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Research Article

The bioavailability of raspberry anthocyanins and ellagitannins in rats

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The fate of anthocyanins and ellagitannins in rats was monitored following ingestion of raspberry juice. After 1 h low nM concentrations of unmetabolised anthocyanins were present in plasma but these declined by 2 h and after 4 h they were no longer detectable. For the first 2 h there was an almost full recovery of anthocyanins as they passed from the stomach through the duodenum/jejunum and into the ileum. After 3 h less than 50% were recovered, and the levels declined rapidly thereafter. Excretion of raspberry anthocyanins in urine over a 24 h period was equivalent to 1.2% of the amount ingested. Trace quantities of anthocyanins were detected in the caecum, colon and faeces and they were absent in extracts of liver, kidneys and brain. Urine also contained a number of phenolic acids but most were present in quantities well in excess of the 918 nmol of anthocyanins present in the ingested juice. These findings indicate that raspberry anthocyanins *per se* are poorly absorbed, probably prior to reaching the ileum, and that substantial amounts pass from the small to the large intestine where they are degraded by colonic bacteria. Ellagitannins disappeared in the stomach without accumulation of ellagic acid.

Keywords: Anthocyanins / Bioavailability / Ellagitannins / Raspberries / Rats

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1 Introduction

Anthocyanins are glycosylated anthocyanidins, one of the major subgroups of the C_6-C_3-C_6 flavonoids. They are red and blue coloured pigments and occur widely in the plant kingdom being present in leaves, flowers and fruits [1]. They are normal dietary components, occurring in a wide range of fruits, vegetables and beverages [2, 3]. Anthocyanins are antioxidants [4, 5] and have anticarcinogenic [6] and anti-inflammatory properties [7]; they are reported to improve vision [8] and enhance memory [9] and may also reduce the incidence of coronary heart disease [10]. The average daily intake of anthocyanins in the USA has been estimated to be 12.5 mg *per* person [3]. However, it is relatively easy for consumers to markedly increase their consumption of anthocyanins as concentrations in red wine of 120 mg/L are not unusual [11], a 100 g serving of some berries can contain >100 mg [3] and there is a report of 200 mL of Austrian elderberry juice containing 2 g of anthocyanins, principally as cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside [12].

In order to better understand the action and potential protective effects of dietary anthocyanins *in vivo* more needs to be known about their fate following ingestion. Although there are exceptions, unlike other flavonoids that have been absorbed and/or excreted [13], most anthocyanins do not undergo extensive metabolic modification of the parent glycosides to glucurono-, sulpho- and methyl derivatives [14–17]. In feeding studies with animals and humans, typically *ca*. 0.1% of the quantities ingested, and sometimes much less, have been detected in plasma and urine [14, 15] implying a low level of absorption compared to other flavonoids [13]. However, *in situ* perfusion studies with rat small intestine indicate that absorption may be of the order of 10–20%, depending upon the aglycone moiety [18, 19].

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The low concentration of anthocyanins in plasma may, therefore, be a consequence of their rapid removal from the circulatory system. In keeping with this possibility, following injection of delphinidin-3-*O*-rutinoside into the neck vein of rats, plasma concentrations fell from 26 nM, 1 min postinjection, to 5 nM after 15 min and were not detectable after 2 h [20]. However, after intravenous administration of cyanidin-3-*O*-glucoside to rats there was a 36% recovery of the glucoside and its metabolites in urine and 12% in bile within 4 h [21]. This implies that the perfusion experiments may have overestimated the extent of anthocyanin absorption from the gastrointestinal tract as if 10–20% of the ingested anthocyanins entered the circulatory system this would be accompanied by the appearance of substantially higher quantities in urine than the *ca*. <0.1% of intake that is routinely obtained in anthocyanin feeding studies [14, 15].

Excretion of <0.1% of intake was obtained with 15 anthocyanins in the urine of rats and humans after supplementation with blueberry, boysenberry, black raspberry and black currant extracts [15]. The ratio of the levels of some of the individual anthocyanins in urine was different to that found in the berries. For instance, after supplementation with a boysenberry extract, the relative concentrations of cyanidin-3-*O*-sophoroside, cyanidin-3-*O*-(^{2}G-*O*-glucosylrutinoside) and cyanidin-3-*O*-rutinoside were comparable to the berry extract indicating that the three anthocyanins behave similarly with respect to absorption and excretion. In contrast, the relative concentration of cyanidin-3-*O*-glucoside was much lower in urine suggesting that the nature of the sugar moiety has an influence on anthocyanin bioavailability. Overall the data obtained in this study suggest that the determinants of absorption and excretion of anthocyanins are influenced by the nature not only of the sugar moiety but also the anthocyanidin structure [15]. This conclusion is supported by data obtained in a recent study in which weanling pigs were fed acute supplements of chokeberry, blackcurrant and elderberry extracts [22].

This paper reports on a study of the bioavailability of anthocyanins and ellagitannins following the ingestion of raspberry juice by rats. The animals were fed with raspberry juice by gavage; after which body tissues and fluids, collected over a 24 h period, were analysed by HPLC with photodiode array (PDA) and MS/MS.

2 Materials and methods

2.1 Chemicals

4-Hydroxybenzoic acid was obtained from Aldrich (Poole, Dorset, UK), 4-hydroxyphenylacetic acid from Fluka (Gillingham, Dorset, UK), hippuric acid and ferulic acid from Sigma (Poole, Dorset, UK), while other phenolic acids were purchased from AASC (Southampton, Hants, UK). Derivatisation reagent *N*,*O*-bis(trimethylsilyl)acetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were also purchased from Sigma, as were formic acid, ellagic acid and cyanidin-3-*O*-glucoside. Methanol and ethyl acetate was obtained from Rathburn Chemicals (Walkerburn, Borders, UK). Cyanidin-3-*O*-sambubiosyl-5-*O*-glucopyranoside was purchased from Polyphenols Laboratories AS (Sandnes, Norway).

2.2 Animal and sample preparation

Sprague–Dawley male rats ($n = 24$), weighing 277 ± 21 g, were housed in metabolic cages allowing the collection of 24 h urine and faecal samples. Rats were deprived of food but did have access to water for 16 h before and 24 h after being fed 2.77 mL of raspberry juice by gavage. The juice was obtained by squeezing raspberries (*Rubus idaeus* var. Glen Ample) and 1 mL of juice corresponded to 1.2 g of raspberries. As a blank, urine was collected for a 3 h period prior to supplementation. Three animals were terminally anaesthetised with pentobarbital at 0, 1, 2, 3, 4, 6, 12 and 24 h after administration of the juice. Blood was removed by cardiac puncture with heparin-moistened syringes and plasma was obtained by centrifugation at $2000 \times g$ for 10 min at 4°C after which 250 μL aliquots were acidified to pH 3.0 with 7.5 μL 50% aqueous formic acid and 25 μL of ascorbic acid (10 mM). Liver, kidney and brain were perfused *in situ* with chilled 0.15 M KCl, and then removed along with stomach, duodenum/jejunum, ileum, caecum and colon, with their contents intact, at each time point. Urine and faeces were collected and acidified to pH 3.0. All samples were frozen in liquid nitrogen and stored at −80°C. With the exception of plasma and urine, all samples were lyophilised prior to analysis.

