JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

Comprehensive Assessment of the Quality of Commercial Cranberry Products. Phenolic Characterization and in Vitro Bioactivity

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Supporting Information

ABSTRACT: Cranberry (*Vaccinium macrocarpon*) products have been widely recommended in traditional American medicine for the treatment of urinary tract infection (UTI). A total of 19 different commercial cranberry products from American and European markets have been analyzed by different global phenolic methods and by UPLC-DAD-ESI-TQ MS. In addition, in vitro antioxidant capacity and uropathogenic bacterial antiadhesion activity tests have been performed. Results revealed that products found in the market widely differed in their phenolic content and distribution, including products completely devoid of flavan-3-ols to highly purified ones, either in A-type proanthocyanidins (PACs) or in anthocyanins. The product presentation form and polyphenolic profile widely affected the antiadhesion activity, ranging from a negative (nulel) effect to a MIC = 0.5 mg/mL for cranberry powders and a MIC=112 mg/mL for gel capsule samples. Only 4 of 19 products would provide the recommended dose of intake of 36 mg total PACs/day. Of most importance was the fact that this dose would actually provide as low as 0.00 and up to 205 μg/g of procyanidin A2, indicating the lack of product standardization and incongruence between global and individual compound analysis.

KEYWORDS: *cranberry, A-type proanthocyanidins, phenolic acids, anthocyanins, UTI, bacterial antiadhesion, ORAC*

INTRODUCTION

Cranberry (*Vaccinium macrocarpon*) and its derived products, including nutraceuticals, are a rich source of polyphenols. Phenolic acids and benzoates, and flavonoid compounds such as anthocyanins, flavonols, and flavan-3-ols, are the most common polyphenols found in cranberries.[1−3] Numerous health effects have been reported for cranberries including[4] in vitro and ex vitro antioxidant activity, antimicrobial activity against bacteria involved in a wide range of diseases (dental caries, gastritis, enteritis, and infections), antiviral activity against A- and B-type influenza virus, anti-inflammatory activity in periodontal disease, antihypertensive activity inhibiting ACE activity, and antiproliferative activity on human oral, colon, and prostate cancer cell lines, among others. Some in vivo trials in humans or animals have also revealed beneficial effects of cranberry juice and powders on oral health,[5] stomach infections,[6] cardiovascular disease,[7] and diabetes.[8] However, the best known bioactivity of cranberry polyphenols is related to their capacity to inhibit the adhesion of pathogenic bacteria to uroepithelial cells of the urinary tract, preventing bacterial colonization and progression of urinary tract infections (UTI),[1,9] activity that has also been extended to pathogens involved in diseases of the oral cavity.[10] The bacterial antiadhesion activity, as well as most of the aforementioned activities or effects of cranberry and derived products, has been mainly attributed to their characteristic polyphenol profile.[4]

Flavan-3-ols in cranberry occur as monomers, and when they are in oligomeric and polymeric forms, they are called proanthocyanidins (PACs). PACs in cranberry vary according to the nature of the interflavan linkage, constitutive units, and degree of polymerization (DP). According to the nature of the intervaflavan linkage, both A- and B-type PACs are found in cranberry. B-type PACs are those in which monomeric units are linked through the C4 position of the upper unit and the C6 or C8 positions of the lower unit, whereas A-type PACs contain an additional ether-type bond between the C2 position of the upper unit and the hydroxyl group at C7 or C5 of the lower unit (C2−*O*−C7 or C2−*O*−C5). It has been estimated that A-type PACs account for ca. 65% of total PACs in cranberry.[1,11] (−)-Epicatechin is more abundant than (+)-catechin as a constitutive unit of cranberry oligomers and polymers (i.e., procyanidins), although traces of (epi)gallocatechins (i.e., prodelphinidins) have also been reported.[9,12−14] Recently, A- and B-type dimers and trimers containing one epigallocatechin unit have been detected in cranberry extracts and derived fractions.[15] The DP of cranberry PACs varies from two to seven monomeric units linked by a least one A-type linkage, although above DP 4 they might contain two A-type linkages.[2,12−14] More recently,

Received: December 1, 2011
Revised: February 9, 2012
Accepted: March 8, 2012
Published: March 22, 2012

dx.doi.org/10.1021/jf204912u | J. Agric. Food Chem. 2012, 60, 3396−3408

Table 1. Global Phenolic Determination and Antioxidant and Antiadhesion Capacities of the Different Commercial Cranberry Products[a]

product	presentation	origin	PT (mg GAE/g)	PAC-butanol/HCl (mg Cy/g)	PAC-DMAC (%, w/w)	ORAC (μmol TE/mg product)	bacterial antiadhesion MIC (mg/mL)
1	syrup	Spain	4.27 ± 0.01	9.19 ± 0.23	0.03	0.11 ± 0.01	326
2	powder	USA	12.62 ± 1.12	32.1 ± 1.8	0.80	0.23 ± 0.02	–
3	powder	Belgium	7.12 ± 0.09	7.67 ± 0.17	0.13	0.22 ± 0.02	60
4	gel capsule	USA	132.09 ± 4.16	9.58 ± 1.22	0.18	0.98 ± 0.01	230
5	gel capsule	USA	139.24 ± 2.47	9.43 ± 0.27	0.21	1.11 ± 0.05	200
6	gel capsule	UK	12.94 ± 1.73	8.48 ± 0.14	0.21	0.21 ± 0.01	–
7	gel capsule	UK	12.80 ± 1.53	6.63 ± 0.24	0.15	0.18 ± 0.01	112
8	powder	UK	39.24 ± 2.65	11.29 ± 0.20	0.09	0.47 ± 0.01	–
9	pill	Italy	46.39 ± 0.03	4.63 ± 0.42	0.21	1.23 ± 0.07	7.5
10	powder capsule	Belgium	69.79 ± 3.77	45.5 ± 3.6	7.20	2.10 ± 0.08	0.9
11	powder capsule	USA	91.67 ± 1.42	13.66 ± 0.50	0.24	1.08 ± 0.04	na
12	powder capsule	France	10.82 ± 0.54	10.95 ± 0.30	0.22	0.35 ± 0.01	60
13	powder	UK	6.49 ± 0.33	3.77 ± 1.31	0.11	0.10 ± 0.01	30
14	powder capsule	USA	3.06 ± 0.19	5.31 ± 1.26	0.28	0.09 ± 0.01	60
15	powder	USA	16.39 ± 0.24	18.15 ± 0.75	0.32	0.44 ± 0.01	15
16	powder	USA	20.72 ± 0.74	30.16 ± 0.92	0.60	0.62 ± 0.01	60
17	powder	USA	165.71 ± 6.02	215.35 ± 3.62	4.7	6.99 ± 0.01	1.9
18	powder	USA	218.91 ± 5.13	313.09 ± 3.33	7.3	9.01 ± 0.92	0.5
19	powder	China	83.54 ± 1.93	159.86 ± 18.07	4.4	2.93 ± 0.15	–

[a]Mean (n = 2) ± standard deviation (SD). MIC, minimum inhibitory concentration; GAE, gallic acid equivalent; Cy, cyanidin; na, not available; –, negative effect.

A-type PACs up to DP 12 have been detected in a high-polymeric fraction of cranberry.[15]

Cranberry products have been widely recommended in traditional American medicine for the prevention of UTI, and in recent years their popularity has considerably increased in the European market. Although a large number of human clinical studies have shown that cranberry products reduce the incidence of UTI in women with recurrent infections, the establishment of recommended intake doses has been one of the limitations of this therapy.[16] This has mainly been due to the lack of standardization of cranberry products used in the different trials and to difficulties with the isolation and analysis of A-type PACs. Recently, the Agence Française de Sécurité Sanitaire des Aliments (AFSSA) has concluded on the basis of the results from randomized clinic studies that the daily intake of 36 mg of PAC in the form of six different cranberry products (fresh/frozen fruits, puree, and four different forms of dried, sugar-added, and/or flavored products) contributes to decreasing adhesion of certain uropathogenic P-fimbriated *Escherichia coli* to the walls of the urinary tract.[17] The method used for the determination of the PAC content to sustain this claim was the DMAC method, which gives a global valorization of both A- and B-type PACs, but which could also lead to an underestimation of the total content in products with higher levels of oligomers and polymers.[18]

Besides the dosage and analytic methodologies best suited for cranberry products, other important issues with cranberry products are those concerning the bioavailability of A-PACs, which remains largely unknown, but which may be partially dependent on their colonic catabolism, as described for B-type PACs.[19] In fact, recent studies have found very small levels of intact procyanidin A2 in rat plasma and urine after the administration of a cranberry concentrate powder[20] to be responsible for the in vivo benefits against UTI. The microbial catabolism of B-type PACs involves the formation of phenyl-propionic, phenylacetic, benzoic, and cinnamic acids of different hydroxylation patterns; however, the A-type bond in cranberry PACs is difficult to break and may result in a very different metabolic profile. Therefore, new studies need to determine the metabolites of A-type PACs to discover their role in preventing UTI.

In view of all these facts, the aim of the present work was to perform a comprehensive assessment of the quality of cranberry products found in American and European markets. For that, global phenolic determinations by different methods, as well as targeted UPLC-DAD-ESI-TQ MS measurement, of a wide range of individual phenolic acids and main flavonoid compounds, including anthocyanins and flavan-3-ols, have been carried out. In addition, in vitro antioxidant capacity and uropathogenic bacterial antiadhesion activity tests have been performed to complement the assessment. Finally, correlation and multivariate statistical analysis have been applied to summarize the data and provide useful conclusions.

