Non-extractable proanthocyanidins from grapes are a source of bioavailable (epi)catechin and derived metabolites in rats

María Luisa Mateos-Martín, Jara Pérez-Jiménez*, Elisabet Fuguet and Josep Lluís Torres

Abstract

The non-extractable fraction of many fruit and vegetables contains putatively bioactive polyphenolic compounds that, in most cases, have not been well characterised structurally. Non-extractable proanthocyanidins (NEPA), of a polymeric nature, are part of the dietary fibre fraction of food. Using liquid chromatography coupled to a mass spectrometer equipped with an electrospray ionisation chamber and a triple quadrupole mass analyser for tandem analysis (HPLC–ESI–QqQ–MS/MS) techniques, we examine the phenolic metabolites present in urine and faeces 24 h after ingestion of an NEPA-rich fraction. We show that NEPA are partially depolymerised during their transit along the intestinal tract, as evidenced by the presence of (epi)catechin (EC) monomers and dimers in faeces and phase II conjugates of EC in urine. Moreover, NEPA are further metabolised by the intestinal microbiota into smaller metabolites including phenolic acids that are present in urine as both free phenolics and conjugates with glucuronate or sulphate moieties. For the first time, we report evidence that NEPA behave in vivo as a source of phenolics that are released progressively and deliver phenolic species that come into contact with the intestinal walls and are bioavailable for at least 24 h after ingestion.

Key words: Bioavailability; MS: Non-extractable proanthocyanidins; Polyphenols

Proanthocyanidins (PA) are a class of dietary polyphenols. They are polymers of flavan-3-ols present in a wide variety of plant-based foodstuffs, such as berries, cocoa or certain nuts (9). Several supplementation studies both in animals and in human subjects using PA-rich products have shown that PA play a preventive role against several conditions including CVD (2–4) or diabetes (1,5,6). Similarly, a recent epidemiological study showed an inverse association between the intake of PA play a preventive role against several conditions including CVD (2–4) or diabetes (1,5,6). Similarly, a recent epidemiological study showed an inverse association between the intake of

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Most studies of PA, including those that address their metabolism, assume that the PA in foodstuffs correspond exclusively to the supernatants obtained after extracting the food with acetone; the most common procedure for their analysis (8). The currently available evidence suggests that NEPA may be as essential constituents of many foodstuffs is still scarce. The currently available evidence suggests that NEPA may be more abundant than EPA in much food (12–14) and, therefore, that significant amounts of NEPA are ingested daily.

Over the last decade, several studies have addressed the metabolism of PA. Although initial studies emphasised fairly poor intestinal absorption that was limited to dimers (15–17), later observations indicate that once intact PA reach the colon they are widely transformed by the colonic microbiota into small phenolic acids (18–21). These metabolites are absorbed, and then transformed in the liver, and the resulting conjugates are transferred to the bloodstream. A recent study
Fate of non-extractable proanthocyanidins

in which $[^{14}C]$procyanidin B2, a labelled PA dimer, was administered to rats, reported bioavailability of around 80%, based on total urinary $^{14}C$ (20).

Nevertheless, these papers have mostly addressed the bioavailability of dimers or trimers, while the most abundant PA in food are polymers (22). Indeed, some recent studies have suggested that PA are also depolymerised into (epi)catechin (EC) units before cleavage into smaller species and further metabolism (21,23–25). These studies suggest that phenolics that are bioavailable after the ingestion of PA-rich foodstuffs must have come from NEPA (24–26), but this has not been proved as the specific metabolism of NEPA has never been reported.

Grape antioxidant dietary fibre (GADF) is a food product obtained from red grapes that is rich in dietary fibre and polyphenols (27). Besides extractable polyphenols, including EPA (28), GADF contains a significant amount (14.8%) of NEPA (27) and was used in the studies which suggest that non-extractable polyphenols are an important source of metabolites that are bioavailable in rats (24–26). To study the contribution of NEPA to the pool of phenolic metabolites from fruit and vegetables, we considered using a NEPA-rich fraction from GADF.

