Research Article

# Human intestinal hydrolysis of phenol glycosides – a study with quercetin and *p*-nitrophenol glycosides using ileostomy fluid

**Bastian Knaup[1], Kathrin Kahle[1], Thomas Erk[2], Anagnostis Valotis[1], Wolfgang Scheppach[3], Peter Schreier[1] and Elke Richling[2]**

[1] Food Chemistry, University of Wuerzburg, Wuerzburg, Germany
[2] Department of Chemistry, Division of Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany
[3] Department of Medicine II, Division of Gastroenterology, University of Wuerzburg, Wuerzburg, Germany

In order to study the influence of sugar moiety, aglycon structure and microflora concentration on the human ileal hydrolysis of phenol glycosides, various quercetin and *p*-nitrophenol glycosides were incubated under anaerobic conditions (37°C for 0, 0.5, 1, 2, 4, 6, 8, 10 and 24 h) with ileostomy fluids from three different donors. The glycosides, *i.e.* β-D-glucopyranosides, β-D-galactopyranosides, α-L-arabinofuranosides, β-D-xylopyranosides and α-L-rhamnopyranosides as well as the liberated aglycones were identified by HPLC-DAD and HPLC-ESI-MS/MS. Among the quercetin glycosides under study, the 3-*O*-β-D-glucopyranoside showed with 0.22 μmol/h the highest hydrolysis rate, followed by the 3-*O*-β-D-galactopyranoside, the 3-*O*-β-D-xylopyranoside and the 3-*O*-α-L-arabinofuranoside (0.04 and each 0.03 μmol/h, respectively). Quercetin 3-*O*-α-L-rhamnopyranoside was found to be stable for the entire incubation period. Using quercetin 3-*O*-β-D-glucopyranoside as a representative example, linear hydrolysis rate was observed from 75 to 2500 μL ileostomy fluid corresponding to its microflora content (log 0.68 up to 21.9 colony forming units). Studies performed in the presence of antibiotics did not reveal any hydrolysis. The *p*-nitrophenol glycosides were hydrolyzed faster than the corresponding quercetin glycosides. The hydrolysis rate decreased from the β-D-glucopyranoside (0.41 μmol/h), to the β-D-galactopyranoside (0.21 μmol/h), the β-D-xylopyranoside (0.12 μmol/h), the α-L-arabinofuranoside (0.09 μmol/h) to the α-L-rhamnopyranoside (0.06 μmol/h). These results demonstrate that the human ileal hydrolysis of phenol glycosides depends on the sugar and the aglycon structure as well as the microflora.

**Keywords:** Ileostomy / Intestinal hydrolysis / Microflora / *p*-Nitrophenol glycosides / Quercetin glycosides

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## 1 Introduction

The flavonol quercetin is one of the most prevalent and thoroughly studied dietary flavonoids. It is present in fruits, vegetables and beverages, mostly bound to one or more sugar molecules [1, 2], and is regarded to exhibit chemopreventive properties [3–6]. The predominant type of glycoside varies among the plant species. For instance, onion contains predominantly quercetin glucosides, while in apple mainly galactosides, rhamnosides and arabinosides have been found [7, 8]. Concerning the bioavailability of quercetin there is evidence from the literature that the sugar moiety is of importance [1, 9–11]. The first step in the metabolic pathway of quercetin is the hydrolysis of the sugar

**Correspondence:** Dr. Elke Richling, Department of Chemistry, Division of Food Chemistry and Toxicology, University of Kaiserslautern, Erwin-Schrödinger-Str. 52, 67663 Kaiserslautern, Germany
**Fax:** +49-631-205-4398
**E-mail**: richling@chemie.uni-kl.de

