JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Bioavailability of Intact Proanthocyanidins in the Rat Colon after Ingestion of Grape Seed Extract

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ABSTRACT: Current evidence shows that monomeric flavonoids are known to be only slightly absorbed in the small intestine, but the metabolism of oligomeric and polymeric proanthocyanidins (PAC) in the colon is poorly understood. The objective of this study was to optimize the analysis of grape seed extract (GSE) in feces and use that method to assess the presence of PAC in the colon after ingestion of GSE. Rats were fed a diet ad libitum containing 0.25% (w/w) GSE for 10 days. Feces were collected daily and colonic contents at sacrifice on day 10, respectively. The recovery of fecal PAC using a solid-phase extraction (SPE) method was >70%. PAC were separated by normal-phase HPLC with fluorescence detection, and subsequent peak confirmation was done by MS-ion trap. The concentration of colonic contents at day 10 was 13.9 mg/kg for monomer, and those for oligomers (dimers−hexamers) were 33.4, 84.6, 87.2, 57.3, and 35.7 mg/kg, respectively. The concentration of monomeric and oligomeric PAC in daily feces was similar among days. In the mass balance analysis, approximately 11% of ingested PAC was recovered in the feces. These findings indicate that ingested PAC were present in the colon as the intact parent compounds and thus may contribute to the health of the gastrointestinal tract.

KEYWORDS: *proanthocyanidins, metabolism, colonic feces, GSE, gastrointestinal health*

INTRODUCTION

Proanthocyanidins (PAC) are secondary plant metabolites present in many different kinds of fruits, vegetables, plant-derived beverages, and foods such as cocoa, grapes, apple, tea, and red wine. PAC fall into the class of condensed tannins and are oligomers and polymers of (+)-catechin and (−)-epicatechin and other related flavonoids, mainly linked by either B-type (C4→C6 or C8) or A-type linkages (C2→O7). Grape seed extract (GSE) falls into the B-type category, and GSE-rich diets have been associated with a reduced risk of chronic cardiovascular diseases[1] and various common cancers, including colorectal cancer.[2] Epidemiological studies have linked the consumption of PAC, but not the monomer, with a decreased risk of colorectal cancer.[3,4] Numerous in vitro and animal models have proposed various mechanisms to explain this association.[5,6] GSE is a rich source of PAC and has various potential biological and physiological effects via its capacity to interact with membranes,[7] protect the intestinal barrier from excess permeability,[8] and possess antioxidant and anti-inflammatory actions.[9]

Monomeric flavonoids including (+)-catechin and (−)-epicatechin have been previously reported to be absorbed in humans and animals, either as parent compounds or conjugated metabolites following the consumption of dietary PAC.[10,11] Although studies of the metabolism and absorption of monomeric flavan-3-ols are numerous, investigations on the fate of oligomeric and polymeric PAC are limited, being mostly focused on the fate of dimeric PAC. Dimers have been found in the urine as well as the colon of rats fed GSE.[12] A low level of PAC dimer B_2 has been reported in human urine and plasma after consumption of cocoa,[13,14] whereas dimer B_1 was found in human plasma following ingestion of GSE.[15] Interestingly, dimer procyanidin B_3 was reported as not bioavailable in rats fed either GSE or the dimer.[16]

The results on the bioavailability of larger PAC in humans and animals obtained thus far have been very sparse and also conflicting. Whereas [^{14}C]PAC from *Desmodium intortum* were reported in the urine of both sheep and goats, the analytical methods detected only phenolics in the urine, and the PAC-sensitive method gave no response.[17] It has been reported that apple procyanidin oligomers up to pentamers were detected in rat plasma 2 h after oral administration,[18] but a number of additional studies report that polymeric PAC were not absorbed.[19,20] Deprez et al. reported that human microbiota degrades PAC to low molecular weight phenolic compounds,[21] suggesting that absorption of the parent compounds is unlikely, and a recent study in humans demonstrated that dietary polymeric PAC do not contribute to the pool of plasma flavanols.[22] Thus, the absorption of larger PAC is unclear at best.

