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Comparing Procyanidins in Selected *Vaccinium* Species by UHPLC-MS^2 with Regard to Authenticity and Health Effects

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ABSTRACT: Cranberry procyanidins have been associated with an effect against urinary tract infections (UTI) for decades, and European health claims are requested. This study compares the procyanidin profiles and concentrations of American cranberry (*Vaccinium macrocarpon* Ait.), European cranberry (*Vaccinium oxycoccus* L.), and lingonberry (*Vaccinium vitis-idaea* L.) analyzed using ultrahigh-performance liquid chromatoraphy coupled to a triple-quadrupole mass spectrometer with electrospray interface (UHPLC-MS^2). Concentrations of A-type trimers, procyanidin A2, catechin, epicatechin, and B-type dimers and trimers have been evaluated and compared for the first time in the three berries. The data clearly show remarkable differences in the procyanidin profiles and concentrations, especially the lack of A-type trimers in *V. oxycoccus*; thus, the effectiveness against UTI may vary among the *Vaccinium* species. These differences can be used to prove authenticity.

KEYWORDS: *Vaccinium macrocarpon, Vaccinium oxycoccus, Vaccinium vitis-idaea, UHPLC-MS^2, A-type procyanidins, authenticity, flavonoids*

INTRODUCTION

Cranberry plants are evergreen shrubs of the family Ericaceae and the genus *Vaccinium*. There are two different kinds of cranberries: the American cranberry, *Vaccinium macrocarpon* (Ait.), and the smaller European cranberry, *Vaccinium oxycoccus* L.[1] Lingonberries (*Vaccinium vitis-idaea* L.), which are often confused with cranberries, belong to the same genus, but not to the subgenus *Oxycoccus*.[2]

V. macrocarpon is a prominent agricultural food crop of the United States and Canada. In 2011 the total production in the United States was about 7.7 million barrels grown on 38.500 acres.[3] American cranberries have been intensively studied and long been considered as a health-promoting food. The native Americans already used cranberries not only as a food but also for meat preservation and as a folk remedy against diverse aliments. These benefits have been linked to its various phenolic phytochemicals such as anthocyanins, phenolic acids, flavonols, flavanols, and tannins.[4]

V. macrocarpon and its juice have been the focus of research for decades because of their most recognized and often proved health benefit to prevent urinary tract infections (UTI).[5] A-type procyanidins have been implicated as important inhibitors of the adhesion of uropathogenic fimbriated *Escherichia coli* to uroepithelial cells in the urinary tract. In several experiments the compounds could be isolated and identified as three trimeric A-type procyanidins and the procyanidin dimer A2.[6−8] Therefore, the European Food Safety Authority (EFSA) is requested to assess health claims in relation to procyanidins from *V. macrocarpon* and defense against bacterial pathogens in the lower urinary tract.[9] The French Food Safety Authority (AFSSA) has already accepted the claim "helps to reduce the adherence of certain *E. coli* bacteria to the urinary tract walls" for *V. macrocarpon* as the first health claim for berry phenolics.[10]

In contrast to *V. macrocarpon*, studies about the bioactive properties of *V. oxycoccus* and *V. vitis-idaea* are rare.[11] Consequently, there are a number of publications showing the inhibition of the attachment of *E. coli* to the uroepithelia cells in relation to procyanidins, especially from *V. macrocarpon*, but there is no clear evidence for *V. oxycoccus* and *V. vitis-idaea*.[12] In the study of Kylli et al.[11] the European cranberry did not inhibit the adhesion of *E. coli*, in contrast to American cranberry, which contains more A-type procyanidins.

The three *Vaccinium* berries examined here have visual and gustatory conformities and are often confused, but the phenolic compositions differ and thus there might be differences in their health benefits. Consequently, authenticity of cranberry products is important and research on the phenolic composition is useful to identify which species is really meant when labeled as "cranberry".[13]

Although much work has been done to characterize the procyanidins in cranberries, a comparison of the three *Vaccinium* species has rarely been performed. The aim of this study is to compare the procyanidin profiles of the *V. macrocarpon*, *V. oxycoccus*, and *V. vitis-idaea* berries and demonstrate the differences. This study focuses on A-type trimer procyanidin pattern and procyanidin A2, which are, besides the high molecular weight procyanidins, related to the prevention of UTI and therefore important information concerning the health claim. Amounts of different procyanidins

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are quantified by UHPLC-MS^2 and compared, especially for *V. oxycoccus* and *V. macrocarpon*, to our knowledge for the first time.

MATERIALS AND METHODS

Chemicals. Ultrahigh-quality (UHQ) water was prepared with a Direct-Q 3 system (Millipore, Billerica, MA, USA). Acetonitrile and methanol were obtained from Fisher Scientific (Loughborough, UK), formic acid was from Merck (Darmstadt, Germany), and water for LC was from VWR International (Leuven, Belgium). All solvents and additives used as eluents were of LC-MS grade. Hydrochloric acid (0.1 M) was from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Procyanidins A2 (>99%), B1 (>80%), and B2 (>90%) were purchased from Extrasynthese (Genay, France). Catechin hydrate (>98%) and epicatechin (>97%) were obtained from Sigma-Aldrich Chemie GmbH.

Samples. Table 1 gives an overview of the analyzed *Vaccinium* berries. A mix of different varieties and single varieties of *V. macrocarpon* and *V. vitis-idaea* from Europe (EU) and China (CN) and *V. oxycoccus* were sponsored and purchased by Rabenhorst O. Lauffs GmbH & Co. KG (Unkel, Germany). The *V. macrocarpon* varieties were all grown in Aylesford, Canada. Additional *V. macrocarpon* berries of the variety Pilgrim were kindly given from a horticulture in Runów near Warsaw, Poland. Berries of *V. oxycoccus* were additionally bought in an organic store in Warsaw. All berries were stored at −20 °C.