**Abbreviations: *p*-np**, *p*-nitrophenol; ***p*-npara**, *p*-nitrophenyl α-L-arabinofuranoside arabinofuranoside; ***p*-npgal**, *p*-nitrophenyl β-D-galactopyranoside; ***p*-npglc**, *p*-nitrophenyl β-D-glucopyranoside; ***p*-nprha**, *p*-nitrophenyl α-L-rhamnopyranoside; ***p*-npxyl**, *p*-nitrophenyl β-D-xylopyranoside; **Q3ara**, quercetin 3-*O*-α-L-arabinofuranoside; **Q3gal**, quercetin 3-*O*-β-D-*p*-npara *p*nitrophenyl α-L-galactoside arabinofuranoside; **Q3glc**, quercetin 3-*O*-β-D-*p*-npgal *p*-nitrophenyl β-D-glucopyranoside galactopyranoside; **Q4'glc**, quercetin 4′-*O*-β-D-*p*-npglc *p*-nitrophenyl β-D-glucopyranoside glucopyranoside; **Q3rha**, quercetin 3-*O*-α-L-*p*-nprha *p*-nitrophenyl α-L-rhamnopyranoside rhamnopyranoside; **Q3rut**, quercetin 3-*O*-β-D-*p*-npxyl *p*nitrophenyl β-D-rutinoside xylopyranoside; **Q3xyl**, quercetin 3-*O*-β-D-Q3ara quercetin 3-*O*-α-L-xyloside arabinofuranoside

link in the small intestine, common to most phenolics [12]. The remaining quercetin glycosides reaching the colon are then completely hydrolyzed by the gut microflora, and the aglycon undergoes further metabolism and degradation to phloroglucinol, 3,4-dihydroxyphenylacetic acid, and 3,4-dihydroxytoluene [13, 14].

Using an *in situ* rat perfusion model, fast hydrolysis of quercetin 3-*O*-β-D-glucopyranoside (Q3glc) and quercetin 4′-*O*-β-D-glucopyranoside (Q4'glc) in the small intestine followed by a rapid absorption of the aglycon has been observed [15]. In contrast, quercetin 3-*O*-α-L-rhamnopyranoside (Q3rha) and quercetin 3-*O*-α-L-arabinofuranoside (Q3ara) were not affected. In their studies with ileostomy fluid, other groups have shown that Q3glc and Q4'glc were rapidly absorbed, while quercetin 3-*O*-β-D-rutinoside (Q3rut) was not [16].

Using an *ex vivo* ileostomy model, it was the aim of our studies to extend our knowledge about the enzymatic hydrolysis of phenol glycosides in the small intestine. Thus, various quercetin and *p*-nitrophenol glycosides, the latter as convenient tools to check glycosidase activity [17], were incubated under anaerobic conditions with ileostomy fluid.

Apart from the expected insight into the influence of sugar moiety and aglycon structure, the effect of microflora was checked by additional experiments using antibiotic supplement.

## 2 Materials and methods

### 2.1 Subjects

Ileostomy effluents were provided by three healthy female subjects (34–39 years old) with a terminal ileostomy undergone colectomy (all suffering of Crohn's disease without an involvement of the ileum) 5–6 years prior to the study. Due to the medical history of each, no ileal resection was performed by surgery. A flyer was handed out to the probands to omit the consumption of food such as tea, coffee, chocolate, vegetables, fruits, onion, soy products, juices, and wine 48 h before the study. No drugs such as antibiotics were administered.

### 2.2 Chemicals

All chemicals and solvents were of analytical grade. Solvents were redistilled before use and water for HPLC analysis was filtered through a Millipore Milli-Q ion exchange system from Millipore S.A. (Molsheim, France). ACN (Lichrosolv®) was from Merck (Darmstadt, Germany), formic acid was purchased from Fluka (Deisenhofen, Germany) and DMSO was obtained from Gruessing (Filsum, Germany). Quercitrin (Q3rha), quercetin (3,5,7,3′,4′-pentahydroxyflavone), *p*-nitrophenol (*p*-np), *p*-nitrophenyl α-L-arabinofuranoside (*p*-npara) and *p*-nitrophenyl *a*-L-rhamnopyranoside (*p*-nprha) were purchased from Sigma (Steinheim, Germany). Isoquercetin (Q3glc) and *p*-nitrophenyl β-D-glucopyranoside (*p*-npglc) were obtained from Fluka (Deisenhofen, Germany).

*p*-Nitrophenyl β-D-galactopyranoside (*p*-npgal) and *p*-nitrophenyl β-D-xylopyranoside (*p*-npxyl) were purchased from Serva (Heidelberg, Germany). Hyperoxide (Q3gal) was purchased from Roth (Karlsruhe, Germany). Avicularin (Q3ara) and reynoutrine (Q3xyl) were kindly provided by H. Becker (Saarbrücken, Germany). The penicillin/streptomycin antibiotic was obtained from Biochrom AG (Berlin, Germany). The internal standard (IS) 3,4,5-trimethoxycinnamic acid was purchased from Sigma (Steinheim, Germany).

