabolism and excretion of phenolic compounds.

2.2. Enterocyte absorption

Although the intestine plays a crucial role in the metabolism and absorption of (poly)phenols, only a small percentage, approximately 5-10 %, of total (poly)phenolic compounds are absorbed in the small intestine, and they may undergo further extensive metabolism. Thus, most (poly)phenols accumulate in the large intestine before being excreted in the faeces [78,79,84]. In the intestinal mucosa, only aglycones and certain glucosides have the potential for absorption [78]. The pH in this region increases to around 7, facilitating the activation of pancreatic and biliary enzymes [79]. Interestingly, studies have consistently demonstrated that anthocyanins undergo significant degradation in the small intestine [79].

Most (poly)phenols require the action of specific hydrolytic enzymes, such as lactase-phlorizin-hydrolase (LPH) and cytosolic β-glucosidase (CBG), to be cleaved from their sugar moiety [78]. Once cleaved into their respective aglycones, (poly)phenols can be absorbed into the enterocytes of the small or large intestine through intestine via passive-facilitated diffusion. Passive diffusion is likely the primary pathway for absorption of low-weight (poly)phenols, including monomeric or dimeric forms [78,79]. On the other hand, certain glycosylated (poly)phenols are actively taken up through sodium-glucose transport proteins, particularly sodium-glucose-linked transporter 1 (SGLT1) [78,79]. After absorption, the aglycones undergo phase-I and II metabolism (in the enterocyte and the liver) to produce glucuronidated, sulfated, and/or methylated conjugates, catalysed respectively by UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), and catechol *O*-methyltransferase (COMT) [85]. Consequently, (poly)phenols are directed to the liver through the portal vein, where a crucial role in the phase-II metabolism of phenolic compounds is exerted.

It has been described that the liver produces a diverse array of phase-II enzymes in significant quantities [86–88]. Following hepatic metabolism, the resulting metabolites enter the systemic circulation. In the plasma, these metabolites are transported through the systemic circulation to various tissues and organs until they eventually reach the kidneys for excretion in the urine [78,79]. It is important to note that not all the liver-conjugated metabolites enter the systemic circulation, a small portion can be solubilized in bile acids and excreted into the colon through enterohepatic circulation. Consequently, along with (poly)phenols with high polymerization degrees that have not been absorbed by the intestine, they undergo microbial metabolism in the colon [78,84,85].

2.3. Microbial fermentation in the colon

Approximately 90-95 % of dietary phenolic compounds are estimated to reach the colon [78,84]. In this region, (poly)phenols can undergo metabolism by the gut microbiota, where a small portion of them is subjected to microbial degradation into small molecules such as phenolic acids or valerolactones [89–91]. The remaining oligomeric and polymeric PACs interacts with gut microbiota, resulting in improved microbial diversity and, thereby, contributing to improved health (e.g., *Akkermansia muciniphila* and butyrate-producing bacteria) [91]. The gut microbiota performs various common processes, including deglycosylation, dehydroxylation, demethylation, deconjugation, as well as epimerization, ring cleavage, hydrolysis, and chain-shortening reactions [79]. The bioavailability and bioactivity of (poly)phenols are heavily influenced by bacterial metabolism, and in turn, they can also modulate the composition of intestinal bacteria [84]. Ultimately, (poly)phenols not absorbed by the colon are excreted through faeces [78,79,84].

3. Identification and quantification methods

It is crucial to identify and quantify as many phenolic compounds present in biological samples (fluids and tissues) as possible in order to understand their physiological activity. Therefore, it is important to have sufficiently developed tools to analyse the (poly)phenolic profile. A scheme of the most common strategies employed in recent years for the pre-extraction, extraction, separation, quantification and characterisation of phenolic compounds is shown in **Figure 4** [92].

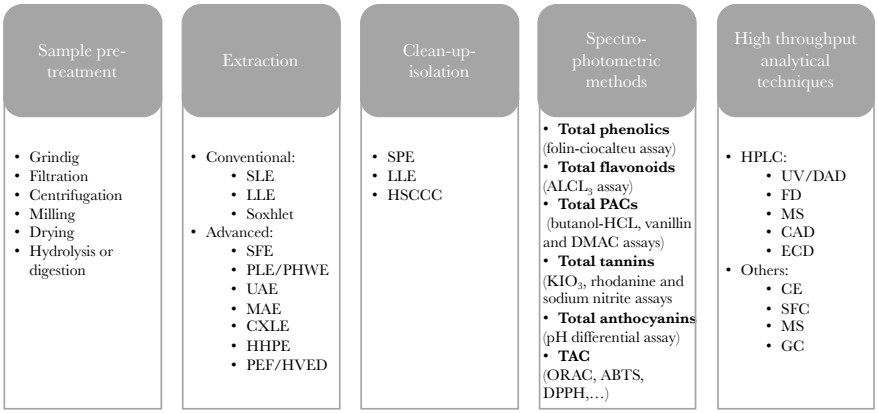


Figure 4. The different steps involved in the analysis of phenolic compounds from plants and food (by-products). ABTS, 2,2ˈazinobis-(3-ethylbenzothiazoline-6-sulphonate; CAD, charged aerosol detection; CE, capillary electrophoresis; CXLE, carbon dioxide-expanded liquid extraction; DAD, diode array detection; DMAC, dimethylaminocinnamaldehyde; DPPH, 2,2-diphenyl-picrylhydrazyl; ECD, electrochemical detection; FD, fluorescence detection; GC, gas chromatography; HHPE, high hydrostatic pressure extraction; HPLC, high performance liquid chromatography; HSCCC, high-speed countercurrent chromatography; HVED, high voltage electrical discharge; LLE, liquid-liquid extraction; MAE, microwave-assisted extraction; MS, mass spectrometry; ORAC, oxygen radical absorbance capacity; PEF, pulse electric field; PHWE, pressurized hot water extraction; PLE, pressurized liquid extraction; SFC, supercritical fluid chromatography; SFE, supercritical fluid extraction; SLE, solid-liquid

extraction; SPE, solid phase extraction; TAC, total antioxidant capacity; UAE, ultrasound assisted extraction; UV, ultraviolet. Modified from Plaza M, *et al* [92].

3.1. Sample processing

Steps for pre-treatment, extraction and clean-up-isolation need to be carried out before identifying and quantifying phenolic compounds in raw plant products or biological samples. Due to the high complexity of the sample matrix, there is no standardised process for the pre-processing of samples rich in phenolic compounds and each method should be optimized for each product [92].

In addition, before extracting flavanols and their metabolites from biological matrices, appropriate sample collection, preservation, and preparation are essential [89,93]. To preserve (poly)phenols and prevent degradation, fast freezing of the biological samples before extraction and quantification is crucial [89,93]. When analysing tissues, lyophilization is employed to prevent analyte degradation and minimize errors due to water content. Factors like temperature, light, and oxygen exposure can also influence (poly)phenol content [94].

3.1.1. Processing of fruit samples

The extraction step is crucial for profiling the phenolic content of fruits or other plant materials. Its outcome determines the release of analytes from the plant matrix into the medium, which allows quantitative determination of the extracts [92]. Solvent extraction involves two main steps: first, the solid phase swells as the solvent is absorbed, resulting in the extraction of solutes from the damaged plant material. Secondly, the solutes diffuse into the plant matrix and then out of the surrounding layer of plant particles [94,95]. Several factors, such as temperature, extraction

solvent, liquid-solid ratio (LSR), extraction time, particle size, pH, number of extraction steps and food matrix, can influence this process and modulate the quantification of phenolic compounds in fruits [94,95].

Therefore, due to the wide range of food matrices and polarities of phenolic compounds, specific and optimised extraction methods are necessary. Two strategies to optimise the extraction of phenolic compounds from food matrices are classical optimisation studies and extraction optimisation by Response Surface Methodology (RSM). Classical approaches, including one-variable-at-a-time, are traditional optimisation methods that change one factor at a time and are slow and costly. These approaches cannot evaluate the interactions between the factors and may lead to wrong conclusions. On the other hand, RSM is a methodology that uses statistical and mathematical techniques to optimize a process where a response is influenced by several factors. It fits a polynomial equation to the data and generates a model that describes the process and its interactions. RSM also allows to design experiments, model processes, verify the significance of the factors and obtain the optimal conditions of the process [94,95].

3.1.2. Processing of biological samples

Several methods for (poly)phenols extraction from biological samples are available [75,92,96–98]. Extracting analytes from solid matrices, like biological tissues, poses a challenge due to the need to break down collagen structures [93]. Common extraction methods involve homogenization in saline or strong acids, followed by organic solvent extraction [99]. However, newer, sensitive methodologies have made these extractions unnecessary by eliminating salts present in the solution [93].

Liquid-liquid extractions (LLE) or liquid-solid extractions (LSE) are widely used as starting points for these procedures. Organic solvents like methanol, ethanol, and acetone aid in metabolite liberation and removal from the matrix, particularly during protein denaturation [75]. Subsequently, samples can be directly analyzed, purified, and concentrated using solid phase extractions (SPE) with conventional cartridges [100]. Nevertheless, traditional SPE has a drawback concerning sample volume, leading to the introduction of µ-SPE methodologies [89,98]. µ-SPE allows for interference removal and analyte concentration, lowering the limits of detection and utilizing ultra-low elution volumes, thereby eliminating time-sensitive evaporation and reconstitution steps [89,98].

3.2. (Poly)phenol detection and quantification methods

Accurate identification and quantification of (poly)phenols and their metabolites in biological samples is crucial for understanding their metabolism and potential health effects. Therefore, in order to detect (poly)phenols and achieve reliable quantification, it is necessary to use a very accurate, sensitive and selective analytical technique. **Figure 4** shows different techniques used to determine the (poly)phenol content of our samples.

3.2.1. Spectrophotometry methods

Spectrophotometry is a rapid and simple method used to quantify phenolic compounds in plant materials [75]. **Figure 4** presents the main assays to quantify phenolic compounds in plants and food by-products and measure their antioxidant capacity. These assays use UV-Vis spectrum to detect electronic transitions within OH-phenolic groups,

allowing determination of the total (poly)phenol content [75]. However, it is essential to note that these methods may overestimate the phenolic content due to the presence of numerous interferents such as proteins and sugars. Despite their simplicity, cost-effectiveness, and rapid analysis capabilities, spectrophotometric methods have limitations in providing specific information about individual phenolic compounds and may lack accuracy [92]. To overcome these limitations, advanced techniques like capillary electrophoresis and LC-MS/MS have emerged. These techniques not only enable the elucidation of complex phenolic structures but also enhance accuracy in phenolic compound quantification.

3.2.2. High throughput analytical techniques

Nowadays, **high throughput analytical techniques** represent an alternative to the traditional methods for identifying and quantifying (poly)phenols and their metabolites. These techniques include among others, capillary electrophoresis (CE), nuclear magnetic resonance (NMR), and chromatographic techniques (gas and liquid chromatography, GC and LC).

Gas chromatography (GC) is a valuable technique employed for separating, identifying, and quantifying certain phenolic compounds in plants, including tannins, flavonoids, and anthocyanins. This method operates on the principle of separating compounds based on their evaporation temperatures. However, it is essential to note that GC is primarily suitable for volatile compounds. Phenolic compounds, due to their non-volatile nature, often require derivatization of the samples before analysis, which can introduce complexities and potential inaccuracies. Consequently, GC is not typically the method of choice for analysing (poly)phenols. Despite its limitations in (poly)phenol analysis,

GC can be effectively coupled with a flame ionization detector (FID) or mass spectrometer (MS) to enhance selectivity and sensitivity for the quantification of certain phenolic compounds in plant samples [75,96].

Liquid chromatography (LC) has emerged as the primary method for analyzing (poly)phenols and their metabolites due to its advantages over GC [75,96]. LC offers improved ionization interfaces and avoids the need for sample derivatization, making it more suitable for phenolic compound analysis [101]. The chromatographic equipment can be coupled with various detectors like ultraviolet (UV), diode array (DAD), fluorescence (FLD), and MS. While UV, DAD, and FLD detectors have limitations in terms of specificity and accuracy, they play a crucial role in separating compounds within the LC system, impacting the overall analysis time [92].

The emergence of **High-Performance Liquid Chromatography** (HPLC) and Ultra High-Performance Liquid Chromatography (UHPLC) revolutionized chromatographic analyses. These advancements involved the use of smaller chromatographic columns and adaptability to high pressures, significantly reducing analysis time, which is crucial when dealing with a large number of samples [75,92,96,98]. Chromatographic separation, often overlooked, is vital for successful analyses. Recent developments in MS technology, in conjunction with high-resolution LC systems, have facilitated the identification and characterization of bioactive compounds with improved specificity. MS detection relies on the measurement of the mass-to-charge ratio (m/z) and abundance of ionized molecules, thereby achieving greater specificity by eliminating other masses. When LC is combined with time-of-flight (TOF) MS, untargeted analysis becomes feasible, permitting detection of any ionizable compound. This method is beneficial in the identification of

unknown compounds in a sample or in the establishment of quantification procedures [97].

The use of MS in tandem mode (MS/MS) had a significant impact because it provides intense ionization, which leads to substantial fragmentation of molecules. This fragmentation offers valuable structural information that facilitates the identification of unknown compounds [102]. This can be done by comparing them with mass spectra libraries obtained under identical conditions. MS/MS reduces detection and quantification limits, which enables the measurement of metabolites even at very low concentrations. Researchers currently use various detectors, including triple quadrupole (QqQ), quadrupole ion trap (Q-TRAP), and quadrupole-time-of-flight (Q-TOF) systems, to study these compounds and conduct targeted or untargeted metabolomics [103–106].

As a general statement, the HPLC–MS/MS is one of the most powerful methods for the correct identification and quantification of (poly)phenols. Recently, several characterizations have been described using this technique, as reported by references [94,95,107–111].

4. Factors influencing bioavailability of phenolic compounds

Despite the multiple biological activities of (poly)phenols, they are of little or no use if negligible amounts of the compound reach the intended tissues. Thus, it is essential to establish definitions of the terms "bioavailability," "bioaccessibility," and "bioactivity" which are frequently used indistinctly to describe similar functions.

Bioavailability is a critical factor in understanding the effectiveness of functional foods, encompassing gastrointestinal (GI) digestion, absorption, metabolism, tissue distribution, and bioactivity. Depending on the context, it can refer to the rate and extent of absorption of therapeutic components in pharmacology or the fraction of nutrients available for physiological functions in nutrition. The interactions between foods and the digestive tract may influence the bioavailability of bioactive compounds. Therefore, bioavailability expresses the fraction of ingested nutrient or bioactive compound that reaches the systemic circulation and is ultimately utilized [112,113].

Bioaccessibility is another essential concept, representing the release of bioactive compounds from the food matrix during digestion, making them available for absorption. It involves transformations in the GI tract, absorption into epithelial cells, and various metabolic processes. *In vitro* digestion procedures are commonly used to assess bioaccessibility such as INFOGEST [112–115].

On the other hand, **bioactivity** is the specific effect of a substance once it is absorbed, leading to physiological responses such as antioxidant or

anti-inflammatory effects. It involves tissue uptake, interactions with biomolecules, metabolism, and the generation of biomarkers. Bioactivity measurements are crucial in supporting health claims for functional foods [112–114].

Recently, Thakur *et al.* (2020) defined bioavailability as the union of the concepts of bioaccessibility and bioactivity. To be bioavailable, in addition to being stable for gastrointestinal digestion, compounds must be released from the matrix, *i.e.* bioaccessible, metabolised and pass through epithelia to exert their bioactivity. Hence, comprehending the bioavailability, bioaccessibility, and bioactivity of phenolic compounds, as well as identifying factors that may alter them, is vital to evaluate the possible health advantages of functional foods [116].

4.1. Host related factors

(Poly)phenol intake is influenced by a multitude of internal factors inherent to the consuming organism.

- **Digestive Factors**: Microbiota and enzymes play a vital role in the modification of (poly)phenols, as detailed in "Section 2". Any pathological disruption within the digestive system significantly affects the subsequent generation of bioactive and bioavailable (poly)phenol metabolites [117,118].

- **Age:** Aging diminishes homeostatic capacity, impairing regulatory processes vital for the functional integration of organs and cells [119]. Liver expression and activity of xenobiotic-metabolizing enzymes are also age-dependent [120]. Gastrointestinal functions are markedly reduced, impacting oral

and oesophageal function, gastric pH, emptying rates, and intestinal transit times [121]. Alterations in bacterial populations further drive biochemical changes in the intestines [122,123]. Margalef *et al.* (2016) demonstrated significant differences in plasma flavanol metabolites with respect to age [124]. Adult rats exhibited reduced flavanol absorption and phase-II flavanol metabolism, in contrast to their younger counterparts.

- **Sex**: Male rats metabolize xenobiotics faster and have higher activity of phase-II detoxification enzymes than females [125,126]. Differences between sexes often arise from COMT polymorphisms, which are transcriptionally regulated by oestrogens [127]. Margalef *et al.* (2016) found that rat flavanol metabolism and distribution were influenced by sex [128]. In the liver and brain, males showed greater methylation of phase-II metabolites. The study postulated that these differences could explain sex-dependent variations in flavanol bioactivities.

- **Health Status:** Obesity is a prominent metabolic disorder that can lead to chronic illnesses like type II diabetes, hypertension, or atherosclerosis. It is also a necessary condition for diagnosing metabolic syndrome [129–131]. Moreover, studies have shown that various metabolic disorders can affect phase-II metabolism. For example, the activity of phase-II enzymes in the intestine, liver, or kidneys can be disrupted [132,133].

- **Strain**: It has been reported that variability is observed not only between animal families (rodents) but also among different strains of rodents [134] . Actually, different results can be obtained for the

same procedure depending on the strain of animal due to physiological and behavioural differences.

4.2. External Factors

- **Food processing related factors**: Within this group, several influential factors such as the **thermal treatment** of the sample is included. This may cause a significant reduction of the (poly)phenol content in foods such as extra virgin olive oil [135], fennel [136], tomato [137] or cocoa [138]. Cooking affects the bioavailability and bioaccessibility of phenolic compounds in foods [118,139]. The preservation or loss of these compounds depends on factors such as cooking temperature, duration, and the type of oil used. To minimize the loss of phenolic compounds, it is recommended to use low cooking temperatures and short cooking times (less than 15 and 6 minutes for standard and microwaving heating, respectively) [140]. Extra virgin olive oil generally offers better protection for phenolic compounds compared to other oils [140]. Cooking with water has minimal impact on phenolic compound concentrations in foods with high phenolic content, but consuming the cooking water can enhance their absorption [140]. Also, **air drying**, **freeze-drying** [141] and **storage** [118] affect the (poly)phenol content. On the other hand, factors such as the **homogenization** of vegetables could help to increase the bioavailability. For instance, tomato puree and tomato paste have been shown to be more bioavailable sources of lycopene than raw tomatoes [142]. Another factor that can decrease (poly)phenols content during storage is **light exposure**. Light can induce photo-oxidation of (poly)phenols, which means that they react

with oxygen and lose their antioxidant activity. This can happen especially with UV light, which has high energy and can break the chemical bonds of (poly)phenols. Some studies have shown that light exposure can reduce the activity of (poly)phenols in natural extracts from spruce bark and grape seed [143,144].

- **Food Matrix interaction**: The food matrix is the structure and composition of the food that contains (poly)phenols, and it can influence their interactions with other food components, such as carbohydrates, proteins, fat and fibre. These interactions can have positive or negative effects on the bioavailability of (poly)phenols (**Figure 5**). For example, some interactions can enhance the stability, solubility, or release of (poly)phenols from the food matrix, while others can reduce their absorption or increase their metabolism and excretion [145]. In the last years, the interaction of (poly)phenols with the food matrix has been studied extensively, showing generally a positive effect on their bioavailability. Therefore, the food matrix is an important factor to consider when evaluating the health effects of (poly)phenols [145–147].

    - **Lipids**: It appears that interactions between lipids and (poly)phenols may play a positive role in reducing the fat absorption process, which may have a positive effect on health. These interactions increase emulsion droplet size and decrease specific surface area. These interactions can form complexes or aggregates that affect lipase activity and fat absorption. In addition, (poly)phenols can reduce the damage caused by lipid oxidation products by

creating an antioxidant environment or by interacting with them [146]. For example, red wine (poly)phenols can prevent the modification of LDL by lipid oxidation products in rats [148]. The mechanisms of this protection may involve the antioxidant properties of (poly)phenols, the formation of complexes between (poly)phenols and lipid oxidation products, or the delay of fat absorption caused by (poly)phenol-lipid interactions. Furthermore, lipid-(poly)phenol interactions may result in the protection of (poly)phenols as they pass through the gastrointestinal tract. This may allow the beneficial effects of phenolic compounds to occur within the gastrointestinal tract [145].

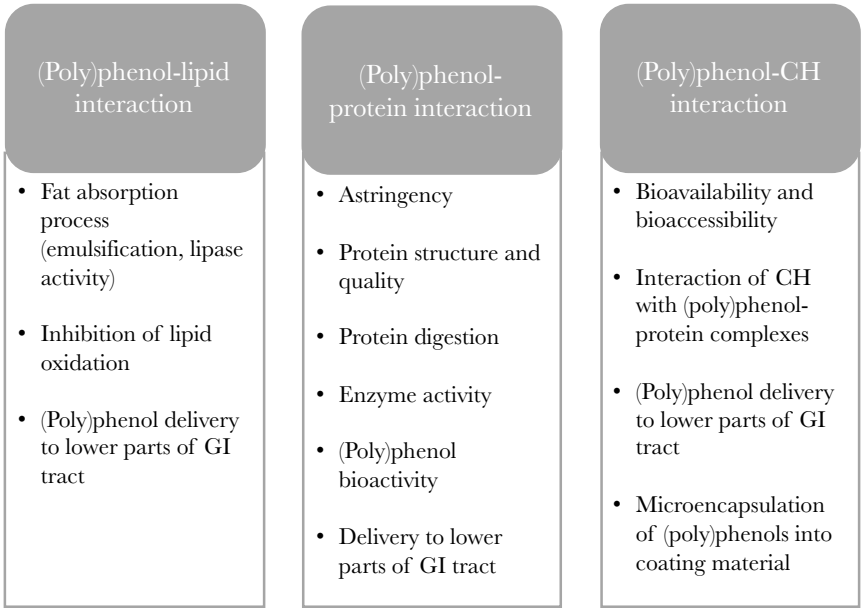


Figure 5. The effects of the interplay between (poly)phenols and lipids, proteins, and carbohydrates. Modified from Jakobek, 2015 [145].

- **Proteins**: The interactions between (poly)phenols and proteins have various effects on the properties and functions of both molecules [146]. One of the effects is the modulation of astringency, which is an oral sensation of dryness and roughness caused by the formation of aggregates between (poly)phenols and salivary proteins [147]. Another effect is the alteration of amino acid availability and protein structure, which can affect the functionality and digestibility of proteins. The strength of this effect may depend on the nutritional quality of the proteins. (Poly)phenols can also interact with enzymes and change their enzymatic activity, which can have positive or negative consequences for health and nutrition. For example, (poly)phenols can inhibit α-amylase activity and prevent dental cavities [145]. Moreover, some (poly)phenol–protein interactions can change the allergenicity of some compounds by modifying their structure and their binding sites with immunoglobulins [146]. On the other hand, they can inhibit digestive enzymes and affect the digestion process [145,146]. Protein–(poly)phenol interactions can also influence the (poly)phenols themselves. They can reduce or "mask" some of their activities, such as their antioxidant activity. Finally, proteins can act as protectors and carriers of (poly)phenols in the gastrointestinal tract and prevent their oxidation reactions [146].

- **Carbohydrates**: (Poly)phenols interact with various carbohydrates, derived from cell wall constituents such as pectin, cellulose, and dietary fibers. These interactions arise due to weak bonds, including hydrogen bonds and hydrophobic interactions [145]. These interactions are essential influencing the bioavailability of (poly)phenols, regulating the amount and rate at which the compound is absorbed in the body. Some studies highlight that these interactions can reduce (poly)phenol absorption by trapping them within carbohydrate structures [145,146]. However, these interactions aid in the transportation of (poly)phenols to the large intestine, where enzymatic and microbial processes result in their release. This phenomenon leads to various results, such as increased (poly)phenol bioavailability, improved colon microflora, production of beneficial metabolites, and the creation of an antioxidant environment. Moreover, dietary fibre associated (poly)phenols contribute to the increased excretion of lipids, proteins, water, and total faecal output. They also have a positive impact on lipid metabolism, total cholesterol, LDL-cholesterol, triacylglycerides, and enhance antioxidant activity in the large intestine [145]. Finally, carbohydrates have the potential to prevent the formation of (poly)phenol-protein complexes, which can lead to unfavourable effects such as enzyme inhibition and astringency perception [149].

- (**Poly**)**phenol Related Factors**: One of the main factors of bioavailability is the chemical structure of the compound. In

foods, most (poly)phenols exist as polymers or glycosylated forms that hinder their absorption [1,77]. Therefore, both their chemical structure and the type of sugar that forms the glycoside determine the rate and extent of absorption [118]. Moreover, the dosage of the (poly)phenol, the duration of the treatment and the model used (*in vitro* or *in vivo*) affect the bioavailability and bioactivity of phenolic compounds [93,150].

- **Harvest and postharvest factors**: The (poly)phenol content of foods is significantly affected by the **harvest** environment, where factors such as sun exposure, rainfall, crop variety, and degree of ripeness can cause variation in the total concentration of (poly)phenols [118,151]. On the other hand, **post-harvest** practices are used by the food industry to maintain the quality and safety of fruits and vegetables. Nevertheless, these practices can also influence the phenolic content of these foods and thus their bioactivity. For instance, depending on the postharvest practice, including factors like UV-B irradiation, temperature, ripening, storage, and pitting on the fruit surface, the phenolic compounds can either increase or decrease in concentration [152–154]

5. Biological Rhythms

Recently, there have been increasing interest and supporting evidence for the importance of food intake timing and dietary patterns in regulating various physiological processes. This has resulted in the emergence of a new field of study called chrononutrition. This novel research field focuses on exploring the complex relationship between biological rhythms, nutrition, and metabolism. As well as their influence on the prevention or development of certain diseases [155,156].

Chronobiology is a field of science that studies the cycles in biological systems caused by environmental changes due to the movement of the Earth. Daily cycles, known as **circadian rhythms,** are influenced by the Earth's rotation and result in variations in light and temperature over a 24-hour period. Annual cycles, known as **circannual or seasonal rhythms,** are influenced by the Earth's movement around the Sun and result in seasonal changes. Many living organisms, including fungi, plants, fish and mammals, exhibit these rhythms [157]. In mammals, these rhythms are controlled by internal clocks (central and peripheral clocks) [158,159]. However, periodic environmental factors, called synchronisers or zeitgebers, can modulate these rhythms by adapting to external conditions such as day length and temperature. The main signal used by organisms to establish daily and seasonal timing is the photoperiod, the light/dark phases of the day. Although there are other environmental factors that can influence rhythms, they are inconsistent and unpredictable [158,159].

5.1. Circadian Rhythms

Circadian clocks, called so from the Latin “circa diem”, meaning “about a day”, have evolved in every organism to adjust their behaviour and

physiology to the relevant time of day, such as predator activity or food availability [160]. In the organisms, circadian rhythms are regulated by various environmental cycles such as sunlight [161], temperature [162], food [163], small molecule modifiers (intrinsic or extrinsic) [164], and drugs [165]. The term "zeitgeber", derived from the German word "timer", is used to describe the external signals mentioned above that influence the internal circadian rhythms, acting as synchronizers [166]. The circadian clock system in mammals relies on a central clock situated in the hypothalamus. However, circadian clocks also occur in peripheral tissues, where the circadian rhythm plays a significant role in preserving body function and homeostasis [160]. This indicates that maintaining proper circadian control is essential for promoting good health throughout the body [167]. In recent years, the serious metabolic implications of circadian disruption have emerged, including neurodegenerative diseases, obesity, metabolic syndrome [167–169], cancer [170], and reproduction issues [171].

The intrinsic circadian rhythm is based on the oscillation of transcription and translation of genes and proteins, known as "Clock genes". These genes form heterodimers such as the one composed by circadian locomotor output cycles kaput (CLOCK) - brain and muscle ARNT-like protein 1 (BMAL1) or occasionally the formed by Neuronal PAS domain protein 2 and BMAL1 (NPAS2 - BMAL1) (see **Figure 6a**). These complexes bind to E-box elements in promoter regions and activate the transcription of genes including their negative regulators *Period* (*PER1*, *PER2* and *PER3*) and *Cryptochrome* (*CRY1* and *CRY2*). Subsequently, PER and CRY proteins enter the nucleus and repress transcription of their own gene loci, which initiates a new circadian cycle. A second feedback loop, consisting of nuclear receptors from the D nuclear receptor subfamily (REV-ERBA) and the RAR-related orphan receptor (ROR) families,

reinforces the oscillatory mechanism. Clock factors also bind to cell-specific enhancers and form complexes with various epigenetic regulators, directing the rhythmic expression of target genes outside the core clock mechanism. Such genes are known as Clock-controlled genes [156,172,173] (see **Figure 6b**).

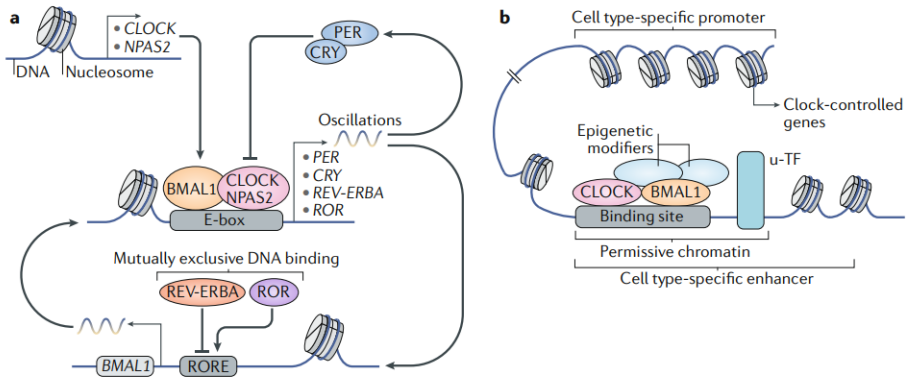


Figure 6. Transcriptional and metabolic circadian oscillators. a) Transcriptional feedback loops in the central clock. b) driving rhythmic expression of target genes. Extracted from Reinke, H. & Asher, G. Crosstalk, (2019) [172].

5.2. Seasonal Rhythms

Both the circadian rhythm and the circannual rhythm are regulated by mechanisms which involve a pacemaker in the brain and long-term timing control in peripheral tissues. The pineal gland is crucial for generating circannual rhythms and inducing photoperiodic transitions [174]. The concentration of melatonin in the pineal gland varies seasonally, leading to a seasonally dependent endocrine response [175]. Several cells in the pineal gland and the third ventricle of the brain act as pacemakers and regulate hormonal and genetic pathways involved in the generation of circannual rhythms. Unlike circadian rhythms, circannual rhythms are capable of self-sustenance, flexibility, and reprogramming. Although

temperature has minimal effect on the duration of the circannual period, circannual rhythms can adjust to environmental signals, mainly photoperiod, to synchronize physiology with seasonal changes [176]. This results in a complex and highly regulated hormonal coordination, which in turn modulates physiological, behavioural and reproductive functions in a seasonal pattern. Because seasons regulate many physiological processes, environmental seasonality results in tissue programming, creating endogenous rhythms that approach an annual cycle. In species where winter represents a higher pathological risk, exposure to short days induces an increase in immune function in anticipation of increased defence needs [177]. Therefore, variations in climate or season duration can greatly impact organismal health due to modifications in yearly rhythms that can take place.

Recently, studies have shown that some dietary components, such as phenolic compounds, can interact with molecular clocks, contributing to the synchronization of biological rhythms [156,178]. These findings are consistent with the xenohormesis hypothesis [179,180], which proposes that heterotrophs are able to detect chemical cues synthesised by plants in response to stress, such as (poly)phenols, and use them to provide warnings to heterotrophs about changes in environmental conditions. **Figure 7** summarises the mechanisms involved in the interaction between seasonal variations in the (poly)phenolic composition of fruits, regulation of gene expression linked to biological rhythms, and the consequential impact on health.

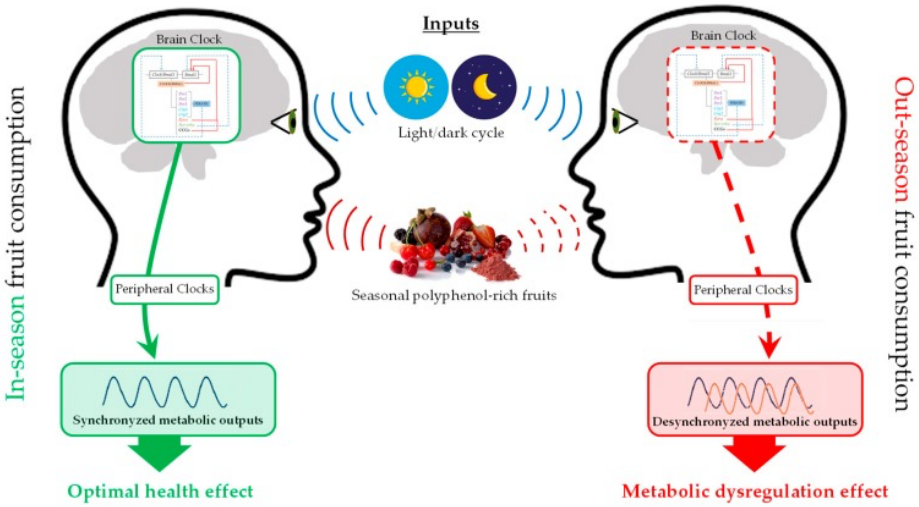


Figure 7. Interaction between gene regulation of biological rhythms, seasonal variation of plant (poly)phenols composition, and health seasonal effects. Extracted from Arola-Arnal *et al.*, (2019) [156].

However, the existing literature on the effect of consuming bioactive compounds under varying photoperiods is rather limited. Thus, to obtain a detailed understanding of this interesting area of research, it is crucial to perform studies in both animals and humans, taking into account factors such as the timing of compound administration and light/dark cycles. This can help us gain well-defined insights into the role of chrononutrition in maintaining optimal biological rhythms and metabolic processes.

6. References

1. Del Rio, D. *et al.* Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal* **18**, 1818–92 (2013).

2. Rana, A., Samtiya, M., Dhewa, T., Mishra, V. & Aluko, R. E. Health benefits of polyphenols: A concise review. *Journal of Food Biochemistry* vol. **46** (2022).

3. Shen, N. *et al.* Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chemistry* vol. **383** (2022).

4. Pisoschi, A. M. & Pop, A. The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry* vol. **97** (2015).

5. Rudrapal, M. *et al.* Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action. *Frontiers in Pharmacology* vol. **13** (2022).

6. Durazzo, A. *et al.* Polyphenols: A concise overview on the chemistry, occurrence, and human health. *Phytotherapy Research* vol. **33** (2019).

7. Rodriguez-Mateos, A. *et al.* Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. *Archives of Toxicology* vol. **88** (2014).

8. Torres-Fuentes, C. *et al.* Cardioprotective Properties of Phenolic Compounds: A Role for Biological Rhythms. *Molecular Nutrition and Food Research* vol. **66** (2022).

9. Wang, S., Du, Q., Meng, X. & Zhang, Y. Natural polyphenols: a potential prevention and treatment strategy for metabolic syndrome. *Food and Function* vol. **13** (2022).

10. Landeka Jurčević, I. *et al.* Wine Lees Polyphenols as a Novel Functional Bioactive Compound in the Protection against Oxidative Stress and Hyperlipidemia. *Food Technol Biotechnol* **55**, 109–116 (2017).

11. Gorzynik-Debicka, M. *et al.* Potential Health Benefits of Olive Oil and Plant Polyphenols. *Int J Mol Sci* **19**, 686 (2018).

12. Domínguez-Avila, J. A. *et al.* Phenolic compounds can induce systemic and central immunomodulation, which result in a neuroprotective effect. *Journal of Food Biochemistry* vol. **46** (2022).

13. Ding, S., Xu, S., Fang, J. & Jiang, H. The Protective Effect of Polyphenols for Colorectal Cancer. *Frontiers in Immunology* vol. **11** (2020).

14. Agunloye, O. M. *et al.* Cardio-protective and antioxidant properties of caffeic acid and chlorogenic acid: Mechanistic role of angiotensin converting enzyme, cholinesterase and arginase activities in cyclosporine induced hypertensive rats. *Biomedicine and Pharmacotherapy* **109**, (2019).

15. González Arbelaéz, L. F. *et al.* Cardioprotection and natural polyphenols: An update of clinical and experimental studies. *Food Funct* **9**, (2018).

16. Patel, R. V. *et al.* Therapeutic potential of quercetin as a cardiovascular agent. *European Journal of Medicinal Chemistry* vol. **155** (2018).

17. Serino, A. & Salazar, G. Protective role of polyphenols against vascular inflammation, aging and cardiovascular disease. *Nutrients* vol. **11** (2019).

18. Vaisman, N. & Niv, E. Daily consumption of red grape cell powder in a dietary dose improves cardiovascular parameters: A double blind, placebo-controlled, randomized study. *Int J Food Sci Nutr* **66**, (2015).

19. Bogdanski, P. *et al.* Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutrition Research* **32**, (2012).

20. Moreno-Luna, R. *et al.* Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension. *Am J Hypertens* **25**, (2012).

21. Renzo, L. Di *et al.* Effects of dark chocolate in a population of normal weight obese women: A pilot study. *Eur Rev Med Pharmacol Sci* **17**, (2013).

22. Khan, N. *et al.* Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces oxidized LDL levels in

subjects at high-risk of cardiovascular disease. *Nutrition, Metabolism and Cardiovascular Diseases* **22**, (2012).

23. Terauchi, M. *et al.* Effects of grape seed proanthocyanidin extract on menopausal symptoms, body composition, and cardiovascular parameters in middle-aged women: A randomized, double-blind, placebo-controlled pilot study. *Menopause* **21**, (2014).

24. Chang, Y. C., Yang, M. Y., Chen, S. C. & Wang, C. J. Mulberry leaf polyphenol extract improves obesity by inducing adipocyte apoptosis and inhibiting preadipocyte differentiation and hepatic lipogenesis. *J Funct Foods* **21**, (2016).

25. Klaus, S., Pültz, S., Thöne-Reineke, C. & Wolfram, S. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. *Int J Obes* **29**, (2005).

26. Noratto, G., Martino, H. S. D., Simbo, S., Byrne, D. & Mertens-Talcott, S. U. Consumption of polyphenol-rich peach and plum juice prevents risk factors for obesity-related metabolic disorders and cardiovascular disease in Zucker rats. *Journal of Nutritional Biochemistry* **26**, (2015).

27. Uchiyama, S., Taniguchi, Y., Saka, A., Yoshida, A. & Yajima, H. Prevention of diet-induced obesity by dietary black tea polyphenols extract in vitro and in vivo. *Nutrition* **27**, (2011).

28. Bird, J. K., Raederstorff, D., Weber, P. & Steinert, R. E. Cardiovascular and antiobesity effects of resveratrol mediated through the gut microbiota. *Advances in Nutrition* **8**, (2017).

29. Boccellino, M. & D'Angelo, S. Anti-obesity effects of polyphenol intake: Current status and future possibilities. *International Journal of Molecular Sciences* vol. **21** (2020).

30. Khoo, H. E., Azlan, A., Tang, S. T. & Lim, S. M. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food and Nutrition Research* vol. **61** (2017).

31. Rózańska, D. & Regulska-Ilow, B. The significance of anthocyanins in the prevention and treatment of type 2 diabetes. *Advances in Clinical and Experimental Medicine* vol. **27** (2018).

32. de Oliveira, M. S., Pellenz, F. M., de Souza, B. M. & Crispim, D. Blueberry Consumption and Changes in Obesity and Diabetes Mellitus Outcomes: A Systematic Review. *Metabolites* vol. **13** (2023).

33. Yin, P. *et al.* Hypoglycemic and hypolipidemic effects of polyphenols from burs of castanea mollissima blume. *Molecules* **16**, (2011).

34. Prabhakar, P. K. & Doble, M. Synergistic effect of phytochemicals in combination with hypoglycemic drugs on glucose uptake in myotubes. *Phytomedicine* **16**, (2009).

35. Takikawa, M., Inoue, S., Horio, F. & Tsuda, T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of amp-activated protein kinase in diabetic mice. *Journal of Nutrition* **140**, (2010).

36. Prasad, C., Davis, K. E., Imrhan, V., Juma, S. & Vijayagopal, P. Advanced Glycation End Products and Risks for Chronic Diseases: Intervening Through Lifestyle Modification. *American Journal of Lifestyle Medicine* vol. **13** (2019).

37. Shahwan, M., Alhumaydhi, F., Ashraf, G. M., Hasan, P. M. Z. & Shamsi, A. Role of polyphenols in combating Type 2 Diabetes and insulin resistance. *International Journal of Biological Macromolecules* vol. **206** (2022).

38. Jin, S., Zhang, Q. Y., Kang, X. M., Wang, J. X. & Zhao, W. H. Daidzein induces MCF-7 breast cancer cell apoptosis via the mitochondrial pathway. *Annals of Oncology* **21**, (2010).

39. Zhao, Q., Huo, X. C., Sun, F. D. & Dong, R. Q. Polyphenol-rich extract of Salvia chinensis exhibits anticancer activity in different cancer cell lines, and induces cell cycle arrest at the G0/G1-phase, apoptosis and loss of mitochondrial membrane potential in pancreatic cancer cells. *Mol Med Rep* **12**, (2015).

40. Yuan, J. P., Wang, J. H. & Liu, X. Metabolism of dietary soy isoflavones to equol by human intestinal microflora - Implications for health. *Molecular Nutrition and Food Research* vol. **51** (2007).

41. Tsai, H. C., Li, Y. C., Hsu, S. H., Young, T. H. & Chen, M. H. Inhibition of growth and migration of oral and cervical cancer cells by citrus polyphenol. *Journal of the Formosan Medical Association* **115**, (2016).

42. Polivka, J. & Janku, F. Molecular targets for cancer therapy in the PI3K/AKT/mTOR pathway. *Pharmacology and Therapeutics* vol. **142** (2014).

43. Nakaso, K., Ito, S. & Nakashima, K. Caffeine activates the PI3K/Akt pathway and prevents apoptotic cell death in a Parkinson's disease model of SH-SY5Y cells. *Neurosci Lett* **432**, (2008).

44. Kinarivala, N., Patel, R., Boustany, R. M., Al-Ahmad, A. & Trippier, P. C. Discovery of Aromatic Carbamates that Confer Neuroprotective Activity by Enhancing Autophagy and Inducing the Anti-Apoptotic Protein B-Cell Lymphoma 2 (Bcl-2). *J Med Chem* **60**, (2017).

