

Adulteration of Cranberry (*Vaccinium macrocarpon*)

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Goal: The goal of this bulletin is to provide timely
information and/or updates on issues of adulteration of
cranberry fruit (*Vaccinium macrocarpon*) extract to the
international herbal products industry and extended
natural products community in general. It is intended
to present the available data on the occurrence of adul-
teration, the market situation, and consequences for the
consumer and the industry.

1. General Information

1.1 Common name: Cranberry¹

1.3 Accepted Latin binomial: *Vaccinium macrocarpon* Aiton¹

Note: Cranberry products on the dietary supplement,
food and beverage markets are predominantly made
from *V. macrocarpon*. However, the second edition
of *American Herbal Products Association's Herbs of
Commerce*,¹ which provides guidance on dietary supple-
ment labeling in the United States, also accepts prod-
ucts derived from *V. oxycoccos* to be labeled as cranberry.



Cranberry
Vaccinium macrocarpon
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1.2 Other common names:

English: American cranberry, large cranberry, North American cranberry²⁻⁵

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 Moosbeere²⁻⁴

Italian: Ossidocco americano, mirtillo rosso canadese, mortella di palude, cranberry⁷

Spanish: Arándano, arándano americano, arándano trepador, arándano rojo²⁻⁴

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

1.5 Botanical family: Ericaceae

1.6 Plant part and form: The vast majority of the cranberry dietary supplements are made from the fruit. The ingredients used in dietary supplements are powdered fruit, dried powdered fruit juice, or extracts made from the whole fruit, fruit juice or from cranberry press cakes, which is the material that remains after the juice has been pressed out. The extraction techniques are often proprietary, and may include an enzymatic or chemical hydrolysis step to release proanthocyanidins (PACs) from cell wall polysaccharides. Further concentration of the phenolic compounds, in particular the PACs, can be achieved by chromatographic techniques, e.g., by using specific resins that retain these phenolic compounds.^{8,9} While most extracts that give a defined amount of cranberry compounds are standardized to PACs, some manufacturers prefer to indicate levels of organic acids or anthocyanins. Depending on the process-

ing method, the PACs present can be soluble, insoluble, or both. Dietary supplements made solely from cranberry seeds or cranberry leaves are not within the scope of this document.

1.7 General use(s): Investigations in the early 20th century reported the effect of cranberries on urinary acidity, i.e., a lowering of the urinary pH after ingestion of cranberries.^{10,11} Since the late 1950s, the beneficial effect of cranberry juice and cranberry juice derivatives in the prevention and adjuvant treatment of recurrent urinary tract infections has been demonstrated in a plethora of pre-clinical and clinical investigations. Critical reviews, including meta-analyses, generally support the efficacy of cranberry in the aforementioned indication.¹²⁻¹⁴ However, a 2012 review indicated that methodological flaws in the design of the clinical studies and insufficient characterization of the administered cranberry products impede a reliable assessment.¹⁵

Other health benefits and actions of cranberry which have been investigated include prevention of gastric ulcers (caused by *H. pylori*), and activities related to periodontal disease, cancer prevention, glycemic response, antiviral activities, and a reduction of cardiovascular risk factors.¹⁶⁻²⁰

The PACs with a specific A-type linkage are considered responsible for the bacterial anti-adhesion activity – the health benefit which relates to urinary tract infections, ulcer prevention, and reduction of periodontal disease. Howell et al. determined that foods containing PACs with only B-type linkages do not provide as substantial bacterial anti-adhesion activity in urine as the cranberry PACs with A-type linkages.²¹ The most common dose used in early and recent studies demonstrating efficacy was approximately 300 mL of cranberry juice cocktail that, when calculated, delivers approximately 36 mg of soluble PACs (as measured with the 4-dimethylaminocinnamaldehyde [DMAC] color reagent using procyanidin A2 as standard; see below).^{19,22,23}

Further compounds of note contained in cranberries and its preparations are PACs with a B-type linkage, anthocyanins, flavonols, organic acids, volatile compounds, sugars, and vitamins. Ratios of A-type PACs to B-type PACs

Table 1: Sales data for cranberry products in the herbal supplement category in the United States from 2012-2016.

Channel	2012		2013		2014		2015		2016	
	Rank	Sales [US\$]	Rank	Sales [US\$]	Rank	Sales [US\$]	Rank	Sales [US\$]	Rank	Sales [US\$]
Natural ^a	10	5,078,657	20	3,855,518	17	4,254,478	13	5,670,347	11	7,513,172
Mainstream Multi-Outlet ^b	3	66,369,322	4	48,575,307	3	56,366,811	2	65,740,231	2	74,020,175

^aAccording to SPINS (SPINS does not track Whole Foods Market sales, which is a major natural products retailer in the United States.)