Table 1. Overview of Variety, Origin, Harvest Year, and Percentage of Water of the Analyzed *Vaccinium* Species

Vaccinium species	variety	origin	harvest year	water (%)
macrocarpon	mixed	North America	2009	88.5
macrocarpon	Grygleski	Aylesford, Canada (CA)	2010	86.9
macrocarpon	Stevens	Aylesford, Canada (CA)	2010	87.7
macrocarpon	Ben Liar	Aylesford, Canada (CA)	2010	87.8
macrocarpon	Pilgrim	Aylesford, Canada (CA)	2010	87.7
macrocarpon	Pilgrim	Runów, Poland (PL)	2010	87.3
oxycoccus	wild	Europe (EU)	2009	88.6
oxycoccus	wild	PL (organic store)	probably 2010	88.6
vitis-idaea	wild	Europe (EU)	2009	83.5
vitis-idaea	wild	China (CN)	2009	85.1

Quantification of Procyanidins by UHPLC-UV-MS^2. *Extraction of the Procyanidins.* Prior to the extraction, the berries were freeze-dried and milled by a ball mill (Retsch, Haan, Germany) to a fine and homogeneous powder. These powders were extracted with pressurized liquid extraction (PLE) performed by an accelerate solvent extractor (ASE 200, Dionex, Idstein, Germany). Therefore, 0.5 g of powdered berry sample was homogenized with 4.5 g of diatomaceous earth (Biotage, Uppsala, Sweden) and packed into 11 mL stainless steel extraction cells after insertion of two cellulose filters (Schleicher & Schuell GmbH, Dassel, Germany). The extraction solvent was acetone/water 70 + 30 (v + v), and the extraction parameters were 10 min, 2 cycles, room temperature, and 50% flush volume. Each sample was extracted in triplicate.[14]

Solid-phase extraction (SPE) was performed using a Gilson ASPEC XL4i system (automated sample preperation with extraction cartridges, Abimed, Langenfeld, Germany). The SPE was carried out on multimode cartridges Chromabond HR-XC (500 mg, 3 mL, Macherey-Nagel, Düren, Germany) consisting of a strong acidic benzosulfonic acid cation exchanger based on polysterene−divinylbenzene. First, the cartridges were conditioned with 10 mL of methanol and 10 mL of UHQ water. The extracts were made up to 60 mL with water to reduce the organic part before loading on the SPE cartridge. The extract was loaded in three steps at 20 mL onto the cartridges. After each loading step, the cartridges were washed with 20 mL of 0.1 M HCl to remove matrix. The elution of the polyphenols was carried out with 3.5 mL of methanol, discarding the first 0.5 mL. Eluates were directly analyzed in duplicate by UHPLC-MS^2.

UHPLC-UV-MS^2. For analysis of the procyanidins an Acquity UPLC system by Waters (Milford, MA, USA) consisting of a binary pump (BSM), an autosampler (SM; cooled to 10 °C), a column oven (CM) set at 40 °C, a diode array detector (PDA), and a triple-quadrupole mass spectrometer (Acquity TQD) with electrospray interface operating in negative mode was used. An Acquity BEH Shield RP18 column (150 mm × 2.1 mm, 1.7 μm; Waters) was used for separation. The whole system was controlled by MassLynx 4.1 software. The solvents were LC-MS grade water with 0.1% (v/v) formic acid (mobile phase A) and acetonitrile with 0.1% (v/v) formic acid (mobile phase B). The UHPLC gradient was as follows: 0−28 min, 98−76% A; 28−29 min, 76−0% A; 29−31 min, 0% A; 31−33 min, 0−98% A; 33−35 min, 98% A; flow rate = 0.4 mL/min. Two microliters of each sample extract was injected.

For quantification of A-type procyanidins, the mass spectrometer was tuned using a standard solution of procyanidin A2. The resulting parameters were as follows: capillary voltage, −2.0 kV; cone voltage, 46 V; extractor voltage, 2.0 V; RF voltage, 0.20 V; source temperature, 150 °C; desolvation temperature, 450 °C; cone gas (nitrogen) flow, 50 L/h; desolvation gas (nitrogen) flow, 800 L/h.

For quantification purposes mass traces of procyanidins m/z were measured by using selected reaction monitoring (SRM) with the following compound-specific transitions of parent and product ions: monomers m/z 289 → 245, dimers m/z 575 → 449 and 577 → 289, trimers m/z 863 → 575, 863 → 573, and 865 → 577. The dimers and trimers were quantified as procyanidin A2 dimer equivalents (A2 equiv) with an external calibration curve of procyanidin A2 in the range of 0.5−70 μg/mL (r^2 = 0.999). To account for the matrix effect the standard was diluted with blank matrix. As a blank matrix a raspberry extract (*Rubus idaeus*), prepared in the same way as the berry samples, was used, which was found to be free of procyanidin A2. Monomeric flavan-3-ols were quantified using a calibration curve of catechin and epicatechin in the range of 0.125−450 μg/mL (r^2 = 0.999). For the identification of procyanidin B5 and C1 a cacao bean extract was injected to compare the retention times. Catechin, epicatechin, and procyanidins B1 and B2 were identified by authentic standards. All UHPLC-MS^2 analyses of each extract were done at least in duplicate. All concentrations are calculated as milligram A2 equivalents in 100 g of fresh berries (mg A2 equiv/100 g FW).

RESULTS AND DISCUSSION

Analysis of Procyanidins in *Vaccinium* Species. *Vaccinium* species show a large number of different polyphenols,[15] which makes the identification and quantification of individual compounds complicated. Proanthocyanidins are difficult to detect by UV, due to many interfering compounds at their absorption maxima at 280 nm. UHPLC-UV-MS^2 was used for analysis to enable identification[16] and quantification of the procyanidins.[17] The important steps for analyzing polyphenols are extraction, cleanup, and enrichment of the minor components. Acetone is commonly used for the procyanidin extraction.[14,16,18] For sample preparation a multimode cartridge with cation exchanger was chosen to detach the anthocyanins for less coelution in the chromatogram and to concentrate the other phenolic compounds by binding to the reversed phase.