On the other hand, metabolism of PAC to simpler compounds and their appearance in plasma and urine have been documented. A number of metabolites have been observed, namely, benzoic acids, phenylpropionic acids, phenylacetic acids, phenylvaleric acids, phenylpropionic acids, and phenylacetic acids, with hydroxylation mostly occurring at the meta position.[23,24] For instance, Urpi-Sarda detected microbial metabolites including 3-hydroxyphenylacetic acid, hippuric acid, and the phenylvalerolactones 5′-(3′,4′-dihydroxyphenyl)-γ-valerolactone and 5′-(3-methoxy-4-hydroxyphenyl)-

Received: May 10, 2012
Revised: November 21, 2012
Accepted: December 4, 2012
Published: December 17, 2012

γ-valerolactone in human urine after cocoa consumption.[13] Other studies have suggested that these microbial metabolites attributed to dietary polyphenol could have important biological effects,[25] but little is known about the fate of intact parent PAC compounds in the gut.[26]

GSE consists of a complex mixture of monomer, oligomers (2−10 subunits), and polymers of flavan-3-ols, which makes the identification and quantitation of these compounds difficult and challenging. To date, normal-phase chromatography utilizing fluorescence detection has been proven to be successful in separating and detecting monomeric and oligomeric fractions of PAC on the basis of the degree of polymerization (DP). In addition, analytical methods using liquid chromatography coupled to electrospray ionization mass spectrometric detection (LC-ESI-MS),[27,28] tandem mass spectrometry including ion trap,[29] and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS)[30] have been described for the analysis of flavan-3-ol compounds and metabolites. The sample preparation step is very important for quantitative and qualitative analyses, particularly working with complex biological matrices. A thorough sample preparation is needed to reduce interference and increase sensitivity when compounds are quantified by sensitive analytical instruments.[13]

As the gastrointestinal fate of PAC with a degree of polymerization higher than two is largely unknown, the present work investigates in rats whether or not oligomeric PAC (DP ≥ 2) are present in the colon after the dietary consumption of GSE. Therefore, the aim of this study is to develop an analytical method to extract fecal PAC and then use this method to measure the presence and concentration of parent PAC compounds in rat feces and colonic contents.

MATERIALS AND METHODS

Chemicals and Reagents. (−)-Epicatechin was purchased from Sigma-Aldrich (St. Louis, MO, USA). GSE was donated by Kikkoman Corp. (Tokyo, Japan). Procyanidin B-type dimers, trimers, pentamers, and hexamers were purified individually and supplied by Mars Inc. (Hackettstown, NJ, USA). All solvents (acetonitrile, methanol, acetic acid, and formic acid) were HPLC grade reagents from Fisher Scientific (Pittsburgh, PA, USA). Water was deionized using a Milli-Q water purification system (Millipore, Bedford, MA). Sep-Pak Vac C18, 3 cm^3/500 mg cartridges were purchased from Waters (Milford, MA, USA); a 0.2 μm PTFE syringe filter and 0.22 μm Ultrafree-MC centrifugal filter units were purchased from Fisher Scientific and Millipore.

Animals and Diets. All procedures were administered under the auspices of the Animal Resource Services of the University of California, Davis, which is accredited by the American Association for the Accreditation of Laboratory Animal Care. Experimental protocols were approved before implementation by the University of California, Davis, Animal Use and Care Administrative Advisory Committee, and were administered through the Office of the Campus Veterinarian. Seven-week-old male Sprague−Dawley rats (Wilmington, MA, USA) (180−200 g) were housed individually in suspended stainless steel cages in a temperature (22−23 °C) and photoperiod (12 h/day)-controlled room. Animals (5 rats/group) were fed ad libitum either a control diet consisting of purified egg white based powder diet (Table 1) or the control diet supplemented with 0.25% (w/w) GSE. Given that the GSE contains 83.4% of PAC, the GSE diet will provide 0.2% of PAC. Body weight gain and food and water consumption were recorded daily and were similar for both dietary groups throughout the study. Fecal samples were collected 24 h after initiation of the diet and subsequently every 24 h. At day 10 on the diets, rats were euthanized and the colonic contents were collected. All samples were immediately frozen in liquid nitrogen and stored at −80 °C until analyzed.

Table 1. Composition of Control Diets

ingredient	%
cerelose	39.5
egg white	21
corn starch	20
alphacel	4
mineral mix[a]	6
vitamin mix[b]	1.5
corn oil	8