MATERIALS AND METHODS

Materials. Standards of phenolic compounds were purchased from Sigma-Aldrich Chemical Co. (St. Loius, MO, USA), Phytolab (Vestenbergsgreuth, Germany), or Extrasynthèse (Genay, France). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2′-azobis(2-methylpropionamidine) dihydrochloride were purchased from Aldrich (St. Louis, MO, USA). Dimethylaminocinnaldehyde was purchased from Sigma (St. Louis, MO, USA). LC grade solvents were purchased from Lab-Science (Sowinskiego, Poland) or from Scharlau (Barcelona, Spain). The remaining chemicals and reagents were obtained either from Sigma-Aldrich Co. Ltd. (Poole, Dorset, U.K.) or from Fisher (Loughborough, Leics, U.K.).

Commercial Cranberry Products. A total of 19 different commercial cranberry products, presented as powder capsules, gel capsules, pills, loose powders, and syrups, were purchased from European and U.S. markets or kindly provided by the suppliers (Table 1). Only product 10 was reported to contain grape seed proanthocyanidins, as indicated on the label. Product 8 also contained probiotics, and

products 3 and 4 included vitamin C in their formulations. Products (0.50 g) were mixed with 10 mL of MeOH/H_2O (20:80, v/v) containing 0.2% HCl, sonicated for 10 min, centrifuged at 3500 rpm during 15 min, and finally filtered through 0.22 μm for UPLC-DAD-ESI-TQ MS determination. For global phenolic and antioxidant activity determinations, cranberry products (0.05 g) were extracted with 10 mL of methanol/HCl (1000:1, v/v) by sonication for 5 min followed by an extra 15 min resting period, followed by centrifugation and filtration, as above. Extractions were performed in duplicate.

Global Phenolic Determinations. Total polyphenols (TP) were determined according to the method of Singleton and Rossi,[21] which is based on the oxidation of the hydroxyl groups of phenols in basic media by the Folin−Ciocalteu reagent (mixture of phosphotungstic and phosphomolybdic acids of yellow color), using gallic acid as a calibration standard. Two different methods were used for the determination of total PACs: (a) the Bate−Smith method, based on the acid-catalyzed oxidative cleavage of the C−C interflavanic bond of PACs in butanol−HCl (PAC-But/HCl),[22] using cyanide chloride as a standard and (b) the dimethylaminocinnaldehyde (DMAC) (PAC-DMAC) method, based on the reactions of aldehydes with the hydroxyl groups in the B ring of flavan-3-ols, following the protocol described by Prior et al.,[23] using procyanidin A2 as a standard. Analyses were performed in duplicate.

Analysis of Phenolic Compounds by UPLC-DAD-ESI-TQ MS. An UPLC system coupled to an Acquity PDA eλ (extended wavelength) photodiode array detector and an Acquity TQD tandem quadrupole mass spectrometer equipped with a Z-spray electrospray interface (UPLC-DAD-ESI-TQ MS) (Waters, Milford, MA, USA) was used. Separation (2 μL) was performed on a Waters BEH C18 column (2.1 × 100 mm; 1.7 μm) at 40 °C. For phenolic acids and flavan-3-ols, a gradient composed of solvent A (water/acetic acid, 98:2, v/v) and solvent B (acetonitrile/acetic acid, 98:2, v/v) was applied at flow rate of 0.5 mL/min as follows:[24] 0.0−1.5 min, 0.1% B; 1.5−11.2 min, 0.1−16.3% B; 11.2−11.5 min, 16.3−18.4% B; 11.5−14.0 min, 18.4% B; 14.0−14.1 min, 18.4−99.9% B; 14.1−15.5 min, 99.9% B; 15.5−15.6 min, 0.1% B; 15.6−18.0 min, 0.1% B. For anthocyanins, a gradient consisting of A (water/formic acid, 90:10, v/v) and B (acetonitrile) was applied at flow rate of 0.5 mL/min as follows: 0−1.0 min, 5−15% B; 1.0−8.09 min, 15−30% B; 8.09−8.67 min, 30−100% B; 8.67−9.84 min, 100−5.0% B; 9.84−12.17 min, 5.0−95% B. The DAD was operated in the 250−420 nm wavelength range (for phenolic acids and flavan-3-ols) and from 260 to 650 nm (for anthocyanins) at a 20 point/s rate and 1.2 nm resolution. The ESI parameters were as follows: capillary voltage, 3 kV; source temperature, 130 °C; desolvation temperature, 400 °C; desolvation gas (N_2) flow rate, 750 L/h; cone gas (N_2) flow rate, 60 L/h. The ESI was operated in negative mode for phenolic acids and flavan-3-ols and in positive mode for anthocyanins. For quantification purposes, data were collected in the multiple reaction monitoring (MRM) mode, tracking the transition of parent and product ions specific for each compound, and using external calibration curves.

The MS parameter optimization, MRM transitions, tested concentration range, and limits of detection and quantification for phenolic acids and flavan-3-ols were those described by Sánchez-Patán et al.[24,25] For procyanidin A2, the optimized MRM [father ion (m/z), daughter ion (m/z), cone energy (V), collision energy (V)] were 575, 449, 50, and 20, respectively. For trimeric A-type procyanidins two different MRM transitions [865/573 (A-type bond in the upper position) and 865/575 (A-type bond in terminal unit)] were screened, using the optimized MS/MS parameters (cone and collision energies) of procyanidin C1. Quantification of procyanidins B2, B3, B4, B5, and B7 was carried out using the calibration curve of procyanidin B1. A-type trimeric procyanidins were quantified using the calibration curve of procyanidin C1.

For anthocyanins, the optimized MRM [father ion (m/z), daughter ion (m/z), cone energy (V), collision energy (V)] were, respectively, cyanidin-3-galactoside (449, 287, 35, 20); cyanidin-3-glucoside (449, 287, 35, 20); peonidin-3-glucoside (463, 301, 35, 20), and malvidin-3-glucoside (493, 331, 35, 20). Cyanidin-3-arabinoside (m/z 419, 287) was quantified using the calibration curve of cyanidin-3-galactoside; peonidin-3-arabinoside (m/z 433, 301) and peonidin-3-galactoside (m/z 463, 301) were quantified as peonidin-3-glucoside; and malvidin-3-arabinoside (m/z 463, 331) was quantified as malvidin-3-glucoside. The limits of detection for the different anthocyanins were between 0.100 and 0.300 μg/mL.

In Vitro Antioxidant Activity. The radical scavenging activity of the extracts was determined by the ORAC method using fluorescein as a fluorescence probe.[26] Briefly, the reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4) and the final assay mixture (200 μL) contained fluorescein (70 nM), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (12 mM), and antioxidant [Trolox (1−8 μM) or sample (at different concentrations)]. The plate was automatically shaken before the first reading, and the fluorescence was recorded every minute for 98 min. A Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with 485-P excitation and 520-P emission filters was used. The equipment was controlled by Fluostar Galaxy software version 4.11-0 for fluorescence measurement. Black 96-well untreated microplates (Nunc, , Roskilde, Denmark) were used. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride and Trolox solutions were prepared daily, and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer (pH 7.4).

All reaction mixtures were prepared in duplicate, and at least three independent runs were performed for each sample. Fluorescence measurements were normalized to the curve of the blank (no antioxidant). From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as

$$\mathrm{AUC} = 1 + \sum_{i=1}^{i=98} f_i/f_0$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The net AUC corresponding to a sample was calculated as follows:

$$\text{net AUC} = \mathrm{AUC}_{\text{antioxidant}} - \mathrm{AUC}_{\text{blank}}$$

The regression equation between net AUC and antioxidant concentration was calculated. The ORAC value was calculated by dividing the slope of the latter equation by the slope of the Trolox line obtained for the same assay. Final ORAC values were expressed as micromoles of Trolox equivalents per milligram of cranberry product.

In Vitro Bacterial Antiadhesion Activity. Samples were tested for in vitro bacterial antiadhesion activity on a per weight basis. Samples were suspended (60 mg/mL) in PBS, neutralized with 1 N NaOH, diluted serially (2-fold), and tested for bacterial antiadhesion activity utilizing an HRBC hemagglutination assay specific for uropathogenic P-fimbriated *Escherichia coli* according to the method of Howell et al.[9] A 30 μL drop of each dilution was incubated with 10 μL of bacterial suspension on a 24-well polystyrene plate for 10 min at room temperature on a rotary shaker. Freshly drawn human red blood cells (HRBCs; A1, Rh+) were suspended (3%) in PBS and added separately (10 μL drops) to test suspensions, which were then incubated for 20 min on a rotary shaker at room temperature and evaluated microscopically for the ability to prevent agglutination. The concentration at which hemagglutination activity was suppressed by 50% (minimum inhibitory capacity, MIC) was recorded as an indicator of the strength of the bacterial antiadhesion activity. Antiadhesion assays were repeated three times and the results averaged. Controls included wells containing bacteria + PBS, HRBC + PBS, bacteria + test compound, HRBC + test compound, and bacteria + HRBC.

Statistical Analysis. The statistical methods used for data processing were simple regression to study the relationship between polyphenols and bacterial antiadhesion activity (MIC values) among commercial cranberry products using the Statgraphics Centurion XV program for Windows, version 15.2.00 (StatPoint Inc., 1982−2006, www.statgraphics.com), and principal component analysis (PCA), from standardized variables, to summarize the global and individual phenolic data as well as the results obtained from in vitro antioxidant

capacity test (ORAC values) using the Statistica program for Windows, version 7.1 (StatSoft Inc., 1984−2006, www.statsoft.com).

