



Sex- and photoperiod-dependent differences in the bioavailability of (poly)phenols from mixtures of seasonal fruit extracts in obese rats

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ARTICLE INFO

Keywords:

Circannual rhythm
Sex differences
Fischer 344 rats
Male
Phenolic metabolites
Microbiota
Polyphenol-rich mixtures

ABSTRACT

Obesity and related metabolic disorders are highly prevalent worldwide. (Poly)phenols are bioactive compounds with health-promoting properties, but their activity depends on the processes of absorption, distribution, metabolism, and excretion (ADME), which are modulated by several individual factors, including sex, health status, and photoperiod. Previous studies revealed that photoperiod can influence the bioavailability and bioactivity of (poly)phenol-rich fruits. However, whether these effects extend to mixtures of seasonal fruit extracts remains unknown.

This study evaluated how sex and photoperiod affect the bioavailability of (poly)phenols from two multi-ingredient mixtures composed of (poly)phenol-enriched fruit extracts: a winter mixture (WM; grape and pomegranate) and a summer mixture (SM; plum and cherry). Both mixtures were administered to cafeteria diet-induced obese Fischer 344 rats chronically exposed to either a short-day (L6) or a long-day (L18) photoperiod.

The two mixtures exhibited markedly different (poly)phenolic profiles, with SM being richer in anthocyanins, hydroxycinnamic acids and total (poly)phenols, and WM containing higher amounts of flavan-3-ols and ellagitannins. The administration of the mixtures to the animals showed that the serum metabolite profiles were dominated by microbiota-derived metabolites. Specific microbial metabolites, unique to each mixture, were detected. Males and females had higher microbial metabolite levels under L6 and L18, respectively, and the administration of WM reduced body weight gain specifically in males under L18. In conclusion, photoperiod and sex jointly influence (poly)phenol bioavailability depending on the mixture of (poly)phenols.

1. Introduction

Obesity has become a major global health concern strongly associated with type 2 diabetes (T2D) and metabolic syndrome (MetS) (Obesity and overweight, 2026; Soares Andrade et al., 2023). The relationship between obesity and MetS is well-established, with excess body fat, particularly abdominal adiposity, contributing to insulin

resistance, dyslipidaemia, and hypertension (Alberti et al., 2009; Gunawan et al., 2021; Tune et al., 2017).

(Poly)phenols, widely distributed in fruits, vegetables, tea, and wine, are known for their beneficial effects in the prevention and management of various chronic diseases, including obesity and MetS (Jardon et al., 2024; Wang et al., 2014). However, their health effects largely depend on their absorption, distribution, metabolism, and excretion (ADME).

Abbreviations: ADME, absorption distribution metabolism and excretion; ANOVA, analysis of variance; BW, body weight; CAF, cafeteria; DAD, photodiode array detector; EICs, extracted ion chromatograms; F344, Fischer 344; GSPE, grape seed proanthocyanidins; HPG axis, hypothalamic-pituitary-gonadal axis; HPLC-MS/MS, high-performance liquid chromatography coupled with tandem mass spectrometry; IT, ion trap; L6, short photoperiod; L12, standard photoperiod; L18, long photoperiod; MetS, metabolic syndrome; PVDF, polyvinylidene difluoride filter; Q1, first quartile; Q3, third quartile; SM, summer mix; T2D, type 2 diabetes mellitus; UPLC-ESI-qTOF-MS, ultra-performance liquid chromatography coupled with electrospray ionisation/quadrupole-time-of-flight-mass spectrometry; WM, winter mix.

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<https://doi.org/10.1016/j.foodres.2026.119110>

Received 14 January 2026; Received in revised form 19 March 2026; Accepted 12 April 2026

Available online 14 April 2026

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After ingestion, only 5%–10% of (poly)phenols are absorbed in the small intestine, with deglycosylation occurring in those that are glycosylated. They are then subjected to phase II metabolism, producing sulfate, methyl, and glucuronide conjugates (Espín et al., 2024; Tomas-Barberan et al., 2018). The remaining (poly)phenols reach the colon, where they are metabolized by the gut microbiota, undergoing hydrolysis, reduction, decarboxylation, demethylation, oxidation, ring fission and dehydroxylation (Catalkaya et al., 2020a; Espín et al., 2024). As a result of those transformations, dietary (poly)phenols are converted to smaller microbial metabolites that can be absorbed and further metabolized in the liver (Cardona et al., 2013a; Iglesias-Aguirre et al., 2021). Ultimately, both phase II and microbial-derived metabolites are distributed to target tissues and then excreted via urine (Espín et al., 2024). Therefore, it is believed that the bioactive forms of (poly)phenols are the products of their metabolism (Iglesias-Aguirre et al., 2021).

Several intrinsic and extrinsic factors can modulate the bioavailability of dietary (poly)phenols (Iglesias-Aguirre et al., 2021). In fact, health status has been shown to influence the bioavailability profile of grape seed proanthocyanidins (GSPE) in male rats, both when comparing standard and cafeteria (CAF) diet-induced obesity (Margalef, Pons, Iglesias-Carres, Bravo, et al., 2017), and between normotensive and spontaneously hypertensive rats (Margalef, Pons, Iglesias-Carres, Quiñones, et al., 2017). Another factor known to modify (poly)phenols bioavailability is sex. In this sense, it has been described that male and female rats exhibit different metabolic profiles in plasma after (poly)phenol administration, also differing in the tissue distribution of phenolic metabolites (Margalef et al., 2016).

Biological rhythms further contribute to variations in (poly)phenol bioavailability. Among these, circannual rhythms refer to changes that occur over one year, including changes in seasonality, temperature, or food availability (Fatima & Rana, 2020; Zucker, 2001). These processes are primarily controlled and synchronized by light, which controls melatonin production during the dark phase (Dardente et al., 2014). Previous studies have shown that photoperiod (day length) can alter the ADME of GSPE linked to rhythmic changes in host metabolism and microbial activity in male obese rats (Arreaza-Gil et al., 2023). In addition, the bioavailability of red grape and tomato (poly)phenols was shown to be affected by different photoperiod conditions in healthy male rats (Cruz-Carrión et al., 2022; Iglesias-Carres, Mas-Capdevila, Bravo, Aragonès, et al., 2019; Iglesias-Carres, Mas-Capdevila, Bravo, Arola, et al., 2019).

Since fruits are rarely consumed in isolation, interactions among bioactive compounds from different fruits may modulate (poly)phenol bioaccessibility and bioactivity (Stelmach-Mardas et al., 2016). For instance, in vitro studies suggest that combining multiple dietary (poly)phenols can improve compound stability, enhancing accessibility and antioxidant activity (Ren et al., 2022).

We hypothesized that sex and photoperiod would differentially influence the bioavailability of seasonal fruit (poly)phenols in obese rats. Therefore, this study aimed to evaluate the influence of sex and photoperiod on the bioavailability of (poly)phenols from two seasonal fruit extract mixtures in CAF diet-induced obese Fischer 344 (F344) rats. A winter-typical mixture of grape and pomegranate extracts (winter mixture, WM) and a summer-typical mixture of plum and cherry extracts (summer mixture, SM) were chronically administered to male and female rats exposed to short-day (L6; 6 h light/day) or long-day (L18; 18 h light/day) photoperiods.

2. Materials and methods

2.1. Fruit extracts

Cherry (*Prunus avium* L. var. lapins; Sierra el Frasno, Zaragoza, Spain), plum (*Prunus domestica* L. var. black diamond; Ruidoms, Tarragona, Spain), grape (*Vitis vinifera* L. var. cabernet; Constantí, Tarragona, Spain), and pomegranate (*Punica granatum* L. var. tendral de Valencia;

Benissanet, Tarragona, Spain) were acquired from local producers in their corresponding season.

Fruit extracts were prepared using ethanol and formic acid, as described by Manocchio et al. (Manocchio et al., 2024). Whole fruits were processed as commonly consumed, including the skin, seeds, and pulp (except for cherry and plum, from which the seeds were removed, and for pomegranate, from which the skin was discarded).

Grape and pomegranate extracts were prepared using 65% ethanol (v/v) and 1% formic acid, with a solvent/solute ratio of 80 mL/g for 100 min at 72 °C. Cherry and plum extracts were performed using 72% ethanol (v/v) and 1% formic acid at a solvent/solute ratio of 20 mL/g at 55 °C for 20 min (for cherry) and 30 min (for plum). After extraction, the mixtures were centrifuged at 7871 xg for 10 min at 4 °C. Ethanol was removed using a Hei-VAP rotary evaporator (Heidolph, Schwabach, Germany) at 40 °C, and the samples were lyophilized. Dried extracts were stored at −20 °C.

The WM was elaborated with grape and pomegranate extracts, while the SM contained cherry and plum extracts. Both mixtures were prepared with the same concentration of each fruit extract and were diluted in water to a final concentration of 100 mg/mL for administration to animals. The proximate composition of each extract is shown in Table 1.

2.2. Fruit extract characterization using HPLC-DAD-IT-MS/MS

Each fruit extract (300 mg) was dissolved in 5 mL of methanol: DMSO:water (40:40:20) containing 0.1% HCl. Samples were mixed until complete solubilization, then centrifuged at 4000 xg for 10 min at room temperature. The supernatant was filtered through a 0.45 µm polyvinylidene difluoride (PVDF) filter before analysis by HPLC-MS/MS. Three replicates were analyzed for each fruit extract.