2.3 Processing rat tissues, plasma and urine

Tissue samples from three individual rats at each time point were combined, extracted by homogenising 200 mg of lyophilised tissue with 2 mL of 50% aqueous methanol containing 1% formic acid using an Ultra-turrax homogeniser and further extracted by continuous shaking for 30 min. The mixture was centrifuged at $2000 \times g$ for 20 min, the supernatant decanted and the pellet re-extracted twice. The three supernatants were combined and reduced to dryness *in vacuo*. The extract was dissolved in 50 μL methanol in 1950 μL aqueous 1% formic acid and loaded on to a 2 g Sep-Pak C_{18} cartridge (Waters, Milford, MA, USA) which was washed with 4 mL acidified water (pH 30) before elution with 4 mL methanol containing 1% formic acid. The methanolic eluates were reduced to dryness and resuspended in 50 μL methanol in 950 μL aqueous 1% formic acid before analysis by HPLC-PDA-MS/MS. Plasma from individual rats was loaded directly onto the Sep-Pak C_{18} cartridge without extraction while urine from each animal was analysed directly without extraction or purification. Cyani-

din-3-*O*-sambubioside-5-*O*-glucoside was used as an internal standard.

2.4 HPLC-PDA-MS/MS

All samples were analysed in triplicate on a Surveyor HPLC system comprising of a HPLC pump, diode array detector scanning from 250 to 700 nm, and an autosampler set at 4°C (Thermo Finnigan, San Jose, USA) with separation carried out using a 250×4.6 mm^2 internal diameter 4 μm Synergi RP-Max column (Phenomenex, Macclesfield, UK) eluted at the flow rate of 1 mL/min. A mobile phase consisting of a 30 min 8–18% gradient of ACN in 1% aqueous formic acid was used for the analysis of all samples. After passing through the flow cell of the diode array detector the column eluate was split and 0.3 mL was directed to a LCQ Deca XP IT mass spectrometer fitted with an electrospray interface (Thermo Finnigan). Analysis was carried out with positive and negative ionisation operating in full-scan mode from 100 to 2000 amu. The tuning of the mass spectrometer was optimised by infusing a standard of cyanidin-3-*O*-glucoside and ellagic acid dissolved in methanol containing 1% formic acid, into the source at a flow rate of 0.25 mL/min. Capillary temperature was 350°C, sheath gas and auxiliary gas were 80 and 20 arbitrary units, respectively, source voltage was 5 kV. Identification of anthocyanins, their metabolites and other compounds in all samples was carried out using full-scan data-dependent MS/MS. Quantitative estimates of the anthocyanins and other phenolic compounds in the raspberries were based on the absorbance response at 520 and 280 nm. Anthocyanins were quantified by reference to cyanidin-3-*O*-glucoside as a standard. Ellagitannins were quantified by reference to a gallic acid standard curve and the data expressed in nmoles of sanguiin H-6 and lambertianin C.

2.5 GC-MS analysis of urine

Urine samples from individual rats were prepared as described by Olthof *et al.* [23] with slight modification. After thawing, 1.0 mL aliquots of urine were added to 4.0 mL of 0.2 M hydrochloric acid (HCl) containing 30 μg of 2,4,5 trimethoxycinnamic acid as an internal standard. A styrene divinyl benzene (SDB-L) (Phenomenex, USA) SPE cartridge was used for purification. Before loading the acidified urine, cartridge was preconditioned with 5 mL of ethyl acetate, followed by methanol (5 mL) and finally 5 mL 0.1 M HCl. After the addition of the extract the cartridge was washed with 5 mL of 0.1 M HCl before elution with 3 mL of ethyl acetate. The upper ethyl acetate phase was separated from the traces of aqueous phase and dried using an activated molecular sieve (Sigma) prior to being reduced to dryness. The extract was then redissolved in ethyl acetate and transferred to a silylated glass vial and further dried with nitrogen gas. Three hundred microliters of BSTFA + 1% TMCS was then added to the vial which was sealed and the sample silylated by heating at 80°C for 80 min. The vials were vortexed every 30 min to ensure complete silylation. Care was taken during preparation as the reagents and silylated derivatives are both highly sensitive to moisture. Samples were cooled in a closed, dry container prior to analysis by GC-MS (Trace DSQ, Thermo Finnigan). Phenolic acids were separated on a ZB-5MS $30\ m \times 0.25\ id \times 0.25\ \mu m$ capillary column (Phenomenex) with helium as a carrier gas (1.0 mL/min). The GC-MS conditions were as follows: injection volume (1 μL), initial temperature 80°C for 5 min then to 160°C at 10°C/min for 10 min and to 235°C at 5°C/min for 10 min; injector temperature (280°C), MS transfer line (290°C), ion source (200°C), split ratio (1:100). Mass spectra were scanned from *m/z* 50–650 at 0.82 scans/s. Electron impact energy was 70 eV. Identification of phenolic compounds in urine was based on the retention time and mass spectra of authentic standards and NIST98 mass spectral library. Quantifications were based on a standard curve of 2,4,5-trimethoxycinnamic acid (internal standard). All standards and samples were analysed in triplicate.

3 Results and discussion

3.1 Analysis of raspberry juice

The HPLC-PDA-MS/MS analysis of the raspberry juice resulted in the identification and quantification of nine anthocyanins, two ellagitannins and ellagic acid. The HPLC profiles of the extract at 280 and 520 nm are shown in Fig. 1. Identifications, which are summarised below were based on published data on the MS/MS fragmentation of raspberry phenolics [24, 25].

Peak 1 correspond to cyanidin-3,5-*O*-diglucoside (*m/z* 625 → 449, 287); *peak 2* correspond to cyanidin-3-*O*-sophoroside (*m/z* 611 → 287); *peak 3* was cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside) (*m/z* 757 → 611, 287); *peak 4* was cyanidin-3-*O*-glucoside (*m/z* 449 → 287); *peak 5* was pelargonidin-3-*O*-sophoroside (*m/z* 595 → 271); *peak 6* was cyanidin-3-*O*-xylosylrutinoside (*m/z* 727 → 581, 287); *peak 7* was cyanidin-3-*O*-rutinoside (*m/z* 595 → 287); *peak 8* correspond to pelargonidin-3-*O*-(2^G-*O*-glucosylrutinoside) (*m/z* 741 → 595, 271); *peak 9* was pelargonidin-3-*O*-glucoside (*m/z* 433 → 271); *peak 10* was the ellagintannin lambertianin C (*m/z* 2801 → 1869, 1567, 1265, 1251, 935, 633); *peak 11* was also an ellagitannin, sanguiin H-6 (*m/z* 1869 → 1567, 1265, 1235, 933, 631) and *peak 12* was ellagic acid (*m/z* 301 → 257).