RESULTS AND DISCUSSION

Global Phenolic Determinations and Antioxidant Capacity. Total polyphenol (PT) and total PAC as determined by both the butanol−HCl (PAC-But/HCl) and DMAC (PAC-DMAC) methods were determined in the different products (Table 1). A large variation (ca. 100-fold) was found among the different products ranging from 4.27 ± 0.01 (product 1) to 219 ± 5 (product 18) mg GAE/g for PT. Product 18 also presented the highest PRO content [313 ± 3 mg cyanidin/g for PAC-But/HCl and 7.3% (or 73 mg/g) for PAC-DMCA], whereas products 13 and 1 presented the lowest values [3.77 ± 1.31 for PAC-But/HCl and 0.03% (0.3 mg/g) for PAC-DMCA, respectively]. Values from the PAC-But/HCl method were higher than those obtained by the PAC-DMAC. However, it should be highlighted that besides the typical interferences/limitations of each method, different standards were used for the concentration calculations (cyanidin chloride for PAC-But/HCl method and procyanidin A2 for the PAC-DMAC method), which may partially have contributed to the differences observed.

According to previous papers[23,27] the presence of cranberry anthocyanins may lead to an overestimation of PACs in the PAC-But/HCl method because the latter compounds are also converted into anthocyanins during acidic hydrolysis. On the other hand, the DMAC method has also the drawback of being more accurate in the estimation of monomeric flavan-ols than for oligomeric and polymeric flavan-3-ols[15,18] and, therefore, would lead to a partial underestimation of the total PAC content. It is also worth mentioning that in both methods, A- and B-type PACs are measured together, not reflecting the real content of A-type PACs, which could be measured only by LC techniques. Although recently the use of the PAC-But/HCl has not been recommended for cranberry products,[23] we also consider it is a useful methodology because the acid-catalyzed oxidative cleavage of the C−C interflavanic bond of PACs is the only reaction that actually breaks the A-type interflavan linkages of PACs. Despite all of these facts, a good correlation (R^2 = 0.7931; DMAC = 0.0208 PAC-But/HCl + 0.0008) was found between both methods taking into consideration the range of concentrations found in most commercial products (PAC-But/HCl < 35 mg/g and DMAC < 0.90% (90 mg/g)). One exception to this correlation trend was product 10 (also containing grape seed PACs, as indicated on the label), which largely deviated from the model. As will be described below (Figure 3), this product contained an extremely high content of monomeric flavan-3-ols, indicating that the flavan-3-ol profile (i.e., monomers-to-oligomers ratio) of a particular sample could influence the DMAC response.

With regard to the in vitro antioxidant, ORAC values varied from 0.11 ± 0.01 (product 1) to 9.01 ± 0.92 μmol TE/mg product (product 18) (Table 1). For whole cranberry and cranberry extract, ORAC values of 0.28 and 0.11 μmol TE/mg, respectively have been reported.[28] In another study, an ORAC value of 0.02 μmol TE/mg was found for a cranberry extract.[29] Of all products analyzed, only products 10 (containing grape seed extract) and 17−19 showed ORAC values in the same range of some grape seed ingredients.[30] A good correlation between ORAC values with the global phenolic determination assays, in particular with PAC-But/HCl (r = 0.97; ORAC = 0.1706 + 0.0276 PAC-But/HCl) and PT (r = 0.84; ORAC = −0.338 + 0.0319 PT) values, was also found, in accordance with previous studies.[30]

Phenolic Acids. A total of 47 different phenolic acids, including phenylpropionic, phenylacetic, mandelic, benzoic, and cinnamic acids, bearing different hydroxylation patterns were targeted by UPLC-DAD-ESI-TQ MS[24] (Figure 1). Among these compounds only 24 phenolic acids were detected in the cranberry samples. Hydroxybenzoic acids (from 48.62 ± 2.13 to 9761.58 ± 261.32 μg/g; 68−96% of total phenolic acids) were found in higher concentration than hydroxycinnamic acids (from 10.59 ± 0.20 to 1353.03 ± 22.4 μg/g; 4−32% of total phenolic acids), both largely varying among the different products (Table 2).

Among hydroxybenzoic acids, benzoic acid [from 0.00 (product 19) to 8317.88 ± 222.31 μg/g (product 18)] was the most abundant compound followed by protocatechuic acid [from 9.99 ± 0.39 (product 8) to 735.12 ± 17.76 μg/g (product 18)] and vanillic acid [from 1.33 ± 0.13 (product 8) to 262.54 ± 10.16 μg/g (product 18)] (Table 2). Acids present in a medium concentration range included gallic, salicylic, and 4-hydroxybenzoic acids. These results are in accordance with previous studies that also found that benzoic acid was the most concentrated hydroxybenzoic acid in cranberry juice.[31,32] Also in line with our results, Prior et al.[33] found that protocatechiuc acid was among the most abundant hydroxybenzoic acids in a commercial cranberry powder (515 μg/g). However, in contrast to this latter study that found high contents of hippuric acid in cranberry powders,[33] we detected it only in products 15, 17, and 18 in very low concentrations, ranging from 0.17 ± 0.02 μg/g (product 15) to 1.26 ± 0.13 μg/g (product 17). Nevertheless, because hippuric acid is also formed in the organism as the result of the glycination of benzoic acid in the liver, the high content of the precursor benzoic acid in cranberry products could result in a high production of hippuric acid in urine, as has been reported in previous studies.[33] In this sense, it is also important to highlight that benzoic and phenolic acids in cranberry occur mainly in bound form, esterified to sugars, cell wall polysaccharides, or other components,[32] which further contribute to the pool of free phenolic acids after hydrolysis and enzymatic reactions occurring during absorption and metabolism in either the small intestine or colon.

Other series of phenolic acids, including 3,4,5-trimethoxybenzoic, 3,4-dihydroxyphenylacetic, 4-hydroxy-3-methoxyphenylacetic, 3,4-dihydroxymandelic, 4-hydroxymandelic, and 4-hydroxy-3-methoxymandelic acids, were detected only in products 9, 10, and 17−19. Also in line with our results, other studies have reported that these acids were among the less abundant hydroxybenzoic acids in cranberry powder.[33] Product 9 showed the highest concentration of 3,4-dihydroxymandelic and, in particular, 4-hydroxymandelic acids. Also of note, this product presented a remarkable content of gallic acid in comparison to the rest of products, a feature that was also observed in product 10 due to the additional presence of grape seed extract.

With regard to hydroxycinnamic acids, *p*-coumaric acid was the most abundant compound [from 7.93 ± 0.02 (product 8) to 844.16 ± 15.20 μg/g (product 18)], followed by *trans*-cinnamic, caffeic, and ferulic acids (Table 2). Isoferulic was present in lower concentration than the rest of the hydroxycinnamic acids. Zuo et al.[32] also found that *p*-coumaric acid was the most abundant hydroxycinnanic acid in cranberry juice. According to Prior et al.,[33] *p*-coumaric acid, followed by caffeic and ferulic acids, was the most abundant

