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REVIEW

# Quantifying and characterizing proanthocyanidins in cranberries in relation to urinary tract health

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**Abstract** The "A-type" proanthocyanidins in cranberry fruit (*Vaccinium macrocarpon* Ait.) are bioactive components associated with prevention of urinary tract infections (UTI). Cranberry juice, fruit (fresh and dried), functional foods, and cranberry dietary supplements are promoted for prevention of UTI and for maintenance of urinary tract health (UTH), on the basis of their content of cranberry proanthocyanidins (c-PAC) with "A-type" interflavan bonds. With increasing consumer use of cranberries for maintenance of UTH and an expanding number of commercial cranberry products of different types, the availability of unified methods for measuring levels of c-PAC is important. This review discusses quantitative and qualitative analysis of c-PAC with "A-type" interflavan bonds in relation to their biological activity for UTI prevention. The integrity (including authenticity, standardization, efficacy, and safety) of cranberry fruit, juices, and dietary supplements may now be measured by using recent advances in mass spectrometry, liquid chromatography, production of c-PAC standards, and improved simple quantitative techniques.

**Keywords** Cranberry · DMAC · Mass spectrometry · Urinary tract health · Proanthocyanidins

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## Introduction

Urinary tract infections (UTI), regarded as among the most pervasive of bacterial infections, are a large economic and medical burden worldwide. In the United States alone, they account for over seven million outpatient hospital visits and one million emergency room visits, with an annual direct cost greater than $2 billion [1]. Cranberry (*Vaccinium macrocarpon* Ait.) fruit has been used for many years for prevention of UTI and for promotion of urinary tract health (UTH). Originally, the ameliorating effect was believed to be because of the acidity of the fruit, but in the last 20 years, research has focused on the effect of cranberry proanthocyanidins (c-PAC) or condensed tannins which are oligomers and polymers of monomeric flavan-3-ols, for example catechin and epicatechin. There are two common series of procyanidin dimers. The "B-type" series are dimers linked either in the C4–C6 or C4–C8 position whereas the "A-type" series are dimers linked in the C4–C8 position with an additional C2–O–C7 ether linkage (Fig. 1). c-PAC oligomers with a DP>2 may incorporate both "A-type" and "B-type" interflavan linkages. By extension of this definition, and for purposes of discussion, c-PAC oligomers that contain one or more "A-type" interflavan linkages in their structure are referred to as "A-type" c-PAC whereas c-PAC oligomers that contain only "B-type" interflavan linkages are referred to as "B-type" c-PAC. In-vitro studies have revealed that c-PAC, specifically those that contain "A-type" interflavan bonds [2], inhibit the adhesion of P-fimbriated uropathogenic *Escherichia coli* (*E. coli*) to uroepithelial cells

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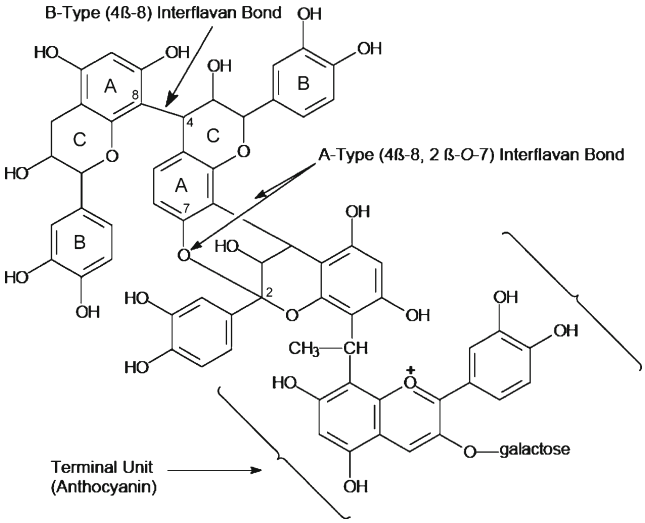


**Fig. 1** Representative structure of a c-PAC dimer linked to an anthocyanin through an ethyl group. Variation in degree of polymerization, position, and number of A-type versus B-type interflavan bonds and substitutions with anthocyanins leads to large structural heterogeneity among c-PAC oligomers [69]

[3–5], which is the first step in the infection process [6]. If bacteria are unable to bind to the bladder wall, they will not grow and progress to a UTI. This mechanism does not kill bacteria, so there is less chance of selection for resistant bacterial strains. c-PAC inhibit adherence of multi-drug-resistant *E. coli* strains to uroepithelial cells by up to 70 % [7]. Foods that contain only proanthocyanidins (PAC) with "B-type" interflavan bonds, for example chocolate and grapes do not seem to be effective in preventing UTI [2].

