UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

Bundesinstitut für Risikobewertung

FOOD INTEGRITY

Challenges in Mass Spectrometry Based Non-Targeted Analysis

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International Symposium on "Standardisation of Non-Targeted Methods for Food Authentication"
November 28, 2016, Berlin

FOOD: A COMPLEX COCKTAIL OF CHEMICALS

Food fiber

Natural toxins / antinutrients

Primary flavour compounds

Biologically active compounds, beneficial (antioxidants...)

NATURAL COMPONENTS – primary and secondary metabolites

Nutrients

Minerals
Vitamins

Fraudulent components - ´UNKNOWNS´

Products originated during processing

CONTAMINANS

Environmental contaminants

esticide / vet rugs residues

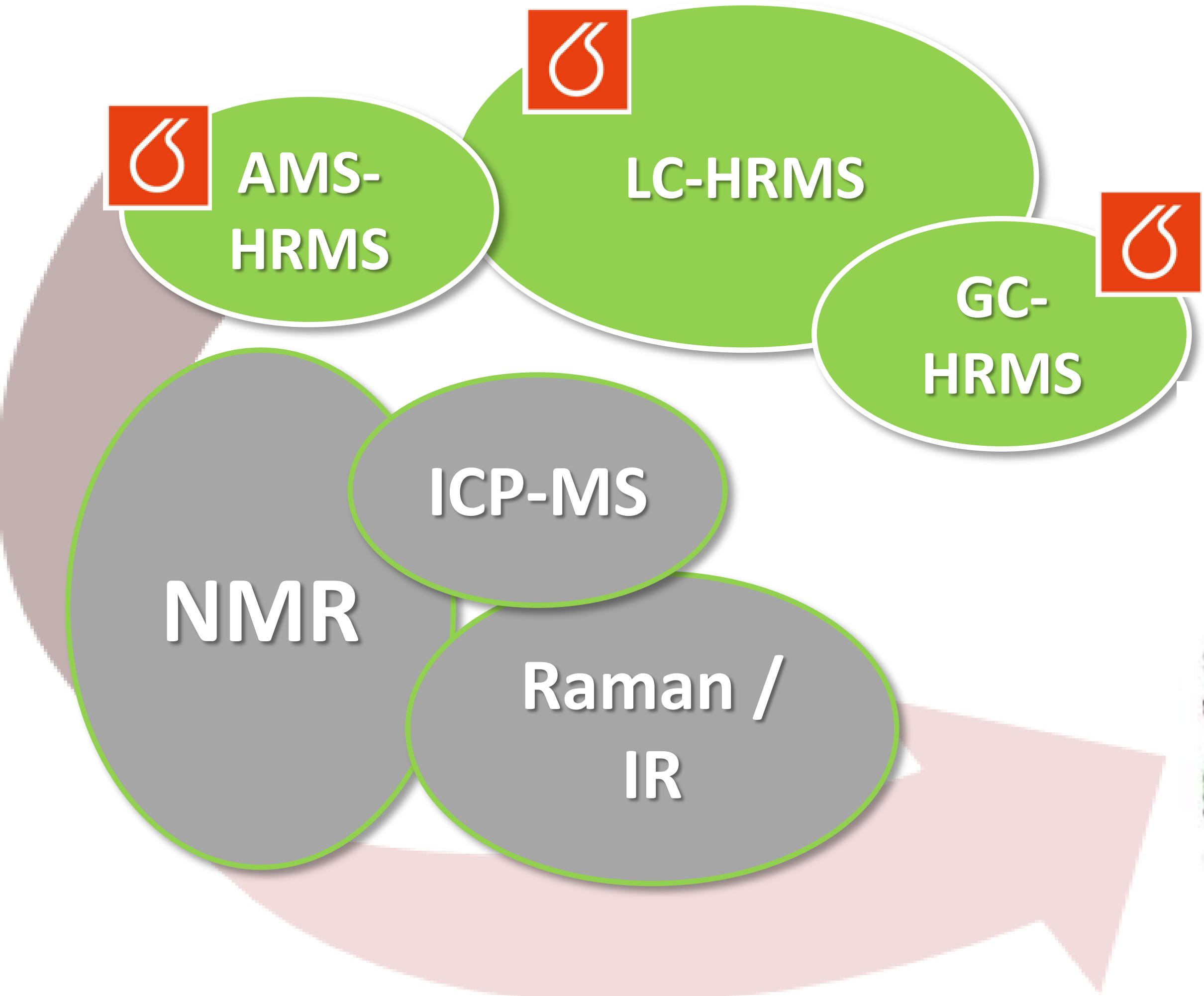
Migrants from food contact materials

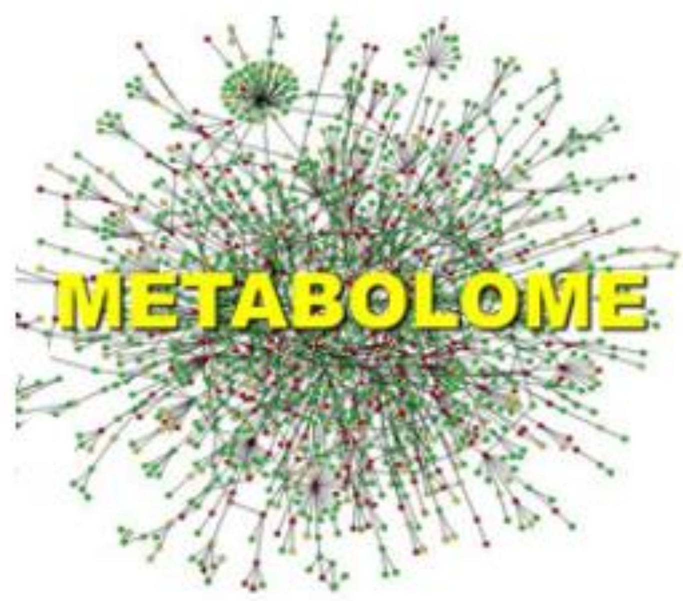
Toxic metals

Food additives

Need for new analytical approaches to detect food fraud

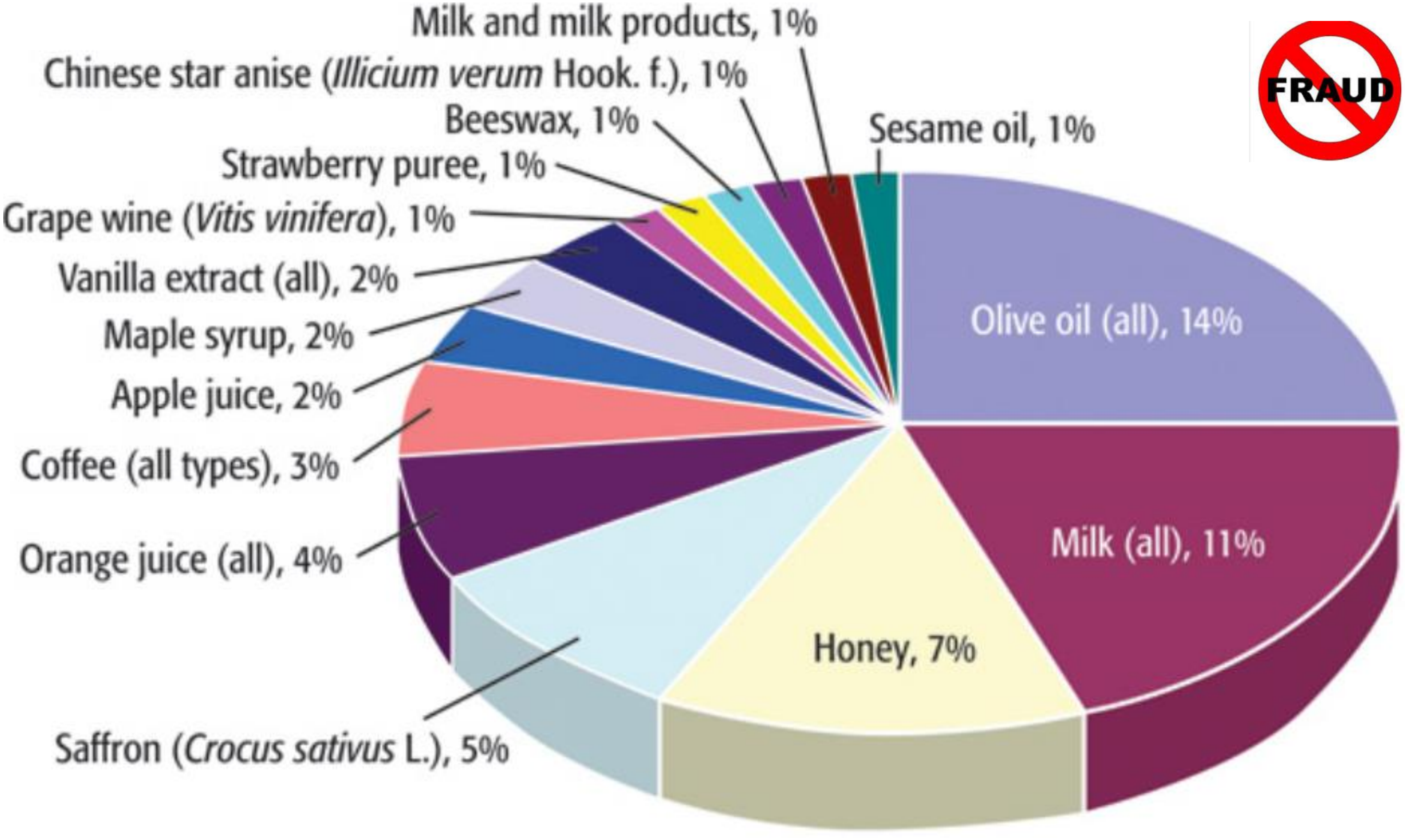
FINGERPRINTING TECHNIQUES FOR FOOD FRAUD DETECTING





UCT Prague – wide platform of cutting edge HRMS technologies for authenticity / safety testing

Fifteen most problematic food commodities for economical adulteration in the previous decade



http://nabc.cals.cornell.edu/Publications/Reports/nabc_23/23_5_3_Moore.pdf

APPLICATION POTENTIAL OF HIGH RESOLUTION MASS SPECTROMETRY

Analytica Chimica Acta 645 (2009) 56–63

Metabolomics (2012) 8:793–803
DOI 10.1007/s11306-011-0371-7

Anal Bioanal Chem (2014) 406:6791–6803
DOI 10.1007/s00216-014-7864-y

PAPER IN FOREFRONT

Metabolic fingerprinting based on high-resolution tandem spectrometry: a reliable tool for wine authentication?

Josep Rubert · Ondrej Lacina · Carsten Fauhl-Hassek ·
Jana Hajslova

origin recognition

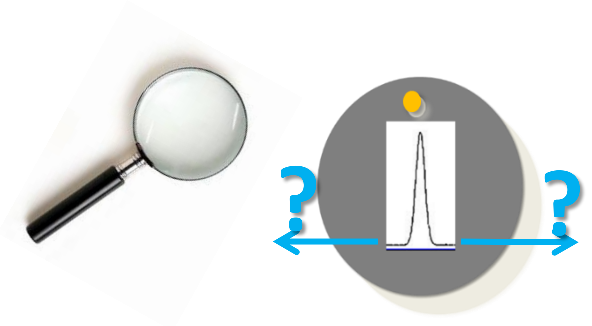
Tomas Cajka · Katerina Riddellova ·
Monika Tomaniova · Jana Hajslova

Metabolomics (2011) 7:500–508
DOI 10.1007/s11306-010-0266-z

Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology, Prague, Technicka 3, 166 28 Prague 6, Czech Republic

Moving ahead with HRMS fingerprints…

´CLASSIC´ APPROACH



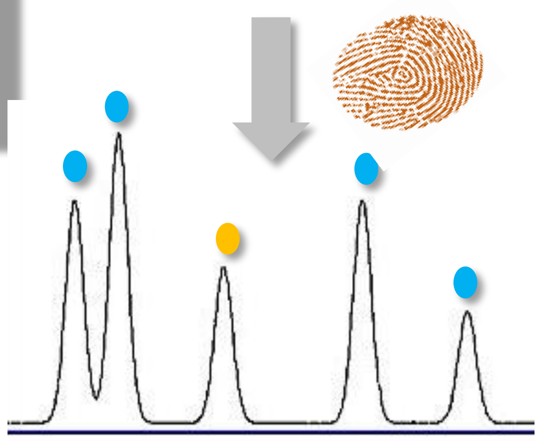
A set of physico-chemical and/or biochemical measurements → TARGET ANALYSIS of one or few markers