Our objective was to evaluate the fate of NEPA in rats 24 h after ingestion of a preparation free from any extractable polyphenols. NEPA metabolites, including hepatic and microbially derived metabolites, were analysed in urine and faeces using liquid chromatography coupled to a mass spectrometer equipped with an electrospray ionisation (ESI) chamber and a triple quadrupole mass analyser for tandem analysis (HPLC–ESI–QqQ–MS/MS).

Experimental methods

Reagents and samples

GADF was obtained from red grapes (Cencibel variety, harvested in 2005 in La Mancha region of Spain) by a patented procedure (29). The NEPA content of GADF has previously been reported to be 14.8 g/100 g of dry weight (27). To obtain an EPA-free (and therefore NEPA-rich) fraction, GADF (4 g) was defatted with hexane (3 × 40 ml), air-dried overnight and the residue was extracted with methanol–water (50:50, v/v, 200 ml) and then with acetone–water–acetic acid (70:29:5:0.5, by vol., 10 ml by nitrogen stream at room temperature). The supernatant was decanted and the residue, including NEPA-rich fraction, was vacuum filtered and lyophilised. The supernatant was decanted and the residue was extracted with methanol–water (50:50, v/v, 40 ml), air-dried overnight and then placed in 1 ml of acid water (addition of phosphoric acid to reach pH 3). Taxifolin (100 μl of a 50 parts per million (ppm) solution) was added as an internal standard, to obtain a final concentration of 5 ppm. Then the samples were subjected to solid phase extraction in Oasis HLB (60 mg) cartridges from Waters Corporation (Mildford, MA, USA). The cartridges were activated with methanol (1 ml) and acid water (2 ml) and the samples loaded. To remove interfering components, the samples were washed with acid water (9 ml) and then the phenolic compounds were eluted with methanol (1 ml). Faeces (0.5 g) were defatted with hexane (10 ml) and the residue was extracted with methanol–water–phosphoric acid (8:1:9:0.1, by vol., 10 ml) and concentrated down to 1 ml by nitrogen stream at room temperature. Taxifolin (100 μl of a 50 ppm solution, final concentration 5 ppm) was added to each sample as an internal standard.

Extracts from both urine and faeces were filtered through a polytetrafluoroethylene 0.45-μm membrane from Waters Corporation into amber vials for HPLC–MS/MS analysis.

Animal experiments

The study was carried out on female Sprague–Dawley rats (n = 10, body weight 235 (sd 9.3) g, 12 weeks of age) provided by Harlan Interfana Ibérica SL (Barcelona, Spain). The animals were fed with a polyphenol-free diet (TD94048), also purchased from Harlan Interfana Ibérica SL, and they were maintained in plastic cages at room temperature (22 ± 2°C) and 55 (sd 10)% relative humidity, with a 12 h light–12 h dark cycle for 1 week, in accordance with European Union regulations. After food deprivation for 12 h with free access to water, a group of animals (n = 5) was administered a suspension of NEPA from GADF in tap water (1 g NEPA-rich fraction/10 ml, 1.6 g NEPA-rich fraction/kg body weight) by oral gavage, while a control group (n = 5) was administered tap water (16 ml/kg body weight). The animals were then placed in metabolism cages and urine and faeces were collected over 24 h and stored at −80°C until extraction and analysis. These experimental protocols were approved by the Experimental Animal Ethical Research Committee of the CSIC in accordance with the current regulations for the use and handling of experimental animals.

