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Botanical Adulterants Prevention Program
American Botanical Council • the American Herbal Pharmacopoeia • the University of Mississippi's National Center for Natural Products Research

Cranberry Products Laboratory Guidance Document

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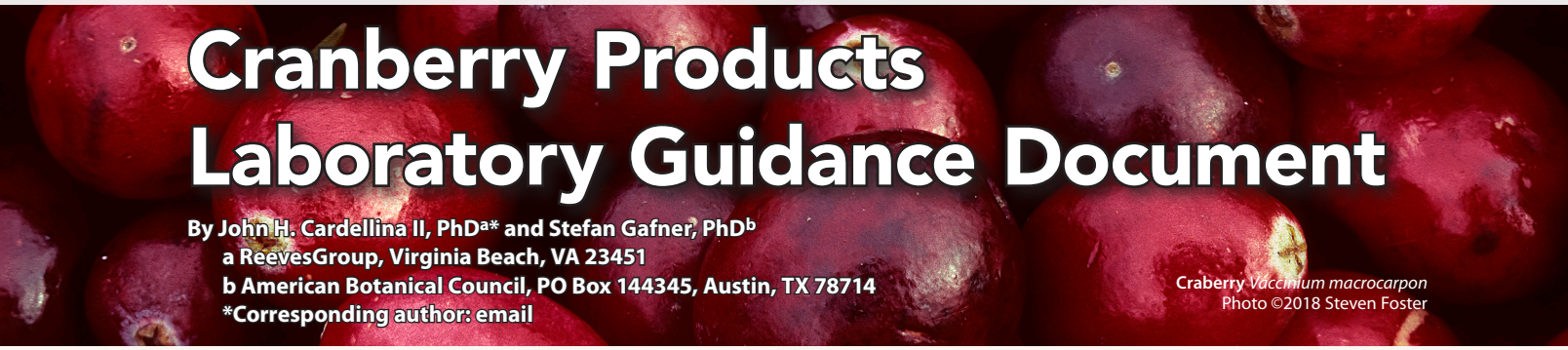
Craberry *Vaccinium macrocarpon*
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Purpose

Cranberry remains one of the most popular of the 'healthy' fruits, with an array of extract products appearing in the botanical dietary supplement markets and a large number of juice products in the beverage industry. There is considerable evidence that both, but especially the extract-based, product categories have been subjected to adulteration.[1] This Laboratory Guidance Document is intended to review the analytical technologies used to determine whether cranberry extract products are authentic and, if not, to identify the adulterants involved. This document should be viewed in conjunction with the corresponding Botanical Adulterants Bulletin on Cranberry published by the ABC-AHP-NCNPR Botanical Adulterants Prevention Program.[1]

Scope

The continued demand for cranberry based supplements and beverages in the marketplace and the rising costs of cranberry raw material have seemingly served as an incentive for economically-motivated adulteration with synthetic colorants and/or anthocyanin- or proanthocyanidin (PAC)-rich extracts or PAC-rich materials (e.g., powders) from other, less expensive botanical sources. While admixture or substitution with synthetic colorants or anthocyanin-containing extracts can be detected rather readily, the inclusion of PACs from, for example, grape seed, peanut skin, or pine species in products purported to be cranberry extract is more difficult to detect and may require more advanced instrumentation, and/or a combination of analytical methods.

The evaluation of a specific analytical method or methods in this Laboratory Guidance Document for testing cranberry materials does not reduce or remove the responsibility of laboratory personnel to demonstrate adequate method performance in their own laboratory using accepted protocols outlined in various domestic (in the United States) or international legal and/or regulatory documents. Such documents include, for example, the 21 CFR Part 111 (Dietary Supplement GMPs, in the U.S. Code of Federal Regulations) and Part 117 (FSMA Final Rulemaking for Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls for Human Food, in the U.S. Code of Federal Regulations), and by AOAC International, International Standards Organization (ISO), World Health Organization (WHO), and the International Council on Harmonisation (ICH).

3. Common and Scientific Names

3.1 Common name: cranberry

3.2 Other common names:

English: American cranberry, large cranberry, North American cranberry[2-5]
Chinese: *da guo yue jie* (大果越桔)[6]
French: canneberge, canneberge d'Amérique, canneberge à gros fruits, atoca, atoka, ronce d'Amérique[2,3]
German: Kranbeere, grosse Moosbeere[2-4]
Italian: ossicocco americano, mirtillo rosso canadese, mortelle di palude, cranberry[7]
Spanish: arándano, arándano americano, arándano trepador, arándano rojo[2-4]
Wampanoag: ibimi, sasumuneash[8]

3.3 Accepted Latin binomial name: *Vaccinium macrocarpon* Aiton

Note: Cranberry products on the dietary supplement, food and beverage markets are predominantly made from

V. macrocarpon. However, the American Herbal Products Association's (AHPA) *Herbs of Commerce, 2nd Edition,* which provides guidance on dietary supplement labeling in the United States, also permits products derived from *V. oxycoccos* to be labeled as cranberry.[9]

3.4 Synonyms: *Oxycoca macrocarpa* (Aiton) Raf., *Oxycoccus macrocarpus* (Aiton) Pers., *Oxycoccus palustris* var. *macrocarpos* (Aiton) Pers., *Schollera macrocarpa* (Aiton) Steud., *Schollera macrocarpos* (Aiton) Britton

3.5 Botanical family: Ericaceae

4. Botanical Description

Vaccinium macrocarpon, which is indigenous to North America, is a fruit bearing, trailing or ascending rhizomatous evergreen shrub that grows 5-20 cm in height. Cranberry plants in the wild are generally associated with bogs, swamps and other low-lying wetland areas; the species has adapted to low nutrient, generally sandy soils.[8] The fruit (berry) is the only component of current interest or importance in trade, although there are some references to Native American use of the stem and leaves for medicinal purposes.[8]

5. Identification and Distinction of Fruit Using Macroanatomical Characteristics

Fresh berries are globose to ellipsoidal; 9 to 20 mm in diameter; red, crimson, burgundy to almost black; glabrous, with a smooth lustrous surface. A more detailed physical description is available in the American Herbal Pharmacopoeia (AHP) monograph on cranberry.[8] The morphological features may allow one to distinguish the fruit of *V. macrocarpon* from fruits of *V. oxycoccos* and *V. vitis-idaea* (the latter two species have berries of smaller size [*V. oxycoccos*: 9-14 mm; *V. vitis-idaea*: 6-12 mm; and *V. macrocarpon*: 9-16 mm] and globose compared to the slightly elongated *V. macrocarpon* berry),[10] but for cranberry powders and extracts, where adulteration issues are most prominent, macroscopic identification is not feasible.

6. Identification and Distinction of Fruit Using Microanatomical Characteristics

The exocarp is comprised of anthocyanin-colored polygonal cells covered by a thick cuticle. Groups of cells are separated by fairly thick, colorless walls, while the walls within the respective groups are rather thin. The mesocarp consists of large, spherical, thin-walled cells in which small bundles of spirally thickened vessels are embedded. As noted above in Section 5, a more detailed description, including figures displaying key anatomical features, is available in the AHP monograph on cranberry, and from other sources.[8,11,12] Microscopic distinction of *V. macrocarpon*, *V. oxycoccos*, and *V. vitis-idaea* may not be feasible, although no papers investigating the topic could be retrieved. Botanical microscopy is not capable of detecting adulteration with extracts from other plant sources.

7. Genetic Identification and Distinction

While there have been a number of genetic studies of *V. macrocarpon* using simple sequence repeats (SSR) in recent years, they have all been focused on identifying genetic characteristics related to fruit quality and breeding programs.[13-17] Researchers in Lithuania and Poland used both SSR and RAPD (random amplified polymorphic DNA) to compare two wild populations of *V. oxycoccos* growing in different nature preserves in Lithuania.[18] The authors reported 71% variation between the two populations, based on RAPD analyses, compared to 97% variation in the SSR comparison. Unfortunately, no genetic comparison of different *Vaccinium* species were conducted in these or other such studies in the literature.

A preliminary report on an assay analyzing DNA by PCR amplification of the *matK* gene was recently presented by Herbst and co-workers. The authors reported successfully discriminating *V. macrocarpon* DNA from DNA of grape (*Vitis vinifera*, Vitaceae), apple (*Malus domestica*, Rosaceae) and pear (*Pyrus* spp., Rosaceae); unfortunately, the primers developed thus far were unable to distinguish cranberry from blueberry (*V. corymbosum*).[19] Further work by this group may lead to a genetic means of distinguishing various *Vaccinium* spp. in commerce.

