Challenges offered by various mass spectrometric techniques in oils authentication

Jana Hajslova, Klára Navrátilová, Vojtech Hrbek, Michal Stupak, Monika Tomaniova

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

EUCHINASAFE 中欧食品安全

Claims on cold pressed oils

Cold pressed oils will provide a vital contribution to your healthier life…

- They are not refined, deodorized or processed in any way
- They contain natural antioxidants such as polyphenols, tocopherols and phosphoplipids
- The natural flavour and odour is retained, enhancing respective favourite recipes
- *They are cholesterol free*
- *They do not contain harmful solvent residues*
- *No added chemicals or preservatives*

Cold pressed oils

Growing demand, high value commodity

→ prone to fraud (?)

Characteristics in any fraud definition

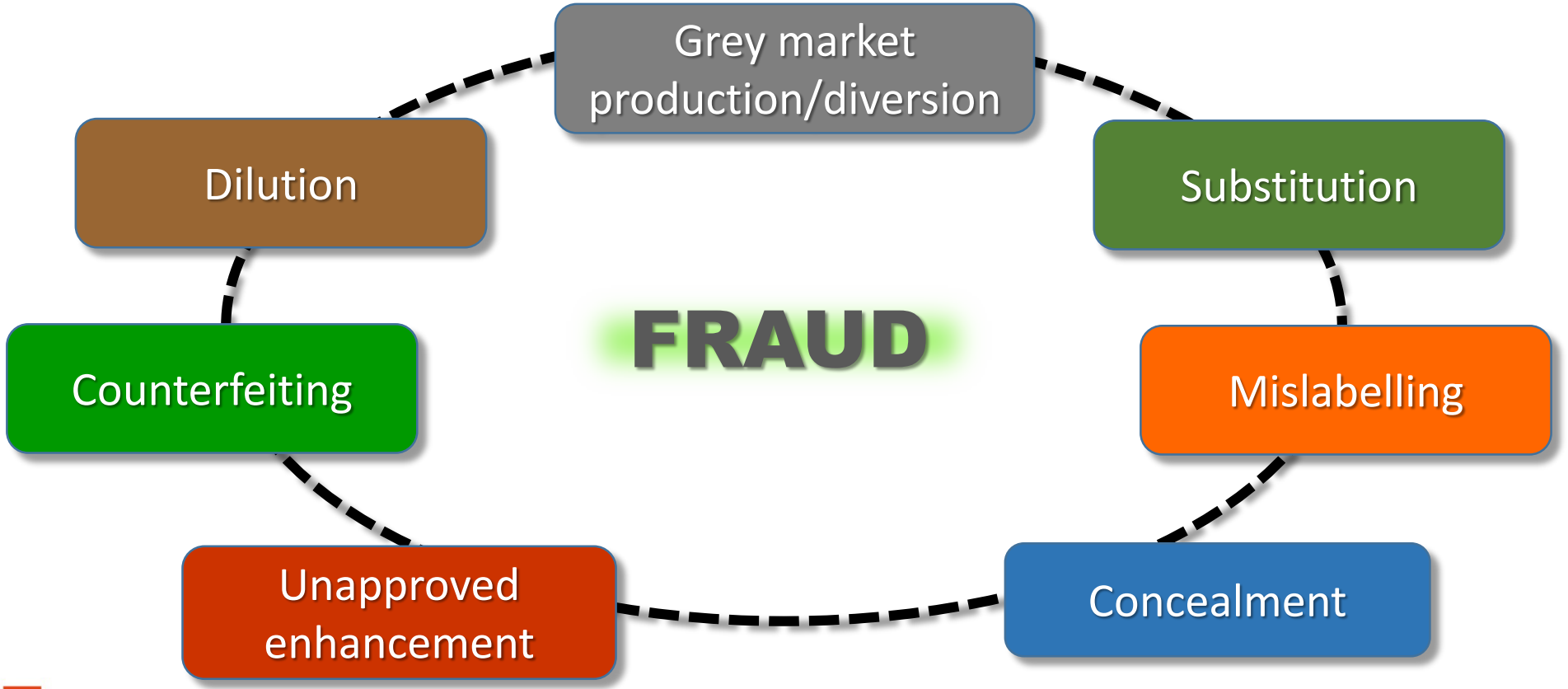
Food law violation

Intention

Economic gain

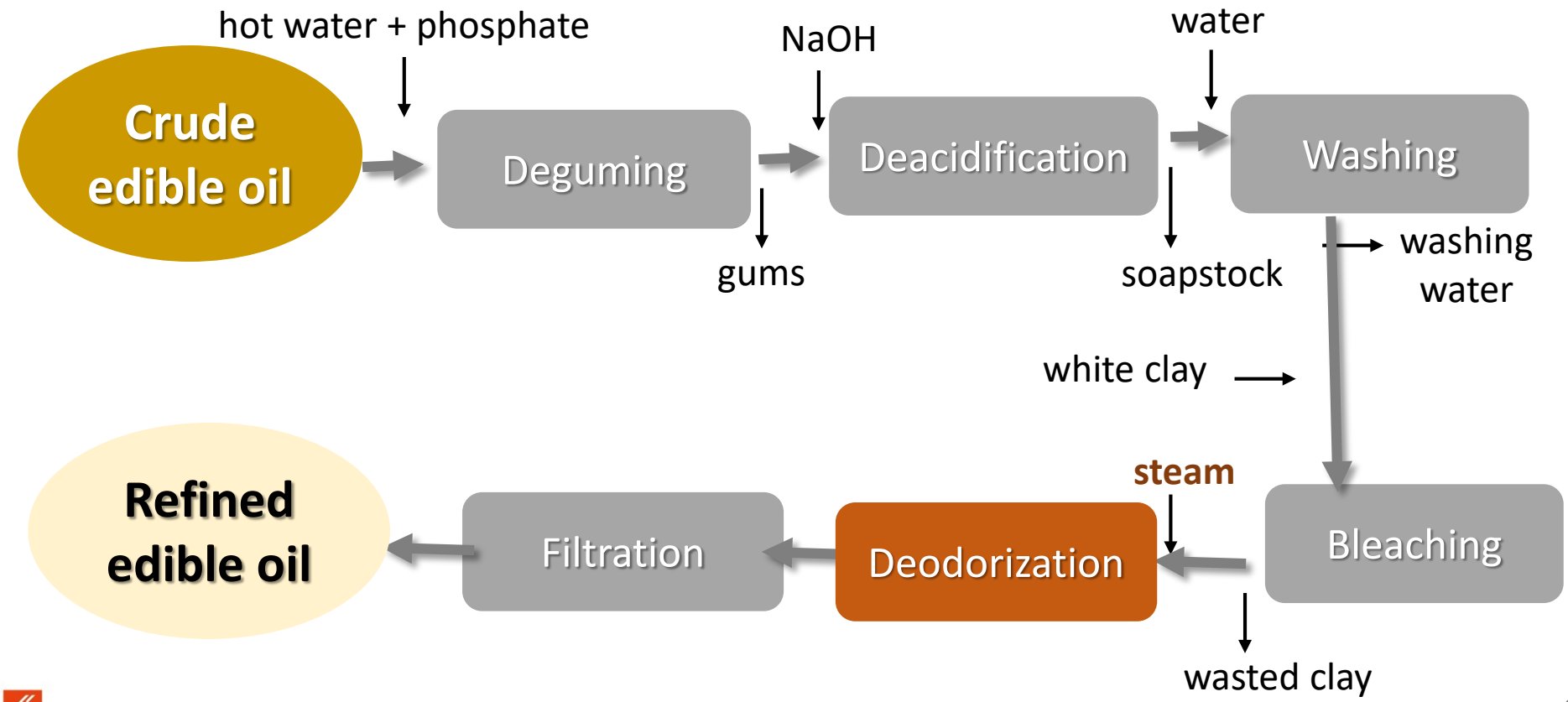
Customer deception

Conceivable types of fraud on plant oils

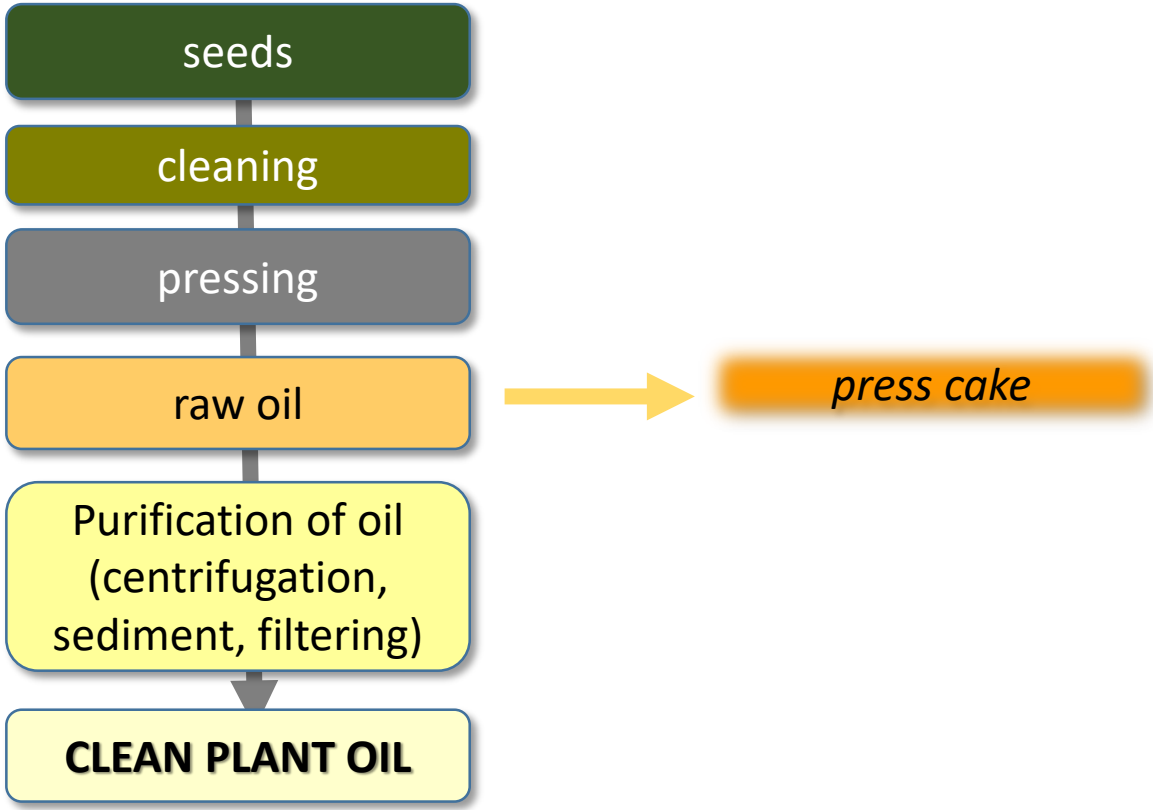


https://knowledge4policy.ec.europa.eu/food-fraud-quality/topic/food-fraud_en#Types

