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The matrix of fruit & vegetables modulates the gastrointestinal bioaccessibility of polyphenols and their impact on dietary protein digestibility

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ABSTRACT

Fruit and vegetables (F & V) polyphenols have numerous positive health effects, ascribed either to their antioxidant activity within the gastrointestinal tract (GIT) or to bioactivity of their absorbed metabolites. The effect of the F & V matrix on the gastrointestinal bioaccessibility of polyphenols was investigated along with its possible interaction with protein digestion. Minipigs were fed a complete meal with either cubed F & V (apple, plum, artichoke) added, or the corresponding phenolic extract (PE). Gastric and ileal chymes were kinetically collected over the postprandial period. The overall polyphenol bioaccessibility in the stomach was found to be 1.5% and 3.1% after F & V and PE consumption, respectively. The lower release rate from artichoke than from apple showed evidence of a plant effect. Flavanol monomers and glucoside conjugates were not recovered in the ileum in agreement with their absorption in the upper GIT. Interestingly, PE, but not F & V, significantly decreased the speed and efficiency of dietary protein digestion.

1. Introduction

The consumption of fruit and vegetables (F & V) is inversely associated with the development of cardiovascular diseases and tends to be protective of major diet-related chronic diseases (Fardet & Boirie, 2014). Part of the cardiovascular health benefit could be ascribed to flavonoids, a class of polyphenols largely distributed in fruit and vegetables (Claude et al., 2014; Kay, Hooper, Kroon, Rimm, & Cassidy, 2012). Studies in ileostomy patients (Borges, Lean, Roberts, & Crozier, 2013; Erk et al., 2012; Kahle, Kraus, Scheppach, & Richling, 2005; Stalmach, Steiling, Williamson, & Crozier, 2010) or in pigs (Bittner et al., 2014; Wu, Pittman, & Prior, 2006) showed that very few phenolic structures can be absorbed in the upper gastrointestinal tract (they are mainly aglycones and glucoside conjugates) when the vast majority of polyphenols (> 90%) pass unaltered into the colon. There they undergo extensive metabolization by the microflora, producing bioactive metabolites which can cross the colon mucosa and be released in the plasma (Del Rio et al., 2013). Upstream in the colon, the bioaccessible polyphenols can exert beneficial effects on health through their antioxidant activity. For example, by limiting the oxidation of fatty acids and cholesterol present in the diet, they could play a role in the prevention of atherosclerosis (Staprans, Pan, Rapp, & Feingold, 2005; Williams et al., 1999). A recent study showed that the lipid oxidation observed during the gastric digestion of a Western type meal can be partially prevented by a supplementation with F & V or the corresponding phenolic extract (Gobert et al., 2014). Moreover, F & V polyphenols could also help to fight against the prooxidant effect of the heme iron of red meat, suspected to be involved in colorectal cancer development (Bouvard et al., 2015). Indeed, the addition of a flavonoid (rutin) to a rich-meat diet was shown to decrease the presence of lipid oxidation markers in urine and faecal waters associated with colon precancerous lesions (Pierre et al., 2013).

A prerequisite step before either the expression of an antioxidant activity in the gastrointestinal tract (GIT) or intestinal absorption is the release of polyphenols from the plant matrix and their solubilization in the gastric and intestinal digesta, which is also termed bioaccessibility. Using *in vitro* digestion systems, it was shown that the polyphenol bioaccessibility from homogenized plant matrices can range from 30 to 100% in the gastric phase whereas that of juices or wines appears total (Bermudez-Soto, Tomas-Barberan, & Garcia-Conesa, 2007; Bouayed,

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Deusser, Hoffmann, & Bohn, 2012; Gumienna, Lasik, & Czarnecki, 2011; Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). The factors affecting the bioaccessibility of polyphenols could primarily be F & V processing, but also the disintegration of the food matrix by mastication, the interactions with constituents from both the bolus and gastrointestinal juices, and physiological digestive conditions (Alminger et al., 2014). In particular, polyphenols are well known for their high affinity for proteins and fibers which can be brought with nutrients.

Although an increase in polyphenol bioaccessibility may have beneficial health effects, it could also potentially have adverse effects. Indeed, binding of polyphenols on proteins is known to decrease protein digestibility, either through interaction with digestive enzymes, or by protecting dietary proteins from enzyme degradation (Jansman, Verstegen, Huisman, & Vandenberg, 1995). For instance, it was shown in pigs that 1.5% of grape tannins in the diet decreases the protein digestibility in the small intestine by 5% (Myrie, Bertolo, Sauer, & Ball, 2008). However, it is unclear whether this effect can be different if the polyphenols are entrapped in the plant matrix, or in the form of extracts.

Although *in vitro* digestion may be used to highlight the impact of numerous parameters on macro- and micronutrient bioaccessibility, only animal studies can take into account the complexity of the digestive processes. In the present *in vivo* study, we intended to give a new insight into the gastric and ileal fates of common polyphenols and whether they are brought in their natural matrix or in the form of extract. Plant polyphenols were ingested with a typical Western diet to take into account molecular interactions with food macronutrients. The concomitant digestibility of dietary proteins was investigated.

2. Materials and methods

2.1. Test meals

2.1.1. Fruit and vegetables and the phenolic extract

Frozen artichoke hearts (Camus de Bretagne var., Picard®) were cooked in a microwave oven for 5 min then cut into 6 pieces and quick-frozen before freezing at −20 °C. Fresh rennet apples were from local market. The central part was removed before cutting apples into 12 or 24 pieces for meal preparation or polyphenol extraction, respectively. Quick freezing of apple pieces was followed by freezing at −20 °C until use. Frozen quetsche plums (halves) were purchased from Picard® and kept at −20 °C until needed. Each F & V portion was made of 120 g of apple, 40 g of artichoke heart and 40 g of plum as prepared above.

The phenolic extract was prepared from frozen F & V as previously described by Gobert et al. (2014). One F & V portion contained the same amount of polyphenols as 22.8 g of the phenolic extract (PE).