FOOD INTEGRITY

NOVEL STRATEGY

Metabolomic fingerprinting

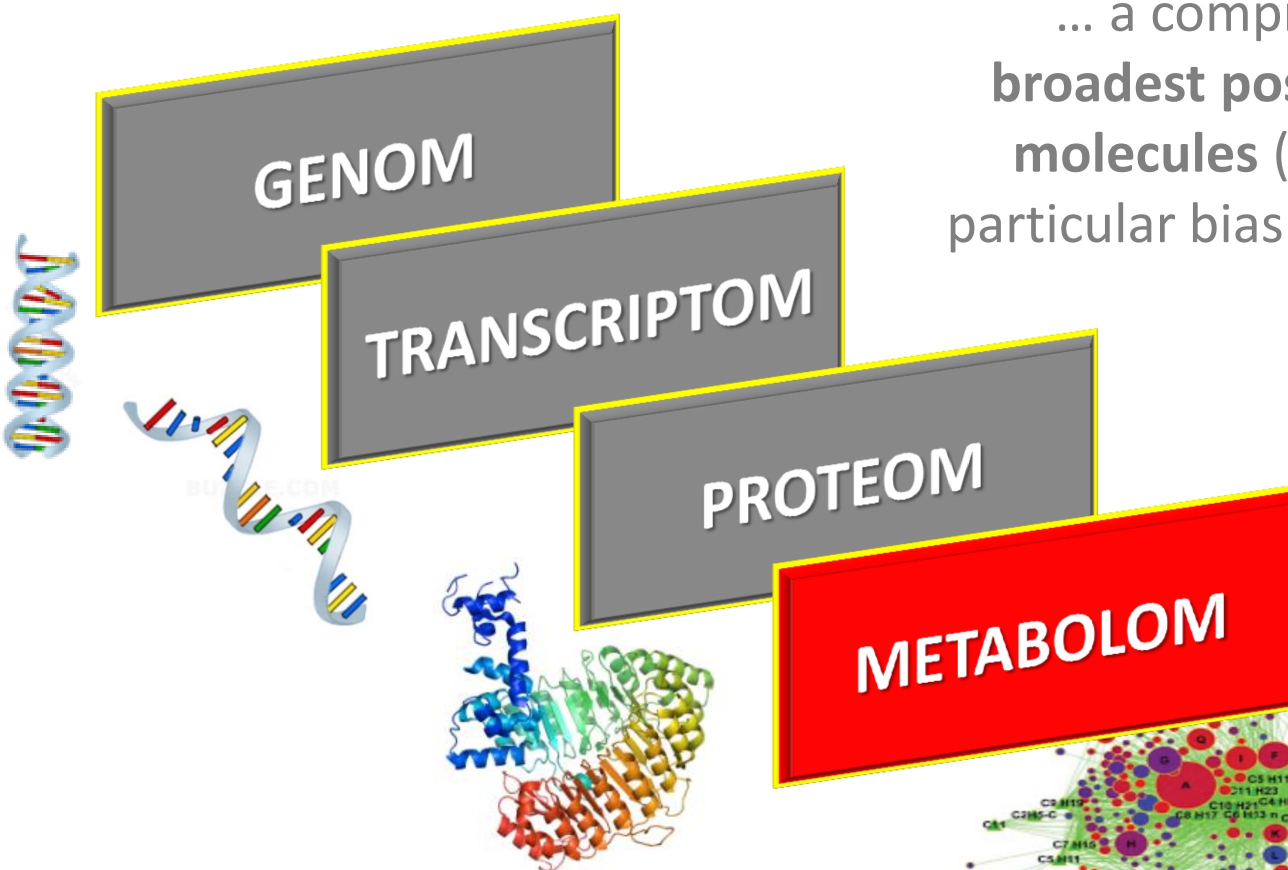
→ NON TARGET SCREENING

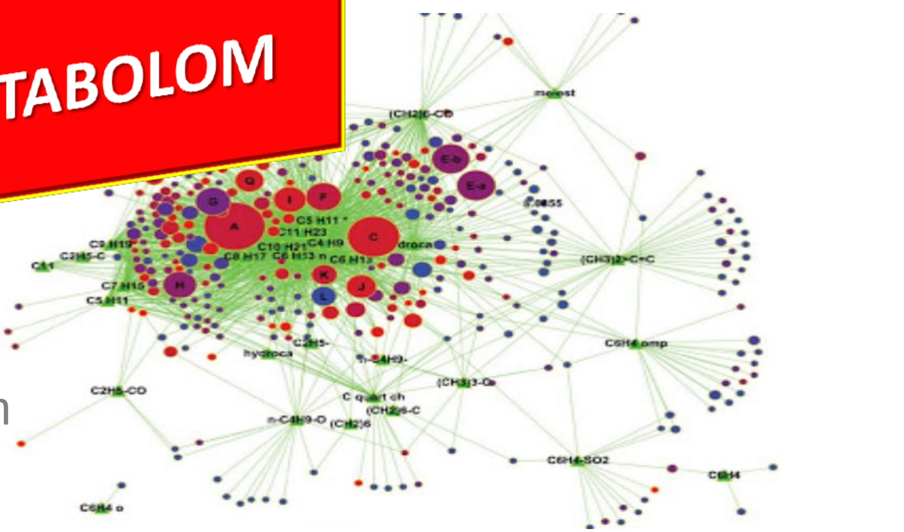


- complex characterization of matrix pattern
- detection / identification of ´unknown´ components (even retrospective)
- identification of a set of composition markers

Metabolomic fingerprinting

… a comprehensive analysis of **broadest possible range of small molecules** (<1200 Da) without a particular bias to specific groups of metabolites





… arround 7,000 - 15,000 metabolites within individual plant species

Typical metabolomic workflow

AUTHENTIC SAMPLES → SAMPLE PREPARATION → **FINGERPRINTING (DATA ACQUISITION)** → **FEATURES (IONS) MINING** → MULTIVARIATE DATA ANALYSIS

HYPOTHESIS?

LLE / LSE

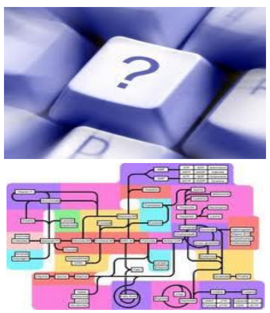
SEPARATION: (U)HPLC, SFC, GC, GCxGC

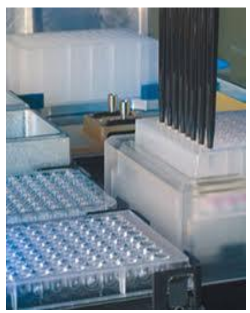
IONIZATION: ESI, APCI, EI, CI, DART, DESI, …

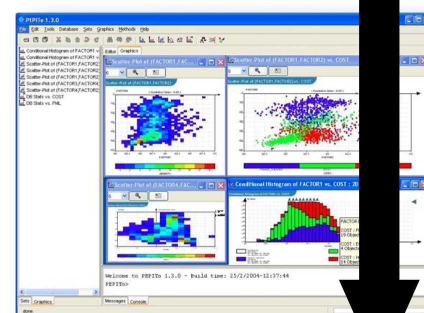
MS ANALYSIS: Scan LR/HR, SIM, SRM, …

DATA FILTERING, ALIGNMENT, NORMALIZATION

PCA, ANOVA, LDA, PLS-DA, …







CRITERIA? STATISTICAL, BIOLOGICAL

MARKER SELECTION → ELEMENTAL COMPOSITION, IDENTIFICATION → CONFIRMATION OF STRUCTURE → **PROFILING** → USE IN PRACTICE

ONLINE/OFFLINE DATABASES

ULTRA-HRMS, HR MS/MS

MS^n, HR MS/MS, STANDARD ANALYSIS

TARGET METABOLOMICS LC(GC)–MS/MS

DIAGNOSTIC purposes, AUTENTICATION

Case study # 1

Distinguishing of botanical origin of berries (Lingonberries vs. Cranberries)

Lingonberries vs. Cranberries

- *Vaccinium vitis-idaea*
- ‚Rock‘ or ‚mountain‘ cranberry
- Berries from Nordic countries, the Baltic states, central and northern Europe
- Size of fruits: 5-10 mm
- Price of fresh berries: 1.3 €/kg
- Up to 2x higher content of phenolics and proanthocianidins

- *Vaccinium macrocarpon*
- ‚Large‘ or ‚American‘ cranberry
- Major commercial crop in the United States and Canada
- Size of fruits: 9-16 mm
- Price of fresh berries : 0.4 €/kg

Sensory and phytochemical similarities may lead to confusion or **intentional substitution** of the two crops (=**adulteration)**

AIM OF THE STUDY

FINGERPRINTING

- Development of extraction procedure
- LC-HRMS analysis, data processing, markers characterization

PROFILING

- Assessment of stability of markers during food processing
- Analysis of markers in processed foods
- Assessment of authenticity of the lingonbery-based products

Samples analyzed within the initial metabolomic fingerprinting (1st step)

- **Fresh samples** (stored in freezer)
  - 15 authentic samples of **lingonberries** *(Vaccinium vitis-idaea)*
  - 10 authentic samples of **cranberries** *(Vaccinium macrocarpon)*

Samples analyzed within the assessment of suitability of markers for processed foods (2nd step)

- **Dried samples**

  3 samples of **lingonberries** *(Vaccinium vitis-idaea)*

  16 samples of **cranberries** *(Vaccinium macrocarpon)*

Sample preparation

1st STEP
Polar extract

1 g of sample with 5 mL of **methanol**
Ultraturrax
Centrifugation

2nd STEP
Non-polar extract

Repeated extraction of solid residue with 5 mL of **hexane/2-propanol**
Vortex
Centrifugation

UHPLC-HRMS analysis

UHPLC-Thermo Dionex UltiMate 3000

Non-polar extract

- **Column:** BEH C18

(2.1x100 mm, 1.7 µm)

Column temperature: 60 °C

- **Mobile phase:**

A: 5 mM ammonium formate in H_2O:MeOH (95:5) + 0,1% formic acid

B: 5 mM ammonium formate in iPrOH:MeOH:H_2O (65:30:5) + 0,1% formic acid

Polar extract

- **Column:** HSS T3

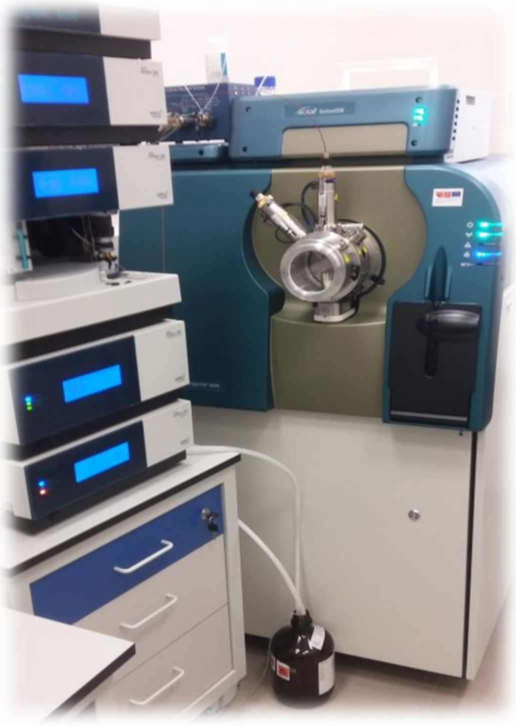
(2.1x100 mm, 1.8 µm)

Column temperature: 40 °C

- **Mobile phase:**

A: 5 mM ammonium formate in H_2O

B: MeOH



MS(MS/MS)-TripleTOF™ 6600 (Sciex)

- m/z range: 100-1200
- Ionisation technique: ESI +/-
- This technology allowed: Sensitivity, Speed, Resolution and Mass Accuracy

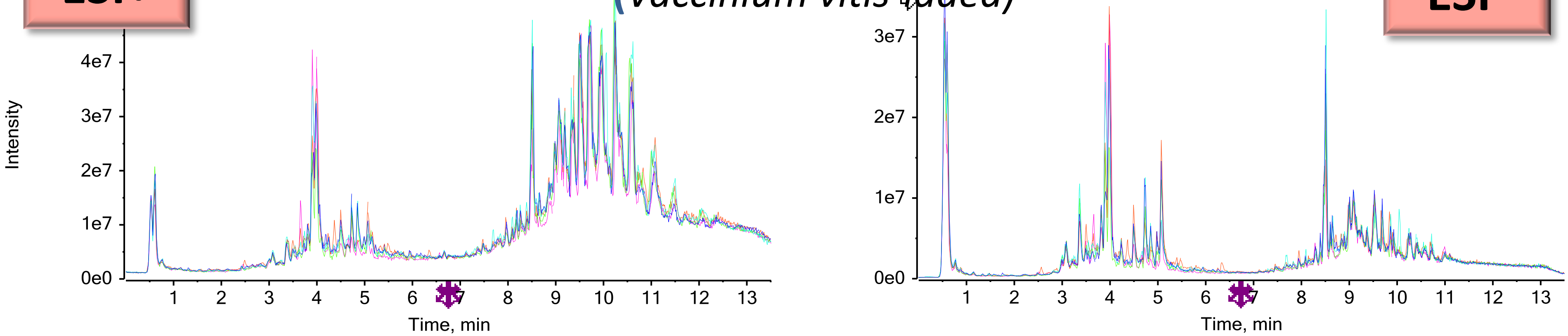
U-HPLC-HRMS/MS metabolomic fingerprint of polar extracts of fruits