The main raspberry anthocyanins are di- and triglycosides (Table 1) with the disaccharide cyanidin-3-sophoroside being the major anthocyanin (56% of the total anthocyanins) in the juice followed by the trisaccharide cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside) (23%) and the monosaccharide cyanidin-3-*O*-glucoside (11.1%). The ellagitannins

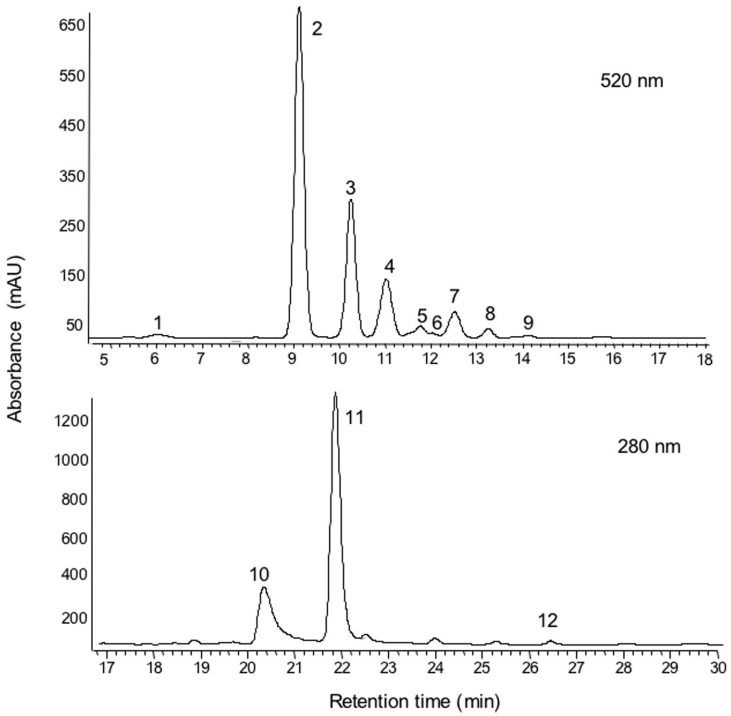

Figure 1. Gradient RP-HPLC of raspberry juice with detection at 520 and 280 nm. For identification by MS/MS and quantification of peaks 1 – 12 see text and Table 1.

Table 1. Quantities of anthocyanins, ellagitannins and ellagic acid in 2.77 mL of raspberry juice fed to rats by gavage[a)]

Peak number	R_t (min)	Compound	nmoles/rat
1	6.1	Cyanidin-3,5-diglucoside	8.7 ± 0.3
2	9.2	Cyanidin-3-sophoroside	513 ± 6
3	10.3	Cyanidin-3-(2^G-glucosylrutinoside)	211 ± 2
4	11.1	Cyanidin-3-glucoside	102 ± 2
5	11.8	Pelargonidin-3-sophoroside	14 ± 1.0
6	12.1	Cyanidin-3-xylosylrutinoside	5.5 ± 0.2
7	12.6	Cyanidin-3-rutinoside	46 ± 1
8	13.3	Pelargonidin-3-(2^G-glucosylrutinoside)	14 ± 1
9	14.2	Pelargonidin-3-glucoside	3.7 ± 0.2
		Total anthocyanins	**918**
10	20.3	Lambertianin C	71 ± 4
11	21.8	Sanguiin H-6	284 ± 5
		Total ellagitannins	**355**
12	26.3	Ellagic acid	36 ± 1

a) For HPLC traces and peak numbers see Fig. 1.

lambertianin C and sanguiin H-6 were also present along with ellagic acid as reported previously [25]. Ellagitannins are powerful antioxidants in raspberries [24] and acid treatment of extracts results in their breakdown and the release of ellagic acid. In addition to the compounds listed in Table 1 the raspberry juice also contained a number of mono-, di- and trisaccharide flavonol conjugates which appeared as HPLC peaks on the 365 nm trace. This is in keeping with the data of Mullen *et al.* [25]. However, these compounds were present in very low concentrations which made it impractical to monitor their fate in rats.

3.2 Ingestion of raspberry juice by rats

Each rat ingested 2.77 mL of raspberry juice containing 8.7 nmol cyanidin-3,5-*O*-diglucoside, 513 nmol cyanidin-3-*O*-sophoroide, 211 nmol cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside), 102 nmol cyanidin-3-*O*-glucoside, 14 nmol pelargonidin-3-*O*-sophoroside, 5.5 nmol of cyanidin-3-*O*-xylosylrutinoside, 46 nmol cyanidin-3-*O*-rutinoside, 14 nmol pelargonidin-3-*O*-(2^G-*O*-glucosylrutinoside) and 3.7 nmol of pelargonidin-3-*O*-glucoside. The 2.77 mL of juice, thus, contained a total of 918 nmol of anthocyanins as well as 355 nmol of ellagitannins and 36 nmol of ellagic

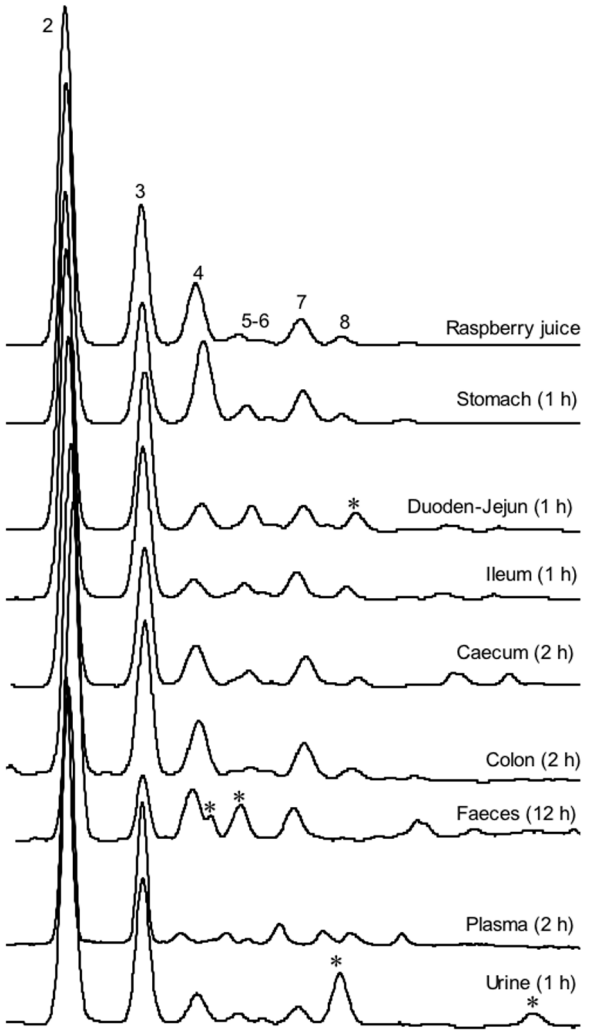

Figure 2. Gradient RP-HPLC with detection at 520 nm of raspberry juice and of rat tissues and fluids after ingestion of raspberry juice. For peak numbers see Table 1. Asterisk indicates a metabolite. Duoden–Jejun, duodenum–jejunum.

acid (Table 1). This supplement is equivalent to a 70 kg human consuming 700 mL of raspberry juice.