2.1.2. Meal preparation

Aside from F & V (or PE), test meals contained 40 g of sunflower oil (Lesieur “Coeur de Tournesol” from local market), 120 g of ground beef meat, 3 g of egg yolk phospholipids (Sigma-Aldrich, St Quentin-Fallavier, France) (Supplemental Table 1). Meat was cooked at 70 °C for 30 min (Gobert et al., 2014). The meals were prepared by mixing for 1 min in a food processor (KM336 Kenwood) the defrosted meat, the sunflower oil, egg yolk phospholipids and either the frozen F & V cut into cubes (5–8 mm-edge lengths) or the phenolic extract. F & V defrosted during the mixing step and were thus protected as long as possible from browning and polyphenol degradation. In the PE meal and in the control meal (without F & V and PE), starch, cellulose, and apple pectin (all from Sigma-Aldrich, St Quentin-Fallavier, France) were added to simulate F & V complex sugars and cell wall materials in addition to water.

2.2. Study design

All procedures were conducted in accordance with the guidelines formulated by the European Community for the use of experimental animals (2010/63/EU), and the study was approved by the Local Committee for Ethics in Animal Experimentation (Approval CE24-10; Comité d’Ethique en Matière d’Expérimentation Animale d’Auvergne, Aubière, France). Bioaccessibility of polyphenols in the stomach (Exp 1) and protein digestion (Exp 2) were investigated in 2 successive experiments.

2.2.1. Animals

Göttingen minipigs (Ellegaard, Denmark) were housed in individual pens, separated by Plexiglass walls, in a ventilated room with controlled temperature (20–23 °C). Apart from sampling days, they were fed once daily, at 08:15, with 400 g of a commercial feed [18% protein, 2% fat, 5% cellulose, 6% ash] (Porcyprima, Sanders Nutrition Animale, France), and had free access to water. Minipigs were accustomed to receive the test meals before starting the experiment in order to ensure a rapid and complete ingestion during the sampling days. Minipigs were surgically prepared at least 3 weeks before initiating the study.

2.2.1.1. Experiment 1. Six female minipigs (12–16 months old; 20–25 kg) were surgically fitted with a permanent cannula (silicone rubber; 12 mm i.d., 17 mm o.d.) on the left flank, in the middle of the long axis of the greater curvature of the stomach, just after the last rib.

2.2.1.2. Experiment 2. Six other female minipigs were surgically fitted with a permanent catheter (polyvinyl chloride; 1.1-mm i.d., 1.9-mm o.d.) in the aorta on the right flank, and a *T*-shaped cannula (silicone rubber; 12-mm i.d., 17-mm o.d.) in the distal ileum on the left flank.

2.2.2. Experimental procedures

2.2.2.1. Experiment 1. F & V and PE test meals were randomly tested on each minipig. Digesta (average volume 60 ml) were gravimetrically collected in a graduated beaker 30 min before and 15, 45, 90, 150, 240 and 330 min after test meal delivery. The exact digesta volume was recorded before mixing with 10 ml of water for a better consistency, then frozen in liquid nitrogen, and stored at −80 °C.

2.2.2.2. Experiment 2. F & V, PE and control meals were randomly tested on each minipig. Ytterbium acetate (450 mg) was added to the test meals as undigestible marker in order to correct for losses of chyme not exported through the cannula. Digesta were continuously collected from 30 min before to 8 h after test meal delivery. Plastic bottles, attached to the cannula, were regularly replaced according to digestive burst. The collected digesta (accumulated over 1-h intervals) were weighed and immediately frozen at −20 °C. At the end of the experiment, digesta were lyophilized, homogenized using a ball mill and stored dry. Along with digesta sampling, blood samples were collected before and after test meal delivery. They were immediately centrifuged at 1500 *g* for 10 min at 4 °C. The resulting supernatant was frozen in liquid nitrogen and stored at −80 °C. Because catheters of 2 minipigs did not work properly, blood samplings were performed on 4 minipigs only.

2.3. Analytical procedures

2.3.1. Materials

(+)-Catechin, 5-caffeoylquinic acid (5-CQA) and rutin were from Sigma-Aldrich (St Quentin-Fallavier, France) while phloridzin and 1,3-dicaffeoylquinic acid (1,3-diCQA or cynarin) from Extrasynthèse (Genay, France). 3-Caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA) and 4,5-dicaffeoylquinic acid (4,5-diCQA) were from Phytolab (Vestenbergsgreuth, Germany). Cyanidin-3-arabinose was purchased from Polyphenols AS (Sandnes, Norway).

2.3.2. F & V and PE

Extraction of phenolic compounds from freeze-dried powders of apple or questche plum or artichoke (18–26 mg) was performed three times by stirring with 0.5 ml of acetone/water (70:30, v/v) for 30 min. After centrifugation (10,860 g, 4 °C, 5 min) and concentration under nitrogen, the resulting aqueous phase was used for analysis. Both the individual extracts of F & V and PE were submitted to UPLC-DAD and UPLC-MS/MS analyses as described below.

Separation and characterization of phenolic compounds were carried out using a Waters UPLC Acquity apparatus equipped with a photodiode array detector and an Acquity BEH C18 column (100 mm × 3.1 mm with 1.7 µm particle size, protected by a guard column of the same material) in a 35 °C heated oven. A binary solvent system was used at a 0.3 ml/min flow rate with solvents A and B being water/formic acid (99.95:0.05, v/v) and methanol, respectively. The elution gradient was as follows: 0–1 min 0% B; 1–2 min, 0–5% B linear; 2–8 min, 5–25% B linear; 8–18 min, 25–60% B linear; 18–19 min, 60–100% B linear, 19–21 min, 100% B isocratic (vol. inj. 2 µl). Anthocyanins were separated using the same gradient system except solvent A, which was water/formic acid (99:1, v/v).

Standards were used for 5-point calibration curves between 0.2 and 100 µg/ml (triplicate experiment) for (+)-catechin and phloridzin at 280 nm and for rutin, 5-CQA, 4-CQA, 3,5-diCQA at 330 nm. Dicaffeoylquinic acids were expressed in 3,5-diCQA equivalent, 3- and 4-*p*-coumaroylquinic acids in 4-CQA equiv., (*cis*)-5-CQA in 5-CQA equiv., phloretin derivatives in phloridzin equiv., flavanol monomers and dimers in (+)-catechin equiv. and quercetin glycosides in rutin equiv.

