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In vitro gastrointestinal digestion and simulated colonic fermentation of pistachio nuts determine the bioaccessibility and biosynthesis of chronobiotics†

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Chronodisruption leads to obesity and other metabolic disorders that can be alleviated by food-derived potential chronobiotics, such as phytomelatonin (PMT), phenolic compounds (PCs) and dietary fiber rich pistachios. Pistachios with (PN + SC) or without (PN) the seed coat were investigated for their *in vitro* chronobiotic potential since they are one of the main reported PMT sources. Consequently we evaluated the bioaccessibility, permeability, and biosynthesis of pistachio chronobiotics, particularly PMT, during gastrointestinal and colonic fermentation. The maximum *in vitro* bioaccessibility and apparent permeability (efflux-prone) of PCs, flavonoids and PMT were sample-specific [~1.3% (both), 27 and 3.4% (PN + SC)], but additional amounts (flavonoids > PCs > PMT) were released under simulated colonic conditions. Short-chain fatty acids (SCFAs; 38 mM; >50% butyrate, PN + SC > PN) and some metabolites (*e.g.*, indole, benzaldehyde, phenolic acids, and aliphatic/aromatic hydrocarbons) were detected depending on the sample. The predominant pistachio butyrate production during *in vitro* colonic fermentation can improve chronodisruption and benefit obese individuals. Pistachio's digestion increases the bioaccessibility and intestinal permeability of potential chronobiotics (PMT and PCs) and the biosynthesis of colonic metabolites (SCFAs, among others) also with chronobiotic potential.

1. Introduction

Obesity is a burden on global public health, including in Mexico where seven out of ten adults (≥20 years) are overweight.[1] It is a multifactorial chronic disease that involves many genetic, physiological, dietary, and environmental traits. However, recent studies have highlighted the role of gut microbiota and chronodisruption as independent factors affecting the body's energy metabolism and weight gain.[2–4] New strategies have therefore emerged to control weight alterations. One of these novel strategies, chrononutrition, is positioned to benefit human health through food components that can positively restore the circadian rhythms of internal organs, modulating various physiological processes and, consequently, be beneficial in conditions such as overweight and obesity.[2,5]

Pistachios (*Pistacia vera* L.) are considered one of the most consumed nuts globally due to their nutritional characteristics, and several international associations recommend its habitual consumption. Pistachios are a good source of proteins, unsaturated fatty acids, and phytochemicals such as phenolic compounds and tocopherols which provide health benefits.[6] Furthermore, phenolic compounds and polyunsaturated fatty acids can restore the synchronization of the internal clock.[7,8]

Pistachios have been reported as one of the dietary sources with highest phytomelatonin (PTM), containing up to 223 μg g^{-1}, depending on the variety.[9] Melatonin is produced endogenously in organisms. However, melatonin production generally decreases in ageing population and societies exposed to prolonged chrono-disruptive conditions (*e.g.* working in rooms lit by artificial light, increased exposure to bright light at night or dim light during the day, and chronic and social jet lag).[10,11] Therefore, exogenous melatonin administration is necessary, which can be through natural sources such as food, thus helping to regulate sleep and to restore the circadian

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rhythm of internal organs, acting as a chronobiotic.[2,12] However, despite the experimental and clinical evidence on melatonin's role as a chronobiotic, information on PMT and its fate during its gastrointestinal (GI) passage is limited.

Tree nuts also stand out for being a good source of dietary fiber. Pistachios contain 5–12 g per 100 g dietary fiber,[13] which increases the colonic production of short-chain fatty acids (SCFAs) with chemopreventive and chronobiotic action.[14,15] However, no studies to date have investigated other colonic metabolites produced from pistachios and their potential as chronobiotics.

Therefore, the aim of this study was to evaluate the *in vitro* chronobiotic potential of pistachios by determining the *in vitro* bioaccessibility, *ex vivo* apparent permeability, fermentative behavior (SCFA production) and potential biotransformation of major PCs and PMT from roasted pistachios with (PN + SC) or without (PN) the seed coat.

2. Material and methods

2.1. Materials

Dry roasted and salted pistachios (*Pistacia vera* L.) were purchased from the local market (Member's Mark, Wal-Mart Stores, Inc. 2019). Whole pistachios comprise 6% seed coat (SC, skin) and 94% nutmeat by weight,[17] and in this study, we evaluated pistachios with (PN + SC) and without (PN) the SC. Both samples were ground and stored at 4 °C until analysis. All chemicals and solvents were purchased from Sigma Chemical Co. and J. T. Baker (Mexico City, Mexico).

2.2. Chemical characterization

The moisture (925.40; convection drying), lipid (948.22; Soxhlet), protein (950.48; Kjeldahl; N × 6.25), and ash (923.03; oven) contents were determined with the standardized AOAC methods, as previously described.[16]

2.3. Phytochemical composition

2.3.1. Phenolic compounds (PCs) and antioxidant capacity. Hydromethanolic extracts from PN and PN + SC (2 : 1 w/v) were obtained according to the procedure of Tomaino *et al.*[18] Total PCs were determined by the Folin–Ciocalteu method using gallic acid as standard, while flavonoids were quantified according to the procedure adapted for microplates described previously.[19–21] Individual PCs were analyzed by high-performance liquid chromatography-diode array detection (HPLC-DAD) in an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) using a Zorbax Eclipse XDB-C18 column (Agilent Technologies, 4.6 × 250 mm, 5 μm); experimental conditions were the same as those previously reported,[22] and quantification was carried out using the external standards, namely (+)-catechin, epigallocatechin gallate and gallic, and protocatechuic and ellagic acids. Finally, antioxidant capacity was measured using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the results were expressed as the Trolox equivalent antioxidant capacity (TEAC) using Trolox as the standard for the calibration curve.[23]

2.3.2. Tocols and carotenoids. PN and PN + SC were individually homogenized twice in hexane (1 : 10 w/v), stirred for 3 minutes, and filtered (Whatman # 4 filter paper) under vacuum. The extracts were dehydrated with anhydrous sodium sulfate (1 g), and both sodium sulfate and hexane were removed by filtration and rotary evaporation at 40 °C, respectively. The resulting oil phase was transferred to an amber bottle, sealed under nitrogen, and kept at −80 °C until analysis. Tocols and carotenoids were determined by HPLC and detected by fluorescence (285/325 excitation/emission) according to Stevens-Barron *et al.*[24] The tocopherol and tocotrienol peaks were identified by comparing their retention time with those of pure standards (α-tocopherol, α-tocotrienol, γ-tocopherol, γ-tocotrienol, and β-tocopherol). The results were expressed in mg of tocols per 100 grams of solid sample. Total carotenoids were determined by UV-VIS spectrophotometry and quantified using the following equation:

$$\beta\,\text{carotene equivalents}(\beta\text{CE})\,\text{mg per } 10\,\text{g} = (A\,\text{mL} \times 106)/(1000\,\text{g} \times 2500)$$

where A = absorbance at 445 nm, mL = volume of the extraction solution (2 mL), g = weight of the sample (0.5 g) and 2500 = average absorption coefficient of a carotenoid molecule.

2.3.3. Phytomelatonin (PMT). PMT was extracted by an ultrasound-assisted solid–liquid phase, using methanol according to a previous report.[25] Quantification was performed in a Varioskan™ Flash Multimode Reader (Thermo Scientific, Waltham, MA) at 275 nm (excitation) and 366 nm (emission).

2.4. *In vitro* gastrointestinal digestion (GI) and bioaccessibility

PN and PN + SC were digested under *in vitro* simulated physiological conditions using a standardized and previously described static method.[26] All participants provided written consent, and the study was approved by the Universidad Autónoma de Querétaro Human Research Internal Committee (CBQ19/013) and complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

- Oral stage: Four participants chewed 1 g of sample 15 times, for approximately 15 s, and expectorated the product into a beaker containing 5 mL of distilled water and rinsed their mouths with another 5 mL of distilled water for 60 s. This procedure was conducted four times for PN and pistachio nut with the seed coat (PN + SC), and saliva, which was used as a blank.
- Gastric stage: Samples from the oral stage were further mixed in a single beaker, and 10 mL aliquots were adjusted to pH 2.0 using HCl solution (150 mM). Pepsin (0.055 g) was dissolved in 0.94 mL of 20 mM HCl solution and added to each aliquot. The samples were incubated for 2 h at 37 °C.
- Intestinal stage: A simulated intestinal premix (3 mg of bovine bile and 2.6 mg of pancreatin) was mixed with 5 mL of Krebs–Ringer buffer [118 mM NaCl, 4.7 mM KCl, 1.2 mM

$MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose and 2.5 mM $CaCl_2$; pH 6.8; gasified (10 : 10 : 80, H_2 : CO_2 : N_2)] freshly prepared 30 min before the assay, and the sample from the gastric stage. The resulting suspension (15 mL) was further transferred to a glass test tube containing a recently dissected and everted gut sac obtained from male Wistar rats (250–300 g body weight, n = 8).

Before the surgical procedure, rats were fasted overnight (16–20 h) with water *ad libitum*. The animals were anesthetized with pentobarbital solution (60 mg per kg body weight, intraperitoneally). The intestine of every rat was exposed by a midline abdominal incision, and a 25–30 cm segment of the proximal rat jejunum was excised and placed in the gasified Krebs–Ringer buffer solution (37 °C). The intestine was washed using the same buffer and everted over a glass rod, divided into 6 cm-segments, and filled in the serosal side with 1 mL of the above-mentioned buffer. This segment was secured and submerged in the Krebs–Ringer buffer at 30 °C to prevent the loss of tissue viability. The test tubes were incubated in an oscillating water bath (Terlab, Mexico) at 80 cycles per min at 30 °C for 2 h. The sacs were removed after incubation, and the digested pistachio fraction (DF) at the basolateral side (inner side of the everted sac) was separated from the non-digestible pistachio fraction (NDPF) at the mucosal side (outside).

The phytochemical bioaccessibility during *in vitro* gastrointestinal digestion and colonic fermentation simulation was estimated with previously described methods for total PCs, flavonoids, and PMT. Finally, the bioaccessibility of PN and PN + SC was calculated using the following equation:

$$\%\text{Bioaccessibility} = \text{concentration at each digestion stage} \times 100/\text{initial concentration(phytochemical profile)}.$$

A complete *in vitro* gastrointestinal digestion was carried out twice, and the experiments were performed per duplicate (n = 4), including a blank prepared only with saliva instead of the sample.