All named *Vaccinium* species contain different A- and B-type procyanidins. Especially in the genus *Vaccinium*, A-type procyanidins are dominant in comparison to other fruits.[19] In

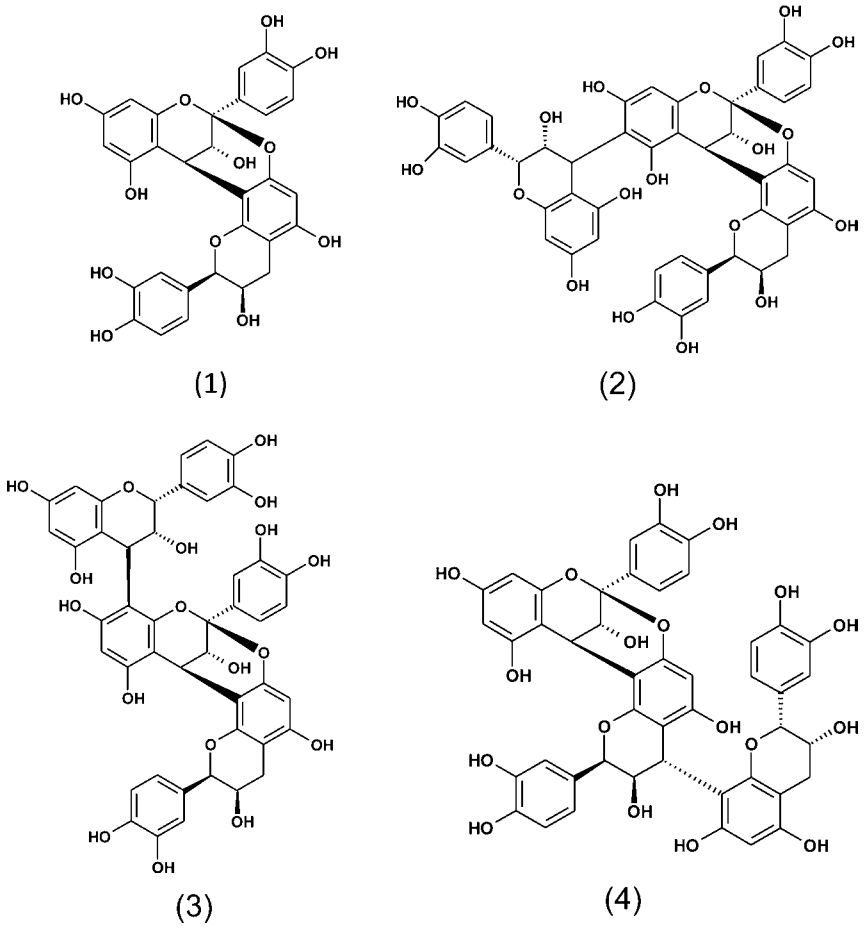

Figure 1. Structures of procyanidins that prevent the adherence of P-fimbriated *E. coli*: (**1**) epicatechin-(4β→8,2β→O→7)-epicatechin (procyanidin A2); (**2**) epicatechin-(4β→6)-epicatechin-(4β→8,2β→O→7)-epicatechin; (**3**) epicatechin-(4β→8)-epicatechin-(4β→8,2β→O→7)-epicatechin; (**4**) epicatechin-(4β→8,2β→O→7)-epicatechin-(4β→8)-epicatechin.

a series of experiments, Foo et al.[8] isolated and identified the following compounds of *V. macrocarpon* that inhibit the adherence of P-fimbriated *E. coli* (see Figure 1): three A-type procyanidin trimers (MW 864.78), epicatechin-(4β→6)-epicatechin-(4β→8,2β→O→7)-epicatechin, epicatechin-(4β→8,2β→O→7)-epicatechin-(4β→8)-epicatechin, and epicatechin-(4β→8)-epicatechin-(4β→8,2β→O→7)-epicatechin; and the A-type dimer epicatechin-(4β→8,2β→O→7)-epicatechin (A2). Therefore, in this study, A-type procyanidin trimers with $[M - H]^-$ ions at m/z 863 and procyanidin A2 ($[M - H]^-$ at m/z 575) are quantified using SRM of suitable product ions, and amounts in the three *Vaccinium* species are compared (see Table 2). Additionally, monomers and B-type procyanidins are quantified. Chromatograms are shown in Figure 4 and amounts in Table 3.

Profile and Quantification of Procyanidin A-Type Dimers and Trimers. Figure 2 shows the SRM chromatograms of the three berries of the transitions at m/z 863 → 573, 863 → 575, and 575 → 449. The A-type trimers were tentatively assigned due to their following characteristic fragments[16] monitored in the SRM mode: the ion m/z 575 or 573, derived from quinone methide cleavage of the interflavan bond indicated that these trimers had a connection sequence of (epi)cat-(epi)cat-A(epi)cat or (epi)cat-A(epi)cat-(epi)cat, respectively. The ion m/z 711 results from the retro-Diels–Alder (RDA) fission.

The pattern of A-type trimers varies in the three berries. In contrast to *V. macrocarpon* and *V. vitis-idaea*, in *V. oxycoccus* only two A-type trimer isomers could be detected. *V. vitis-idaea* bears the greatest variety and is closer to the pattern of *V. macrocarpon* than to that of *V. oxycoccus*. In all of the studied berries the A-type trimers 2 and 3 are present. Procyanidin A2 is present in all berries.

Table 2 shows that the numbers of isomers as well as their concentrations differ in the berries. The procyanidin A2 content of *V. macrocarpon* varieties ranges from 4.10 to 5.49 mg/100 g fresh berries. In *V. vitis-idaea* procyanidin A2 concentration is 2.11 ± 0.27 mg/100 g FW in the European and 7.98 ± 0.72 mg/100 g FW in the Chinese berries, respectively. *V. oxycoccus* clearly shows the lowest amount of procyanidin A2 with 0.13 −0.21 mg/100 g FW. Besides procyanidin A2 other A-type dimers were detected in the berries (see Table 3). In all berries *V. macrocarpon* and *V. vitis-idaea* (CN) procyanidin A2 was quantified as the main dimer. In *V. oxycoccus* both A-type dimers show nearly the same concentration. Only in *V. vitis-idaea* (EU) is the amount of A2 smaller than that of the A-type dimer 1. Overall, *V. vitis-idaea* has the highest A-type dimer concentration followed by *V. macrocarpon* and at last *V. oxycoccus*.