[a]Mineral mix (g/kg of mixture): calcium carbonate, 139.7; calcium phosphate dibasic anhydrous, 166.6; magnesium sulfate anhydrous, 49.5; potassium phosphate dibasic, 133.6; sodium chloride, 21.2; ferrous sulfate-7-hydrate, 6.2; zinc carbonate, 0.8; manganous sulfate monohydrate, 0.61; cupric sulfate-5-hydrate, 0.66; potassium iodide, 0.0033; chromium potassium sulfate dodecahydrate, 0.048; sodium selenite, 0.015; sodium molybdate, 0.0063; and cerelose, 481.06. [b]Vitamin mix (g/kg of mixture): inositol, 25; ascorbic acid, 5; calcium pantothenate, 0.67; thiamin hydrochloride, 0.53; pyridoxine hydrochloride, 0.53; nicotinic acid, 1; menadione, 0.25; riboflavin, 0.27; *p*-aminobenzoic acid, 0.5; folic acid, 0.07; biotin, 0.26; rovimix E-50%, 3.6; rovimix A-100%, 0.16; rovimix D3−500, 0.13; vitamin B12, 3.33; choline chloride, 71.5; and cerelose, 887.5.

Sample Preparation for the Identification of PAC in Rat Feces. Feces from rats fed the control diet were pooled from five animals. To determine spike recovery, 0.1 g of blank feces was spiked with 20 μL of a GSE solution (10 mg/mL), and 100 μL of 1 mM ascorbic acid was added to the feces to prevent oxidation during extraction. Different volumes of acid (1 or 10% (v/v) formic acid) and ratios of methanol/water were studied in the extraction step to optimize the extraction efficiency. Briefly, the mixture was homogenized with 3 mL of formic acid/water/methanol (1:49.5:49.5, v/v/v). GSE was allowed to interact with samples for 10 min and sonicated to allow PAC to bind to the fecal matrix. The samples were vortexed and centrifuged at 4 °C for 10 min at 4200*g* on a refrigerated Eppendorf centrifuge 5804 R (Westbury, NY, USA). The supernatant was collected, and the pellet was re-extracted following the same extraction procedure described above. The supernatants were combined and evaporated in a Buchi rotary evaporator at 30 °C to almost dryness, and samples were reconstituted with 1% (v/v) formic acid in water. Samples were loaded into solid-phase extraction (SPE) cartridges (Sep-Pak Vac C18, 3 cm^3/500 mg) previously conditioned with 5 mL of 100% methanol and followed by 1% (v/v) formic acid in water. The cartridge was washed with 5% aqueous methanol to remove carbohydrates, proteins, and other impurities. After the washing step, GSE was eluted with formic acid/water/methanol (1:49.5:49.5, v/v/v). The eluate was evaporated at 30 °C to almost dryness and resuspended to a final volume of 0.5 mL with the elution solvent, formic acid/water/methanol (1:49.5:49.5, v/v/v). All standard solutions and samples were filtered through 0.2 μm PTFE filters (Fisher Scientific, Houston TX, USA) or 0.22 μm Ultrafree-MC centrifugal filter units (Millipore) prior to HPLC injection.

Normal-Phase LC-ESI-MS Ion Trap Analysis. Chromatographic conditions were adapted and modified from previous papers.[31,32] Separations were conducted on an Agilent HPLC 1100 series (Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler, binary pump, column heater, and diode array (DAD) and fluorescence (FLD) detectors. The column used was Develosil diol 100 (250 mm × 4.6 mm i.d., 5 μm) from Phenomenex (Torrance, CA, USA). The binary mobile phase consisted of (A) acetonitrile/acetic acid (98:2, v/v) and (B) methanol/water/acetic acid (95:3:2, v/v/v). The elution was performed with 7% B for 3 min and increased to 37.6% B for 50 min and to 100% B for the next 3 min. The elution was held for 13 min at 100% B and returned to 7% B over 6 min with an equilibration time under initial conditions for 10 min. The UV detector was set to monitor from 200 to 400 nm. FLD was conducted with excitation and emission wavelengths at 231 and 320 nm, respectively. FLD was employed because it is more sensitive than UV for PAC identification.

The flow rate and injection volume were 1 mL/min and 15 μL, respectively, with a total analysis time of 76 min. After passing through the flow cells of the DAD and FLD, about 400 μL/min of the eluant was split to the Agilent 6330 Ion Trap MSD (Agilent Technologies). The ionization technique was electrospray ionization (ESI). The mass spectrometer was operated in negative ionization mode with full scan range from m/z 100 to 2200. The capillary temperature was maintained at 350 °C, and the ESI needle voltage was 4 kV. Helium gas was used as a nebulizer gas at a setting of 30 psi, and high-purity nitrogen was used as collision gas (8 L/min).

Statistical Analysis. One-way analysis of variance (ANOVA) followed by Fisher's least significance difference (LSD) test was used to determine significant differences of PAC in the feces between days, and differences were considered to be statistically significant at $p < 0.05$. Statistical analyses were conducted using Statview 512+ (Brainpower Inc., Calabasas, CA, USA).