Further characterization of the phenolic and anthocyanin composition was achieved through the coupling of UPLC with an ion trap mass spectrometer (Bruker Daltonics HCT ultra) equipped with an ESI source. For polyphenol and anthocyanin characterization, a capillary voltage of 2 kV was used in the negative and positive ion modes, respectively. Nitrogen was used as the drying and nebulizing gas with a flow rate of 9 L min^{-1}. The desolvation temperature was set at 365 °C and the nebulization pressure at 35 psi. The ion trap was operated in the Ultrascan mode from *m/z* 100 to 800. Content in flavanol oligomers and the average degree of polymerization were obtained after thiolysis (Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998).

2.3.3. Gastric digesta

Gastric digesta (ca. 5 g) were defrosted and centrifuged (10 min, 4 °C, 10,860 g). After weighing the whole supernatant, one ml was shaken with 2 ml of ice-cold acetone (30 s) before centrifugation (5 min, 4 °C, 10,000 rpm). Acetone from the supernatant was then evaporated under vacuum and the residual aqueous phase increased to 1 ml with water before UPLC analysis.

2.3.4. Ileal digesta

For Ytterbium determination, samples were mineralized at 550 °C for 6 h. Ashes were dissolved in nitric acid and Ytterbium concentration was determined by atomic absorption spectrometry (AAnalyst 400, Perkin-Elmer) using a nitrous oxide-acetylene flame. Standards for Yb analysis were prepared by adding known amounts of Yb to ileal samples obtained before Yb was dosed. Total nitrogen levels in the meals and the gastrointestinal effluents were determined using an elemental analyser (Vario Isotope cube, Elementar). For phenolic compounds analysis, 200 mg of freeze-dried ileal material were shaken for 15 min with 1 ml of acetone/H_2O (70:30, v/v), centrifuged (10,860 g, 15 min, 4 °C), filtered (0.45 µm) before UPLC/MS analysis.

2.3.5. Plasma

Plasma (250 µl) was deproteinized with sulfosalicylic acid (4% wt/vol), after addition of norleucine (50 µl, 1 mM) used as an internal marker of amino acid concentration. After centrifugation (4 °C, 10,000 *g*, 15 min), supernatant (200 µl) was diluted with the injection buffer (100 µl). Amino acids were measured by ion exchange chromatography, with ninhydrine post-column detection (Amino acid auto-analyser L8900, Hitachi).

2.4. Calculations

Apparent ileal digestibility (AID) of proteins was calculated from the cumulative amounts of nitrogen recovered at the ileal level, corrected by the percentage of Ytterbium recovery, using the following equation:

$$AID(\%) = ((N_{intake} - (N_{ileum} \times Yb_{intake}/Yb_{ileum}))/N_{intake}) \times 100$$

where N_{intake} is the amount of nitrogen in the test meal, N_{ileum} is the total amount of nitrogen collected in the ileum, and Yb_{intake} and Yb_{ileum} are the amount of Ytterbium added to the test meal and recovered in the ileum, respectively.

2.5. Statistical analysis

Repeated measures analysis of the variance (ANOVA) was used to evaluate time-dependent changes in the studied parameters after the meal intake (time and meal as fixed effects). When statistical significance was reached for the interaction between meal and time ($p < 0.05$), *post hoc* analysis was used to compare data at a given time.

3. Results

3.1. Identification and quantification of polyphenols in F & V and the phenolic extract

The separate quantitative analyses of low-molecular weight phenolic compounds led to the respective contribution of each plant material to PE (Table 1, Suppl. Table 2). Five major polyphenol classes were present. Main flavanols were identified as (+)-catechin, (−)-epicatechin, dimer B1, dimer B2 and three additional dimers. Apple and plum contributed to the (+)-catechin and dimer B1 pool while only apple provided (−)-epicatechin and dimer B2.

In apple and plum, flavonols were mainly present as quercetin-3-O-galactoside and quercetin-3-O-glucoside.

Hydroxycinnamoylquinic acids represent 75% (w/w) of all low-molecular weight phenolic compounds detected in the extract among which 74% are caffeoylquinic acids. Contents of the most abundant *trans* mono- and dicaffeoylquinic acids were in the decreasing order: 5-CQA ≫ 3,5-diCQA = 1,5-diCQA > 3-CQA ≫ 4,5-diCQA > 4-CQA > 3,4-diCQA > 1,3-diCQA. Artichoke was the only source for dicaffeoylquinic acids and 4-CQ, when artichoke and apple contributed to 5-CQ and plum principally to 3-CQ.

Phloretin-2′-O-xyloglucoside and phloridzin (phloretin-2′-O-Glc) are typical of apple and their respective levels are in agreement with those reported for Reinette du Canada (Ceymann, Arrigoni, Schaerer, Nising, & Hurrell, 2012).

The overall content of low-molecular weight phenolic compounds was 154 mg. Proanthocyanidin analysis by thioacidolysis revealed 96 mg of monomeric and oligomeric structures with an average degree of polymerization of 3. The epicatechin/catechin ratio was 5.9. Lastly, plum and apple brought 89 and 11% of the anthocyanin pool (34 mg), respectively. Cyanidin-3-O-galactose was the sole anthocyanin for apple whereas cyanidin-3-O-glucose, a cyanidin-3-hexosyldeoxyhexoside and a peonidin-3-hexosyldeoxyhexoside were the main contributors to plum. After subtraction of the flavanol monomers and dimers quantified twice in UPLC and thioacidolysis, the total polyphenol amount ingested by minipigs weighing 20–25 kg is ca. 260 mg. It is consistent with a human daily consumption of 1.2 g (Pérez-Jiménez et al., 2011).

Table 1
Recovery yields for the bioaccessible phenolic compounds in the initial stage of gastric digestion after consumption of the F & V and PE meals.

