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Fast and accurate detection of adulteration in cranberry ingredients through PAC analysis by MALDI-TOF

Cranberry is a widely used fruit-crop marketed as a food, beverage, and dietary supplement. Protecting this product against fraud and adulteration is of high economic importance. MALDI-TOF provides a fast, robust and confident monitoring of cranberry products for potential adulteration.

Abstract

Cranberry (*Vaccinium macrocarpon* Aiton) is an economically important fruit crop that is marketed as a food, beverage, and dietary supplement. Health benefits associated with cranberries include prevention of gastric ulcers and activities related to peridontal disease, cancer prevention, glycemic response, antiviral activity, and a reduction of cardiovascular risks. The health benefits associated with consumption of cranberry products are attributed to the presence of A-type proanthocyanidins (PAC). Further compounds of note in cranberries are PACs with a B-type linkage like anthocyanins, flavanols, organic acids sugars and vitamins.

Ratios of A-type PACs to B-type PACs in cranberry products may be used for authentication since cranberry products are susceptible to adulteration with lower cost sources of PAC. This study reports the result of a close cooperation with the Association of Official Agricultural Chemists (AOAC) to identify stakeholder concerns, set guidelines based on Standard Method Performance Requirements (SMPR), and develop an **AOAC First Action Method (2019.05)** to identify A-type PAC in cranberry-based foods and dietary supplements by MALDI-TOF (Matrix-Assisted Laser Desorption/ ionization Time-of-Flight mass spectrometry).

Keywords:
MALDI-TOF, food adulteration, autoflex maX

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Introduction

The cranberry market has seen a steady growth in sales in the past decade. Cranberry products are among the most popular herbal dietary supplements in the US with more than $80m sales (2016). Adulteration is identified as a serious issue. Jointly with AOAC, a working group was established to address the demand for proving authenticity analytically. The AOAC working group put out an SMPR and call for a method to identify A-type PAC in cranberry products. CPS (Complete Phytochemical Solutions LLC, Cambridge, WI) submitted a MALDI TOF MS method using the Bruker autoflex Max MALDI-TOF in response to the SMPR, and the AOAC granted first action status to the method. MALDI-TOF is considered the MS method of choice for the analysis of PACs with structural heterogeneity and compliments spectrophotometric methods to quantify total PACs. It provides high mass resolution and allows for a very rapid measurement of the relative ratios of A-type and B-type PAC in cranberry oligomers, useful to accurately identify A-type PACs and other PAC compositional differences in a high throughput fashion. As well, MALDI-TOF is an extremely robust technology with maximum uptime, easy to use by everyone even without a high level of mass spectrometric experience and is highly cost-effective. The study was performed on a Bruker autoflex Max which has a market-leading combination of mass resolution, mass accuracy and analysis speed, which is ideally suited for the demands of robust, high-throughput analyses with high spectral quality. The subsequent adoption and modification of the MALDI-TOF method on the autoflex Max enabled to the development of an application that goes beyond just identification of A-type PAC. Semi-quantitative data can be provided on the relative ratios of A-type PAC to support the quality control of ingredients and finished products, i.e., identify adulteration down to a very low percentage of illegal additions. Furthermore, the method can be used to determine relative proportions of two known ingredients (modeled by purified PAC from cranberry and apples) to show how formulated ingredients and products could be characterized.

Materials and methods – Sample preparation

PAC isolated from cranberry and apple fruit were mixed to obtain 21 different mixture ratios ranging from 0 to 95% by weight. [1,2]. The MALDI-TOF analysis was performed on a Bruker autoflex maX in positive ionization reflectron mode at m/z 800 – 3500. 0.5 µL of samples were spotted onto the stainless-steel MALDI plate followed by addition of 1 µL of the matrix DHB (2,5-dihydroxybenzoic acid, at a concentration of 1.3 M in methanol). Signals from 200 laser shots were accumulated at 10 different locations (2000 shots in total) across the individual sample well. PAC are detected as $[M+Na]^+$ due to the natural abundance of sodium. No additional sodium was added.

Data analysis

Deconvolution of overlapping isotope distributions of PAC at each degree of polymerization was accomplished by methods of matrix algebra [3]. Statistical treatment such as principal component analysis (PCA), was utilized for the discrimination and classification of the various samples.

Results and discussion – Cranberry PAC

Figure 1 shows a typical MALDI mass spectrum of cranberry PACs These appear in different multimers ranging from dimers to undecamers. All signal peaks are fully isotopically resolved, providing exact monoisotopic masses. Figure 2 shows the simulated mass spectra of dimers of the two A- and B-types of PAC. The A-type dimer has a monoisotopic mass of 599 Da (fig 2a) while the B-type dimer has 601 Da due to the addition of two hydrogen atoms (fig 2b) Fig 2c shows a simulation of a 1:1 mixture of A- and B-type PAC with an equal signal intensity. Both species can be distinguished by the 2 Da mass difference and show only minimal overlap in their isotopic patterns.
Pure cranberry contains a high percentage of A-type PAC while other fruits like apple have a lower abundance of A-type versus a higher abundance of the B-type. These relative ratios of A- and B-types can be used to distinguish both and to develop a reliable AOAC First Action MALDI-TOF MS Method (2019.05) for identifying A-type PAC bonds with a probability of >90% and a confidence of 95% for following potential cranberry adulteration.