In total, the content of A-type trimers (m/z of 863) in both *V. oxycoccus* is 0.14 or 0.48 mg A2 equiv/100 g FW, whereas in *V. macrocarpon* the A-type trimer content ranges from 4.66 to 6.99 mg A2 equiv/100 g fresh berries, which is at minimum a

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Table 2. Concentrations of Procyanidin A2 (mg/100 g) and A-Type Procyanidin Dimers (m/z 575) and Trimers m/z 863 (mg A2 equiv/100 g) in Fresh Berry Samples

peak[b]	RT (min)	compd	mg (A2 equiv)/100 g fresh weight (FW)[a]								
			V. macrocarpon (mixed)	*V. macrocarpon* (Ben Lear)	*V. macrocarpon* (Grygleski)	*V. macrocarpon* (Stevens)	*V. macrocarpon* (Pilgrim, CA)	*V. macrocarpon* (Pilgrim, PL)	*V. oxycoccus* (EU)	*V. oxycoccus* (PL)	*V. vitis-idaea* (EU)	*V. vitis-idaea* (CN)
6	19.81	dimer 1	0.12 ± 0.02	0.10 ± 0.02	0.13 ± 0.02	0.13 ± 0.01	0.10 ± 0.03	0.20 ± 0.04	0.18 ± 0.02	0.09 ± 0.02	5.94 ± 0.79	1.77 ± 0.20
8	21.30	A2	4.57 ± 0.34	4.14 ± 0.37	4.66 ± 0.78	4.71 ± 0.12	4.10 ± 0.12	5.49 ± 0.61	0.21 ± 0.03	0.13 ± 0.02	2.11 ± 0.27	7.98 ± 0.72
sum of A-type dimers (m/z 575)			**4.69 ± 0.36**	**4.24 ± 0.39**	**4.79 ± 0.80**	**4.84 ± 0.13**	**4.20 ± 0.15**	**5.69 ± 0.65**	**0.39 ± 0.05**	**0.22 ± 0.04**	**8.05 ± 1.06**	**9.75 ± 0.92**
1	14.99	trimer 1	nd	nd	nd	nd	nd	nd	nd	nd	1.55 ± 0.22	0.29 ± 0.07
2	17.31	trimer 2	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.00	0.08 ± 0.01	0.11 ± 0.01	0.28 ± 0.05	0.09 ± 0.01	1.03 ± 0.16	0.32 ± 0.02
3	17.45	trimer 3	0.68 ± 0.06	0.62 ± 0.06	0.67 ± 0.08	0.68 ± 0.04	0.71 ± 0.10	1.05 ± 0.14	0.20 ± 0.01	0.05 ± 0.01	0.22 ± 0.03	0.98 ± 0.12
4	18.59	trimer 4	1.82 ± 0.16	2.35 ± 0.32	3.30 ± 0.41	2.83 ± 0.30	2.49 ± 0.34	2.96 ± 0.49	nd	nd	0.82 ± 0.14	3.18 ± 0.77
5	19.60	trimer 5	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	nd	nd	1.10 ± 0.18	0.27 ± 0.05
7	20.85	trimer 6	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.07 ± 0.02	nd	nd	3.40 ± 0.45	0.60 ± 0.11
9	21.80	trimer 7	1.03 ± 0.08	1.14 ± 0.15	1.35 ± 0.21	1.37 ± 0.10	1.17 ± 0.18	1.40 ± 0.20	nd	nd	0.29 ± 0.04	1.59 ± 0.33
10	23.10	trimer 8	0.98 ± 0.09	1.04 ± 0.11	1.43 ± 0.015	1.29 ± 0.09	1.09 ± 0.13	1.35 ± 0.22	nd	nd	1.60 ± 0.31	0.46 ± 0.08
sum of A-type trimers (m/z 863)			**4.66 ± 0.42**	**5.30 ± 0.67**	**6.93 ± 0.88**	**6.32 ± 0.55**	**5.62 ± 0.78**	**6.99 ± 1.09**	**0.48 ± 0.09**	**0.14 ± 0.02**	**10.01 ± 1.53**	**7.69 ± 1.55**

[a]Values are given as the mean ± standard deviations (n = 6). Procyanidin A2 calibration curve was used for quantification. nd, not detected. [b]Peak numbers of trimers refer to Figure 2.

15-fold higher concentration than in *V. oxycoccus*. European and Chinese *V. vitis-idaea* show the highest amounts with 10.01 ± 1.53 and 7.69 ± 1.55 mg A2 equiv/100 g FW, respectively. The concentration of the A-type trimers in sum and in the single amounts in *V. oxycoccus* is the lowest in comparison to those of the other berries. The amounts of single isomers and in sum differ between the selected *V. macrocarpon* varieties, but the relative amounts of the single trimers (see Figure 3) are quite the same. Similar results were already shown by Rzeppa et al.[18] for procyanidins in different apple cultivars.

There are many studies about the total procyanidin content or sum parameters of the berries,[11,19] but only a few about the concentration of single isomers. To our knowledge, a direct comparison of the three berries relating to the A-type trimers and procyanidin A2 has never been published. Rzeppa et al.[18] evaluated different procyanidins in selected fruits. In cranberries, concentrations of procyanidin A2 of 6.90 ± 0.25 mg/100 g and cinnamtannin B1 of 2.16 ± 0.05 mg/100 g fresh weight were determined. The procyanidin A2 concentration is in accordance with results of the *V. macrocarpon* (4.10−5.49 mg/100 g fresh berries) in our study. As an exact structure elucidation was not performed and no authentic procyanidin trimer standard was available, an assignment of the isomers to defined formulas cannot be presented here.

The effectiveness of A-type procyanidins to prevent urinary tract infection seems clear, but many questions remain.[4] The A-type procyanidins from *V. macrocarpon* are supposed to be a key parameter in bacterial antiadhesion activity,[7,20] but the molecular mechanism is not fully understood.[21] The higher molecular weight procyanidins show the greatest effect in vitro, but also dimers and trimers have antiadhension activity.[7] However, little is known about the absorption and metabolism of cranberry procyanidins. Ou et al.[20] demonstrated on Caco-2-human intestinal cells that A-type dimers, trimers, and tetramers were transported across the cells at low rates, suggesting that they could be absorbed intact by humans after cranberry consumption. The transport ratio decreased with increasing molecular weight. Further research is especially needed to elucidate the bioavailability and pharmacokinetics and thereby the influence of the molecular weight, position of the A-type bond, and absorption of cranberry procyanidins.[7]

