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Bioaccessible and dialysable polyphenols in selected apple varieties following *in vitro* digestion vs. their native patterns

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ABSTRACT

To assess bioaccessible and dialysable apple polyphenols available for potential uptake by intestinal epithelial cells, an *in vitro* gastrointestinal (GI) digestion method was developed and main polyphenols investigated by UPLC. Polyphenolic profiles in the gastric medium were similar to those natively occurring in apples; however, bioaccessible polyphenols were at lower concentrations than those in the apples. The polyphenolic profile was altered during intestinal digestion, with a considerable decrease of total polyphenols. Flavan-3-ols were completely unstable in the intestinal medium, owing to their pH sensitivity. In addition, 41–77% of bioaccessible chlorogenic acid, the major abundant hydroxycinnamic acid in apples, was degraded during intestinal digestion, with partial isomerisation to cryptochlorogenic acid and neochlorogenic acid. All polyphenols found in the intestinal medium were dialysable, but were present at lower concentrations, suggesting that dialysable polyphenols can potentially be taken up by the enterocytes. These results highlight that GI digestion may substantially affect native apple-derived polyphenolic patterns and concentrations.

1. Introduction

Polyphenols constitute a large group of secondary plant metabolites, with over 8000 compounds. The diet is the principal human source of polyphenolic compounds. Polyphenol-rich foods include fruits, vegetables and whole grains. Polyphenol consumption has been related to several health beneficial effects, such as reduced incidence of cancer and cardiovascular diseases (Bouayed, 2010; Bouayed & Bohn, 2010). Polyphenols may exert their bioactive properties either via their antioxidant properties, or via additional mechanisms, such as affecting intracellular signalling or gene expression (Bouayed, 2010; Bouayed & Bohn, 2010; Williams, Spencer, & Rice-Evans, 2004). However, several reports have highlighted poor bioavailability of several groups of polyphenols, reflected for example by their typical low plasma concentration (Halliwell, Rafter, & Jenner, 2005; Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Bioavailability of polyphenols depends on a variety of factors, including the release from the matrix during gastrointestinal digestion, i.e. bioaccessibility, and cellular uptake, metabolism, and further transport in the circulatory system.

Digestion is a physiological process that permits the extraction of macronutrients (e.g., carbohydrates, proteins), their respective basic units (e.g., monosaccharides, amino acids), micronutrients (e.g., vitamins and dietary minerals, such as Zn, Fe and Na) and phytochemicals (e.g., polyphenols) from the food matrix, for subsequential absorption (Hinsberger & Sandhu, 2004; Pedersen, Bardow, Jensen, & Nauntofte, 2002; Saura-Calixto, Serrano, & Goni, 2007). Substances found predominantly in the small intestine that are extractable (Hinsberger & Sandhu, 2004) constitute the bioaccessible fraction, soluble in gastrointestinal (GI) media; while non-released compounds (non-bioaccessible fraction) will be excreted in the faeces (Bohn et al., 2007; Cilla, González-Sarrías, Tomás-Barberán, Espín, & Barberá, 2009; Saura-Calixto et al., 2007; Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). It is widely accepted that not all constituents present in the food matrix may be completely bioaccessible (Kulkarni, Acharya, Rajurkar, & Reddy, 2007; Saura-Calixto et al., 2007), depending on several parameters, including the initial concentration in the food matrix, the composition of the matrix and host-related factors, such as enzyme concentrations. In humans, the digestive process starts in the mouth where the initial degradation of polysaccharides and triglycerides during mastication, under the effect of salivary α-amylase and lingual lipase, occurs (Hinsberger & Sandhu, 2004; Pedersen et al., 2002). Subsequently, the food bolus is subjected to GI digestion, where digestive enzymes of the stomach and the small intestine, i.e. via secretions from liver/biliary system and pancreas, and later also colonic bacterial fermentation in the large intestine, together play a key role in the release of nutrients and non-nutrients (Biehler & Bohn, 2010; Hinsberger & Sandhu,

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2004; Pedersen et al., 2002; Saura-Calixto et al., 2007), making them available for absorption through the gut barrier, especially in the proximal intestine.

During GI digestion, polyphenols may either interact with other food constituents (e.g., chelation of ions), be further degraded (such as anthocyanins in the small intestine), or metabolised, such as by hydrolysis via, e.g., deglycosylation or cleavage by esterases (Argyri, Komaitis, & Kapsokefalou, 2006; Cilla et al., 2009; McDougall, Dobson, Smith, Blake, & Stewart, 2005; Saura-Calixto et al., 2007). These structural changes could affect both their further uptake and their bioactivity. For instance, the antioxidant activity of free phenols is higher than their glycosides and iron-phenol chelates (Argyri et al., 2006; El Hajji, Nkhili, Tomao, & Dangles, 2006; Lee, Kim, Kim, Lee, & Lee, 2003).