45. Smolensky, D. *et al.* High-polyphenol sorghum bran extract inhibits cancer cell growth through ROS induction, cell cycle arrest, and apoptosis. *J Med Food* **21**, (2018).

46. Chen, B. T. *et al.* Anti-inflammatory effects of a polyphenols-rich extract from tea (camellia sinensis) flowers in acute and chronic mice models. *Oxid Med Cell Longev* (2012).

47. Agarwal, B. *et al.* Resveratrol for primary prevention of atherosclerosis: Clinical trial evidence for improved gene expression in vascular endothelium. *Int J Cardiol* **166**, (2013).

48. Koczka, N., Stefanovits-Bányai, É. & Ombódi, A. Total Polyphenol Content and Antioxidant Capacity of Rosehips of Some Rosa Species. *Medicines* **5**, (2018).

49. Kumar, S., Sharma, U. K., Sharma, A. K. & Pandey, A. K. Protective efficacy of Solanum xanthocarpum root extracts against free radical damage: Phytochemical analysis and antioxidant effect. *Cell Mol Biol* **58**, (2012).

50. Johnson, S. L. *et al.* Polyphenol microbial metabolites exhibit gut and blood–brain barrier permeability and protect murine microglia against lps-induced inflammation. *Metabolites* **9**, (2019).

51. Gaballah, H. H., Zakaria, S. S., Elbatsh, M. M. & Tahoon, N. M. Modulatory effects of resveratrol on endoplasmic reticulum stress-associated apoptosis and oxido-inflammatory markers in a rat model of rotenone-induced Parkinson's disease. *Chem Biol Interact* **251**, (2016).

52. Si, T. L. *et al.* Enhanced anti-inflammatory effects of DHA and quercetin in lipopolysaccharide-induced RAW264.7 macrophages by inhibiting NF-κB and MAPK activation. *Mol Med Rep* **14**, (2016).

53. Kurutas, E. B. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. *Nutrition Journal* vol. **15** (2016).

54. Wang, W. *et al.* Resveratrol: Multi-Targets Mechanism on Neurodegenerative Diseases Based on Network Pharmacology. *Front Pharmacol* **11**, (2020).

55. Ortiz-López, L. *et al.* Green tea compound epigallo-catechin-3-gallate (EGCG) increases neuronal survival in adult hippocampal neurogenesis in vivo and in vitro. *Neuroscience* **322**, (2016).

56. Ward, R. J., Dexter, D. T. & Crichton, R. R. Ageing, neuroinflammation and neurodegeneration. *Frontiers in Bioscience - Scholar* vol. **7**S (2015).

57. Di Meo, F., Margarucci, S., Galderisi, U., Crispi, S. & Peluso, G. Curcumin, gut microbiota, and neuroprotection. *Nutrients* vol. **11** (2019).

58. Di Meo, F. *et al.* New Therapeutic Drugs from Bioactive Natural Molecules: The Role of Gut Microbiota Metabolism in Neurodegenerative Diseases. *Curr Drug Metab* **19**, (2018).

59. Moosavi, F., Hosseini, R., Saso, L. & Firuzi, O. Modulation of neurotrophic signaling pathways by polyphenols. *Drug Design, Development and Therapy* vol. **10** (2015).

60. Milbury, P. E., Graf, B., Curran-Celentano, J. M. & Blumberg, J. B. Bilberry (Vaccinium myrtillus) anthocyanins modulate heme oxygenase-1 and glutathione S-transferase-pi expression in ARPE-19 cells. *Invest Ophthalmol Vis Sci* **48**, (2007).

61. Xu, J. Y. *et al.* Green tea polyphenols attenuating ultraviolet B-induced damage to human retinal pigment epithelial cells in vitro. *Invest Ophthalmol Vis Sci* **51**, (2010).

62. Beracochea, D. *et al.* Intake of Wild Blueberry Powder Improves Episodic-Like and Working Memory during Normal Aging in Mice. *Planta Med* **82**, (2016).

63. Krikorian, R. *et al.* Blueberry supplementation improves memory in older adults. *J Agric Food Chem* **58**, (2010).

64. Chen, B. H. *et al.* Anti-allergic activity of grapeseed extract (GSE) on RBL-2H3 mast cells. *Food Chem* **132**, (2012).

65. Panche, A. N., Diwan, A. D. & Chandra, S. R. Flavonoids: An overview. *Journal of Nutritional Science* vol. **5** (2016).

66. Khan, J. *et al.* Dietary flavonoids: Cardioprotective potential with antioxidant effects and their pharmacokinetic, toxicological and therapeutic concerns. *Molecules* vol. **26** (2021).

67. Manach, C., Scalbert, A., Morand, C., Rémésy, C. & Jiménez, L. Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition* vol. **79** 727–747 (2004).

68. Khan, M. K., Zill-E-Huma & Dangles, O. A comprehensive review on flavanones, the major citrus polyphenols. *Journal of Food Composition and Analysis* vol. **33** (2014).

69. Crozier, A., Jaganath, I. B. & Clifford, M. N. Dietary phenolics: Chemistry, bioavailability and effects on health. *Nat Prod Rep* **26**, (2009).

70. Orlikova, B., Tasdemir, D., Golais, F., Dicato, M. & Diederich, M. Dietary chalcones with chemopreventive and chemotherapeutic potential. *Genes Nutr* **6**, (2011).

71. Chen, S., Wang, X., Cheng, Y., Gao, H. & Chen, X. A Review of Classification, Biosynthesis, Biological Activities and Potential Applications of Flavonoids. *Molecules* **28**, (2023).

72. Prior, R. L. & Wu, X. Anthocyanins: Structural characteristics that result in unique metabolic patterns and biological activities. in *Free Radical Research* vol. **40** (2006).

73. Pandey, K. B. & Rizvi, S. I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity* vol. **2** (2009).

74. Havsteen, B. H. The biochemistry and medical significance of the flavonoids. *Pharmacology and Therapeutics* vol. **96** (2002).

75. Zhang, Y., Cai, P., Cheng, G. & Zhang, Y. A Brief Review of Phenolic Compounds Identified from Plants: Their Extraction, Analysis, and Biological Activity. *Natural Product Communications* vol. **17** (2022).

76. Ignat, I., Volf, I. & Popa, V. I. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry* vol. **126** (2011).

77. Xiao, J. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? *Critical Reviews in Food Science and Nutrition* vol. **57** (2017).

78. Ozdal, T. *et al.* The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrients* vol. **8** (2016).

79. Bohn, T. Dietary factors affecting polyphenol bioavailability. *Nutr Rev* **72**, (2014).

80. Kamiloglu, S., Capanoglu, E., Grootaert, C. & van Camp, J. Anthocyanin absorption and metabolism by human intestinal Caco-2 cells—A review. *International Journal of Molecular Sciences* vol. **16** (2015).

81. Passamonti, S., Vrhovsek, U., Vanzo, A. & Mattivi, F. The stomach as a site for anthocyanins absorption from food. *FEBS Lett* **544**, (2003).

82. Konishi, Y., Zhao, Z. & Shimizu, M. Phenolic acids are absorbed from the rat stomach with different absorption rates. *J Agric Food Chem* **54**, (2006).

83. Gamel, T. H. *et al.* Absorption and metabolites of anthocyanins and phenolic acids after consumption of purple wheat crackers and bars by healthy adults. *J Cereal Sci* **86**, (2019).

84. Wan, M. L. Y., Co, V. A. & El-Nezami, H. Dietary polyphenol impact on gut health and microbiota. *Critical Reviews in Food Science and Nutrition* vol. **61** (2021).

85. Monagas, M. *et al.* Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food and Function* vol. **1** 233–253 (2010).

86. Myöhänen, T. T., Schendzielorz, N. & Männistö, P. T. Distribution of catechol-O-methyltransferase (COMT) proteins and enzymatic activities in wild-type and soluble COMT deficient mice. *J Neurochem* **113**, (2010).

87. Ohno, S. & Nakajin, S. Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. *Drug Metabolism and Disposition* **37**, (2009).

88. Riches, Z., Stanley, E. L., Bloomer, J. C. & Coughtrie, M. W. H. Quantitative evaluation of the expression and activity of five major sulfotransferases (SULTs) in human tissues: The SULT 'pie'. *Drug Metabolism and Disposition* **37**, (2009).

89. Margalef, M., Pons, Z., Muguerza, B. & Arola-Arnal, A. A rapid method to determine colonic microbial metabolites derived from grape flavanols in rat plasma by liquid chromatography-tandem mass spectrometry. *J Agric Food Chem* **62**, 7698–7706 (2014).

90. Margalef, M., Pons, Z., Bravo, F. I., Muguerza, B. & Arola-Arnal, A. Plasma kinetics and microbial biotransformation of grape seed flavanols in rats. *J Funct Foods* **12**, 478–488 (2015).

91. Niwano, Y., Kohzaki, H., Shirato, M., Shishido, S. & Nakamura, K. Metabolic Fate of Orally Ingested Proanthocyanidins through the Digestive Tract. *Antioxidants* vol. **12** (2023).

92. Plaza, M., Domínguez-Rodríguez, G., Castro-Puyana, M. & Marina, M. L. Polyphenols analysis and related challenges. in *Polyphenols: Properties, Recovery, and Applications* (2018).

93. Margalef, M., Pons, Z., Bravo, F. I., Muguerza, B. & Arola-Arnal, A. Tissue distribution of rat flavanol metabolites at different doses. *J Nutr Biochem* **26**, 987–995 (2015).

94. Iglesias-Carres, L. *et al.* Optimization and characterization of Royal Dawn cherry (Prunus avium) phenolics extraction. *Sci Rep* **9**, (2019).

95. Iglesias-Carres, L. *et al.* Optimized extraction by response surface methodology used for the characterization and quantification of phenolic compounds in whole red grapes (Vitis vinifera). *Nutrients* **10**, (2018).

96. Shi, L., Zhao, W., Yang, Z., Subbiah, V. & Suleria, H. A. R. Extraction and characterization of phenolic compounds and their

potential antioxidant activities. *Environmental Science and Pollution Research* vol. **29** (2022).

97. Sun, M. F. *et al.* Recent Advances in Analytical Methods for Determination of Polyphenols in Tea: A Comprehensive Review. *Foods* vol. **11** (2022).

98. López-fernández, O. *et al.* Determination of polyphenols using liquid chromatography–tandem mass spectrometry technique (LC–MS/MS): A review. *Antioxidants* vol. **9** (2020).

99. Miyazawa, T., Nakagawa, K., Kudo, M., Muraishi, K. & Someya, K. Direct intestinal absorption of red fruit anthocyanins, cyanidin-3- glucoside and cyanidin-3,5-diglucoside, into rats and humans. *J Agric Food Chem* **47**, (1999).

100. Bajkacz, S., Baranowska, I., Buszewski, B., Kowalski, B. & Ligor, M. Determination of Flavonoids and Phenolic Acids in Plant Materials Using SLE-SPE-UHPLC-MS/MS Method. *Food Anal Methods* **11**, (2018).

101. Bajoub, A. *et al.* Comparing two metabolic profiling approaches (liquid chromatography and gas chromatography coupled to mass spectrometry) for extra-virgin olive oil phenolic compounds analysis: A botanical classification perspective. *J Chromatogr A* **1428**, (2016).

102. Pitt, J. J. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. *Clin Biochem Rev* **30**, (2009).

103. Ali, A., Bashmil, Y. M., Cottrell, J. J., Suleria, H. A. R. & Dunshea, F. R. Lc-ms/ms-qtof screening and identification of phenolic compounds from australian grown herbs and their antioxidant potential. *Antioxidants* **10**, (2021).

104. Chou, O. *et al.* Lc-esi-qtof-ms/ms characterisation of phenolics in herbal tea infusion and their antioxidant potential. *Fermentation* **7**, (2021).

105. Iqbal, Y., Ponnampalam, E. N., Suleria, H. A. R., Cottrell, J. J. & Dunshea, F. R. Lc-esi/qtof-ms profiling of chicory and lucerne polyphenols and their antioxidant activities. *Antioxidants* **10**, (2021).

106. Du, J. *et al.* Lc-esi-qtof-ms/ms profiling and antioxidant activity of phenolics from custard apple fruit and by-products. *Separations* **8**, (2021).

107. Abdelghffar, E. A., El-Nashar, H. A. S., Al-Mohammadi, A. G. A. & Eldahshan, O. A. Orange fruit (Citrus sinensis) peel extract attenuates chemotherapy-induced toxicity in male rats. *Food Funct* **12**, (2021).

108. Angeloni, S. *et al.* An analytical method for the simultaneous quantification of 30 bioactive compounds in spent coffee ground by HPLC-MS/MS. *Journal of Mass Spectrometry* **55**, (2020).

109. Boke Sarikahya, N., Goren, A. C. & Kirmizigul, S. Simultaneous determination of several flavonoids and phenolic compounds in nineteen different Cephalaria species by HPLC-MS/MS. *J Pharm Biomed Anal* **173**, (2019).

110. Dabetic, N., Todorovic, V., Malenovic, A., Sobajic, S. & Markovic, B. Optimization of Extraction and HPLC–MS/MS Profiling of Phenolic Compounds from Red Grape Seed Extracts Using Conventional and Deep Eutectic Solvents. *Antioxidants* **11**, (2022).

111. Pan, X. *et al.* Phenolic characterization of fermented jujube puree by HPLC-MS/MS and their release during in vitro dynamic digestion. *Food Chem* **413**, (2023).

112. Fernández-García, E., Carvajal-Lérida, I. & Pérez-Gálvez, A. In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research* vol. **29** (2009).

113. Angelino, D. *et al.* Bioaccessibility and bioavailability of phenolic compounds in bread: A review. *Food and Function* vol. **8** (2017).

114. Di Lorenzo, C., Colombo, F., Biella, S., Stockley, C. & Restani, P. Polyphenols and human health: The role of bioavailability. *Nutrients* vol. **13** (2021).

115. Brodkorb, A. *et al.* INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nat Protoc* **14**, (2019).

116. Thakur, N. *et al.* Recent updates on bioaccessibility of phytonutrients. *Trends in Food Science and Technology* vol. **97** (2020).

117. Pasinetti, G. M. *et al.* The Role of the Gut Microbiota in the Metabolism of Polyphenols as Characterized by Gnotobiotic Mice. *J Alzheimers Dis* **63**, 409–421 (2018).

118. D'Archivio, M., Filesi, C., Varì, R., Scazzocchio, B. & Masella, R. Bioavailability of the polyphenols: status and controversies. *Int J Mol Sci* **11**, 1321–42 (2010).

119. Mangoni, A. A. & Jackson, S. H. D. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. *Br J Clin Pharmacol* **57**, 6–14 (2004).

120. Lee, J. S. *et al.* Coordinated Changes in Xenobiotic Metabolizing Enzyme Gene Expression in Aging Male Rats. *Toxicological Sciences* **106**, 263–283 (2008).

121. Gidal, B. E. Drug absorption in the elderly: Biopharmaceutical considerations for the antiepileptic drugs. *Epilepsy Res* **68**, 65–69 (2006).

122. Hopkins, M. J., Sharp, R. & Macfarlane, G. T. Age and disease related changes in intestinal bacterial populations assessed by cell culture, 16S rRNA abundance, and community cellular fatty acid profiles. *Gut* **48**, 198–205 (2001).

123. MACFARLANE, G. T. & HOPKINS, M. J. Changes in predominant bacterial populations in human faeces with age and with Clostridium difficile infection. *J Med Microbiol* **51**, 448–454 (2002).

124. Margalef, M. *et al.* Age related differences in the plasma kinetics of flavanols in rats. **29**, 90–96 (2016).

125. Soldin, O. P., Chung, S. H. & Mattison, D. R. Sex Differences in Drug Disposition. *J Biomed Biotechnol* **2011**, 1–14 (2011).

126. Schwartz, J. B. The Influence of Sex on Pharmacokinetics. *Clin Pharmacokinet* **42**, 107–121 (2003).

127. Jiang, H., Xie, T., Ramsden, D. B. & Ho, S. L. Human catechol-O-methyltransferase down-regulation by estradiol. *Neuropharmacology* **45**, 1011–8 (2003).

128. Margalef, M. *et al.* Gender-related similarities and differences in the body distribution of grape seed flavanols in rats. *Mol Nutr Food Res* **60**, 760–772 (2016).

129. Sampey, B. P. *et al.* Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: Comparison to high-fat diet. *Obesity* **19**, 1109–1117 (2011).

130. Dobrian, A. D., Davies, M. J., Schriver, S. D., Lauterio, T. J. & Prewitt, R. L. Oxidative stress in a rat model of obesity-induced hypertension. *Hypertension* **37**, 554–60 (2001).

131. Tesauro, M. & Cardillo, C. Obesity, blood vessels and metabolic syndrome. *Acta Physiologica* **203**, 279–286 (2011).

132. Merrell, M. D. & Cherrington, N. J. Drug metabolism alterations in nonalcoholic fatty liver disease. *Drug Metab Rev* **43**, 317–334 (2011).

133. Jancova, P., Anzenbacher, P. & Anzenbacherova, E. Phase II drug metabolizing enzymes. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* **154**, 103–16 (2010).

134. Carere, C. & Maestripieri, D. *Animal Personalities*. *Animal Personalities* (University of Chicago Press, 2013).

135. Carrasco-Pancorbo, A. *et al.* Evaluation of the Influence of Thermal Oxidation on the Phenolic Composition and on the Antioxidant Activity of Extra-Virgin Olive Oils. *J Agric Food Chem* **55**, 4771–4780 (2007).

136. Rawson, A., Hossain, M. B., Patras, A., Tuohy, M. & Brunton, N. Effect of boiling and roasting on the polyacetylene and polyphenol content of fennel (Foeniculum vulgare) bulb. *Food Research International* **50**, 513–518 (2013).

137. D'Evoli, L., Lombardi-Boccia, G. & Lucarini, M. Influence of Heat Treatments on Carotenoid Content of Cherry Tomatoes. *Foods* **2**, 352–363 (2013).

138. Tomas-Barberán, F. A. *et al.* A New Process To Develop a Cocoa Powder with Higher Flavonoid Monomer Content and Enhanced Bioavailability in Healthy Humans. *J Agric Food Chem* **55**, 3926–3935 (2007).

139. Cattivelli, A., Conte, A., Martini, S. & Tagliazucchi, D. Influence of cooking methods on onion phenolic compounds bioaccessibility. *Foods* **10**, (2021).

140. Ambra, R., Lucchetti, S. & Pastore, G. A Review of the Effects of Olive Oil-Cooking on Phenolic Compounds. *Molecules* vol. 27 Preprint at https://doi.org/10.3390/molecules27030661 (2022).

141. Michalczyk, M., Macura, R. & Matuszak, I. The Effect Of Air-Drying, Freeze-Drying And Storage On The Quality And Antioxidant Activity Of Some Selected Berries. *J Food Process Preserv* **33**, 11–21 (2009).

142. Porrini, M., Riso, P. & Testolin, G. Absorption of lycopene from single or daily portions of raw and processed tomato. *Br J Nutr* **80**, 353–61 (1998).

143. Volf, I., Ignat, I., Neamtu, M. & Popa, V. I. Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols. *Chemical Papers* **68**, (2014).

144. Antony, A. & Farid, M. Effect of Temperatures on Polyphenols during Extraction. *Applied Sciences (Switzerland)* vol. **12** (2022).

145. Jakobek, L. Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chem* **175**, 556–567 (2015).

146. Cianciosi, D. *et al.* The reciprocal interaction between polyphenols and other dietary compounds: Impact on bioavailability, antioxidant capacity and other physico-chemical and nutritional parameters. *Food Chem* **375**, (2022).

147. Kardum, N. & Glibetic, M. Polyphenols and Their Interactions With Other Dietary Compounds: Implications for Human Health. *Adv Food Nutr Res* **84**, 103–144 (2018).

148. Gorelik, S., Kanner, J., Schurr, D. & Kohen, R. A rational approach to prevent postprandial modification of LDL by dietary polyphenols. *J Funct Foods* **5**, (2013).

149. Soares, S., Mateus, N. & De Freitas, V. Carbohydrates inhibit salivary proteins precipitation by condensed tannins. *J Agric Food Chem* **60**, (2012).

150. Margalef, M. *et al.* A dose–response study of the bioavailability of grape seed proanthocyanidin in rat and lipid-lowering effects of

generated metabolites in HepG2 cells. *Food Research International* **64**, 500–507 (2014).

151. Gómez-Rico, A., Salvador, M. D., La Greca, M. & Fregapane, G. Phenolic and Volatile Compounds of Extra Virgin Olive Oil (*Olea europaea* L. Cv. Cornicabra) with Regard to Fruit Ripening and Irrigation Management. *J Agric Food Chem* **54**, 7130–7136 (2006).

152. Blando, F. & Oomah, B. D. Sweet and sour cherries: Origin, distribution, nutritional composition and health benefits. *Trends in Food Science and Technology* vol. **86** (2019).

153. Lavelli, V. & Giovanelli, G. Evaluation of heat and oxidative damage during storage of processed tomato products. II. Study of oxidative damage indices. *J Sci Food Agric* **83**, (2003).

154. Toivonen, P. M. A., Kappel, F., Stan, S., McKenzie, D. L. & Hocking, R. Firmness, Respiration, and Weight Loss of `Bing', `Lapins' and `Sweetheart' Cherries in Relation to Fruit Maturity and Susceptibility to Surface Pitting. *HortScience* **39**, 1066–1069 (2004).

155. Ruddick-Collins, L. C., Johnston, J. D., Morgan, P. J. & Johnstone, A. M. The Big Breakfast Study: Chrono-nutrition influence on energy expenditure and bodyweight. *Nutr Bull* **43**, (2018).

156. Arola-Arnal, A. *et al.* Chrononutrition and polyphenols: Roles and diseases. *Nutrients* vol. **11** (2019).

157. Oike, H., Oishi, K. & Kobori, M. Nutrients, Clock Genes, and Chrononutrition. *Current Nutrition Reports* vol. **3** (2014).

158. Reppert, S. M. & Weaver, D. R. Coordination of circadian timing in mammals. *Nature* vol. **418** (2002).

159. Dibner, C., Schibler, U. & Albrecht, U. The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. *Annual Review of Physiology* vol. **72** (2009).

160. Sahar, S. & Sassone-Corsi, P. Regulation of metabolism: the circadian clock dictates the time. *Trends Endocrinol Metab* **23**, 1–8 (2012).

161. Zylka, M. J., Shearman, L. P., Weaver, D. R. & Reppert, S. M. Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. *Neuron* **20**, 1103–10 (1998).

162. Poletini, M. O., Moraes, M. N., Ramos, B. C., Jerônimo, R. & Castrucci, A. M. de L. TRP channels: a missing bond in the entrainment mechanism of peripheral clocks throughout evolution. *Temperature (Austin)* **2**, 522–34 (2015).

163. Hamaguchi, Y., Tahara, Y., Kuroda, H., Haraguchi, A. & Shibata, S. Entrainment of mouse peripheral circadian clocks to. *Sci Rep* **5**, 14207 (2015).

164. Chen, Z., Yoo, S.-H. & Takahashi, J. S. Small molecule modifiers of circadian clocks. *Cell Mol Life Sci* **70**, 2985–98 (2013).

165. Moriya, S. *et al.* Effect of quetiapine on Per1, Per2, and Bmal1 clock gene expression in the mouse amygdala and hippocampus. *J Pharmacol Sci* **125**, 329–32 (2014).

166. Grandin, L. D., Alloy, L. B. & Abramson, L. Y. The social zeitgeber theory, circadian rhythms, and mood disorders: Review and evaluation. *Clin Psychol Rev* **26**, 679–694 (2006).

167. Delezie, J. & Challet, E. Interactions between metabolism and circadian clocks: reciprocal disturbances. *Ann N Y Acad Sci* **1243**, 30–46 (2011).

168. Garaulet, M. & Madrid, J. A. Chronobiological aspects of nutrition, metabolic syndrome and obesity☆. *Adv Drug Deliv Rev* **62**, 967–978 (2010).

169. Qi, G. *et al.* Tea polyphenols ameliorates neural redox imbalance and mitochondrial dysfunction via mechanisms linking the key circadian regular Bmal1. *Food and Chemical Toxicology* **110**, 189–199 (2017).

170. Sahar, S. & Sassone-Corsi, P. Metabolism and cancer: the circadian clock connection. *Nat Rev Cancer* **9**, 886–896 (2009).

171. Tamura, H. *et al.* Melatonin and female reproduction. *Journal of Obstetrics and Gynaecology Research* **40**, 1–11 (2014).

172. Reinke, H. & Asher, G. Crosstalk between metabolism and circadian clocks. *Nat Rev Mol Cell Biol* **20**, 227–241 (2019).

173. Robinson, I. & Reddy, A. B. Molecular mechanisms of the circadian clockwork in mammals. *FEBS Letters* vol. **588** (2014).

174. Lincoln, G. A. Melatonin entrainment of circannual rhythms. in *Chronobiology International* vol. 23 (2006).

175. Gorman, M. R. Temporal organization of pineal melatonin signaling in mammals. *Molecular and Cellular Endocrinology* vol. **503** (2020).

176. Lincoln, G. A brief history of circannual time. *Journal of Neuroendocrinology* vol. **31** (2019).

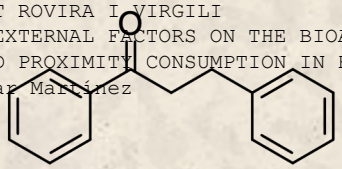
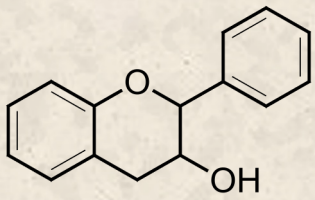
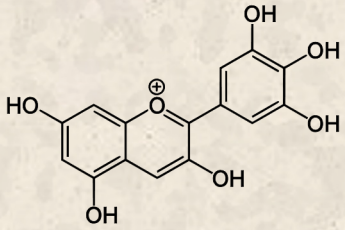
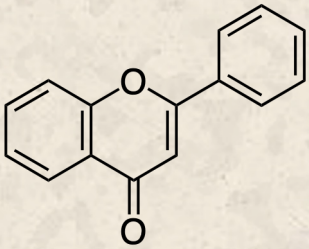
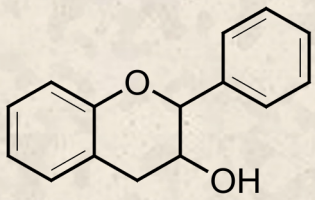
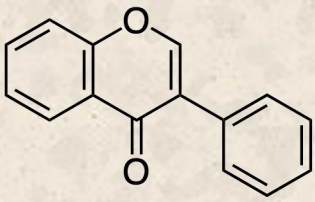
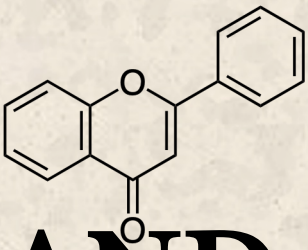
177. Stevenson, T. J. *et al.* Disrupted seasonal biology impacts health, food security and ecosystems. *Proceedings of the Royal Society B: Biological Sciences* vol. **282** (2015).

178. Ribas-Latre, A. *et al.* Dietary proanthocyanidins modulate melatonin levels in plasma and the expression pattern of clock genes in the hypothalamus of rats. *Mol Nutr Food Res* **59**, 865–878 (2015).

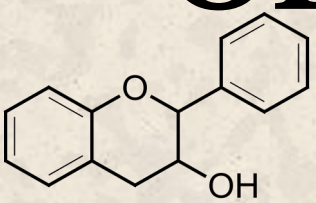
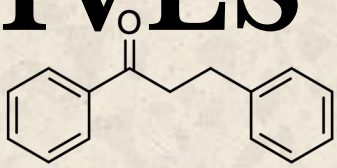
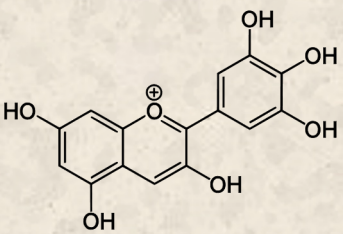
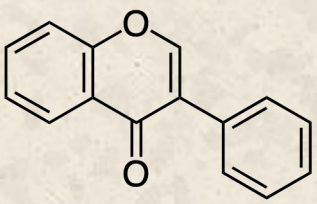
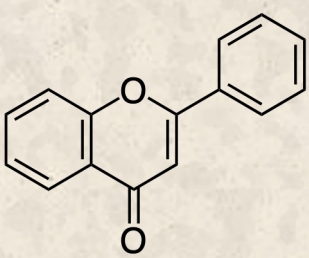
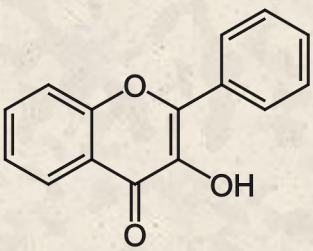
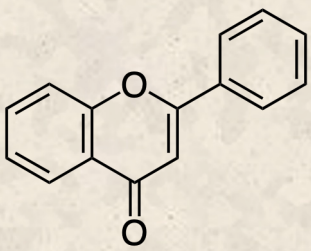
179. Baur, J. A. & Sinclair, D. A. What is xenohormesis? *Am J Pharmacol Toxicol* **3**, 149–156 (2008).

180. Howitz, K. T. & Sinclair, D. A. Xenohormesis: Sensing the Chemical Cues of Other Species. *Cell* **133**, 387–391 (2008).

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

HYPOTESIS AND OBJECTIVES

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Contemporary societies have seen significant lifestyle changes, which have been linked to the rise of metabolic disorders, including diabetes, obesity, and metabolic syndrome. These changes are primarily driven by disruptions in biological rhythms, such as exposure to artificial light, jet lag, and shift work, as well as alterations in dietary habits, such as the consumption of high-fat and high-sugar foods. Additionally, globalization has disrupted traditional practices of seasonal and local fruit consumption, challenging their natural seasonal cycles. Furthermore, these changes have an impact on bioactive compounds found in fruits and vegetables, known as (poly)phenols. These compounds possess antioxidant and anti-inflammatory properties and have been linked to various health benefits, including cardiovascular health and neuroprotection. However, the bioactivity of these compounds depends on their absorption and metabolism. Despite the growing recognition of the role of (poly)phenols in health, there is a significant gap in the literature regarding their bioavailability and metabolism in relation to different biological rhythms. The limited available literature highlights the need for further research into how current lifestyle patterns affect the bioavailability and metabolism of these bioactive compounds.

Therefore, we hypothesize that different factors including biological rhythms, such as circadian and seasonal rhythms, as well as agronomic and post-harvesting practices, linked to crop production location, influence the bioavailability and metabolization of dietary (poly)phenols.

Considering all this, the main objective of this thesis was to evaluate whether the metabolization and bioavailability of (poly)phenols can be

conditioned by different biological rhythm patterns and fruit production origin in the context of healthy and altered dietary habits.

To achieve this general objective, **specific objectives** were proposed:

1. **To evaluate the influence of biological rhythms on the bioavailability and metabolism of dietary (poly)phenols in the context of healthy and altered dietary habits (Chapter 1).**

To this aim, chronic studies were carried out in which grape seed proanthocyanidins extract (GSPE) was administered as a source of (poly)phenols to Fischer 344 rats. A cafeteria diet was used to induce metabolic syndrome. To assess this objective two goals were proposed:

1.1 To elucidate whether GSPE administration time (circadian rhythms) significantly affects the bioavailability and metabolism of phenolic compounds **[Manuscript 1].**

1.2 To evaluate the circannual rhythms effect on the bioavailability of phenolic compounds **[Manuscript 2]**.

2. **To determine if proximity consumption impacts on the bioactivity and bioavailability of phenolic compounds from sweet cherry (Chapter 2).**

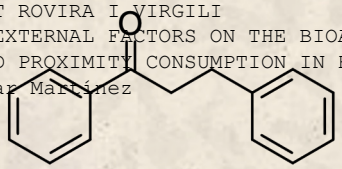
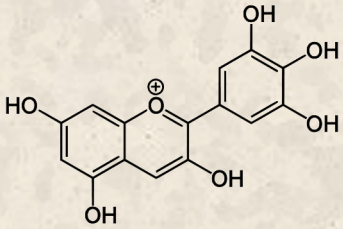
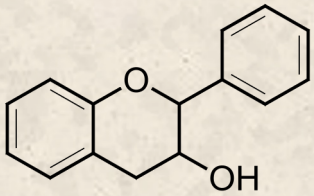
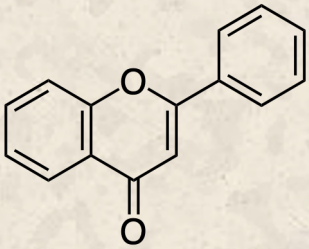
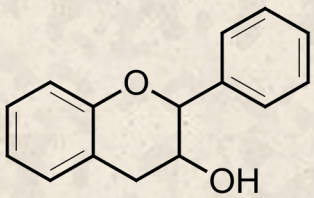
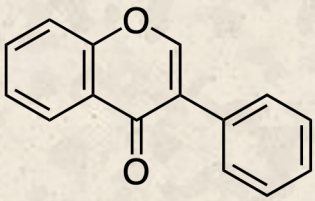
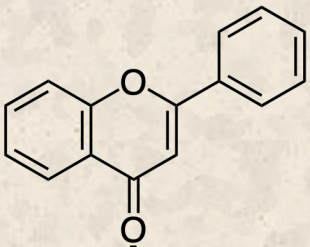
To achieve this aim, two tasks were proposed:

2.1 To characterize the phenolic profile of sweet cherry cv. from two geographical origins of cultivations **[Manuscript 3].**

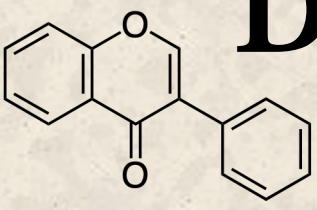
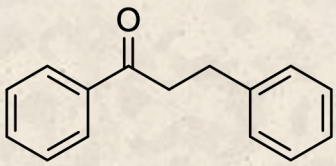
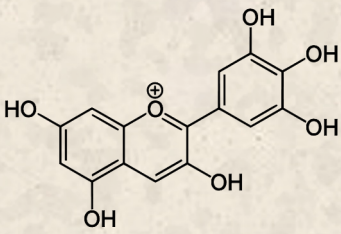
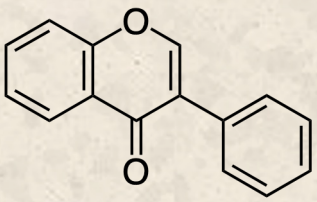
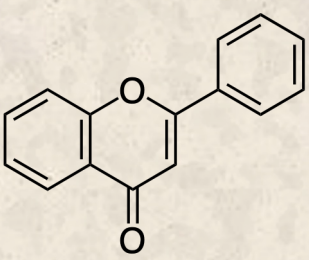
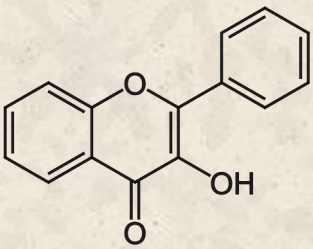
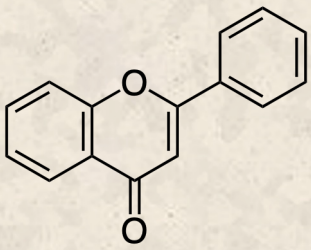
2.2 To evaluate the bioavailability and pharmacokinetic profiles of phenolic compounds after acute consumption of sweet cherry cv. from two geographical origins of cultivation in rats **[Manuscript 3].**

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

EXPERIMENTAL DESIGNS

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Different experimental designs were used to assess the main hypothesis and reach the experimental objectives previously stated in this thesis.

1- Effect of administration time on plasma bioavailability of grape seed proanthocyanidins extract in healthy and obese Fischer 344 rats.

To elucidate whether GSPE administration time (circadian rhythms) significantly affects the bioavailability and metabolism of phenolic compounds [Manuscript 1] (Figure 8). Sixty-four 8-week-old Fischer 344 rats (32 males and 32 females) were randomly divided into eight groups based on treatment time (ZT), sex, and diet (standard and cafeteria). They were fed standard chow diet (ST) or cafeteria diet (CAF) for 9 weeks. During the last 4 weeks, they received a daily dose of GSPE equivalent to 25 mg/kg body weight. The GSPE was administered orally in condensed milk diluted in water. Control animals received only vehicle. Treatments were given at ZT-0 (8 a.m.) or ZT-12 (8 p.m.). Rats were sacrificed 3 hours after the last dose, and plasma samples were collected for chromatographic analysis.

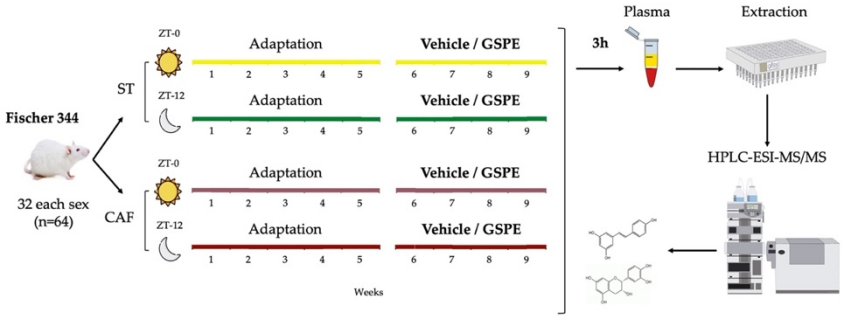


Figure 8. Experimental design for the objective 1.1. CAF, cafeteria diet; GSPE, grape seed proanthocyanidin extract; ST, standard chow diet; ZT-0, GSPE administration time at 8 a.m.; ZT-12, GSPE administration time at 8 p.m.

2- Effect of circannual rhythms on the bioavailability of phenolic compounds from grape seed proanthocyanidins extract differently in healthy and obese Fischer 344 rats

To evaluate the circannual rhythms effect on the bioavailability of phenolic compounds [Manuscript 2] (Figure 9). Ninety-six male Fischer 344 rats (F344), aged thirteen weeks, were obtained from Janvier Laboratories (France) and housed in pairs under standard conditions: temperature of 22 ± 1 ºC, relative humidity of 50-55%, and a 12:12 hour light/dark cycle, with free access to water and a standard chow diet (ST) for one week as an acclimation period. Rats were randomly assigned to twelve groups (n = 8) based on their diet, photoperiod (L6, L12, L18), and treatment (GSPE or vehicle). Throughout the experimental period, rats were fed either the ST diet or CAF, and during the last 4 weeks, they received a daily oral dose of GSPE (25 mg/kg of body weight) diluted in condensed milk and water, while the vehicle group received only the vehicle, both at 8 a.m. Rats were sacrificed 3 hours after the last dose, and serum samples were obtained for chromatographic analysis and stored at -80 ºC.

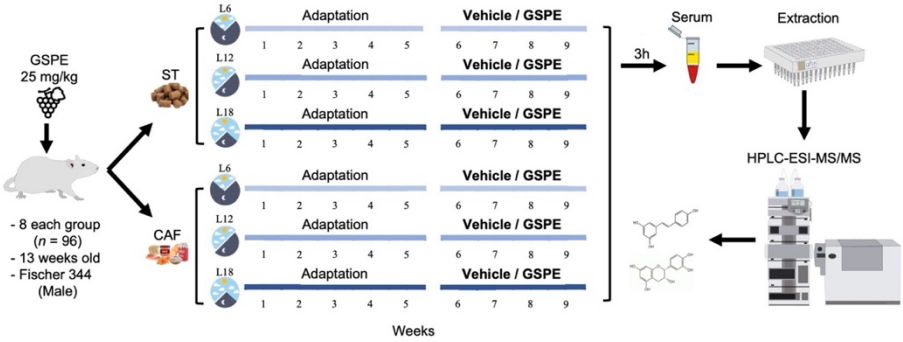


Figure 9. Experimental design for the objective 1.2. ST, standard chow diet; CAF, cafeteria diet; L6, short photoperiod (6 h light/18 h dark); L12, standard photoperiod (12 h light/12 h dark); L18, long photoperiod (18 h light/6 h dark); GSPE, grape seed proanthocyanidins extract.

3. Effect of proximity on bioavailability of (poly)phenols from sweet cherry

To determine if proximity consumption impacts on the bioactivity and bioavailability of phenolic compounds from sweet cherry [Manuscript 3] (Figure 10). Eighteen-week-old male Wistar rats (537 ± 26 g) were obtained from Janvier-Labs (Le Genest Saint Isle, France) and housed in pairs at 22 ºC with a 12-hour light/dark cycle (lights on at 8:00 a.m.), with ad libitum access to tap water and standard chow diet (Safe-A04c, Germany) throughout the experiment. The rats were randomly divided into two groups (n = 7 each): Local and Non-Local cherry (LC and NLC). Both groups received an oral dose of 65 mg gallic acid equivalent (GAE)/kg body weight, determined by the Folin-Ciocalteu method. Before oral administration by intragastric intubation, rats were fasted for 12 hours. Blood samples were collected from the saphenous vein to obtain baseline (0 h) measurements between 9:00 and 9:45 a.m. Additional samples were collected at 2, 4, 7, 24, and 48 hours post-

administration. Serum samples were obtained by centrifugation, pooled to ensure sufficient volume for duplicated chromatographic analyses, and stored at -80 ºC until analysis.