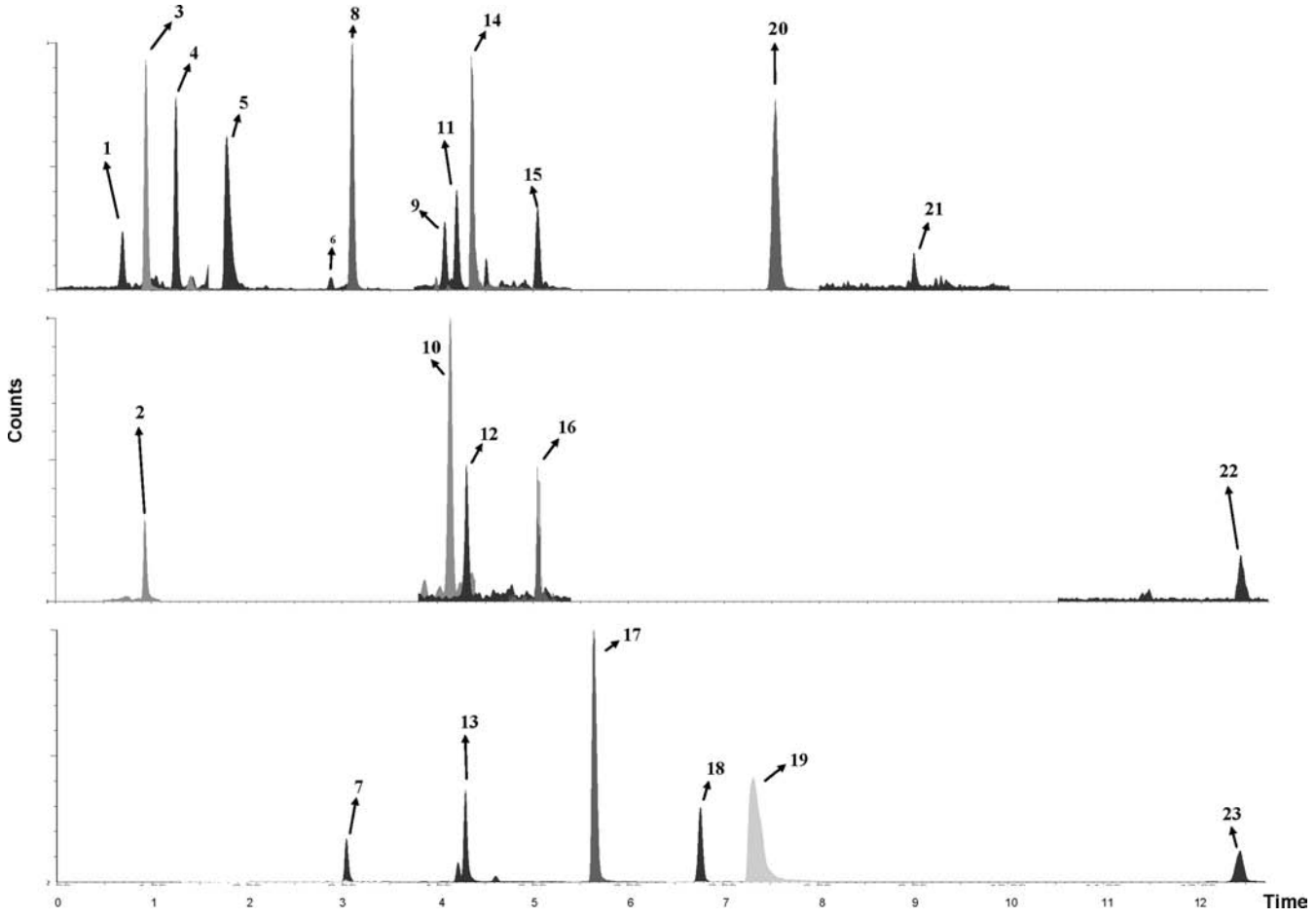


Figure 1. MRM chromatograms of phenolic acids of commercial product 18: (1) 3,4,-hydroxymandelic acid A; (2) 4-hydroxymandelic acid; (3) gallic acid; (4) 4-hydroxy-3-methoxymandelic acid; (5) protocatechuic acid; (6) 3,4-dihydroxyphenylacetic acid; (7) 3-*O*-methylgallic acid; (8) 4-hydroxybenzoic acid; (9) 4-hydroxyphenylacetic acid; (10) 3-(3,4-dihydroxyphenyl)propionic acid; (11) 3-hydroxybenzoic acid; (12) hippuric acid; (13) caffeic acid; (14) vanillic acid; (15) syringic acid; (16) 4-hydroxy-3-methoxyphenylacetic acid; (17) *p*-coumaric acid; (18) ferulic acid; (19) benzoic acid; (20) salycilic acid; (21) 3,4,5-trimethoxybenzoic acid; (22) 3,4,5-trimethoxycinnamic acid; (23) *trans*-cinnamic acid.

hydroxycinnamic acid in cranberry powder. Zheng and Wang[29] also found that *p*-coumaric and caffeic acids were in very similar proportion in cranberry extracts.

Flavan-3-ols. Monomers (MRM 289/245), B-type procyanidin dimers (MRM 577/289) and trimers (MRM 865/577), and A-type procyanidin dimers (MRM 575/449) and trimers (MRM 863/575 and 863/573) were targeted in the different products. Although (epi)gallocatechins (i.e., prodelphinidins) have also been found at trace levels as a structural monomeric unit of cranberry's PAC, as well as polymers containing more than one A-type linkages,[12−15] we focused on the analysis of only the most abundant A- and B-type PACs for comparison purposes among the different products. Figure 2 illustrates a representative MRM chromatogram of the different transitions for product 18. Flavan-3-ols identified included monomers (+)-catechin and (−)-epicatechin, B-type dimeric procyanidins (B1, B2, B3, B4, B5, B6, and B7), and A-type procyanidins dimers (A2 and an unknown dimer at Rt = 6.43 min, possibly A1) (Figure 2). For A-type procyanidin trimers, 10 different isomers (Rt = 4.99, 5.46, and 6.76 min for MRM 863/573 and Rt = 4.56, 4.94, 6.31, 6.49, 6.83, 7.81, and 8.30 min for MRM 863/575) were quantified. As observed in Figure 2, isotopes of A-type trimers with MRM 863/575 are reflected in the chromatograms of B-type trimers with MRM 865/577, procyandin C1 and one unknown trimer at Rt = 4.30 min being clearly distinguished as the main B-type trimeric procyanidins in the cranberry products. The mass spectra of A-type dimers and trimers are presented in the Supporting Information (Figures 1−3-OSM).

Differences were found among products not only in the total content of flavan-3-ols (0.00−2111.54 ± 130.33 μg/g) but also in their distribution (Figure 3). Some products, such as product 1, were completely devoid of monomeric, dimeric, and trimeric flavan-3-ols [although they may contain high molecular weight PACs, as measured by global phenolic determinations (see Table 1)], whereas others (i.e., product 19) contained only B-type PACs. Among monomers, (−)-epicatechin was more abundant than (+)-catechin (data not shown), whereas procyanidins B2 and C1 were the most abundant B-type oligomers (Figure 2). The large concentration of monomers in product 10 (1348.99 ± 73.08 μg/g), representing up to 37% of total flavan-3-ols, was in line with the additional presence of grape seed extract. Similar was the case of B-type dimers and trimers in this product, representing up to 55 and 3.7% of total flavan-3-ols, respectively. Excluding product 10, the highest concentrations of monomers and B-type dimers and trimers found among products were 80.70 ± 0.50 μg/g (38% of total flavan-3-ols) in product 14 and 201.87 ± 5.66 μg/g (9.6%) and 34.11 ± 1.36 μg/g (1.6%) both in product 18, respectively. In general, B-type trimers were considerably lower than B-type

dx.doi.org/10.1021/jf204912u | *J. Agric. Food Chem.* 2012, 60, 3396−3408

Table 2. Concentration of Hydroxybenzoic Acids in the Different Commercial Cranberry Products[a]

	concentration (mg/g) in product																		
compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
hydroxybenzoic acids																			
gallic acid	1.54	1.94	2.73	0.95	0.68	4.55	2.84	0.58	729.38	267.66	3.37	6.56	1.81	0.54	2.10	3.30	92.43	136.16	9.72
SD	0.07	0.13	0.02	0.09	0.02	0.18	0.26	0.01	72.94	1.14	0.13	0.44	0.02	0.05	0.13	0.33	2.23	1.50	0.40
3-*O*-methylgallic acid	0.65	0.48	0.62	0.15	0.09	0.63	0.36	nd	0.40	2.04	0.36	1.57	0.28	0.12	0.56	0.75	21.08	30.05	0.65
SD	0.06	0.02	0.03	0.01	0.01	0.06	0.01		0.04	0.04	0.04	0.05	0.03	0.01	0.04	0.02	0.32	0.64	0.01
protocatechuic acid	98.24	90.82	71.45	61.13	25.68	102.51	70.14	9.99	50.27	130.63	95.09	155.02	26.66	25.77	112.61	110.18	608.16	735.12	583.22
SD	1.40	5.36	2.56	3.13	2.57	10.25	0.30	0.39	5.03	0.94	1.04	0.73	0.29	0.58	6.91	3.69	5.09	17.76	0.87
vanillic acid	11.38	19.08	13.84	5.92	4.16	8.83	7.38	1.33	3.15	7.46	11.34	21.43	8.63	4.88	18.48	14.13	214.74	262.54	56.63
SD	0.87	1.91	1.38	0.59	0.42	0.88	0.54	0.13	0.26	0.28	0.09	0.78	0.86	0.28	1.08	0.19	7.06	10.16	0.65
syringic acid	0.74	0.61	0.21	nd	nd	0.10	0.24	nd	0.83	1.99	0.33	0.32	0.29	0.17	0.24	1.18	11.07	11.80	2.60
SD	0.07	0.06	0.02			0.01	0.02		0.01	0.20	0.03	0.03	0.03	0.01	0.02	0.12	0.69	1.18	0.19
salicylic acid	7.31	0.68	4.86	2.08	1.21	4.80	3.26	0.45	15.11	4.79	6.31	5.21	0.85	1.35	8.69	2.29	65.56	91.05	54.55
SD	0.27	0.07	nd	0.21	0.10	0.48	0.14	0.04	1.51	0.15	0.01	0.13	0.03	0.03	0.17	0.18	0.66	2.16	0.02
4-hydroxybenzoic acid	3.14	2.93	2.26	1.14	0.78	2.43	1.57	0.42	8.16	4.73	2.51	4.12	0.90	1.09	2.67	4.06	65.93	94.81	7.82
SD	0.11	0.10	0.15	0.06	0.08	0.24	0.05	0.01	0.82	0.19	0.12	0.08	0.11	0.01	0.02	0.06	1.19	2.23	0.26
3-hydroxybenzoic acid	3.13	nd	1.66	nd	nd	nd	nd	nd	nd	nd	1.70	1.77	nd	nd	1.63	1.56	9.70	11.58	1.10
SD	0.34		0.02								0.17	0.06			0.04	0.16	0.43	0.01	0.01
3,4,5-trimethoxybenzoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.17	0.32	nd
SD																		0.03	
benzoic acid	453.83	194.28	194.26	157.16	140.47	207.24	187.07	35.84	115.35	75.53	255.42	189.71	154.01	196.03	493.32	434.19	6011.14	8317.88	nd
SD	12.02	15.43	9.65	15.76	14.05	6.61	12.10	1.55	11.54	6.90	3.96	6.32	1.59	16.64	20.23	28.03	225.46	222.31	
hippuric acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.17	nd	1.26	1.14	nd
SD															0.02		0.13	0.10	
3-(3,4-dihydroxyphenyl)-propionic acid	nd	nd	nd	nd	nd	nd	nd	nd	3.49	0.66	nd	1.58	nd	nd	nd	nd	7.42	9.61	0.69
SD									0.35	0.01		0.10					0.30	0.16	0.04
3,4-dihydroxyphenyl-acetic acid	nd	nd	nd	nd	nd	nd	nd	nd	3.79	0.08	nd	nd	nd	nd	nd	nd	0.57	1.05	nd
SD									0.06	0.01							0.06	0.10	