HPLC analyses were performed on an Agilent 1200 HPLC system with a photodiode array detector (DAD) (Agilent Technologies, Waldbronn, Germany) and an ion trap (IT) mass spectrometer detector in series (Bruker Daltonik, Bremen, Germany). A reverse-phase Poroshell 120 C18 column (100 × 3.0 mm, 2.7 µm) (Agilent Technologies) was used for the chromatographic separation with water:formic acid (99:1) (phase A) and acetonitrile (phase B) as mobile phases, delivered at a flow rate of 0.5 mL/min with the following elution profile: 0 min 1% phase B, 0–35 min, 1–30% phase B; 35–40 min, 30–90% phase B, 40–42 min, 90–1% B. Finally, the phase B content was decreased to the initial condition (1%) in 2 min, and the column was re-equilibrated for 4 min. The sample injection volume was 10 µL. The UV–Vis spectra were acquired in the range of 200–600 nm.

Nitrogen was used as both the drying and nebulizing gas at 65 psi, a flow rate of 11 L/min, and a temperature of 350 °C. The capillary voltage was set at 4000 V. MS spectra were recorded in negative and positive mode (for anthocyanins) with a full scan acquisition in the *m/z* range 100–1100 with a target mass of 500 *m/z*. The Auto-MSn mode was used to obtain information about the fragmentation patterns. The compounds were identified by analyzing their elution order, UV spectra, molecular weights, MS/MS fragmentation patterns, and, when possible, chromatographic comparison with authentic standards. The (poly)phenol compounds detected in the extracts are presented in Table S1.

Hydrolyzable tannins and ellagic acid derivatives were quantified at 280 nm using ellagic acid. Isoflavones were quantified at 260 nm using genistein. Flavan-3-ols were quantified at 280 nm using catechin. Hydroxycinnamic acids were quantified at 320 nm using 3,4-dihydroxycinnamic acid (caffeic acid). Flavanones were quantified at 340 nm using hesperidin. Flavonols were quantified at 360 nm using quercetin 3-O-rutinoside (rutin). Anthocyanins were quantified at 520 nm using cyanidin 3-O-rutinoside.

Proanthocyanidin products in grape extract were analyzed after phloroglucinolysis by HPLC-DAD-IT-MS/MS. The compounds detected in this extract are presented in Table S2. The same procedure was also applied to plum extract. However, no additional relevant information was obtained beyond the initial phenolic characterization (data not

Table 1

Proximate composition of the (poly)phenol-enriched fruit extracts.

	Grape	Pomegranate	Winter Mixture	Cherry	Plum	Summer Mixture	p-value
Protein	0.18 ± 0.02	0.48 ± 0.08	0.33 ± 0.04	0.85 ± 0.02	0.42 ± 0.01	0.64 ± 0.01	0.0002
Fat	9.82 ± 0.25	2.46 ± 0.30	6.10 ± 0.20	4.19 ± 2.50	6.27 ± 0.87	5.23 ± 1.32	0.3221
Total carbohydrates, by difference	77.14 ± 3.58	90.63 ± 1.52	83.89 ± 1.95	89.86 ± 2.77	88.60 ± 1.76	89.23 ± 1.64	0.0222
Insoluble fiber	9.21 ± 3.39	2.41 ± 0.12	5.81 ± 1.70	2.13 ± 0.96	2.59 ± 0.98	2.36 ± 0.69	0.0312
Soluble fiber	2.49 ± 1.12	2.95 ± 1.48	2.72 ± 0.93	1.95 ± 0.67	1.22 ± 1.14	1.59 ± 0.66	0.1613
Ash	1.16 ± 0.01	1.07 ± 0.02	1.12 ± 0.01	1.02 ± 0.27	0.90 ± 0.31	0.96 ± 0.21	0.2579

Values are expressed as the mean (g/100 g dried extract) ± standard deviation ($n = 3$). p -value column shows the comparisons between Winter Mixture and Summer Mixture using two-tailed Student's t -test. The total carbohydrate content was determined by subtracting the sum of the other compounds. The composition of grape, pomegranate, cherry and plum were previously determined by Manocchio et al. (Manocchio et al., 2024). The total composition of the Winter Mixture and Summer Mixture was calculated as the arithmetic mean of the individual proximal compositions of grape and pomegranate (Winter Mixture) and cherry and plum (Summer Mixture), assuming equal proportional contribution of each extract.

shown).

2.3. Animal experimental design

A total of 120 F344 rats (60 males and 60 females), 12 weeks of age, with body weight (BW) between 251 ± 15 g for males and 155 ± 7 g for females, were purchased from Charles River Laboratories (Barcelona, Spain) and housed by pairs at 22 °C and 50% humidity under standard light-darkness conditions (L12; 12 h light/day) under a reverse light cycle (lights off at 8:00 a.m.). The number of animals per group ($n = 10$) was determined by a priori power analysis ($\alpha = 0.05$, $\beta = 0.2$), ensuring 80% power to detect relevant physiological differences between experimental conditions (McConnell et al., 2019).

The animals were fed a CAF diet and tap water ad libitum for 10 weeks (Fig. 1). Diet was prepared daily and administered 1 h after the lights were turned off. The CAF diet is a high-caloric and palatable diet composed of human foods (58% carbohydrates, 11% protein, and 31% lipids) (Gomez-Smith et al., 2016). The CAF diet consisted of milk with 22% sucrose (w/v), a biscuit with pâté and cheese, pork bacon, ensaimada (a type of pastry), carrots, and a standard chow diet. After 3 weeks of dietary adaptation, the animals were exposed to either a short photoperiod (L6; 6 h light/day) or a long photoperiod (L18; 18 h light/day), simulating the light hours during the winter and summer seasons,

respectively. Following 3 additional weeks of photoperiod adaptation (week 6 of the experiment), 60 rats per photoperiod ($n = 10$ each sex) were administered 100 mg/kg bw of WM, SM, or water (vehicle) daily for 30 days. The selected dose was based on a previous study conducted by our group using the individual extracts under L6 and L18 photoperiods, in which this dose showed significant metabolic effects (Manocchio et al., 2024). This dose was approximately equivalent to 1.46 g of extracts for a 70 kg person, according to the conversion proposed by Reagan-Shaw et al. (Reagan-Shaw et al., 2008). Dose administration was performed by voluntarily licking a syringe 1 h after the lights were turned off, following administration of the CAF diet. BW and food intake were recorded weekly during the experiment. For food intake, the weight of each dietary component was measured over 24 h, assuming equal consumption by both rats within each cage, and the total kcal was calculated based on the manufacturer's nutritional values.

After 30 days of oral mixture administration, animals were deprived of food at 8:00 a.m. (lights off), and each mixture was administered 1 h later. At 11:00 a.m., blood from the saphenous vein was collected in non-heparinized tubes and centrifuged at 1200 $\times$ g for 15 min to separate the serum.

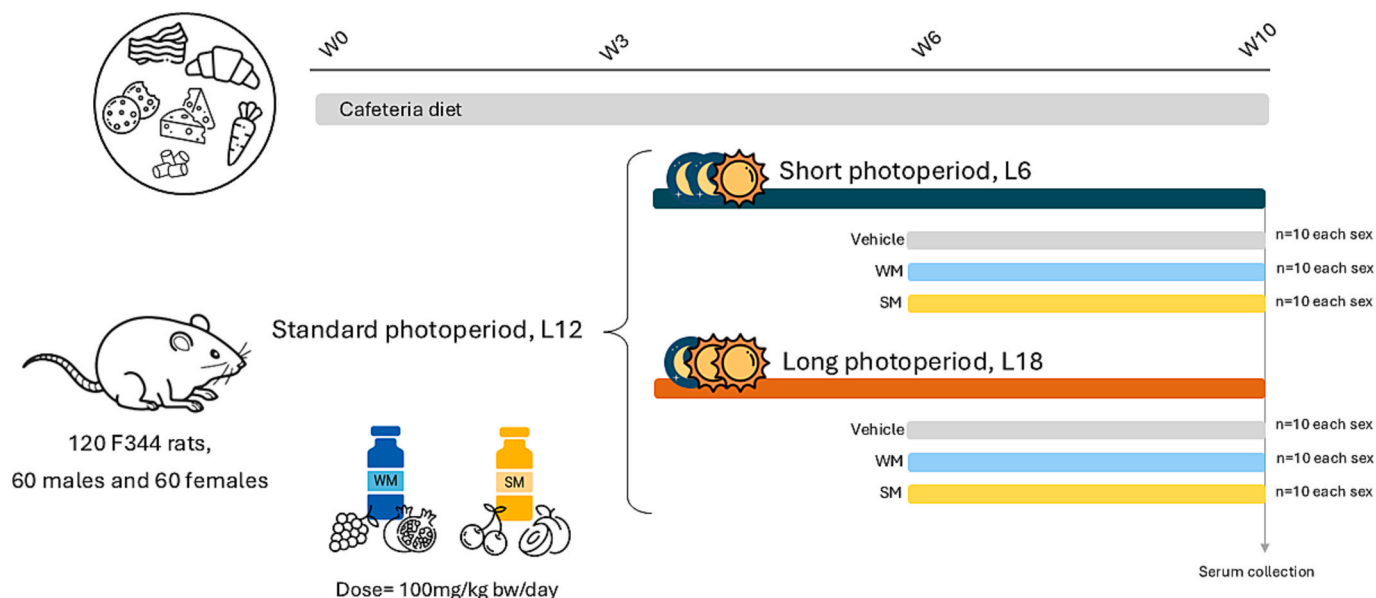


Fig. 1. Experimental design used in the study. Rats were fed a cafeteria (CAF) diet for 10 weeks and exposed to either a short photoperiod of 6/18 h light/darkness (L6) or a long photoperiod of 18/6 h light/darkness (L18) for the last 8 weeks. A winter mixture (WM) of phenolic-enriched grape and pomegranate extracts, and a summer mixture (SM) of phenolic-enriched cherry and plum extracts, were administered orally once daily at a dose of 100 mg/kg of body weight for the last 4 weeks, coinciding with light onset. L12, 12 h of light and 12 h of darkness.

