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Metabolism and bioavailability aspects of natural products of plant origin using mass spectrometry-based and metabolomic approaches

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The therapeutic value of natural products (NPs) is well established, as evidenced by their rich ethnopharmacological history and the significant proportion of marketed drugs derived from natural sources. Despite their notable advantages such as structural versatility and scaffold diversity, NPs have been increasingly sidelined by the pharmaceutical industry due to the labor-intensive nature of their isolation and structural elucidation as well as issues related to patenting, sustainable sourcing and preclinical evaluation. Moreover, current bioavailability research focuses predominantly on well-known medicinal and edible plants or specific compound classes, leaving many other promising candidates underexplored. The interplay between the gut microbiota and NPs, which is critical for pharmacokinetics and ADME (absorption, distribution, metabolism, and excretion), is also overlooked. Numerous *in vitro* and *in vivo* models have been developed to study the ADME properties of xenobiotics, while human clinical trials remain scarce in the field of NPs. Recent technological advancements, including innovations in mass spectrometry (MS), smart library screening, dereplication, molecular networking, and metabolomics, have significantly improved the NP research pipeline, offering faster and more accurate compound identification. High-resolution instruments like Orbitrap, QTOF, FT-ICR, and MRMS, alongside IMS and advanced data acquisition techniques (DDA and DIA), now offer deeper insights into complex mixtures. Despite MS being a cornerstone of pharmacokinetics–pharmacodynamics (PK/PD) studies, the integration of metabolomics and big data analytics remains underutilized, particularly in NP prioritization. This review aims to explore the evolution of MS in NP metabolism studies, from early investigations to current multidisciplinary approaches, proposing a critical reflection on the challenges in NP drug development.

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1. Introduction

The therapeutic value of natural products (NPs) is well established, as justified by their impressive ethnopharmacological track record as well as the high percentage of marketed drugs derived directly or indirectly from nature.[1] Nevertheless, due to numerous reasons, the valorization of NPs by the

pharmaceutical industry has fallen severely behind, despite their significant advantages such as extensive scaffold diversity and structural versatility.[2] This can be partially attributed to the often laborious process of extraction, isolation and structure elucidation of NPs, which inevitably leads to an evident gap in the NP drug development pipeline, especially regarding sustainable sourcing and preclinical steps.[3] Specifically in the latter case, despite the plethora of traditional phytotherapy data available from multiple origins throughout the world,[4] most studies related to the bioavailability of natural bioactives focus either on the well-known medicinal plants (*e.g. Echinacea*, cannabis, and St. John's wort) and edible (*e.g.* grapevine, citrus fruits, olive, coffee, and cocoa) plants or on specific chemical categories, with flavonoids, polyphenols, cannabinoids, stilbenes (resveratrol) and lignans getting the lion's share of the literature space, thus significantly limiting the exploitation potential of other promising candidates.

Regarding the established NP discovery pipeline, so far, most approaches in the NP field are still largely based on *in vitro* screening assays to identify the pharmacological properties of potentially active principles.[2,5] However, the fact that any observed activity could be attributed not only to the parent molecules but also to their metabolites in humans is highly neglected, especially considering the important interplay between the gut microbiota and natural bioactives.[6] Additionally, other pharmacokinetic (PK)/ADME properties immediately linked to bioavailability are overlooked, thus increasing the gap between basic and translational research, and further hindering the "drugability" of NPs, particularly regarding polyherbal formulations.[7] In fact, it appears that for most NPs, the research on bioavailability and metabolism seldom goes beyond biochemical, cell-based or *in vivo* assays, given that clinical trials involving human participants are relatively limited due to their high cost, difficulties in participant recruitment and formulation standardization, as well as issues arising from patent submission. Therefore, to date, most phytotherapeutics are treated as supplements and the full potential of active compounds is significantly underexploited, further disorienting the NP drug discovery process.[8]

Thus far, many *in vitro* and *in vivo* models have been developed to assess the ADME properties of xenobiotics including drugs, food components and NPs. *In vitro* models that aim to assess the oral bioavailability of active principles can be largely classified into three major categories, namely traditional 2D cell monolayers, with the most common employing the Caco-2 and HT-29 cell lines, 3D cellular systems and fluid dynamic platforms, with the latter providing the most promising potential for the study of the intestinal activity in a human-mimicking environment.[9,10] Evidently, these models are often associated with certain limitations; for instance, the failure to address

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issues such as population variability or the intricate interaction between different systems of a complete organism. On the other hand, *in vivo* animal models still constitute the golden standard of preclinical research given that they are the closest model to human physiology. Despite the plethora of available platforms, rodent models represent the majority for both pharmaceutical and nutritional studies. However, due to the differences in inter-species biochemical processes, the use of laboratory animals in metabolism research has been widely criticized.[11]

In parallel, the impressive technological advancement in recent years, which has also permeated the field of NPs, drastically facilitates the entire pipeline from natural sources to a single compound.[12] New methods, concepts and approaches such as smart library screening, dereplication studies, molecular networking workflows and metabolomic approaches significantly accelerate the entire process making it much more rational, sensitive, and specific. This is particularly true in the field of mass spectrometry (MS), where the enormous progress achieved in instrumentation as well as in the incorporation of bioinformatics tools under the metabolomics concept, has completely revolutionized the metabolite annotation process.[13] In fact, the emergence of high-resolution mass analyzers such as the Orbitrap, quadrupole time-of-flight (QTOF), and more recently Fourier-transform ion cyclotron resonance (FT-ICR) or magnetic resonance-MS (MRMS) instruments has provided a significant boost in the analysis and characterization of complex mixtures such as plant extracts, given their profound versatility, increased sensitivity, high resolving power, wide detection dynamic range and ability for hyphenation with chromatographic and spectroscopic techniques.[14,15] More recently, ion mobility spectrometry (IMS), which uses an electric field to separate analytes through a buffer gas, has been increasingly employed to assist in the identification of isobaric compounds.[16] Moreover, the diverse possibilities that are currently available for the acquisition of fragmentation spectra such as data-dependent acquisition (DDA) and data-independent acquisition (DIA) techniques provide a rich diversity of information that scientists need to handle and interpret, thus generating novel challenges along the way.[14] Interestingly, although MS from its early days has been an integral part of PK–PD research, the true potential of modern tools such as metabolomics and big data analysis has not yet been fully utilized in these studies, a fact which is particularly evident where NP prioritization is concerned.

Taking the above into consideration, the current review attempts to offer an inquisitive view on the available literature regarding the use of MS to study plant-derived NP metabolism, from the first papers published in the early 1980s to the latest reports on multidisciplinary studies combining the expertise of analytical chemistry, bioinformatics, combinatorial chemistry, pharmacology and systems biology, among others. Through this journey, we will try to briefly address the challenges faced by modern scientists working in this field and offer our viewpoint on the future of NPs in drug development, with the hope to ignite a fruitful discussion towards an alternative workflow in the bioactive NP discovery process.

2. Consideration points

When setting out on our quest to map the metabolism of NPs with MS, it was evident that a vast amount of information was available that needed to be sorted. Soon, it was made clear that the preliminary literature search had to involve the combination of the terms "bioavailability" and "mass spectrometry", given that at this stage, we considered that limiting our search with the keywords "natural products" would seriously restrain our findings and lead to the loss of valuable information. Nevertheless, the terms "metabolism" and "biotransformation" were not employed because they primarily diverted the query to purely clinical and biotechnological fields, respectively. Also, to restrict the vast volume of results generated, several exclusion criteria were adopted. Articles discussing synthetic or semi-synthetic drugs, inorganic materials including minerals as part of the diet, dissolved organic matter (DOM), amino acids, peptides, proteins, hormones, sugars and polysaccharides, nucleotides, lipids (even of natural origin), vitamins, widely used antibiotics and mycotoxins were excluded from the literature survey. Additionally, novel delivery systems such as nanocarriers and cocrystals were not considered in the current review, unless they were tested in a clinical study involving human participants.

In silico studies were also excluded from the literature survey, unless their results were examined in conjunction with actual MS analyses. Furthermore, "microbiome" was only considered if referring to the human or animal microbiome, regardless of the testing platform (*in vitro*, *in vitro–ex vivo*, *in vivo*, and clinical studies) and only if the research was conducted under a pharmaceutical development concept. Veterinary research articles and studies dealing with ecotoxicity (*e.g.* bioavailability in fish and crustaceans, unless they were used as model organisms) were excluded from our query. In this case, if for some reason, the line between drug development and environmental impact was not clear, the authors decided case by case. Moreover, only papers employing MS for monitoring compounds in physiological fluids were considered. Articles only discussing profiling of extracts by MS were not included, even if they covered ADMET/PK–PD aspects with other assays and analytical techniques. Papers discussing herb–drug interactions and those that did not provide any data on MS parameters employed were also excluded. Articles not written in the English language were not considered in the current review, including those whose abstract was also published in English. Finally, due to the vast number of articles available on this topic, the final selection of articles that were included was based on our critical opinion. Recognizing this shortcoming, where available, we have tried to refer the reader to more specialized reviews that provide a deeper insight into the PK, bioavailability and metabolism of certain classes of compounds. The chemical classes and/or compounds discussed in greater detail in this review were selected based on their frequent occurrence in the literature, the novelty of the MS approach, and the comprehensiveness of the work presented. This selection aims to inform the reader about the current state of research in this field. We have to note

Table 1 Definition of certain terms regarding bioavailability and metabolism

Term	Definition	Bibliography
Bioavailability	"The rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action"	17
Bioaccessibility	"The fraction that is soluble in the gastrointestinal (GI) environment and is available for absorption"	18
Pharmacokinetics	"All physiological processes related to the absorption, distribution, metabolism and excretion (ADME)" of a xenobiotic	19
Pharmacodynamics	The relationship between the measured drug concentration and the resulting effect, including the time course and intensity of therapeutic and adverse effects	20
Biotransformation	"Is the chemical conversion of substances by living organisms or enzyme preparations", while "in medicinal chemistry the term metabolism refers to the biotransformation of xenobiotics and particularly drugs" with metabolites being intermediates or products of metabolic reactions	21
Metabolomics	"The comprehensive analysis of all metabolites in a given sample"	22
Untargeted metabolomics	The analysis of all the detectable metabolites in a sample, including chemical unknowns	23
Targeted metabolomics	The measurement of defined metabolites or chemical groups thereof	

that even though the term "natural products" is extensively used in this review, we recognize that our search was limited to plant specialized metabolites. Evidently, NPs encompass a greater deal of chemical space; however, for brevity and clarity herein, we will not deal with NPs from microbial, marine or animal sources, although we acknowledge this shortcoming.

Medicinal plants, phytotherapeutics, and herbal products that have seen little progress over the past 25 years or do not focus on metabolism with the use of MS are also not included. Alternatively, certain studies on less common compounds are discussed, highlighting their novelty and/or the completeness and rigor of their design and implementation. It is worth noting as a conclusion of this search that it is very difficult to examine the literature with a strict focus on NPs with biological and/or pharmacological action. Generally, the relevant information is obscured by the huge number of studies related to nutrition or various types of supplements. In our opinion, this is an interface that most probably cannot be avoided but tends to hide the substantial potential of NPs. Furthermore, it is important to highlight that very often in the literature, there are studies where the exact composition of the formulation administrated is neglected or partially given. However, this could be greatly misleading given that several precursors, even minor ones, could contribute to the formation of the same metabolite(s). In this review, we also tried to consider this aspect in our article selection.

Another point that needs to be made is the apparent inconsistency in terms of liquid chromatography (LC)-MS terminology employed by different authors working in this field. The lack of accurate method reporting, particularly in earlier publications, the confusion between vendor-specific and generic terms, and the often-observed contradictions within the same publication on MS parameters in conjunction with reported results are only a few of the problems that can be encountered in published articles. Thus, to counter these issues in the current review, we have tried to unify to a certain degree the employed terminology without heavily interfering with the phrasing chosen by the authors to describe their own methods.

Finally, going through the available literature, as authors we felt that several terms related to the subjects of bioavailability and metabolism are often confused or used interchangeably, even though they might constitute distinct notions, destined to describe discrete proesses. Therefore, the definition of certain terms is summarized in Table 1.

3. Key players in metabolism investigation

3.1 Phenolics

3.1.1 Citrus flavonoids. One of the most well-studied classes of compounds regarding metabolism and bioavailability is the constituents of various citrus fruits, likely due to their long history of regular intake worldwide. The consumption of fruits from the *Citrus* genus (Rutaceae) is increasing, given that they are linked to several health benefits, including a reduced risk of diabetes, cardiovascular diseases, and cancer. The most abundant compounds in citrus fruits are the flavanones naringin **(1)** (aglycone), hesperidin **(2)** and narirutin **(3)** (glycosides) with certain polymethoxyflavones and flavonols found in lower concentrations.[24–26] Due to the widespread consumer interest in citrus fruits, several studies have been published regarding their metabolism, and particularly for the flavanones hesperetin **(4)**, naringenin **(5)** and their glycosylated derivatives. Unlike other chemical groups, several studies have been conducted in humans, primarily in the form of juices. There are numerous animal studies, mainly in rats, investigating various biological fluids and tissues for metabolite detection. However, studies involving pure compounds are considerably fewer, though this is a common practice across all chemical categories. Fig. 1 illustrates the main citrus flavonoids and their metabolism pathways based on certain representative works.[26–28] For a more detailed account on the bioavailability and metabolism of citrus phenolics, readers are encouraged to refer to the works by Zhang *et al.*, Visvanathan *et al.*, and Karn *et al.*[25–27]

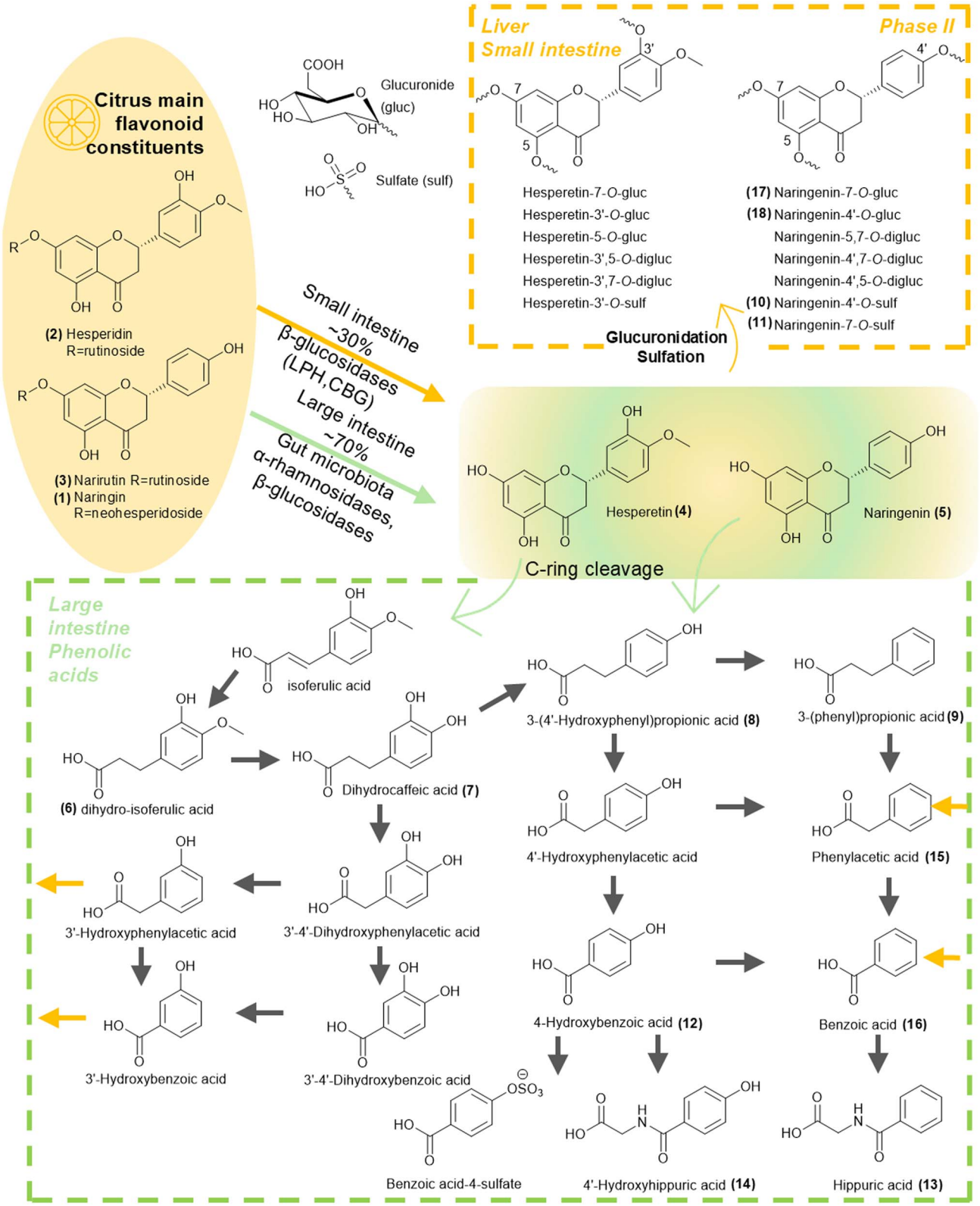

Fig. 1 Three major citrus flavonoids are hesperidin, narirutin and naringin. They are metabolized by approx. 30% in small intestine mainly through the activity of β-glucosidase and lactase phlorizin hydrolase (CBG, LPH, accordingly). The metabolization degree is higher in the large intestine (approx. 70%) due to the activity of α-rhamnosidase and β-glucosidase. In both cases, aglycone forms are liberated. Different glucuronides and sulfates are formed based on the basic scaffold structural motifs mainly in the liver and through phase II metabolization. During catabolism, phenolic acids are the main metabolites after the cleavage of C ring of the flavanones. Different derivatives of ferulic, phenylacetic and benzoic acids are produced together with hippuric acid and its derivatives.[26–28]

The work of Pereira-Caro *et al.* presented the *in vitro* colonic catabolism of citrus flavanones to investigate the effect of the gut microbiota on the native compounds. Pure hesperetin and naringenin were incubated overnight with human fecal slurries from healthy male and female volunteers and metabolite detection was carried out by gas chromatography (GC)-MS and LC-photodiode array detection (PDA)-MS/MS. Hesperetin was converted to 3-(3′-hydroxy-4′-methoxyphenyl)propionic acid (dihydro-isoferulic acid **(6)**), 3-(3′,4′-dihydroxyphenyl)propionic acid (dihydrocaffeic acid **(7)**), and 3-(3′-hydroxyphenyl)propionic acid, while the major end product derived from naringenin was 3-(4′-hydroxyphenyl)propionic acid **(8)** followed by 3-(phenyl)propionic acid **(9)**.[29] It should be noted that although this is an *in vitro* study, albeit employing human biofluids, and its findings may be difficult to translate to human conditions, it provides valuable information. This is especially true given the use of pure compounds, which is rarely seen in human studies where whole foods or mixtures are typically used. In a continuation study published by the same group, plasma and urinary metabolites of orange juice polyphenols were detected and tentatively identified in samples of male human volunteers, this time employing an LC-Orbitrap-high resolution (HR)MS platform. Through this effort, 19 flavanone metabolites and 65 colon-derived phenolic acid catabolites were detected in both matrices. Furthermore, among the compounds tentatively annotated, two urinary flavanone metabolites, namely naringenin-4′-*O*-sulfate **(10)** and an eriodictyol-*O*-glucuronyl-sulfate, and more than 40 phenolic catabolites that had not previously been described were reported, thus highlighting the value of high-resolution MS data acquisition in these studies.[28]

In animal set-ups, Guo and coworkers investigated the intestinal absorption and distribution of pure naringin, hesperidin administered orally, as well as their metabolites in male KM mice. The intestinal tissues of the animals were collected and divided into duodenum, jejunum, ileum, cecum, and colon and the samples were analyzed on an LC-QTOF-MS/MS platform. The authors report the detection of 7 metabolites of naringin and 13 of hesperetin, which have previously been described following citrus flavanone consumption and widely distributed in the different intestinal tissues.[30] Similarly, Zeng *et al.* described the distribution of naringin and its metabolites in fourteen male and female Sprague-Dawley rat tissues following a single oral administration of the compound. In this case an LC-TripleTOF-HRMS platform was employed for analyte detection, with a total of 23 flavonoid metabolites and 15 phenolic catabolites being tentatively annotated in all samples. Additionally, naringenin glucuronides were the predominant metabolites in plasma, naringenin and naringenin-7-*O*-sulfate **(11)** were the prevalent forms in most tissues, and phenolic catabolites derived from naringin were mainly found in liver and kidney samples.[31] Alternatively, Jiao *et al.* presented the metabolic biotransformation of hesperetin and its rutinoside hesperidin after oral administration in male Sprague-Dawley rat plasma and fecal and urine samples using LC-Orbitrap MS instrumentation. Consequently, 17 metabolites of hesperetin and 52 of hesperidin were detected in total with mono- and di-glucuronidation, as well as sulfation being the main observed metabolic reactions.[32]

In 2011, Manthey and coworkers investigated the PK and metabolism of the polymethoxylated flavones tangeretin and nobiletin after gavage and IP administration in male Wistar rats. The analyte levels in rat serum were monitored by LC-PDA-MS, while isolation and structure elucidation of metabolites was also carried out from urine samples. Two metabolites of tangeretin and eight metabolites of nobiletin were detected and measured over a 24 h period including glucuronidated and demethylated derivatives of the parent compounds.[33] Similarly, Ferreira and colleagues examined the metabolic biotransformation of eriocitrin, the so-called lemon or citrus flavonoid, in male Wistar rats with the collection of plasma, urine and tissue samples at different time-points over a 24 h period, analyzed in an LC-PDA-MS system. 9 metabolites were detected in urine and 7 in tissue samples, while notably the authors proceeded with the isolation and structure elucidation of urine metabolites, including eriodictyol, homoeriodictyol and their glucuronidated conjugates. According to the authors, the most abundant metabolite of eriocitrin was homoeriodictyol-7-*O*-glucuronide and its respective aglycone, homoeriodictyol, together amounting to a 73% of the total eriocitrin metabolites in the studied tissues.[34] Notably, the isolation and structural determination of metabolites using other spectroscopic methods, such as nuclear magnetic resonance (NMR) spectroscopy, is of utmost importance. Unexpected metabolites can often be formed, and unambiguous identification based solely on MS may not be possible, especially for compounds with multiple isomers or chiral centers, as is commonly the case with NPs.

Recently, Bai *et al.* described the PK and metabolism of naringin through oral and IV administration in different biological systems, namely Sprague-Dawley rats, beagle dogs and healthy human volunteers. In all cases, plasma samples were collected, while human participants also provided urine and fecal specimens. All matrices were analyzed in an LC-tQ-MS/MS system. The reported glucuronidation and sulfate conjugation reactions were noted in all biological substrates, while methylation reactions were only observed in rats and dogs. Finally, certain metabolites such as 2,4,6-trihydroxybenzoic acid, 4-hydroxybenzoic acid **(12)**, hippuric acid **(13)**, and 4-hydroxyhippuric acid **(14)** were only detected in traces in the human urine and fecal samples.[35] In this comparative study, the interspecies variation in metabolic biotransformations is nicely discussed.

Aiming to investigate the impact of various factors on metabolism, Fraga *et al.* investigated the metabolic profiles of citrus phenolics in a healthy volunteer cohort that consumed 500 mL of orange juice daily for 60 days. Urine samples were collected at three time points and analytical measurements were performed by LC-PDA-MS. 15 flavanone phase II conjugates were detected, including 9 hesperetin, 3 naringenin, and 3 eriodictyol derivates, mainly glucuronide and sulfate conjugates. Additionally, 78 phenolic acids, derived from phenylpropanoid, phenyl propionic, phenylacetic **(15)**, benzoic **(16)**, benzenetriol, benzoylglycine, and hydroxycarboxylic acids were

also observed, with glucuronide and sulfate derivatives being the most common. Remarkably, the authors did not detect any discrimination between the BMI or sex of the participants, but rather proposed a stratification based on the metabolic profiles of the volunteers.[36] On a similar note, Nishioka *et al.* examined the interindividual differences in 24 h urine metabolite profiles of healthy volunteers following "Pera" and "Moro" orange juice consumption with the help of an LC-tQ-MS platform. 10 phase II metabolites of hesperetin and naringenin were identified in urine samples at different times after the consumption of both orange juices, including five hesperetin and five naringenin phase II metabolites characterized as glucuronide and sulfate conjugates. The authors proposed two distinct stratifications of volunteers based on their excretion profiles, while an attempt was made to associate these differences with the gut microbiota profiles.[37] Similarly, Ávila-Gálvez *et al.* investigated the differences in the plasma and urine metabolic markers detected in male and female healthy volunteers, following either an eriocitrin-rich lemon extract or hesperidin-rich orange extract. The samples were analyzed with an LC-QTOF-HRMS system using a targeted screening strategy. Overall, a total of 17 metabolites were detected, and remarkably the authors observed that hesperetin metabolites were almost 11-fold higher after the consumption of lemon *vs.* orange extract.[38] In a study conducted by Castello *et al.*, an LC-electrospray ionization (ESI)-MS^n-targeted approach was implemented to measure the plasma and urine metabolite levels of volunteers receiving two orange juice preparations (fresh and fermented). The authors provide a detailed account of all biotransformation products detected in the physiological fluids of participants with phase II conjugates of hesperetin and naringenin being the dominant metabolites in plasma, while phenolic acids, primarily hydroxybenzoic acids, were the main urinary metabolites observed.[39]

A noteworthy study was performed by Mullen *et al.* in 2008 describing the bioavailability and metabolism of hesperidin and naringin found in orange juice co-administered with a full fat yogurt, in healthy volunteers of both sexes. The impact of the food matrix on the bioavailability of orange juice flavanones has garnered significant attention. Plasma and urine samples were collected at different timepoints and analyzed by LC-PDA-MS, fitted with an ESI source and operated in SIM mode. Yogurt consumption appeared to have little effect on the bioavailability of flavanone over the 24 h window, while several metabolites were detected in both plasma and urine samples. Two hesperetin-*O*-glucuronides were detected in both matrices, while urine also contained a third hesperetin-*O*-glucuronide, two hesperetin-*O*-glucuronide-*O*-sulfates, a hesperetin-*O*-diglucuronide, a naringenin-*O*-diglucuronide, as well as tentatively identified naringenin-7-*O*-glucuronide **(17)** and naringenin-4′-*O*-glucuronide **(18)**. Interestingly, the authors highlighted the superior performance of direct LC-MS analysis and metabolites identification compared to traditional approaches that involve prior hydrolysis and determination of total aglycones.[40]

A noteworthy concept was investigated Spigoni *et al.*, aiming to explore the possible activity of metabolites compared to parent molecules. Specifically, the bioavailability of bergamot flavanones in plasma and urine samples of healthy volunteers was examined, thus shedding light on the metabolic fate of a somewhat neglected member of the *Citrus* genus. Similar to previous reports, mainly sulfates and glucuronides of hesperetin, naringenin and eriodictyol were detected and quantified in the biofluids of participants using an LC-MS platform, while importantly the bioactivity of certain metabolites was also tested on the gene expression of inflammatory markers and apoptosis in myeloid angiogenic cells exposed to stearic acid.[41]

Finally, in a study published by Tomás-Navarro *et al.* in 2021, a combination of untargeted and targeted metabolomics approaches was implemented to examine the metabolic fate of orange juice consumption in urine samples from healthy volunteers using an LC-QTOF-MS analytical platform. Using MVA statistical analysis, the authors were able to discriminate the biomarkers related to either fresh and industrially processed juice intake, with the latter leading to an increase in hydroxy-polymethoxyflavone sulfates, abscisic acid, and sinapic acid 4′-glucuronide in the physiological fluids of the participants. Moreover, this research highlighted the interindividual variability in the excretion profiles of volunteers, thus adopting an overall holistic approach on the investigation of citrus polyphenol metabolism, taking advantage of novel tools in instrumentation and data processing workflows.[42] This study is one of the few to use metabolomics to explore metabolism, an area of research that is still overlooked but, in our opinion, holds great potential in the future.

3.1.2 Curcumin and curcuminoids. Curcumin is the most abundant phenol, and more specifically diarylheptanoid, found in the rhizome of *Curcuma longa*, Zingiberaceae (commonly referred to as turmeric) and its keto form **(19)** exists in tautomeric equilibrium with its enol form **(20)**. Curcumin has attracted considerable attention due to its impressive biological properties in *in vitro* assays, including a strong antioxidant, anticancer, antibiotic, anti-inflammatory, neuroprotective and anti-aging activity.[43–45] However, these positive effects have largely failed to translate to *in vivo* effects or in clinical practice (curcumin has been characterized as a pan-assay interference compound – PAIN), thus rendering curcumin one of the most famous and at the same time controversial known NPs. Nevertheless, this compound continues to spark interest from scientists and the industry, with several papers being published annually on the topic, and supplements containing either turmeric extracts or pure curcumin flooding the global market.[46] It has been hypothesized that the main culprit behind the poor performance of curcumin *in vivo* is its low solubility and bioavailability, and thus in recent years many research groups have dedicated considerable effort to increasing its absorption through the development of novel delivery systems such as liposomes, micelles, and nanoparticles. However, apart from curcumin, turmeric also contains two additional structurally related congeners that lack either one or both methoxy groups in its phenyl rings, namely demethoxycurcumin **(21)** and bisdemethoxycurcumin **(22)**, respectively, which are collectively referred to as curcuminoids.[45] Fig. 2 demonstrates their chemical structure and basic metabolic pathways.[45,47] Despite the contradiction surrounding these compounds, their popularity

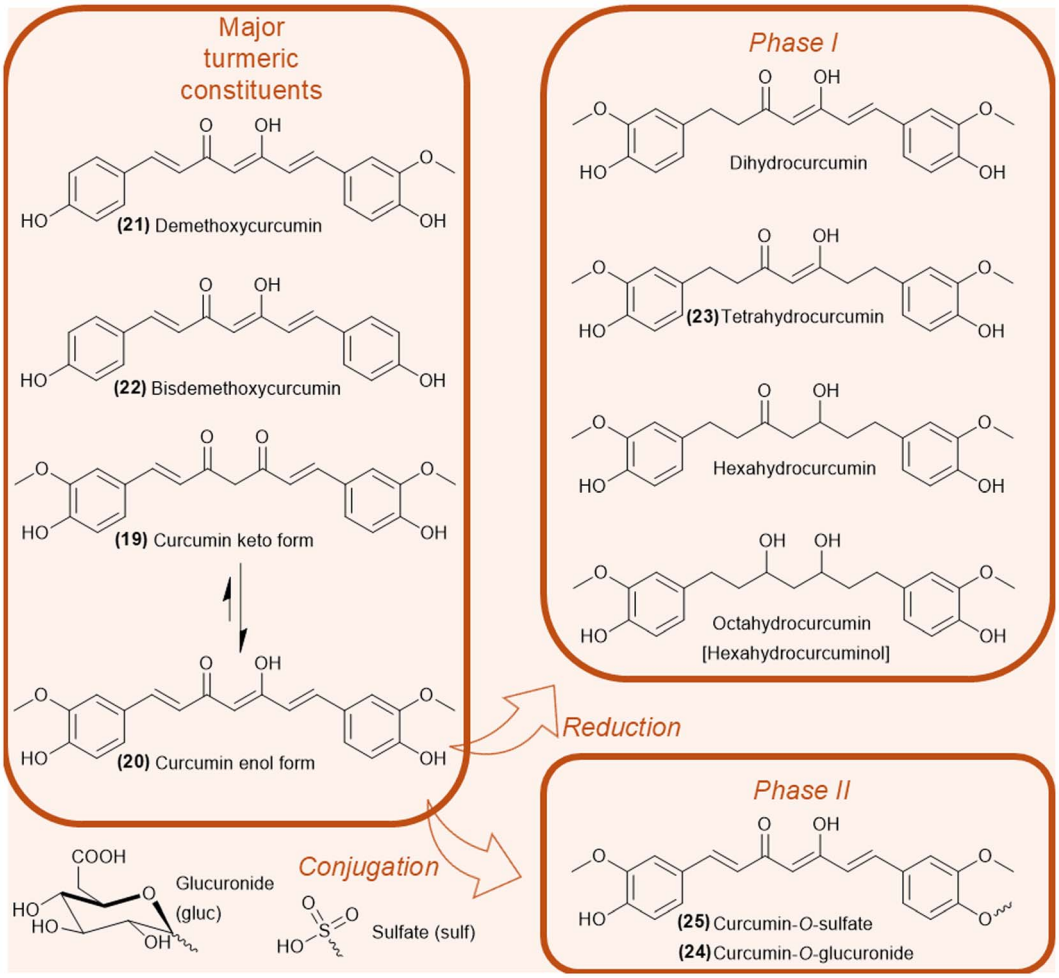

Fig. 2 Major curcuminoids are curcumin and its derivatives missing one or both methoxy groups. Curcumin diketone structure exists in a tautomeric equilibrium with enol structure through intramolecular hydrogen transfer, with the enol form predominating in most solvents. Through phase I metabolism *via* reduction reaction, multiple hydro metabolites are formed, while through phase II metabolism it forms conjugated derivatives (sulfates and glucuronides).[45,47]

cannot be ignored, and therefore selected publications related to their bioavailability and PK utilizing MS approaches will be presented. For deeper insight into the bioavailability and metabolism of curcuminoid, readers are referred to the following work.[45]

One of the first thorough studies regarding curcumin was conducted in 2014 by Cao and coworkers proposing a validated LC-tQ-MS/MS method for the simultaneous quantification of curcumin, demethoxycurcumin, bisdemethoxycurcumin and their metabolic products, namely tetrahydrocurcumin **(23)**, curcumin-*O*-glucuronide **(24)** and curcumin-*O*-sulfate **(25)** in human plasma samples. The authors presented a detailed view of the fragmentation patterns of the compounds in ESI-positive mode, and overall the method demonstrated a good performance.[48] The same year, Ramanilgam *et al.* presented an LC-tQ-MS/MS method for monitoring the curcumin levels in plasma and brain tissue samples of male Balb/c mice. The animals received two curcumin formulation IV (pure curcumin and solid lipid curcumin nanoparticles) and the PK parameters were assessed and found to differ significantly between the two formulations.[49] It is important to highlight here the high number of studies related to curcumin formulations using different experimental designs, set-ups and analytical methods.