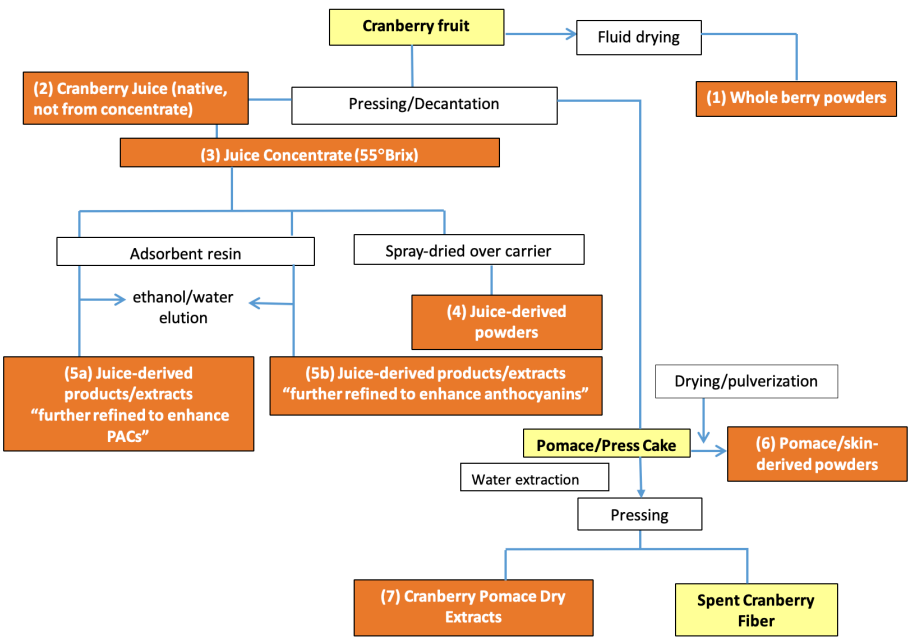


Figure 1. Flow diagram of cranberry fruit processing, illustrating various ingredients and products of cranberry (modified by the author and used with permission of USP).

8. Cranberry Products Description

Cranberry products may be comprised of powdered whole berries, juice, juice concentrate, juice powder, powdered pomace (residue after juice pressing), dried pomace extracts, and processed juice fractions. A flow diagram of the various processing steps for cranberries is provided in Figure 1.

This diversity of product materials makes selection and validation of an analytical method an important consideration for any product manufacturer in the food or supplement arenas.

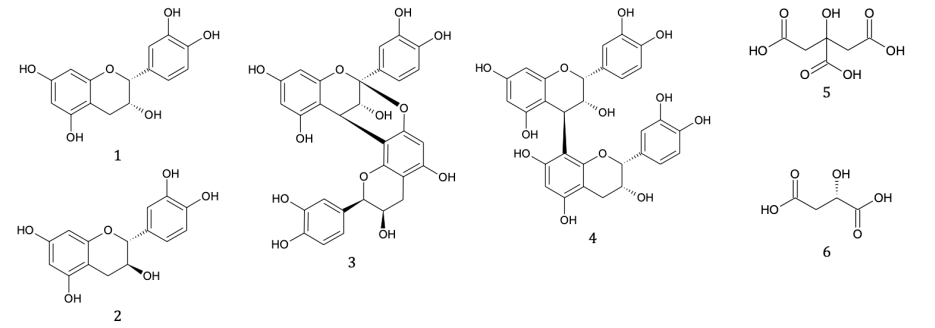
9. Chemical Identification and Distinction

9.1 Chemistry of *V. macrocarpon* fruit

A good summary of the chemistry of cranberry is provided in the AHP monograph.[8] The chemistry is dominated by phenols and polyphenolics, notably anthocyanins and procyanidins‡. The procyanidins are oligomers† and polymers of catechins, each connected to another by either two bonds (A type) or one bond (B type); the A type procyanidins have been identified as the putative bacterial anti-adhesion compounds in cranberry, while the B type PACs have been shown to be inactive as anti-adhesion agents.[20] There are four known catechin (flavan-3-ol) building blocks in cranberry, and oligomers of three or more catechin units are considered the pharmacologically active procyanidins; the challenge of rigorously identifying the complete structure and absolute configuration of a PAC is directly proportional to the degree of polymerization (DP), i.e., the number of catechin units present, as the number of possible isomers increases with increasing DP. The anthocyanins provide cranberry with its red color; there are six major anthocyanins in cranberry, which are glycosides of two anthocyanidin aglycones (cyanidin and peonidin) and three sugars (galactose, arabinose and glucose, listed here in order of abundance in cranberry anthocyanins).

Also abundant in cranberry are flavonols. While more than 20 flavonol glycosides have been identified in cranberry, the primary flavonol glycosides are galactosides, arabinosides, and rhamnosides of quercetin, myricetin, and kaempferol. Certain processing operations can release the flavonol aglycones and free sugars in the final product or ingredient, e.g., via hydrolysis. Another important group of compounds, from the standpoint of identification and adulteration, is the organic acids, mainly quinic, citric, and malic acids. Particularly noteworthy is the high relative level of quinic acid in cranberries; analysts can make use of the ratios of quinic to the other acids to glean insight into potential adulteration of cranberry juice or dietary ingredients derived from juice. Triterpenes are also found in cranberry; ursolic acid is the most abundant of these, although a number of structurally related pentacyclic triterpenes are also present in the fruit and leaves. Figure 2 illustrates the most important chemical classes found in cranberry.

Figure 2. Representatives of the main classes of secondary metabolites in cranberry

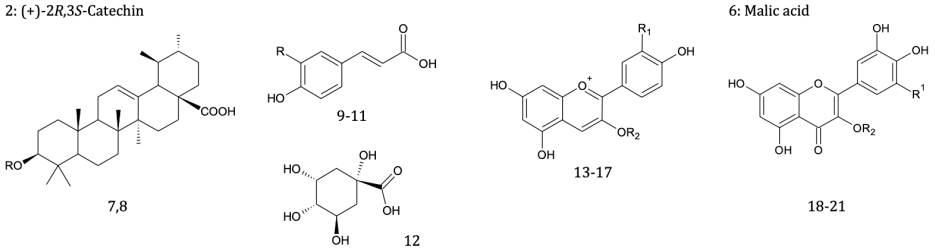


1: (-)-2*R*,3*R*-Epicatechin
2: (+)-2*R*,3*S*-Catechin

3: Procyanidin A2

4: Procyanidin B2

5: Citric acid
6: Malic acid



7: R = H: Ursolic acid
8: R = Coumaroyl: 3-*O*-*p*-coumaroylursolic acid

9: R = H: *p*-Coumaric acid
10: R = OH: Caffeic acid
11: R = OCH_3: Ferulic acid
12: Quinic acid

13: R_1 = H, R_2 = galactose: Cyanidin-3-*O*-glactoside
14: R_1 = H, R_2 = glucose: Cyanidin-3-*O*-glucoside
15: R_1 = H, R_2 = arabinose: Cyanidin-3-*O*-arabinoside
16: R_1 = OCH_3, R_2 = galactose: Peonidin-3-*O*-glactoside
17: R_1 = OCH_3, R_2 = arabinose: Peonidin-3-*O*-arabinoside

18: R_1 = H, R_2 = galactose: Hyperoside
19: R_1 = H, R_2 = arabinose: Avicularin
20: R_1 = OH, R_2 = galactose: Myricetin-3-*O*-galactoside
21: R_1 = OH, R_2 = arabinose: Myricetin-3-*O*-arabinoside

‡ The terms proanthocyanidin and procyanidin seem to be used interchangeably in the literature. However, proanthocyanidin is a generic term for a family of structurally related polyphenolic compounds comprised of the procyanidins, prodelphinidins, propelargonidins, etc. The different proanthocyanidin classes are distinguished by the specific flavan-3-ol hydroxylation pattern, e.g., 3,3′,4′,5,7-pentahydroxyflavan-3-ol in case of the procyanidins, or 3,4′,5,7-tetrahydroxyflavan-3-ol for the propelargonidins. The name "proanthocyanidin" is derived from the fact that these compounds produce anthocyanidins when treated with a mineral acid. Specifically, a procyanidin will produce the anthocyanidin cyanidin, a prodelphinidin will yield the anthocyanidin delphinidin, a propelargonidin will be converted into pelargonidin, etc.

† According to the International Union of Pure and Applied Chemistry (IUPAC), the term "oligomer" is defined as a substance composed of a few molecules repetitively linked to each other. The addition of another unit leads to a notable change in the physical properties of the molecule. While there is no universally accepted number of flavan-3-ol units that make up an oligomeric PAC, for the purpose of this document, the term "oligomer" describes PACs having 3-10 units.

9.2 Chemistry of potential cranberry adulterants

While anthocyanins from grape (*Vitis vinifera*, Vitaceae) seed and skin extracts were detected in cranberry juice over 30 years ago, more accurate labeling of juice products to acknowledge admixture of other fruit juices has reduced the problem of adulteration of juices. However, there remains the possibility that other fruit juices can be masqueraded as cranberry juice by the addition of anthocyanins and, perhaps, quinic acid, from exogenous sources.