Estimations of the nutritional value of plant foods are usually based on native concentrations of nutrients and phytochemicals, their profile, and biological activity such as their total antioxidant activity. These data are commonly obtained by direct extraction using aqueous-organic solvents (Bouayed, Hoffmann, & Bohn, 2011a; Bouayed, Rammal, Dicko, Younos, & Soulimani, 2009; Brat et al., 2006; Lamperi et al., 2008), being substantially different from extraction conditions in the digestive tract.

Using fresh apples, we have previously reported that hydro-methanolic extraction overestimates total available polyphenol concentration and antioxidant activity compared to *in vitro* GI digestion (Bouayed, Hoffmann, & Bohn, 2011b). In the present study, the bioaccessibility of individual polyphenols following apple digestion during different phases of GI digestion (gastric and intestinal phase) was estimated by a UPLC method. In addition, free individual soluble polyphenols potentially available for further uptake, following dialysis through a semipermeable cellulose membrane, were determined and quantified.

2. Materials and methods

2.1. Chemicals

Solvents (of analytical grade or superior) and formic acid were obtained from Biosolve (Valkenswaard, The Netherlands). Polyphenol standards (+)-catechin, caffeic acid, *p*-coumaric acid, cryptochlorogenic acid (4-caffeoylquinic acid), neochlorogenic acid (3-caffeoylquinic acid), quercetin, rutin (quercetin 3-*O*-rutinoside), quercitrin (quercetin 3-*O*-rhamnoside), quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, phloridzin, phloretin, procyanidins B1 and B2, and (−)-epicatechin were obtained from Sigma–Aldrich (St. Louis, MO). Chlorogenic acid (5-caffeoylquinic acid) was purchased from Merck (Darmstadt, Germany). Porcine pepsin (800–2500 units/mg protein), porcine bile mixture, porcine pancreatin (4× US Pharmacopeia specifications) and sodium carbonate ($NaHCO_3$) were purchased from Sigma–Aldrich. Dialysis bags (pre-washed and wetted, type P/N 128418, with a molecular weight cut-off of 10,000 Da, thickness of 0.002 inches and a flat width of 18 mm) and clips (P/N 132735 with a width of 23 mm) were obtained from Spectrum Laboratories, Inc. (Rancho Dominguez, CA). For all analyses, 18 MΩ water (Millipore, Brussels, Belgium) was used throughout.

2.2. Sample preparation

Four apple varieties grown in Luxembourg were procured at commercial maturity from a local farmer (Kehlen, Luxembourg) during February 2010: Jonaprinz, Jonagold, Golden Delicious and Mutzu, the first two varieties being of red, the others of green skin. For each variety, six apples were rapidly cut into thin slices with peel, put into transparent polyethylene bags, dipped into liquid nitrogen, and frozen at −80 °C. These pre-sliced samples were then further homogenised at 4 °C with a Gastroback robot mixer (Gastroback GmbH, Hollenstedt, Germany), and ground aliquots stored at −80 °C in 50-mL plastic centrifuge tubes until analysis.

2.3. Extraction of phenolics

2.3.1. Hydro-methanolic extraction

Three independent extractions for each cultivar were carried out as described previously (Bouayed et al., 2011b), using ultrasound-assisted liquid extraction. For this objective, polyphenolics were extracted from ca. 10 g of the stored, homogenised, ground fruit samples. In brief, the samples were extracted by a mixture of methanol/water (80/20, v/v), and the extracts were evaporated at 40 °C to approximately 4 mL. This remaining phenolic concentrate was first dissolved in an additional 10 mL of 100% methanol and then diluted to a final volume of 20 mL with water, to reach a final concentration of methanol/water (50/50, v/v). Aliquots from hydro-methanolic extracts were filtered through a 0.2-μm PVDF syringe filter (Pall Corporation, Ann Arbor, MI) prior to UPLC-DAD analysis.

2.3.2. In vitro simulated gastrointestinal (GI) digestion

To monitor the release of individual polyphenols from apple matrices at different stages of digestion, aliquots from gastric digesta (ca. 30 mL), GI digesta (ca. 30 mL) and dialysable content (ca. 14 mL) were analysed by UPLC ($n = 3$ independent replicas per variety). Individual experiments were conducted to measure bioaccessible polyphenols at each of the different stages (gastric, intestinal and dialysable stage, Fig. 1) of digestion, in a similar way to that described recently (Bouayed et al., 2011b). For all analyses, blanks were prepared with identical chemicals but without food matrix, and underwent the same conditions as the samples.

Aliquots from hydro-methanolic extraction and various phases of digestion were diluted in a mixture of water/methanol (90:10, v/v), except for dialysed samples, which were taken undiluted, and filtered through a 0.2-μm PVDF syringe filter prior to UPLC-DAD analysis.