A lack of the A-type procyanidin trimer (m/z 863) in *V. oxycoccus* seems to be a possible reason for the absence of a preventive effect against UTI, especially because also a difference by the higher molecular weight procyanidins is imaginable. There is no clear information about synergistic effects of the isomers, thus also the procyanidin pattern has to be considered. Furthermore, information about the dosage and duration of supplementation is not conclusively studied. A current recommended daily dosage of cranberry (*V. macrocarpon* Ait.) procyanidins for UTI prevention is based on the efficacious levels that have been administered in human intervention trails. Three hundred milliliters of cranberry juice cocktail (27% juice) containing 36 mg of cranberry procyanidins showed a reduction of bacteria pyuria[22] and a dosage dependence[23] in clinical trial. This dosage is also recommended in the health claim of the French Food Safety Authority (AFSSA) for the bacterial antiadhesion activity. The amount is determined by the 4-dimethylaminocinnamaldehyde (DMAC) assay (colorimetric method), which gives the total content of procyanidins in samples. Prior et al.[24] suggested an improved DMAC assay as a worldwide standard reference method for quantification of cranberry procyanidins but with

Table 3. Concentrations of Monomeric Flavan-3-ols (mg/100 g FW) and Different Procyanidins (mg A2 equiv/100 g FW) in Fresh Berry Samples

			mg (A2 equiv)/100 g fresh weight (FW)[a]									
peak[b]	RT (min)	compd	*V. macrocarpon* (mixed)	*V. macrocarpon* (Ben Liar)	*V. macrocarpon* (Grygleski)	*V. macrocarpon* (Stevens)	*V. macrocarpon* (Pilgrim, CA)	*V. macrocarpon* (Pilgrim, PL)	*V. oxycoccus* (EU)	*V. oxycoccus* (PL)	*V. vitis-idaea* (EU)	*V. vitis-idaea* (CN)
1	9.27	catechin	0.35 ± 0.05	0.33 ± 0.05	0.61 ± 0.08	0.37 ± 0.07	0.38 ± 0.06	0.54 ± 0.07	1.21 ± 0.33	0.33 ± 0.04	13.01 ± 2. 77	7.18 ± 1.70
4	11.99	epicatechin	2.45 ± 0.43	2.84 ± 0.66	4.44 ± 0.82	2.72 ± 0. 48	3.10 ± 0.76	4.46 ± 0.59	0.73 ± 0.21	0.22 ± 0.03	2.47 ± 0.77	10.68 ± 0.77
sum of monomers (*m/z* 289)			**2.80 ± 0.48**	**3.17 ± 0.71**	**5.05 ± 0.90**	**3.09 ± 0.55**	**4.48 ± 0.82**	**5.00 ± 0.66**	**1.94 ± 0.54**	**0.55 ± 0.07**	**15.48 ± 3.54**	**17.86 ± 2.47**
2	10.04	dimer B1	0.19 ± 0.02	0.28 ± 0.06	0.39 ± 0.06	0.27 ± 0.04	0.22 ± 0.06	0.39 ± 0.06	1.27 ± 0.22	0.29 ± 0.06	14.22 ± 2.51	6.66 ± 1.89
3	10.81	dimer B-type	nd	nd	nd	nd	nd	nd	0.13 ± 0.02	0.03 ± 0.01	8.02 ± 1.13	1.97 ± 0.53
5	13.01	dimer B2	2.12 ± 0.10	3.14 ± 0.55	3.81 ± 0.50	3.13 ± 0.41	3.10 ± 0.65	4.12 ± 0.02	0.68 ± 0.14	0.14 ± 0.02	1.47 ± 0.23	9.40 ± 2.68
8	15.01	dimer B-type	nd	nd	nd	nd	nd	nd	nd	nd	0.88 ± 0.10	0.36 ± 0.10
11	17.38	dimer B-type	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.26 ± 0.06	0.07 ± 0.02	2.55 ± 0.48	1.22 ± 0.31
15	20.50	dimer B5	0.36 ± 0.04	0.52 ± 0.08	0.57 ± 0.08	0.50 ± 0.06	0.47 ± 0.09	0.64 ± 0.12	0.11 ± 0.03	0.03 ± 0.01	0.36 ± 0.06	1.50 ± 0.46
sum of B-type dimers (*m/z* 577)			**2.67 ± 0.16**	**3.94 ± 0.69**	**4.77 ± 0.64**	**3.90 ± 0.51**	**3.71 ± 0.80**	**5.15 ± 0.20**	**2.45 ± 0.47**	**0.56 ± 0.12**	**27.50 ± 4.51**	**21.11 ± 5.97**
6	13.75	trimer B-type	<0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.09 ± 0.03	0.01 ± 0.00	1.07 ± 0.20	0.53 ± 0.16
7	14.92	trimer B-type	0.09 ± 0.02	0.16 ± 0.03	0.13 ± 0.02	0.15 ± 0.02	0.15 ± 0.04	0.19 ± 0.04	0.03 ± 0.01	<0.01	0.16 ± 0.04	0.39 ± 0.11
9	15.30	trimer B-type	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.04 ± 0.02	0.01 ± 0.00	0.41 ± 0.06	0.20 ± 0.07
10	16.86	trimer C1	0.19 ± 0.04	0.31 ± 0.04	0.33 ± 0.05	0.30 ± 0.04	0.30 ± 0.08	0.40 ± 0.08	0.07 ± 0.02	0.01 ± 0.00	0.22 ± 0.04	0.95 ± 0.27
12	17.66	trimer B-type	nd	nd	nd	nd	nd	nd	0.03 ± 0.01	<0.01	0.38 ± 0.06	0.17 ± 0.04
13	18.55	trimer B-type	0.13 ± 0.01	0.19 ± 0.03	0.25 ± 0.03	0.20 ± 0.03	0.18 ± 0.04	0.21 ± 0.04	nd	nd	0.20 ± 0.04	0.24 ± 0.06
14	18.99	trimer B-type	0.04 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.08 ± 0.01	0.01 ± 0.00	<0.01	0.06 ± 0.01	0.17 ± 0.06
16	20.79	trimer B-type	nd	nd	nd	nd	nd	nd	nd	nd	0.29 ± 0.04	nd
17	21.65	trimer B-type	0.07 ± 0.01	0.09 ± 0.02	0.10 ± 0.02	0.10 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	nd	nd	nd	0.15 ± 0.03
18	23.15	trimer B-type	0.09 ± 0.01	0.10 ± 0.01	0.12 ± 0.02	0.09 ± 0.01	0.10 ± 0.02	0.11 ± 0.02	nd	nd	0.03 ± 0.02	0.13 ± 0.07
sum of B-Type trimers (*m/z* 865)			**0.61 ± 0.10**	**0.93 ± 0.15**	**1.01 ± 0.16**	**0.92 ± 0.13**	**0.88 ± 0.22**	**1.12 ± 0.22**	**0.27 ± 0.09**	**0.04 ± 0.00**	**2.28 ± 0.51**	**2.93 ± 0.87**

[a]The data represent mean values of the samples ± standard deviations ($n = 6$). Procyanidin A2 calibration curve was used for quantification of dimers and trimers; (epi)catechin calibration curve was used for quantification of monomers. nd, not detected. [b]Peak numbers refer to Figure 4.

