High resolution mass spectrometry based metabolomics - an effective tool for fruit products authentication

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UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

EUCHINASAFE 中欧食品安全

Red and blue berries

- **Source of bioactive substances**
  - Flavonoids (anthocyanins, flavonols, flavanols)
  - Tannins (proanthocyanidins)
  - Phenolic acids

Health promoting properties

- Antioxidant activity
- Anti-inflammatory activity
- Prevention of weight gain
- Preventive and therapeutic effects against UTI

Popular high value fruit products

Lingonberries vs. Cranberries

Vaccinium vitis-idaea

- " Rock or mountain cranberry "
- Berries from North and Central Europe, Balstic countries
- Size of berries: 5-10 mm
- **Price of fresh berries: 1.3 €/kg**
- Up to twice the total phenolics and proanthocianidins content (prevention of urinary tract infection)

Vaccinium macrocarpon

- " Large or American cranberry "
- Major commercial crop in the United States and Canada
- Size of berries: 9-16 mm
- **Price of fresh berries : 0.4 €/kg**
- More prelevant A-type proanthocianidins

Sensory and phytochemical simmilarities may lead to confusion or intetional substitution of the two crops

Adulteration practices: partial or total replacement of lingonberries by cranberries

Adulteration of red and blue berries

- **Substitution** of high value berries (lingonberry, bilberry or black currant) by less expensive ingredients (swamp cranberry, chokeberry)
- **Admixtures** were reported mainly in case of
  - Fruit juices
  - Jams and purees
  - Food supplements

➡ **Determination of fruit authenticity**

- **Quality control**
- **Consumer protection**
- **Unfair competition avoidance**

Aim of the study

To investigate the applicability of HRMS baaed

METABOLOMIC FINGERPRINTING

for berries authentication (according to species)

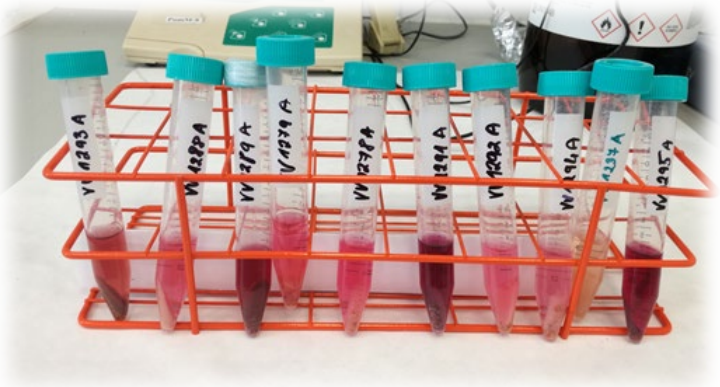
Analyzed samples

11 fruit species

altogether **90** authentic samples

(frozen and dried samples)

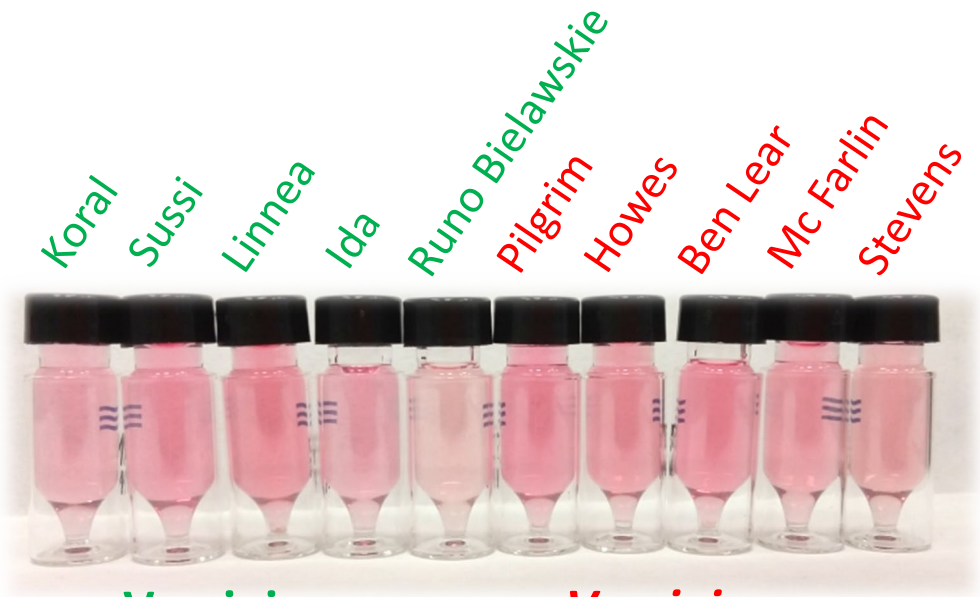
- Cranberry
- Lingonberry
- Raspberry
- Red currant
- Strawberry
- Swamp cranberry
- Bilberry
- Black currant
- Blackberry
- Chokeberry
- Elderberry



Optimization of sample preparation

1st STEP
Polar extract

1 g of sample extracted with 5 mL of **methanol**
Ultraturrax (1 min)
Centrifugation (5 min, 5 °C, 10,000 rpm)



2nd STEP
Non-polar extract

repeated extraction of solid residue with 5 mL of **hexane/2-propanol (50:50, *v/v*)**
Vortex (1 min)
Centrifugation (5 min, 5 °C, 10,000 rpm)

U-HPLC-HRMS/MS conditions

U-HPLC-Thermo Dionex UltiMate 3000

- **Column:** HSS T3

(2.1x100 mm, 1.8 µm)