RESULTS AND DISCUSSION

Optimization of the Extraction Method. The methodology for analysis of high molecular weight PAC in feces has not been previously reported. Because feces represent a more complex matrix than other biological samples such as plasma, urine, or tissue, a sample pretreatment step is crucial for subsequent qualitative and quantitative analyses.[33] Thus, we initially optimized an extraction method for the analysis of PAC in colonic content and feces. To determine the PAC extraction efficiency and recovery, GSE was spiked into pooled feces pooled from animals fed the control diet. Ascorbic acid was added to the samples to increase the stability of monomer and PAC and to prevent oxidation during extraction.[34]

After loading of the SPE cartridge, it was washed with 5% methanol to remove interferences, and then the analytes were eluted with a formic acid/water/methanol mixture (1:49.5:49.5, v/v/v), which gave the best recoveries as elution solvent. The recoveries for each DP fraction using liquid–solid extraction (LSE) followed by SPE are shown in Table 2. Recovery from rat feces spiked with GSE varied depending on the DP, with lower recovery for higher DP (see Figure 1 and Table 2). These lower recovery rates from feces are likely due to increasing adsorption of the larger oligomers to the complex polar matrix present in the feces.

Table 2. Recovery of Monomer and Oligomers PAC from Feces Spiked with Grape Seed Extract (GSE)

degree of polymerization (DP)	recovery[a] (%)
monomer	115 ± 28
dimers	84 ± 32
trimers	84 ± 33
tetramers	72 ± 24
pentamers	61 ± 11
hexamers	51 ± 9

[a]Recovery of PAC from feces spiked with GSE after liquid–solid extraction (LSE) and solid-phase extraction (SPE). Results are shown as the mean of three replicates.

Identification of Flavan-3-ols and Oligomeric GSE in Rat Colonic Content. HPLC analysis using a normal-phase column with fluorescence detection has been widely used for the analysis of PAC in plants, foods, and beverages.[28,32] Oligomer PAC with different DP values can be separated using a diol column (Figure 2) and florescence detection, which is more sensitive than UV detection and thus preferred as PAC have a low UV extinction coefficient.[29] We subsequently analyzed the concentration of PAC in rat colonic content and feces by using FLD and included MS detection to help validate peak identity. Both negative and positive ion modes can be used to detect molecular ions of PAC, although negative ion mode yields ions more effectively, due to the weak acidic nature of PAC.[27,28] Therefore, the MS analyses of monomeric flavan-3-ols and oligomeric PAC (DP ≥ 2) were characterized using ESI-ion trap-MS in negative ion mode. Figure 3a shows the extracted ion chromatograms (EIC) from the total ion chromatogram (TIC) of GSE via negative ESI-MS.

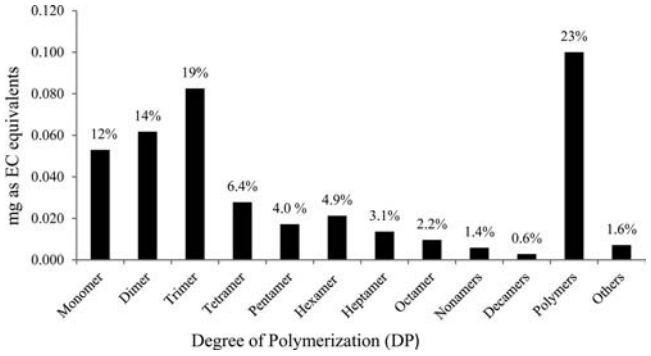

Figure 1. Concentration of monomer and oligomer PAC of GSE. Compounds were separated according to their DP using a diol stationary phase with fluorescence detection. PAC composition is expressed as epicatechin equivalents. Values on top of each column indicate the percentage of each compound in the mixture.

