BERRY-BASED PRODUCTS CLASSIFICATION BY FIA–HRMS FINGERPRINTING AND CHEMOMETRIC ANALYSIS

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1. INTRODUCTION

In recent years, nutraceuticals prepared with cranberry (*Vaccinium macrocarpon*) have gained special attention because of their beneficial effects on human health (*e.g.*, antioxidant activity and antimicrobial activity against bacteria involved in a wide range of diseases), which are mainly attributed to the high content of specific polyphenols in cranberry. However, these products present a risk of fraud consisting of the total or partial substitution of cranberry extracts with cheaper or more abundant fruit extracts.

In this study, flow injection analysis coupled to high-resolution mass spectrometry (FIA–HRMS) fingerprinting was proposed as a rapid high-throughput analytical approach to address the classification of berry-based products through chemometrics, focusing on cranberry-based products authentication. Thus, several berry-based natural products and cranberry-based nutraceuticals were analysed. After both negative and positive electrospray ionisation FIA–HRMS sample analysis, four different data matrices —including negative, positive, low-level data fusion (LLDF), and mid-level data fusion (MLDF) FIA–HRMS fingerprints— were then subjected to principal component analysis (PCA) and partial least squares regression-discriminant analysis (PLS-DA).

2. INSTRUMENTATION

FIA SYSTEM

Accela autosampler and Accela 1250 Pump (Thermo Fisher Scientific)

Injection volume: 10 µL
Carrier phase:
50% water 0.1% formic acid (*v/v*)
50% acetonitrile
Flow rate: 150 µL·min^{-1}

MASS SPECTROMETRY

Q-Exactive Orbitrap (Thermo Fisher Scientific)

Ionisation source: H-ESI (±)
Acquisition mode: Full Scan
***m/z*:** 100 - 1500

S-Lens: 50 V
Vaporizer T: 250 °C
Sheath gas: 40 a.u.
Spray voltage: ±3.0 kV
Capillary T: 350 °C
Auxiliary gas: 12 a.u.

DATA ANALYSIS

- mzMine 2.53 software [1]
- Solo 8.6 chemometrics software (Eigenvector Research)

3. SAMPLES AND SAMPLE TREATMENT

49 Cranberry (CB)

Natural products
14 juices
10 raisins
4 fruits

Nutraceuticals
11 capsules
4 extracts
4 sachets
2 syrups

12 Raspberry (RB)

Natural products
12 fruits

18 Blueberry (BB)

Natural products
12 juices
6 fruits

25 Grape (G)

Natural products
13 juices
8 raisins
4 fruits

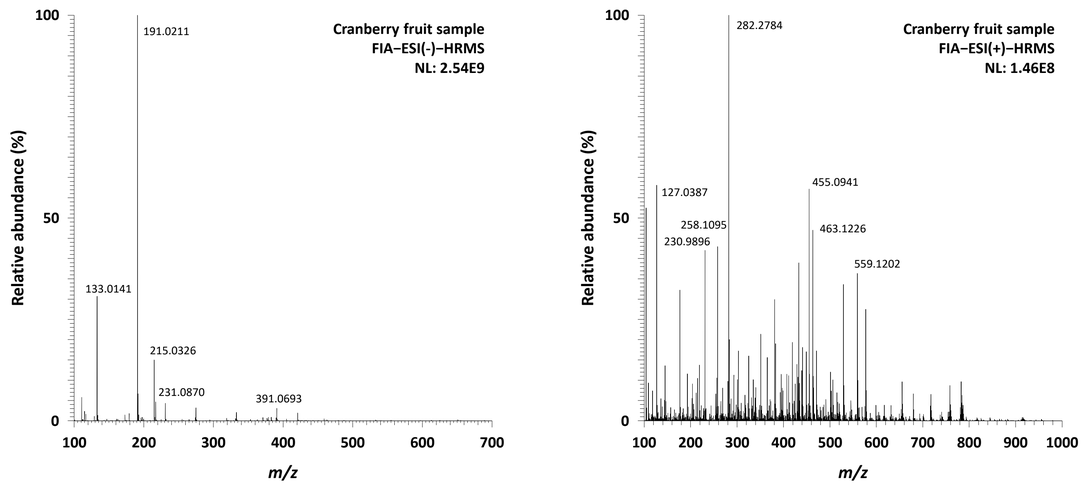
Grinding → Lyophilisation → Ultrasound-assisted solid-liquid extraction (USLE) → Centrifugation (15 min, 3500 rpm) → FIA–HRMS analysis

Fruits and raisins

Juices and nutraceuticals

0.10 g sample
+
10 mL acetone:water:hydrochloric acid 70:29.9:0.1 (*v/v/v*)
+
Sonication (10 min)

4. FIA–HRMS BERRY FINGERPRINTS



Negative (on the left) and positive (on the right) ionisation mode FIA–HRMS fingerprints for a cranberry fruit sample. MS spectrum are displayed in the *m/z* range from 100 to 700 and from 100 to 1000, respectively.

Remarkable differences in the FIA–HRMS fingerprints depending on the berry sample matrix were obtained.