2.4. Ethical statement

All experimental procedures involving animals were conducted in accordance with the ARRIVE guidelines. The study was conducted following the European Communities Council Directive (86/609/EEC) and approved by the Animal Ethics Review Committee for Animal Experimentation of the Universitat Rovira i Virgili (Tarragona, Spain) and Generalitat de Catalunya (permission number 11610). All efforts were made to minimize animal suffering and to reduce the number of animals used. Both male and female Fischer 344 rats were included in the study, and the influence of sex on the outcomes was considered in the analysis.

2.5. Determination and quantification of serum (poly)phenol metabolites using UPLC-ESI-qTOF-MS

Phenolic compounds and derived metabolites from serum samples (200 μ L) were extracted using 600 μ L of acetonitrile:formic acid (98:2). Hesperetin (20 μ L of 1 μ M) was added as an internal standard. Samples were vortexed for 1 min and centrifuged at 14,000 $\times$ g for 15 min at 4 °C. The supernatant was evaporated to dryness using a speed-vacuum concentrator, and the residue was reconstituted in 100 μ L of methanol followed by filtration through a 0.22 μ m PVDF filter. For injection, 50 μ L of the reconstituted sample was transferred to a vial and spiked with 5 μ L of isourulithin A 3-O-glucuronide (internal standard for injection control). The analyses were performed on an Agilent 1290 Infinity UPLC system coupled to a 6550 Accurate-Mass quadrupole-time-of-flight (qTOF) mass spectrometer (Agilent Technologies, Waldbronn, Germany) using an electrospray interface (Jet Stream Technology). The parameters of the analysis method are detailed elsewhere (Ávila-Gálvez et al., 2019).

The metabolites were quantified whenever possible with their corresponding standards. When authentic standards were unavailable, the metabolites were tentatively identified and expressed as peak areas of extracted ion chromatograms (EICs). Positional isomers were assigned only when confirmed with an authentic standard or, in specific cases, when comparison with an available isomeric standard allowed exclusion of an alternative structure. Accordingly, some metabolites were tentatively annotated by exclusion of the alternative isomeric option. Serum metabolites monitored by UPLC-qTOF-MS are presented in Table S3 and those metabolites identified in serum are presented in Table S4. Mass Hunter Qualitative Analysis software version 08.00 (Agilent Technologies) was employed for data analysis.

2.6. Statistical analysis

Data in the area under the curve (AUC) graph of BW gain are presented as median as box-and-whisker plots showing the median, interquartile range (first quartile (Q1) and third quartile (Q3)), and minimum to maximum values ($n = 10$). Proximal composition of fruit extracts and (poly)phenol concentrations in each fruit extract are presented as mean $\pm$ standard deviation ($n = 3$). Concentrations of (poly)phenol metabolites in serum are presented as median (Q1 – Q3).

Two- and three-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test, were performed to evaluate differences among more than two groups. To analyse the effect of each treatment on body weight gain, an unpaired two-tailed Student's *t*-test was performed between each treatment and its corresponding control group. BW evolution was analyzed using a two-way repeated-measures ANOVA with photoperiod, sex, and treatment as factors. Post hoc multiple comparisons were performed using the Tukey method.

Statistical analysis was performed using GraphPad Prism software (V9; Dotmatics). Two-sided *p*-values <0.05 were considered statistically significant, while *p*-values between 0.1 and 0.05 were interpreted as a trend.

3. Results

3.1. (Poly)phenolic composition of the fruit mixtures

The total content of (poly)phenols varied between the two different fruit mixtures, with the SM being richer in (poly)phenols than the WM. This difference was mainly driven by the lower amount of total (poly)phenols contained in the pomegranate extract (10.66 mg/g extract) (Fig. 2). In addition, each mixture showed a distinct phenolic profile (Fig. 2 and Table 2). The WM was particularly enriched in flavan-3-ols, whereas the SM contained higher concentrations of anthocyanins and hydroxycinnamic acids. The SM had higher concentrations of anthocyanidins than WM, but with less diversity. In this regard, the SM contained three different anthocyanidins (cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside, and peonidin 3-O-rutinoside, being the most abundant cyanidin 3-O-rutinoside), which were present in both cherry and plum. In contrast, 8 different anthocyanins were present in WM, with only cyanidin 3-O-glucoside being common to both WM and SM.

Ellagic acid and its derivatives were exclusively found in the WM, specifically from pomegranate extracts.

Regarding flavan-3-ols, they were detected in both WM and SM, but were identified and quantified only in the grape and plum extracts. In the case of the grape extract, a phloroglucinolysis analysis was performed to detect procyanidins not identifiable via HPLC-DAD-IT-MS/MS. This extract had a high concentration of polymerized catechin with a mean degree of polymerization of 7.5 (Table 2). Catechin was the most abundant (adduct 19.28 ± 5.09 mg/g and monomer 1.64 ± 0.25 mg/g), followed by lower amounts of catechin gallate (0.34 ± 0.02 mg/g adduct and monomer 0.26 ± 0.03 mg/g). Several B-type procyanidin dimers and trimers were identified in both WM and SM. In contrast, procyanidin A was detected only in the SM, and epicatechin was present only in the WM.

Naringin, the only flavanone identified in the WM grape, and genistein 7-O-glucoside, the only isoflavone quantified, were found in the SM, present in cherry extract. Flavonol content was higher in SM, with characteristic compounds including kaempferol 3-O-rutinoside, quercetin 3-O-xyloside, quercetin-deoxyhexoside, quercetin 3-O-rutinoside-7-O-glucoside, and quercetin 3-O-glucoside. In contrast, the concentration of flavonols in WM was lower, as these compounds were absent in the pomegranate extract. However, laricitrin 3-O-hexoside, myricetin, and quercetin 3-O-glucoside were specific to the WM grape extract. Quercetin 3-O-rutinoside was detected in both mixtures.

Interestingly, hydrolyzable tannins, specifically punicalagin α and punicalagin β , were only detected in the WM. Finally, hydroxycinnamic acids were the only phenolic subclass present in both extracts of the SM, but they were not detected in WM.

3.2. Impact of the mixtures on the body weight gain

The evolution of BW in male and female F344 rats across the experimental weeks is shown in Fig. 3A (*p*-values from repeated measures analysis are provided in Table S5). The analysis revealed a strong effect of sex and photoperiod on BW progression, whereas no significant effect of the mixtures was detected on a week-by-week basis.

However, when BW gain was evaluated as AUC, a significant reduction was observed in males under the L18 photoperiod following WM administration compared with their vehicle-treated counterparts (Fig. 3B). Consistent with the evolution analysis, a significant interaction between these factors was also evident, with lower BW gain in females compared to males and in males exposed to L6 relative to L18. No statistical differences in food intake were observed (data not shown).