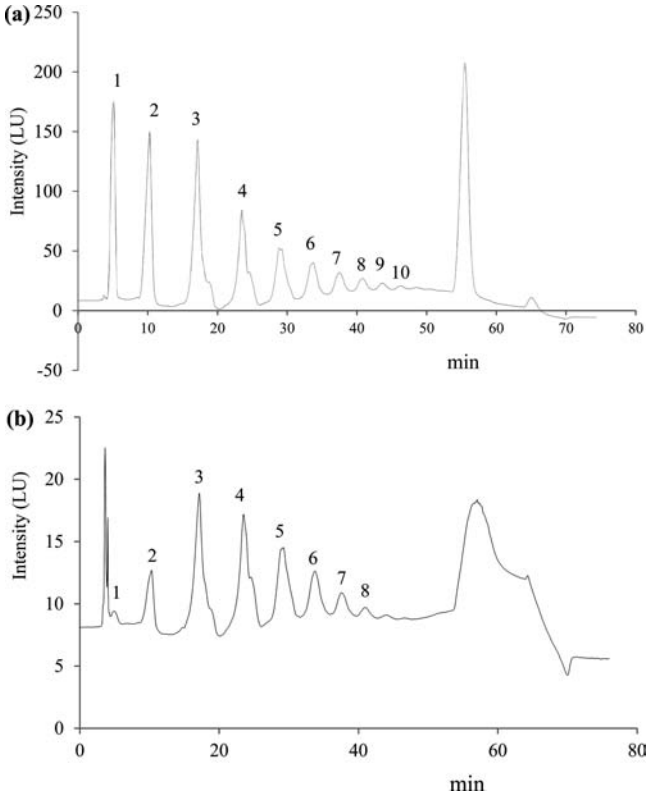

Figure 2. (a) NP-HPLC chromatogram utilizing fluorescence (FLD) detection of GSE and (b) colonic content from rats fed a diet containing 0.25% (w/w) GSE for 10 days. Separations were conducted using a diol phase column in a binary gradient of mobile phases A, acetonitrile/acetic acid (98:2), and B, methanol/H_2O/acetic acid (95:3:2), respectively. The numbers indicate the DP (1 = monomer, 2 = dimers, 3 = trimers, etc.).