Phenolic compound	Recovery yield from F & V meal (% w/w)	Recovery yield from PE meal (% w/w)	Contributor
Hydroxycinnamic acids			
3-Caffeoylquinic acid	2.6 ± 2.4	6.9 ± 1.7*	Plum (91%) > Artichoke (9%)
4-Caffeoylquinic acid	2.2 ± 1.0	5.4 ± 1.5*	Artichoke
5-Caffeoylquinic acid	1.6 ± 0.9	5.5 ± 1.5*	Artichoke (71%) > Apple (28%)
5-Caffeoylquinic acid (*Cis*)	2.9 ± 1.4	2.9 ± 0.6	Apple (72%) > Artichoke (28%)
4-*p*-Coumaroylquinic acid	9.4 ± 4.0	6.5 ± 2.1	Apple
3,5-Dicaffeoylquinic acid	0.49 ± 0.17	0.60 ± 0.26	Artichoke
1,5-Dicaffeoylquinic acid	0.86 ± 0.53	0.90 ± 0.37	Artichoke
Dihydrochalcones			
Phloridzin	4.2 ± 1.5	2.5 ± 0.6	Apple
Phloretin-2′-Xyl-Glc	3.8 ± 1.1	4.1 ± 1.3	Apple
Flavonols			
Quercetin-3-Glc	9.7 ± 4.5	3.4 ± 1.1*	Apple
Flavanols			
Catechin	ND[a]	D[b]	Apple (86%) > Plum (14%)
Epicatechin	ND	D	Apple
Dimer B1	ND to D	D	Apple (93%) > Plum (7%)
Dimer B2	ND	ND	Apple
Anthocyanins	ND	ND	Plum (89%) > Apple (11%)

[a] ND: not detectable in MS.
[b] D: detected but not quantifiable. Recovery is estimated as the following ratio: (mean level of a polyphenol at 15 min × meal size)/quantity brought by the extract. (n = 6, mean ± SD, differences between meals at $p < 0.05$ marked by an asterisk).

3.2. Polyphenol bioaccessibility in the stomach

3.2.1. Composition of the gastric digesta

Contributions of the liquid and solid phases to the gastric digesta were obtained after centrifugation of the thawed samples. The liquid phase, representing 41% and 39% just 15 min after the ingestion of the F & V and PE meals, respectively, only slightly increased during the first 240 min (Fig. 1). Towards the end of the digestion, this contribution was however significantly higher reaching 61 and 58% ($p < 0.05$). Meal solids were diluted by half during the 330 min-long period of study. It is worth noting that meal size and composition did not play a role in the water content, although the F & V meal was heavier by 26%, had an apparent dryer consistency although a lower dry matter than the PE meal (Suppl. Table 1).

3.2.2. Bioaccessibility at the beginning of the digestion

Based on the hypotheses of a zero dilution by gastric juices at 15 min and a density of 1 for the gastric digesta, one can calculate the bioaccessibility of phenolic compounds at the very beginning of gastric digestion (Table 1). Bioaccessible phenolic compounds are defined as the molecules present in the aqueous phase of the digesta after centrifugation. Higher recovery yields were significantly observed for 3-, 4- and 5-CQA when provided by the PE meal rather than the F & V meal. However, no difference was found between meals for the other phenolic compounds except for quercetin-3-Glc, which oppositely appeared at a threefold greater content in the F & V meal. Additionally, dicaffeoylquinic acids (0.49–0.90%) were recovered less compared to monocaffeoylquinic acids (1.6–6.9%) after ingestion of both meals. DiCQA are larger and more hydrophobic molecules than CQA. They may thus be poorly soluble and rather interact with the hydrophobic sites of macromolecules such as proteins or fibers. Dihydrochalcones, phloridzin and phloretin-2′-XylGlc, as well as the flavonol quercetin-3-Glc were also weakly recovered (< 9.7%). Lastly, flavanol monomers and dimers were mostly not detected in the aqueous phase.

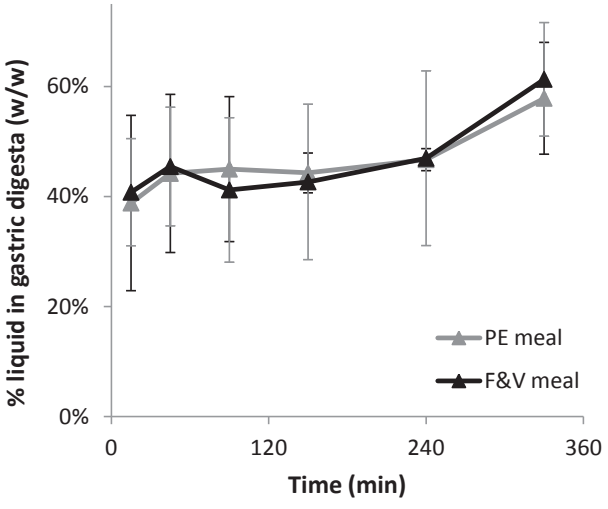

Fig. 1. Liquid contribution to gastric digesta of minipigs fed a complete meal containing diced F & V or the corresponding phenolic extract (mean ± SD, n = 6).

3.2.3. Kinetics

Bioaccessible phenolic compounds were kinetically monitored in the gastric digesta following ingestion of both F & V and PE meals. Compounds such as 3-CQA, 4-CQA, 5-CQA, (*cis*)-5-CQA, 4-*p*-coumaroylquinic acid, 1,5-diCQA, 3,5-diCQA, phloridzin, phloretin-2′-XylGlc and quercetin-3-Glc were bioaccessible during the 330 min-long gastric digestion whereas anthocyanins, flavanol monomers and dimers were not recovered. As illustrated in Fig. 2, two different patterns were mainly exhibited. For 3-CQA, 4-CQA, 5-CQA, 3,5-diCQA, 1,5-diCQA, the bioaccessibility was significantly higher for PE than for F & V at most digestion times. By contrast, levels in 4-*p*-coumaroylquinic acid, (*cis*)-5-CQA, phloridzin, phloretin-2′-XylGlc and quercetin-3-Glc were similar when comparing the PE meal to the F & V meal. This last pattern reveals a release of the phenolic compounds prior to the digestion step. Indeed, diffusion could start readily when homogenizing the diced frozen F & V in the meal preparation.