### 2.3 Preparation of inoculum

After removal, the ileostomy bag was immediately placed in an anaerobic jar containing AnaeroGen™ from Oxoid Limited (Hampshire, UK) to create an anaerobic atmosphere. It was transported straight into the laboratory where the jar was transferred immediately into an anaerobic chamber (self-constructed) flushed with a $N_2/CO_2$ gas mixture (80:20 v/v). The ileostomy fluid was diluted with the same volume of anaerobic carbonate-phosphate buffer (pH 6.3) according to [18]. The ileostomy fluid was mixed and coarse particles were removed by filtration using glass wool. The filtrate was used as inoculum.

### 2.4 Incubation conditions

Aliquots of 2.5 mL inoculum were added to each pregassed (with $N_2$) incubation vessel containing an aliquot of 20 μL from a stock solution of each substrate (50 mM in DMSO). The incubation vessels were sealed tightly and stored at 37°C for 0, 0.5, 1, 2, 4, 6, 8, 10, and 24 h. To stop the enzymatic reaction the vessels were placed in liquid nitrogen and the samples were lyophilized immediately. As control, samples with 20 μL DMSO and inoculum as well as samples with flavonoid and buffer were prepared and subjected to the procedure described above. In addition, stability studies of each substance were conducted using anaerobic carbonate-phosphate buffer (pH 6.3) without inoculum as described above. All experiments were performed in triplicate.

### 2.5 Incubation conditions for inoculum concentration-dependent assay

Different inoculum volumes (75, 312.5, 625, 1250, and 2500 μL) were added to each pregassed (with $N_2$) incubation vessel containing an aliquot of 20 μL from a Q3glc stock solution (50 mM in DMSO) as described previously. At defined time points (0, 30, 60, 90, and 120 min), the enzymatic reaction of each volume of inoculum was stopped by freezing with liquid nitrogen followed by lyophilization.

The same assays were performed after addition of 1% penicillin/streptomycin antibiotic to the anaerobic carbonate-phosphate buffer before mixing with the ileostomy fluid.

### 2.6 Determination of intestinal microflora

Microbiological analyses were carried out in a Don Whitley MAKS VA500 anaerobic chamber under anaerobic conditions. The entire growing colonies were determined by conventional plating techniques on Brain Heart Infusion Agar (BHI) and standard quantification methods [19]. For homogenization, the samples were shaken for 30 min. Each working step, inclusive the incubation of the plates at 37°C for 4 days, was performed in the anaerobic chamber. After counting the colonies, typical isolates were identified by phase-contrast-microscopy, gram, oxidase and catalase tests.

### 2.7 Sample preparation

The freeze-dried samples were extracted twice, using 1.25 mL 70% (v/v) methanol in water containing 1% (v/v) acetic acid. After centrifugation (5000 × g for 10 min), the supernatants were pooled and filtered (polyvinylidene difluoride, 0.45 µm). Blanks (without substrates and inoculum, respectively) were treated identically. Aliquots of the extracts (50 µL) were measured by HPLC-DAD and HPLC-ESI-MS/MS.

### 2.8 HPLC- DAD analysis

The HPLC system used was a Hewlett-Packard 1100 HPLC gradient pump and a Hewlett-Packard 1100 photodiode array detector (DAD) (Waldbronn, Germany), equipped with a Wisp 712b autosampler (Waters, Eschborn, Germany). Data acquisition and evaluation were performed with Hewlett-Packard ChemStation software. A Hypersil™ Gold $C_{18}$ column, 100 × 4.6 mm, with 3 µm particle size (Thermo, Runcorn, UK) was used. The mobile phase consisted of aqueous 0.1% (v/v) formic acid (A) and ACN (B). The gradient applied was 1 to 50% B in 40 min at a flow rate of 1 mL/min. The peaks were identified by comparison of retention time and UV spectra (200–600 nm) with authentic references. Quercetin and quercetin glycosides were determined at 360 nm, *p*-npara at 306 nm, *p*-nprha at 304 nm, *p*-npxyl, *p*-npgal and *p*-npglc at 302 nm. Calibration curves (at the appropriate wavelengths according to the absorption maximum of the compounds) were used for quantification. Compounds were quantified by means of calibration curves (peak area divided by IS area *vs*. quotient of substance and IS concentration). All values are given as mean values of triplicate analyses, represented by ± SD.