2.4. Polyphenolic identification using UPLC

Quantification of polyphenols was done using a Waters Acquity UPLC® system (Milford, MA) equipped with a photodiode array detector. For separation, an Acquity UPLC® HSS T3 column (2.1 × 100 mm, 1.8-μm particle size, Waters) with a flow rate of 0.75 mL/min at 50 °C was used. The eluents were 0.1% formic acid in water (**A**) and 0.1% formic acid in acetonitrile (**B**) and the gradient was as follows: 0 min, 3% **B**; 5 min, 5% **B**; 13 min, 14% **B**; 20 min, 29% **B**; 21 min, 100% **B**; 26 min, 100% **B**; 27 min, 3% **B**. The injection volume was 10 μL. Polyphenols were identified according to retention time of standards and literature (Nakatani et al., 2000; Tomás-Barberán et al., 2001), and their respective absorption spectra.

Polyphenols were detected at 280 nm (flavan-3-ols and dihydrochalcones), 320 nm (hydroxycinnamic acids), or 350 nm (flavonols) according to their absorption maxima. Linear calibration curves were prepared with external standards for each compound, ranging from 0.01 to 50 mg/L ($n > 5$ concentrations per calibration curve). Limits of detection ranged between 0.01 and 0.1 mg/L; limits of quantification (LOQ) were between 0.05 and 0.5 mg/L.

2.5. Statistical comparison

Unless otherwise stated, all data are reported as mean ± SD. Equality of variances was verified by box plots, normality of distribution by normality plots. To compare recoveries of total polyphenols across different phases of digestion, a linear mixed model was

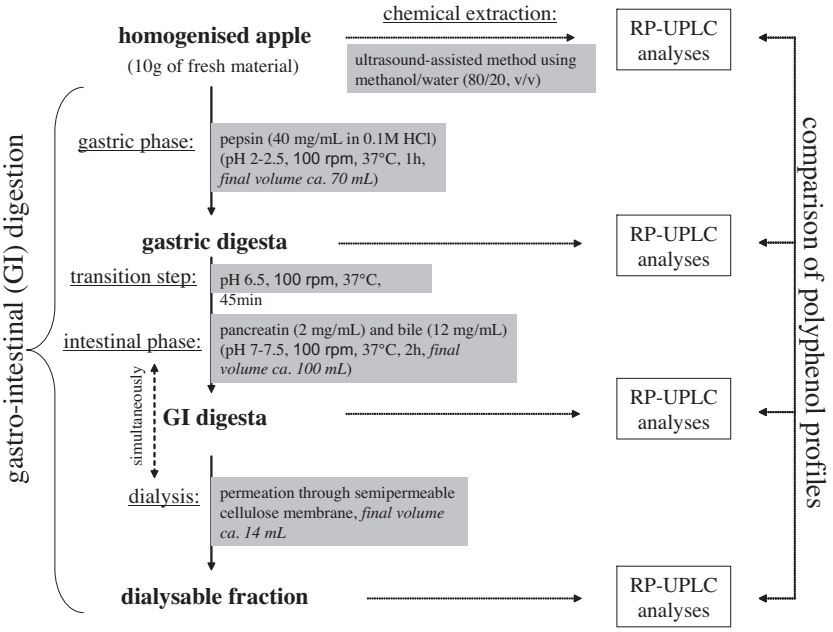

Fig. 1. Simulation of gastrointestinal digestion of apple varieties ($n = 4$), including gastric phase, intestinal phase and dialysis through a semipermeable membrane. For the latter, cellulose segments of 15.5 cm were filled bubble-free with 5.5 mL NaCl 0.9% and 5.5 mL $NaHCO_3$ (0.5 M), sealed with clips, and immersed into the gastric digesta immediately after digestion for a total of 2.75 h.

developed with apple variety and phase of digestion as fixed factor, and concentration of phenolics as observed factor. Following significant *F*-values, Bonferroni post-hoc tests were conducted for individual comparisons. A *p*-value below 0.05 (2-sided) was considered as statistically significant.

3. Results

3.1. Hydro-methanolic extraction

Native polyphenolic profiles of apples encompassed hydroxycinnamic acids, flavonols, flavan-3-ols and dihydrochalcones (Table 1). Chlorogenic acid was the most abundant apple polyphenol (11.8–16.3 mg/100 g), and depending on the apple variety was followed by either epicatechin (4.8–7.8 mg/100 g) or procyanidin B2 (5.0–7.1 mg/100 g), and then by quercetin 3-*O*-galactoside (3.6–6.6 mg/100 g). With respect to polyphenol classes, hydroxycinnamic acids (13.0–17.5 mg/100 g) constituted the major group, followed by flavonols (10.6–16.8 mg/100 g), flavan-3-ols (9.8–14.9 mg/100 g) and dihydrochalcones (2.2–2.8 mg/100 g).

3.2. Simulated GI digestion including dialysability

Table 2 shows soluble individual polyphenols accessible in both simulated gastric and intestinal digesta, as well as dialysable polyphenols from the intestinal medium across a semipermeable membrane, used as a simplified mechanical model for the epithelial barrier. Concerning bioaccessible polyphenols in the gastric phase,

Table 1
Individual polyphenols as determined from fresh apple matrices after hydro-methanolic (2:8, v/v) extraction using a UPLC method. Data are expressed as mg/100 g apple and represent means ± SD ($n = 3$ independent experiments).