ESI+

ESI-

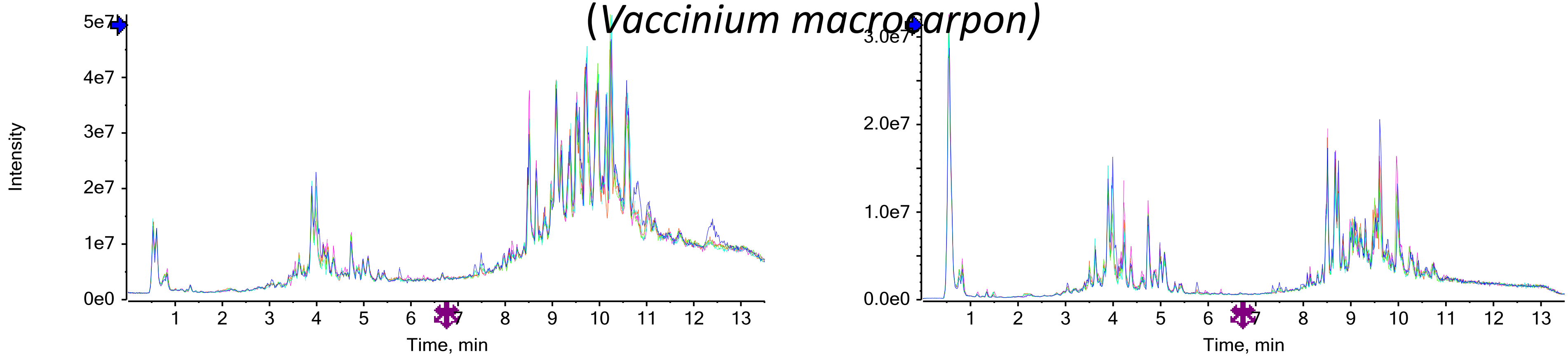
Lingonberries

(Vaccinium vitis-idaea)

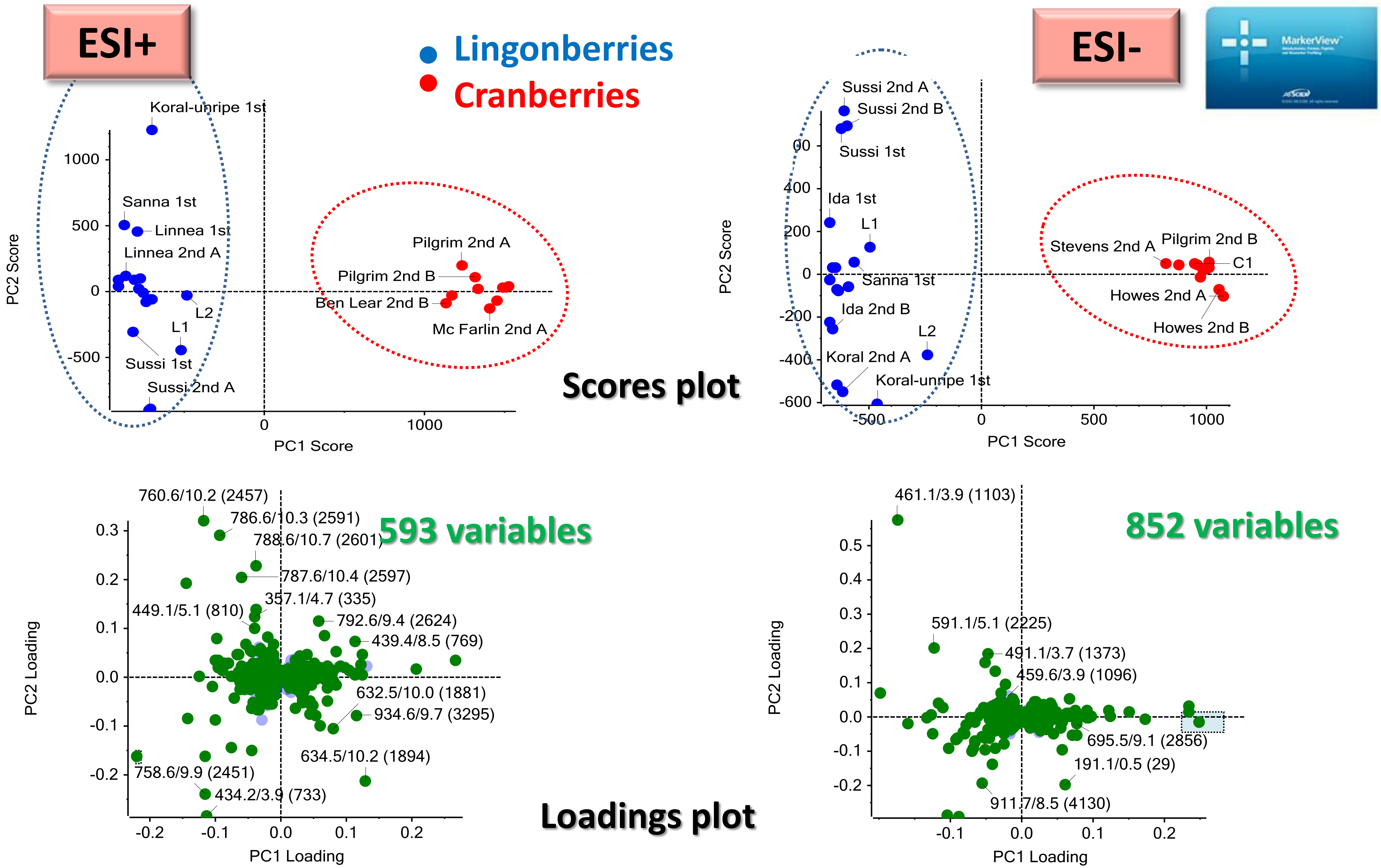


Cranberries

(Vaccinium macrocarpon)



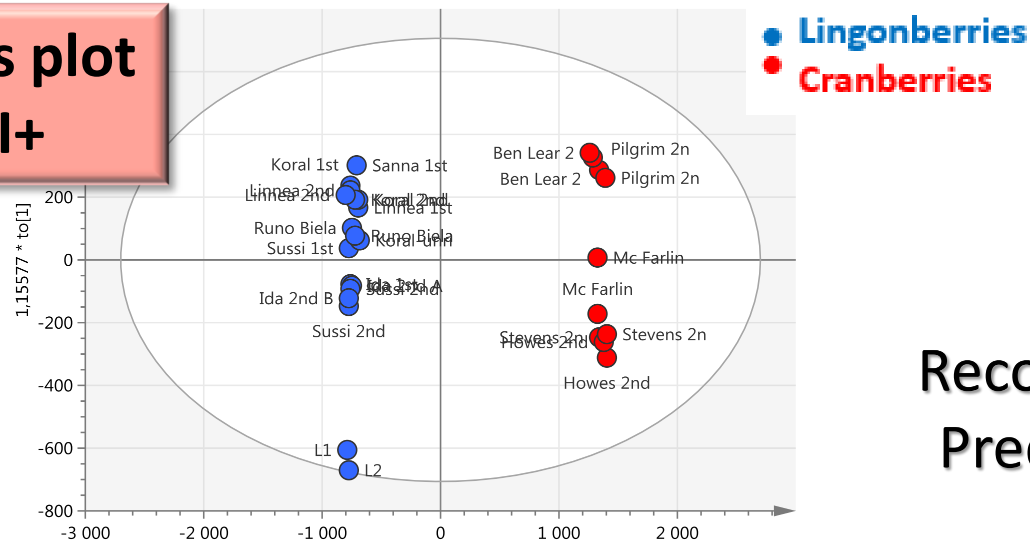
Multivariate PCA analysis of polar extract



Multivariate OPLS-DA analysis of polar extract

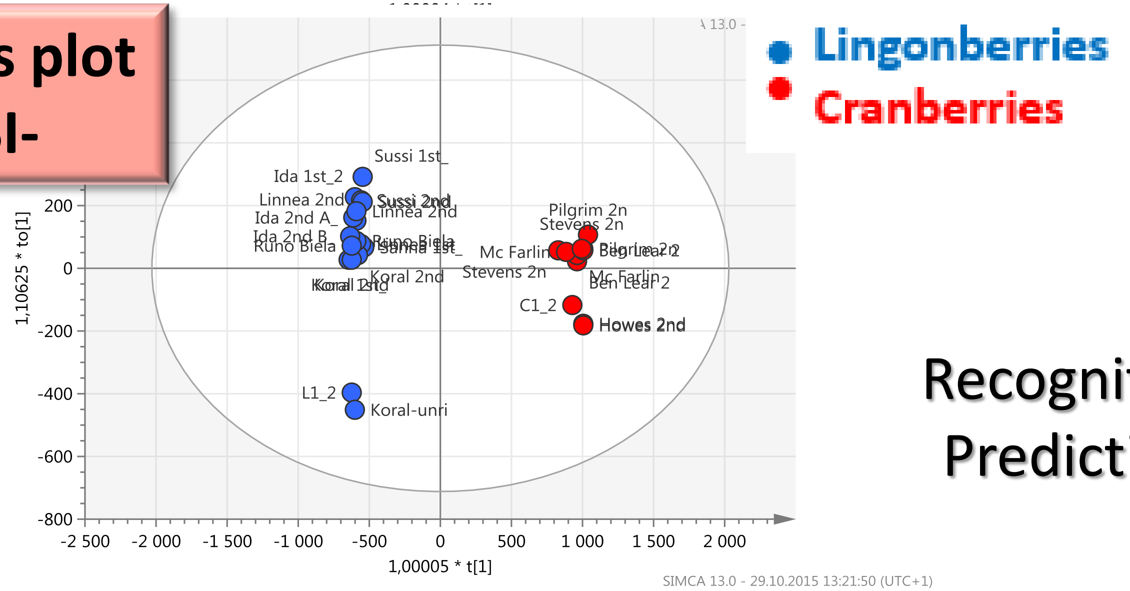
SIMCA
BY UMETRICS

Scores plot ESI+



Recognition ability: 100 %
Prediction ability: 100 %

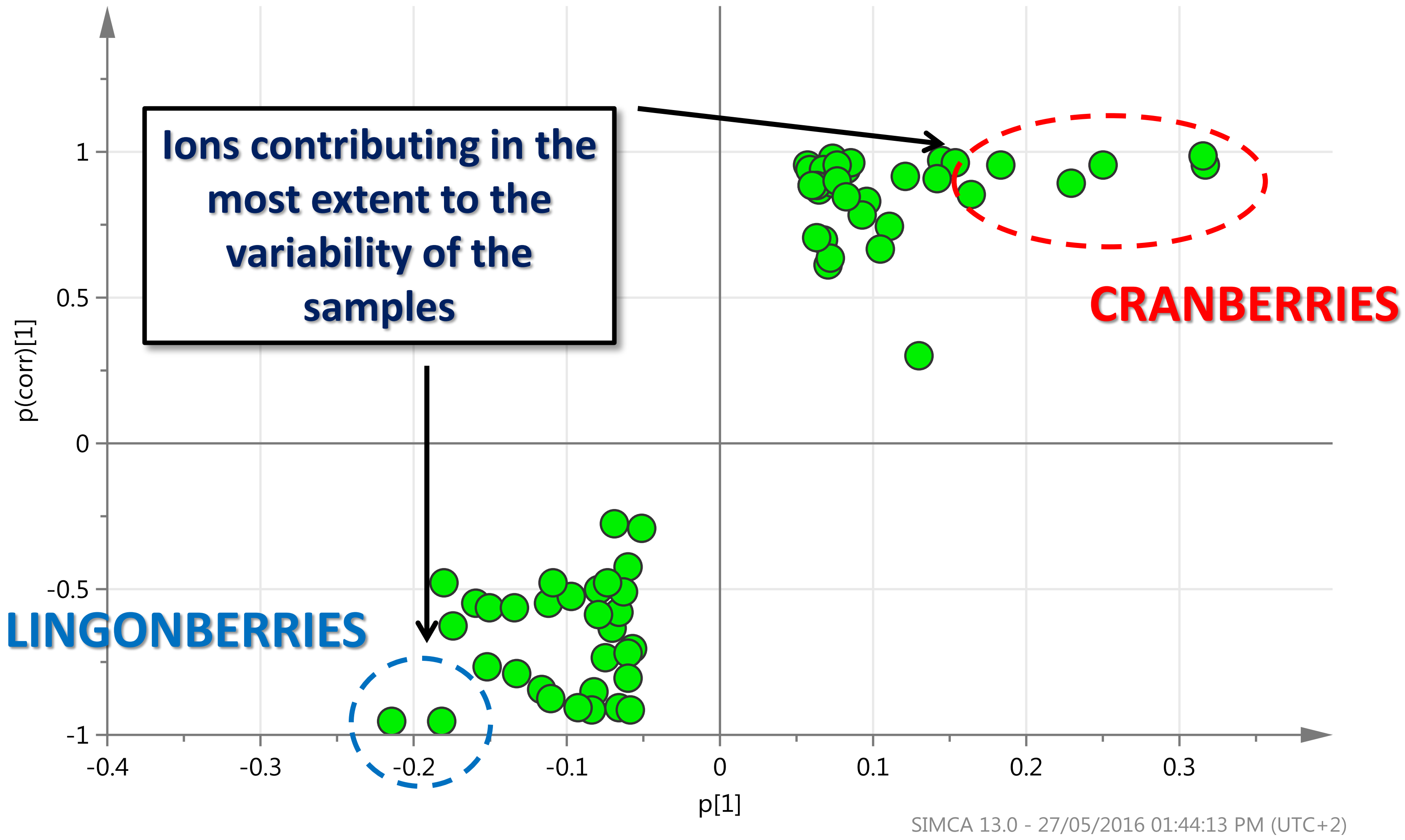
Scores plot ESI-



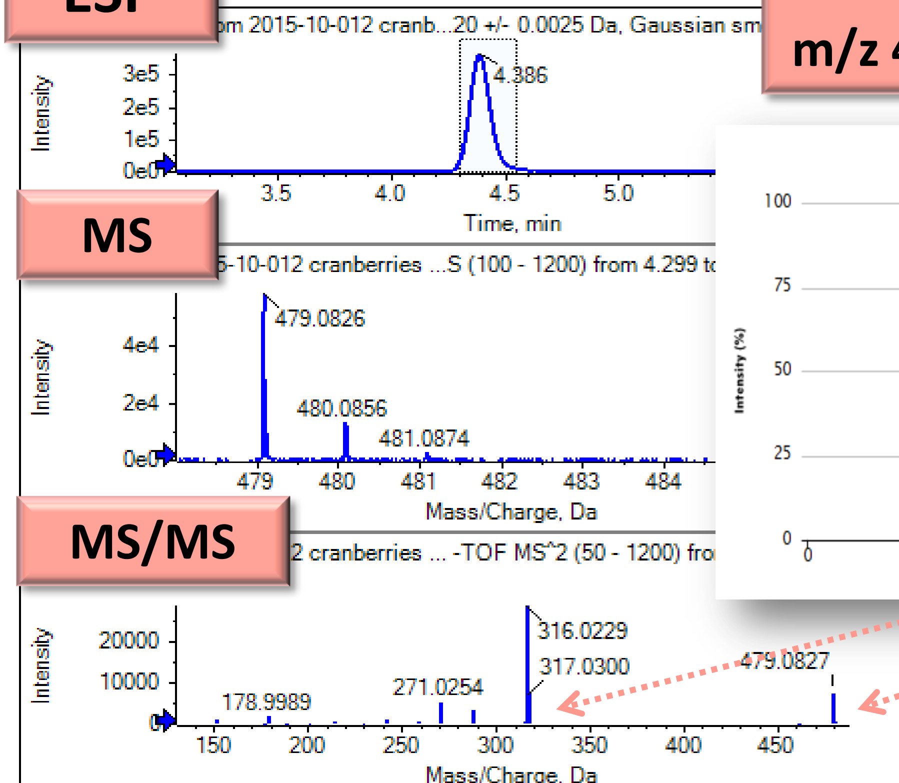
Recognition ability: 100 %
Prediction ability: 100 %