In the study published by Tan *et al.*, a mixture containing the three major native curcuminoids was incubated for a 24 h period with human fecal slurry *in vitro*. The samples were analyzed with an LC-IT-MS platform and the metabolites were annotated based on their tandem MS spectra. According to the authors, up to 24% of curcumin, 61% of demethoxycurcumin and 87% of bisdemethoxycurcumin were degraded by the fecal microbiota, while the 3 main metabolites, namely tetrahydrocurcumin, dihydroferulic acid and a metabolite tentatively annotated as 1-(4-hydroxy-3-methoxyphenyl)-2-propanol, were detected in the fermentation cultures.[50]

From a different perspective, Gopi *et al.* described an open-label parallel-arm study, aiming to assess the oral absorption of curcumin in healthy male volunteers receiving a single dose of three distinct formulations. The plasma samples were analyzed with an LC-tQ-MS system, and the PK parameters were assessed for the three major curcuminoids. Notably, the natural

turmeric extract exhibited enhanced bioavailability compared to the other two formulations.[51] More recently, Fança-Berthon *et al.* conducted a human crossover study, whereby healthy volunteers of both sexes consumed a single oral dose of different curcuminoid formulations including a standard turmeric extract. Plasma samples were collected at different time points within a 24 h window and analyzed through an LC-tQ-MS platform, either in their native form or after incubation with β-glucuronidase or sulfatase. The developed method allowed the quantification of 15 curcuminoids (3 parent compounds and 12 metabolites), while significant interindividual variability was observed among the participants.[47] Finally, Kroon *et al.* described an LC-tQ-MS/MS method for the simultaneous quantification of curcumin, demethoxycurcumin, bisdemethoxycurcumin, their metabolite tetrahydrocurcumin as well as piperine, an alkaloid often used as an absorption enhancer in turmeric preparations, in diverse physiological fluids from human participants. The method was tested in a small-scale PK study with a single dose curcuminoid administration in healthy male volunteers donating plasma, urine and fecal samples.[52]

3.1.3 Resveratrol and other stilbenes. Stilbenes are a broad class of compounds encountered in various plant families, many of which produce edible products. Stilbenes are synthesized by plants as a defense mechanism against UV radiation and infections by several pathogens. The structure of stilbene is based on a C6–C2–C6 backbone, comprising two aromatic rings connected through an ethylene bridge.[53] The compound that has gained the most attention in the stilbene group is resveratrol (*trans* **(26)** and *cis* **(27)** isomers). Numerous health effects have been attributed to resveratrol including antioxidant, anti-ageing, anti-inflammatory, and anti-cancer activities. Resveratrol is mainly found in the human diet through the consumption of grape products (primarily red wine), berries and peanuts. However, despite the promising bioactivity exhibited by resveratrol, *in vitro* and *in vivo* experiments and clinical trials with resveratrol-containing formulations did not produce the same favorable results. Similarly to the case of other NPs such as curcumin, the low bioavailability of resveratrol has often been blamed for this phenomenon.[54] Also, it is classified amongst PAINS as a compound with nonspecific global interference properties.[55] Furthermore, a lot of criticism and an open discussion exist regarding resveratrol and its drugability, connected most probably to the above-mentioned aspects.[56] In the current review, a selection of articles was employed to highlight the analysis of resveratrol (and other stilbenes) using LC-MS platforms and methods in physiological fluids; however, the reader is referred to the following works for a more comprehensive overview of stilbene bioavailability and metabolism.[53,54] The chemical structures of stilbenoids and their biotransformation pathways are demonstrated in Fig. 3.[57,58]

In 2004, Walle *et al.* published a study, attempting to measure the levels of ^{14}C resveratrol in healthy male and female volunteers receiving oral and IV single doses of resveratrol. Plasma and urine samples were collected and analyzed with an LC-tQ-MS/MS platform. The authors found that the oral bioavailability of resveratrol was negligible in plasma with a fast sulfate conjugation, occurring in the intestine/liver, being the rate-limiting step, while the principal conjugates of resveratrol detected in the plasma and urine samples were glucuronidated and sulfated derivatives.[59] Azorín-Ortuño and coworkers utilized an interesting biological platform to investigate the PK parameters of *trans*-resveratrol in the plasma samples of pigs, following a single oral administration (5.9 mg kg^{-1} of body weight). Metabolite annotation was performed with an LC-PDA-MS/MS system, with the most abundant being resveratrol-3-*O*-glucuronide **(28)**, followed by a resveratrol sulfate isomer. Additionally, a resveratrol-diglucuronide, two isomers of resveratrol-sulfoglucuronide, another isomer of resveratrol glucuronide as well as the parent compound were detected.[60] In a follow-up study by the same group using the same model and dosage, forty-seven tissues, organs and fluids were analyzed 6 h after intragastric resveratrol administration, employing different MS platforms, including ion trap, tQ and QTOF mass analyzers coupled with LC systems. In total, 12 resveratrol and 7 dihydroresveratrol **(29)** metabolites were detected, with the latter being the major metabolites detected in the cecum, colon and rectum tissues, while resveratrol-3-*O*-glucuronide was the most abundant one in physiological fluids and organ tissues.[61]

Moreover, Rotches-Ribalta *et al.* described the PK and metabolic profile of male healthy volunteers following a single dose of red wine (375 mL) or grape extract tablets (15 tablets of Resverasor®). Plasma and urine samples were collected at different time points during a 24 h window and analyses were performed with an LC-tQ-MS/MS platform. 17 metabolites, including conjugates of resveratrol, piceid and dihydroresveratrol, were detected. Interestingly, resveratrol glucosides were observed in the plasma, while increased plasma concentrations and t_{max} values were reported for resveratrol glucuronides and dihydroresveratrol-glucuronides **(30)**, with the same trend being seen in the urine.[62] Additionally, the same team performed a study on high cardiovascular risk patients aiming to investigate the metabolic profiles of volunteers after a prolonged red wine or dealcoholized red wine administration (272 mL daily for 4 weeks). 24 h urine samples were analyzed by means of LC-tQ-MS/MS and up to 21 resveratrol metabolites, including those formed by the gut microbiota, were detected, while the beverage alcohol content did not seem to affect the bioavailability of the compounds.[57]

Iwuchukwu *et al.*, in a study published in 2012, attempted to resolve a common issue faced by scientists working in the NP field, namely the lack of available reference standards, by synthesizing 4 *trans*-resveratrol metabolites, *i.e.* resveratrol-3-sulfate **(31)** and resveratrol-4′-sulfate **(32)** and 3- and 4′-monoglucuronides **(33)**. The pure compounds were later used for the development and validation of an LC-MS^n method employing a tQ mass analyzer, and applied to an *in vivo* study involving male C57BL/6 mice, receiving a single intra-arterial dose of resveratrol (60 mg kg^{-1}).[63] In another study involving a lesser-known stilbene, namely pterostilbene, Azzolini *et al.* reported the PK and tissue distribution of the compound in male Wistar rats, following IV or oral administration (88 mol kg^{-1}). Selected analyses were performed with an LC-MS system on blood samples and various animal tissues. Pterostilbene sulfate, the

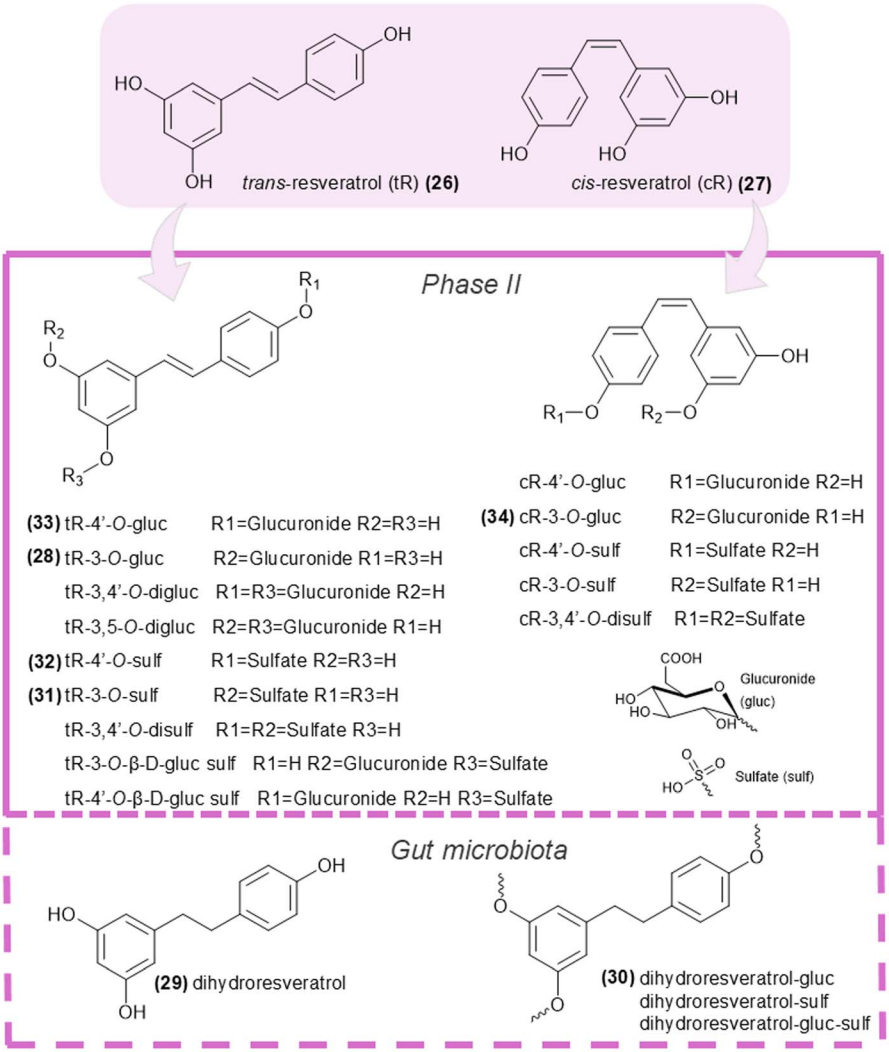

Fig. 3 Resveratrol is found in nature in two geometrical isomers, namely, *cis*- and *trans*-, with the latter being the most abundant. Through phase II metabolism, sulfation and glucuronidation conjugation reactions occur to all available hydroxyl groups and in different combinations. During catabolism by the gut microbiota, dihydroresveratrol and its glucuronidated and sulfated derivatives are the most characteristic metabolites.[57,58]

major metabolite detected in all fluids and tissues except for brain samples, was chemically synthesized to serve as a reference standard.[64] On a different note, Murakami and colleagues examined the metabolism of resveratrol in male and female mice after oral (40 mg per kg body weight) and dermal (1 mg in EtOH) administration routes. Additionally, three different cell lines (HepG2, HaCaT, and C2C12) were employed for the investigation of resveratrol metabolism. LC-MS analyses were performed using an Orbitrap mass analyzer, while for compound separation, a ZIC-pHILIC method was employed. The authors detected several metabolites of resveratrol, including resveratrol sulfate, *trans*-resveratrol-3-*O*-glucuronide, *cis*-resveratrol-3-*O*-glucuronide **(34)**, and dihydroresveratrol conjugates. Interestingly, according to the authors, the metabolite distribution of resveratrol conjugates after oral and dermal administration did not present significant differences.[65] Finally, in the work of Svilar *et al.*, resveratrol and its metabolites were measured in the plasma samples of healthy male volunteers, receiving a single oral dose of resveratrol (200 mg), co-administered with 20 mg of piperine for enhanced bioavailability. Analytical measurements were performed on an LC-Orbitrap-MS platform where several metabolites of resveratrol, including glucuronide and sulfate isomers, were detected and quantified at different timepoints following ingestion.[58]

3.1.4 Anthocyanins. Anthocyanins are the most widespread class of water-soluble plant pigments, particularly found in flowers and fruits of all types of vascular plants. Chemically, they belong to the class of flavonoids, whereby a core anthocyanidin (aglycone) moiety is often glycosylated at the C3, C5 or C7 positions, thus offering the compound increased solubility and stability. To date, more than 700 structurally distinct anthocyanin derivatives of 27 aglycones, known as "anthocyanidins," have been identified from natural sources. Six anthocyanidins pelargonidin **(35)**, cyanidin **(36)**, peonidin **(37)**, delphinidin **(38)**, petunidin **(39)** and malvidin **(40)** dominate in nature, accounting for approximately 90% of all anthocyanins identified thus far. In addition to their natural diversity, anthocyanins can undergo acylation, where a cinnamic or

aliphatic acid is esterified to one or more of the sugar substitutions. Around 50% of anthocyanins in nature is acylated. Their enormous diversity makes them a very complex and fascinating group. Numerous studies suggest that this family of flavonoids may help reduce the incidence of cardiovascular disease, cancer, hyperlipidemia, and other chronic conditions through the consumption of anthocyanin-rich foods.[66,67]

Despite their established health benefits, the extremely low bioavailability of anthocyanins is probably the core reason for their relatively poor performance in *in vivo* and clinical trials. Even the nature and position of the glycosidic groups on the basic scaffold seem to influence their absorption.

Nevertheless, these compounds present distinct biological interest, and therefore in the last few years, several studies have emerged attempting to propose ways of increasing the bioavailability of anthocyanins.[68,69] Given that anthocyanins are one the most extensively studied groups of plant phenolics, for a more comprehensive overview of their bioavailability and metabolism, readers are referred to the following recent review articles by Lila *et al.*, Alvarez-Suarez *et al.*, Hahm *et al.*, and Kay *et al.*[69–72] The most abundant anthocyanins and their metabolic pathways are demonstrated in Fig. 4.[69,71,73]

In 2003, a study by Felgines *et al.* investigated the metabolic biotransformations of anthocyanins in healthy subjects of both sexes, following the consumption of 200 g of strawberry fruits (179 μmol of pelargonidin-3-glucoside). Urine samples were collected at different time points within 24 h post-ingestion and the samples were analyzed by means of LC-ESI-MS/MS. Apart from pelargonidin-3-glucoside, 5 anthocyanin metabolites were detected in the urine, including 3 pelargonidin glucuronides, pelargonidin sulfate and pelargonidin aglycone.[74] Although the study focused on accurately identifying metabolites, the low-resolution analyzer used made this difficult, and it was nearly impossible without reference standards. As a result, alternative methods, such as hydrolysis, were employed to uncover the identity of some metabolites. However, even with these methods, the substitution positions remained unclear. Later on, the same group, utilizing the same MS platform, conducted an *in vivo* study with male Wistar rats receiving a daily dose of blackberry extract (14.8 mmol anthocyanins per kg for 15 days). Different types of biological fluids and tissues were collected, with the stomach presenting only native anthocyanins, while in the jejunum, liver, and kidney, native and methylated anthocyanins as well as conjugated derivatives thereof (cyanidin and peonidin monoglucuronides) were annotated. Interestingly, anthocyanins were also detected in brain tissue samples, thus hinting the BBB permeability of these molecules.[75]

Along similar lines, Marczylo *et al.* investigated the PK and metabolism of cyanidin-3-glucoside (more accurately cyanidin-3-*O*-glucoside) in C57BL6J mice receiving either 500 mg kg^{-1} by gavage or 1 mg kg^{-1} *via* tail vein injection. Plasma, urine and tissue samples were collected, while biomatrix analysis was carried out either with an LC-UV/Vis system or LC-MS/MS system for metabolite detection. Notably, in many studies on anthocyanins, the atom conjugated with sugars, either oxygen or carbon, is often omitted, assuming that the compound is an *O*-glycoside. Eight distinct metabolic products were observed in all biospecimens, along with the parent compound. Most biotransformation products were either the result of methylation and/or glucuronidation reactions.[76] A similar concept was followed by Vanzo *et al.*, who assessed the blood to tissue uptake of cyanidin 3-glucoside in an *in vivo* study with male Wistar rats (0.67 μmol, diluted in 0.3 mL of PBS injected into the iliac vein). Plasma, urine, bile and tissue samples were analyzed with an LC-DAD-tQ-MS/MS platform. The native compound and its methylated derivative (peonidin 3-glucoside) were rapidly detected in plasma, kidney and liver samples, with the latter demonstrating a fast excretion rate in bile and urine. Moreover, three methylated derivatives, delphinidin 3-glucoside, petunidin 3-glucoside, and malvidin 3-glucoside, were observed, albeit in traces.[77] Similarly, more informative and accurate results could have been obtained in this study if a high-resolution analyzer was used.

In an important study by Ichiyanagi *et al.*, the structural elucidation of two glucuronyl metabolites of pelargonidin 3-*O*-β-D-glucopyranoside was investigated based on an *in vivo* male Wistar rat model. The animals received the pure compound either through IV or *per os* administration and plasma and urine samples were collected for metabolite detection. Even though an LC-PDA method was initially employed, the structural elucidation of isolated metabolites was carried out by both HRMS/MS experiments (with a QTOF mass analyzer) as well as through NMR spectroscopy. The structures of the two prevailing metabolites were determined to be pelargonidin 3-*O*-β-D-glucuronide and pelargonidin 3-*O*-β-D-glucuronyl-(1→2)-β-D-glucoside.[78] Another common issue in identifying unknown metabolites in biological matrices is the vague or imprecise description of sugar moieties. For this reason, we chose to highlight this study, where the authors focused on the accurate identification of metabolites, taking into account the challenging structure of sugar moieties.

Acknowledging that valuable information can be obtained from urine analysis in metabolism studies, Kalt and coworkers performed a clinical study involving healthy volunteers of both sexes, who were asked to consume blueberry juice containing 216 mg of cyanidin-3-glucoside equivalents. Urine samples were collected prior and during a 24 h window after the intervention and analyzed by means of LC-ESI-tQ-MS/MS. The authors detected multiple anthocyanin biotransformation products in the urine of the subjects, mainly aglycones, especially aglycone glucuronides, possibly including positional isomers of anthocyanin conjugates and chalcones (**41**). Moreover, the authors reported the persistent excretion of anthocyanin metabolites, which should be taken into account for the design of future clinical trials.[79] Some years later, in 2017, the same research group published two follow-up studies involving human participants consuming different doses of anthocyanin-rich blueberry juice. In the first case, the effect of intake duration and dosing was investigated, and the authors reported high interindividual variability among volunteers, as well as the slow and persistent excretion of anthocyanins. Finally, the 24–0 h excretion was higher for total anthocyanins but not for parent anthocyanins when daily blueberry juice was administered as a single dose, which also led to the increased retention of the

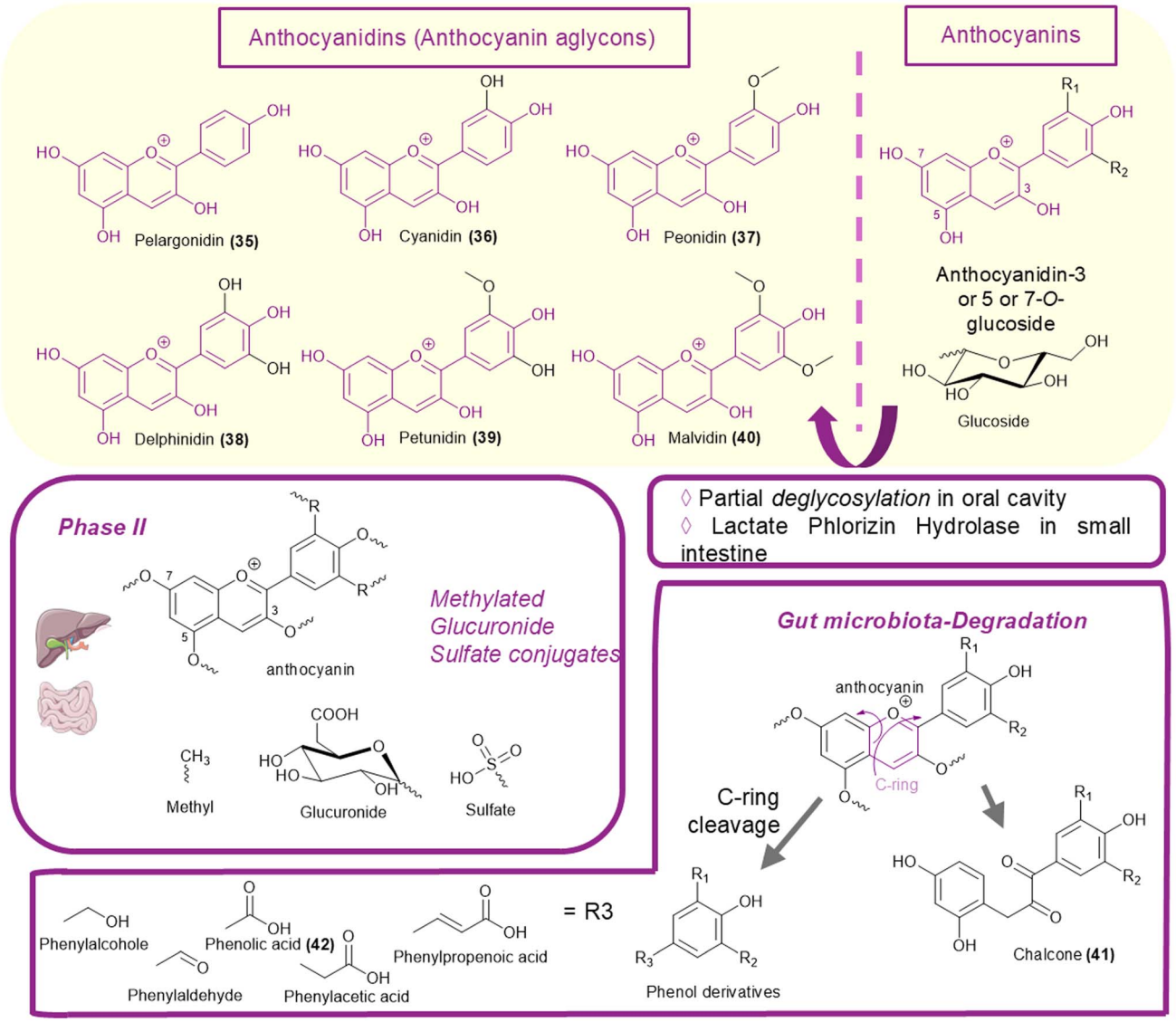

Fig. 4 Most abundant aglycones of anthocyanins (anthocyanidins) are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. The most common site of glycosylation is the hydroxyl groups at positions 3, 5 and 7 on the basic aglycone core structure. Deglycosylation is the main biotransformation route that occurs in the small intestine by hydrolases (*e.g.* lactase phlorizin hydrolase) and partially in the oral cavity. Phase II metabolic reactions include methylation, glucuronidation and sulfation. The catabolic reactions in the colon involve C-ring cleavage and formation of chalcones, simple phenols and short chain fatty acids (SCFA) and their derivatives.[69,71,73]

parent anthocyanins.[80] In the second case and using a similar study design, urine samples of volunteers consuming blueberry juice for 28 days after a 7 day washout period were analyzed with an LC-ESI-tQ-MS/MS platform. Overall, the authors processed a total of 664 samples, monitoring 18 parent anthocyanins and 42 predicted biotransformation products, which yielded a total of 371 products. Flavonoid metabolites were found to be 20-times higher than the parent compounds, while 55 major anthocyanins accounted for about 80% of the total anthocyanins detected.[81] In these reports, the authors nicely discussed the challenges in the accurate identification as well as level determination with the LC-MS instrumentation used.

In a study by Kamonpatana *et al.* thinking out of the box, the metabolic fate of anthocyanins in the buccal mucosa was investigated. For this purpose, healthy human subjects were instructed to retain either grape or chokeberry juice in their mouth after a washout period and buccal scrapings along with the retained juice were collected, and the samples were analyzed by means of LC-PDA-ESI-MS. According to the findings, for the grape juice, loss of delphinidin-3-glucoside surpassed that of other anthocyanin-glucosides, possibly due to degradation, while for chokeberry juice, the decrease in cyanidin-3-xyloside was greater, and cyanidin-3-glucoside showed an increased accumulation in epithelium cells.[82] Ludwig *et al.* examined the bioavailability of anthocyanins and ellagitannins after red raspberry ingestion by male and female healthy volunteers. The subjects received a total of 300 g of homogenized raspberries, and plasma and urine samples were

collected at different timepoints prior to and after ingestion. Analysis was carried out with an LC-PDA-MS/MS system, with the latter operated in SRM for metabolite detection. A total of 27 compounds was detected in the urine over the 48 h window following ingestion and a total of 15 compounds in the plasma. High levels of phase II metabolites of ferulic acid and isoferulic acid, along with 4′-hydroxyhippuric acid, possibly a product from the degradation of cyanidin in the proximal GI tract, appeared in both physiological fluids.[83] Xie *et al.* investigated the bioavailability and colonic metabolism of anthocyanins following the consumption of aronia berry extract (500 mg of extract) by healthy volunteers. Plasma and urine samples were collected at regular intervals 24 h post-ingestion and analyzed by LC-MS. The authors reported that protocatechuic acid, hippuric acid, 3-(4-hydroxyphenyl)propionic acid, cyanidin-3-*O*-glucoside, and peonidin-3-*O*-galactoside were detected in the plasma samples, while apart from these, 3,4-dihydroxyphenylacetic acid, ferulic acid, cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside were also observed in the urine.[84] Following a somewhat similar approach, Zhong *et al.* examined the bioavailability and kinetic profile of anthocyanins, chlorogenic acids and their metabolites in healthy subjects that consumed a wild blueberry beverage. Plasma samples were collected at different time points over a 24 h period and analyzed by means of LC-QTOF-MS/MS. According to the findings, the parent anthocyanins reached the maximum at around 2 h post-ingestion, while phase II metabolites, such as glucuronide conjugates of peonidin, delphinidin, cyanidin and petunidin achieved a maximum concentration later (2.6 to 8.8 h).[85] Although the above-mentioned studies were well-designed and executed, a relevant question that could be raised generally in similar future studies concerns the variability of the administered product. In our view, this is where natural variability, bioaccessibility, and bioavailability intersect.

Highlighting the role of the gut microbiome, in 2017, Mueller and colleagues published the results of a clinical study involving both healthy subjects and ileostomy volunteers who were asked to consume a bilberry extract consisting of 24% ± 1% of 15 different anthocyanins. Plasma, urine and ileostomy fluids were collected at regular intervals post-ingestion and analyzed by LC-ESI-MS/MS for metabolite detection. The comparison of the bioavailability of anthocyanins in healthy subjects *versus* ileostomists revealed critically higher levels of anthocyanins and their degradation products in the plasma/urine of volunteers with an intact gut.[73] In a comprehensive *in vivo* study published by Han *et al.* four years later, pure cyanidin-3-glucoside (500 mg kg^{-1}) was intubated into the stomach of male Sprague-Dawley rats and their whole blood, gastric juice, and 5 cm of duodenum, jejunum, ileum, and colon samples were collected and analyzed with an LC-PDA-ESI-MS/MS platform. Overall, 12 compounds were annotated in the serum, gastric juice, duodenum, jejunum, ileum, and colon samples over a 24 h window, while cyanidin and phenolic acids **(42)** were the main biotransformation products absorbed, mainly in the jejunum and ileum, between 1 and 5 h post-ingestion.[86] In a recent study by Victoria-Campos *et al.*, selected anthocyanins isolated from fruits were subjected to an *in vitro* gastric and small intestine digestion model and the samples were analyzed by LC-tQ-MS/MS for metabolite detection. The tested anthocyanins appeared to be highly stable in the gastric environment, while their recovery decreased in the small intestine dialysates. Anthocyanins as flavylium cations were detected after both phases of digestion and in dialysis sacs, while chalcone forms, as sodium adducts, of the parent anthocyanins were the main metabolites detected.[87] Finally, in another *in vitro* study recently published by Chan *et al.*, the metabolic fate of black raspberry polyphenols was investigated through anaerobic fermentation with the microbiota from human adults, infants, rats, and mice under a metabolomics concept. Sample analysis was carried out with a LC-QTOF-MS/MS platform, while targeted phenolic analysis was also performed with an LC-tQ-MS/MS system. In human adult and rat groups, the most prominent anthocyanin peaks were replaced by pelargonidin conjugates. Interestingly, human adult and rat samples demonstrated a greater quantity and diversity in terms of phenolic metabolites compared to that originating from infants and mice.[88] Although many studies have been conducted in various settings, including human trials, there is still a long way to go in unraveling the complex puzzle of anthocyanin metabolism. Technological advancements in MS will undoubtedly play a crucial role in this effort.

3.1.5 Isoflavones. Isoflavones are a subclass of flavonoids, often referred to as phytoestrogens, due to their estrogenic bioactivity. They are abundant in legumes, mainly in soybeans (*Glycine max*) and soy by-products, which have been a staple in the traditional diet of numerous Asia-Pacific countries. Genistein **(43)** and daidzein **(44)** are the major isoflavones in soybean. Many studies have highlighted the health benefits of isoflavones regarding chronic diseases in humans, including relief of menopausal symptoms, breast and prostate cancer and cardiovascular diseases. Daidzein and genistein are considered the most significant phytoestrogens for human health.[89]

One of the earliest cases of a confirmed NP metabolite is related to isoflavones and regards the fascinating story of equol. Equol was first isolated from the urine of pregnant mares in 1932. Its presence was later confirmed in many animal species, and fifty years later, equol was identified in human blood as a metabolite of the soybean isoflavones daidzin **(45)** and daidzein. Its identification was performed by GC-MS and NMR.[90] Interestingly, it is produced by intestinal bacteria in some, but not all, adults. This observation gave rise to the term 'equol-producers,' referring to adults who can produce equol in response to consuming soy isoflavones. It also led to the hypothesis that the health benefits of soy-based diets might be more pronounced in equol-producers than in nonproducers. Since then, many studies have been performed investigating the biological role of equol, which is mostly associated with hormone-related complications and diseases, and especially hormone-dependent malignances.[91]

Fig. 5 shows the main constituents of soy products as well as their biotransformation to aglycone forms and glucuronidation and sulfation steps, based on the following works.[92,93] For more detailed information regarding the metabolism of phytoestrogens, the reader is encouraged to refer to the recent review

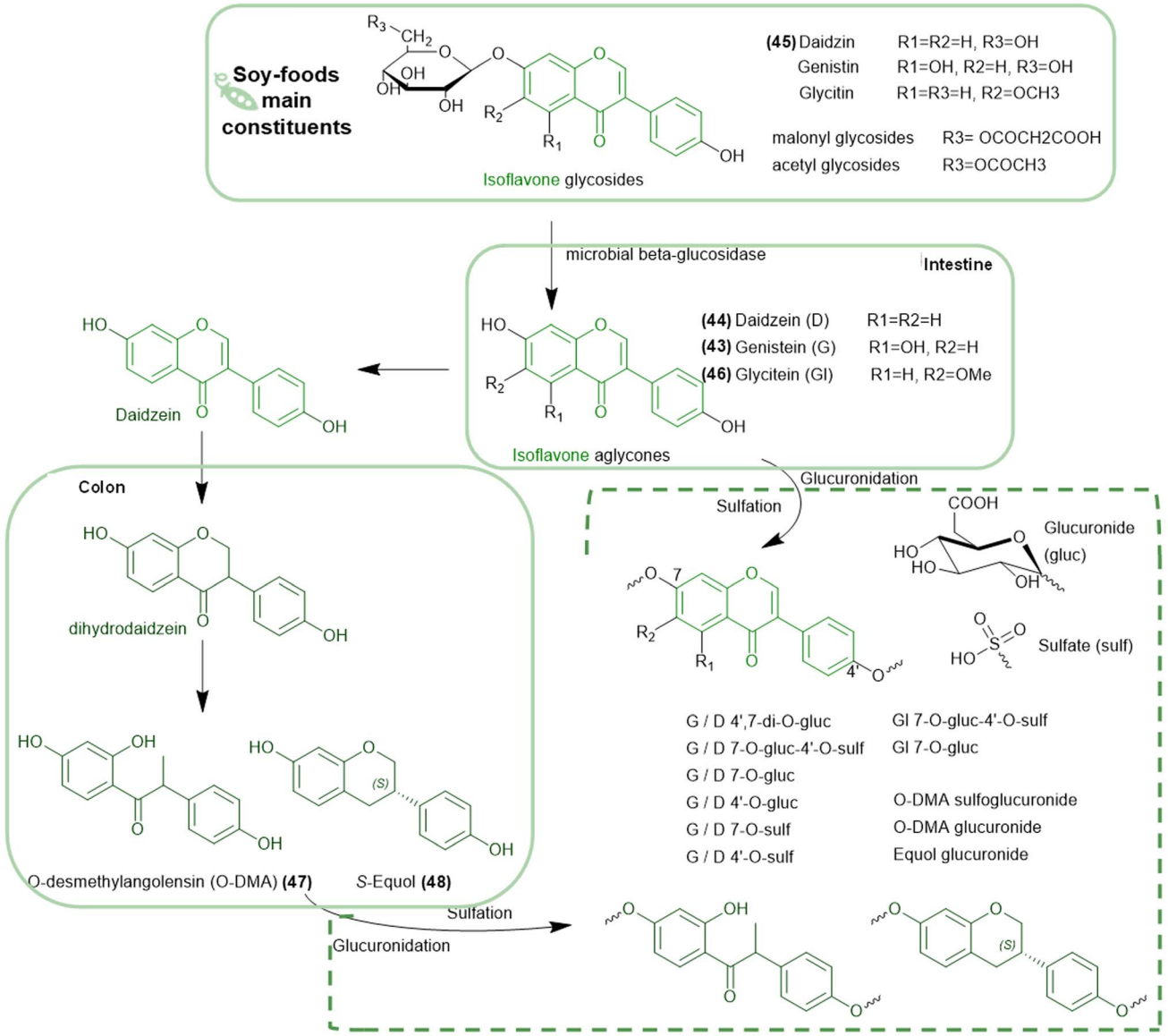

Fig. 5 Daidzein, genistein and glycitin are the main isoflavones of soy and soy-products, together with malonyl and acetyl glycosides. Glycosylation typically occurs on the hydroxyl groups at position 7 of the A ring and rarely on the hydroxyl moiety of the B ring or other positions. In the small intestine, aglycones are formed, and the most popular metabolite is daidzein. Conjugation reactions occur during phase II biotransformations, affording sulfate and glucuronide derivatives. In the colon, dihydro derivatives are produced, which are catabolized further to *S*-equol and derivatives after reducing the C ring such as O-DMA. The catabolism products also undergo glucuronidation and sulfation reactions.[92,93]

articles by Larkin *et al.*, Aboushanab *et al.*, and Sánchez-Calvo *et al.*[92,94,95]

In the significant effort by Koh and colleagues, performed in 2011, ten healthy Koreans consumed 52 g of soybean paste or 12.2 g soy flour, and urine samples were collected for 24 h. Twenty urinary isoflavone metabolites, mainly glucuronide conjugates, were identified using LC-(ESI)-MS/MS.[93] To delve more into additional metabolites, a sensitive LC-MS/MS method was developed by Fang and colleagues to characterize 17 conjugates of isoflavones in female rat urine samples collected for 24 h after the consumption of soy protein isolate (SPI) preparations, *i.e.* SPI 670 and IF-SPI, containing different levels of major isoflavones. The estimated daily intake of genistein and daidzein equivalents was 19.3 and 9.2 mg per kg body weight after the consumption of SPI 670 and less than 0.1 mg of genistein or daidzein for IF-SPI, respectively, which was used as the control.[96] It is worth noting that the authors of this study focused extensively on metabolite identification using a combination of LC-UV and LC-MS/MS methods, as well as reference standards. They also placed significant emphasis on MS/MS fragmentation patterns for identifying unknown metabolites.

In another study, four new isoflavonoid metabolites were annotated using GC-MS by Heinonen and coworkers who investigated the *in vitro* metabolism of genistein and daidzein

and their hydroxylated analogues in fecal fermentation and human urine samples from 6 volunteers after soy supplementation.[97] A work to investigate chronic soy consumption as well as sex influence in isoflavones bioavailability was performed by Wiseman *et al.* in 76 healthy adults who consumed high-soy diet (104 ± 24 mg total isoflavones per day) or low-soy diet (0.54 ± 0.58 mg total isoflavones per day) for ten weeks. The metabolites genistein, daidzein, glycitein **(46)**, equol and *O*-desmethylangolensin (O-DMA) **(47)** were measured, using GC-MS, in plasma, urine and feces and found in higher concentrations in the high-soy diet, while interestingly differences between the two sexes were observed only in terms of O-DMA levels.[98] Moreover, Chen *et al.* investigated the bioavailability of isoflavones in a 5 week soy nutritional supplementation period. Eight volunteers consumed a supplement containing 98% acetyl glucoside isoflavones and their urine samples were collected regularly. The analysis was performed using an LCQ MS in negative APCI mode and the authors proposed a fragmentation pattern for O-DMA.[99] More recently, aiming to explore the impact of fermentation, the work conducted by Jang and coworkers in 2020 investigated the effect of soybean fermentation on isoflavone bioavailability and metabolism. 10 participants were included in the study and on the day of the experiment were provided with specific prepared meals either fermented or not, both containing 33 g of soybean. Plasma and urine samples were collected and analyzed using LC coupled to a DAD detector and a QTOF MS. The results indicated that glucuronide isoflavones were more dominant than sulfate or aglycone metabolites *in vivo*, while fermentation showed to increase absorption and bioavailability.[100] In a well-designed and focused study, Setchell *et al.* investigated for the first time the PK of oral administrated [^{13}C]daidzein or [^{13}C]genistein. Sixteen healthy women were given a low dose (0.4 mg kg^{-1} of body weight), a moderate dose (0.8 mg kg^{-1} of body weight) and a low dose after eating soy containing food for one week. Serum samples were analyzed using GC-MS, while urine samples were analyzed using LC-ESI-MS. The serum PK of isoflavones was consistent among the subjects, where genistein was found to be more bioavailable than daidzein. Equol was identified in 37.5% of the subjects in a time-dependent manner.[101] A couple of years later, the same group established *S*-equol **(48)** as the sole enantiomeric form produced by human intestinal bacterial flora through *in vitro* studies on cultured fecal flora from a healthy human.[102] In another PK study by Rüfer and coworkers, the bioavailability of daidzein and its metabolites was explored by oral administration of its pure aglycone (1 mg per kg body weight) and glucoside form in seven male volunteers. Plasma from twelve time points and urine samples from four time points in a 24 h window were collected and analyzed using GC-MS after derivatization. Bacterial metabolites appeared after 6–8 h in plasma samples and between 6–12 h in urine samples. The oxidative metabolites recovered after 2 h in plasma samples and between 6–12 h in urine samples. Finally, the glucoside form was found to be more bioavailable as measured in urine samples.[103] In this study, the difference in metabolism when a sole compound is administrated *vs.* embedded in a matrix such as food or food products is highlighted.