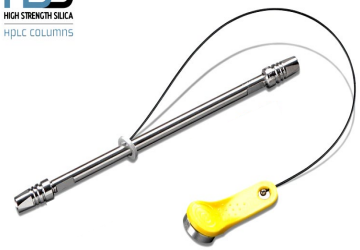
Column temperature: 40 °C

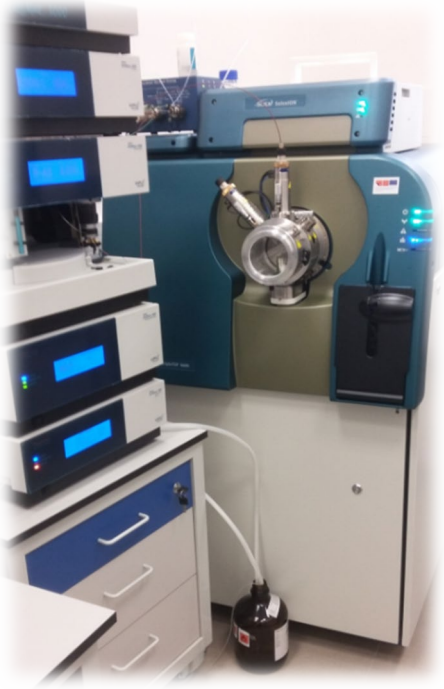
- **Mobile phase:**

A: 5 mM ammonium formate in H_2O

B: 5 mM ammonium formate in MeOH

- **Injection volume:** 4 µL

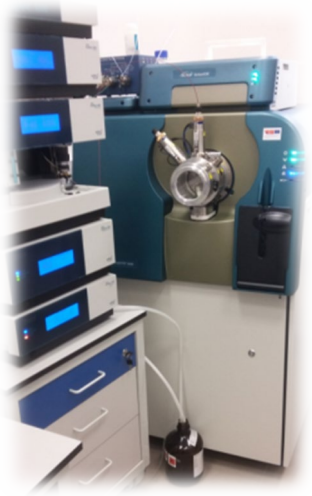


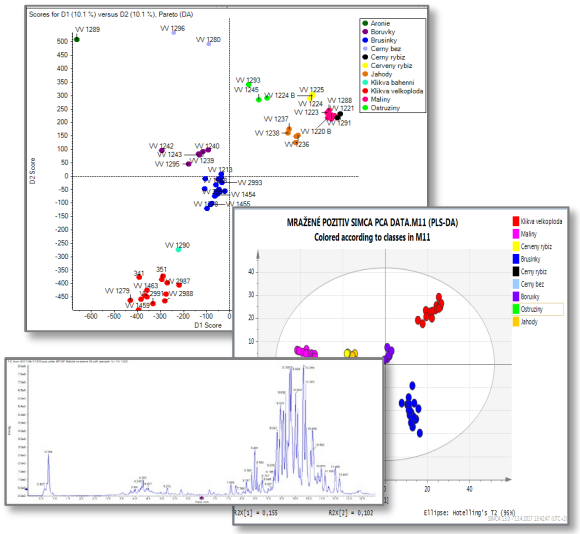


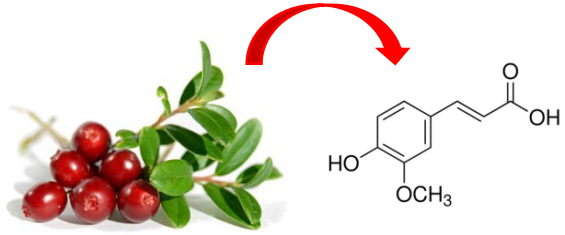
HRMS(MS/MS)-TripleTOF™ 6600 (Sciex)

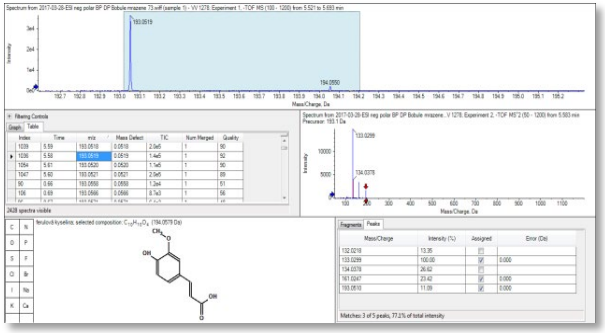
- *m/z* range: 100-1200
- Ionization technique: ESI +/-
- Ion source temperature: 480 °C
- Capillary voltage: +5000 V/-4500 V
- Collision energy: 35 eV (+/- 15 eV)

Metabolomic fingerprinting strategy







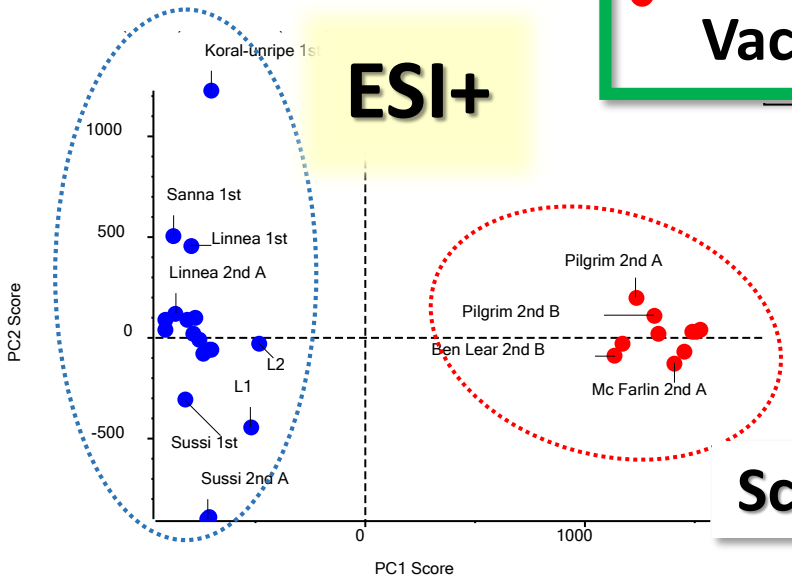


Extract analysis → **Chemometric data processing** → **Marker identification**

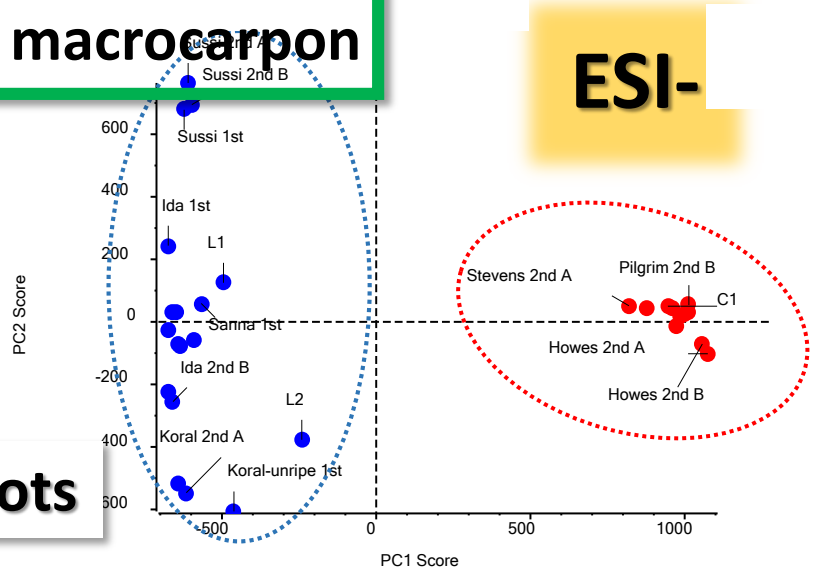
Multivariate analysis of polar extract: PCA