Polyphenol class	Compound	Apple variety			
		Jonaprinz	Jonagold	Mutzu	Golden Delicious
Hydroxycinnamic acids	Chlorogenic acid	16.33 ± 1.01	11.78 ± 0.35	15.5 ± 0.67	12.77 ± 1.11
	Neochlorogenic acid[a]	BLQ	BLQ	BLQ	ND
	Cryptochlorogenic acid[a]	0.04 ± 0.01	0.09 ± 0.04	0.01 ± 0.02	BLQ
	p-Coumaric acid	1.14 ± 0.01	1.11 ± 0.01	1.13 ± 0.01	1.10 ± 0.01
	Caffeic acid	0.059 ± 0.003	0.060 ± 0.008	ND	0.058 ± 0.004
Flavonols	Quercetin 3-*O*-galactoside	6.63 ± 0.43	3.58 ± 0.8	6.27 ± 0.53	4.89 ± 0.46
	Rutin	0.46 ± 0.026	0.32 ± 0.04	1.16 ± 0.09	0.59 ± 0.05
	Quercetin 3-*O*-glucoside	0.45 ± 0.03	0.34 ± 0.02	0.91 ± 0.06	0.52 ± 0.04
	Quercetin 3-*O*-rhamnoside	3.65 ± 0.21	2.21 ± 0.33	3.21 ± 0.17	2.13 ± 0.18
	Other quercetin glycosides[b]	5.58 ± 0.26	4.20 ± 0.24	4.28 ± 0.15	3.32 ± 0.24
	Quercetin aglycone	ND	ND	ND	ND
Flavan-3-ols	Catechin	BLQ	BLQ	0.53 ± 0.08	BLQ
	Epicatechin	7.83 ± 0.79	4.83 ± 0.16	7.05 ± 0.39	6.41 ± 0.97
	Procyanidin B1	ND	ND	ND	ND
	Procyanidin B2	7.10 ± 0.51	5.00 ± 0.12	6.89 ± 0.13	6.15 ± 0.39
Dihydrochalcones	Phloridzin	2.18 ± 0.11	2.81 ± 0.63	2.80 ± 0.47	2.24 ± 0.23
	Phloretin	ND	ND	ND	ND

[a] Expressed as chlorogenic acid.
[b] Expressed as quercetin 3-*O*-glucoside; ND = not detected; BLQ: below limit of quantification.

Table 2
Bioaccessible individual polyphenolics in the gastric and intestinal phases, and dialysable individual polyphenolics as determined from fresh apple matrices after simulated gastrointestinal (GI) digestion using a UPLC method. Data are expressed as mg/100 g fresh apple and represent means ± SD (n = 3 independent experiments).