dx.doi.org/10.1021/jf204912u | J. Agric. Food Chem. 2012, 60, 3396–3408

Table 2. continued

compound	concentration (mg/g) in product																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
4-hydroxyphenylacetic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.81	nd	nd	nd	nd	2.53	3.20	1.10
SD					0.10							0.12					0.18	0.41	0.24
4-hydroxy-3-methoxyphenyl-acetic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.20	nd	nd	nd	nd	nd	nd	4.81	6.55	0.62
SD										0.02							0.48	0.66	0.04
3,4-dihydroxymandelic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.48	3.56	0.39
SD																	0.05	0.31	0.04
4-hydroxymandelic acid	nd	nd	nd	nd	nd	nd	nd	nd	598.17	144.64	nd	nd	nd	nd	nd	nd	21.87	30.84	nd
SD									59.82	12.41							2.11	1.14	
4-hydroxy-3-methoxymandelic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.29	nd	nd	nd	nd	nd	nd	9.43	14.33	0.46
SD										0.03							0.64	0.45	0.05
total	579.96	310.82	291.90	228.52	173.08	331.09	272.86	48.62	1528.11	640.72	376.44	388.12	193.43	229.94	640.47	571.64	7148.36	9761.58	719.56
SD	15.21	23.07	13.84	19.85	17.34	18.72	13.43	2.13	152.36	22.31	5.59	8.84	2.97	17.60	28.65	32.77	247.07	261.32	2.82
hydroxycinnamic acids																			
p-coumaric acid	71.12	60.91	71.58	28.70	26.67	57.84	45.64	7.93	17.86	41.97	61.27	141.02	21.62	20.10	107.42	65.21	710.84	844.16	8.67
SD	0.08	3.80	3.26	2.35	2.67	5.78	nd	0.02	1.79	0.44	0.34	1.83	0.50	0.13	3.58	1.32	20.06	15.20	0.25
caffeic acid	6.24	9.99	11.05	4.13	2.40	11.10	5.66	1.18	32.01	10.05	7.44	24.40	2.49	3.12	26.83	10.80	116.54	133.67	20.25
SD	0.49	0.55	0.75	0.38	0.14	1.11	0.12	0.01	3.20	0.07	0.20	0.97	0.01	0.04	0.10	0.33	0.52	2.52	0.33
ferulic acid	8.21	5.53	5.55	2.76	2.07	4.75	4.33	0.56	3.65	3.67	7.88	9.19	2.49	3.92	11.62	17.02	87.55	111.92	10.52
SD	0.32	0.44	0.36	0.16	0.13	0.48	0.04	0.05	0.36	0.15	0.43	0.37	0.10	0.10	0.03	0.42	1.09	4.38	0.16
isoferulic acid	1.41	2.08	2.67	1.12	0.81	1.64	1.53	0.60	0.52	0.48	3.15	2.46	1.96	2.23	3.98	4.69	2.56	nd	nd
SD	0.14	0.12	0.10	0.11	0.08	0.16	0.05	0.06	0.05	0.05	0.32	0.12	0.20	0.22	0.38	0.13	0.02		
trimethoxycinnamic acid	2.36	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.11	2.72	nd
SD	0.24																0.21	0.27	
trans-cinnamic acid	14.17	7.89	6.71	2.52	1.57	3.50	3.42	0.33	8.96	0.90	6.55	9.77	5.28	2.35	10.94	5.94	211.24	260.55	nd
SD	0.05	0.65	0.17	0.01	0.07	0.35	0.03	0.05	0.90	0.08	0.20	0.09	0.53	0.23	0.02	0.41	1.95	0.04	
total	103.51	86.40	97.56	39.22	33.52	78.83	60.57	10.59	63.00	57.06	86.29	186.85	33.85	31.72	160.79	103.66	1130.84	1353.03	39.45
SD	1.32	5.56	4.64	3.00	3.09	7.88	0.25	0.20	6.30	0.80	1.49	3.37	1.33	0.73	4.12	2.60	23.86	22.41	0.74
total phenolic acids	683.47	397.22	389.46	267.75	206.60	409.93	333.43	59.20	1591.11	697.78	462.74	574.96	227.28	261.66	801.26	675.30	8279.20	11114.61	759.01
SD	16.53	28.63	18.48	22.85	20.44	26.60	13.69	2.33	158.66	23.10	7.08	12.21	4.29	18.32	32.77	35.37	270.93	283.73	3.55

[a]Mean (n = 2) ± standard deviation (SD); nd, not detected.

dx.doi.org/10.1021/jf204912u | J. Agric. Food Chem. 2012, 60, 3396–3408

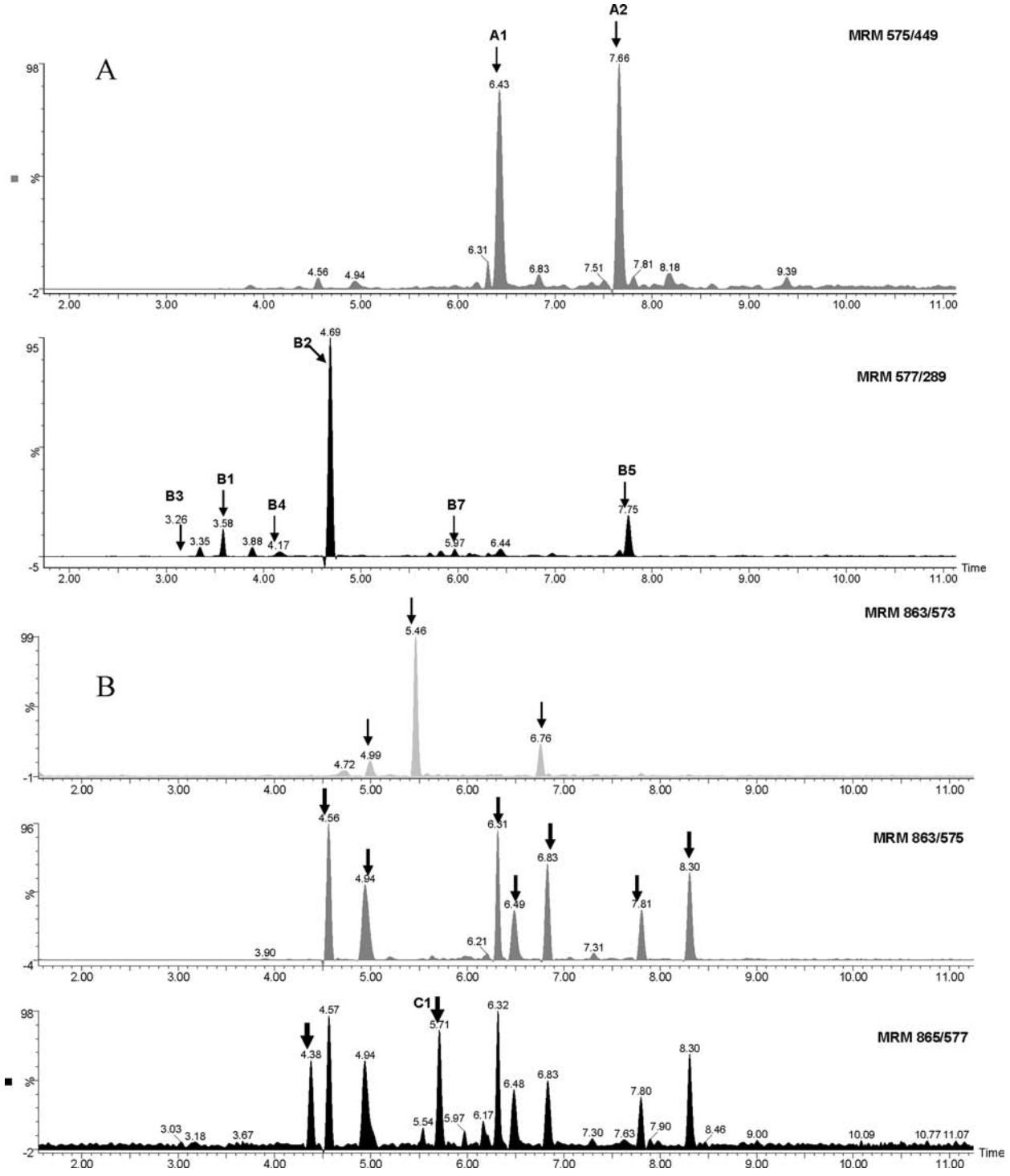
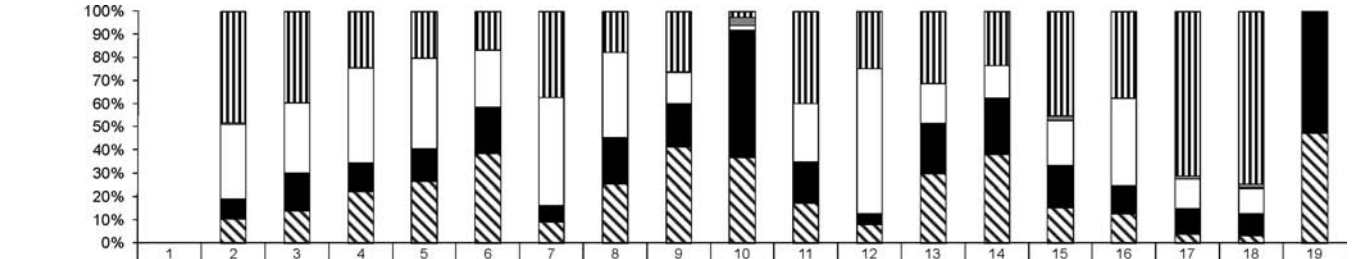


Figure 2. MRM chromatograms of flavan-3-ols in commercial product 18: (A) A-type dimers (MRM 575/449) and B-type dimers (MRM 577/289); (B) A-type trimers (MRM 863/573 and 863/575) and B-type trimers (MRM 865/577).

dimers among cranberry products and even were not detected in some products (products 3−8, 11−14, 16, and 19). This seems to be in line with previous studies on cranberry pomace, for which B-type dimers and trimers accounted for ca. 2.6 and 0.96% of total flavan-3-ols (DP 1−6), respectively, as measured by normal phase HPLC.[2]