- Vaccinium vitis-idaea
- Vaccinium macrocarpon

ESI+

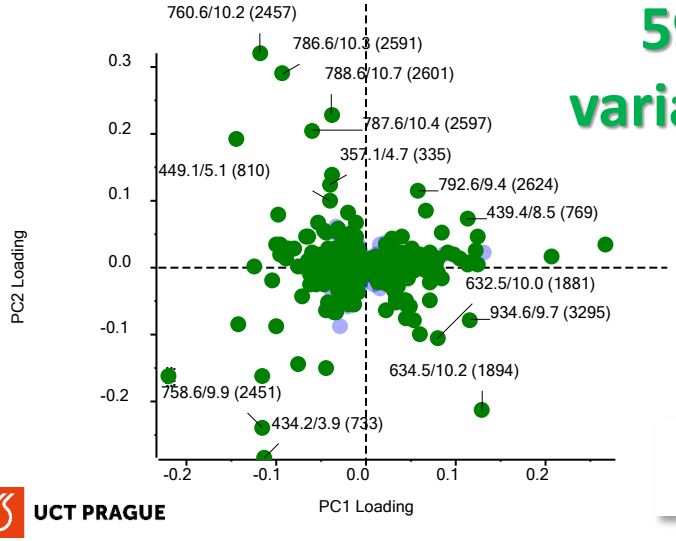


ESI-

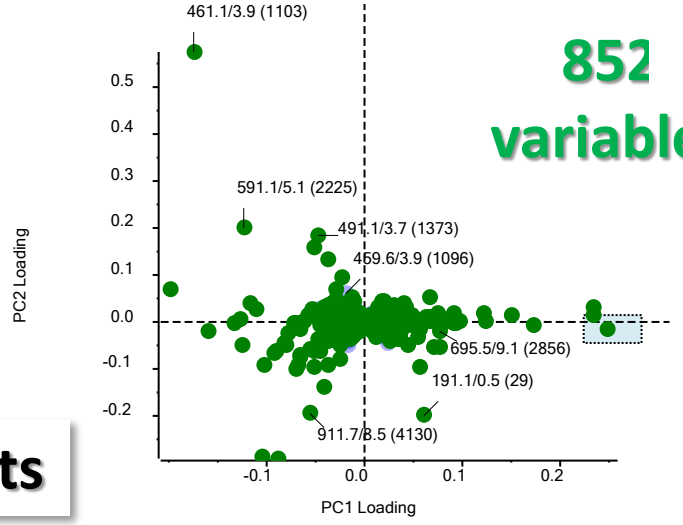


Scores plots

593 variables

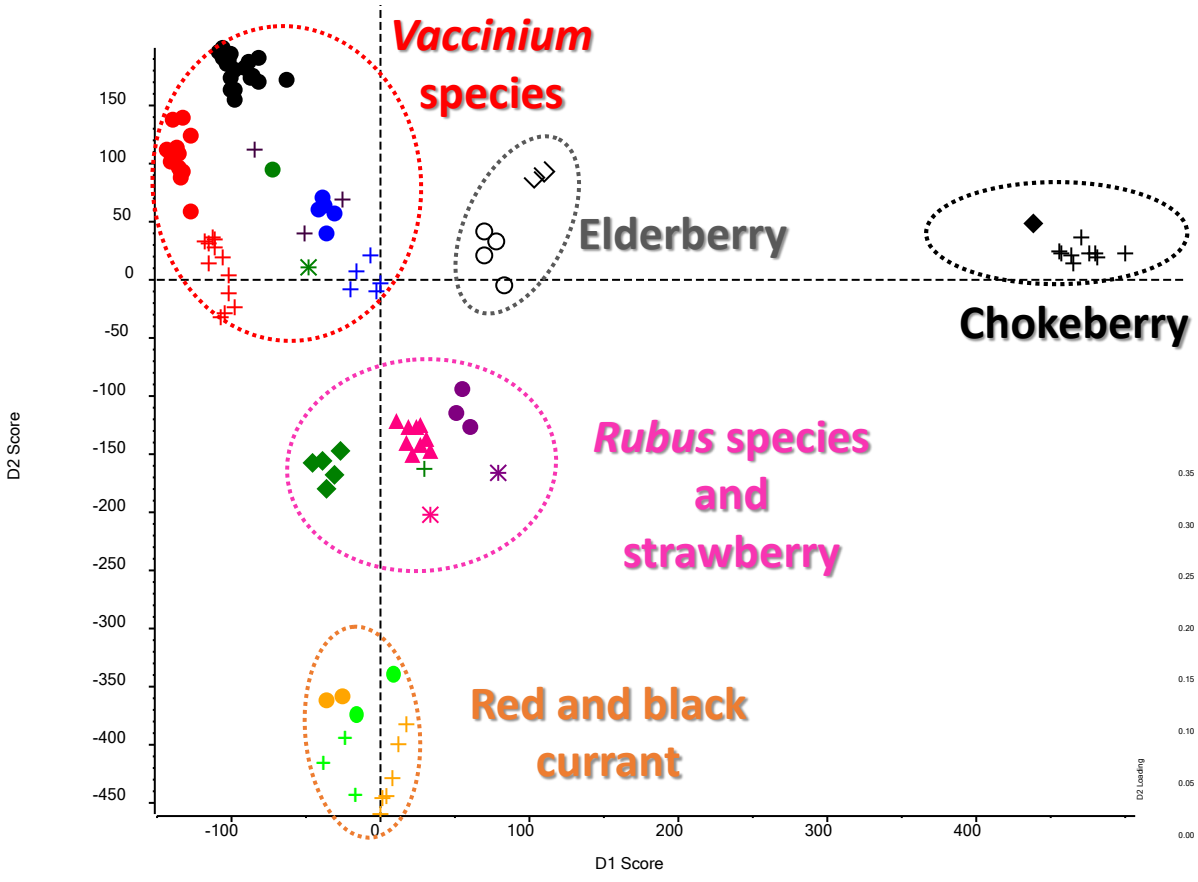


852 variables



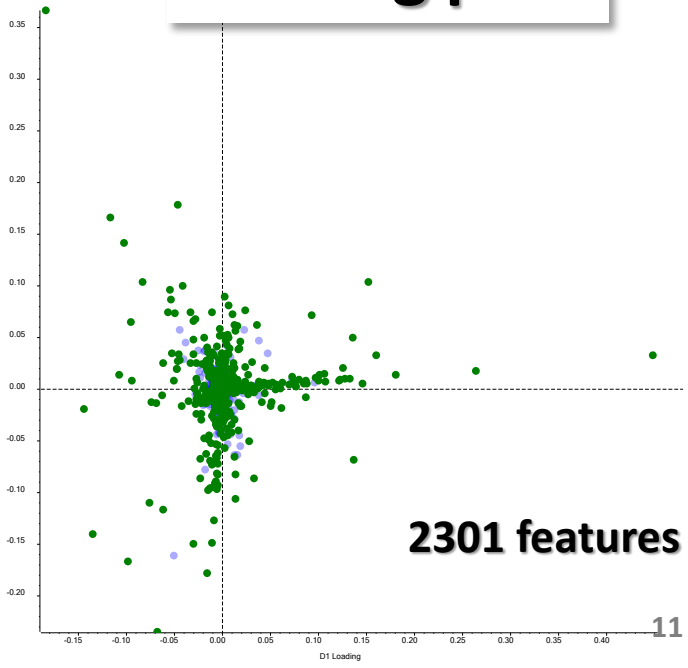
Loadings plots