2.4.1. Apparent permeability coefficients (P_{app}). The apparent permeability coefficient (P_{app}) was determined using the following equation: $P_{app} = (dQ/dt)(1/AC_0)$, where P_{app} (cm s^{-1}) is the apparent permeability coefficient, dQ/dt (mg s^{-1}) is the amount of drug transported across the membrane (everted gut sac) per unit time, A (cm^2) is the surface area available for permeation and C_0 (mg mL^{-1}) represents the initial concentration of the drug outside the everted gut sacs.[27]

2.5. *In vitro* colonic fermentation

The human gut microbiota fermentation method suggested by Campos-Vega *et al.*[28] was followed. Briefly, fresh fecal samples were collected from one obese donor [Mexican, male, 23 years, body mass index (BMI): 32 kg m^{-2}] who had not consumed antibiotics for at least 3 months and had no history of gastrointestinal or other metabolic diseases. The NDPF (100 μg) from the simulated intestinal digestion of PN and PN + SC described above was used as a substrate, and inulin (100 mg) as a reference fermentable carbohydrate. Two independent assays per duplicate (n = 4) were carried out at 37 °C simulating human colonic conditions. During fermentation, pH and SCFAs produced from all samples were monitored at 2, 4, 6, 12, and 24 h. At the end of the incubation period, the tubes were stored in a freezer at −70 °C for further analysis.

2.5.1. Short-chain fatty acids (SCFAs) and metabolite analysis. Identification and quantification of SCFAs and other colonic metabolites (colonic fermentation samples) were carried out by gas chromatography-mass spectrometry (GC-MS) and solid-phase microextraction (SPME), according to Zamora-Gasga *et al.*[29] Data analysis was performed using the MSD ChemStation software. SCFAs were identified by comparing the mass spectra with those of the NIST/EPA/NIH Mass Spectral Library (NIST, USA) and the retention times of chemical standards. The molar mass ratio (MR) was calculated using the following equation:

$$\text{MR} = (\text{SCFAs}/\text{SCFAT})$$

where SCFAs and SCFAT represent individual SCFA concentration and the total amount of SCFAs (mmol L^{-1}; 100 μg of sample). The spectrometric signals of any other potential compound (parent molecule or metabolite) were identified and confirmed by the NIST/EPA/NIH Mass Spectral Library. The metabolites were analyzed using the MetaboAnalyst Platform for metabolomic analysis and interpretation (https://www.metaboanalyst.ca).

2.6. Statistical analysis

All measurements were carried out as independent experiments at least in triplicate and experimental data were expressed as mean ± (SD). Pairwise comparisons were performed using Student's "*t*"-test and multiple comparisons (*e.g.* time-trend metabolite changes) were performed using one-way ANOVA and *post hoc* Tukey's test. Statistical analyses were performed using JMP 10.0 software and statistical differences were considered at $p \leq 0.05$. Spearman rank correlation (r) was used to seek any plausible relationship (magnitude & direction) between PMT and antioxidant activity. Partial Least Squares Discriminant Analysis (PLSA-DA) was used to rank each volatile metabolite (detected by GC-MS) according to the number of components and variables in each model and their specific *Variable Importance Projection* (VIP) scores, as previously reported.[30] Metabolomic data derived from *in vitro* (faecal) fermentation was corrected for blank (only feces) to eliminate the matrix effect, as suggested by Rocchetti *et al.*[31] and a metabolite was considered important when VIP ≥ 2.[32]

3. Results and discussion

3.1. Chemical composition

PN + SC had significantly ($p < 0.05$) lower lipid (−7.9) and protein (−1.1) contents, but higher carbohydrate (+8.4) and total dietary fiber (+4.8, twice as much as soluble and insoluble) contents (g per 100 g) than PN (Table 1). A similar

Table 1 Phytochemical composition of pistachio nut (*Pistacia vera* L.)

	PN + SC	PN
Lipids (g)	45.4 ± 0.5	53.3 ± 0.2*
Proteins (g)	23.9 ± 0.2	25.0 ± 0.1*
Carbohydrates (g)	24.8 ± 0.5*	16.4 ± 0.2
Total dietary fiber (g)	13.2 ± 0.2*	8.4 ± 0.3
Soluble dietary fiber (g)	12.6 ± 0.2*	6.3 ± 0.2
Insoluble dietary fiber (g)	0.60 ± 0.0*	0.3 ± 0.0
Ash (g)	4.3 ± 0.1*	3.2 ± 0.0
Moisture (g)	1.6 ± 0.1	2.2 ± 0.1*
TEAC (mg TE)	638.2 ± 62.3*	155.6 ± 26.5
Total polyphenols (mg GAE)	3.4 ± 0.4*	2.4 ± 0.2
Flavonoids (mg RE)	1.7 ± 0.3*	1.1 ± 0.1
Gallic acid (mg)	43.3 ± 0.8*	9.3 ± 1.7
Protocatechuic acid (mg)	0.35 ± 0.0*	0.07 ± 0.0
Ellagic acid (mg)	0.02 ± 0.0*	—
Catechin (mg)	0.6 ± 0.0*	0.4 ± 0.0
Epigallocatechin gallate (mg)	0.2 ± 0.0	0.1 ± 0.0
Total tocols (mg)	11.8 ± 1.3	13.1 ± 2.3
α-Tocopherol (mg)	1.8 ± 0.1	2.0 ± 0.3
β-Tocopherol (mg)	1.5 ± 0.0	1.5 ± 0.2
γ-Tocopherol (mg)	8.2 ± 1.0	9.3 ± 2.0
γ-Tocotrienol (mg)	0.3 ± 0.1	0.3 ± 0.1
Total carotenoids (mg βCE)	0.02 ± 0.01	0.03 ± 0.02
Phytomelatonin (mg ME)	17.2 ± 0.0	21.6 ± 0.0*

Values are expressed as mean ('units' per 100 g) ± standard deviation ($n \geq 6$); pistachio nuts with (PN + SC)/without (PN) the seed coat, gallic acid (GAE), rutin (RE), melatonin (ME), beta-carotene (βCE), and trolox equivalent (TE), antioxidant capacity (TEAC), below the detection limit (—). *values are statistically significant ($p < 0.05$) based on the *t*-test.

trend was reported for dry-roasted cashew nuts with and without seed coat.[32] Raw and processed pistachios are good quality protein sources[16,24,31,33,34] with digestible indispensable amino acid score >75%,[35] and well-known carbohydrate and dietary fiber sources.[36] A similar dietary fiber profile for roasted pistachios [11 g per 100 g, soluble (SDF): insoluble (IDF) ratio of 1 : 3 w/w] was reported by Rocchetti *et al.*,[31] but a total dietary fiber content from PN + SC (13.2 g per 100 g) was lower than that previously reported for Italian pistachio *cv.* Bianca;[37] nevertheless, fiber-rich foods facilitate SCFA production by the colonic microbiota. SCFAs and, in turn, these organic acids can synchronize peripheral clocks in the liver, kidneys, and submandibular gland.[15] Also, short term consumption of pistachios modifies the murine intestinal microbiota characterized by a higher number and diversity of firmicutes including *Turicibacter*[38] that expresses a neurotransmitter sodium symporter-related protein homologous to mammalian serotonin[39] potentially acting as a diurnal sleep–wake synchronizer.[10,11]

The main macronutrients in both pistachio samples (PN > PN + SC) were lipids (~50%), where oleic (C18:1n9*c*) and linoleic (C18:2n6*c*) acids together account for 76–85% of the total fatty acids, followed by palmitic acid (C16:0) with 13 g per 100 g oil.[16,24] Polyunsaturated fatty acids (PUFAs) have an important role in the circadian system through melatonin secretion[7] where a n3-PUFA deficient diet alters daily melatonin oscillations and their functional outcomes *in vivo* including striatal dopaminergic activity, hyperactivity and sleep disturbances.[40] Furthermore, prolonged palmitate exposure modifies *Bmal1-dLuc* circadian rhythms and inflammatory response, whereas docosahexanoic acid (C22:6, n3-PUFA) does not.[41]

3.2. Phytochemical composition and antioxidant capacity

PN + SC had a higher content of total phenols (+42%), total flavonoids (+54%), gallic and protocatechuic acids (>350%), catechin (+50%), and PMT (+21%) compared to PN ($p \leq 0.05$; Table 1). Similar results have been reported for pistachios *cv.* Bronte and in raw pistachios *cv.* Kerman.[16,24] Liu *et al.*[17] observed that the dominant PCs in raw, non-dried, shelled Kerman pistachios (μg per g fresh weight) were flavan-3-ols (catechins; ~200), myricetin (135), and cyanidin-3-galactose (38) and most PCs were present in the skin, while Grace *et al.*[42] found similar results in roasted unsalted Kerman pistachios. Many external factors influence the content of functional phytochemicals in pistachios including genetic and environmental factors, geographical location, and post-harvest treatments.[16,37]

Dietary polyphenols improve some metabolic and physiological processes *via* a circadian-clock-related mechanism.[43] For example, PCs positively affect lipid metabolism regulation in hepatic cells, mediated by brain-and-muscle-ARNT-like-1 (BMAL-1), a key transcription factor in the molecular machinery of the circadian clock.[44] Gallic acid, the main PC found in both samples, was over 3-fold higher in PN + SC (43.3 mg per 100 g) than in PN (Table 1). Gallic acid is involved in various signaling pathways that regulate many biological functions including pro- and anti-inflammatory, NO signaling, intrinsic and extrinsic apoptosis, and NF-κB signaling pathways.[45] Flavonoids also have an important health contribution. Epigallocatechin gallate (EGCG), found in PN and PN + SC, improves insulin resistance, prevents adiposity, and maintains lipid homeostasis caused by *in vivo* circadian desynchronization induced by a fructose and fat rich diet.[8] Other health benefits of pistachios are related to their fat-soluble phytochemicals, including tocols and carotenoids,[16,24] that exert a positive effect on circadian rhythm.[36] In this study, γT > αT > βT > γT3 (12–13 mg kg^{-1}) and total carotenoids (~0.025 mg kg^{-1}) were detected with no significant differences between PN and PC + SC (Table 1). We reported similar results in two preceding studies evaluating raw (untreated) PN + SC *cv.* Kerman[16,24] and according to Liu *et al.*,[17] pistachios are a very rich source (μg per g fresh weight) of lipophilic bioactives including γ-tocopherol (182), and lutein (42). High γT plasma levels have been positively associated with reduced risk for all-cause cancer and cardiovascular disease mortality,[46] while lutein (main pistachio carotenoid), zeaxanthin, and meso-zeaxanthin increase the concentration of macular pigments, lowering the UV light induced photo-oxidative stress due to their antioxidant action.[47]

Melatonin (aka. *N*-acetyl-5-methoxytryptamine, circadin; $C_{13}H_{16}N_2O_2$, Pubchem CID: 896) commonly known as "the darkness hormone" is produced from tryptophan (Trp) in the human pineal gland. Its function is essential in age-related

disturbances including chronodisruption and cell senescence.[48] It is produced by various body tissues, but the intestine produces 400 times more melatonin than the pineal gland.[49] However, the amount of physiologically produced melatonin decreases with age and may be related to the development of pathological conditions. In this case, PMT from plant foods can be used.