3.3 Fate of anthocyanins

Figure 2 provides an overview of the fate of raspberry juice anthocyanins after being fed to rats by gavage. The HPLC-520 nm anthocyanin profiles of plasma, urine, faeces and the gastrointestinal organs are very similar to that of the ingested juice, containing principally cyanidin-3-*O*-sophoroside, cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside) and cyanidin-3-*O*-glucoside. Putative metabolites were also present, but in trace amounts which, in most instances, precluded identification. Mass spectral information was, however, obtained on two minor urinary metabolites. The larger, earlier eluting 520 nm peak had a positively charged molecular ion ($[M + H]^+$) at *m*/*z* 625 which on MS/MS fragmented with a loss of 324 amu (cleavage of a sophorosyl unit) to yield an ion at 301 *m*/*z* which corresponds with methylcyanidin. In view of the presence of cyanidin-3-*O*-sophoroside in raspberries, this minor metabolite is probably its 3′-methylated derivative peonidin-3-*O*-sophoroside. The second metabolite in urine had a $[M + H]^+$ at *m*/*z* 463 which ionised with a 164 amu loss to produced an MS/MS fragment at *m*/*z* 301. This again is in keeping with a 3′-methylation resulting in the conversion of cyanidin-3-*O*-glucoside to peonidin-3-*O*-glucoside.

Quantitative estimates of the overall levels of anthocyanins in the rat tissues and fluids over a 24 h period after ingestion are presented in Table 2. Within the first hour 59.4% of the ingested anthocyanins were found in the ileum with 31.5% remaining in the stomach. After 2 h almost all the anthocyanins had left the stomach and moved to the ileum (85.9%). At the 1 and 2 h time points the overall recoveries of anthocyanins, almost exclusively from the gastrointestinal tract, were high at 99.6 and 97.0%, respectively, of intake. Three hours after ingestion more than 50% of the anthocyanins had disappeared and of the original dose 40.3% was still in the ileum with 6.5% distributed in the rest of the digestive tract. After 6 h only 2% of the anthocyanins remained principally in the caecum and colon in their native forms. Over a 24 h postingestion period, 1.5% of the anthocyanins appeared in faeces, 1.2% was excreted in urine and only traces were detected in plasma (Table 2).

The percentage recoveries of the three main raspberry anthocyanins, cyanidin-3-*O*-sophoroside, cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside) and cyanidin-3-*O*-glucoside in rat tissues, plasma, faeces and urine over a 24 h postgavage period are shown in Table 3. After 2 h there were only trace losses of the sophoroside and the glucosylrutinoside while cyanidin-3-*O*-glucoside declined by almost 40%. Arguably, this suggests that the monosaccharide may be more readily metabolised and/or absorbed that the di- and trisaccharide. However, there was no evidence of preferential increases in the low levels of cyandin-3-*O*-glucoside in either plasma or urine compared to those of cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside) (Table 4) implying that the initial decline in the glucoside in the gastrointestinal tract may due to metabolism, possibly conversion to peonidin-3-*O*-glucoside which is excreted in urine (see Table 5), rather than preferential absorption into the circulatory system.

Three hours after feeding, anthocyanin levels in the ileum had declined without concomitant increases further down the gastrointestinal tract in the caecum and colon (Table 3). Between 2 and 3 h there was also a fall in the overall recovery of the individual anthocyanins with values of 50% or less being obtained (Table 3). This trend continued with *ca.* 10% recoveries at 4 h, and at 6, 12 and 24 h losses were of the order of >98%; a pattern in keeping with

Table 2. Overall recovery of anthocyanins from organs, faeces, urine and plasma of rats after the ingestion of raspberry juice[a)]

Tissue/fluid	1 h	2 h	3 h	4 h	6 h	12 h	24 h
Stomach	289.6 ± 8.0	36.7 ± 3.9	33.3 ± 7.5	1.3 ± 0.0	0.1 ± 0.0	n.d.	n.d.
	(31.5 ± 0.9)	*(4.0 ± 0.4)*	*(3.6 ± 0.8)*	*(0.1 ± 0.0)*	*(0.0 ± 0.0)*	–	–
Duodenum/jejunum	78.7 ± 2.1	34.7 ± 2.3	8.8 ± 0.2	2.0 ± 0.1	n.d.	n.d.	n.d.
	(8.6 ± 0.2)	*(3.8 ± 0.2)*	*(1.0 ± 0.0)*	*(0.2 ± 0.0)*	–	–	–
Ileum	545.3 ± 12.5	788.7 ± 12.3	369.6 ± 25.1	65.5 ± 7.4	0.9 ± 0.0	0.6 ± 0.0	0.8 ± 0.0
	(59.4 ± 0.5)	*(85.9 ± 1.4)*	*(40.3 ± 2.8)*	*(7.1 ± 0.8)*	*(0.1 ± 0.0)*	*(0.1 ± 0.0)*	*(0.1 ± 0.0)*
Caecum	0.2 ± 0.0	24.8 ± 0.9	1.8 ± 0.1	21.3 ± 3.6	9.0 ± 2.2	1.0 ± 0.2	n.d.
	(0.0 ± 0.0)	*(2.7 ± 0.1)*	*(0.2 ± 0.0)*	*(2.3 ± 0.4)*	*(1.0 ± 0.2)*	*(0.1 ± 0.0)*	–
Colon	0.4 ± 0.2	1.5 ± 0.2	17.1 ± 1.0	10.2 ± 0.3	7.6 ± 1.5	0.3 ± 0.0	n.d.
	(0.0 ± 0.0)	*(0.2 ± 0.0)*	*(1.9 ± 0.1)*	*(1.1 ± 0.0)*	*(0.8 ± 0.2)*	*(0.0 ± 0.0)*	–
Faeces	n.d.	n.d.	n.d.	n.d.	n.d.	6.1 ± 2.7	6.9 ± 1.3
	–	–	–	–	–	*(0.7 ± 0.3)*	*(0.8 ± 0.1)*
Urine	0.8 ± 0.2	4.3 ± 1.9	0.5 ± 0.4	1.8 ± 0.5	1.1 ± 0.1	1.4 ± 0.4	0.7 ± 0.1
	(0.1 ± 0.0)	*(0.5 ± 0.2)*	*(0.0 ± 0.0)*	*(0.2 ± 0.1)*	*(0.1 ± 0.0)*	*(0.2 ± 0.1)*	*(0.1 ± 0.0)*
Plasma[b)]	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	n.d.	n.d.	n.d.	n.d.
	(0.0 ± 0.0)	*(0.0 ± 0.0)*	*(0.0 ± 0.0)*	–	–	–	–
Total	915.3 ± 12.5	890.8 ± 12.3	431.2 ± 25.1	102.1 ± 7.4	18.7 ± 0.0	9.4 ± 0.0	8.4 ± 0.0
	(99.6 ± 0.1)	*(97.0 ± 1.4)*	*(47.0 ± 2.8)*	*(11.1 ± 0.8)*	*(2.0 ± 0.0)*	*(1.0 ± 0.0)*	*(0.9 ± 0.0)*

a) Data presented as mean values in nmoles ± standard error ($n = 3$) and in italicised parentheses as a percentage of total anthocyanins ingested.

b) Data for plasma calculated on the basis of 12 mL of plasma *per* rat. n.d., not detected. No anthocyanins detected in brain, liver or kidney.