3.3. Serum bioavailability

The (poly)phenols detected in serum reflect a combination of metabolites derived from the last administered dose (2 h before blood

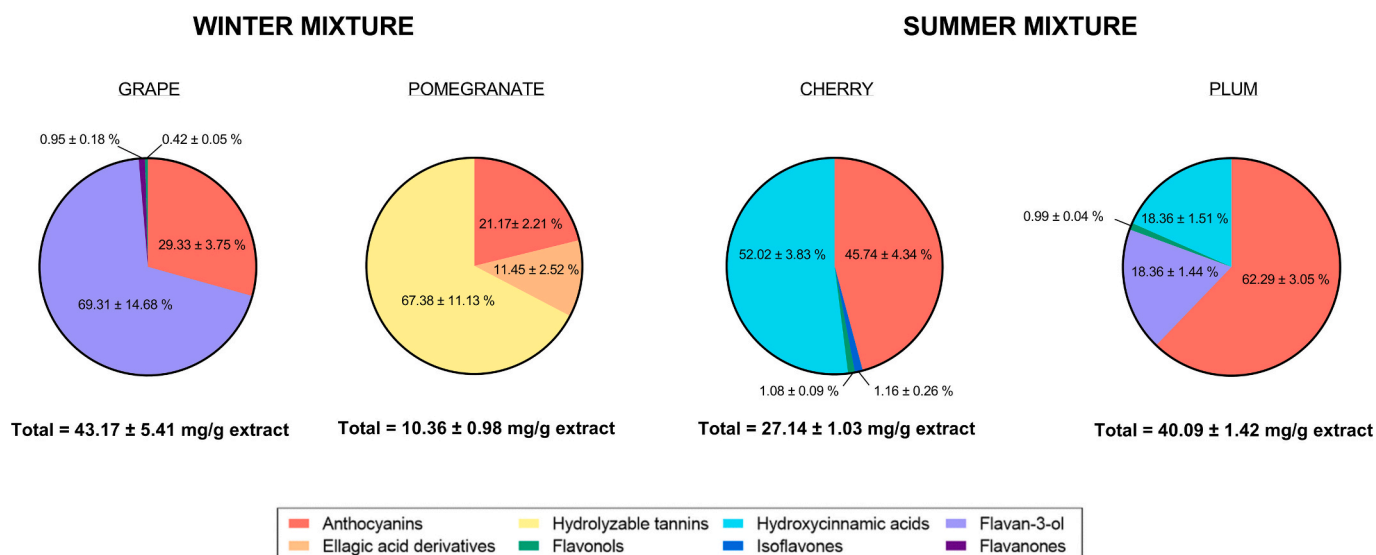


Fig. 2. The (poly)phenolic composition in each phenolic-enriched fruit extract was quantified by HPLC-DAD-IT-MS/MS. Values are represented as percentages ± standard deviation ($n = 3$).

extraction) and those resulting from the dose administered the day before (26 h prior). Interestingly, chronic administration of either WM or SM resulted in distinct (poly)phenolic metabolite serum profiles, which were influenced by the photoperiod exposure (L6 or L18) and sex (Table 3, Fig. 4).

A total of 17 microbial-derived metabolites were identified and quantified in serum samples, with their presence and levels varying across experimental groups. Most of them were microbial metabolites, including phase II conjugates (such as sulfates and glucuronides).

Notably, certain compounds were found exclusively in specific experimental conditions, while others were broadly distributed across groups. Moreover, metabolite diversity and concentration patterns revealed a marked influence of photoperiod and sex.

3.3.1. Winter mixture serum metabolites: Effect of sex and photoperiod

The analysis of serum metabolites in male and female rats fed the CAF diet under both L6 and L18 conditions, when WM was administered, revealed a clear interaction between sex and photoperiod. WM administration in male rats under L6 photoperiod resulted in the formation of 13 metabolites, whereas only 3,4-dimethoxybenzoic acid was detected under L18 conditions. Conversely, in female rats, the metabolic pattern was reversed; i.e., under L6, only 3,4-dimethoxybenzoic acid, 2-hydroxybenzene-1-sulfate, and hippuric acid were found, while L18 exposure led to the detection of 11 different metabolites.

Among the 13 metabolites detected in male rats under L6 conditions, phenylacetic acid sulfate, 3-(3'-hydroxyphenyl)propanoic acid, 5-(hydroxyphenyl)valeric acid-glucuronide (isomer), and 3-(4'-methoxyphenyl)propanoic acid-3'-sulfate were only detected under this photoperiod condition. In contrast, 2,6-dihydroxybenzoic acid, hydroxyphenyl propanoic acid sulfate, (R/S)-5-(3',4'-dihydroxyphenyl)valeric acid, 4-hydroxybenzaldehyde, 1,3,5-trihydroxybenzene, and 1-(hydroxyphenyl)-3-(2'',4'',6''-trihydroxyphenyl)-propan-2-ol were found in female rats under L18 conditions.

2-Hydroxybenzene-1-sulfate and hippuric acid were present in all groups except L18-exposed males. Interestingly, 3,4-dimethylbenzoic acid was detected in all WM groups, with differences in abundance depending on sex and photoperiod. However, no statistical interaction was observed between these factors.

3.3.2. Summer mixture serum metabolites: Effect of sex and photoperiod

Similar sex-photoperiod interactions were observed in the serum metabolites following SM administration. The most diverse serum

metabolite profiles were found in males under the L6-exposed photoperiod and females under the L18 photoperiod. In contrast, only a few metabolites were identified in males under L18 or females under L6 photoperiod conditions. Specifically, the SM administered in male rats exposed to L6 conditions generated 11 different metabolites, whereas only 4'-methoxycinnamic acid-3'-glucuronide was detected under L18 photoperiod exposure. Otherwise, in females under L6, only 3 metabolites were found: hydroxyphenyl propanoic acid sulfate, hippuric acid, and 4'-methoxycinnamic acid-3'-glucuronide.

From the 11 metabolites detected in males under L6 photoperiod conditions, 3,4-dimethoxybenzoic acid, phenylacetic acid sulfate, 3-(3'-hydroxyphenyl)propanoic acid, (R/S)-5-(3',4'-dihydroxyphenyl)valeric acid, 4-hydroxybenzaldehyde, 1,3,5-trihydroxybenzene were only present in this group, while 2,6-dihydroxybenzoic acid, 5-(hydroxyphenyl)valeric acid-glucuronide (isomer) and 2-hydroxybenzene-1-sulfate were also detected in females exposed to L18 photoperiod. Interestingly, all the above-mentioned metabolites presented a strong interaction between sex and photoperiod when SM was administered. The two ferulic acid metabolites, 3-(4'-methoxyphenyl)propanoic acid-3'-sulfate and 3'-methoxycinnamic acid-4'-sulfate, were only detected in females exposed to L18 photoperiod, and hydroxyphenyl propanoic acid sulfate was detected in all groups but males L18. All those metabolites also showed an interaction between sex and photoperiod. Interestingly, hippuric acid was detected only in females after SM administration, regardless of the photoperiod, although an interaction between the two factors was observed. Notably, 4'-methoxycinnamic acid-3'-glucuronide was the only SM-derived metabolite consistently detected across all experimental groups, irrespective of sex or photoperiod.

3.3.3. Interactions between mixture extracts, sex, and photoperiod in serum metabolites

Serum metabolite profiles revealed correlations between sex, photoperiod, and treatments. Several metabolites showed a three-way interaction involving those three parameters, including hydroxybenzoic acid glucuronide (isomer), dihydroxybenzoic acid sulfate (isomer), 3,4-dimethoxybenzoic acid, 3'-methoxycinnamic acid-4'-sulfate, 1,3,5-trihydroxybenzene, 1-(hydroxyphenyl)-3-(2'',4'',6''-trihydroxyphenyl)-propan-2-ol, and 4'-methoxycinnamic acid-3'-glucuronide (Table 3). For instance, the benzoic acid derivatives, hydroxybenzoic acid glucuronide (isomer) and dihydroxybenzoic acid sulfate (isomer), were only present in females exposed to the L18 photoperiod condition and treated with WM. In contrast, 3'-Methoxycinnamic acid 4'-sulfate

Table 2

(Poly)phenolic compounds in dried phenolic-enriched fruit extracts quantified by HPLC-DAD-IT-MS/MS, expressed as mg/g of dry extract.