Adulteration of dried cranberry concentrates and powdered extracts is considered more common, driven by increasing consumer demand for and rising prices of cranberries, and abetted by a dearth of suitable, broadly applicable analytical methods and lack of reference compounds. Potential cranberry adulterants will likely mimic either the anthocyanin fraction or the PAC fraction, the focus of most marketing efforts. It thus follows that reported adulterants include grape seed and skin extracts, red peanut (*Arachis hypogaea*, Fabaceae) skin extracts, plum (*Prunus domestica*, Rosaceae) extracts, and, to a lesser extent, extracts of maritime pine (*Pinus pinaster*, Pinaceae) and Masson pine (*P. massoniana*) bark, black bean (*Phaseolus vulgaris*, Fabaceae) skins, black rice (*Oryza sativa*, Poaceae), mulberries (*Morus* spp., Moraceae), and other parts of cranberry plants.[8]

***Vitis vinifera*:** Grape seed extract (GSE) is almost exclusively supplied to dietary supplement manufacturers in the form of a dry extract. The extract contains polyphenolic compound concentrations in a range of 50-90% of the extract. The main phenolic compounds are flavan-3-ol monomers and polymers and their gallic acid esters. Grape seeds contain predominantly B-type PACs, which are flavan-3-ol polymers where the units are linked by a single bond. Appeldoorn et al.[21] isolated procyanidin B1, B2, B3, and B4 from a commercial GSE, accounting for 3.2%, 7.1%, 1.5%, and 1.2% of the extract. Similar results were reported by Weseler and Bast,[22] with concentrations of 7.7%, 8.3%, 2.8%, and 1.6% of procyanidins B1, B2, B3, and B4, respectively. The presence of B-type dimers, trimers, tetramers and polymers of up to the size of a dodecamer trigallate was described by Weber et al.,[23] who analyzed four commercial GSEs by HPLC-APCI/MS, and MALDI-TOF/MS and found that the molecular weight distribution varied substantially depending on the product. Average degrees of polymerization (DP) for commercial GSE were reportedly between 3-11,[24,25] although the DP may deviate substantially from these values, depending on processing.

***Arachis hypogaea*:** Peanut skin extracts contain both A-type and B-type PACs.[26,27] Appledoorn isolated a number of PACs from peanut skin, with A-type dimers procyanidin A1 and A2 as most abundant (6.9% and 2.1%, respectively). Procyanidin B7 was present at 0.2%.[21] Dudek et al.[28] confirmed the presence of procyanidins A1 and A2, and isolated four trimers and two tetramers, named peanut procyanidins A-F. Besides procyanidin A1, peanut procyanidin E was the most abundant in a 70% aqueous acetone extract of the skins. Other phenolic compounds in peanut skin include flavonols (quercetin, kaempferol, isorhamnetin, and their glycosides), the isoflavone genistein, the flavanone hesperetin, anthocyanins (cyanidin, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-sophoroside, peonidin-3-*O*-galactoside, and petunidin-3-*O*-galactoside), and the stilbene resveratrol.[29]

Pinus spp.: Weber et al.[23] also (see ***Vitis vinifera***, above) investigated the PAC type and size in extracts from *P. pinaster* and *P. massoniana*. From an economic perspective, Masson pine extracts are 5-10 fold less expensive than Maritime pine bark extracts, making Masson pine more attractive as an economic adulterant (Yannick Piriou [les Dérivés Résiniques et Terpéniques] email to Maria J. Monagas [USP], May 3, 2018). Contrary to Maritime pine, Masson

Table 1: Proanthocyanidin characteristics of low-cost, non-cranberry botanical materials containing condensed tannins

Ingredient	Monomer(s)	Galloylation	PAC-type	Average degree of polymerization[a,b]
grape seed	catechin, epicatechin	Yes	B-type	2-12[24,25,35]
almond skin	afzelechin, catechin, gallocatechin, epiafzelechin, epicatechin, epigallocatechin	No	A-type, B-type	no data
apple	catechin, epicatechin	No	mainly B-type	3-10[36,37]
green tea	catechin, epicatechin, epiafzelechin, epigallocatechin, gallocatechin	Yes	B-type	1-1.1[38]
maritime pine	catechin, epicatechin, epigallocatechin, gallocatechin	No	B-type	3-7[32]
Masson pine	catechin, epicatechin, epigallocatechin, gallocatechin	Yes	mainly B-type	no data
peanut skin	catechin, epicatechin	No	A-type, B-type	1-9[39]

[a] Measured by thiolysis

[b] Degree of polymerization determined depends on the processing method; for grape seed, degrees of polymerization between 1 and 37 have been reported on isolated fractions[40-42]

pine contains some galloylated PACs.[23] The monomer units consist mainly of catechin and epicatechin, although small amounts of epigallocatechin and gallocatechin have also been reported.[30,31] Typically, pine bark extracts contain only B-type PACs. The average degree of polymerization of a hot water extract of *P. pinaster* is between 6 and 7.[30,32] Similar results were reported for Scots pine (*Pinus sylvestris*) by Bianchi et al.[33] The PAC fraction of a hot water extract consisted of exclusively of B-type procyanidins with average degree of polymerization of 6.7. A comparison of HPLC-UV fingerprints between grape seed and Masson pine extract did not show a substantial difference, except that the Masson pine extract had a larger concentration of more highly polymerized PACs and exhibited the peak of an A-type dimer.[34] Table 1 lists the key characteristics of the PAC constituents of the adulterant botanicals described above.

9.3 Laboratory methods

There are various reports in the literature on analytical methods to identify cranberry, assess its quality, and/or disclose evidence of adulteration. Analytical methods for the analysis of main cranberry polyphenols (monomeric flavan-3-ols and flavan-3-ol glycosides, anthocyanins, and PACs), sugars, and organic acids have been developed. Not all the reported methods are suitable for all these purposes or all forms of cranberry ingredients in the marketplace. The selection of anti-adulteration analytical methods is largely dependent on the composition of each ingredient or finished product, which at the same time defines testing requirements for quality assurance purposes. Cranberry juice or juice concentrate could be assessed following official juice standards—European Juice Association (AIJN)-Code of Practice. Reference Guideline for Cranberry Juice;[43] USDA Commodity Specification Bottled Juices – Cranberry Juice Concentrate 3+1 (commercial name: Cranberry Juice Cocktail) and Cranberry Juice Concentrate 55-gallon drum (commercial name: Cranberry Juice Concentrate 50 Brix);[44] USP-NF Cranberry Liquid Preparation; Codex General Standard for Fruit Juices and Nectars (CODEX STAN 247-2005)[45]—by considering the ratio of organic acids and sugars, as well as the anthocyanin profile. Some of these tests could be also applied to cranberry spray-dried juice powders and whole berry powders, as the original fruit identity is still present in this type of ingredient. However, when juices are further processed and purified into cranberry extracts (for example, by industrial resin adsorption) the original juice identity (chemical profile) is altered and other tests are required to properly characterize the ingredient. This also the case of ingredients derived from aqueous extraction from the pomace remaining after juice extraction (cranberry pomace extracts) and the skin-derived powders. In these latter cases, the proper characterization of the PAC fraction becomes critical to the detection of adulteration.

Table 2 provides a selection of representative analytical methods used to analyze commercial cranberry products that seem most adaptable for investigations of adulteration and considers the key advantages and disadvantages of each.

The challenge with juices is determining what adulterant juices or additives (e.g., sugars, organic acids, pigments) are present. A variety of options is available to researchers/analytical groups, but the most useful of these appear to be analysis of sugar content (notably glucose and fructose) and organic acid content (quinic, malic, and citric). Hong and Wrolstad used HPLC-RI and HPLC-UV to identify anomalies in the sugar and acid constitution of purported

Table 2. Comparison of different analytical approaches to determine adulterants in cranberry products

Reference	Sample Set	Method	Analyte(s)#	Pro	Contra
Hong (1986a, 1986b)[46,47]	1986a: 8 samples of whole berries 1986b: 31 juice samples, 1 juice concentrate	HPLC-RI HPLC-UV HPLC-UV/Vis	sugars organic acids anthocyanidins	equipment not costly and is available in most labs; standards available for acids, sugars	anthocyanidins not well resolved; there are now far better approaches for the pigments
Prior (2010)[48]	11 commercial juices or powdered cranberries (5 US, 6 European)	colorimetric (DMAC)[a]	PACs	simple, inexpensive; validated across 5 labs; fast, reproducible when 96 well plate readers are used	useful within a given food type or preparation; sample prep will vary with product type; color yield varies w/structure; 96 well plate readers not available in all analytical labs
Krueger (2016)[49]	juice, juice blends, powders, extracts	colorimetric (DMAC)[a]	PACs (c-PAC[b] vs ProA2[c] as standard)	simple, inexpensive; ProA2[c] reference standard available	c-PAC[b] has to be prepared or established as certified reference standard, but is superior to ProA2[c]
Sintara (2017)[50]	cranberry extract, capsules, juice conc.	colorimetric (DMAC)[a]	PACs	simple, fast, more reproducible, inexpensive; ProA2[c] reference standard available	only single laboratory validation thus far
Boudesocque (2013)[51] Boudesocque-Delaye (2018)[52]	1 fresh cranberry sample;[23] 1 juice sample and 10 commercial products[24]	HPTLC-densitometry	catechin[48] or epicatechin;[49] ProA2[c] and ProB2[c]	3 key reference standards available; results confirmed by UPLC-MS; most useful for lower MW polyphenols	specialized equipment required, not as generally available as HPLC; not useful for polymeric PACs (DP >4)