Besides, the kinetic behaviour of the bioaccessible phenolic compounds clearly depends on the meal ingested. For the PE meal, the levels of bioaccessible phenolic compounds appear relatively stable over the first 150 min for 3-CQA and 4-*p*-coumaroylquinic acid or even slightly increase for 4-CQA, 5-CQA, 1,5-diCQA, 3,5-diCQA, quercetin-3-Glc, phloridzin and phloretin-2′-XylGlc. Phenolic compounds from the PE meal are thus chemically stable in the gastric environment and likely not evacuated through gastric emptying until 150 min. After this time,

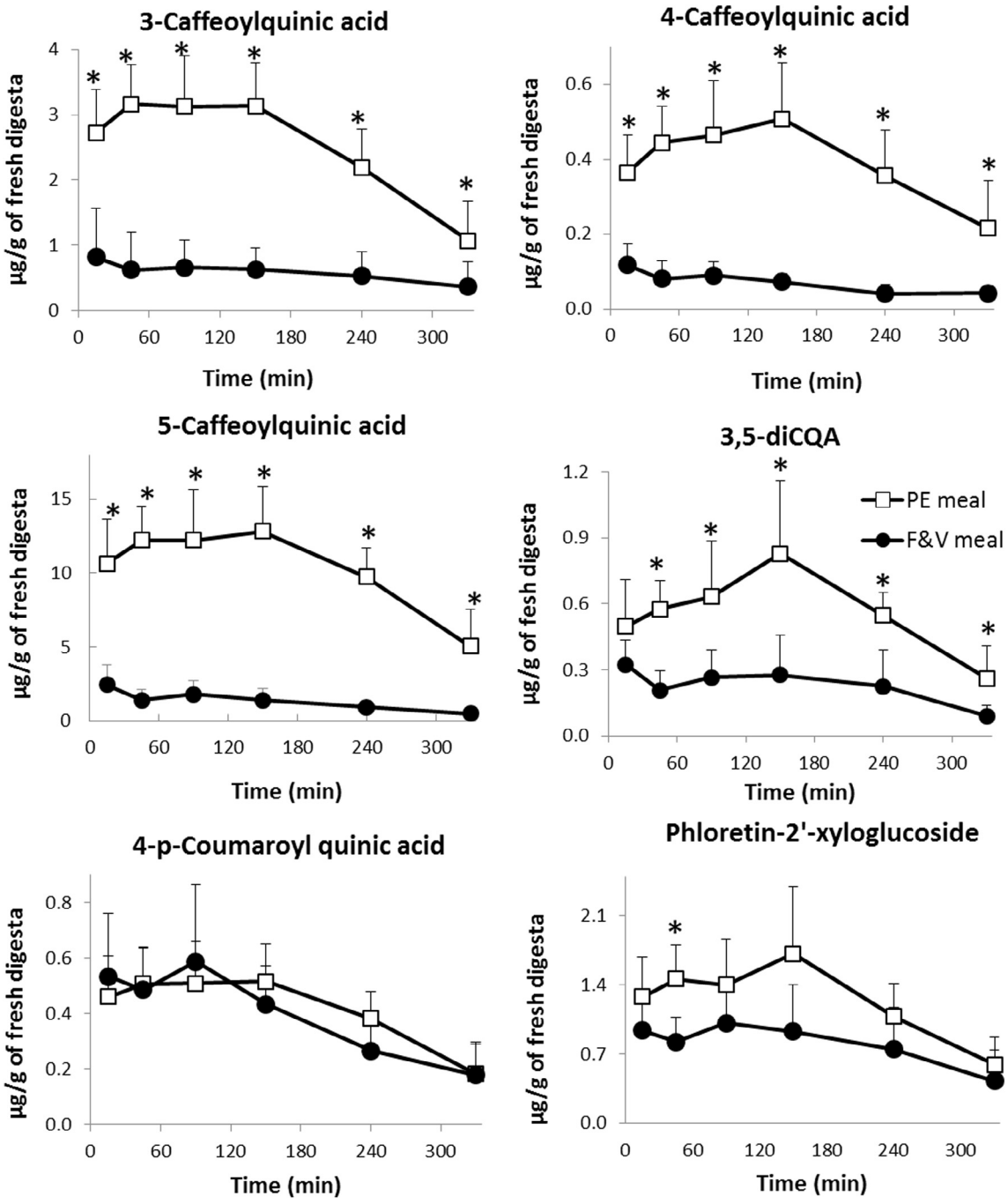

Fig. 2. Bioaccessible phenolic compounds in gastric digesta for digestion of meals containing diced fruit & vegetables (F & V meal, ●) or the phenolic extract (PE meal, □) (n = 6, mean ± SD, differences at $p < 0.05$ marked by an asterisk).

they decrease sharply and almost linearly in agreement with dilution by gastric juices (Fig. 1) and gastric emptying. By contrast, the levels in bioaccessible polyphenols for the F & V meal are maximum at 15 min and then regularly decrease over the digestion time. Because dilution by gastric juices and gastric emptying are weak over the first 150 min, this suggests a poor diffusion of phenolic compounds from the plant matrix during the digestion step.

3.3. Bioaccessible polyphenol in the ileal digesta

The presence of the native polyphenols provided by F & V was investigated in the soluble phase of the ileal digesta using MS and MS^2 fragmentations. After both F & V and PE meal consumption, hydroxycinnamoylquinic acids, 3-CQA, 4-CQA, 5-CQA, 3,5-diCQA, 3-*p*-coumaroylQA, and 4-*p*-coumaroylQA were detected along with dimer B1, dimer B2 and phloretin-2′-XylGlc whereas phloridzin, catechin, epicatechin, 1,5-diCQA, quercetin-3-Glc and quercetin-3-Gal remained undetectable (data not shown). Less than 5% of total polyphenols could be recovered with a high inter-individual variability (Suppl. Fig. 2) from both F & V and PE meals.