To evaluate the hydrolysis rates for each substrate in µmol/h a tangent was drawn through the linear domain of the hydrolysis curves. By the resulting slope of each linear regression line the hydrolysis rate in µmol/h was achieved.

In case of the inoculum concentration dependent assay with and without antibiotic, the hydrolysis rates were additionally set versus the inoculum volumes.

### 2.9 HPLC-ESI-MS/MS analysis

HPLC-ESI-MS/MS was performed with a TSQ 7000 MS/MS spectrometer system equipped with an ESI interface (Finnigan MAT, Bremen, Germany) and an Applied Biosystems 140b pump (BAI, Bensheim, Germany). Data acquisition and evaluation were conduced using Xcalibur Qual Browser Software 1.2/1.3 (Thermo Electron, Dreieich, Germany). HPLC chromatographic separations were carried out on a Hypersil™ Gold $C_{18}$ column, 100 × 2.1 mm, with 3-µm particle size (Thermo, Runcorn, UK). The mobile phase consisted of aqueous 0.1% formic acid (A) (v/v) and ACN (B). The gradient applied was 5 – 99% B in 40 min at a flow rate of 0.2 mL/min, and 10 µL injection volume. The analysis was performed in the negative ionization mode. The spray capillary voltage was set to 3.2 kV, and the temperature of the heated capillary was 200°C. Nitrogen served as both sheath (70 psi) and auxiliary gas (10 units). The mass spectrometer was operated in the full-scan mode, *m/z* 150–700, with a total scan duration of 1.0 s. MS/MS experiments were performed at a collision energy of 20–40 eV, with argon (2.0 mTorr) serving as collision gas. The obtained deprotonated ions and product ion spectra were compared to those of references measured before.

## 3 Results

Quercetin glycosides, *i.e*. Q3glc, Q3gal, Q3ara, Q3xyl, and Q3rha (Fig. 1) were incubated under anaerobic conditions at 37°C for 24 h using human ileostomy fluid from three different donors. Q3glc was almost completely hydrolyzed into its aglycon quercetin and glucose within 2 h (Fig. 2). The aglycon appeared rapidly and reached its maximum of 0.3 µmol after 6 h. Quercetin was almost stable until the end of the incubation (24 h). The liberation rate recorded for Q3glc (0.22 µmol/h) was determined to be the highest among the quercetin glycosides under study (Table 1). Q3rha was found to be stable for the entire incubation period as shown in Fig. 3. With 0.04 and each 0.03. µmol/h, respectively, Q3gal, Q3xyl and Q3ara exhibited nearly identical hydrolysis rates (Table 1). No other metabolites than quercetin were detectable during the incubation period (data not shown). In addition, in experiments using buffer solution (pH 6.3) without ileostomy content, quercetin and its corresponding glycosides were found to be stable during the incubation time of 24 h.

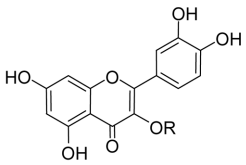
Synthetic *p*-np glycosides (Fig. 1) were treated identically using human ileostomy fluid. After 1 h of incubation,

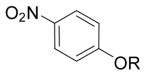
**Table 1.** Hydrolysis rates [μmol/h] of quercetin and *p*-np glycosides under study incubated with human ileostomy fluids from three different donors[a)]

| Quercetin glycoside | Hydrolysis rate [μmol/h] | *p*-Nitrophenyl glycoside | Hydrolysis rate [μmol/h] |
|---|---|---|---|
| Q3glc | 0.22 ± 0.017 | *p*-npglc | 0.41 ± 0.017 |
| Q3gal | 0.04 ± 0.006 | *p*-npgal | 0.21 ± 0.012 |
| Q3xyl | 0.03 ± 0.007 | *p*-npxyl | 0.12 ± 0.005 |
| Q3ara | 0.03 ± 0.012 | *p*-npara | 0.09 ± 0.066 |
| Q3rha | 0 | *p*-nprha | 0.06 ± 0.006 |

a) Values are means of threefold determinations (SD are given).