Researchers are currently examining the hypothesis that the urinary anti-adhesion effect may result from an innate immune response in the urinary tract that is induced by interaction of c-PAC with gut-associated lymphoid tissue. Research demonstrates that there are bacterial anti-adhesion effects in urine after consumption of cranberry products [2, 8–13]. Although dimers of c-PAC (procyanidin A2) have been detected in urine [14], c-PAC as a class of compounds are minimally absorbed because of non-hydrolyzable bonds between monomeric subunits and a propensity to bind proteins by hydrogen bonding [15, 16]. c-PAC complex with salivary glycoproteins, a process that causes astringency in the oral cavity when many fruits and beverages are ingested [17]. Astringency stimulates increased production of saliva, hypertrophy of the parotid gland, and a shift in salivary composition to proline-rich glycoproteins in rodents [18, 19]. Because of poor absorption, >95 % of c-PAC remain in the intestinal lumen during transit [20, 21], suggesting that beneficial dietary effects of c-PAC may occur via interactions at the mucosal surface of the gastrointestinal tract [22], for example, by affecting secretion of mucins, a class of glycoproteins, in the small intestine [23–25].

Evidence from a meta-analysis of clinical studies indicates that cranberry juice and dietary supplements reduce the number of symptomatic UTI over a 12-month period for women with recurrent UTI [26]. However, the usefulness of these clinical studies is limited because they used different doses and products, lacked compositional analysis of putative bioactive components, and lacked mechanistic guidance for selection of subjects and study design. Although it is now widely accepted that c-PAC are the putative bioactive component, research shows that there are substantial differences among products in c-PAC content and structural heterogeneity, including number of "A-type" linkages, which affect their bioactivity [27]. Cranberry fruit is typically made into 27 % juice drinks containing 36 mg PAC and dosed daily at 300 mL for recurrent UTI prevention, although consumption of cranberry encapsulated powder has become a popular alternative to juice. Accurate determination of the amount of PAC in cranberry products is required to ensure correct consumer product labeling, efficacy monitoring, shelf-life determination, and formulation of standardized materials for research studies. This review discusses quantitative and qualitative analysis of c-PAC with "A-type" interflavan bonds in relation to their biological activity for UTI prevention.

## Quantitative analysis of cranberry proanthocyanidins

### 4-(Dimethylamino)cinnamaldehyde (DMAC)

Rapid analytical methods to determine the concentration of c-PAC are essential to research on the integrity of cranberry products. In this regard, the 4-(dimethylamino)cinnamaldehyde (DMAC) method has great potential as a rapid analytical method [28]. DMAC is an aromatic aldehyde that reacts with flavan-3-ols and c-PAC to form a green chromophore with maximum absorbance at approximately 640 nm [29]. This wavelength effectively excludes the spectra of anthocyanidins which are a source of interference in other assays for quantification of c-PAC [30], for example the vanillin and the butanol–HCl assays. In strongly acidic solutions, DMAC is highly reactive via the formation of a reactive electrophilic carbocation [31]. Because of delocalization of the positive charge on the DMAC molecule and consequent reduced electrophilicity, the reaction is specific for phenolic compounds with meta-oriented di or trihydroxy phenols, as found in c-PAC [32, 33]. Post-column derivatization with DMAC has been used to detect PAC by high-performance liquid chromatography (HPLC) [29, 34, 35] and by chromatography on Sephadex G-25 [36] and to detect accumulation of PAC in plant seeds [37]. Cell-specific localization of PAC is possible by DMAC staining of plant tissues [38]. DMAC does not react with hydroxycinnamic acids, hydroxybenzoic acids, flavones, and flavonols [29, 36] and is more

accurate and sensitive for PAC than the acid–butanol and the vanillin assays [31].

## Adoption of DMAC method by cranberry industry

Currently, the cranberry industry is using DMAC in a standard method, with a procyanidin A2 dimer standard, to measure the c-PAC content of products. This method seems to be more accurate than other colorimetric methods, is inexpensive, rapid, and simple to perform, and is less likely to suffer from interference from other sample components [28]. Overall results suggest that DMAC may be useful for quantification of c-PAC, but may not be as accurate for comparing levels among different types of cranberry product, especially if they contain highly polymerized c-PAC. The degree of polymerization (DP) of c-PAC is affected by the extraction process. Powders that originate from cranberry juice tend to contain oligomers with a lower degree of polymerization (2–10 DP) than powders that originate from whole cranberry fruit or press cake (2 to >30 DP). Thus, the use of A2 dimer standard is biased toward providing more accurate quantification of products that contain lower c-PAC oligomers because these products more closely reflect the structure and reaction kinetics of the A2 dimer standard.