PMT-containing foods include tree nuts, cereals, fruits and vegetables, coffee, green tea, and animal origin products, among others.[2] Pistachios have one of the highest PMT contents above other foods such as roasted coffee beans, mushrooms, and lentils (6500, 6400 and 1089 ng g^{-1}, respectively).[9,50] Pistachio seed coat contributes almost 20% (43.3 mg ME per g sample) of the total pistachio PMT content (215.6 mg ME per g, PN + SC) (Table 1). The Mediterranean diet recommends daily pistachio intake of 32 g, which provides 6.4 mg of PMT.[51] Recently, PMT has been proposed as a key coordinator of the core biological clock-redox network[50] and according to Reiter *et al.*,[10] melatonin exerts its antioxidant activity by the following mechanisms: (A) direct detoxification of reactive oxygen (ROS) and nitrogen (NOS) species, (B) stimulation of antioxidant enzymes and (C) suppressing the activity of pro-oxidant enzymes.

Finally, most pistachio phytochemicals exhibit strong antioxidant activity although it is considered intermediate compared to other edible nuts.[31,52] This antioxidant activity has been associated with the chemopreventive, cardiopreventive, and vasoprotective action of pistachios.[36] Thus, pistachios ranked among the 50 food products with the highest antioxidant potential.[53] In this study, TEAC was 3-fold higher in PN + SC than in PN (638 and 156 μM TE per 100 g, respectively: $p \leq 0.05$). The overall antioxidant capacity in PN + SC correlated positively with PMT ($r = 0.657$, $p \leq 0.05$) while the latter was inversely associated with PN ($r = -0.989$, $p \leq 0.05$). PMT has higher antioxidant capacity than classic antioxidants including ascorbic acid and vitamin E.

3.3. Bioaccessibility of bioactive compounds from pistachios during *in vitro* gastrointestinal digestion and colonic fermentation

3.3.1. Total phenolic compounds and flavonoids. The ultimate health benefits of pistachios depend partly on the effective gastrointestinal delivery (bioaccesibility) of its phytochemicals. The structure-specific bioaccessibility of pistachios' phytochemicals is influenced by the amount and type of dietary fibers and fats (unsaturated fatty acids) more than its protein fraction.[24] In particular, the PCs of pistachios apparently link covalently to non-digestible matrix components like polysaccharides, structural proteins, and highly polymerized phenols resulting in partial bioaccessibility of phenolic compounds (~50%, oral + gastric + intestinal: Stevens-Barron *et al.*[24]). In contrast, Mandalari *et al.*[19] reported that pistachios PCs are highly bioaccessible during simulated GI digestion and therefore available for absorption in the upper GI tract, other authors argue otherwise.[54] However, bioaccessible polyphenols in the upper GI tract (oral-gastric-small intestine) can be efficiently absorbed and metabolized (first-pass metabolism) while those covalently linked to dietary fiber can metabolize further under colonic conditions.[13,55]

Chewing and saliva mixing are crucial for the initial bioaccessibility of nut-derived phytochemicals by reducing their particle size and humectation. According to Fig. 1a, ~1.3% (~0.03 to 0.05 μg g^{-1}) of the original PCs amount present in PN and PN + SC was bioaccessible under oral conditions. Mechanical chewing preliminary breaks down and grinds the matrix of pistachios, partially releasing medium-to-low molecular weight phytochemicals such as lipids (*e.g.* triacylglycerides and sterols), micronutrients (*e.g.* fat-soluble vitamins) and xenobiotic phytochemicals (*e.g.* phenolic acids; Stevens-Barron *et al.*).[24] Incomplete grinding may in turn affect the observed bioaccessibility, despite the protective effect of transient micelle formation that lipids exert on PCs.[55] Moreover, pistachios' PCs are very likely bound to dietary fiber since their bioaccessibility from PN and PN + SC did not increase significantly after drastic acidification and partial proteolysis (pepsin) during simulated gastric conditions. This confirms our recent report that 27% of the associated variance observed in the apparent bioaccessibility of PCs from several edible nuts and peanuts is inversely related to their dietary fiber content.[24]

The *in vitro* bioaccessibility of PCs from both samples (PN > PN + SC) was more pronounced under simulated intestinal conditions (Fig. 1a): A bi-phasic trend was observed for PCs from PN + SC, characterized by a simultaneous release ability of PC concurrent with their apparent permeability predisposed to an efflux-type behavior (Table 2). PCs are partially absorbed in the small intestine after the complete breakdown of digestible carbohydrates and proteins except those bound to undigestible matrix components under colonic conditions;[54] colonic PCs are further degraded and biotransformed into other metabolites including short-chain fatty acids by beneficial bacteria lowering the pH and preventing the pathogenic microbial growth.[56] Since the activity of colon microbiota on PCs increases (PN) or decreases (PN + SC) the chemical nature and contribution of SCs' dietary fiber to PCs' bioaccessibility deserve further study.

On the other hand, the bioaccessibility of flavonoids (mg ER per g) was lower ($p \leq 0.05$) than that observed for PCs in both samples (Fig. 1b). The highest bioaccessibility of flavonoids from PN + SC was achieved under simulated intestinal conditions (30 min, 27%) while that for PN was under colonic conditions (24 h, 73.6%; Fig. 1b). Contrary to our results, Mandalari *et al.*[19] reported that 90% of flavonoids are released from pistachios under gastric conditions and we have previously reported the same for several tree nuts (including pistachios) and peanuts.[24] Under gastric conditions, the maximum relaxation of several macromolecules present in the food matrices occur although they can also establish transient interactions with smaller molecules present in both the food matrix and with the GI tract, limiting their luminal releasability and further absorption under intestinal conditions.[24]

Under colonic conditions, the bioaccessibility of flavonoids decreased further (Fig. 1b) to reach the maximum after 24 h of

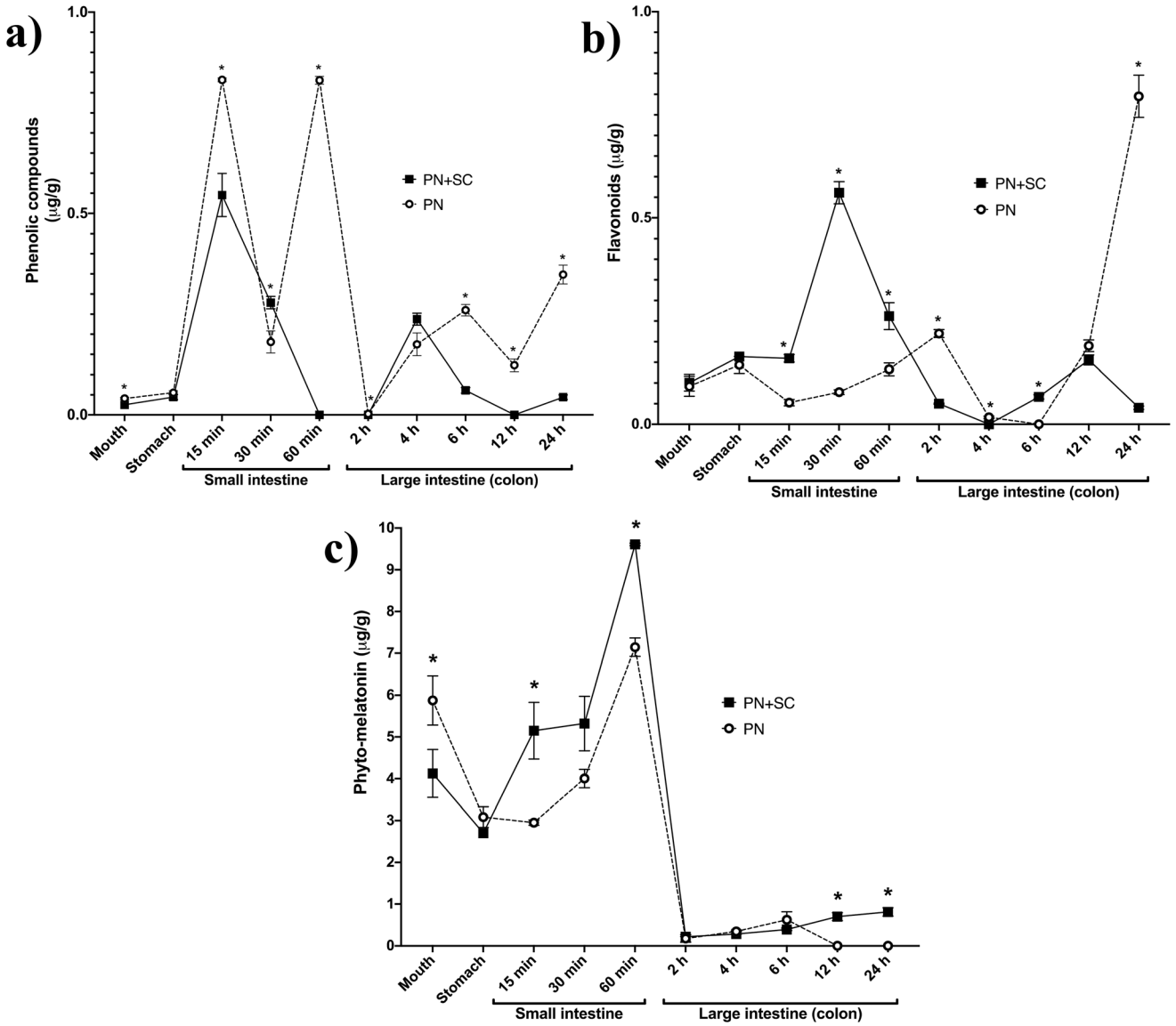

Fig. 1 Bioaccessibility of phytochemicals during *in vitro* gastrointestinal digestion of pistachio nuts with (PN + SC) and without (PN) the seed coat (SC). (a) Total polyphenols (GAE), (b) flavonoids [rutin equivalents (RE)] (c) phytomelatonin [μg melatonin (ME)]. Results are expressed as the mean ± the standard deviation of two independent experiments per duplicate. * Indicates a significant difference between samples (PN *vs.* PN + SC) by stage from Student's *t*-test for unpaired data $p < 0.05$.