Table 2 continued on next page

Table 2 continued. Comparison of different analytical approaches to determine adulterants in cranberry products

Reference	Sample Set	Method	Analyte(s)#	Pro	Contra
Upton (2016)[8]	dried, powdered fruit, extracts, juice	HPTLC-densitometry	hyperoside, anthocyanin-3-*O*-glucoside	specific for different type of low MW polyphenols; reference standards available; can detect ≥ 15% grape skin	specialized equipment required, not as generally available as HPLC
Brown (2011)[53]	fruit, juice, juice cocktail, extract powder	HPLC-DAD	5 major cranberry anthocyanins	specific for detection of anthocyanins; 5 reference standards available; common laboratory equipment	one commercial ref std not pure; method not validated for analysis of PACs; one of 6 major cranberry anthocyanins omitted from analysis
Gao et al (2018)[54]	dried cranberries, cranberry juice cocktail, partially purified PACs, dietary supplements containing cranberry extract	before/after thiolysis: HPLC-FD; thiolysis-HPLC-UV-ESI/TOF	PACs, after depolymerization by reaction with acid and cysteamine	specific for cranberry PACs; provides total PACs content, ratio of A-type linkages and A-type PAC equivalents	multiple analytical methods; laborious sample preparation sensitive to many variables; needs careful validation and calibration curve prep based on thiolysis products; only single laboratory validation thus far
Puigventos (2017)[55]	extracts of fresh and dried cranberries and grapes	HPLC-DAD, with PCA and PLS[d]	comparison of total phenolic profiles	common laboratory equipment	grape phenolic profile reportedly much weaker than that of cranberry
Navarro (2014)[56]	fruit, juice, extracts, products	HPLC-DAD, CZE[e]-DAD, with PCA	8 CZE[e] peaks selected as relevant	CZE[e] a potentially complementary approach to HPLC	equipment not widely available; complex sample preparation
Bakhytkyzy (2018)[57]	2 cranberry extracts and 17 anti-cystitis phytomedicines and dietary supplements	HPLC-UV-FD, with PCA	catechin, epicatechin and related PACs (ProA2, B2, C1)[c]	improved sensitivity and selectivity compared with UV methods; reference standards available for the three PACs of interest	FD detection not as widely used as UV
Prior (2001)[58]	blueberries, cranberries, juice, spray-dried extract	HPLC-DAD-FD-MS	PACs - normal phase; anthocyanins -reversed phase	normal phase allows separation of PAC oligomers/polymers by DP and linkage type; common laboratory equipment	some equipment is moderately expensive; lack of reference standards for PACs
Gu (2003)[59]	88 different foods	HPLC-MS/MS (normal phase)	PACs - oligomeric and polymeric, A and B types	normal phase allows separation of PACs oligomers/polymers by DP and linkage type; method applicable to many food sources/forms	long HPLC run time; extensive sample prep; qualitative method as reported; lack of reference standards for PACs
Sánchez-Patán (2012)[60]	19 commercial products US and EU	UPLC-DAD-ESI/TQ/MS[f]	phenolic acids, anthocyanins, flavan-3-ols, including PACs	applicable to various low MW polyphenols, including A-type dimers and trimers; short experimental run times	equipment rather costly; method not useful for polymeric PACs (DP >4)
Feliciano (2012)[61]	cranberry press cake	MALDI-TOF-MS	cPAC[b]	relatively facile determination of A type to B type PAC ratio	expensive equipment; complex calculations; not quantitative
Jungfer (2012)[62]	fruit: *V. macrocarpon*, *V. oxycoccos*, *V. vitis-idaea*	UPLC-TQ/MS[f]	compares profiles of monomers, dimers, and trimers of A and B type PACs	focused on oligomeric (lower MW) PACs; distinguishes three species of *Vaccinium*	expensive equipment; complex data processing; not useful for polymeric PACs (DP >4)
Barbosa (2018)[63]	cranberry: 21 juices, 4 fruits, 8 raisins, 5 raw extracts, 11 encapsulated, 4 sachets, 2 syrups; grape: 17 juices, 4 fruits, 8 raisins; blueberry: 6 juices, 6 fruits; raspberry: 10 fruits	UPLC-HRMS (orbitrap), with PCA[g] and PLS[d]	compares broad phenolic profile of 53 compounds (using reference standards)	many phenolic compounds can be analyzed in a single chromatographic run	equipment is expensive, not available in every laboratory, but is becoming increasingly available

[a] 4-(dimethylamino)cinnamaldehyde
[b] cranberry fruit proanthocyanidin extract as described by Feliciano et al.[61]
[c] procyanidin (A2 or B2 or other, if specified)
[d] partial lease square regression
[e] capillary zone electrophoresis
[f] tandem quadrupole mass spectrometry (double or triple not specified)
[g] principal component analysis

Comments:

The methods (and attendant literature citations) in Table 1 can be divided into groups based on the analytical methodology employed. One entry deals with juices only and reports well established methods for analyzing sugar and non-volatile organic acid content.

Several abbreviations for the same molecule can be found in the literature. Procyanidin A2 is written as PAC-A_2 by Boudescoque et al.,[51] and Boudescoque-Delaye et al.,[52] as A2 or procyanidin A2 by Bakhytkyzy et al.,[57] or as ProA2 by Krueger et al.[49] Similar inconsistencies occur for other procyanidins. For this laboratory guidance document, the terminology by Krueger et al.[49] has been followed.

cranberry juices over three decades ago, coupled with HPLC-UV analyses of the anthocyanidin profiles. They identified 20 of 31 juice samples that they analyzed as adulterated.[46,47] Reviewing these two articles is informative, as the sugar and organic acid methodologies are still useful today, offering good resolution, but the separation of the anthocyanidins as presented in the paper seems quite dated by today's standards, and yet the researchers could readily distinguish cranberry juice from those of blackberry and mango. Current HPLC column technology and the use of MS as the detection mode permit direct analysis of the anthocyanins in cranberry and other fruits.

It is important to note that analyzing the anthocyanin profile is an excellent check for adulteration by color, i.e., adding exogenous colored materials to present an apparent cranberry color. Brown and Shipley[53] developed and validated, via single laboratory protocol,[64] a quantitative HPLC-DAD analysis of the five major anthocyanins of cranberry as a quality control tool. Reference standards of those anthocyanins are commercially available, permitting verification of identity and quantitative measurements of content in fruit, juice, juice cocktail, and dried extracts. This method is an interesting complement to the numerous methods to measure the content of the PAC in cranberry and can be used to assess overall quality and composition of cranberry products.

More recently, $^{12}C/^{13}C$ ratios have been increasingly used to identify cases where synthetic or exogenous sugars have been added to a juice product.[65]

The rest of the entries are focused on the polyphenolics (PAC or total phenolic profile) in cranberry; analytical approaches include colorimetric (DMAC), HPTLC-densitometry, HPLC-UV and/or FD, HPLC-MS, and MS (MALDI TOF, Orbitrap).

The DMAC assay, which involves the reaction of 4-dimethylamino cinnamaldehyde at a hydrogen-bearing aromatic carbon with two free phenolic hydroxyl groups positioned ortho- or ortho-/para- in the flavanol portion of a PAC molecule to form an intensely green- or blue-colored compound, has been the subject of investigation as a potential quantitative assay for PAC for about two decades. The three DMAC papers listed in Table 1 highlight recent developments with this assay.

Prior et al.[48] validated a DMAC method across five laboratories, using a sample set (n = 11) consisting of juices or powdered fruit or extract. They used commercially available procyanidin A2 as a standard; cranberry powders (dried, ground berries) were extracted in a protocol that required 1-1.5 hours of effort, while the PAC fraction of juice was obtained by quick chromatography on C_{18} cartridges. The DMAC reaction was conducted and the color read and evaluated in a 96 well plate format. Krueger et al.[49] followed the Prior study with a comparison of the use of procyanidin A2 vs c-PAC, a standardized total PAC fraction from cranberry press cake (pomace) extracts, as a standard for the DMAC assay. Their investigation revealed more accurate results with the use of c-PAC, but the preparation of c-PAC was labor intensive, involving a triple extraction and gel permeation chromatography. The challenge, then, would be to create a significant, sustained supply of certified reference standard c-PAC, in order for results from different laboratories to be compared. Very recent work by Sintara et al.[50] reports a single laboratory validation of a DMAC method using procyanidin A2 as a reference standard. Improvements over previous methods include changing extraction solvent to methanol for better reproducibility, changing solvent for DMAC reagent from hydrochloric acid in ethanol to sulfuric acid in methanol for higher sensitivity, and using a UV/Vis spectrophotometer instead of a plate reader for wider availability. The precision of the DMAC method was improved from 16.5% (RSD for Prior[48]) to an RDS of less than 5%. However, the DMAC method, as well as other spectrophotometric methods, is not appropriate for the detection of adulteration since it is not specific enough to differentiate among cranberry PACs, and those from potential adulterants.