	Winter mixture		Summer mixture		p-value
	Grape	Pomegranate	Cherry	Plum	
Σ Anthocyanins	12.66 ± 0.32	2.25 ± 0.11	12.42 ± 1.08	24.93 ± 0.81	0.5380
Cyanidin 3-O-glucoside	n.d.	1.53 ± 0.13	n.d.	16.11 ± 0.46	<0.0001
Cyanidin 3-O-rutinoside	n.d.	n.d.	10.97 ± 1.20	8.82 ± 0.44	<0.0001
Delphinidin 3,5-O-diglucoside	n.d.	0.37 ± 0.01	n.d.	n.d.	<0.0001
Delphinidin 3-O-galactoside	0.68 ± 0.03	n.d.	n.d.	n.d.	<0.0001
Malvidin 3-O-(6'-p-coumaroyl)-glucoside	1.91 ± 0.07	n.d.	n.d.	n.d.	<0.0001
Malvidin 3-O-acetylmonoglucoside	3.20 ± 0.05	n.d.	n.d.	n.d.	<0.0001
Malvidin 3-O-glucoside	6.22 ± 0.20	n.d.	n.d.	n.d.	<0.0001
Pelargonidin 3-O-glucoside	n.d.	0.35 ± 0.03	n.d.	n.d.	<0.0001
Peonidin 3-O-rutinoside	n.d.	n.d.	1.45 ± 0.24	n.d.	<0.0001
Petunidin 3-O-glucoside	0.65 ± 0.03	n.d.	n.d.	n.d.	<0.0001
Σ Ellagic acid derivatives	n.d.	1.22 ± 0.24	n.d.	n.d.	0.0002
Ellagic acid	n.d.	0.24 ± 0.04	n.d.	n.d.	<0.0001
Ellagic acid derivative	n.d.	0.49 ± 0.15	n.d.	n.d.	<0.0001
Ellagic acid hexoside	n.d.	0.49 ± 0.12	n.d.	n.d.	<0.0001
Σ Flavan-3-ols	3.86 ± 0.36	n.d.	n.d.	7.37 ± 0.54	<0.0001
Epicatechin	0.54 ± 0.18	n.d.	n.d.	n.d.	<0.0001
Catechin	0.63 ± 0.14	n.d.	n.d.	0.63 ± 0.05	<0.0001
Procyanidin A	n.d.	n.d.	n.d.	0.92 ± 0.31	<0.0001
Procyanidin A-type dimer	n.d.	n.d.	n.d.	2.03 ± 0.08	<0.0001
Procyanidin B	0.28 ± 0.04	n.d.	n.d.	0.56 ± 0.21	0.0005
Procyanidin B	0.28 ± 0.10	n.d.	n.d.	n.d.	<0.0001
Procyanidin B	n.d.	n.d.	n.d.	0.68 ± 0.18	<0.0001
Procyanidin B	0.52 ± 0.20	n.d.	n.d.	n.d.	<0.0001
Procyanidin B	0.39 ± 0.11	n.d.	n.d.	1.32 ± 0.16	<0.0001
Procyanidin C	n.d.	n.d.	n.d.	0.61 ± 0.11	<0.0001
Procyanidin C	1.22 ± 0.13	n.d.	n.d.	0.62 ± 0.05	<0.0001
Σ Flavanones	0.41 ± 0.06	n.d.	n.d.	n.d.	<0.0001
Naringin	0.41 ± 0.06	n.d.	n.d.	n.d.	<0.0001
Σ Isoflavones	n.d.	n.d.	0.32 ± 0.07	n.d.	<0.0001
Genistein 7-O-glucoside	n.d.	n.d.	0.32 ± 0.07	n.d.	<0.0001
Σ Flavonols	0.18 ± 0.004	n.d.	0.28 ± 0.021	0.40 ± 0.005	0.2138
Kaempferol 3-O-rutinoside	n.d.	n.d.	0.14 ± 0.03	n.d.	<0.0001
Laricitrin 3-O-hexoside	0.01 ± 0.002	n.d.	n.d.	n.d.	<0.0001
Myricetin	0.03 ± 0.006	n.d.	n.d.	n.d.	<0.0001
Quercetin 3-O-glucoside	0.09 ± 0.002	n.d.	n.d.	n.d.	<0.0001
Quercetin 3-O-xyloside	n.d.	n.d.	n.d.	0.12 ± 0.003	<0.0001
Quercetin 3-O-rutinoside	0.05 ± 0.001	n.d.	0.10 ± 0.01	0.09 ± 0.002	<0.0001
Quercetin-deoxyhexoside	n.d.	n.d.	n.d.	0.02 ± 0.0003	<0.0001
Quercetin 3-O-glucoside	n.d.	n.d.	n.d.	0.17 ± 0.05	<0.0001
Quercetin 3-O-rutinoside-7-O-glucoside	n.d.	n.d.	0.04 ± 0.005	n.d.	<0.0001
Σ Hydrolyzable tannins	n.d.	7.16 ± 0.98	n.d.	n.d.	0.0003
Bis-HHDP-glucose	n.d.	0.61 ± 0.08	n.d.	n.d.	<0.0001
Chebularic acid	n.d.	0.42 ± 0.14	n.d.	n.d.	<0.0001
Granatin A	n.d.	1.35 ± 0.44	n.d.	n.d.	<0.0001
Punicalagin α	n.d.	2.24 ± 0.45	n.d.	n.d.	<0.0001
Punicalagin β	n.d.	2.54 ± 0.12	n.d.	n.d.	<0.0001
Σ Hydroxycinnamic acids	n.d.	n.d.	14.12 ± 0.87	7.39 ± 0.49	0.1730
n-Caffeoylquinic acid	n.d.	n.d.	1.52 ± 0.11	n.d.	<0.0001
3-Caffeoylquinic acid	n.d.	n.d.	9.82 ± 0.51	3.00 ± 0.18	<0.0001
p-Coumaroylquinic acid	n.d.	n.d.	2.78 ± 0.33	n.d.	<0.0001
Caffeic acid	n.d.	n.d.	n.d.	0.78 ± 0.09	<0.0001
p-Coumaroyl hexose	n.d.	n.d.	n.d.	3.61 ± 0.29	<0.0001
Σ Total (Poly)phenols	17.11 ± 0.48	10.63 ± 0.98	27.14 ± 1.03	40.09 ± 1.42	<0.0001

(Poly)phenols were quantified as described in the Materials and Methods section. Values are expressed as the mean (mg/g extract) ± standard deviation (n = 3). p-value column shows the differences between individual (poly)phenol composition in each extract using one-way ANOVA. n.a., not analyzed; n.d., not detected.

was only found in females under L18 conditions treated with the SM. These findings highlight the combined influence of treatment type, sex, and photoperiod on the bioavailability of specific metabolites.

Additionally, phenylacetic acid sulfate (isomer) and 3-(3-hydroxyphenyl)propionic acid were consistently detected in males exposed to L6, regardless of treatment, suggesting regulation primarily driven by sex and photoperiod. Similarly, 3-(4'-methoxyphenyl)propanoic acid-3'-sulfate showed a distinct pattern, being detected only in WM-treated males under L6 and in SM-treated females under L18, further illustrating a sex-, photoperiod-, and treatment-specific modulation of systemic bioavailability.

4. Discussion

(Poly)phenol consumption has been shown to exert several beneficial effects on obesity and MetS development (Wang et al., 2014). However, their bioactivity largely depends on their ADME (Del Rio et al., 2013). Hence, the study of factors that are affecting bioavailability and (poly)phenol metabolism is essential. Several intrinsic factors have been identified as capable of modulating host physiology and, consequently, (poly)phenols' ADME (Margalef et al., 2016; Margalef, Pons, Iglesias-Carres, Bravo, et al., 2017; Margalef, Pons, Iglesias-Carres, Quinones, et al., 2017). Moreover, photoperiod exposure is increasingly recognized as a relevant environmental variable influencing (poly)phenol bioavailability and activity (Baur & Sinclair, 2008). In this

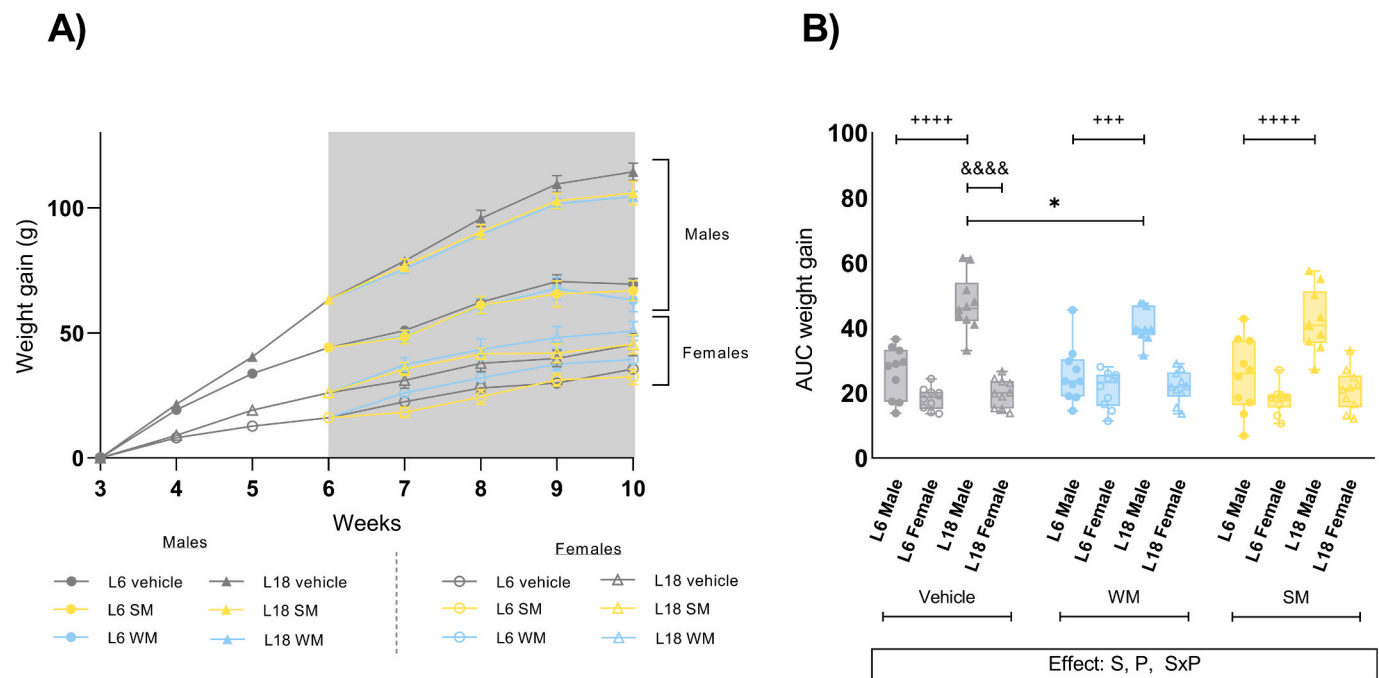


Fig. 3. Effect of mixtures and photoperiod on evolution of body weight gain (A) and AUC (Area Under the Curve) (B) of F344 rats fed with CAF diet for 10 weeks, exposed for 8 weeks to short (L6) or long (L18) photoperiod, and daily administered for 4 weeks with water as a vehicle, Winter Mixture (WM) or Summer Mixture (SM). The AUC was calculated for the 4 weeks of treatment. Data in AUC are presented as box-and-whisker plots showing the median, interquartile range (Q1–Q3), and minimum to maximum values ($n = 10$). P -values from repeated measures analysis of body weight gain evolution in rats from week 4 to week 10 are in Table S4. P, photoperiod effect; S, sex effect; SxP, interaction between sex and photoperiod using 3-way ANOVA. + Indicates significant differences between photoperiods ($+++ p < 0.001$, $++++ p < 0.0001$) in each sex and treatment, and & indicates significant differences between sex (&&& $p < 0.0001$) using 3-way ANOVA and Tukey as post-hoc test. * Indicates significant differences by treatment (WM or SM) and its corresponding vehicle group (* $p < 0.05$) using an unpaired two-tailed Student's t -test.