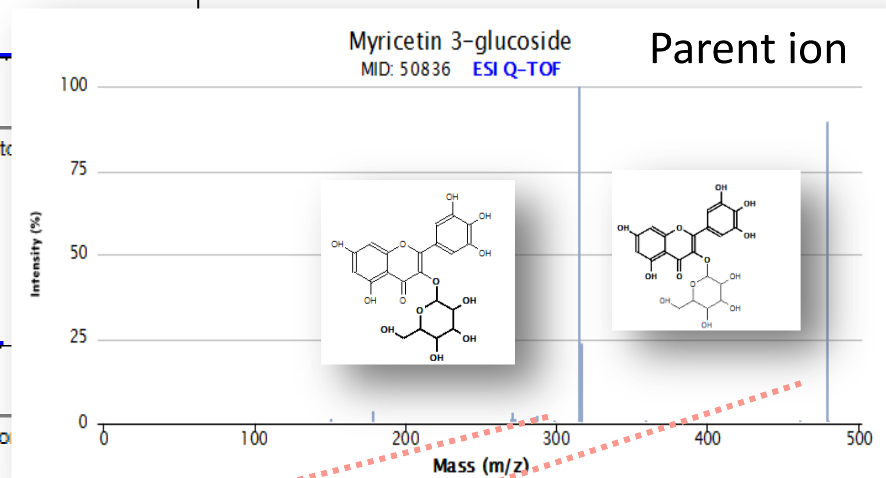
Multivariate analysis of polar extract

S-plot (ESI-)



Marker identification: cranberries *(Vaccinium macrocarpon)*





Found elemental compositions

Find Any | Find

Hit	Formula	m/z	RDB	ppm	MS Rank	MSMS ppm	MSMS Rank	Foun...
4	C21H20O13	479.0831	12.0	-1.1	5 (2)	1.3 (8)	9	NA/N...
5	C17H16N6O11	479.0804	13.0	4.5	7	2.0 (9)	10	NA/N...

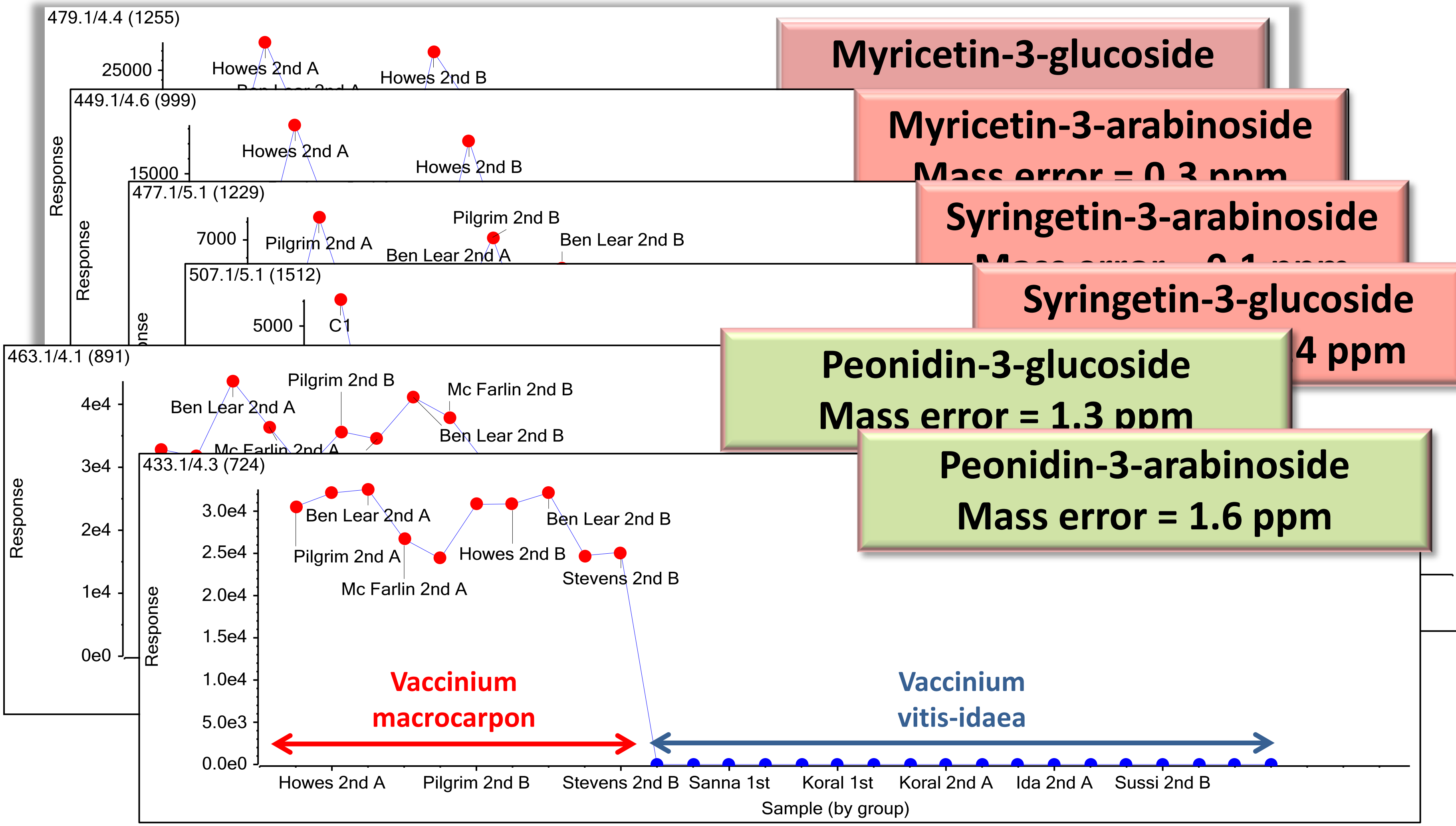
Formula finder

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

myricetin-3-glucoside

Mass error <3 ppm

Plot profiles of markers of cranberries (*Vaccinium macrocarpon*), ESI- / ESI+



CHARACTERISTIC MARKERS FOR DIFFERENTIATION OF CRANBERRIES AND LINGONBERRIES

Markers for *Vaccinium macrocarpon* (cranberry)	Summary formula	Ion	*m/z*
Myricetin-3-arabinoside	$C_{20}H_{18}O_{12}$	$[M-H]^-$	449,0726
Myricetin 3-glucoside	$C_{21}H_{20}O_{13}$	$[M-H]^-$	479,0831
Syringetin-3-glucoside	$C_{23}H_{24}O_{13}$	$[M-H]^-$	507,1144
Peonidin 3-arabinoside	$C_{21}H_{20}O_{10}$	$[M+H]^+$	[illegible]
Peonidin 3-glucoside	$C_{22}H_{22}O_{11}$	$[M+H]^+$	[illegible]

MARKERS SHOULD BE PRESENT IN THE TYPICAL RATIOS

Markers for *Vaccinium vitis-idaea* (lingonberry)	Summ[illegible]	[illegible]	*m/z*
Catechin	[illegible]	$[M-H]^-$	290,0790
Procyanidin B	$C_{30}H_{26}O_{12}$	$[M-H]^-$	577,1351
1-O-feruloylglucose	$C_{16}H_{20}O_9$	$[M-H]^-$	355,1034

CHARACTERISTIC MARKERS FOR DIFFERENTIATION OF CRANBERRIES AND LINGONBERRIES

Markers for *Vaccinium macrocarpon* (cranberry)	Summary formula	Ion	*m/z*
Myricetin-3-arabinoside	[illegible]	[illegible]	[illegible]
Myricetin 3-glucoside	[illegible]	[illegible]	[illegible]
Syringetin-3-glucoside	[illegible]	[illegible]	[illegible]
Peonidin 3-arabinoside	$C_{21}H_{20}O_{10}$	$[M+H]^+$	433,1129
Peonidin 3-glucoside	$C_{22}H_{22}O_{11}$	$[M+H]^+$	463,1235

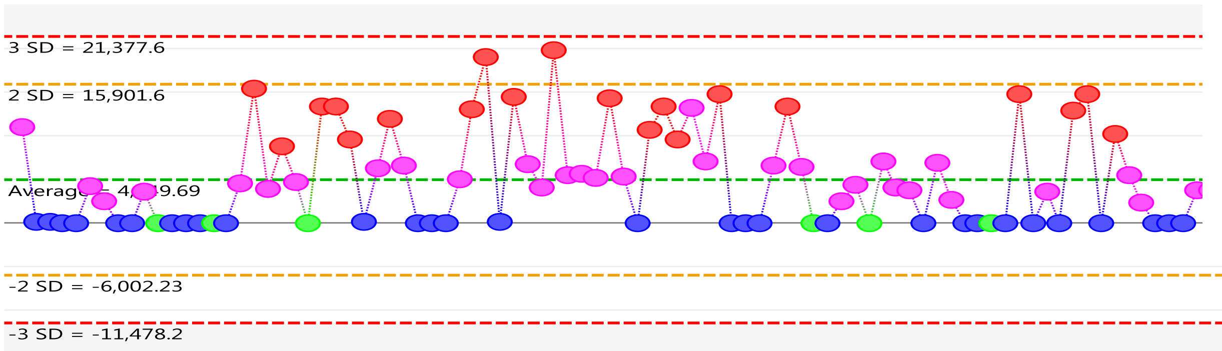
SOME MARKERS CAN BE PRESENT IN OTHER BERRIES

Markers for *Vaccinium vitis-idaea* (lingonberry)	Summary formula	Ion	*m/z*
Catechin	$C_{15}H_{14}O_6$	$[M-H]^-$	290,0790
Procyanidin B	$C_{30}H_{26}O_{12}$	$[M-H]^-$	577,1351
1-O-feruloylglucose	$C_{16}H_{20}O_9$	$[M-H]^-$	355,1034

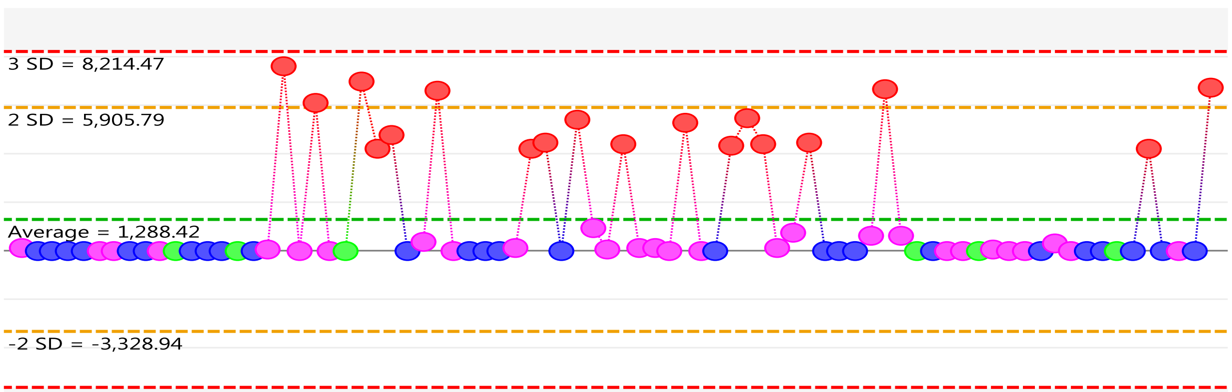
FRESH VS. DRIED FRUITS, markers stability during the food processing