Two papers illustrate the evolution of an HPTLC-densitometry approach by Boudesocque-Delaye et al.[51,52] In the first, catechin, procyanidin A2 and procyanidin B2 served as reference standards, while epicatechin replaced catechin in the more recent iteration of the methodology. In the latter paper, the authors report that the sample preparation protocol, which required several liquid/solid extractions to isolate fully the polyphenolic fraction, was crucial to obtaining a meaningful comparison of product quality and pharmacological activity. The HPTLC-densitometry results, which indicated that only two of the 10 products tested were high quality cranberry formulations, were confirmed by both UPLC and DMAC analyses. The recently revised AHP monograph on cranberry fruit by Upton and Brendler[8] provides a richly detailed description of the sample preparation, execution and review of an HPTLC analysis of various forms of cranberry, including samples adulterated with 15% grape skin extract by weight; a series of informative color plates are included. HPTLC is a good screening technique, allowing the detection of most types of adulteration, although mixtures/substitutions between cranberry and certain other PAC-rich extracts can represent a problem. However, some of these materials may be distinguished from cranberry based on the general fingerprint, or by comparing the flavan-3-ol monomer, dimer, and trimer pattern (Figures 3-5, see page 8-9).

Table 2 includes two publications on analytical methods employing HPLC-UV and four others focused on HPLC-MS, with or without UV monitoring.

Brown and Shipley's[53] single laboratory validated quantitative HPLC-DAD analysis of the five major anthocyanins of cranberry as a quality control tool is discussed above in regard to juice and juice products. This method is an interesting complement to the numerous methods to measure the content of PACs in cranberry. In a quite different approach, Puigventos et al.[55] used HPLC-DAD, followed by PCA and PLS data analyses, to compare the entire polyphenolic profiles of extracts of both fresh and dried cranberries and grapes. The grape polyphenolic profile was significantly weaker than the cranberry profile at the three wavelengths evaluated (280, 370 and 520 nm), but was most prominent at 370 nm. Application of PCA and PLS data mining allowed distinction of test mixtures containing 50 or 10% grape juice (in cranberry juice), but 2.5, 5 and

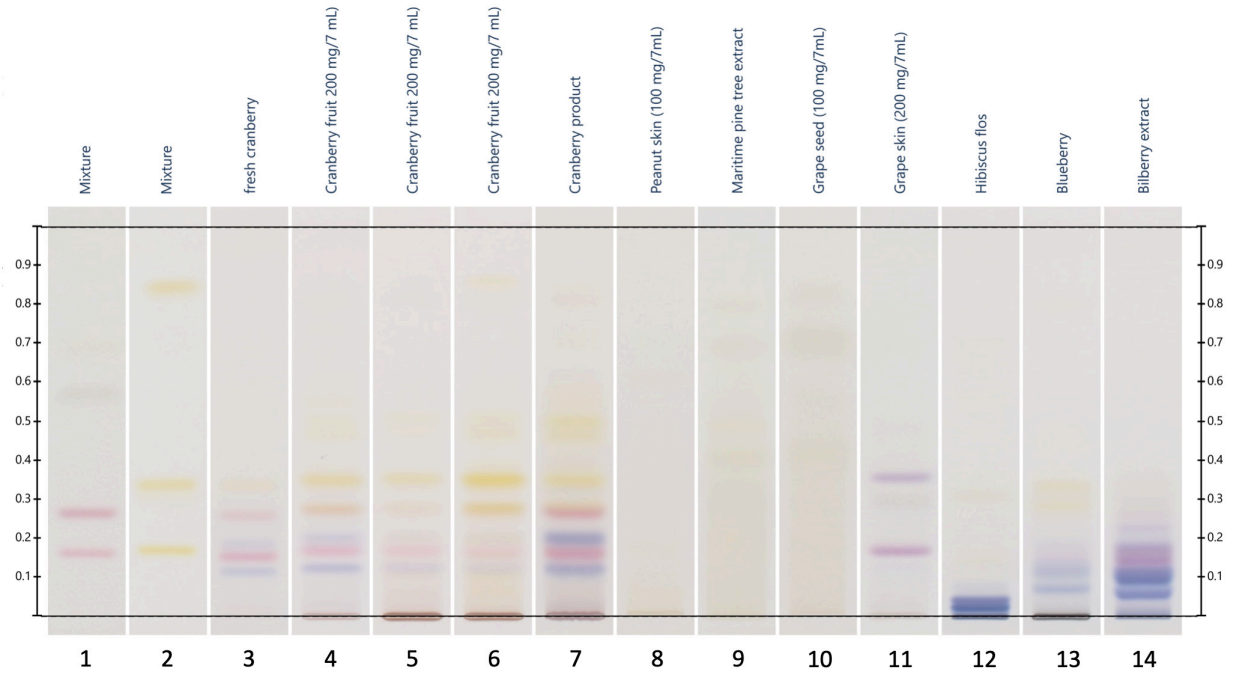


Figure 3: HPTLC analysis of cranberry, related and and adulterating species. Lane 1: peonidin-3-*O*-galactoside and peonidin-3-*O*-arabinoside, with increasing Rf value; Lane 2: rutin, chlorogenic acid, hyperoside, and quercetin, with increasing Rf value; Other lanes as indicated above. Stationary phase: silica gel 60 F254; Mobile phase: toluene-water-formic acid-ethyl formate (3:6:8:60); Detection: Natural product reagent, viewed under white light. Image provided by Camag AG; Switzerland.

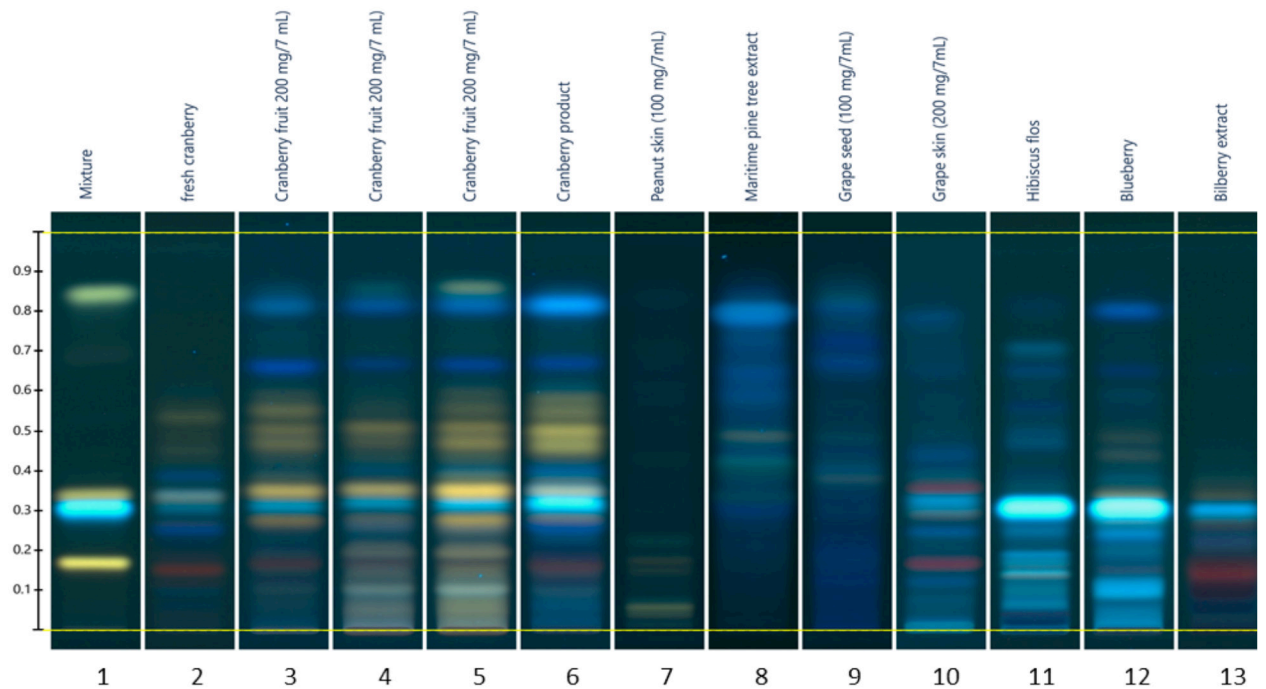


Figure 4: HPTLC analysis of cranberry, related and adulterating species. Lane 1: rutin, chlorogenic acid, hyperoside, and quercetin, with increasing Rf value; Other lanes as indicated above. Stationary phase: silica gel 60 F254; Mobile phase: toluene-water-formic acid-ethyl formate (3:6:8:60); Detection: Natural product reagent, viewed using UV light at 366 nm. Image provided by Camag AG; Switzerland.