Sample preparation

The biological samples were prepared according to previously described procedures for the extraction of phenolic metabolites (21,24,25). Briefly, urine samples were concentrated via a nitrogen stream at room temperature and then resuspended in 1 ml of acid water (addition of phosphoric acid to reach pH 3). Taxifolin (100 μl of a 50 parts per million (ppm) solution) was added as an internal standard, to obtain a final concentration of 5 ppm. Then the samples were subjected to solid phase extraction in Oasis HLB (60 mg) cartridges from Waters Corporation (Mildford, MA, USA). The cartridges were activated with methanol (1 ml) and acid water (2 ml) and the samples loaded. To remove interfering components, the samples were washed with acid water (9 ml) and then the phenolic compounds were eluted with methanol (1 ml).
**HPLC–electrospray ionisation–MS/MS analysis**

A Quatro LC from Waters Corporation triple quadrupole mass spectrometer with an electrospray source was used in negative mode to obtain MS and MS/MS data. Liquid chromatography separations were performed using an Alliance 2695 system from Waters Corporation equipped with a Phenomenex (Torrance, CA, USA) Luna C18 (50 × 2.1 mm internal diameter) 3.5-μm particle size column and a Phenomenex Securityguard C18 (4 × 3 mm internal diameter) column. Gradient elution was performed with a binary system consisting of (A) 0.1% aqueous formic acid and (B) 0.1% formic acid in CH3CN. An increasing linear gradient (v/v) was used (t (min), %B): 0, 8; 10, 23; 15, 50; 20, 50; 21, 100, followed by a re-equilibration step.

Metabolites were detected in multiple reaction monitoring (MRM) experiments, and their identity was confirmed by product ion scan experiments. The dwell time for the MRM experiments was 100 ms and the cycle time for all the experiments was 2 s. Cone energy and collision energy in MRM experiments was 100 ms and the cycle time for all the experiments was 2 s. Cone energy and collision energy in MRM mode were optimised for each group of metabolites: 30 V and 10 eV for taxifolin, 30 V and 15 eV for microbially derived proanthocyanidin metabolites detected in urine included valerolactones, phenylvaleriacids, phenylpropionic acids, phenylacetic acids, benzoic acid, cinnamic acids and lignans. Microbially derived proanthocyanidin metabolites were initially identified by previously reported MRM transitions corresponding to the main fragments and their identity was confirmed by a second MRM transition and/or by product ion scan experiments.

The EC metabolites included three glucuronidated forms (465 → 289), two sulphated forms (369 → 289), a monoconjugated metabolite with glutathione (594 → 289), two methylated and glucuronidated forms (479 → 303), a di-glucuronidated form (614 → 289) and a tri-conjugated metabolite (397 → 289) corroborated by MS/MS experiments. All these derivatives were detected in urine samples and the di-glucuronidated conjugate was also detected in faeces.

Fig. 1 shows the product ion spectrum of glucuronide (GlcA)-EC-3 (m/z 465). Fragments at m/z 289 and m/z 245, corresponding to the loss of the conjugate moiety and the respective cleavage of CO2 from the free EC unit, were observed; as were two fragments at m/z 175 and 113, from the fragmentation of the GlcA moiety. The MS/MS spectra of other conjugates showed characteristic fragments corresponding to a B-ring retro Diels–Alder fission of the EC, such as the fragment at m/z 137 for Me-GlcA-EC, which corresponds to a B-ring fragment with attached methyl group and GlcA moieties, indicating that the conjugation was located on the B-ring.

### Microbiologically derived proanthocyanidin metabolites
A total of twenty microbiologically derived PA metabolites were identified in urine from rats fed the NEPA-rich fraction; two of them were also detected in faeces (Table 2). All these metabolites were either not detected in the control group or detected at concentrations that were at least 10-fold lower. The metabolites included three glucuronidated forms (465 → 289), two sulphated forms (369 → 289), a monoconjugated metabolite with glutathione (594 → 289), two methylated and glucuronidated forms (479 → 303), a di-glucuronidated form (614 → 289) and a tri-conjugated metabolite (397 → 289).