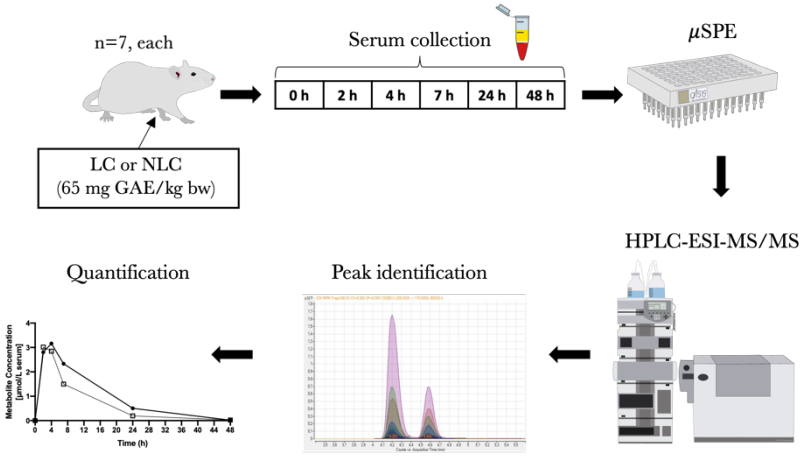
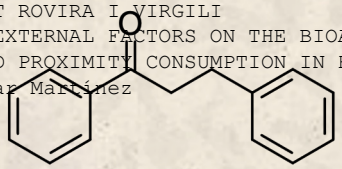
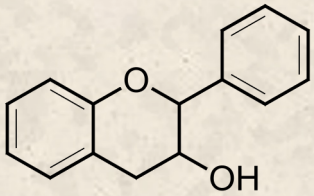
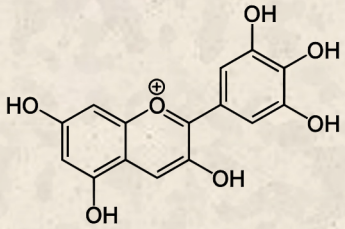
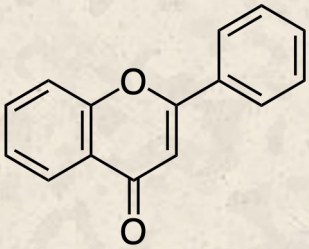
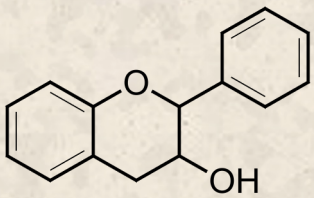
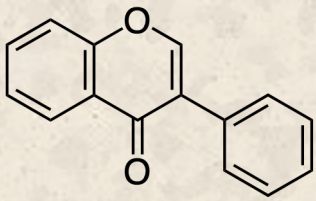
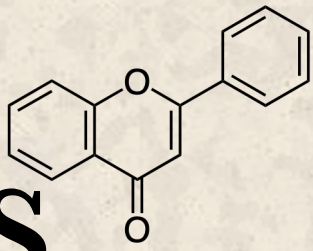


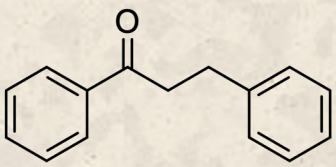
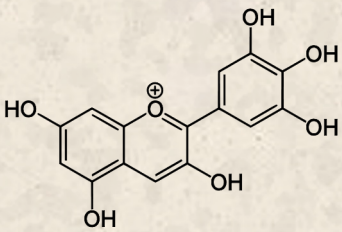
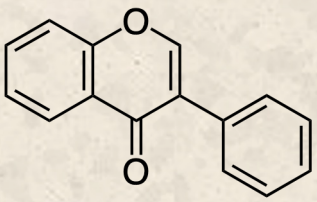
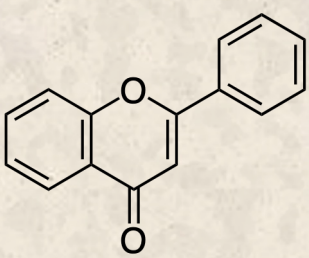
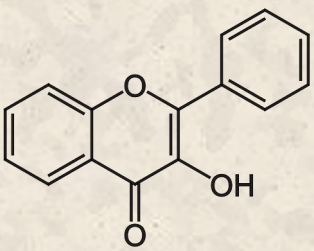
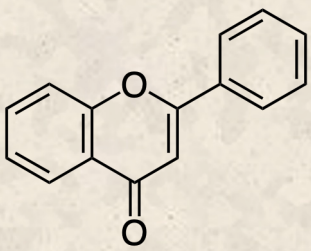
Figure 10. Experimental design for the objectives 2.1 and 2.2. LC, local sweet cherry; NLC, non-local sweet cherry; GAE, gallic acid equivalent; bw, body weight.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

RESULTS

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

CHAPTER 1

To evaluate the influence of biological rhythms on the bioavailability and metabolism of dietary (poly)phenols in the context of healthy and altered dietary habits.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

RESEARCH ARTICLE
Molecular Nutrition & Food Research
www.mnf-journal.com

Administration Time Significantly Affects Plasma Bioavailability of Grape Seed Proanthocyanidins Extract in Healthy and Obese Fischer 344 Rats

Iván Escobar-Martínez, Verónica Arreaza-Gil, Begoña Muguerza, Anna Arola-Arnal, Francisca Isabel Bravo, Cristina Torres-Fuentes, and Manuel Suárez*

Scope: **Phenolic compounds are bioactive molecules that are associated with several health benefits. Metabolization and absorption are the main determinants of their bioavailability and bioactivity. Thus, the study of the factors that modulate these processes, such as sex or diet is essential. Recently, it has been shown that biological rhythms may also play a key role. Hence, the aim of this study is to evaluate if the bioavailability of a grape proanthocyanidin extract (GSPE) is affected by the administration time in an animal model of metabolic syndrome (MetS).**

Methods and Results: **Female and male Fischer 344 rats are fed either a standard or a cafeteria diet (CAF) for 9 weeks, and an oral dose of GSPE (25 mg kg^{-1}) is daily administered either at 8:00 am (zeitgeber time (ZT)-0) or at 8:00 pm (ZT-12) during the last 4 weeks. Plasma phenolic compounds are then quantified by liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). Phase-II and gut microbiota-derived phenolic metabolites are affected by ZT in all conditions or only in obese rats, respectively. CAF feeding affected the bioavailability of phenolic acids and free flavan-3-ols. Differences due to sex are also observed.**

Conclusion: **These findings demonstrate that ZT, diet, and sex are key factors influencing phenolic compounds bioavailability.**

1. Introduction

Phenolic compounds, known as polyphenols when including more than one aromatic ring, are a group of bioactive molecules produced by plants in response to stress.[1] These compounds can be classified into four main groups: phenolic acids, flavonoids, stilbenes, and lignans. This family of compounds is highly distributed in plants, being flavonoids the most consumed. Flavonoids comprise flavonols, flavones, flavan-3-ols, isoflavones, flavanones, and anthocyanidins.[2] Consumption of phenolic compounds has been associated with several health benefits in cardiovascular and metabolic disorders and in some cancers.[1]

It has been estimated that only a small part of the ingested polyphenols is absorbed by the small intestine (5–10%). The rest (90–95%) reach the colon, where they are subjected to extensive microbial metabolism. For example, high-molecular-weight proanthocyanidins are transformed into small phenolic acid microbial derived metabolites.[3] Once absorbed, phenolic compounds and their metabolites are transported through the systemic circulation to the different tissues and organs where they are recognized as xenobiotics and undergo extensive phase II reactions including glucuronidation, sulfation, and/or methylation, all of them catalyzed by the action of uridine 5'-diphospho-glucuronyltransferase (UGTs), sulphotransferases (SULTs), and catechol-*O*-methyltransferase (COMT), respectively.[4] In addition, these metabolites may be transferred back into the intestine through the enterohepatic cycle together. Finally, the metabolites transported via systemic circulation reach the kidneys and are excreted through the urine. Non-absorbed polyphenols are excreted through feces.[4]

Several factors affect the metabolism and bioavailability of these bioactive compounds.[5] Actually, it has been shown that external and internal factors such as the environment,[6] polyphenol structure,[6] age,[7] sex,[8] gut microbiota,[3] and animal strain[9] determine the bioavailability of phenolic compounds.

In addition to these factors, circadian rhythm has emerged as potential key modulator of bioactivity due to its effect on metabolism, this could be caused by changes in bioavailability. Circadian clocks have evolved in each organism so that they can adapt their behavior and physiology to the most appropriate time of day. The central system of the circadian clock depends on a

I. Escobar-Martínez, V. Arreaza-Gil, B. Muguerza, A. Arola-Arnal, F. I. Bravo, C. Torres-Fuentes, M. Suárez
Universitat Rovira i Virgili
Departament de Bioquímica i Biotecnologia
Nutrigenomics Research Group
Tarragona 43007, Spain
E-mail: cristina.torres@urv.cat

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202100552

DOI: 10.1002/mnfr.202100552

central clock located in the hypothalamus and secondary clocks placed in peripheral tissues.[10] The intrinsic circadian clock is based on the oscillation of the transcription and translation of the clock genes. In this regard, it is known that there are certain external signals such as light[11] and food[12] that regulate metabolism by modulating circadian clocks. The term "zeitgeber time" (ZT), which in German means "timer," is used to describe a unit of time based on the period of a zeitgeber, such as the 12:12 light:dark cycle.[13]

Several studies have demonstrated that circadian rhythms can influence the bioactivity of phenolic compounds and, at the same time, the intake of these compounds can modulate biological rhythms.[14] For example grape seed proanthocyanidins extract (GSPE) has been shown to exert properties by modulating the level of melatonin and the expression of Clock genes in the hypothalamus depending on the administration time.[15] This extract is rich in flavonoids including flavan-3-ols such as catechin, epicatechin and their polymeric forms, proanthocyanidins. Furthermore, GSPE contain a wide variety of phenolic acids.[7] In this regard, GSPE has been shown to exert several beneficial effects including, protection against weight gain,[16] decrease in inflammation,[17] restoration of blood pressure, improvement of the diversity of gut microbiota and regulation of circadian rhythms.[18]

Considering all this evidence, we hypothesize that metabolism and absorption of phenolic compounds may be influenced by circadian rhythms. Therefore, the aim of this study was to evaluate if the plasma bioavailability of phenolic compounds from GSPE is affected by the administration time in an animal model of metabolic syndrome (MetS) induced by cafeteria diet feeding.

2. Experimental Section

2.1. Grape Seed Polyphenol Extract (GSPE)

GSPE was obtained from white grape seed and provided by *Les Dérives Résiniques et Terpéniques* (Dax, France). The main phenolic compounds (flavan-3-ols and phenolic acids) present in the extract used for this study were shown in **Table 1**. Nomenclature according to Kay et al.[19]

2.2. Chemicals and Reagents

Acetone, acetonitrile and phosphoric acid (Sigma-Aldrich, Madrid, Spain), glacial acetic acid (Panreac, Barcelona, Spain), and methanol (Scharlab S.L., Barcelona, Spain) were all of HPLC analytical grade. Ultrapure water was obtained from a MilliQ Advantage A10 system (Millipore, Madrid, Spain).

Individual standard stock solutions of 2000 mg L^{-1} (+) -catechin, (−) epicatechin, epigallocatechin gallate (EGCG), 3,4,5-trihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 3-hydroxybenzoic acid, 3″-hydroxyphenylacetic acid, 4″-hydroxyphenylacetic acid, 3″,4″-dihydroxyphenylacetic acid, 3-(4′-hydroxyphenyl)propanoic acid, benzoic acid, hippuric acid, 4'-hydroxy-3'-methoxycinnamic acid and benzene-1,2-diol (internal standard; IS) (all purchased from Fluka/Sigma-Aldrich, Madrid, Spain), and proanthocyanidin B2 (Extrasynthese Lyon, France), were prepared in methanol and stored in dark glass flasks at −20 °C. In addition, a stock solution containing all individual compounds was prepared weekly at a concentration of 20 mg L^{-1} in methanol. This standard solution was diluted to the desired concentration using a solution of acetone: water: acetic acid (70:29.5:0.5, v:v:v) and 40 µL IS at 20 ppm, in order to obtain straight standards at different concentrations of the pure metabolites. This solution was stored in dark glass containers at −20 °C until chromatographic analysis.

Table 1. Main phenolic compounds (flavan-3-ols and phenolic acids) of the grape seed polyphenol extract (GSPE) used in this study, analyzed by HPLC-MS / MS.

Compound	Concentration [mg g^{-g}]
3,4,5-trihydroxybenzoic acid	31.07 ± 0.08
3,4-dihydroxybenzoic acid	1.34 ± 0.02
4-hydroxy-3-methoxybenzoic acid	0.77 ± 0.04
PC dimer B2	33.24 ±1.39
PC dimer B1[a)]	88.80 ± 3.46
PC dimer B3[a)]	46.09 ± 2.07
Catechin	121.32 ± 3.41
Epicatechin	93.44 ± 4.27
Dimer gallate[a)]	8.86 ± 0.14
Epicatechin gallate	21.24 ± 1.08
Epigallocatechin gallate	0.03 ± 0.00
Epigallocatechin[b)]	0.27 ± 0.03
PC trimer[a)]	4.90 ± 0.47
PC tetramer [a)]	0.05 ± 0.01

Adapted from Margalef, Pons, Iglesias-Carres et al. (2017). The results are expressed on a wet basis as the means ± SD ($n = 3$) in mg of phenolic compound per g of GSPE. PC, proanthocyanidin. [a)] Quantified using the calibration curve of proanthocyanidin B2. [b)] Quantified using the calibration curve of epigallocatechin gallate.

2.3. Experimental Procedure in Rats

Sixty-four 8-week-old male ($n = 32$) and female ($n = 32$) Fischer 344 rats (Charles River Laboratories, Barcelona, Spain), weighing 236.36 ± 30.55 and 181.94 ± 19.14 g, respectively, were housed in pairs at 22 °C with ad libitum access to tap water and food under a light/dark cycle of 12 h (light from 8:00 a.m. to 8:00 p.m.). The animals were randomly divided into eight groups according to time of treatment (ZT), sex, and diet (standard and cafeteria) (**Figure 1**).

Standard chow diet (ST) (72% carbohydrates (CH) (being 4% sugars), 8% lipid, and 19% protein; Safe-A04c, Germany) or cafeteria diet (CAF) (55% CH (being 35% sugars), 34% lipid, and 11% protein) were administered for 9 weeks. CAF was a hypercaloric diet, highly palatable, that induced hyperphagia and obesity.[20] This length of CAF administration had been shown to be adequate for a proper development of metabolic syndrome.[21] CAF diet was freshly prepared every day and included the following regular human food products (g per rat): bacon (12–15 g), biscuits with pâté (12 g), biscuits with cheese (14 g), sweet roll (8–10 g), carrots (8–10 g) and sweetened milk (20% sucrose (w/v)), and standard chow diet (10–12 g).

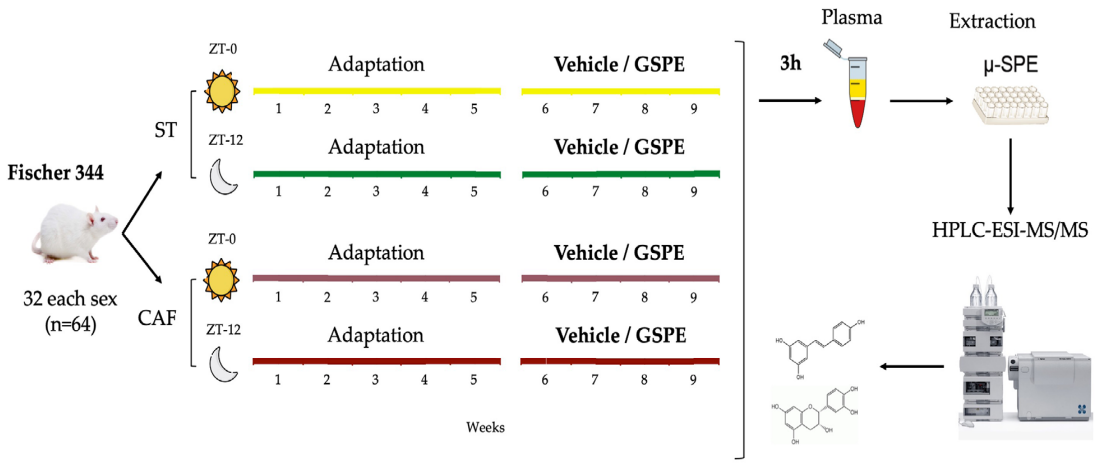


Figure 1. Graphical representation of the experimental design used in this study. CAF, cafeteria diet; GSPE, grape seed proanthocyanidin extract; ST, standard chow diet; ZT-0, GSPE administration time at 8 a.m.; ZT-12, GSPE administration time at 8 p.m. Copyright white laboratory rat: Rosa Jay/Shutterstock.com.

2.3.1. Dosage Information / Dosage Regimen

During the last 4 weeks of the experiment, a daily dose of GSPE equivalent to dietary intake levels (25 mg kg^{-1} of body weight) was orally administered in condensed milk diluted in water (1:4 v:v) by allowing rats to drink it from the tip of a syringe. This dose has been widely used by the group and had been shown to be the lowest most effective dose in modulating many central metabolic pathways in healthy rats.[22] In addition, taking into account a translation of animal to human doses and estimating the daily intake for a 70 kg human,[23] the phenol extract dose of 25 mg kg^{-1} per day corresponds to an intake of approximately 370 mg of phenols per day. This amount of phenolic compounds can be easily achieved in humans with a polyphenol-rich diet. Animals receiving only vehicle were included as controls. Vehicle (condensed milk diluted in water (1:4 v:v)) and GSPE treatments were administrated either at ZT-0 (8 a.m.; light or resting phase) or at ZT-12 (8 p.m.; dark or active phase). The sacrifice of the rats was 3 h after the last dose.

2.3.2. Sacrifice and Plasma Collection

After sacrifice by decapitation, plasma samples were obtained by centrifugation (2000 x *g*, 15 min, 4 °C) in heparinized tubes (Started, Barcelona, Spain) and stored at −80 °C until chromatographic analysis was performed.

All procedures were performed in accordance with the guidelines for the care and use of laboratory animals, and the experimental procedure was approved by the Ethical Committee for Animal Experimentation of the Universitat Rovira i Virgili (reference number 9495 by Generalitat de Catalunya) in accordance with the EU Directive 2010/63/EU.

2.4. Micro-Solid Phase Plasma Phenolic Metabolites Extraction

In order to analyze the content of polyphenols and its derivatives of the plasma samples it was necessary to make an extraction to eliminate possible interferences. For this purpose, the previous developed methodology based on micro solid-phase extraction (μSPE) was used using 30 µm OASIS HLB μ-Elution Plates (Waters, Barcelona, Spain).[24]

Briefly, the micro-cartridges were sequentially conditioned with 250 µL of methanol and 250 µL of 0.2% acetic acid. Plasma samples (250 µL) were mixed with 300 µL 4% H_3PO_4 and 50 µL IS at 20 ppm, and this mixture was loaded into the plates. Subsequently, a washing process was carried out to remove interferences that may have been retained in the plates with 200 µL Milli-Q water and then 200 µL acetic 0.2%. Finally, the samples were eluted twice with 50 µL of acetone: water: acetic acid (70:29.5:0.5, v:v:v). The eluted solutions were directly injected into the chromatography equipment.

2.5. Chromatographic Analysis (HPLC-ESI-MS/MS)

Chromatographic separation of phenolic compounds in µ-SPE eluted solutions was performed using an Agilent 1290 LC Series and Zorbax SB-Aq chromatographic column (150 mm x 21 mm i.d., 3.5 µm particle size, Agilent Technologies Palo Alto, CA, USA). The mobile phase consisted of 0.2% acetic acid in water (solvent A) and 100% acetonitrile (solvent B) with the following elution gradient: initial conditions started at 5% of eluent B and was linearly increased to 55% after 10 min, further increased to 80% B in 2 min. Then, it was kept isocratic for 3 min and back to initial conditions for 1 min. A post run of 10 min was applied for column equilibration. The flow rate was set at 0.4 mL min^{-1} and the injection volume at 2.5 µL. Quantification was performed by coupling the LC system to a 6490 (MS/MS) tandem mass spectrometer (Agilent Technologies) following the method described by Margalef et al.[24] Electrospray ionization (ESI) was performed at 350 °C and 12 L min^{-1} with 45 psi nebulizer gas pressure and 4000 V capillary voltage. The mass spectrometer operated in negative mode and MS/MS data was acquired in "Multiple Reaction Monitoring" (MRM) mode. Optimized MRM conditions for the analysis were performed as previously reported for the quantifica-

tion of phase-II and microbial flavan-3-ols metabolites in plasma and tissues.[24]

2.6. Sample Quantification

For the quantification of the samples, control group blank plasma was spiked with standard compounds at nine different concentrations to obtain calibration curves in both ZT-0 and ZT-12 for each group (CAF and ST). Compounds present at the 0 ppb were subtracted from the plasma concentration at all other concentration-points. Samples were quantified by interpolating the analyte/IS peak abundance ratio in the standards curves. All quality parameters are presented in Tables S1 and S2 (Supporting Information). Data acquisition was performed by using MassHunter Software (Agilent Technologies, Palo Alto, CA, USA).

2.7. Statistics

Statistical analysis was performed using SPSS Software (SPSS Inc., Chicago, IL, USA). The differences among groups were assessed using one- and three-way ANOVA. First, three-way ANOVA was carried out to evaluate the effects of diet, ZT, sex, and their interactions. The results were reported in tables and figures with italic capital letters indicating significant effects of diet (*D*), GSPE administration time (*ZT*), sex (*S*), or their interaction (*DxZT, DxS, ZTxS, DxZTxS*). When one or more main effects were statistically significant, one-way ANOVA was used to determine the differences between the means. When only the interactions were statistically significant according to the three-way ANOVA model, a one-way ANOVA was performed followed by multiple comparisons. The assumption of normality was determined using the Shapiro-Wilk test, and the homoscedasticity between groups was determined using Levene's test. LSD *post hoc* contrast was used when variances between groups were similar, and Tamhane's T2 test was used if this assumption was not fulfilled. Non-normally distributed data were analyzed by non-parametric multiple comparisons Kruskal-Wallis test. All results represent the mean ± SD from N independent experiments (see figure and table legends for statistical details for each case). Principal component analysis (PCA) was performed to evaluate, under a multivariate approach, the influence of different factors on the metabolization of phenolic compounds using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/).

3. Results

The effects of sex and time of administration on phenolic compounds plasma bioavailability were investigated under healthy and obesogenic conditions induced by CAF feeding. To this aim, circulating levels of phenolic compounds were analyzed in plasma samples obtained from ST- and CAF-fed rats of both sexes after chronic administration at different daytime of a proanthocyanidins extract. A three-way analysis of variance was conducted to analyze the main effect of diet (*D*), administration time (*ZT*), sex (*S*) and their interaction on the plasma bioavailability of the main phenolic compounds metabolites (flavan-3-ols and phenolic acids, phase-II and microbial colonic metabolites).

3.1. Flavan-3-ols and Phenolic Acids

Figure 2a and **Table 2** show the quantified phenolic compounds belonging to the group of flavan-3-ols and phenolic acids. Remarkably, although CAF-fed animals presented decreased plasma total levels, a wider variability of this group of compounds was observed. Indeed, all the members of this group of phenolic metabolites were significantly ($p < 0.001$) influenced by diet except for 4-hydroxy-3-methoxybenzoic acid. Catechin, epicatechin, and procyanidin dimers levels were detected only in CAF-fed animals. 3,4,5-trihydroxybenzoic acid was the most abundant phenolic acid, and its concentration was significantly higher in ST-fed animals ($p < 0.001$).

Overall, from a multivariate approach, diet was the main factor affecting the flavan-3-ols and phenolic acids overall profile (F (1, 51) = 1358.307, $p < 0.001$) (Table 2). Indeed, groups clustered differently accordingly to diet when analyzed by PCA (Figure 2b). No significant differences due to sex were observed for any compound in this large group.

3.2. Phase-II Flavan-3-ols Metabolites

Figure 2c and Table 2 show the different metabolites identified and quantified from phase II metabolism. The majority of the compounds of this group were methyl-epicatechin glucuronide, epicatechin glucuronide and catechin glucuronide. A significant overall impact of GSPE administration time (F (1, 51) = 33.326, $p < 0.001$) was observed. Indeed, this factor significantly impacted on the plasma bioavailability of all the analyzed metabolites in this group except for the sulfate forms. Thus, metabolites levels were higher when GSPE was administered at ZT-0 ($p < 0.001$). In addition, diet did also affect the plasma levels of most of these phase II metabolites. Methyl-epicatechin glucuronide ($p < 0.01$) showed higher values in rats fed a cafeteria diet, while catechin glucuronide, methyl-catechin glucuronide, catechin sulphate, methyl-catechin sulphate, and methyl-epicatechin sulphate ($p < 0.001$) were found at higher levels in the plasma from rats fed a standard diet. Epicatechin sulphate levels were not altered by any of the studied factors. When analyzed by PCA, groups clustered together accordingly to administration time and type of diet (Figure 2d). No significant differences due to sex were observed.

3.3. Microbial Colonic Metabolites

Microbiota metabolites were the second largest group of compounds detected in the samples. Figure 2e and Table 2 show all the quantified phenolic compounds belonging to this group. An overall significant effect of GSPE administration time (F (1, 51) = 11.180, $p < 0.01$) as well as a significant interaction effect of diet and administration time (F (1, 51) = 6.133, $p < 0.01$) were observed when analyzing plasma levels of these metabolites. Thus, significant higher overall levels of microbial-derived metabolites were found when GSPE was administered at ZT-12 in CAF-fed rats. This ZT effect was not observed in ST-fed rats. In addition, diet significantly altered the overall levels of these metabolites only when GSPE was administered at ZT-0 (see Figure 2e). Moreover, this group was the only one showing a significant overall

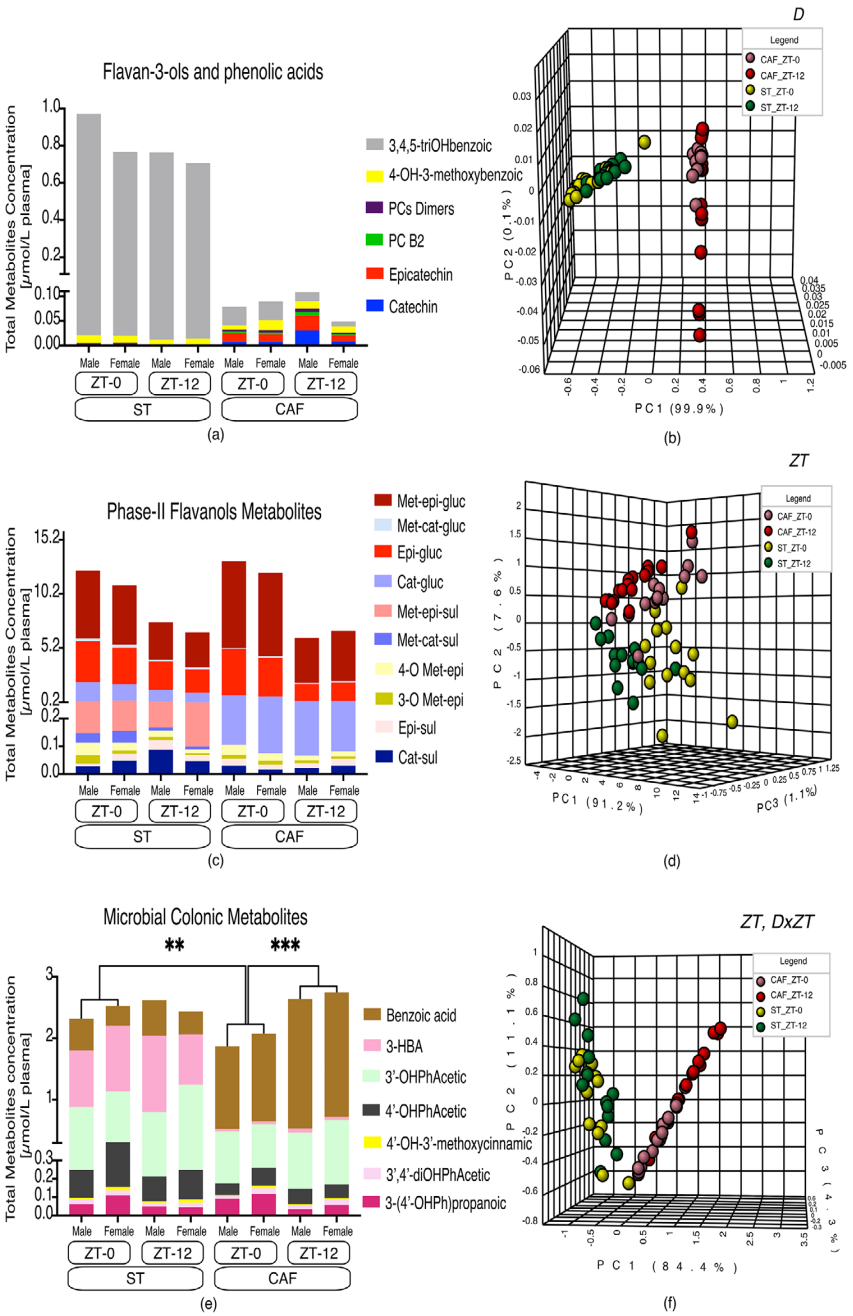


Figure 2. Distribution of phenolic compounds and their derivatives quantified in plasma 3 h after the last dose (4 weeks) of GSPE (25 mg kg^{-1}) by HPLC-ESI-MS/MS in Flavan-3-ols and phenolic acids a), Phase II metabolites c) and microbiota-derived metabolites e). Rats were divided into eight groups, depending on diet (ST and CAF), sex (male and female), and time of administration of GSPE (ZT-0 and ZT-12). Data are presented as means (n = 8). Principal component analysis (PCA) b, d, f) graphs show the statistical effect of diet (*D*), GSPE administration time (*ZT*), sex (*S*) or their interaction (*DxZT, DxS, ZTxS, DxZTxS*). The entire statistical procedure is described in the Section 2.7 Statistics. *Indicates significant effects (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). 3-HBA, 3-hydroxybenzoic acid; cat, catechin; epi, epicatechin; gluc, glucuronide; met, methyl; OH, hydroxyl; OHPh, hydroxyphenyl; PC, Procyanidin; sul, sulfate.

Table 2. Phenolic compounds and their derivatives quantified in plasma (μM) 3 h after the last acute dose for 4 weeks of GSPE (25 mg kg^{-1}) by HPLC-ESI-MS/MS.

Compound	Effect	Standard diet				Cafeteria diet			
		ZT-0		ZT-12		ZT-0		ZT-12	
		Male	Female	Male	Female	Male	Female	Male	Female
Σ Flavan-3-ols and phenolic acids	*D**	0.973 ± 0.033	0.768 ± 0.152	0.764 ± 0.113	0.707 ± 0.040	0.079 ± 0.030	0.089 ± 0.024	0.108 ± 0.051	0.049 ± 0.024
Catechin	*D**	n.d.	n.d.	n.d.	n.d.	0.007 ± 0.001	0.007 ± 0.004	0.031 ± 0.021	0.009 ± 0.006
Epicatechin	*D**	n.d.	n.d.	n.d.	n.d.	0.018 ± 0.008	0.016 ± 0.004	0.029 ± 0.013	0.012 ± 0.007
Procyanidin B2	*D**	0.002 ± 0.001	0.002 ± 0.001	n.q.	n.q.	0.003 ± 0.002	0.003 ± 0.001	0.008 ± 0.005	0.003 ± 0.001
Procyanidin dimers[a]	*D**	0.004 ± 0.001	0.004 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.004 ± 0.001	0.005 ± 0.002	0.007 ± 0.003	0.003 ± 0.001
3,4,5-trihydroxybenzoic acid	*D**	0.952 ± 0.032	0.747 ± 0.153	0.752 ± 0.110	0.693 ± 0.040	0.038 ± 0.022	0.038 ± 0.022	0.019 ± 0.010	0.010 ± 0.005
4-hydroxy-3-methoxybenzoic acid		0.016 ± 0.006	0.015 ± 0.004	0.011 ± 0.005	0.012 ± 0.005	0.009 ± 0.001	0.020 ± 0.010	0.015 ± 0.004	0.013 ± 0.004
Σ Phase-II flavan-3-ols	***ZT*** *	**12.339 ± 5.626**	**11.005 ± 2.383**	**7.599 ± 1.780**	**6.647 ± 3.504**	**13.217 ± 4.262**	**12.134 ± 3.074**	**6.136 ± 2.378**	**6.796 ± 4.378**
Catechin gluc[b]	*ZT**, *D**	1.784± 0.774	1.530 ± 0.0.466	1.119 ± 0.241	0.885 ± 0.572	0.757 ± 0.252	0.660 ± 0.176	0.266 ± 0.120	0.263 ± 0.163
Epicatechin gluc[c]	*ZT**	3.751 ± 2.139	3.336 ± 1.033	2.626 ± 0.763	2.128 ± 1.166	4.254 ± 1.600	3.569 ± 1.304	1.591 ± 0.935	1.690 ± 1.294
Methyl-catechin gluc[b]	*ZT**, *D**	0.256 ± 0.139	0.289 ± 0.049	0.138 ± 0.049	0.194 ± 0.066	0.103 ± 0.037	0.144 ± 0.032	0.065 ± 0.025	0.112 ± 0.044
Methyl-epicatechin gluc[c]	*ZT**, *D**	6.249 ± 2.506	5.483 ± 1.038	3.479 ± 0.702	3.231 ± 1.665	7.999 ± 2.485	7.686 ± 1.652	4.149 ± 1.284	4.649 ± 2.894
Catechin sulphate[b]	*D**	0.027 ± 0.021	0.048 ± 0.013	0.087 ± 0.049	0.046 ± 0.026	0.030 ± 0.013	0.016 ± 0.009	0.022 ± 0.009	0.030 ± 0.023
Epicatechin sulphate[c]		0.009 ± 0.008	0.024 ± 0.006	0.035 ± 0.021	0.022 ± 0.013	0.024 ± 0.010	0.016 ± 0.009	0.016 ± 0.007	0.025 ± 0.017
3-O Methyl epicatechin[c]	*ZT**	0.031 ± 0.017	0.013 ± 0.010	0.011 ± 0.005	0.007 ± 0.003	0.014 ± 0.007	0.015 ± 0.004	0.012 ± 0.006	0.009 ± 0.004
4-O Methyl epicatechin[c]	*ZT**	0.045 ± 0.028	0.028 ± 0.012	0.023 ± 0.011	0.014 ± 0.009	0.036 ± 0.009	0.027 ± 0.006	0.017 ± 0.005	0.018 ± 0.010
Methyl-catechin sulphate[b]	*D**	0.034 ± 0.024	0.043 ± 0.029	0.011 ± 0.006	0.009 ± 0.004	n.d.	n.d.	n.d.	n.d.
Methyl-epicatechin sulphate[c]	*D**	0.153 ± 0.097	0.210 ± 0.107	0.070 ± 0.032	0.111 ± 0.088	n.d.	n.d.	n.d.	n.d.
Σ Microbial metabolism	***ZT*** * ***DxZT*** *	**2.318 ± 0.397#**	**2.521 ± 0.561#**	**2.621 ± 0.547**	**2.433 ± 0.482**	**1.870 ± 0.262$**	**2.075 ± 0.453$**	**2.637 ± 0.382**	**2.747 ± 0.592**
3-(4′-hydroxyphenyl) propanoic acid	*ZT**	0.063 ± 0.020	0.111 ± 0.051	0.051 ± 0.018	0.045 ± 0.019	0.092 ± 0.041	0.118 ± 0.019	0.034 ± 0.009	0.059 ± 0.020
3′,4′-dihydroxyphenylacetic acid	*S**, *SxD**	0.026 ± 0.012	0.027 ± 0.007	0.019 ± 0.008	0.025 ± 0.008	0.014 ± 0.003†	0.032 ± 0.011	0.020 ± 0.005†	0.026 ± 0.004
3′-hydroxyphenylacetic acid	*S**, *D**	0.633 ± 0.137	0.825 ± 0.196	0.582 ± 0.197	0.994 ± 0.224	0.311 ± 0.066	0.343 ± 0.164	0.324 ± 0.094	0.500 ± 0.187
4′-hydroxyphenylacetic acid	*D**	0.152 ± 0.013	0.155 ± 0.047	0.134 ± 0.034	0.160 ± 0.020	0.062 ± 0.031	0.099 ± 0.035	0.082 ± 0.019	0.075 ± 0.019
3-hydroxybenzoic acid	*D**	0.918 ± 0.439	1.067 ± 0.354	1.244 ± 0.370	0.817 ± 0.277	0.045 ± 0.018	0.051 ± 0.016	0.066 ± 0.012	0.055 ± 0.019
Benzoic acid	*D**, *DxZT**	0.518 ± 0.083	0.318 ± 0.054	0.581 ± 0.090	0.373 ± 0.070	1.339 ± 0.229$	1.420 ± 0.409$	2.101 ± 0.346	2.022 ± 0.675
4′-hydroxy-3′-methoxycinnamic acid	*S**	0.009 ± 0.004	0.019 ± 0.006	0.009 ± 0.006	0.020 ± 0.014	0.007 ± 0.002	0.013 ± 0.004	0.008 ± 0.002	0.010 ± 0.007

gluc, glucuronide; n.d., not detected; n.q., not quantified; ZT-0, GSPE administration time at 8 a.m.; ZT-12, GSPE administration time at 8 p.m. [a] Quantified using the calibration curve of procyanidin dimer B2. [b] Quantified using the calibration curve of catechin. [c] Quantified using the calibration curve of epicatechin. The "effect" column represents the statistical results of diet (*D*), GSPE administration time (*ZT*), sex (*S*) or their interaction (*DxZT, DxS, ZTxS, DxZTxS*). #, $ and † indicate D, ZT and S effects respectively by one- and three-way ANOVA followed by LSD or Tamhane's T2 post hoc test, $p < 0.05$. Further details are described in the Section 2.7 Statistics. Results are expressed as μM ± SD ($n = 8$). *Indicates significant effects (*$p < 0.01$, **$p < 0.001$).

impact of sex for some of the analyzed metabolites. When analyzing by PCA, groups clustered together according to diet and, in the case of metabolites from CAF-fed rats, according to ZT (Figure 2f). Therefore, ZT seems to have an effect on microbial-derived metabolites plasma levels only in CAF-fed rats but not in ST-fed rats.

Plasma levels of benzoic acid were significantly increased ($p < 0.001$) by cafeteria diet. In addition, benzoic acid levels were also affected by *ZT* ($p < 0.001$), being higher in ZT-12 compared to ZT-0 in animals fed with cafeteria diet. On the other hand, 3-hydroxybenzoic acid was significantly increased ($p < 0.001$) in rats fed a standard diet. 3′-hydroxyphenylacetic acid was also significantly reduced ($p < 0.001$) in CAF-fed rats compared to ST and was increased in females compared to males ($p < 0.001$). The remaining metabolites described in Table 2 represent a small part of the microbiota-derived metabolite group and are mainly influenced by diet and sex (see Table 2).

4. Discussion

Consumption of phenolic compounds has been associated with several health benefits.[1] However, their bioactivity is dependent on bioavailability, including both metabolization and absorption. Thus, the study of the factors that modulate the bioavailability and metabolism of phenolic compounds, such as the gut microbiota, sex, or diet, is essential.[3,8] In addition to these factors, it has recently been shown that biological rhythms may also play a key role on phenolic compounds bioavailability.[14] However, little is known about the relationships between flavonoids and circadian rhythms. Therefore, the aim of this study was to evaluate if the plasma bioavailability of phenolic compounds from GSPE is affected by the administration time in healthy rats and in a model of metabolic syndrome (MetS) induced by cafeteria diet feeding. Fischer 344 rats were selected because they are characterized by a high degree of sensitivity to circadian rhythms.[9] The administered dose of GSPE (25 mg kg^{-1} BW per day) is equivalent to a human dose of 370 mg per day.[23] Considering that it is estimated that daily intake of total polyphenols in humans can be up to 1 g per day and doses up to 1 g per day GSPE are not associated with detectable adverse effects, this dose of 370 mg per day is appropriate for long-term administrations in humans.[25]

In this chronic study we investigated the main GSPE-derived metabolites, including flavan-3-ols and phenolic acids, phase II metabolites and gut microbiota-derived metabolites at 3 h after the last GSPE administration. Phase II-metabolites were the most abundant while microbiota-derived metabolites were detected at lower levels, corresponding to the remaining in the blood from the chronic administration of GSPE in the previous days. This is in accordance with previous studies that have shown that flavan-3-ols and their phase II metabolites reach their maximum concentration in tissue and plasma during the first hours after consumption,[4,26,27] while colonic microbial metabolites reach the maximum concentration from 7 to 24 h after consumption, in some cases remaining up to 48 h in the blood.[28]

Regarding flavan-3-ols and phenolic acids metabolites, they were detected at higher levels in ST-fed rats compared to CAF-fed rats, especially in the case of 3,4,5-trihydroxybenzoic acid. This may be due to the fact that 3,4,5-trihydroxybenzoic acid may be mainly produced as a result of microbial metabolism since its intestinal absorption is very low.[3] CAF diet is known to alter gut microbiota composition[29] and consequently this can determine a lack of enzymes involved in polyphenols metabolism such as microbial esterases. These enzymes are responsible for the rapid cleavage of the 3,4,5-trihydroxybenzoic acid ester from the galloylated monomeric flavan-3-ols,[30] thus explaining the lower values observed in the animals fed CAF diet. Besides, free sugar rich-foods such as white bread and grape fruit juice has been shown to significantly increase monomeric flavonols bioavailability, specifically epicatechin and catechin.[31] This is in accordance with our results, as we found increased absorption of these phenolic compounds while reduced absorption of galloylated monomeric flavan-3-ols (EGC, ECG, EGCG) was observed. This may translate into a reduction of 3,4,5-trihydroxybenzoic acid in animals with metabolic syndrome.[32] Also, the higher presence of flavan-3-ols in CAF but not in ST may be caused by increased intestinal permeability due to metabolic syndrome.[27] On the other hand, CAF diet is rich in fat which can also have an impact on polyphenols bioavailability. Thus, it has been shown that dietary fats may alter the transit of polyphenols through gastrointestinal tract impacting the absorption of more hydrophobic polyphenols.[33] However, our study is mainly focused on hydrophilic polyphenols, so the fat content has a lower impact on these. This study shows a strong influence of circadian rhythm on the plasma bioavailability of phase-II metabolites. Many biological processes involved in metabolism and absorption of functional food factors follow circadian rhythm.[34] According to the time of the day, metabolization and absorption processes change, being most optimal during the active phase of the organism[35] and reaching the highest levels of most intermediate metabolites. In the case of the rodents, the maximum level of these metabolites is observed at night. In addition, Arola-Arnal et al.[14] described the "timing" of obtaining food as being closely related to metabolism. Examples of this are the rhythmic fluctuations of metabolites such as butyrate in response to circadian rhythms,[36] variation in the expression of hexose carriers SGLT1, GLUT2, and GLUT5[37] or the gastric emptying time of anthocyanins, which was significantly faster when administered in the dark period (active phase) than in the light period (inactive phase).[34] Our results show that the group treated during its resting phase (ZT-0) showed a higher average concentration of total phenolic compounds compared to those treated during the active phase (ZT-12). In the same line Zhang et al[38] described in C57BL/6 mice a higher expression of carriers and phase II enzymes during the light phase, or what is the same in rodents, their resting phase. In this line, Zmrzljak and Rozman observed that mRNA levels of UGTs were maximal during the light time of the day and SULTs during light to dark transition.[39] Similarly, in our study the levels of phase 2-derived metabolites are higher during the day phase. In addition, a higher concentration of glucuronide metabolites is observed compared to sulfated and methylated metabolites, which could be attributed to a higher expression of UGTs at early light hours compared to SULTs and COMTs. Furthermore, these results could indicate that expression of SULTs is not as sensitive to zeitgeber time as in other enzymes. An increase in epicatechin:catechin (EPI:CAT) ratio was

observed for both free and glucuronide forms. In the same way, the absorption rate of four different types of monomeric proanthocyanidins in human at 2–4 h has been shown to have a higher EPI:CAT ratio.[32] This increase in the EPI:CAT could be the consequence of the depolymerization of GSPE into monomeric forms, which is mainly composed of epicatechin units.[26]

On the other hand, it is known that gut microbiota is altered in obesity and related disorders, and that diet is one of the main factors shaping the gut microbiota composition.[29] Moreover, gut bacteria directly affect the metabolism of phenolic compounds and as a result their bioavailability and composition.[3] The results obtained from microbiota-derived metabolites show a variation in the concentration of metabolites such as benzoic acid, 3-hydroxybenzoic acid, and 3′-hydroxyphenylacetic acid according to the type of diet consumed. This may be caused by changes in the relative abundance of different strains in charge of the bacterial β-oxidation to produce hydroxybenzoic acids.[30] Furthermore, we observed an influence of ZT in animals treated with a cafeteria diet. Unpublished results from our group observed differences due to ZT in the ratio of Bacteroidetes/Firmicutes only in animals that have consumed a cafeteria diet. This could explain the difference by ZT found only in CAF group.