PN fermentation (0.8 μg ER per g). Under colonic conditions, flavonoids are broken down into low molecular weight PCs and/or biotransformed into other non-phenolic metabolites by the microbiota.[55] Rocchetti *et al.*[31] reported that simultaneous *in vitro* digestion and fermentation processes time dependently degrade flavonoids, lignans, and hydroxycinnamic acids and form lower molecular weight compounds, namely hydroxycinnamic, hydroxybenzoic, and hydroxyphenylacetic acids and several tyrosols, alkylphenols and hydroxyphenylpropenes.

3.3.2. Phytomelatonin. This is the first report of PMT bioaccessibility. In the oral stage, bioaccessibility was low for both pistachio samples (1.92 and 3.41%, PN + SC and PN) (Fig. 1c). The low bioaccessibility during this stage may be due to its rapid absorption in the epithelium of the oral mucosa. PMT is an amphipathic molecule enabling it to cross lipid membranes thereby making it is accessible to almost all somatic cells. Also, this compound interacts with melatonin membrane receptors 1 and 2 (MT1 and MT2) which are coupled to G proteins and are present in salivary glands.[57] In this study, PMT bioaccessibility was affected by gastric conditions; at this stage, both pistachio samples had the lowest values. The acidic pH induces the polymerization of indoleamines, limiting their detection.[58]

The highest bioaccessibility of PMT for both samples occurred during the intestinal stage, increasing stepwise with the incubation time. However, PN + SC displayed the highest

Table 2 *Ex vivo* apparent permeability coefficients (P_{app}) of the selected phytochemicals from pistachio samples: time-trend changes

		PN + SC			PN		
Component	Time (min)	A → B	B → A	ER	A → B	B → A	ER
Total polyphenols	15	—	41.7^{a*}	—	27.6^{x*}	27.4^{x}	1.0^{y*}
	30	2.5^{b}	9.1^{b*}	3.7^{a*}	6.3^{y*}	14^{y}	2.2^{x}
	60	17.6^{a*}	41.6^{a*}	2.4^{b*}	10^{y}	—	—
Flavonoids	15	0.9^{b}	0.7^{b*}	0.9^{b}	0.34^{y}	2.2^{z}	6.4^{y*}
	30	0.5^{c}	1.1^{b}	2.2^{a}	0.42^{y}	7.6^{x*}	18.6^{x*}
	60	1.7^{a}	1.8^{a}	1.2^{b}	2.0^{x}	3.6^{y*}	1.9^{z}
Phytomelatonin	15	2.8^{a*}	3.7^{b*}	1.3^{b}	2.0^{y}	2.1^{z}	1.1^{z}
	30	3.5^{a}	3.8^{b}	1.1^{b}	1.3^{z}	2.9^{y}	2.2^{x*}
	60	3.2^{a}	6.9^{a*}	2.2^{a}	2.8^{x}	5.2^{x}	1.9^{y}

P_{app} values are expressed as mean (cm $s^{-1} \times 10^{-3}$) ± standard deviation ($n \geq 4$). Transport from apical (A) to basolateral (B) chamber (A → B) and the other way around (B → A), efflux ratio [ER = (B → A)/(A → B)]; below the detection limit (—). * Statistical differences (*t*-test, $p < 0.05$) between samples (PN *vs.* PN + SC) and between incubation times (superscript letters) for the same component and sample.

bioaccessibility (4.46%, 60 min). Furthermore, for both samples, all the bioaccessible PMTs were permeated (small intestine) (data not shown). Melatonin has a relatively high lipid partition coefficient (1570 M^{-1}) and can easily cross the lipid bilayers and sub-cellular organelles such as mitochondria.[59] Melatonin is considered within the category of highly permeable compounds,[60] which indicates that PMT from pistachios may become bioavailable and maintain the good function of the clock system, as the body's melatonin.[2]

The time of colonic fermentation had a significant effect on PMT release from PN + SC (Fig. 1c). After 6 h of incubation, PN reached the highest PMT bioaccessibility (0.36% at 6 h), which was undetected after 12 h of incubation. However, PMT release for PN + SC was higher after 6 h of incubation, presumably from the skin during microbial dietary fiber fermentation. Thus, bioaccessible PMT reaches the colon, and another part is released from the food matrix through microbial activity. Furthermore, the dynamic changes of PMT from PN suggest that it is used as a substrate by some microbial communities, as reported previously for synthetic melatonin.[11] Moreover, PMT may improve the composition and diurnal rhythmicity of some specific gut microbiota species/genera in obese individuals, as has been demonstrated in obese mice.[61] The potential effects of PMT on gut microbiota circadian rhythms must be further explored.

3.4. Apparent permeability of pistachio bioactives

The apparent permeability is defined as the accumulation rate of a compound in apical and basolateral chambers, normalized by superficial tissue area, where the basolateral side is considered the one to blood flux, and the intestinal lumen is the apical side.[26] Table 2 shows the time-trend (15–60 min) changes in the apparent permeability coefficients (P_{app}) for selected phytochemicals (PCs, flavonoids, and PMT). P_{app} values closely mimic their *in vivo* absorption process and further bioavailability of pistachio bioactives,[17,19] reflecting their absorption in the small intestine stage.

P_{app} values (A → B and B → A) change over time in a sample-specific and phytochemical-specific manner. P_{app} A → B for PCs increased (PN + SC) or decreased (PN); those for flavonoids increased (both), whereas their corresponding P_{app} B → A remained practically unchanged resulting in efflux ratios [ER = (B → A)/(A → B)] favoring efflux. The time-trend ER values for PMT were generally stable, suggesting an intra/extracellular equilibrium. Together the data suggest passive rather than active transport mechanisms, as previously reported for other food matrixes.[26]

3.5. SCFA production

The highest levels of SCFAs were produced at 12 h during *in vitro* colonic fermentation for both pistachio samples, where PN + SC displayed the highest ($p \leq 0.05$) value (51 mmol L^{-1}) (Fig. 2a), followed by PN (25.9 mmol L^{-1}) and control (2.06 mmol L^{-1}). Moreover, the results indicated that all fermented substrates were fit with a polynomial (cubic) response ($r^2 \geq 0.96$), reaching its maximum at 12 (control) and 16 (PN + SC, PN) h, respectively. SCFAs are the main metabolites derived from bacterial fermentation of dietary fibers, the level of which was higher for PN + SC (13.2%) (Table 1).

Pistachio samples (PN + SC – black triangles, and PN – grey squares) were highly effective in producing SCFAs, as well as shifting their molar ratios, especially to butyric production than the positive control (inulin, white circles) (Fig. 2a) as previously reported for nuts compared to a recognized prebiotic fiber (Synergy1®).[62] The net ratios of SCFA production (Ac : But : Prop) at 16 h were as follows: inulin (1 : 9.2 : 4.7), PN (1 : 0.2 : 1) and PN + SC (1 : 1.5 : 1.3). The SCFA production from PN + SC at 12 h was 1 (acetate)-, 0.7 (butyrate)-, and 1.5 (propionate)-fold higher than that from PN. The significant SCFA increase, especially butyrate, from pistachio may indicate their potential to improve health in obese subjects through an increased proportion of beneficial butyrate-producing bacteria observed in a randomized cross-over human feeding study.[63]

The SCFA molar ratio (4–12 h fermentation) remained almost constant for the positive control and both pistachio samples: butyric > propionic > acetic (Fig. 2b). The relatively higher butyric acid production may be attributed to the donor characteristics; obesity causes dysbiosis in the colonic micro-