Quercetin glycosides




*p*-Nitrophenyl glycosides

| | R |
|---|---|
| Q3glc | glc |
| Q3gal | gal |
| Q3ara | ara |
| Q3xyl | xyl |
| Q3rha | rha |

| | R |
|---|---|
| *p*-np3glc | glc |
| *p*-np3gal | gal |
| *p*-np3ara | ara |
| *p*-np3xyl | xyl |
| *p*-np3rha | rha |

**Figure 1**. Chemical structures of the quercetin and *p*-np glycosides under study.

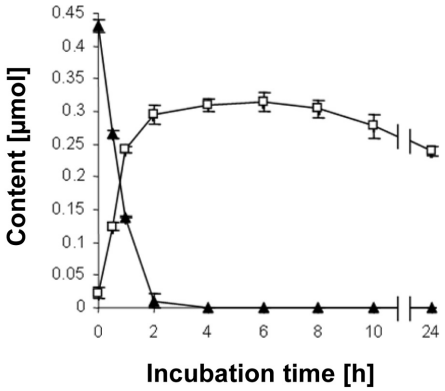



**Figure 2**. Time course of Q3glc (20 μL, 50-mM stock solution) incubation with human ileostomy fluid of one representative donor measured by HPLC-DAD (360 nm). Q3glc (▲); quercetin (□); values are means of threefold determinations. SD are given.

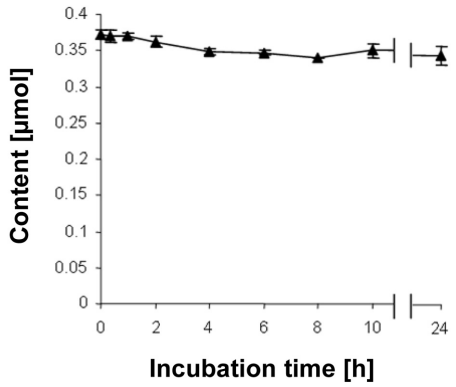



**Figure 3**. Time course of Q3rha (20 μL, 50-mM stock solution) incubation with human ileostomy fluid of one representative donor measured by HPLC-DAD (360 nm). Q3rha (▲); values are means of threefold determinations. SD are given.

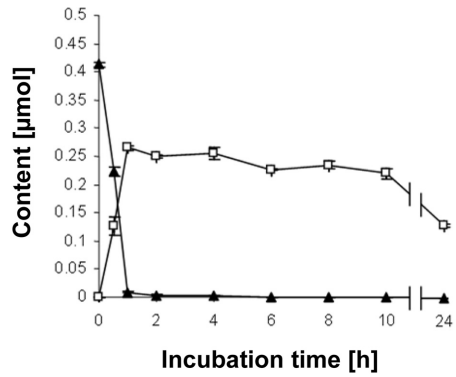



**Figure 4**. Time course of *p*-npglc (20 μL, 50-mM stock solution) incubation with human ileostomy fluid of one representative donor measured by HPLC-DAD (302 nm). *p*-Npglc (▲); *p*-np (□); values are means of threefold determinations. SD are given.

*p*-npglc was completely cleaved into *p*-np and glucose exhibiting a hydrolysis rate of 0.41 μmol/h (Fig. 4). The aglycon increased up to 0.27 μmol after 1 h and was almost stable within 10 h of incubation. In contrast to Q3rha, the *p*-nprha was hydrolyzed by the applied human ileostomy fluids with a hydrolysis rate of 0.06 μmol/h (Fig. 5). After 8 h, most of *p*-nprha was hydrolyzed to *p*-np and rhamnose. After 0.5 h, *p*np was already detectable, reaching its maximum concentration (0.21 μmol) after 4 h and being stable within 10 h of incubation. As shown in Fig. 6 *p*-npglc exhibited the highest hydrolysis rate (Table 1), followed by *p*-npgal (0.21 μmol/h), *p*-npxyl (0.12 μmol/h) and *p*-npara

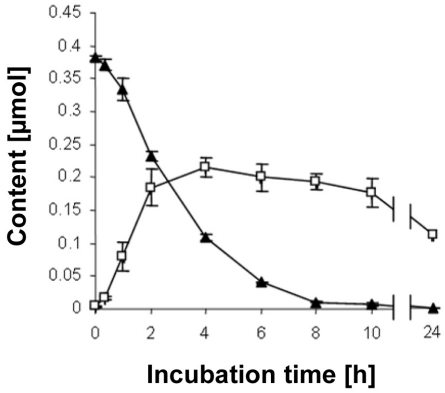



**Figure 5**. Time course of *p*-nprha solution (20 µL, 50-mM stock solution) incubation with human ileostomy fluid of one representative donor measured by HPLC-DAD at 304 nm. *p*-Nprha (▲); *p*-np (▫); values are means of threefold determinations. SD are given.