Figure 1
MALDI-TOF spectra for Cranberry PAC
Mass spectrum and associated mass list of cranberry proanthocyanidins in positive reflectron mode

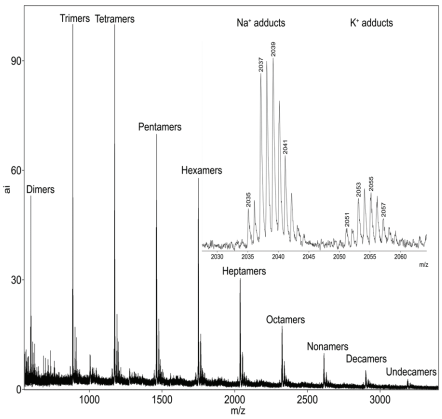
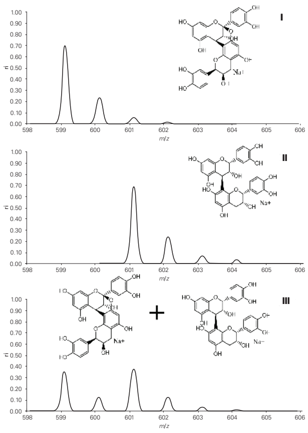


Figure 2
Isotopic Distribution of PAC
Theoretical isotopic distribution of procyanidin A2 (I), procyanidin B2 (II) and 1:1 ratio of procyanidins A2 and B2 (III)



A-type Bond
Monoisotope mass at m/z 599. Two Hydrogen (Δ2 amu) less than B-type

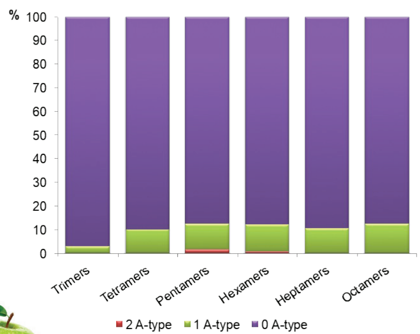
B-type Bond
Monoisotope mass at m/z 601. Two Hydrogen (Δ2 amu) more than A-type

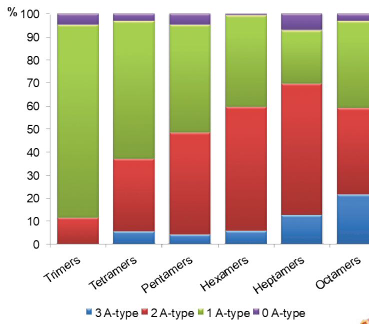
1:1 Ratio of A and B types
Overlap of both isotope patterns

Deconvolution using matrix algebra

Deconvolution by methods of matrix algebra was developed to determine the percentage of A-type to B-type interflavan bonds of each apple and cranberry PAC individually. Figure 3 shows the abundance of A-type species in apple (left) and cranberries (right) for the different detected multimers, here trimers to octamers. While apples show a significantly lower abundance of A-type PAC (mostly 0 A-type, in purple), the cranberries consist of almost only A-type in different ranges from to 1x A-type (green) to 3x A-type (blue), depending on the multimer number. These differences can be used in a statistical analysis not only to separate cranberries from apples but also to determine the percentage of mixing apple into cranberry products.

Figure 3
Isotopic Distribution Results
Deconvolution of apple and cranberry A-type PAC content

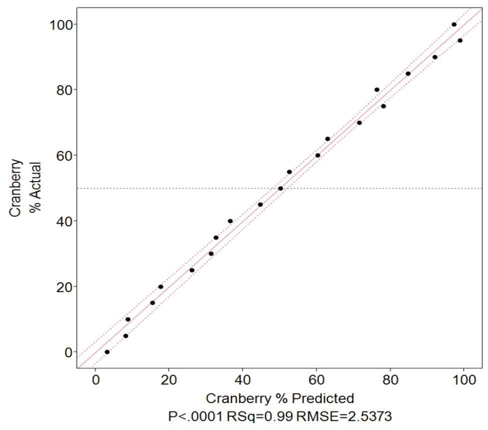




Deconvolution analysis

Figure 4 shows the deconvolution of the MALDI-TOF analysis of 21 different ratios (0 to 95%) of isolated cranberry and apple PAC by the statistical tools. The experimentally determined percentage of cranberry PAC (x-axis) follows the perfect diagonal to the actual values (y-axis): the experimentally determined ratios are within a deviation of 3.9% of actual mixed ratios.

This proves this method to be sufficiently accurate for the practical purpose of testing cranberry products for food fraud even at very low mixing ratios of other fruits



Conclusion

- A First Action MALDI-TOF MS Method (2019.05) for identifying A-type PAC bonds with a probability of >90% and a confidence of 95% was developed.
- MALDI-TOF Polyphenol FingerprintingSM enables reliable detection of cranberry PAC adulteration and proved to be a powerful analytical technique for the food, beverage, and dietary supplement markets.
- MALDI-TOF MS is a fast, robust and easy to use methods for food authenticity and consistency in manufacturing.

References

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