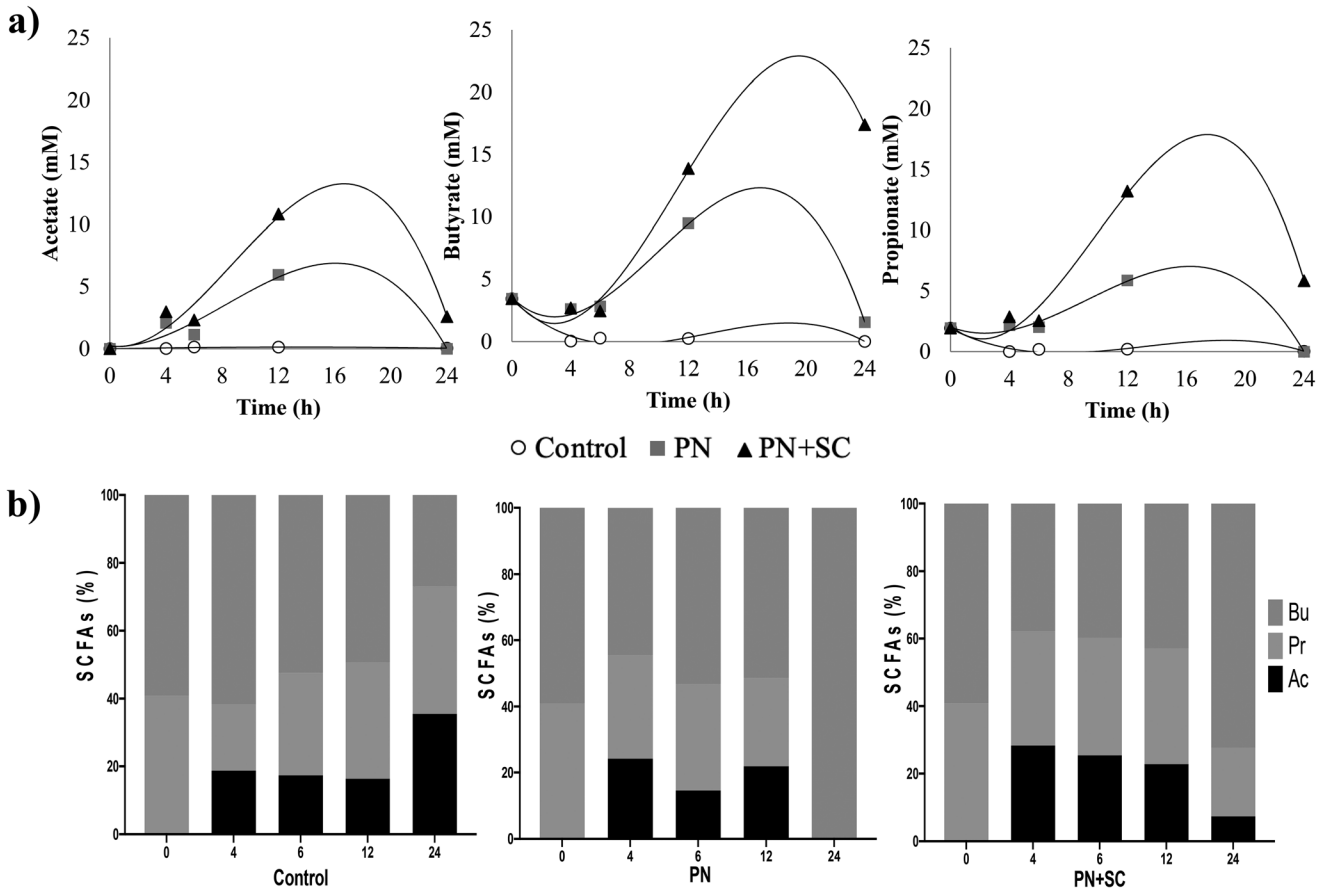

Fig. 2 Time-trend (a) and molar ratio (b) of short-chain fatty acid (SCFA) production during *in vitro* colonic fermentation of pistachio nuts with (PN + SC) and without (PN) the seed coat (SC) and a positive control (inulin). Results are expressed as the mean of two independent experiments per duplicate (n = 4).

biota leading to higher butyric acid production.[4,14,64] However, at 24 h, the molar ratio differed among samples; PN and PN + SC predominantly produced butyric acid, whereas inulin generated acetic acid. Regular pistachio consumption positively modifies the composition of colonic microbiota in obese mice, decreasing the inflammation associated genera like *Desulfovibrio*, responsible for hydrogen sulfide (H_2S) production in the colon.[65] The enhanced output of butyric acid at 24 h may be associated with H_2S decrease, also an indirect marker of improved intestinal health and inflammation in obese individuals,[4] as previously reported in mice.[44]

The most significant changes (PN > PN + SC > control) in the SCFA molar ratio were observed between 12 and 24 h, with butyrate being the only SCFA produced from PN at 24 h (Fig. 2b). The molar ratio observed at 24 h from PN and PN + SC suggests that acetic and propionic acids are mainly produced from the skin, and butyric acid from the seed. Although PN had a lower dietary fiber content, as well as its fractions, their individual structures can be used by primary degrading populations that mainly establish cross-feeding reactions with butyrogenic populations. The products of SC primary degradation could be (a) directly acetic and propionic acids and (b) used by primary degrading populations that establish cross-feeding reactions with acetogenic, propiogenic, and butyrogenic populations.[66] The SCFA production is the link between host nutrition and intestinal homeostasis maintenance.[67] Furthermore, the administration of dietary fiber (cellobiose) upon fermentation produces SCFAs, which modulate the peripheral clock present in the liver, kidneys, and sub-mandibular gland in an *in vivo* model,[11] highlighting the potential of pistachios in improving chronodisruption in obese individuals.

3.6. Untargeted metabolomic analysis

According to Rocchetti *et al.*,[31] information is still limited regarding the metabolic fate of edible nut-derived bioactives during their GI passage and colonic fermentation as affected by their particular macromolecular composition (food matrix) and luminal factors, further influencing their bioavailability. In this study, an untargeted metabolomic approach was used to track the biotransformation of parent compounds into several volatile metabolites during the *in vitro* (fecal) colonic fermentation of PN and PN + SC.

3.6.1. Unsupervised statistics. The samples were clustered in a heatmap by fermentation times, according to their metab-

olite composition grouping 50 metabolites found in both fermented pistachio samples. The metabolite profile at the beginning of the fermentation (0 h) was the same for both samples; however, with time their pattern and abundance were entirely different (ESI Fig. S1a†).

The abundance of selected compounds (furans, alcohols, aldehydes, and aromatic hydrocarbons) increased, indicating their resistance to GI digestion and fermentation. Caryophyllene is one of the major components of pistachio essential oil, with biological activities.[68] Other identified compounds included eicosane, 2,4-dimethyl-furan, tetradecane,[69] and copaene.[70] 1-Hexadecanol, 1-decanol, and 1-octanol have been identified as components of the essential oil of other nuts,[71] whereas *trans*-α-bergamotene has been identified in other plants such as cinnamon.[72] Furthermore, other compounds such as 3-methyl-1-butanol benzaldehyde, 1-octen-3-ol, and octanal surely originated from the microbial biotransformation of the parent molecules of pistachios.[73,74]

The metabolic pathways were also evaluated for metabolites derived from *in vitro* PN and PN + SC fermentation (ESI Fig. S1b†). Butyric acid and sphingolipid metabolisms were the main metabolic pathways for PN and PN + SC, respectively. Certainly, PN contributes to almost 70% of the butyrate produced by PN + SC at 12 h fermentation (Fig. 2a). Ceramides, the molecular base of sphingolipids, are abundant in pistachios, with C16:0 being the most representative species, followed by 18:1, 18:0 and 14:0;[34] *Bacteroides* and *Bifidobacterium* metabolize dietary sphingolipids.[75] Gut-derived bacterial sphingolipids positively affect host lipid metabolism that may contribute to reverse the chronodisruption induced lipid dysfunction.[73]

3.6.2. Supervised statistics (PLS-DA). Maintaining a healthy microbiota is key to combat dysbiosis-related chronodisruption in obese people. Gut bacteria influence host physiology through various signaling molecules and pathways, some like SCFAs are involved in host circadian clock activity in different tissues.[76] PN showed significant changes (VIP scores ≥ 2) (1.5–3.0) than PN + SC (1.4–2.2) (Fig. 3). Sample-specific metabolites with VIP scores ≥2.0 were (Pubchem CID): PN [1,4-benzodioxan-6-amine (89 148), indole (798), 2,4-dihydroxybenzaldehyde (7213), 4-hydroxymandelic acid (328), nonylcyclopropane (522 556), and 7-methyl-1-undecene (522 554)]; PN + SC [benzaldehyde (240), 2,4-dimethyl-6-tertbutylphenol (15 884), 1,1-dimethyl-2-propyl-cyclohexane (549 978), trichloromethane (6212), 10-methylnonadecane (530 070), hexylchloroformate (22 466), and 1-butanol (263)] (Fig. 4).

These compounds exert beneficial health effects. For example, 2,4-dihydroxybenzaldehyde (PN) is a product of the colonic degradation of flavonoids such as quercetin and rutin, both present in pistachios. Other compounds derived from flavonoids, lignans and phenolic acids have been found (UHPLC-Orbitrap) after *in vitro* colonic fermentation of edible nuts, among them, 3,4-dihydroxyphenylacetic acid (predominantly from pistachios), 4-hydroxybenzoic acid, hippuric acid, caffeic acid, protocatechuic acid and protocatechuic aldehyde.[76] Flavonoids and other PCs positively influence the circadian

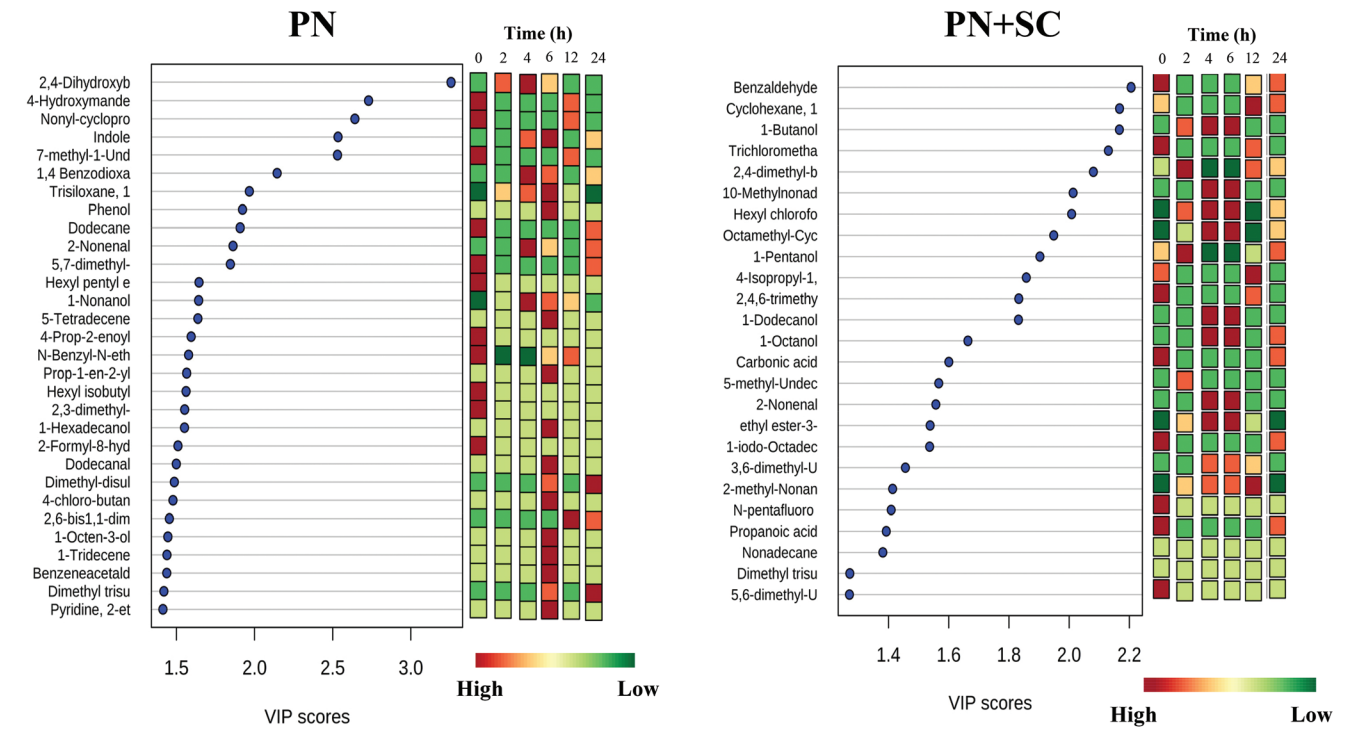

Fig. 3 VIP scores of major metabolites produced during the *in vitro* colonic fermentation of pistachio nuts (PN) with (PN + SC) or without (PN) the seed coat (SC).