dx.doi.org/10.1021/jf301939e | J. Agric. Food Chem. 2013, 61, 121–127

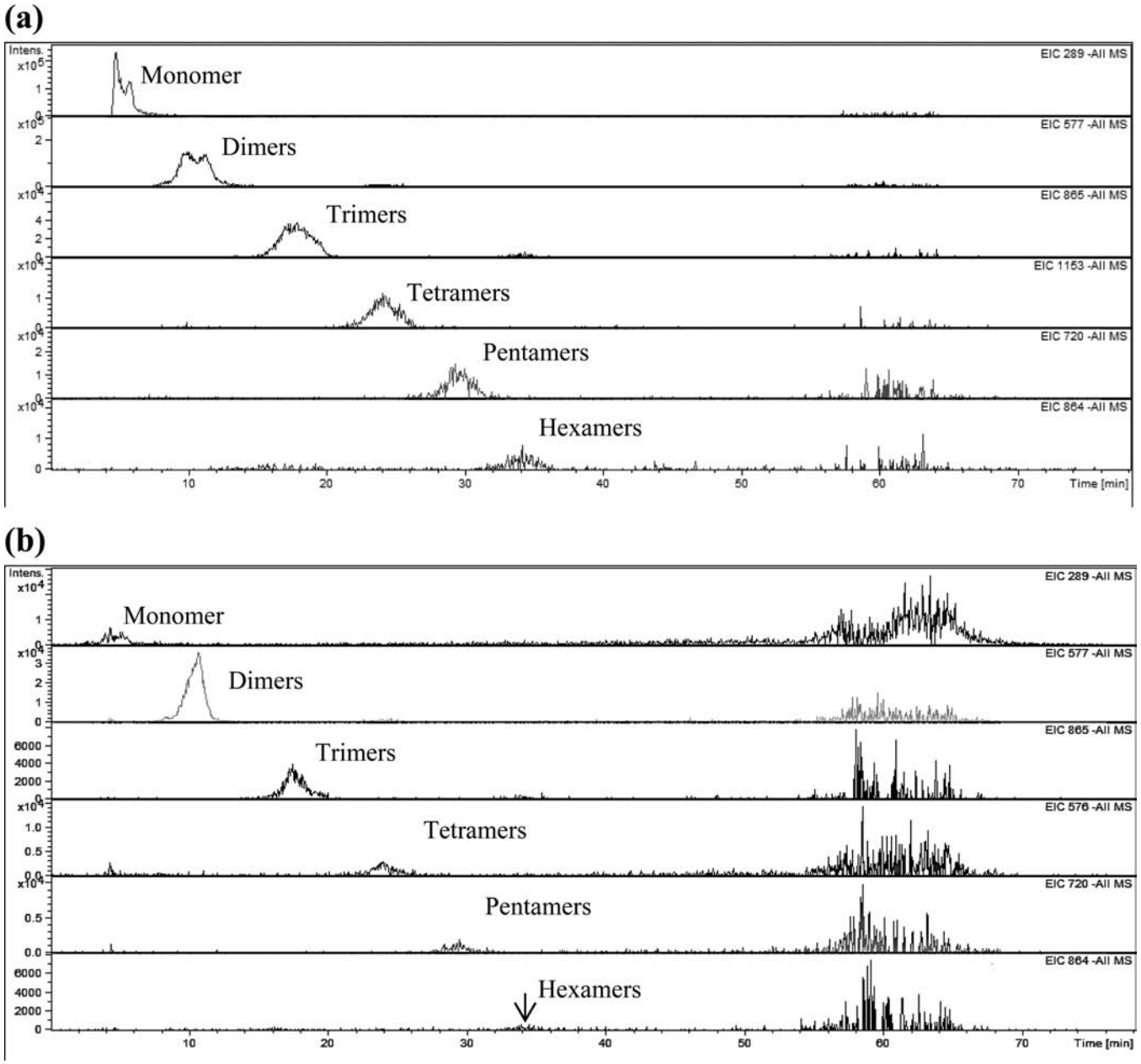

Figure 3. (a) Extracted ion chromatogram (EIC) derived from total ion chromatogram (TIC) on negative ESI-MS ion trap of GSE and (b) EIC of colonic content from rats fed a diet containing GSE 0.25% (w/w) for 10 days. Both $[M - H]^-$ and $[M - 2H]^{2-}$ ions were used for identification of monomer (m/z 289), dimers (m/z 577), trimers (m/z 865), tetramers (m/z 1153 and 576), pentamers (m/z 720), and hexamers (m/z 864).

The above method was used to analyze PAC colonic content in rats consuming a diet that contained 0.25% (w/w) GSE for 10 days. The FLD and EIC data show trace amounts of monomer and a substantial presence of GSE PAC up to hexamers. Monomer and dimer contents (13.9 and 33.4 mg/kg wet feces, respectively) were low compared to the proportion of these fractions in the GSE, suggesting that more intestinal absorption or metabolism has occurred on the smaller flavan-3-ols. Our findings are consistent with previous studies showing that (+)-catechin and (−)-epicatechin are absorbed or degraded rapidly in the gastrointestinal tract.[10]

The identity of the peaks was accomplished by the molecular weight observed in the mass spectra and by comparison with the retention times of individual purified PAC standards with FLD (data not shown) and comparison with other published data. For instance, the singly charged $[M - H]^-$ ions were the most abundant ions for DP 1−4 at m/z 289, 577, 865, and 1153. It is also well-known that higher oligomeric PAC are susceptible to forming multiply charged ions. For DP 5 and 6, the doubly charged $[M - 2H]^{2-}$ ions were the most intense, so m/z 720 and 864 were selected for identification. These singly and doubly charged ions are in accordance with previously published data.[28] Whereas the molecular ion was present for DP 4, the doubly charged ion at m/z 576 was more intense and used for identification. Additionally, FLD suggested the separation of oligomeric GSE up to about decamers; however, the EIC of PAC derived from the TIC shows only up to hexamers. The lack of sensitivity for the higher DP PAC may be due to poorer ionization but is also likely due to the larger number of galloylated oligomers at higher DP values,[28] yielding

ions with masses different from those expected. A summary of the most abundant ions corresponding to different oligomers of GSE in feces is shown in Table 3.

Table 3. Most Abundant Ions for GSE Detected by ESI-Ion Trap-MS in Negative Mode

DP	t_R (min)	$[M - H]^-$	$[M - 2H]^{2-}$
1	4.9	289	
2	10.2	577	
3	17.3	865	
4	23.9	1153	576
5	28.9		720
6	33.4		864

Composition of PAC in Rat Feces and Colonic Content. We observed that within 24 h post-GSE ingestion, significant amounts of PAC are present in the feces, whereas control rats had no detectable PAC. Our observations are comparable to previous studies which showed that approximately 40−45% of the total radioactivity of dimeric [^{14}C]PAC is excreted in rat feces following the administration of radiolabeled PAC.[35] The DP of PAC seems to play an important role in their metabolic fate in the gastrointestinal tract, owing to a poor absorption of large PAC through the gut barrier.[24] As shown in Figure 4, the level of oligomeric PAC tends to increase slightly with additional days of GSE treatment, although differences were not significant except for dimers between days 1 and 7.

