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Research Article

Yoghurt impacts on the excretion of phenolic acids derived from colonic breakdown of orange juice flavanones in humans

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Human urine was collected over a 24 h period after the consumption of 250 mL of (i) water, (ii) orange juice, and (iii) orange juice plus 150 mL of full fat natural yoghurt. The orange juice contained 168 μmol of hesperetin-7-*O*-rutinoside and 18 μmol of naringenin-7-*O*-rutinoside. GC-MS analysis of the urine identified nine phenolic acids, five of which, 3-hydroxyphenylacetic acid, 3-hydroxyphenylhydracrylic acid, dihydroferulic acid, 3-methoxy-4-hydroxyphenylhydracrylic acid and 3-hydroxyhippuric acid, were associated with orange juice consumption indicating that they were derived from colonic catabolism of hesperetin-7-*O*-rutinoside. The overall 0 – 24 h excretion of the five phenolic acids was 6.7 ± 1.8 μmol after drinking water and this increased significantly ($p < 0.05$) to 62 ± 18 μmol, equivalent to 37% of the ingested flavanones, following orange juice consumption. When the orange juice was ingested with yoghurt excretion fell back markedly to 9.3 ± 4.4 μmol. This was not due to a difference in mouth to caecum transit time, as measured with breath hydrogen production, though possibly there may have been a slowing of the bulk of the meal reaching the large intestine which may then have altered the catabolism of the flavanones to phenolic acids by the colonic microbiota.

Keywords: Colonic catabolism / Flavanones / Orange juice / Urinary phenolic acids / Yoghurt

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1 Introduction

Flavanones occur predominantly in citrus species. Their presence in fruits of orange (*Citrus sinensis*), lemon (*C. limon*) lime (*C. aurantifolia*) and grapefruit (*C. paradisi*) [1], as well as many commercial fruit juices and drinks [2], almost certainly results in the daily intake of flavanones for some individuals being well in excess of the estimated 37 mg *per* person *per* day in Finland [3]. Citrus consumption has been associated with reduced risk of cancers [4, 5] and cardiovascular disease [6]. These protective effects could be due to the antioxidant properties of flavanones [7] as well as their platelet anti-aggregation and anti-adhesive activity [8] and the modulation of cell signalling pathways involved in neuronal apoptosis [9], cancer, atherosclerosis and various inflammatory conditions [10]. In many parts of the world the most commonly consumed citrus fruit are oranges. The main flavanones in oranges are hesperetin-7-*O*-rutinoside (aka hesperidin) and naringenin-7-*O*-rutinoside (aka narirutin) (Fig. 1) [11].

The bioavailability of the flavanones in humans has been investigated by a number of groups [12 – 16]. Following the ingestion of orange juice hesperetin-7-*O*-rutinoside appears not to be absorbed in the small intestine but passes to the large intestine where the sugar moiety is cleaved by the action of colonic bacteria. Some of the released aglycone is absorbed into the circulatory system where sub-micromolar concentrations of hesperetin-7-*O*-glucuronide and an uncharacterised hesperetin-*O*-glucuronide appear transitorily, reaching a peak plasma concentration around 5 h after consumption of the juice [17]. The same glucuronides, along with diglucuronide and sulphoglucuronide metabolites are excreted in urine 0 – 24 h after ingestion, in quantities corresponding to *ca.* 5% of flavanone intake [17]. Fla-

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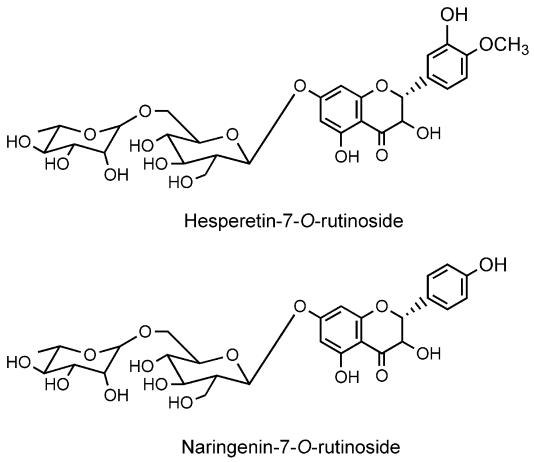

Figure 1. Structures of orange juice flavanones.

vanone aglycones remaining in the large intestine are degraded by the colonic microflora [18 – 21] with an undetermined portion of the resultant phenolic acids being absorbed into the circulatory system via the portal vein prior to urinary excretion.