### Results

#### (Epi)catechin and its phase II metabolites

Free EC (MRM transition 289 → 245) and a signal corresponding to a dimer (577 → 289) were detected in faeces by liquid chromatography–ESI–MS/MS. The product ion spectrum of this dimer provided characteristic fragments at m/z 425 and m/z 405 originated by cleavage of the C-ring of one of the EC units through a retro Diels–Alder reaction and consecutive loss of water. A fragment at m/z 451, caused by heterolytic ring fission, was also observed.

A total of ten EC conjugates derived from the activity of phase-II enzymes in both the intestinal tract and liver were detected in urine samples (Table 1). All these compounds were either not detected in the control group or detected at concentrations that were at least 10-fold lower. The metabolites were initially identified by previously reported MRM transitions corresponding to the main fragments and their identity was confirmed by a second MRM transition and/or by product ion scan experiments.

#### Microbiologically derived proanthocyanidin metabolites
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<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MRM parent</th>
<th>Identification</th>
<th>Urine</th>
<th>Faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>(−)-EC</td>
<td>289 → 245</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>PC dimer</td>
<td>577 → 289</td>
<td>MS/MS: 575; 451; 424; 405; 327; 289; 123</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Mono-conjugated metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GlcA-EC-1</td>
<td>465 → 289</td>
<td>MS/MS: 465; 289; 245; 137; 113</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>GlcA-EC-2</td>
<td>465 → 289</td>
<td>MS/MS: 465; 289; 257; 175;113</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>GlcA-EC-3</td>
<td>465 → 289</td>
<td>MS/MS: 465; 289; 245; 175; 113</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Sulf-EC-1</td>
<td>369 → 289</td>
<td>MS/MS: 369; 289; 245; 228; 184; 113</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Sulf-EC-2</td>
<td>369 → 289</td>
<td>MRM daughter 289 → 245</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>GSH-EC-1</td>
<td>594 → 289</td>
<td>MRM daughter 289 → 245</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Di-conjugated metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me-GlcA-EC-1</td>
<td>479 → 303</td>
<td>MS/MS: 479; 303; 289; 245; 173; 137; 113</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Me-GlcA-EC-2</td>
<td>479 → 303</td>
<td>MS/MS: 479; 303; 285; 259; 173; 137; 113</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Di-GlcA-EC-1</td>
<td>641 → 289</td>
<td>MS/MS: 641; 465; 289</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Tri-conjugated metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di-Me-Sulf-EC-1</td>
<td>397 → 289</td>
<td>MRM daughter 289 → 245</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

MRM, multiple reaction monitoring; PC, procyanidins; GlcA, glucuronide; Sulf, sulphate; GSH, glutathione; Me: methyl group.
3-hydroxyphenylacetic acid and 4-hydroxyphenylacetic acid (151→107), which were also detected. Similarly, 3,4-dihydroxyphenylpropionic acid, 3-hydroxyphenylpropionic acid and 4-hydroxyphenylpropionic acid were detected in urine. Both 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylpropionic acid were absorbed and later conjugated in the liver as shown by the detection of the derivatives GlcA-3 or 4-hydroxyphenylacetic acid (327→151), Sulf-3, 4-dihydroxyphenylpropionic acid (261→181) and Sulf-3 or 4-hydroxyphenylpropionic acid (245→165). Conjugated forms of phenylvaleric and hippuric acid were detected in urine.

Two microbially derived phenolic metabolites were identified in the faeces from rats fed the NEPA-rich fraction: 4-hydroxyphenylpropionic acid (165→121) and 3,4-dihydroxyphenylpropionic acid (181→137).

Phenolic acids generated fragments corresponding to the successive loss of two CO2 molecules. Similarly, MS/MS spectra of sulphated forms showed signals corresponding to the loss of sulphate and CO2. These fragments confirm the identity of phenolic acids and their conjugated forms.