Common refining process of edible oils



Cold pressed plant oils preparation

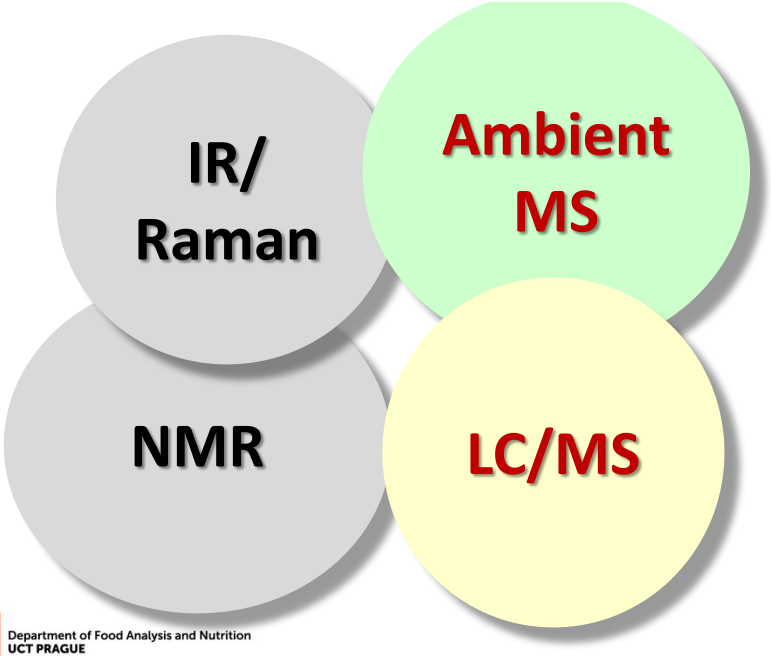


Recent authentication strategies?

Advanced techniques in edible oil authentication: A systematic review and critical analysis

Anjali Sudhakar, Subir Kumar Chakraborty, Naveen Kumar Mahanti & Cinu Varghese

https://doi.org/10.1080/10408398.2021.1956424 (2021)



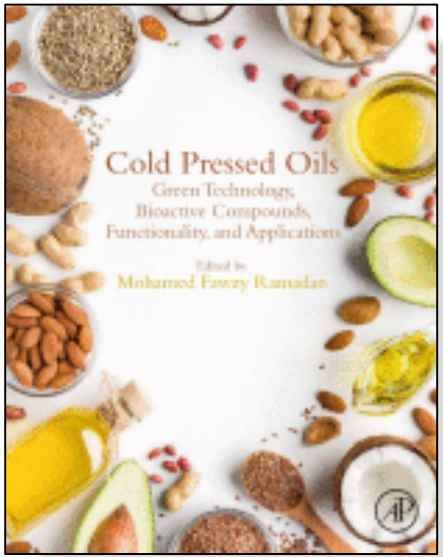
Most often employed techniques for authentication of olive oil, but also sesame oil, flaxseed, walnut, borage..

However, what about other rarer plant oils?