^bAccording to SPINS/IRI (the Mainstream Multi-Outlet channel was formerly known as food, drug and mass market channel [FDM]; possible sales at Walmart and Club stores are excluded in 2013-2016).

Source: Smith et al.²⁴ T. Smith (American Botanical Council) e-mail to S. Gafner, September 2, 2015 and September 3, 2015.

in cranberry products may be used for authentication. PACs can be soluble and insoluble (the latter are insoluble because they are bound to cell wall components such as polysaccharides or proteins), depending on the preparation and extraction method (e.g., juice and juice derivatives contain mostly soluble PACs, while whole cranberry powder, press cake, and press cake derivatives contain mostly insoluble PACs).¹⁹

Apart from the medicinal and supplement use, cranberry and its derivatives are widely consumed as foods and food ingredients.

2. Market

2.1 Importance in the trade: Cranberry has seen a steady growth in sales in the past decade. Cranberry products are among the most popular herbal dietary supplements in the United States with over US \$80 million in sales (Table 1) in 2016, combining both the Natural retail channel and the Mainstream Multi-Outlet retail channel (excluding sales data from Walmart and Club stores in 2013-2016, which were not available).

2.2 Supply sources: The majority of fresh cranberries is grown in northern North America (United States and Canada), followed by Chile and smaller producers in Europe and China.¹⁹

2.3 Market dynamics: The majority of cranberry supplements in the United States are sold in the Mainstream Multi-Outlet retail channel. A comparison with sales data from 2000 (US \$9,616,326),²⁵ 2005 (\$15,839,160),²⁶ 2010 (\$35,806,000)²⁷ and 2015 (\$65,740,231) in this retail channel shows the consistent growth of the cranberry market. This is in line with increases in North American cranberry production from approximately 300,000 metric tons in 2000 to 500,000 in 2013.²⁸ According to a report commissioned by the Cranberry Marketing Committee USA, Cranberry Institute, and British Columbia Cranberry Marketing Commission, the most important importers of cranberry products are the United Kingdom, Germany, France, Mexico, and Australia.²⁸ However, while the dietary supplement sales are growing, sales in other cranberry categories, in particular cranberry juice concentrates, have been falling recently, leading to a systemic oversupply of cranberry in 2014 and to low prices for growers.²⁹

According to an informal inquiry by the authors, market prices for dried press cake (containing 0.8 to 1.5% PACs) are around US \$50-75/kg; these dominate the bulk cranberry ingredient market with over 50% of the market share. Whole berry extracts, and blends of juice extracts with berry extracts (3 to 5% PACs) cost around US \$150-300/kg, while prices for pure juice extracts (12 to 24% PACs) range from US \$400-600/kg.

PAC-rich extracts from other plant sources, which are used to inflate PAC values of lower cost cranberry extracts or to substitute for cranberry extracts altogether, are available at much lower cost: For example, the price for peanut skin extract in 2015 was at US \$10-13/kg, while pine bark extracts were sold for US \$20-22/kg.³⁰ In 2017, pricing for

peanut skin extract with a PAC content of 80-90% was priced between US \$30-50.

3. Adulteration

With one notable exception dated around Thanksgiving 1959[†], adulteration of cranberry products is a relatively recent phenomenon, the first evidence for which appearing in the mid-1980s when grape anthocyanidins (a mixture of grape skin pigments collectively called enocyanin) were detected in cranberry juice.³²⁻³⁴ As the demand for cranberry products increased, incidents of admixture of lower cost and more readily available sources of anthocyanins, PACs, and flavanols were more frequently observed. One primary way of authenticating cranberry preparations is by determining the presence of A-type linkages in the PACs, and establish the ratio between A-type and B-type PACs. This guards against adulteration of cranberry products with materials from less expensive food sources characterized by their B-linked PACs and/or flavanols (epicatechin or catechin).³⁴

3.1 Known adulterants: Historically, American high-bush blueberry (*V. corymbosum*) and alpine blueberry, bog bilberry or bog blueberry (*V. uliginosum*) have occasionally been noted as adulterants.¹⁹ Grape anthocyanins were detected in cranberry juice in the 1980s (see above).³²⁻³⁴ More commonly than in juice products, adulteration is detected in dry concentrates and powdered extracts, likely due to the absence of standard analytical methods, difficulties in measuring complex PACs, and a lack of readily available reference standards. Most prevalent adulterants include grape (*Vitis vinifera*, Vitaceae) seed and skin extracts, red peanut (*Arachis hypogaea*, Fabaceae) skin extracts, but also maritime pine (*Pinus pinaster*, Pinaceae) bark extracts, and extracts of black bean (*Phaseolus vulgaris*, Fabaceae) skins, black rice (*Oryza sativa*, Poaceae), plum (*Prunus domestica*, Rosaceae), mulberries (*Morus* spp., Moraceae), other berries, and other parts of cranberry.^{19,36-43}