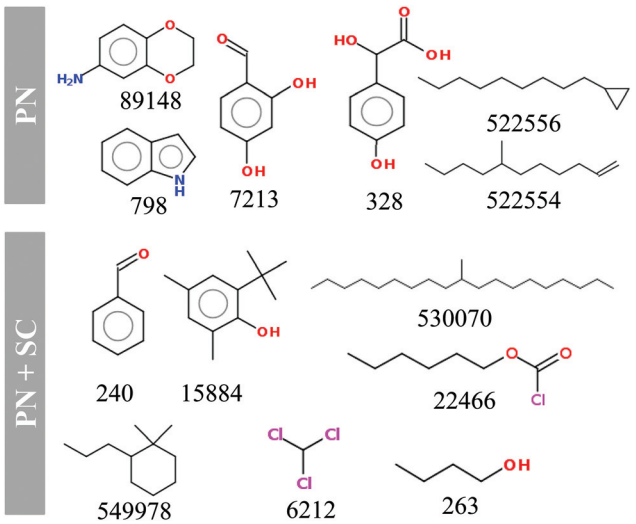

Fig. 4 Main metabolites produced during *in vitro* colonic fermentation of pistachio nuts (PN) with (PN + SC) or without (PN) the seed coat (SC). [1,4-Benzodioxan-6-amine (89 148), indole (798), 2,4-dihydroxybenzaldehyde (7213), 4-hydroxymandelic acid (328), nonyl-cyclopropane (522 556), and 7-methyl-1-undecene (522 554)]; PN + SC [benzaldehyde (240), 2,4-dimethyl-6-tertbutylphenol (15 884), 1,1-dimethyl-2-propylcyclohexane (549 978), trichloromethane (6212), 10-methylnonadecane (530 070), hexyl-chloroformate (22 466), and 1-butanol (263)].

rhythm *in vitro* and *in vivo* that may be helpful for patients with high amplitude circadian rhythm disorders.[77] Hydroxymandelic acid (PN) is a phenolic metabolite whose plasma and urinary concentration increases after consuming orange juice.[78] This phenolic compound has also been recovered from the fecal matter, after the human microbial fermentation of grape seed flavan-3-ols,[79] a compound accumulated in the pistachio seed coat.[41] Benzaldehyde (PN + SC) is the gut product of cyanidin, an anthocyanin present in the pistachio seed coat.[80] Polyphenols may improve circadian rhythms by regulating the colonic microbiota and by the local and systemic effects of their derived microbial metabolites.[77]

The significant increase of indole, the main Trp colonic metabolite, by PN, may signify better health benefits for obese subjects who display an altered colonic tryptophan metabolic pathway. This is reflected by the low production of indole and derivatives which correlated with obesity-related systemic inflammation.[4,81] It is unknown if indolamines could be metabolized to melatonin, increasing the plasmatic levels of this hormone.

Edible nuts, including pistachios, are rich in Trp (0.78 g per 100 g protein[82]) and its GI microbial-derived metabolite indole enhances the epithelial cell barrier function and reduces the IL-10 related inflammatory process, reducing the odds for chronic inflammatory diseases.[83,84] Some Trp metabolites have been identified after walnut consumption, including kynurenic acid, a product with anti-inflammatory and immunosuppressive properties.[85] Trp has been proposed as one of the key molecules to be evaluated in the chrononutrition field to improve circadian rhythms because it is both a serotonin and melatonin precursor.[85] Nonyl-cyclopropane and 10-methylnonadecane have been identified as a component of the essential oils from other plant sources.[86,87] In fact, omega-3 and omega-6 fatty acids, including docosahexaenoic acid and arachidonic acid, as well as several metabolites of linoleic acid, were marked elevated (LC-HRAM analysis) in fecal samples after two weeks of walnut consumption (mice).[85] PUFAs modulate miRNAs that regulate the circadian system although information on the effects of essential oils on the regulation of the biological clock is limited.[88,89]

On the other hand, the concentration of 1-butanol from PN + SC reached its maximum between 2 and 6 h fermentation and then decreased, suggesting positive changes in the metabolic activity of the microbiota or a shift in their composition. However, further studies on the microbiome changes are required.

4. Conclusions

Our results suggest that the functional phytochemicals of pistachios are bioaccessible through GI digestion; some can reach the colon, where they can be used by the microbiota. Therefore, pistachios may exert chronobiotic effects due to the potential bioavailability of their functional phytochemicals, particularly PMT, and the production of SCFAs and derived metabolites in the colon. These are relevant for overweight and obese populations, helping them alleviate some adverse effects since they present low melatonin secretion, altered SCFA production, circadian alterations, and dysbiosis. This is the first report of PTM bioaccessibility, and further studies are required for in-depth identification of compounds, antioxidant capacity, and microbiota composition and to evaluate their *in vivo* chronobiological effect.

Author contributions

Elisa Dufoo-Hurtado: Data curation, writing – original draft. Rocío Olvera-Bautista: Data curation. Abraham Wall-Medrano: Writing-review and editing. Ma. Guadalupe Flavia Loarca-Piña: Writing-review and editing. Rocio Campos-Vega: Conceptualization, Methodology, Software, Writing-review and editing.

Conflicts of interest

The authors declare no conflict of interest.

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References

1 T. Shamah-Levy, I. Campos-Nonato, L. Cuevas-Nasu, L. Hernández-Barrera, M. D. C. Morales-Ruán, J. Rivera-Dommarco and S. Barquera, Overweight and obesity in Mexican vulnerable population. Results of Ensanut 100k, *Salud Publica Mexico*, 2020, **61**, 852–865.

2 E. Dufoo-Hurtado, A. Wall-Medrano and R. Campos-Vega, Naturally-derived chronobiotics in chrononutrition, *Trends Food Sci. Technol.*, 2020, **95**, 173–182.

3 F. F. Anhê, T. V. Varin, M. Le Barz, Y. Desjardins, E. Levy, D. Roy and A. Marette, Gut microbiota dysbiosis in obesity-linked metabolic diseases and prebiotic potential of polyphenol-rich extracts, *Curr. Obes. Rep.*, 2015, **4**, 389–400.

4 F. J. S. Muniz and C. S. Martin, Clock Genes, Chronodisruption, Nutrition and Obesity, *Curr. Res. Diabetes Obes. J.*, 2017, **3**, 1–6.

5 Y. Tahara and S. Shibata, Chrono-biology, chrono-pharmacology, and chrono-nutrition, *J. Pharmacol. Sci.*, 2014, **124**, 320–335.

6 M. L. Dreher, Pistachio nuts: composition and potential health benefits, *Nutr. Rev.*, 2012, **70**(4), 234–240.

7 M. Lavialle, G. Champeil-Potokar, J. M. Alessandri, L. Balasse, P. Guesnet, C. Papillon, P. Pévet, S. Vancassel, B. Vivien-Roels and I. Denis, An (n-3) polyunsaturated fatty acid–deficient diet disturbs daily locomotor activity, melatonin rhythm, and striatal dopamine in syrian hamsters, *J. Nutr.*, 2008, **138**, 1719–1724.

8 Y. Mi, G. Qi, R. Fan, X. Ji, Z. Liu and X. Liu, EGCG ameliorates diet-induced metabolic syndrome associating with the circadian clock, *Biochim. Biophys. Acta, Mol. Basis Dis.*, 2017, **1863**, 1575–1589.

9 X. Meng, Y. Li, S. Li, Y. Zhou, R. Y. Gan, D. P. Xu and H. B. Li, Dietary sources and bioactivities of melatonin, *Nutrients*, 2017, **9**, 367–431.

10 R. J. Reiter, J. C. Mayo, D. X. Tan, R. M. Sainz, M. Alatorre-Jimenez and L. Qin, Melatonin as an antioxidant: under promises but over delivers, *J. Pineal Res.*, 2016, **61**, 253–278.

11 D. X. Tan, L. C. Manchester, E. Esteban-Zubero, Z. Zhou and R. J. Reiter, Melatonin as a potent and inducible endogenous antioxidant: synthesis and metabolism, *Molecules*, 2015, **20**, 18886–18906.

12 K. Peuhkuri, N. Sihvola and R. Korpela, Diet promotes sleep duration and quality, *Nutr. Res. Rev.*, 2012, **32**, 309–319.

13 R. M. Lamuel-Raventos and M. P. S. Onge, Prebiotic nut compounds and human microbiota, *Crit. Rev. Food Sci. Nutr.*, 2017, **57**, 3154–3163.

14 A. C. G. De Cosío-Barrón, A. M. Hernández-Arriaga and R. Campos-Vega, Spent coffee (*Coffea arabica* L.) grounds positively modulate indicators of colonic microbial activity, *Innovative Food Sci. Emerging Technol.*, 2020, **60**, 102286.

15 Y. Tahara, M. Yamazaki, H. Sukigara, H. Motohashi, H. Sasaki, H. Miyakawa, A. Haraguchi, Y. Ikeda, S. Fukuda and S. Shibata, Gut microbiota-derived short chain fatty acids induce circadian clock entrainment in mouse peripheral tissue, *Sci. Rep.*, 2018, **8**, 1–12.

16 P. E. Garcia-Moreno, L. A. De la Rosa, J. C. Stevens-Barron, R. Rodriguez-Ramirez, B. Corral-Diaz, E. Alvarez-Parrilla, F. J. Olivas-Aguirre and A. Wall-Medrano, Dehiscence and prolonged storage of 'Kerman' Pistachios: Effects on morphometry and nutraceutical value, *J. Food Sci. Technol.*, 2020, **57**, 1–11.

17 Y. Liu, J. B. Blumberg and C. Y. O. Chen, Quantification and bioaccessibility of California pistachio bioactives, *J. Agric. Food Chem.*, 2014, **62**, 1550–1556.