Recently, a multi-laboratory validation study was conducted to evaluate the use of procyanidin A2 standards in the DMAC method for determination of the c-PAC content of cranberry powders. The results of this study revealed intra-laboratory variation of 16 % and inter-laboratory variation of 32 % [28]. The greatest variation was evident for cranberry powders with a low total c-PAC content (<2 % *w*/*w*). Conditions that affect the DMAC reaction can be found in the publication of Wallace and Giusti, who investigated the effects of acid concentration, temperature, reaction time, water content, and DMAC concentration [30]. The laboratories participating in the validation study used a common procedure controlling for these conditions. However, it was noted that, depending upon the particular brand of plate reader, some laboratories used an automatic pipettor in the plate reader to add the DMAC solution and the laboratories were not given specific instructions about dilutions to use, both of which may account for the variability among laboratories [28].

## Improved accuracy of the DMAC assay as a result of the development of c-PAC standards

To enable more accurate quantification of c-PAC in cranberry powders and juices, Feliciano et al. [39] investigated the suitability of c-PAC isolated from cranberry press cake as a standard in the DMAC assay. Mass spectrometric analysis corroborated c-PAC composition and confirmed cranberry-specific structures, i.e. "A-type" PAC. It has been shown that PAC DP affects the stoichiometry of the reaction with DMAC, leading to underestimation of the PAC oligomer content when using a commercially available "A-type" dimer (procyanidin A2) as a standard [39]. Thus, use of monomers and procyanidin dimers as DMAC standards to estimate the c-PAC oligomer content is inaccurate and greatly underestimates the c-PAC content of cranberry products [31].

The slope of the c-PAC regression curve ($y$=0.1406$x$+0.0101) was a factor of 7.1 lower than that for catechin and a factor of 2.5 lower than those for procyanidin A2 and B2 standards (Fig. 2). Prior et al. [28] previously speculated that the response per unit weight may be lower for large polymeric PAC compounds than for monomers or dimeric procyanidins. Use of PAC oligomers as standards was suggested as a better approach for estimating the PAC content of chocolate and confectionary products rich in high-molecular-weight PAC [32]. The reaction seems to be limited to the $C_8$ position of the A-ring of PAC terminal units. The reduced response of c-PAC is likely to be because of the proportion of $C_8$ reactive sites on the PAC that are available to participate in the DMAC reaction. If, as previously reported, the $C_8$ terminal unit is the only position available for DMAC reaction, then as the DP of PAC increases, each additional flavan-3-ol adds weight but no additional DMAC reactivity.

Increasing the accuracy of the DMAC assay by development of a more robust standard will improve the marketing and regulation of cranberry products. Ideally, standards should express the complex nature of the specific food component being assayed. Commercially available flavan-3-ols and procyanidin dimers are not representative of the structural heterogeneity of c-PAC. As reference standards for PAC with higher DP are not yet commercially available, isolation of PAC from the food being studied is recommended to obtain accurate results [31]. For this specific application, a

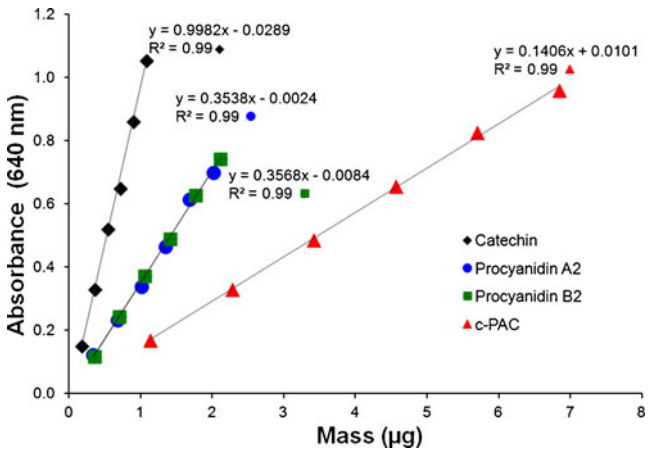


**Fig. 2** Regression curves for catechin, procyanidin A2, procyanidin B2, and c-PAC after reaction with 4-(dimethylamino)cinnamaldehyde [39]

standard for the DMAC assay would be most accurate when based on purification of c-PAC oligomers from cranberries [40].