The objective of this study was to investigate the catabolism of hesperetin-7-*O*-rutinoside following acute supplementation with orange juice, with and without yoghurt. The phenolic acid content of urine samples collected 0 – 24 h after ingestion was analysed by GC-MS. This was part of an investigation into the influence of other food components on the absorption of dietary flavonoids, the so-called matrix effect, with parallel research having determined that while yoghurt caused a significant delay in the initial 0 – 5 h excretion of flavanone metabolites, overall it did not have a statistically significant impact on either plasma pharmacokinetic profiles or 0 – 24 h urinary excretion [17]. An earlier publication has provided information on the bioavailability of pelargonidin-3-*O*-glucoside following the ingestion of strawberries with and without cream [22] while there has been much debate, and disagreement, about the impact of milk on plasma flavan-3-ol and antioxidant levels after the consumption of tea and chocolate products [23 – 31].

2 Materials and methods

2.1 Materials

Own brand full-fat natural yoghurt (3.8% fat), and orange juice were purchased from a local supermarket (Somerfield's, Byres Rd, Glasgow, UK). 4-Hydroxybenzoic acid was obtained from Aldrich (Poole, Dorset, UK), 3-hydroxyphenylacetic acid and 4-hydroxyphenylacetic acid from Fluka (Gillingham, Dorset, UK), and 2,4,5-trimethoxycinnamic acid, hippuric acid and 3-methoxy-4-hydroxyphenylacetic acid from Sigma (Poole, Dorset, UK). Molecular sieves and derivatisation reagent (*N*,*O*-bis(trimethylsilyl)-acetamide (BSTFA) + 1% trimethylchlorosilane (TMCS)) were also purchased from Sigma. Dihydroferulic acid was obtained from Alfa Aesar (Heysham, Lancs, UK). Methanol and ethyl acetate were obtained from Rathburn Chemicals (Walkerburn, Borders, UK). Hesperetin-7-*O*-rutinoside was purchased from Extrasynthase (Genay, France).

2.2 Study design

Five healthy volunteers participated in each of the three aspects of this study. The study protocol was approved by the Glasgow Royal Infirmary Ethics Committee. Subjects (21 – 50 years of age, mean BMI 23.1, range 19.9 – 27.2, healthy, nonsmokers, not pregnant and not on any medication), were required to follow a low flavonoid diet for 2 days prior to the study, avoiding fruits, vegetables, high fibre products and beverages such as tea, coffee, fruit juice and wine. Three sets of experiments were carried out with subjects ingesting either (i) orange juice (ii) orange juice with yoghurt or (iii) a control with water. Following two days on the low flavonoid diet and after an overnight fast, volunteers consumed 250 mL of orange juice fortified with 131 μmol of hesperetin-7-*O*-rutinoside and 4 wk later under identical conditions ingested the same supplement together with 150 mL of natural yoghurt. In the control study, a different group of volunteers (31 – 38 years of age, mean BMI 30.9, range 24.2 – 46.9, healthy, nonsmokers and not on any medication), followed a low flavonoid diet for 2 days, and after an overnight fast drank 250 mL of water.

Urine was collected prior to supplementation and over four time periods, 0 – 2, 2 – 5, 5 – 10 and 10 – 24 h, after the ingestion of the orange juice. The total volume for each period was recorded. After collection, urine samples were acidified to pH 3 with formic acid and aliquots stored at –80 °C prior to analysis.