A sensitive and robust LC-MS/MS method for the simultaneous determination of genistein and its four main metabolites in mouse blood after both oral and intravenous administration of pure genistein at dose 20 mg kg^{-1} was developed by Yang *et al.*[104] Lee and coworkers designed a study to determine whether ovariectomy affects the nutrikinetics of genistein and its metabolites. The female sham-operated and OVX mice were administrated once, 5 mg per kg genistein orally and blood samples were collected. Thirteen metabolites, mainly glucuronides, were identified using LC-QTOF-MS, while according to the results of the study, the authors suggest that gut microbiota alteration after ovariectomy could affect the bioavailability of genistein.[105] Finally, Wang *et al.* studied the PK profile of tectorigenin, a less investigated isoflavone. A dose of 130 mg kg^{-1} was administered to six rats by oral gavage and blood samples were collected at 17 time points up to 72 h. The analysis was performed using an accurate LC-QTOF-MS method for the simultaneous characterization of nine metabolites and quantitation of six metabolites, mainly sulfates and glucuronides.[106]

3.1.6 Tea, cocoa and grape polyphenols. Although the term 'polyphenols' is often associated with polyhydroxylated compounds such as tannins, lower molecular weight compounds with less hydroxyl groups are also classified as polyphenols. Depending on the research field, different terminologies are used, making it a challenge to categorize polyphenols. One effective approach is to classify them into flavonoids and non-flavonoids. Their various health benefits, particularly in relation to cardiovascular diseases, obesity, cancer and inflammatory bowel disease, are well known and readers are encouraged to refer to the informative recent review articles by LIczbiński and Bukowska, Rana *et al.*, Liu *et al.*, and Di Pede *et al.*, also focusing on bioavailability aspects.[107–110] Based on the number of available reports and due to their chemical similarity, we chose to discuss the polyphenols of tea, cocoa and grape together. Flavan-3-ols represent a major source of (poly)phenols in the Western diet, with tea, cocoa and grape products being the most common sources.[108] The primary constituents of these products include (epi)-catechins, procyanidins, phenolic acids, and ellagitanins. Given the broad scope of this category, the reader is referred to the following works for a more detailed account on their metabolism and bioavailability.[107,108,111–113] A noteworthy mention should be given to phenyl-γ-valerolactones (PVLs) and phenylvaleric acids (PVAs), which are the main colonic metabolites of flavan-3-ols. PVLs were first reported in 1958 as metabolites of (+)-catechin in rabbits. After their synthesis together with their detection using radiolabeled precursor compounds, it was demonstrated that they are gut microbiota metabolites. Recently, the interest in both classes of metabolites was renewed based on the established knowledge that they comprise the major metabolites of flavan-3-ols. We suggest that readers refer to the excellent review reported by Mena *et al.* in 2019, which covers several topics related to these metabolites.[114]

In a study published in 2001, Li *et al.* reported for the first time the simultaneous analysis of multiple tea polyphenol

conjugates in urine samples. Human, mice and rat urine samples were collected after the administration of a single dose of green tea and analyzed by LC-ESI-MS. The major metabolites in human urine were phase II metabolic products such as monoglucuronides and monosulfates of (−)-epigallocatechin **(49)**, (−)-epicatechin **(50)**, (−)-5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone **(51)** and (−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone **(52)**, *O*-methyl-epigallocatechin-*O*-glucuronides and -*O*-sulfates and *O*-methyl-epicatechin-*O*-sulfates. Many of these were also detected in mice urine, unlike the rat urine profile, which was differentiated.[115] The following year, the same group conducted an expanded similar study investigating the metabolic fate of green tea in humans, mice and rats, collecting not only urine samples, but also other biological fluids and tissues, which were analyzed by LC-MS/MS. In addition, human volunteers were given a single dose of (−)-epigallocatechin-3-gallate **(53)** (2 mg kg^{-1}) after the wash-out period. Most of the results were aligned with the previous study, while the investigation of ring-fission products led the authors to propose a metabolic pathway for (−)-epigallocatechin-3-gallate.[116] In this study, it should be noted that a combination of analytical methods and platforms was used for the precise identification of metabolites *i.e.* LC-UV, LC-MS/MS and LC-MS^n. Moreover, the use of enzymes and reference standards was extensive, while in some cases isolation from urine or synthesis of certain metabolites took place for metabolite structure verification.

In 2003, Natsume *et al.* examined the metabolism of pure (−)-epicatechin after it was orally administrated to human volunteers (1 g per person) and rats (20 mg kg^{-1} or 500 mg kg^{-1}). Blood and urine samples were collected and analyzed by LC-MS and six metabolites from urine samples were purified and elucidated, including (−)-epicatechin-3′-*O*-glucuronide, 4′-*O*-methyl-(−)-epicatechin-3′-*O*-glucuronide, and 4′-*O*-methyl-(−)-epicatechin-5 or 7-*O*-glucuronide from human urine samples and 3′-*O*-methyl-(−)-epicatechin, (−)-epicatechin-7-*O*-glucuronide, and 3′-*O*-methyl-(−)-epicatechin-7-*O*-glucuronide from rat urine samples. The results agree with previous studies reporting a different metabolic profile in humans and rats.[117] In another study, Auger and coworkers investigated the small intestine absorption of flavan-3-ols in human volunteers with an ileostomy. Five participants consumed a capsule containing 200 mg of polyphenol E, a green tea extract, on its own, or in another case, alongside different food matrixes. In addition, they consumed capsules with different contents (200, 500 or 1500 mg of polyphenol E), and finally they were given a 200 mg capsule of (−)-epigallocatechin gallate with 3% (−)-epigallocatechin impurity. Ileal fluid and urine were collected over a 24 h period and were analyzed by LC-IT-MS adapted for MS^n analysis. Fourteen urinary sulfate, glucuronide and methylated (epi)catechin or (epi)gallocatechin metabolites were identified and quantified. The results indicated that almost 40% of green tea extract intake is recovered in ileal fluid, while increasing doses led to increased urinary excretion of only (epi)catechin metabolites, and finally administration alongside the food matrixes did not affect the ADME properties of flavan-3-ols.[118]

Amongst the intervention studies conducted in healthy volunteers, the work of Del Rio *et al.* should be featured. This study involved 20 healthy participants who drank 400 mL of a green tea beverage containing 400 μmol of flavan-3-ols to investigate their metabolism. Plasma and urine samples were collected for the first 4 and 24 h, respectively, and analyzed by LC-MS/MS. In total, 39 metabolites were identified including (−)-epigallocatechin and (−)-epicatechin conjugates and poly-hydroxyphenyl-γ-valerolactones, as reported previous studies.[119] In 2014, Clarke and colleagues developed a method to extract and monitor both free and conjugated forms of catechins and metabolites for the first time. In a 3 month double-blind randomized controlled trial, 50 participants received an encapsulated placebo or green tea supplement equivalent to 5 cups of tea daily, along with 50 mg of ascorbic acid. Urine samples were collected pre-supplementation, on day 1, week 6 and week 12 and were analyzed using an LC-tQ-MS/MS. The authors achieved good recovery percentages, and 55 catechin metabolites were investigated, including 26 conjugated and 3 free-form compounds, which were identified.[120] This study highlights a common challenge in metabolite identification due to the limited availability of reference standards. Despite using MS/MS acquisition and fragmentation pattern analysis, determining a definitive structure remains challenging, particularly for flavan-3-ols, where chirality and various isomers come into play.

Investigating cocoa, Urpi-Sarda *et al.* performed a long-term feeding trial with 42 subjects at high risk of cardiovascular disease. The participants consumed two sachets of 20 g of cocoa powder daily along with 250 mL or 500 mL of skimmed milk for two 4 week periods. Fasting plasma and 24 h urine samples were analyzed by LC-tq-MS/MS and an MRM method. The results highlighted 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and hydroxyphenylacetic acids as possible biomarkers of cocoa or flavanol-rich foods consumption.[121] In a similar human intervention study published by Wiese *et al.*, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone is also proposed as an important metabolite, given that its formation varied largely between the subjects. The authors investigated the metabolic fate of pure (−)-epicatechin, procyanidin B1 and polymeric procyanidins in seven male volunteers. Blood, urine and feces samples were collected and analyzed by GC-MS and LC-MS. The blood and urine main metabolites were glucuronidated, sulfated and methylated products, in agreement with the bibliography.[122] In another study, Gomez-Juaristi and coworkers explored the absorption and metabolism of cocoa polyphenols after the consumption of a conventional cocoa product or flavanol-rich product (19.80 mg and 68.25 mg of flavanols, respectively) by 13 healthy volunteers. Blood and urine samples were collected before and after the administration and were analyzed by LC-QTOF-MS, leading to the identification of 10 and 30 metabolites, respectively. Cocoa flavanols showed extensive metabolism by colonic microbiota with the main metabolites phase II derivatives of phenyl-γ-valerolactone and phenylvaleric acid.[123] Hakeem Said and colleagues used LC-MS-based metabolomics to investigate the urinary excretion of cocoa phenolics. Thirteen participants divided into three groups (high consumption-2

bars of 100 g of chocolate, low consumption-1 bar, no consumption, per day for 48 h), and urine samples were collected from 8 time points. In agreement with previous studies, phase-II conjugated epicatechin metabolites were found in high concentration, and derivatives of phenyl-γ-valerolactone conjugates were the most significant.[124] In a more recent study, Becerra *et al.* performed an *in vitro* digestion experiment based on the INFOGEST protocol (*in vitro* model simulating oral, gastric and intestinal digestion) using chocolate, where the bioactive compounds were monitored during the oral, gastric and intestinal phases to investigate their absorption course. The metabolic profile was evaluated using LC-QTOF-MS analysis alongside statistical and molecular networking platforms. Most compounds were found in the intestinal phase, while the metabolic fingerprint analysis revealed the presence of various families of chemical compounds, mainly flavonoids and peptides.[125]

Masumoto and colleagues published a study using metabolomics and multivariate statistical analyses to elucidate the metabolic differences among flavan-3-ols/procyanidins based on an *in vivo* Wistar rat model. The animals were divided into four groups, *i.e.*, control, (epi)catechin, epigallocatechin gallate and procyanidin dimer (1 g per kg dosage) groups. Urine samples were collected for 48 h in 3 distinguished periods and were analyzed by LC-QTOF-MS, leading to the annotation of 156 metabolites in total. Glucuronide and sulfate conjugates were the major metabolites in the first 24 h, and the administration of flavan-3-ols/procyanidins altered the gut microbiota environment, given that the authors established an effect regarding endogenous metabolites, such as amino acids and bile acid derivatives.[126] Mena *et al.* carried out an important study incorporating unsupervised and supervised multivariate statistical analysis to evaluate the inter-individual variability and urinary excretion of (poly)phenols after the administration of green tea extract (GTE) and green coffee beans extract (GCE) without dietary restrictions. Eleven participants enrolled in a 12 week trial, where they were given daily, and for the first 8 weeks, 6 GTE and 3 GCE tablets. 24 h urine samples were collected at 5 different time points and were analyzed by LC-MS^n. In total, 32 compounds were identified, with methyl, sulfate and glucuronide conjugates of (epi)catechins and phenyl-γ-valerolactones being the predominant metabolites. Based on the high inter-individual variation among metabolites from the colonic degradation of GTE flavan-3-ols and the associations conducted for the participants of the study, the authors defined, for the first time, three distinct metabotypes based on the production of valerolactones.[127]

Similarly, Li and coworkers studied the inter-individual differences in the human microbial metabolism of pure (+)-catechin **(54)** utilizing an *in vitro* fecal model. Fecal samples from 12 participants were donated and enriched with 200 mg L^{-1} of (+)-catechin daily to mimic everyday green tea consumption. Samples were collected at 0, 4, 24, 48, 72 and 96 h and analyzed by LC-IMS-QTOF-MS. The 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone metabolite contributed mainly to the differentiation of the examined metabolic profiles, while based on the rate of (+)-catechin metabolism, the authors stratified three types of converters, fast, medium and slow, highlighting the importance of the metabotype.[128] The following year, Krueger *et al.* investigated the effects of metabolite mixtures extracted from rat urines after the oral gavage of either Vitaflavan® GSE flavonol supplement (grape seed extract) or mixture of (+)-catechin hydrate and (−)-epicatechin supplement on INS-1 832/13 β-cell glucose stimulated insulin secretion (GSIS) capacity. Urine samples were collected for 48 h, analyzed by LC-MS/MS and used to treat the cell line for 24 h, before measuring 4 classic GSIS parameters. Interestingly, the results indicated variability in the response, based on the unique microbiome or metabolome of each animal, with valerolactones having the strongest effect.[129]

To delve deeper into catabolic biotransformations, Pereira-Caro *et al.* conducted an *in vitro* fecal model, and a human feeding study to explore the catabolism of black tea theaflavin **(55)** and its galloyl derivatives. Theaflavin and its derivatives are dimeric forms of flavan-3-ols, which are mainly produced during the fermentation of green tea to produce black tea (Fig. 6) and its main catabolic pathways are demonstrated in Fig. 7. Two healthy volunteers consumed one capsule containing 1 g theaflavin extract and their urine samples (0–30 h) were collected over 4 periods prior to and following supplementation. Fecal samples were provided by three volunteers in which 10 μmol/50 mL of the extract was added, and samples were collected at 7 time points in a 24 h window. The analysis of the samples was performed by LC-HRMS using an Orbitrap mass spectrometer. In the urine samples, 3-(4′-hydroxyphenyl)propionic acid **(8)** was identified as the main metabolite, while in the fecal samples, theaflavin gave a 67% recovery, after 24 h, showing resistance to colonic bacteria degradation, while 21 phenolic and aromatic catabolites were identified and the authors provided a proposed pathway for the catabolism of theaflavin.[130]

Sun and colleagues also incorporated an *in vitro* anaerobic human gut microbiota model to investigate the biotransformation of theaflavins. Fecal samples from 8 Chinese volunteers were used for the 48 h fermentation process, in which 0.1 mg mL^{-1} of theaflavins was added. Samples were collected at seven time points and were analyzed using an LC-Orbitrap-MS/MS. Based on the 17 metabolites identified, the authors provided a comprehensive potential metabolic pathway, where theaflavins undergo the initial metabolism steps, such as ester hydrolysis, C-ring cleavage and oxidation, followed by further degradation reactions, such as A-ring fission, dehydroxylation and aliphatic chain shortening, finally producing several phenolic metabolites.[131] The same *in vitro* model was used by Liu *et al.*, who focused on the metabolism of a specific catechin dimer of black tea, theasinensin, alongside epigallocatechin and procyanidin B2 at concentrations of 50, 100 and 50 μmol L^{-1}, respectively. The pure compounds were incubated with human fecal microbiota, provided by 4 healthy volunteers, under anaerobic conditions, and the samples were collected at various time points for 48 h. The analysis was performed using LC-Orbitrap-MS and 26 metabolites were identified. The degradation process for theasinensin was slower compared to the other two compounds, giving degalloylation product

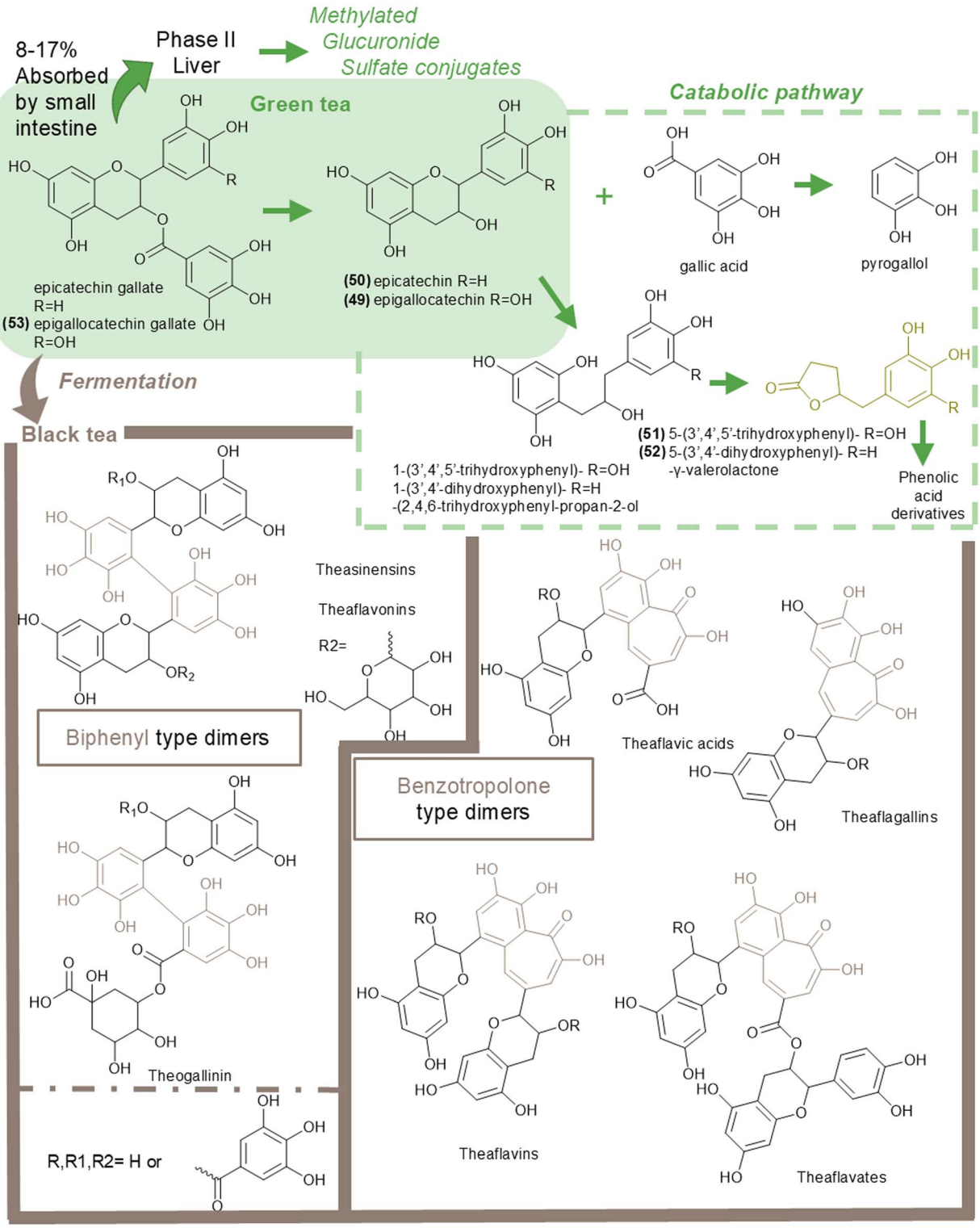

Fig. 6 Epigallocatechin, epicatechin and their gallate derivatives are the most abundant catechins in green tea. They are partially absorbed in the small intestine and pass through the liver, where they are metabolized to methylated, glucuronidated and sulfated products by phase II reactions. Given their poor bioavailability, catabolic biotransformation is their major metabolism route. Following the galloyl ester hydrolysis, the gallate derivatives give epicatechin or epigallocatechin along with gallic acid, which can further be decarboxylated to pyrogallol, while following the C-ring opening and further modifications, epicatechin and epigallocatechin eventually afford γ-valerolactones derivatives. With the lactone ring opening, hydroxyphenyl valeric, hydroxyphenyl propionic, hydroxyphenylacetic and hydroxybenzoic acids are formed. During the manufacturing of black tea, fresh tea leaves undergo fermentation, which is the oxidation of flavan-3-ols, resulting in complex mixtures of dimeric, oligomeric or polymeric flavan-3-ols. Biphenyl and benzotropolone-type dimers represent the major categories of black tea constituents, for which the most characteristic compounds are illustrated.[107,111]

theasinensin C as its main metabolite. Based on computational studies by the authors, this can be attributed to the shorter length and higher strength of the isoflavonic bond in theasinensin.[132] Carry *et al.* investigated microbial-derived phenolic metabolites in an *in vitro* fermentation model involving the human gut microbiota employing, for the first time, GC coupled with tQ-MS. Derivatization, MRM method and extended-use reference standards were combined in this study. Samples from two donors were enriched with (+)-catechin and (−)-epicatechin, and analysis led to the identification of 16 phenolic acid metabolites.[133]

Stalmach *et al.* investigated the metabolic fate of grape juice (Concord grape variety) constituents after the acute consumption of 350 mL by 8 healthy volunteers. Blood and urine samples were collected in a 24 h window and were analyzed by LC-PDA-MS^n. Sulfated, glucuronidated and methylated (epi)catechin and one methyl-(epi)gallocatechin-sulfate were detected in urine and 3-*O*-glucoside anthocyanins and methyl, sulfate and glucuronide metabolites of phenolic acids were detected in the plasma and urine samples.[134] Tourino *et al.* explored the polyphenol metabolism in the urine of rats after the administration of grape antioxidant dietary fiber (GADF) or pure (−)-epicatechin (1 and 1.6 g kg^{-1}, respectively). Samples were collected at six time points and analyzed by LC-tQ-MS/MS. The authors detected 18 mono-, di- and tri-conjugates of epicatechin with glucuronide, methyl and sulfate moieties, as well as free and conjugated phenolic acids, including sulfated and glucuronidated conjugates of hydroxyphenyl acids (Sulf-DHPhPA and Sulf-HphPA), respectively, which are reported for the first time.[135] In a follow up study, the same group aimed to determine the colonic catabolism of GADF polyphenols by analyzing fecal and cecal content and colonic tissue samples from rats, employing the same experimental design. Proanthocyanidin polymers were found to be gradually (more than 24 h) cleaved into monomeric epicatechins and smaller phenolics along the intestinal tract.[136] The next year, the same research team examined the metabolism of non-extractable proanthocyanidins (NEPA), isolated form GADF, after their oral administration in female rats. Analysis of urine and feces samples by LC-tQ-MS/MS led to the identification of (epi)catechin monomers and dimers and phase II conjugates, as products of the depolymerization of NEPA and this was the first report of their behavior *in vivo*.[137]

In another study, Wang *et al.* incorporated an *in vitro* gastric–pancreatic digestion model and *in vitro* colonic model combined with Caco-2 cell permeability studies to investigate the metabolism of grape seed phenolic extract (GSPE) using LC-DAD-MS. Phenolic compounds were highly recovered after gastric digestion, even though the monomers increased and polymers decreased, and they showed less stability after pancreatic digestion. Furthermore, phenolic acid metabolites were recovered in the apical compartment except for two intestinal microbiota metabolites that passed the Caco-2 cell monolayer.[138] A few years later, the same group investigated the bioavailability of GSPE-derived phenolic acids after oral administration (25 or 250 mg per kg body weight per day for 11 days) on rats. The authors monitored 12 phenolic acids by LC-tQ-MS/MS, which were found in increased concentrations in urine, plasma, cecum and colon tissues. Two of them, 3-hydroxybenzoic acid and 3-(3′-hydroxyphenyl)propionic acid, were also found in brain tissues, and *in vitro* bioactivity assays indicated their interference with the assembly of β-amyloid peptides into neurotoxic β-amyloid aggregates, which play key roles in the pathogenesis of Alzheimer's disease.[139]

In 2014, Margalef and colleagues developed an LC-MS method for the determination of 30 different colonic derivatives in rat plasma after the administration of 1000 mg of GSPE per kg of body weight.[140] The following year, the same group investigated the tissue distribution of orally administered GSPE in male rats at different dosages (125, 250, 375 and 1000 mg kg^{-1}). Quantification of the metabolites was performed by LC-MS/MS and the results showed a tissue-specific distribution pattern, which agrees with previous studies.[141] Similar research was performed by Serra *et al.*, who studied the behavior of flavanols and their metabolites in rat plasma, adipose tissues, muscle and liver after the administration of GSPE (5, 25 and 50 mg per kg body weight doses). Analysis was performed using LC-MS/MS and the results showed that each of the studied organs has a specific metabolite accumulation and response to each given dose.[142] Margalef *et al.* performed a follow up study aiming to evaluate whether GSPE flavonols are tissue-accumulated after a long-term administration period (12 weeks), and whether they would differ in a cafeteria-diet-obesity state. The authors used a Wistar rat *in vivo* model and collected plasma, liver, mesenteric white adipose tissue, brain and aorta samples, which were analyzed by LC-MS/MS. The results showed that GSPE flavanols do not accumulate in rat tissues, indicating their daily clearance, suggesting they would be beneficial in a disease state, while the metabolism and bioavailability of flavanols were influenced by the obesity-induced diet.[143] In 2016, Margalef and coworkers published two more research articles studying age-related differences and gender-related differences in the body distribution of grape seed flavanols in rats. In the first case, five 8 week-old and five 24 week-old rats were administrated 1000 mg per kg GSPE by oral gavage, while each age group included also one control rat. The plasma samples collected at 0, 2, 4, 7, 24 and 48 h were analyzed by LC-MS/MS. The results showed a lower flavanol absorption and phase II metabolism in adult rats, who consequently had higher concentrations of microbial metabolites.[144] In the second case, the same dosage of GSPE was administrated to 18 male and 18 female rats, which were further categorized in groups depending on the time of sacrifice and sample collection (1, 2 or 4 h). Their plasma and various tissues were analyzed by the same MS platform, leading to important sex-related quantitative differences mainly between the plasma and brain and liver and brain biological samples.[145]

Continuing with animal models, Pereira-Caro and colleagues explored the ADME properties of flavan-3-ols in proanthocyanidin-rich extracts from grape seeds and red wine (GSP and RWP, respectively) using an *in vivo* rat model. Forty male rats ($n = 3$ per time point, per treatment), were administrated 50 mg of RWP or GSP and euthanized at 1, 2, 4, 8 or 24 h. Plasma, urine and feces samples were analyzed and the authors

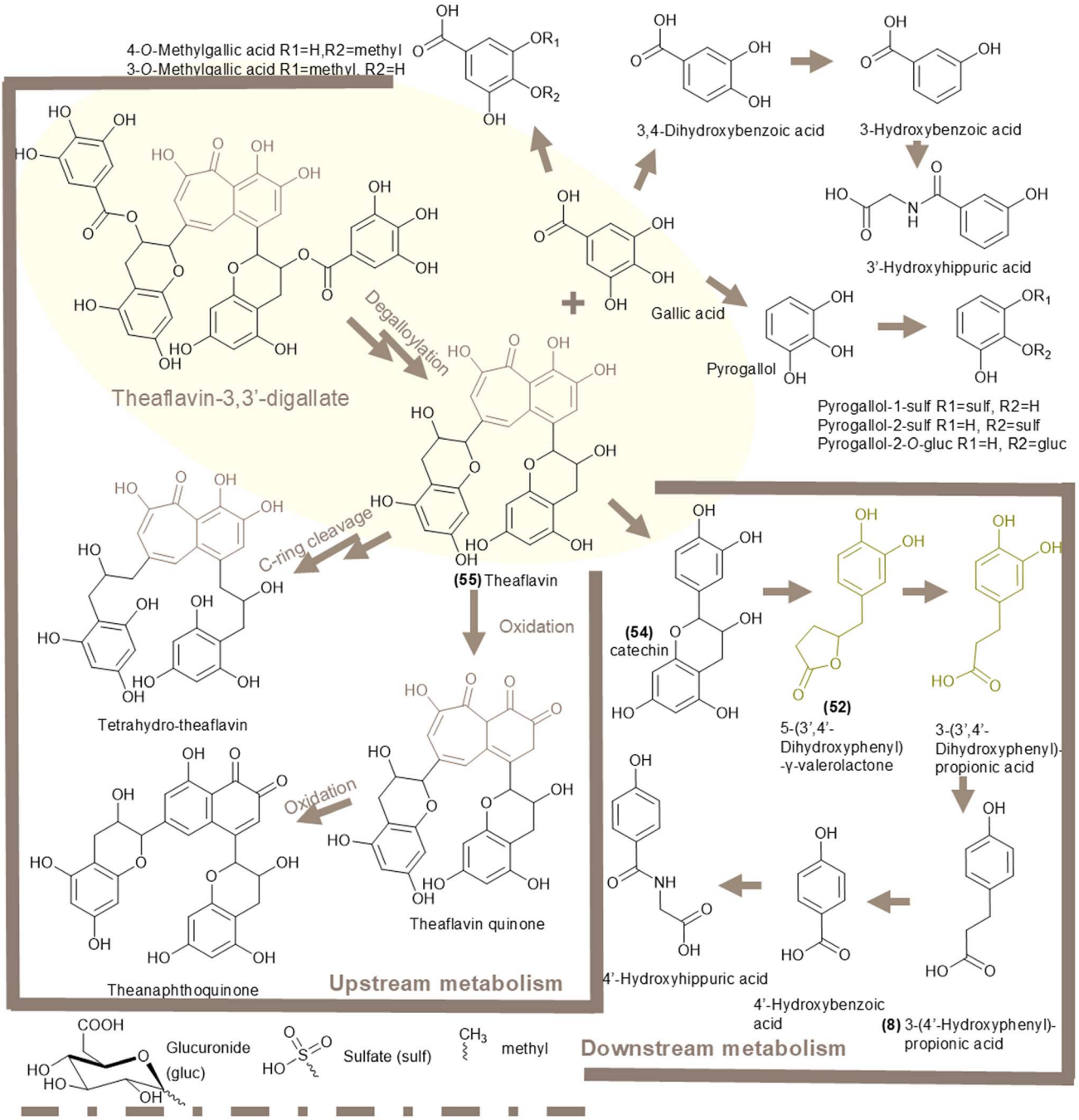

Fig. 7 Catabolic pathways of theaflavin-3,3′-digallate, which is used as a representative of benzotropolone-type dimer phenolics found in black tea, include degalloylation processes to afford theaflavin along with gallic acid, as the first step. The latter is further transformed to provide benzoic acid products, together with pyrogallol and its sulfate and glucuronide conjugates. In the upstream metabolism, theaflavin is further metabolized to C-ring cleavage derivatives, such as tetrahydro-theaflavin, as well as to oxidation products. In the downstream metabolism, theaflavin affords catechin analogues, which eventually give rise to γ-valerolactones and phenolic catabolites, similar to epigallocatechin and epicatechin degradation metabolites.[107,111,130,131]

identified more than 50 metabolites in total. The results showed that RWP consumption led to the formation of flavan-3-ol monomers, while GSP intake increased 5-carbon side chain ring fission metabolites. Finally, some phenyl-γ-valerolactones and phenylvaleric acids were proposed as potential markers of proanthocyanidin intake by rats. The analysis was performed using a sensitive LC-HRMS method developed by the same group two years prior.[146] In this publication, the authors quantified three parent flavan-3-ols, four 5-carbon side chain ring fission metabolites and 27 phenolic acid and aromatic catabolites in urine, plasma and feces samples from rats after the administration of 50 mg of a red wine proanthocyanidin extract.[147] Alternatively, Cerdá and coworkers investigated the metabolism of ellagitannins after the intake of common food

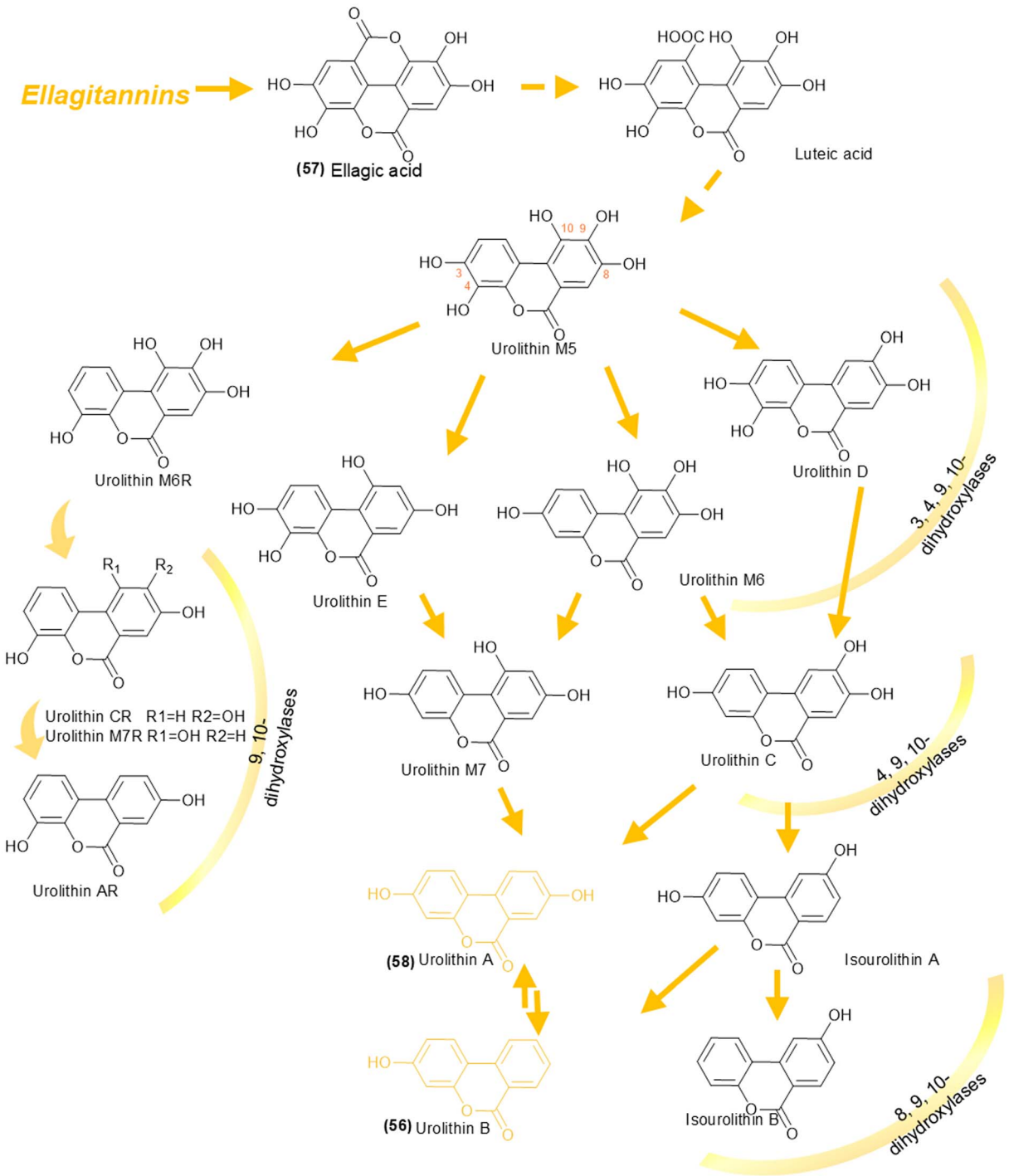

Fig. 8 Ellagitannins and ellagic acid undergo extensive metabolism by the gut microbiota, including lactone-ring cleavage, decarboxylation and dihydroxylation reactions to produce 6*H*-dibenzo[*b*,*d*]pyran-6-one derivatives or urolithins.[149,150]

products consumed in the Western diet. Forty volunteers consumed red wine, strawberries, red raspberries or walnuts and their urine samples were collected at 5 different time points and analyzed by LC-MS/MS. The results indicated the presence of "high and low metabolite excreters" based on the interindividual differences, while urolithin B (**56**) conjugated with glucuronic acid was detected among all subjects and was proposed as a possible biomarker.[148]

Here, it is worth noting another group of hallmark metabolites of active NPs, urolithins. They are catabolites mainly of ellagitannins (ETs) and ellagic acid (EA) (**57**) discovered as bioavailable metabolites almost 20 years ago in animal models and humans.[149] Especially, urolithin B (Uro B) has recently

garnered considerable attention owing to its wide range of beneficial effects. According to several studies, Uro B prevents the development of hyperlipidemia, cardiovascular disease (CVD) and tumors due to its strong antioxidant and anti-inflammatory properties (Fig. 8).

Regarding the metabolism of ellagitannins, Espín *et al.* used an *in vivo* Iberian pig model and analyzed through LC-MS/MS various biological fluids and tissues after the consumption of either cereal fodder or an ellagitannin-rich source. Thirty-one metabolites were detected, including 25 urolithin and 6 ellagic acid derivatives. Ellagic acid was detected in bile and urine samples but not in intestinal tissue, suggesting its absorption in the stomach, while urolithin A **(58)** was the sole metabolite identified in feces, and along with its glucuronide derivative were the main metabolites were found in urine.[151] The same research group published a study focusing on the production of urolithin derivatives in different animals (humans, pigs, sheep, birds, among others) after the consumption of ellagitannin-rich products (oak leaves, blackberries, acorns, *etc.*). The analysis of various collected samples was performed using LC-DAD-MS/MS and LC-TOF-MS/MS. Interestingly, the results showed that all the mammals produced urolithins and their glucuronyl and sulfate conjugates, while insects and birds released ellagic acid. The hydroxylation patterns differ between species and beavers and pigs produced dehydroxyellagic acid derivatives.[150] In another study, van't Slot *et al.* used an *in vitro* pig cecum model to study five flavan-3-ol *C*-glycosides and three B-type procyanidins. The analysis was performed using both GC-MS and HRMS, and the results showed that the compounds with a *C*-glycosidic bond at C8 were not degraded, while the degradation of procyanidins took place up to dimer but because of the interindividual differences the authors could not state a specific trend.[152] The same group incorporating the same *in vitro* model and analytical platforms investigated the microbial catabolism of two A-type procyanidins, procyanidin A2 and cinnamtannin B1. Phenolic compounds and two new unknown catabolites were identified as the degradation products.[153]

3.1.7 Olive polyphenols. *Olea europaea* L., Oleaceae is widely abundant in the Mediterranean region and its products, olive oil, table olives, and leaves have been used for years through history for food, cosmetic and medicinal applications. In 2011, the European Food Safety Authority (EFSA) stated that "olive oil polyphenols contribute to the protection of blood lipids from oxidative stress".[154,155] This fact together with a published research some years earlier about the ibuprofen-like activity of an olive phenol renewed interest from scientists.[156] The most studied olive oil polyphenols are hydroxytyrosol **(59)**, tyrosol **(60)** and the secoiridoids oleacein **(61)**, oleocanthal **(62)** and oleuropein **(63)** and their derivatives.[155,157] They are responsible for many beneficial health effects such as preventing hypertension, reducing pro-inflammatory state and oxidative stress.[157] Given that olive oil products are a daily part of the diets of many consumers, several studies have been published regarding their metabolism. Fig. 9 demonstrates the main constituents of olive and their metabolic pathways inspired by the works of Nikou *et al.*, Nikou *et al.* and López de las Hazas *et al.*[157–159] For a more detailed intake of the bioavailability and metabolism of olive polyphenols, readers are referred to the comprehensive review articles by Žugčić *et al.*, Galmés *et al.*, and Nikou *et al.*[154,155,157]

In 2001, Mikró-Casas and colleagues suggested an analytical method to quantitate hydroxytyrosol and tyrosol in human urine. Eleven participants of both sexes consumed 50 mL virgin olive oil after four days of a low phenolic diet and urine samples were collected before and after, within 24 h window. The samples were analyzed by capillary GC-MS and the results indicated that hydroxytyrosol and tyrosol are mainly excreted in conjugated form. The authors highlighted the difficulty of estimating the actual dose of the phenolic compounds present in foods as well as the importance of the GI microflora in the metabolism of olive polyphenols.[160] It is important to emphasize that determining the detailed composition of edible oils, particularly the non-fatty acid fractions, is challenging due to the significant variability, lack of reference standards, and influence of multiple factors on their composition. As a result, standardizing the product used in human intervention studies can be difficult, and working with pure compounds is almost impossible.