7.5% grape juice 'adulteration' could not be differentiated from pure cranberry juice.

Gao et al.[54] revived and modified a 45-year-old thiolysis method[66,67] and combined that with HPLC-FD detection to develop a method to quantitate total procyanidins, average degree of polymerization, ratio of A-type linkages, and A-type procyanidin equivalents in cranberries, cranberry juice, partially purified PACs and dietary supplements containing cranberry extracts. While the sample preparation is sensitive to a number of variables, the method has been through an AOAC single laboratory validation and offers the distinct advantage of an ability to focus on the A-type PACs, because the thiolysis reaction is blocked from cleaving the carbon-carbon bond that distinguishes A-type PACs. The authors used HPLC-ESI/TOF to verify the composition of the various thiolysis products. Bakhytkyzy et al.[57] established an HPLC method using fluorescence detection (FD) to separate and identify A, B and C type PACs; one of the keys to success in this method was the availability of authentic reference standards for procyanidin A2, B2, and C1, along with catechin and epicatechin. FD gave better sensitivity and selectivity than UV detection. The authors found that the two extracts and 17 market products they analyzed fell into three groups: a) extracts rich in procyanidin A2; b) extracts enriched in monomeric species; and c) extracts rich in procyanidin B2. These results indicated that a significant number of the analytical samples did not conform to expectations of a cranberry profile.

Prior et al.[58] used HPLC-DAD-FD-MS to profile both the PAC (normal phase HPLC) and anthocyanin (reverse phase HPLC) content of cranberries and blueberries, along with their juices and extracts. The authors observed the best separation/resolution of the PACs by normal phase HPLC, while the anthocyanins were readily resolved by the more commonly used reverse phase columns. The compounds were detected by both UV (DAD) and FD, while compound identities were confirmed by comparison of retention time and mass spectral data, when reference compounds were available, or were proposed by comparison of UV and mass spectral data with literature reports, when standards were not available.

Gu et al.[59] used a similar normal phase HPLC-MS/MS method to analyze different food forms for oligomeric and polymeric PACs; the complexity of the sample preparation and the long HPLC run times may limit adaptation of this method to the food industry. Sánchez-Patán et al.[60] applied different reverse phase UPLC-DAD methods for the separation and analysis of phenolic acids/flavan-3-ols (including PACs) and anthocyanins by tandem quadrupole MS; this approach allowed the researchers to demonstrate that only four of 19 commercial extract products examined delivered the requisite daily dose of 36 mg of PACs. The method has a relatively straightforward sample preparation and short run time, but the latter is offset by having to run two separate UPLC analyses to account for all the analytes of interest.

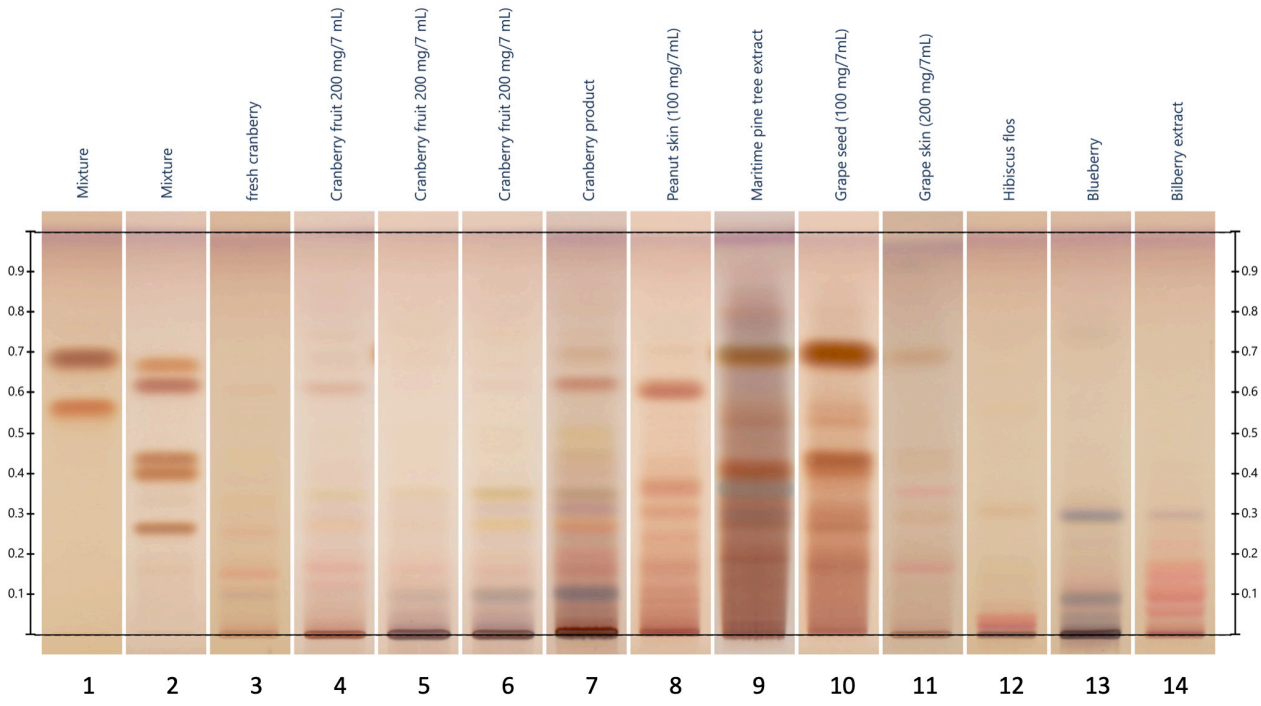


Figure 5: HPTLC analysis of cranberry, related and adulterating species. Lane 1: epigallocatechin-3-*O*-gallate and epicatechin, with increasing Rf value; Lane 2: procyanidin C1, procyanidin B1, procyanidin B2, procyanidin A2, and epicatechin-3-*O*-gallate, with increasing Rf value; Other lanes as indicated above. Stationary phase: silica gel 60 F254; Mobile phase: toluene-water-formic acid-ethyl formate (3:6:8:60); Detection: Natural product reagent, followed by anisaldehyde reagent, viewed under white light. Image provided by Camag AG; Switzerland.

Feliciano et al.[61] used MALDI-TOF MS to determine the ratios of PAC-A to PAC-B in cranberry press cake and juice; however, the method is not quantitative and requires extensive calculations. Even though no commercial products were analyzed, the contribution from Jungfer et al.[62] is of considerable potential value, because it compares the profiles of the monomers, dimers, and trimers of A and B type PACs in three species of *Vaccinium*: *V. macrocarpon*, *V. oxycoccos*, *V. vitis-idaea*. The researchers used UPLC and triple quadrupole MS to establish the unique profiles in each species and the variation observed in samples of different origin. This method would seem to have great usefulness in species verification at the raw material acquisition stage. However, analysts must remember that the total amount of PACs as monomers, dimers and trimers represents only a fraction of the total PACs in any cranberry product. In a very recent paper, Barbosa et al.[63] utilized UPLC-HRMS (Orbitrap system) to create and compare phenolic profiles, using 53 reference standard compounds, of cranberry, grape, raspberry and blueberry fruit, dried fruit, juice, extracts and finished products. As might be expected, grape was easily differentiated from cranberry, with raspberry showing similar, but not as significant differences. Blueberry and cranberry were closely related in the PCA analyses illustrated in the manuscript. Cranberry extracts and encapsulated products showed significant differences from fruit, juice and dried fruit; this is not surprising, given the alteration of chemical profile brought on by extraction and other processing steps. PLS regression analyses were efficient in identifying the level (%) adulteration in mixtures of grape and cranberry juices. This method also has considerable potential for the detection and identification of adulteration of cranberry products. HPLC-UV/MS is appropriate for the detection of most types of adulteration if it is based on a fingerprint of flavan-3-ol monomers, dimers, smaller oligomers, and other relevant compounds. One advantage having a UV/Vis detector as part of a hyphenated system is that it can measure anthocyanins at the same time. Based on the paper by Ye et al.,[68] distinction between cranberry and peanut skin extracts may be challenging, even using a MALDI-TOF MS fingerprint. The latter is nevertheless ideal for distinguishing PAC-rich materials from various sources, but may not be optimal for materials with little to no PACs, such as green tea. In addition, adulteration of cranberry extracts with anthocyanin-rich materials, or with food colorants may go undetected using MALDI-TOF MS.

Navarro et al.[56] compared HPLC to CZE (capillary zone electrophoresis), both linked to diode array detectors, to determine the applicability of CZE to the analysis and authentication of cranberry in fruit, juice, and extract forms. CZE was shown to be complementary to HPLC in this report and may be an alternative approach for some analytical groups.