In contrast to the profile of B-type oligomers, which reflected the presence of other flavan-3-ol sources, the profile of A-type oligomers actually revealed the real content of cranberry active ingredients. A-type dimers ranged from 0.00 (products 1 and 19) to 230.95 ± 9.32 μg/g (product 18) (Figure 3), procyanidin A2 showing higher concentration than dimer A1, which was detected only in products 16−18) (data not shown). With the exception of some products (9, 10, 13, 14, and 19), total A-type dimers were higher than B-type dimers in all samples. With regard to A-type trimeric procyanidins, the concentration ranged from 0.00 μg/g (products 1 and 19) to 1578.76 ± 111.47 μg/g (product 18). Among the different A-type trimeric procyanidins detected, only four species were commonly found in most of the samples (Rt = 5.46 min for MRM 863/573 and

dx.doi.org/10.1021/jf204912u | J. Agric. Food Chem. 2012, 60, 3396−3408



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A-TYPE TRIMERS	0.00	113.33	43.49	14.00	8.06	7.58	18.80	2.91	27.61	96.32	72.28	18.65	34.03	50.11	218.69	48.96	1223.05	1578.76	0.00
B-TYPE TRIMERS	0.00	1.67	0.00	0.00	0.00	0.00	0.00	0.00	0.53	134.80	0.00	0.00	0.00	0.00	8.02	0.00	24.15	34.11	0.00
A-TYPE DIMERS	0.00	75.07	33.10	23.38	15.63	10.97	23.44	6.02	14.13	67.99	45.58	47.34	18.84	29.56	94.09	48.81	212.48	230.95	0.00
B-TYPE DIMERS	0.00	19.88	17.57	6.97	5.62	8.97	3.55	3.33	19.99	2027.40	31.73	3.70	23.64	51.38	86.95	15.26	191.58	201.87	8.13
MONOMERS	0.00	24.76	15.42	12.69	10.47	17.11	4.61	4.11	43.63	1348.99	30.97	5.81	32.23	80.70	72.85	16.57	64.82	65.85	7.32

Figure 3. Concentration (μg/g) of flavan-3-ols and distribution according to their degree of polymerization (monomers, dimers, and trimers) and type of interflavan linkage (A- or B-type) in the different commercial cranberry products.

Rt = 6.31, 6.83, 8.30 min for MRM 863/575), whereas the remaining six isomers were found only in products 16−18 (data not shown), indicating possible differences in purification among products. According to Tarascou et al.,[15] five different trimers [two containing the A-type bond between the intermediate and lower units (i.e, m/z 863/575) and three between upper and intermediate units (i.e, m/z 863/573)] were detected in whole cranberry extract.

Also of note was the distribution of A-type dimers and trimers (Figure 3). For some products (4, 5, 7, 8, and 12) the percentage of A-type dimers was higher than that of A-type trimers (37−63% dimers vs 18−37% trimers); however, for other products (2, 3, 11, 13, 15−18) the opposite (31−75% trimers vs 11−38% dimers) was found. In crude cranberry pomace, A-type dimers have been reported to account ca. 50% of total flavan-3-ols (DP 1−6) followed by A-type trimers (ca. 18%).[2] Differences in the distribution of A-type dimers and trimers may be due to possible differences in the level of PAC purification among products and/or differences in the composition of the cranberry fruit used for processing.

Anthocyanins. The total anthocyanin content considerably differed (~10000-fold) among products (Table 3). Some products were practically devoid of anthocyanins (1.74 μg/g for product 13), whereas others contained up to 15116.61 μg/g (product 19). With regard to the individual anthocyanin profile, peonidin-3-galactoside (Pn-3-gal) was the most abundant anthocyanin in cranberry products (30−52% of total anthocyanins), followed by cyanidin-3-galactoside (Cy-3-gal, 17−30%), and finally by the -3-arabinoside derivatives of cyanidin (Cy-3-arb; 12−25%) and peonidin (Pn-3-arb; 8−21%), which were presented in very similar amounts. After peonidin-3-glucoside (Pn-3-glc; 3−10%), cyanidin-3-glucoside (Cy-3-glc; 0.34−1.6%) and malvidin-3-arabinoside (Mv-3-arb; 0.21−1.0%) were the minor anthocyanins in cranberry products. This profile, which was found in most of the products, seems to be in agreement with previous papers.[3,28,29] However, for products 17 and 18 the profile was inverted, and higher contents of Cy- and Pn-3-arb than of Cy- and Pn-3-gal were found, as has been reported to occur in cranberry pomace.[2] Finally, product 19, which contained an extremely high anthocyanin concentration in comparison to the rest of the products, did not fit in any of the two former profiles, presenting Pn-3-glc (90%) as the major anthocyanin followed by Cy-3-arab (10%) and Cy-3-glc (0.06%) and the absence of Cy-3-gal, Pn-3-gal, Pn-3-arb, and Mv-3-arb. This difference in profile could be due to changes occurring during extraction and purification of anthocyanins and/or the use of a different anthocyanin source other than cranberry.

Overall Assessment of the Phenolic Composition of Commercial Cranberry Products. To summarize the global and individual phenolic data as well as the results obtained from in vitro antioxidant, a principal component analysis (PCA) was applied. Two principal components (PC1 and PC2), which explained 77% of the total variance of the data, were obtained (Figure 4).

PC1, explaining 58% of the total variance, was negatively correlated (loadings ≤ -0.7) with all of the phenolic acids (except 4-hydroxymandelic, gallic, 3,4-dihydroxyphenylacetic, and isoferulic acids) and all A-type dimers (A1 and A2) and trimers (seven isomers with MRM 863/575 and three isomers with MRM 863/573), PT, PAC-But/HCl, PAC-DMAC, and ORAC (Figure 4A). PC2, explaining 19% of the total variance, was negatively correlated (loadings ≤ -0.7) with monomeric flavan-3-ols [(+)-catechin and (−)-epicatechin], dimeric procyanidins (B1−B5), and trimeric procyanidins (C1 and unknown trimer B). In summary, PC1 was mainly correlated with phenolic acids and A-type PACs, whereas PC2 was mainly correlated with monomeric flavan-3-ols and B-type PACs. Most of the cranberry samples of the market were clustered together, indicating similar phenolic profile and ORAC values; however, products 2, 15, and 19 were slightly differentiated from the rest of the products. Products 17 and 18 were characterized by presenting high values in variables correlated in PC1, indicating their abundance in A-type PACs. On the other hand, product 10 (containing a mixture of cranberry and grape seed extract) clearly differentiated from all products by presenting extremely high PC2 values, indicating higher contents of monomeric flavan-3-ols and B-type PACs. This was also the case of products 2 and 15, which presented higher (negative) values in PC2.

When product 10 was removed from the list of samples, a different PCA result was obtained (Figure 4B). In this case PC1 explained 72% of the total variance and was negatively correlated (loadings ≤ -0.7) with all variables [with the exception of 4-hydroxymandelic, gallic, 3,4-dihydroxyphenylacetic, and isoferulic acids and (−)-epicatechin]. PC2 explained 11% of total variance and was positively correlated (loadings ≥ 0.7) with all anthocyanin compounds (with the exception of Cy-3-glc and Pn-3-glc). The plane defined by the first two principal components indicated that products 2 and 15 not only presented a different content of B-type PACs (as shown in Figure 3A) from the rest of the products but also showed a high content of the anthocyanins correlated with this component

Table 3. Concentration of Anthocyanins in the Different Commercial Cranberry Products[a]

	concentration (μg/g) in product																		
compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Cy-3-gal	1.43	447.34	86.61	28.26	16.12	75.58	102.39	19.74	71.90	223.50	42.88	80.21	0.42	44.15	156.45	99.66	9.50	0.19	0.00
SD	0.07	21.32	1.19	0.31	0.38	3.81	2.28	0.38	4.23	37.84	4.20	3.30	0.02	2.21	3.52	0.79	0.95	0.01	0.00
Cy-3-glc	0.02	13.63	2.84	1.09	0.64	3.20	3.06	0.60	2.28	14.92	1.06	2.71	0.01	0.60	5.89	1.81	0.62	0.31	1544.18
SD	0.00	1.36	0.09	0.01	0.06	0.06	0.09	0.01	0.18	1.49	0.11	0.08	0.00	0.03	0.30	0.07	0.06	0.02	95.88
Cy-3-arab	0.88	398.84	83.42	35.09	19.64	72.24	70.95	14.88	59.43	219.56	38.64	95.16	0.26	20.31	216.58	37.70	38.74	15.01	9.27
SD	0.01	39.88	0.47	1.18	0.75	6.66	1.91	0.06	5.94	7.35	3.86	4.08	0.01	1.02	2.42	3.39	3.87	0.05	0.93
Pn-3-gal	3.46	611.21	131.25	58.08	28.23	119.42	150.15	32.83	154.31	280.55	96.66	137.70	0.77	50.62	263.93	141.36	25.76	1.03	0.00
SD	0.17	61.12	3.78	2.27	0.70	8.23	1.13	1.45	15.43	28.06	7.93	6.17	0.04	2.53	5.07	7.45	2.58	0.09	0.00
Pn-3-glc	0.26	92.74	19.69	8.49	7.00	23.52	24.00	5.73	26.02	92.94	11.13	20.54	0.06	4.63	45.91	18.45	8.12	4.84	13563.17
SD	0.00	9.27	0.09	0.10	0.54	1.96	0.16	0.19	2.60	9.29	1.11	1.08	0.00	0.23	2.64	0.18	0.81	0.48	1019.30
Pn-3-arb	0.51	291.16	57.61	30.89	15.74	56.44	53.14	11.91	53.55	112.94	37.08	69.48	0.24	16.73	187.14	24.79	56.76	32.73	0.00
SD	0.01	29.12	0.09	3.09	1.09	3.45	0.32	0.54	5.35	11.29	3.71	0.68	0.01	0.84	3.05	0.08	5.68	3.27	0.00
Mv-3-arb	0.05	9.57	1.81	0.75	0.59	1.72	1.03	0.24	1.43	2.17	2.32	1.95	0.00	0.64	7.51	0.77	1.97	1.16	0.00
SD	0.00	0.96	0.12	0.05	0.05	0.17	0.07	0.00	0.14	0.22	0.00	0.13	0.00	0.03	0.49	0.01	0.10	0.02	0.00
total	6.62	1864.49	383.24	162.64	87.96	352.12	404.72	85.93	368.92	946.58	229.77	407.76	1.74	137.67	883.41	324.54	141.46	55.27	15116.61
SD	0.25	163.03	5.83	7.01	3.58	24.33	5.95	2.63	33.88	95.55	20.93	15.53	0.09	6.88	17.49	11.98	14.05	3.95	1116.10