**Table 2. Detection of microbially derived proanthocyanidin metabolites in urine and faeces from rats fed a non-extractable proanthocyanidin-rich fraction from grape antioxidant dietary fibre**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MRM parent</th>
<th>Identification</th>
<th>Urine</th>
<th>Faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valerolactones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulf-dihydroxyphenylvalerolactone</td>
<td>287→207</td>
<td>MRM daughter 207→163</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Phenyvaleric acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulf-dihydroxyphenylvaleric acid</td>
<td>289→209</td>
<td>MRM daughter 209→165</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Phenylpropionic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Hydroxyphenylpropionic acid</td>
<td>165→121</td>
<td>MS/MS: 165; 121; 93</td>
<td>×</td>
<td>×</td>
</tr>
<tr>
<td>3,4-Dihydroxyphenylpropionic acid</td>
<td>181→137</td>
<td>Standard retention time</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Sulf-3,4-dihydroxyphenylpropionic acid</td>
<td>261→181</td>
<td>MRM daughter 181→137</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Sulf-3 or 4-hydroxyphenylpropionic acid</td>
<td>245→165</td>
<td>MS/MS: 245; 165; 121</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Phenylacetic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Hydroxyphenylacetic acid</td>
<td>151→107</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>4-Hydroxyphenylacetic acid</td>
<td>151→107</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>GlcA-3 or 4-hydroxyphenylacetic acid</td>
<td>327→151</td>
<td>MS/MS: 327; 151; 107</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>3,4-Dihydroxyphenylacetic acid</td>
<td>167→123</td>
<td>MS/MS: 167; 123; 105; 95</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Benzoic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzoeic acid</td>
<td>121→77</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid</td>
<td>137→93</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>3,4-Dihydroxybenzoic acid</td>
<td>153→109</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>178→134</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Me-hippuric acid</td>
<td>193→178</td>
<td>MS/MS: 193; 178; 134</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Cinnamic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m-Coumaric acid</td>
<td>163→119</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>163→119</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>193→134</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Lignans</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterodiol</td>
<td>301→107</td>
<td>Standard retention time</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Sulf-enterolactone</td>
<td>377→297</td>
<td>MRM daughter 297→253</td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>

MRM, multiple reaction monitoring; Sulf, sulphate; GlcA, glucuronide; Me, methyl group.
of some of the microbially derived metabolites; others were confirmed by the use of standards, i.e. 4-hydroxybenzoic acid (Fig. 2).

**Discussion**

Several studies have addressed the metabolism of dietary oligomeric PA, mostly dimers. That work provides a quite clear picture of the different steps in the metabolism of PA dimers in laboratory animals and human subjects\(^{(18–21)}\). This process comprises the absorption of monomers and small oligomers (dimers) of PA in the small intestine and the absorption of microbially derived metabolites in the large intestine, after direct fermentation of the oligomers by microbiota without prior depolymerisation into EC. The absorbed metabolites may be conjugated in the liver, mostly resulting in GlcA, sulphates and methyl derivatives which pass to the bloodstream and eventually reach other tissues. Finally, the metabolites are excreted in urine and the fraction of PA that is not absorbed is excreted in faeces.

The metabolic fate of larger PA polymers is believed to follow the same pattern as that of dimers and trimers: essentially, direct cleavage of the EC units into smaller phenolic acids by the intestinal microbiota. By examining the metabolic fate of GADF, we have recently suggested that polymeric PA undergo depolymerisation into EC units during their transit along the intestinal tract\(^{(24,25)}\). This is important because it implies that the polymers may gradually release EC moieties during the postprandial period. Using a NEPA-rich fraction, devoid of EC monomers and extractable oligomers, we show here that this is indeed occurring. The faeces of rats fed with NEPA contained monomeric and dimeric EC and

![Fig. 2. HPLC–electrospray ionisation-MS profile corresponding to the detection by multiple reaction monitoring of 4-hydroxybenzoic acid (transition 137 → 93): (a) urine from rats fed non-extractable proanthocyanidins from grape antioxidant dietary fibre, (b) 4-hydroxybenzoic acid standard, (c) urine from rats fed water.](https://www.cambridge.org/core/journals/british-journal-of-nutrition/issue/B9F8B99E04B6A6C9F3F7C6D258B270F4)