18 A. Tomaino, M. Martorana, T. Arcoraci, D. Monteleone, C. Giovinazzo and A. Saija, Antioxidant activity and phenolic profile of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins, *Biochimie*, 2010, **92**, 1115–1122.

19 G. Mandalari, C. Bisignano, A. Filocamo, S. Chessa, M. Sarò, G. Torre, R. M. Faulks and P. Dugo, Bioaccessibility of pistachio polyphenols, xanthophylls, and tocopherols during simulated human digestion, *Nutr. J.*, 2013, **29**, 338–344.

20 B. D. Oomah, A. Cardador-Martínez and G. Loarca-Piña, Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L), *J. Sci. Food Agric.*, 2005, **85**, 935–942.

21 V. L. Singleton and J. A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Vitic.*, 1965, **16**, 144–158.

22 A. K. Ramírez-Jiménez, R. Reynoso-Camacho, S. Mendoza-Díaz and G. Loarca-Piña, Functional and technological potential of dehydrated *Phaseolus vulgaris* L. flours, *Food Chem.*, 2014, **161**, 254–260.

23 L. R. Fukumoto and G. Mazza, Assessing antioxidant and prooxidant activities of phenolic compounds, *J. Agric. Food Chem.*, 2000, **48**, 3597–3604.

24 J. C. Stevens-Barron, L. A. De la Rosa, A. Wall-Medrano, E. Álvarez-Parrilla, R. Rodríguez-Ramirez, R. E. Robles-Zepeda and H. Astiazaran-García, Chemical composition and *in vitro* bioaccessibility of antioxidant phytochemicals from selected edible nuts, *Nutrients*, 2019, **11**, 2303–2326.

25 E. Oladi, M. Mohamadi, T. Shamspur and A. Mostafavi, Spectrofluorimetric determination of melatonin in kernels of four different pistacia varieties after ultrasound-assisted solid–liquid extraction, *Spectrochim. Acta, Part A*, 2014, **132**, 326–329.

26 R. Campos-Vega, K. Vázquez-Sánchez, D. López-Barrera, G. Loarca-Piña, S. Mendoza-Díaz and B. D. Oomah, Simulated gastrointestinal digestion and *in vitro* colonic fermentation of spent coffee (*Coffea arabica* L.): Bioaccessibility and intestinal permeability, *Food Res. Int.*, 2015, **77**, 156–161.

27 M. A. Lassoued, F. Khemiss and S. Sfar, Comparative study of two *in vitro* methods for assessing drug absorption: Sartorius SM 16750 apparatus versus everted gut sac, *Int. J. Pharm. Pharm. Sci.*, 2011, **14**, 117–127.

28 R. Campos-Vega, R. Reynoso-Camacho, G. Pedraza-Aboytes, J. A. Acosta-Gallegos, S. H. Guzman-Maldonado, O. Paredes-Lopez, B. D. Oomah and G. Loarca-Piña,

Chemical composition and *in vitro* polysaccharide fermentation of different beans (*Phaseolus vulgaris* L.), *J. Food Sci.*, 2009, **74**, 59–65.

29 V. M. Zamora-Gasga, G. Loarca-Piña, P. A. Vázquez-Landaverde, R. I. Ortiz-Basurto, J. Tovar and S. G. Sáyago-Ayerdi, *In vitro* colonic fermentation of food ingredients isolated from *Agave tequilana* Weber var. azul applied on granola bars, *LWT–Food Sci. Technol.*, 2015, **60**, 766–772.

30 L. A. Herrera-Cazares, A. K. Ramírez-Jiménez, A. Wall-Medrano, R. Campos-Vega, G. Loarca-Piña, M. L. Reyes-Vega, P. A. Vázquez-Landaverde and M. Gaytán-Martínez, Untargeted metabolomic evaluation of mango bagasse and mango bagasse based confection under in vitro simulated colonic fermentation, *J. Funct. Foods*, 2019, **54**, 271–280.

31 G. Rocchetti, G. Chiodelli, G. Giuberti and L. Lucini, Bioaccessibility of phenolic compounds following *in vitro* large intestine fermentation of nuts for human consumption, *Food Chem.*, 2018, **245**, 633–640.

32 S. RoyChoudhury, T. H. More, R. Chattopadhyay, I. Lodh, C. D. Ray, G. Bose, H. S. Sarkar, B. Chakravarty, S. Rapole and K. Chaudhury, Polycystic ovary syndrome in Indian women: a mass spectrometry-based serum metabolomics approach, *Metabolomics*, 2017, **13**, 115.

33 L. E. Griffin and L. L. Dean, Nutrient composition of raw, dry-roasted, and skin-on cashew nuts, *J. Food Res.*, 2017, **6**, 13–28.

34 R. Paroni, M. Dei Cas, J. Rizzo, R. Ghidoni, M. T. Montagna, F. M. Rubino and M. Iriti, Bioactive phytochemicals of tree nuts. Determination of the melatonin and sphingolipid content in almonds and pistachios, *J. Food Compos. Anal.*, 2019, **82**, 103227.

35 H. M. Bailey and H. H. Stein, Raw and roasted pistachio nuts (*Pistacia vera* L) are "Good" sources of protein based on their digestible indispensable amino acid score (DIAAS) as determined in pigs, *J. Sci. Food Agric.*, 2020, **100**, 3878–3885.

36 C. Alasalvar, J. S. Salvadó and E. Ros, Bioactives and health benefits of nuts and dried fruits, *Food Chem.*, 2020, **314**, 126192.

37 L. D'Evoli, M. Lucarini, P. Gabrielli, A. Aguzzi and G. Lombardi-Boccia, Nutritional value of Italian pistachios from Bronte (*Pistacia vera*, L.), their nutrients, bioactive compounds and antioxidant activity, *J. Food Nutr. Sci.*, 2015, **6**, 1267–1276.

38 A. E. Yanni, G. Mitropoulou, I. Prapa, G. Agrogiannis, N. Kostomitsopoulos, E. Bezirtzoglou, Y. Kourkoutas and V. T. Karathanos, Functional modulation of gut microbiota in diabetic rats following dietary intervention with pistachio nuts (*Pistacia vera* L.), *Metab. Open*, 2020, **7**, 100040.

39 T. C. Fung, H. E. Vuong, C. D. Luna, G. N. Pronovost, A. A. Aleksandrova, N. G. Riley, A. Vavilina, J. McGinn, T. Rendon, L. R. Forrest and E. Y. Hsiao, Intestinal serotonin and fluoxetine exposure modulate bacterial colonization in the gut, *Nat. Microbiol.*, 2019, **4**, 2064–2073.

40 M. Lavialle, G. Champeil-Potokar, J. M. Alessandri, L. Balasse, P. Guesnet, C. Papillon, P. Pévet, S. Vancassel, B. Vivien-Roels and I. Denis, An (n-3) polyunsaturated fatty acid–deficient diet disturbs daily locomotor activity, melatonin rhythm, and striatal dopamine in syrian hamsters, *J. Nutr.*, 2008, **138**(9), 1719–1724.

41 S. M. Kim, N. Neuendorff, R. S. Chapkin and D. J. Earnest, Role of inflammatory signaling in the differential effects of saturated and poly-unsaturated fatty acids on peripheral circadian clocks, *EBioMedicine*, 2016, **7**, 100–111.

42 M. H. Grace, D. Esposito, M. A. Timmers, J. Xiong, G. Yousef, S. Komarnytsky and M. A. Lila, *In vitro* lipolytic, antioxidant and anti-inflammatory activities of roasted pistachio kernel and skin constituents, *Food Funct.*, 2016, **7**(10), 4285–4298.

43 T. Guo, C. T. Ho, X. Zhang, J. Cao, H. Wang, X. Shao, D. Pan and Z. Wu, Oolong tea polyphenols ameliorate circadian rhythm of intestinal microbiome and liver clock genes in mouse model, *J. Agric. Food Chem.*, 2019, **67**, 11969–11976.

44 J. Guo, X. Han, J. Zhan, Y. You and W. Huang, Vanillin alleviates high fat diet-induced obesity and improves the gut microbiota composition, *Front. Microbiol.*, 2018, **9**, 2733.

45 N. Kahkeshani, F. Farzaei, M. Fotouhi, S. S. Alavi, R. Bahramsoltani, R. Naseri, S. Momtaz, Z. Abbasabadi, R. Rahimi, M. H. Farzaei and A. Bishayee, Pharmacological effects of gallic acid in health and diseases: A mechanistic review, *Iran. J. Basic Med. Sci.*, 2019, **22**, 225–237.

46 W. Chai, G. Maskarinec, A. A. Franke, K. R. Monroe, S. Y. Park, L. N. Kolonel, L. R. Wilkens, L. Le Marchand and R. V. Cooney, Association of serum γ-tocopherol levels with mortality: the Multiethnic Cohort Study, *Eur. J. Clin. Nutr.*, 2020, **74**, 87–96.

47 M. F. Culver, J. Bowman and V. Juturu, Lutein and Zeaxanthin Isomers Effect on Sleep Quality: A Randomized Placebo-Controlled Trial, *Biomed. J. Sci. Tech. Res.*, 2018, **9**, 7018–7024.

48 D. P. Cardinali, Melatonin as a chronobiotic/cytoprotector: its role in healthy aging, *Biol. Rhythm Res.*, 2019, **50**, 28–45.

49 C. Q. Chen, J. Fichna, M. Bashashati, Y. Y. Li and M. Storr, Distribution, function, and physiological role of melatonin in the lower gut, *World J. Gastroenterol.*, 2011, **17**, 3888–3898.

50 M. B. Arnao and J. Hernández-Ruiz, Is phytomelatonin a new plant hormone?, *Agron. J.*, 2020, **10**, 95.

51 A. Bach-Faig, E. M. Berry, D. Lairon, J. Reguant, A. Trichopoulou, S. Dernini, F. X. Medina, M. Battino, R. Belahsen, G. Miranda and L. Serra-Majem, Mediterranean diet pyramid today. Science and cultural updates, *Public Health Nutr.*, 2011, **14**, 2274–2284.

52 P. Rosales-Martínez, S. Arellano-Cárdenas, L. Dorantes-Álvarez, F. García-Ochoa and M. D. S. López-Cortez, Comparison between antioxidant activities of phenolic extracts from Mexican peanuts, peanuts skins, nuts and pistachios, *J. Mex. Chem. Soc.*, 2014, **58**, 185–193.