Finally, sex effects were only observed for some microbiota-derived metabolites depending on the diet. In addition, no significant intra-group differences in the bioavailability of phenolic compounds were observed in female rats. Therefore, we assume that if there were differences in the estrous cycle this did not impact on polyphenols bioavailability. Previous studies have shown increased phase-II enzymatic activity in male rats compared to female rats. Margalef et al.[8] demonstrated differences in phase-II enzymatic activity at the level of tissues such as the brain and liver, however the total amount of flavan-3-ols and their metabolites in liver tissues were not affected by gender differences. However, those results were obtained in adult rats from a different strain (Wistar) and diet. Several studies such as those published by Margalef et al.[7] and Lee et al.[40] concluded that the age factor plays a key role in sex differences, being the hormonal development one of the main responsible for differences in metabolism between males and females. The age of the animals in this study (8 weeks) may indicate that they are still growing and therefore attenuate the difference between sexes.

This study clearly shows a strong influence of administration time on the plasma bioavailability of phase-II metabolites and gut microbiota-derived phenolic metabolites treated with cafeteria diet. Levels of phase 2-derived metabolites showed a higher average concentration of total polyphenols during the day phase (ZT-0) compared to the active phase (ZT-12). In addition, a higher concentration of glucuronide metabolites was observed compared to sulfated and methylated metabolites. Furthermore, we observed an influence of ZT in an animal model of metabolic syndrome (MetS) induced by cafeteria diet feeding. This could be due to the alteration in the rhythms of both gut microbiota and circadian clock hosts induced by cafeteria diet feeding. Further studies about gut microbiota changes mediated by diet and circadian rhythms are needed in order to elucidate which bacterial taxa are linked to specific polyphenols metabolites. Diet-induced obesity also affected the plasma bioavailability of phenolic acids and free flavan-3-ols. Finally, the levels of some microbiota-derived metabolites were affected by sex.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

Special thanks go to Melina Rojas-Criollo, Niurka Llópiz, Rosa M. Pastor, Pol Herrero and Antoni del Pino for their assistance and technical support. This work was supported by the *Ministerio de Economía, Industria y Competitividad* (AGL2016-77105-R). I.E.,-M. was supported by *Estrategia de Emprendimiento y Empleo Joven* (PEJ2018-002778-A) and the financial contributions of the following entities and programmes: Youth Employment Initiative from the European Social Fund, *Ministerio de Ciencia e Innovación*, *Agencia Estatal de Investigación* and *Universitat Rovira i Virgili*. A.A.-A. and F.I.B. are *Serra Húnter* fellows. V.A.-G. is beneficiary of scholarship from Universitat Rovira i Virgili – Martí I Franquès (2018-PMF-PIPF-35).

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

I.E.-M.: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing – original draft. V.A.-G.: Formal analysis; Investigation. B.M.: Conceptualization; Funding acquisition; Supervision. A.A.-A.: Conceptualization; Funding acquisition; Supervision. F.I.B.: Conceptualization; Funding acquisition; Supervision. C.T.-F.: Conceptualization; Funding acquisition; Supervision; Investigation; Methodology; Writing – Reviewing and Editing. M.S.: Conceptualization; Funding acquisition; Supervision; Investigation; Methodology; Writing – Reviewing and Editing.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

bioavailability, circadian rhythms, GSPE, metabolic syndrome, phenolic compounds

Received: June 10, 2021
Revised: October 7, 2021
Published online: December 26, 2021

[1] D. del Rio, A. Rodriguez-Mateos, J. P. E. Spencer, M. Tognolini, G. Borges, A. Crozier, *Antioxid. Redox Signaling* **2013**, *18*, 1818.
[2] C. Manach, A. Scalbert, C. Morand, C. Rémésy, L. Jiménez, *Am. J. Clin. Nutr.* **2004**, *79*, 727.
[3] T. Ozdal, D. A. Sela, J. Xiao, D. Boyacioglu, F. Chen, E. Capanoglu, *Nutrients* 78, **2016**, *8*.
[4] M. Monagas, M. Urpi-Sarda, F. Sánchez-Patán, R. Llorach, I. Garrido, C. Gómez-Cordovés, C. Andres-Lacueva, B. Bartolomé, *Food Funct.* **2010**, *1*, 233.
[5] M. Porrini, P. Riso, *Nutrition* **2008**, *18*, 647.
[6] M. D'Archivio, C. Filesi, R. Varì, B. Scazzocchio, R. Masella, *Int. J. Mol. Sci.* **2010**, *11*, 1321.

[7] M. Margalef, L. Iglesias-Carres, Z. Pons, F. I. Bravo, B. Muguerza, *A. Arola-Arnal* **2016**, *29*, 90.
[8] M. Margalef, Z. Pons, L. Iglesias-Carres, L. Arola, B. Muguerza, A. Arola-Arnal, *Mol. Nutr. Food Res.* **2016**, *60*, 760.
[9] C. Carere, D. Maestripieri, *Animal Personalities, Behavior, Physiology, and Evolution*, The University of Chicago Press, Chicago and London **2013**.
[10] S. Sahar, P. Sassone-Corsi, *Trends Endocrinol. Metab.* **2012**, *23*, 1.
[11] M. J. Zylka, L. P. Shearman, D. R. Weaver, S. M. Reppert, *Neuron* **1998**, *20*, 1103.
[12] Y. Hamaguchi, Y. Tahara, H. Kuroda, A. Haraguchi, S. Shibata, *Sci. Rep.* **2015**, *5*, 14207.
[13] I. N. Karatsoreos, R. Silver, P. M. Conn, *Conn's Translational Neurosciences*, Academic Press, San Diego, CA **2017**, pp. 599–615.
[14] A. Arola-Arnal, Á. Cruz-Carrión, C. Torres-Fuentes, J. Ávila-Román, G. Aragonès, M. Mulero, F. I. Bravo, B. Muguerza, L. Arola, M. Suárez, *Nutrients* 2602, **2019**, *11*.
[15] A. Ribas-Latre, J. M. Del Bas, L. Baselga-Escudero, E. Casanova, A. Arola-Arnal, M.-J. Salvadó, L. Arola, C. Bladé, *Mol. Nutr. Food Res.* **2015**, *59*, 865.
[16] J. Serrano, À. Casanova-Martí, A. Gual, A. M. Pérez-Vendrell, M. T. Blay, X. Terra, A. Ardévol, M. Pinent, *Eur. J. Nutr.* **2017**, *56*, 1629.
[17] X. Terra, V. Pallarés, A. Ardèvol, C. Bladé, J. Fernández-Larrea, G. Pujadas, J. Salvadó, L. Arola, M. Blay, *J. Nutr. Biochem.* **2011**, *22*, 380.
[18] A. Ribas-Latre, L. Baselga-Escudero, E. Casanova, A. Arola-Arnal, M. J. Salvadó, L. Arola, C. Bladé, *J. Nutr. Biochem.* **2015**, *26*, 112.
[19] C. D. Kay, M. N. Clifford, P. Mena, G. J. McDougall, C. Andres-Lacueva, A. Cassidy, D. Del Rio, N. Kuhnert, C. Manach, G. Pereira-Caro, A. Rodriguez-Mateos, A. Scalbert, F. Tomás-Barberán, G. Williamson, D. S. Wishart, A. Crozier, *Am. J. Clin. Nutr.* **2020**, *112*, 1051.
[20] B. P. Sampey, A. M. Vanhoose, H. M. Winfield, A. J. Freemerman, M. J. Muehlbauer, P. T. Fueger, C. B. Newgard, L. Makowski, *Obesity* **2011**, *19*, 1109.
[21] A. Mas-Capdevila, L. Iglesias-Carres, A. Arola-Arnal, M. Suárez, F. I. Bravo, B. Muguerza, *Food Funct.* **2020**, *11*, 8735.
[22] G. Aragonès, M. Suárez, A. Ardid-Ruiz, M. Vinaixa, M. A. Rodríguez, X. Correig, L. Arola, C. Bladé, *Sci. Rep.* **2016**, *6*, 24977.
[23] S. Reagan-Shaw, M. Nihal, N. Ahmad, *FASEB J.* **2008**, *22*, 659.
[24] M. Margalef, Z. Pons, B. Muguerza, A. Arola-Arnal, *J. Agric. Food Chem.* **2014**, *62*, 7698.
[25] D. Wang, L. Ho, J. Faith, K. Ono, E. M. Janle, P. J. Lachcik, B. R. Cooper, A. H. Jannasch, B. R. D'Arcy, B. A. Williams, M. G. Ferruzzi, S. Levine, W. Zhao, L. Dubner, G. M. Pasinetti, *Mol. Nutr. Food Res.* **2015**, *59*, 1025.
[26] A. Serra, A. Mací, M. P. Romero, J. Valls, C. Bladé, L. Arola, M. J. Motilva, *Br. J. Nutr.* **2010**, *103*, 944.
[27] M. Margalef, Z. Pons, L. Iglesias-Carres, F. I. Bravo, B. Muguerza, A. Arola-Arnal, *Food Chem.* **2017**, *231*, 287.
[28] M. Margalef, Z. Pons, F. I. Bravo, B. Muguerza, A. Arola-Arnal, *J. Funct. Foods* **2015**, *12*, 478.
[29] R. E. Ley, P. J. Turnbaugh, S. Klein, J. I. Gordon, *Nature* **2006**, *444*, 1022.
[30] M. Dueñas, C. Cueva, I. Muñoz-González, A. Jiménez-Girón, F. Sánchez-Patán, C. Santos-Buelga, M. V. Moreno-Arribas, B. Bartolomé, *Antioxidants* **2015**, *4*, 1.
[31] D. D. Schramm, M. Karim, H. R. Schrader, R. R. Holt, N. J. Kirkpatrick, J. A. Polagruto, J. L. Ensunsa, H. H. Schmitz, C. L. Keen, *Life Sci.* **2003**, *73*, 857.
[32] W. Tao, Y. Zhang, X. Shen, Y. Cao, J. Shi, X. Ye, S. Chen, *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 971.
[33] N. Kardum, M. Glibetic, *Adv. Food Nutr. Res.* **2018**, *84*, 103.
[34] T. Tsurusaki, H. Sakakibara, Y. Aoshima, S. Yamazaki, M. Sakono, K. Shimoi, *J. Clin. Biochem. Nutr.* **2013**, *52*, 208.
[35] H. Reinke, G. Asher, *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 227.
[36] L. Zhou, L. Kang, X. Xiao, L. Jia, Q. Zhang, M. Deng, *Int. J. Endocrinol.* **2019**, 5893028.
[37] J. Fatima, C. W. Iqbal, S. G. Houghton, M. S. Kasparek, J. A. Duenes, Y. Zheng, M. G. Sarr, *J. Gastrointest. Surg.* **2009**, *13*, 634.
[38] Y. K. J. Zhang, R. L. Yeager, C. D. Klaassen, *Drug Metab. Dispos.* **2009**, *37*, 106.
[39] U. P. Zmrzljak, D. Rozman, *Chem. Res. Toxicol.* **2012**, *25*, 811.
[40] S. M. Lee, N. Kim, H. Yoon, R. H. Nam, D. H. Lee, *Front. Microbiol.* **2018**, *9*, 2236.

Table S1: HPLC-ESI-MS/MS method quality parameters for the studied phenolic compounds in GSPE in CAF-fed animals

Compound	RT (min)	Calibration curve (ppb)	Determination coefficient (R2)	Working linearity range (ppb)	LOD (ppb)	LOQ (ppb)
ZT-0						
Catechin	4,20	y = 116,66x	0,9906	10-1000	0,739	2,462
Epicatechin	4,57	y = 26,534x	0,9936	2-1000	0,202	0,674
Procyanidin B2	4,38	y = 179,01x	0,9702	10-1000	0,189	0,631
3,4,5-trihydroxybenzoic acid	2,45	y = 335,92x	0,9511	10-1000	0,194	0,645
4-hydroxy-3-methoxybenzoic acid	4,64	y = 199,39x	0,997	2-1000	0,061	0,202
3-(4'-hydroxyphenyl)propanoic acid	5,08	y = 11,5x	0,9621	10-1000	0,573	1,911
3',4'-dihydroxyphenylacetic acid	4,64	y = 53,909x	0,9957	2-1000	0,280	0,935
3'-hydroxyphenylacetic acid	4,10	y = 3,3396x	0,9758	10-1000	0,000	0,000
4'-hydroxyphenylacetic acid	4,30	y = 2,0941x	0,9948	50-1000	3,125	10,417
3-hydroxybenzoic acid	4,50	y = 604,75x	0,9845	2-1000	0,044	0,148
Benzoic acid	5,80	y = 140,08x	0,9678	250-1000	1,022	3,408
4'-hydroxy-3'-methoxycinnamic acid	5,90	y = 188,56x	0,9956	10-1000	0,160	0,532
ZT-12						
Catechin	4,20	y = 76,473x	0,9905	50-1000	0,491	1,638
Epicatechin	4,57	y = 19,045x	0,9799	20-1000	2,045	6,818
Procyanidin B2	4,38	y = 137,39x	0,9968	50-1000	0,456	1,520
3,4,5-trihydroxybenzoic acid	2,45	y = 168,44x	0,9939	10-1000	0,429	1,429
4-hydroxy-3-methoxybenzoic acid	4,64	y = 138,03x	0,9797	50-1000	0,194	0,648
3-(4'-hydroxyphenyl)propanoic acid	5,08	y = 6,88x	0,9949	50-1000	1,293	4,310
3',4'-dihydroxyphenylacetic acid	4,64	y = 38,311x	0,9522	50-1000	1,480	4,932
3'-hydroxyphenylacetic acid	4,10	y = 2,0972x	0,9918	100-1000	0,000	0,000
4'-hydroxyphenylacetic acid	4,30	y = 1,4836x	0,9775	50-1000	0,000	0,000
3-hydroxybenzoic acid	4,50	y = 359,1x	0,9883	20-1000	0,648	2,159
Benzoic acid	5,80	y = 89,482x	0,9932	50-1000	0,319	1,064
4'-hydroxy-3'-methoxycinnamic acid	5,90	y = 157,46x	0,9979	50-1000	0,243	0,810

Table S2: HPLC-ESI-MS/MS method quality parameters for the studied phenolic compounds in GSPE in ST-fed animals

Compound	RT (min)	Calibration curve (ppb)	Determination coefficient (R2)	Working linearity range (ppb)	LOD (ppb)	LOQ (ppb)
ZT-0						
Catechin	4,10	y = 53,422x	0,9985	2-2000	1,368	4,561
Epicatechin	4,50	y = 38,639x	0,9932	2-2000	0,280	0,935
Procyanidin B2	4,30	y = 148,24x	0,9982	2-2000	0,174	0,580
3,4,5-trihydroxybenzoic acid	2,40	y = 171x	0,9987	25-2000	0,196	0,655
4-hydroxy-3-methoxybenzoic acid	4,60	y = 190,15x	0,9986	1-2000	0,205	0,684
3-(4'-hydroxyphenyl)propanoic acid	5,00	y = 53,951x	0,9994	1-2000	0,289	0,964
3',4'-dihydroxyphenylacetic acid	4,60	y = 87,943x	0,9988	1-2000	0,286	0,952
3'-hydroxyphenylacetic acid	4,10	y = 49,079x	0,99	25-2000	1,368	4,561
4'-hydroxyphenylacetic acid	4,30	y = 57,722x	0,9923	25-2000	2,373	7,911
3-hydroxybenzoic acid	4,50	y = 382,8x	0,9983	25-2000	3,576	11,921
Benzoic acid	5,80	y = 91,074x	0,9985	25-2000	2,385	7,949
4'-hydroxy-3'-methoxycinnamic acid	5,90	y = 404,03x	0,9975	2-2000	0,549	1,829
ZT-12						
Catechin	4,10	y = 56,248x	0,9974	10-2000	2,671	8,904
Epicatechin	4,50	y = 38,52x	0,9951	10-2000	2,804	9,346
Procyanidin B2	4,30	y = 143,44x	0,9966	25-2000	4,141	13,804
3,4,5-trihydroxybenzoic acid	2,40	y = 185,36x	0,9721	50-2000	0,439	1,462
4-hydroxy-3-methoxybenzoic acid	4,60	y = 202,11x	0,9992	25-2000	1,575	5,249
3-(4'-hydroxyphenyl)propanoic acid	5,00	y = 57,781x	0,9982	25-2000	4,393	14,644
3',4'-dihydroxyphenylacetic acid	4,60	y = 95,292x	0,9992	25-2000	5,556	18,519
3'-hydroxyphenylacetic acid	4,10	y = 49,283x	0,9739	50-2000	2,243	7,478
4'-hydroxyphenylacetic acid	4,30	y = 64,376x	0,9983	25-2000	1,657	5,523
3-hydroxybenzoic acid	4,50	y = 405,41x	0,9993	10-2000	1,063	3,543
Benzoic acid	5,80	y = 92,383x	0,9953	50-2000	2,182	7,274
4'-hydroxy-3'-methoxycinnamic acid	5,90	y = 420,35x	0,9992	10-2000	1,505	5,016

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Manuscript 2

Objective: To evaluate the circannual rhythms effect on the bioavailability of phenolic compounds

Circannual rhythms affect the bioavailability of phenolic compounds from grape seed proanthocyanidins extract differently in healthy and obese Fischer 344 rats

Iván Escobar-Martínez, Verónica Arreaza-Gil[§], Anna Arola-Arnal, Begoña Muguerza, Miquel Mulero, Manuel Suárez* and Cristina Torres-Fuentes.

Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, 43007 Tarragona, Spain.

Nutrigenomics Research Group, Institut d'Investigació Sanitària Pere Virgili (IISPV), C/ Marcel·lí Domingo 1, 43007 Tarragona, Spain.

Center of Environmental Food and Toxicological Technology (TecnATox). C/ Marcel·lí Domingo 1, 43007 Tarragona, Spain.

[§]Current affiliation: Disease Biomarkers and Molecular Mechanisms Group (DIBIOMEC), Institut d'Investigació Sanitària Pere Virgili (IISPV), C/Dr. Mallafré Guash, 4, 43005 Tarragona, Spain.

*Corresponding author.

Manuscript in preparation for *Journal of Agricultural and Food Chemistry*.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Abstract

(Poly)phenols are plant-derived bioactive molecules that are associated with several health benefits. Therefore, it is essential to study the factors that affect the bioavailability of these phenolic compounds. Recently, circannual rhythms have been identified as one of the factors that may affect the bioavailability of these compounds. Hence, this study evaluates the impact of circannual rhythms on grape seed proanthocyanidins extract (GSPE) bioavailability in healthy and obesogenic contexts. Male Fischer 344 rats, fed standard (ST) or cafeteria (CAF) diets, were housed under different photoperiod conditions (6, 12, or 18 h of light per day) during 9 weeks and an oral dose of GSPE (25 mg/kg) was daily administered for the last 4 weeks. Serum GSPE-derived metabolites were then quantified by HPLC-ESI-MS/MS. A higher bioavailability was observed in rats exposed to a 12-hour photoperiod and fed ST diet. However, this pattern was altered in CAF-fed rats, suggesting an attenuate influence of photoperiod under obesogenic conditions. These findings contribute to a better understanding of the complex relationships between diet, photoperiod, and serum metabolites.

1. Introduction

Phenolic compounds, widely known as (poly)phenols, constitute a group of natural chemical compounds found in vegetables, fruits, grains, and their derived beverages [1,2]. These compounds are synthesised by most plants in response to stress [2]. (Poly)phenols can range from simple molecules to high molecular weight polymers and can be classified into two main groups: flavonoids and non-flavonoids [3]. In turn, flavonoids can be classified into different subclasses based on the degree of oxidation in the heterocyclic ring, including flavan-3-ols, flavonols, flavones, isoflavones, flavanones and anthocyanidins [4]. Non-flavonoids include phenolic acids, lignans, stilbenes, tannins, and xanthones [1]. Consumption of these compounds has been associated with several beneficial effect in cardiovascular, metabolic disorders and some cancers [1,5–8].

In order to understand the full spectrum of benefits offered by (poly)phenols, it is necessary to explore the concept of bioavailability. This term expresses the fraction of ingested nutrient or bioactive compound that reaches the systemic circulation and is ultimately utilized [9,10]. This intricate process of absorption, distribution, metabolization and excretion of (poly)phenolic compounds takes place through a complex net of biological interactions that influence the degree to which these compounds can exert their beneficial effects [9,10].

(Poly)phenols are commonly found in dietary sources as esters, glycosides or polymeric forms that are not directly absorbed [11]. In this regard, it has been estimated that only a small fraction of the consumed polyphenols is absorbed within the small intestine, constituting about 5-10 %. The others, reach the colon, where undergo extensive microbial transformations [12]. After absorption, phenolic compounds and their

resulting metabolites are transported through the systemic circulation to the different tissues and organs. Within these tissues, they are identified as foreign substances, or xenobiotics, and subsequently undergo a series of comprehensive phase-II reactions. These reactions, involving glucuronidation, sulfation, and methylation, are catalyzed by specific enzymes, including uridine 5'-diphospho-glucuronyltransferase (UGTs), sulphotransferases (SULTs), and catechol-*O*-methyltransferase (COMT). Furthermore, these metabolites may undergo re-circulation into the intestine through the enterohepatic cycle. Ultimately, the journey of these metabolites, which are transported via the systemic circulation, are excreted through the kidneys via urine. For (poly)phenols that remain unabsorbed, they are excreted through faeces [12,13].

The bioavailability and metabolism of these bioactive substances are subject to a multitude of factors. In fact, it has been shown that both external and internal elements, such us (poly)phenol structure [14], environmental conditions [14], sex [15], age [16], gut microbiota-composition [12,17], and the specific animal strain [18], affect the bioavailability of phenolic compounds. In addition to these factors, circadian and seasonal rhythms have emerged as a key potential modulators of (poly)phenols bioactivity [19]. This effect of biological rhythms in (poly)phenols bioactivity may be due to alterations in their bioavailability. Indeed, we have recently demonstrated how the time of administration can influence the bioavailability of phenolic compounds in both healthy and obese rats [20]. Moreover, we have also recently shown that exposure to different photoperiods, which mimics different seasons, leads to changes in the bioavailability of these compounds in obese rats and that gut microbiota may be playing an important role [17]. However, the impact of biological rhythms on (poly)phenols bioavailability, especially in the case of

circannual rhythms, has not been sufficiently investigated yet and more studies are needed. Thus, the aim of this study was to evaluate whether circannual rhythms affect serum bioavailability of phenolic compounds differently depending on metabolic status. Hence, we evaluated the bioavailability of (poly)phenols from GSPE in healthy and obese rats.

2. Experimental Section

2.1. Grape seed proanthocyanidins extract (GSPE)

GSPE was obtained from white grape seeds and provided by *Les Dérives Résiniques et Terpéniques* (Dax, France). The main phenolic compounds (flavan-3-ols and phenolic acids) present in the extract are listed in **Table 1.**

Table 1. Main phenolic compounds (flavan-3-ols and phenolic acids) of the grape seed proanthocyanidins extract used in this study, analysed by HPLC-MS/MS.

Phenolic compound	Concentration (mg/g)
3,4-Dihydroxybenzoic acid	1.40 ± 0.25
(+)-Catechin	51.88 ± 5.56
(-)-Epicatechin	62.86 ± 8.32
3,4,5-Trihydroxybenzoic acid	44.66 ± 7.76
Kaempferol-3-O-glucoside	0.50 ± 0.02
Naringenin-7-glucoside	0.64 ± 0.08
p-Coumaric acid	0.09 ± 0.01
Quercetin	0.05 ± 0.01
Quercetin-3-O-galactoside	0.43 ± 0.05
4-Hydroxy-3-methoxybenzoic acid	0.09 ± 0.01
Procyanidin dimer	76.84 ± 15.76
Procyanidin trimer	13.04 ± 0.64
Procyanidin tetramer	5.14 ± 0.28
Dimer gallate	15.22 ± 2.72
Epicatechin gallate	14.24 ± 2.76
Epigallocatechin gallate	0.06 ± 0.01

Adapted from Rodríguez et al. 2022 [21] Concentrations are expressed as mg of compound per gram of fresh extract (means ± standard desviation).

2.2. Chemical and Reagents

The chemical substances and reagents used in the present study have been previously documented by our group [20]. Acetone, acetonitrile, phosphoric acid (Sigma-Aldrich, Madrid, Spain), glacial acetic acid (Panreac, Barcelona, Spain) and methanol (Scharlab S.L., Barcelona, Spain) were all HPLC analytical quality. Ultrapure water was obtained from a MilliQ Advantage A10 system (Millipore, Madrid, Spain).

Individual stock standard solutions were prepared at a concentration of 2,000 mg/L for various compounds including (+)-catechin, epigallocatechin gallate (EGCG), 3,4,5-trihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 3-hydroxybenzoic acid, 3'-hydroxyphenylacetic acid, 3′,4′-dihydroxycinnamic acid, 3-(4'-hydroxyphenyl)propanoic acid, benzoic acid, hippuric acid, 4'-hydroxy-3'-methoxycinnamic acid and benzene-1,2-diol (internal standard; IS) (all purchased from Fluka / Sigma-Aldrich, Madrid, Spain), and proanthocyanidin B2 (Extrasynthese Lyon, France), were prepared using methanol as the solvent and stored in dark glass flasks at -20 °C.

Furthermore, a weekly stock solution containing all the individual compounds was prepared at a concentration of 2,000 ppm in methanol. To establish the calibration curve, the standards were combined in a mixture of acetone/water/acetic acid (70/29.5/0.5, v/v/v). This solution was stored in dark glass containers at -20 °C until chromatographic analysis.

2.3. Experimental design

Ninety-six male Fischer 344 rats (F344) at the age of thirteen weeks were obtained from Janvier Laboratories (France). Rats were housed in pairs

under standard conditions, including a temperature of 22 ± 1 ºC, relative humidity of 50-55 %, and a 12:12 hour light/dark cycle. They had free access to water and a standard chow diet (ST) consisting of 72% carbohydrates, 8% lipids and 19% protein (Safe-A04c, Barcelona, Spain) for one week as an acclimation period.

Rats were weighed and randomly assigned to twelve groups (n = 8) based on their diet (standard and cafeteria), photoperiod (L12, L6, L18) and treatment (GSPE and vehicle) (see **Figure 1**). From then on, rats were fed either ST or cafeteria (CAF) diet throughout the entire experimental period. The CAF diet consisted of highly palatable and energy-dense human foods known to induce hyperphagia and obesity [22,23] and had a composition of 58 % carbohydrates, 31 % lipids, and 11 % protein. The CAF diet was freshly prepared daily and included the following components per rat per day: biscuits with pâté and cheese (15-17 g), bacon (7-10 g), *ensaimada* (pastry) (10-15 g), carrots (11-12 g), standard chow (20-25 g), and milk containing 22 % sucrose (w/v). It has previously been shown that the duration of CAF administration used in this study was suitable for the development of metabolic syndrome [24]. In addition, throughout the experimental procedure, rats were assigned to three different photoperiods for a period of nine weeks: short photoperiod (L6, 6 hours of light and 18 hours of darkness), standard photoperiod (L12, 12 hours of light and 12 hours of darkness), or long photoperiod (L18, 18 hours of light and 6 hours of darkness).

2.4.Dosage Information / Dosage Regimen

During the last 4 weeks of the experiment, rats were administered a daily oral dose of GSPE equivalent to dietary intake levels (25 mg/kg of body weight) dissolved in condensed milk diluted with water (1/4, v/v),

allowing the rats to drink it from the tip of a syringe. This dose has been extensively used by the research group and has been shown to be the lowest and most effective dose in modulating various central metabolic pathways in rats [25]. Furthermore, considering the translation of animal doses to humans and estimating the daily intake for a 70 kg human [26], the dose of 25 mg/kg GSPE corresponds to an intake of approximately 370 mg of phenolics per day. This amount of phenolic compounds can be easily obtained by humans from a diet rich in polyphenols. Control animals received the vehicle only (condensed milk diluted in water, 1/4 v/v). The vehicle and GSPE treatments were administered at 8 a.m. Rats were sacrificed 3 hours after the last dose.

2.5. Sacrifice and Serum Collection

After sacrifice by decapitation, serum samples were obtained from the collected blood in non-heparinised tubes. The blood was incubated at room temperature for 1 hour and then immediately centrifuged at 1,200 x g for 15 minutes to isolate the serum fraction. The obtained serum samples were subsequently stored at -80 ºC until ready for chromatographic analysis, as shown in **Figure 1**. All procedures performed in this study were approved by the Animal Ethics Committee of the University Rovira i Virgili (Tarragona, Spain) and the Generalitat de Catalunya, in accordance with the EU Directive 2010/63/EU on animal experimentation, under reference number 9495.

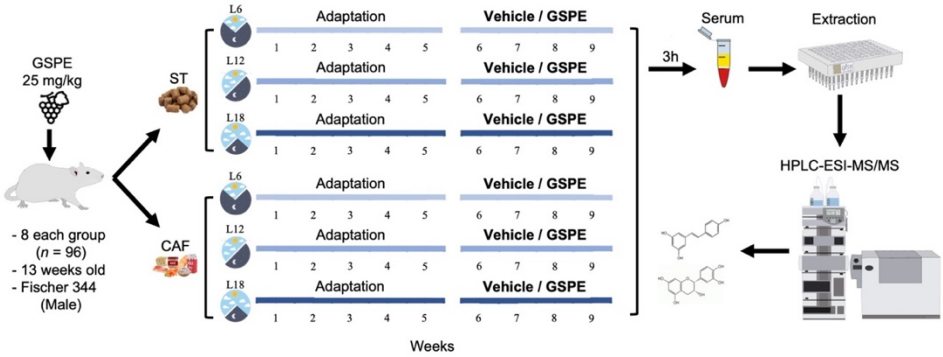


Figure 1. Graphical representation of the experimental design used in this study. 13-weeks-old male Fischer 344 rats were randomly divided into twelve groups (n = 8) based on diet (standard or cafeteria), photoperiod (L12, L6, L18) and treatment (GSPE or vehicle). During the last 4 weeks, the GSPE group received a daily oral dose of GSPE (25 mg/kg) diluted in condensed milk and water, while the vehicle group received only the vehicle. Serum samples were collected by decapitation, and the collected blood was centrifuged to obtain serum, which was stored for further analysis. *Abbreviations*: ST, standard chow diet; CAF, cafeteria diet; L6, short photoperiod (6 h light/18 h dark); L12, standard photoperiod (12 h light/12 h dark); L18, long photoperiod (18 h light/6 h dark); GSPE, grape seed proanthocyanidins extract.

2.6. Micro-solid phase serum phenolic metabolites extraction

Prior to the analysis of phenolic metabolites in serum, the micro-solid phase extraction (μSPE) method was employed to pre-treat the samples. Serum samples were cleaned and concentrated using 30 μm OASIS HLB μ-Elution Plates (Waters, Barcelona, Spain) following the procedures described in our previous research studies [20].

2.7. Chromatographic analysis (HPLC-ESI-MS/MS)

Chromatographic separation of phenolic compounds in μ-SPE eluted solutions was performed using an Agilent 1290 LC Series and a Zorbax SB-Aq chromatographic column (150 mm x 21 mm i.d., 3.5 μm particle

size, Agilent Technologies Palo Alto, CA, USA) at room temperature. The mobile phase consisted of 0.2 % acetic acid in water (solvent A) and 100 % acetonitrile (solvent B). A specific elution gradient was employed: starting with 5 % solvent B, the proportion of solvent B was linearly increased to 55 % over a period of 10 minutes, further increased to 80 % B in 2 minutes, maintained at 80 % B for 3 minutes, and finally returned to 5 % solvent B for 1 minute. Following elution, a post run of 10 minutes was applied for column equilibration. The flow rate was set at 0.4 mL/min, and the injection volume for all runs was 2.5 μL. Mass spectrometry analysis was conducted in negative electrospray (ESI) mode at unit resolution. The electrospray capillary voltage was set to 3,000 V, the source temperature was maintained at 200 ºC and the flow rate was set to 14 L/min with a nebulizer gas pressure of 20 psi. The MS/MS data were acquired in "Multiple Reaction Monitoring" (MRM) mode. Optimised MRM conditions for the analysis were performed as previously reported for the quantification of phase-II and microbial flavan-3-ols metabolites in plasma [17,20].

2.8. Sample quantification

For sample quantification, a calibration curve was constructed by spiking standard compounds into blank serum from the control group at eight different concentrations ranging from 20 to 5,000 ppb. To determine the concentrations of metabolites in the GSPE groups, the concentrations of compounds quantified in the VH group within each photoperiod and diet were subtracted. Sample quantification was achieved by interpolating the analyte/internal standard (IS) peak abundance ratio in the calibration curves. The optimised MRM conditions used in the analysis are given in **Table S1**. To validate the quantitative method, various parameters

including calibration curves, linearity, limit of detection (LOD), limit of quantification (LOQ) and accuracy were assessed (**Table S2**). Standard curves were constructed for each analyte using peak area data and linear least-squares regression was employed to calculate the slope, intercept, and correlation coefficient (R^2), all of which exhibited R^2 values exceeding 0.975. The sensitivity of the analytical method was evaluated by determining the LOD, defined as the concentration corresponding to three times the signal-to-noise ratio, and the LOQ, defined as the concentration corresponding to ten times the signal-to-noise ratio. The detection and quantification limits are detailed in **Table S2**. Data acquisition was performed using MassHunter Software (Agilent Technologies, Palo Alto, CA, USA).

2.9. Statistical analysis

Statistical analyses were conducted using SPSS software (SPSS Inc., Chicago, IL, USA). Differences between groups were examined through one-way and two-way analysis of variance (ANOVA). Initially, the suitability of parametric or non-parametric tests was determined based on the nature of the data. The assessment of normality was conducted using the Shapiro-Wilk test, while Levene's test was employed to evaluate homoscedasticity across groups. Grubbs test was used to check for outliers at a significance level of $\alpha = 0.05$. Two-way ANOVA (for parametric analyses) or Krustal-Wallis (for non-parametric analyses) were carried out to evaluate the effects of diet, photoperiod and their interactions. The results were reported in tables and figures with italic capital letters indicating a significant effect of diet (*D*), photoperiod (*L*) or their interaction (*LxD*). Following the identification of statistically significant main effects or their combinations, further analyses were performed. For

dichotomous variables, T-Student test (parametric) or Mann-Whitney U test (non-parametric) were used. For factors with more than two levels, one-way ANOVA or Kruskal-Wallis test followed by multiple comparisons was employed. *Post hoc* contrasts using BSD (Bonferroni significant difference) method were employed when variances between groups were comparable, whereas the Tamhane's T2 test was used if this assumption was not fulfilled. Results are presented as means with their corresponding standard deviations (SD). Specific statistical tests employed for each analysis are provided in the figure legends. Partial least squares discriminant analysis (PLS-DA) was performed to evaluate, under multivariate approach, the influence of different factors on the metabolization of phenolic compounds using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/).

3. Results

To evaluate whether administration of GSPE under exposure to different photoperiods affects its bioavailability and metabolism in both healthy and diet-induced obesogenic conditions, the levels of circulating phenolic compounds were analysed by HPLC-ESI-MS/MS (**Table 2**). In order to identify differences in concentration due to GSPE consumption, the net increase in phenolic compound concentrations relative to their baseline or dietary levels was calculated. Specifically, the concentration of metabolites in the vehicle-treated groups was subtracted from that in the GSPE-treated groups. The raw concentration data are presented in supplementary material (see **Table S3** and **S4)**. Finally, a multivariate analysis was performed to analyse the main effect of photoperiod (*L*), diet (*D*) and their interaction on the serum bioavailability of the main phenolic metabolites (flavan-3-ols and phenolic acids, phase-II and microbial colonic metabolites).

First, partial least squares discriminant analysis (PLS-DA) was employed as the primary analytical approach, aiding in the identification of differences in total (poly)phenols bioavailability across the treatment groups. The PLS-DA score plot revealed that samples clustered differently depending on the diet, indicating that (poly)phenols bioavailability is different in healthy rats compared to obese rats (**Figure 2A**). In contrast, differences were less evident for the photoperiod factor (**Figure 2B**), although it displayed a lower level of data dispersion under L12 conditions in comparison to L6 or L18. To examine the primary contributors to the separation observed in the PLS-DA components, we compared the metabolic loadings within the Variable Importance in Projection (VIP) for

the respective treatments. **Figure 2C** and **2D** present the top 15 compounds that significantly contributed to separate the treatments.

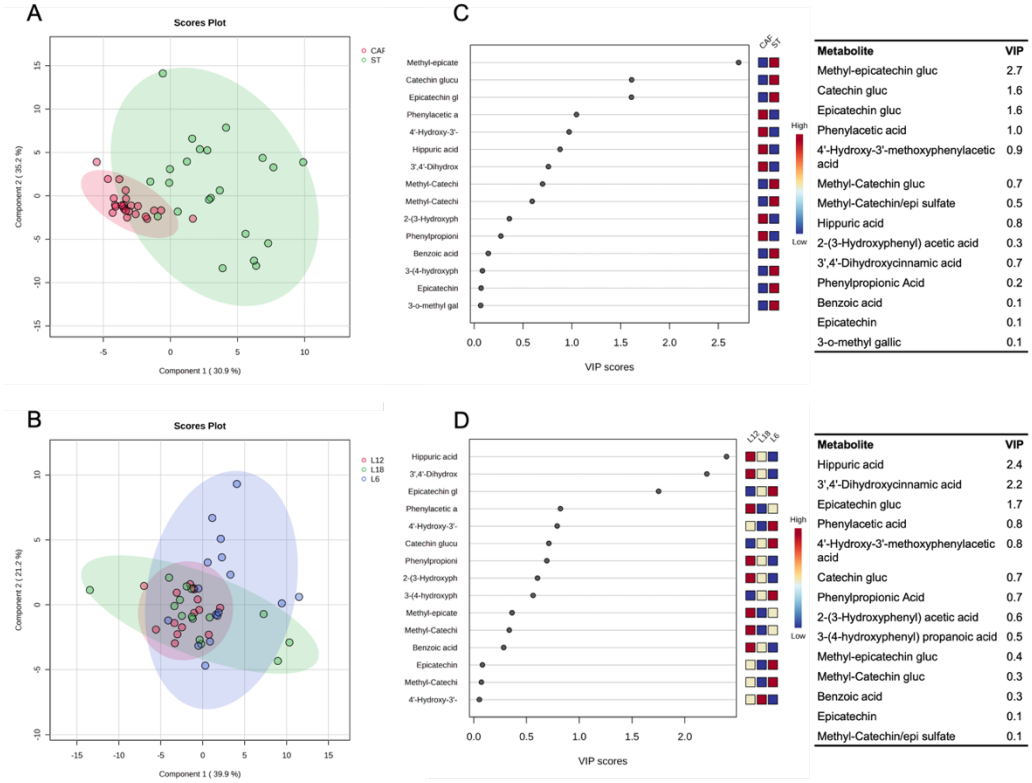


Figure 2. Score plots derived from Partial Least Squares Discriminant Analysis (PLS-DA) (**A** and **B**) and Variable of Importance (VIP) scores (**C** and **D**). In panel **A**, the colour coding distinguishes between diet treatments, with red dots representing the CAF group and green dots signifying the ST group. The X-axis and Y-axis are labelled as the first and second principal components, accounting for 30.9% and 35.2% of the total variation, respectively. Plot **B**, correspond to photoperiod treatment, where red, green, and blue dots correspond to L12, L18, and L6, respectively. Component 1 accounts for 39.9% of the total variation, while Component 2 contributes 21.2%. Tables on the right report the highest VIP values for Component 2 (**C**) and Component 1 (**D**).

3.1. Total polyphenolic compounds

Figure 3A illustrates the total content of identified and quantified polyphenolic compounds. Differences in photoperiod effects were observed depending on the diet. Interestingly, rats fed the ST and housed under L12 conditions had higher levels of metabolites than those housed under L6 and L18 conditions. This photoperiod effect in ST-fed rats was primarily driven by a group of microbiota-derived metabolites that only exceeded baseline levels in L12 conditions. Conversely, this pattern was not observed in the CAF group. Actually, the concentration levels of phenolic compounds in CAF-fed rats housed under L12 conditions were significantly lower than those fed the ST. Additionally, a trend was observed in rats fed with CAF and housed in L6 conditions, as they had higher metabolite levels than those housed in L12 and L18 conditions, which is consistent with our previous study [17]. Finally, among the different groups of metabolites listed in **Table 2**, phase-II derived metabolites were the most abundant, followed by microbiota-derived metabolites. Flavan-3-ols and phenolic acids were the least abundant.