2.3 GC-MS analysis of urine

After thawing, 1.0 mL aliquots of urine were added to 4.0 mL of 0.2 M HCl containing 30 μg of 2,4,5-trimethoxycinnamic acid as an internal standard. A styrene divinylbenzene (SDB-L) (Phenomenex, Macclesfield, UK) SPE cartridge was used for purification. Before loading the acidified urine, the 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 activated molecular sieves 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 microlitre 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 was vortexed every 30 min to ensure

Table 1. GC retention time and characteristic MS ions of phenolic compounds in urine samples of humans after drinking 250 mL of orange juice containing 168 μmol hesperetin-7-*O*-rutinoside and 12 μmol naringenin 7-*O*-rutinoside

Peak	Compounds	t_R (min)	Base ion (*m/z*)	Qualifier ion (*m/z*)	Basis of identification
1	3-Hydroxyphenylacetic acid	17.47	164	281; 296	Standard, NIST
2	4-Hydroxybenzoic acid	18.06	267	223; 193	Standard, NIST
3	4-Hydroxyphenylacetic acid	18.47	296	281; 252	Standard, NIST
4	3-Methoxy-4-hydroxyphenylacetic acid	24.13	209	326; 267	Standard
5	Hippuric acid	26.88	105	206; 308	Standard, NIST
6	3-Hydroxyphenylhydracrylic acid	27.89	267	398; 147	NIST
7	Dihydroferulic acid	28.71	340	209; 192	Standard, NIST
8	3-Methoxy-4-hydroxyphenylhydracrylic acid	31.59	297	298; 73	NIST
9	3-Hydroxyhippuric acid	35.09	294	193; 73	NIST

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 × 0.25 mm (i.d.) 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.

2.4 Statistical analysis

Each sample was analysed in triplicate and data are presented as mean values ± standard error ($n = 3$). Where appropriate, data were subjected to statistical analysis using paired and unpaired *t*-test with Minitab software, version 13 (Minitab, Addison-Wesley Publishing, Reading, MA, USA)

3 Results

3.1 Flavanones in orange juice

Data on the flavanone content of the orange juice was obtained from Mullen *et al*. [17]. A 250 mL sample of juice contained 168 μmol of hesperetin-7-*O*-rutinoside and 12 μmol of naringenin-7-*O*-rutinoside of which 131 μmol hesperetin-7-*O*-rutinoside was added to make a total flavanone content of 180 μmol. This is broadly similar to anthocyanin, flavonol and flavan-3-ol intakes in feeding studies previously carried out with strawberries [22], onions [32], tomato juice [33] and green tea [34].

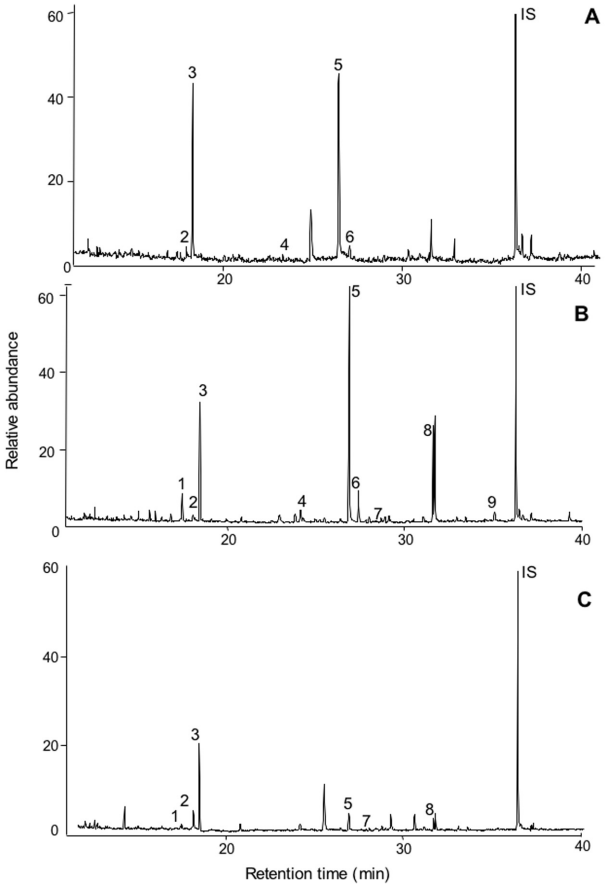

Figure 2. GC-MS traces of phenolic acids in human urine collected 10 – 24 h after supplementation with (A) – 250 mL water (B) – 250 mL orange juice with 150 mL yoghurt and (C) – 250 mL orange juice. The orange juice contained 168 μmol hesperetin-7-*O*-rutinoside and 12 μmol naringenin 7-*O*-rutinoside. For identification of peaks, refer to Table 1.