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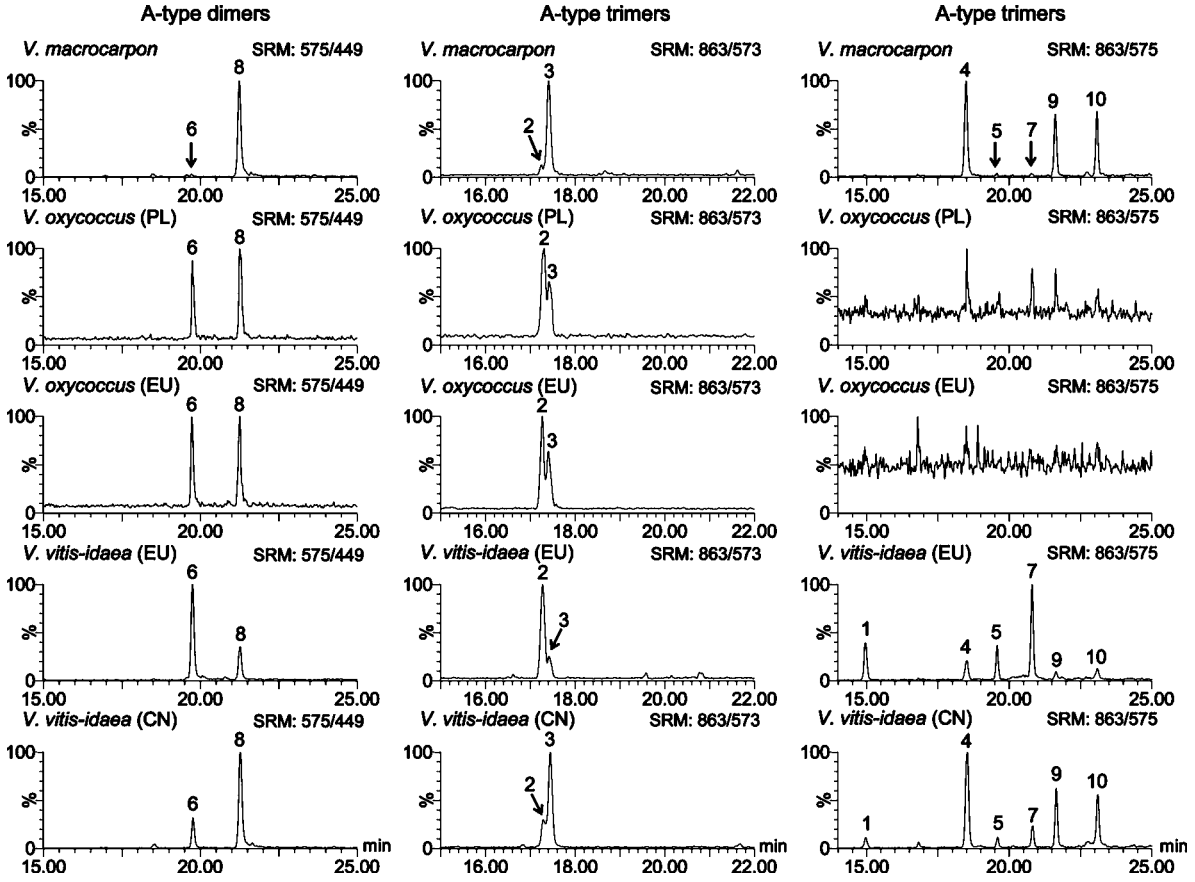

Figure 2. SRM chromatograms at m/z 863 → 573, 863 → 575, and 575 → 449, acquired from the acetone extract of the different *Vaccinium* berries. Peak numbers refer to Table 2.

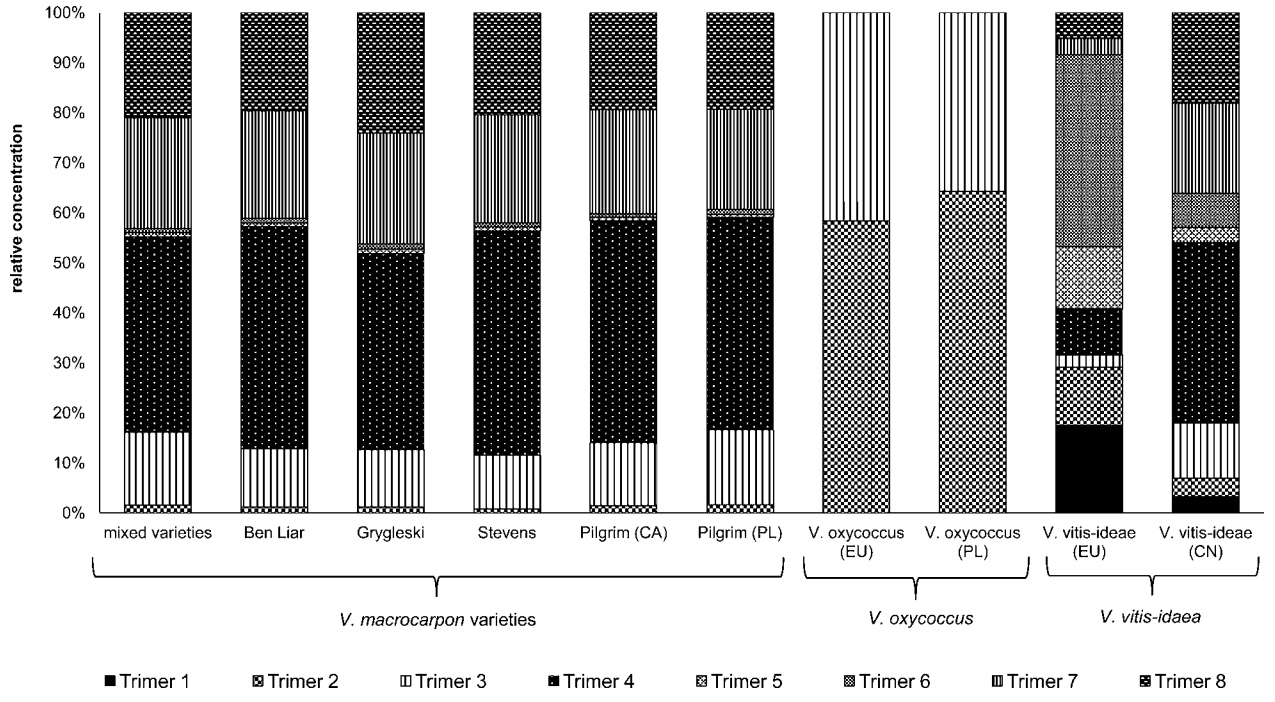

Figure 3. Relative concentrations of procyanidin A-type trimers in different varieties of *V. macrocarpon*, *V. oxycoccus*, and *V. vitis-idaea*.