Table 3. Recovery of the three main anthocyanins from the gastrointestinal tract, faeces, plasma and urine of rats after ingestion of raspberry juice[a)]

Time	Anthocyanin	Stomach	Duod/jej	Ileum	Caecum	Colon	Faeces	Urine	Plasma	Total recovered
1 h	Cyan-3-Soph	29.9 ± 0.0	8.4 ± 0.0	61.6 ± 0.0	n.d.	0.1 ± 0.0	n.d.	0.09 ± 0.02	0.038 ± 0.008	100
	Cyan-3-Glc-Rut	26.5 ± 0.0	9.6 ± 0.0	66.5 ± 0.3	n.d.	n.d.	n.d.	0.10 ± 0.02	0.044 ± 0.012	103
	Cyan-3-Glc	43.8 ± 0.1	4.0 ± 0.0	19.1 ± 0.1	n.d.	n.d.	n.d.	0.04 ± 0.01	0.004 ± 0.001	67
2 h	Cyan-3-Soph	3.4 ± 0.0	3.5 ± 0.0	87.5 ± 0.5	2.6 ± 0.0	0.2 ± 0.0	n.d.	0.48 ± 0.21	0.019 ± 0.002	98
	Cyan-3-Glc-Rut	2.6 ± 0.0	3.5 ± 0.0	89.3 ± 0.5	2.6 ± 0.0	0.1 ± 0.0	n.d.	0.52 ± 0.23	0.022 ± 0.012	99
	Cyan-3-Glc	7.1 ± 0.0	3.1 ± 0.0	49.1 ± 0.2	1.9 ± 0.0	0.1 ± 0.0	n.d.	0.15 ± 0.07	0.000 ± 0.000	61
3 h	Cyan-3-Soph	3.4 ± 0.1	1.0 ± 0.0	42.4 ± 2.9	0.2 ± 0.0	2.5 ± 0.1	n.d.	0.06 ± 0.04	0.014 ± 0.004	50
	Cyan-3-Glc-Rut	2.4 ± 7.2	0.8 ± 0.0	39.7 ± 2.7	0.1 ± 0.0	2.7 ± 0.1	n.d.	0.06 ± 0.04	0.011 ± 0.001	46
	Cyan-3-Glc	7.2 ± 0.1	1.0 ± 0.0	28.8 ± 1.6	0.3 ± 0.0	3.0 ± 0.3	n.d.	0.02 ± 0.02	0.005 ± 0.005	40
4 h	Cyan-3-Soph	0.2 ± 0.0	0.3 ± 0.0	7.3 ± 0.8	2.2 ± 0.0	1.4 ± 0.1	n.d.	0.19 ± 0.06	n.d.	12
	Cyan-3-Glc-Rut	0.1 ± 0.0	0.2 ± 0.0	6.6 ± 0.7	1.8 ± 0.0	0.5 ± 0.0	n.d.	0.22 ± 0.08	n.d.	9.4
	Cyan-3-Glc	0.2 ± 0.0	0.1 ± 0.0	6.5 ± 0.8	2.6 ± 0.0	1.2 ± 0.1	n.d.	0.05 ± 0.02	n.d.	11
6 h	Cyan-3-Soph	n.d.	n.d.	0.1 ± 0.0	1.2 ± 0.0	1.0 ± 0.1	n.d.	0.13 ± 0.01	n.d.	2.4
	Cyan-3-Glc-Rut	n.d.	n.d.	0.1 ± 0.0	0.5 ± 1.9	0.4 ± 0.1	n.d.	0.15 ± 0.01	n.d.	1.1
	Cyan-3-Glc	n.d.	n.d.	0.1 ± 0.0	n.d.	1.3 ± 0.2	n.d.	0.06 ± 0.01	n.d.	1.5
12 h	Cyan-3-Soph	n.d.	n.d.	0.1 ± 0.0	0.1 ± 0.0	n.d.	0.7 ± 0.0	0.16 ± 0.08	n.d.	1.1
	Cyan-3-Glc-Rut	n.d.	n.d.	n.d.	n.d.	n.d.	0.3 ± 0.0	0.16 ± 0.08	n.d.	0.5
	Cyan-3-Glc	n.d.	n.d.	n.d.	0.2 ± 0.0	0.1 ± 0.0	1.5 ± 0.5	0.04 ± 0.02	n.d.	1.8
24 h	Cyan-3-Soph	n.d.	n.d.	0.1 ± 0.0	n.d.	n.d.	0.8 ± 0.0	0.08 ± 0.02	n.d.	1.0
	Cyan-3-Glc-Rut	n.d.	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.0	0.09 ± 0.01	n.d.	0.5
	Cyan-3-Glc	n.d.	n.d.	n.d.	n.d.	n.d.	0.7 ± 0.4	0.04 ± 0.01	n.d.	0.7

Cyan-3-Soph, cyanidin-3-*O*-sophoroside; Cyan-3-Glc-Rut, cyanidin-3-*O*-(2^{G}-*O*-glucosylrutinoside); Cyan-3-Glc, cyanidin-3-*O*-glucoside; Duod/jej, duodenum/jejunum; n.d., not detected.

a) Data for the three individual anthocyanins presented as a percentage of the amount ingested ± standard error ($n = 3$). Figures for plasma based on a total of 12 mL of plasma *per* rat.

Table 4. Concentration of anthocyanins from plasma of rats 0–24 h after the ingestion of raspberry juice[a)]

Anthocyanin	0 h	1 h	2 h	3 h	4, 6, 12 and 24 h
Cyan-3-Soph	n.d.	16.4 ± 3.4	8.2 ± 1.1	5.8 ± 1.5	n.d.
		(0.04 ± 0.008)	*(0.019 ± 0.002)*	*(0.014 ± 0.004)*	–
Cyan-3-Glc-Rut	n.d.	7.7 ± 2.1	3.8 ± 2.1	2.0 ± 0.2	n.d.
		(0.044 ± 0.012)	*(0.022 ± 0.012)*	*(0.011 ± 0.001)*	–
Cyan-3-Glc	n.d.	0.3 ± 0.1	n.d.	0.4 ± 0.4	n.d.
		(0.004 ± 0.001)	–	*(0.005 ± 0.005)*	–
Total	n.d.	24.4 ± 6.5	12.0 ± 2.8	8.2 ± 1.3	n.d.
		(0.04 ± 0.009)	*(0.017 ± 0.004)*	*(0.012 ± 0.002)*	–

Cyan-3-Soph, cyanidin-3-*O*-sophoroside; Cyan-3-Glc-Rut, cyanidin-3-*O*-(2^{G}-*O*-glucosylrutinoside); Cyan-3-Glc, cyanidin-3-*O*-glucoside; n.d., not detected.

a) Data presented as mean values in nM ± standard error ($n = 3$). Figures in italicised parentheses are data expressed as a percentage of the amount ingested calculated on the basis of a total of 12 mL of plasma in the circulatory system of each rat.