3.2 Excretion of phenolic acids

Typical GC traces obtained with urine collected after ingestion of water, orange juice with and without yoghurt are illustrated in Fig. 2. The basis of the identification of nine phenolic acids is summarised in Table 1 with quantitative

Table 2. Quantities of phenolic acids excreted in human urine 0 – 2, 2 – 5, 5 – 10 and 10 – 24 h after drinking 250 mL of either water or 250 mL of orange juice, containing 168 μmol hesperetin-7-*O*-rutinoside and 12 μmol naringenin-7-*O*-rutinoside, with or without 150 mL of natural yoghurt[a)]

GC peak	Compounds	Drink	0 – 2 h	2 – 5 h	5 – 10 h	10 – 24 h	Total
1	3-Hydroxyphenylacetic acid	W	n.d.	n.d.	n.d.	n.d.	n.d.
		O	0.1 ± 0.1	0.3 ± 0.3	1.8 ± 0.8	5.1 ± 2.0	7.3 ± 3.0
		O – Y	0.3 ± 0.3	0.2 ± 0.2	0.3 ± 0.3	3.2 ± 2.6	3.9 ± 2.6
2	4-Hydroxybenzoic acid	W	2.9 ± 1.6	3.2 ± 0.9	3.8 ± 1.9	3.5 ± 1.7	13 ± 2
		O	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.5 ± 0.4	0.7 ± 0.4
		O – Y	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
3	4-Hydroxyphenylacetic acid	W	80 ± 48	70 ± 26	57 ± 23	64 ± 13	271 ± 106
		O	7.3 ± 3.8	5.5 ± 2.3	73.2 ± 28.0	67.1 ± 15.2	153 ± 38
		O-Y	5.2 ± 2.9	2.7 ± 0.8	9.0 ± 4.2	31.2 ± 15.5	48 ± 15
4	3-Methoxy-4-hydroxyphenylacetic acid	W	4.3 ± 1.9	4.6 ± 2.0	3.6 ± 1.6	5.6 ± 1.8	18 ± 7
		O	0.4 ± 0.3	0.5 ± 0.3	3.6 ± 0.8	7.2 ± 1.7	11.7 ± 2.6
		O – Y	n.d.	n.d.	n.d.	n.d.	n.d.
5	Hippuric acid	W	27 ± 10	29 ± 11	26 ± 9	56 ± 21	137 ± 43
		O	5.5 ± 4.3	4.4 ± 2.4	52.1 ± 19.2	93.1 ± 32.2	155 ± 53
		O – Y	15.1 ± 11.3	5.8 ± 2.9	2.9 ± 2.2	15.7 ± 8.8	39.4 ± 15.2
6	3-Hydroxyphenylhydracrylic acid	W	1.8 ± 0.7	1.1 ± 0.5	1.2 ± 0.2	2.7 ± 0.8	6.7 ± 1.8
		O	0.1 ± 0.1	0.3 ± 0.2	5.5 ± 1.7	11.4 ± 3.5	17.2 ± 4.2
		O – Y	0.3 ± 0.3	n.d.	0.3 ± 0.3	0.7 ± 0.7	1.2 ± 0.6
7	Dihydroferulic acid	W	n.d.	n.d.	n.d.	n.d.	n.d.
		O	0.1 ± 0.1	1.0 ± 0.4	2.8 ± 1.2	2.4 ± 1.4	6.2 ± 2.0
		O – Y	0.2 ± 0.2	n.d.	0.0 ± 0.0	1.8 ± 1.7	2.0 ± 1.8
8	3-Methoxy-4-hydroxyphenylhydracrylic acid	W	n.d.	n.d.	n.d.	n.d.	n.d.
		O	n.d.	0.2 ± 0.2	13.8 ± 6.7	11.9 ± 5.6	25.9 ± 11.4
		O – Y	n.d.	0.3 ± 0.3	0.9 ± 0.5	1.0 ± 0.4	2.2 ± 1.1
9	3-Hydroxyhippuric acid	W	n.d.	n.d.	n.d.	n.d.	n.d.
		O	0.2 ± 0.2	n.d.	1.9 ± 0.7	2.9 ± 1.0	5.0 ± 1.4
		O – Y	n.d.	n.d.	n.d.	n.d.	n.d.