Multivariate analysis of polar extract (ESI-) PCA-DA



Score plot

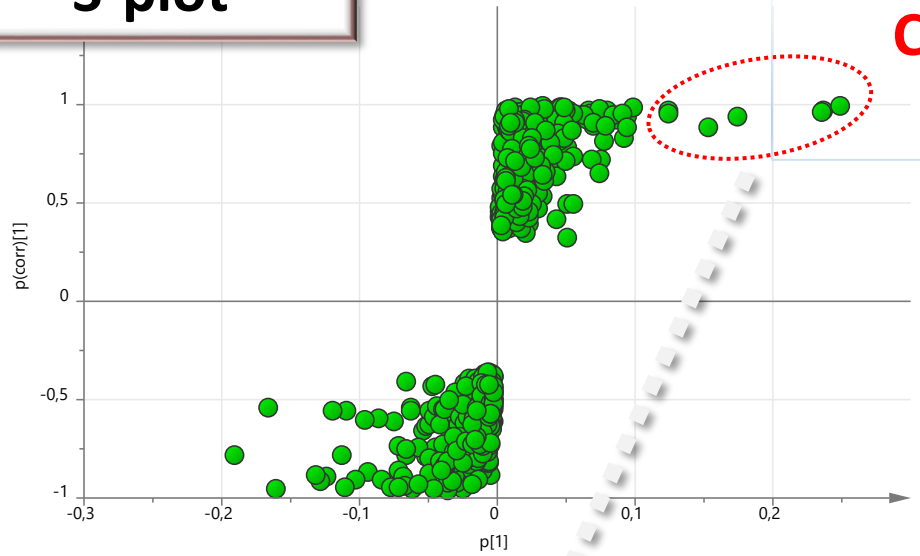
Loading plot



2301 features

Marker identification: *Vaccinium macrocarpon*

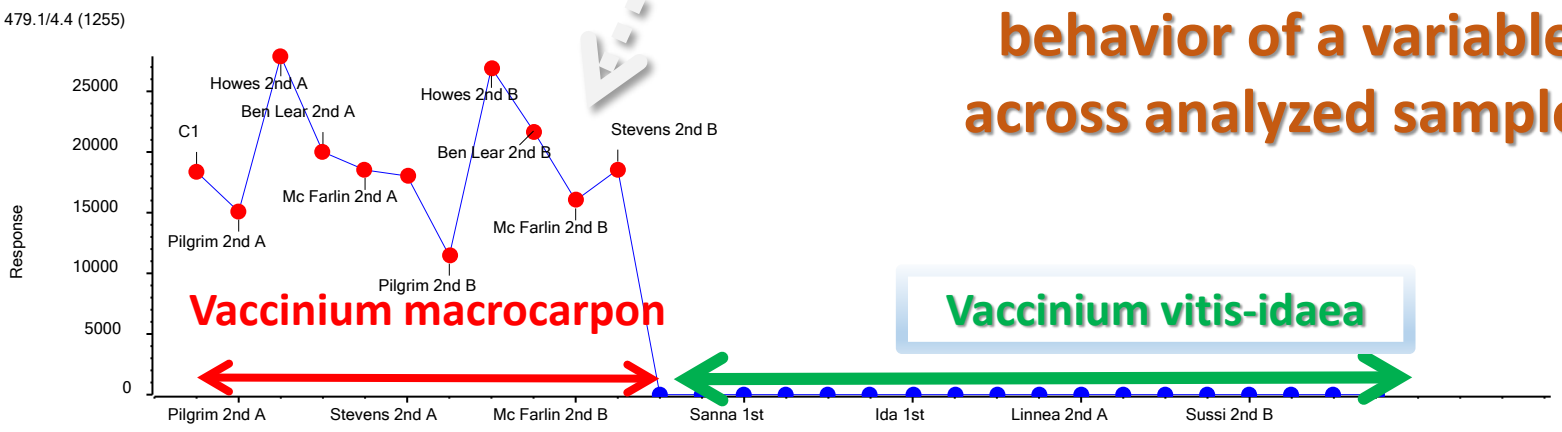
S-plot



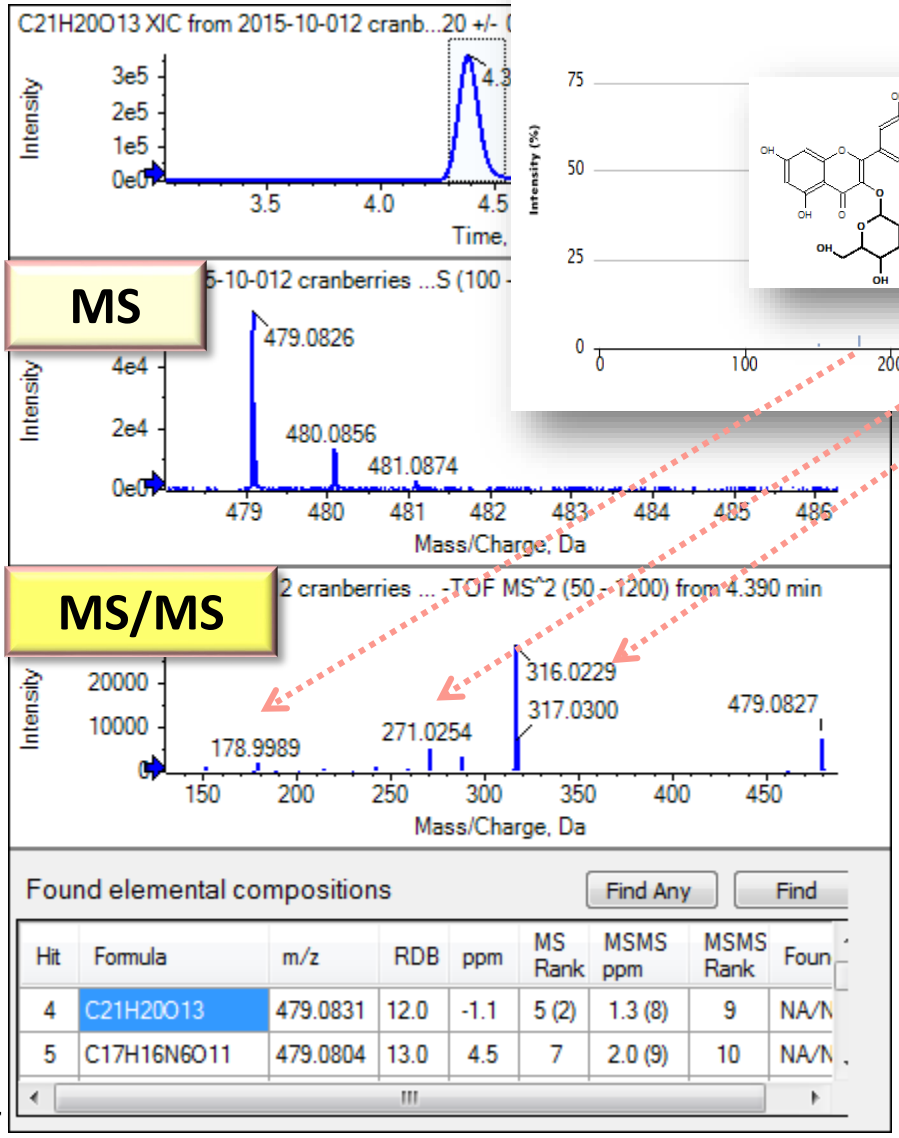
Characteristic markers for *Vaccinium macrocarpon*