FRESH VS. DRIED FRUITS, markers stability

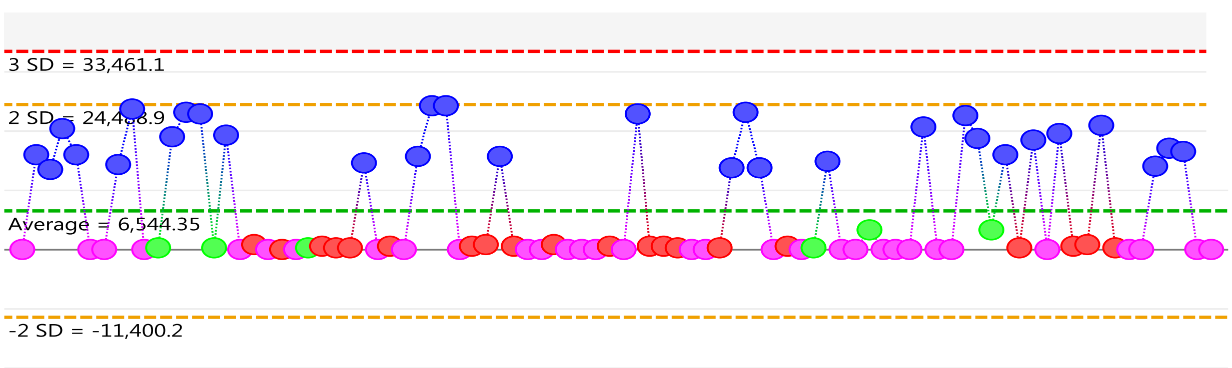
Lingonberries fresh
Lingonberries dried
Cranberries fresh
Cranberries dried



m/z 479.0836
Myricetin-3-glucoside



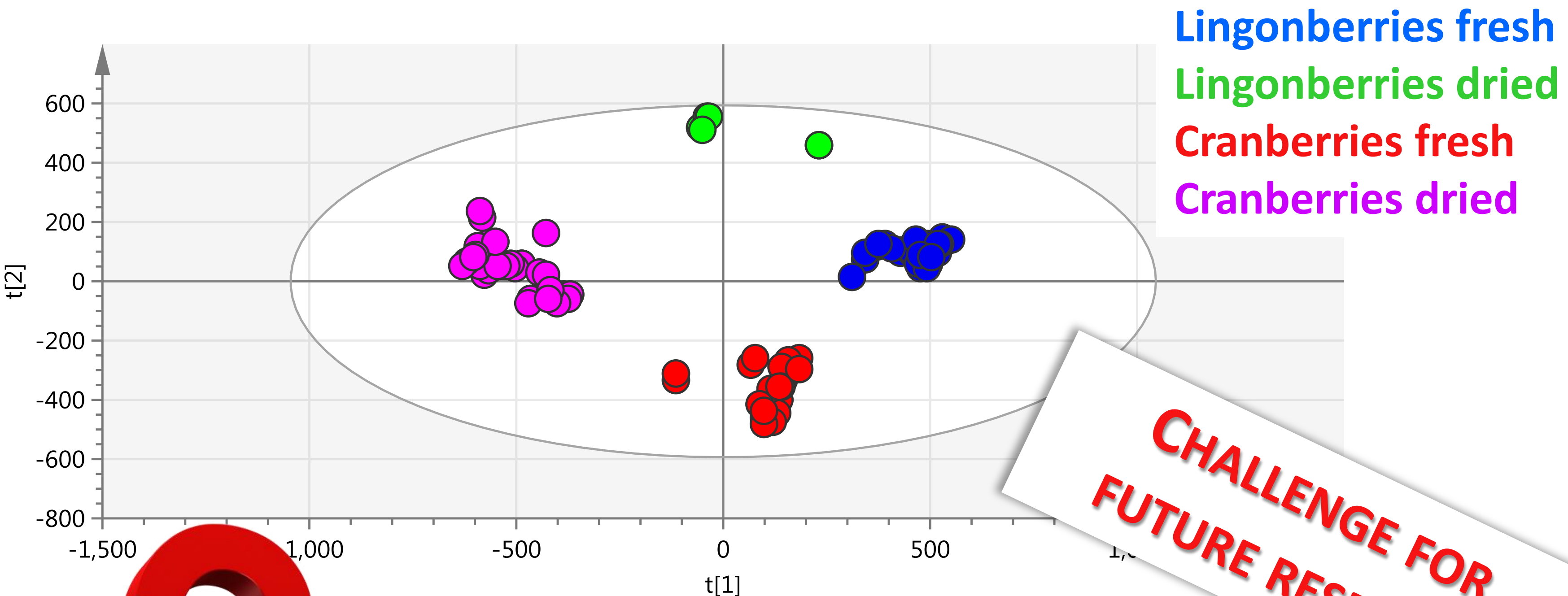
m/z 463.1240
Peonidin-3-glucoside



m/z 289.0730
Katechin

Fresh vs. dried fruits

Multivariate PCA analysis of polar extract



Challenge for future research

→ **Different markers for dried fruits?**

→ **Reaction (thermal degradation) products of original markers?**

METABOLOMIC PROFILING (MARKERS ANALYSIS) IN PROCESSED FOODS

Metabolomic fingerprinting rather unsuitable for multicomponent foods

→ **Cranberries / lingonberries are often the minor component of foods**

→ **High variability in food composition - difficult to create multivariate models from fingerprints obtained**

RESULTS: CONFORMITY WITH DECLARATION, REVEALING OF FRAUD (fruit juices)

Case study # 2

AUTHENTICATION OF COLD PRESSED OILS OF VARIOUS BOTANICAL ORIGIN

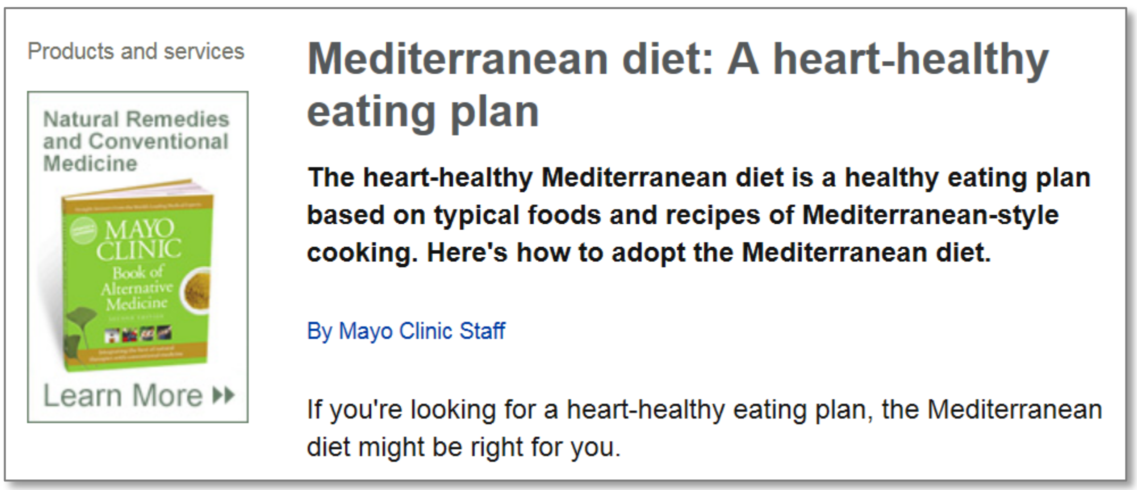
Cold pressed (virgin) oils

- Gentle pressing at low temperatures
- Advantages compared to refined oils
  - Delicious taste and smell
  - Natural color
  - Presence of valuable biologically active components

- Composition:
  - Lipids - mainly TAGs, free FA, minority of DAGs, MAGs
  - Lipid accompanying compounds: alcohols, terpenoids, vitamins

Cold pressed (virgin) oils

- Protective effects against lifestyle diseases (e.g. Cardiovascular)
- Results of epidemiological studies - healthy Mediterranean diet with a high use of olive oil



MAYO CLINIC

- **Expensive commodity – subject for frauds!**

AIM OF THE STUDY

FINGERPRINTING

- Development of extraction procedure
- LC-HRMS analysis, multivariate analysis
- Markers characterization
- Assessment of stability of markers during oils oxidation

Samples analyzed

12 types of cold pressed plant oils:

1. Argan (roasted seeds) (ROA)
2. Argan (raw seeds) (RA)
3. Gold flax (GF)
4. Brown flax (BF)
5. White poppy (WP)
6. Blue poppy (BP)
7. Nigella sativa (NS)
8. Red pumpkin (RP)
9. White sesame (WS)
10. Black sesame (BS)
11. Milk thistle (MT)
12. Hemp (HE)

Fzesh vs. oxidized (10 days at 60°C)

Workflow of sample preparation and data processing

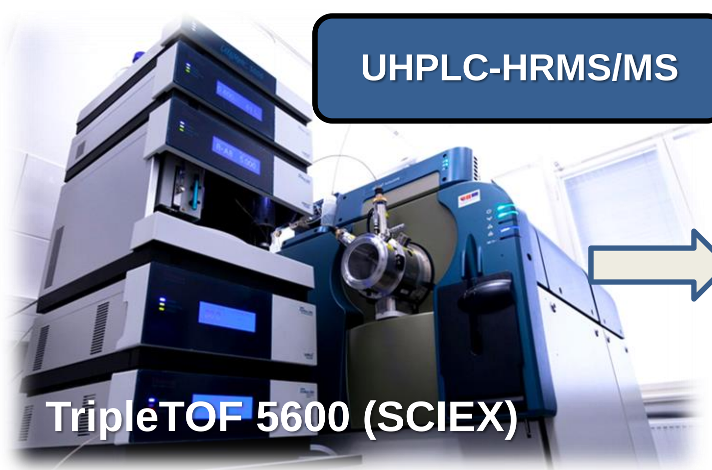
1 g of sample

3 mL MeOH:H2O (80:20 (*v/v*))

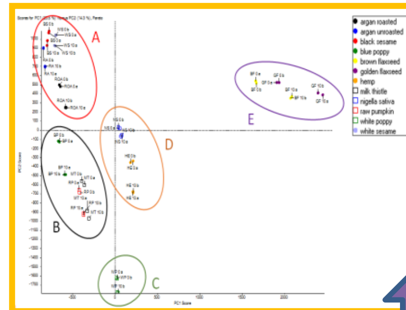
shaking

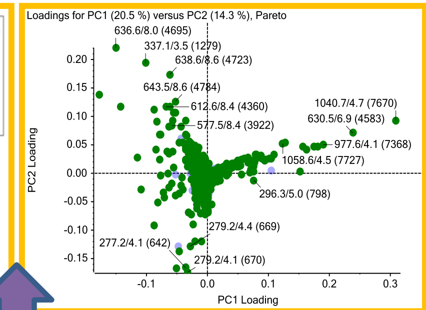
Centrifugation

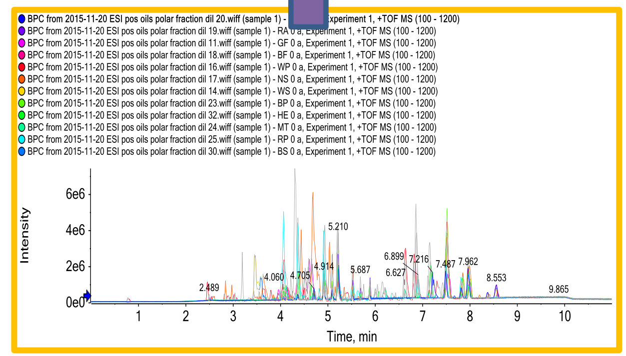
UHPLC-HRMS/MS



Data processing







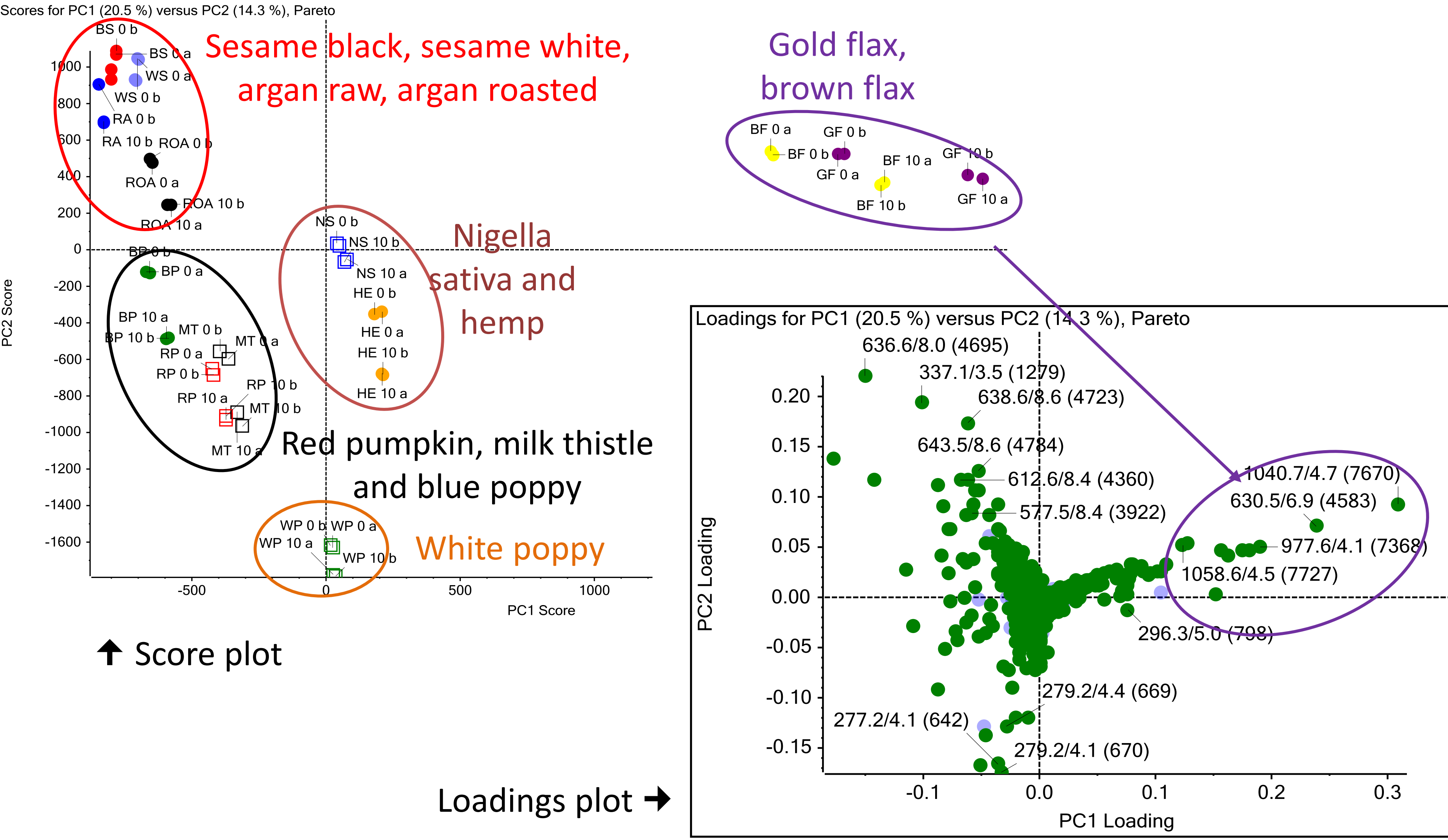
Multivariate analysis - PCA

MarkerView (SCIEX)

