Workflow for Rapid Identification of Oligomeric Polyphenols in Foods, Beverages and Dietary Supplements Using Advanced Chemometric Software

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Christian G. Krueger[1]; Jess D. Reed[1]; Pierre Mbarushimanar[2];
Complete Phytochemical Solutions, LLC, Cambridge, WI, USA; [2]Bruker Daltonics, Billerica, MA, USA.

Introduction

Oligomeric polyphenols, including proanthocyanidins (PAC) and hydrolysable tannins, are heteropolymers that vary in degree of polymerization, nature of phenolic substitutions and terminal units. Oligomeric polyphenols are found in many foods, beverages and dietary supplements that are associated with, and promoted for their health benefits. MALDI-TOF MS is uniquely suited to characterize complex polymeric mixtures. We have developed a rapid workflow for the identification of oligomeric polyphenols in botanicals. The workflow involves sample clean-up with C18 pipette tips, deionization with strong cation exchange tips, spiking with Cs^+, MALDI-TOF MS data acquisition and data processing with advanced chemometric software.

Methods

- Samples were prepared with C18 pipette tips, deionized with strong cation resin tips and spiked with cesium trifluoroacetate (0.01M).
- MALDI-TOF analysis was performed on an Autoflex Max (Bruker Daltonics) in positive ionization reflectron mode (800 – 3500 Da).
- Samples (0.5 μL) were spotted on the stainless steel target followed by addition of 1.0 μL of the matrix 2,5-dihydroxybenzoic acid (DHB).
- Data analysis and processing with advanced chemometric software can be visualized in the form of Kendrick Mass Defect (KMD) plots.
- Statistical treatments using advanced in-house developed **Polyphenol Fingerprinting™**, such as principal component analysis (PCA) were utilized for the discrimination/classification of the samples.

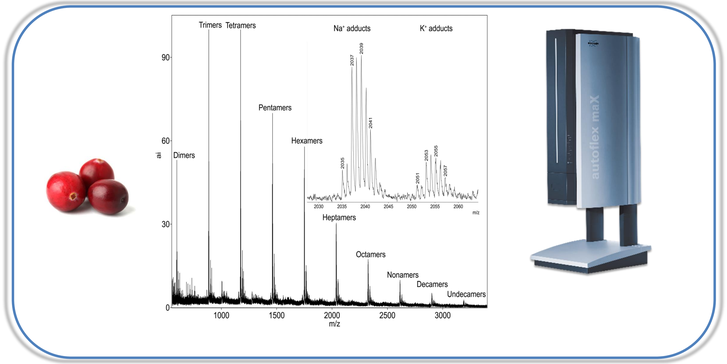

Fig. 1 MALDI-TOF mass spectrum of cranberry proanthocyanidins in positive reflectron mode, showing a series from dimers to undecamers.

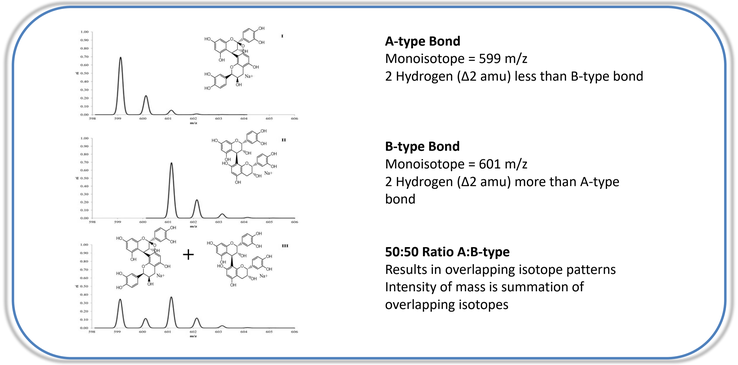

Fig. 2: . Isotopic distribution of procyanidin A2 (I), procyanidin B2 (II) and 1:1 ratio of procyanidins A2 and B2 (III). Cranberry PAC contain predominantly 1 or more A-type bonds at each degree of polymeriazation.

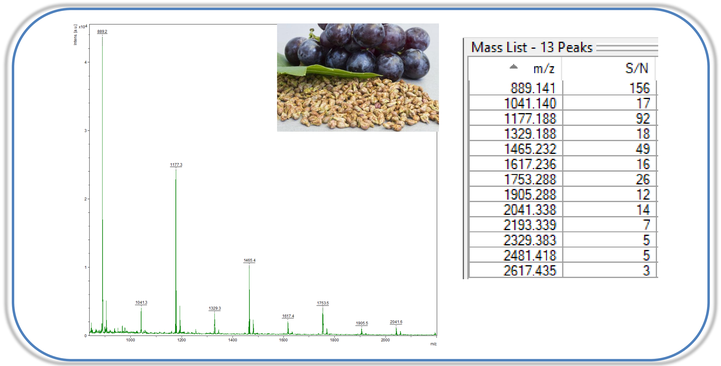

Fig. 3: MALDI-TOF mass spectrum and associated mass list of grape seed extract proanthocyanidins in positive reflectron mode.

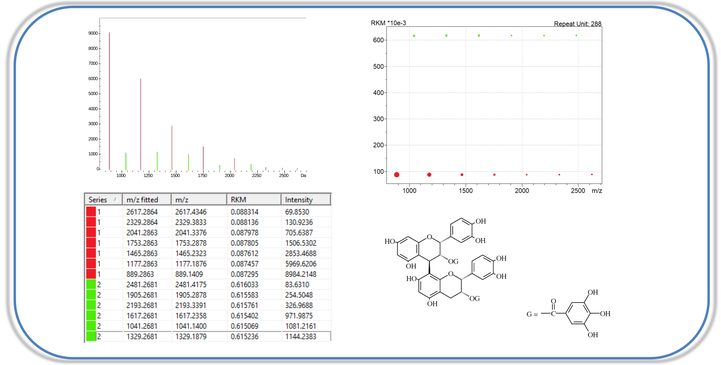

Fig. 4: Advanced chemometric analysis of grape seed extract mass spectra identified an oligomeric PAC series differing in number of catechin units (288 amu) with (green) and without (red) gallic acid end group. Residual Kendrick Mass Defect plot graphically represents the two series.

Results

- Cranberry extract contains an oligomeric PAC series differing in the number of catechin units (Δ288 amu) and containing predominantly 1 or more A-type bonds at each degree of polymerization. The presence of A-type bonds is one principal component that differentiates cranberry PAC from grape seed PAC.
- Grape seed extract contains an oligomeric PAC series differing in the number of catechin units (Δ288 amu) containing predominantly all B-type interflavan bonds and a second oligomeric PAC substituted with gallic acid (Δ152 amu).

References

- Esquivel-Alvarado *et. al* Journal AOAC International. 2021. 104(1):223-231
- Esquivel-Alvarado *et. al* Food Chemistry. 2021; 336:127667

Conclusions

- MALDI-TOF mass spectral Polyphenol Fingerprinting™, enables rapid identification of oligomeric polyphenols in foods, beverages and dietary supplements
- Peak lists generated during post acquisition data processing are uploaded into advanced chemometric software packages where average molecular weights M_n and M_w as well as observed end group combinations can directly be compared with botanical reference materials.
- Visualization in the form of Kendrick mass defect (KMD) plots allows for easy data interpretation and subsequent botanical categorization.

MALDI / Polyphenol Fingerprinting™

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