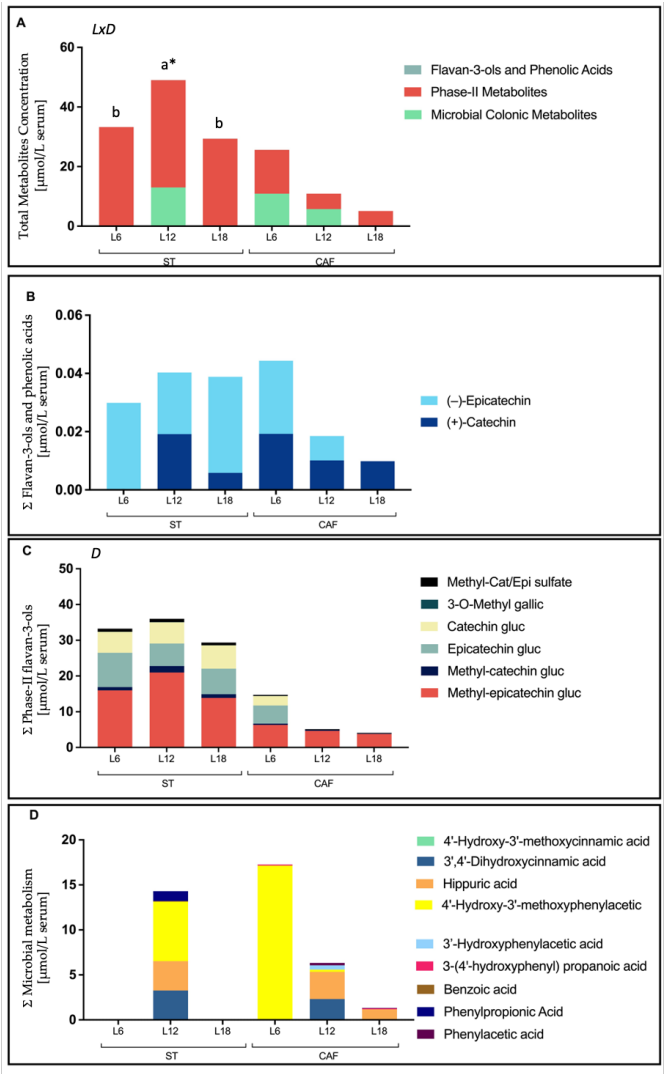


Figure 3. Distribution of quantified phenolic compounds and their derivatives in serum 3 hours after the last dose of GSPE (25 mg/kg) (4 weeks) analysed by HPLC-ESI-MS/MS and with VH levels subtracted. The figure shows the levels of total polyphenolic compounds (A), flavan-3-ols and phenolic acids (B), phase II flavan-3-ols metabolites (C), and microbial colonic metabolites (D). Rats were divided into twelve groups (n=8), according to diet (ST and CAF), photoperiod (L12, L6, L18) and treatment (GSPE or vehicle). Statistical comparisons between the groups were conducted using two- and one-way ANOVA. Graphs show the statistical effect ($p < 0.05$) for diet (D), photoperiod (L),

and their interaction (LxD). Where one-way ANOVA was significant, post hoc tests (BSD or Tamhane's T2) were performed to determine differences between the means. Lowercase letters (a, b, c) represent significant differences in photoperiods for rats fed the ST diet, while uppercase letters (A, B, C) represent significant differences for rats fed the CAF diet. When there was no statistically significant difference between groups, the letters are not shown. * Indicates significant variance between the different diets for the same photoperiod. Abbreviations used are L6 for short photoperiod (6 hours of light / 18 hours of darkness), L12 for standard photoperiod (12 hours of light / 12 hours of darkness), L18 for long photoperiod (18 hours of light / 6 hours of darkness), and glucuronide (gluc).

Table 2. (Poly)phenols and their derivatives quantified in serum (μM) 3 h after the last acute dose for 4 weeks of GSPE (25 mg/kg) by HPLC-ESI-MS/MS.

Compound	Effect	L6 (ST)	L12 (ST)	L18 (ST)	L6 (CAF)	L12 (CAF)	L18 (CAF)
S Flavan-3-ols and phenolic acids		**0.03 ± 0.031**	**0.04 ± 0.022**	**0.039 ± 0.027**	**0.044 ± 0.044**	**0.018 ± 0.007**	**0.01 ± 0.007**
(+)-Catechin	*LxD*	0,004 ± 0,004(b)	0.019 ± 0.004(a)	0.006 ± 0.006(b)	0.019 ± 0.018	0.01 ± 0.006	0.01 ± 0.007
(−)-Epicatechin		0.03 ± 0.03	0.021 ± 0.02	0.033 ± 0.028	0.025 ± 0.028	0.008 ± 0.007	n.q.
Procyanidin dimer B1 [a)]		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Procyanidin dimer B2		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3,4,5-Trihydroxybenzoic acid		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxy-3-methoxybenzoic acid		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S Phase-II flavan-3-ols	**D**	**33.256 ± 17.062**	**36.004 ± 11.42**	**29.344 ± 13.696**	**14.729 ± 9.1**	**5.137 ± 1.991**	**4.105 ± 1.969**
(+)-Catechin gluc [b)]		5.857 ± 4.446	5.917 ± 4.014	6.492 ± 4.275	2.633 ± 2.613	n.d.	n.d.
(−)-Epicatechin gluc [c)]		9.577 ± 8.81	6.294 ± 4.213	7.17 ± 4.162	5.054 ± 5.233	n.d.	n.d.
Methyl-catechin gluc [b)]	*D*	0.944 ± 0.792	1.832 ± 0.862	1.021 ± 1.072	0.37 ± 0.148	0.242 ± 0.196	0.112 ± 0.116
Methyl-epicatechin gluc [c)]	*D*	15.974 ± 5.819	20.978 ± 6.327	13.881 ± 7.222	6.32 ± 1.71	4.648 ± 1.696	3.804 ± 1.839
3-O-methylgallic acid [d)]	*D*	0.034 ± 0.019	0.057 ± 0.029	0.049 ± 0.036	0.037 ± 0.023	0.034 ± 0.026	0.016 ± 0.007
Methyl-cate/epi sulphate [b) c)]	*D*	0.869 ± 0.499	0.925 ± 0.081	0.731 ± 0.453	0.314 ± 0.16	0.214 ± 0.098	0.173 ± 0.104
S Microbial metabolism		**>BL**	**13.002 ± 15.559**	**>BL**	**10.895 ± 21.437**	**5.775 ± 16.349**	**>BL**
Phenylacetic acid [e)]	*D, LxD*	>BL	>BL	>BL	>BL	0.267 ± 1.263	0.054 ± 1.657(*)
3-(4'-hydroxyphenyl) propanoic acid	*L, LxD*	0.023 ± 0.11	>BL	>BL	0.121 ± 0.429(A)	>BL (AB)	0.087 ± 0.172(A)
3',4'-dihydroxyphenylacetic acid		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3'-hydroxyphenylacetic acid	*D, L, LxD*	>BL	>BL	>BL	>BL	0.473 ± 0.432(*)	>BL

4'-hydroxyphenylacetic acid		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4'-Hydroxy-3'-methoxyphenylacetic acid [f]	*LxD*	>BL	6.621 ± 8.388	>BL	17.143 ± 19.256(*A)	0.247 ± 9.192(AB)	>BL(B)
Hippuric acid		>BL	3.257 ± 5.288	>BL	>BL	3.035 ± 7.242	1.136 ± 5.372
3',4'-Dihydroxycinnamic acid		BL	3.262 ± 3.791	BL	BL	2.311 ± 5.697	BL
4'-Hydroxy-3'-methoxycinnamic acid	*D, L, LxD*	BL	0.001 ± 0.021	BL	BL(B)	BL(AB)	0.044 ± 0.059(A)
Benzoic acid		BL	0.067 ± 0.335	BL	BL	BL	BL
3-Hydroxybenzoic acid		n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Phenylpropionic Acid [g]		BL	1.102 ± 1.498	BL	n.d.	n.d.	n.d.

Abbreviations: BL, baseline level; L6, short photoperiod (6 h light / 18 h dark); L12, standard photoperiod (12 h light / 12 h dark); L18, long photoperiod (18 h light / 6 h dark); not detected (n.d.); not quantified (n.q.); glucuronide (gluc). [a] Quantified using the calibration curve of procyanidin dimer B2; [b] Quantified using the calibration curve of catechin; [c] Quantified using the calibration curve of epicatechin; [d] Quantified using the calibration curve of 3,4,5-trihydroxybenzoic acid; [e] Quantified using the calibration curve of 3'-hydroxyphenylacetic acid; [f] Quantified using the calibration curve of 4-Hydroxy-3-methoxybenzoic acid; [g] Quantified using the calibration curve of 3-(4'-hydroxyphenyl) propanoic acid. The entire statistical procedure is described in the Section 2.9 Statistical analysis. "Effect" column represents the statistical results ($p < 0.05$) of diet (*D*), photoperiod (*L*) or their interaction (*LxD*). When interaction was also significant, BSD or Tamhane's T2 *post hoc* test was performed. When comparing the photoperiods in each diet, lowercase letters (a, b, c) indicate significant differences in photoperiods for rats fed the ST diet and uppercase letters (A, B, C) for rats fed the CAF diet. Same letters indicate no significant difference; different letters indicate statistically significant differences. * Indicates significant variance between the different diets for the same photoperiod. Where there was no statistically significant difference between groups, the letters are not shown. Results are expressed as µM ± SD (n=8). The significance level was $p < 0.05$.

3.2. Flavan-3-ols and Phenolic Acids

Figure 3B and **Table 2** show the quantification and identification of different GSPE non-metabolized metabolites (group of flavan-3-ols and phenolic acids) present in serum. Within this group, there was no apparent effect or rhythmicity due to photoperiod variations in rats fed with ST. However, a significant difference was observed in (+)-catechin levels, with higher concentrations in L12 compared to L6 and L18 among rats on the standard diet. In contrast, a significant photoperiod-dependent trend was observed in rats fed a CAF diet, with the highest concentration found under L6 conditions.

Additionally, the correlation heatmap showed a positive correlation (coefficient > 0.5) between phase-II derived metabolites and epicatechin. These findings highlight the importance of (−)-epicatechin in the metabolic profile (**Figure S1A**).

3.3. Phase-II Flavan-3-ols Metabolites

Figure 3C and **Table 2** show the metabolites identified and quantified from phase-II metabolism. This group comprises the highest concentration of bioavailable metabolites in serum. The compounds that significantly contributed the most were methyl-epicatechin glucuronide (2.7 VIP score), (−)-epicatechin glucuronide (1.6 VIP score) and (+)-catechin glucuronide (1.6 VIP score). This large group is clearly influenced by dietary factors, exhibiting significantly higher bioavailability in rats fed the ST diet compared to the CAF diet ($p < 0.001$). Similarly, pattern hunter analysis (**Figure S1B**) identified a positive correlation (>

0.5) among ST consumption and serum bioavailability of these metabolites.

3.4.Microbial Colonic Metabolites

Finally, the bioavailability of microbiota-derived compounds, which is the second-largest group identified in the samples, is shown in **Figure 3D** and **Table 2**. The behaviour of this group is highly diverse, suggesting a complex interplay of factors influencing their production. After subtracting the basal concentrations used as a blank, we identified groups of rats that had concentrations below basal levels, suggesting that both photoperiod and dietary stress cause a change in the metabolic environment of the microbiota. Within the ST-fed group, the L12 photoperiod was found to be the most favourable to produce microbiota-derived metabolites, consistently exceeding basal levels. In contrast, the concentrations were below the basal level in ST-fed rats housed under L6 or L18 conditions. However, rats on the cafeteria diet showed concentrations above basal levels, although each photoperiod exhibited different serum metabolite profiles. In particular, CAF-fed rats housed under L12 conditions showed a similar pattern to that observed in ST diet-fed rats but with a significant reduction in the concentration of these metabolites. Thus, compounds such as 4'-hydroxy-3'-methoxyphenylacetic acid, was significantly higher under L6 photoperiod compared to any of the other groups. All other differences belonging to the concentration of the individual microbiota-derived compounds are shown in **Table 2.**

4. Discussion

Phenolic compounds, referred to as (poly)phenols, are natural chemicals present in various foods, produced by plants in response to stress. Their consumption has been associated with several health benefits, including improvements in cardiovascular health and metabolism [1]. However, to fully understand these benefits, it is crucial to investigate their bioavailability. Various factors, including compound structure, environmental conditions, sex, age, gut microbiota and diet have been shown to affect how these compounds are absorbed and metabolized [12,14–16,18]. Biological rhythms, including circadian and seasonal rhythms, are emerging as important factors affecting the bioactivity of (poly)phenols [19]. Annual cycles, known as circannual or seasonal rhythms, are influenced by the Earth's movement around the sun, resulting in seasonal changes [27]. In this context, exposure to different photoperiods (the number of light hours per day) significantly influences the behaviour, physiology, and metabolism of mammals, leading to changes in physical activity, energy expenditure, and body fat, particularly in regions with pronounced seasons [28,29]. Disruptions in these rhythms are linked to disease development, affecting physiological aspects like plasma estradiol levels, kidney glomerular filtration rate, metabolic rate, and gene expression governing physiological processes, thus potentially influencing polyphenol bioavailability [30,31]. Additionally, fluctuations in light/dark patterns are associated with metabolic disturbances, contributing to the onset of metabolic syndrome [32,33]. This exposure to varied photoperiods has demonstrated effects on serum lipid levels, insulin sensitivity, body mass, and adiposity in both human [34] and animal studies [35–37].

Hence, the present study aimed to evaluate whether circannual rhythms affect serum bioavailability of phenolic compounds differently depending on health conditions. Fischer 344 rats were selected because they are characterised by a high sensitivity to biological rhythms [18]. According to Togo *et al.*, this metabolic response to daylength is evident in this strain which show a particular adaptability to photoperiod variations [38]. Furthermore, our recent findings indicate an effect on bioavailability due to the timing of dose administration in Fischer 344 [20].

In this chronic study, we analyzed the main families of (poly)phenols and their metabolized derivatives, including flavan-3-ols and phenolic acids, phase-II derivatives and microbial colonic metabolites at 3 hours after the last GSPE administration. Phase-II metabolites were the most abundant, followed by microbiota-derived metabolites, with flavan-3-ols and phenolic acids being the least abundant. This aligns with our previous studies suggesting that the highest levels of flavan-3-ols and their phase-II metabolites are achieved during the initial hours after ingestion [20,39,40]. At the same time, microbial metabolites reach their peak concentration between 7 to 24 hours after consumption and can remain in the blood for up to 48 hours in some cases [41].

A multivariate approach was employed to assess the combined influence of diet and photoperiods on the bioavailability of phenolic compounds derived from GSPE consumption. Initially, this chronic study demonstrates how metabolic disruption resulting from the diet contributes to the onset of metabolic syndrome. In the CAF diet-fed rat group, a unification of diversity and a reduction in the bioavailability of total metabolites, flavan-3-ols, phenolic acids and transformed by phase-II, were observed. This phenomenon was not observed in the ST diet-fed rat

groups, where greater bioavailability and variability of phenolic compounds were found. This discrepancy may be attributed to the absence of a unifying element such as the alteration caused by the diet. In contrast, the difference in photoperiod, regardless of diet, did not result in a clear separation between groups in the PLS-DA multivariate analysis.

The interaction of both factors (diet and photoperiod) on the bioavailability of total metabolites was analyzed, concluding that within a "standard" health state (ST), differences in light exposure hours significantly affected the bioavailability of phenolic compounds. Hence, rats fed a standard diet and exposed to a 12-hour photoperiod (L12) exhibited higher levels of total metabolites compared to those under shorter (L6) or longer (L18) photoperiods.

However, CAF group did not exhibit the same pattern. The cafeteria diet, comprising highly palatable and energy-dense foods, appears to alter the relationship between photoperiod and metabolite levels [22]. Thus, in a state of obesity and metabolic disturbance, there is an initial alteration resulting in lower bioavailability, translating to a reduced influence of photoperiods. In the same way as in our previous work, rats fed a CAF diet showed higher bioavailability and variability of total phenolic compounds under the shorter photoperiod or, equivalently, a longer duration of the active phase (L6), compared to other photoperiods (L12, L18) [17]. This finding aligns with Larkin *et al.* work, which demonstrated that rats consume most of their daily food intake during the dark phase [42]. We believe that a longer active phase leads to increased food consumption and, consequently, greater bioavailability of polyphenols from the diet. This aligns with recent results from Soliz-Rueda *et al.*, reporting differences in energy intake in CAF-fed rats depending on the

photoperiod, with a tendency to increase food and energy intake under short light conditions [43]. Notably, this difference in energy intake was mainly attributed to increased carbohydrate intake, possibly linked to higher insulin levels observed in CAF-fed rats under L6 conditions [44].

However, there is a discrepancy in the literature on this topic. Hence, previous studies have linked short photoperiods to alterations in fat content in rodents [38,45,46]. Gibert-Ramos *et al.* evaluated the role of photoperiod on the metabolism of WAT and BAT in Fischer 344 rats and did not observe differences in body weight, only noting a trend of reduced fat in rats fed with an ST on the short photoperiod [35]. This discrepancy persists in the literature, where uncertainties remain regarding the impact of photoperiod on body weight and energy intake. Some studies suggest that changes in body mass may be linked to fluctuations in food intake [38,47], while others, note variations in fat composition without parallel modifications in food consumption [46,48]. Shoemaker *et al.* suggested a complex relationship where decreases in food intake may follow reductions in body weight [49].

Concerning flavan-3-ols and phenolic acids, our data revealed a significant photoperiod-dependent trend in cafeteria diet-fed rats, with the highest concentration found in the L6 photoperiod. This emphasizes the influence of photoperiod in conjunction with dietary stress on the production of flavan-3-ols and phenolic acids. However, this pattern was not evident in rats fed a standard diet, indicating a complex interplay between diet, photoperiod, and the metabolite profiles of these compounds. Interestingly, (+)-catechin levels exhibited significant differences across photoperiods in rats on a standard diet, with higher concentrations in the L12 group. On the other hand, phase-II-derived

metabolites constituted the most abundant group among the analysed compounds, and their levels were significantly influenced by dietary factors, with higher bioavailability observed in rats fed a standard diet compared to those fed a cafeteria diet. The dominance of these metabolites underscores the importance of metabolic pathways involving glucuronidation and other phase-II processes in the bioavailability of phenolic compounds. Notably, we identified a positive correlation between (−)-epicatechin and phase-II metabolites, emphasizing the central role of this compound in the metabolic profile. Additionally, similar to the flavan-3-ols and phenolic acids group, although variations in photoperiod did not produce significant differences in this group, the metabolite bioavailability levels of the rats exposed under L6 conditions and fed CAF seem to stand out from the other photoperiods. These results are consistent with our previous work [17]. Similarly, Iglesias-Carres *et al.* also showed that the bioavailability of grape seed was higher in rats exposed to L6 conditions compared to rats under L18 conditions [50]. This finding reinforces the theory that the diurnal rhythmicity of phase-II enzymes could influence the bioavailability and metabolism of these phenolic compounds, with greater activity during the dark phase when rats are active.

The study found that microbial colonic metabolites exhibited notably diverse behaviour, with evidence of complex interactions between photoperiod and diet [20]. Interestingly, concentrations of these metabolites were below basal levels in several rat groups, suggesting that both photoperiod and dietary stress can alter the metabolic environment of the microbiota [22]. In standard diet-fed rats, the L12 photoperiod appeared to promote the production of microbiota-derived metabolites, consistently exceeding basal levels. In contrast, rats on a cafeteria diet showed

concentrations above basal levels, but with different serum metabolite profiles for each photoperiod. L6, like the others, had the highest concentrations due to increased food intake during the active phase.

In conclusion, this study emphasises how the influence of circannual rhythms on the bioavailability of (poly)phenols varies between different dietary health states. A pronounced effect on bioavailability levels was revealed in rats exposed to a 12-hour photoperiod and fed with a standard diet. However, this pattern was altered in rats fed with cafeteria diet, suggesting an attenuated influence of photoperiod under obesogenic conditions. These findings contribute to a better understanding of the complex relationships between diet, photoperiod, and serum metabolites.

Acknowledgements

This work was supported by MCIN/AEI/10.13039/501100011033/ FEDER "Una manera de hacer Europa" (AGL2016-77105-R). I.E.-M. was supported by the Youth Employment Initiative from the European Social Fund, Ministry of Science, The State Research Agency and Universitat Rovira i Virgili (PEJ2018-002778-A); V.A.-G. was supported by the Martí i Franquès Doctoral Fellowships Programme, Universitat Rovira i Virgili (PMF-PIPF-35). The authors would like to thank Niurka Dariela Llópiz and Rosa Pastor for their assistance.

References

1. Del Rio, D. *et al.* Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal* **18**, 1818–92 (2013).

2. Rana, A., Samtiya, M., Dhewa, T., Mishra, V. & Aluko, R. E. Health benefits of polyphenols: A concise review. *Journal of Food Biochemistry* vol. **46** (2022).

3. Durazzo, A. *et al.* Polyphenols: A concise overview on the chemistry, occurrence, and human health. *Phytotherapy Research* vol. **33** (2019).

4. Shen, N. *et al.* Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chemistry* vol. **383** (2022).

5. Torres-Fuentes, C. *et al.* Cardioprotective Properties of Phenolic Compounds: A Role for Biological Rhythms. *Molecular Nutrition and Food Research* vol. **66** (2022).

6. Chang, Y. C., Yang, M. Y., Chen, S. C. & Wang, C. J. Mulberry leaf polyphenol extract improves obesity by inducing adipocyte apoptosis and inhibiting preadipocyte differentiation and hepatic lipogenesis. *J Funct Foods* **21**, (2016).

7. Shahwan, M., Alhumaydhi, F., Ashraf, G. M., Hasan, P. M. Z. & Shamsi, A. Role of polyphenols in combating Type 2 Diabetes and insulin resistance. *International Journal of Biological Macromolecules* vol. **206** (2022).

8. Ding, S., Xu, S., Fang, J. & Jiang, H. The Protective Effect of Polyphenols for Colorectal Cancer. *Frontiers in Immunology* vol. **11** (2020).

9. Fernández-García, E., Carvajal-Lérida, I. & Pérez-Gálvez, A. In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research* vol. **29** (2009).

10. Angelino, D. *et al.* Bioaccessibility and bioavailability of phenolic compounds in bread: A review. *Food and Function* vol. **8** (2017).

11. Xiao, J. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? *Critical Reviews in Food Science and Nutrition* vol. **57** (2017).

12. Ozdal, T. *et al.* The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrients* vol. **8** (2016).

13. Bohn, T. Dietary factors affecting polyphenol bioavailability. *Nutr Rev* **72**, (2014).

14. D'Archivio, M., Filesi, C., Varì, R., Scazzocchio, B. & Masella, R. Bioavailability of the polyphenols: status and controversies. *Int J Mol Sci* **11**, 1321–42 (2010).

15. Margalef, M. *et al.* Gender-related similarities and differences in the body distribution of grape seed flavanols in rats. *Mol Nutr Food Res* **60**, 760–772 (2016).

16. Margalef, M. *et al.* Age related differences in the plasma kinetics of flavanols in rats. **29**, 90–96 (2016).

17. Arreaza-Gil, V. *et al.* Gut Microbiota Influences the Photoperiod Effects on Proanthocyanidins Bioavailability in Diet-Induced Obese Rats. *Mol Nutr Food Res* **67**, (2023).

18. Carere, C. & Maestripieri, D. *Animal Personalities*. *Animal Personalities* University of Chicago Press, (2013).

19. Arola-Arnal, A. *et al.* Chrononutrition and polyphenols: Roles and diseases. *Nutrients* vol. **11** (2019).

20. Escobar-Martínez, I. *et al.* Administration Time Significantly Affects Plasma Bioavailability of Grape Seed Proanthocyanidins Extract in Healthy and Obese Fischer 344 Rats. *Mol Nutr Food Res* **66**, 2100552 (2022).

21. Rodríguez, R. M. *et al.* Grape-seed proanthocyanidin extract (GSPE) modulates diurnal oscillations of key hepatic metabolic genes and metabolites alleviating hepatic lipid deposition in cafeteria-fed obese rats in a time-of-day-dependent manner. *bioRxiv* (2022)

22. Sampey, B. P. *et al.* Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: Comparison to high-fat diet. *Obesity* **19**, 1109–1117 (2011).

23. Lalanza, J. F. & Snoeren, E. M. S. The cafeteria diet: A standardized protocol and its effects on behavior. *Neuroscience and Biobehavioral Reviews* vol. **122** (2021).

24. Mas-Capdevila, A. *et al.* Changes in arterial blood pressure caused by long-term administration of grape seed proanthocyanidins in

rats with established hypertension. *Food Funct* **11**, 8735–8742 (2020).

25. Aragonès, G. *et al.* Dietary proanthocyanidins boost hepatic NAD + metabolism and SIRT1 expression and activity in a dose-dependent manner in healthy rats. *Sci Rep* **6**, 1–12 (2016).

26. Reagan-Shaw, S., Nihal, M. & Ahmad, N. Dose translation from animal to human studies revisited. *The FASEB Journal* **22**, 659–661 (2008).

27. Oike, H., Oishi, K. & Kobori, M. Nutrients, Clock Genes, and Chrononutrition. *Current Nutrition Reports* vol. **3** (2014).

28. Prendergast, B. J. Internalization of seasonal time. *Horm Behav* **48**, (2005).

29. Korf, H. W. Signaling pathways to and from the hypophysial pars tuberalis, an important center for the control of seasonal rhythms. *General and Comparative Endocrinology* vol. **258** (2018).

30. Liu, J. *et al.* Seasonal Variation Influences on Intestinal Microbiota in Rats. *Curr Microbiol* **75**, (2018).

31. Bailey, M. T., Walton, J. C., Dowd, S. E., Weil, Z. M. & Nelson, R. J. Photoperiod modulates gut bacteria composition in male Siberian hamsters (Phodopus sungorus). *Brain Behav Immun* **24**, (2010).

32. Zimmet, P. *et al.* The Circadian Syndrome: is the Metabolic Syndrome and much more! *Journal of Internal Medicine* vol. **286** (2019).

33. Coskun, A., Zarepour, A. & Zarrabi, A. Physiological Rhythms and Biological Variation of Biomolecules: The Road to Personalized Laboratory Medicine. *International Journal of Molecular Sciences* vol. **24** (2023).

34. Yoshimura, E., Tajiri, E., Hatamoto, Y. & Tanaka, S. Changes in season affect body weight, physical activity, food intake, and sleep in female college students: A preliminary study. *Int J Environ Res Public Health* **17**, (2020).

35. Gibert-Ramos, A., Ibars, M., Salvadó, M. J. & Crescenti, A. Response to the photoperiod in the white and brown adipose tissues of Fischer 344 rats fed a standard or cafeteria diet. *Journal of Nutritional Biochemistry* **70**, (2019).

36. Xie, X. *et al.* Effects of altered photoperiod on circadian clock and lipid metabolism in rats. *Chronobiol Int* **34**, (2017).

37. Navarro-Masip, È. *et al.* Vitis vinifera L. Bioactive Components Modulate Adipose Tissue Metabolic Markers of Healthy Rats in a Photoperiod-Dependent Manner. *Mol Nutr Food Res* **67**, (2023).

38. Togo, Y., Otsuka, T., Goto, M., Furuse, M. & Yasuo, S. Photoperiod regulates dietary preferences and energy metabolism in young developing Fischer 344 rats but not in same-age Wistar rats. *Am J Physiol Endocrinol Metab* **303**, (2012).

39. Monagas, M. *et al.* Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food and Function* vol. **1** 233–253 (2010).

40. Margalef, M. *et al.* Flavanol plasma bioavailability is affected by metabolic syndrome in rats. *Food Chem* **231**, 287–294 (2017).

41. Margalef, M., Pons, Z., Bravo, F. I., Muguerza, B. & Arola-Arnal, A. Plasma kinetics and microbial biotransformation of grape seed flavanols in rats. *J Funct Foods* **12**, 478–488 (2015).

42. Larkin, L. M., Moore, B. J., Stern, J. S. & Horwitz, B. A. Effect of photoperiod on body weight and food intake of obese and lean Zucker rats. *Life Sci* **49**, (1991).

43. Soliz-Rueda, J. R. *et al.* Grape Seed Proanthocyanidins Mitigate the Disturbances Caused by an Abrupt Photoperiod Change in Healthy and Obese Rats. *Nutrients* **14**, (2022).

44. Soliz-Rueda, J. R. *et al.* Metabolism disturbance by light/dark cycle switching depends on the rat health status: the role of grape seed flavanols. *Food Funct* **14**, (2023).

45. Bartness, T. J. Photoperiod, sex, gonadal steroids, and housing density affect body fat in hamsters. *Physiol Behav* **60**, (1996).

46. Kooijman, S. *et al.* Prolonged daily light exposure increases body fat mass through attenuation of brown adipose tissue activity. *Proc Natl Acad Sci U S A* **112**, (2015).

47. Tavolaro, F. M., Thomson, L. M., Ross, A. W., Morgan, P. J. & Helfer, G. Photoperiodic Effects on Seasonal Physiology, Reproductive Status and Hypothalamic Gene Expression in Young Male F344 Rats. *J Neuroendocrinol* **27**, (2015).

48. Boon, P., Visser, H. & Daan, S. Effect of photoperiod on body mass, and daily energy intake and energy expenditure in young rats. *Physiol Behav* **62**, (1997).

49. Shoemaker, M. B. & Heideman, P. D. Reduced body mass, food intake, and testis size in response to shortphotoperiod in adult F344 rats. *BMC Physiol* **2**, (2002).

50. Iglesias-Carres, L. *et al.* Exposure of Fischer 344 rats to distinct photoperiods influences the bioavailability of red grape polyphenols. *J Photochem Photobiol B* **199**, (2019).

Table S1. Retention Times, Exact Mass, and Optimized MRM conditions

Compound	MW	RT (min)	MS/MS quantification		MS/MS confirmation	
			MRM_1	CE (V)	MRM_2	CE(V)
(+)-Catechin	290.27	4.8	289.1 > 203	12	289.1 > 245	10
Procyanidin dimer B2	578.52	4.9	577.5 > 407	24	577.5 > 425	16
3,4,5-Trihydroxybenzoic acid	170.12	2.7	169 > 125	12	169 > 79	28
4-Hydroxy-3-methoxybenzoic acid	168.15	3.8	167 > 123	12	167 >108	20
3-(4'-hydroxyphenyl) propanoic acid	182.17	5.6	181 > 163	10	181 > 134	20
3'-hydroxyphenylacetic acid	152.15	2.5	151 > 107	12	151 > 93.1	20
Hippuric acid	179.17	4.2	178 > 134	8	178 > 132	16
3',4'-Dihydroxycinnamic acid	180.16	4.2	179 > 135	16	179 > 107	24
4'-Hydroxy-3'-methoxycinnamic acid	194.18	6.5	193 > 193	0	193 > 175	10
Benzoic acid	122.12	6.3	121 > 77	8	121 > 59	4
3-Hydroxybenzoic acid	138.12	4.6	137 > 65	36	137 > 93	20

All compounds were analyzed in negative ion mode. Fragmentor used in all compounds was 380 V. *Abbreviations*: MW, Molecular weight; RT, retention time; MRM, Multiple Reaction Monitoring; CE, collision energy; V, Volts.

Table S3. HPLC-ESI-MS/MS method quality parameters for the different phenolic compounds in sweet cherry extracts

Compound	Calibration curve	R^2	Linearity (ppb)	LOD (ppb)	LOQ (ppb)
(+)-Catechin	y = 41.98x	0.991	23-5750	1.812	6.039
Procyanidin dimer B2	y = 32.21x	0.997	15-3750	0.445	1.482
3,4,5-Trihydroxybenzoic acid	y = 331.87x	0.998	28-3500	1.316	4.386
4-Hydroxy-3-methoxybenzoic acid	y = 16.01x	0.997	22-2750	24.665	82.217
3-(4'-hydroxyphenyl) propanoic acid	y = 19.03x	0.996	20-2500	2.132	7.106
3'-hydroxyphenylacetic acid	y = 3.01x	0.975	25-3125	21.33	71.101
Hippuric acid	y = 163.28x	0.998	27-3375	2.635	8.782
3',4'-Dihydroxycinnamic acid	y = 7.39x	0.995	20-2500	33.574	111.912
4'-Hydroxy-3'-methoxycinnamic acid	y = 148.6x	0.997	22-2750	1.037	3.457
Benzoic acid	y = 53.60x	0.986	22-2750	1.703	5.678
3-Hydroxybenzoic acid	y = 253.87x	0.998	40-5000	12.275	40.917

Abbreviations: RT, retention time; R^2, determination coefficient; LOD, limit of detection; LOQ, limit of quantification.

Table S3. Phenolic compounds and their derivatives quantified in serum (μM) 3 h after the last acute dose for 4 weeks of GSPE (25 mg/kg) by HPLC-ESI-MS / MS.

VH Compound	Standard Diet (ST)			Cafeteria Diet (CAF)		
	L6	L12	L18	L6	L12	L18
Σ Flavan-3-ols and phenolic acids	**0.01 ± 0.001**	**n.d.**	**0.007 ± 0.004**	**n.d.**	**n.d.**	**n.d.**
(+)-Catechin	0.01 ± 0.001	n.q.	0.007 ± 0.004	n.q.	n.q.	n.d.
(−)-Epicatechin	n.d.	n.d.	n.d.	n.q.	n.q.	n.q.
Procyanidin dimer B1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Procyanidin dimer B2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3,4,5-Trihydroxybenzoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxy-3-methoxybenzoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Phase-II flavan-3-ols	**1.658 ± 0.708**	**1.981 ± 0.73**	**3.244 ± 1.687**	**0.2 ± 0.131**	**0.18 ± 0.071**	**0.528 ± 0.22**
(+)-Catechin gluc [b]	0.419 ± 0.301	0.538 ± 0.405	0.923 ± 0.533	n.d.	n.d.	n.q.
(−)-Epicatechin gluc [c]	n.d.	n.q.	n.q.	n.d.	n.d.	n.d.
Methyl-catechin gluc [b]	0.294 ± 0.169	0.185 ± 0.074	0.882 ± 0.854	0.014 ± 0.018	0.035 ± 0.044	0.097 ± 0.06
Methyl-epicatechin gluc [c]	0.941 ± 0.38	1.25 ± 0.444	1.433 ± 0.556	0.184 ± 0.117	0.143 ± 0.055	0.428 ± 0.211
3-*O*-methylgallic acid	0.004 ± 0.003	0.007 ± 0.004	0.005 ± 0.002	0.002 ± 0.001	0.002 ± 0.003	0.002 ± 0.001
Methyl-cate/epi sulphate [b,c]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Microbial metabolism	**128.837 ± 11.918**	**120.207 ± 34.718**	**130.577 ± 20.502**	**62.794 ± 12.04**	**72.762 ± 9.865**	**64.811 ± 10.923**
Phenylacetic acid	11.525 ± 4.409	12.363 ± 3.272	9.815 ± 5.103	4.432 ± 1.631	4.789 ± 1.542	3.145 ± 1.437
3-(4'-hydroxyphenyl) propanoic acid	0.093 ± 0.041	0.184 ± 0.087	0.305 ± 0.238	0.244 ± 0.243	0.536 ± 0.392	0.113 ± 0.174
3',4'-dihydroxyphenylacetic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

3'-hydroxyphenylacetic acid	2.155 ± 0.683	2.375 ± 0.461	2.004 ± 0.862	0.877 ± 0.397	0.811 ± 0.153	0.946 ± 0.465
4'-hydroxyphenylacetic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4'-Hydroxy-3'-methoxyphenylacetic acid	47.077 ± 9.904	45.502 ± 3.807	47.552 ± 9.1	44.138 ± 7.192	55.968 ± 8.546	46.411 ± 6.753
Hippuric acid	33.913 ± 4.955	29.408 ± 16.281	34.055 ± 14.896	6.59 ± 3.497	5.035 ± 2.408	6.648 ± 2.262
3',4'-Dihydroxycinnamic acid	27.244 ± 4.918	23.324 ± 13.072	28.443 ± 12.353	5.02 ± 2.594	3.961 ± 1.815	6.237 ± 3.379
4'-Hydroxy-3'-methoxycinnamic acid	0.031 ± 0.011	0.038 ± 0.013	0.042 ± 0.012	0.016 ± 0.011	0.012 ± 0.003	0.014 ± 0.007
Benzoic acid	1.433 ± 0.134	1.428 ± 0.399	1.407 ± 0.192	1.476 ± 0.714	1.65 ± 0.461	1.299 ± 0.4
3-Hydroxybenzoic acid	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Phenylpropionic Acid	5.367 ± 1.015	5.584 ± 2.796	6.955 ± 3.293	n.d.	n.d.	n.d.

Abbreviations: L6, short photoperiod (6 h light / 18 h dark); L12, standard photoperiod (12 h light / 12 h dark); L18, long photoperiod (18 h light / 6 h dark); not detected (n.d.); not quantified (n.q.); glucuronide (gluc). [a)] Quantified using the calibration curve of procyanidin dimer B2; [b)] Quantified using the calibration curve of catechin; [c)] Quantified using the calibration curve of epicatechin; [d)] Quantified using the calibration curve of 3,4,5-trihydroxybenzoic acid; [e)] Quantified using the calibration curve of 3'-hydroxyphenylacetic acid; [f)] Quantified using the calibration curve of 4-Hydroxy-3-methoxybenzoic acid; [g)] Quantified using the calibration curve of 3-(4'-hydroxyphenyl) propanoic acid. Results are expressed as μM ± SD (n=8). The significance level was $p < 0.05$.

Table S4. Phenolic compounds and their derivatives quantified in serum (μM) 3 h after the last acute dose for 4 weeks of GSPE (25 mg/kg) by HPLC-ESI-MS / MS.

GSPE Compound	Standard Diet (ST)			Cafeteria Diet (CAF)		
	L6	L12	L18	L6	L12	L18
Σ Flavan-3-ols and phenolic acids	**0.04 ± 0.031**	**0.04 ± 0.022**	**0.046 ± 0.027**	**0.044 ± 0.044**	**0.018 ± 0.007**	**0.01 ± 0.007**
(+)-Catechin	0.01 ± 0.004	0.019 ± 0.004	0.013 ± 0.006	0.019 ± 0.018	0.01 ± 0.006	0.01 ± 0.007
(−)-Epicatechin	0.03 ± 0.03	0.021 ± 0.02	0.033 ± 0.028	0.025 ± 0.028	0.008 ± 0.007	n.q.
Procyanidin dimer B1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Procyanidin dimer B2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3,4,5-Trihydroxybenzoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxy-3-methoxybenzoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Phase-II flavan-3-ols	**34.914 ± 17.062**	**37.985 ± 11.42**	**32.588 ± 13.696**	**14.93 ± 9.1**	**5.317 ± 1.991**	**4.632 ± 1.969**
(+)-Catechin gluc [b]	6.276 ± 4.446	6.455 ± 4.014	7.415 ± 4.275	2.633 ± 2.613	n.d.	n.d.
(−)-Epicatechin gluc [c]	9.577 ± 8.81	6.294 ± 4.213	7.17 ± 4.162	5.054 ± 5.233	n.d.	n.d.
Methyl-catechin gluc [b]	1.238 ± 0.792	2.017 ± 0.862	1.903 ± 1.072	0.384 ± 0.148	0.277 ± 0.196	0.209 ± 0.116
Methyl-epicatechin gluc [c]	16.915 ± 5.819	22.229 ± 6.327	15.315 ± 7.222	6.505 ± 1.71	4.79 ± 1.696	4.232 ± 1.839
3-*O*-methylgallic acid	0.039 ± 0.019	0.065 ± 0.029	0.054 ± 0.036	0.039 ± 0.023	0.036 ± 0.026	0.019 ± 0.007
Methyl-cate/epi sulphate [b,c]	0.869 ± 0.499	0.925 ± 0.081	0.731 ± 0.453	0.314 ± 0.16	0.214 ± 0.098	0.173 ± 0.104
Σ Microbial metabolism	**106.633 ± 22.81**	**133.209 ± 15.559**	**116.833 ± 52.279**	**73.689 ± 21.437**	**78.536 ± 16.349**	**62.372 ± 17.852**
Phenylacetic acid	8.965 ± 2.066	11.294 ± 1.263	6.349 ± 1.534	3.866 ± 0.73	5.056 ± 1.263	3.199 ± 1.657
3-(4'-hydroxyphenyl) propanoic acid	0.116 ± 0.11	0.137 ± 0.131	0.223 ± 0.103	0.365 ± 0.429	0.138 ± 0.141	0.2 ± 0.172
3',4'-dihydroxyphenylacetic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3'-hydroxyphenylacetic acid	1.748 ± 0.395	2.185 ± 0.478	1.699 ± 0.537	0.752 ± 0.189	1.285 ± 0.432	0.844 ± 0.122

4'-hydroxyphenylacetic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4'-Hydroxy-3'-methoxyphenylacetic acid	42.011 ± 6.847	52.123 ± 8.388	43.688 ± 8.481	61.282 ± 19.256	56.215 ± 9.192	43.122 ± 9.255
Hippuric acid	26.886 ± 11.156	32.665 ± 5.288	30.841 ± 22.21	3.525 ± 1.755	8.07 ± 7.242	7.783 ± 5.372
3',4'-Dihydroxycinnamic acid	21.369 ± 8.74	26.585 ± 3.791	26.264 ± 18.902	2.675 ± 1.398	6.273 ± 5.697	5.991 ± 4.19
4'-Hydroxy-3'-methoxycinnamic acid	0.018 ± 0.006	0.038 ± 0.021	0.037 ± 0.019	0.008 ± 0.005	0.009 ± 0.006	0.058 ± 0.059
Benzoic acid	1.261 ± 0.269	1.495 ± 0.335	1.218 ± 0.321	1.216 ± 0.139	1.49 ± 0.412	1.176 ± 0.244
3-Hydroxybenzoic acid	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
Phenylpropionic Acid	4.259 ± 2.035	6.686 ± 1.498	6.514 ± 5.062	n.d.	n.d.	n.d.

Abbreviations: L6, short photoperiod (6 h light / 18 h dark); L12, standard photoperiod (12 h light / 12 h dark); L18, long photoperiod (18 h light / 6 h dark); not detected (n.d.); not quantified (n.q.); glucuronide (gluc). [a)] Quantified using the calibration curve of procyanidin dimer B2; [b)] Quantified using the calibration curve of catechin; [c)] Quantified using the calibration curve of epicatechin; [d)] Quantified using the calibration curve of 3,4,5-trihydroxybenzoic acid; [e)] Quantified using the calibration curve of 3'-hydroxyphenylacetic acid; [f)] Quantified using the calibration curve of 4-Hydroxy-3-methoxybenzoic acid; [g)] Quantified using the calibration curve of 3-(4'-hydroxyphenyl) propanoic acid. Results are expressed as µM ± SD (n=8). The significance level was $p < 0.05$.