A similar problem was encountered when use of gallic acid as a standard in the Folin–Ciocalteu assay resulted in the total polyphenol content of pomegranate (*Punica granatum* L.) powder being underestimated by up to 30 %. Pomegranates contain hydrolyzable tannins classified as gallotannins (1,2,4,6-tetra-*O*-galloyl-D-glucose and 1,2,3,4,6-penta-*O*-galloyl-D-glucose), ellagitannins (ellagic acid esters of D-glucose with one or more galloyl substituents), and the much less common gallagyl esters with glucose, for example punicalagin and punicalin. Pomegranates also contain oligomeric ellagitannins with two to five glucose core molecules cross-linked by dehydrodigalloyl esters. Collectively, these pomegranate hydrolyzable tannins contain approximately 20 % glucose by weight. The Folin–Ciocalteu reagent does not react with glucose and thus the choice of gallic acid as a standard leads to underestimation of total polyphenol content. This issue was addressed by developing and validating a pomegranate standard [41]. The standard contained a complex mixture of oligomeric ellagitannins that are found in pomegranate and improved the accuracy of quantification of polyphenols in pomegranate powder by the Folin–Ciocalteu assay in comparison with use of gallic acid.

### Thiolysis

Thiolysis and phloroglucinolysis are used to determine monomeric composition and average DP of PAC [42–47] and have also been used to determine the presence of "A-type" interflavan bonds [43, 47, 48]. However, several problems with these techniques are unresolved. Thiolysis and phloroglucinolysis depends on cleavage by auto-oxidation of the interflavan bond in the presence of strong acid and subsequent reaction of extension units with a nucleophile [42].

Thiolysis is performed on the basis of the assumptions that cleavage of the interflavan bond is complete, the reaction follows 1:1 stoichiometry [42], and flavan-3-ol benzylthioethers have the same molar absorptivities as their respective flavan-3-ol monomers [49]. Furthermore, other PAC sources, for example quebracho, lack 5-OH groups in their fisetinidol units, which makes determination of average DP by thiolysis impossible [50]. Therefore, use of thiolysis and phloroglucinolysis to estimate DP and the presence of "A-type" interflavan bonds may be inaccurate when these assumptions are incorrect. Other disadvantages of this method include the use of benzyl mercaptan, the most commonly used nucleophile agent, which is highly flammable and has an unpleasant odor, and the need to use strong acid at high temperatures.

### Mass spectrometric methods for characterization of PAC

#### *Application of LC–ESI–MS for quantification of c-PAC*

Atmospheric-pressure ionization [51] and liquid chromatography with electrospray ionization (LC–ESI) mass spectrometry (MS) have been used to characterize PAC oligomers in foods [52–63]. A method for analysis of procyanidins in cocoa, by normal-phase HPLC coupled with detection by UV absorbance, fluorescence, or mass spectrometry, was developed by researchers at M&M Mars [52, 55, 64]. Individual procyanidin oligomers up to a DP of 10 were isolated by preparative HPLC and their masses were confirmed by HPLC–MS and used as quantitative standards for fluorescence detection [65]. The isolated oligomers were then used as standards for quantification of PAC in samples of chocolate, wine, cranberry juice, and apples [53]. Although the method is an excellent example of the application of modern chromatography to the analysis of PAC, there are several problems in its application for quantification of PAC in a variety of samples. The research of Hammerstone et al. indicates that the PAC in chocolate are exclusively "B-type" procyanidin homopolymers with a DP of 1 to 10 [52]. Thus they are quite homogeneous when compared with c-PAC with "A-type" linkages.

Although ESI–MS is capable of detecting intact molecular ions with high molar mass (>100,000 Da), ESI is best suited to analysis of monodispersed biopolymers because of complications arising from the formation of multiply charged ions [66]. The use of oligomeric procyanidins from chocolate as standards for quantification of PAC from cranberries is therefore inaccurate. Accurate quantification of c-PAC would require preparation of appropriate standards by isolation and characterization of the individual oligomers, in a manner similar to that used for chocolate. However, because the number and structural diversity of c-PAC is much greater than for chocolate, isolation of each oligomer would be difficult. For instance, Yang and Chien [67] concluded that as the DP of galloylated procyanidins in grape seeds increased, separation and detection of individual isomers solely by normal phase HPLC becomes impossible.