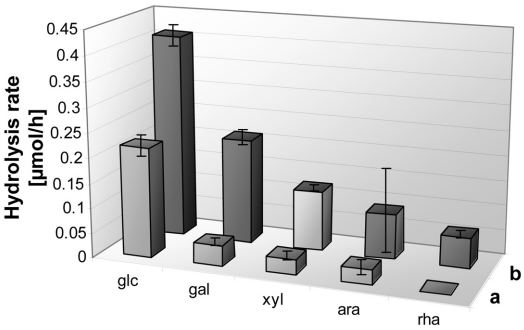



**Figure 6**. Hydrolysis rates [µmol/h] of quercetin- and *p*-np glycosides in ileostomy fluids from three different donors between 0 and 24 h with different quercetin and *p*-np glycoside stock solutions (50 mM). glc: glucopyranoside; gal: galactopyranoside; ara: arabinofuranoside; xyl: xylopyranoside; rha: rhamnopyranoside. a: quercetin glycosides, b: *p*-np glycosides. Mean values of triplicate determinations with SD.

(0.09 µmol/h), whereas *p*-nprha showed the lowest rate with 0.06 µmol/h. *p*-Np was the only metabolite detectable during the incubation. The slight decrease of the *p*-np concentration between 10 and 24 h of incubation (Figs 4 and 5) might be caused by microbial degradations, however, no attempt was made to investigate this effect.

Comparing the degradation rates of quercetin and *p*-np glycosides (Table 1), it is obvious that the decomposition of the investigated *p*-np glycosides was much faster than that of the corresponding quercetin glycosides (Fig. 6).

Additional studies were performed using a constant Q3glc concentration (20 µL of 50-mM stock solution) but different amounts of inoculum volumes (75, 312.5, 625, 1250 and 2500 µL) with and without addition of penicillin/streptomycin antibiotic. As shown in Fig. 7, the hydrolysis rates of Q3glc was observed to be linear from 75 to 2500 µL ileostomy fluid, corresponding to its microflora content. In 1 mL ileostomy fluid an average content of log

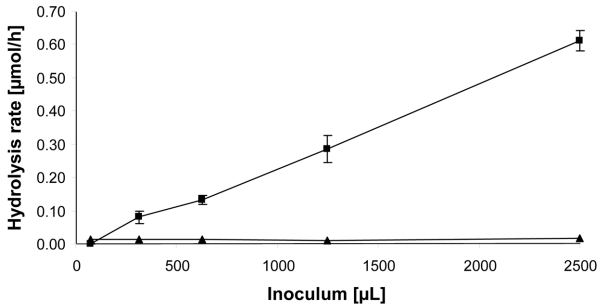



**Figure 7**. Hydrolysis rates [µmol/h] of Q3glc solution (20 µL, 50-mM stock solution) using different amounts of three human ileostomy fluids (75 µL, 312.5 µL, 625 µL, 1250 µL, 2500 µL); without antibiotic (■), with penicillin/streptomycin antibiotic (▲).

8.75 (± 0.37) colony-forming units (cfu) were determined. Thus, in the volumes of 75 to 2500 µL applied in our study total average values varying from log 0.68 up to 21.9 cfu were calculated. The samples predominantly consisted of facultative anaerobic bacteria and largely contained gram negative and oxidase negative rods. After the addition of antibiotics, no hydrolysis of Q3glc occurred (Fig. 7).

## 4 Discussion

Despite of a number of chemopreventive *in vitro* effects of dietary flavonoids, the *in vivo* situation remains still unclear [3–6]. The actual situation has recently been reviewed [4, 20]. Most of the orally administered polyphenols are absorbed or metabolized in the small intestine. In our previous studies carried out with apple polyphenols and ileostomy subjects, between 0 and 33% of the oral dose was recovered in the ileostomy bag [21]. There is evidence from the literature that quercetin glucosides are completely hydrolyzed to quercetin in the small intestine and are mostly absorbed (64.5–80.7%) [12, 22] before conjugation and circulation in the plasma [23]. At this stage, microbial decomposition of the aglycon obviously does not occur [4, 24] as confirmed by our present study.