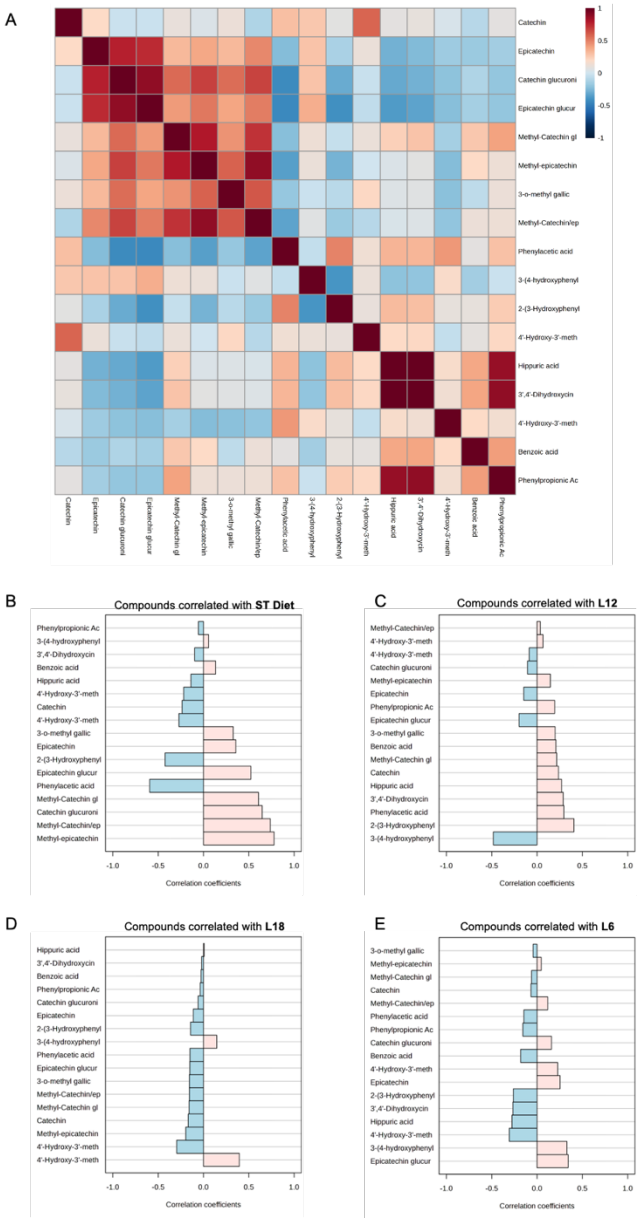


Figure S1. Correlation Analysis of Serum Metabolites from GSPE Consumption with Photoperiod and Diet Factors. (**A**) A correlation heatmap displays Pearson r coefficients representing associations between various metabolites, with red indicating positive correlations and blue indicating negative ones. (**B, C, D, E**) Correlation analyses, conducted using Pattern Hunter (Pearson r), between diet groups and photoperiod conditions, are visualized in these panels. In each panel, the color scheme remains consistent, with red indicating positive correlations and blue representing negative associations.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

CHAPTER 2

To determine if proximity consumption impacts on the bioactivity and bioavailability of phenolic compounds from sweet cherry

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Manuscript 3

Objective: To characterize the phenolic profile of sweet cherry cultivars from two geographical origins and evaluate their bioavailability and pharmacokinetic profiles in rats after acute consumption.

Impact of proximity on bioavailability of (poly)phenols from sweet cherry

Iván Escobar-Martínez, Verónica Arreaza-Gil[§], Gerard Aragonès, Miquel Mulero, Anna Arola-Arnal, Cristina Torres-Fuentes* and Manuel Suárez

Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, 43007 Tarragona, Spain.

Nutrigenomics Research Group, Institut d'Investigació Sanitària Pere Virgili (IISPV), C/ Marcel·lí Domingo 1, 43007 Tarragona, Spain.

Center of Environmental Food and Toxicological Technology (TecnATox). C/ Marcel·lí Domingo 1, 43007 Tarragona, Spain.

[§]Current affiliation: Disease Biomarkers and Molecular Mechanisms Group (DIBIOMEC), Institut d'Investigació Sanitària Pere Virgili (IISPV), C/Dr. Mallafré Guash, 4, 43005 Tarragona, Spain.

*Corresponding author.

Manuscript in preparation for *Journal of the Science of Food and Agriculture*.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Abstract

Sweet cherries (*Prunus avium*) are a source of phenolic compounds, which have several beneficial effects such as anti-inflammatory and antioxidant. However, the phenolic profile is highly dependent on post-harvest conditions, such us transport, ripening in the storage or surface damage. Therefore, the objective of this work was to characterize the phenolic composition of local and non-local cherries and to evaluate the impact of proximity on bioavailability of (poly)phenols in rats. To this aim local and non-local sweet cherry were characterized and administered to Wistar rats (65 mg GAE/kg bw). Serum was collected at different time points and quantified by HPLC-ESI-MS/MS. The results showed significant differences depending on the geographical origin, mainly attributed to the post-harvest processes followed to reach commercial maturity. The (poly)phenol content of local cherries significantly higher compared to non-local. Furthermore, changes in the pharmacokinetics of phenolic compounds according to the geographical origin of the cherries were observed.

Keywords: bioavailability; cherries; HPLC-ESI-MS/MS; pharmacokinetics; phenolic profile; post-harvest.

1. Introduction

The daily intake of fruits and vegetables is recognized to be fundamental in the prevention of chronic diseases such as diabetes, cancer, neurodegenerative disorders and cardiovascular diseases [1,2]. Many of these benefits are mainly attributed to their high content in phenolic compounds, which are produced as a response to environmental stress [3]. Within this context, it has been observed that animals possess the capability to utilize these specific responses as a means of anticipating impending seasonal changes, thus facilitating the development of adaptive survival skills [4]. These phenolic compounds can be classified into four main groups: flavonoids, phenolic acids, stilbenes and lignans. Among these groups, flavonoids are the most widely consumed and comprise flavonols, flavones, flavan-3-ols, isoflavones, flavanones, and anthocyanidins [5]. In this context, it has been reported that sweet cherries are rich in anthocyanins, hydroxycinnamic acids, flavonols and flavan-3-ols [6–8]. Thus, sweet cherry consumption has been associated with several beneficial effects such as anti-inflammatory, antioxidant [9], neuroprotective [10], antimicrobial and antifungal [11] effects.

Nowadays, globalization allows the easy access to several fruit varieties from different parts of the world throughout the year. These have shown high levels of variation in agromorphological, genetic and organoleptic traits. In addition, it has recently been studied fruits from different geographical origins under different climatic conditions shown differences in phenolic profile giving a particular response towards evaluated biomarkers [12]. Consequently, it is important to note that the composition and levels of phenolic compounds in fruits and vegetables could be directly

affected by diverse external factors such as agronomic conditions, harvest time, storage conditions, ripening stages and extraction processes [13].

Taking all together, the phenolic profile of fruits plays a key role in terms of health benefits. It has been estimated that only a limited portion of ingested (poly)phenols is absorbed by the small intestine (5–10%) [14]. The rest (90–95%) reaches the colon, where they are broken down by microbial metabolism. Hence, (poly)phenols can undergo metabolism by the gut microbiota and a small portion of them is degraded into small molecules such as phenolic acids or valerolactones [15]. The remaining oligomeric and polymeric forms interacts with gut microbiota, resulting in improved microbial diversity and, thereby, contributing to improved health bacteria) [15]. Once absorbed, phenolic compounds and their metabolites are sent through the systemic circulation to the various tissues and organs where they are seen as foreign substances and undergo extensive phase II reactions including glucuronidation, sulfation, and/or methylation, catalyzed by the action of uridine 5'-diphospho-glucuronyltransferase (UGTs), sulphotransferases (SULTs), and catechol-*O*-methyltransferase (COMT), respectively [16]. Moreover, these metabolites may be transported back into the intestine through the enterohepatic cycle. Ultimately, the metabolites sent via systemic circulation reach the kidneys and are excreted through the urine. Non-absorbed (poly)phenols are released through feces [16]. The wide diversity of structures of cherries demands HPLC-ESI-MS/MS methodologies able to accurately determine all these compounds. However, most studies have only focused on anthocyanins and their derivatives, while other relevant (poly)phenols are not considered [17]. Therefore, it is necessary to study the broad spectrum of (poly)phenols available in cherry including both anthocyanin and non-anthocyanin derived metabolites.

Considering all this, the aim of this work was to determine the effect of proximity on the phenolic composition of cherries, as well as to evaluate its impact on the bioavailability and metabolism of phenolic compounds.

2. Materials and Methods

2.1. Chemicals and Reagents

All water used was ultrapure and obtained from Mili-Q Advantage A10 system (Milipore, Madrid, Spain). Acetone, acetonitrile and phosphoric acid (Sigma-Aldrich, Madrid, Spain), glacial acetic acid (Panreac, Barcelona, Spain), and methanol (Scharlab S.L., Barcelona, Spain) were all HPLC analytical grade. Folin–Ciocalteu reagent was acquired from Fluka/Sigma-Aldrich (Madrid, Spain). The standard compounds (+) – catechin, (-) epicatechin, quercetin, 3,4,5-trihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 3′,4′-dihydroxycinnamic acid, *p*-coumaric acid, 4′-hydroxy-3′-methoxycinnamic acid, 4′-hydroxyphenylacetic acid, 3′-hydroxyphenylacetic acid, 3-(4′-hydroxyphenyl)propanoic acid were purchased from Fluka/Sigma-Aldrich (Madrid, Spain). The standard compounds procyanidin dimer B2, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, 3-*O*-caffeoylquinic acid, were purchased from Extrasynthese (Lyon, France) and quercetin-3-*O*-rutinoside was provided by Nutrafur (Murcia, Spain). The standard anthocyanin compounds cyanidin-3-*O*-rutinoside, malvidin-3-*O*-glucoside and peonidin-3-*O*-rutinoside were purchased from PhytoLab (Vestenbergsgreuth, Germany).

2.2. Plant and fruit material

Samples of sweet cherries (*Prunus avium* cv. Brooks) were obtained at their commercial maturity from two different geographical origins: local sweet cherries (LC) from Tarragona, Spain (41°24'04.8"N, 1°03'09.4"E, at 480

m altitude), which were donated by the producer in June 2018 (in-season consumption), and non-local sweet cherries (NLC) from Cachapoal, Chile (34°15'35.8"S, 70°47'40.8"W, at 450 m altitude), which were purchased in a local market in December 2018 (out-of-season consumption) (**Figure S1**).

2.3. Proximate Composition

Proximate composition of the sweet cherries (*Prunus avium* cv. Brooks) was previously determined [18] (**Table S1**). Soluble solids content was measured in reception time as indicator of maturity, being 8.43 ºBrix for LC and 9.13 ºBrix for NLC. All dietary components of LC and NLC were characterized according to the official methods of the Association of Official Analytical Chemists (AOAC) [19]. The ash content was determined by subjecting them to heating at 550 ºC for 24 hours, which allowed for the removal of water and organic matter. The remaining inorganic residue was then quantified. The total protein content was measured using the Kjeldahl method, employing a conversion factor of 6.25. The total lipid content was assessed through continuous extraction with *n*-hexane utilizing a Soxhlet extractor. The determination of total dietary fiber (TDF) involved subjecting the samples to treatment with heat-stable α-amylase, proteases from *Bacillus licheniformis*, and amyloglucosidase from *Aspergillus niger*. Subsequently, the dry residue was weighed sequentially. The total carbohydrate content was calculated by subtracting the nutrient contents described above from the weight of both LC and NLC samples.

2.4. Experimental procedure for rat serum collection

Eighteen-week-old male Wistar rats (537 ± 26 g), were obtained from Janvier-Labs (Le Genest Saint Isle, France). All the animals were housed

in pairs at 22 ºC with a light/dark cycle of 12 h (lights on at 8:00 a.m.) and were provided tap water and standard chow diet (Safe-A04c, Germany) *ab libitum* during the experiment. The rats were randomly divided into two groups (n = 7, each): LC and NLC. In both groups, rats were orally administrated a dose of 65 mg gallic acid equivalent (GAE)/ kg body weight (bw), as determined by the Folin-Ciocalteu method [20].

Rats were fasted for 12 h before the day of the experiment prior to oral administration by intragastric intubation. In order to obtain 0 h (blank), blood samples were collected from the saphenous vein using nonheparinized vials (Sarstedt, Barcelona, Spain) previous to oral administration (between 9:00 and 9:45 a.m.). Afterwards, samples at 2, 4, 7, 24 and 48 h were collected (**Figure 1**). At each extraction point samples (n = 7) were maintained at room temperature for 1 h and then centrifuged (2,000 x g, 15 min, 4 ºC) to obtain the serum. Then serum samples were pooled to obtain sufficient volume to perform duplicated chromatographic analyses. The pooled serum samples were stored at − 80 ºC until chromatographic analysis was performed.

All procedures were conducted in accordance with the guidelines for the care and use of laboratory animals, and the experimental procedure was approved by the Ethical Committee for Animal Experimentation of the Universitat Rovira i Virgili (reference number 9495 by Generalitat de Catalunya) in accordance with the EU Directive 2010/63/EU.

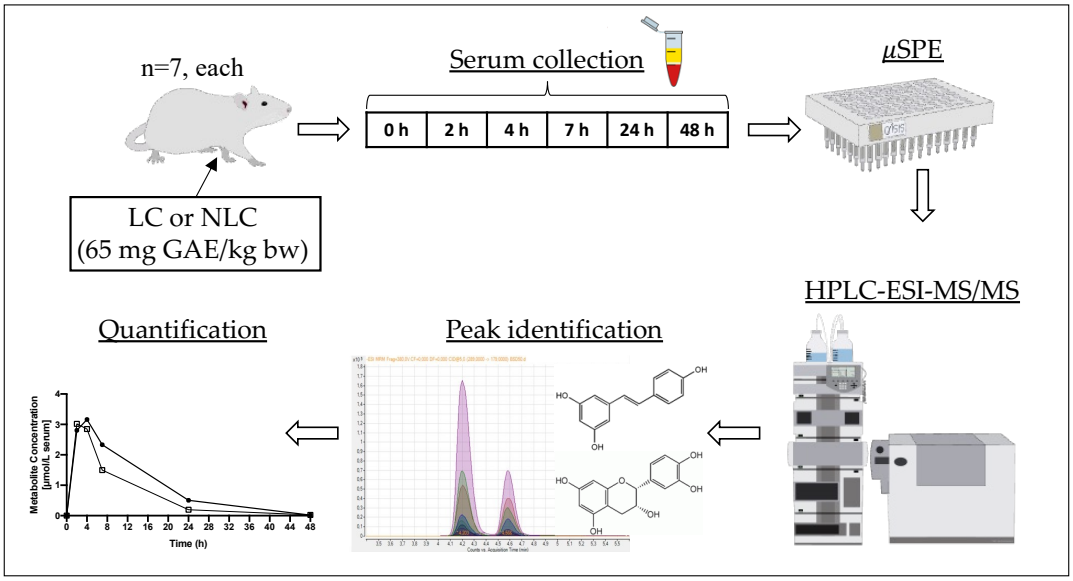


Figure 1. Graphical representation of the experiment design used in this study. LC, local sweet cherry; NLC, non-local sweet cherry; GAE, gallic acid equivalent; bw, body weight.

2.5. Phenolic compounds extraction

2.5.1. From fruits extracts (phenolic compounds from cherries)

De-stoned cherries were frozen in liquid nitrogen and grounded. Then, the samples were freeze-dried for one week in a Telstar LyoQuest freeze-dryer (Thermo Fisher Scientific, Madrid, Spain) at −55 °C and grounded (Moulinette 1, 2, 3, Moulinex). The resulting powder was then stored in amber flasks at room temperature until further analysis or use in subsequent experiments. The phenolic compounds in LC and NLC were extracted as previously described [6]. Briefly, the sweet cherry powder was mixed with pre-heated (55 ºC) extraction solvent (MeOH of 72 % including 1 % formic acid) in a 12 mL/g of liquid-to-solid-ratio (LSR) and immediately centrifuged (9,500 x g, 10 min, 4 ºC). Pellets were re-

extracted under the same conditions three more times and supernatants were collected and stored for (poly)phenols content analysis.

2.5.2. From serum samples (micro-solid phase extraction)

Prior to analysing the phenolic metabolites in the serum, the samples were pre-processed using a method based on micro-solid phase extraction (μSPE). The serum samples were cleaned and concentrated using 30 μm OASIS HLB μ-Elution Plates (Waters, Barcelona, Spain) as described in our previous studies [21].

2.6. Chromatographic analysis (HPLC-ESI-MS/MS)

Sweet cherry extracts and serum samples were directly analyzed using a 1200 LC series instrument coupled to a 6490 MS/MS (Agilent Technologies, Palo Alto, CA, USA). Two different HPLC-ESI-MS/MS procedures were used to separate, detect, and quantify non-anthocyanin and anthocyanin phenolic compounds. The nomenclature adopted follows the one defined by Kay and colleagues [22] to harmonize the chemical names of (poly)phenols and their metabolites.

2.6.1. Non-anthocyanin

Chromatographic analysis was performed using a Zorbax eclipse XDB-C18 column (150 mm x 2.1 mm, 5 μm particle size) equipped with a Narrow-Bore guard column (2.1 mm x 12.5 mm, 5 μm particle size) from Agilent Technologies (Palo Alto, CA, USA). The mobile phase consisted of 0.2% acetic acid in water (solvent A) and 100% acetonitrile (solvent B). The elution gradient followed a specific pattern: initial conditions started at 100% of eluent A during 0.5 min and was linearly decreased to 60% after 14.5 min, further decreased to 0% A in 1 min. Then it was kept

isocratic for 4 min and back to the initial conditions for 1 min. A post-run of 10 minutes was employed to ensure column equilibration. The flow rate was set at 0.4 mL/min, and the injection volume for all runs was 2.5 μL. For mass spectrometry analysis, negative electrospray (ESI) mode at unit resolution was selected. The electrospray capillary voltage was set to 3000 V, the source temperature was maintained at 200 ºC, and the flow rate was set to 14 L/min with a nebulizer gas pressure of 20 psi. The acquisition of MS/MS data was performed in Dynamic-Multiple-reaction monitoring (MRM) mode, allowing for precise and targeted analysis.

2.6.2. Anthocyanin

The separation of anthocyanin was achieved using an Acquity BHE C18 (100 mm x 2.1 mm, 1.7 μm particle size) as a chromatographic column (Waters, Milford, MA, USA) at room temperature. The mobile phase consisted of a mixture of water and formic acid in a ratio of 9:1 (*v:v*) (solvent A) and 100% acetonitrile (solvent B). The elution gradient employed was as follows: initially, eluent A was utilized at 100% and gradually reduced to 91% after 5 minutes, then decreased to 85% after an additional 2 minutes, and ultimately dropped to 0% A in 1 minute. This composition was maintained isocratically for 2 minutes before returning to the initial conditions for 3 minutes. To ensure proper column equilibration, a post-run of 10 minutes was employed. The flow rate was set at 0.4 mL/min, and the injection volume for all runs was 2.5 μL. For mass spectrometry analysis, the electrospray ionization (ESI) capillary voltage was set to 3000 V, the source temperature was maintained at 200 ºC, and the flow rate was set to 14 L/min with a nebulizer gas pressure of 20 psi. The mass spectrometer operated in positive mode, and the

acquisition of MS/MS data was conducted using Dynamic-Multiple-reaction monitoring (MRM) mode.

2.7. Standard solutions (HPLC-ESI-MS/MS quantification)

In order to quantify the phenolic compounds, control group blank plasma was spiked with standard compounds at nine different concentrations (20-10,000 parts per billion (ppb)) to obtain calibration curves. Compounds present at the 0 ppb were subtracted from the plasma concentration at all other concentration-points. The quantification of samples was performed by estimating the analyte/internal standard (IS) peak abundance ratio using standard curves and interpolating the results. All optimized MRM conditions are presented in **Table S2**. To validate the quantitative method, the calibration curves, linearity, LOD, LOQ and accuracy were studied (**Table S3**). For each analyte, standard curves were constructed using peak areas data, and linear least-squares regression was used to calculate the slope, intercept, and correlation coefficient (R^2) with $R^2 >$ 0.971 in all cases. the sensitivity of the analytical method was assessed by determining the limit of detection (LOD), which is the concentration corresponding to 3 times the signal-to-noise ratio, and the limit of quantification (LOQ), which is the concentration corresponding to 10 times the signal-to-noise ratio. The ranges of detection and quantification limits are presented in **Table S3.** To evaluate the accuracy of the method, the relative error (RE) was calculated for three different concentrations of each analyte within the linear range: 100 ppb, 2,500 ppb, and 5,000 ppb. The resulting fluctuations for accuracy were within limits recommended by most of the guidelines (IUPAC, FDA and SANCO, where 15–20 % is given as an acceptable level of variation). In both methodologies, data

acquisition was performed using MassHunter Software (Agilent Technologies, Palo Alto, CA, USA).

2.8. Pharmacokinetic data analysis

The pharmacokinetic parameters were estimated using the non-compartmental analysis with the validated PKSolver software [23]. Area under serum concentration-time curve for 48 h ($AUC_{0\text{-}48h}$) was calculated with the help of the linear trapezoid method. The time point where maximum of serum concentration (C_{max}) reached was defined as t_{max}. The elimination constant (λ_Z) was obtained from the slope of the regression line obtained from the terminal segment, and the elimination half-life ($t_{1/2}\ \lambda_Z$) was calculated as ln (2)/ λ_Z. The mean residence time (MRT) was calculated according to the equation $MRT = AUMC\ 0 - \infty / AUC\ 0 - 48h$, where $AUMC_{0\text{-}\infty}$ is the area under the plasma concentration curve multiplied by time as a function of time, calculated according to the trapezoid method and extrapolated to infinity. The volume of distribution during the terminal elimination phase (Vz/F) was as $Vz/F = dose/[\lambda Z\ (AUC\ 0 - \infty)]$. The apparent total clearance of drug from serum (Cl/F) was calculated using the equation $Cl/F = dose/AUC\ 0 - \infty$.

2.9. Statistics

Statistical analysis was performed using RStudio software (RStudio Inc.; Boston, MA, USA). The assumption of normality was determined using the Shapiro-Wilk test and the homoscedasticity between groups was determined using F-test. Student's or Welch's *t*-test were used depending on the homogeneity of variance to estimate differences ($p < 0.05$) in the phenolic composition of LC and NLC as normally distributed data. Non-normally distribution was analyzed by nonparametric Wilcox's test.

3. Results

3.1. Phenolic profile of local and distant sweet cherries (Prunus avium cv. Brooks)

A total of 27 phenolic compounds, anthocyanins (8), flavan-3-ols (3), flavonols (5), hydroxybenzoic acids (4), hydroxycinnamic acids (4), hydroxyphenylacetic acids (2) and hydroxyphenylpropanoic acids (1), were identified and quantified in sweet cherry cultivars. **Table 1** shows the concentration values as mg of phenolic components per 100 grams of dry weight for each phenolic compound. In addition, the percentage of the phenolic profile of each type of cherry was represented in **Figure S2**. The total content of phenolics in sweet cherries ranged from 275.3 (LC) mg/100g dw to 126.7 (NLC) mg/100g dw (**Table 1**). These results are similar to those reported by Kelebek & Selli, 2011 where where the total phenolic content in sweet cherry cultivars ranged from 88.72 to 239.54 mg/100g dw.

Table 1. Phenolic compounds in local cherry (LC) and non-local cherry (NLC) quantified by HPLC-ESI-MS/MS.

Compound	LC	NLC	p-Value
Σ Anthocyanins	**205.67**	**66.11**	
Cyanidin-3-O-glucoside d2 [a]	0.24 ± 0.06	0.30 ± 0.03	$p = .209$
Cyanidin-3-O-rutinoside	177.75 ± 43.98	51.90 ± 4.29	$p = .008$
Cyanidin-3-O-glucoside d1 [a]	16.80 ± 4.42	2.92 ± 0.22	$p = .006$
Delphinidin O-coumaroylglucose d1 [a]	0.46 ± 0.13	0.21 ± 0.02	$p = .029$
Delphinidin O-coumaroylglucose d2 [a]	0.46 ± 0.12	0.19 ± 0.04	$p = .022$
Delphinidin O-coumaroylglucose d3 [a]	8.39 ± 2.40	9.99 ± 1.16	$p = .378$
Malvidin O-coumaroylglucose	0.006 ± 0.001	0.002 ± 0.00	$p = .003$
Peonidin-3-O-rutinoside	1.57 ± 0.42	0.59 ± 0.06	$p = .019$
Σ Flavan-3-ols	**23.29**	**5.75**	
(+)-Catechin	11.63 ± 1.61	3.64 ± 0.58	$p = .002$
Procyanidin dimer B2	3.50 ± 0.25	0.78 ± 0.10	$p < .001$
(-)-Epicatechin	8.15 ± 1.25	1.33 ± 0.22	$p < .001$
Σ Flavonols	**26.60**	**32.25**	
Quercetin-3-O-rutinoside	20.46 ± 4.13	24.13 ± 3.98	$p = .366$
Quercetin-3-O-glucoside	1.80 ± 0.36	2.31 ± 0.34	$p = .174$
Kaempferol-3-O-rutinoside	3.92 ± 0.80	5.25 ± 0.60	$p = .107$
Kaempferol-3-O-glucoside	0.13 ± 0.03	0.21 ± 0.03	$p = .038$
Quercetin	0.29 ± 0.06	0.35 ± 0.06	$p = .311$
Σ Hydroxybenzoic acids	**10.74**	**11.64**	
3,4,5-Trihydroxybenzoic acid	0.36 ± 0.03	0.73 ± 0.12	$p = .033$
2,5-Dihydroxybenzoic acid	0.71 ± 0.15	1.18 ± 0.20	$p = .043$
4-Hydroxybenzoic acid	8.77 ± 1.24	9.13 ± 0.91	$p = .703$
4-Hydroxy-3-methoxybenzoic acid	0.90 ± 0.15	0.61 ± 0.07	$p = .047$
Σ Hydroxycinnamic acids	**8.49**	**10.39**	
3ˈ,4ˈ-Dihydroxycinnamic acid	0.43 ± 0.09	1.73 ± 0.06	$p < .001$
3-O-Caffeoylquinic acid	6.20 ± 1.42	4.67 ± 1.02	$p = .241$
p-Coumaric acid	1.37 ± 0.20	3.27 ± 0.52	$p = .007$
4ˈ-Hydroxy-3ˈ-methoxycinnamic acid d1	0.49 ± 0.10	0.72 ± 0.13	$p = .104$
Σ Hydroxyphenylacetic acids	0.42	0.45	
4'-Hydroxyphenylacetic acid d1	0.18 ± 0.06	0.31 ± 0.09	$p = .128$
3/4'-Hydroxyphenylacetic acid d3	0.25 ± 0.07	0.14 ± 0.04	$p = .095$
Σ Hydroxyphenylpropanoic acids	0.05	0.12	
3-(4-Hydroxyphenyl) propionic acid	0.05 ± 0.03	0.12 ± 0.04	$p = .032$
Σ Total (poly)phenols	**275.3**	**126.7**	

Results are expressed as mg of phenolic components per 100 grams of dry weight (mg/100g ± SD (n=6). $p < 0.05$ indicates a significant difference between LC and NLC by Student's or Welch's *t*-test depending on homogeneity of variance. Anthocyanins were analyzed in the positive ion mode. Different isomers are indicated by d1, d2 and d3. [a] Quantified using the calibration curve of cyanidin-3-*O*-rutinoside**.**

In relation to anthocyanins**,** eight different structures were identified by HPLC-ESI-MS/MS (**Table 1**). The highest total anthocyanin content was detected in LC (205.67 mg/100g dw vs 66.11 mg/100g dw in NLC). These results are in agreement with others published by several researchers. For example, Gao & Mazza reported that the total anthocyanin content ranged from 2 to 41 mg/100 g dw for light-colored cherries and from 82 to 297 mg/100g dw for dark cherries [25]. Among anthocyanins, cyanidin-3-*O*-rutinoside is by far the main one representing 79-87% of total contents of anthocyanins, followed by cyanidin-3-*O*-glucoside 4-8%, delphinidin *O*-coumaroylglucose d3 4-15% and peonidin-3-*O*-rutinoside 1% (**Figure S2**). The specific concentrations showed for cyanidin-3-*O*-rutinoside d2, cyanidin-3-*O*-glucoside d1, delphinidin *O*-coumaroylglucose d1, delphinidin *O*-coumaroylglucose d2, malvidin *O*-coumaroylglucose and peonidin-3-*O*-rutinoside presented significant differences (**Table 1**), being LC the group with highest concentrations.

Regarding flavan-3-ols, three different compounds were identified. In general, the concentration ranges expressed as a percentage of our results agree with other authors [6,26]. Comprising a range of 50-63% for (+)-catechin, 35-23% for (-)-epicatechin and 13-16% for procyanidin dimer B2, of total contents of flavan-3-ols (**Figure S2**). The specific concentrations showed that (+)-catechin was the dominant flavan-3-ol. Nevertheless, (-)-epicatechin is usually the main flavan-3-ol in sweet cherry [6,7,24]. However, in this study, (+)-catechin was shown to have the highest amount compared to the other compounds in the group. All compounds belonging to the flavan-3-ols family showed significantly higher concentrations in the

LC group (23.29 mg/100g dw) compared to the NLC group (5.75 mg/100g dw) (**Table 1**).

Concerning the family of flavonols, five different compounds were identified. Compounds such as quercetin and kaempferol belong to this group. However, they are normally found in glycosylated form. As other authors have described for sweet cherry [7,26], quercetin-3-*O*-rutinoside is the main flavonol detected, ranging from 75-77 %, followed by kaempferol-3-*O*-rutinoside 15-16 %, quercetin-3-*O*-glucoside 6-7 %, kaempferol-3-*O*-glucoside 0.50-0.64% and quercetin 1 % (**Figure S2**). The highest total flavonol content was detected in NLC (32.25 mg/100g dw vs 26.60 mg/100g dw in LC). The concentrations expressed in **Table 1** as mg/100g dw, are similar to the values identified by Martini et al. (2017), except for quercetin-3-*O*-rutinoside and quercetin-3-*O*-glucoside which are more similar to those of Serra et al. (2011). Overall, there are no significant differences, although an upward trend was observed for the NLC metabolites. There are only significant differences (p = 0.038) in kaempferol-3-*O*-glucoside, but it is at residual levels (**Table 1**).

In relation to the phenolic acids family, they are further distinguished into two subgroups, the hydroxybenzoic acids (HBA) (C_6–C_1) and the hydroxycinnamic acids (HCA) (C_6–C_3) 3. In this regard, four different compounds were identified in the samples. Contrary to the anthocyanin and flavan-3-ols groups, the highest total phenolic acids content was detected in NLC (22.03 mg/100g dw vs 19.23 mg/100g dw in LC). The percentage composition of HBA and HCA is reported in **Figure S2.** Among HBA, 4-

hydroxybenzoic acid (76-80%) is the majority, followed by 2,5-dihydroxybenzoic acid (9-14%), 4-hydroxy-3-methoxybenzoic acid (5-8%) and 3,4,5-trihydroxybenzoic acid (3-5%). On the other hand, the most abundant compounds of the HCA group are 3-*O*-caffeoylquinic acid (44-73%) and 3′,4′-dihydroxycinnamic acid (6-20%) followed by *p*-coumaric acid (15-29%) and 4′-hydroxy-3′-methoxycinnamic acid d1 (5-6%). The specific concentrations of phenolic acids showed for 3,4,5-trihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 3′,4′-dihydroxycinnamic acid and *p*-coumaric acid presented significant differences (**Table 1**), being NLC the factor with highest concentrations, except 4-hydroxy-3-methoxybenzoic acid.

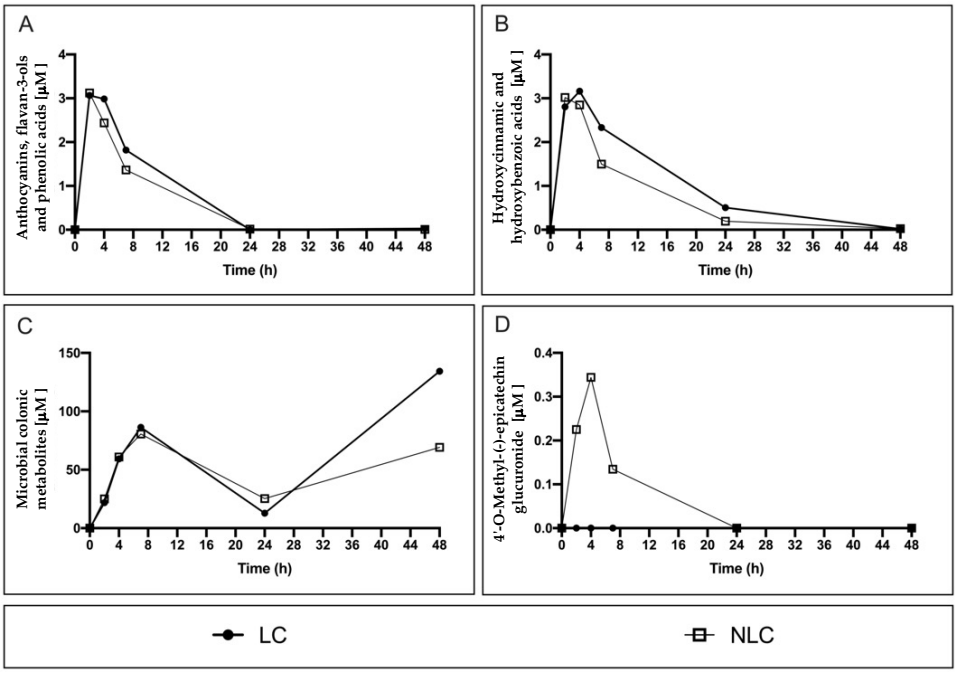


Figure 2. Kinetic profile of local (LC) and non-local (NLC) sweet cherries metabolites in rat serum. A) Anthocyanins, flavan-3-ols and phenolic acids; B) Hydroxycinnamic and hydroxybenzoic acids; C) Microbial colonic metabolites; D) 4'-*O*-Methyl-(-)-epicatechin glucuronide. Concentrations (µM) were quantified by HPLC-ESI-MS/MS in duplicate rat pooled serum (n = 7, each) at 0, 2, 4, 7, 24 and 48 h after the ingestion of sweet cherries at a dose of 65 mg GAE/kg bw.

Table 2. Pharmacokinetic parameters of phenolic compounds in rats pooled serum after administration of 65 mg GAE/kg bw of local cherry (LC) and non-local cherry (NLC).

Compound	Origin	λ_z (h^{-1})	$t_{1/2\ \lambda z}$ (h)	t_{max} (h)	C_{max} (µmol/L)	AUC_{0-48h} (µmol·h/L)	$AUC_{0-\infty}$ (µmol·h/L)	$MRT_{0-\infty}$ (h)	Vz/F (g/kg)/(µmol/L)	Cl/F (g/kg)/(µmol/L)/h
Anthocyanins										
Cyanidin-3-*O*-glucoside d1 [a)]	LC	0.652	1.063	2	0.003	0.031	0.031	5.601	494.887	322.706
	NLC	0.574	1.207	4	0.001	0.006	0.006	4.362	3063.478	1758.580
Cyanidin-3-*O*-glucoside d2 [a)]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Cyanidin-3-*O*-rutinoside	LC	0.790	0.877	2	0.065	0.376	0.376	4.227	33.613	26.555
	NLC	0.680	1.019	2	0.025	0.089	0.089	3.140	164.481	111.853
Delphinidin *O*-coumaroylglucose d1 [a)]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Delphinidin *O*-coumaroylglucose d2 [a)]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Delphinidin *O*-coumaroylglucose d3 [a)]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Malvidin *O*-coumaroylglucose	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Peonidin-3-*O*-rutinoside	LC	0.608	1.140	2	0.001	0.011	0.011	4.865	1422.734	865.149
	NLC	0.561	1.235	2	0.001	0.005	0.005	5.085	3394.780	1905.350
Flavan-3-ols and derivatives										
(+)-Catechin	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Procyanidin dimer B2	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
(-)-Epicatechin	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Methyl cate/epi glucuronide [b)]	NLC	0.875	0.792	4	0.344	1.678	1.678	4.119	6.810	5.959
Methyl cate/epi [b)]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Cate/epi glucuronide [b)]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Methyl cate/epi sulphate [b)]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Cate/epi sulphate [b)]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Flavonols										
Quercetin-3-*O*-rutinoside	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*

Quercetin-3-*O*-glucoside	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Kaempferol-3-*O*-rutinoside	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Kaempferol-3-*O*-glucoside	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Quercetin	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Hydroxybenzoic acids										
3,4,5-Trihydroxybenzoic acid	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
3,4-Dihydroxybenzoic acid	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*	*n.q.*
4-Hydroxybenzoic acid	LC	0.435	1.594	4	2.708	43.798	43.798	10.028	0.525	0.228
	NLC	0.406	1.706	2	2.464	24.610	24.610	7.072	1.000	0.406
4-Hydroxy-3-methoxybenzoic	LC	3.420	0.203	2	0.119	0.448	0.448	2.941	6.528	22.328
	NLC	0.818	0.847	4	0.107	0.590	0.590	4.125	20.720	16.945
Benzoic acid	LC	0.049	14.001	7	2.796	63.620	71.110	22.113	2.842	0.141
	NLC	0.492	1.408	7	3.646	41.492	41.492	7.176	0.490	0.241
4-Hydroxybenzoic acid-3 glucuronide [c]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
4-Hydroxybenzoic acid-3-sulphate [c]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Hippuric acid	LC	0.551	1.258	7	31.062	423.708	423.708	8.560	0.043	0.024
	NLC	0.551	1.257	7	26.943	538.895	538.895	12.831	0.034	0.018
3-Methoxybenzoic acid-4-glucuronide [d]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
3-Methoxybenzoic acid-4-sulphate [d]	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Hydroxycinnamic acids										
3-*O*-Caffeoylquinic acid	LC	0.656	1.057	4	0.003	0.029	0.029	5.406	518.200	339.941
	NLC	0.660	1.049	2	0.003	0.034	0.034	5.430	438.127	289.442
3′,4′-Dihydroxycinnamic acid	LC	0.904	0.767	4	0.369	2.962	2.962	4.980	3.734	3.376
	NLC	0.932	0.744	4	0.506	5.465	5.465	5.457	1.964	1.830
4′-Hydroxy-3′-methoxycinnamic acid d1	LC	0.759	0.914	2	0.037	0.210	0.210	4.086	62.835	47.668
	NLC	0.784	0.884	2	0.040	0.332	0.332	4.995	38.400	30.104
4′-Hydroxy-3′-methoxycinnamic acid d2	LC	0.343	2.021	4	0.040	0.358	0.358	6.058	81.479	27.947
	NLC	0.344	2.016	4	0.035	0.478	0.478	6.602	60.911	20.938

p-Coumaric acid	LC	1.023	0.677	4	2.894	30.932	30.932	5.373	0.316	0.323
	NLC	0.258	2.683	2	2.897	24.812	24.865	5.304	1.557	0.402
Hydroxyphenylacetic acids										
3′,4′-Dihydroxyphenylacetic	LC	0.445	1.557	4	4.431	53.764	53.764	9.338	0.418	0.186
	NLC	0.458	1.512	4	9.766	70.390	70.390	7.792	0.310	0.142
4′-Hydroxyphenylacetic acid	NLC	0.992	0.699	4	5.041	15.210	15.210	4.152	0.663	0.657
5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
Hydroxyphenylpropanoic acids										
3-(4-Hydroxyphenyl)propionic	LC	0.546	1.269	7	23.439	369.282	369.282	10.320	0.049	0.027
	NLC	0.546	1.270	7	21.831	404.962	404.962	12.713	0.045	0.025
3-Phenylpropanoic acid [e]	LC	0.549	1.264	7	25.936	408.638	408.638	10.320	0.045	0.024
	NLC	0.548	1.264	7	24.157	448.120	448.120	12.713	0.041	0.022

Abbreviations: *n.d.,* not detected*; n.q.,* not quantified*;* λz, elimination constant; $t_{1/2}$ λz, elimination half-life; t_{max}, time of peak serum concentration; C_{max}, maximum serum concentration; AUC, area under serum concentration-time curve; MRT, mean residence time; Vz/F, volume of distribution during the terminal elimination phase; Cl/F, apparent total clearance; epi, (-)-Epicatechin; cate, (+)-Catechin. a) Quantified using the calibration curve of Cyanidin-3-O-rutinoside, b) Quantified using the calibration curve of (-)-Epicatechin; c) Quantified using the calibration curve of 4-Hydroxybenzoic acid; d) Quantified using the calibration curve of 4-Hydroxy-3-methoxybenzoic; e) Quantified using the calibration curve of 3-(4-Hydroxyphenyl)propionic.

3.2 Serum pharmacokinetic profile of sweet cherries metabolites depending on the origin

After oral administration of LC and NLC at the same phenolic dose (65 mg GAE/kg bw) to rats, which corresponds to 9.8 g dw LC/kg bw and 10.6 g dw NLC/kg bw, the pharmacokinetic profile of the sum of all detected cherry phenolic metabolites in rat serum was grouped by similar origin or metabolization (**Table 2, Figure 2**).

Total free absorption metabolites group are constituted by anthocyanins, flavan-3-ols, 3-*O*-caffeoylquinic acid and *p*-coumaric acid. The kinetic profile of this group for both LC and NLC reached its maximum peak concentration at 2 hours (**Figure 2A**). However, for LC this difference was not so remarkable because the hydroxycinnamic acids that form this group (namely 3-*O*-caffeoylquinic acid and *p*-coumaric acid) reached their maximum at 4 hours as opposed to NLC, that reach their maximum at 2 hours.

The group of hydroxycinnamic and hydroxybenzoic acids is composed of metabolites that are present in sweet cherry and can also be identified in blood as produced by the action of colonial microbiota. These metabolites are: 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid, 4′-hydroxy-3′-methoxycinnamic acid and 3′,4′-dihydroxycinnamic acid. Their behaviour among the different types of cherry was different, reaching their maximum at 4 h for LC and 2 h for NLC (**Figure 2B**).

Figure 2C, represents metabolites generated by microbiota. The behaviour of the whole group was similar, with the maximum point of concentration at 7 hours and a second stage of growth after 24

hours. Finally, within the group of phase-II metabolites, only 4'-*O*-methyl-(-)-epicatechin glucuronide was detected at very low concentrations and exclusively in the NLC group (**Figure 2D**).

3.2.1. Anthocyanins, flavan-3-ols and phenolic acids.

Among this group of metabolites, the anthocyanin family is included, being the t_{max} of these metabolites at 2 h (**Table 2**). As in the fruit extracts, the group that was administered with LC showed higher anthocyanin values, being cyanidin-3-*O*-rutinoside the most abundant in both fruits (**Figure 3**). Finally, 3-*O*-Caffeoylquinic acid and *p*-Coumaric acid showed similar behaviour in the kinetic profile of both fruits.