#### *MALDI–TOF-MS qualitative analysis of c-PAC*

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF-MS) methods have been developed to characterize the structural features of PAC and other tannins in fruit used to produce dietary supplements [2, 27, 49, 68–77]. MALDI–TOF-MS is ideally suited to characterization of polydispersed oligomers and is regarded as the mass spectral method of choice for analysis of PAC with large structural heterogeneity [27, 78]. MALDI–TOF-MS produces only a singly charged molecular ion for each

parent molecule and enables detection of high mass with precision [66]. Use of MALDI–TOF-MS to characterize the heterogeneity of c-PAC is possible by using published structures of isolated dimers and trimers as determined by nuclear magnetic resonance (NMR) spectroscopy, MS, chemical degradation, and MS fragmentation studies. MALDI–TOF-MS separates molecular ions in the flight tube and enables baseline detection of a broad range of c-PAC oligomers over the DP range of 2 to 26 [27, 39].

*Determining ratios of "A-type" to "B-type" interflavan bonds by use of MALDI–TOF-MS*

A series of c-PAC which vary only in the ratio of "A-type" to "B-type" interflavan bonds produces a MALDI–TOF mass spectrum with overlapping isotope patterns for each individual oligomer. An understanding of the natural abundance of C, H, and O isotopes within c-PAC oligomers enabled Feliciano et al. [79] to develop a novel MALDI–TOF-MS isotope deconvolution method. They were able to quantify ratios of "A-type" to "B-type" interflavan bonds on the basis of theoretical isotope distributions and application of matrix algebra to the experimental spectra (Fig. 3). As an example, experimental and theoretical isotope clusters for c-PAC trimer $[M + Na]^+$ ions revealed remarkably high correlation of isotope patterns between predicted and estimated percentages of "A-type" and "B-type" bonds, indicating the method is simple, repeatable, robust, precise, and accurate. It was shown that the characteristic isotope clusters for the c-PAC were quantifiable from DP 2 to DP 11 (Fig. 4). The ability to separate overlapping isotopic patterns provides another method of determination of the structural diversity of c-PAC in food products and dietary supplements. Improved accuracy in determining "A-type" to "B-type" interflavan ratios can be accomplished by refinement of instrument settings, including:

1. low-mass gating (i.e., suppression of matrix ion adducts);
2. accelerating voltage;
3. reflectron voltage; and
4. intra and inter-well shot accumulation.

*MALDI–TOF-MS for detection of pigmented polyflavan-3-ols (i.e. derived PAC)*

Recent advances in mass spectrometry have also enabled the characterization of complex mixtures of anthocyanin–polyflavan-3-ol pigments in wine [80–83]. The pigmentation of such fruit as cranberries and grapes is primarily attributed to anthocyanins. However, results indicate that cranberries contain oligomeric pigments that are similar to structures found in wine (Fig. 5) [69]. Kennedy et al. [84] reported that anthocyanins are incorporated into PAC during fruit ripening. Although there are few reports of anthocyanin–polyflavan-3-ol oligomers occurring in fruit and unfermented beverages, there are well-documented accounts of complex pigments forming in such alcoholic beverages as red wine [85–87] and rose cider [88, 89]. During the aging and storage of red wines, anthocyanins are converted to new pigments by reactions with other phenolics, for example polyflavan-3-ols. Condensation of an anthocyanin and a polyflavan-3-ol via an ethyl bridge, arising from acetaldehyde, is one mechanism by which anthocyanin–polyflavan-3-ol oligomers may occur (Fig. 1) [85, 86, 90–93]. Acetaldehyde is found naturally in wine, either as a by-