[a]Mean (n = 2) ± standard deviation (SD); nd, not detected.

dx.doi.org/10.1021/jf204912u | J. Agric. Food Chem. 2012, 60, 3396–3408

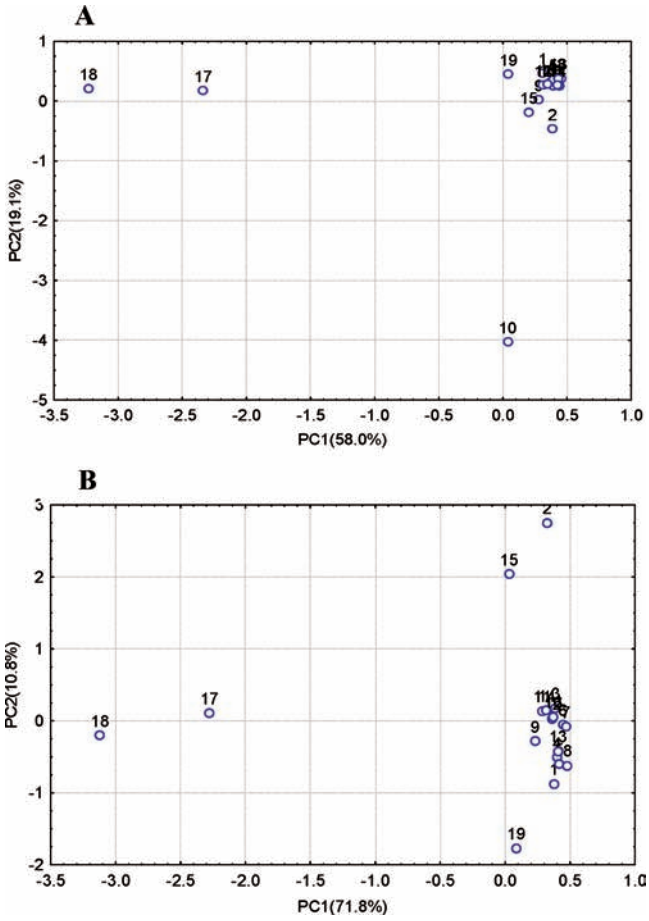


Figure 4. Representation of the different commercial cranberry products in the plane defined by principal component 1 (PC1) and principal component 2 (PC2) that resulted from the application of the principal component analysis: (A) all products; (B) all products except product 10.

(i.e., Cy-3-gal, Cy-3-arb, Pn-3-gal, Pn-3-arb, and Mv-3-arb). Finally, product 19 was completely separated from the rest by presenting very low PC2 values, which indicated a very different anthocyanin profile in comparison to the remaining products.

When considering the issue of 36 mg of total PACs as the daily recommended intake dose of cranberry PACs (based on the DMCA method),[17] only four products (10 and 17−19) would provide this amount, considering the daily intake dose indicated on the product's label. Of most importance was the fact that this dose would provide as low as 0.00 (product 19) and up to 205 μg/g (product 18) of procyanidin A2. Therefore, the actual PAC-But/HCl and DMAC values do not indicate the actual degree of structural heterogeneity of PACs present in commercial cranberry products or the presence of other natural sources of PACs, such as grape seed extract (product 10), as was indicated by the UPLC analysis.

Antiadhesion Effects on Uropathogenic Bacteria. The in vitro antiadhesion activity against uropathogenic *E. coli* ranged from a negative (null) effect (products 2, 6, 8, and 19) to a MIC = 0.5 mg/mL (product 18) for cranberry powders and a MIC = 112 mg/mL (product 7) for gel capsule or syrup samples (Table 1). In the HRBC hemagglutination assay, a MIC value of ≤15 mg/mL is considered to be efficient.[9] In general, no statistical correlation was found between global phenolic content and MIC values when considering all of the products in their different presentation forms. However, when the powder and powder capsules products given positive MIC values were considered, a second-order polynomic type relationship was found between PT and MIC values (Figure 5A).

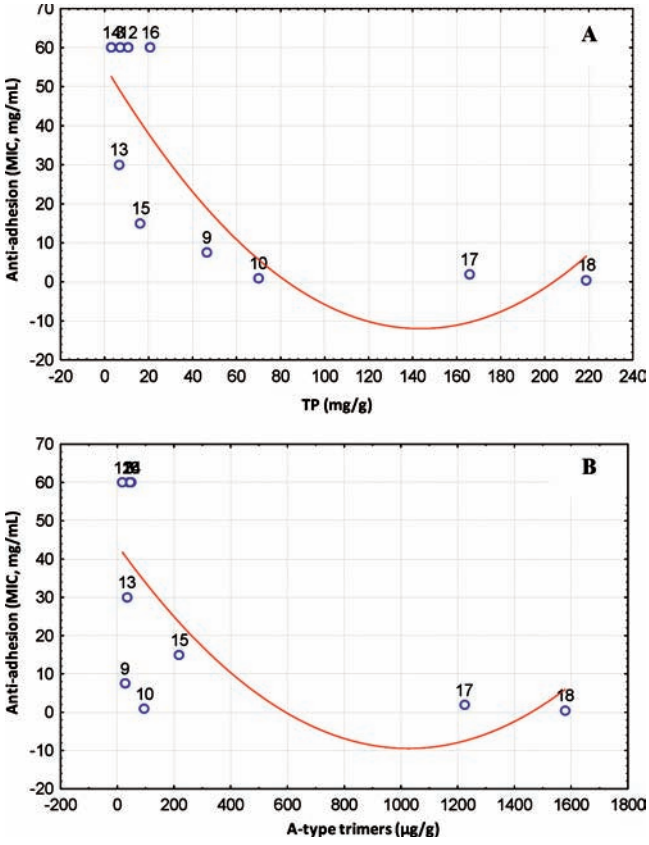


Figure 5. Relationship between polyphenols and bacterial antiadhesion capacity: (A) total polyphenols (PT) versus MIC values; (B) $\sum$ A-type trimers versus MIC values.

Despite products 3, 12, 14, and 16 showing PT values similar to those of products 13 and 15, the latter products presented lower MIC values than the former ones. The opposite was observed for products 10, 17, and 18, which despite showing very different PT contents presented very similar MIC values. These findings indicate that the in vitro antiadhesion activity of cranberry products not only depends on the total PAC content but also on the PAC structure and size. This fact is of note when the MIC values and total A-type trimer contents are compared (Figure 5B). Products such as product 10 (containing grape seed extract) and product 9 (containing a high content of gallic acid) were now less fitted into the model. On the other hand, the small differences in A-type trimer content observed between products 3, 12, 14, and 16 may help to explain their similar MIC value (60 mg/mL). Although recent studies have shown that A-type trimers could be transported across Caco-2 cells at a low rate (0.4%),[34] only a very little amount of A-type proanthocyanidins (i.e., dimer A2) has been found in biological fluids[20] to be responsible for the in vivo effects at the urinary tract level. Nevertheless, it is also important to highlight that the exact microbial catabolic pathway of these compounds is still under elucidation,[35] and they may exert local effects at the gut level or modulate the

dx.doi.org/10.1021/jf204912u | J. Agric. Food Chem. 2012, 60, 3396−3408

microbiota composition before being catabolized, also resulting in benefits against UTI.

Finally, the negative antiadhesion activity found for powder products 2, 8, and 19 may also be explained by their particular composition (Table 1). In fact, products 2 and 19 presented the highest anthocyanin content of all products (Table 3), whereas product 8 also contained probiotics, which could have produced some kind of interference in the antiadhesion test. Similarly, products 4 and 5, which also contained vitamin C, showed MIC values (230 and 200 mg/mL, respectively) that were not in line with their high PT contents (132 and 139 mg gallic acid/g) due to the reaction of vitamin C with the Folin−Ciocalteu reagent. Therefore, many variables seem to contribute to or affect the in vitro antiadhesion activity of cranberry products against *E. coli* and its correlation with phenolic content.

In summary, the present work provides a comprehensive and valuable assessment of the quality of cranberry products based on global phenolic determination, targeted UPLC-DAD-ESI-TQ MS phenolic characterization, and in vitro bioactivity tests. Products found in the market largely differed in their phenolic content and distribution, including products completely devoid of flavan-3-ols to highly purified ones, either in A-type PACs or in anthocyanins. To our knowledge, this work also constitutes the most complete study of free phenolic acids in cranberries. Besides the phenolic composition, factors such as the presentation (syrup, powder or gel capsule, etc.) and inclusion of other antioxidants or botanical ingredients have a profound influence in the in vitro bioactivity tests assayed. In the case of the antiadhesion test, no linear correlation was found between MIC values and global or individual phenolic contents, but the best correlation model obtained indicated that antiadhesion activity may vary on the basis of differences in structure and size of the PAC molecules. Among these compounds, A-type trimers help to better explain the tendency observed for total polyphenols.