Polyphenolic constituent	Gastric phase Apple variety				Intestinal phase Apple variety				Dialysis Apple variety			
	Jonaprinz	Jonagold	Mutzu	Golden Delicious	Jonaprinz	Jonagold	Mutzu	Golden Delicious	Jonaprinz	Jonagold	Mutzu	Golden Delicious
Chlorogenic acid	17.88 ± 1.32	12.21 ± 0.58	14.27 ± 0.60	10.72 ± 1.52	1.92 ± 0.34	1.69 ± 0.33	4.25 ± 1.05	1.76 ± 0.45	1.19 ± 0.38	BLQ	BLQ	0.11 ± 0.04
Neochlorogenic acid[a]	BLQ	BLQ	BLQ	BLQ	1.80 ± 0.23	1.28 ± 0.05	2.39 ± 0.16	1.37 ± 0.31	1.20 ± 0.28	BLQ	BLQ	0.10 ± 0.06
Cryptochlorogenic acid[a]	BLQ	BLQ	BLQ	BLQ	1.44 ± 0.17	1.06 ± 0.11	2.13 ± 0.24	1.12 ± 0.25	1.00 ± 0.21	0.06 ± 0.01	0.09 ± 0.01	0.12 ± 0.04
p-Coumaric acid	0.52 ± 0.01	0.44 ± 0.03	0.44 ± 0.02	0.44 ± 0.02	0.78 ± 0.10	0.62 ± 0.04	0.61 ± 0.05	0.64 ± 0.01	0.17 ± 0.00	0.20 ± 0.07	0.16 ± 0.00	0.19 ± 0.08
Caffeic acid	0.03 ± 0.00	0.03 ± 0.00	ND	0.03 ± 0.01	ND	ND	ND	ND	ND	ND	ND	ND
Quercetin 3-O-galactoside	5.43 ± 0.63	2.84 ± 0.12	4.58 ± 0.45	3.70 ± 0.59	5.23 ± 0.28	3.20 ± 0.07	4.11 ± 2.79	4.44 ± 0.12	3.69 ± 0.22	1.25 ± 0.05	2.35 ± 0.07	2.24 ± 0.10
Rutin	0.33 ± 0.04	0.21 ± 0.01	0.64 ± 0.06	0.34 ± 0.05	0.19 ± 0.01	0.13 ± 0.01	0.45 ± 0.01	0.23 ± 0.00	0.05 ± 0.00	ND	0.08 ± 0.00	0.05 ± 0.00
Quercetin 3-O-glucoside	0.91 ± 0.51	0.97 ± 0.03	1.37 ± 0.05	1.09 ± 0.04	1.50 ± 0.02	1.29 ± 0.01	1.65 ± 0.29	1.48 ± 0.16	0.55 ± 0.01	0.39 ± 0.01	0.60 ± 0.01	0.47 ± 0.01
Quercetin 3-O-rhamnoside	3.11 ± 0.29	1.94 ± 0.04	2.55 ± 0.20	1.87 ± 0.14	4.02 ± 0.13	2.71 ± 0.02	1.63 ± 0.29	2.75 ± 0.04	2.46 ± 0.07	1.22 ± 0.04	1.88 ± 0.04	1.32 ± 0.06
Other quercetin-glycosides[b]	5.21 ± 0.31	3.38 ± 0.66	3.48 ± 0.71	2.86 ± 0.67	7.51 ± 0.18	4.25 ± 0.07	6.27 ± 0.09	3.98 ± 0.06	3.51 ± 0.09	2.19 ± 0.26	2.29 ± 0.02	1.93 ± 0.06
Quercetin aglycone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Catechin	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND	ND	ND	ND	ND
Epicatechin	6.28 ± 0.85	3.17 ± 0.15	2.76 ± 0.27	1.94 ± 0.22	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Procyanidin B1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Procyanidin B2	5.51 ± 0.40	4.07 ± 0.09	3.69 ± 0.18	3.86 ± 0.22	ND	ND	ND	ND	ND	ND	ND	ND
Phloridzin	2.11 ± 0.34	2.43 ± 0.24	2.21 ± 0.31	1.95 ± 0.29	3.06 ± 0.16	4.65 ± 0.43	3.91 ± 0.11	3.22 ± 0.23	2.32 ± 0.12	3.36 ± 0.29	2.87 ± 0.08	2.15 ± 0.16
Phloretin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] Expressed as chlorogenic acid.
[b] Expressed as quercetin 3-O-glucoside; ND = not detected; BLQ: below limit of quantification.

chlorogenic acid (10.7–17.9 mg/100 g) was the most abundant polyphenol released from apple matrices. Depending on the apple variety, chlorogenic acid was followed in concentration either by epicatechin (1.9–6.3 mg/100 g), procyanidin B2 (3.7–5.5 mg/100 g) or quercetin 3-O-galactoside (2.8–5.4 mg/100 g). Considering polyphenolic classes, hydroxycinnamic acids (11.2–18.4 mg/100 g) represented the major group, followed by flavonols (9.3–15.0 mg/100 g), flavan-3-ols (5.8–11.8 mg/100 g) and dihydrochalcones (2.2–2.8 mg/100 g).

As for bioaccessible polyphenols in the intestinal juice, the polyphenolic profile was somewhat changed compared to the polyphenolic pattern in the gastric medium, as procyanidin B2 and caffeic acid were no longer detected, and chlorogenic acid no longer constituted the major abundant polyphenol (1.7–4.2 mg/100 g). However, chlorogenic acid isomers, i.e. cryptochlorogenic acid and neochlorogenic acid, were detected in the intestinal phase at concentrations almost comparable to that of chlorogenic acid. With regard to the polyphenol abundance, generally, quercetin 3-O-galactoside (3.2–5.2 mg/100 g) was followed by phloridzin (3.1–4.6 mg/100 g) and quercetin 3-O-rhamnoside (1.6–4.0 mg/100 g). Flavonols (11.6–18.4 mg/100 g) constituted the major group of polyphenols, followed by hydroxycinnamic acids (4.6–9.4 mg/100 g) and dihydrochalcones (3.1–4.6 mg/100 g). However, no flavan-3-ols were detected in the intestinal juice.

With regard to dialysable polyphenols, all polyphenols found in the intestinal medium were also found after dialysis, albeit at lower concentrations, with phloridzin (3.1–4.6 mg/100 g) and quercetin 3-O-galactoside (2.3–3.7 mg/100 g) being the most abundant; however, the ranking depended on the apple variety. Flavonols constituted the major group of dialysable polyphenols, followed by dihydrochalcones and hydroxycinnamic acids.

3.3. Total chlorogenic acid recovery

Total chlorogenic acid, including its isomers neochlorogenic and cryptochlorogenic acid, was almost completely recovered in the gastric phase (84–109%, Table 3). However, during intestinal digestion, simultaneously carried out with dialysis, lower recoveries were recorded in the intestinal medium (31.6–56.5%) and after dialysis (0.5–20.7%). Thus, 41–77% of chlorogenic acid was degraded in the comparatively alkaline pH of the small intestine (Table 3).