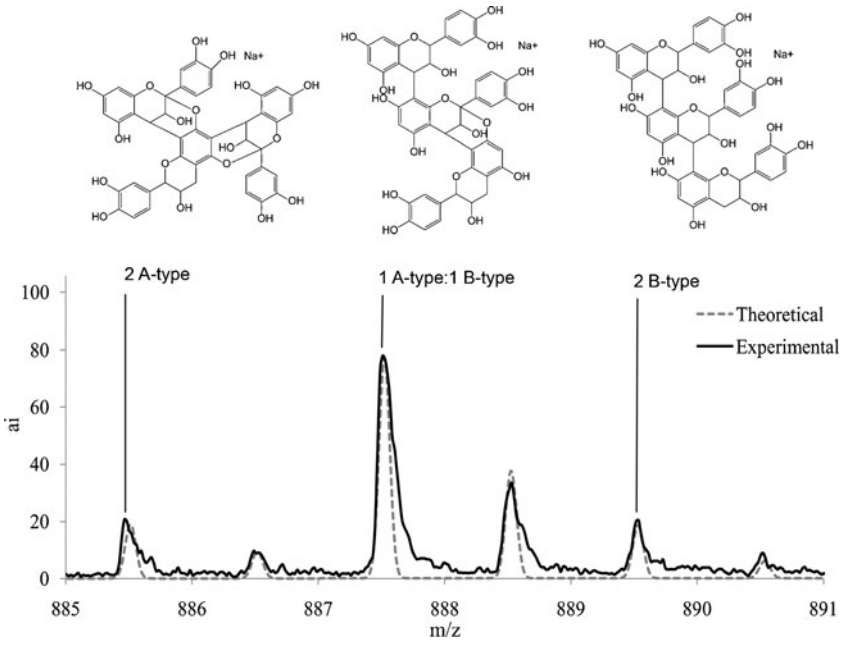


**Fig. 3** Comparison of a trimer PAC series MALDI-TOF mass spectrum obtained with c-PAC (experimental) and generated with IsoPro (theoretical) for the same composition as determined by the deconvolution method [79]

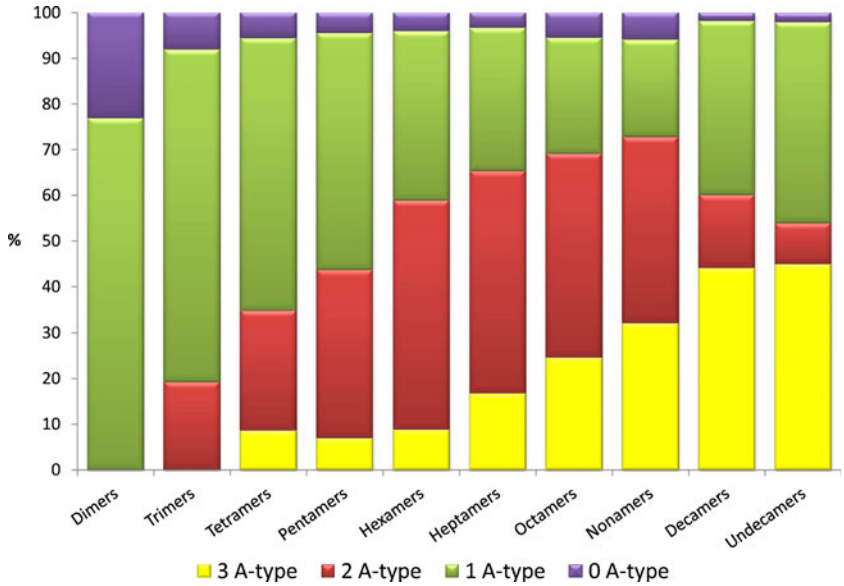


**Fig. 4** Percentage of A-type and B-type interflavan bonds in c-PAC from dimers to undecamers analyzed by using matrix algebra deconvolution for overlapping isotopic peaks after MALDI–TOF-MS analysis [79]

product of yeast metabolism or as an oxidation product of ethanol [85]. Using a rose cider model, Shoji et al. [88] elucidated the structure of such oligomeric pigments by high-resolution fast-atom-bombardment mass spectrometry (FAB–MS) and $^{1}H$ and $^{13}C$ (NMR) analysis. The dimeric pigments consisted of an anthocyanin linked by a $CH_3$–CH bridge to a flavan-3-ol. In addition, direct linkages between anthocyanins and PAC in wines have been described [87]. Anthocyanin–vinyl–PAC linkages have also been discovered and may occur as a result of the acetaldehyde condensation reaction [83].