Table 5. Recovery of anthocyanins in the urine of rats 0–24 h after the ingestion of raspberry juice[a)]

Anthocyanin	0–1 h	1–2 h	2–3 h	3–4 h	4–6 h	6–12 h	12–24 h	Total
Cyan-3,5-diGlc	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cyan-3-Soph	0.44 ± 0.10	2.39 ± 1.04	0.25 ± 0.21	0.95 ± 0.29	0.55 ± 0.04	0.81 ± 0.22	0.36 ± 0.05	5.24 ± 1.17
	(0.09 ± 0.02)	*(0.48 ± 0.21)*	*(0.06 ± 0.04)*	*(0.19 ± 0.06)*	*(0.13 ± 0.01)*	*(0.16 ± 0.08)*	*(0.08 ± 0.02)*	*(1.17 ± 0.16)*
Cyan-3-Glc-Rut	0.20 ± 0.04	1.07 ± 0.47	0.11 ± 0.09	0.44 ± 0.16	0.25 ± 0.01	0.32 ± 0.09	0.16 ± 0.01	2.43 ± 0.55
	(0.10 ± 0.02)	*(0.52 ± 0.23)*	*(0.03 ± 0.01)*	*(0.22 ± 0.08)*	*(0.15 ± 0.01)*	*(0.16 ± 0.08)*	*(0.09 ± 0.01)*	*(1.25 ± 0.22)*
Cyan-3-Glc	0.04 ± 0.01	0.15 ± 0.07	0.03 ± 0.02	0.06 ± 0.02	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.01	0.39 ± 0.07
	(0.04 ± 0.01)	*(0.15 ± 0.07)*	*(0.02 ± 0.02)*	*(0.05 ± 0.02)*	*(0.06 ± 0.01)*	*(0.04 ± 0.02)*	*(0.04 ± 0.01)*	*(0.40 ± 0.04)*
Pel-3-Soph	0.01 ± 0.00	0.04 ± 0.02	0.01 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.01		0.10 ± 0.02
	(0.05 ± 0.02)	*(0.27 ± 0.11)*	*(0.10 ± 0.07)*	*(0.11 ± 0.02)*	*(0.13 ± 0.06)*	*(0.11 ± 0.06)*	n.d.	*(0.74 ± 0.07)*
Cyan-3-Xylo-Rut	0.00 ± 0.00	0.01 ± 0.01	n.d.	0.01 ± 0.00	n.d.	0.00 ± 0.01	n.d.	0.01 – 0.01
	(0.08 ± 0.06)	*(0.21 ± 0.23)*		*(0.19 ± 0.04)*		*(0.09 ± 0.22)*		*(0.36 ± 0.14)*
Cyan-3-Rut	0.03 ± 0.01	0.12 ± 0.05	0.01 ± 0.01	0.05 ± 0.02	0.03 ± 0.00	0.03 ± 0.01	n.d.	0.25 ± 0.06
	(0.05 ± 0.02)	*(0.23 ± 0.10)*	*(0.03 ± 0.02)*	*(0.10 ± 0.03)*	*(0.07 ± 0.01)*	*(0.07 ± 0.03)*		*(0.54 ± 0.08)*
Pel-3-Glc-Rut	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Peon-3-Soph (M)	0.07 ± 0.02	0.41 ± 0.18	0.05 ± 0.04	0.20 ± 0.04	0.17 ± 0.02	0.16 ± 0.04	0.11 ± 0.01	1.11 ± 0.21
Pel-3-Glc	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Peon-3-Glc (M)	0.026 ± 0.007	0.150 ± 0.074	0.070 ± 0.056	0.045 ± 0.009	0.015 ± 0.011	0.006 ± 0.012	n.d.	0.308 ± 0.040
Total	0.81 ± 0.18	4.34 ± 1.90	0.52 ± 0.37	1.76 ± 0.53	1.07 ± 0.07	1.40 ± 0.39	0.67 ± 0.07	9.71 ± 2.07
	(0.10 ± 0.02)	*(0.48 ± 0.21)*	*(0.04 ± 0.04)*	*(0.20 ± 0.06)*	*(0.14 ± 0.01)*	*(0.18 ± 0.06)*	*(0.08 ± 0.01)*	*(1.22 ± 0.17)*

Cyan-3-Soph, cyanidin-3-*O*-sophoroside; Cyan-3-Glc-Rut, cyanidin-3-*O*-(2^{G}-*O*-glucosylrutinoside; Cyan-3-Glc, cyanidin-3-*O*-glucoside; Cyan-3,5-diGlc, cyanidin-3,5,–*O*-diglucoside; Cyan-3-Rut, cyanidin-3-*O*-rutinoside; Cyan-Xylo-Rut, cyanidin-3-*O*-xylosylruinoside; Pel-3-Glc, pelargonidin-3-*O*-glucoside; Pel-3-Glc-Rut, pelargonidin-3-*O*-(2^{G}-*O*-glucosylrutinoside); Peon-3-Glc, peonidin-3-*O*-glucoside; M, metabolite; n.d., not detected.

a) Data presented as means values in nmoles ± standard error ($n = 3$) and in italicised parentheses as a percentage of the amounts of anthocyanin ingested.

bacterial degradation of anthocyanins in the colon and, possibly, also the caecum.

Analysis of purified extracts of brain, liver and kidneys of rats did not detect the presence of any anthocyanins or anthocyanin metabolites. Plasma contained low nM concentrations of cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-(2^{G}-*O*-glucosylrutinoside) and sub-nM levels of cyanidin-3-*O*-glucoside (Table 4). The levels of the cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-(2^{G}-*O*-glucosylrutinoside) were highest (T_{max}) 1 h after ingestion of the raspberry juice with respective C_{max} values of 16.4 ± 3.4 and 7.7 ± 2.1 nM. As the highest plasma anthocyanin concentrations occurred at the first time point, if earlier samples had been collected it is possible that a T_{max} of less than 1 h would have been obtained and that the C_{max} values may have been slightly higher. After 1 h the concentration of both anthocyanins declined rapidly. The elimination half-life ($T_{1/2}$) values were 1.33 h for cyanidin-3-*O*-sophoroside and 1.03 h for cyanidin-3-*O*-(2^{G}-*O*-glucosylrutinoside), with no anthocyanins being detected in plasma at the 4, 6, 12 and 24 h time points. The amounts of anthocyanins detected in plasma were very low indeed. On the basis of each rat containing a total of 12 mL of plasma, the 16.4 nM concentration of cyanidin-3-*O*-sophoroside present at T_{max} is equivalent to 0.038% of the

amount ingested and the figure for cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside) is 0.044%. The level of cyanidin-3-glucoside was *ca.* 10-fold lower (Table 4).

In keeping with the pharmacokinetic data obtained with plasma, the highest level of anthocyanin excretion in urine, 4.34 ± 1.90 nmol, occurred 1 – 2 h after feeding the raspberry juice (Table 5). The total urinary excretion over a 24 h period was 9.7 ± 2.1 nmol corresponding to 1.2 ± 0.2% of the anthocyanins ingested, a figure somewhat higher than that obtained in many anthocyanin-feeding studies. As mentioned previously, the urinary anthocyanin profile was very similar to that of raspberries with the exception that three minor components, cyanidin-3,5-*O*-diglucoside, pelargonidin-3-*O*-(2^G-*O*-glucosylrutinoside) and pelargonidin-3-*O*-glucoside which were not detected, while the methylated metabolites, peonidin-3-*O*-sophoroside and peonidin-3-*O*-glucoside were present in quantifiable amounts (Table 5). Overall, however, absorption and excretion were not associated with extensive metabolism of the raspberry anthocyanins.

It is of note that the levels of anthocyanins in plasma were highest 1 h after supplementation with the raspberry juice (Table 4) at which point most of the anthocyanins in the gastrointestinal tract were in the stomach (31.5%) and the ileum (59.4%) (Tables 2 and 3). Over the next hour the levels declined in the stomach and rose in the ileum to 85.9% of intake while the low concentrations in the bloodstream declined. This suggests that the albeit low absorption of anthocyanins occurs before reaching the ileum. This is in keeping with evidence obtained in other studies with rats and mice indicating that the stomach [26, 27] and the jejunum [28] are sites of anthocyanin absorption.