An interesting case of a scientific investigation inadvertently carried out with an adulterated reference material is provided by Wei and co-workers,⁴⁴ who isolated delphinidin-3-O-sambubioside, cyanidin-3-O-sambubioside, and *p*-coumaric acid from a crude "cranberry" extract purchased from Tiancheng (Shaanxi, China) using high-performance counter-current chromatography. In fact, those anthocyanidins do not occur in *V. macrocarpon* or *V. oxycoccos*, but are known to be the main anthocyanins in hibiscus (*Hibiscus sabdariffa*, Malvaceae) calyces.

3.2 Accidental or intentional adulteration: While historically adulteration of cranberry products may be considered primarily accidental (see above), more recent cases are more likely intentional, i.e., for economic reasons.

3.3 Frequency of occurrence: There is no comprehensive published study on the frequency of cranberry adulteration.

3.4 Possible safety/therapeutic issues: Adulterants

which are commonly detected in cranberry products generally do not pose a safety risk, although the allergenic potential of peanut skin extract is a matter of concern. Efficacy, however, is believed to depend on quantity and bioavailability specifically of A-type PACs, thus adulteration with other, lower-cost PACs may substantially reduce efficacy of cranberry products.

While peanut skin contains most of the known peanut allergens, these are bound and rendered insoluble by the phenolic compounds (particularly procyanidins, which are proanthocyanidins formed exclusively from catechin and epicatechin) present in peanut skin. Furthermore, proteins extracted from peanut skins do not appear to bind peanut specific IgE in the presence of phenolic compounds.⁴⁵ The allergenicity of spray-dried peanut skin extracts (using 70% aqueous ethanol as the extraction solvent) was evaluated by Constanza using a peanut allergen test kit. While no allergenic proteins were found in the spray-dried materials containing only the extract, peanut allergens were detected in a peanut skin extract spray-dried onto maltodextrin.⁴⁶ Since the manufacturing process for peanut skin extracts may vary from one supplier to another, elimination of the allergenic peanut proteins is not guaranteed. Therefore, an allergic reaction to peanut skin extracts used to adulterate cranberry extracts cannot be excluded.

3.5 Analytical methods to detect adulteration:

Depending on the level of detail required in terms of presence, qualification, and quantification of adulterants, and the analytical endpoint, several analytical methods of increasing complexity can be utilized in a step-by-step fashion:

- a. Quinic acid and the ratio of quinic to malic acid can be used to calculate the cranberry juice content; however, these acids are not unique to cranberry, and while they may suffice to determine a certain quality, reliance solely on the assessment of these organic acids for identification of cranberry will not rule out adulteration.^{47,48} The organic acids are predominantly used as chemical markers for authentication of cranberry juice and juice-derived products.
- b. Anthocyanins: Where identity and purity of the tested material are known, spectrophotometric analysis can be used to estimate total anthocyanins. However, this approach is non-specific and thus susceptible to adulteration with anthocyanin-rich or proanthocyanidin-rich materials from other sources. A more reliable approach to authenticate cranberry extracts is to use the HPLC-Vis anthocyanin fingerprint. Cranberry has a unique qualitative anthocyanin profile.^{48,49} Comparing HPTLC and HPLC fingerprints to an authentic reference material are standard techniques used for the identification of anthocyanin-containing cranberry extracts. These methods can also detect the presence of some adulterants (e.g., black rice extracts, hibiscus extracts, and admixture of up to 15% grape skins or grape seeds).^{19,50} However, this method is not suitable to

detect admixture of PAC-containing materials that are devoid of anthocyanins. A validated HPLC-Vis method was published to quantify five of the six predominant cranberry anthocyanins (cyanidin-3-*O*-galactoside, cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-glucoside, peonidin-3-*O*-galactoside, and peonidin-3-*O*-arabinoside) in commercial cranberry fruit products.⁵¹ Puigventos and co-workers utilized HPLC-ESI-MS to create "polyphenolic fingerprints" of cranberry products to elucidate adulteration with grape. Their results were clearly distributed in relation to the extent of grape adulteration with overall quantification errors of <5%.⁵²