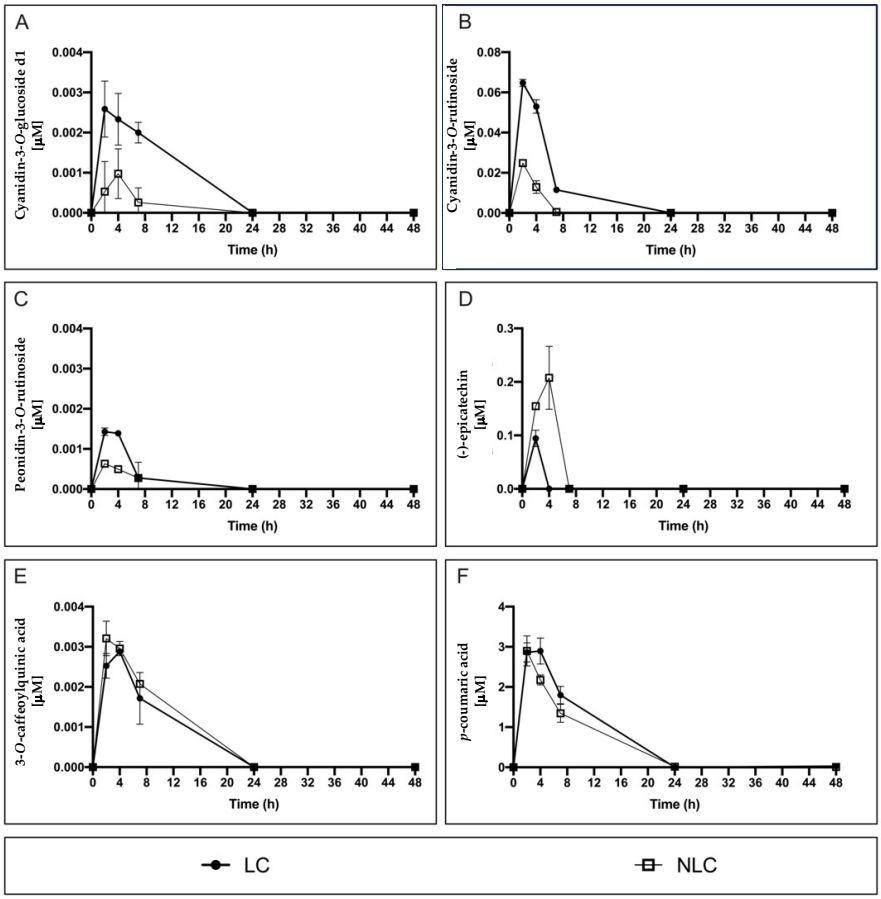


Figure 3. Kinetic behavior of anthocyanins, flavan-3-ols and phenolic acids of local (LC) and non-local (NLC) sweet cherries in rat serum. A) cyanidin-3-*O*-glucoside d1; B) cyanidin-3-*O*-rutinoside; C) peonidin-3-*O*-rutinoside; D) (-)-epicatechin; E) 3-*O*-caffeoylquinic acid; F) *p*-coumaric acid. Concentrations (µM) were quantified by HPLC-ESI-MS/MS in duplicate rat pooled serum (n = 7, each) at 0, 2, 4, 7, 24 and 48 h after the ingestion of sweet cherries at a dose of 65 mg GAE/kg bw.

3.2.2. Hydroxycinnamic and hydroxybenzoic acids.

Figure 4 shows a specific group of compounds that are present in the fruit extract (**Table 1**) and can also be generated by metabolism, making them bioavailable in serum from both sources. In the case of 4-hydroxybenzoic acid, we found its maximum concentration at t_{max} of 4 for LC. For 4-hydroxy-3-methoxybenzoic acid, the maximum concentration of LC was at 2 h compared to 4 h for NLC. The 4′-hydroxy-3′-methoxycinnamic acid did not show a differentiated behavior according to the geographic origin of the samples. Finally, regarding 3′,4′-dihydroxycinnamic acid, as in the extract, its values were higher in NLC and its maximum serum concentration was found at 4 h after administration.

3.2.3. Microbial colonic metabolites

Finally, **Figure 5** shows the kinetics of the compounds derived from microbiota, which in turn is the largest bioavailable group. The behavior of this group is highly diverse, although they have in common a t_{max} of 7 h. For hippuric Acid, 3-(4-hydroxyphenyl)propionic acid and 3-phenylpropanoic acid, which in turn are the majority of this group, we found an increase in the LC-administered group concentration after 24 h. On the other hand, benzoic acid, 3′,4′-dihydroxyphenylacetic acid and 4′-hydroxyphenylacetic acid showed a higher concentration in the group administered with NLC before 24 h.

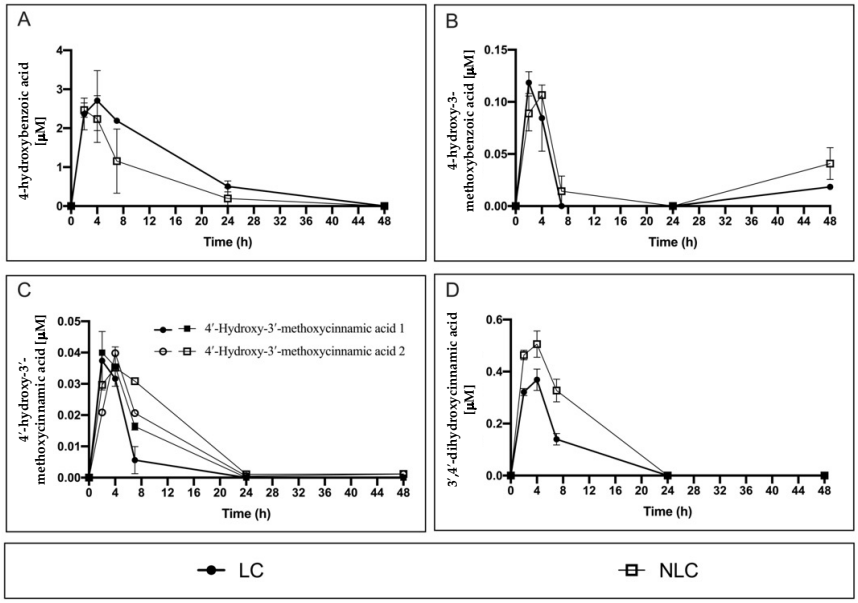


Figure 4. Kinetic behavior of hydroxycinnamic and hydroxybenzoic acids of local (LC) and non-local (NLC) sweet cherries in rat serum A) 4-hydroxybenzoic acid; B) 4-hydroxy-3-methoxybenzoic acid; C) 4′-hydroxy-3′-methoxycinnamic acid; D) 3′,4′-dihydroxycinnamic acid. Concentrations (μM) were quantified by HPLC-ESI-MS/MS in duplicate rat pooled serum (n = 7, each) at 0, 2, 4, 7, 24 and 48 h after the ingestion of sweet cherries at a dose of 65 mg GAE/kg bw.

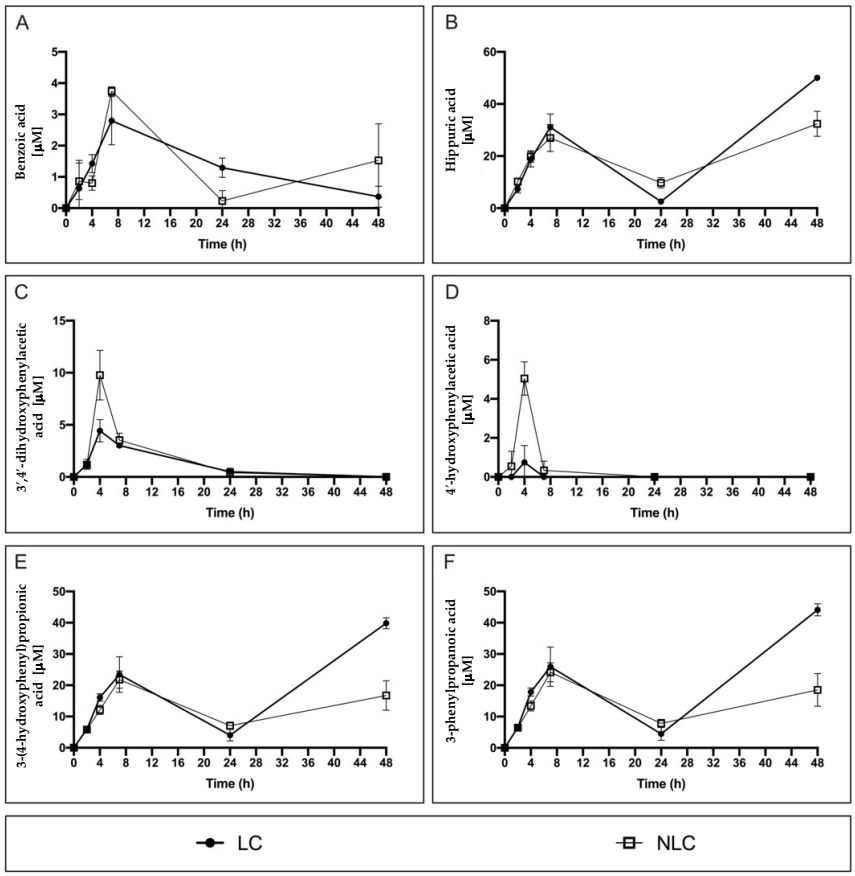


Figure 5. Kinetic behavior of microbial colonic metabolites. A) benzoic acid; B) hippuric Acid; C) 3′,4′-dihydroxyphenylacetic acid; D) 4′-hydroxyphenylacetic acid; E) 3-(4-hydroxyphenyl)propionic acid; F) 3-phenylpropanoic acid. Concentrations (µM) were quantified by HPLC-ESI-MS/MS in duplicate rat pooled serum (n = 7, each) at 0, 2, 4, 7, 24 and 48 h after the ingestion of sweet cherries at a dose of 65 mg GAE/kg bw.

4. Discussion

(Poly)phenols are one of the most important groups of bioactive metabolites in plants. Moreover, sweet cherry phenolic compounds are associated with a multitude of beneficial effects. Thus, it is important to characterize the profile of these compounds accurately. To this aim, in this study a standardized method for the extraction, detection and quantification of these compounds was carried out. Moreover, (poly)phenol profile and bioavailability of local and non-local fruits were evaluated.

Local and non-local sweet cherry extracts were analysed by HPLC-ESI-MS/MS to accurately quantify phenolic compounds. This procedure included two specific protocols that allowed us to quantify both anthocyanins and non-anthocyanins. This is an advantage in comparison with other studies where only one family of compounds such as non-anthocyanins is analysed [8,27]. On the other hand, Hu et al. (2021) worked with a hybrid method with both positive and negative ionization but the extraction conditions were not optimal for the anthocyanin family and was only able to detect petunidin 3-*O*-(6″acetyl-glucoside).

Regarding the effects of cherries origin on the content of phenolic compounds, when measured by HPLC-ESI-MS/MS a higher amount of total (poly)phenol content in the LC extract was observed. This is in accordance with our previous results where we identified differences in the (poly)phenol profile of tomatoes from two different geographical origins [29]. These differences are important as they may translate in different health effects [12]. Indeed, our studies about local

and non-local cherries have shown differential effects on plasma metabolic parameters and gene expression of key enzymes of lipid metabolism [30] and oxidative stress [18]. This variation may be caused by different factors related to the origin of the samples such as difference in the state of ripening or stress caused by surface damage and temperature. Thus, as shown in **Figure S1,** LC has an adequate commercial ripening degree and no surface damage is visible. However, NLC has a lower ripening stage and some surface damage, known as pitting. This is in line with other studies that showed that fruits in unripe stage are more susceptible to surface pitting than fruits at an advanced stage of ripening [31]. This can be linked with some of the reported consequences of long-distance transporting of sweet cherries, which include cell wall degradation and moisture loss, leading to a decrease in firmness [32]. It has been shown that mechanical damage in conjunction with cold storage could have a synergistic effect on the degree of homogalacturonan methylation. In addition, some authors have observed a decrease in pectin methylesterase (PME) activity during cold storage of pitted sweet cherries. The activity of PME is regulated by endogenous PME inhibitors, which have been reported in sweet cherry and several fruits [33]. For all the above reasons, surface pitting may be one of the most important consequences of long-distance transport, which in turn causes changes in the phenolic profile of the fruit [34].

Regarding anthocyanins, the results are in accordance with our previous studies that shown that local sweet cherry had higher contents of total anthocyanins than non-local sweet cherry [18]. These results are in agreement with the color of this fruit (darker in LC) (**Figure S1**), since the contents of anthocyanin correlate with the

color of sweet cherries, being also the most important indicator of quality and ripeness [35]. Recently Fuentealba *et al.* (2021) evaluated different varieties of sweet cherries under different conditions of developmental stage and pitting. Similar to our results, anthocyanin levels were found to be increased in fruits with a higher degree of ripeness and color. This may be due to the fact that during ripening an increase in fruit weight and soluble solids content occurs and phenolic compounds, such as chlorogenic acid, are transformed into anthocyanins [34]. Regarding phenolic acids, HBA and HCA groups showed a significantly higher concentration in NLC (surface pitting) (**Table 2**). These results are in agreement with Fuentealba *et al.* (2021) where sweetheart cherries with surface pitting presented higher contents of this phenolic acid than non-pitted samples. Moreover, Bunsiri *et al.* (2003) previously reported phenolic acid synthesis after mechanical impact in mangosteen (*Garnicia mangostana L.*). This is because storage combined with pitting increases phenylalanine ammonia-lyase (PAL) activity which is responsible for the first step of the phenylpropanoid pathway, which is involved in the synthesis of hydroxycinnamic acid and their derivatives [37]. These metabolites may act as antiradicals neutralizing the damage in the cherry skin tissue due to oxidative stress [33].

The bioavailability and pharmacokinetic of cherry phenolics from LC and NLC were studied in rat serum at different times after acute administration. The amount of (poly)phenols administered to the rats was carefully chosen to ensure that the bioavailability of the phenolic compounds was not impacted by dose differences. The quantity of 65 mg GAE/ kg bw, was determined by the Folin-Ciocalteu method [20]. This dose was shown to lead to the correct

detection of the (poly)phenol metabolites in serum after intake [38]. The decision to collect blood at specific points in time was based on the understanding that earlier collection points (i.e., 2–4 h) would provide insight into how the phenolic compounds were being absorbed in the small intestine and their phase-II metabolites [16], while later points (i.e., 7–48 h) would reveal how the compounds were being processed by the colon [38].

Similar to the results presented in this study for sweet cherry characterization, previous studies have shown that anthocyanins and hydroxycinnamic acids are the main phenolic compounds present in sweet cherries [6,39]. However, in terms of bioavailability, the most relevant are those that come from the microbiota. Recently, the study performed by Boskov *et al.* (2023) using an in-vitro digestion study highlighted the concentration of most of the native cherry (poly)phenols decreased significantly under simulated stomach and small intestine conditions [39]. Furthermore, as our results show, native cherry (poly)phenols decrease constantly over time from 7h onwards, confirming that bioconversion measured by the microbiota is the main activity of all phenolic compounds reaching the colon.

This study has shown how the bioavailability profile of phenolic compounds from sweet cherry administration varies according to origin. The first group to show this difference was the anthocyanin family, which consistent with the fruit characterization results, serum concentration levels for this family were significantly higher in rats administered with LC. Moreover, 4-hydroxybenzoic acid, one of the main degradation products of cyanidins [14], was significantly found more bioavailable in the serum of rats administered with LC.

Concerning 4-hydroxy-3-methoxybenzoic acid, it could be observed that during the first 2 h the circulating levels were mainly derived from direct absorption from the fruit. Therefore, in line with the results of the phenolic fruit profile, its point of highest concentration was in LC. However, in NLC its concentration increases according with the time, reaching it maximum value at 4 h. This is because the levels of 4-hydroxy-3-methoxybenzoic acid come from the direct metabolization of 3′,4′-dihydroxycinnamic acid [40], which is a compound found in higher concentration in NLC in both cherries and serum. Likewise, the microbiota-derived metabolites 3′,4′-dihydroxyphenylacetic acid and 4′-hydroxyphenylacetic acid are also derived from 3′,4′-dihydroxycinnamic acid [41] and are therefore found at higher concentration levels in the NLC-administered rats. Among the microbiota-derived metabolites, hippuric Acid, 3-(4-hydroxyphenyl)propionic acid and 3-phenylpropanoic acid are derived from (-)-epicatechin [14]. Therefore, their concentration increases significantly after 24 h for animals administered with LC.

5. Conclusions

In conclusion, the results presented in this study demonstrate that changes in the phenolic profile of sweet cherries from distinct geographical origins and post-harvest conditions result in different sweet cherry phenolic serum metabolite kinetic profile in rats. The main difference between LC and NLC extracts is mainly dominated by anthocyanins group, being cyanidin-3-*O*-rutinoside the major one.

References

1. Boeing, H. *et al.* Critical review: vegetables and fruit in the prevention of chronic diseases. *European Journal of Nutrition 2012 51:6* **51**, 637–663 (2012).

2. Slavin, J. L. & Lloyd, B. Health Benefits of Fruits and Vegetables. *Advances in Nutrition* **3**, 506–516 (2012).

3. Del Rio, D. *et al.* Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal* **18**, 1818–92 (2013).

4. Howitz, K. T. & Sinclair, D. A. Xenohormesis: Sensing the Chemical Cues of Other Species. *Cell* **133**, 387–391 (2008).

5. Manach, C., Scalbert, A., Morand, C., Rémésy, C. & Jiménez, L. Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition* vol. **79** 727–747 (2004).

6. Iglesias-Carres, L. *et al.* Optimization and characterization of Royal Dawn cherry (Prunus avium) phenolics extraction. *Sci Rep* **9**, (2019).

7. Martini, S., Conte, A. & Tagliazucchi, D. Phenolic compounds profile and antioxidant properties of six sweet cherry (Prunus avium) cultivars. *Food Research International* **97**, 15–26 (2017).

8. Peixoto, J. *et al.* Cherry stem infusions: antioxidant potential and phenolic profile by UHPLC-ESI-QTOF-MS. *Food Funct* **11**, 3471–3482 (2020).

9. Kelley, D. S., Rasooly, R., Jacob, R. A., Kader, A. A. & Mackey, B. E. Consumption of Bing Sweet Cherries Lowers Circulating Concentrations of Inflammation Markers in Healthy Men and Women. *J Nutr* **136**, 981–986 (2006).

10. Kim, D. O., Ho, J. H., Young, J. K., Hyun, S. Y. & Lee, C. Y. Sweet and Sour Cherry Phenolics and Their Protective Effects on Neuronal Cells. *J Agric Food Chem* **53**, 9921–9927 (2005).

11. Daglia, M. Polyphenols as antimicrobial agents. *Curr Opin Biotechnol* **23**, 174–181 (2012).

12. Cruz-Carrión, Á. *et al.* Tomatoes consumed in-season prevent oxidative stress in Fischer 344 rats: impact of geographical origin. *Food Funct* **12**, 8340–8350 (2021).

13. Garcia, E. & Barrett, D. M. Evaluation of processing tomatoes from two consecutive growing seasons: Quality attributes, peelability and yield. *J Food Process Preserv* **30**, 20–36 (2006).

14. Ozdal, T. *et al.* The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrients* vol. **8** (2016).

15. Niwano, Y., Kohzaki, H., Shirato, M., Shishido, S. & Nakamura, K. Metabolic Fate of Orally Ingested Proanthocyanidins through the Digestive Tract. *Antioxidants* vol. **12** (2023).

16. Monagas, M. *et al.* Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food and Function* vol. **1** 233–253 (2010).

17. Blackhall, M. L., Berry, R., Davies, N. W. & Walls, J. T. Optimized extraction of anthocyanins from Reid Fruits' Prunus avium 'Lapins' cherries. *Food Chem* **256**, 280–285 (2018).

18. Cruz-Carrión, Á., de Azua, M. J. R., Mulero, M., Arola-Arnal, A. & Suárez, M. Oxidative stress in rats is modulated by seasonal consumption of sweet cherries from different geographical origins: Local vs. non-local. *Nutrients* **12**, 1–15 (2020).

19. Association of Official Analytical Chemist. *A.O.A.C. Official Methods of Analysis.* (Washington DC, 1990).

20. Iglesias-Carres, L. *et al.* Optimized extraction by response surface methodology used for the characterization and quantification of phenolic compounds in whole red grapes (Vitis vinifera). *Nutrients* **10**, (2018).

21. Escobar-Martínez, I. *et al.* Administration Time Significantly Affects Plasma Bioavailability of Grape Seed Proanthocyanidins Extract in Healthy and Obese Fischer 344 Rats. *Mol Nutr Food Res* **66**, 2100552 (2022).

22. Kay, C. D. *et al.* Recommendations for standardizing nomenclature for dietary (poly)phenol catabolites. *Am J Clin Nutr* **112**, 1051–1068 (2020).

23. Zhang, Y., Huo, M., Zhou, J. & Xie, S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput Methods Programs Biomed* **99**, 306–314 (2010).

24. Kelebek, H. & Selli, S. Evaluation of chemical constituents and antioxidant activity of sweet cherry (Prunus avium L.) cultivars. *Int J Food Sci Technol* **46**, 2530–2537 (2011).

25. Gao, L. & Mazza, G. Characterization, Quantitation, and Distribution of Anthocyanins and Colorless Phenolics in Sweet Cherries. *J Agric Food Chem* **43**, 343–346 (1995).

26. Serra, A. T., Duarte, R. O., Bronze, M. R. & Duarte, C. M. M. Identification of bioactive response in traditional cherries from Portugal. *Food Chem* **125**, 318–325 (2011).

27. Nastić, N., Lozano-Sánchez, J., Borrás-Linares, I., Švarc-Gajić, J. & Segura-Carretero, A. New technological approaches for recovering bioactive food constituents from sweet cherry (Prunus avium L.) stems. *Phytochemical Analysis* **31**, 119–130 (2020).

28. Hu, T. *et al.* Determination and Characterization of Phenolic Compounds from Australia-Grown Sweet Cherries (Prunus avium L.) and Their Potential Antioxidant Properties. *ACS Omega* **6**, 34687–34699 (2021).

29. Cruz-Carrión, Á. *et al.* (Poly)phenolic composition of tomatoes from different growing locations and their absorption in rats: A comparative study. *Food Chem* **388**, 132984 (2022).

30. Ruiz de Azua, M. J., Cruz-Carrión, Á., Muguerza, B., Arola-Arnal, A. & Suarez, M. Seasonal consumption of cherries from different origins affects metabolic markers and gene expression of lipogenic enzymes in rat liver: A preliminary study. *Nutrients* **13**, 3643 (2021).

31. Toivonen, P. M. A., Kappel, F., Stan, S., McKenzie, D. L. & Hocking, R. Firmness, Respiration, and Weight Loss of `Bing', `Lapins' and `Sweetheart' Cherries in Relation to Fruit Maturity and Susceptibility to Surface Pitting. *HortScience* **39**, 1066–1069 (2004).

32. Correia, S., Schouten, R., Silva, A. P. & Gonçalves, B. Factors affecting quality and health promoting compounds during growth and postharvest life of sweet cherry (Prunus avium L.). *Front Plant Sci* **8**, 2166 (2017).

33. Ponce, E. *et al.* Biochemical and phenotypic characterization of sweet cherry (Prunus avium L.) cultivars with induced surface pitting. *Postharvest Biol Technol* **175**, 111494 (2021).

34. Fuentealba, C. *et al.* Cell wall and metabolite composition of sweet cherry fruits from two cultivars with contrasting susceptibility to surface pitting during storage. *Food Chem* **342**, 128307 (2021).

35. Habib, M., Bhat, M., Dar, B. N. & Wani, A. A. Sweet cherries from farm to table: A review. *http://dx.doi.org.sabidi.urv.cat/10.1080/10408398.2015.1005831* **57**, 1638–1649 (2017).

36. Bunsiri, A., Ketsa, S. & Paull, R. E. Phenolic metabolism and lignin synthesis in damaged pericarp of mangosteen fruit after impact. *Postharvest Biol Technol* **29**, 61–71 (2003).

37. KEGG. 2022 KEGG. https://www.genome.jp/kegg/ (2022).

38. Iglesias-Carres, L. *et al.* A comparative study on the bioavailability of phenolic compounds from organic and nonorganic red grapes. *Food Chem* **299**, 125092 (2019).

39. Boskov, D. *et al.* The Phenolic Profile of Sweet Cherry Fruits Influenced by Cultivar/Rootstock Combination. *Plants* **12**, (2023).

40. Gonthier, M. P. *et al.* Microbial aromatic acid metabolites formed in the gut account for a major fraction of the polyphenols excreted in urine of rats fed red wine polyphenols. *J Nutr* **133**, 461–467 (2003).

41. Williamson, G., Kay, C. D. & Crozier, A. The Bioavailability, Transport, and Bioactivity of Dietary Flavonoids: A Review from a Historical Perspective. *Compr Rev Food Sci Food Saf* **17**, 1054–1112 (2018).

Figure S1. Representative images of Brooks sweet cherries from two different geographical origins: (**a**) local cherry (LC) from Tarragona, Spain; (**b**) non-local cherry (NLC) from Cachapoal, Chile.

Table S1. Proximate composition of local cherry (LC) and non-local cherry (NLC).

Nutrients	**LC**	**NLC**
Ash	2.05	2.11
Protein	6.41	5.51
Total lipids (fat)	1.01	0.70
Fiber, total dietary	10.95	10.74
Total carbohydrates, by difference	79.58	80.94

Adapted from Cruz-Carrión et al. (2020).The results are expressed as percentage in dry weight (dw).

Table S2. Retention Times, Exact Mass, and Optimized MRM conditions

Compound	MW	RT (min)	MS/MS quantification		MS/MS confirmation	
			MRM_1	CE (V)	MRM_2	CE(V)
Anthocyanins						
Cyanidin-3-*O*-glucoside d2	449.40	8.3	449 > 287	40	449 > 137	20
Cyanidin-3-*O*-rutinoside	595.53	6.2	595 > 287	40	595 > 449	40
Cyanidin-3-*O*-glucoside d1	449.40	5.6	449 > 287	31	449 > 137	20
Delphinidin *O*-coumaroylglucose d1	611.10	6.6	611 > 303	25	611 > 449	25
Delphinidin *O*-coumaroylglucose d2	611.10	7.3	611 > 303	25	611 > 449	25
Delphinidin *O*-coumaroylglucose d3	611.10	7.8	611 > 303	25	611 > 449	25
Malvidin *O*-coumaroylglucose	639.20	6.2	639 > 331	25	639 > 449	25
Peonidin-3-*O*-rutinoside	609.20	7.3	609 > 301	30	609 > 463	30
Flavan-3-ols and derivates						
(+)-Catechin	290.27	5.3	289 > 203	12	289 > 245	10
Procyanidin dimer B2	578.52	6.1	577 > 407	28	577 > 425	12
(-)-Epicatechin	290.27	6.3	289 > 203	12	289 > 245	10

Methyl cate/epi glucuronide	480,40	5.7	479 > 303	20	479 > 289	20
Methyl cate/epi	304.29	10.8	303 > 137	20	303 > 285	10
Cate/epi glucuronide	466.40	13.3	465 > 289	20	465 > 203	40
Methyl cate/epi sulphate	384.40	16.3	383 > 303	20	383 > 245	10
Cate/epi sulphate	370.30	16.3	369 > 289	20	369 > 245	20
Flavonols						
Quercetin-3-*O*-rutinoside	610.52	8.3	609.> 299	40	609 > 271	60
Quercetin-3-*O*-glucoside	464.38	8.4	463 > 299	32	463 > 271	48
Kaempferol-3-*O*-rutinoside	594.52	9.0	593 > 255	32	593 > 285	60
Kaempferol-3-*O*-glucoside	448.38	9.3	447 > 255	28	447 > 284	40
Quercetin	302.24	11.6	301 > 151	20	301 > 179	20
Hydroxybenzoic acids						
3,4,5-Trihydroxybenzoic acid	170.12	2.3	169 > 125	12	169 > 79	24
3,4-Dihydroxybenzoic acid	154.12	3.5	153 > 109	16	153 > 62	40
4-Hydroxybenzoic acid	138.12	4.6	137 > 65	36	137 > 93	20
4-Hydroxy-3-methoxybenzoic acid	168.15	5.4	167 > 152	10	167 >108	10
Benzoic acid	122.12	7.6	121 > 77	8	121 > 59	8

4-Hydroxybenzoic acid-3 glucuronide	330.24	3.5	329 > 153	20	329 > 153	20
4-Hydroxybenzoic acid-3-sulphate	234.18	10.3	233 > 153	20	233 > 153	20
Hippuric acid	179.17	5.3	178 > 77	10	178 > 134	5
3-Methoxybenzoic acid-4-glucuronide	342.27	5.4	341 > 167	20	341 > 167	20
3-Methoxybenzoic acid-4-sulphate	248.21	12	247 > 167	20	247 > 167	20
Hydroxycinnamic acids						
3′,4′-Dihydroxycinnamic acid	180.16	5.7	179 > 135	16	179 > 107	24
3-*O*-Caffeoylquinic acid	354.31	5.7	353 > 191	16	353 > 85	16
p-Coumaric acid	164.16	7.1	163 > 119	16	163 > 93	36
4′-Hydroxy-3′-methoxycinnamic acid	194.18	7.6	193 > 178	10	193 > 134	12
Hydroxyphenylacetic acids						
4'-Hydroxyphenylacetic acid	152.15	3.40	151 > 107	5	151 > 93	20
3',4'-Hydroxyphenylacetic acid	152.15	5.40	151 > 107	5	151 > 93	20
5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	208.10	11.2	207 > 207	10	207 > 207	10
Hydroxyphenylpropanoic acids						
3-Phenylpropanoic acid	150.10	10	149 > 105	10	149 > 149	10
3-(4-Hydroxyphenyl)propionic acid	166.17	6.20	165 > 59	10	165 > 121	10

Anthocyanins were analyzed in the positive ion mode. Fragmentor used in all compounds was 380 V. Different isomers are indicated by d1, d2 and d3. *Abbreviations*: MW, Molecular weight; RT, retention time; MRM, Multiple Reaction Monitoring; CE, collision energy; V, Volts.

Table S3. HPLC-ESI-MS/MS method quality parameters for the different phenolic compounds in sweet cherry extracts

Compound	Calibration curve	R^2	Linearity (ppb)	Relative Error (RE) %			LOD (ppb)	LOQ (ppb)
				100 (ppb)	2500 (ppb)	5000 (ppb)		
Anthocyanins								
Cyanidin-3-*O*-glucoside d2	y=195.50x	0.998	20 – 10000	3.2	4.7	2.4	0.20	0.67
Cyanidin-3-*O*-rutinoside	y=195.50x	0.998	20 – 10000	3.2	4.7	2.4	0.20	0.67
Cyanidin-3-*O*-glucoside d1	y=195.50x	0.998	20 – 10000	3.2	4.7	2.4	0.25	0.84
Delphinidin *O*-coumaroylglucose d1	y=195.50x	0.998	20 – 10000	3.2	4.7	2.4	0.20	0.67
Delphinidin *O*-coumaroylglucose d2	y=195.50x	0.998	20 – 10000	3.2	4.7	2.4	0.20	0.67
Delphinidin *O*-coumaroylglucose d3	y=195.50x	0.998	20 – 10000	3.2	4.7	2.4	0.20	0.67
Malvidin *O*-coumaroylglucose	y=2582.90x	0.999	20 – 10000	3.5	2.0	4.5	0.25	0.83
Peonidin-3-*O*-rutinoside	y=1187.40x	0.999	20 – 10000	0.7	6.8	2.3	0.04	0.13
Flavan-3-ols								
(+)-Catechin	y=20.35x	0.971	80 – 40340	6.6	13.5	6.2	24.85	82.84

Procyanidin dimer B2	y=27.97x	0.996	20 – 10000	4.7	6.2	2.2	46.55	115.18
(-)-Epicatechin	y=35.09x	0.999	20 – 10000	14.1	1.3	0.7	5.34	17.78
Flavonols								
Quercetin-3-*O*-rutinoside	y=126.30x	0.979	40 – 20454	1.6	5.1	9.7	0.30	1.00
Quercetin-3-*O*-glucoside	y=179.52x	0.994	20 – 10000	0.6	9.1	6.4	2.10	6.99
Kaempferol-3-*O*-rutinoside	y=113.45x	0.996	21 – 10606	7.1	13.6	6.6	2.30	7.66
Kaempferol-3-*O*-glucoside	y=406.24x	0.989	20 – 10000	3.8	6.7	4.9	1.02	3.40
Quercetin	y=218.69x	0.995	20 – 10000	2.5	3.1	0.9	21.64	72.12
Hydroxybenzoic acids								
3,4,5-Trihydroxybenzoic acid	y=15.70x	0.999	107 – 53535	2.8	2.4	0.5	28.41	94.70
3,4-Dihydroxybenzoic acid	y=184.35x	0.960	98 – 49242	4.8	1.0	7.6	2.35	7.82
4-Hydroxybenzoic acid	y=3.40x	0.973	20 – 10000	0.9	0.4	6.8	43.68	145.61
4-Hydroxy-3-methoxybenzoic acid	y=144.60x	0.988	56 – 28472	2.9	4.3	0.2	1.15	3.83
Hydroxycinnamic acids								
3′,4′-Dihydroxycinnamic acid	y=337.14x	0.995	110 – 55176	5.8	8.7	3.2	1.96	6.54
3-*O*-Caffeoylquinic acid	y=2176.70x	0.999	20 – 10000	3.3	0.4	2.9	0.31	1.03
p-Coumaric acid	y=396.89x	0.993	94 – 47285	0.5	4.5	13.1	1.98	6.58

4´-Hydroxy-3´-methoxycinnamic acid	y=284.16x	0.997	20 - 10000	2.7	8.2	6.4	0.49	1.64
Hydroxyphenylacetic acids								
4'-Hydroxyphenylacetic acid	y=359.10	0.976	20 – 10000	3.0	8.3	2.8	38.59	128.62
3,4'-Hydroxyphenylacetic acid	y=59.15x	0.994	20 – 10000	10.5	1.3	12.8	16.06	53.53
Hydroxyphenylpropanoic acids								
3-(4-Hydroxyphenyl)propionic acid	y=6.17x	0.998	20 – 10000	0.4	5.4	2.4	54.67	182.25

Anthocyanins were analyzed in the positive ion mode. Different isomers are indicated by d1, d2 and d3. *Abbreviations*: RT, retention time; R^2, determination coefficient; LOD, limit of detection; LOQ, limit of quantification.

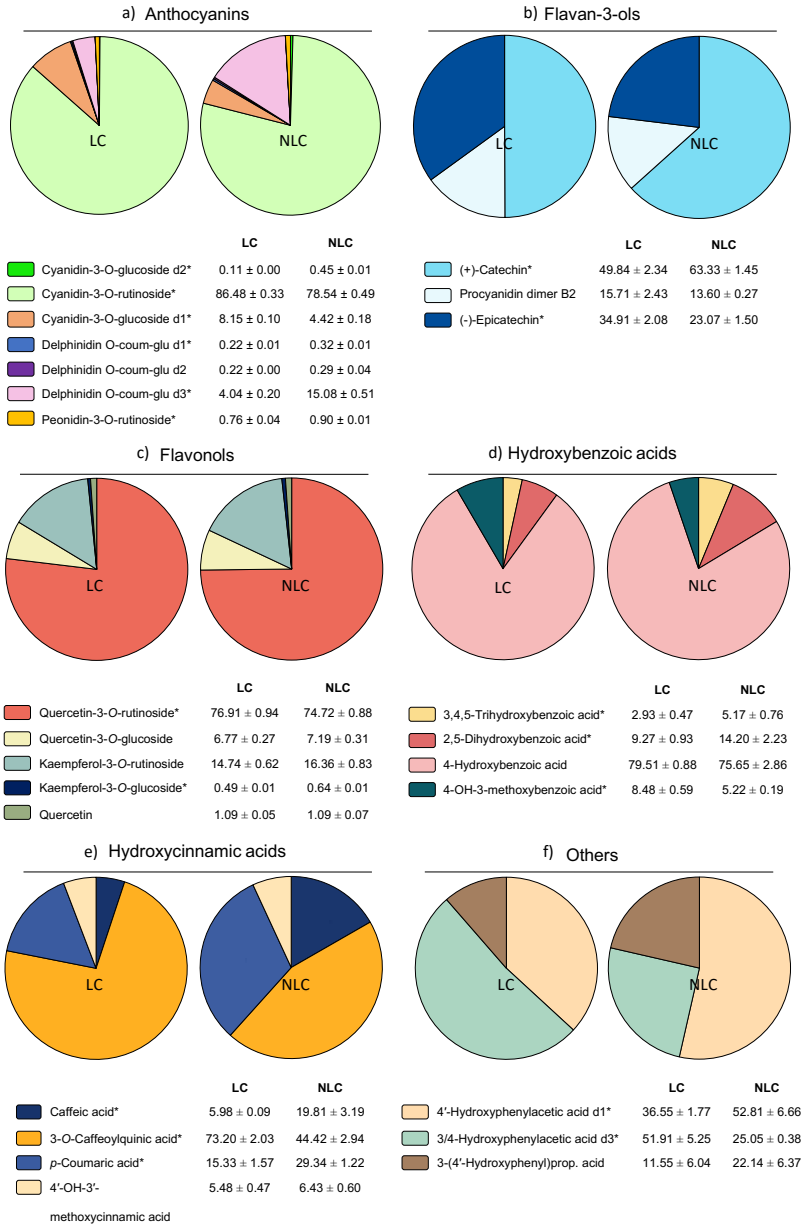
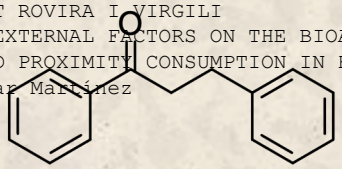
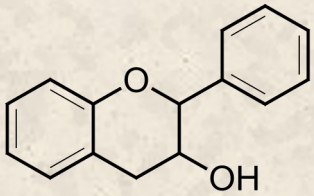
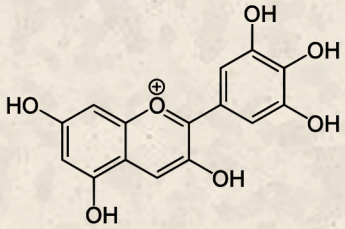
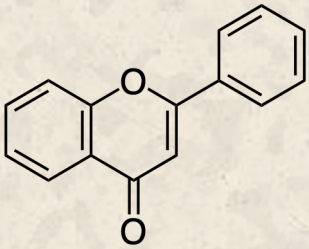
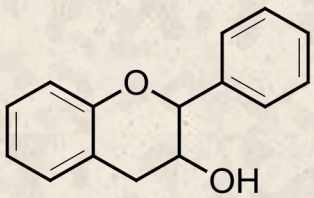
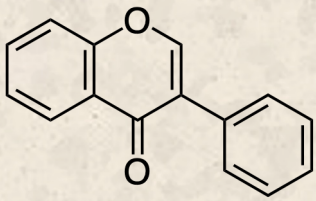
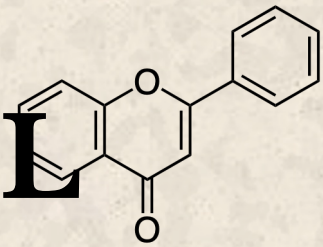
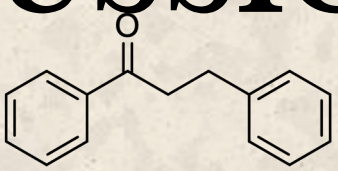
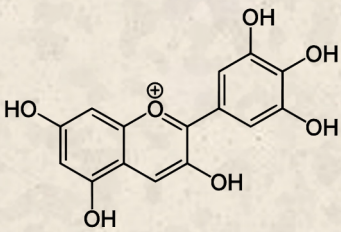
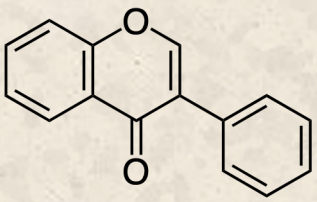
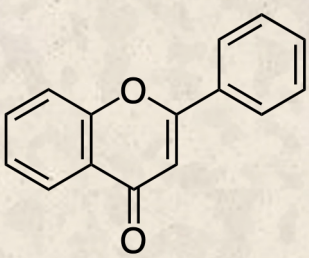
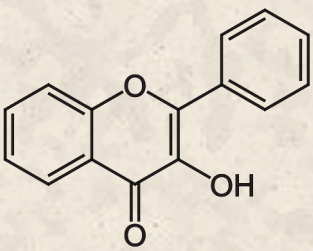
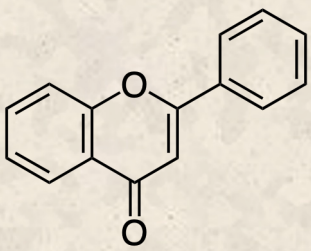


Figure S2. Distribution of phenolic compounds in local cherry (LC) and non-local cherry (NLC) quantified by HPLC-ESI-MS/MS grouped by a) anthocyanins, b) flavan-3-ols, c) flavonols, d) hydroxybenzoic acid, e) hydroxycinnamic acid and f) others. Data are presented as means ± SD (n=6) and expressed as percentages. * Indicates a significant difference ($P < 0.05$) between LC and NLC by Student's or Welch's *t*-test depending on homogeneity of variance.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

GENERAL DISCUSSION

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Phenolic compounds, commonly referred to as (poly)phenols, are natural chemicals synthesized by plants as a response to stress. These compounds exhibit potent antioxidant and anti-inflammatory properties, and their consumption has been linked to various health benefits, including the promotion of cardiovascular health and neuroprotection [1]. However, to fully understand these benefits, it is crucial to investigate their bioavailability. In this regard, several factors such as compound structure, environmental conditions, sex, age, gut microbiota and diet have been identified as critical determinants influencing the absorption and metabolism of these compounds [2–6]. In addition to these factors, biological rhythms, including circadian and seasonal rhythms, are emerging as important factors affecting the bioavailability and bioactivity of (poly)phenols [7]. Thus, disruption in these rhythms linked to lifestyle in contemporary societies such as exposure to artificial light, jet lag, and shift work, have been shown to affect metabolic status and gut microbiota composition, which therefore may affect (poly)phenols bioavailability.

Considering the previous background, the first objective of this thesis was to evaluate the influence of biological rhythms on the bioavailability and metabolism of (poly)phenols in the context of healthy and altered dietary habits (Chapter 1). Achieving this objective required careful considerations, including the selection of the appropriate animal strain, the determination of the adequate (poly)phenol dose, the selection of a robust model for metabolic syndrome (MetS), and the precise simulation of both circadian **[Manuscript 1]** and seasonal **[Manuscript 2]** rhythms. Fischer 344 rats were selected because they are characterized by a high degree of sensitivity to circadian rhythms [6]. The administered dose of GSPE (25 mg kg−1 BW per day) is equivalent to mirror 370 mg per day for an adult of 70 kg [8]. This dose falls within the

range of total (poly)phenol intake considered safe for humans, with doses up to 1 g per day of GSPE showing no detectable adverse effects [9]. To induce obesity and MetS, the cafeteria diet (CAF) model was employed [10]. This robust model involved the administration of high-palatable, high-caloric, and unhealthy human foods, such as bacon, biscuits with cheese or pâté, and pastries. This diet contributes to the development of insulin resistance (IR), dyslipidaemia, obesity and other comorbidities [11,12].