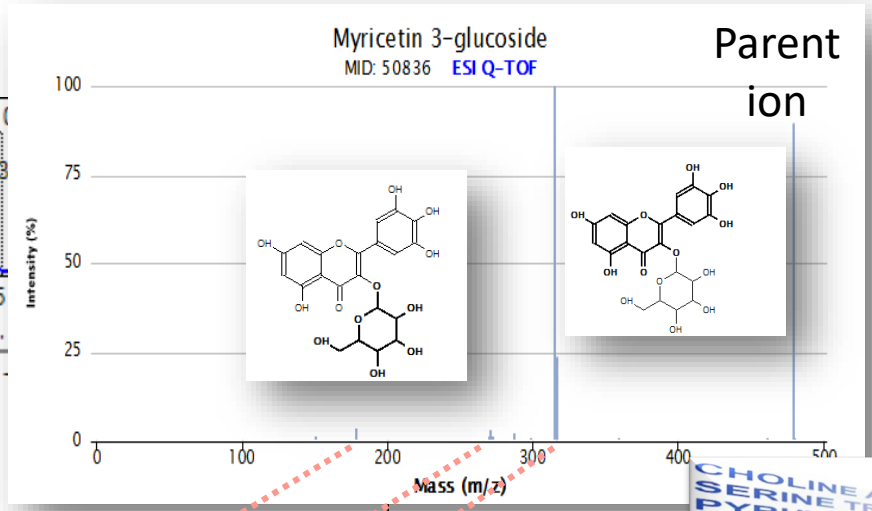
Plot profile
m/z 479.0857
RT: 4.4 min

Plot profile expresses behavior of a variable across analyzed samples

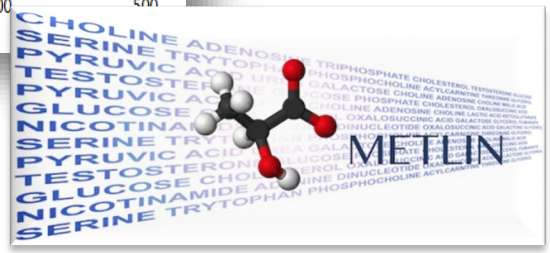


Marker identification: *Vaccinium macrocarpon*





ESI-



Formula finder

$C_{21}H_{20}O_{13}$

myricetin-3-glucoside

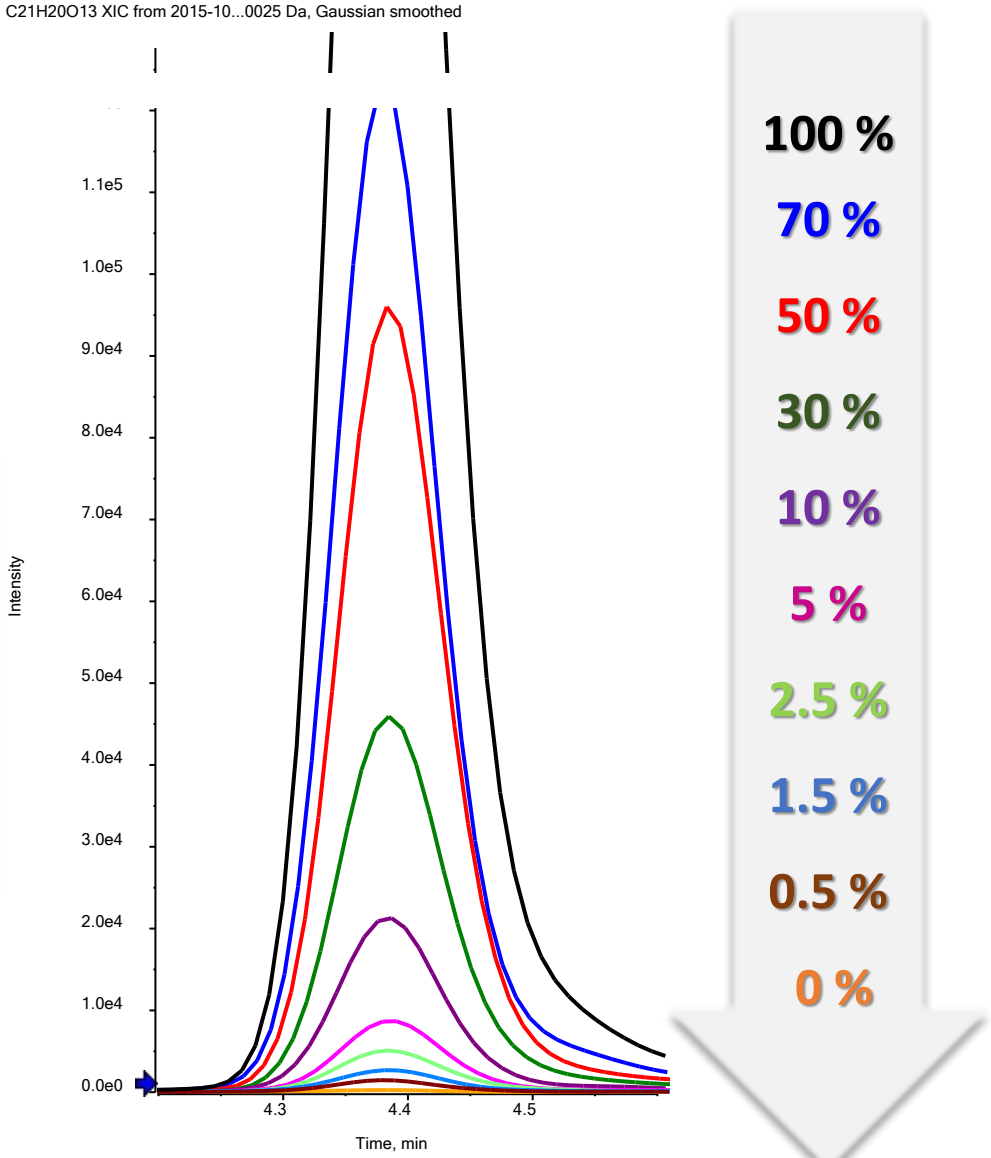
Mass error <3 ppm

Admixtures 100- 0 % of *Vaccinium macrocarpon*

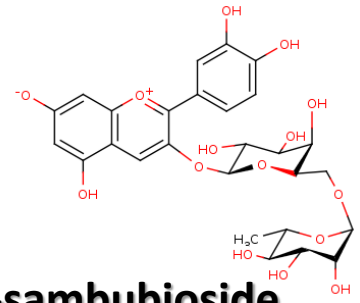
XIC of myricetin-3-glucoside/galactoside, $C_{21}H_{20}O_{13}$

ESI-

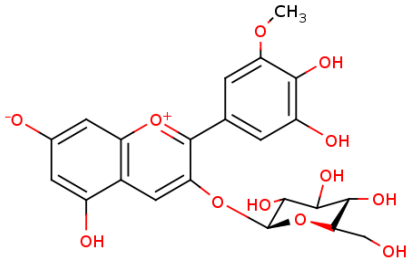
marker detected with addition of only 0.5 % of *Vaccinium macrocarpon*



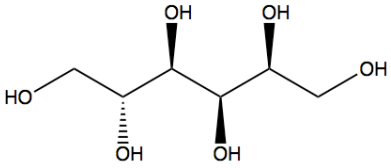
Identified characteristic markers



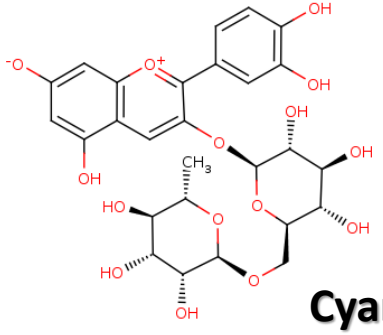
Cyanidin-3-O-sambubioside
***m/z* 579.1368; [M-H]⁻**



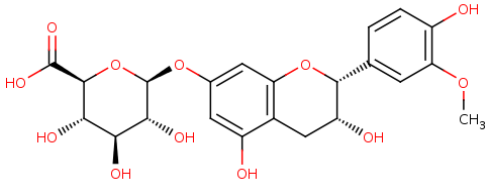
Petunidin-3-O-glucoside
***m/z* 479.1185; [M+H]⁺**



Sorbitol
***m/z* 181.0732; [M-H]⁻**

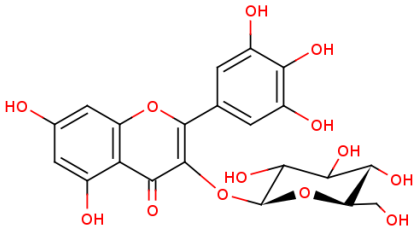


Cyanidin-3-O-rutinoside
***m/z* 593.1525; [M-H]⁻**

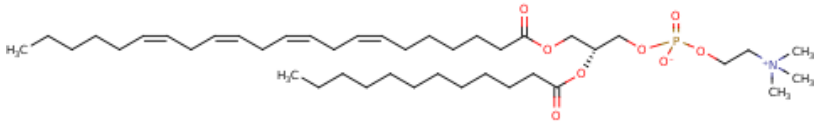


3-O-methyl-epicatechin-7-O-glucuronide
***m/z* 525.1256; [M-H]⁻**

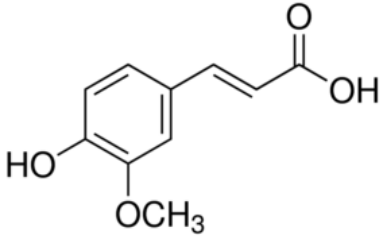
Identified characteristic markers



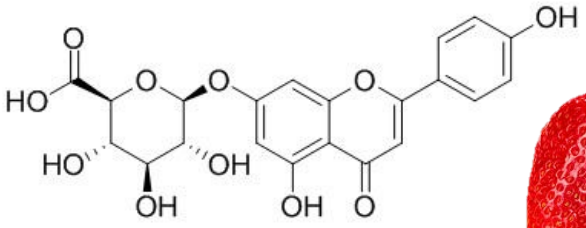
Myricetin -3-arabinoside
***m/z* 449.0733; [M-H]⁻**