3.4. Protein digestion

While F & V addition to the control meal did not affect the postprandial N flux at the ileum ($P > 0.05$), PE significantly increased it ($P < 0.05$) (Fig. 3A). Consequently, the apparent ileal digestibility

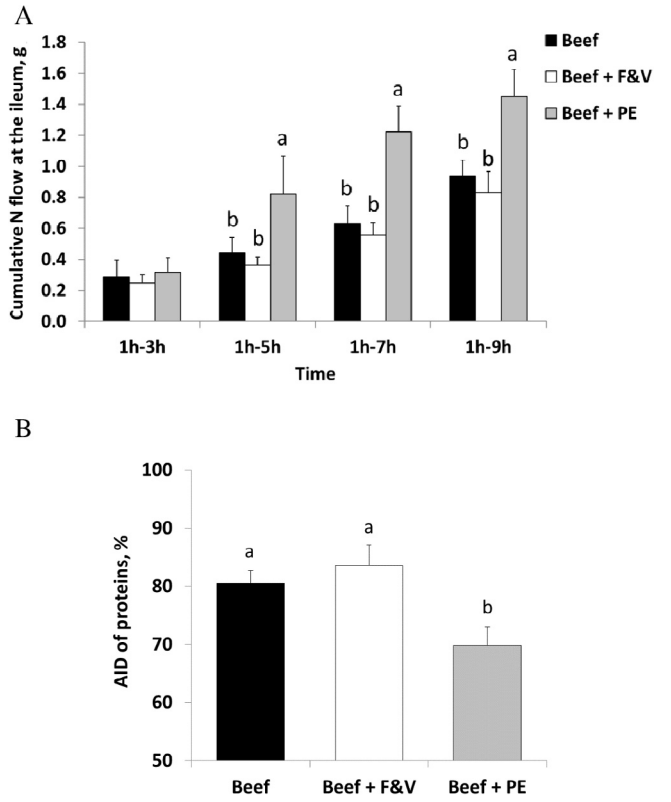

Fig. 3. Ileal cumulative flux (A) and apparent ileal digestibility (AID) (B) of nitrogen in minipigs ($n = 6$) fed a control meal (Beef) and the same meal added with either Fruit & Vegetables (Beef + F & V) or the Phenolic Extract (Beef + PE). Values are means ± SEM. Fig. 3A: data were analyzed by repeated measures ANOVA. Time*meal interaction was observed ($P < 0.001$). At a time point, different letters indicate values significantly different ($P < 0.05$). Fig. 3B: data were analyzed by ANOVA. Meal effect was significant ($P < 0.01$). Values with different letters differs ($P < 0.05$).

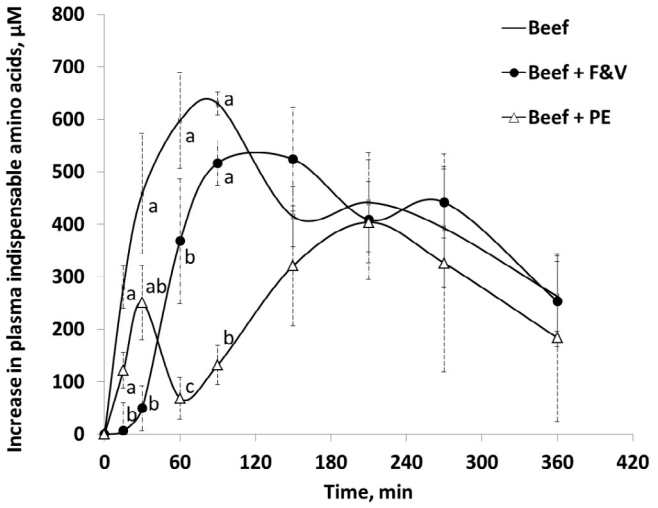

Fig. 4. Posprandial increase in the sum of plasma indispensable amino acids in minipigs ($n = 4$) fed a control meal (Beef) and the same meal with either Fruit & Vegetables (Beef + F & V) added or the phenolic extract (Beef + PE). Values are means ± SEM. Data were analyzed by repeated measures ANOVA. Time*meal interaction was observed ($P < 0.001$). At a time point, different letters indicate values significantly different ($P < 0.05$).

significantly decreased with PE ($P < 0.05$) (Fig. 3B).

Compared to the control and PE meals, the addition of F & V created a 15–30 min lag in the postprandial increase in plasma aminoacidemia, suggesting that the incorporation of these solid foods to the meal has slowed the gastric emptying (Fig. 4). Apart from this lag, the kinetics for the control and F & V meals were not greatly different. Conversely, with PE, after a rapid increase during the first 30 min following the meal, the plasma aminoacidemia tended to decrease before starting to increase again about 90 min later. The area under the curve was significantly lower ($P < 0.05$) for the PE meal than for the control and F & V meals (average postprandial increase in indispensable amino acid concentration: 250 ± 63 vs 413 ± 59 and 364 ± 69 µM, respectively).

4. Discussion

This study is the first one to investigate the *in vivo* polyphenol behaviour in the stomach according to their matrix environment, and the consequences on the digestive processes in the small intestine. It clearly highlights that the potential health benefit of an increase in polyphenol bioaccessibility in the GIT may be accompanied by unsuitable effects on protein digestion.

4.1. Bioaccessibility of the phenolic compounds in the stomach

In this intervention study, minipigs were fed with a Western-type diet associated with half of the recommended portion of F & V, i.e. 2.5 servings or 200 g. Apple is a rich source of monomeric and oligomeric flavanols as well as of dihydrochalcones, while artichoke heart mostly provides hydroxycinnamic acids, and quetsche plum all classes of phenolic compounds. Actually, hydroxycinnamic acids are the main contributors (45%, p/p) to the F & V and PE meals with 5-CQA being the most abundant compound (20%). Proanthocyanidins and catechins were brought into the meals at the level of 37%. This is consistent with intakes in the French diet where hydroxycinnamic acids and total proanthocyanidins represent 51% and 28% of the daily consumed polyphenols, respectively (Pérez-Jiménez et al., 2011).