- c. For (relative) quantitation of soluble PACs, spectrophotometric DMAC methods using a procyanidin A2²² standard or using a c-PAC standard⁵³ are employed, whereas the butanol-HCl method can be used for insoluble PACs (e.g., from press cake).⁵⁴ The DMAC method was recently modified for better results with cranberry juice extracts in terms of intermediate precision (RSD \leq 5%), repeatability (RSD \leq 3%), robustness (\leq 3%) and linearity (R² \geq 0.995).^{19,23} However, these colorimetric assays are not suitable for authentication of cranberry.
- d. The determination of the compounds obtained after a reaction of cranberry PACs with benzyl mercaptan (thiolysis) by HPLC-UV provides information about the structure and average size (degree of polymerization) of these molecules. While data on the ability to detect adulteration of cranberries with other PAC-containing materials are lacking, thiolysis can provide additional evidence for the authenticity of a material.
- e. Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is an analytic tool that is considered the MS method of choice for analysis of PACs with structural heterogeneity and complements spectrophotometric methods to quantify total PACs. MALDI-TOF MS provides superior mass resolution and allows measuring the relative ratios of A-type and B-type PAC in cranberry oligomers, useful when trying to accurately identify A-type PACs and other PAC compositional differences.⁵⁴⁻⁵⁶
- f. An assay analyzing DNA by PCR amplification of the *MatK* gene was recently presented by Herbst and co-workers. The assay successfully discriminated *Vaccinium* DNA from grape, apple (*Malus domestica*, Rosaceae) and pear (*Pyrus* spp., Rosaceae); however, the primers developed were unable to discriminate among *Vaccinium* spp.⁵⁷ With the selection of one/several appropriate genomic region(s), distinction among *Vaccinium* species should be possible by genetic means. Nevertheless, genetic methods cannot distinguish among plant parts and may be of limited use in processed materials. Depending on the nature and extent of the processing, cranberry extracts may not contain DNA of sufficient quality

to enable the identification of adulteration by genetic means.

- g. Recently, it has been suggested to utilize anti-adhesion activity of cranberry products in order to detect adulteration. The approach is based on the distinct 'A-Type' linkage proanthocyanidins (PAC's), which have been shown to inhibit the adhesion of *E. coli* bacteria to the walls of the urinary tract and do not occur in PACs from many other tannin-rich substances. However, adulterants containing A-type PACs, such as peanut skin extracts, may provide false positive results. In addition, the assay would neither identify the adulterant nor quantify the PAC concentration.⁵⁸

3.6 Perspectives: Considering the rapidly-growing market for cranberry products and the many promising health benefits of cranberry that still await further elucidation, it can be expected that cases of adulteration will not disappear anytime soon. Confusion about the exact content in certain cranberry products due to discrepancies in labeling of cranberry ingredients may also lead to instances where a consumer may not purchase the intended product. Therefore, a clear indication what the cranberry supplement is made from, (i.e., dried whole berries, juice, or press cake) is crucial to meet consumers' expectations about the product. A good source of information on quality control issues associated with cranberry, cranberry extracts, and the need

for differentiation of the various types of cranberry products is the extensive cranberry monograph of the American Herbal Pharmacopoeia.¹⁹

Emerging new analytical methods, as well as available methods' becoming more accessible and widely available, will contribute to making successful intentional adulteration not only more difficult but also less economically viable. Recent initiatives by organizations like USP (United States Pharmacopeia) and AOAC have renewed efforts to evaluate current analytical methods for cranberry to produce more widely accepted analytical tests. The efforts rely on stakeholder, i.e., industry, consensus[†] to choose the most suitable approaches for qualitative and quantitative analysis of cranberry and cranberry-derived ingredients.

4. Conclusions

The success and comparatively high cost of cranberry extracts has provided an incentive for economically-motivated adulteration with anthocyanin- or proanthocyanidin-rich extracts from other botanical sources, as well as synthetic colorants. Data on the problem are fragmented, and mainly based on a small number of published papers and case reports from industry members. While admixture or substitution with anthocyanin-containing extracts is readily detected, the inclusion of proanthocyanidins from, for example, grape seed, peanut skin, or pine species masquerading as cranberry extract is more difficult to detect and may require more advanced instrumentation,



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and/or a combination of analytical methods. In order to provide the desired health benefits from a cranberry supplement, it is imperative to follow an appropriate test protocol including adequate methods to verify the authenticity of all cranberry-derived ingredients.

†What was initially considered an adulterant turned out to be a contamination with an herbicide.³¹

‡For example, a June 2017 USP round-table discussion on cranberry standards with more than 40 participants from industry concluded to recommend the DMAC method with the procyanidin A2 standard for USP cranberry fruit juice concentrate/powder/dry extract monographs.

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Vaccinium macrocarpon
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REVISION SUMMARY

Version # , Author,	Date Revised	Section Revised	List of Changes
Version 1, T. Brendler, S. Gafner	n/a	n/a	none

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