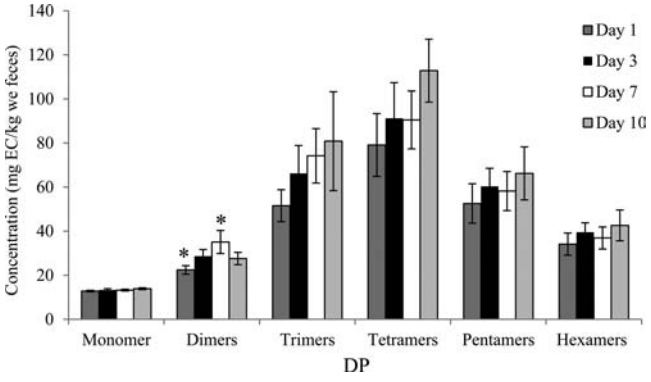

Figure 4. Profiles of monomer and oligomer PAC in rat feces collected at days 1, 3, 7, and 10 after initiating GSE dietary supplementation containing 0.25% (w/w) GSE. Results are expressed as mg (−)-epicatechin/kg wet feces. Bars represent the mean ± SEM of two to four animals with two replications each. * indicates significant difference between days ($P < 0.05$, ANOVA).

PAC analysis in the colonic content is of particular interest given that it provides information on the concentration of PAC in contact with the colon epithelial cells after dietary GSE supplementation. Figure 5 shows the presence of oligomeric PAC in the colonic content from rats fed a diet containing 0.25% (w/w) GSE for 10 days. From the LC-ESI-ion trap-MS analysis, oligomeric PAC up to hexamers were detected as parent compounds. As expected, the profiles and distribution of colonic content were similar to those observed in feces. This result is in contrast to Tsang et al.,[12] who found only trace amounts of PAC in the colon after 3−12 h of GSE ingestion in rats. To our knowledge, this is the first study to detect and quantify the concentrations of the larger PAC in the colon. As

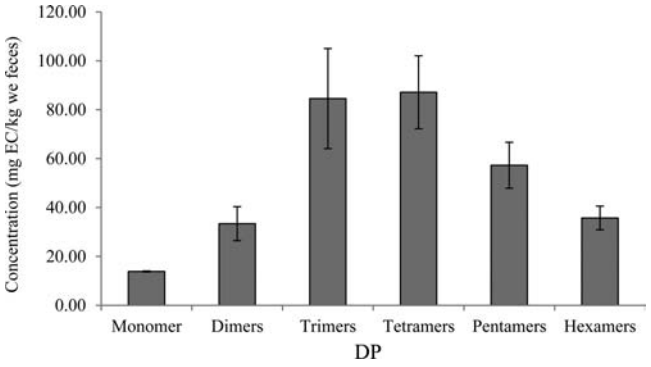

Figure 5. Profiles of monomer and oligomer PAC in rat colonic content from rats fed a diet containing 0.25% (w/w) GSE for 10 days. Results are expressed as mg (−)-epicatechin/kg wet feces. Bars represent the mean ± SEM of five animals with two replications each.

noted above, previous studies were mostly done on the bioavailability and absorption of smaller PAC (i.e., dimers), but the fate of higher molecular weight PAC, the major dietary polyphenols in our diet, has not been closely investigated.[15,24] Here we found the nonabsorbable larger PAC compounds in the colon, indicating that they are not heavily degraded in the stomach and small intestine and do reach the colon within 24 h of GSE ingestion. In support of our findings, dietary cocoa procyanidins were not a significant source of plasma and urine flavanols in humans, indicating very poor absorption of these compounds.[22] In the same study, the contribution of dietary PAC to a microbiome-generated metabolite in plasma and urine was much lower than that originated from monomer, suggesting a lower susceptibility of PAC to gut flora metabolism compared to monomer.

Mass Balance Analysis of PAC. To make an approximate calculation for the mass balance of PAC, we assessed the 24 h PAC food consumption and fecal output in rats fed the diet containing 0.25% (w/w) GSE for 10 days (Table 4). Data

Table 4. Recovery of Dietary PAC in Rat Feces

DP	PAC ingested (mg EC)/day[a]	fecal output (mg EC)/day[b]
monomer	2.44	0.05
dimers	2.84	0.11
trimers	3.80	0.26
tetramers	1.28	0.35
pentamers	0.79	0.23
hexamers	0.98	0.15
total (DP ≥ 2)	12.1	1.15

[a]The calculations of mass balance are based on average food consumption ($n = 5$) = 22.5 ± 1.5 g/day. [b]The calculations of mass balance are based on average fecal output ($n = 4$) = 3.8 ± 0.25 g/day.