The bioaccessibility of the main phenolic compounds was estimated after 15 min of digestion when dilution by gastric juice and gastric emptying are rather limited. The recovery in the aqueous phase of hydroxycinnamic acids, dihydrochalcones and flavonols was found to be low, in the ranges of 0.6–6.9% and 0.5–9.7% after the PE and F & V meal ingestion, respectively. Additionally, flavanol monomers and dimers were only detected in traces amounts whereas anthocyanins were absent. By contrast, all the phenolic compounds were present in greater amounts in the initial meals although the extraction yields (20–95%) were structure- and meal-dependent (data not shown).

In the plant matrix, polyphenols, proteins and cell wall polysaccharides are present in different intracellular organites. Upon thermal or mechanical processing, polyphenols diffuse out of the vacuolar frontiers and noncovalently bind to proteins and polysaccharides (Dangles & Dufour, 2008; Le Bourvellec & Renard, 2011). Among polyphenols, only procyanidins are known to strongly bind apple cell walls. Besides, *in vitro* digestion of apples of different varieties showed a greater gastric bioaccessibility of caffeoylquinic acids, phloridzin, and quercetin glycosides (73–100%), than of epicatechin and dimer B2 (30–80%) (Bouayed et al., 2012). The discrepancy between these results and the low bioaccessibilities we observed at 15 min, points to the binding of polyphenols to the solid part of the digesta and further elimination upon centrifugation. Thus, dietary proteins may markedly decrease polyphenol bioaccessibility through extensive binding. During digestion, meat proteins are hydrolyzed by pepsin releasing small-size peptides with less affinity for polyphenols. This could explain the slight increase in concentration observed in the present study for most polyphenols in the PE meal with time.

4.2. Matrix and F & V effects on polyphenol bioaccessibility

A plot of the relative bioaccessibility, which is defined as the ratio between the content from the F & V meal and the content from the PE meal for a given phenolic compound, can be used to give a deeper

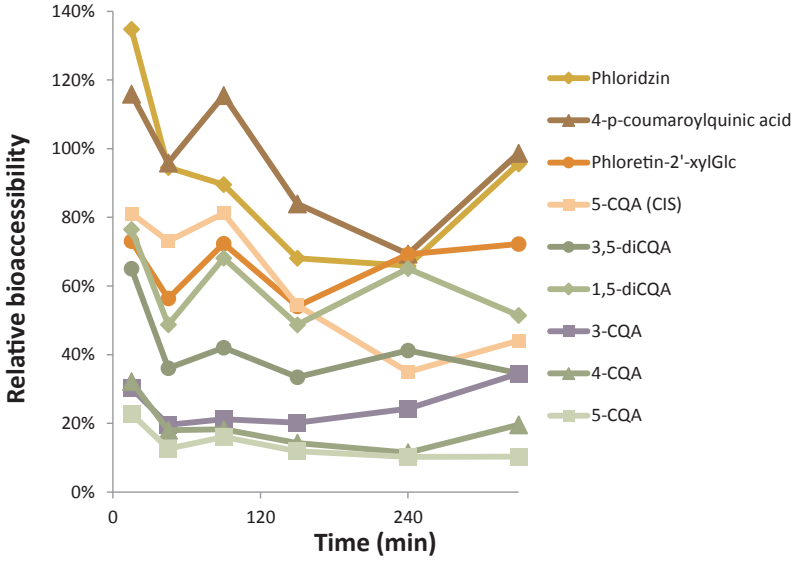

Fig. 5. Relative bioaccessibility (content in F & V digesta/content in PE digesta) for the main phenolic compounds provided by plum, apple and artichoke.

insight into the kinetic data (Fig. 5). Relative bioaccessibilities were found in the range 10–135% and remained relatively stable over time. It is noteworthy that contrasted relative bioaccessibilities were exhibited for structurally similar compounds. For example, (*trans*)-5-CQA was 4–5 times less bioaccessible than (*cis*)-5-CQA although these compounds only differ by the stereochemistry of the caffeoyl group double bond. In the same manner, 4-*p*-coumaroylquinic acid, which only lacks a 3-hydroxyl group compared to 4-caffeoylquinic acid, was ca. 5 times more bioaccessible than the latter. Bioaccessibility appears thus not to be purely driven by the physicochemical properties of polyphenols. Indeed, five phenolic compounds had a high relative bioaccessibility (> 50%), namely 4-*p*-coumaroylquinic acid, (*cis*)-5-CQA, phloridzin, phloretin-2′-XylGlc and quercetin-3-Glc. Additionally, the relative bioaccessibility of quercetin-3-Glc was unexpectedly high, between 110 and 200% (not shown). Extra quercetin-3-Glc may have resulted from glycosidase activities on the flavonol pool in freshly cut apple. Actually, all the compounds with a high relative bioaccessibility were provided by the apple contributor. By contrast, lower relative bioaccessibilities were observed for 4-CQA and 5-CQA (10–30%) while dicaffeoylquinic acids, 1,5-diCQA, and 3,5-diCQA, appeared slightly more bioaccessible (35–75%). Artichoke was the sole or the main plant contributor for these compounds. Lastly, quetsche plum exclusively provided 3-CQA. Its relative bioaccessibility remained low (30%) during the whole digestion step. A plant effect was thus outlined. Phenolic compounds appear to have been released from the apple skin and flesh as early as meal preparation and kept into the chyme at levels observed after PE meal consumption. By contrast, incomplete polyphenol release was observed from artichoke and plum.

Cooking of artichoke and freezing of all three F & V prior to meal preparation may have contributed to disruption of the F & V matrix. Mastication is the next step involved in polyphenol diffusion. Besides the mechanical action, the oral bacterial flora are known to operate rapidly through various enzymatic activities. Indeed, hydrolysis of flavonoid β-monoglycosides and oxidation by peroxidase have been demonstrated during this short-time period (Hirota et al., 2001). In pig, mastication time is short, but its efficiency is probably high. Actually, studies on the structure of swallowed food bolus by these animals are lacking.