PeakView (SCIEX)

Markers identification

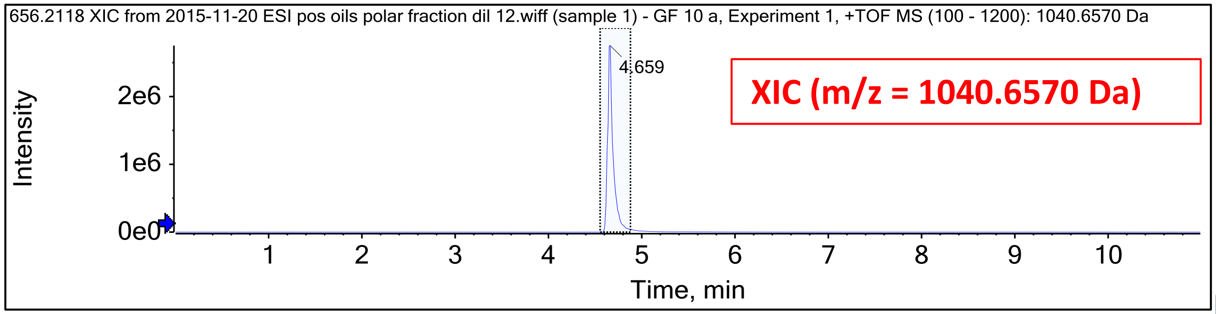
Multivariate PCA analysis (ESI+)

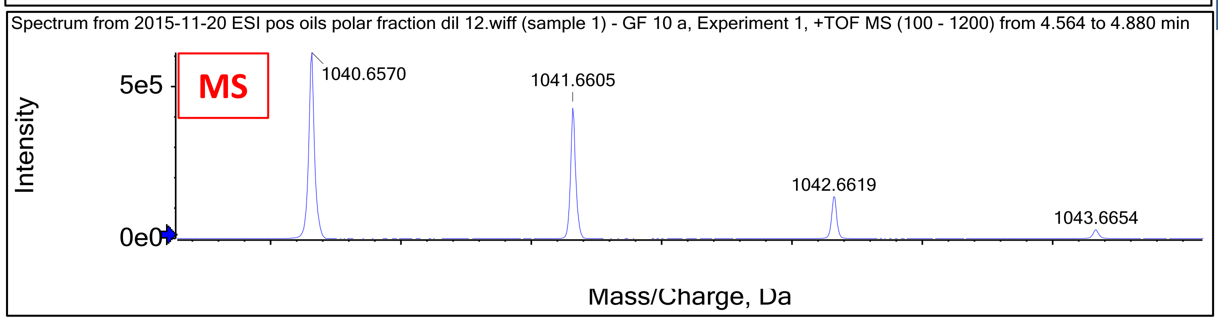


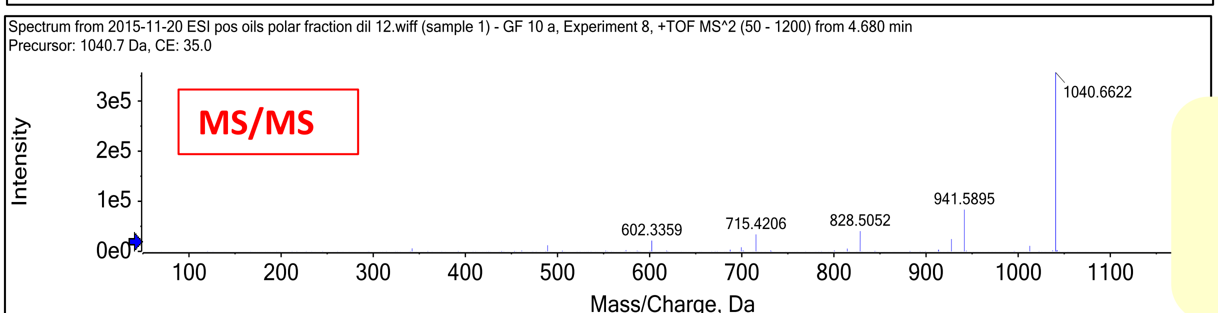
Markers identification

Example for gold flax seed oil

PeakView



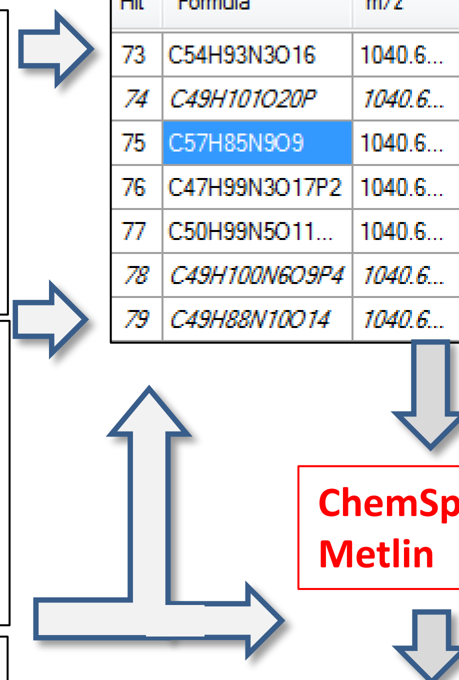




Navržené sumární vzorce

Hit	Formula	m/z	RDB	ppm	MS Rank
73	C54H93N3O16	1040.6...	10.0	-5.6	73
74	C49H101O20P	1040.6...	0.5	-4.6	74
75	C57H85N9O9	1040.6...	20.0	2.6	75
76	C47H99N3O17P2	1040.6...	1.0	4.6	76 ...
77	C50H99N5O11...	1040.6...	5.0	-3.8	76 ...
78	C49H100N6O9P4	1040.6...	5.5	7.0	78
79	C49H88N10O14	1040.6...	11.5	9.0	79