A general note about HPLC columns may be helpful to readers. Some of the more recent articles reviewed herein used core-shell columns for the HPLC analyses; the authors of those articles noted that better resolution and peak shapes were obtained with these columns, compared to conventional packed columns. One might hypothesize that such column architecture lends itself to partition chroma-

Craberry *Vaccinium macrocarpon*
Photo ©2018 Steven Foster

tography, rather than adsorption mechanisms.

Readers less familiar with cranberry analysis would benefit from first studying the AHP monograph on cranberry,[8] since it reviews most of the studies and methods listed in Table 1, with the exception of the very recent (2018) publications.

9. Conclusions

There is a growing body of reliable data indicating that cranberry juice and extract products are frequently adulterated. Possibly driven by supply/demand issues and/or financial incentives, such fraudulent products likely deprive consumers of the perceived and documented health benefits of cranberry.

A number of analytical methods are reviewed in this guidance document, with the seemingly most broadly applicable and useful of those highlighted for the benefit of readers. In general, methods most useful for checking juices for quality and lack of adulteration include analyses for organic acids (HPLC-RI or UV) sugars (HPLC-RI or $^{12}C/^{13}C$ ratios by MS) and anthocyanin pigments (HPLC-Vis). For fruits and fruit-derived extracts and powders, the higher resolution separation techniques like HPTLC and HPLC/UPLC give better separation of the complex mixtures present. HPLC would need to be coupled to a specific detection methodology, like Vis (anthocyanins), FD (procyanidins), or MS (all compounds). HPTLC-MS systems have been developed to address this and other challenges.

It should be noted that none of the adulterating materials, whether they be other fruit juices or exogenous substances, represent an apparent safety concern to consumers, although the possible presence of peanut allergens from peanut skins could be of concern to a subset of the general population.

10. References

1. Brendler T, Gafner S. *Adulteration of Cranberry (Vaccinium macrocarpon) – Botanical Adulterants Bulletin.* Austin, TX: ABC-AHP-NCNPR Botanical Adulterants Prevention Program; Botanical Adulterants Bulletin. 2017;1-8.
2. *Thesaurus of Agricultural Organisms: Pests, Weeds and Diseases*. Volume One: A to M. New York, NY: Chapman and Hall/CRC Press; 1990.
3. Goetz P, Ghedira K. *Phytothérapie Anti-infectieuse.* Paris, France: Springer-Verlag; 2012.
4. United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Genetic Resources Program. Germplasm Resources Information Network (GRIN) Online Database. Beltsville, MD: National Germplasm Resources Laboratory. http://www.ars-grin.gov. Accessed 24 October 2018.
5. Murray MT, Pizzorno JE. *The Encyclopedia of Healing Foods*. New York, NY: Atria Books. 2005:268-271.
6. Flora of China. eFloras.org website. http:/www.efloras.org. Accessed August 21, 2018.
7. *Oxycoccus***.** *Wikipedia database***.** Available at: https:/it.wikipedia.org/wiki/*ghorn*. Accessed August 21, 2018.
8. Upton R, Brendler T (Eds.). *American Herbal Pharmacopoeia and Therapeutic Compendium: Cranberry Fruit: Vaccinium macrocarpon* Aiton. Scotts Valley, CA: American Herbal Pharmacopoeia. Monograph revision; 2016.
9. McGuffin M, Kartesz JT, Leung AY, Tucker AO. *Herbs of Commerce*. *2nd ed.* Silver Spring, MD: American Herbal Products Association; 2000.
10. Vander Kloet SP. *The Genus Vaccinium in North America*. Ottawa, Ontario: Agriculture Canada. 1988: 107-118.
11. Upton R, Graff A, Jolliffe G , Länger R, Williamson E, eds. *American Herbal Pharmacopoeia: Botanical Pharmacognosy—Microscopic Characterization of Botanical Medicines.* Boca Raton, FL: CRC Press; 2011: 689-691.
12. Khaneja M, Gupta S, Sharma A. Pharmacognostical and preliminary phytochemical investigations on fruit of *Vaccinium macrocarpon* Aiton. *Pharmacogn J*. 2015;7: 333-338.
13. Schlautman B, Bolivar-Medina J, Hodapp S, Zalapa J. Cranberry SSR multiplexing panels for DNA horticultural fingerprinting and genetic studies *Scientia Hort.* 2017; 219: 280-286.
14. Schlautman B, Fajardo D, Bougie T, Wiesman E, Polashock J, Vorsa N, Steffan S, Zalapa J. Development and validation of 697 novel polymorphic genomic and EST-SSR markers in the American cranberry (*Vaccinium macrocarpon* Ait.). *Molecules*. 2015; 20: 2001-2013.
15. Fajardo D, Morales J, Zhu H, Steffan, S, Harbut R, Bassil N, Hummer K, Polashock J, Vorsa N, Zalapa J. Discrimination of American cranberry cultivars and assessment of clonal heterogeneity using microsatellite markers. *Plant Mol Biol Rep*. 2013; 31: 264-271.
16. Georgi L, Johnson-Cicalese J, Honig J, Das SP, Rajah VD, Bhattacharya D, Bassil N, Rowland LJ, Polashock J, Vorsa N. The first genetic map of the American cranberry: exploration of synteny conservation and quantitative trait loci. *Theor Appl Genetics* 2013; 126: 673-692.
17. Zhu H, Senalik D, McCown BH, Zeldin EL, Speers J, Hyman J, Bassil N, Hummer K, Simon PW, Zalapa JE. Mining and validation of pyrosequenced simple sequence repeats (SSRs) from American cranberry (*Vaccinium macrocarpon* Ait.) *Theor Appl Genetics* 2012; 124: 87-96.
18. Cesoniene L, Daubaras R, Paulauskas A, Zukauskiene J, Zych M. Morphological and genetic diversity of European cranberry (*Vaccinium oxycoccus* L., Ericaceae) clones in Lithuanian reserves. *Acta Soc Bot Poloniae* 2013; 82: 211-217.
19. Herbst N, Wilson T, Klein J, Cooper S. Detection of cranberry and blueberry (*Vaccinium* spp.) DNA by PCR amplification of the MatK gene. *FASEB J*. 2014; 28(1 Supplement): LB386.
20. Howell AB, Reed JD, Krueger CG, Winterbottom R, Cunningham DG, Leahy M. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. *Phytochemistry* 2005; 66: 2281-2291.
21. Appeldoorn MM, Vincken J-P, Sanders M, Hollman PCH, Gruppen H. Combined normal-phase and reversed-phase liquid chromatography/ESI-MS as a tool to determine the molecular diversity of A-type procyanidins in peanut skins. *J Agric Food Chem.* 2009; 57: 6007-6013.
22. Weseler AR, Bast A. Masquelier's grape seed extract: from basic flavonoid research to a well-characterized food supplement with health benefits. *Nutr J.* 2017; 16: Article #5, 19 pp.
23. Weber HA, Hodges AE, Guthrie JR, O'Brien BM, Robaugh D, Clark AP, Harris RK, Algaier JW, Smith CS. Comparison of proanthocyanidins in commercial antioxidants: Grape seed and pine bark extracts. *J Agric Food Chem.* 2007; 55: 148-156.
24. La VD, Bergeron C, Gafner S, Grenier D. Grape seed extract suppresses lipopolysaccharide induced matrix metalloproteinase (MMP) secretion by macrophages and inhibits human MMP-1 and -9 activities. *J Periodontol.* 2009; 80: 1875-1882.
25. Monagas M, Hernández-Ledesma B, Garrido I, J Martín-Alvarez P, Gómez-Cordovés C, Bartolomé B. Quality assess-