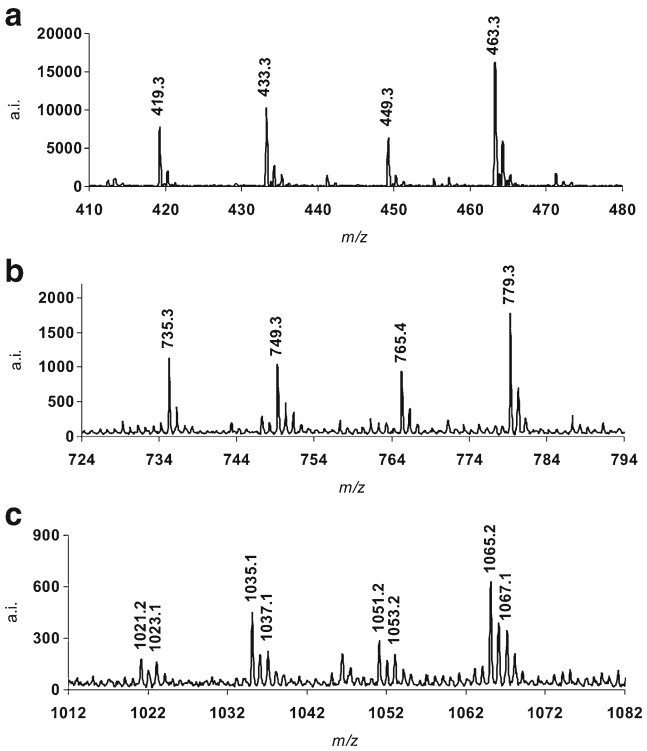


**Fig. 5** MALDI-TOF positive reflectron mode mass spectra of the anthocyanin–polyflavan-3-ol oligomers of "HyRed" cranberry fruit and spray-dried juice. (**a**) Anthocyanins $[M]^+$. (**b**) Anthocyanin linked to a single flavan-3-ol through a $CH_3$–CH bridge $[M]^+$. (**c**) Anthocyanin linked to a polyflavan-3-ol of two degrees of polymerization through a $CH_3$–CH bridge, containing either an A-type or a B-type interflavan bond $[M]^+$ [69]

*MALDI–FT-ICR-MS for improved detection of pigmented polyflavan-3-ols (i.e. derived PAC)*

MALDI–Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry is superior to MALDI–TOF-MS in its ability to resolve c-PAC that are substituted with anthocyanins [39]. Use of MALDI–TOF-MS revealed an unresolved mass series between the c-PAC oligomers. Analysis of the same sample by MALDI-FT-ICR identified a series of c-PAC substituted with anthocyanins, that were well resolved above baseline (Fig. 6). These compounds had *m*/*z* that were consistent with pyranoanthocyanidins and anthocyanidin moieties linked with PAC either directly or via a vinyl bridge, as described by Tarascou et al. [43].

FT-ICR-MS has also been used to study PAC in lychees [94], because of the high resolution and mass accuracy associated with the superconducting magnet, which is more stable than rf voltage control. When dealing with complex mixtures, for example c-PAC, higher resolution (narrow peak width) enables signals of two ions of similar *m*/*z* to be detected as distinct ions. FT-ICR MS differs substantially from other MS techniques, in that ions are not detected by hitting a detector, for example an electron multiplier. Rather they are detected by passing near detection plates. Masses are not resolved in space or time (TOF) but only by cyclotron (rotational) frequency that each ion possesses as it rotates in a magnetic field [95]. All ions are detected simultaneously over a given period of time. The attributes of FT-