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their urine contained 10 phase II EC metabolites. These results clearly show that this fraction of dietary fibre generates bioavailable derivatives of EC. The di-glucuronidated EC derivative detected in faeces further demonstrates that those monomers that are released from NEPA efficiently enough to reach the liver suffer conjugation and are transported back to the intestine via bile. Our evidence for intestinal depolymerisation is consistent with a recent observation by Jove et al. (32) who report a 600% recovery of free EC in the caecal content of rats after providing them with a single dose of PA-rich

Fig. 3. Metabolism of non-extractable proanthocyanidins (NEPA).
almond extract. Moreover, the more than twenty EC-derived smaller metabolites detected in urine are consistent with the previous description of microbial fermentation and absorption. Our results also corroborate NEPA as PA, since direct evidence of the structure of these insoluble polymers is scarce. In fact, the residue after extraction with 70% acetone is commonly not considered to be a source of polyphenols. Our results agree with those which report that the residues of the common extraction with 70% acetone contain significant amounts of PA.\(^{12-14}\)

The transformation of NEPA (Fig. 3) differs in part from the process suggested for the transformation of small EPA, previously described. In the case of NEPA, a proportion of the larger PA polymers appears to be depolymerised during their transit along the intestinal tract, resulting in delivery of EC monomers and possibly oligomers. The posterior degradation by the intestinal microbiota into small units may also differ between NEPA and EPA. As NEPA are associated with the food matrix in foodstuffs, particularly with other insoluble polymers constitutive of dietary fibre, their conversion may be slow compared to that of EPA. This deferred release would make NEPA metabolites bioavailable for particularly long times after intake and may result in them having health effects for a long time. This may explain the previously reported delay in the increase of plasma antioxidant capacity after the intake of GADF by human subjects compared to that observed after the intake of food rich in EPA, such as red wine.\(^{35}\) In addition, the metabolites detected in faeces prove that putatively active species remain in contact with the colonic epithelium for at least 24 h after ingestion. Indeed, the intake of PA, and particularly PA with a high degree of polymerisation, has been associated with a reduced risk of colorectal cancer\(^{37}\) and our results suggest that food sources of NEPA could provide such putative cancer-preventative PA.

Previous nutritional studies have considered the extractable fraction of PA as the only source of dietary polyphenols. We show here that NEPA should be taken into account as most of the food in these studies contains significant amounts of NEPA. Further work is needed, both on the systematic analysis of NEPA in foodstuffs and on the metabolism of NEPA from different food sources, in order to unravel the contribution of this fraction of dietary PA to the health-promoting effects of fruit and vegetables.

In conclusion, we show here that NEPA are a source of polymeric PA that are progressively depolymerised during their transit along the intestinal tract into EC monomers and dimers, and later metabolised by the intestinal microbiota into smaller units. As a result, EC, phenolic acids and their phase II metabolites are in contact with the intestinal tract and bioavailable for at least 24 h after ingestion.

Acknowledgements

The present work was supported by the Spanish Ministry of Education and Science (AGL2009-12374-C03-03/ALI). J. P.-J. thanks the Spanish Ministry of Science and Innovation for granting her a Sara Borrell postdoctoral contract (CD09/00068). GADF was a generous gift from Professor Fulgencio Saura-Calixto, ICTAN-CSIC. None of the authors had any conflict of interest. J. L. T., J. P.-J. and M. L. M.-M. designed the research. M. L. M.-M. and J. P.-J. carried out the experimental work. M. L. M.-M., J. P.-J. and E. F. analysed the data. M. L. M.-M. and J. P.-J. wrote the first version of the manuscript. All the authors contributed to writing the manuscript and approved the final version. Language revision by Christopher Evans is appreciated.

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