53 B. L. Halvorsen, M. H. Carlsen, K. M. Phillips, S. K. Bøhn, K. Holte, D. R. Jacobs Jr. and R. Blomhoff, Content of redox-active compounds (ie, antioxidants) in foods con-

sumed in the United States, *Am. J. Clin. Nutr.*, 2006, **84**, 95–135.
54 F. Shahidi and H. Peng, Bioaccessibility and bioavailability of phenolic compounds, *J. Food Bioact.*, 2018, **4**, 11–68.
55 J. A. Domínguez-Ávila, A. Wall-Medrano, G. R. Velderrain-Rodríguez, C. Y. O. Chen, N. J. Salazar-López, M. Robles-Sánchez and G. A. González-Aguilar, Gastrointestinal interactions, absorption, splanchnic metabolism and pharmacokinetics of orally ingested phenolic compounds, *Food Funct.*, 2017, **8**, 15–38.
56 F. Shahidi and J. Yeo, Insoluble-Bound Phenolics in Food, *Molecules*, 2016, **21**, 1216–1237.
57 M. Emet, H. Ozcan, M. Yayla, Z. Halici and A. Hacimuftuoglu, A review of melatonin, its receptors and drugs, *Eurasian J. Med.*, 2016, **48**, 135–141.
58 H. J. Shin, N. H. Park, W. Lee, M. H. Choi, B. C. Chung and J. Hong, Metabolic profiling of tyrosine, tryptophan, and glutamate in human urine using gas chromatography–tandem mass spectrometry combined with single SPE cleanup, *J. Chromatogr. B: Biomed. Sci. Appl.*, 2017, **1051**, 97–107.
59 J. C. Mayo, R. M. Sainz, P. González-Menéndez, D. Hevia and R. Cernuda-Cernuda, Melatonin transport into mitochondria, *Cell. Mol. Life Sci.*, 2017, **74**, 3927–3940.
60 H. Yu, E. J. Dickson, S. R. Jung, D. S. Koh and B. Hille, High membrane permeability for melatonin, *J. Gen. Physiol.*, 2016, **147**, 63–76.
61 J. Yin, Y. Li, H. Han, J. Ma, G. Liu, X. Wu, X. Huang, R. Fang, K. Baba, P. Bin, G. Zhu, W. Ren, B. Tan, G. Tosini, X. He, T. Li and Y. Yin, Administration of exogenous melatonin improves the diurnal rhythms of the gut microbiota in mice fed a high-fat diet, *mSystems*, 2020, **5**, e00002–e00020.
62 W. Schlörmann, M. Birringer, A. Lochner, S. Lorkowski, I. Richter, C. Rohrer and M. Glei, *In vitro* fermentation of nuts results in the formation of butyrate and c9, t11 conjugated linoleic acid as chemopreventive metabolites, *Eur. J. Nutr.*, 2016, **55**, 2063–2073.
63 M. Ukhanova, X. Wang, D. J. Baer, J. A. Novotny, M. Fredborg and V. Mai, Effects of almond and pistachio consumption on gut microbiota composition in a randomised cross-over human feeding study, *Br. J. Nutr.*, 2014, **111**, 2146–2152.
64 S. Rahat-Rozenbloom, J. Fernandes, G. B. Gloor and T. M. Wolever, Evidence for greater production of colonic short-chain fatty acids in overweight than lean humans, *Int. J. Obes.*, 2014, **38**, 1525–1531.
65 S. Terzo, F. Mulè, G. F. Caldara, S. Baldassano, R. Puleio, M. Vitale, G. Cassata, V. Ferrantelli and A. Amato, Pistachio consumption alleviates inflammation and improves gut microbiota composition in mice fed a high-fat diet, *Int. J. Mol. Sci.*, 2020, **21**, 365.
66 N. T. Baxter, A. W. Schmidt, A. Venkataraman, K. S. Kim, C. Waldron and T. M. Schmidt, Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers, *mBio*, 2019, **10**, e02566–e02518.
67 C. Martin-Gallausiaux, L. Marinelli, H. M. Blottière, P. Larraufie and N. Lapaque, SCFA: mechanisms and functional importance in the gut, *Proc. Nutr. Soc.*, 2020, 1–13.
68 S. F. Taghizadeh, G. Davarynejad, J. Asili, B. Riahi-Zanjani, S. H. Nemati and G. Karimi, Chemical composition, antibacterial, antioxidant and cytotoxic evaluation of the essential oil from pistachio (*Pistacia khinjuk*) hull, *Microb. Pathog.*, 2018, **124**, 76–81.
69 M. Z. Ozel, F. Gogus, J. F. Hamilton and A. C. Lewis, The essential oil of *Pistacia vera* L. at various temperatures of direct thermal desorption using comprehensive gas chromatography coupled with time-of-flight mass spectrometry, *Chromatographia*, 2004, **60**, 79–83.
70 M. R. Nargesi, F. Bagheri, S. Shahriari and R. Tahvilian, Comparison of ultrasonic assist head space solid-phase microextraction and classic hydrodistillation methods for the identification of essential oil in fruits and leaves of *Pistacia atlantica*, *Indian J. Nat. Prod. Resour.*, 2018, **9**, 28–33.
71 J. Mata and G. C. Villegas-Castro, Costa Rican cashew (*Anacardium occidentale* L.): Essential oils, carotenoids and bromatological analysis, *Am. J. Essent. Oil Nat. Prod.*, 2018, **6**, 01–09.
72 A. Adarsh, B. Chettiyar, B. M. Kanthesh and N. Raghu, Phytochemical screening and antimicrobial activity of "*Cinnamon zeylanicum*", *Int. J. Innovative Pharm. Sci. Res.*, 2020, **13**, 22–33.
73 N. Fatima and S. Rana, Metabolic implications of circadian disruption, *Pfluegers Arch.*, 2020, 1–14.
74 Y. Feng, C. Cui, H. Zhao, X. Gao, M. Zhao and W. Sun, Effect of koji fermentation on generation of volatile compounds in soy sauce production, *Int. J. Food Sci. Technol.*, 2013, **48**, 609–619.
75 M. T. Lee, H. H. Le and E. L. Johnson, Dietary sphinganine is selectively assimilated by members of the mammalian gut microbiome, *J. Lipid Res.*, 2021, **62**, 100034.
76 G. Rocchetti, S. R. Bhumireddy, G. Giuberti, R. Mandal, L. Lucini and D. S. Wishart, Edible nuts deliver polyphenols and their transformation products to the large intestine: An *in vitro* fermentation model combining targeted/untargeted metabolomics, *Food Res. Int.*, 2019, **116**, 786–794.
77 D. Song, C. S. Yang, X. Zhang and Y. Wang, The relationship between host circadian rhythms and intestinal microbiota: A new cue to improve health by tea polyphenols, *Crit. Rev. Food Sci. Nutr.*, 2020, **61**, 1–10.
78 G. Pereira-Caro, I. A. Ludwig, T. Polyviou, D. Malkova, A. García, J. M. Moreno-Rojas and A. Crozier, Identification of plasma and urinary metabolites and catabolites derived from orange juice (poly) phenols: analysis by high-performance liquid chromatography–high-resolution mass spectrometry, *J. Agric. Food Chem.*, 2016, **64**, 5724–5735.
79 P. Mena, L. Calani, R. Bruni and D. Del Rio, *Bioactivation of high-molecular-weight polyphenols by the gut microbiome, Diet-microbe interactions in the gut*, Academic Press, 2015.

80 M. G. Bellomo and B. Fallico, Anthocyanins, chlorophylls and xanthophylls in pistachio nuts (*Pistacia vera*) of different geographic origin, *J. Food Compos. Anal.*, 2007, **20**, 352–359.
81 S. Cussotto, I. Delgado, A. Anesi, S. Dexpert, A. Aubert, C. Beau, D. Forestier, P. Ledaguenel, E. Magne, F. Mattivi and L. Capuron, Tryptophan metabolic pathways are altered in obesity and are associated with systemic inflammation, *Front. Immunol.*, 2020, **11**, 1–7.
82 M. Venkatachalam and S. K. Sathe, Chemical composition of selected edible nut seeds, *J. Agric. Food Chem.*, 2006, **54**, 4705–4714.
83 E. E. Alexeev, J. M. Lanis, D. J. Kao, E. L. Campbell, C. J. Kelly, K. D. Battista, M. E. Gerich, B. R. Jenkins, S. T. Walk, D. J. Kominisky and S. P. Colgan, Microbiota-derived indole metabolites promote human and murine intestinal homeostasis through regulation of interleukin-10 receptor, *Am. J. Pathol.*, 2018, **188**, 1183–1194.
84 T. Bansal, R. Alaniz and A. Jayaraman, Role for the bacterial signal indole in promoting epithelial cell barrier function, *J. Epithelial Biol. Pharmacol.*, 2012, **5**, 32–38.
85 M. Nakanishi, A. Matz, C. Klemashevich and D. W. Rosenberg, Dietary walnut supplementation alters mucosal metabolite profiles during DSS-induced colonic ulceration, *Nutrients*, 2019, **11**, 1118.
86 R. Bravo, L. Franco, A. B. Rodríguez, L. Ugartemendia, C. Barriga and J. Cubero, Tryptophan and hops: Chrononutrition tools to improve sleep/wake circadian rythms, *Ann. Clin. Nutr.*, 2018, **1**, 1004–1011.
87 G. Zahra, B. Khadijeh, D. Hossein and S. Ali, Essential oil composition of two Scutellaria species from Iran, *J. Tradit. Chin. Med. Sci.*, 2019, **6**, 244–253.
88 N. O. Avoseh, I. A. Ogunwande, P. A. Afolabi, O. A. Lawal, T. D. Thang, R. Ascrizzi and F. Guido, Essential oil of Cordia millenii from Nigeria, *Am. J. Essent. Oil Nat. Prod.*, 2018, **6**, 13–17.
89 L. Daimiel-Ruiz, M. Klett-Mingo, V. Konstantinidou, V. Micó, J. F. Aranda, B. García, J. Martínez-Botas, A. Dávalos, C. Fernández-Hernando and J. M. Órdovás, Dietary lipids modulate the expression of miR–107, a miRNA that regulates the circadian system, *Mol. Nutr. Food Res.*, 2015, **59**, 1865–1878.