n.d., not detected; W, water; O, orange juice; O–Y, orange juice with yoghurt.
a) Data expressed in micromoles as mean values ± standard error ($n = 5$).

estimates of the amounts excreted 0 – 24 h after supplementation presented in Table 2. Excretion of 4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid were lower in subjects who drank orange juice than occurred with volunteers who ingested only water, indicating that these phenolic acids are not derived from the breakdown of flavanones. Hippuric acid was present in substantial amounts in urine collected after the ingestion of water and the quantity excreted after drinking orange juice increased, but was not significantly higher, indicating that it was not a major flavanone catabolite. Yoghurt did, however, impact on the excretion of hippuric acid as a much reduced amount was detected in urine after the ingestion of orange juice with yoghurt.

Despite the high background of phenolic acids, it was possible to establish that 3-hydroxyphenylacetic acid, 3-hydroxyphenylhydracrylic acid, dihydroferulic acid, 3-methoxy-4-hydroxyphenylhydracrylic acid and 3-hydroxyhippuric acid were all present in greater quantities in urine collected after drinking orange juice than after supplementation with water and the levels fell when orange juice was consumed with yoghurt (Table 2). When only orange juice was ingested the main excretion period for all five of these phenolic acids was 5 – 10 h and 10 – 24 h which is in keeping with a colonic origin.

The overall 0 – 24 h excretion of the five phenolic acids identified as being derived principally from hesperetin-7-*O*-rutinoside was 6.7 ± 1.8 μmol following consumption of water and this increased significantly ($p < 0.05$) to 62 ± 18 μmol after drinking orange juice. When orange juice and yoghurt were ingested together phenolic acid excretion fell to 9.3 ± 4.4 μmol, a value which was not significantly different from that obtained after subjects consumed water (Table 3).

4 Discussion

In a parallel study in which orange juice containing similar quantities of flavanones were ingested with and without yoghurt, data were obtained indicating that hesperetin-7-*O*-rutinoside was absorbed in the large intestine and appeared in plasma in sub-micromolar concentrations as hesperetin-7-*O*-glucuronide and an uncharacterised hesperetin-*O*-glucuronide [17]. The same glucuronides, along with diglucuronide and sulphoglucuronide metabolites are excreted in

Table 3. Quantities of key phenolic acids excreted in human urine 0–24 h after drinking 250 mL of water or 250 mL of orange juice, containing 168 μmol hesperetin-7-*O*-rutinoside and 12 μmol naringenin-7-*O*-rutinoside, with and without 150 mL of yoghurt[a)]

	0–2 h	2–5 h	5–10 h	10–24 h	Total (0–24 h)
Water	1.8 ± 0.4	1.1 ± 0.2	1.2 ± 0.2	2.7 ± 0.5	6.7 ± 1.8[A]
Orange juice	0.5 ± 0.0	1.7 ± 0.2	26 ± 2	34 ± 12	62 ± 18[B]
Orange juice with yoghurt	0.7 ± 0.1	0.4 ± 0.1	1.5 ± 0.2	6.7 ± 0.5	9.3 ± 4.4[A]

a) Data were expressed in μmol as mean values ± standard error ($n = 5$). Quantifications based on the combined levels of 3-hydroxyphenylacetic acid, 3-hydroxyphenylhydracrylic acid, dihydroferulic acid and 3-methoxy-4-hydroxyphenylhydracrylic acid and 3-hydroxyhippuric acid presented in Table 2. Means followed by different superscript letters are significantly different at $p < 0.05$.

urine 0–24 h after ingestion in quantities corresponding to *ca.* 5% of flavanone intake. Except for an initial delay in excretion, yoghurt did not have a significant effect on either plasma or urinary levels of the hesperetin metabolites. In contrast, the present study has demonstrated that yoghurt has a marked impact on the excretion of phenolic acids originating from the breakdown of the orange juice flavanones by the colonic microflora.