Twenty-three years later, Luque-Córdoba *et al.* used the method proposed by Mikró-Casas with some modifications to quantitate hydroxytyrosol and tyrosol in human urine using tQ-MS. Twenty volunteers, after a 2 day phenol free diet, consumed 40 g of extra virgin olive oil containing 500 mg mL^{-1} total phenols. The quantitation results showed a 5-fold increase in hydroxytyrosol and tyrosol. This group also performed a qualitative analysis using LC-QTOF-MS/MS, leading to the annotation of ligstroside **(64)** aglycone, oleuropein aglycone, sulfate and glucuronide conjugates of hydroxytyrosol and tyrosol, catechol and 3-methyl-catechol, among others.[161] Bazoti *et al.* developed a method for the simultaneous quantification of oleuropein and its main metabolites, hydroxytyrosol, 2-(3,4-dihydroxyphenyl)acetic acid, homovanillyl (homovanillic) alcohol **(65)**, homovanillic acid **(66)**, and elenolic acid **(67)** in the plasma of female rats. The rats were given a supplement containing 0.33 mg per kg oleuropein or 1.1 g kg^{-1} of extra virgin olive oil for 80 days. The samples were pre-treated with enzymatic hydrolysis and SPE steps and were analyzed by an LC-tQ-MS/MS system.[162] In another study, García-Villalba and coworkers investigated the oral bioavailability of oleuropein through an olive leaf extract matrix. Eight premenopausal women and eight postmenopausal women consumed 250 mg of the oleuropein-rich extract (40%), and their blood samples were collected at various time points in a 24 h period alongside urine samples divided in five fractions. The analysis was performed using LC-QTOF-MS and LC-tQ-MS. The main metabolites found in plasma were hydroxytyrosol and oleuropein aglycone derivatives, with hydroxytyrosol sulfoglucuronide being reported for the first time in human plasma, and 2 derivatives of homovanillic acid. A similar profile of metabolites was found in urine, except for homovanillic alcohol sulfate, elenolic acid and elenolic glucuronide, which were exclusively excretion metabolites. The hydroxytyrosol sulfate levels were inversely correlated with the malondialdehyde (MDA) levels, which were found to be lower in postmenopausal women, and thus the authors suggest

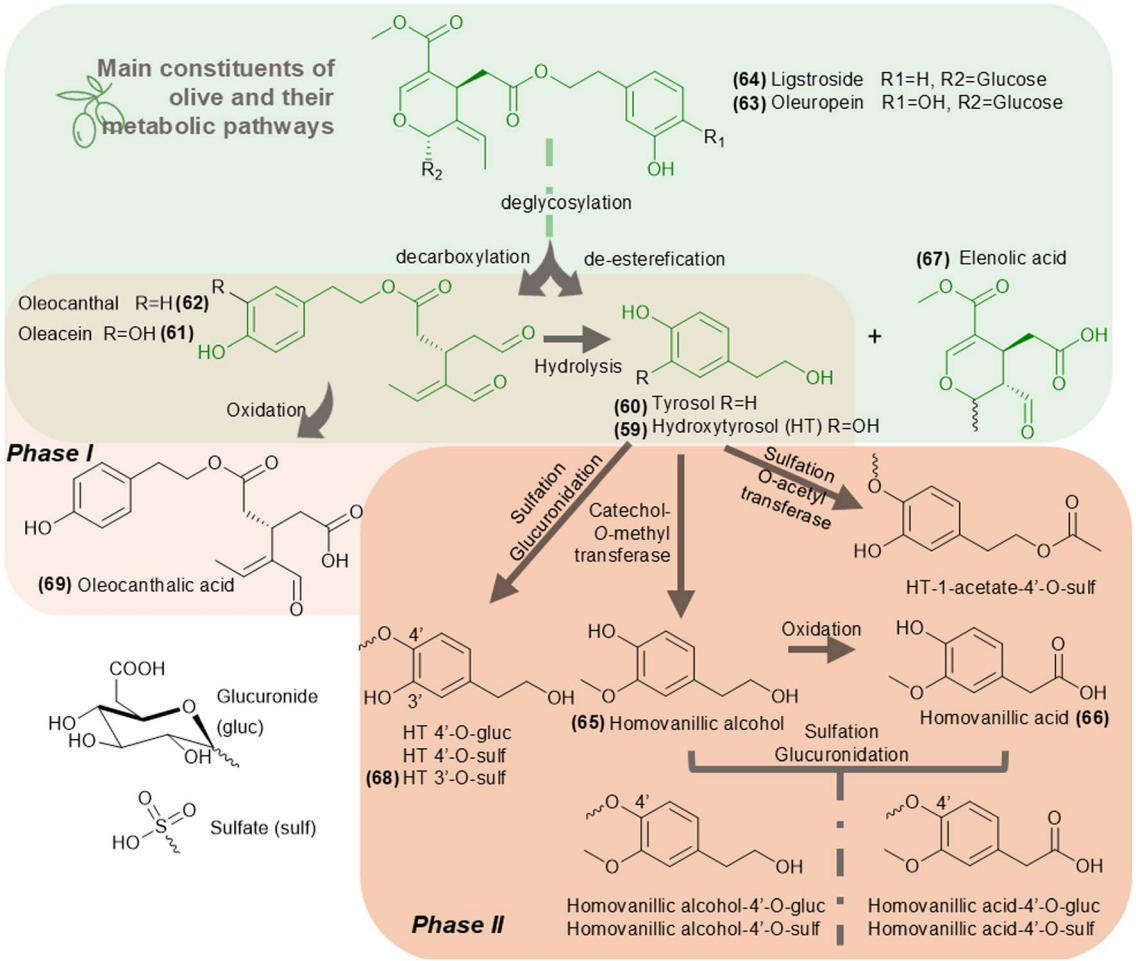

Fig. 9 Main polyphenols or biophenols in olive and olive oil products are the phenyl alcohols tyrosol and hydroxytyrosol, the secoiridoids oleocanthal and oleacein as well as their corresponding glycosylated forms ligstroside and oleuropein, respectively. Oleocanthal and oleacein are formed through the esterification of elenolic acid with tyrosol and hydroxytyrosol, respectively. The aglycone forms of secoiridoids are rapidly oxidized to the respective acid (oleocanthalic acid) during phase I metabolism. Phase II reactions such as sulfation, glucuronidation, acetylation and methylation occur either on native phenyl alcohols or on phenolic compounds released after the hydrolysis of secoiridoids. Homovanillic alcohol and the respective acid together with different glucuronides have been also reported.[157–159]

they could be a target population to prevent stress-related processes.[163] In a more recent study by Polia *et al.*, a new analytical methodology was proposed for the more accurate quantification of oleuropein metabolites based on the calculation of a response factor in QTOF-MS for each metabolite, comparing their quantification in UV and MS. Fifteen healthy adults consumed one capsule of Bonolive® (olive leaf polyphenol mixture, with 40% oleuropein), under controlled meals, and blood samples were collected at 0 and 2 h, while urine samples were collected after 24 h. One extra volunteer consumed 2 capsules, and their urine samples were used for the calculation of the response factor. The authors quantified with accuracy glucuronide and sulfate conjugates of hydroxytyrosol, homovanillyl alcohol and oleuropein aglycone. For the first time, glucuronides of oleuropein derivatives were quantified in human plasma and urine samples.[164] Moreover, García-Villalba *et al.* analyzed the urine samples of ten healthy volunteers of both sexes after the consumption of 50 mL virgin olive oil, along with 30 g of bread, after 2 days of a phenol-free diet. Samples were collected before and after within a 6 h window and they were analyzed using LC-TOF-MS, resulting in the identification of more than 60 metabolites of all the compounds described in olive oil, for the first time.[165]

The metabolic fate of olive oil polyphenols after the administration of 50 mL of extra virgin olive oil with 30 g of bread to 9 volunteers was also explored by Silva *et al.* After two days of phenol-free diet, the participants consumed a single dose containing 322 mg kg^{-1} total phenolic content and 6 mg/20 g hydroxytyrosol and its derivatives. Urine and plasma samples from different time points were collected and analyzed by LC-Orbitrap-MS. The authors identified the products of phase I hydrogeneration and phase II glucuronidation as major metabolites.[166] Rubió *et al.* studied how the intake of olive oil (30 mL) containing different amounts of total phenols (250, 500, and 750 mg kg^{-1}) affects the PK of olive oil polyphenols. Plasma samples were collected from the twelve participants and analyzed by LC-MS/MS. The results showed a dose–response relationship, while the main metabolites detected were

hydroxytyrosol sulfate and hydroxytyrosol acetate sulfate, which were reported for the first time and their metabolic pathway was proposed *via* homovanillic acid and homovanillic acid sulfate, respectively.[167,168] A few years later, the same group published a differentiated study analyzing hydroxytyrosol and its conjugates in red blood cells for the first time. Male Wistar rats were fasted for 16–17 h, and then intragastrically gavaged with 1.5 g per kg body weight of the olive oil extract. Blood was collected at sacrifice at 1, 2, 4 and 6 h post-gavage, and the samples were analyzed using LC-MS/MS. Sulfated and methyl-sulfated derivatives were the main metabolites detected, maybe forming complexes with the red blood cells, which the authors suggest play an important role in the bioavailability of phenol.[169] Kundisová and colleagues performed a sensitive LC-MS/MS analysis to determine the olive polyphenols in male rat plasma after a single dose of table olives (7.7 g per kg body weight) administrated by gavage. Samples were collected half an hour after the administration and their analysis led to the determination of 16 phenolic compounds, such as salidroside, *p*-coumaric acid, hydroxytyrosol, verbascoside, tyrosol, luteolin, and luteolin-7-*O*-glucoside.[170] To discover the influence of the food matrix on the PK and bioavailability of hydroxytyrosol and its metabolite, Alemán-Jiménez *et al.* performed a double-blind study with 20 participants, of both sexes, who consumed a single dose of 5 mg of hydroxytyrosol through diverse sources (extra virgin olive oil, refined olive oil, flax oil, grapeseed oil, margarine and pineapple juice). Blood samples were collected at 0, 30, 60, 120 and 240 min and urine samples were collected at 0 and 24 h and they were analyzed by LC-tQ-MS/MS. In the case of hydroxytyrosol, the intake of extra virgin olive oil showed a higher plasma concentration after 30 min and was the best dietary matrix owing to its bioavailability.[171] Khymenets *et al.* studied the absorption and bioavailability of pure hydroxytyrosol administrated in two doses (5 or 25 mg day^{-1}), alongside a placebo group, in a randomized, double-blind study including 21 participants. After a week of olive-free diet, the volunteers consumed the corresponding dose for seven days and urine samples were collected. This cycle repeated another two times and 105 samples in total were analyzed by LC-MS/MS. Hydroxytyrosol-sulphate-3′ **(68)** was detected as the major metabolite in urine and proposed as a biomarker, and dose-dependent excretion was confirmed for both hydroxytyrosol and its metabolites.[172] The same year, López de las Hazas and colleagues investigated the metabolism of hydroxytyrosol administrated either in its pure form or through its precursors, such as oleuropein and secoiridoids. Thirty-two male and female rats were separated in four groups, *i.e.*, one control group and 5 mg kg^{-1} of hydroxytyrosol, oleuropein and secoiridoids groups. Biological fluids (plasma and urine), GI contents (stomach, small intestine, caecum, and faeces) and metabolic tissues (liver and kidneys) were collected, pre-treated and analyzed by LC-MS/MS. Based on the results and the metabolites detected, the authors proposed catabolism pathways for hydroxytyrosol, oleuropein and the main constituents of secoiridoid extract, while the maximum bioavailability of hydroxytyrosol was achieved through oleuropein administration.[159] In another study, Domínguez-Perles *et al.* studied the oral bioavailability of hydroxytyrosol, hydroxytyrosol acetate and 2,3-dihydroxyphenylacetic acid (DOPAC) in male and female rats. The pure compounds were administrated at two doses (1 and 5 mg kg^{-1}) and blood and urine samples were collected at different time points for 24 h and they were analyzed using LC-tQ-MS/MS. For all three compounds, the highest plasma concentration occurred between 30 min and 2 h, while different dosages did not provide a linear, dose-dependent plasma concentration or urine excretion. The results indicated that gender affects the bioavailability of hydroxytyrosol derivatives, from which tyrosol was found as the second main metabolite in both sexes.[173] Although the vast majority of studies have focused on olive and olive oil, it is worth highlighting the work of Garcia-Villalba and colleagues, who investigated the secoiridoids of *Fraxinus angustifolia* found in its seeds and fruits, a species within the Oleaceae family. They applied targeted and untargeted metabolomics to investigate the bioavailability of secoiridoids present in an encapsulated (334 mg per capsule) *F. angustifolia* extract. Nine healthy participants consumed a single acute dose of 3 hard capsules. LC-QTOF-MS analyses of plasma and urine samples revealed the limited bioavailability of secoiridoids and highlighted tyrosol and ligstroside-aglycones as main metabolites, along with phenolic conjugates including ferulic acid, caffeic acid sulfates, and hydroxybenzyl and hydroxyphenylacetaldehyde sulfate derivatives.[174]

Recently, a study performed by Sakavitsi and coworkers explored the metabolism of pure hydroxytyrosol and tyrosol through a continuous dialysis *in vitro* model with a colon phase simulating gastric, intestinal and gut conditions (GI dialysis, GIDM-colon model) of the human GI tract, using one pooled human fecal sample. The samples collected at different time points were analyzed by LC-Orbitrap-MS and an HRMS/MS-based untargeted metabolomic approach was applied. Statistical analysis offered a clear classification of the metabolites between the different compartments and a total of 27 metabolites of hydroxytyrosol and 13 metabolites of tyrosol were identified. The results established, for the first time, the polymerization of phenolic alcohols as an autooxidation reaction.[175] Finally, two less investigated olive constituents were studied more recently by López-Yerena *et al.* and Nikou *et al.* In 2021, López-Yerena and colleagues investigated the intestinal permeation and metabolism of oleacein using an *in situ* technique, the single-pass intestinal perfusion (SPIP) model in rats. Intestinal fluid, mesenteric blood and ileum segment were collected and analyzed by LC-LTQ-Orbitrap-MS. The main metabolites are products of hydrolysis and hydroxylation, indicating that the small intestine is very important in the bioavailability of oleacein, given that it appears to be highly permeable. The metabolic pathway for oleacein is proposed and the structures of its derivatives are reported for the first time by the authors.[176] Three years later, in 2024, Nikou and coworkers investigated for the first time the PK of oleocanthal and its metabolism in mice. Oleocanthal was administrated intraperitoneally at dose of 5 mg kg^{-1} and plasma samples were collected at ten time points in a 24 h window. Thirteen metabolites were identified using LC-HRMS/MS, while pure

oleocanthal was not detected. Oxidized derivatives were present immediately after administration and for 30 min, while conjugation derivatives appeared after 8 h. Tyrosol sulfate and oleocanthalic acid **(69)**, which is associated with oleocanthal for the first time *in vivo*, were the most demonstrative metabolites and were proposed as biomarkers.[158] Finally, the same group investigated the metabolic fate of hydroxytyrosol on obese women through a randomized double-blind prospective design. Two different doses of hydroxytyrosol (15 and 5 mg day^{-1}) and a placebo capsule were administered to 29 women with overweight/obesity for six months and they were monitored at three time points (baseline, 4, 12 and 24 weeks). Urine samples were collected during the intervention, analyzed *via* LC-HRMS/MS under untargeted metabolomic purposes and correlated with weight loss. 10 statistically significant metabolites were detected and identified including hippuric acid, glutamine and *p*-cresol sulfate for hydroxytyrosol groups.[177] These studies are amongst the few using the holistic approach of metabolomics to explore metabolism pathways and reveal biomarkers. In our opinion, further research should be conducted on the metabolism and PK of olive secoiridoids, given their notable biological activity profile.

3.1.8 Hydroxycinnamates. Hydroxycinnamates (HCA) are some of the most broadly distributed phenolic compounds in plants. They are biosynthesized from phenylalanine and tyrosine, and they all possess a C_6–C_3 carbon skeleton with a double bond on their side chain. Hydroxycinnamates can be found in a variety of fruits, vegetables and beverages and they have received particular attention, being the most abundant antioxidants in the human diet.[178] Due to their wide distribution, several articles have dealt directly or indirectly with the metabolism of hydroxycinnamates. Given the vast amount of information encountered in the literature, the current review will present selected articles for this diverse compound category. For further information on the bioavailability and metabolism of hydroxycinnamates, readers are advised to read the comprehensive review articles, which also include detailed metabolic pathways, by El-Seedi *et al.* and Clifford *et al.*[178–180]

The thorough study by Gonthier *et al.* described the bioavailability and metabolism of pure compounds, *i.e.* chlorogenic, caffeic and quinic acids in a male Wistar rat model. The animals were fed with 250 μmol in 20 g diet of pure compounds per day, while plasma and urine samples were collected and analyzed with an LC-MS/MS system. Importantly, metabolites were analyzed after deconjugation by glucuronidase and sulfatase enzymes. The microbial-derived metabolites *m*-coumaric acid and derivatives of phenylpropionic, benzoic and hippuric acids were the major compounds detected in both biofluids, with the latter originating from the quinic acid moiety and the rest from caffeic acid.[181] In another study published the same year by Kern *et al.*, the metabolic fate of the major hydroxycinnamates, namely ferulic, sinapic, *p*-coumaric and caffeic acids, was investigated in an *in vitro* Caco-2 model with a 24 h incubation time. Once more, the samples were treated with glucuronidase and sulfatase prior to analysis with an LC-tQ-MS/MS system, which was used for metabolite annotation. The metabolites detected were products of de-esterification, glucuronidation, sulfation, and *O*-methylation; however, only sulfate conjugates of ferulate, sinapate and *p*-coumarate could be detected after 24 h, while ferulic and isoferulic acids were the only metabolic products observed following incubation with caffeic acid.[182] In another study utilizing a model cell line, Mateos and colleagues reported the uptake and metabolism of chlorogenic, caffeic and ferulic acids in a HepG2 cell line after 2 and 18 h of incubation with the selected compounds. Analyses were performed on an LC-PDA-MS system, and according to the authors, a low absorption of chlorogenic acid was observed compared to the other compounds, probably due to its esterification with the caffeic acid moiety. Moreover, methylated derivatives were the most abundant metabolites detected for caffeic acid, followed by glucuronidation and sulfation, while for the ferulate parent molecule, glucuronidation was the only observed metabolic reaction taking place.[183]

In 2009, Stalmach *et al.* investigated the metabolic fate of hydroxycinnamates in male and female volunteers following a single administration of an instant coffee beverage (3.4 g of coffee powder in 200 mL of boiled water, containing 412 μmol of chlorogenic acids). Plasma and urine samples were collected within a 24 h window and analyzed with an LC-PDA-IT-MS^n platform. The authors reported the detection of 21 metabolites, mainly sulfates and glucuronides. Additionally, the authors proposed urinary dihydrocaffeic acid-3-*O*-sulfate and feruloylglycine as potentially very sensitive biomarkers for the intake of relatively small amounts of coffee.[184] In a follow-up study by the same group, a single intake of instant coffee containing 385 μmol of chlorogenic acids was administered to an ileostomy patient cohort and ileal effluent was collected 0–24 h post ingestion. The samples were analyzed with the same MS platform, while the main metabolites observed in the ileal fluid were ferulic acid-4-*O*-sulfate, caffeic acid-3-*O*-sulfate, isoferulic acid-3-*O*-glucuronide and dihydrocaffeic acid-3-*O*-sulfate. The differences observed between the two studies highlighted the importance of colonic metabolism but also assisted the distinction between the small and large intestine absorption of specific compounds.[185] In a paper published in 2011 following a similar research concept, Nagy and coworkers investigated the metabolism of coffee components in a human cohort consuming 400 mL of 1% (w/v) soluble coffee. Plasma samples were collected at regular intervals and analyzed by LC-HRMS. The authors reported, for the first time, the *in vivo* presence of 3,4-dimethoxycinnamic acid and its dihydro-derivative, 3,4-dimethoxy-dihydrocinnamic acid, a fact which was further confirmed by synthesizing and analyzing the respective reference standards for comparison.[186] In another article published by the same research group, healthy subjects of both sexes were administered 400 mL of an instant coffee beverage, and their plasma samples were collected at multiple time points within 24 h. Analysis was carried out with an LC-HRMS system and a total of 34 coffee metabolites were reported, among which 19 were detected for the first time in plasma after coffee consumption. Through their findings, the authors questioned the usefulness of glucuronidase and sulfatase treatment of samples prior to analysis, a method that had widely been employed for this compound class in the past. Also, the authors

detected, for the first time, chlorogenic acid lactone derivatives and their sulfated and glucuronidated analogues, hinting an increase in absorption due to the lactone moiety.[187]

Continuing the reported works related to coffee, in 2013 Ludwig *et al.* investigated the metabolic fate of coffee chlorogenic acids after incubation of freeze-dried espresso powder (500 mg containing 80 μmol of caffeoylquinic acids, CGAs) with human fecal samples. The samples were collected at several timepoints within a 6 h window and analyzed by both LC-PDA-MS^n and GC-MS platforms. In total, 11 catabolites were annotated, with dihydrocaffeic acid, dihydroferulic acid, and 3-(3′-hydroxyphenyl)propionic acid being the most abundant end products, comprising 75–83% of the total catabolites.[188] On a related note, Stalmach and coworkers investigated the impact of dose on the bioavailability of coffee CGAs. Coffee preparations containing different amounts of total CGAs were consumed by a healthy human cohort and plasma and urine samples were collected at regular intervals within a 24 h period. Analysis was performed with an LC-PDA-IT-MS^n platform and a total of 21 metabolites, primarily glucuronide and sulfate conjugates, were detected in the biofluids of the volunteers, along with some of their parent compounds.[189]

In the work by Farrell *et al.*, which contributed to the accurate identification of metabolites, a sensitive LC-PDA-MS/MS method was developed to characterize the fragmentation patterns of hydroxycinnamic acid conjugates including sulfates and glucuronides, which were chemically synthesized prior to the method development. The method was then applied to a Caco-2 cell culture, incubated with the parent compounds, while for the first time, four hydroxycinnamic acid conjugates were unequivocally identified as novel Caco-2 monoculture intestinal metabolites, namely ferulic acid-4-*O*-glucuronide, dihydroferulic acid-4-*O*-sulfate, caffeic acid-4-*O*-sulfate, and caffeic acid-3-*O*-sulfate. Through this effort, the authors attempted to stress the importance of using authentic analytical standards for analyte detection and quantification to evade errors such as over- or under-estimation of physiological concentrations if similar-structure compounds are utilized.[190]

In 2018, Gómez-Juaristi *et al.*, working with a different HCA-rich beverage, namely yerba mate, investigated the absorption and metabolism of several phenolic compounds in healthy subjects of both sexes administered an infusion prepared from 4.91 g of yerba mate in 250 mL of boiling water. Plasma and urine samples were collected at distinct timepoints within a 12 h and 24 h window, respectively, and all analyses were carried out on an LC-QTOF-MS system. The authors reported that a total of 45 metabolites were detected after the administration of yerba mate, 31 of which were present in the plasma, while only 16 had a concentration above the LOQ of the method. Also, 34 metabolism products were quantified in urine, 15 new metabolites were detected in urine but not in plasma.[191] The same research group performed a noteworthy study examining the factors that might affect the stability, transport and metabolism of 5-caffeoylquinic and 3,5-dicaffeoylquinic acids (along with 3 flavanols) in a Caco-2 model system incubated with 100 μM of pure standard for 4 h. Apart from the use of LC-PDA, extracellular culture samples and cell lysates were also analyzed with an LC-QTOF instrument for metabolite annotation. The authors noted that the compounds were scarcely absorbed and minimally metabolized by the intestinal epithelium model, while the observed metabolites were mainly the products of hydrolysis and methylation and no glucuronides were detected in the current study.[192]

In 2015, Breynaert *et al*. developed an *in vitro* experimental GIDM with colon phase, to study colonic metabolism,[193] which was later employed by Mortelé and colleagues, to investigate the effect of obesity on the metabolic biotransformation of chlorogenic acid.[194] For this purpose, 78 mg of chlorogenic acid was introduced into the GIDM system, whose ultimate stage included the ultrafiltration of cells containing human fecal slurry samples, collected from healthy volunteers. All collected dialysates were analyzed on an LC-QTOF platform and a total of 24 biotransformation products were annotated in both the lean and obese populations. Remarkably, based on the findings, several differences could be observed between the study groups, with 11 metabolites being unique for the lean population, while only 1 for the obese samples.[194] On a related subject, Seguido *et al.* examined the effect of a prolonged decaffeinated green coffee supplement in the metabolic profiles of an overweight/obese population. In brief, the volunteers consumed a polyphenol-rich nutraceutical from green coffee for a total duration of two months (600 mg per day phenols, 300 mg HCAs), and plasma, urine and fecal samples were collected at diverse timepoints on the first and last days of the trial and analyzed by LC-QTOF. Overall, 54 biotransformation products were detected in physiological fluids, while regular consumption of the supplement led to increased levels of reduced forms of caffeic, ferulic and coumaric acids in urine or 3-(3′-hydroxypenyl) propanoic, and 3,4-dihydroxybenzoic acids in feces after 8 weeks and decreased concentrations of coumaroylquinic and dihydrocoumaroylquinic acids in urine.[195] In our opinion, despite the already existing data regarding the metabolism of HCAs and given their abundance in the diet, it is important to continue related research, focusing on the precise structure determination of metabolites. In another study, Wang *et al.* investigated the metabolism of lithospermic acid **(70)**, a component of *Salvia miltiorrhiza* (Lamiaceae), by intravenous injection and oral administration (20 mg kg^{-1}) in rats. Lithospermic acid is a phenylpropanoid oligomer and it is commonly classified in the chemical group of chlorogenic acid derivatives. Bile tissues and serum samples from various time points were collected and analyzed by LC-MS/MS. The results indicated that methylation is the main metabolic pathway for lithospermic acid and two metabolites, 3′-monomethyl- and 3′,3″-dimethyl-lithospermic acid, were detected in all samples.[196]

3.1.9 Other phenolics. In addition to the main categories of phenolic compounds previously discussed, there exists a diverse range of other phenolics that do not fit neatly into these classifications, such as flavones, flavanols, dihydroflavones, and coumarins. This section explores various phenolic compounds (Fig. 10 and 11) and categories that have been studied but fall outside the defined groups that exhibit a wide array of structures and biological activities, contributing to the overall complexity and significance of phenolics in NP

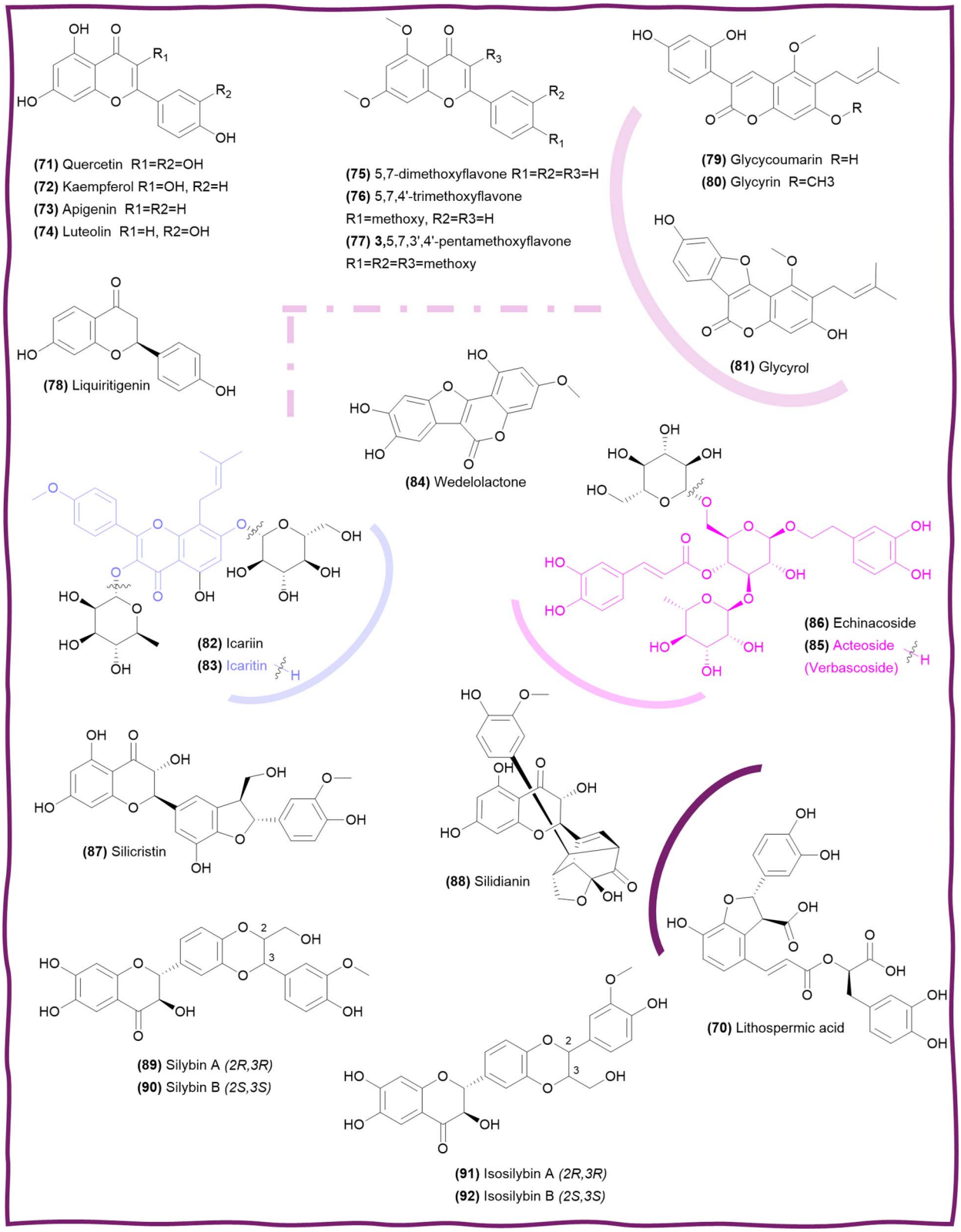

Fig. 10 Chemical structures of compounds (70)–(92).

and health research. The discussed studies have been selected based on the coherence and originality of either the reported metabolites or the approach used.

Hong *et al.* monitored the urine metabolic profile of certain flavonols after the consumption of 200 g of cooked onions by healthy human volunteers. The biotransformation products of

quercetin (**71**) and kaempferol (**72**) were identified using LC-MS/MS including quercetin monoglucuronides and their methyl and sulfate derivatives, quercetin diglucuronides and one methyl derivative, quercetin glucoside sulfates along with methyl quercetin and quercetin and kaempferol monoglucuronides.[197] In a similar concept, Mullen *et al.* followed the metabolic fate of flavonols (275 μmol) in human plasma and urine through the consumption of light fried onions. Quercetin-3′-sulfate, quercetin-3-glucuronide, isorhamnetin-3-glucuronide, a quercetin diglucuronide and a quercetin glucuronide sulfate were detected in the plasma, while the urine profile was differentiated, containing quercetin-3′-glucuronide, two quercetin glucoside sulfates and a methylquercetin diglucuronide, as identified by LC-MS/MS.[198] The same group performed a PK study to monitor a glycosylated derivative of quercetin and specifically [2-^{14}C]quercetin-4′-glucoside, which was fed by gavage to rats (4 mg per kg body weight). At each point, in a 72 h period, the corresponding rat group was euthanized, and various biological fluids and tissues were collected and analyzed by LC-PDA-RC-MS/MS. Among the metabolites detected, phenolic acids were identified for the first time, and overall the authors provided a detailed metabolism and its catabolism pathway.[199] In another study, Borges *et al.* investigated the bioavailability of apigenin (**73**), a flavone occurring in plants of dietary interest, in an open-label, crossover, single intake study in which male participants consumed different apigenin-containing products such as an apigenin capsule, dried parsley leaves, infused in hot water, alone or mixed with yogurt and three bags of chamomile tea. Plasma and urine samples were collected and analyzed by LC-MS/MS. Apigenin-4′-glucuronide, apigenin-7-glucuronide and apigenin-7-sulfate were identified as metabolites and the intake of the parsley drink provided information on their intra- and inter-individual variations at their excretion levels.[200] Luteolin (**74**), another well-known flavone found in many natural plants, has low bioavailability due to the glucuronidation process. Wu *et al.* conducted a study aiming at the exploration of its glucuronidation time-course by 12 human UDP-glucuronosyltransferases and its intestinal first-pass metabolism in mice. LC-HRMS analysis led to the identification of six metabolites, including two novel diglucuronides, while their formation was shown to be time-dependent and isoform-specific. Moreover, their metabolism in the intestine of mice differed from that in humans.[201]

In another study, Mekjaruskul and coworkers investigated the metabolic fate of *Kaempferia parviflora* (Zingiberaceae) constituents, which are mainly methoxyflavones, after the oral or intravenous administration of the ethanolic extract in male rats. A dose of 250 mg kg^{-1} was administrated, and blood samples were collected at various time points to examine the PK profile of 5,7-dimethoxyflavone (DMF) (**75**), 5,7,4′-trimethoxyflavone (TMF) (**76**), and 3,5,7,3′,4′-pentamethoxyflavone (PMF) (**77**) for the first time, while a higher dose (750 mg kg^{-1}) was employed for the distribution and excretion studies by analyzing various biological tissues and fluids. The analysis was performed by LC-UV, and for urine and feces samples an additional LC-MS/MS analysis was performed. The results showed low oral bioavailability of the methoxyflavones, which reached maximum concentration levels within 1–2 h. They were found mainly in liver and kidney tissues but also detected in the lungs and brain. They were eliminated through urine in the forms of demethylated, sulfated and glucuronidated derivatives and as demethylated products through feces.[202]

A structurally similar compound, liquiritigenin (**78**), a dihydroflavonoid found in licorice (*Glycyrrhiza uralensis*, Fabaceae) was studied by Keranmu and colleagues in the gut microbiota and hepatic metabolism in *in vitro* models. Samples were collected in various time points during the incubation period and were analyzed by LC-MS/MS and LC/MS^n-IT-TOF, leading to the identification of three possible metabolites of the gut microbiota, phloretic acid, resorcinol and the third one speculated to be davidigenin.[203] Other major bioactive compounds from *Glycyrrhiza uralensis* were also investigated by Wang *et al.* in two separate publications. In 2014, the metabolism of the major bioactive coumarin of licorice glycycoumarin (**79**) and one of the most popular herbal medicines worldwide, was investigated after its oral administration (40 mg kg^{-1}) in rats. Plasma and urine samples were analyzed by LC-DAD-MS^n and LC-IT-TOF-MS and 14 metabolites were identified in total. Two metabolites from the microbial transformation of *Syncephalastrum racemosum* AS 3.264, 4″-hydroxyl glycycoumarin and 5″-hydroxyl glycycoumarin, were reported for the first time.[204] The following year, the authors monitored the metabolism of glycyrin (**80**) and glycyrol (**81**) after their oral administration (40 mg kg^{-1}) in rats, and plasma and urine samples were analyzed using the same analytical platforms. The two coumarins showed poor oral bioavailability, while a new compound, 4″-hydroxyl glycyrin, was found to be the major metabolite from the microbial transformation of *S. racemosum* AS 3.264.[205]

Cheng *et al.* studied the metabolism of icariin (**82**), one of the major constituents of *Epimedium brevicornum* (Berberidaceae), after the administration of a single oral dose (150 mg kg^{-1}) in rats. Plasma, bile, urine and feces samples were collected and analyzed by LC-QTOF-MS. Five metabolites, demethylicariin, icariside I-3-*O*-glucuronide, demethylicariside II, demethylicariside II-7-*O*-glucuronide, and dehydroxyicaritin-glucuronide, were reported for the first time, while most metabolites were detected in the bile, revealing the main excretion pathway for icariin.[206] On a similar note, Zhang *et al.* investigated the metabolism of another major constituent of *Epimedium brevicornu*, icaritin (**83**). The pure compound was administrated in a single dose to rats *via* the intravenous route (2 mg kg^{-1}) and oral administration (40 mg kg^{-1}) and plasma samples from various time points along with various biological tissues and fluids were analyzed by LC-MS/MS. C-7 glucuronidated icaritin was identified as the major metabolite in plasma after oral administration, and along with the parent compound were mainly distributed in the kidney and liver, respectively.[207] Li and colleagues investigated the metabolic fate of a medicinal plant-derived coumestan, wedelolactone (**84**), after its oral administration (50 mg kg^{-1}) to rats. Plasma, urine, feces and bile samples were collected and analyzed by LC-QTOF-

MS with the identification of 17 metabolites, mainly glucuronidation and methylation products.[208]

In 2013 Qi and colleagues studied acteoside (or verbascoside) **(85)**, a polyphenolic compound composed of four chemical moieties, *i.e.* caffeic acid, glucose, rhamnose and phenylethanoid aglycone. A dose of 100 mg kg^{-1} was administrated by oral gavage to six rats and prior to and post dose urine samples were collected and analyzed by LC-QTOF-MS combined with an automated MS^E technique. In total 35 metabolites were detected, and according to the metabolic pathways proposed by the authors, acteoside functioned as a prodrug and underwent hydrolysis before its blood absorption.[209] A few years later, Cui *et al.* investigated the catabolic fate of acteoside using an *in vitro* fecal model. Human and rat feces along with 0.80 mM of acteoside were incubated under anaerobic conditions, and samples were collected at 6, 12, 24 and 36 h, and were analyzed by LC-QTOF-MS. A total of 14 metabolites were detected and hydrolyzation was found as the main metabolic pathway.[210] Using the same *in vitro* model and samples from six healthy donors, Li and coworkers monitored the metabolism of three relevant phenylethanoid glycosides. The analysis was performed by LC-QTOF-MS with MS^E, leading to the identification of 11 metabolites of acteoside, 7 metabolites of isoacteoside and 11 metabolites of 2′-acetylacteoside, including the degradation products hydroxytyrosol and 3-hydroxyphenylpropionic acid.[211] The same research group incorporating the same *in vitro* model and analytical platforms studied the metabolism of another phenylethanoid glycoside, echinacoside **(86)**. Three new metabolites were reported among the 13 detected in total, including, once more, hydroxytyrosol and 3-hydroxyphenylpropionic acid.[212]