As noted above, anthocyanins were not detected in the brain of rats following the ingestion of the 2.77 mL supplement of raspberry juice that is equivalent to a 70 kg human consuming 700 mL of juice. Other studies have detected anthocyanins in rat brains after supplementation with berry and grape extracts. Andres-Lacueva *et al.* [29] daily fed rats a blueberry extract, containing an uncited amount of anthocyanins, for 8 – 10 weeks after which the animals exhibited enhanced special learning and memory in the Morris water maze test and trace levels of anthocyanins, which could not be quantified, were detected in the cerebellum, cortex and hippocampus, regions of the brain important for learning and memory. Extremely low concentrations of anthocyanins, 0.25 nmol/g, were also detected in rat brains after feeding a blackberry extract for 15 days [30]. The daily dose of anthocyanins was of the order of 318 μmol *per* rat which is equivalent to a 70 kg human eating a daily serving of *ca.* 18 kg of blackberries. It has also been reported that within 10 min of the introduction of a red grape extract, containing 3.8 μmol of anthocyanins (2 mg), into the stomach of rats, unmetabolised anthocyanins were detected in plasma (176 ng/mL) and also in the brain (192 ng/g) [31]. This intake corresponds to a 70 kg human consuming *ca.* 300 g of red grapes, an amount that is an intake in keeping with a normal dietary intake.

3.4 Fate of ellagitannins

Along with the anthocyanins, the raspberry juice fed to rats contained substantial amounts of the ellagitannins lambertianin C and sanguiin H-6 together with a smaller quantity of ellagic acid (Fig. 1 and Table 1). The ellagitannins disappeared rapidly and were not detected in plasma, urine, faeces, the stomach, duodenum/jejunum, ileum and other parts of the gastrointestinal tract, 1 h after ingestion and at all later time points. This contrasts with the anthocyanins which at 1 h were recovered intact, mainly in the stomach and ileum (Table 2). The absence of ellagitannins in the rats implies that breakdown of sanguiin H-6 and lambertianin C occurred in the acidic conditions of the stomach to such an extent that they had disappeared after 1 h. HPLC-MS/MS analysis of urine demonstrated an absence of urolithin B, hydroxyurolithin B and their glucuronide conjugates, compounds excreted by rats after a 37-day intake of a pomegranate husk extract containing very high quantities of the ellagitannin punicalagin [32].

Ellagic acid recovered in the stomach after 1 h was 9.6% of the amount present in the raspberry juice and after 2 h only traces were detected (Fig. 3). Except for these small amounts in the stomach, no ellagic acid was detected in any of the rat organs/tissues or fluids collected over a 24 h period after consumption of the raspberry juice. Degradation of the ellagitannins has the potential to release substantial amounts of ellagic acid. In theory, the ingested 71 nmol of lambertianin C and 284 nmol of sanguiin H-6 could give rise to 426 and 1136 nmol of ellagic acid, respectively. This would appear not to have occurred. An accurate picture of the fate of the ellagitannins is unlikely to be ascertained until radiolabelled derivatives become available.

3.5 GC-MS analysis of phenolic acids in urine

In view of the likely involvement of colonic bacteria in the disappearance of anthocyanins after the ingestion of raspberry juice a search was made of urine HPLC profiles within the 240 – 365 nm range for the accumulation of putative catabolites. Most of the traces were very complex and no clear-cut evidence of the presence of catabolites derived from raspberry anthocyanins was obtained. Protocatechuic acid (3,4-dihydroxybenzoic acid), vanillic acid (3-methoxy-4-hydroxybenzoic acid) and syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid) have been proposed as colonic breakdown products of anthocyanins [33 – 36] but HPLC-MS/MS analysis revealed that they were not present in detectable amounts in any of the urine samples collected in the present study. An alternative analytical strategy was therefore employed, based on the methods of Olthof *et al.* [23], which involved forming trimethylsilyl derivatives and

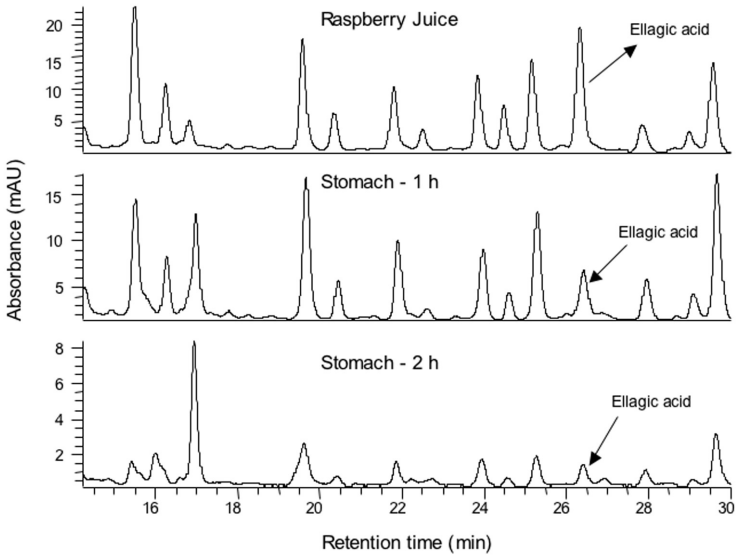

Figure 3. Gradient RP-HPLC-365 nm profile of raspberry juice and extracts of rat stomachs collected 1 and 2 h after the ingestion of the raspberry juice.

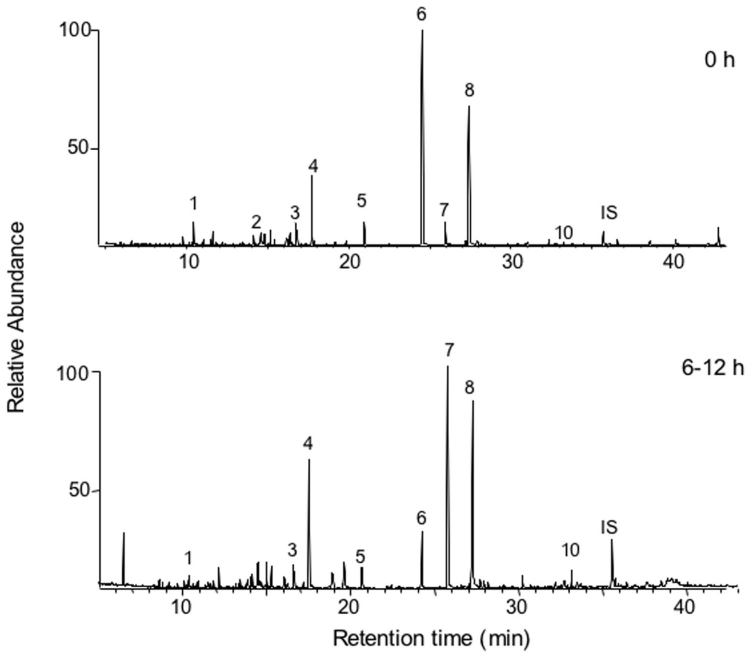

Figure 4. GC-MS TIC traces of urine collected from rats prior to feeding raspberry juice by gavage supplementation (blank) and 6–12 h after supplementation. For identity of peaks see Table 6. IS, internal standard.

analysing samples by capillary GC-MS. Representative traces obtained are presented in Fig. 4, and the identification of the compounds detected is summarised in Table 6, together with quantitative estimates of the amounts excreted over a 24 h post-gavage period.