ChemSpider
Metlin



Formula finder

$C_{47}H_{85}N_9O_9$

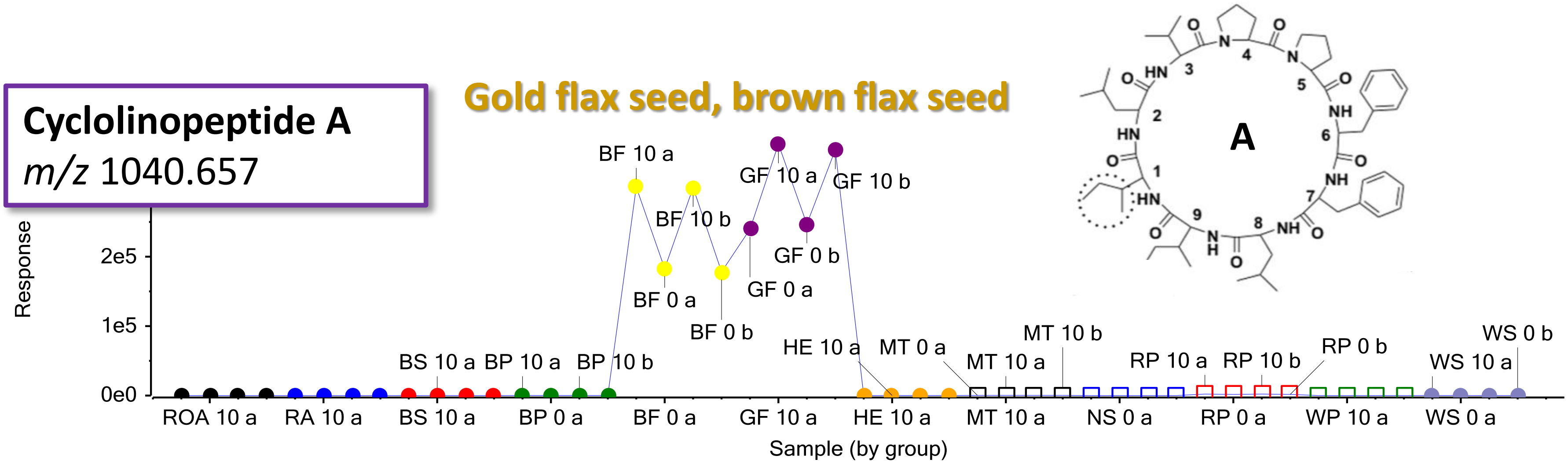
Cyclolinopeptide A

Mass error = 2.6 ppm

Plot profiles of markers of gold flax seed oil

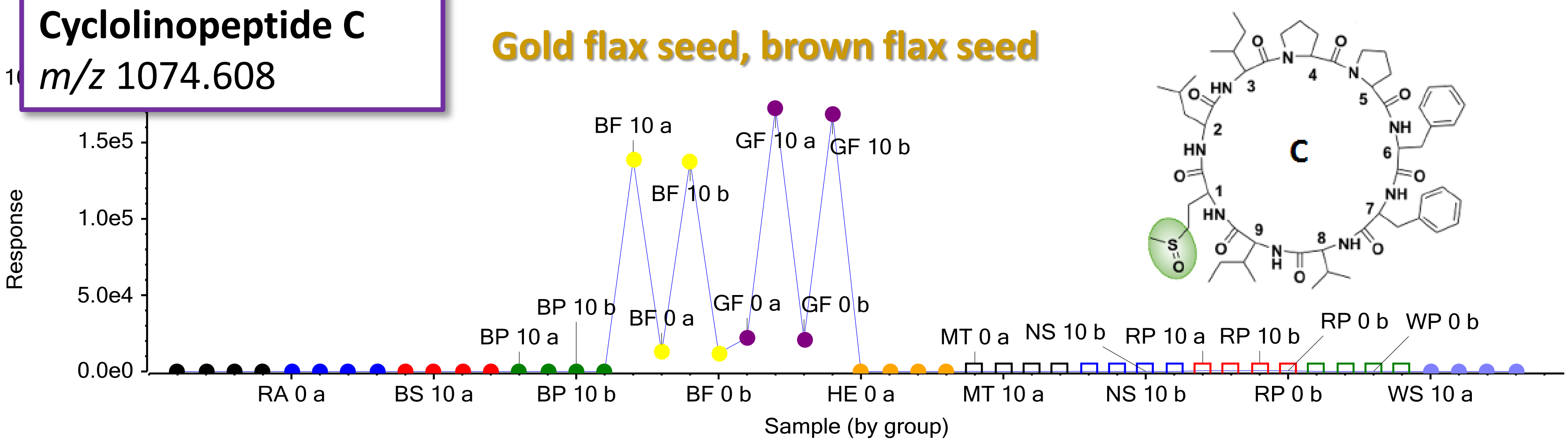
Cyclolinopeptide A
m/z 1040.657

Gold flax seed, brown flax seed

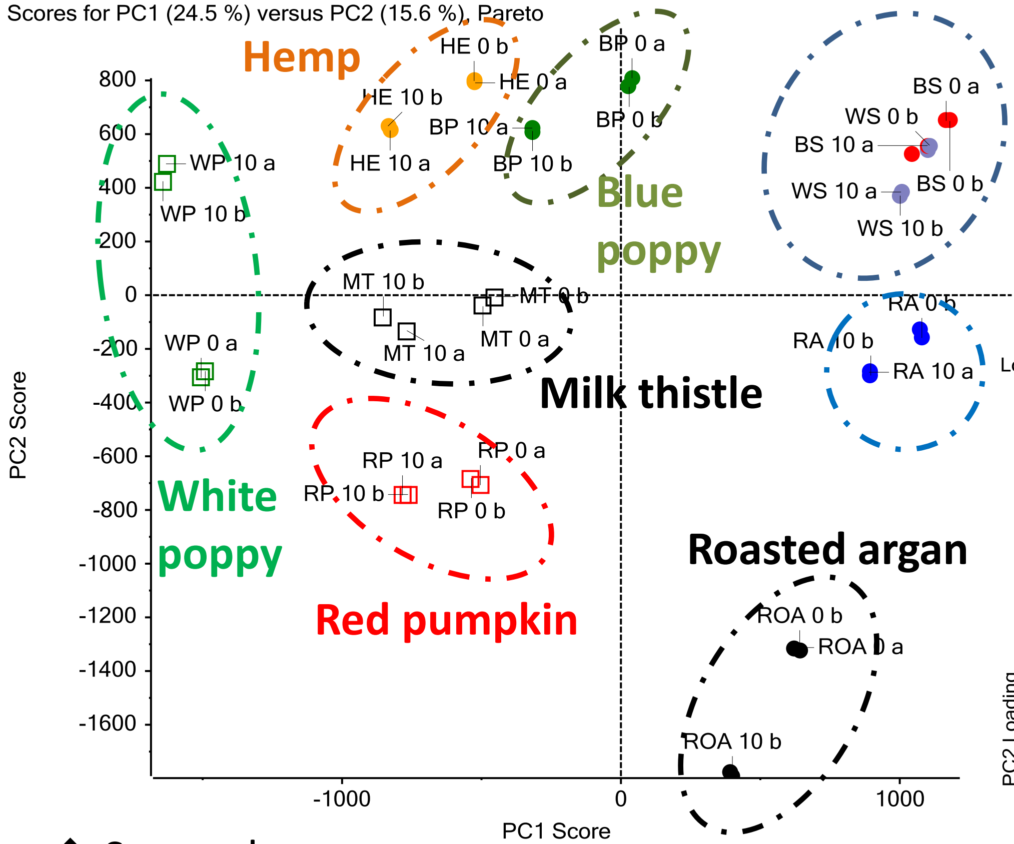


Cyclolinopeptide C
m/z 1074.608

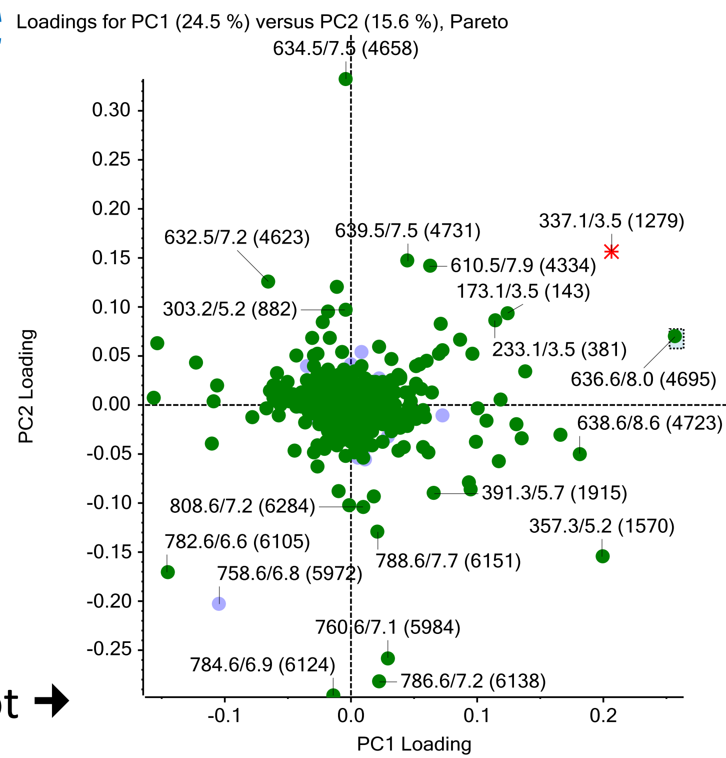
Gold flax seed, brown flax seed



Multivariate PCA analysis, without flax seed oils (ESI+)

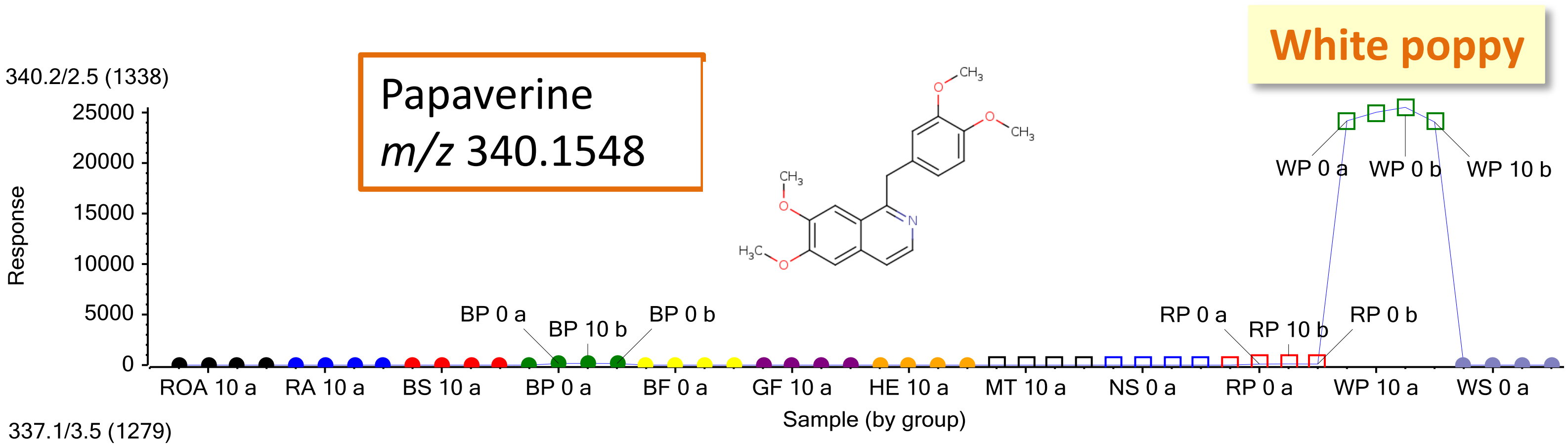


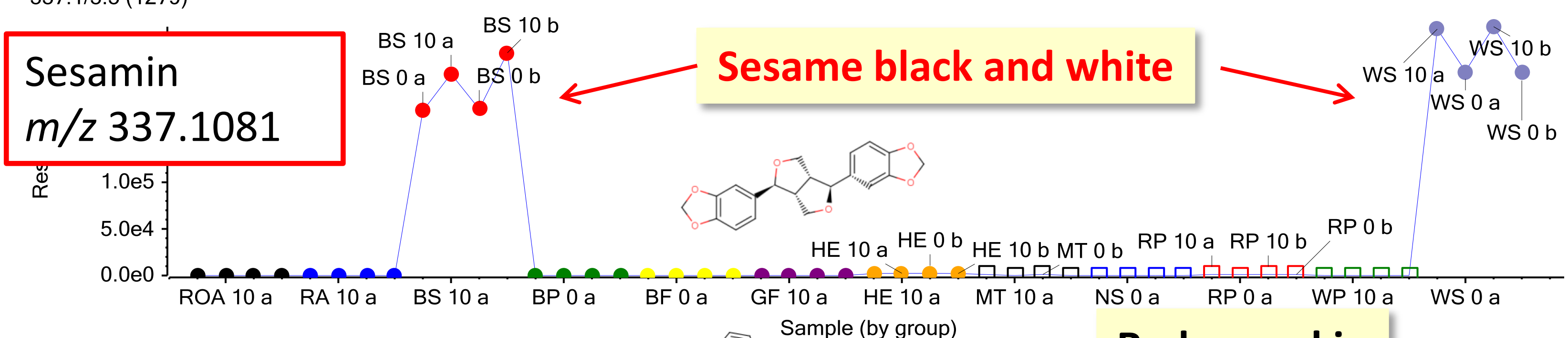
Score plot

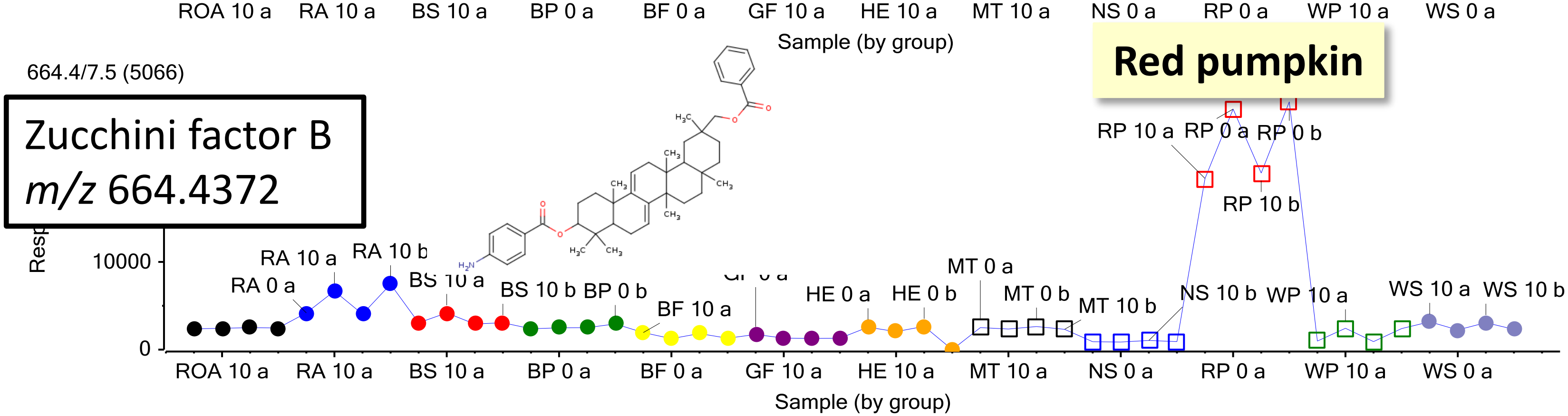


Loadings plot

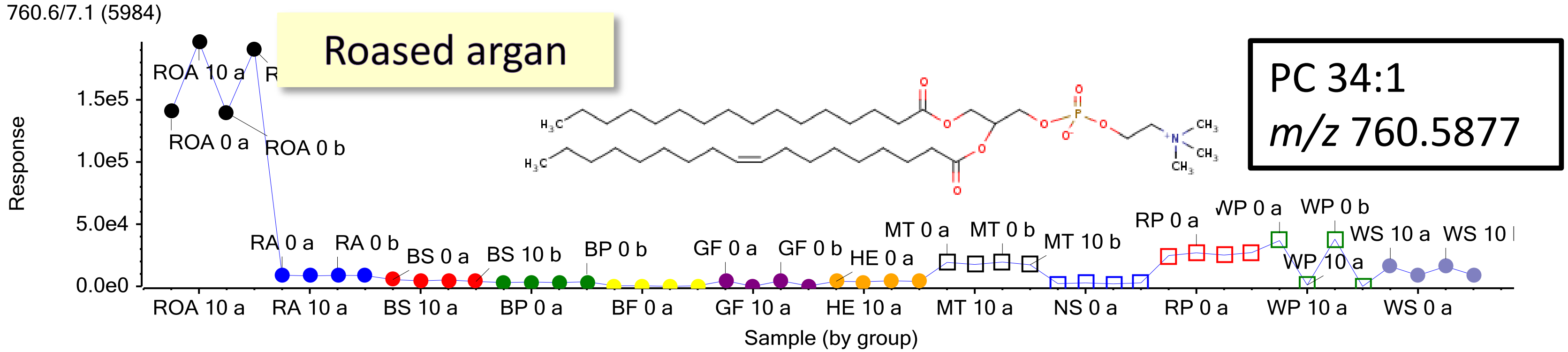
Plot profiles of markers of other cold pressed oils