Delving into the metabolism of a different chemical class, Calani *et al.* evaluated the absorption and metabolism of the flavonolignans silychristin **(87)**, silydianin **(88)**, silybin A **(89)**, silybin B **(90)**, isosilybin A **(91)** and isosilybin B **(92)**. *Silybum marianum* (L.), commonly known as milk thistle, is one of the most prominent medicinal plants of the Asteraceae family. The lipophilic extract derived from its seeds is known as silymarin, a complex mixture of compounds primarily composed of structurally related flavonolignan isomers. Silymarin has been reported to exhibit a wide range of biological activities, including hepatoprotective, anti-inflammatory, immunomodulatory, neuroprotective, and lactogenic effects, as well as anticancer potential against various cancer types. In this study, fourteen participants consumed 8 g of milk thistle formulation, following a specific diet, and urine samples were collected at various times for 48 h. 99% of the absorbed fraction was recovered as sulfo- and glucuronyl-conjugates and 31 metabolites were identified by LC-MS/MS.[213–215] Marhol and colleagues focused on the PK study of the diastereoisomers silybin A and silybin B after their separate administration in rats (200 mg kg^{-1} of body weight) and the collection of plasma samples. Silybin B showed a faster absorption rate, and SPE-LC-MS/MS analysis led to the identification of glucuronides and sulfates as its main metabolites.[216] In addition, in a more recent study, Pferschy-Wenzig and coworkers investigated the gut biotransformation of silybins A and B and silymarin extract using an *in vitro* human fecal model. A mixture (1 : 1, 80 mg) of the two diastereoisomers was incubated with the fecal suspension of one human donor for 24 h and analyzed by LC-DAD-HRMS. Also, the extract was incubated with a human fecal suspension for 24 h and samples from three time points were analyzed by LC-HRMS. Demethylation, reduction/ring cleavage and combination products, as well as low molecular weight aromatic metabolites were identified.[217]

Upadhyay *et al.* performed *in vitro* metabolic studies on rat liver microsomes and primary hepatocytes and *in vivo* studies to investigate the PK and metabolic profile of picroside I **(93)** and II **(94)**, iridoid glycosides found in the roots and rhizomes of *Picrorhiza kurroa* (Plantaginaceae). A sensitive LC-MS method was developed to analyze rat plasma samples and overall, eight metabolites of picroside I and six metabolites of picroside II were identified *in vitro*, out of which four metabolites for each compound were also detected *in vivo*.[218]

Exploring simpler phenols, Zhang *et al.* performed the first PK study of ginger (*Zingiber officinale*, Zingiberaceae) root constituents. 6-Gingerol **(95)** and 6-shogaol **(96)** are the major bioactives of ginger and evidence suggest their high instability and interconvertibility. Interestingly, there is limited data on their *in vivo* interconversion influencing their PK profiles. In this study, asthma patients consumed twice per day and for 56 days a capsule containing 1.0 g of the root extract or a placebo capsule. Serum samples were collected at 0–8 h on days 0, 28 and 56 and were analyzed by LC-MS/MS. The results showed that one-third of 6-gingerol was metabolized to 6-gingerdiols, while more than 90% of 6-shogaol was metabolized to phase I and cysteine-conjugated derivatives. All metabolites reached their peak concentration in less than 2 h, but long-term intake enhanced their accumulation.[219,220] The following year, Zagorska *et al.* investigated the bioavailability of selected ginger phenols in the presence of various types of diets incorporating an *in vitro* digestion model. A ginger extract (0.2 g) was used for the experiment and samples were analyzed by LC-QTOF-MS. The results indicated that 6-gingerol has the highest bioavailability, followed by 10-gingerdione **(97)**, 8-gingerdione **(98)** and 8-shogaol **(99)**, while the high-residue diet enhanced the bioavailability of these compounds.[221]

In another study, Zhang *et al.* investigated the PK profile of methyl salicylate **(100)**-2-*O*-β-D lactoside (MSL) after its tail vail injection (15 mg kg^{-1}) or oral administration (300 mg kg^{-1}) in rats. Blood samples from various time points as well as urine, feces and various organs were collected and analyzed by LC-QTOF-MS/MS. MSL was detected at the highest levels 2 h post-administration in liver and spleen samples, while salicylic acid, its main metabolite, was found in higher concentration in the liver and kidney. Both compounds were also detected in the brain.[222]

Wu *et al.* studied the PK and metabolism of a specific dianthrone extracted from senna, sennoside B **(101)**, after its oral (50 mg kg^{-1}) or intravenous (5 mg kg^{-1}) administration in rats. Plasma, urine, bile and feces samples were analyzed by LC-QTOF-MS, resulting in the identification of 14 metabolites, which are mainly the products of hydrolysis to aglycones, formation of rhein-type anthrones and extensive conjugation

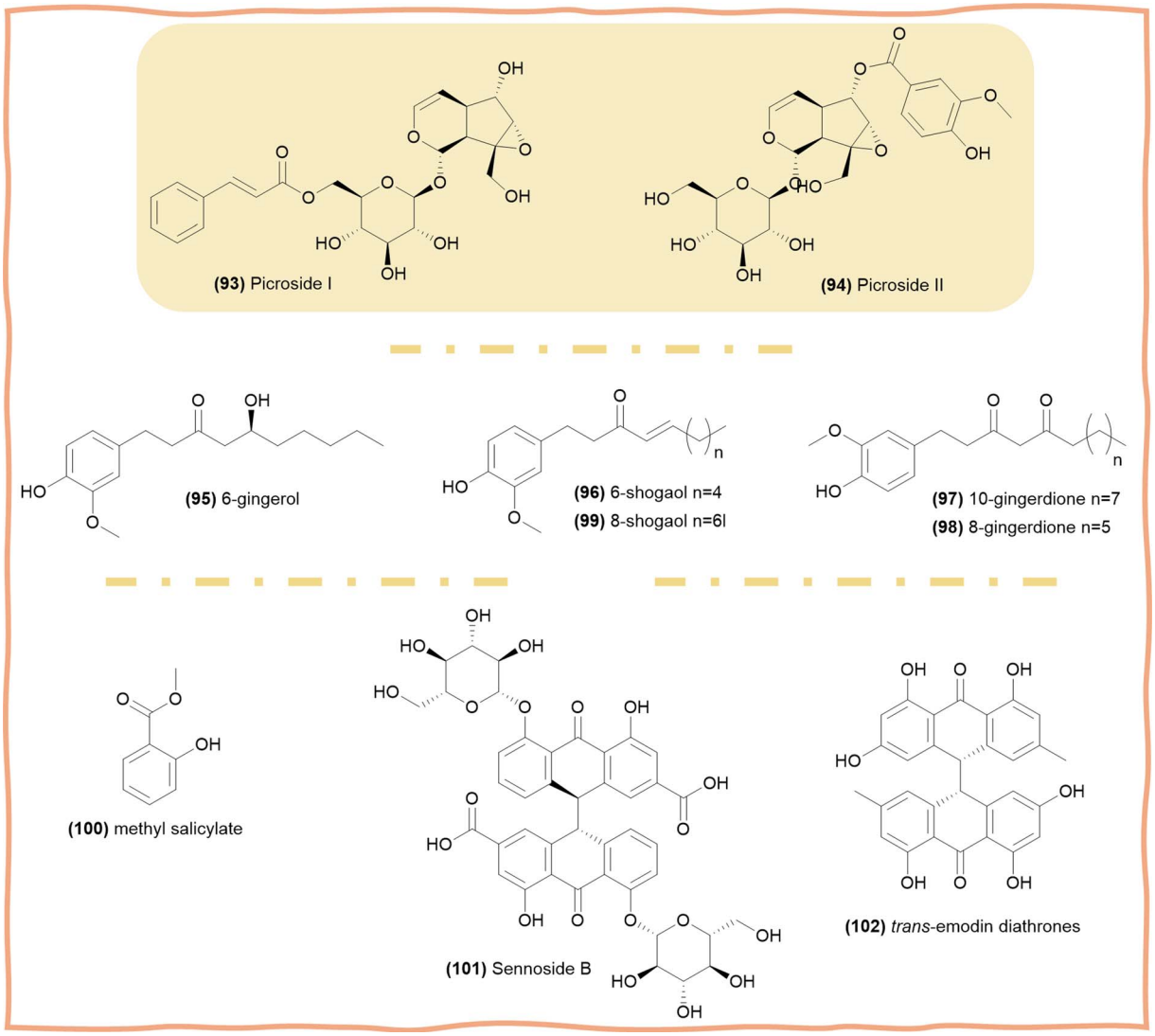

Fig. 11 Chemical structures of compounds (93)–(102).

pathways.[223] Song *et al.* studied the PK and bioavailability of *trans*-emodin dianthrones **(102)** after their administration (20 mg kg^{-1}) in rats by gavage. The analysis of plasma, urine and feces samples by LC-tQ-MS/MS revealed their low oral bioavailability, while LC-QTOF-MS analysis led to the identification of 21 metabolites mainly glucuronidation, oxidation, carbonylation, (de)methylation, sulfation and hydrogenation products.[224]

Finally, Yuan *et al.* developed a targeted LC-tQ-MS/MS method to analyze the raspberry ketone (4-(4-hydroxyphenyl)-2-butanone) and 25 associated metabolites in mouse plasma and brain samples, and one year later developed a high-throughput sample preparation method with an improved micro-scale QuEChERS approach to monitor the same compounds in white adipose tissues with LC-tQ-MS/MS analysis.[225,226] However, although these works are interesting and useful, they are mainly focused on method development for monitoring certain metabolites.

3.2 Alkaloids

Alkaloids are abundant in nature (plants, fungi, and bacteria) primarily as a class of nitrogen-containing organic molecules, exhibiting various health beneficial activities such as anti-inflammatory and central nervous system effects as well as anti-cancer and hypoglycemic effects.[227] Given the great variety and classification of alkaloids, this review discusses some of the most well-known and studied alkaloid representatives as examples (Fig. 12).

Martínez-Lopez *et al.* studied the bioavailability of methylxanthines **(103)** in a crossover, randomized, controlled, single-blind study, where 13 healthy volunteers consumed either 15 g of cocoa product dissolved in semi-skimmed milk, or 25 g of enriched in methylxanthine cocoa product. On each intervention day, blood samples were collected at various time points up to 8 h, and urine samples were collected up to 24 h. In total, 12 metabolites were identified by LC-QTOF and quantified by LC-DAD. Theobromine **(104)** and its biotransformation

products 3- and 7-monomethylxanthine were the major metabolites detected, while a strong dose–response effect took place.[228] The same research group published a similar study investigating the metabolism of methylxanthines, after the intake of a soluble green/roasted coffee blend containing 70.69 mg caffeine and 0.119 mg theobromine by 12 healthy participants. The plasma and urine samples collected at various time points were analyzed by LC-QTOF-MS and seven metabolites were detected in plasma, with paraxanthine, 1-methyluric acid and 1-methylxanthine, which are reported for the first time in plasma, being the major metabolites. In the urine samples, eleven metabolites were identified, with 1-methyluric acid being the major one.[229]

Wang and colleagues studied the metabolism of rhynchophylline **(105)**, a tetracyclic oxindole alkaloid composed of an indole moiety and an indolizidine moiety, isolated mainly from *Uncaria* sp. (Rubiaceae). The study was conducted using an *in vitro* rat model and analysis by LC-MS showed the detection of the pure compound in the plasma, bile, brain, urine and feces samples, with 78% found to be excreted in feces. The metabolites 11-hydroxyrhynchophylline 11-*O*-β-D-glucuronide and 10-hydroxyrhynchophylline 10-*O*-β-D-glucuronide were detected in bile and 11-hydroxyrhynchophylline and 10-hydroxyrhynchophylline in urine and feces.[230] Wang *et al.* evaluated the differences of first-pass metabolism in the liver and intestine between the epimers rhynchophylline and isorhynchophylline **(106)** through *in vitro* incubation with rat liver microsomes. Also, their excretion *via* feces and urine was examined. All sample analyses were performed by LC-MS/MS. The total and hepatic bioavailability of **(105)** was higher than that of **(106)**, while the intestinal bioavailability was similar for the two epimers and the excretion of both compounds was negligible.[231]

In another study, Li and coworkers investigated the PK and metabolic profile using an *in vitro* rat model, of isoboldine **(107)**, an aporphine alkaloid known for its abundance in the Papaveraceae family and opium. Twelve male rats were fasted for 12 h, and then were administrated isoboldine by oral gavage (30 mg kg^{-1}) or by tail vein injection (10 mg kg^{-1}). Blood samples were collected at various time points up to 180 min, and along with bile, urine and feces samples were analyzed by LC-TQ-(ESI+)-MS/MS in MRM mode. A total of five phase II metabolites were detected, while isoboldine showed very low oral bioavailability.[232,233]

Li *et al.* developed a sensitive LC-MS/MS method for the simultaneous determination of the β-carboline alkaloid harmane **(108)** and 10 metabolites in the plasma of rats, following its intravenous (1 mg kg^{-1}) and oral (30 mg kg^{-1}) administration. The phase II metabolites were detected at higher levels with the sulfation pathway being the main metabolic route in both administration ways.[234] The same year, Han and coworkers published a study focusing for the first time on the *in vivo* PK and metabolism of the polycyclic alkaloid protostemonine **(109)**. The pure compound was administrated to rats intravenously (2 mg kg^{-1}) or orally (10, 20 and 50 mg kg^{-1}) and blood and urine samples were collected and analyzed by LC-QTOF-MS. The results indicated dose-dependent PK characteristics, while up to 10 metabolites were detected in urine samples with demethylation, hydrolysis and oxygenation being the proposed metabolic pathways.[235]

In another study, Feng *et al.* followed the PK profile of berberine **(110)** and nine of its metabolites, for which they provide detailed metabolic pathways, after its single intravenous administration (4 mg kg^{-1}) or oral administration (48.2, 120 or 240 mg kg^{-1}) in rats. Blood samples were collected at various time points up to 48 h. In addition, the authors followed excretion profile of berberine after its intragastric administration (48.2 mg kg^{-1}) to rats, and urine and feces samples were collected up to 84 h along with bile tissues. All samples were analyzed by LC-MS/MS and the results showed that berberine has low oral bioavailability and rapid metabolism with phase II metabolites found in higher levels compared to phase I metabolites. The total recovery of berberine and its nine metabolites from urine, feces and bile was 41.2%.[236] The following year, Liu and colleagues aimed to investigate berberine metabolites in HepG2 cells and their distribution and concentration differences in intracellular and extracellular fluids. For this purpose, the authors incorporated LC/MS^n-IT-TOF and LC-MS/MS methodologies. Among the metabolites detected, jatrorrhizine + columbamine (appeared as a single chromatographic peak) were the major phase I derivatives, followed by demethyleneberberine, while phase II metabolites were sulfate and glucuronide conjugates of phase I metabolites. Demethyleneberberine was highly abundant in cells, whereas other metabolites were mainly located outside the cells.[237] Finally, Huang *et al.* investigated simultaneously two extrahepatic pathways for the metabolism of berberine to oxyberberine through intestine metabolism and blood metabolism, performing *in vivo* and *in vitro* studies in rats using a sensitive LC/MS-IT-TOF method.[238] Interestingly, berberine has been one of the most extensively studied alkaloids in recent years, with particular attention given to developing various formulations aimed at improving its performance and enhancing its bioavailability.

3.3 Cannabinoids

The term cannabinoids traditionally refers to a group of compounds found in *Cannabis sativa* (Cannabaceae), which is known both for its recreational and medicinal uses. Even though the recreational use of cannabis products is illegal in many countries, in recent years many states have introduced laws to permit the medical use of this plant and related formulations, particularly for the pain management of chronically ill patients.[239] Even though the plant contains a myriad of diverse cannabinoids, the most abundant and relevant today are Δ^9-tetrahydrocannabinol (Δ^9-THC) **(111)**, which is responsible for the exhibited psychoactive activity, and cannabidiol (CBD) **(112)**, which, although lacks a psychoactive effect, has been extensively studied for its potential benefits in numerous disorders.[240] Fig. 13 illustrates the knowledge on Δ^9-THC and CBD metabolism.[241–243] In the current review article, a small number of references is included discussing the PK properties of major cannabinoids; however, for further information on the

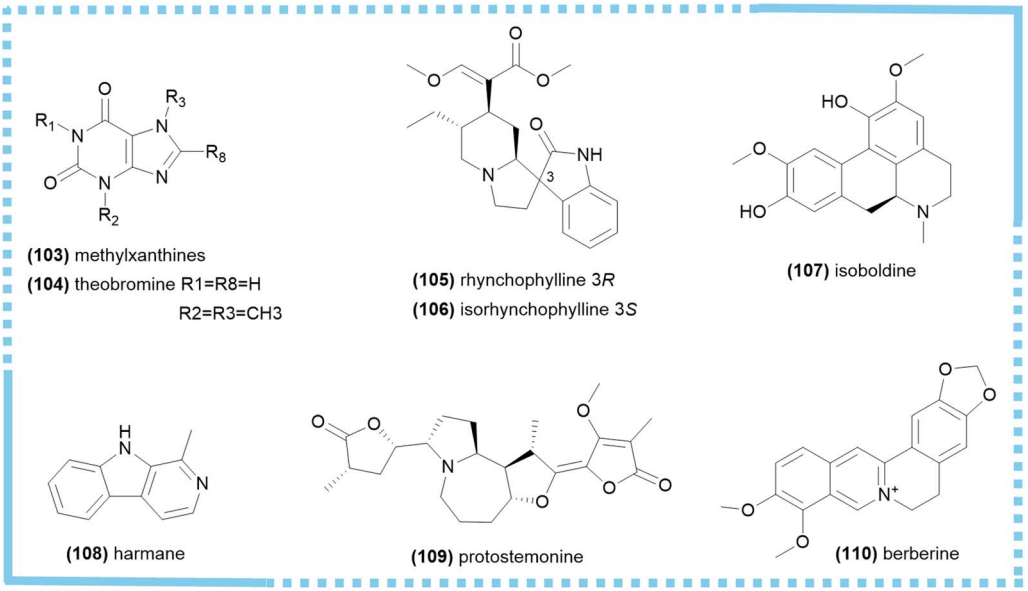

Fig. 12 Chemical structures of alkaloids (103)–(110).

topic, the reader is referred to the following works from Citti *et al.* and Lucas *et al.*[241,242]

Lowe *et al.* described a 2D GC-EI-MS method for the simultaneous quantification of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) **(113)**, and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) **(114)** in human plasma. Following development and validation, the method was applied to plasma samples of healthy chronic cannabis users, and overall the authors reported acceptable assay characteristics and increased analytical sensitivity with improved S/N and detection limits, reaching picogram concentrations.[244] In 2011, Gronewold *et al.* reported an LC-MS/MS method for the detection of THC, 11-OH-THC, THC-COOH and its glucuronide **(115)** as well as CBD and cannabinol (CBN) in post-mortem physiological fluids and tissues of different cases of toxicological investigations. Even though the exact ingested dose is not known, the authors reported several interesting observations. In fact, gall bladder fluid displayed the maximum concentrations of all the analytes except THC, which was also detectable in the muscle and lung specimens. THC-COOH-gluc was found at high levels in the liver and kidney samples, while results from bile measurements suggested an increased enterohepatic circulation of this metabolite.[245] Similar observations were made by Fabritius and colleagues, who described an LC-MS/MS method for the measurement of various cannabinoids (THC, 11-OH-THC, THC-COOH, CBN, CBD, D^9-tetrahydrocannabinolic acid A (THC-A) and THC-COOH-gluc) in post-mortem bile samples. Apart from increased concentrations of THC-COOH-glucuronide, the authors reported relatively elevated levels of THC-COOH and THC-gluc **(116)**.[246]

Schwope and coworkers developed and LC-tQ-MS/MS method for the quantification of THC-glucuronide, 11-OH-THC, CBD, CBN, THC, THC-COOH and THC-COOH-glucuronide in whole blood of cannabis users, using an SPE sample clean-up. According to the authors, this method can find extensive applications in clinical trials and toxicological studies owing to its robustness, sensitivity, and specificity.[247] Pérez-Acevedo *et al.* performed a study on healthy male and female volunteers, treated first with 100 mL of cannabis decoction and afterwards with 0.45 mL of cannabis oil, both containing defined amounts of THC, CBD and their respective acids. Serum, oral fluid, sweat and urine samples were collected at regular intervals within a 24 h window, while analysis was performed using an LC-MS/MS platform. 11-OH-THC was only detected in the serum, THC, CBD, THCA-A and CBDA were the only compounds observed in the oral fluid, and THC and CBD were the only cannabinoids detected in the sweat. In urine samples, only THC-COOH, THC-gluc and THC-COOH-gluc could be detected. Finally, according to the authors, cannabinoid absorption was higher with the decoction compared to the oil.[248] In another article, the same research group, working with the same sample set, attempted to investigate the CBD metabolites in serum and urine samples of participants. 7-COOH-CBD **(117)** was the main serum metabolite, followed by 7-OH-CBD **(118)**, 6-β-OH-CBD **(119)**, and 6-α-OH-CBD **(120)**. Although 7-OH-CBD and 6-α-OH-CBD demonstrated similar PK properties for both cannabis preparations, 7-COOH and 6-α-OH-CBD displayed higher bioavailability after decoction administration.[243] Moreover, Williams and coworkers described a clinical study involving healthy male and female subjects, administered five different preparations containing a standardized CBD dose of 30 mg. The plasma samples of the participants collected at different time points within 240 min after CBD ingestion were analyzed with an LC-tQ-MS/MS platform. The authors reported that both the type of CBD preparation, as well as body composition, appear to have an effect on the bioavailability of CBD.[249] More recently, in 2022, Clarke *et al.* published a clinical study

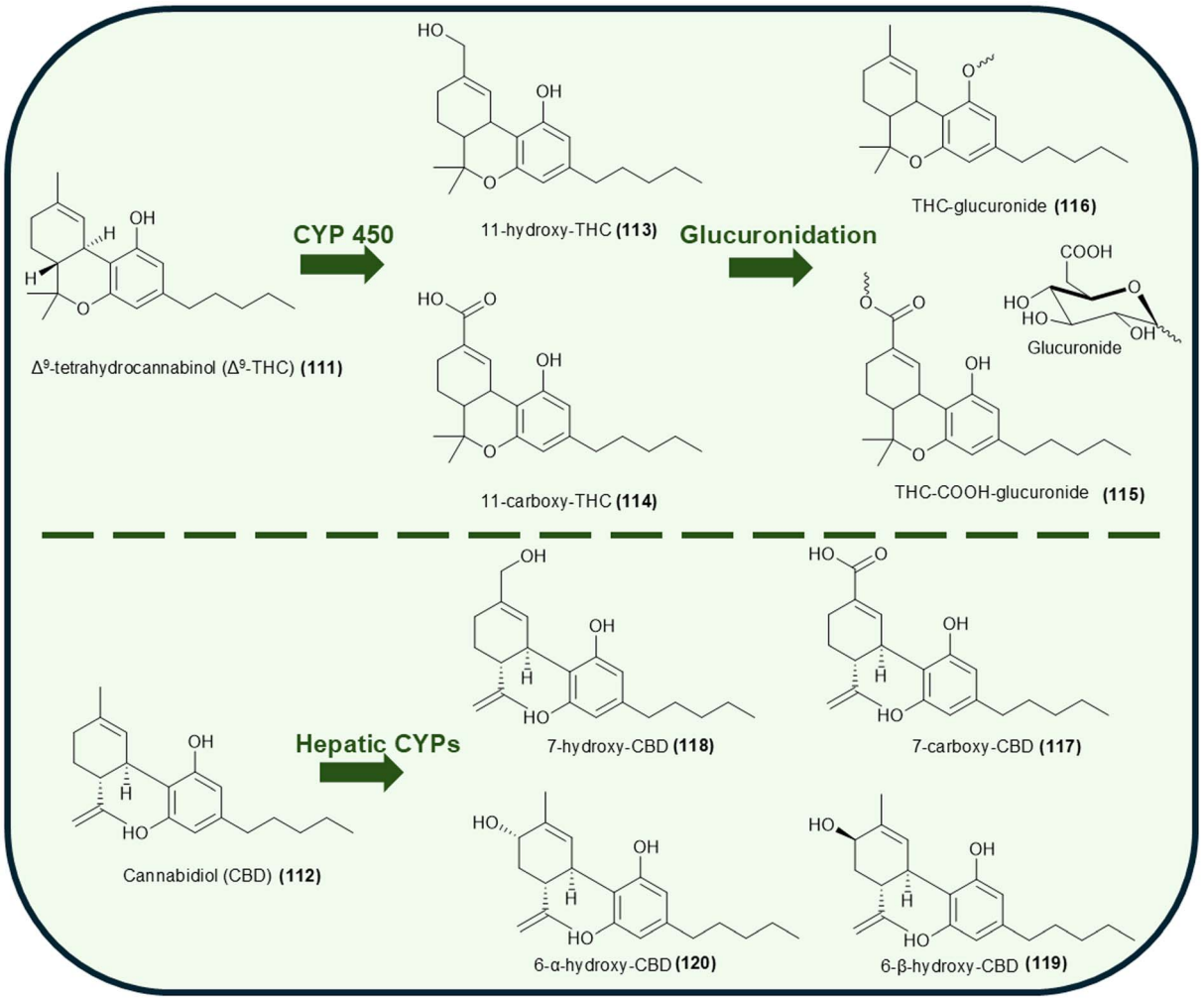

Fig. 13 Psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC) is metabolized in the liver to afford hydroxyl- and carboxyl-derivatives, with glucuronidation being described as the main metabolic reaction. The non-psychoactive cannabidiol (CBD) is prone to similar reactions by hepatic CYPs.[241–243]

investigating the effect of a THC/CBD oro-buccal spray on the pain response of advanced cancer patients. PK parameters were assessed over 2 days, whereby subjects were administered 2 dosing schemes (2.5 mg THC and 2.5 mg of CBD in 300 μL on the first day and 7.5 mg of THC and 7.5 mg of CBD in 900 μL on the second). Blood (plasma) samples were collected at regular intervals and assayed for THC, CBN, CBD, 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THC-COOH) using an LC-tQ-MS/MS platform, with median plasma concentrations found to increase in a dose-dependent manner.[250] Finally, Dumbraveanu and colleagues conducted an *in vivo* study in male C57BL/6J mice administered either a cannabinoid-rich extract or a cannabinoid-depleted extract in two distinct doses (2 *vs.* 40 mg per kg bodyweight and 40 *vs.* 1560 mg per kg bodyweight, respectively), while a third group received a pure THC preparation (1 *vs.* 20 mg per kg bodyweight). Plasma, spinal cord and brain tissue were collected and analyzed by means of LC-tQ-MS/MS, whereby THC, 11-OH-THC, THC-COOH and CBD were monitored. In plasma, both THC-COOH and 11-OH-THC were present for all three THC formulations, whereas 11-OH-THC was the predominant metabolite detected in the brain.[251]

Despite the increasing therapeutic and economic interest of this class of compounds, in our opinion, the available literature data on their bioavailability and metabolism are rather disproportionate, particularly for the so-called "minor cannabinoids". This observation could be partly attributed to the focus of most pharmaceutical companies on THC and CBD alone, as well as the fact that most clinical research related to cannabinoids remains patent protected. Interestingly, also the respective acidic forms Δ^9-tetrahydrocannabinolic acid and cannabidiolic acid have scarcely been investigated for their metabolism despite being the major cannabinoids of the cannabis plant.

3.4 Terpenes and terpenoids

Terpenes are probably the most diverse and most abundant class of NPs, which are found in the majority of terrestrial and marine organisms. Terpenes are classified based on the number

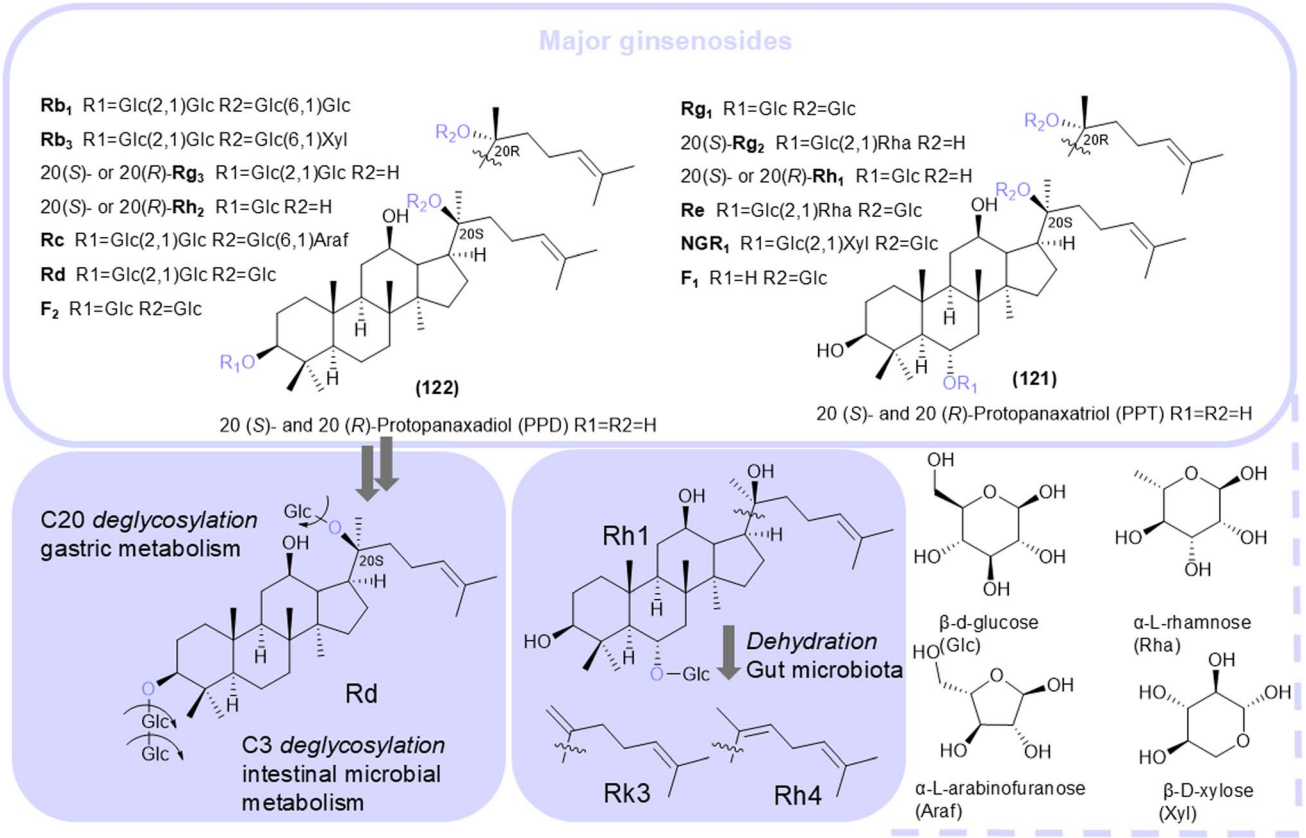

Fig. 14 Major ginsenosides are classified according to their structures, as either protopanaxadiol (PPD)- or protopanaxatriol (PPT)-type saponins with the deglycosylation reaction being their main metabolic pathway. Under acidic conditions deglycosylation of Rd, a PPD-type ginsenoside, is more facile at the C-20 position than in the C-3 position, while C-3 deglycosylation is catalyzed by intestinal microbial strains. Another metabolic pathway is dehydration at C-20/21 or C-20/22, generating a double bond. Rh1, a PPT-type ginsenoside, undergoes dehydration reactions to afford Rk3 and Rh4 metabolites.[253,256–258]

of isoprene units that make up their structure, and they have exhibited an extremely wide array of bioactivities such as anti-insecticidal, antimicrobial, anti-inflammatory, antidiabetic, and anticancer.[252] Given the extensive literature published thus far on this particular compound category, and due to their high structural diversity and complexity, the current review can only attempt to provide certain examples on their bioavailability and metabolism, focusing mainly on some more widely studied representatives.

Probably one of the most well-known groups of compounds are ginsenosides (Fig. 14), the triterpene saponins isolated from the plants of the genus *Panax* from the Araliaceae family. Kong *et al.* used an LC-FTICR-MS platform to investigate the metabolic bioconversion of saponins from red ginseng in an *in vitro* GI simulation model. Under acidic (gastric) conditions, the main reactions taking place were deglycosylation (particularly in the C20 position) and hydration, while intestinal microbial metabolism mainly led to ginsenoside deglycosylation at the C3 position.[253] Kim *et al.*, following a very similar approach, assessed the metabolic fate of red and white ginseng saponins in a Caco-2 cell culture model coupled with oral, gastric, and small intestinal simulated digestion, and sample analyses were performed using an LC-IT-MS instrument. Based on the findings, the bioaccessibility of protopanaxatriol-type ginsenosides **(121)** in the early food phase was greater than that of the protopanaxadiol-type **(122)** and all the ginsenosides demonstrated poor absorption in the Caco-2 cell monolayers.[254] In 2015, He and coworkers performed a PK investigation of notoginsenoside Fc in plasma samples of male Sprague-Dawley rats receiving either 10, 25, or 50 mg kg^{-1} of the compound orally. Also, six rats were administrated a dose of 5 mg kg^{-1} intravenously. In parallel, metabolism investigation was carried out in animals receiving a dose of 100 mg kg^{-1}, whereby urine and stool samples were collected from 0 h to 36 h post-administration. The PK study was carried out using an LC-TQ-MS instrumentation, while for metabolite detection and annotation, an LC-QTOF-MS system was employed. The authors reported the detection of 10 metabolic products of notoginsenoside Fc, with deglycosylation being once more the main reaction taking place.[255]

Chen and coworkers examined the metabolic biotransformation of ginsenosides from a *P. notoginseng* hydroalcoholic extract, following fermentation with gut microbiota from a healthy male volunteer. Sample analysis by LC-DAD-QTOF-MS/MS disclosed the presence of 45 metabolites, with deglycosylation highlighted again as the major metabolic pathway,

while isomerization, hydration, dehydration, hydroxylation, oxidation, glycosylation, hydrogenation, dehydrogenation, acetylation, decarboxylation, methylation and dehydroxymethyl reactions were also detected to occur.[256] Zhang and colleagues aimed to investigate the PK parameters of ginsenosides through different routes of administration (IV and intramuscular – IM) of a TCM medicine, namely XueShuanTong, comprised of the lyophilized extract of *P. notoginseng* roots. For this purpose, an open-label study with healthy human volunteers receiving either a 1.5 h IV infusion of XueShuanTong at 150 mg per person or the same dosage dissolved in 4 mL of the 0.9% NaCl IM injection. Plasma and urine samples were collected at regular intervals over a 72 h period and analyzed by means of LC-QTRAP-MS. Based on the results, following both administration routes, the unchanged ginsenosides were the major circulating forms, while 20(*S*)-protopanaxadiol-type ginsenosides exhibited considerably longer terminal half-lives than the 20(*S*)-protopanaxatriol-type ginsenosides.[259]

The same year, Guo *et al.* utilized an *in vivo* male Sprague-Dawley rat model to examine the gut microbiota-mediated metabolic profiles of the same compound group. The animals received an oral dose of 1.535 g kg^{-1} of *P. notoginseng* saponin extract twice a day for 5 days and fecal samples were collected at different timepoints over 72 h and analyzed with an LC-QTOF-MS/MS platform. Remarkably, 73 saponin metabolites were detected in the normal control group, while only 11 were observed in the pseudo germ-free (GF) group, with hydrolysis and dehydration being the main metabolic reactions detected.[257] Finally, Fu *et al.* investigated the oral bioavailability and PK of *P. notoginseng* saponins in a Sprague-Dawley rat model, where the animals received either an oral dose of extract at 40 mg kg^{-1} or IV injection of 10 mg kg^{-1}. Plasma samples were collected at regular intervals and analyzed by LC-MS/MS with a validated method monitoring a total of 28 compounds. Among their findings, the authors noted the particularly low oral bioavailability of *P. notoginseng* saponins, and also that 20(*S*)-protopanaxadiol, 20(*S*)-protopanaxatriol, and ginsenoside CK were detected as the major metabolites *in vivo*.[258] Regarding saponin metabolism, Li *et al.* examined the PK and metabolism of pure α-hederin in an *in vivo* female Sprague-Dawley rat model. Rats were administered either a *per os* (10 mg kg^{-1}) or an IV dose (2 mg kg^{-1}) of the compound, and serial blood collections were performed to obtain the plasma samples. Then, the samples were analyzed by LC-MS/MS, and based on the results, apart from the poor oral bioavailability of the saponin, low systemic exposure of the aglycone (hederagenin) was also noted after IV administration. Finally, in plasma samples, the deglycosylated metabolite and its sulfate conjugate as well as hederagenin sulfate were detected.[260]

Another terpene group that has gained considerable attention is ginkgolides, the diterpenic lactones found in the leaves of *Ginkgo biloba* (Ginkgoaceae). In a study by Huang and coworkers, the effect of food and gender on the PK of specific ginkgo terpene lactones (bilobalide **(123)**, ginkgolide A **(124)**, B **(125)** and C **(126)** (Fig. 15)) was evaluated after their oral administration. In this case, both male and female Sprague-Dawley rats received a ginkgo terpene lactone extract (6 mg of extract per kg) and plasma samples were collected over 24 h and analyzed by LC-QTRAP-MS/MS with a validated method. Based on the findings, oral doses of the ginkgo extract should be lowered for fasted and female animals, compared to the fed and male subjects.[261] Wang *et al.* investigated the PK and tissue distribution of four ginkgolides after IV infusion in a Sprague-Dawley model. The animals were administered either a single dose of 1, 3 or 9 mg kg^{-1} of a ginkgo diterpene lactone meglumine injection or 3 mg kg^{-1} of the same formulation on seven consecutive days and plasma samples were analyzed with a validated method on an LC-tQ-MS/MS apparatus. The authors report that ginkgolides A, B and K **(127)** were mainly detected as hydrolyzed forms in the plasma, while also demonstrating an extensive tissue distribution, with the exception of brain samples.[262] Lastly, in a study by Cao *et al.*, a metabolomics approach was utilized to investigate trace-level ginkgo metabolites in intestinal mucosa samples of male Sprague-Dawley rats. The animals were intragastrically administered a *G. biloba* extract at a dose of 600 mg kg^{-1}, and plasma, brain, liver and intestinal mucosa samples were collected 3 h post-administration. For the sample analysis, an LC-TripleTOF-HRMS system was employed, while a "nontargeted diagnostic ion network analysis" (NINA) and a "chemicalome to metabolome matching approach" (CMMA) were used for metabolite detection and annotation. Also, the trace-level metabolites in

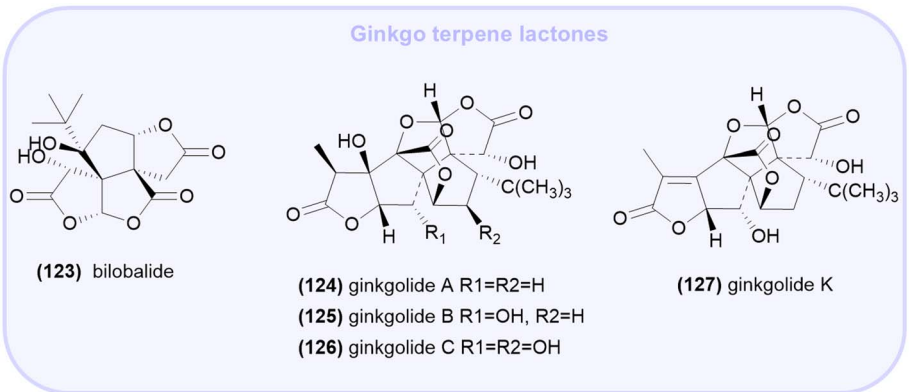

Fig. 15 Chemical structures of ginkgo terpene lactones (123)–(127).