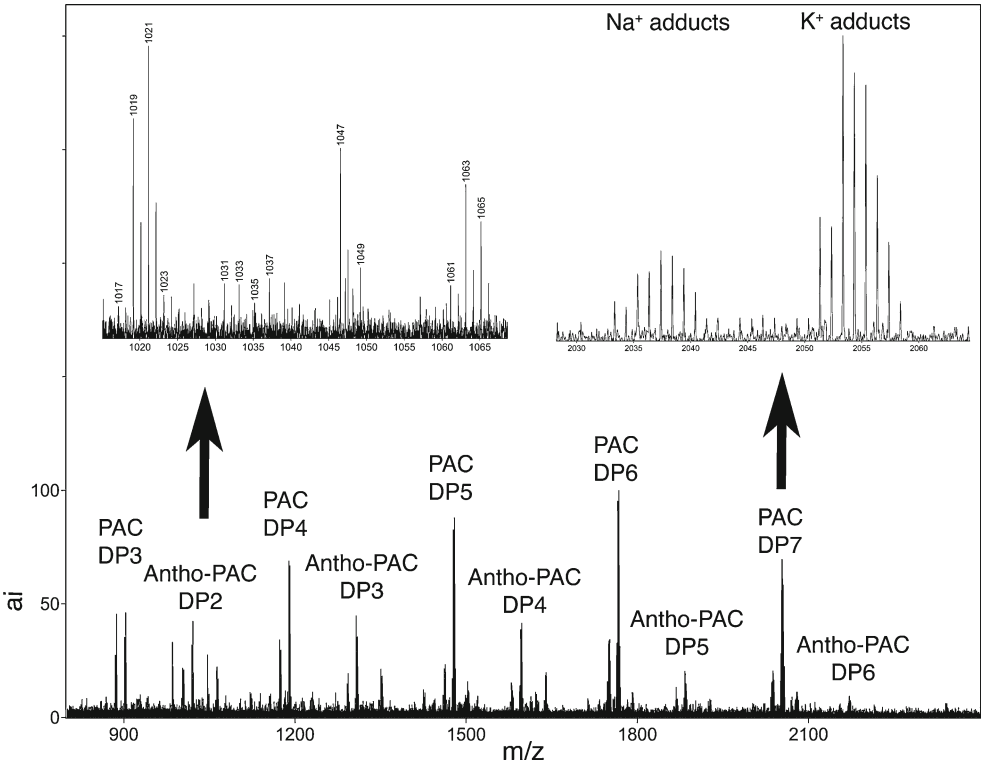


**Fig. 6** MALDI–FT-ICR mass spectrum, showing PAC and antho-PAC. *m/z*=1017: pyranocyanidin-arabinoside-A2, *m/z*=1019: pyranoanthocyanidin-arabinoside-DP2B, *m/z*=1021: A2-ethyl-cyanidin-arabinoside, *m/z*=1023: DP2B-ethyl-cyanidin-arabinoside, *m/z*=1031: pyranopeonidin-arabinoside-A2, *m/z*=1033: pyranopeonidin-arabinoside-DP2B, *m/z*=1035: A2-ethyl-peonidin-arabinoside, *m/z*=1037: DP2B-ethyl-peonidin-arabinoside, *m/z*=1047: pyranocyanidin-hexoside-A2, *m/z*=1049: pyranocyanidin-hexoside-DP2B, *m/z*=1061: pyranopeonidin-hexoside-A2, *m/z*=1063: pyranopeonidin-hexoside-DP2B, *m/z*=1065: A2-ethyl-peonidin-hexoside [39]

ICR MS make it ideal for high-resolution detection of PAC with large structural heterogeneity.

## Effects of cranberry processing on c-PAC composition

Conventional harvest and storage of cranberries for juicing involves cleaning, sorting, and then freezing in 500-kg bins [96]. The rate of freezing in these bins is highly variable and it may take longer than 60 days for the temperature at the center of the bin to reach −18 °C. During this period, the fruit will continue to respire and then shift to anaerobic metabolism until frozen. Some microbial fermentation may also occur. Both anaerobic plant metabolism and fermentation generate acetaldehyde [97], which participates in the reaction between anthocyanins and c-PAC [98]. Slow freezing increases solute-induced, osmotic, and structural fruit damage [99]. This damage causes anthocyanins from the exocarp to "bleed" into the mesocarp and most likely increases reactions between anthocyanins and c-PAC.

Research applying MALDI–TOF-MS indicates that fruit processing changes the structure of c-PAC (i.e., DP, types of interflavan bonds, and substitution with anthocyanins) [27, 69]. For instance, juice extraction affects the distribution of c-PAC in juice and press cake, with c-PAC in press cake of higher DP than those in juice.

Storage and juice extraction also increases the extent to which anthocyanins are linked to PAC through acetaldehyde derived covalent bonds in both juice and press cake [69]. Cranberry juice is acidic and interflavan bonds are susceptible to acid-catalyzed autoxidation that cleaves the bond, and intermediates form new bonds with anthocyanins. This rearrangement reaction is accelerated by heat. Appearance of c-PAC-anthocyanin oligomers with low degree of polymerization have been detected in cranberry juice by MALDI–TOF-MS [69]. Appearance of c-PAC–anthocyanin oligomers with a high degree of polymerization were detected in the press cake by MALDI–TOF-MS [27, 69].

## Conclusion

The National Institute for Standards and Technology (NIST), the National Center for Complementary and Alternative Medicine (NCCAM), and the Office of Dietary Supplements (ODS) are calling for validation and optimization of analytical methods for quantitative determination of bioactive components in dietary supplements. Polyphenolic compounds, for example c-PAC, are of specific interest to NIST, NCCAM, and ODS because polyphenols are present in many natural products of plant origin that are used in complementary and alternative medicine. Standardization of c-PAC levels and structures is critical for products used in clinical trials to ensure that participants are receiving bioactive test products, and to enable accurate meta-analysis of intervention trials. Accurate quantitative determination of c-PAC of all molecular sizes is important, not only to regulators and manufacturers, but to consumers who depend on the potency and bioactivity of the different cranberry products they purchase for maintenance of

urinary tract health. As is apparent from this review, the structural heterogeneity of "A-type" c-PAC makes accurate quantification a difficult undertaking, requiring use of appropriate standards and a knowledge of how c-PAC structure affects bioactivity. However, as a result of recent advances in MALDI-TOF and MALDI-FT-ICR mass spectrometry, generation of c-PAC standards, and improved understanding of the DMAC reaction kinetics, the integrity (including authenticity, standardization, efficacy, and safety) of cranberry fruit, juice and dietary supplements can now be determined.

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