limitation to authentic cranberry (*V. macrocarpon*) products. This limitation is founded in the disadvantage of the assay that the presence of the A-type procyanidins is not guaranteed. Sánchez-Patán et al.[25] showed in a study that procyanidin profiles of commercial cranberry products are very variable, especially by the A-type procyanidins, which is not detectable

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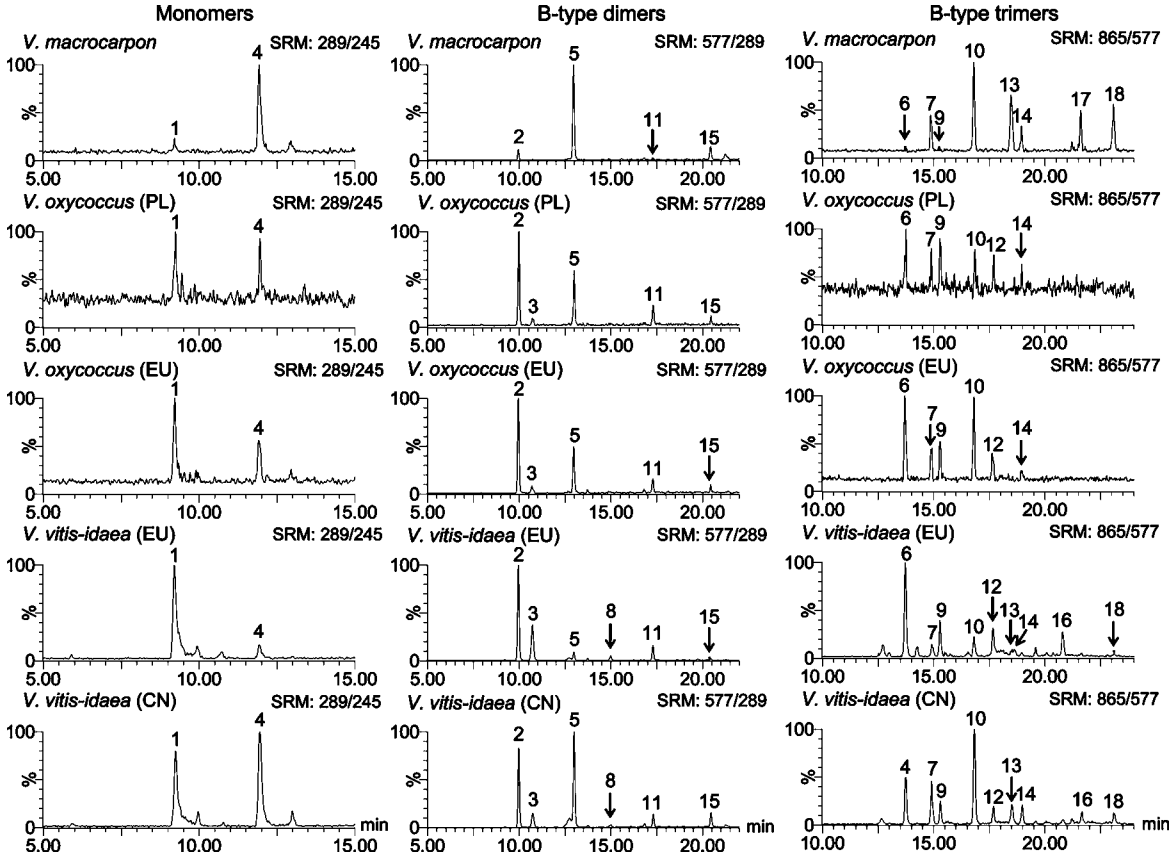

Figure 4. SRM chromatograms of monomers *m/z* 289, dimers *m/z* 577, and trimers *m/z* 865 acquired from the acetone extract of the different *Vaccinium* berries. Peak numbers refer to Table 3.

by DMAC assay and gives doubts about the authenticity of the products. In light of the data generated in this study, not only an adulteration with food containing flavan-3-ol and B-type procyanidins should be considered but also the determination of the variety of the berry is an essential requirement. Conclusively, dosage recommendation and the DMAC assay as a standard method are suitable only for authentic *V. macrocarpon* products, due to the different patterns and concentrations of A-type trimers and the dimer A2. Research is needed to illuminate the difference in higher A-type procyanidins and other flavonoids and if all *Vaccinium* berries have the same antiadhesion effect or if it depends on the dosage of A-type procyanidin.

Profile and Quantification of Flavan-3-ols and Other Procyanidins. Using UHPLC-MS^2, monomeric flavan-3-ols and different B-type procyanidins in addition to the A-type dimers and trimers were detected and quantified using external standards. The single isomers of the procyanidins were quantified up to trimers as procyanidin A2 equivalents and the monomeric flavan-3-ols by catechin and epicatechin calibration curves. Figure 4 presents the SRM chromatograms of different monomeric flavan-3-ols and B-type procyanidins. In Table 3 the concentrations of these monomers, dimers, and trimers are reported. Procyanidin contents can be influenced by different environmental factors, so variations in the contents are expected.[19,26] However, comparing the concentrations revealed clear differences between the species.