regard, a previous study demonstrated that (poly)phenol-enriched grape and pomegranate extracts (representing winter fruits) showed more positive effects in male rats under L6 conditions. In contrast, (poly)phenol-enriched cherry and plum extracts (typically summer fruits) exerted more pronounced effects under long photoperiod (L18) conditions (Manocchio et al., 2024). These results agree with the xenohormesis theory. This theory proposes that phytochemicals, such as (poly)phenols, synthesized by stressed plants can act as signals to heterotrophs that consume them, serving as molecular messengers, preparing consumers for upcoming adverse environmental changes (Baur & Sinclair, 2008). However, there is a lack of understanding about how these fruit extracts interact when consumed together, as humans typically consume them, and how these potential interactions depend on the sex and photoperiod in which they are consumed.

(Poly)phenol-enriched fruit extracts for the mixture preparation were prepared from the fruits as commonly consumed, including the skin, seeds, and pulp, except for cherry and plum, whose seeds were removed, and pomegranate, from which the membranes surrounding the arils were removed. Each mixture was thoroughly characterized to establish its specific (poly)phenol fingerprint. The lower concentration of (poly)phenols in pomegranate extract compared to previous reports can be attributed to the omission of the peel from the extraction process. It has been demonstrated that pomegranate peel contains a higher concentration of (poly)phenols compared to the pulp (Malviya et al., 2013). As expected, anthocyanins were present in all four extracts, being the main pigments responsible for color in fruits (Sunil & Shetty, 2022).

In line with the literature, catechin was the most abundant compound in the grape extract, which coincides with other reported extracts, although their concentrations differed (Šikuten et al., 2020). Grape seeds are a great source of flavan-3-ols, especially catechin (Padilla-González et al., 2022), but this compound is also present in the pulp and skin (Feng et al., 2023; Prakash & Kudachikar, 2020).

Similarly, in pomegranate, ellagitannins were the most relevant class, particularly punicalagins as the most predominant (poly)phenols, aligned with other research that also examined pulp extract (Díaz-Mula et al., 2019). The results obtained with the plum extract differ from those reported in previous literature. In our study, the most predominant (poly)phenol was cyanidin-3-*O*-glucoside, whereas some studies reported that this could be quercetin-3-*O*-rutinoside (Hameed et al., 2022), or chlorogenic acid (Jaiswal et al., 2013; Zheng et al., 2023), both of which were also detected and quantified in our study, albeit in lower concentrations. The differences observed in the phenolic profile may be attributed to the use of a different variety (i.e., *Prunus domestica* var. black diamond) (Hameed et al., 2022; Jaiswal et al., 2013).

Additionally, variations in phenolic composition have been reported across different stages of fruit ripening, which could further contribute to these discrepancies (Zheng et al., 2023). In fact, the phenolic composition analysis of the mixtures revealed notable differences in both concentration and phenolic profile composition. The SM was richer in anthocyanins and hydroxycinnamic acids, while the WM showed higher levels of flavan-3-ols, ellagitannins, and ellagic acid derivatives. These compounds are associated with well-documented antioxidant, anti-inflammatory, and metabolic benefits (Cremonini et al., 2020; F. Fu et al., 2021; Kao et al., 2000; Sova & Saso, 2020; Speer et al., 2020).

The different profiles of (poly)phenols in fruit extracts typical of winter or summer may give distinct signals to heterotrophs that consume them, resulting in different bioactivities due to variations in bioavailability, depending on the photoperiod conditions of exposure. To evaluate this, male and female F344 rats fed a CAF diet and exposed to L6 or L18 conditions were chronically administered the mixtures at a dose of 100 mg/kg BW. This is a dose equivalent to 1.46 g/day in a 70 kg person, which aligns with reported daily (poly)phenol intakes up to 1.70 g/day (Grosso et al., 2014). In this study, a CAF diet was used to develop obesity and MetS in rats, as it has been proven to be a suitable

Table 3

Quantification of serum (poly)phenol metabolites using UPLC-ESI-qTOF-MS in Fischer 344 rats after 30 days of daily oral administration of a winter mixture (grape and pomegranate extracts) or a summer mixture (cherry and plum extracts) under short (L6) or long (L18) photoperiods. Data is expressed in $\mu\text{mol/L}$, when authentic standards were available, or as the integrated extracted ion chromatograms.

	Winter mixture					Summer mixture					EFFECT ²
	Short photoperiod (L6)		Long photoperiod (L18)		EFFECT ₁	Short photoperiod (L6)		Long photoperiod (L18)		EFFECT ₁	
	Male	Female	Male	Female		Male	Female	Male	Female		
Microbial metabolites											
Benzoic acids (C6–C1)											
Hydroxybenzoic acid glucuronide (isomer)*	n.d.	n.d.	n.d.	37,980 (0–89,120) && ++	PxS	n.d.	n.d.	n.d.	n.d. \$\$		P, S, T, PxS, PxSxT
Dihydroxybenzoic acid sulfate(isomer)*	n.d.	n.d.	n.d.	6329 (244–50,252) && ++	PxS	n.d.	n.d.	n.d.	n.d. \$\$		P, S, T, PxS, PxSxT
2,6-Dihydroxybenzoic acid ^a	0.03 (0.00–0.10)	n.d. &&	n.d. ++	0.01 (0.00–0.03) & +	PxS	0.03 (0.02–0.08)	n.d. &&&	n.d. +++	0.03 (0.00–0.08) &&& +++	PxS	
3,4-Dimethoxybenzoic acid*	398 (0–6468)	4850 (0–10,078)	17,475 (15427–19,503) +	1019 (0–2141) &&		802 (0–7129)	n.d. \$\$&&	n.d. \$\$\$++	n.d. \$\$	PxS	S, T, PxSxT
Benzaldehydes (C6–C1)											
4-Hydroxybenzaldehyde ^a	0.02 (0.00–0.07)	n.d. &	n.d. +	0.01 (0.00–0.12) && ++		0.03 (0.00–0.08)	n.d. &&	n.d. ++	n.d. \$\$	PxS	PxS
Phenylacetic acids (C6–C2)											
Phenylacetic acid sulfate (isomer)*	2090 (226–5620)	n.d. &&	n.d. ++	n.d.	PxS	1013 (0–8299)	n.d. &	n.d. +	n.d.	PxS	PxS
Phenylpropanoic acids (C6–C3)											
3-(3'-Hydroxyphenyl) propanoic acid ^a	0.07 (0.03–0.10)	n.d. &&	n.d. +++	n.d.	PxS	0.01 (0.00–0.05)	n.d.	n.d. ++	n.d.	PxS	P, S, PxS
Hydroxyphenyl propanoic acid sulfate (isomer)*	272,639 (196614–914,600)	n.d. &&&	n.d. +++	27,155 (6994–130,207) & +	PxS	303,646 (26967–490,607)	5480 (0–100,240) ^{sc}	n.d. ++	86,444 (9721–207,662) &&	PxS	P, S, PxS
3-(4'-Methoxyphenyl) propanoic acid-3'-sulfate *	1402 (0–10,815)	n.d. &	n.d. ++	n.d.	PxS	n.d. \$\$	n.d.	n.d.	8505 (112–54,979) \$\$\$& +	PxS	PxS
4-Hydroxy-5-(phenyl)valeric acids											
(R/S)-5-(3',4'-Dihydroxyphenyl)valeric acid *	119,077 (4326–190,777)	n.d. &&	n.d. ++	7821 (724–250,608) && ++	PxS	95,403 (32165–219,629)	n.d. &&&	n.d. +++	n.d. \$\$	PxS	PxS
5-(Hydroxyphenyl)valeric acid-glucuronide (isomer)*	1913 (0–17,221)	n.d. &&	n.d. ++	n.d.	PxS	1214 (0–5815)	n.d.	n.d.	4345 (421–20,003)	PxS	PxS
Cinnamic acids (C6–C3 unsaturated)											
3'-Methoxycinnamic acid-4'-sulfate ^a	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	0.10 (0.00–0.77) ^s &&& ++	PxS	S, PxSxT
4'-Methoxycinnamic acid-3'-glucuronide*	n.d.	n.d.	n.d.	n.d.		13,501 (8453–42,884) \$\$\$	11,389 (7145–13,480) ^s \$\$	8595 (4361–10,095) ^s \$\$\$d	16,576 (8807–19,787) \$\$\$c		T, PxSxT
Hydroxybenzenes (C6–C0)											
2-Hydroxybenzene-1-sulfate *	180,579 (46892–256,109)	38,680 (0–86,314)	n.d. ++	89,423 (15909–217,349) &&		11,055 (0–118,478)	n.d. \$\$&	n.d. +	144,075 (61741–443,300) &&& +++	PxS	PxS
1,3,5-trihydroxybenzene ^a	0.18 (0.00–1.00)	n.d. &&	n.d. ++	0.03 (0.00–1.30) & +	PxS	0.22 (0.04–0.31)	n.d. &&	n.d. ++	n.d.	PxS	T, PxS, PxSxT
Hydroxy-3-(phenyl)propanoic acids (C6–C3)											