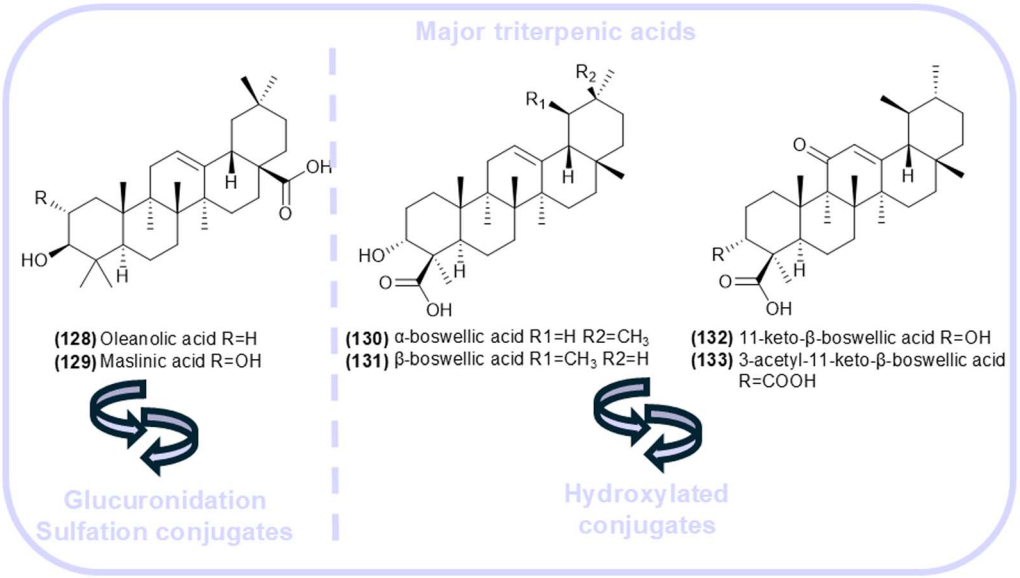

Fig. 16 Major triterpenic acids (oleanolic and maslinic acids) are mainly biotransformed to their glucuronidated and sulfated conjugates, while boswellic acids afford hydroxylated conjugates as their major metabolite porducts.[264–266]

plasma, liver and brain samples were further investigated using the "intestinal mucosal metabolites-guided detection" (IMMD) approach. By employing these workflows, the authors reported the detection of 39, 45 and 6 metabolites in plasma, liver and brain respectively, thus proposing an improved protocol to the DDA approach.[263]

Moving on to a different class of terpenes, namely triterpenic acids (Fig. 16), Pozo *et al.* examined the metabolic disposition of oleanolic acid **(128)** and maslinic acid **(129)**, two compounds found in high amounts in olive drupes and oil, in plasma and urine samples of human volunteers using an LC-tQ-MS system. To aid their effort, the authors proceeded to the synthesis of the metabolic products of these acids such as sulfates and glucuronides, which were later used as analytical standards. Based on the results, oleanolic acid appeared to be metabolized as both sulfate and glucuronide conjugates, whereas maslinic acid was mainly excreted in the form of glucuronide, with the highest amount of triterpenes being excreted between 2 and 4 h after olive oil consumption.[264]

Working with the same two triterpenic acids, de la Torre and coworkers reported the PK parameters of these two compounds based on the results of a randomized, controlled, dose–response study, involving the administration of olive oils with different terpene contents in a healthy adult population. Plasma and urine samples were analyzed by means of LC-MS/MS, and according to the results, the concentrations of both compounds in biological fluids increased in a dose-dependent manner and the bioavailability of maslinic acid appears to be greater to that of oleanolic acid.[266] Moreover, in a thorough study by Krüger and colleagues, the metabolism of an important group of triterpenic acids, namely boswellic acids (α-boswellic acid **(130)**, β-boswellic acid **(131)**) was investigated both in *in vitro* and *in vivo* models. In their *in vitro* study, the authors worked with both rat and human liver microsomes, as well as rat hepatocytes, while in the *in vivo* experiments, female Wistar rats were administered an oral dose of 12.5 mg kg^{-1} of either 11-keto-β-boswellic acid (KBA) **(132)** or 3-acetyl-11-keto-β-boswellic acid (AKBA) **(133)** and brain, liver and plasma samples were collected 2 h after administration. Analyses were carried out with an LC-MS/MS system, while identification was performed by direct comparison with available standards. Based on the results, oxidation to hydroxylated metabolites was found to be the major metabolic route, while *in vitro*, the metabolic profile of KBA was similar to that obtained from the *in vivo* experiments in plasma and liver, whereas no metabolites of AKBA could be identified in rat biospecimens.[265]

3.5 Glucosinolates and other sulfur-containing compounds

Glucosinolates **(134)** are sulfur-rich, anionic secondary metabolites found almost exclusively within the cruciferous plants such as those of the *Brassica* genus (Brassicaceae). They have gained considerable attention due to their health-promoting properties including antimicrobial, antioxidant and anti-inflammatory activities and cardiovascular, central nervous system and neuropathy protection.[267,268] Glucosinolates are the native forms found in plant tissues; however, upon tissue damage (*e.g.* cooking and mastication), a β-thioglucosidase enzyme, namely myrosinase, is released and triggers the conversion of glucosinolates to other metabolites, the most important of which are isothiocyanates (ITCs) **(135)** (Fig. 17). ITCs are highly reactive, and they have exhibited potent bioactivity both *in vitro* and *in vivo* as inhibitors of mitosis and stimulators of apoptosis in tumor cell lines, as antimicrobial agents against diverse pathogens, *etc.*[269,270] However, despite the increasing interest in this chemical class, bioavailability studies, particularly through human clinical trials remain scarce, possibly due to the unstable nature of these compounds,

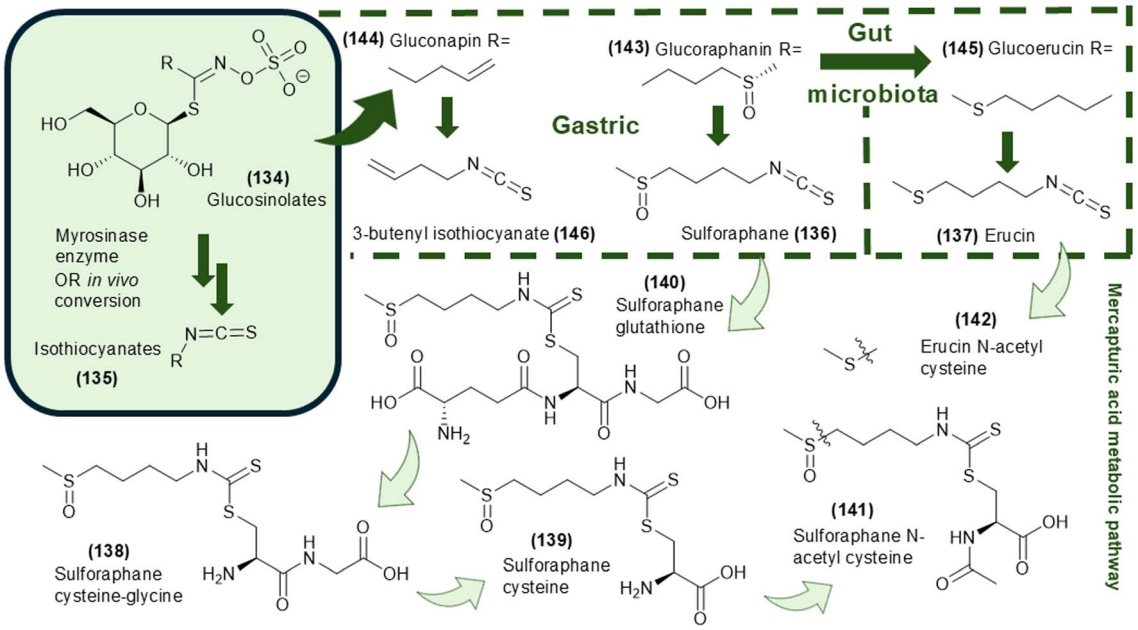

Fig. 17 Glucosinolates are converted through myrosinase enzyme or *in vivo* to afford isothiocyanates. In the gastric environment, gluconapin and glucoraphanin are biotransformed through hydrolysis to 3-butenyl isothiocyanate and sulforaphane, respectively. Sulforaphane enters the mercapturic acid metabolic pathway to afford more metabolites. Glucoraphanin can be further converted to glucoerucin through the gut microbiota, which can then be hydrolysed to erucin.[270,275,278]

which poses certain challenges from an analytical point of view. Nevertheless, for a more thorough overview of the bioavailability and metabolism of glucosinolates, readers are referred to the works by Barba *et al.*, Oliviero *et al.*, and Holst *et al.*[270–272] In 2005, Song *et al.* published a study on the quantitative analysis of glucosinolates, and their metabolic derivatives, in plasma samples of healthy volunteers, administered 200 g of raw broccoli, with an LC-MS/MS method. Glucosinolates were readily detected in negative mode, while ITCs and their amine degradation products required a previous derivatization step with ammonia and acetylation to produce thiourea and *N*-acetamides, respectively.[273] Clarke and coworkers investigated the bioavailability of two ITCs, namely sulforaphane **(136)** and erucin **(137)**, in healthy subjects receiving either broccoli sprouts or a commercially available broccoli supplement. Urine and plasma samples were collected at different timepoints and analyzed by means of LC-MS/MS. The authors reported a higher bioavailability of ITCs when volunteers consumed the broccoli sprouts compared to the nutraceutical, while sulforaphane and erucin were also observed to interconvert *in vivo*.[274] A similar observation was made by Saha *et al.* who performed a clinical study with healthy volunteers consuming broccoli soups produced from either fresh or frozen broccoli sprouts (100 g each). Plasma and urine samples were collected at regular intervals within a 48 h period and analyzed by LC-MS/MS. Several sulforaphane conjugates were detected in biofluids of participants such as sulforaphane-cysteine-glycine **(138)**, sulforaphane-cysteine **(139)**, sulforaphane-glutathione **(140)**, and sulforaphane-*N*-acetylcysteine **(141)**. Moreover, the authors reported the detection of an erucin *N*-acetyl-cysteine **(142)** conjugate in urine samples as well as that the gut microbiota could produce sulforaphane, erucin, and their nitriles from glucoraphanin **(143)**.[275] Dominguez-Perles and coworkers proposed an LC-tQ-MS/MS method for the accurate measurement of glucoraphanin, sulforaphane and their metabolites in human urine samples. Following its development and validation, the method was applied to urine samples of healthy subjects of both sexes who ingested 30 and 60 g of fresh broccoli sprouts. According to the findings, this method could serve as a sensitive and robust alternative for clinical trials involving glucosinolates and isothiocyanates.[276] Moreover, Hwang *et al.*, following a somewhat different approach, assessed the bioaccessibility and bioavailability of glucosinolates from whole kale in an *in vitro* digestion model. Gastric and intestinal digesta were analyzed with an LC-QTOF-MS/MS platform, while the transport of the detected glucosinolates and their products in the digesta was evaluated in Caco-2 cell monolayers differentiated into intestinal epithelial cells. After 2 h of gastric digestion, the concentrations of glucoraphanin, sinigrin, and gluconapin **(144)** decreased, and no glucoerucin **(145)** or glucobrassicin was detected, whereas a related increase in allyl nitrile, 3-butenyl isothiocyanate **(146)**, phenylacetonitrile, and sulforaphane was noted.[277]

In a work published by Charron *et al.*, the effect of BMI on the presence of glucosinolate in plasma and urine following the consumption of cooked broccoli was investigated. Healthy subjects were divided into high- and low-BMI groups and were instructed to consume 200 g of previously blanched and frozen broccoli, reheated to an acceptable temperature, providing a total of 147.6 μmol of glucoraphanin and 3.6 μmol of glucoerucin, respectively. Plasma and urine samples were collected at different timepoints on the last day of the trial and analyzed

on an LC-tQ-MS/MS platform, whereby several metabolites were measured. The presence of glucosinolate metabolites in the plasma and urine greatly increased in the high-BMI group compared to the low-BMI participants.[279] Finally, in a recent work by Zhu *et al.*, an LC-ESI(+)-MS/MS method was developed and validated for the accurate measurement of glucoraphanin and sulforaphane, along with its metabolic conjugates (glutathione, cysteine, and *N*-acetylcysteine) in diverse biological matrices, including digesta, cell culture medium, cell lysates (both from a Caco-2 cell monolayer culture) and urine samples, thus proposing an efficient method with potential applications in clinical trial measurements.[278]

Another group of sulfur-containing compounds are those found in the *Allium* species (Amaryllidaceae), a genus comprised of multiple edible species such as garlic, onion and leeks. *Allium* species are not only revered for their use in food preparations throughout the world, but also for their potent biological properties that have been known since antiquity.[280] For a comprehensive outline of the secondary metabolites of *Allium* and their bioavailability and metabolism, readers are referred to the review articles by Putnik *et al.*, Rahman *et al.*, and Deng *et al.*[280–282] Remarkably, despite the scientific interest in this group of compounds, studies examining their metabolic fate, particularly with the use of MS, remain extremely scarce. Probably the most comprehensive study was recently published by Moreno-Ortega *et al.*, whereby the bioavailability of organosulfur compounds following the intake of black garlic by healthy human volunteers of both sexes was investigated. The participants received a total of 20 g of black garlic, and their urine samples were collected at several timepoints within a 24 h time frame and analyzed on an LC-Orbitrap-HRMS platform. Overall, 33 organosulfur compounds were determined in the human urine, with methiin, isoalliin, *S*-(2-carboxypropyl)-L-cysteine and *S*-propyl-L-cysteine (deoxypropiin), accounting for 75.9% of the total organosulfur compounds excreted after the intake of black garlic.[283]

4. Future perspectives and conclusions

Looking forward, the future of NP metabolism research can be shaped by the integration of advanced MS techniques with powerful computational tools. Traditional identification methods that rely on the empirical knowledge of each analyst along with the existing substantial inter-laboratory variations leading to false quantification assessments due to the use of different HPLC methods, columns and MS instruments, are limiting factors regarding time consumption and quality of data obtained in many studies. Owing to these reasons, Ottaviani and colleagues suggest the use of validated LC-MS methods in combination with the use of authentic standards. Alternatively, Tian *et al.*, taking full advantage of data processing and data-mining technologies, propose a software-assisted LC-QTOF strategy for the identification of metabolites, incorporating as well shifting among three data acquisition modes, aiming to adjust, if needed, to any QTOF user. This methodology is an effort towards simplifying complicated metabolite elucidation processes, and at the same time aids in the construction of databases for large-scale metabolite screening.[284,285] Biological databases are essential for efficiently retrieving relevant information from the vast and ever-growing body of scientific literature. Rothwell *et al.* used the web database Phenol-Explorer to describe and visualize for the first time the polyphenol metabolome, important knowledge to understand *in vivo* bioactivity and search for biomarkers.[286]

Targeted metabolomics based on MS has emerged as one of the most powerful tools in nutrition research given that it enables the highly sensitive and specific quantification of metabolites that serve as direct indicators of food intake, while also providing valuable insights into the metabolic fingerprint and dietary patterns. Gonzalez-Dominguez *et al.* provided significant advancement in this field, with the development and validation of a quantitative targeted urinary nutrimetabolomic platform known as quantitative dietary fingerprinting (QDF), which enabled for the first time the simultaneous quantitation of 350 urinary metabolites. This method was later enlarged and modified to achieve the analysis of 450 plasma metabolites at the same time. This approach enables precise dietary assessment and its correlation with metabolic health, further strengthening the role of MS in personalized nutrition and public health research.[287,288] As these technologies continue to evolve, their integration with NP metabolism studies will drive innovation in drug discovery, systems biology, and precision medicine.

Despite their significant therapeutic potential, NPs are often overshadowed by their association with dietary supplements and functional foods. This widespread perception risks diminishing their credibility as serious contenders in drug development. As a result, many valuable bioactive compounds remain overlooked or underexploited, followed by a lack of rigorous PK studies, mechanistic insights, and clinical validation. Thus, to overcome the limitations of this "supplement" label and fully harness the pharmacological promise of NPs, a more structured and scientifically rigorous approach is essential.

Metabolism and PK are critical components of the preclinical phase of drug development, serving as key steps for advancing from basic to translational research of NPs, yet they remain unexplored areas requiring extensive further investigation. Particularly, in light of recent advancements in analytical methodologies, especially MS, now is most probably the right timing. The development and integration of powerful tools, most notably in the field of MS, have revolutionized our capacity to investigate complex biological matrices and trace the fate of NPs within the human body. These technological improvements now make it possible to not only detect NPs and their metabolites with remarkable sensitivity and specificity, but also identify and characterize them with a high degree of structural confidence. This is especially important considering that metabolites, rather than their parent compounds, are often the true bioactive entities circulating in organisms. Accordingly, without a clear understanding of their structures, it becomes difficult to fully comprehend the pharmacodynamics of NPs,

their mechanism of action, and ultimately their therapeutic potential. Accurate metabolite profiling is essential to bridge the gap between *in vitro* observations and real-world biological outcomes. Furthermore, the rising awareness of the crucial role of the gut microbiota in maintaining homeostasis, supporting immune function, and influencing disease development has brought renewed attention to biotransformation reactions. The interaction between NPs and the gut microbiota significantly affects their bioavailability, metabolism, and biological activity.[289–291] Interestingly, multi-compartment dynamic simulation systems of the GI tract including the human gut (*in vitro–ex vivo* systems) typically used in nutritional studies and from big pharma, are greatly missing from NPs.

Thus, understanding how microbial enzymes modify these compounds and how the resulting metabolites behave in the human system is critical for evaluating their pharmacological relevance. This perspective shifts the research focus toward a more integrative and system-level approach, where metabolic pathways, microbial contributions, and host responses are all considered essential components of the NP action mechanism.

In this context, holistic methodologies such as metabolomics emerge as ideal tools for studying the multifaceted interactions between NPs and human metabolism. Metabolomics, when combined with cutting-edge MS platforms, enables the comprehensive profiling of small molecules and provides insights into both endogenous metabolic responses and exogenous compound transformations. The unmatched sensitivity, resolution, and dynamic range of modern MS instruments make them indispensable in tracing metabolites across various biological systems. This powerful combination offers a pathway toward the more rational, evidence-based integration of NPs into modern pharmacology, potentially paving the way for the next generation of nature-derived therapeutics. Embracing these comprehensive strategies will not only enhance our understanding of NPs but also contribute to closing the translational gap that has long hindered their development into approved and effective drugs.

5. Author contributions

Conceptualization is attributed to MH. EVM and AB participated in writing the original draft, and all authors contributed to review and editing.

6. Conflicts of 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.

7. Data availability

No primary research results, software or code have been included, and no new data were generated or analyzed as part of this review.

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9. References

1 D. J. Newman and G. M. Cragg, Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019, *J. Nat. Prod.*, 2020, **83**(3), 770–803, DOI: **10.1021/acs.jnatprod.9b01285**.

2 A. G. Atanasov, S. B. Zotchev, V. M. Dirsch, *et al.*, Natural products in drug discovery: advances and opportunities, *Nat. Rev. Drug Discovery*, 2021, **20**(3), 200–216, DOI: **10.1038/s41573-020-00114-z**.

3 A. G. Atanasov, B. Waltenberger, E.-M. Pferschy-Wenzig, *et al.*, Discovery and resupply of pharmacologically active plant-derived natural products: A review, *Biotechnol. Adv.*, 2015, **33**(8), 1582–1614, DOI: **10.1016/j.biotechadv.2015.08.001**.

4 D. A. Dias, S. Urban and U. Roessner, A Historical overview of natural products in drug discovery, *Metabolites*, 2012, **2**(2), 303–336, DOI: **10.3390/metabo2020303**.

5 J. W.-H. Li and J. C. Vederas, Drug Discovery and Natural Products: End of an Era or an Endless Frontier?, *Science*, 2009, **325**(5937), 161–165, DOI: **10.1126/science.1168243**.

6 Y. Zhao, X. Zhong, J. Yan, C. Sun, X. Zhao and X. Wang, Potential roles of gut microbes in biotransformation of natural products: An overview, *Front. Microbiol.*, 2022, **13**, 956378, DOI: **10.3389/fmicb.2022.956378**.

7 R. P. Dash and M. Nivsarkar, in *Pharmacokinetics of Phytopharmaceuticals: A Peek into Contingencies and Impediments in Herbal Drug Development BT – Medicinal Plants – Recent Advances in Research and Development*, ed. H.-S. Tsay, L.-F. Shyur, D. C. Agrawal, Y.-C. Wu and S.-Y. Wang, Springer, Singapore, 2016, pp. 297–308, DOI: **10.1007/978-981-10-1085-9_12**.

8 C. J. Paller, S. R. Denmeade and M. A. Carducci, Challenges of conducting clinical trials of natural products to combat cancer, *Clin. Adv. Hematol. Oncol.*, 2016, **14**(6), 447–455.

9 A. Fedi, C. Vitale, G. Ponschin, S. Ayehunie, M. Fato and S. Scaglione, In vitro models replicating the human intestinal epithelium for absorption and metabolism studies: A systematic review, *J. Controlled Release*, 2021, **335**, 247–268, DOI: **10.1016/j.jconrel.2021.05.028**.

10 A. Hevia, P. Ruas-Madiedo, M. A. Faria, *et al.*, A Shared Perspective on in Vitro and in Vivo Models to Assay Intestinal Transepithelial Transport of Food Compounds, *J. Agric. Food Chem.*, 2023, **71**(49), 19265–19276, DOI: **10.1021/acs.jafc.3c05479**.

11 M. J. Motilva, A. Serra and L. Rubió, Nutrikinetic studies of food bioactive compounds: From in vitro to in vivo approaches, *Int. J. Food Sci. Nutr.*, 2015, **66**, S41–S52, DOI: **10.3109/09637486.2015.1025721**.

12 J. L. Wolfender, M. Litaudon, D. Touboul and E. F. Queiroz, Innovative omics-based approaches for prioritisation and

targeted isolation of natural products-new strategies for drug discovery, *Nat. Prod. Rep.*, 2019, **36**(6), 855–868, DOI: 10.1039/c9np00004f.

13 J. L. Wolfender, J. M. Nuzillard, J. J. J. Van Der Hooft, J. H. Renault and S. Bertrand, Accelerating Metabolite Identification in Natural Product Research: Toward an Ideal Combination of Liquid Chromatography-High-Resolution Tandem Mass Spectrometry and NMR Profiling, in Silico Databases, and Chemometrics, *Anal. Chem.*, 2019, **91**(1), 704–742, DOI: 10.1021/acs.analchem.8b05112.

14 G. Alvarez-Rivera, D. Ballesteros-Vivas, F. Parada-Alfonso, E. Ibañez and A. Cifuentes, Recent applications of high resolution mass spectrometry for the characterization of plant natural products, *TrAC, Trends Anal. Chem.*, 2019, **112**, 87–101, DOI: 10.1016/j.trac.2019.01.002.

15 A. Bouslimani, L. M. Sanchez, N. Garg and P. C. Dorrestein, Mass spectrometry of natural products: Current, emerging and future technologies, *Nat. Prod. Rep.*, 2014, **31**(6), 718–729, DOI: 10.1039/c4np00044g.

16 V. Gabelica and E. Marklund, Fundamentals of ion mobility spectrometry, *Curr. Opin. Chem. Biol.*, 2018, **42**, 51–59, DOI: 10.1016/j.cbpa.2017.10.022.

17 Food and Drug Administration (FDA), https://www.ecfr.gov/current/title-21/chapter-I/subchapter-D/part-314/subpart-A/section-314.3.

18 C. Cardoso, C. Afonso, H. Lourenço, S. Costa and M. L. Nunes, Bioaccessibility assessment methodologies and their consequences for the risk-benefit evaluation of food, *Trends Food Sci. Technol.*, 2015, **41**(1), 5–23, DOI: 10.1016/j.tifs.2014.08.008.

19 V. Kantae, E. H. J. Krekels, M. J. V. Esdonk, *et al.*, Integration of pharmacometabolomics with pharmacokinetics and pharmacodynamics: towards personalized drug therapy, *Metabolomics*, 2016, **13**(1), 9, DOI: 10.1007/s11306-016-1143-1.

20 W. Kielbasa and D. L. Helton, A new era for migraine: Pharmacokinetic and pharmacodynamic insights into monoclonal antibodies with a focus on galcanezumab, an anti-CGRP antibody, *Cephalalgia*, 2019, **39**(10), 1284–1297, DOI: 10.1177/0333102419840780.

21 IUPAC, *Glossary of terms used in medicinal chemistry*, 1998.

22 R. J. Bino, R. D. Hall, O. Fiehn, *et al.*, Potential of metabolomics as a functional genomics tool, *Trends Plant Sci.*, 2004, **9**(9), 418–425, DOI: 10.1016/j.tplants.2004.07.004.

23 T. Cajka and O. Fiehn, Toward Merging Untargeted and Targeted Methods in Mass Spectrometry-Based Metabolomics and Lipidomics, *Anal. Chem.*, 2016, **88**(1), 524–545, DOI: 10.1021/acs.analchem.5b04491.

24 L. Yi, S. Ma and D. Ren, *Phytochemistry and Bioactivity of Citrus Flavonoids: A Focus on Antioxidant, Anti-Inflammatory, Anticancer and Cardiovascular Protection Activities*, Springer, Netherlands, 2017, vol. 16, DOI: 10.1007/s11101-017-9497-1.

25 M. Zhang, S. Zhu, W. Yang, Q. Huang and C. T. Ho, The biological fate and bioefficacy of citrus flavonoids: Bioavailability, biotransformation, and delivery systems, *Food Funct.*, 2021, **12**(8), 3307–3323, DOI: 10.1039/d0fo03403g.

26 R. Visvanathan and G. Williamson, Review of factors affecting citrus polyphenol bioavailability and their importance in designing in vitro, animal, and intervention studies, *Compr. Rev. Food Sci. Food Saf.*, 2022, **21**(6), 4509–4545, DOI: 10.1111/1541-4337.13057.

27 A. Karn, C. Zhao, F. Yang, *et al.*, In-vivo biotransformation of citrus functional components and their effects on health, *Crit. Rev. Food Sci. Nutr.*, 2021, **61**(5), 756–776, DOI: 10.1080/10408398.2020.1746234.

28 G. Pereira-Caro, I. A. Ludwig, T. Polyviou, *et al.*, 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**(28), 5724–5735, DOI: 10.1021/acs.jafc.6b02088.

29 G. Pereira-Caro, G. Borges, I. Ky, *et al.*, In vitro colonic catabolism of orange juice (poly)phenols, *Mol. Nutr. Food Res.*, 2015, **59**(3), 465–475, DOI: 10.1002/mnfr.201400779.

30 X. Guo, K. Li, A. Guo and E. Li, Intestinal absorption and distribution of naringin, hesperidin, and their metabolites in mice, *J. Funct. Foods*, 2020, **74**, 104158, DOI: 10.1016/j.jff.2020.104158.

31 X. Zeng, H. Yao, Y. Zheng, *et al.*, Tissue distribution of naringin and derived metabolites in rats after a single oral administration, *J. Chromatogr. B:Anal. Technol. Biomed. Life Sci.*, 2020, **1136**(135), 121846, DOI: 10.1016/j.jchromb.2019.121846.

32 Q. Jiao, L. Xu, L. Jiang, Y. Jiang, J. Zhang and B. Liu, Metabolism study of hesperetin and hesperidin in rats by UHPLC-LTQ-Orbitrap MS n, *Xenobiotica*, 2020, **50**(11), 1311–1322, DOI: 10.1080/00498254.2019.1567956.

33 J. A. Manthey, T. B. Cesar, E. Jackson and S. Mertens-Talcott, Pharmacokinetic study of nobiletin and tangeretin in rat serum by high-performance liquid chromatography-electrospray ionization-mass spectrometry, *J. Agric. Food Chem.*, 2011, **59**(1), 145–151, DOI: 10.1021/jf1033224.

34 P. S. Ferreira, J. A. Manthey, M. S. Nery and T. B. Cesar, Pharmacokinetics and Biodistribution of Eriocitrin in Rats, *J. Agric. Food Chem.*, 2021, **69**(6), 1796–1805, DOI: 10.1021/acs.jafc.0c04553.

35 Y. Bai, W. Peng, C. Yang, *et al.*, Pharmacokinetics and Metabolism of Naringin and Active Metabolite Naringenin in Rats, Dogs, Humans, and the Differences Between Species, *Front. Pharmacol*, 2020, **11**, 1–16, DOI: 10.3389/fphar.2020.00364.

36 L. N. Fraga, C. P. Coutinho, A. C. Rozenbaum, E. D. C. Tobaruela, F. M. Lajolo and N. M. A. Hassimotto, Blood pressure and body fat % reduction is mainly related to flavanone phase II conjugates and minor extension by phenolic acid after long-term intake of orange juice, *Food Funct.*, 2021, **12**(22), 11278–11289, DOI: 10.1039/d1fo02664j.

37 A. Nishioka, E. de C. Tobaruela, L. N. Fraga, F. A. Tomás-Barberán, F. M. Lajolo and N. M. A. Hassimotto, Stratification of volunteers according to flavanone metabolite excretion and phase ii metabolism profile after single doses of 'pera' orange and 'moro' blood orange juices, *Nutrients*, 2021, **13**(2), 1–21, DOI: **10.3390/nu13020473**.

38 M. Á. Ávila-gálvez, J. A. Giménez-bastida, A. González-sarrías and J. C. Espín, New insights into the metabolism of the flavanones eriocitrin and hesperidin: A comparative human pharmacokinetic study, *Antioxidants*, 2021, **10**(3), 1–20, DOI: **10.3390/antiox10030435**.

39 F. Castello, M. S. Fernández-Pachón, I. Cerrillo, *et al.*, Absorption, metabolism, and excretion of orange juice (poly)phenols in humans: The effect of a controlled alcoholic fermentation, *Arch. Biochem. Biophys.*, 2020, **695**, 108627, DOI: **10.1016/j.abb.2020.108627**.

40 W. Mullen, M. A. Archeveque, C. A. Edwards, H. Matsumoto and A. Crozier, Bioavailability and metabolism of orange juice flavanones in humans: Impact of a full-fat yogurt, *J. Agric. Food Chem.*, 2008, **56**(23), 11157–11164, DOI: **10.1021/jf801974v**.

41 V. Spigoni, P. Mena, F. Fantuzzi, *et al.*, Bioavailability of bergamot (Citrus bergamia) flavanones and biological activity of their circulating metabolites in human pro-angiogenic cells, *Nutrients*, 2017, **9**(12), 1328, DOI: **10.3390/nu9121328**.

42 M. Tomás-Navarro, J. L. Navarro, F. Vallejo and F. A. Tomás-Barberán, Novel Urinary Biomarkers of Orange Juice Consumption, Interindividual Variability, and Differences with Processing Methods, *J. Agric. Food Chem.*, 2021, **69**(13), 4006–4017, DOI: **10.1021/acs.jafc.0c08144**.

43 R. R. Kotha and D. L. Luthria, Curcumin: Biological, Pharmaceutical, Nutraceutical, and Analytical Aspects, *Molecules*, 2019, **24**(16), 2930, DOI: **10.3390/molecules24162930**.

44 B. Scazzocchio, L. Minghetti and M. D'Archivio, Interaction between Gut Microbiota and Curcumin: A New Key of Understanding for the Health Effects of Curcumin, *Nutrients*, 2020, **12**(9), 2499, DOI: **10.3390/nu12092499**.

45 W. W. Quitschke, in *Bioavailability and Metabolism of Curcuminoids BT – Natural compounds as inducers of cell death: volume 1*, ed. M. Diederich and K. Noworyta, Springer, Netherlands, 2012, pp. 95–124, DOI: **10.1007/978-94-007-4575-9_5**.

46 K. M. Nelson, J. L. Dahlin, J. Bisson, J. Graham, G. F. Pauli and M. A. Walters, The Essential Medicinal Chemistry of Curcumin, *J. Med. Chem.*, 2017, **60**(5), 1620–1637, DOI: **10.1021/acs.jmedchem.6b00975**.

47 P. Fança-Berthon, M. Tenon, S. L. Bouter-Banon, *et al.*, Pharmacokinetics of a Single Dose of Turmeric Curcuminoids Depends on Formulation: Results of a Human Crossover Study, *J. Nutr.*, 2021, **151**(7), 1802–1816, DOI: **10.1093/jn/nxab087**.

48 Y. Cao, R. X. Xu and Z. Liu, A high-throughput quantification method of curcuminoids and curcumin metabolites in human plasma via high-performance liquid chromatography/tandem mass spectrometry, *J. Chromatogr. B:Anal. Technol. Biomed. Life Sci.*, 2014, **949–950**, 70–78, DOI: **10.1016/j.jchromb.2013.12.039**.

49 P. Ramalingam and Y. T. Ko, A validated LC-MS/MS method for quantitative analysis of curcumin in mouse plasma and brain tissue and its application in pharmacokinetic and brain distribution studies, *J. Chromatogr. B:Anal. Technol. Biomed. Life Sci.*, 2014, **969**, 101–108, DOI: **10.1016/j.jchromb.2014.08.009**.

50 S. Tan, L. Calani, L. Bresciani, *et al.*, The degradation of curcuminoids in a human faecal fermentation model, *Int. J. Food Sci. Nutr.*, 2015, **66**(7), 790–796, DOI: **10.3109/09637486.2015.1095865**.

51 S. Gopi, J. Jacob, K. Varma, *et al.*, Comparative Oral Absorption of Curcumin in a Natural Turmeric Matrix with Two Other Curcumin Formulations: An Open-label Parallel-arm Study, *Phytother Res.*, 2017, **31**(12), 1883–1891, DOI: **10.1002/ptr.5931**.

52 M. A. G. M. Kroon, H. W. M. van Laarhoven, E. L. Swart, E. M. Kemper and O. van Tellingen, A validated HPLC-MS/MS method for simultaneously analyzing curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and piperine in human plasma, urine or feces, *Heliyon*, 2023, **9**(5), e15540, DOI: **10.1016/j.heliyon.2023.e15540**.

53 T. El Khawand, A. Courtois, J. Valls, T. Richard and S. Krisa, A review of dietary stilbenes: sources and bioavailability, *Phytochem. Rev.*, 2018, **17**(5), 1007–1029, DOI: **10.1007/s11101-018-9578-9**.

54 J. Gambini, M. Inglés, G. Olaso, *et al.*, Properties of Resveratrol: In Vitro and In Vivo Studies about Metabolism, Bioavailability, and Biological Effects in Animal Models and Humans, *Oxid. Med. Cell. Longevity*, 2015, **2015**(1), 837042, DOI: **10.1155/2015/837042**.