In this regard, we first aimed to evaluate whether administration time (circadian rhythms) significantly affects the bioavailability of GSPE-derived (poly)phenols [Manuscript 1]. To achieve this goal, female and male Fischer 344 rats were fed either a standard (ST) or a CAF diet for 9 weeks, and an oral dose of 25 mg/kg body weight of GPSE was daily administered either at ZT-0 (8 a.m.; light or resting phase) or at ZT-12 (8 p.m.; dark or active phase) during the last 4 weeks. After sacrifice by decapitation, plasma was obtained 3h after the last dose and consequently, phenolic compounds were quantified by liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). This chronic study highlights a significant impact of administration time on the plasma bioavailability of phase-II metabolites and their interactions with gut microbiota-derived phenolic metabolites in CAF-fed rats. Notably, higher concentrations of phase-II derived metabolites were observed when the GSPE was administered during the day phase (ZT-0) compared to the dark phase (ZT-12), with glucuronide metabolites being predominant. This aligns with previous research by Zhang *et al.*, which observed increased expression of carriers and phase-II enzymes during the light phase (ZT-0) in C57BL/6 mice, corresponding to their resting phase [13]. Similarly, Zmrzljak and Rozman noted maximal mRNA levels of UGTs during the light phase and SULTs

during the light to dark transition in rodents [14]. On the other hand, gut microbiota plays a crucial role in metabolizing phenolic compounds, thereby affecting their bioavailability and composition [15,16]. Variations in microbiota-derived metabolites, such as benzoic acid and its derivatives, were noted based on diet type, potentially stemming from shifts in bacterial strains involved in β-oxidation. Furthermore, the time of administration (ZT) was found to influence animals treated with a cafeteria diet. The increase could be due to the fact that the consumption of CAF diet strongly increased the Bacteroidetes/Firmicutes ratio exclusively during the active phase (ZT-12), but not during the resting phase (ZT-0), in F344 rats [17]. Thus, the CAF diet was able to significantly disrupt microbial rhythmicity. Furthermore, diet-induced obesity was found to impact the plasma bioavailability of phenolic acids and free flavan-3-ols, exhibiting higher levels in ST-fed rats compared to CAF-fed rats. Additionally, sex differences were only noted in the levels of certain microbiota-derived metabolites. Taken together, these findings underscore the intricate interplay between administration time, diet, metabolic syndrome, and microbiota composition in shaping phenolic compound metabolism.

Hence, following the study of circadian rhythms, **our subsequent goal was to evaluate the effect of circannual rhythms on the bioavailability of phenolic compounds [Manuscript 2].** To achieve this aim, male Fischer 344 rats were randomly allocated to various groups based on their respective diets (ST and CAF), photoperiods (L12, L6, L18), and treatments (GSPE and vehicle). The different photoperiod conditions were strategically chosen to mimic seasonal rhythms, with short photoperiods representing winter hours of light (L6, 6 hours of light), standard photoperiods simulating spring and autumn hours of light (L12,

12 hours of light), and long photoperiods emulating summer hours of light (L18, 18 hours of light). These photoperiods have been widely used, becoming a precise model to study seasonal rhythms effects on metabolic and physiologic processes [16,18–21]. The results showed that under standard health conditions, differences in light exposure significantly affected phenolic compound bioavailability, with rats under a 12-hour photoperiod (L12) exhibiting higher levels of total metabolites compared to shorter (L6) or longer (L18) photoperiods. However, this pattern was not observed in CAF-fed rats, suggesting that the influence of photoperiods is attenuated by the impact of obesogenic conditions. Flavan-3-ols and phenolic acids exhibited a photoperiod-dependent trend in CAF-fed rats, with the highest concentration found in the L6 photoperiod. This indicates that a longer active phase with expected increased food intake, may at some extent lead to higher (poly)phenol bioavailability. In this regard, recent findings by Soliz-Rueda *et al.* showed differences in energy intake among CAF-fed rats based on photoperiod, with greater intake under short light conditions [22]. This increase was primarily attributed to higher food consumption in rats under L6 conditions [23]. Phase-II metabolites were significantly influenced by dietary factors, showing higher bioavailability in ST-fed rats compared to CAF-fed rats. While variations in photoperiod did not produce significant differences in the ST group, rats on a CAF diet displayed notably higher bioavailability levels when exposed to L6 conditions. These findings are consistent with prior research [16,19] suggesting that metabolic and absorption processes are optimized during the active phase. Our results highlight the importance of longer active phase (L6) in maximizing metabolite levels, contrasting with lower concentrations observed during the longer resting phase (L18).

Moreover, it's noteworthy to consider the theory of xenohormesis, which proposes that phytochemicals in plants provide signals for organisms to adapt to external conditions, supporting the consumption of locally grown products [24–26]. In this context, grapes are fruits that typically grow under short photoperiods during their season, thus it is plausible that the bioavailability of (poly)phenols from grape seed proanthocyanidin extract (GSPE) is maximized during 6 and 12-hour light phase [16]. This aligns with the concept of xenohormesis, suggesting that organisms may adapt to environmental cues through the consumption of locally available produce, thereby enhancing the bioavailability of beneficial phytochemicals. Finally, microbial colonic metabolites showed diverse behaviour, indicating complex interactions between photoperiod and diet. These findings revealed that both photoperiod and dietary stress can alter the microbiota metabolic environment, influencing phenolic compound bioavailability and metabolism. Thus, this study showed that rats exposed to a 12-hour photoperiod and fed a standard diet had significantly higher bioavailability levels. However, this pattern was altered in rats fed with cafeteria diet, suggesting an attenuated influence of photoperiod under obesogenic conditions.

In these manuscripts, the impact of both circadian and seasonal rhythms on the bioavailability of phenolic compounds was evaluated. We found similarities between them, such as the high bioavailability of phase-II-derived metabolites following the ingestion of phenolic compounds, suggesting that the highest levels of flavan-3-ols and their phase II metabolites are achieved during the initial hours after ingestion [27–29]. Moreover, due to the high content of catechin and epicatechin in GSPE, the glucuronidated compounds derived from them were the most abundant, exerting the greatest influence on the group of phase-II

metabolites. Additionally, there is a common observation of the diverse behavior of colonic microbial metabolites, mainly influenced by metabolic stress impacting the microbiota and resulting in varied concentrations of bioavailable metabolites, indicating complex interactions between photoperiod and diet. However, despite both experiments sharing common features, they also present differences in the bioavailability and metabolite profile. Firstly, the concentration of bioavailable metabolites in both experiments is not entirely comparable as **Manuscript 1** is based on plasma while **Manuscript 2** is based on serum. This slight difference may lead to variations in the final quantification due to the extraction processes. Another important distinction is the impact of rhythms on phase-II metabolites metabolism. **Manuscript 1** demonstrated an influence of circadian rhythms on metabolites belonging to this group, whereas the seasonal rhythms in **Manuscript 2** showed no influence on the bioavailability of phase-II-derived metabolites. This can be due to UGT expression being mediated by light input rather than by number of active light hours, which may explain the absence of differences between photoperiods but variations due to the administration time. Additionally, the influence of health status on the bioavailability of phase-II metabolisms also exhibits discrepancies. In **Manuscript 2**, unlike **Manuscript 1**, significant differences were observed in the concentrations of phase-II metabolites among different health statuses, with a significantly lower bioavailability in the diet-induced obese rat group. These findings are consistent with those reported by other authors. Zhang, L *et al.* showed a decrease in the expression and activity of phase-II enzymes in the livers of diet-induced obese rats compared to lean control rats [30]. Similarly, Benjamin W Redan *et al.* highlighted the influence of obesity and diabetes on the expression and activity of UGT

enzymes, indicating complex and differential regulation of these enzymes under pathological conditions [31]. Therefore, as demonstrated in **Manuscript 2**, health status plays a fundamental role in the expression of these phase-II enzymes. Thus, the lack of significant differences in rats from **Manuscript 1** may be due to the early age of the rats, affecting the acquisition of the metabolic syndrome model. This theory is consistent with the findings of Souza *et al.* which reported a significant increase in weight in elderly mice fed with a high-fat diet compared to young mice, suggesting greater susceptibility to age- and diet-related metabolic changes [32].

After demonstrating how external factors such as circadian and circannual rhythms affect the metabolism and bioavailability of phenolic compounds, it becomes crucial to consider other potential factors. Among these are environmental conditions intrinsic to the growing location, which play a significant role in shaping the phenolic compounds biosynthetic pathway in plants [33–36]. When fruits are consumed, they carry a chemical signature reflecting the environmental state of their cultivation [25]. This concept aligns with the xenohormesis theory, suggesting that these signals provide beneficial responses, serving as an early warning system for external environmental conditions [24,25]. However, globalization has disrupted traditional practices of seasonal and local fruit consumption, disturbing natural cycles and leading to misguided xenohormetic responses. Consequently, these changes profoundly impact the bioactive compounds present in fruits and vegetables, particularly (poly)phenols [21,37–39]. Hence, we also planned the second objective of this thesis, which was to assess **if proximity consumption of cherries can influence the bioactivity and bioavailability of phenolic compounds** (**Chapter 2**)**.** In this context, **we first optimised the process of characterising the**

phenolic profile of sweet cherry cultivars from two different geographical origins [Manuscript 3]. To this purpose, the analysis of anthocyanins and non-anthocyanins in both local (LC) and non-local (NLC) sweet cherry extracts was optimised using HPLC-ESI-MS/MS. This allowed for the precise quantification of phenolic compounds, which is an advantage over studies that focus on only one family of compounds [40,41]. Our findings revealed a higher total content of (poly)phenols in local cherry extract, consistent with previous research demonstrating geographic variations in (poly)phenol profiles and their potential health effects [42,43]. Importantly, cherries sourced from distant locations, such as Chile, undergo extensive post-harvest treatments and long-distance transportation, significantly differing from locally sourced cherries, which do not undergo such treatments. Thus, the variations in phenolic profiles among extracts can be primarily attributed to surface damage caused by long-distance transportation and unripe stage, known as pitting [44,45]. This surface damage can lead to changes in phenolic profiles due to cell wall degradation and moisture loss [46]. These changes can potentially impact metabolic parameters and gene expression related to lipid metabolism [47] and oxidative stress [48]. Furthermore, non-local cherries with surface pitting exhibited significantly higher concentrations of phenolic acid. This could be attributed to the increased activity of phenylalanine ammonia-lyase (PAL) in response to mechanical damage, leading to the synthesis of hydroxycinnamic acids and derivatives that act as antioxidants. Moreover, anthocyanin levels are closely associated with fruit color, serving as a vital indicator of quality and ripeness in sweet cherries [49]. This relationship underscores the importance of optimal ripening conditions, which local cherries benefit from, unlike fruits subjected to ripening during long-distance transportation. Thus, these results highlight the

influence of geographic origin, ripening stage, and surface damage on the phenolic profiles of sweet cherries.

Once these two extracts from different origin were characterised, we evaluated the bioavailability and pharmacokinetic profiles of phenolic compounds after their acute consumption in rats [Manuscript 3]. To ensure that phenolic compound bioavailability was not affected by dose variations, we administered a carefully determined dose of 65 mg GAE/kg body weight. This dose was chosen to neutralize potential differences in phenolic content between cherries from different sources. The aim was to investigate whether, at an equal phenolic dose, the metabolization process differed. This approach allowed us to observe potential differences in metabolite levels, which would be more pronounced when comparing equal fruit quantities [50]. Our findings reveal that while previous research has emphasized anthocyanins and hydroxycinnamic acids as primary phenolic compounds in sweet cherries [51,52], microbiota-derived metabolites play a key role in after metabolization. Notably, rats administered with LC exhibited higher serum concentrations of anthocyanins and 4-hydroxybenzoic acid compared to those administered with NLC, suggesting a substantial impact of geographical origin on phenolic compound absorption and metabolism. These observations emphasize the importance of considering the intricate interplay between fruit origin, phenolic composition, and microbiota activity in shaping bioavailability in dietary strategies aimed at promoting human health. Our findings offer a novel perspective on phenolic compounds bioavailability variability and highlight the significance of fruit geographical origin in health-promoting dietary formulations.

Overall, the comprehensive investigation carried out in this thesis sheds light on the complex interplay between biological rhythms, dietary habits and geographical origin of fruits in shaping the bioavailability and metabolism of phenolic compounds. The first objective focused on elucidating the influence of circadian and seasonal rhythms on the bioavailability of (poly)phenols, revealed significant variations depending on the time of administration and exposure to photoperiod, especially under obesogenic conditions induced by a cafeteria diet. The second objective focused on the effect of the geographical origin of the fruit on the bioavailability of phenolic compounds, demonstrated significant differences in phenolic profiles and microbiota-derived metabolites between local and non-local cherry cultivars. These differences highlight the importance of fruit origin and ripening stage in shaping the absorption and metabolism of phenolic compounds. In conclusion, this work contributes to a better understanding of the multiple factors that influence the bioavailability, metabolism and thus bioactivity of phenolic compounds. It provides valuable information that will allow, firstly, to increase the knowledge in this area and, secondly, to suggest dietary strategies to take advantage of the health benefits of these bioactive compounds.

References

1. Del Rio, D. *et al.* Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal* **18**, 1818–92 (2013).

2. D'Archivio, M., Filesi, C., Varì, R., Scazzocchio, B. & Masella, R. Bioavailability of the polyphenols: status and controversies. *Int J Mol Sci* **11**, 1321–42 (2010).

3. Margalef, M. *et al.* Gender-related similarities and differences in the body distribution of grape seed flavanols in rats. *Mol Nutr Food Res* **60**, 760–772 (2016).

4. Margalef, M. *et al.* Age related differences in the plasma kinetics of flavanols in rats. **29**, 90–96 (2016).

5. Ozdal, T. *et al.* The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrients* vol. **8** (2016).

6. Carere, C. & Maestripieri, D. *Animal Personalities*. *Animal Personalities* University of Chicago Press, (2013).

7. Arola-Arnal, A. *et al.* Chrononutrition and polyphenols: Roles and diseases. *Nutrients* vol. **11** (2019).

8. Reagan-Shaw, S., Nihal, M. & Ahmad, N. Dose translation from animal to human studies revisited. *The FASEB Journal* **22**, 659–661 (2008).

9. Wang, D. *et al.* Role of intestinal microbiota in the generation of polyphenol-derived phenolic acid mediated attenuation of

Alzheimer's disease β-amyloid oligomerization. *Mol Nutr Food Res* **59**, 1025–1040 (2015).

10. Gual-Grau, A., Guirro, M., Mayneris-Perxachs, J., Arola, L. & Boqué, N. Impact of different hypercaloric diets on obesity features in rats: a metagenomics and metabolomics integrative approach. *Journal of Nutritional Biochemistry* **71**, (2019).

11. Sampey, B. P. *et al.* Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: Comparison to high-fat diet. *Obesity* **19**, 1109–1117 (2011).

12. Lalanza, J. F. & Snoeren, E. M. S. The cafeteria diet: A standardized protocol and its effects on behavior. *Neuroscience and Biobehavioral Reviews* vol. **122** (2021).

13. Zhang, Y. K. J., Yeager, R. L. & Klaassen, C. D. Circadian expression profiles of drug-processing genes and transcription factors in mouse liver. *Drug Metabolism and Disposition* **37**, 106–115 (2009).

14. Zmrzljak, U. P. & Rozman, D. Circadian regulation of the hepatic endobiotic and xenobitoic detoxification pathways: The time matters. *Chemical Research in Toxicology* vol. **25** 811–824 (2012).

15. Margalef, M., Pons, Z., Bravo, F. I., Muguerza, B. & Arola-Arnal, A. Plasma kinetics and microbial biotransformation of grape seed flavanols in rats. *J Funct Foods* **12**, 478–488 (2015).

16. Arreaza-Gil, V. *et al.* Gut Microbiota Influences the Photoperiod Effects on Proanthocyanidins Bioavailability in Diet-Induced Obese Rats. *Mol Nutr Food Res* **67**, (2023).

17. Gual Grau, A. Gut microbiota dysbiosis in diet-induced obesity. A focus on the influence of genetics, circadian rhythms and potential prebiotics. *TDX (Tesis Doctorals en Xarxa)* (2019).

18. Tavolaro, F. M., Thomson, L. M., Ross, A. W., Morgan, P. J. & Helfer, G. Photoperiodic Effects on Seasonal Physiology, Reproductive Status and Hypothalamic Gene Expression in Young Male F344 Rats. *J Neuroendocrinol* **27**, (2015).

19. Iglesias-Carres, L. *et al.* Exposure of Fischer 344 rats to distinct photoperiods influences the bioavailability of red grape polyphenols. *J Photochem Photobiol B* **199**, (2019).

20. Arreaza-Gil, V. *et al.* The effects of grape seed proanthocyanidins in cafeteria diet-induced obese Fischer 344 rats are influenced by faecal microbiota in a photoperiod dependent manner. *Food Funct* **13**, (2022).

21. Ibars, M. *et al.* Seasonal consumption of polyphenol-rich fruits affects the hypothalamic leptin signaling system in a photoperiod-dependent mode. *Sci Rep* **8**, (2018).

22. Soliz-Rueda, J. R. *et al.* Grape Seed Proanthocyanidins Mitigate the Disturbances Caused by an Abrupt Photoperiod Change in Healthy and Obese Rats. *Nutrients* **14**, (2022).

23. Soliz-Rueda, J. R. *et al.* Metabolism disturbance by light/dark cycle switching depends on the rat health status: the role of grape seed flavanols. *Food Funct* **14**, (2023).

24. Baur, J. A. & Sinclair, D. A. What is xenohormesis? *Am J Pharmacol Toxicol* **3**, 149–156 (2008).

25. Howitz, K. T. & Sinclair, D. A. Xenohormesis: Sensing the Chemical Cues of Other Species. *Cell* **133**, 387–391 (2008).

26. Hooper, P. L., Hooper, P. L., Tytell, M. & Vígh, L. Xenohormesis: Health benefits from an eon of plant stress response evolution. *Cell Stress and Chaperones* vol. **15** 761–770 (2010).

27. Monagas, M. *et al.* Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food and Function* vol. **1** 233–253 (2010).

28. Margalef, M. *et al.* Flavanol plasma bioavailability is affected by metabolic syndrome in rats. *Food Chem* **231**, 287–294 (2017).

29. Escobar-Martínez, I. *et al.* Administration Time Significantly Affects Plasma Bioavailability of Grape Seed Proanthocyanidins Extract in Healthy and Obese Fischer 344 Rats. *Mol Nutr Food Res* **66**, 2100552 (2022).

30. Zhang, L. *et al.* Diet-induced obese alters the expression and function of hepatic drug-metabolizing enzymes and transporters in rats. *Biochem Pharmacol* **164**, (2019).

31. Redan, B. W., Buhman, K. K., Novotny, J. A. & Ferruzzi, M. G. Altered transport and metabolism of phenolic compounds in obesity and diabetes: Implications for functional food development and assessment. *Advances in Nutrition* vol. **7** (2016).

32. Nunes-Souza, V. *et al.* Aging Increases Susceptibility to High Fat Diet-Induced Metabolic Syndrome in C57BL/6 Mice: Improvement in Glycemic and Lipid Profile after Antioxidant Therapy. *Oxid Med Cell Longev* **2016**, (2016).

33. Heimler, D., Romani, A. & Ieri, F. Plant polyphenol content, soil fertilization and agricultural management: a review. *European Food Research and Technology* vol. **243** (2017).

34. Asensio, E., Sanvicente, I., Mallor, C. & Menal-Puey, S. Spanish traditional tomato. Effects of genotype, location and agronomic conditions on the nutritional quality and evaluation of consumer preferences. *Food Chem* **270**, (2019).

35. Ballistreri, G. *et al.* Fruit quality and bioactive compounds relevant to human health of sweet cherry (Prunus avium L.) cultivars grown in Italy. *Food Chem* **140**, (2013).

36. Tomás-Barberán, F. A. *et al.* Health Benefits from Pomegranates and Stone Fruit, Including Plums, Peaches, Apricots and Cherries. in *Bioactives in Fruit* (2013).

37. Mariné-Casadó, R. *et al.* Cherry consumption out of season alters lipid and glucose homeostasis in normoweight and cafeteria-fed obese Fischer 344 rats. *Journal of Nutritional Biochemistry* **63**, (2019).

38. Gibert-Ramos, A., Palacios-Jordan, H., Salvadó, M. J. & Crescenti, A. Consumption of out-of-season orange modulates fat accumulation, morphology and gene expression in the adipose tissue of Fischer 344 rats. *Eur J Nutr* **59**, (2020).

39. Gibert-Ramos, A., Crescenti, A. & Salvadó, M. J. Consumption of cherry out of season changes white adipose tissue gene expression and morphology to a phenotype prone to fat accumulation. *Nutrients* **10**, (2018).

40. Nastić, N., Lozano-Sánchez, J., Borrás-Linares, I., Švarc-Gajić, J. & Segura-Carretero, A. New technological approaches for recovering bioactive food constituents from sweet cherry (Prunus avium L.) stems. *Phytochemical Analysis* **31**, 119–130 (2020).

41. Peixoto, J. *et al.* Cherry stem infusions: antioxidant potential and phenolic profile by UHPLC-ESI-QTOF-MS. *Food Funct* **11**, 3471–3482 (2020).

42. Cruz-Carrión, Á. *et al.* (Poly)phenolic composition of tomatoes from different growing locations and their absorption in rats: A comparative study. *Food Chem* **388**, 132984 (2022).

43. Cruz-Carrión, Á. *et al.* Tomatoes consumed in-season prevent oxidative stress in Fischer 344 rats: impact of geographical origin. *Food Funct* **12**, 8340–8350 (2021).

44. Toivonen, P. M. A., Kappel, F., Stan, S., McKenzie, D. L. & Hocking, R. Firmness, Respiration, and Weight Loss of `Bing', `Lapins' and `Sweetheart' Cherries in Relation to Fruit Maturity and Susceptibility to Surface Pitting. *HortScience* **39**, 1066–1069 (2004).

45. Fuentealba, C. *et al.* Cell wall and metabolite composition of sweet cherry fruits from two cultivars with contrasting susceptibility to surface pitting during storage. *Food Chem* **342**, 128307 (2021).

46. Correia, S., Schouten, R., Silva, A. P. & Gonçalves, B. Factors affecting quality and health promoting compounds during growth and postharvest life of sweet cherry (Prunus avium L.). *Front Plant Sci* **8**, 2166 (2017).

47. Ruiz de Azua, M. J., Cruz-Carrión, Á., Muguerza, B., Arola-Arnal, A. & Suarez, M. Seasonal consumption of cherries from different origins affects metabolic markers and gene expression of lipogenic enzymes in rat liver: A preliminary study. *Nutrients* **13**, 3643 (2021).

48. Cruz-Carrión, Á., de Azua, M. J. R., Mulero, M., Arola-Arnal, A. & Suárez, M. Oxidative stress in rats is modulated by seasonal consumption of sweet cherries from different geographical origins: Local vs. non-local. *Nutrients* **12**, 1–15 (2020).

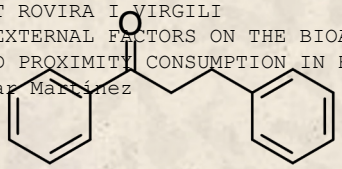
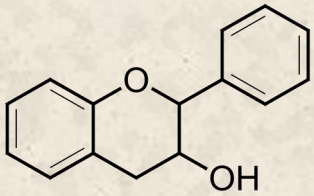
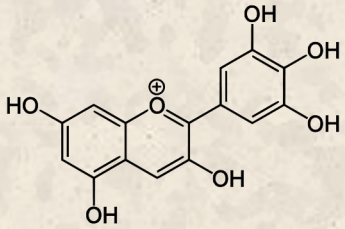
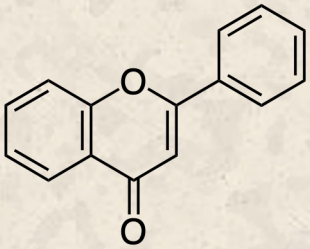
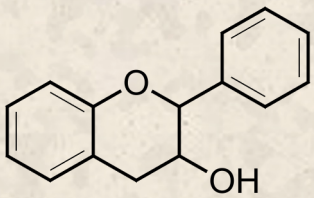
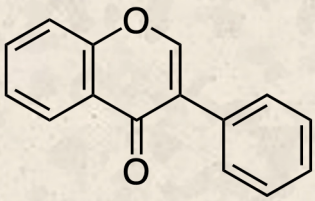
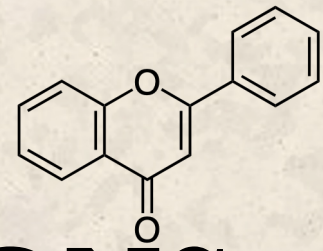
49. Habib, M., Bhat, M., Dar, B. N. & Wani, A. A. Sweet cherries from farm to table: A review. **57**, 1638–1649 (2017).

50. Iglesias-Carres, L. *et al.* A comparative study on the bioavailability of phenolic compounds from organic and nonorganic red grapes. *Food Chem* **299**, 125092 (2019).

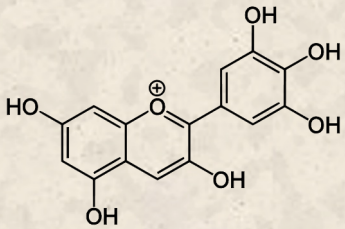
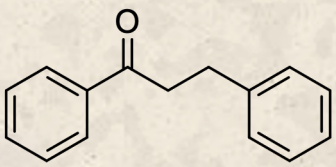
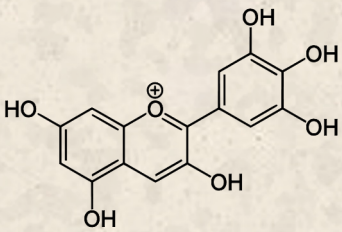
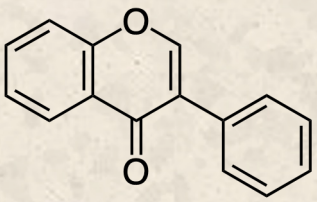
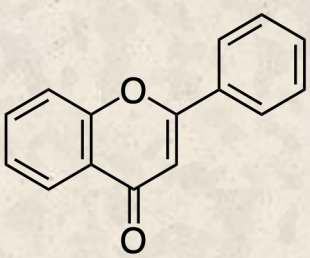
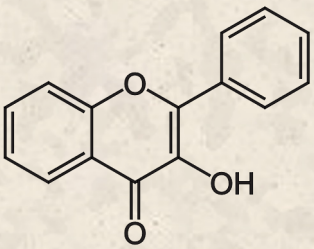
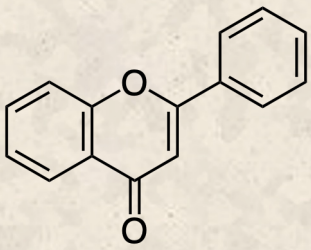
51. Iglesias-Carres, L. *et al.* Optimization and characterization of Royal Dawn cherry (Prunus avium) phenolics extraction. *Sci Rep* **9**, (2019).

52. Boskov, D. *et al.* The Phenolic Profile of Sweet Cherry Fruits Influenced by Cultivar/Rootstock Combination. *Plants* **12**, (2023).

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Considering all the results obtained in the different experiments carried out in this Doctoral Thesis the main conclusions drawn are presented below:

1. To evaluate the influence of biological rhythms on the bioavailability and metabolism of dietary (poly)phenols in the context of healthy and altered dietary habits (Chapter 1)

1.1. To elucidate whether GSPE administration time (circadian rhythms) significantly affects the bioavailability and metabolism of phenolic compounds [Manuscript 1].

- Administration timing (ZT) of phenolic compounds affects plasma bioavailability, especially in CAF diet-fed rats, highlighting the interaction between dietary habits and timing of administration.
- There is a significant influence of administration time on the plasma bioavailability of phase-II metabolites and gut microbiota-derived phenolic metabolites in cafeteria diet-fed rats.
- Phase-II derived metabolites exhibit higher average concentrations of total (poly)phenols during the resting phase (ZT-0) compared to the active phase (ZT-12).
- ZT influences the metabolites derived from gut microbiota, particularly in animals fed a cafeteria diet, possibly correlating with changes in the Bacteroidetes/Firmicutes ratio.

1.2. To evaluate the circannual rhythms effect on the bioavailability of phenolic compounds [Manuscript 2].

- Seasonal rhythms impact the bioavailability of phenolic compounds differently depending on health status and diet.

- In standard health conditions, differences in light exposure significantly affect phenolic compound bioavailability, with rats under a 12-hour photoperiod (L12) showing higher levels of total metabolites compared to shorter (L6) or longer (L18) photoperiods.
- The influence of photoperiods on phenolic compound bioavailability is diminished by the impact of obesogenic conditions in cafeteria diet-fed rats.
- Dietary factors significantly affect phase-II metabolites, with higher bioavailability in standard diet-fed rats compared to cafeteria diet-fed rats.
- The active phase (L6) within obesity status maximizes metabolite levels, contrasting with lower concentrations observed during the longer resting phase (L18). This suggests that (poly)phenol bioavailability from grape seed proanthocyanidin extract (GSPE) may be optimized during the 6-hour light phase (L6).
- Circadian rhythms influence the content of phase-II metabolites, while seasonal rhythms have no significant effect on phase-II-derived metabolites bioavailability. This indicates that UGTs expression may depend on light inputs rather than active light hours.

2. To determine if proximity consumption impacts on the bioactivity and bioavailability of phenolic compounds from sweet cherry (Chapter 2)

2.1. To characterize the phenolic profile of sweet cherry cv. from two geographical origins of cultivations [Manuscript 3].

- The phenolic profile of sweet cherries varies significantly due to factors such as geographical origin, ripening stage, and surface

damage, impacting the total content of (poly)phenols and specific phenolic compounds.

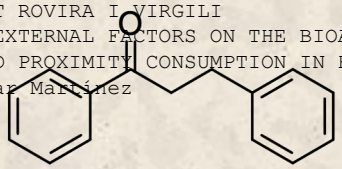
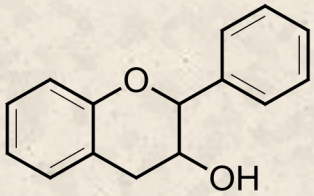
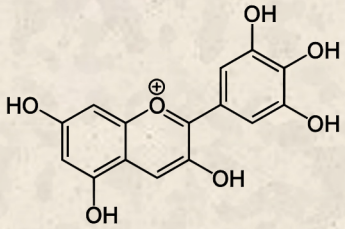
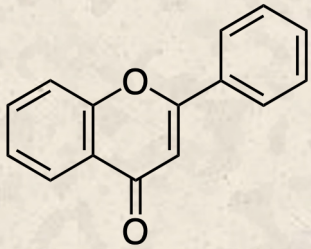
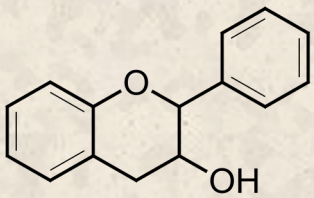
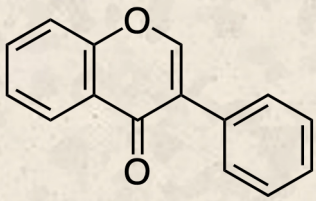
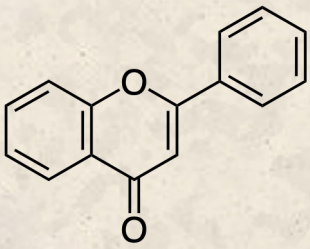
- Local cherries demonstrate higher phenolic content compared to non-local cherries, underscoring the importance of optimal ripening conditions and minimizing surface damage during transportation.
- The superior total (poly)phenol content in local cherry extract is mainly attributed to the absence of surface damage caused by long-distance transportation and an unripe stage known as "pitting".

2.2. To evaluate the bioavailability and pharmacokinetic profiles of phenolic compounds after acute consumption of sweet cherry cv. from two geographical origins of cultivation in rats [Manuscript 3].

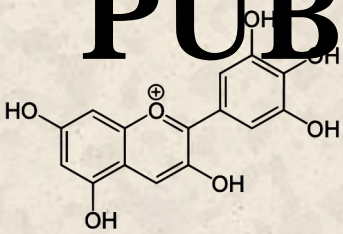
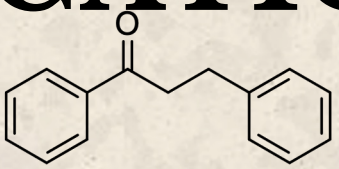
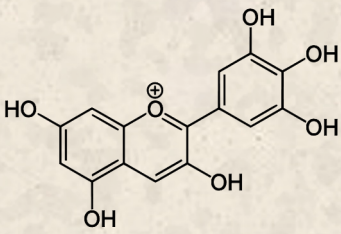
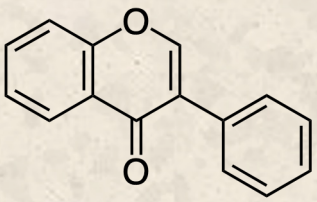
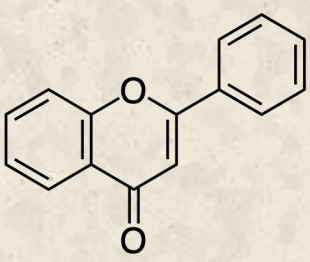
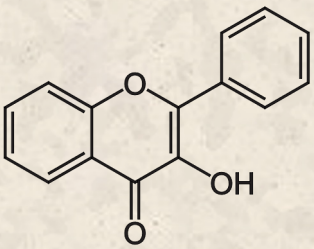
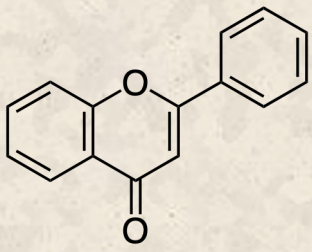
- Variations in the phenolic profile of sweet cherries from different geographical origins result in varying sweet cherry phenolic serum metabolite kinetic profiles in rats.
- The main discrepancies between local and non-local cherry extracts are predominantly observed in the anthocyanin group, with cyanidin-3-*O*-rutinoside being the major component.

The hypothesis presented in this doctoral thesis has been corroborated by the results obtained in the various experiments conducted. The factors studied, including biological rhythms such as circadian and seasonal rhythms, as well as the proximity of food consumption, have been demonstrated to significantly influence the bioavailability and metabolization of dietary (poly)phenols. Consequently, this research has contributed to a more comprehensive understanding of the mechanisms regulating the absorption and metabolism of (poly)phenols. It has also highlighted the importance of considering these factors in the design of dietary and agronomic interventions aimed at enhancing the bioavailability and, consequently, the bioactivity of polyphenols, which are linked to beneficial health effects.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

LIST OF PUBLICATIONS

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

PUBLISHED PAPERS

- **Escobar-Martínez, I.,** Arreaza-Gil, V., Muguerza, B., Arola-Arnal, A., Bravo, F. I., Torres-Fuentes, C*., Suárez, M. Administration Time Significantly Affects Plasma Bioavailability of Grape Seed Proanthocyanidins Extract in Healthy and Obese Fischer 344 Rats. *Molecular Nutrition & Food Research.* 2022 Feb, 66(3):e2100552. DOI: 10.1002/mnfr.202100552. IF (2021): 6.575. SI Journal Citation Reports © Ranking 21/143 (Q1) in Food Science & Technology

- Arreaza-Gil V., **Escobar-Martínez I.**, Muguerza B., Aragonès G., Suárez M., Torres-Fuentes C.*, Arola-Arnal A. The effects of Grape Seed Proanthocyanidins in cafeteria diet-induced obese Fischer 344 rats are influenced by faecal microbiota in a photoperiod dependent manner. *Food & Function*. 2022 Aug, 13(16):8363. DOI: 10.1039/D2FO01206E. IF (2021): 6.317. SI Journal Citation Reports © Ranking 67/296 (Q1) in Biochemistry & Molecular Biology and 24/143 (Q1) in Food Science & Technology.

- Arreaza-Gil V., **Escobar-Martínez I.**, Suárez M., Bravo F.I., Muguerza B., Arola-Arnal A.*, Torres-Fuentes C. Gut seasons: Photoperiod effects on fecal microbiota in healthy and cafeteria-induced obese Fisher 344 Rats. *Nutrients*. 2022 Feb, 14(3):722. DOI: 10.3390/nu14030722. IF (2021): 6.706. SI Journal Citation Reports © Ranking 15/90 (Q1) in Nutrition & Dietetics.

- Arreaza-Gil, V., **Escobar-Martínez, I**., Mulero, M., Muguerza, B., Suárez, M., Arola-Arnal, A., & Torres-Fuentes, C.

Gut Microbiota Influences the Photoperiod Effects on Proanthocyanidins Bioavailability in Diet-Induced Obese Rats. *Molecular Nutrition & Food Research*, 2022, *67*(9), 2200600. DOI: 10.1002/mnfr.202200600. IF (2022): 5.2. SI Journal Citation Reports © Ranking 34/142 (Q1) in Food Science & Technology.

- Arreaza-Gil, V., Ávila-Román, J., **Escobar-Martínez, I**., Muguerza, B., Suárez, M., Arola-Arnal, A., & Torres-Fuentes, C. (2023). Photoperiod Conditions Modulate Serum Oxylipins Levels in Healthy and Obese Rats: Impact of Proanthocyanidins and Gut Microbiota. *Nutrients*, *15*(3), 707. IF (2021): 5.9. SI Journal Citation Reports © Ranking 17/88 (Q1) in Nutrition & Dietetics.

- Zhang, Z., **Escobar-Martínez, I**., Malik, Z., Torres-Fuentes, C., Suárez, M., Bajka, B., Ellis P.R., & Rodriguez-Mateos, A. (2023). Oral and gastrointestinal bioaccessibility of anthocyanins in fresh, frozen, and blended blueberries. *Proceedings of the Nutrition Society*, *82*(OCE1), E10. IF (2022): 3.12.

IN PREPARATION/SUBMITTED PAPERS

- **Escobar-Martínez, I.,** Arreaza-Gil, V., Muguerza, B., Mulero, M., Arola-Arnal, A., Suárez, M.*, Torres-Fuentes, C. Circannual rhythms affect the bioavailability of phenolic compounds from grape seed proanthocyanidins extract

differently in healthy and obese Fischer 344 rats. [In preparation for J*ournal of Agricultural and Food Chemistry*].

- **Escobar-Martínez, I.,** Arreaza-Gil, V., Aragonès G., Mulero, M., Arola-Arnal, A., Torres-Fuentes C.*, Suárez, M. Impact of proximity on bioavailability of (poly)phenols from sweet cherry. [In preparation for *Journal of the Science of Food and Agriculture*].

- Arreaza-Gil V., **Escobar-Martínez I.**, Soliz-Rueda JR, Suárez M., Schellekens H.*, Torres-Fuentes C.*, Arola-Arnal A. Photoperiod effects on corticosterone and seasonal clocks in obese Fischer 344 rats are influenced by gut microbiota. [Submitted to *Scientific Reports*].

POSTER COMMUNICATIONS

- **Escobar-Martínez, I.,** Arreaza-Gil, V., Arola-Arnal, A., Torres-Fuentes, C., Suárez, M. Biological rhythms affect the bioavailability of phenolic compounds from grape seed proanthocyanidins extract in healthy and obese Fischer 344 rats. *The 18th International Conference on Food bioactives for diseases prevention-chrononutrition (NuGOweek)*. Tarragona (Spain), **2022**.

- Z. Zhang, **I. Escobar-Martínez**, Z. Malik, C. Torres-Fuentes, M. Suárez, B. Bajka, P.R. Ellis and A. Rodriguez-Mateos. Effects of mastication on anthocyanin bioaccesibility in fresh blueberries: application of harmonised INFOGEST protocol. *The 7th International Conference on Food Digestion (ICFD)*. Cork (Ireland), **2022**.

- Z. Zhang, **I. Escobar-Martínez**, Z. Malik, C. Torres-Fuentes, M. Suárez, B. Bajka, P.R. Ellis and A. Rodriguez-Mateos. Oral and gastrointestinal bioaccessibility of anthocyanins in fresh, frozen, and blended blueberries using the INFOGEST protocol. *The 10th International Conference on Polyphenols and Health (ICPH).* London (UK), **2022**.

- **Escobar-Martínez, I**., Rojas-Criollo, M., Cruz-Carrión, A., Bravo, F., Arola-Arnal. A., Suárez, M. Administration time significantly affects bioavailability of grape seed proanthocyanidins extract in Fischer 344 rats. I*CPH2019 KOBE The 9th International Conference on Polyphenols and Health*. Kobe (Japan), **2019.**

- Arreaza-Gil V, **Escobar-Martínez I**, Suárez M, Arola-Arnal A, Torres-Fuentes C. Gut microbiota dysbiosis in cafeteria fed rats under different photoperiods. *International Human Microme Consortium (IHMC), 8th* congress. Barcelona (España), **2021.**

- Arreaza-Gil V, **Escobar-Martínez I**, Suárez M, Torres-Fuentes C, Arola-Arnal A. Effects of a grape seed proanthocyanidin extract (GSPE) on cafeteria diet-induced obese rats: role of seasonal rhythms and gut microbiota. *I Jornadas Nutracéutica: Compuestos bioactivos y Nutracéuticos.* Tarragona (España), **2022.**

ORAL COMMUNICATIONS

- **Escobar-Martínez, I.,** Arreaza-Gil, V., Arola-Arnal, A., Torres-Fuentes, C., Suárez, M. Biological rhythms affect the bioavailability of phenolic compounds from grape seed proanthocyanidins extract in healthy and obese Fischer 344 rats. *The 18th International Conference on Food bioactives for diseases prevention-chrononutrition (NuGOweek).* Tarragona (Spain), **2022**.

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF EXTERNAL FACTORS ON THE BIOAVAILABILITY OF (POLY)PHENOLS: FOCUS ON BIOLOGICAL RHYTHMS AND PROXIMITY CONSUMPTION IN HEALTHY AND OBESE RATS
Iván Escobar Martínez

Phenolic compounds are substances produced by plants in response to stress. These compounds possess antioxidant and anti-inflammatory properties, and their consumption has been associated with various health benefits. However, a comprehensive understanding of these benefits requires an investigation into their bioavailability. Several factors, such as compound structure, environmental conditions, sex, gut microbiota, and diet, have been identified as influencing their absorption and metabolism. In addition to these factors, biological rhythms, including circadian and seasonal patterns, are emerging as crucial determinants in their effects. Disruptions in these rhythms associated with contemporary lifestyle factors have been shown to impact metabolic status, potentially influencing (poly)phenol bioavailability. Considering all of this, the main objective of this thesis is to evaluate whether the metabolism and bioavailability of (poly)phenols can be influenced by different biological rhythm patterns and proximity consumption in the context of healthy and altered dietary habits.

To achieve this, we first investigated the influence of circadian and seasonal rhythms on the bioavailability of phenolic compounds derived from grape seed proanthocyanidins extract (GSPE) in healthy and diet-induced obese rats. Subsequently, we characterized the phenolic profile of sweet cherry cultivars from two geographical origins and evaluated their bioavailability after acute consumption.

The results revealed significant effects of administration time and photoperiod on phenolic compound bioavailability highlighting the interplay between dietary habits and time of administration. Moreover, geographical origin significantly influenced the phenolic profile of sweet cherries, exhibiting the local cherries higher phenolic content. These findings underscore the importance of considering biological rhythms and environmental factors in the bioavailability of (poly)phenols, which may contribute to enhancing food consumption strategies and fostering a deeper understanding of the relationship between diet and health.