3.4. Total polyphenolic comparisons

Total polyphenolics determined by UPLC in native apple varieties (36.3–51.4 mg/100 g) were significantly higher than those found following the gastric digestion (28.8–47.3 mg/100 g, $p < 0.001$), except for Jonaprinz ($p = 0.085$, Table 4). A further significant decrease was observed following the intestinal digestion (18.0–27.4 mg/100 g, $p < 0.001$) and dialysis (8.7–16.1 mg/100 g, $p < 0.001$), in comparison to the native concentrations. Total polyphenolics (for all apple varieties combined) in the intestinal medium were also significantly lower than in the gastric medium, by approximately 40% ($p < 0.001$) (Table 4).

4. Discussion

In the present study, the release of major apple polyphenols was monitored during *in vitro* digestion, i.e. gastric and intestinal phases, and compared to the polyphenolic profile natively present in apples as assessed by hydro-methanolic extraction. In addition, the free soluble polyphenols dialysable through a cellulose membrane, chosen as a model of mechanical aspects of the epithelial

Table 3
Recovery of total chlorogenic acids in gastric and intestinal digesta and after dialysis, following simulated gastrointestinal (GI) digestion, compared to native concentrations found by hydro-methanolic extraction (considered as 100%), and estimation of chlorogenic acid degradation (%) following the intestinal phase.

Apple variety	Total chlorogenic acids[a]					
	Hydro-methanolic extraction (mg/100 g)	Gastric recovery (%)	Intestinal recovery (%)	Recovery after dialysis (%)	Total recovery after GI digestion/ dialysis (%)	Degradation during intestinal digestion[b](%)
Jonaprinz	16.37 ± 1.02	109.47 ± 8.11	31.61 ± 4.54	20.74 ± 5.34	31.40 ± 2.63	77.60 ± 5.48
Jonagold	11.86 ± 0.39	103.67 ± 4.90	34.19 ± 4.21	0.52 ± 0.05	31.23 ± 3.79	72.44 ± 1.11
Mutzu	15.51 ± 0.69	92.07 ± 3.84	56.52 ± 9.40	0.60 ± 0.08	50.98 ± 8.25	41.09 ± 4.41
Golden Delicious	12.77 ± 1.11	83.93 ± 11.90	33.31 ± 7.86	2.58 ± 1.04	30.58 ± 7.26	53.35 ± 4.64

[a] Chlorogenic acids correspond to the sum of chlorogenic acid and its isomers (neochlorogenic acid and cryptochlorogenic acid, expressed as chlorogenic acid equivalents).
[b] Including combined intestinal and dialysis phases.

Table 4
Total native, gastric and intestinal bioaccessible, and dialysable apple polyphenolics* as measured by UPLC (mg/100 g fresh weight) after simulated gastrointestinal (GI) digestion.

Variety	Hydro-methanolic extraction (mg/100 g)	GI digestion with dialysis (mg/100 g)		
		Gastric phase	Intestinal phase	Dialysis
Jonaprinz	51.45 ± 3.40[a]	47.32 ± 4.36[a]	27.45 ± 1.62[b]	16.14 ± 1.38[b]
Jonagold	36.34 ± 2.74[a]	31.69 ± 1.95[b]	20.88 ± 1.14[c]	8.67 ± 0.73[d]
Mutzu	49.74 ± 2.77[a]	35.99 ± 2.85[b]	18.02 ± 5.08[c]	10.32 ± 0.23[d]
Golden Delicious	40.18 ± 3.63[a]	28.80 ± 3.77[b]	20.99 ± 1.63[c]	8.68 ± 0.61[d]
Average	44.42 ± 7.32[a]	35.95 ± 8.14[b]	21.84 ± 3.99[c]	10.95 ± 3.54[d]

* Total polyphenolics always constitute the sum of all individual polyphenolic compounds as detected by UPLC. Total polyphenolics in apple matrices were determined using hydro-methanolic extraction (20:80, v/v). Total bioaccessible polyphenolics (i.e. in the gastric and intestinal phases) as well as dialysable polyphenolics were determined using an *in vitro* GI digestion model including dialysability through a semipermeable cellulose membrane. Data shown represent means ± SD ($n = 3$ independent experiments). Values in one row not sharing the same superscript are significantly different ($p < 0.05$).

barrier, were identified and quantified. Following GI digestion, 0–380% and 0–120% of polyphenols were recovered in the small intestine and the dialysable fraction, respectively. Considerable degradation of polyphenolic constituents and formation of additional isomers such as from chlorogenic acid were observed, highlighting the manifold changes occurring during GI digestion that impact availability and possibly bioactivity of polyphenols.