Considering the complete hydrolysis of glucosides it would be interesting to study the effect of sugar moiety, as recently observed in animal studies and *ex vivo* experiments [15, 25], on the human intestinal hydrolysis rate. In our recent ileostomy study only Q3rha and traces of Q3ara, but no Q3glc, Q3gal or Q3xyl were recovered in the ileostomy bag after ingestion and therefore would reach the colon under physiological circumstances [21]. In our present study, in which effects that happen in the oral cavity and in the stomach [26] were not considered, we observed a clear-cut difference in the hydrolysis rates of quercetin glycosides depending on the sugar moiety, which is in agreement with [15] and [25]. Most of the Q3glc was rapidly cleaved, while the Q3rha remained unaffected by the microflora. Consequently, glucosides are hydrolyzed in the small intestine

and the aglycon will be absorbed and reach the blood circulation [12] or the colon. The rhamnosides reach the colon unaffected and will be hydrolyzed by the colonic microflora to rhamnose and quercetin, the latter is then degraded to phloroglucinol and 3,4-dihydroxyphenylacetic acid [13, 27–29].

The glycosidic activities were monitored using synthetic *p*-np glycosides (*p*-npgal, *p*-npara, *p*-nprha, *p*-npxyl, and *p*-npglc) as substrates. The microbial release of the used substrates into the *p*-np aglycon was also depending on the sugar moiety as shown by the quercetin analogues.

Comparing the hydrolysis rates of quercetin with that of the synthetic *p*-np glycosides (Table 1) it becomes clear that the cleavage of *p*-np glycosides was much faster than that of the corresponding quercetin glycosides (Fig. 6). Thus, the aglycon moiety has an additional effect on the human ileal hydrolysis rate. Berrin and coworkers [30] showed that a human cytosolic β-glucosidase hydrolyzed flavonoid glucosides depending on their aglycone structure. Four single mutant proteins ($Val^{168} \rightarrow Tyr$, $Phe^{225} \rightarrow Ser$, $Tyr^{308} \rightarrow Ala$ and $Tyr^{308} \rightarrow Phe$) homologue to the substrate (aglycone) binding site residue of the human cytosolic *β*-glucosidase in *Pichia pastoris*, were expressed, purified and characterized. The $Val^{168} \rightarrow Tyr$ mutation did not affect $K_m$ on *p*-np glycosides, but increased $K_m$ fivefold on flavonoid glucosides, providing the first biochemical evidence for a distinct role of this residue in aglycon binding of the substrate. Our results fit well with this observation.

Owing to the *β*-glycosidic structure of the investigated quercetin glycosides, they are resistant to hydrolysis by pancreatic enzymes and, therefore, it was thought that such glycosides could not be absorbed [31]. However, it was demonstrated that after ingestion of quercetin glucoside-rich foods, quercetin occurred in the plasma rapidly, indicating a fast absorption in the small intestine [3, 23]. Two hypotheses on the absorption mechanism of flavonoid glucosides across the small intestine have been proposed, the active uptake of the quercetin glucoside by the sodium-dependent glucose transporter (SGLT1) with subsequent deglycosylation within the enterocyte by cytosolic *β*-glucosidase (CBG), or the luminal hydrolysis of the glucoside by lactase phlorizin hydrolase (LPH) and absorption by passive diffusion of the released aglycon. Evidently, it is becoming clear that LPH is the main determinant of uptake [32]. LPH is a membrane-bound *β*-glycosidase found on the brush border of the mammalian small intestine. The ability of LPH to deglycosylate dietary flavonoid glycosides suggests a possible role for this enzyme in the metabolism of these biologically active compounds [33]. Nevertheless, LPH is, as mentioned before, membrane-bound, hence LPH should only to a low extent via abrasion be involved in the hydrolase activity of the used ileostomy fluid. Therefore, we suppose that the cleavage rate is dependent on the hydrolytic activity of the small intestinal microflora. This opinion is supported by the results of our present study, in which a distinct dependence of the hydrolysis rate and the applied microflora was observed. This was proven by the lack of any Q3glc hydrolysis after addition of antibiotics (Fig. 7).

In spite of the inter-individual variability in the microbial composition of ileostomy effluent that was considered only by the use of three samples, it can be summarized that the human ileal hydrolysis of phenol glycosides depends not only on the sugar and aglycon structures but also on the microflora.

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