(continued on next page)

Table 3 (continued)

	Winter mixture				Summer mixture				EFFECT ²	
	Short photoperiod (L6)		Long photoperiod (L18)		Short photoperiod (L6)		Long photoperiod (L18)		EFFECT ¹	
	Male	Female	Male	Female	Male	Female	Male	Female		
1-(Hydroxyphenyl)-3-(2',4',6'-trihydroxyphenyl)-propan-2-ol *	2942 (0–24,047)	n.d. &&	n.d. ++	2088 (280–16,581) &+++	n.d. \$\$	n.d.	n.d.	n.d. \$		T, PxS, PxSXT
Hippuric acids (C6–C1–N<) and other amino acid conjugates										
Hippuric acid ^a	2.09 (0.26–9.80)	0.93 (0.00–3.06)	n.d. ++	1.74 (0.01–2.75) &&	n.d. \$\$	0.39 (0.00–5.31) &	n.d.	1.36 (0.00–6.14) &&	PxS	PxS

^aThe identification is tentative due to the lack of authentic standards, and values are expressed as the integrated extracted ion chromatograms (EICs). ¹Compounds identified and quantified (μmol/L) with their corresponding authentic standards. Results are expressed as median (first quartile (Q1) – third quartile (Q3)) (n = 10). 2-way ANOVA analysis: The “EFFECT¹” column represents the statistical results of the interaction between photoperiod (P) and sex (S) in each treatment, or their interaction (SxP). 3-way ANOVA analysis: The “EFFECT²” column represents the statistical results of photoperiod (P), sex (S), treatment (T), or their interaction (SxP; PxSXT). Tukey post-hoc analysis: \$, Significant differences (¹p < 0.05; ²\$p < 0.01; ³\$\$\$p < 0.001) between mixtures (Winter mixture vs Summer mixture) in each photoperiod and sex, and letter b indicates tendency (¹p < 0.1); &, Significant differences (¹p < 0.05; ²&&p < 0.01; ³&&&p < 0.001) between sexes (males vs females) in each photoperiod and mixture, and letter c indicates tendency; +, Significant differences (¹p < 0.05; ²++p < 0.01; ³+++p < 0.001) between photoperiods (short photoperiod (L6) vs long photoperiod (L18)) in each sex and mixture, and letter d indicates tendency. n.d., not detected; L6, 6 h light and 18 h darkness; L18, 18 h light and 6 h darkness. The nomenclature of microbial and phase II (poly)phenol metabolites was revised according to the extended recommendations of Curti et al. (Curti et al., 2025). When positional isomers could not be assigned with sufficient confidence, a more conservative nomenclature was adopted.

model for studying the changes in rodents (Sampey et al., 2011). F344 rats, due to their sensitivity to variations in day length, are commonly used to study the effects of photoperiod changes (Heideman & Sylvester, 1997). No standard L12 photoperiod was studied, as exposure to long photoperiods (more than 12 h of light per day) has been shown to exert a stimulatory response in F344 rats, including body weight gain, food intake, and reproductive status. In contrast, photoperiods with less than 10 h of light per day, considered short photoperiods, exert an inhibitory effect in rodents (Tavolero et al., 2015). Interestingly, WM reduced body weight gain only in males exposed to the L18 photoperiod compared to the vehicle L18 group, as assessed by AUC. These differences in BW gain across mixtures, sexes, and photoperiods could be attributed to distinct profiles of circulating metabolites (Cardona et al., 2013b). Therefore, to evaluate the potential involvement of these circulating metabolites in these health outcomes, metabolites derived from chronic consumption of WM or SM were identified and quantified in serum at 2 h after the last dose and 26 h after the previous dose. The WM-L18 group, which reduced body weight gain, showed the lowest diversity of circulating phenolic metabolites, as only 3,4-dimethoxybenzoic acid was detected. This metabolite likely derives from the gut microbial degradation of flavan-3-ols, such as catechins and proanthocyanidins, which are well-documented for their capacity to reduce body weight as well as to improve different obesity related pathologies, such as lipid metabolism in obesity rodent models (Chen et al., 2016; Liu et al., 2020). However, it should not be excluded that the local actions of unabsorbed (poly)phenols in the gut, including modulation of nutrient absorption, microbiota composition, and gut-derived signaling, can influence energy balance independently of systemic bioavailability (Cardona et al., 2013b).

Saura-Calixto et al. (Saura-Calixto et al., 2010) discussed how dietary fibers and (poly)phenols interact during colonic fermentation, leading to the production of various microbial metabolites. In the present study, the predominance of microbial-derived phenolic metabolites detected in plasma may be partly explained by the high fiber content of the administered mixtures (Edwards et al., 2017). Dietary fiber can modulate (poly)phenol bioavailability by limiting the absorption of parent compounds in the small intestine, thereby increasing their delivery to the colon, where they undergo extensive microbial biotransformation (Catalkaya et al., 2020b). This mechanism is consistent with the relatively low detection of phase II conjugated metabolites in circulation. In this regard, in previous studies analyzing (poly)phenols bioavailability in serum after the administration of organic and conventional red grapes found lower accumulation of phase II metabolites in rats treated with conventional grapes (Iglesias-Carres, Mas-Capdevila, Bravo, Aragonès, et al., 2019; Iglesias-Carres, Mas-Capdevila, Bravo, Arola, et al., 2019), both after 1 h and 25 h of the last dose administration, with levels of dietary fiber similar to those present in the mixtures used in this study. Microbial-derived metabolites are likely formed later in the digestive process, around 24 h after the last administration. Hence, in this study, we are detecting bacterial-derived metabolites from the previous day's dose administration, 26 h prior to sampling. This finding is also consistent with a previous study (Pereira-Caro et al., 2023), which attributed the reduction in phase II metabolites, along with an increase in microbial metabolites, to the higher fiber content of β-glucan-rich oat bran.

The predominance of microbial-derived metabolites in serum suggests a strong interplay between (poly)phenols and the gut microbiota, potentially acting as environmental messengers modulated by photoperiods (Howitz & Sinclair, 2008). The CAF diet has been shown to reduce microbial diversity after 9 weeks of administration (Arreaza-Gil et al., 2022). Moreover, the composition of gut microbiota is influenced by changes in photoperiod exposure. For instance, it has been shown that altered photoperiods, either long or short, can reduce the gut microbiota α-diversity when compared to the standard photoperiod in male rats fed a CAF diet, and, consequently, the metabolism of (poly)phenols may differ (Arreaza-Gil et al., 2023). In the present study, the role of photoperiod in the bioavailability of serum (poly)phenols was