Phosphatidylcholine (34:4)
***m/z* 754.5379; [M+H]⁺**



Ferulic acid
***m/z* 193.0520; [M-H]⁻**



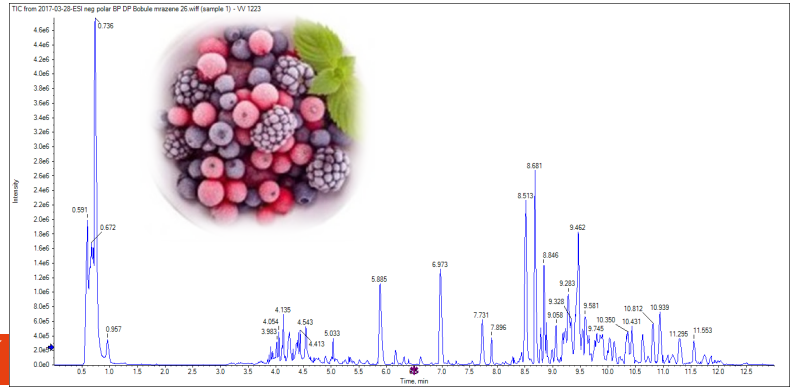
Apigenin-7-O-glucuronide
***m/z* 433.1120; [M+H]⁺**

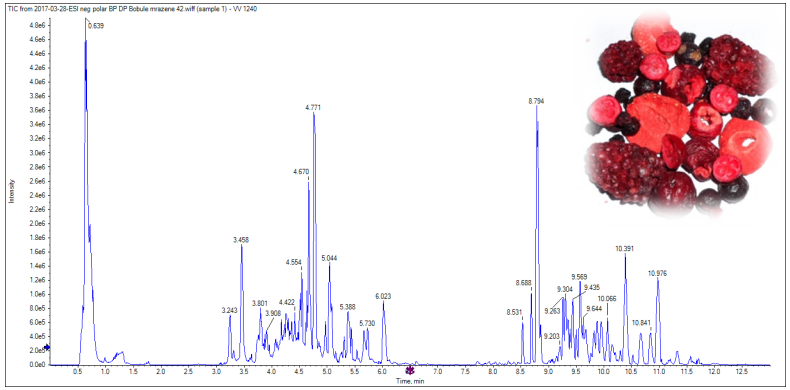
Flavonol triglycoside
C32H38O19
m/z 725.1963; [M-H]⁻

How does the drying process influence the metabolome?

Thermal treatment induces a lot of changes (interactions with amino acids, Maillard reaction…)

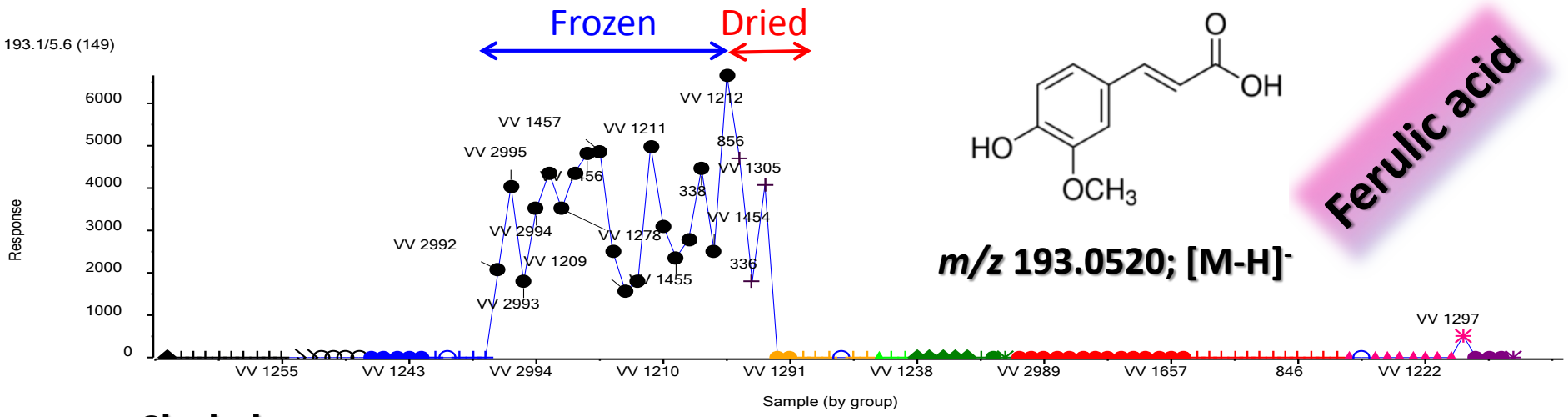
Does it affect stability of identified characteristic markers?





Markers stabilty: trend diagrams

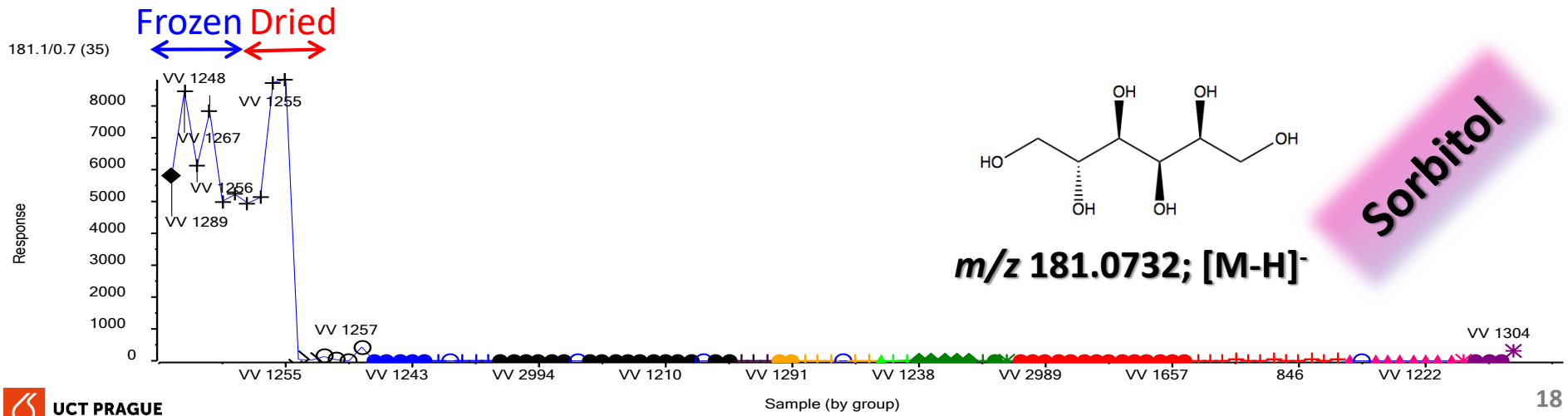
Lingonberry



Ferulic acid

***m/z* 193.0520; [M-H]⁻**

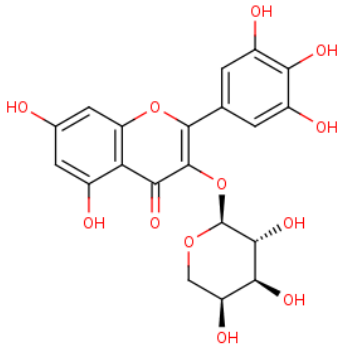
Chokeberry



Sorbitol

***m/z* 181.0732; [M-H]⁻**

Trend diagrams: Myricetin-arabinoside



Cranberry

Frozen

Dried

449.1/5.3 (1049)