55 J. B. Baell, Feeling Nature's PAINS: Natural Products, Natural Product Drugs, and Pan Assay Interference Compounds (PAINS), *J. Nat. Prod.*, 2016, **79**(3), 616–628, DOI: **10.1021/acs.jnatprod.5b00947**.

56 G. J. Soleas, E. P. Diamandis and D. M. Goldberg, in *The World of Resveratrol BT – Nutrition and Cancer Prevention: New Insights into the Role of Phytochemicals*, Springer US, 2001, pp. 159–182, DOI: **10.1007/978-1-4615-1283-7_13**.

57 M. Rotches-Ribalta, M. Urpi-Sarda, R. Llorach, *et al.*, Gut and microbial resveratrol metabolite profiling after moderate long-term consumption of red wine versus dealcoholized red wine in humans by an optimized ultra-high-pressure liquid chromatography tandem mass spectrometry method, *J. Chromatogr. A*, 2012, **1265**, 105–113, DOI: **10.1016/j.chroma.2012.09.093**.

58 L. Svilar, J. C. Martin, C. Defoort, C. Paut, F. Tourniaire and A. Brochot, Quantification of trans-resveratrol and its metabolites in human plasma using ultra-high performance liquid chromatography tandem quadrupole-orbitrap mass spectrometry, *J. Chromatogr. B:Anal. Technol. Biomed. Life Sci.*, 2019, **1104**, 119–129, DOI: **10.1016/j.jchromb.2018.11.016**.

59 T. Walle, F. Hsieh, M. H. DeLegge, J. E. Oatis and U. K. Walle, High absorption but very low bioavailability of oral resveratrol in humans, *Drug Metab. Dispos.*, 2004, **32**(12), 1377–1382, DOI: **10.1124/dmd.104.000885**.

60 M. Azorín-Ortuno, M. J. Yanéz-Gascón, F. J. Pallarés, *et al.*, Pharmacokinetic study of trans-resveratrol in adult pigs, *J. Agric. Food Chem.*, 2010, **58**(20), 11165–11171, DOI: **10.1021/jf102799m**.

61 M. Azorín-Ortuño, M. J. Yáñez-Gascón, F. Vallejo, *et al.*, Metabolites and tissue distribution of resveratrol in the pig, *Mol. Nutr. Food Res.*, 2011, **55**(8), 1154–1168, DOI: **10.1002/mnfr.201100140**.

62 M. Rotches-Ribalta, C. Andres-Lacueva, R. Estruch, E. Escribano and M. Urpi-Sarda, Pharmacokinetics of resveratrol metabolic profile in healthy humans after moderate consumption of red wine and grape extract tablets, *Pharmacol. Res.*, 2012, **66**(5), 375–382, DOI: **10.1016/j.phrs.2012.08.001**.

63 O. F. Iwuchukwu, S. Sharan, D. J. Canney and S. Nagar, Analytical method development for synthesized conjugated metabolites of trans-resveratrol, and application to pharmacokinetic studies, *J. Pharm. Biomed. Anal.*, 2012, **63**, 1–8, DOI: **10.1016/j.jpba.2011.12.006**.

64 M. Azzolini, M. La Spina, A. Mattarei, C. Paradisi, M. Zoratti and L. Biasutto, Pharmacokinetics and tissue distribution of pterostilbene in the rat, *Mol. Nutr. Food Res.*, 2014, **58**(11), 2122–2132, DOI: **10.1002/mnfr.201400244**.

65 I. Murakami, R. Chaleckis, T. Pluskal, *et al.*, Metabolism of skin-absorbed resveratrol into its glucuronized form in mouse skin, *PLoS One*, 2014, **9**(12), 1–20, DOI: **10.1371/journal.pone.0115359**.

66 S. de Pascual-Teresa and M. T. Sanchez-Ballesta, Anthocyanins: from plant to health, *Phytochem. Rev.*, 2008, **7**(2), 281–299, DOI: **10.1007/s11101-007-9074-0**.

67 Y. Wu, T. Han, H. Yang, L. Lyu, W. Li and W. Wu, Known and potential health benefits and mechanisms of blueberry anthocyanins: A review, *Food Biosci.*, 2023, **55**, 103050, DOI: **10.1016/j.fbio.2023.103050**.

68 A. Smeriglio, D. Barreca, E. Bellocco and D. Trombetta, Chemistry, Pharmacology and Health Benefits of Anthocyanins, *Phytother Res.*, 2016, **30**(8), 1265–1286, DOI: **10.1002/ptr.5642**.

69 J. M. Alvarez-Suarez, C. Cuadrado, I. B. Redondo, F. Giampieri, A. M. González-Paramás and C. Santos-Buelga, Novel approaches in anthocyanin research - Plant fortification and bioavailability issues, *Trends Food Sci. Technol.*, 2021, **117**, 92–105, DOI: **10.1016/j.tifs.2021.01.049**.

70 T. H. Hahm, M. Tanaka and T. Matsui, Current Knowledge on Intestinal Absorption of Anthocyanins, *J. Agric. Food Chem.*, 2022, **70**(8), 2501–2509, DOI: **10.1021/acs.jafc.1c08207**.

71 M. A. Lila, B. Burton-Freeman, M. Grace and W. Kalt, Unraveling Anthocyanin Bioavailability for Human Health, *Annu. Rev. Food Sci. Technol.*, 2016, **7**, 375–393, DOI: **10.1146/annurev-food-041715-033346**.

72 C. D. Kay, G. Pereira-Caro, I. A. Ludwig, M. N. Clifford and A. Crozier, Anthocyanins and Flavanones Are More Bioavailable than Previously Perceived: A Review of Recent Evidence, *Annu. Rev. Food Sci. Technol.*, 2017, **8**, 155–180, DOI: **10.1146/annurev-food-030216-025636**.

73 D. Mueller, K. Jung, M. Winter, D. Rogoll, R. Melcher and E. Richling, Human intervention study to investigate the intestinal accessibility and bioavailability of anthocyanins from bilberries, *Food Chem.*, 2017, **231**, 275–286, DOI: **10.1016/j.foodchem.2017.03.130**.

74 C. Felgines, S. Talavéra, M. P. Gonthier, *et al.*, Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans, *J. Nutr.*, 2003, **133**(5), 1296–1301, DOI: **10.1093/jn/133.5.1296**.

75 S. Talavéra, C. Felgines, O. Texier, *et al.*, Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain, *J. Agric. Food Chem.*, 2005, **53**(10), 3902–3908, DOI: **10.1021/jf050145v**.

76 T. H. Marczylo, D. Cooke, K. Brown, W. P. Steward and A. J. Gescher, Pharmacokinetics and metabolism of the putative cancer chemopreventive agent cyanidin-3-glucoside in mice, *Cancer Chemother. Pharmacol.*, 2009, **64**(6), 1261–1268, DOI: **10.1007/s00280-009-0996-7**.

77 A. Vanzo, U. Vrhovsek, F. Tramer, F. Mattivi and S. Passamonti, Exceptionally fast uptake and metabolism of cyanidin 3-glucoside by rat kidneys and liver, *J. Nat. Prod.*, 2011, **74**(5), 1049–1054, DOI: **10.1021/np100948a**.

78 T. Ichiyanagi, Y. Kashiwada, Y. Shida, *et al.*, Structural elucidation and biological fate of two glucuronyl metabolites of pelargonidin 3-O β-d-glucopyranoside in rats, *J. Agric. Food Chem.*, 2013, **61**(3), 569–578, DOI: **10.1021/jf3032793**.

79 W. Kalt, Y. Liu, J. E. McDonald and M. R. Vinqvist-Tymchuk, Fillmore SAE. Anthocyanin metabolites are abundant and persistent in human urine, *J. Agric. Food Chem.*, 2014, **62**(18), 3926–3934, DOI: **10.1021/jf500107j**.

80 W. Kalt, J. E. McDonald, M. R. Vinqvist-Tymchuk, Y. Liu and S. A. E. Fillmore, Human anthocyanin bioavailability: effect of intake duration and dosing, *Food Funct.*, 2017, **8**(12), 4563–4569, DOI: **10.1039/C7FO01074E**.

81 W. Kalt, J. E. McDonald, Y. Liu and S. A. E. Fillmore, Flavonoid Metabolites in Human Urine during Blueberry Anthocyanin Intake, *J. Agric. Food Chem.*, 2017, **65**(8), 1582–1591, DOI: **10.1021/acs.jafc.6b05455**.

82 K. Kamonpatana, M. L. Failla, P. S. Kumar and M. M. Giusti, Anthocyanin structure determines susceptibility to microbial degradation and bioavailability to the buccal mucosa, *J. Agric. Food Chem.*, 2014, **62**(29), 6903–6910, DOI: **10.1021/jf405180k**.

83 I. A. Ludwig, P. Mena, L. Calani, *et al.*, New insights into the bioavailability of red raspberry anthocyanins and ellagitannins, *Free Radical Biol. Med.*, 2015, **89**, 758–769, DOI: **10.1016/j.freeradbiomed.2015.10.400**.

84 L. Xie, S. G. Lee, T. M. Vance, *et al.*, Bioavailability of anthocyanins and colonic polyphenol metabolites following consumption of aronia berry extract, *Food Chem.*, 2016, **211**, 860–868, DOI: **10.1016/j.foodchem.2016.05.122**.

85 S. Zhong, A. Sandhu, I. Edirisinghe and B. Burton-Freeman, Characterization of Wild Blueberry Polyphenols Bioavailability and Kinetic Profile in Plasma over 24-h Period in Human Subjects, *Mol. Nutr. Food Res.*, 2017, **61**(12), 1–13, DOI: **10.1002/mnfr.201700405**.

86 H. Han, C. Liu, W. Gao, *et al.*, Anthocyanins Are Converted into Anthocyanidins and Phenolic Acids and Effectively Absorbed in the Jejunum and Ileum, *J. Agric. Food Chem.*, 2021, **69**(3), 992–1002, DOI: **10.1021/acs.jafc.0c07771**.

87 C. I. Victoria-Campos, J. d. J. Ornelas-Paz, N. E. Rocha-Guzmán, *et al.*, Gastrointestinal metabolism and bioaccessibility of selected anthocyanins isolated from commonly consumed fruits, *Food Chem.*, 2022, **383**, 132451, DOI: **10.1016/j.foodchem.2022.132451**.

88 Y. T. Chan, J. Huang, H. C. Wong, J. Li and D. Zhao, Metabolic fate of black raspberry polyphenols in association with gut microbiota of different origins in vitro, *Food Chem.*, 2023, **404**(PB), 134644, DOI: **10.1016/j.foodchem.2022.134644**.

89 M. S. Swallah, X. Yang, J. Li, *et al.*, The Pros and Cons of Soybean Bioactive Compounds: An Overview, *Food Rev. Int.*, 2023, **39**(8), 5104–5131, DOI: **10.1080/87559129.2022.2062763**.

90 M. Axelson, D. N. Kirk, R. D. Farrant, G. Cooley, A. M. Lawson and K. D. R. Setchell, The identification of the weak oestrogen equol [7-hydroxy-3-(4′-hydroxyphenyl) chroman] in human urine, *Biochem. J.*, 1982, **201**(2), 353.

91 J. Lv, S. Jin, Y. Zhang, Y. Zhou, M. Li and N. Feng, Equol: a metabolite of gut microbiota with potential antitumor effects, *Gut Pathog.*, 2024, **16**(1), 35, DOI: **10.1186/s13099-024-00625-9**.

92 J. M. Sánchez-Calvo, M. A. Rodríguez-Iglesias, J. M. G. Molinillo and F. A. Macías, Soy isoflavones and their relationship with microflora: beneficial effects on human health in equol producers, *Phytochem. Rev.*, 2013, **12**(4), 979–1000, DOI: **10.1007/s11101-013-9329-x**.

93 E. Koh and A. E. Mitchell, Characterization of urinary isoflavone metabolites excreted after the consumption of soy flour or soybean paste using lc-(esi)ms/ms, *J. Food Biochem.*, 2011, **35**(5), 1474–1485, DOI: **10.1111/j.1745-4514.2010.00469.x**.

94 T. Larkin, W. E. Price and L. Astheimer, The Key Importance of Soy Isoflavone Bioavailability to Understanding Health Benefits, *Crit. Rev. Food Sci. Nutr.*, 2008, **48**(6), 538–552, DOI: **10.1080/10408390701542716**.

95 S. A. Aboushanab, S. M. Khedr, I. F. Gette, *et al.*, Isoflavones derived from plant raw materials: bioavailability, anti-cancer, anti-aging potentials, and microbiome modulation, *Crit. Rev. Food Sci. Nutr.*, 2022, **63**(2), 261–287, DOI: **10.1080/10408398.2021.1946006**.

96 N. Fang, S. Yu and T. M. Badger, Characterization of isoflavones and their conjugates in female rat urine using LC/MS/MS, *J. Agric. Food Chem.*, 2002, **50**(9), 2700–2707, DOI: **10.1021/jf011384v**.

97 S. M. Heinonen, K. Wähälä, K. H. Liukkonen, A. M. Aura, K. Poutanen and H. Adlercreutz, Studies of the In Vitro Intestinal Metabolism of Isoflavones Aid in the Identification of Their Urinary Metabolites, *J. Agric. Food Chem.*, 2004, **52**(9), 2640–2646, DOI: **10.1021/jf030681s**.

98 H. Wiseman, K. Casey, E. A. Bowey, *et al.*, Influence of 10 wk of soy consumption on plasma concentrations and excretion of isoflavonoids and on gut microflora metabolism in healthy adults1234, *Am. J. Clin. Nutr.*, 2004, **80**(3), 692–699, DOI: **10.1093/ajcn/80.3.692**.

99 L. Chen, X. Zhao, L. Fang and D. E. Games, Quantitative determination of acetyl glucoside isoflavones and their metabolites in human urine using combined liquid chromatography-mass spectrometry, *J. Chromatogr. A*, 2007, **1154**(1–2), 103–110, DOI: **10.1016/j.chroma.2007.03.010**.

100 H. H. Jang, H. Noh, H. W. Kim, *et al.*, Metabolic tracking of isoflavones in soybean products and biosamples from healthy adults after fermented soybean consumption, *Food Chem.*, 2020, **330**, 127317, DOI: **10.1016/j.foodchem.2020.127317**.

101 K. D. R. Setchell, M. S. Faughnan, T. Avades, *et al.*, Comparing the pharmacokinetics of daidzein and genistein with the use of 13C-labeled tracers in premenopausal women, *Am. J. Clin. Nutr.*, 2003, **77**(2), 411–419, DOI: **10.1093/ajcn/77.2.411**.

102 K. D. R. Setchell, C. Clerici, E. D. Lephart, *et al.*, S-equol, a potent ligand for estrogen receptor β, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora, *Am. J. Clin. Nutr.*, 2005, **81**(5), 1072–1079, DOI: **10.1093/ajcn/81.5.1072**.

103 C. E. Rüfer, A. Bub, J. Möseneder, P. Winterhalter, M. Stürtz and S. E. Kulling, Pharmacokinetics of the soybean isoflavone daidzein in its aglycone and glucoside form: A randomized, double-blind, crossover study, *Am. J. Clin. Nutr.*, 2008, **87**(5), 1314–1323, DOI: **10.1093/ajcn/87.5.1314**.

104 Z. Yang, W. Zhu, S. Gao, *et al.*, Simultaneous determination of genistein and its four phase II metabolites in blood by a sensitive and robust UPLC-MS/MS method: Application to an oral bioavailability study of genistein in mice, *J. Pharm. Biomed. Anal.*, 2010, **53**(1), 81–89, DOI: **10.1016/j.jpba.2010.03.011**.

105 D. H. Lee, M. J. Kim, E. J. Song, *et al.*, Nutrikinetic study of genistein metabolites in ovariectomized mice, *PLoS One*, 2017, **12**(10), 1–12, DOI: **10.1371/journal.pone.0186320**.

106 S. Wang, T. Gong, J. Lu, Y. Kano and D. Yuan, Simultaneous determination of tectorigenin and its metabolites in rat plasma by ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry, *J. Chromatogr. B:Anal. Technol. Biomed. Life Sci.*, 2013, **933**, 50–58, DOI: **10.1016/j.jchromb.2013.06.009**.

107 Z. Liu, J.-P. Vincken and W. J. C. de Bruijn, Tea phenolics as prebiotics, *Trends Food Sci. Technol.*, 2022, **127**, 156–168, DOI: **10.1016/j.tifs.2022.06.007**.

108 P. G. Di, P. Mena, L. Bresciani, *et al.*, Revisiting the bioavailability of flavan-3-ols in humans: A systematic review and comprehensive data analysis, *Mol. Aspects Med.*, 2023, **89**, 101146, DOI: **10.1016/j.mam.2022.101146**.

109 A. Rana, M. Samtiya, T. Dhewa, V. Mishra and R. E. Aluko, Health benefits of polyphenols: A concise review, *J. Food Biochem.*, 2022, **46**(10), e14264, DOI: **10.1111/jfbc.14264**.

110 P. LIczbiński and B. Bukowska, Tea and coffee polyphenols and their biological properties based on the latest in vitro investigations, *Ind. Crops Prod.*, 2022, **175**, 114265, DOI: **10.1016/j.indcrop.2021.114265**.

111 Z. Liu, M. E. Bruins, L. Ni and J.-P. Vincken, Green and Black Tea Phenolics: Bioavailability, Transformation by Colonic Microbiota, and Modulation of Colonic Microbiota, *J. Agric. Food Chem.*, 2018, **66**(32), 8469–8477, DOI: **10.1021/acs.jafc.8b02233**.

112 P. G. Di, P. Mena, L. Bresciani, *et al.*, Human colonic catabolism of dietary flavan-3-ol bioactives, *Mol. Aspects Med.*, 2023, **89**, 101107, DOI: **10.1016/j.mam.2022.101107**.

113 J. Oracz, E. Nebesny, D. Zyzelewicz, G. Budryn and B. Luzak, Bioavailability and metabolism of selected cocoa bioactive compounds: A comprehensive review, *Crit. Rev. Food Sci. Nutr.*, 2020, **60**(12), 1947–1985, DOI: **10.1080/10408398.2019.1619160**.

114 P. Mena, L. Bresciani, N. Brindani, *et al.*, Phenyl-γ-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: synthesis, analysis, bioavailability, and bioactivity, *Nat. Prod. Rep.*, 2019, **36**(5), 714–752, DOI: **10.1039/C8NP00062J**.

115 C. Li, X. Meng, B. Winnik, *et al.*, Analysis of Urinary Metabolites of Tea Catechins by Liquid Chromatography/Electrospray Ionization Mass Spectrometry, *Chem. Res. Toxicol.*, 2001, **14**(6), 702–707, DOI: **10.1021/tx0002536**.

116 X. Meng, S. Sang, N. Zhu, *et al.*, Identification and Characterization of Methylated and Ring-Fission Metabolites of Tea Catechins Formed in Humans, Mice, and Rats, *Chem. Res. Toxicol.*, 2002, **15**(8), 1042–1050, DOI: **10.1021/tx010184a**.

117 M. Natsume, N. Osakabe, M. Oyama, *et al.*, Structures of (−)-epicatechin glucuronide identified from plasma and urine after oral ingestion of (−)-epicatechin: differences between human and rat, *Free Radical Biol. Med.*, 2003, **34**(7), 840–849, DOI: **10.1016/S0891-5849(02)01434-X**.

118 C. Auger, W. Mullen, Y. Hara and A. Crozier, Bioavailability of Polyphenon E Flavan-3-ols in Humans with an Ileostomy1234, *J. Nutr.*, 2008, **138**(8), 1535S–1542S, DOI: **10.1093/jn/138.8.1535S**.

119 D. Del Rio, L. Calani, C. Cordero, S. Salvatore, N. Pellegrini and F. Brighenti, Bioavailability and catabolism of green tea flavan-3-ols in humans, *Nutrition*, 2010, **26**(11), 1110–1116, DOI: **10.1016/j.nut.2009.09.021**.

120 K. A. Clarke, T. P. Dew, R. E. B. Watson, *et al.*, High performance liquid chromatography tandem mass spectrometry dual extraction method for identification of green tea catechin metabolites excreted in human urine, *J. Chromatogr. B*, 2014, **972**, 29–37, DOI: **10.1016/j.jchromb.2014.09.035**.

121 M. Urpi-Sarda, M. Monagas, N. Khan, *et al.*, Targeted metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography–tandem mass spectrometry, *J. Chromatogr. A*, 2009, **1216**(43), 7258–7267, DOI: **10.1016/j.chroma.2009.07.058**.

122 S. Wiese, T. Esatbeyoglu, P. Winterhalter, *et al.*, Comparative biokinetics and metabolism of pure monomeric, dimeric, and polymeric flavan-3-ols: A randomized cross-over study in humans, *Mol. Nutr. Food Res.*, 2015, **59**(4), 610–621, DOI: **10.1002/mnfr.201400422**.

123 M. Gómez-Juaristi, B. Sarria, S. Martínez-López, L. Bravo Clemente and R. Mateos, Flavanol Bioavailability in Two Cocoa Products with Different Phenolic Content. A Comparative Study in Humans, *Nutrients*, 2019, **11**(7), 1441, DOI: **10.3390/nu11071441**.

124 I. Hakeem Said, C. Heidorn, D. D. Petrov, *et al.*, LC-MS based metabolomic approach for the efficient identification and relative quantification of bioavailable cocoa phenolics in human urine, *Food Chem.*, 2021, **364**, 130198, DOI: **10.1016/j.foodchem.2021.130198**.

125 L. D. Becerra, M. X. Quintanilla-Carvajal, S. Escobar and R. Y. Ruiz Pardo, From controlled transformed cocoa beans to chocolate: Bioactive properties, metabolomic profile, and in vitro bioaccessibility, *Food Chem.*, 2024, **433**, 137321, DOI: **10.1016/j.foodchem.2023.137321**.

126 S. Masumoto, S. Aoki, T. Miura and T. Shoji, Flavan-3-ol/Procyanidin Metabolomics in Rat Urine Using HPLC-Quadrupole TOF/MS, *Mol. Nutr. Food Res.*, 2018, **62**(19), 1700867, DOI: **10.1002/mnfr.201700867**.

127 P. Mena, I. A. Ludwig, V. B. Tomatis, *et al.*, Inter-individual variability in the production of flavan-3-ol colonic metabolites: preliminary elucidation of urinary metabotypes, *Eur. J. Nutr.*, 2019, **58**(4), 1529–1543, DOI: **10.1007/s00394-018-1683-4**.

128 Q. Li, F. Van Herreweghen, S. O. Onyango, M. De Mey and T. Van de Wiele, In Vitro Microbial Metabolism of (+)-Catechin Reveals Fast and Slow Converters with Individual-Specific Microbial and Metabolite Markers, *J. Agric. Food Chem.*, 2022, **70**(34), 10405–10416, DOI: **10.1021/acs.jafc.2c00551**.

129 E. S. Krueger, L. E. Griffin, J. L. Beales, *et al.*, Bioavailable Microbial Metabolites of Flavanols Demonstrate Highly Individualized Bioactivity on In Vitro β-Cell Functions Critical for Metabolic Health, *Metabolites*, 2023, **13**(7), 801, DOI: **10.3390/metabo13070801**.

130 G. Pereira-Caro, J. M. Moreno-Rojas, N. Brindani, *et al.*, Bioavailability of Black Tea Theaflavins: Absorption, Metabolism, and Colonic Catabolism, *J. Agric. Food Chem.*, 2017, **65**(26), 5365–5374, DOI: **10.1021/acs.jafc.7b01707**.

131 L. Sun, Y. Su, K. Hu, D. Li, H. Guo and Z. Xie, Microbial-Transferred Metabolites of Black Tea Theaflavins by Human Gut Microbiota and Their Impact on Antioxidant Capacity, *Molecules*, 2023, **28**(15), 5871, DOI: **10.3390/molecules28155871**.

132 Z. Liu, W. J. C. de Bruijn, M. G. Sanders, S. Wang, M. E. Bruins and J.-P. Vincken, Insights in the Recalcitrance of Theasinensin A to Human Gut Microbial Degradation, *J. Agric. Food Chem.*, 2021, **69**(8), 2477–2484, DOI: **10.1021/acs.jafc.1c00727**.

133 E. Carry, D. Zhao, I. Mogno, *et al.*, Targeted analysis of microbial-generated phenolic acid metabolites derived from grape flavanols by gas chromatography-triple quadrupole mass spectrometry, *J. Pharm. Biomed. Anal.*, 2018, **159**, 374–383, DOI: 10.1016/j.jpba.2018.06.034.

134 A. Stalmach, C. A. Edwards, J. D. Wightman and A. Crozier, Identification of (Poly)phenolic Compounds in Concord Grape Juice and Their Metabolites in Human Plasma and Urine after Juice Consumption, *J. Agric. Food Chem.*, 2011, **59**(17), 9512–9522, DOI: 10.1021/jf2015039.

135 S. Touriño, E. Fuguet, M. P. Vinardell, M. Cascante and J. L. Torres, Phenolic Metabolites of Grape Antioxidant Dietary Fiber in Rat Urine, *J. Agric. Food Chem.*, 2009, **57**(23), 11418–11426, DOI: 10.1021/jf901972c.

136 S. Touriño, J. Pérez-Jiménez, M. L. Mateos-Martín, *et al.*, Metabolites in Contact with the Rat Digestive Tract after Ingestion of a Phenolic-Rich Dietary Fiber Matrix, *J. Agric. Food Chem.*, 2011, **59**(11), 5955–5963, DOI: 10.1021/jf200159f.

137 M. L. Mateos-Martín, J. Pérez-Jiménez, E. Fuguet and J. L. Torres, Non-extractable proanthocyanidins from grapes are a source of bioavailable (epi)catechin and derived metabolites in rats, *Br. J. Nutr.*, 2012, **108**(2), 290–297, DOI: 10.1017/S0007114511005678.

138 D. Wang, B. A. Williams, M. G. Ferruzzi and B. R. D'Arcy, Microbial metabolites, but not other phenolics derived from grape seed phenolic extract, are transported through differentiated Caco-2 cell monolayers, *Food Chem.*, 2013, **138**(2), 1564–1573, DOI: 10.1016/j.foodchem.2012.09.103.

139 D. Wang, L. Ho, J. Faith, *et al.*, Role of intestinal microbiota in the generation of polyphenol-derived phenolic acid mediated attenuation of Alzheimer's disease β-amyloid oligomerization, *Mol. Nutr. Food Res.*, 2015, **59**(6), 1025–1040, DOI: 10.1002/mnfr.201400544.

140 M. Margalef, Z. Pons, B. Muguerza and A. Arola-Arnal, A Rapid Method to Determine Colonic Microbial Metabolites Derived from Grape Flavanols in Rat Plasma by Liquid Chromatography–Tandem Mass Spectrometry, *J. Agric. Food Chem.*, 2014, **62**(31), 7698–7706, DOI: 10.1021/jf5019752.

141 M. Margalef, Z. Pons, F. I. Bravo, B. Muguerza and A. Arola-Arnal, Tissue distribution of rat flavanol metabolites at different doses, *J. Nutr. Biochem.*, 2015, **26**(10), 987–995, DOI: 10.1016/j.jnutbio.2015.04.006.

142 A. Serra, C. Bladé, L. Arola, A. Macià and M.-J. Motilva, Flavanol metabolites distribute in visceral adipose depots after a long-term intake of grape seed proanthocyanidin extract in rats, *Br. J. Nutr.*, 2013, **110**(8), 1411–1420, DOI: 10.1017/S0007114513000706.

143 M. Margalef, Z. Pons, L. Iglesias-Carres, F. I. Bravo, B. Muguerza and A. Arola-Arnal, Lack of Tissue Accumulation of Grape Seed Flavanols after Daily Long-Term Administration in Healthy and Cafeteria-Diet Obese Rats, *J. Agric. Food Chem.*, 2015, **63**(45), 9996–10003, DOI: 10.1021/acs.jafc.5b03856.

144 M. Margalef, L. Iglesias-Carres, Z. Pons, F. I. Bravo, B. Muguerza and A. Arola-Arnal, Age related differences in the plasma kinetics of flavanols in rats, *J. Nutr. Biochem.*, 2016, **29**, 90–96, DOI: 10.1016/j.jnutbio.2015.11.007.

145 M. Margalef, Z. Pons, L. Iglesias-Carres, L. Arola, B. Muguerza and A. Arola-Arnal, Gender-related similarities and differences in the body distribution of grape seed flavanols in rats, *Mol. Nutr. Food Res.*, 2016, **60**(4), 760–772, DOI: 10.1002/mnfr.201500717.

146 G. Pereira-Caro, S. Gaillet, J. L. Ordóñez, *et al.*, Bioavailability of red wine and grape seed proanthocyanidins in rats, *Food Funct.*, 2020, **11**(5), 3986–4001, DOI: 10.1039/D0FO00350F.

147 G. Pereira-Caro, J. L. Ordóñez, I. Ludwig, *et al.*, Development and validation of an UHPLC-HRMS protocol for the analysis of flavan-3-ol metabolites and catabolites in urine, plasma and feces of rats fed a red wine proanthocyanidin extract, *Food Chem.*, 2018, **252**, 49–60, DOI: 10.1016/j.foodchem.2018.01.083.

148 B. Cerdá, F. A. Tomás-Barberán and J. C. Espín, Metabolism of Antioxidant and Chemopreventive Ellagitannins from Strawberries, Raspberries, Walnuts, and Oak-Aged Wine in Humans: Identification of Biomarkers and Individual Variability, *J. Agric. Food Chem.*, 2005, **53**(2), 227–235, DOI: 10.1021/jf049144d.

149 R. García-Villalba, J. A. Giménez-Bastida, A. Cortés-Martín, *et al.*, Urolithins: a Comprehensive Update on their Metabolism, Bioactivity, and Associated Gut Microbiota, *Mol. Nutr. Food Res.*, 2022, **66**(21), 2101019, DOI: 10.1002/mnfr.202101019.

150 R. González-Barrio, P. Truchado, H. Ito, J. C. Espín and F. A. Tomás-Barberán, UV and MS Identification of Urolithins and Nasutins, the Bioavailable Metabolites of Ellagitannins and Ellagic Acid in Different Mammals, *J. Agric. Food Chem.*, 2011, **59**(4), 1152–1162, DOI: 10.1021/jf103894m.

151 J. C. Espín, R. González-Barrio, B. Cerdá, C. López-Bote, A. I. Rey and F. A. Tomás-Barberán, Iberian Pig as a Model To Clarify Obscure Points in the Bioavailability and Metabolism of Ellagitannins in Humans, *J. Agric. Food Chem.*, 2007, **55**(25), 10476–10485, DOI: 10.1021/jf0723864.

152 G. van't Slot, W. Mattern, S. Rzeppa, D. Grewe and H.-U. Humpf, Complex Flavonoids in Cocoa: Synthesis and Degradation by Intestinal Microbiota, *J. Agric. Food Chem.*, 2010, **58**(15), 8879–8886, DOI: 10.1021/jf1012242.

153 A. Engemann, F. Hübner, S. Rzeppa and H.-U. Humpf, Intestinal Metabolism of Two A-type Procyanidins Using the Pig Cecum Model: Detailed Structure Elucidation of Unknown Catabolites with Fourier Transform Mass Spectrometry (FTMS), *J. Agric. Food Chem.*, 2012, **60**(3), 749–757, DOI: 10.1021/jf203927g.

154 T. Žugčić, R. Abdelkebir, C. Alcantara, *et al.*, From extraction of valuable compounds to health promoting benefits of olive leaves through bioaccessibility, bioavailability and impact on gut microbiota, *Trends Food Sci. Technol.*, 2019, **83**, 63–77, DOI: 10.1016/j.tifs.2018.11.005.

155 S. Galmés, B. Reynés, M. Palou, A. Palou-March and A. Palou, Absorption, Distribution, Metabolism, and Excretion of the Main Olive Tree Phenols and Polyphenols: A Literature Review, *J. Agric. Food Chem.*, 2021, **69**(18), 5281–5296, DOI: **10.1021/acs.jafc.1c00737**.

156 G. K. Beauchamp, R. S. J. Keast, D. Morel, *et al.*, Ibuprofen-like activity in extra-virgin olive oil, *Nature*, 2005, **437**(7055), 45–46, DOI: **10.1038/437045a**.

157 T. Nikou, M. E. Sakavitsi, E. Kalampokis and M. Halabalaki, Metabolism and Bioavailability of Olive Bioactive Constituents Based on In Vitro, In Vivo and Human Studies, *Nutrients*, 2022, **14**(18), 3773, DOI: **10.3390/nu14183773**.

158 T. Nikou, K. V. Karampetsou, O. S. Koutsoni, A.-L. Skaltsounis, E. Dotsika and M. Halabalaki, Pharmacokinetics and Metabolism Investigation of Oleocanthal, *J. Nat. Prod.*, 2024, **87**(3), 530–543, DOI: **10.1021/acs.jnatprod.3c00422**.

159 M.-C. López de las Hazas, C. Piñol, A. Macià, *et al.*, Differential absorption and metabolism of hydroxytyrosol and its precursors oleuropein and secoiridoids, *J. Funct. Foods*, 2016, **22**, 52–63, DOI: **10.1016/j.jff.2016.01.030**.

160 E. Miró-Casas, M. Farré Albaladejo, M.-I. Covas, *et al.*, Capillary Gas Chromatography–Mass Spectrometry Quantitative Determination of Hydroxytyrosol and Tyrosol in Human Urine after Olive Oil Intake, *Anal. Biochem.*, 2001, **294**(1), 63–72, DOI: **10.1006/abio.2001.5160**.

161 D. Luque-Córdoba, C. A. Ledesma-Escobar and F. Priego-Capote, Qualitative and quantitative determination of phenols and their metabolites in urine by in-syringe solid-phase extraction and LC–MS/MS analysis for evaluation of virgin olive oil metabolism, *Talanta*, 2024, **266**, 125029, DOI: **10.1016/j.talanta.2023.125029**.

162 F. N. Bazoti, E. Gikas and A. Tsarbopoulos, Simultaneous quantification of oleuropein and its metabolites in rat plasma by liquid chromatography electrospray ionization tandem mass spectrometry, *Biomed. Chromatogr.*, 2010, **24**(5), 506–515, DOI: **10.1002/bmc.1319**.

163 R. García-Villalba, M. Larrosa, S. Possemiers, F. A. Tomás-Barberán and J. C. Espín, Bioavailability of phenolics from an oleuropein-rich olive (Olea europaea) leaf extract and its acute effect on plasma antioxidant status: comparison between pre- and postmenopausal women, *Eur. J. Nutr.*, 2014, **53**(4), 1015–1027, DOI: **10.1007/s00394-013-0604-9**.

164 F. Polia, M. Horcajada, L. Poquet, F. A. Tom and R. García-villalba, A novel combined analytical UV and MS approach for the quantification of oleuropein metabolites in human biological samples when authentic standards are not available, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2022, **1210**, 123457, DOI: **10.1016/j.jchromb.2022.123457**.

165 R. García-Villalba, A. Carrasco-Pancorbo, E. Nevedomskaya, *et al.*, Exploratory analysis of human urine by LC–ESI-TOF MS after high intake of olive oil: understanding the metabolism of polyphenols, *Anal. Bioanal. Chem.*, 2010, **398**(1), 463–475, DOI: **10.1007/s00216-010-3899-x**.

166 S. Silva, M. Garcia-Aloy, M. E. Figueira, E. Combet, W. Mullen and M. R. Bronze, High Resolution Mass Spectrometric Analysis of Secoiridoids and Metabolites as Biomarkers of Acute Olive Oil Intake—An Approach to Study Interindividual Variability in Humans, *Mol. Nutr. Food Res.*, 2018, **62**(2), 1700065, DOI: **10.1002/mnfr.201700065**.

167 L. Rubió, A. Macià, R. M. Valls, *et al.*, A new hydroxytyrosol metabolite identified in human plasma: Hydroxytyrosol acetate sulphate, *Food Chem.*, 2012, **134**(2), 1132–1136, DOI: **10.1016/j.foodchem.2012.02.192**.

168 L. Rubió, R.-M. Valls, A. Macià, *et al.*, Impact of olive oil phenolic concentration on human plasmatic phenolic metabolites, *Food Chem.*, 2012, **135**(4), 2922–2929, DOI: **10.1016/j.foodchem.2012.07.085**.

169 L. Rubió, A. Serra, A. Macià, C. Piñol, M.-P. Romero and M.-J. Motilva, In vivo distribution and deconjugation of hydroxytyrosol phase II metabolites in red blood cells: A potential new target for hydroxytyrosol, *J. Funct. Foods*, 2014, **10**, 139–143, DOI: **10.1016/j.jff.2014.06.001**.

170 I. Kundisová, M. E. Juan and J. M. Planas, Simultaneous Determination of Phenolic Compounds in Plasma by LC-ESI-MS/MS and Their Bioavailability after the Ingestion of Table Olives, *J. Agric. Food Chem.*, 2020, **68**(37), 10213–10222, DOI: **10.1021/acs.jafc.0c04036**.

171 C. Alemán-Jiménez, R. Domínguez-Perles, S. Medina, *et al.*, Pharmacokinetics and bioavailability of hydroxytyrosol are dependent on the food matrix in humans, *Eur. J. Nutr.*, 2021, **60**(2), 905–915, DOI: **10.1007/s00394-020-02295-0**.

172 O. Khymenets, M. C. Crespo, O. Dangles, N. Rakotomanomana, C. Andres-Lacueva and F. Visioli, Human hydroxytyrosol's absorption and excretion from a nutraceutical, *J. Funct. Foods*, 2016, **23**, 278–282, DOI: **10.1016/j.jff.2016.02.046**.