UPLC analyses showed that native fresh apple polyphenols or those released during simulated GI digestion could be divided into four major groups, including hydroxycinnamic acids, flavonols, flavan-3-ols and dihydrochalcones (Table 1). This is in agreement with the literature, which further considers anthocyanins as the fifth major group of native apple-derived polyphenols. Our previous work has shown that some apple varieties, e.g., the green-skinned Golden Delicious and Mutzu, do not contain detectable levels of anthocyanins; whereas the red-skinned Jonagold and Jonaprinz contain low but detectable concentrations (ca. 1 mg/100 g) (Bouayed et al., 2011b). Due to their instability in the mild alkaline intestinal environment, these pigments are found only in the gastric phase.

From the present study, it appears that all individual polyphenols extractable by the hydro-methanolic method were also detectable in the gastric phase, except for catechin, which was below the LOQ (Tables 1 and 2). Both, epicatechin (a catechin isomer) and its dimer (procyanidin B2) are main apple flavan-3-ols. However, in a preceding study simulating pH conditions of the gastric medium (without using digestive enzymes), almost 40% of procyanidin B2 (epicatechin-(4β-8)-epicatechin) was degraded within 30 min, highlighting its susceptibility to gastric pH conditions (Zhu et al., 2002). In another study, the majority of apple-derived polyphenols were stable within the gastric phase over 0–4 h, except for procyanidin B2, which was almost completely degraded into the monomeric (−)-epicatechin within 1.5 h (Kahle et al., 2011). In our study, procyanidin B2 was detectable in the gastric phase (1 h) at concentrations lower (ca. 3.7–5.5 mg/100 g) than those found natively in the fresh apple varieties (ca. 5.0–7.1 mg/100 g). We noted that epicatechin was measurable in all apple varieties (ca. 4.8–7.8 mg/100 g); however following simulated GI digestion, it was found only in the gastric phase at 1.9–6.3 mg/100 g (Tables 1 and 2). Nevertheless, neither procyanidin B2 nor epicatechin were found following the intestinal digestion. These findings rule out considerable decomposition of procyanidin B2 to epicatechin or the conversion of the latter into catechin. Thus, degradation to unknown products after the transition from the acidic gastric to the mild alkaline intestinal environment could be suggested, perhaps under the impact of bile acids and pancreatin. The degradation of procyanidin B2 and the epimerisation of (−)-epicatechin into (−)-catechin have been observed earlier in artificial intestinal medium (Kahle et al., 2011; Zhu et al., 2002). However, Zhu et al. (2002) reported complete decomposition of procyanidin B2 within 2 h into unknown degradation products other than epicatechin or catechin. In addition, the instability of monomeric flavan-3-ols (catechin and epicatechin) under intestinal conditions was suggested (ca. 50% degradation within 2 h). Thus, the instability of procyanidin B2, epicatechin and catechin at increased pH may explain their occurrence in human plasma at only low concentrations, at ca. 0.04, 5.92 and 0.16 μM, respectively, following consumption of flavanol-rich cacao (Holt et al., 2002).

Chlorogenic acid, the most abundant hydroxycinnamic acid in apples, was also decreased in concentration during the transition from the gastric (11–18 mg/100 g) to the intestinal environment (1.7–4.2 mg/100 g), with gastric concentrations resembling those found by hydro-methanolic extraction (ca. 12–16 mg/100 g) (Tables 1 and 2). In addition, chlorogenic acid isomers, including neochlorogenic acid (1.3–2.4 mg/100 g) and cryptochlorogenic acid (1.0–2.1 mg/100 g) were found in the intestinal phase, suggesting isomerisation of chlorogenic acid (Table 2). However, low amounts of chlorogenic acids found in the intestinal medium suggested considerable chlorogenic acid degradation, ca. 41–77% (Table 3). Previous reports have shown isomerisation of chlorogenic acid, an ester of caffeic acid with quinic acid, and even its hydrolysis into its free acids in both simulated and human duodenal juices (Farah, Guigon, & Trugo, 2006; Kahle et al., 2011). In humans, chlorogenic acid can

be absorbed intact from the small intestine, as can caffeic acid following colonic hydrolysis of chlorogenic acid by microflora esterase (Olthof, Hollman, & Katan, 2001), unlike in rats, where chlorogenic acid can only be absorbed in the colon as caffeic acid (Azuma et al., 2000). In the present study, the hydrolysis of chlorogenic acid was unlikely, due to the absence of esterase activity in our assay, also indicated by the fact that caffeic acid was not detectable in its free form in the intestinal phase. It is worth mentioning that caffeic acid was present only at lower concentration in the gastric phase (0.03 mg/100 g), possibly due to its low free amount in the studied apples (ca. 0.06 mg/100 g), except for Mutzu, in which it was unquantifiable (Tables 1 and 2). The substantial degradation of chlorogenic acid in combination with its possible isomerisation suggests that potential attributed health benefits, e.g., anti-anxiety effects following its intraperitoneal administration (Bouayed, Rammal, Dicko, Younos, & Soulimani, 2007), cannot be extrapolated to oral uptake. *p*-Coumaric acid, another abundant hydroxycinnamic acid, was bioaccessible from the gastric phase (ca. 0.4–0.5 mg/100 g); a further release was observed in the intestinal phase (ca. 0.6–0.8 mg/100 g). However, concentrations were still below those found in the original apples (ca. 1.1 mg/100 g, Tables 1 and 2).