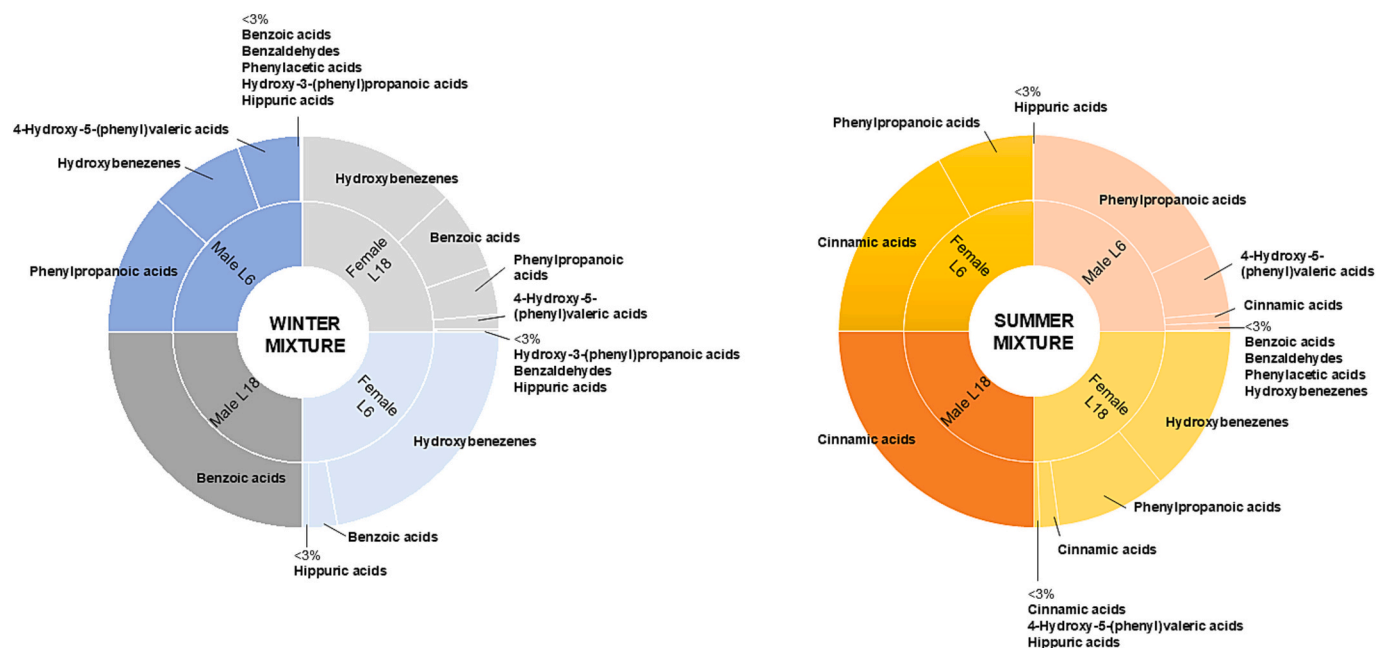


Fig. 4. Sunburst plots showing the relative abundance of serum (poly)phenol metabolite families in Fischer 344 rats after winter mixture or summer mixture administration, under short (L6) and long (L18) photoperiods. For each condition (L6 male, L6 female, L18 female, and L18 male), the contribution of each metabolite family was calculated as the sum of all detected metabolites belonging to that family, based on the data shown in Table 3. The size of each sector reflects the relative abundance of each metabolite family within each experimental condition, allowing visualization of sex- and photoperiod-dependent differences according to the seasonal mixture consumed.

also noticeable. For instance, males, whether treated with WM or SM, presented a higher diversity of microbial metabolites when exposed to the L6 photoperiod than to the L18 photoperiod. Interestingly, 3-(3-hydroxyphenyl)propionic acid was detected only in the serum of males exposed to the L6 photoperiod, regardless of whether they were treated with WM or SM, corroborating that this variation was due to the photoperiod rather than the treatment administered. These results are consistent with a previous study by Arreaza et al., which showed that the bioavailability of GSPE in male rats fed a CAF diet was also lower in the L18 photoperiod compared to L6, attributed to differences in gut microbial β -diversity (Arreaza-Gil et al., 2022).

In contrast to males, female rats had higher levels of microbial metabolites when exposed to L18 photoperiods, regardless of the mixture. This reaffirms sex differences in the ADME of (poly)phenols (Margalef et al., 2016) and in responses to photoperiods (Gómez-Roncal et al., 2025). The microbial composition was affected by photoperiod conditions, diet, and sex, potentially leading to variations in plasma (poly)phenol bioavailability and, consequently, in health outcomes (C. Fu et al., 2023). In fact, male and female subjects may exhibit different metabolic responses to (poly)phenol intake, potentially due to hormonal influences on gut microbiota composition and function (Kosmalski et al., 2022). For instance, sex-dependent differences in (poly)phenol metabolism have been reported, resulting in distinct serum concentrations of their derived metabolites (Stevens & Maier, 2016). This sex dimorphism is thought to stem from the modulatory role of sex hormones on gut microbiota, as previously reported (Haindl et al., 2024).

Some of the detected metabolites were characteristic of a specific mixture administered to rats, which confer specific health benefits to each mix. For instance, 4'-methoxycinnamic acid 3'-glucuronide and 3'-methoxycinnamic acid 4'-sulfate were detected only in SM-treated animals, likely derived from bacterial conjugation of hydroxycinnamic acids, such as chlorogenic or caffeic acids, which are only present in SM (Kawabata et al., 2019). These hydroxycinnamic acids have been reported to exert several benefits for human health, highlighting the antioxidant capacity of caffeic acid, as corroborated by both in vivo and in vitro studies (Agudelo-Ochoa et al., 2016; Sato et al., 2011).

Conversely, 1-(hydroxyphenyl)-3-(2',4',6"-trihydroxyphenyl)-propan-2-ol, dihydroxybenzoic acid sulfate (isomer), and hydroxybenzoic acid glucuronide (isomer) were characteristic of WM-treated groups, likely originating from flavan-3-ols. Ellagic acid, which is degraded to benzoic acid and subsequently serves as a precursor to hydroxybenzoic acid glucuronide (an isomer), has been associated with enhanced insulin sensitivity, improved lipid metabolism, and strong antioxidant activity (Kang et al., 2016).

In this study, several metabolites were produced after the administration of either of the two mixtures or were characteristic of one photoperiod condition, supporting the xenohormesis theory (Baur & Sinclair, 2008). Moreover, a sex-specific seasonal response was observed: males showed greater serum metabolite diversity under WM administration during L6, while females exhibited greater metabolite diversity under SM treatment during L18, suggesting that (poly)phenol bioavailability is synchronized with seasonal physiology (Ren et al., 2022; Stelmach-Mardas et al., 2016). In males, short photoperiods have been linked to a catabolic and energetically efficient state by the activation of AMPK, which has been reported to support gut barrier integrity and modulate the composition and activity of the gut microbiota, potentially facilitating the transformation and absorption of (poly)phenols into bioavailable forms (Mariné-Casadó et al., 2018). On the other hand, under L18 photoperiod, the melatonin secretion is reduced, and, as a consequence, estrogen levels in females are increased, as melatonin acts as a repressor of this hormone secretion in the hypothalamic-pituitary-gonadal (HPG) axis (Shi et al., 2013). Higher levels of estrogens, compared to short photoperiods, are associated with improved uptake of hepatic and intestinal phenolic metabolites (Rettberg et al., 2014; Shchulkin et al., 2023), corroborating the fact that females showed greater bioavailability under L18 photoperiod when SM was administered.

Overall, this study provides novel insights into how biological sex and photoperiod interact to shape the systemic bioavailability of (poly)phenol-derived metabolites after chronic intake of seasonal fruit extract mixtures. The differential metabolite profiles observed between males and females, and across photoperiods, support the hypothesis that both

intrinsic and environmental factors modulate (poly)phenol metabolism. The identification of mix- and condition-specific metabolites reinforces the concept of xenohormesis and highlights the potential of tailoring nutritional strategies to seasonal cues. These findings may contribute to the development of more personalized dietary recommendations to optimize (poly)phenol bioefficacy based on fruit seasonality, sex, and photoperiod. However, this study has limitations, such as the simultaneous analysis of three variables (sex, photoperiod, and classes of (poly)phenol from seasonal fruits and varieties) in a context of diet-induced obesity, a condition that, per se, alters metabolic homeostasis and phenolic handling. This multifactorial approach provides a broader perspective, but also adds complexity to interpreting the results. In this context, it is difficult to determine which factor contributes most to the changes observed in (poly)phenol bioavailability. In addition, exploring gut microbiota composition would be particularly valuable. Therefore, despite the relevance of the combined effects observed, future studies using more targeted or factorial designs will be necessary to clarify the specific contribution and mechanisms of each variable.

5. Conclusion

This study is the first to demonstrate in a diet-induced obese rat model that the combined interaction of photoperiod and sex influences the bioavailability of (poly)phenols following consumption of seasonal fruit mixtures enriched with (poly)phenols, thereby potentially modulating their bioactivity and health outcomes. Moreover, some circulating microbial metabolites derived from health-promoting (poly)phenols were specific to a particular fruit mixture. Further investigations should focus on studying the gut microbiota composition and functionality in these animals to elucidate the mechanisms underlying the observed variations.

Author contribution

S.G.R.: investigation, formal analysis, writing–original draft, writing–review. M.A.A.-G.: investigation, validation, writing–review. M.A.-F.: investigation. J.R. S.-R.: investigation. B.M.: conceptualization, funding acquisition. J.C.E.: funding acquisition, validation, writing–review. A. A.-A.: conceptualization, funding acquisition, supervision, writing–review.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used generative AI technologies for content and editing assistance. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRedit authorship contribution statement

Saioa Gómez-Roncal: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **María Ángeles Ávila-Gálvez:** Writing – review & editing, Validation, Investigation. **Marc Alemany-Fornés:** Investigation. **Jorge R. Soliz-Rueda:** Investigation. **Begoña Muguerza:** Funding acquisition, Conceptualization. **Juan Carlos Espín:** Writing – review & editing, Validation, Funding acquisition. **Anna Arola-Arnal:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Funding

This work was supported by projects PID2020-113739RB-I00 and PID2022-136419OB-I00 (Ministerio de Ciencia, Innovación y Universidades, MICIU, AEI/10.13039/501100011033, Spain) with co-funding from the EU “ERDF A way of making Europe”, and the

AGROALNEXT programme (PRTR-C17-I1, MICIU, Spain) with co-funding from the NextGenerationEU and Fundación Séneca (Comunidad Autónoma Región de Murcia, Spain). S.G.R. is the recipient of a grant for the hiring of predoctoral research staff (Grant number: PRE-2021-097300) from the Spanish Ministry of Science and Innovation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Authors would like to thank Niurka Dariela Llópiz and Rosa Pastor for their technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2026.119110>.

Data availability

Data will be made available on request.

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