5. CHEMOMETRIC ANALYSIS

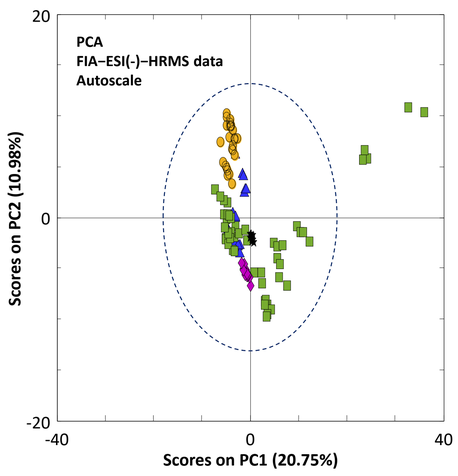
DATA MATRICES (samples × variables)

1. Negative ionisation mode FIA–HRMS fingerprints: 104 × 253
2. Positive ionisation mode FIA–HRMS fingerprints: 104 × 1012
3. LLDF FIA–HRMS fingerprints: 104 × 1265

   Concatenation of negative and positive ionisation mode FIA–HRMS fingerprints
4. MLDF FIA–HRMS fingerprints: 104 × 40

   Selection of the 10 most discriminant variables —those presenting the highest selectivity ratio and VIP values— in the individual PLS-DA models of each berry matrix against the others (*e.g.*, cranberry *vs*. others)

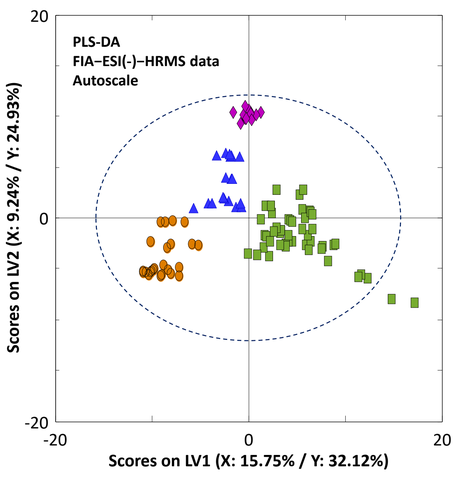
PCA AND PLS-DA



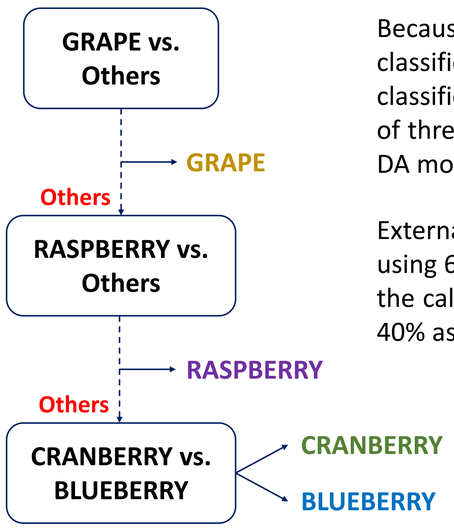
★ QC ■ Cranberry ◆ Raspberry ● Grape ▲ Blueberry

PCA allowed the identification of sample groups and trends as well as checking the correct behaviour of QC samples.

An excellent sample classification according to berry matrices was observed in the PLS-DA scores plots.



CLASSIFICATION DECISION TREE AND EXTERNAL VALIDATION



Because of the complexity of the classification under study, a classification decision tree consisting of three consecutive rule nodes (PLS-DA models) was proposed.

External validation was carried out, using 60% of the analysed samples as the calibration set and the remaining 40% as the validation set.

External validation parameters —class sensitivity (%), class specificity (%), and global accuracy (%)— obtained by the proposed classificatory strategy for all the used data matrices.

	CRANBERRY		RASPBERRY		BLUEBERRY		GRAPE		
	SENS.	SPEC.	SENS.	SPEC.	SENS.	SPEC.	SENS.	SPEC.	ACCURACY
FIA–HRMS (-)	85.0	100.0	100.0	100.0	57.1	100.0	100.0	100.0	85.7
FIA–HRMS (+)	75.0	95.5	100.0	100.0	85.7	100.0	100.0	100.0	85.7
LLDF FIA–HRMS	80.0	90.9	100.0	100.0	71.4	100.0	100.0	100.0	85.7
MLDF FIA–HRMS	95.0	100.0	100.0	100.0	85.7	100.0	100.0	100.0	95.2

6. CONCLUSIONS

- FIA–HRMS fingerprinting, combined with chemometrics, proved to be a suitable analytical approach to address berry-based products classification, independently of their type.
- The classification decision tree strategy provided excellent class sensitivity, class specificity, and global accuracy values in the external validation, especially when applying the MLDF approach.
- Further investigation through liquid chromatography coupled to high-resolution mass spectrometry (LC–HRMS) of the found discriminant ions could lead to their identification.

7. REFERENCES

[1] Pluskal, T.; Castillo, S.; Villar-Briones, A.; Orešič, M. (2010). Mzmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics, 11, 395. https://doi.org/10.1186/1471-2105-11-395

8. ACKNOWLEDGEMENTS

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