Increased excretion of five phenolic acids, 3-hydroxyphenylacetic acid, 3-hydroxyphenylhydracrylic acid, dihydroferulic acid, 3-methoxy-4-hydroxyphenylhydracrylic acid and 3-hydroxyhippuric acid, was associated with orange juice consumption (Tables 2 and 3). The main excretion period for all five of these phenolic acids was 10–24 h after juice consumption (Table 2), which is in keeping with colonic bacteria-mediated degradation of hesperetin-7-*O*-rutinoside with the resultant phenolic acids being absorbed into the portal vein and subsequently excreted in urine. The overall level of the five phenolic acids excreted 0–24 h after drinking water was 6.7 ± 1.8 μmol and this rose to 62 ± 18 μmol, equivalent to 37% of the ingested flavanones, following orange juice consumption (Table 3). When the orange juice was ingested with yoghurt, phenolic acid excretion fell back markedly to 9.3 ± 4.4 μmol. This did not appear to be due to a difference in mouth to caecum transit time as measured previously by breath hydrogen production [17]. However, as reported earlier, there was a significant delay in the initial appearance of flavanone metabolites in urine [17], so arguably there may have been a slowing of the bulk of the meal reaching the large intestine which may then have altered the catabolism of the flavanones to phenolic acids by the colonic microbiota. Exactly how this is brought about is a topic that requires further investigation.

High amounts of hippuric acid, 137 ± 43 μmol, were detected in 0–24 h urine from subjects who consumed only water and there was slight but nonsignificant rise to 155 ± 53 μmol following ingestion of orange juice (Table 2). Against the high background of the water control, it is not possible to ascertain whether or not a small portion of the hippuric acid was derived from catabolism of hesperetin-7-*O*-rutinoside when orange juice was ingested. The high excretion of hippuric acid in the water controls is probably due to the production of hippuric acid from compounds such as benzoic acid, quinic acids [35], tryptophan, tyrosine and phenylalanine [36–38]. Yoghurt impacted on hippuric acid excretion when it was consumed with orange juice with levels falling to 39.4 ± 15.2 μmol.

In vitro faecal breakdown and *in vivo* animal studies involving the ingestion of hesperetin, has been associated with the production of 3-(3-hydroxy-4-methoxy)phenylpropionic acid, 3-(3,4-dihydroxyphenyl)propionic acid, phloroglucinol (1,3,5-trihydroxybenzene) 3-(3-hydroxyphenyl)-propionic acid [21, 39]. However, in the present human feeding study, none of these compounds were detected in urine. The phenolic acids that accumulated in urine after orange juice consumption may have originated from breakdown of hesperetin-7-*O*-rutinoside via the pathways illustrated in Fig. 3. In this scheme, degradation of hesperetin-7-*O*- catabolism starts with deglycosylation to form hesperetin [40]. The C-ring is then opened by cleavage of the ether-*O*-linkage followed by dehydrogenation resulting in the formation of 3-methoxy-4-hydroxyphenylhydracrylic acid. This C–C cleavage probably occurs between the ether-*O*-linkage and the A-ring and between C4 and the A ring as illustrated in Fig. 3. As no phloroglucinol was detected in urine, cleavage of the ether-*O*-linkage and C2, and of C4 and the A ring, are unlikely. 3-Hydroxyphenylhydracrylic acid may be produced from the same C–C cleavage of the hesperetin C-ring followed by *O*-demethylation. Alternatively, it could also arise from *O*-demethylation of 3-methoxy-4-hydroxyphenylhydracrylic acid. These two compounds may then link to dihydroferulic acid, 3-hydroxyhippuric acid and 3-hydroxyphenylacetic acid via the routes indicated in Fig. 3. Most of these steps probably occur in the large intestine mediated by the colonic microflora. *Enterobacter* species are among the colonic bacteria that are able to hydrolyse a rhamnosyl moiety by releasing the α- and β-rhamnosidases to cleave the sugar moiety [20], while a number of human intestinal bacteria such as *Eubacterium limosum* and members of genera *Enterobacter* and *Escherichia* have *O*-demethylase activity [41–43]. However, some postabsorption metabolism may also occur as a result of the involvement of hepatic enzymes such as *O*-methyltransferases.