173 R. Domínguez-Perles, D. Auñón, F. Ferreres and A. Gil-Izquierdo, Gender differences in plasma and urine metabolites from Sprague–Dawley rats after oral administration of normal and high doses of hydroxytyrosol, hydroxytyrosol acetate, and DOPAC, *Eur. J. Nutr.*, 2017, **56**(1), 215–224, DOI: **10.1007/s00394-015-1071-2**.

174 R. García-Villalba, F. A. Tomás-Barberán, P. Fança-Berthon, *et al.*, Targeted and Untargeted Metabolomics to Explore the Bioavailability of the Secoiridoids from a Seed/Fruit Extract (Fraxinus angustifolia Vahl) in Human Healthy Volunteers: A Preliminary Study, *Molecules*, 2015, **20**(12), 22202–22219, DOI: **10.3390/molecules201219845**.

175 M. E. Sakavitsi, A. Breynaert, T. Nikou, *et al.*, Availability and Metabolic Fate of Olive Phenolic Alcohols Hydroxytyrosol and Tyrosol in the Human GI Tract Simulated by the In Vitro GIDM–Colon Model, *Metabolites*, 2022, **12**(5), 391, DOI: **10.3390/metabo12050391**.

176 A. López-Yerena, M. Pérez, A. Vallverdú-Queralt, E. Miliarakis, R. M. Lamuela-Raventós and E. Escribano-Ferrer, Oleacein Intestinal Permeation and Metabolism in Rats Using an In Situ Perfusion Technique,

Pharmaceutics, 2021, **13**(5), 719, DOI: 10.3390/pharmaceutics13050719.

177 C. Fytili, T. Nikou, N. Tentolouris, *et al.*, Effect of Long-Term Hydroxytyrosol Administration on Body Weight, Fat Mass and Urine Metabolomics: A Randomized Double-Blind Prospective Human Study, *Nutrients*, 2022, **14**(7), 1525, DOI: 10.3390/nu14071525.

178 H. R. El-Seedi, A. M. A. El-Said, S. A. M. Khalifa, *et al.*, Biosynthesis, Natural Sources, Dietary Intake, Pharmacokinetic Properties, and Biological Activities of Hydroxycinnamic Acids, *J. Agric. Food Chem.*, 2012, **60**(44), 10877–10895, DOI: 10.1021/jf301807g.

179 M. N. Clifford, I. B. Jaganath, I. A. Ludwig and A. Crozier, Chlorogenic acids and the acyl-quinic acids: discovery, biosynthesis, bioavailability and bioactivity, *Nat. Prod. Rep.*, 2017, **34**(12), 1391–1421, DOI: 10.1039/C7NP00030H.

180 M. N. Clifford, A. Kerimi and G. Williamson, Bioavailability and metabolism of chlorogenic acids (acyl-quinic acids) in humans, *Compr. Rev. Food Sci. Food Saf.*, 2020, **19**(4), 1299–1352, DOI: 10.1111/1541-4337.12518.

181 M. P. Gonthier, M. A. Verny, C. Besson, C. Rémésy and A. Scalbert, Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats, *J. Nutr.*, 2003, **133**(6), 1853–1859, DOI: 10.1093/jn/133.6.1853.

182 S. M. Kern, R. N. Bennett, P. W. Needs, F. A. Mellon, P. A. Kroon and M. T. Garcia-Conesa, Characterization of Metabolites of Hydroxycinnamates in the in Vitro Model of Human Small Intestinal Epithelium Caco-2 Cells, *J. Agric. Food Chem.*, 2003, **51**(27), 7884–7891, DOI: 10.1021/jf030470n.

183 R. Mateos, L. Goya and L. Bravo, Uptake and metabolism of hydroxycinnamic acids (chlorogenic, caffeic, and ferulic acids) by HepG2 cells as a model of the human liver, *J. Agric. Food Chem.*, 2006, **54**(23), 8724–8732, DOI: 10.1021/jf061664g.

184 A. Stalmach, W. Mullen, D. Barron, *et al.*, Metabolite profiling of hydroxycinnamate derivatives in plasma and urine after the ingestion of coffee by humans: Identification of biomarkers of coffee consumption, *Drug Metab. Dispos.*, 2009, **37**(8), 1749–1758, DOI: 10.1124/dmd.109.028019.

185 A. Stalmach, H. Steiling, G. Williamson and A. Crozier, Bioavailability of chlorogenic acids following acute ingestion of coffee by humans with an ileostomy, *Arch. Biochem. Biophys.*, 2010, **501**(1), 98–105, DOI: 10.1016/j.abb.2010.03.005.

186 K. Nagy, K. Redeuil, G. Williamson, *et al.*, First identification of dimethoxycinnamic acids in human plasma after coffee intake by liquid chromatography-mass spectrometry, *J. Chromatogr. A*, 2011, **1218**(3), 491–497, DOI: 10.1016/j.chroma.2010.11.076.

187 K. Redeuil, C. Smarrito-Menozzi, P. Guy, *et al.*, Identification of novel circulating coffee metabolites in human plasma by liquid chromatography-mass spectrometry, *J. Chromatogr. A*, 2011, **1218**(29), 4678–4688, DOI: 10.1016/j.chroma.2011.05.050.

188 I. A. Ludwig, M. Paz de Peña, C. Concepción and C. Alan, Catabolism of coffee chlorogenic acids by human colonic microbiota, *BioFactors*, 2013, **39**(6), 623–632, DOI: 10.1002/biof.1124.

189 A. Stalmach, G. Williamson and A. Crozier, Impact of dose on the bioavailability of coffee chlorogenic acids in humans, *Food Funct.*, 2014, **5**(8), 1727–1737, DOI: 10.1039/c4fo00316k.

190 T. Farrell, L. Poquet, F. Dionisi, D. Barron and G. Williamson, Characterization of hydroxycinnamic acid glucuronide and sulfate conjugates by HPLC-DAD-MS2: Enhancing chromatographic quantification and application in Caco-2 cell metabolism, *J. Pharm. Biomed. Anal.*, 2011, **55**(5), 1245–1254, DOI: 10.1016/j.jpba.2011.03.023.

191 M. Gómez-Juaristi, S. Martínez-López, B. Sarria, L. Bravo and R. Mateos, Absorption and metabolism of yerba mate phenolic compounds in humans, *Food Chem.*, 2018, **240**, 1028–1038, DOI: 10.1016/j.foodchem.2017.08.003.

192 M. Gómez-Juaristi, B. Sarria, L. Goya, L. Bravo-Clemente and R. Mateos, Experimental confounding factors affecting stability, transport and metabolism of flavanols and hydroxycinnamic acids in Caco-2 cells, *Food Res. Int.*, 2020, **129**, 108797, DOI: 10.1016/j.foodres.2019.108797.

193 A. Breynaert, D. Bosscher, A. Kahnt, *et al.*, Development and Validation of an in vitro Experimental GastroIntestinal Dialysis Model with Colon Phase to Study the Availability and Colonic Metabolisation of Polyphenolic Compounds, *Planta Med.*, 2015, **81**(12–13), 1075–1083, DOI: 10.1055/s-0035-1546154.

194 O. Mortelé, B. B. Xavier, C. Lammens, *et al.*, Obesity influences the microbiotic biotransformation of chlorogenic acid, *J. Pharm. Biomed. Anal.*, 2022, **211**, 114550, DOI: 10.1016/j.jpba.2021.114550.

195 M. Á. Seguido, R. M. Tarradas, S. González-Rámila, *et al.*, Sustained Consumption of a Decaffeinated Green Coffee Nutraceutical Has Limited Effects on Phenolic Metabolism and Bioavailability in Overweight/Obese Subjects, *Nutrients*, 2022, **14**(12), 2445, DOI: 10.3390/nu14122445.

196 L. Wang, Q. Zhang, X. Li, *et al.*, Pharmacokinetics and metabolism of lithospermic acid by LC/MS/MS in rats, *Int. J. Pharm.*, 2008, **350**(1), 240–246, DOI: 10.1016/j.ijpharm.2007.09.001.

197 Y.-J. Hong and A. E. Mitchell, Metabolic Profiling of Flavonol Metabolites in Human Urine by Liquid Chromatography and Tandem Mass Spectrometry, *J. Agric. Food Chem.*, 2004, **52**(22), 6794–6801, DOI: 10.1021/jf040274w.

198 W. Mullen, C. A. Edwards and A. Crozier, Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions, *Br. J. Nutr.*, 2006, **96**(1), 107–116, DOI: 10.1079/BJN20061809.

199 W. Mullen, J.-M. Rouanet, C. Auger, *et al.*, Bioavailability of [2-14C]Quercetin-4′-glucoside in Rats, *J. Agric. Food Chem.*, 2008, **56**(24), 12127–12137, DOI: 10.1021/jf802754s.

200 G. Borges, R. Y. Fong, J. L. Ensunsa, *et al.*, Absorption, distribution, metabolism and excretion of apigenin and its glycosides in healthy male adults, *Free Radical Biol. Med.*, 2022, **185**, 90–96, DOI: 10.1016/j.freeradbiomed.2022.04.007.

201 L. Wu, J. Liu, W. Han, *et al.*, Time-Dependent Metabolism of Luteolin by Human UDP-Glucuronosyltransferases and Its Intestinal First-Pass Glucuronidation in Mice, *J. Agric. Food Chem.*, 2015, **63**(39), 8722–8733, DOI: 10.1021/acs.jafc.5b02827.

202 C. Mekjaruskul, M. Jay and B. Sripanidkulchai, Pharmacokinetics, bioavailability, tissue distribution, excretion, and metabolite identification of methoxyflavones in Kaempferia parviflora extract in rats, *Drug Metab. Dispos.*, 2012, **40**(12), 2342–2353, DOI: 10.1124/dmd.112.047142.

203 A. Keranmu, L.-B. Pan, J. Fu, *et al.*, Biotransformation of Liquiritigenin into Characteristic Metabolites by the Gut Microbiota, *Molecules*, 2022, **27**(10), 3057, DOI: 10.3390/molecules27103057.

204 Q. Wang, X. Qiao, C. Liu, *et al.*, Metabolites identification of glycycoumarin, a major bioactive coumarin from licorice in rats, *J. Pharm. Biomed. Anal.*, 2014, **98**, 287–295, DOI: 10.1016/j.jpba.2014.06.001.

205 Q. Wang, X. Qiao, Y. Qian, *et al.*, Metabolites identification of glycyrin and glycyrol, bioactive coumarins from licorice, *J. Chromatogr. B*, 2015, **983–984**, 39–46, DOI: 10.1016/j.jchromb.2014.12.028.

206 T. Cheng, T. Sheng, Y. Yi, T. Zhang and H. Han, Metabolism profiles of icariin in rats using ultra-high performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry and in vitro enzymatic study, *J. Chromatogr. B*, 2016, **1033–1034**, 353–360, DOI: 10.1016/j.jchromb.2016.09.010.

207 S.-Q. Zhang and S.-Z. Zhang, Oral absorption, distribution, metabolism, and excretion of icaritin in rats by Q-TOF and UHPLC–MS/MS, *Drug Test. Anal.*, 2017, **9**(10), 1604–1610, DOI: 10.1002/dta.2188.

208 L. Li, X. Huang, J. Peng, *et al.*, Wedelolactone metabolism in rats through regioselective glucuronidation catalyzed by uridine diphosphate-glucuronosyltransferases 1As (UGT1As), *Phytomedicine*, 2016, **23**(4), 340–349, DOI: 10.1016/j.phymed.2016.01.007.

209 M. Qi, A. Xiong, P. Li, Q. Yang, L. Yang and Z. Wang, Identification of acteoside and its major metabolites in rat urine by ultra-performance liquid chromatography combined with electrospray ionization quadrupole time-of-flight tandem mass spectrometry, *J. Chromatogr. B*, 2013, **940**, 77–85, DOI: 10.1016/j.jchromb.2013.09.023.

210 Q. Cui, Y. Pan, X. Xu, *et al.*, The metabolic profile of acteoside produced by human or rat intestinal bacteria or intestinal enzyme in vitro employed UPLC-Q-TOF–MS, *Fitoterapia*, 2016, **109**, 67–74, DOI: 10.1016/j.fitote.2015.12.011.

211 Y. Li, G. Zhou, Y. Peng, P. Tu and X. Li, Screening and identification of three typical phenylethanoid glycosides metabolites from Cistanches Herba by human intestinal bacteria using UPLC/Q-TOF-MS, *J. Pharm. Biomed. Anal.*, 2016, **118**, 167–176, DOI: 10.1016/j.jpba.2015.10.038.

212 Y. Li, G. Zhou, S. Xing, P. Tu and X. Li, Identification of Echinacoside Metabolites Produced by Human Intestinal Bacteria Using Ultraperformance Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry, *J. Agric. Food Chem.*, 2015, **63**(30), 6764–6771, DOI: 10.1021/acs.jafc.5b02881.

213 V. Valková, H. Ďúranová, J. Bilčíková and M. Habán, Milk thistle (Silybum marianum): a valuable medicinal plant with several therapeutic purposes, *J. Microbiol., Biotechnol. Food Sci.*, 2020, **9**(4), 836.

214 S. A. Emadi, M. G. Rahbardar, S. Mehri and H. Hosseinzadeh, A review of therapeutic potentials of milk thistle (Silybum marianum L.) and its main constituent, silymarin, on cancer, and their related patents, *Iran. J. Basic Med. Sci.*, 2022, **25**(10), 1166.

215 L. Calani, F. Brighenti, R. Bruni and D. Del Rio, Absorption and metabolism of milk thistle flavanolignans in humans, *Phytomedicine*, 2012, **20**(1), 40–46, DOI: 10.1016/j.phymed.2012.09.004.

216 P. Marhol, P. Bednář, P. Kolářová, *et al.*, Pharmacokinetics of pure silybin diastereoisomers and identification of their metabolites in rat plasma, *J. Funct. Foods*, 2015, **14**, 570–580, DOI: 10.1016/j.jff.2015.02.031.

217 E.-M. Pferschy-Wenzig, O. Kunert, T. Thumann, C. Moissl-Eichinger and R. Bauer, Characterization of metabolites from milk thistle flavonolignans generated by human fecal microbiota, *Phytochemistry*, 2023, **215**, 113834, DOI: 10.1016/j.phytochem.2023.113834.

218 D. Upadhyay, S. Anandjiwala, H. Padh and M. Nivsarkar, In vitro – In vivo metabolism and pharmacokinetics of picroside I and II using LC-ESI-MS method, *Chem.-Biol. Interact.*, 2016, **254**, 83–92, DOI: 10.1016/j.cbi.2016.05.031.

219 P. Songvut, W. Nakareangrit, W. Cholpraipimolrat, *et al.*, Unraveling the interconversion pharmacokinetics and oral bioavailability of the major ginger constituents: [6]-gingerol, [6]-shogaol, and zingerone after single-dose administration in rats, *Front. Pharmacol*, 2024, **15**, 1391019, DOI: 10.3389/fphar.2024.1391019.

220 S. Zhang, E. DiMango, Y. Zhu, T. K. Saroya, C. W. Emala and S. Sang, Pharmacokinetics of Gingerols, Shogaols, and Their Metabolites in Asthma Patients, *J. Agric. Food Chem.*, 2022, **70**(31), 9674–9683, DOI: 10.1021/acs.jafc.2c03150.

221 J. Zagórska, K. Pietrzak, W. Kukula-Koch, M. Czop, J. Laszuk and W. Koch, Influence of Diet on the Bioavailability of Active Components from Zingiber officinale Using an In Vitro Digestion Model, *Foods*, 2023, **12**(21), 3897, DOI: 10.3390/foods12213897.

222 D. Zhang, C. Huang, W. Xin, *et al.*, Preclinical pharmacokinetic evaluation and metabolites identification of methyl salicylate-2-O-β-d-lactoside in rats using LC–MS/MS and Q-TOF-MS methods, *J. Pharm. Biomed. Anal.*, 2015, **109**, 1–10, DOI: 10.1016/j.jpba.2015.02.022.

223 H. Wu, F. Feng, X. Jiang, *et al.*, Pharmacokinetic and metabolic profiling studies of sennoside B by UPLC-MS/MS and UPLC-Q-TOF-MS, *J. Pharm. Biomed. Anal.*, 2020, **179**, 112938, DOI: 10.1016/j.jpba.2019.112938.

224 Y. Song, J. Yang, X. Wang, *et al.*, Pharmacokinetics and metabolism of trans-emodin dianthrones in rats, *J. Ethnopharmacol.*, 2022, **290**, 115123, DOI: 10.1016/j.jep.2022.115123.

225 B. Yuan, D. Zhao, W. Lyu, *et al.*, Development and validation of a micro-QuEChERS method with high-throughput enhanced matrix removal followed with UHPLC-QqQ-MS/MS for analysis of raspberry ketone-related phenolic compounds in adipose tissues, *Talanta*, 2021, **235**, 122716, DOI: 10.1016/j.talanta.2021.122716.

226 B. Yuan, D. Zhao, D. Kshatriya, N. T. Bello, J. E. Simon and Q. Wu, UHPLC-QqQ-MS/MS method development and validation with statistical analysis: Determination of raspberry ketone metabolites in mice plasma and brain, *J. Chromatogr. B*, 2020, **1149**, 122146, DOI: 10.1016/j.jchromb.2020.122146.

227 S. Qiu, H. Sun, A.-H. Zhang, *et al.*, Natural alkaloids: basic aspects, biological roles, and future perspectives, *Chin. J. Nat. Med.*, 2014, **12**(6), 401–406, DOI: 10.1016/S1875-5364(14)60063-7.

228 S. Martínez-López, B. Sarriá, M. Gómez-Juaristi, L. Goya, R. Mateos and L. Bravo-Clemente, Theobromine, caffeine, and theophylline metabolites in human plasma and urine after consumption of soluble cocoa products with different methylxanthine contents, *Food Res. Int.*, 2014, **63**, 446–455, DOI: 10.1016/j.foodres.2014.03.009.

229 S. Martínez-López, B. Sarriá, G. Baeza, R. Mateos and L. Bravo-Clemente, Pharmacokinetics of caffeine and its metabolites in plasma and urine after consuming a soluble green/roasted coffee blend by healthy subjects, *Food Res. Int.*, 2014, **64**, 125–133, DOI: 10.1016/j.foodres.2014.05.043.

230 W. Wang, C.-M. Ma and M. Hattori, Metabolism and Pharmacokinetics of Rhynchophylline in Rats, *Biol. Pharm. Bull.*, 2010, **33**(4), 669–676, DOI: 10.1248/bpb.33.669.

231 X. Wang, M. Zheng, J. Liu, *et al.*, Differences of first-pass effect in the liver and intestine contribute to the stereoselective pharmacokinetics of rhynchophylline and isorhynchophylline epimers in rats, *J. Ethnopharmacol.*, 2017, **209**, 175–183.

232 E. Brochmann-Hanssen, B. Nielsen and K. Hirai, Opium alkaloids IV. Isolation of isoboldine, *J. Pharm. Sci.*, 1967, **56**(6), 754–756, DOI: 10.1002/jps.2600560621.

233 Y. Li, R. Zeng, J. Chen, *et al.*, Pharmacokinetics and metabolism study of isoboldine, a major bioactive component from Radix Linderae in male rats by UPLC–MS/MS, *J. Ethnopharmacol.*, 2015, **171**, 154–160, DOI: 10.1016/j.jep.2015.05.042.

234 S. Li, L. Teng, W. Liu, *et al.*, Pharmacokinetic study of harmane and its 10 metabolites in rat after intravenous and oral administration by UPLC-ESI-MS/MS, *Pharm. Biol.*, 2016, **54**(9), 1768–1781.

235 H. Han, L. Yang, Y. Ding, G. Ji and T. Zhang, Pharmacokinetics and metabolism profiles of protostemonine in rat by liquid chromatography combined with electrospray ionization tandem mass spectrometry, *J. Pharm. Biomed. Anal.*, 2016, **117**, 266–275, DOI: 10.1016/j.jpba.2015.09.006.

236 X. Feng, K. Wang, S. Cao, L. Ding and F. Qiu, Pharmacokinetics and Excretion of Berberine and Its Nine Metabolites in Rats, *Front. Pharmacol*, 2021, **11**, 594852, DOI: 10.3389/fphar.2020.594852.

237 X. Liu, W. Li, H. Zhang, *et al.*, Biodistribution and pharmacokinetic profile of berberine and its metabolites in hepatocytes, *Phytomedicine*, 2022, **104**, 154288.

238 Z. Huang, M. Li, Z. Qin, *et al.*, Intestines-erythrocytes-mediated bio-disposition deciphers the hypolipidemic effect of berberine from Rhizoma Coptidis: A neglected insight, *J. Ethnopharmacol.*, 2023, **314**, 116600, DOI: 10.1016/j.jep.2023.116600.

239 P. F. Whiting, R. F. Wolff, S. Deshpande, *et al.*, Cannabinoids for Medical Use: A Systematic Review and Meta-analysis, *JAMA, J. Am. Med. Assoc.*, 2015, **313**(24), 2456–2473, DOI: 10.1001/jama.2015.6358.

240 A. I. Fraguas-Sánchez and A. I. Torres-Suárez, Medical Use of Cannabinoids, *Drugs*, 2018, **78**(16), 1665–1703, DOI: 10.1007/s40265-018-0996-1.

241 C. Citti, D. Braghiroli, M. A. Vandelli and G. Cannazza, Pharmaceutical and biomedical analysis of cannabinoids: A critical review, *J. Pharm. Biomed. Anal.*, 2018, **147**, 565–579, DOI: 10.1016/j.jpba.2017.06.003.

242 C. J. Lucas, P. Galettis and J. Schneider, The pharmacokinetics and the pharmacodynamics of cannabinoids, *Br. J. Clin. Pharmacol.*, 2018, **84**(11), 2477–2482, DOI: 10.1111/bcp.13710.

243 A. P. Pérez-Acevedo, F. P. Busardò, R. Pacifici, *et al.*, Disposition of cannabidiol metabolites in serum and urine from healthy individuals treated with pharmaceutical preparations of medical cannabis, *Pharmaceuticals*, 2020, **13**(12), 1–9, DOI: 10.3390/ph13120459.

244 R. H. Lowe, E. L. Karschner, E. W. Schwilke, A. J. Barnes and M. A. Huestis, Simultaneous quantification of Δ9-tetrahydrocannabinol, 11-hydroxy-Δ9-tetrahydrocannabinol, and 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid in human plasma using two-dimensional gas chromatography, cryofocusing, and electron impact-mass spectrometry, *J. Chromatogr. A*, 2007, **1163**(1–2), 318–327, DOI: 10.1016/j.chroma.2007.06.069.

245 A. Gronewold and G. Skopp, A preliminary investigation on the distribution of cannabinoids in man, *Forensic Sci. Int.*, 2011, **210**(1–3), e7, DOI: 10.1016/j.forsciint.2011.04.010.

246 M. Fabritius, C. Staub, P. Mangin and C. Giroud, Distribution of free and conjugated cannabinoids in human bile samples, *Forensic Sci. Int.*, 2012, **223**(1–3), 114–118, DOI: 10.1016/j.forsciint.2012.08.013.

247 D. M. Schwope, K. B. Scheidweiler and M. A. Huestis, Direct quantification of cannabinoids and cannabinoid

glucuronides in whole blood by liquid chromatography-tandem mass spectrometry, *Anal. Bioanal. Chem.*, 2011, **401**(4), 1273–1283, DOI: 10.1007/s00216-011-5197-7.

248 A. P. Pérez-Acevedo, R. Pacifici, G. Mannocchi, *et al.*, Disposition of cannabinoids and their metabolites in serum, oral fluid, sweat patch and urine from healthy individuals treated with pharmaceutical preparations of medical cannabis, *Phytother Res.*, 2021, **35**(3), 1646–1657, DOI: 10.1002/ptr.6931.

249 N. N. B. Williams, T. R. Ewell, K. S. S. Abbotts, *et al.*, Comparison of Five Oral Cannabidiol Preparations in Adult Humans: Pharmacokinetics, Body Composition, and Heart Rate Variability, *Pharmaceuticals*, 2021, **14**(1), 35, DOI: 10.3390/ph14010035.

250 S. Clarke, B. E. Butcher, A. J. McLachlan, *et al.*, Pilot clinical and pharmacokinetic study of Δ9-Tetrahydrocannabinol (THC)/Cannabidiol (CBD) nanoparticle oro-buccal spray in patients with advanced cancer experiencing uncontrolled pain, *PLoS One*, 2022, **17**, 1–23, DOI: 10.1371/journal.pone.0270543.

251 C. Dumbraveanu, K. Strommer, M. Wonnemann, *et al.*, Pharmacokinetics of Orally Applied Cannabinoids and Medical Marijuana Extracts in Mouse Nervous Tissue and Plasma: Relevance for Pain Treatment, *Pharmaceutics*, 2023, **15**(3), 853, DOI: 10.3390/pharmaceutics15030853.

252 D. Cox-Georgian, N. Ramadoss, C. Dona and C. Basu, in *Therapeutic and Medicinal Uses of Terpenes BT – Medicinal Plants: From Farm to Pharmacy*, ed. N. Joshee, S. A. Dhekney and P. Parajuli, Springer International Publishing, 2019, pp. 333–359, DOI: 10.1007/978-3-030-31269-5_15.

253 H. Kong, M. Wang, K. Venema, *et al.*, Bioconversion of red ginseng saponins in the gastro-intestinal tract in vitro model studied by high-performance liquid chromatography-high resolution Fourier transform ion cyclotron resonance mass spectrometry, *J. Chromatogr. A*, 2009, **1216**(11), 2195–2203, DOI: 10.1016/j.chroma.2008.11.030.

254 E. O. Kim, K. H. Cha, E. H. Lee, *et al.*, Bioavailability of ginsenosides from white and red ginsengs in the simulated digestion model, *J. Agric. Food Chem.*, 2014, **62**(41), 10055–10063, DOI: 10.1021/jf500477n.

255 C. He, J. Li, N. Xu, *et al.*, Pharmacokinetics, bioavailability, and metabolism of Notoginsenoside Fc in rats by liquid chromatography/electrospray ionization tandem mass spectrometry, *J. Pharm. Biomed. Anal.*, 2015, **109**, 150–157, DOI: 10.1016/j.jpba.2015.02.038.

256 M. Y. Chen, L. Shao, W. Zhang, *et al.*, Metabolic analysis of Panax notoginseng saponins with gut microbiota-mediated biotransformation by HPLC-DAD-Q-TOF-MS/MS, *J. Pharm. Biomed. Anal.*, 2018, **150**, 199–207, DOI: 10.1016/j.jpba.2017.12.011.

257 Y. P. Guo, Y. P. Guo, Y. P. Guo, *et al.*, In Vivo Metabolic Profiles of Panax notoginseng Saponins Mediated by Gut Microbiota in Rats, *J. Agric. Food Chem.*, 2020, **68**(25), 6835–6844, DOI: 10.1021/acs.jafc.0c01857.

258 X. Fu, K. Chen, Z. Li, *et al.*, Pharmacokinetics and Oral Bioavailability of Panax Notoginseng Saponins Administered to Rats Using a Validated UPLC-MS/MS Method, *J. Agric. Food Chem.*, 2023, **71**(1), 469–479, DOI: 10.1021/acs.jafc.2c06312.

259 H. Y. Zhang, W. Niu, O. E. Olaleye, *et al.*, Comparison of intramuscular and intravenous pharmacokinetics of ginsenosides in humans after dosing XueShuanTong, a lyophilized extract of Panax notoginseng roots, *J. Ethnopharmacol.*, 2020, **253**, 112658, DOI: 10.1016/j.jep.2020.112658.

260 H. L. Li, Z. M. Qin, H. D. Cai, *et al.*, Determination of α-hederin in rat plasma using liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) and its application to a pharmacokinetic study, *Anal. Methods*, 2015, **7**(5), 2155–2161, DOI: 10.1039/c4ay02837f.

261 P. Huang, L. Zhang, C. Chai, *et al.*, Effects of food and gender on the pharmacokinetics of ginkgolides A, B, C and bilobalide in rats after oral dosing with ginkgo terpene lactones extract, *J. Pharm. Biomed. Anal.*, 2014, **100**, 138–144, DOI: 10.1016/j.jpba.2014.07.030.

262 S. Wang, B. Ouyang, J. Aa, *et al.*, Pharmacokinetics and tissue distribution of ginkgolide A, ginkgolide B, and ginkgolide K after intravenous infusion of ginkgo diterpene lactones in a rat model, *J. Pharm. Biomed. Anal.*, 2016, **126**, 109–116, DOI: 10.1016/j.jpba.2016.04.035.

263 G. Cao, N. Wang, D. He, *et al.*, Intestinal mucosal metabolites-guided detection of trace-level ginkgo biloba extract metabolome, *J. Chromatogr. A*, 2019, **1608**, 460417, DOI: 10.1016/j.chroma.2019.460417.

264 O. J. Pozo, M. Pujadas, S. B. Gleeson, *et al.*, Liquid chromatography tandem mass spectrometric determination of triterpenes in human fluids: Evaluation of markers of dietary intake of olive oil and metabolic disposition of oleanolic acid and maslinic acid in humans, *Anal. Chim. Acta*, 2017, **990**, 84–95, DOI: 10.1016/j.aca.2017.07.041.

265 P. Krüger, R. Daneshfar, G. P. Eckert, *et al.*, Metabolism of boswellic acids in vitro and in vivo, *Drug Metab. Dispos.*, 2008, **36**(6), 1135–1142, DOI: 10.1124/dmd.107.018424.

266 R. de la Torre, M. Carbó, M. Pujadas, *et al.*, Pharmacokinetics of maslinic and oleanolic acids from olive oil – Effects on endothelial function in healthy adults. A randomized, controlled, dose–response study, *Food Chem.*, 2020, **322**, 126676, DOI: 10.1016/j.foodchem.2020.126676.

267 S. Maina, G. Misinzo, G. Bakari and H.-Y. Kim, Human, Animal and Plant Health Benefits of Glucosinolates and Strategies for Enhanced Bioactivity: A Systematic Review, *Molecules*, 2020, **25**(16), 3682, DOI: 10.3390/molecules25163682.

268 N. Miękus, K. Marszałek, M. Podlacha, A. Iqbal, C. Puchalski and A. H. Świergiel, Health Benefits of Plant-Derived Sulfur Compounds, Glucosinolates, and Organosulfur Compounds, *Molecules*, 2020, **25**(17), 3804, DOI: 10.3390/molecules25173804.

269 A. Costa-Pérez, V. Núñez-Gómez, N. Baenas, *et al.*, Systematic Review on the Metabolic Interest of Glucosinolates and Their Bioactive Derivatives for Human Health, *Nutrients*, 2023, **15**(6), 1424, DOI: **10.3390/nu15061424**.

270 F. J. Barba, N. Nikmaram, S. Roohinejad, A. Khelfa, Z. Zhu and M. Koubaa, Bioavailability of Glucosinolates and Their Breakdown Products: Impact of Processing, *Front. Nutr.*, 2016, **3**, 24, DOI: **10.3389/fnut.2016.00024**.

271 T. Oliviero, R. Verkerk and M. Dekker, Isothiocyanates from Brassica Vegetables—Effects of Processing, Cooking, Mastication, and Digestion, *Mol. Nutr. Food Res.*, 2018, **62**(18), 1701069, DOI: **10.1002/mnfr.201701069**.

272 B. Holst and G. Williamson, A critical review of the bioavailability of glucosinolates and related compounds, *Nat. Prod. Rep.*, 2004, **21**(3), 425–447, DOI: **10.1039/b204039p**.

273 L. Song, J. J. Morrison, N. P. Botting and P. J. Thornalley, Analysis of glucosinolates, isothiocyanates, and amine degradation products in vegetable extracts and blood plasma by LC-MS/MS, *Anal. Biochem.*, 2005, **347**(2), 234–243, DOI: **10.1016/j.ab.2005.09.040**.

274 J. D. Clarke, A. Hsu, K. Riedl, *et al.*, Bioavailability and inter-conversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or broccoli supplement in a cross-over study design, *Pharmacol. Res.*, 2011, **64**(5), 456–463, DOI: **10.1016/j.phrs.2011.07.005**.

275 S. Saha, W. Hollands, B. Teucher, *et al.*, Isothiocyanate concentrations and interconversion of sulforaphane to erucin in human subjects after consumption of commercial frozen broccoli compared to fresh broccoli, *Mol. Nutr. Food Res.*, 2012, **56**(12), 1906–1916, DOI: **10.1002/mnfr.201200225**.

276 R. Dominguez-Perles, S. Medina, D. Á. Moreno, C. García-Viguera, F. Ferreres and Á. Gil-Izquierdo, A new ultra-rapid UHPLC/MS/MS method for assessing glucoraphanin and sulforaphane bioavailability in human urine, *Food Chem.*, 2014, **143**, 132–138, DOI: **10.1016/j.foodchem.2013.07.116**.

277 E. S. Hwang, G. M. Bornhorst, P. I. Oteiza and A. E. Mitchell, Assessing the Fate and Bioavailability of Glucosinolates in Kale (Brassica oleracea) Using Simulated Human Digestion and Caco-2 Cell Uptake Models, *J. Agric. Food Chem.*, 2019, **67**(34), 9492–9500, DOI: **10.1021/acs.jafc.9b03329**.

278 W. Zhu, L. A. Lerno, E. Cremonini, *et al.*, Robust UHPLC-(ESI+)-MS/MS Method for Simultaneous Analysis of Glucoraphanin, Sulforaphane, and Sulforaphane Metabolites in Biological Samples, *ACS Food Sci. Technol.*, 2023, **3**(7), 1300–1310, DOI: **10.1021/acsfoodscitech.3c00173**.

279 C. S. Charron, B. T. Vinyard, E. H. Jeffery, S. A. Ross, H. E. Seifried and J. A. Novotny, BMI Is Associated With Increased Plasma and Urine Appearance of Glucosinolate Metabolites After Consumption of Cooked Broccoli, *Front. Nutr.*, 2020, **7**, 1–10, DOI: **10.3389/fnut.2020.575092**.

280 P. Putnik, D. Gabrić, S. Roohinejad, *et al.*, An overview of organosulfur compounds from Allium spp.: From processing and preservation to evaluation of their bioavailability, antimicrobial, and anti-inflammatory properties, *Food Chem.*, 2019, **276**, 680–691, DOI: **10.1016/j.foodchem.2018.10.068**.

281 M. S. Rahman, Allicin and Other Functional Active Components in Garlic: Health Benefits and Bioavailability, *Int. J. Food Prop.*, 2007, **10**(2), 245–268, DOI: **10.1080/10942910601113327**.

282 Y. Deng, C.-T. Ho, Y. Lan, J. Xiao and M. Lu, Bioavailability, Health Benefits, and Delivery Systems of Allicin: A Review, *J. Agric. Food Chem.*, 2023, **71**(49), 19207–19220, DOI: **10.1021/acs.jafc.3c05602**.

283 A. Moreno-Ortega, G. Pereira-Caro, I. A. Ludwig, M. J. Motilva and J. M. Moreno-Rojas, Bioavailability of Organosulfur Compounds after the Ingestion of Black Garlic by Healthy Humans, *Antioxidants*, 2023, **12**(4), 925, DOI: **10.3390/antiox12040925**.

284 X. Tian, Y. Zhang, Z. Li, *et al.*, Systematic and comprehensive strategy for metabolite profiling in bioanalysis using software-assisted HPLC-Q-TOF: magnoflorine as an example, *Anal. Bioanal. Chem.*, 2016, **408**, 2239–2254.

285 J. I. Ottaviani, R. Y. Fong, G. Borges, H. Schroeter and A. Crozier, Use of LC-MS for the quantitative analysis of (poly) phenol metabolites does not necessarily yield accurate results: Implications for assessing existing data and conducting future research, *Free Radical Biol. Med.*, 2018, **124**, 97–103.

286 J. A. Rothwell, M. Urpi-Sarda, M. Boto-Ordoñez, *et al.*, Systematic analysis of the polyphenol metabolome using the Phenol-Explorer database, *Mol. Nutr. Food Res.*, 2016, **60**(1), 203–211.

287 R. González-Domínguez, O. Jáuregui, P. Mena, *et al.*, Quantifying the human diet in the crosstalk between nutrition and health by multi-targeted metabolomics of food and microbiota-derived metabolites, *Int. J. Obes.*, 2020, **44**(12), 2372–2381.

288 R. González-Domínguez, M. Urpi-Sarda, O. Jáuregui, P. W. Needs, P. A. Kroon and C. Andrés-Lacueva, Quantitative dietary fingerprinting (QDF)—A novel tool for comprehensive dietary assessment based on urinary nutrimetabolomics, *J. Agric. Food Chem.*, 2019, **68**(7), 1851–1861.

289 A. Roichman, Q. Zuo, S. Hwang, *et al.*, Microbiome metabolism of dietary phytochemicals controls the anticancer activity of PI3K inhibitors, *Cell*, 2025, **188**(11), 3065–3080.e21, DOI: **10.1016/j.cell.2025.04.041**.

290 E. J. Culp, N. T. Nelson, A. A. Verdegaal and A. L. Goodman, Microbial transformation of dietary xenobiotics shapes gut microbiome composition, *Cell*, 2024, **187**(22), 6327–6345.e20, DOI: **10.1016/j.cell.2024.08.038**.

291 G. A. Kuziel, G. L. Lozano, C. Simian, *et al.*, Functional diversification of dietary plant small molecules by the gut microbiome, *Cell*, 2025, **188**(7), 1967–1983.e22, DOI: **10.1016/j.cell.2025.01.045**.