ment of commercial dietary antioxidant products from *Vitis vinifera* L. grape seeds. *Nutr Cancer.* 2005; 53: 244-254.
26. O'Keefe SF, Wang H. Effects of peanut skin extract on quality and storage stability of beef products. *Meat Sci.* 2006; 73: 278-286.
27. Constanza KE, White BL, Davis JP, Sanders TH, Dean LL. Value-added processing of peanut skins: Antioxidant capacity, total phenolics, and procyanidin content of spray-dried extracts. *J Agric Food Chem.* 2012; 60: 10776-10783.
28. Dudek MK, Gliński VB, Davey MH, Sliva D, Kaźmierski S, Gliński JA. Trimeric and tetrameric A-type procyanidins from peanut skins. *J Nat Prod*. 2017; 80: 415-426.
29. Bansode RR, Randolph P, Ahmedna M, Hurley S, Hanner T, Baxter SA, Johnston TA, Su M, Holmes BM, Yu J, Williams LL. Bioavailability of polyphenols from peanut skin extract associated with plasma lipid lowering function. *Food Chem.* 2014; 148: 24-29.
30. Kim SM, Kang S-W, Jeon J-S, Um B-H. A comparison of Pycnogenol® and bark extracts from *Pinus thunbergii* and *Pinus densiflora*: Extractability, antioxidant activity and proanthocyanidin composition. *J Med Plants Res.* 2012; 6: 2839-2849.
31. Navarrete P, Pizzi A, Pasch H, Rode K, Delmotte L. MALDI-TOF and ^{13}C NMR characterization of maritime pine industrial tannin extract. *Ind Crops Prod.* 2010; 32: 105-110.
32. Jerez M, Pinelo M, Sineiro J, Núñez MJ. Influence of extraction conditions on phenolic yields from pine bark: assessment of procyanidins polymerization degree by thiolysis. *Food Chem.* 2006; 94: 406-414.
33. Bianchi S, Kroslakova I, Janzon R, Mayer I, Saake B, Pichelin F. Characterization of condensed tannins and carbohydrates in hot waterbark extracts of European softwood species. *Phytochemistry.* 2015; 120: 53-61.
34. Villani TS, Reichert W, Ferruzzi MG, Pasinetti GM, Simon JE, Wu Q. Chemical investigation of commercial grape seed derived products to assess quality and detect adulteration. *Food Chem.* 2015; 170: 271-280.
35. Labarbe B, Cheynier V, Brossaud F, Souquet J-M, Moutounet M. Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J Agric Food Chem.* 1999; 47: 2719-2723.
36. Oszmiański J, Wolniak M, Wojdyło A, Wawer I. Influence of apple pure´e preparation and storage on polyphenol contents and antioxidant activity. *Food Chem.* 2008; 107: 1473-1484.
37. Pastene E, Troncoso M, Figueroa G, Alarcón J, Speisky H. Association between polymerization degree of apple peel polyphenols and inhibition of *Helicobacter pylori* urease. *J Agric Food Chem.* 2009; 57: 416-424.
38. Jiang X, Liu Y, Wu Y, Tan H, Meng F, Wang YS, Li M, Zhao L, Liu L, Qian Y, Gao L, Xia T. Analysis of accumulation patterns and preliminary study on the condensation mechanism of proanthocyanidins in the tea plant [*Camellia sinensis*]. *Sci Rep.* 2015; 5: 8742-8756.
39. Sarnoski PJ, Johnson JV, Reed KA, Tanko JM, O'Keefe SF. Separation and characterisation of proanthocyanidins in Virginia type peanut skins by LC–MS^n. *Food Chem.* 2012; 131: 927-939.
40. Prieur C, Rigaud J, Cheynier V, Moutounet M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry.* 1994; 36: 781-784.
41. Sun B, Leandro C, Ricardo da Silva JM, Spranger I. Separation of grape and wine proanthocyanidins according to their degree of polymerization. *J Agric Food Chem.* 1998; 46: 1390-1396.
42. Spranger I, Sun B, Mateus AM, Freitas Vd, Ricardo-da-Silva JM. Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. *Food Chem.* 2008; 108: 519-532.
43. European Fruit Juice Association. Reference guidelines for cranberry juice: http://www.aijn.org/publications/code-of-practice/individual-reference-guidelines. Accessed September 17, 2018 [Note: must be a subscriber to accept the document(s)].
44. Commodity specifications for bottled juices. United States Department of Agriculture. June 2014. Available at: https://www.ams.usda.gov/sites/default/files/media/Commodity%20Specification%20for%20Bottled%20Juices%2C%20June%202014.pdf, pp 11-12. Accessed October 11, 2018.
45. *USP 41-NF 36*. Rockville, MD: United States Pharmacopeial Convention; 2018:4554-4555.
46. Hong V, Wrolstad RE. Cranberry juice composition. *J AOAC Int.* 1986; 69: 199-207.
47. Hong V, Wrolstad RE. Detection of adulteration in commercial cranberry juice drinks and concentrates. *J AOAC Int.* 1986; 69: 208-213.
48. Prior RL, Fan E, Ji H, Howell A, Nio C, Payne MJ, Reed J. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *J Sci Food Agric.* 2010; 90: 1473-1478.
49. Krueger CG, Chesmore N, Chen X, Parker J, Khoo C, Marias JPJ, Shanmuganayagam D, Crump P, Reed JD. Critical reevaluation of the 4-(dimethylamino) cinnamaldehyde assay:

cranberry proanthocyanidin standard is superior to procyanidin A2 dimer for accurate quantification of proanthocyanidins in cranberry products. *J Funct Foods*. 2016; 22: 13-19.
50. Sintara M, Li L, Cunningham DG, Prior RL, Wu X, Chang T. Single-laboratory validation for determination of total soluble proanthocyanidins in cranberry using 4-dimethylaminocinnamaldehyde. *JAOAC Int.* 2018; 101: 805-809.
51. Boudesocque L, Dorat J, Pothier J, Gueiffier A, Enguehard-Gueiffier C. High-performance thin-layer chromatography-densitometry: A step further for quality control of cranberry extracts. *Food Chem.* 2013; 136: 866-871.
52. Boudesocque-Delaye L, Arnaud Lanoue A, Dorat J, Bruyère F, Gueiffier A, Enguehard-Gueiffier C. Quality control of commercial cranberry products: HPTLC-densitometry a new deal. *Food Control* 2018; 86: 214-223.
53. Brown PN, Shipley PR. Determination of anthocyanins in cranberry fruit and cranberry fruit products by high-performance liquid chromatography with ultraviolet detection: single-laboratory validation. *J AOAC Int*. 2011; 94: 459-466.
54. Gao C, Cunningham DG, Liu H, Kho C, Gu L. Development of a thiolysis HPLC method for the analysis of procyanidins in cranberry products. *J Agric Food Chem.* 2018; 66: 2159-2167.
55. Puigventos L, Nuñez O, Saurina J. HPLC fingerprints for the authentication of cranberry-based products based on multivariate calibration approaches. *Curr Anal Chem*. 2017; 13: 256-261.
56. Navarro M, Nuñez O, Saurina J, Hernández-Cassou S, Puignou L. Characterization of fruit products by capillary zone electrophoresis and liquid chromatography using the compositional profiles of polyphenols: application to authentication of natural extracts. *J Agric Food Chem.* 2014; 62: 1038-1046.
57. Bakhytkyzy I, Nuñez O, Saurina J. Determination of flavanols by liquid chromatography with fluorescence detection. Application to the characterization of cranberry-based pharmaceuticals through profiling and fingerprinting approaches. *J Pharm Biomed Anal.* 2018; 156: 206-213.
58. Prior RL, Lazarus SA, Cao G, Muccitelli H, Hammerstone JF. 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.
59. Gu L, Kelm MA, Hammerstone JF, Beecher G, Holden J, Haytowitz D, Prior RL. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J Agric Food Chem.* 2003; 51: 7513–7521.
60. Sánchez-Patán F, Bartolomé B, Martín-Alvarez PJ, Anderson M, Howell A, Monagas M. Comprehensive assessment of the quality of commercial cranberry products. Phenolic characterization and in vitro bioactivity. *J Agric Food Chem.* 2012; 60: 3396-3408.
61. Feliciano RP, Krueger CG, Shanmuganayagam D, Vestling MM, Reed JD. Deconvolution of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry isotope patterns to determine ratios of A-type to B-type interflavan bonds in cranberry proanthocyanidins. *Food Chem*. 2012; 135: 1485–1493.
62. Jungfer E, Zimmermann BF, Ruttkat A, Galensa R. Comparing procyanidins in selected Vaccinium species by UHPLC-MS^2 with regard to authenticity and health effects. *J Agric Food Chem.* 2012; *60*: 9688-9696.
63. Barbosa S, Pardo-Mates N, Hidalgo-Serrano M, Saurina J, Puignou L, Nunez O. Detection and quantitation of frauds in the authentication of cranberry-cased extracts by UHPLC-HRMS (Orbitrap) polyphenolic profiling and multivariate calibration methods. *J Agric Food Chem.* 2018; 66: 9353-9365.
64. Horwitz, W. AOAC Guidelines for single laboratory validation of chemical methods for dietary supplements and botanicals; AOAC International: Gaithersburg, MD, 2002.
65. Zhang Y, Krueger D, Durst R, Lee R. Wang D, Seeram N, Heber D. International multidimensional authenticity specification (IMAS) algorithm for detection of commercial pomegranate juice adulteration. *J Agric Food Chem.* 2009; 57: 2550-2557.
66. Thompson RS, Jacques D, Haslam E, Tanner RJN. Plant proanthocyanidins. Part I. Introduction; the isolation, structure, and distribution in nature of plant procyanidins. *J Chem Soc Perkin Trans I*. 1972; 1387-1399.
67. Torres J, Selga A. Procyanidin size and composition by thiolysis with cysteamine hydrochloride and chromatography. *Chromatographia*. 2003; 57: 441-445.
68. Ye L, Neilson A, Sarnoski P, Ray WK, Duncan S, Boyer R, O'Keefe SF. Comparison of A-type proanthocyanidins in cranberry and peanut skin extracts using matrix assisted laser desorption ionization-time of flight mass spectrometry. *J Mol Genet Med*. 2016; 10(2): 1000209.