It is of interest to note that in contrast to phenolic acids derived from hesperetin-7-*O*-rutinoside, potential catabolites of naringenin-7-*O*-rutinoside, the minor flavanone in the orange juice, such as 4-hydroxyphenylpropionic acid,

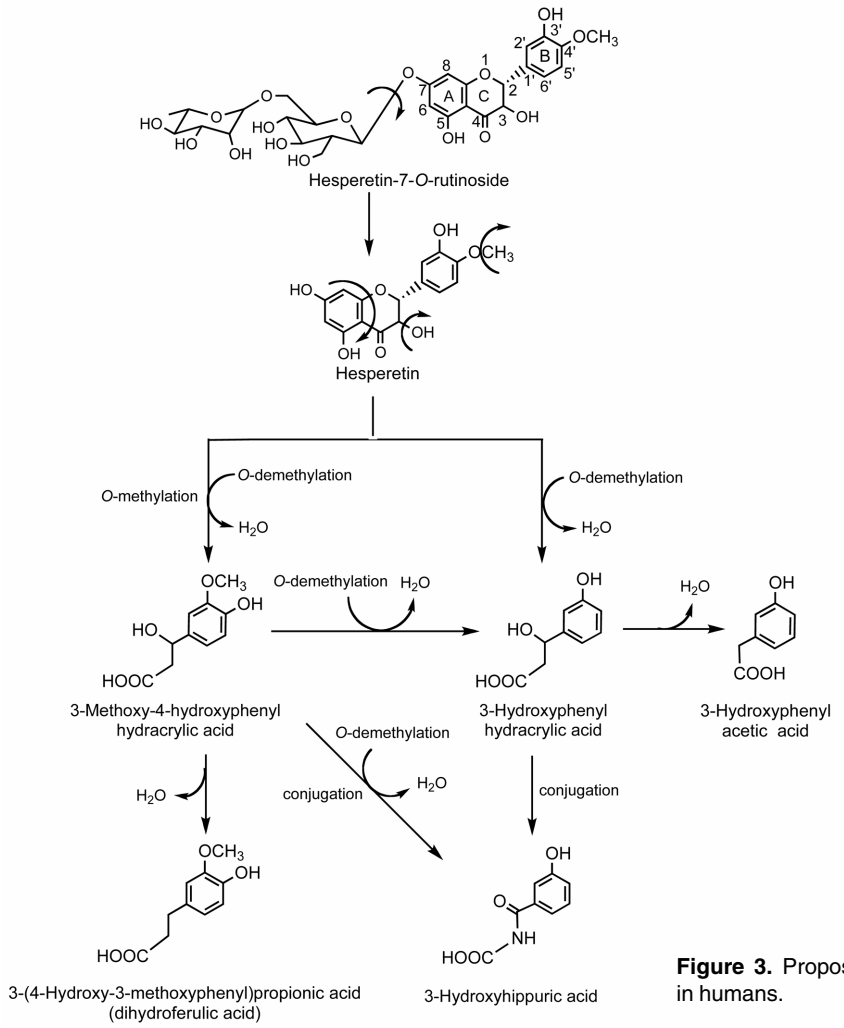

Figure 3. Proposed catabolism of hesperetin-7-*O*-rutinoside in humans.

4-coumaric acid and 4-hydroxybenzoic acid [44], were not detected in urine samples from any of the five volunteers.

3-Methoxy-4-hydroxyphenylhydracrylic acid and 3-hydroxyphenylhydracrylic acid found in this study may have beneficial health effects. According to Karlsson *et al*. [45] phenylpropionic acid found in human faecal water can decrease cyclooxygenase-2 (COX-2) protein levels. COX-2 plays an important role in regulating inflammation involved in colon cancer development. The synthetic amide derivative of 3-(3-hydroxyphenyl)-3-hydroxypropionic acid or 3-hydroxyphenylhydracrylic acid is also effective in lowering plasma and hepatic cholesterol and triglyceride levels in humans [46].

It is possible that excretion of 3-hydroxyphenylhydracrylic acid and 3-methoxy-4-hydroxyphenylhydracrylic acid derived from hesperetin-7-*O*-rutinoside could be used as biomarkers orange juice consumption. However, before any firm conclusions are reached on this point more detailed analysis of phenolic acids excreted following the ingestion of foods rich in other flavonoids is required.

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