Nine compounds were identified in the urine samples but, in most instances, the amounts excreted over the 0–24 h collection period were well in excess of the 0.9 μmol of ingested anthocyanins (Table 6). While 4-hydroxybenzoic acid and 4-hydroxyphenylacetic acid may be anthocyanin catabolites, this is speculative and definitive information will require feeding studies to be carried out using ring-labelled ^{14}C-anthocyanins. As far as ellagitannins are concerned, each rat consumed 71 nmol of lambertianin C and 284 nmol of sanguiin H-6 but neither of these compounds entered the large intestine and are, therefore, unlikely to be degraded to phenolic acids by the colonic microflora.

3.6 Stability of anthocyanins in the gastrointestinal tract

Anthocyanins are readily distinguished from other flavonoids as they undergo rearrangements in response to pH. The red flavylium cation predominates at pH 1–3 but as the pH increases to 4 and above the colourless carbinol pseudo-

Table 6. Identification and quantification of phenolic compounds and other products in rat urine 0 – 24 h after ingestion of raspberry juice[a)]

Peak number	t_R (min)	Compound	Blank	0 – 1 h	1 – 2 h	2 – 4 h	4 – 6 h	6 – 12 h	12 – 24 h	Total (0 – 24 h)
1	14.11	*n*-Glycine	0.6 ± 0.2	n.d.	n.d.	0.1 ± 0.1	n.d.	n.d.	1.2 ± 0.1	1.3 ± 0.3
2	17.24	4-Hydroxybenzoic acid	0.2 ± 0.0	0.2 ± 0.0	0.8 ± 0.1	n.d.	0.3 ± 0.1	0.5 ± 0.2	n.d.	1.8 ± 0.2
3	17.61	4-Hydroxyphenylacetic acid	1.9 ± 0.6	0.6 ± 0.0	2.9 ± 1.0	0.3 ± 0.1	1.6 ± 0.3	3.0 ± 0.6	1.8 ± 0.1	10.2 ± 0.6
4	20.69	Benzenepropanoic acid	0.9 ± 0.4	n.d.	1.9 ± 1.2	0.0 ± 0.0	n.d.	0.5 ± 0.0	0.5 ± 0.2	2.9 ± 0.4
5	24.25	Hippuric acid (tautomeric form)	19.2 ± 13.2	n.d.	0.7 ± 0.5	0.3 ± 0.1	2.0 ± 0.7	5.7 ± 3.2	7.5 ± 1.7	16.2 ± 1.8
6	25.76	Hippuric acid	1.5 ± 0.7	0.2 ± 0.0	9.2 ± 4.4	0.3 ± 0.1	1.7 ± 0.5	3.5 ± 1.0	0.6 ± 0.0	15.5 ± 2.0
7	27.23	Phenylacetylaminoacetic acid	6.9 ± 2.1	0.3 ± 0.0	6.8 ± 5.9	0.6 ± 0.3	10.1 ± 1.9	11.0 ± 3.0	9.7 ± 1.2	38.5 ± 2.8
8	30.87	1,2-Benzenedicarboxylic acid	n.d.	0.6 ± 0.1	1.5 ± 0.4	n.d.	n.d.	n.d.	n.d.	2.1 ± 0.4
9	33.11	Ferulic acid	0.2 ± 0.1	0.1 ± 0.0	1.0 ± 0.8	n.d.	n.d.	0.4 ± 0	n.d.	1.5 ± 0.2

a) Data for individual expressed as μmol ± standard error ($n = 3$). Blank is urine collected for a 3 h period prior to supplementation. For peak numbers and GC-MS traces see Fig. 4. t_R (min), GC retention time in minutes; n.d. not detected.

base is the major component along with smaller amounts of the colourless chalcone pseudobase and the blue quinoidal base [37]. Anthocyanins are traditionally extracted and analysed in acidic medium as the red flavylium cation as this is the most stable form. However, it is not known what forms predominate *in vivo*. The limited available experimental evidence indicates that in the acidic conditions that prevail in the stomach anthocyanins are in the red flavylium form but once they enter more basic conditions in the small intestine the carbinol pseudobase is likely to predominate [15]. To what extent this occurs and what influence it has on absorption remains to be determined. In the present study, extracting the gastrointestinal tract and its contents with acidic methanol resulted in high recoveries of the flavylium cation from the duodenum, jejunum and ileum in the initial 2 h after ingestion of the raspberry juice (Tables 2 and 3). However, it does not follow that *in vivo* the anthocyanins were in this form and, due to an absence of appropriate analytical procedures, nothing is known about either the metabolism or absorption of the pseudobases or the quinoidal base in the gastrointestinal tract. It could be that after anthocyanins leave the stomach, the colourless carbinol pseudobase becomes the main form, in the small intestine where it undergoes very limited absorption. As a consequence, significant amounts pass into the large intestine where degradation, to as yet undetermined products, occurs due to the action of colonic bacteria. This would be in keeping with the data obtained in this study but other more subtle scenarios may exist.

4 Concluding remarks

Rats were fed by gavage a single 2.77 mL supplement of raspberries' juice, containing anthocyanins and ellagitannins – a dose equivalent to a 70 kg human drinking 700 mL of juice. One hour after feeding the ellagitannins, sanguiin H-6 and lambertianin C had disappeared with only traces of ellagic acid being detected in the stomach. Up to 2 h after supplementation there was a very high recovery of unmetabolised anthocyanins, principally cyanidin-3-*O*-sophoroside, cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside) and cyanidin-3-*O*-glucoside, as they passed from the stomach to the duodenum/jejunum and ileum. After 3 h, less than 50% was recovered, after 4 h this declined to 11% of intake and after 6 h only 2% remained. Only trace quantities of anthocyanins were detected in the caecum, colon and faeces and they were absent in extracts of liver, kidneys and brain.

After 1 h low nM concentrations of cyanidin-3-*O*-sophoroside, cyanidin-3-*O*-(2^G-*O*-glucosylrutinoside) and cyanidin-3-*O*-glucoside were detected in plasma but these declined by 2 h and were not present in detectable quantities 4 h after feeding. Excretion of the three main raspberry anthocyanins in urine over a 24 h period after feeding was equivalent to 1.2% of the amounts ingested. These findings imply anthocyanins are poorly absorbed and that this occurs before they reach the ileum, in keeping with evidence indicating that the stomach [26, 27] and the jejunum [28] are sites of anthocyanin absorption in mice and rats. Because anthocyanins are poorly absorbed substantial amounts pass from the small to the large intestine where their rapid disappearance suggests they are degraded by faecal bacteria. GC-MS analysis of urine detected a number of phenolic acids but it was not possible to determine which were catabolites derived from the raspberry juice as most were present in quantities well in excess of the 918 nmol of anthocyanins that were ingested.

More complete information on what happens to anthocyanins and ellagitannins after ingestion would be greatly assisted by the availability of radiolabelled derivatives which would enable the fate of these compounds to be monitored in animal test systems in much the same way as

[2-^{14}C]quercetin-4′-*O*-glucoside has been used to provide a detailed insight of flavonol glucoside bioavailability in rats [38 – 40].

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