The flavan-3-ols catechin and epicatechin were identified in all of the berry samples. *V. vitis-idaea* (EU and CN), with 15.48 ± 3.54 and 17.68 ± 2.47 mg/100 g FW, respectively, bear the highest amounts of monomers followed by *V. macrocarpon* with 2.80−5.05 mg/100 g FW and *V. oxycoccus* with 0.55−1.94 mg/100 g FW. The ratios of catechin and epicatechin in the species are different. *V. macrocarpon* (all varieties) show higher amounts of epicatechin (2.45 ± 0.43 − 4.46 ± 0.59 mg/100 g FW) than catechin (0.33 ± 0.00 − 0.61 ± 0.04 mg/100 g FW), in contrast to *V. oxycoccus*, which shows nearly the ratio 1:1 of the two monomers. The ratios of the monomers in lingonberries are not uniform: Whereas the Chinese lingonberry shows 10.68 ± 0.77 mg/100 g FW higher amounts of epicatechin than catechin, the ratio in the European lingonberry is reversed. Määttä-Riihinen et al.[27] described that different ratios of the flavan-3-ols might depend on the origin of the berries. This is in accordance with our findings by comparison of *V. macrocarpon* to *V. oxycoccus*, but varieties of the species show in each case the same ratio of the monomers independent of the origin (Poland or Canada). Because in several studies the species of cranberry is not mentioned, a comparison is difficult and the differences reported there might be due to the different species.

B-type dimers with *m/z* 577 were detected in all berries by the typical fragments at *m/z* 407 and 425. In *V. macrocarpon* procyanidin B2 is the main dimer in all of the different varieties with contents from 2.12 ± 0.10 to 4.12 ± 0.50 mg A2 equiv/100 g FW. Rzeppa et al.[18] quantified different dimers in cranberries, with 2.945 ± 0.132 mg/100 g procyanidin B2 as the main B-type dimer, which is in accordance with our studies. By comparison, the dimers of *V. vitis-idaea* (EU) and *V. macrocarpon*, which have reversed ratios of monomers, variations in the isomers are remarkable. *V. vitis-idaea* (EU),

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Table 4. Overview of Differences between the Analyzed *Vaccinium* Species that Allow Proof of Authenticity

common names	*Vaccinium* species	variety	ratio[a] epicatechin/catechin	no. of A-type trimers	ratio[a] dimer A2/A-type dimer 1	ratio[a] procyanidin B1/B2
American cranberry, large cranberry, Großfrüchtige Moosbeere, Kranbeere	*Vaccinium macrocarpon*	mixed	7.00	7	38.08	0.09
		Ben Liar	7.35	7	41.40	0.10
		Grygleski	8.61	7	35.85	0.10
		Stevens	7.28	7	36.23	0.09
		Pilgrim	8.16	7	41.00	0.07
		Pilgrim (PL)	8.26	7	27.45	0.09
European cranberry, small cranberry, (kleinfrüchtige) Moosbeere	*Vaccinium oxycoccus*	wild (EU)	0.60	2	1.17	1.87
		wild (PL)	0.67	2	1.44	2.07
lingonberry, cowberry, mountain cranberry, Mos cranberry, Moosbeere, Preiselbeere	*Vaccinium vitis-idaea*	wild (EU)	0.19	8	0.35	9.67
		wild (CN)	1.49	8	4.51	0.71

[a]Ratios are calculated on the basis of the concentrations in Table 3.

for example, has 10 times higher contents of procyanidin B1 than B2. In *V. macrocarpon* it is the opposite. In *V. oxycoccus* procyanidin B1 is the main dimer with a concentration of 0.29 ± 0.06 or 1.27 ± 0.22 mg A2 equiv/100 g FW, which is about 2 times higher than procyanidin B2 (0.14 ± 0.02 or 0.68 ± 0.14 mg A2 equiv/100 g FW). Procyanidin B5 was detected in all berries, but in *V. oxycoccus* and *V. vitis-idaea* (EU) only in traces. In all, the different *Vaccinium* species bear different amounts of isomers and the total contents vary. *V. viti- idaea* (EU and CN) shows the highest B-type dimer concentration with 27.50 ± 4.51 and 21.11 ± 5.957 mg A2 equiv/100 g FW, respectively.

All berries contain B-type trimers (m/z 865). The peaks showed in the SRM the characteristic fragments at m/z 575 and 695. In both cranberries and lingonberries different isomers were detected, of which only some contribute very little to the total procyanidin content. All varieties of *V. macrocarpon* and *V. oxycoccus* berries contain higher amounts of A-type than B-type trimers and dimers. This is in accordance with previous studies, which showed that these berries contain a high percentage of A-type procyanidins.[19]

There are a number of studies about the procyanidin contents of different foods.[19,28] As mentioned by Hellström et al.,[19] the content is highly connected to environmental influences and chosen varieties, and in numerous studies only sum parameters are evaluated. Studies about concentration of single isomers are rare. Consequently, a comparison of absolute concentrations is difficult. Nevertheless, in our study remarkable differences in composition and concentration among the three species are shown, taking into account different origins (Poland, Canada, Europe, China). Conclusively, comparing the three *Vaccinium* species, *V. vitis-idaea* shows the highest amount of monomers, dimers, and trimers followed by *V. macrocarpon* and at least *V. oxycoccus*. Not only the amounts differ among the species, but also the number and the ratio of the single isomers, while the varieties of the same species are similar. This study shows marked differences in the procyanidin profile of this *Vaccinium* berries. Especially, *V. oxycoccus* (European cranberry) and *V. macrocarpon* (American cranberry) are not as similar as they seem to be.

Proof of Authenticity. Premium fruits demand high prices; hence, producers of fruit products search for cheaper ingredients for admixture or substitution. To protect the consumer from various admixtures or inaccurate declaration of fruit products as well as to avoid unfair competition, it is essential to check the composition and authenticity of a food.[29] Polyphenol analysis is a suitable way to prove the authenticity of foods of plant origin. Due to the genetic fixation of the biosynthesis of the polyphenols in plants, the qualitative pattern of the flavonoids of each species is identical. Therefore, changes in the flavonoid pattern can give an indication of adulteration. This has been shown for different issues.[26]

The three *Vaccinium* species focused on in this study are botanically of the same family and have many qualitative and sensory similarities. Because of the resemblance and the similar common names, the berries are often confused and likely to be mixed or even used instead of each other. Considering the procyanidin pattern, an identification of the berries is possible; especially in the case of a wrong declaration, an opportunity to prove the authenticity is given (see Table 4). To use the DMAC assay for the dosage determination of cranberry products, the *Vaccinium* species needs to be known to take the different A-type procyanidin proportion into account.

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Notes

The authors declare no competing financial interest.

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