Quercetin and its derivatives are among the polyphenols, which have been associated in several reports with beneficial health effects, including antioxidant, antidepressant and anticarcinogenic effects (Bouayed, 2010; Bouayed et al., 2010). Quercetin itself was not detectable in the original apples (Table 1). It thus appears that quercetin glycosides are resistant to acid hydrolysis in the stomach and as a consequence reach the intestine intact, as quercetin aglycone was also not present in the gastric and intestinal phases (Table 2). This seems also true during gastric digestion for all flavonoid glycosides (Manach et al., 2004), which do not form corresponding aglycones. Cleavage of quercetin glycosides by intestinal fluids however has been reported in an ileostomy study, depending on the incubation time with ileostomy fluid. However, in this study it cannot be excluded that some of the cleavage occurred by bacterial metabolism (Kahle et al., 2011).

Generally, in our study, it was observed that bioaccessible fractions of polyphenols in the gastric or intestinal medium were approximately alike (e.g., chlorogenic acid) or lower (e.g., *p*-coumaric acid) than those natively found in fresh apples. However, bioaccessible phloridzin (ca. 3.1–4.6 mg/100 g) and quercetin 3-*O*-glucoside (ca. 1.3–1.6 mg/100 g) in the intestinal medium were at higher levels than their native amounts (ca. 2.2–2.8 and 0.3–0.9 mg/100 g, respectively) in all apple varieties (Tables 1 and 2), suggesting efficient extraction and potential availability of these flavonoids under intestinal conditions.

A further decrease of polyphenol concentration was observed during dialysis through the semipermeable cellulose membrane. Nevertheless, all polyphenols found in the intestinal medium were also found to be dialysable (Table 2), which could be regarded as a crude estimate for uptake by the epithelial membrane, at least from a simple mechanical point of view, indicative of passive diffusion, one of the important mechanisms for cellular polyphenol uptake, at least for several aglycones (Manach et al., 2004). However, other mechanisms such as facilitated diffusion (Poquet, Clifford, & Williamson, 2008) or active transport have also been reported (Manach et al., 2004). Prior to absorption of polyphenol glycosides or esters, hydrolysis by intestinal or microbial enzymes may have to occur. For example, extracellular hydrolysis of glucosides by the brush-border-active phloridzin hydrolase has been proposed (Day et al., 2000). However, absorption of glucosides has also been suggested to occur in their native form by the sodium-dependent glucose transporter SGLT1, prior to their further cleavage by cytosolic β-glucosidase (Hollman, de Vries, van Leeuwen, Mengelers, & Katan, 1995; Manach et al., 2004). Furthermore, polyphenols not absorbed in the small intestine, such as rhamnose glycosides (e.g., quercetin 3-*O*-rhamnoside) may be hydrolysed by rhamnosidases of the colonic microflora (Manach et al., 2004), which may then be available for further absorption.

Total dialysable polyphenols were lower than total soluble polyphenols in the intestinal phase (Table 4), indicating that amounts of polyphenols potentially available for further uptake and absorption were lower than those bioaccessible in the intestinal digesta, by approximately 50%, even though it cannot be completely excluded that the conditions chosen, such as representing a static, not dynamic digestion system, dialysis time, etc., further impacted the availability of polyphenols. The trend of total phenolic decrease during dialysis was also observed earlier using Folin–Ciocalteu's assay (Bouayed et al., 2011b). It is interesting to note that total phenolics measured earlier by Folin–Ciocalteu's assay were markedly higher than those found in the present study, by approximately 2–8 times, with highest divergence found in the dialysable fraction. Folin–Ciocalteu's assay possibly also measured polyphenols unidentified by UPLC, phenolics resulting from the degradation of polyphenols during digestion, and macromolecule-bound polyphenols; also it is not very specific for polyphenols, as sugar, proteins, vitamin C and pigments (non-phenolic substances) could interfere during total phenolic content evaluation, reducing the Folin–Ciocalteu reagent.

5. Conclusion

Bioactivity of food ingredients is primarily dependent on their bioaccessibility in the GI tract, and secondly on their further bioavailability for different organs after their enterocyte uptake and passage into the blood stream. The present study determined the amounts of bioaccessible apple polyphenols in gastric and intestinal phases, using an *in vitro* model simulating GI digestion including dialysability. Results showed that GI digestion, in addition to a certain impact of the food matrix, may substantially impact concentration and pattern of native apple polyphenols, with higher concentrations of native compounds compared to those bioaccessible in the GI phases. This was likely due to several factors, including the pH-dependent stability of several polyphenols, such as flavan-3-ols and chlorogenic acid, the most abundant apple polyphenols. In addition, isomerisation of chlorogenic acid at intestinal pH into cryptochlorogenic acid and neochlorogenic acid was also observed, but to a lesser extent.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2011.10.030.

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