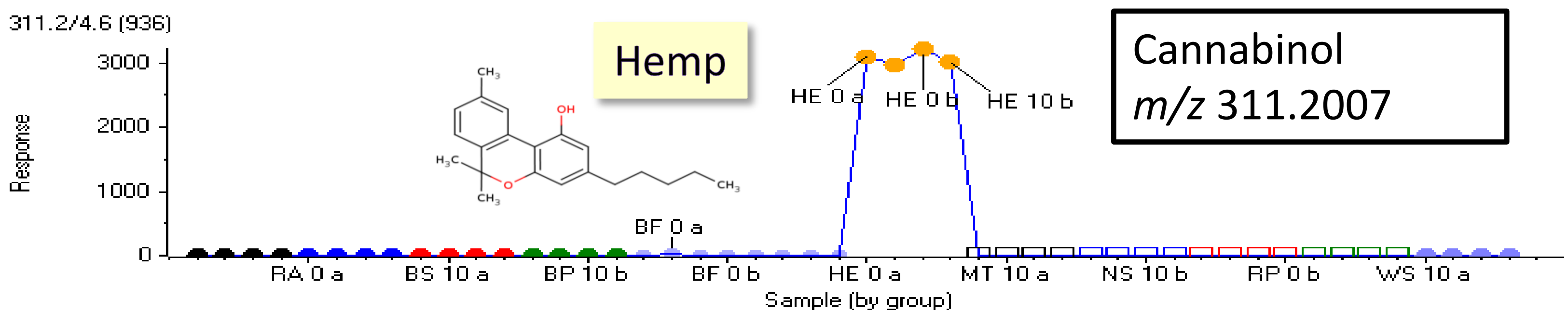


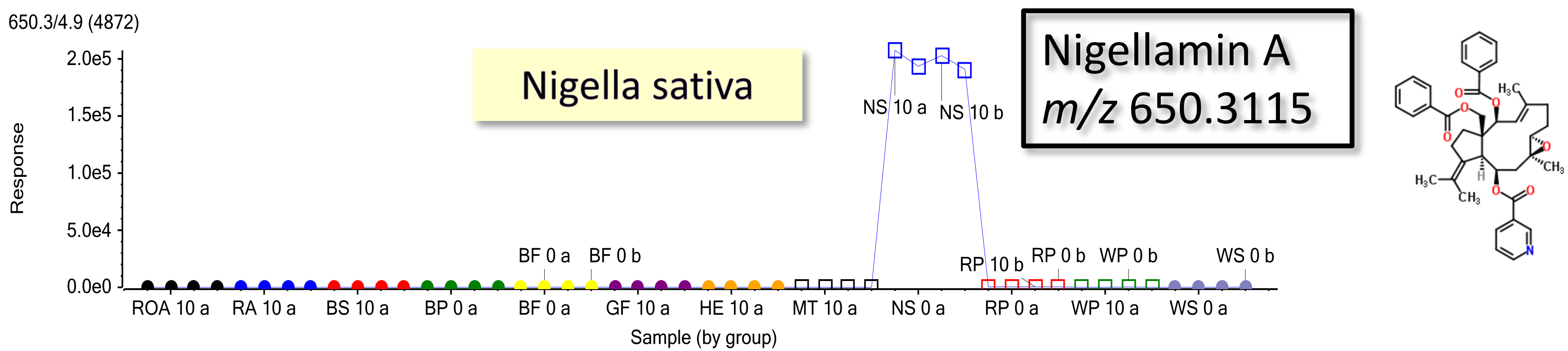




Plot profiles of markers of other cold pressed oils

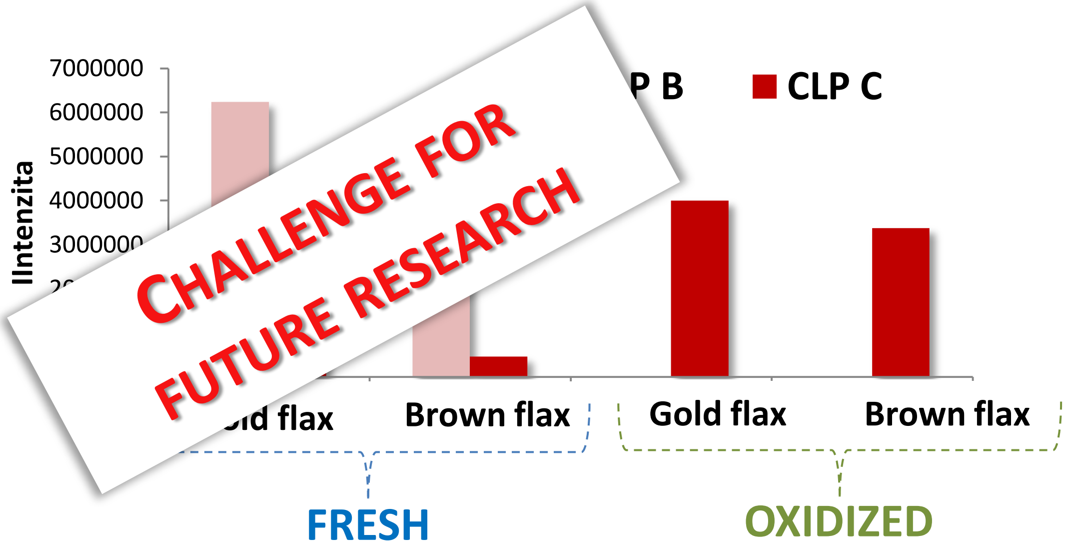


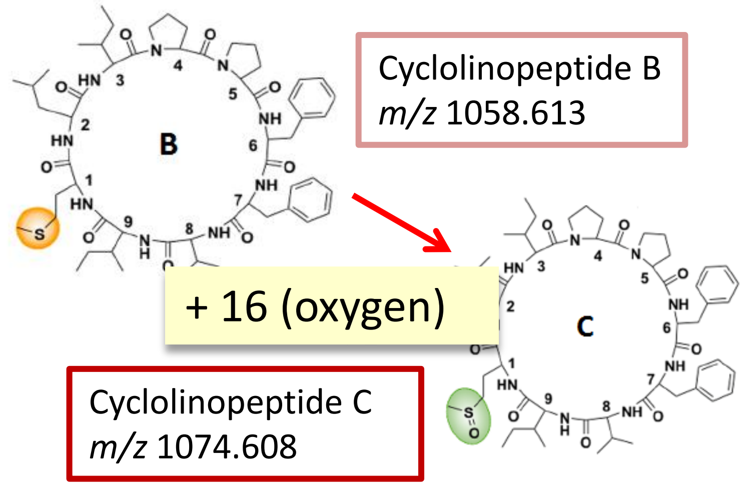


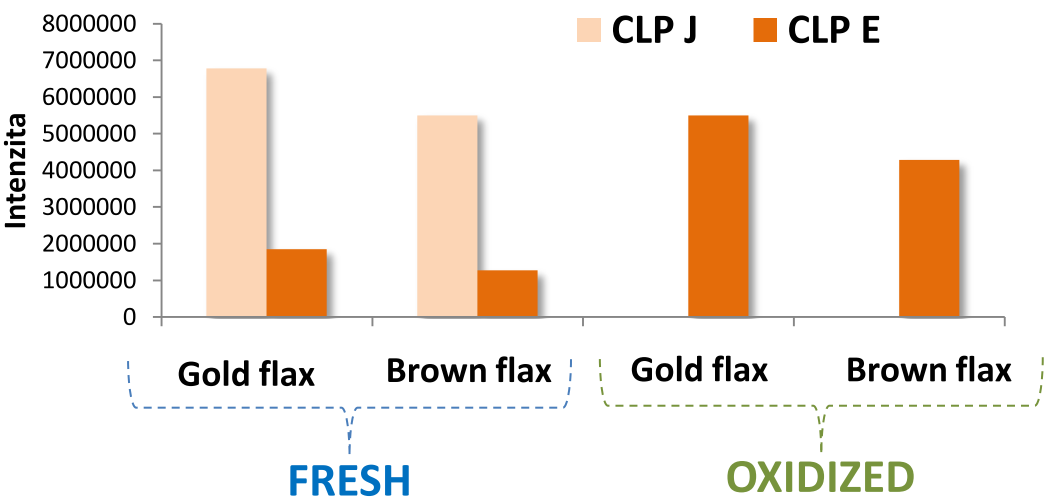


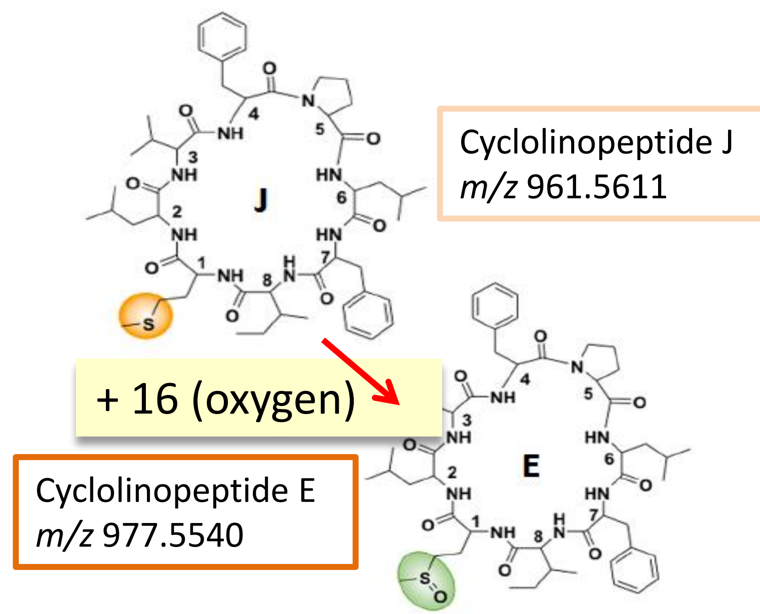
FRESH VS. OXIDIZED LIPIDS, stability of the markers during the flax seed oil oxidation

FRESH VS. OXIDIZED LIPIDS, stability of the markers





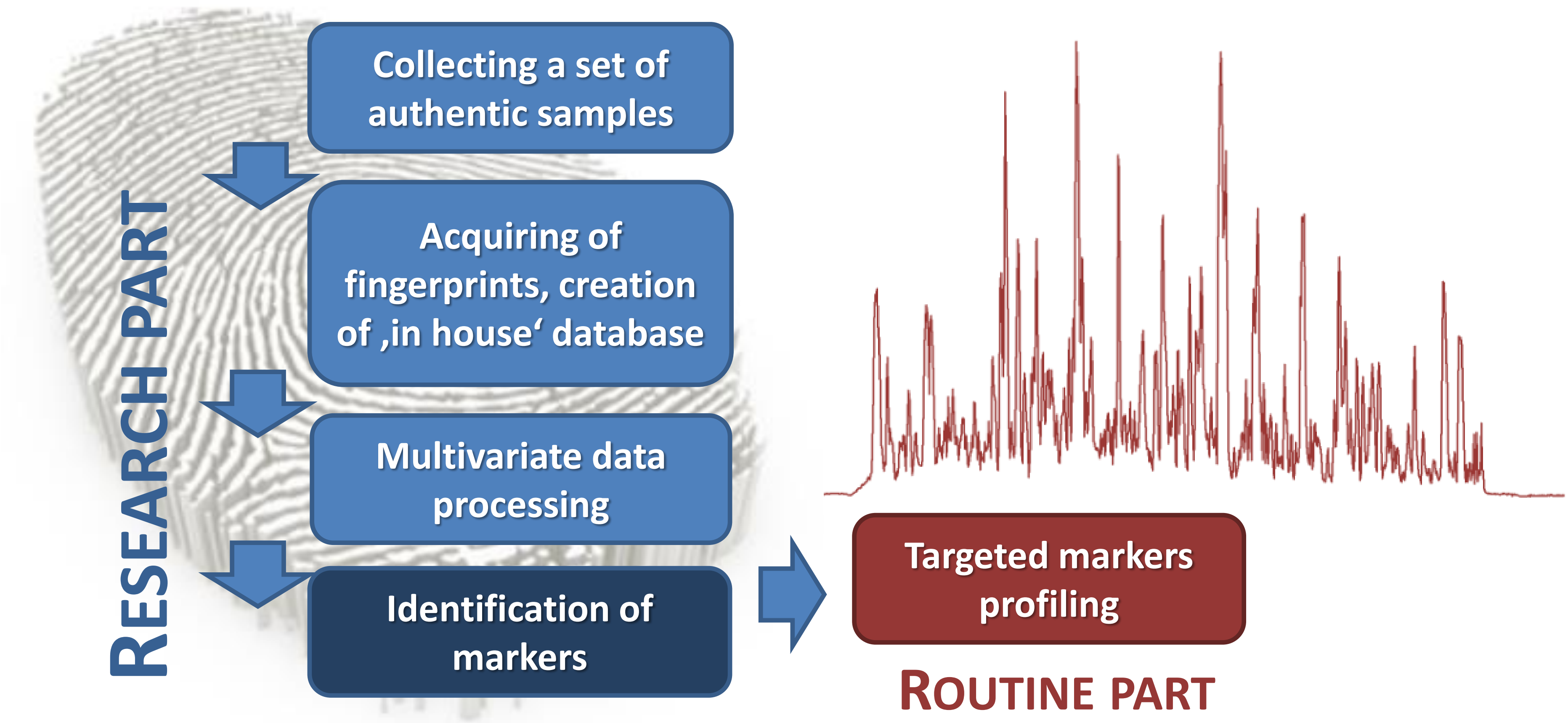




Approaching to the end...

CONCLUDING REMARKS

- **Broad application potential of LC-HRMS non-target metabolomic fingerprinting of small molecules**



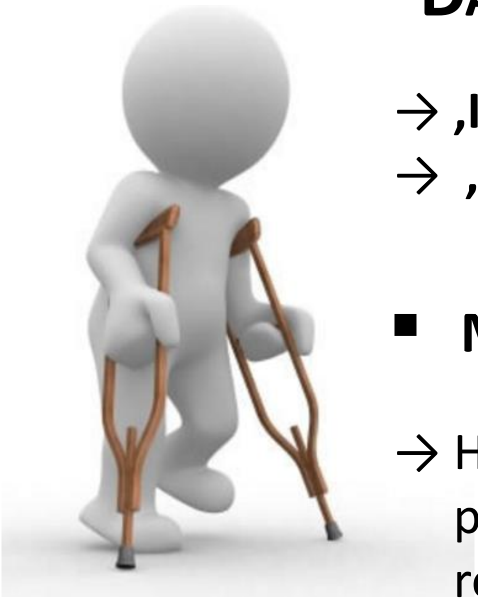
Concluding remarks

▪ Main advantages of LC-HRMS:

- **Low detection limits achievable** compared to other instrumental methods (IR or NMR), hence possibility to identify unique markers occurring at trace level
- **Structural identification of markers is feasible** in most cases

Concluding remarks

- **Main bottlenecks and challenges – building of harmonized LC-HRMS databases:**



- **Data handling**

→ **‚In house‘** databases - inter-temporal data transfer
→ **‚Joint‘** databases - interlaboratory data harmonization

- **METHODS TRANSFER**

→ Harmonized sample preparation protocols assuring uniformity and representativness of metabolomic fingerprints are necessary

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FOOD INTEGRITY

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Thank you for your attention…

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CONCLUDING REMARKS

- **MAIN CHALLENGES FOR OVERCOMING THE HURDLES:**

To assure harmonization of input data as much as possible

- Define requirements for MS instrumentation used (e.g. minimal mass resolving power)
- Assure high quality data alignment
- Define minimal treshold for intensity of input features
- Develop the algorithms for compensating the differences in response of different instruments (by using of suitable matrix reference material)