The recommended intake dose of cranberry products is 36 mg of total PACs/day by the DMAC method), and it was found that products providing this dose will actually provide very different A-type dimer levels due to difference in factors such as the source and nature of PACs (addition of B-type PAC sources) and degree of purification of the cranberry extract used as ingredient in the formulation of the products. Therefore, if A-type PACs are considered the active ingredients in cranberry products for sustaining beneficial claims against UTI, a total PAC measurement either by the butanol/HCl or by the DMAC methods is not specific enough to sustain the claim of the recommended dose of intake of 36 mg total PAC/day. Further determination of the degrees of polymerization of the A-type PACs may be necessary to more completely explain the antiadhesion activity of the recommended dosage. However, these methods could be used as quality control measurements when the individual composition is well standardized. Finally, the bioavailability issue, in particular, the unraveling of the catabolism of A-type PACs and its difference from that of B-type ones, may help to explain the role of phenolic acids, also very abundant in cranberry products, in the benefits against UTI. Our current studies are focused in that direction.

■ ASSOCIATED CONTENT

Supporting Information

Mass spectra of the different A-type dimeric procyanidins (MRM 575 > 449, Figure 1-OSM) and the different A-type trimeric procyanidins (MRM 863 > 573, Figure 2-OSM; MRM 863 > 575, Figure 3-OSM). This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This work has been funded by the Spanish Ministry for Science and Innovation (AGL2010-17499) and the Spanish Research Council (CSIC) (Programa Intramural Especial 201070I028). F.S.-P. is the recipient of a contract from the JAE-Doc Program (CSIC). M.M. is grateful to CSIC for granting permission as a Visiting Researcher at the Diet, Genomic and Immunology Lab of USDA-Beltsville (MD, USA).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Foo, L. Y.; Lu, Y.; Howell, A. B.; Vorsa, N. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli* in vitro. *Phytochemistry* **2000**, *54*, 173−181.

(2) White, B. L.; Howard, L. R.; Prior, R. L. Proximate and polyphenolic characterization of cranberry pomace. *J. Agric. Food Chem.* **2010**, *58*, 4030−4036.

(3) Borges, G.; Degeneve, A.; Mullen, W.; Crozier, A. Identification of flavonoid and phenolic antioxidants in black currants, blueberries, raspberries, red currants, and cranberries. *J. Agric. Food Chem.* **2010**, *58*, 3901−3909.

(4) Pappas, E.; Schaich, K. M. Phytochemicals of cranberries and cranberry products: characterization, potential health effects, and processing stability. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 741−781.

(5) Weiss, E. I.; Houri-Haddad, Y.; Greenbaum, E.; Hochman, N.; Ofek, I.; Zakay-Rones, Z. Cranberry juice constituents affect influenza virus adhesion and infectivity. *Antiviral Res.* **2005**, *66*, 9−12.

(6) Zhang, L.; Ma, J.; Pan, K.; Go, V. L.; Chen, J.; You, W. C. Efficacy of cranberry juice on *Helicobacter pylori* infection: a double-blind, randomized placebo-controlled trial. *Helicobacter* **2005**, *10*, 139−145.

(7) Ruel, G.; Pomerleau, S.; Couture, P.; Lamarche, B.; Couillard, C. Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. *Metabolism* **2005**, *54*, 856−861.

(8) Wilson, T.; Singh, A. P.; Vorsa, N.; Goettl, C. D.; Kittleson, K. M.; Roe, C. M.; Kastello, G. M.; Ragsdale, F. R. Human glycemic response and phenolic content of unsweetened cranberry juice. *J. Med. Food* **2008**, *11*, 46−54.

(9) Howell, A. B.; Reed, J. D.; Krueger, C. G.; Winterbottom, R.; Cunningham, D. G.; Leahy, M. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. *Phytochemistry* **2005**, *66*, 2281−2291.

(10) Weiss, E. I.; Kozlovsky, A.; Steinberg, D.; Lev-Dor, R.; Bar Ness Greenstein, R.; Feldman, M.; Sharon, N.; Ofek, I. A high molecular mass cranberry constituent reduces mutans streptococci level in saliva and inhibits in vitro adhesion to hydroxyapatite. *FEMS Microbiol. Lett.* **2004**, *232*, 89−92.

(11) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003**, *51*, 7513−7521.

(12) Porter, M. L.; Krueger, C. G.; Wiebe, D. A.; Cunningham, D. G.; Reed, J. D. Cranberry proanthocyanidins associate with low-density lipoprotein and inhibit in vitro Cu^{2+}-induced oxidation. *J. Sci. Food Agric.* **2001**, *81*, 1306−1313.

(13) Neto, C. C. Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. *Mol. Nutr. Food Res.* **2007**, *51*, 652−664.
(14) Reed, J. D.; Krueger, C. G.; Vestling, M. M. MALDI-TOF mass spectrometry of oligomeric food polyphenols. *Phytochemistry* **2005**, *66*, 2248−2263.
(15) Tarascou, I.; Mazauric, J. P.; Meudec, E.; Souquet, J. M.; Cunningham, D.; Nojeim, S.; Cheynier, V.; Fulcrand, H. Characterisation of genuine and derived cranberry proanthocyanidins by LC-ESI-MS. *Food Chem.* **2011**, *128*, 802−810.
(16) Guay, D. R. P. Cranberry and urinary tract infections. *Drugs* **2009**, *69*, 775−807.
(17) A. f. d. s. s. d. Aliments. Extension de l'allégation "contribue à diminuer la fixation de certaines bactéries *E. coli* sur les parois des voies urinaires", sur l'emploi de la "cranberry/canneberge" ou "*Vaccinium macrocarpon* dans la canneberge fraîche et la canneberge congelée, la purée de canneberge, les canneberges séchées/sucrées et les canneberges séchées/sucrées et aromatisées". Afssa-Saisine n 2006-SA-0256, June 2007.
(18) McMurrough, I.; McDowell, J. Chromatographic separation and automated analysis of flavanols. *Anal. Biochem.* **1978**, *91*, 92−100.
(19) Monagas, M.; Urpi-Sarda, M.; Sanchez-Patan, F.; Llorach, R.; Garrido, I.; Gomez-Cordoves, C.; Andres-Lacueva, C.; Bartolome, B. Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food Funct.* **2010**, *1*, 233−253.
(20) Rajbhandari, R.; Peng, N.; Moore, R.; Arabshahi, A.; Wyss, J. M.; Barnes, S.; Prasain, J. K. Determination of cranberry phenolic metabolites in rats by liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* **2011**, *59*, 6682−6688.
(21) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolibdicphosphotungstic acid reagent. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(22) Ribéreau-Gayon, P.; Stonestreet, E. Dósage des tannins du vin rouges et determination du leur structure. *Chem. Anal.* **1966**, *48*, 188−196.
(23) Prior, R. L.; Fan, E.; Ji, H.; Howell, A.; Nio, C.; Payne, M. J.; Reed, J. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *J. Sci. Food Agric.* **2010**, *90*, 1473−1478.
(24) Sánchez-Patán, F.; Monagas, M.; Moreno-Arribas, M. V.; Bartolomé, B. Determination of microbial phenolic acids in human faeces by UPLC-ESI-TQ MS. *J. Agric. Food Chem.* **2011**, *59*, 2241−2247.
(25) Sánchez-Patán, F.; Cueva, C.; Monagas, M.; Walton, G. E.; Gibson, G. R.; Martín-Álvarez, P. J.; Moreno-Arribas, M.-V.; Bartolomé, B. Gut microbial catabolism of grape seed flavan-3-ols by human faecal microbiota. Targeted analysis of precursor compounds, intermediate metabolites and end products. *Food Chem.* **2012**, *131*, 337−347.
(26) Davalos, A.; Gomez-Cordoves, C.; Bartolome, B. Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *J. Agric. Food Chem.* **2004**, *52*, 48−54.
(27) Waterhouse, A. L.; Ignelzi, S.; Shirley, J. R. A comparison of methods for quantifying oligomeric proanthocyanidins from grape seed extracts. *Am. J. Enol. Vitic.* **2000**, *51*, 383−389.
(28) Prior, R. L.; Lazarus, S. A.; Cao, G.; Muccitelli, H.; Hammerstone, J. F. Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium* spp.) using high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **2001**, *49*, 1270−1276.
(29) Zheng, W.; Wang, S. Y. Oxygen radical absorbance capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J. Agric. Food Chem.* **2003**, *51*, 502−509.
(30) Monagas, M.; Hernandez-Ledesma, B.; Garrido, I.; Martin-Alvarez, P. J.; Gomez-Cordoves, C.; Bartolome, B. Quality assessment of commercial dietary antioxidant products from *Vitis vinifera* L. grape seeds. *Nutr. Cancer.* **2005**, *53*, 244−254.
(31) Chen, H.; Zuo, Y.; Deng, Y. Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performanc liquid chromatography. *J. Chromatogr., A* **2001**, *913*, 387−395.
(32) Zuo, J.; Wang, C.; Zhan, J. Separation, characterization, and quantification of benzoic and phenolic antioxidants in American cranberry fruit by GC-MS. *J. Agric. Food Chem.* **2002**, *50*, 3789−3794.
(33) Prior, R. L.; Rogers, T. R.; Khanal, R. C.; Wilkes, S. E.; Wu, X.; Howard, L. R. Urinary excretion of phenolic acids in rats fed cranberry. *J. Agric. Food Chem.* **2010**, *58*, 3940−3949.
(34) Ou, K.; Percival, S. S.; Zou, T.; Khoo, C.; Gu, L. Transport of cranberry A-type procyanidin dimers, trimers, and tetramers across monolayers of human intestinal epithelial Caco-2 cells. *J. Agric. Food Chem.* **2012**, *60*, 1390−1396.
(35) Engemann, A.; Hubner, F.; Rzeppa, S.; Humpf, H. U. Intestinal metabolism of two a-type procyanidins using the pig cecum model: detailed structure elucidation of unknown catabolites with Fourier transform mass spectrometry (FTMS). *J. Agric. Food Chem.* **2012**, *60*, 749−757.