showed that only 1.7% of the ingested monomeric PAC was present in the feces, followed by 3.9% dimers and 6.8% trimers, respectively. Importantly, the larger PAC were degraded very poorly, with a significant fraction of PAC retained throughout the colon, so that 27% tetramers, 29% pentamers, and 15% hexamers were present as intact parent compounds in the feces. The approximate total PAC (DP ≥ 2) recovery of the ingested GSE in feces was 11%, which is substantially lower than the 32 and 35% observed in sheep and goats, respectively, although these numbers were for retained radioactivity and likely

dx.doi.org/10.1021/jf301939e | J. Agric. Food Chem. 2013, 61, 121−127

included metabolites.[17] A possible explanation for these differences is that rats have a different digestive system and microbiota compared to sheep and goats.

In terms of total fecal output, Roffey et al.[36] reported that a total of 10 g/day feces is typically excreted by rats. Nevertheless, coprophagia frequently occurs in rodents, including rats, and it has been estimated that they may consume about 34−50% of their total feces.[37] Although the use of wire-bottom cages, such as those used here, should reduce coprophagia, it does not prevent it. This phenomenon may have resulted in an underestimate of fecal output here, measured as 3.8 ± 0.25 g/day. Regardless, the mass balance experiment indicates that a significant amount of PAC persists through the colon, with most of the monomer being absorbed or metabolized. The unabsorbed larger PAC, however, are only partially degraded by gut microbiota and are present in substantial amounts in the feces. Our data suggest that PAC with more than three subunits could be more resistant to degradation due to their chemical structure and, therefore, higher levels are observed in the colon. Conversely, other flavonoids including anthocyanins are rapidly metabolized in the gut to simple phenolic acids and related metabolites.[38]

Potential Bioactivity. Flavonoids in the gut are reported to exert positive health effects. For instance, previous studies by He and co-workers[39] showed that high levels of anthocyanins in the fecal content may play an important role in colonic health. It has also been proposed that a high concentration of phenols could exert local beneficial effects within the gastrointestinal tract.[40] Because PAC are widely present in human diets, the metabolism of PAC is of major importance to understand their biological activities. There is mounting evidence from in vitro and in vivo studies of potential health benefits of PAC consumption linked to protection against colorectal cancer (CRC).[2,6,41] Currently, CRC is the third most diagnosed cancer in the United States. Epidemiological and clinical studies indicate that high consumption of fats and red meat increases the risk of CRC.[42] On the other hand, the consumption of fruits and vegetables is linked to lower CRC risk, and their high content of flavonoids may be responsible for these beneficial effects.[4,42] Among the flavonoids, PAC (≥2 subunits) consumption was found to be inversely related to the decreased risk of CRC in an Italian population.[3] Given the correlation of PAC with lower incidence of CRC, a better understanding of the metabolic fate of PAC in the colon is essential to understand their potential colonic health benefits.

The persistence of larger PAC in the colon would allow these compounds to exert local beneficial biological actions, particularly on the colon epithelial cells, and prevent oxidant accumulation in the feces. This could result in protective effects against inflammation-mediated diseases including CRC. Future work will focus on understanding the molecular mechanisms by which GSE compounds and their metabolites could affect the inflammatory responses in colonic cells, leading to protective effects against the development of chronic diseases with an inflammatory background, including CRC.[43] In addition, it is of great interest to understand the effect of these bioactives on gut microbiota, as well as to better understand the impact of foods rich in PAC, such as wine, apples, and chocolate, and their effects on human gastrointestinal health.

In conclusion, this is the first study assessing the presence of oligomeric PAC (DP ≥ 2) in the rat colon; LC-ESI-ion trap-MS data confirmed the presence of oligomers with DP 2−6 in rat colonic content and feces. From the mass balance analysis, the recovery of parent PAC (DP ≥ 2) compounds in feces was approximately 11%, indicating PAC undergo extensive but incomplete biotransformation during transit through the intestinal tract by the gut microbiota. Thus, these important dietary components may directly contribute to the health status of the colon, and future studies need to address the bioactivity of PAC on colonic tissues.

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Funding

This work was supported by the Wine Spectator Scholarship Foundation, the Adolf L. & Richie C. Heck Research Scholarship, a Horace O. Lanza Scholarship, and the CHNR−State of California Vitamin Price Fixing Consumer Settlement Fund, USA.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Mars Inc., USA, for kindly providing pure procyanidin oligomeric fractions and Kikkoman Corp. (Tokyo, Japan) for the grape seed extract.

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