4.3. Bioaccessible polyphenols at the ileum

No bioaccessible catechin and epicatechin were detected in the minipig ileal digesta. In agreement, 0–17% of these flavanols were found in ileal fluids from ileostomists after the consumption of apple-derived beverages, supporting their absorption in the small intestine (Borges et al., 2013; Kahle et al., 2005). Besides, bioaccessible procyanidin dimers were detected in the ileal digesta although they were almost undetectable in the aqueous phase of the gastric digesta. This supports our hypothesis of the binding of procyanidin dimers by dietary fibers and proteins in gastric digestion. Most studies with ileostomists also show the absorption in the upper GI tract of quercetin-3-O-Glc, quercetin-3-O-Gal, and phloridzin whereas part of phloretin-2′-O-XylGlc and mono- and dihydroxycinnamoylquinic acids usually reaches the colon (Borges et al., 2013; Kahle et al., 2005; Stalmach et al., 2010). This is in agreement with our results where only phloretin-2′-O-XylGlc and trace amounts of CQA and diCQA could be recovered in this series.

Gastric digestion of F & V and the extract took place in oxidative conditions as demonstrated by the formation of lipid oxidation markers (Gobert et al., 2014). The most abundant 5-CQ and the other phenolic antioxidants containing the 1,2-dihydroxyphenyl moiety may have disappeared when protecting polyunsaturated lipids from hypervalent iron forms. Lastly, in a recent *in vitro* digestion study, a protein- and lipid-rich cream was co-digested with crude cabbage and plum. Only 6.3% and 0.26% of the polyphenols were bioaccessible after both gastric and intestinal steps (Kaulmann, Andre, Schneider, Hoffmann, & Bohn, 2016). More work is therefore required to assess the effect of specific nutrients on the bioaccessibility and further bioavailability of plant phenolic compounds.

4.4. Impact of gastric polyphenol bioaccessibility on protein digestion

In animal nutrition, polyphenols, mostly tannins, are considered as anti-nutritional factors, and efforts have been carried out to reduce their content in feed. Indeed, because of their affinity for proteins, they are known to reduce the efficiency of digestion. In human nutrition, in which food is much more diverse, polyphenols are conversely considered as bioactive molecules with health benefits. In the present study, we questioned not only the effect of food matrix on polyphenol bioaccessibility (potential beneficial effect), but also the effect of this bioaccessibility on the digestion of dietary proteins (potential negative effect). We showed evidence of significant differences depending on whether polyphenols were ingested in the F & V or extract form. Whereas polyphenols in the F & V matrix did not affect protein digestion, the use of extracts, as they could be found in dietary supplements, significantly decreased the speed and efficiency of dietary protein digestion.

A decrease in apparent ileal digestibility of proteins (−5%) has previously been observed in pigs with a high level of grape tannin

addition (8 g/100 g proteins) (Myrie et al., 2008). Similarly, apparent ileal digestibility decreased (−15 to 20%) when comparing diets with low or high condensed tannin levels (0.5–3 g tannins/100 g proteins) (Jansman et al., 1995). Compared to these studies, the present one used lower levels of polyphenols (0.9 g/100 g of proteins) among which hydroxycinnamic acids were the major component. Nevertheless, the PE decreased the intestinal apparent digestibility of proteins by 15%. The lack of decrease in digestibility observed in our study with F & V could be mainly explained by the food structure. Indeed, in studies in growing pigs all the ingredients were ground, whereas in the present work F & V were cut in pieces, a process which has limited the polyphenol bioaccessibility.

With the experimental design we used it was not possible to determine whether the decrease in apparent digestibility observed with PE was due to a decrease in dietary protein digestibility or to an increase in endogenous protein secretion, but whatever the mechanism, the increase in protein flowing to the colon will reduce the availability of amino acids for peripheral tissues.

Meat proteins have been reported to be rapidly digested in human and minipigs (Bax et al., 2013; Remond et al., 2007). In agreement, a sharp increase in plasma aminoacidemia was observed in the present study with the control diet. The sarcoplasmic proteins of meats, which are rapidly released in the liquid phase of the stomach, and consequently the first to flow down to the small intestine, probably contribute to the very rapid increase in plasma amino acid concentration (Sayd, Chambon, & Santé-Lhoutellier, 2016). Structural proteins (actin and myosin) probably take more time to be released from the matrix in the stomach. As a consequence, they could be the main target of polyphenols, which would explain why the postprandial increase of plasma aminoacidemia was biphasic with the phenolic extract. Finally, the lower gastric bioaccessibility of polyphenols from the sliced fruits and vegetables matches well with the lack of effect of the F & V meal on amino acid absorption.

The decrease in apparent ileal digestibility observed with the phenolic extract may have little consequences in people having high protein intake levels; however, in people with inadequate protein intake, it would significantly worsen the health state. The use of supplements with free polyphenols, as antioxidants, should therefore be considered carefully in the elderly, a population in which malnutrition is the most often observed. Furthermore, for this population the slower digestion rate we observed with the phenolic extract would negatively impact the assimilation of absorbed amino acids. Indeed rapidly digested proteins are better suited to boost the postprandial protein anabolism in the elderly, which could help them to fight more efficiently against sarcopenia (Dardevet et al., 2012). Of course all these implications can be modulated according to the polyphenol structure and its relative affinity for proteins.

5. Conclusion

Finally, this serving of F & V provided ca. 154 mg of low-molecular weight phenolic compounds among which only 1.5 and 3.1% were readily bioaccessible in the gastric tract after the consumption of the F & V and the PE meals. Flavanol dimers, which were below the quantification limits in the gastric digesta, were partly recovered in the ileal digesta in agreement with their extensive binding by dietary proteins and digestion enzymes in the gastric digesta. The kinetic approach developed in this study further demonstrated relatively stable levels of polyphenols during 150 min before gastric emptying.

Second, the presence of polyphenols at a nutritional dose had no negative effect on protein digestibility when ingested as diced fruit & vegetables. By contrast, the consumption of a phenolic extract may appear detrimental to humans with low protein intake. Special care should then be given to the risk/benefit balance when consuming polyphenol supplements.

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Conflict of interest statement

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.07.104.

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