´Plot profile´

Cranberry

Swamp cranberry

449.1/5.3 (1049)

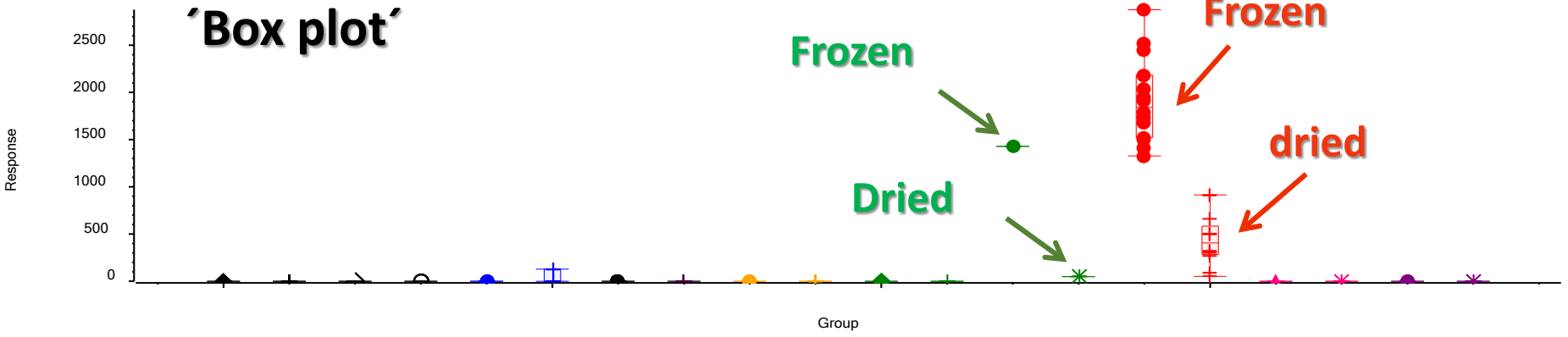
´Box plot´

Frozen

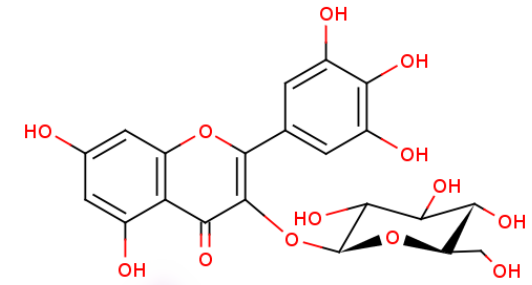
Frozen

dried

Dried



Influence of drying on flavonoids: Myricetin



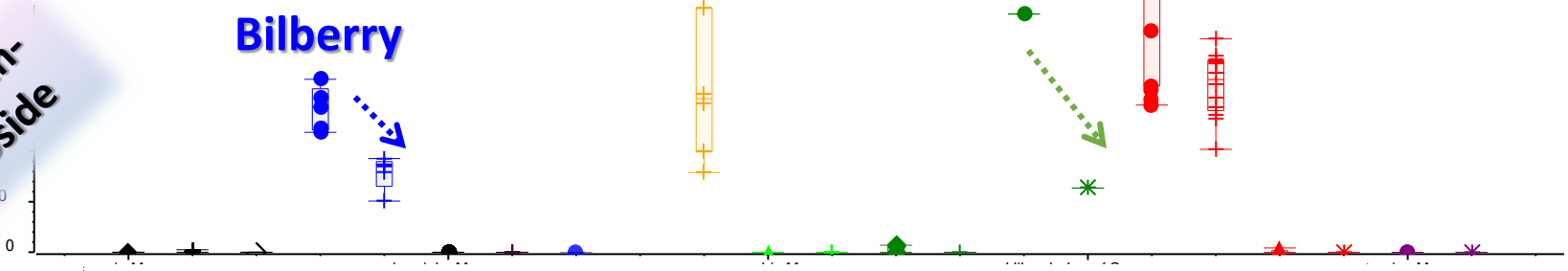
Black currant

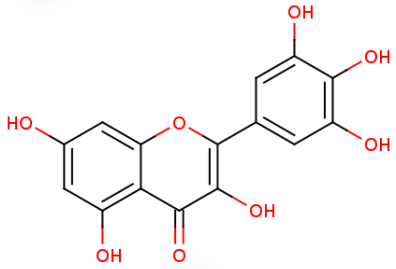
Crenberry

Swamp cranberry

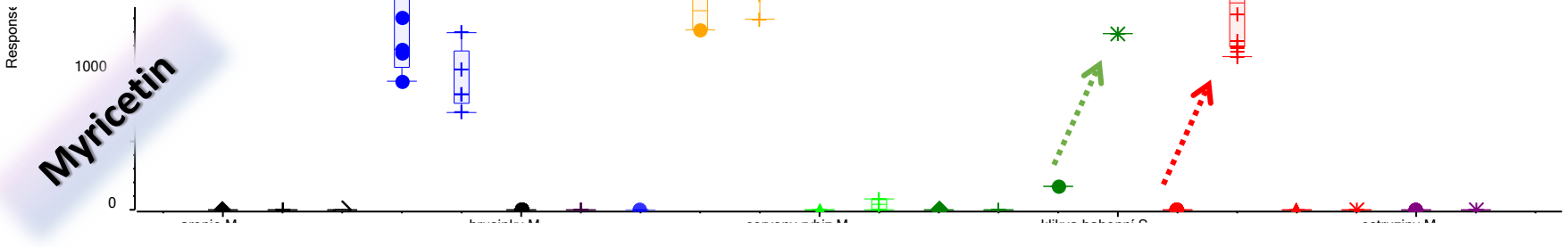
Bilberry

Myricetin-glucoside

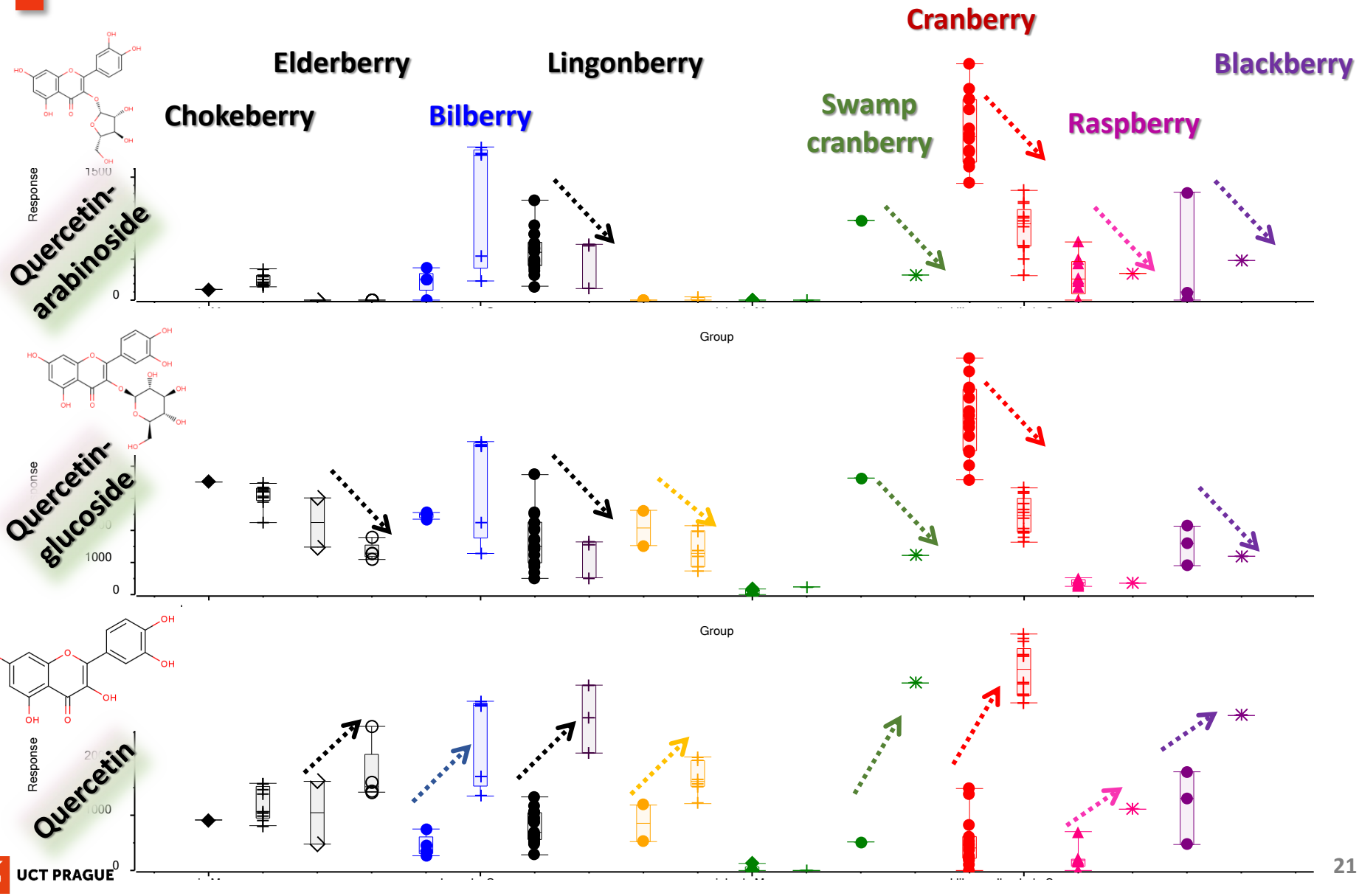




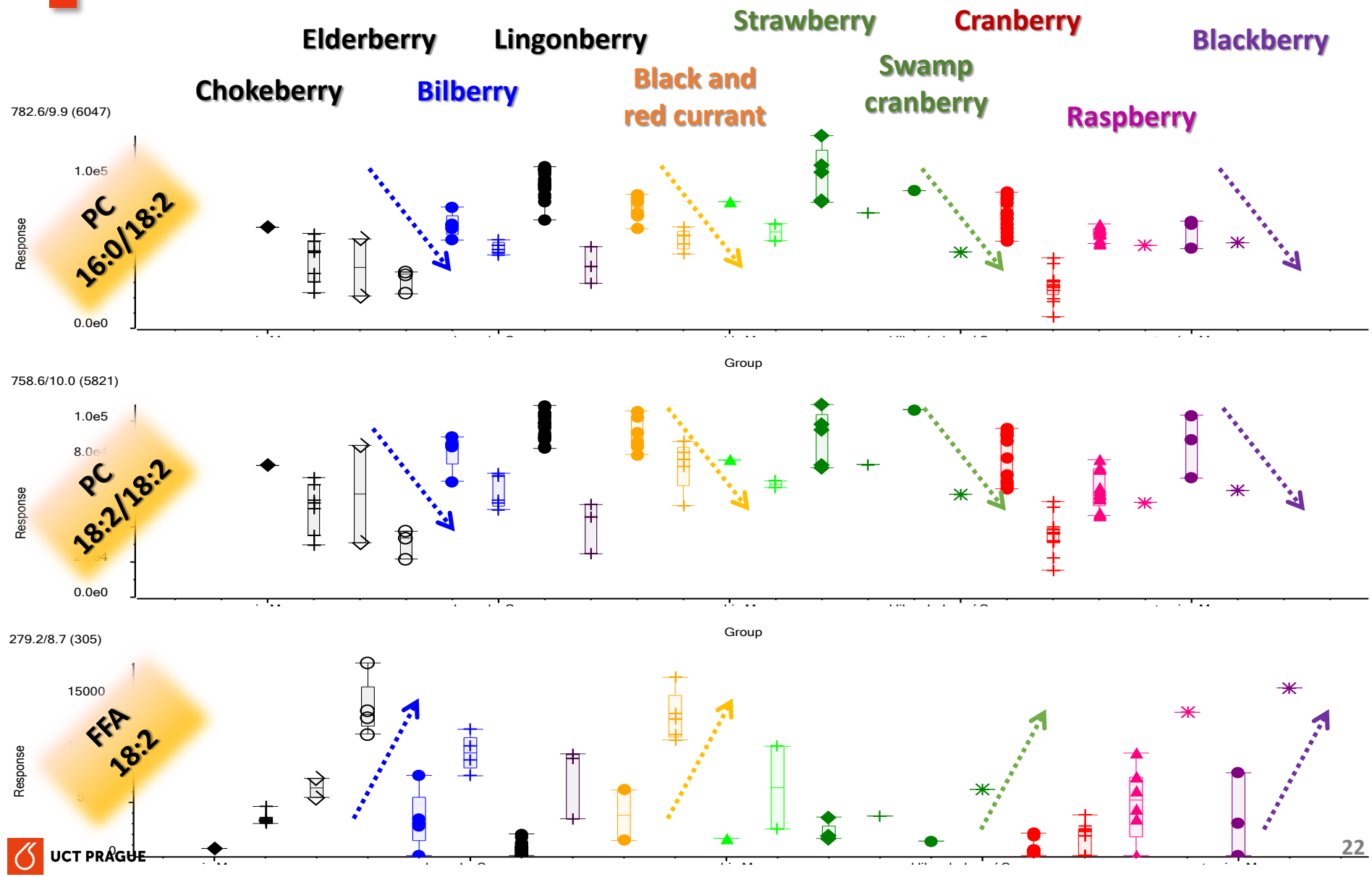
Myricetin



Influence of drying on flavonoids : Quercetin



Influence of drying on phospholipids



Conclusions

- **U-HPLC-HRMS/MS** (6600 Sciex) technique was successfully used for metabolomic fingerprinting, **11 species** of red and blue berries involved
- **MarkerView (Sciex)** allowed data processing, pre-treatment and analysis to be carried out in order to find characteristic markers.
- **Phenolic compounds and lipids** proved to be the most significant markers enabling discrimination of berries (methanolic extracts)
- Drying process was shown to **influence the stability** of certain markers
- As several markers disappear there is a great chance of finding **new markers**, which are originated during the heat treatment