Complex composition of cold pressed oils

MAJOR COMPONENTS - triacylglycerols, phytosterols, tocopheols hydrocarbons, carrotenoids….mostly unspecific

MINOR (BIOACTIVE) COMPONENTS - polar seed / fruits species specific secondary metabolites → AUTHENTICITY MARKERS ?



https://doi.org/10.1016/C2018-0-03151-5

Case study no.1

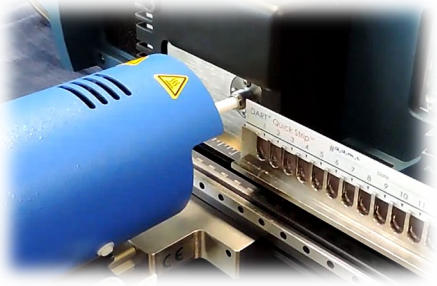
Starting with authentication of plant oils by ambient mass spectrometry (DART-MS)

Ambient mass spectrometry, DART-TOFMS

GENERAL

- Minimal sample preparation
- Rapid analysis → high sample throughput

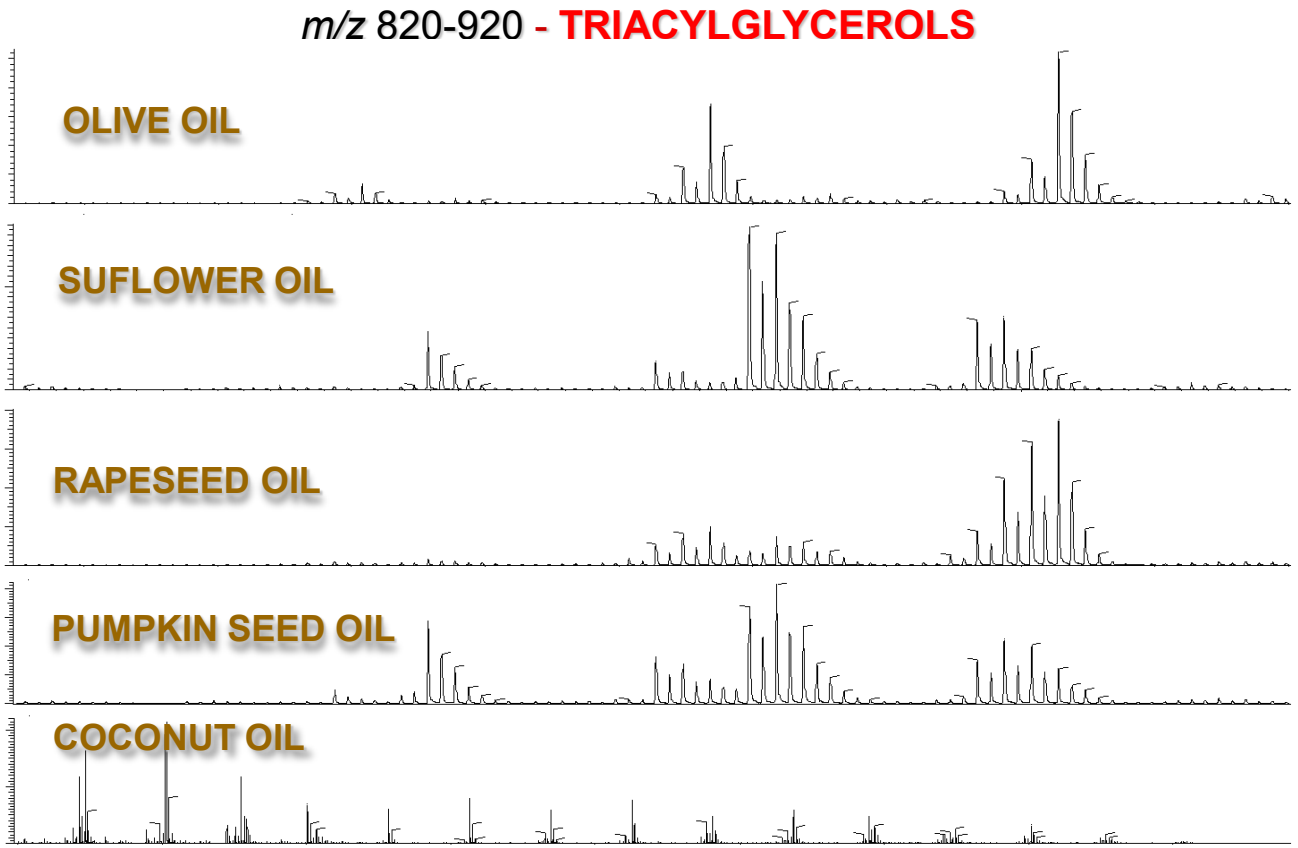
- Isomers cannot be recognized
- Intensive matrix effects (signal supression)



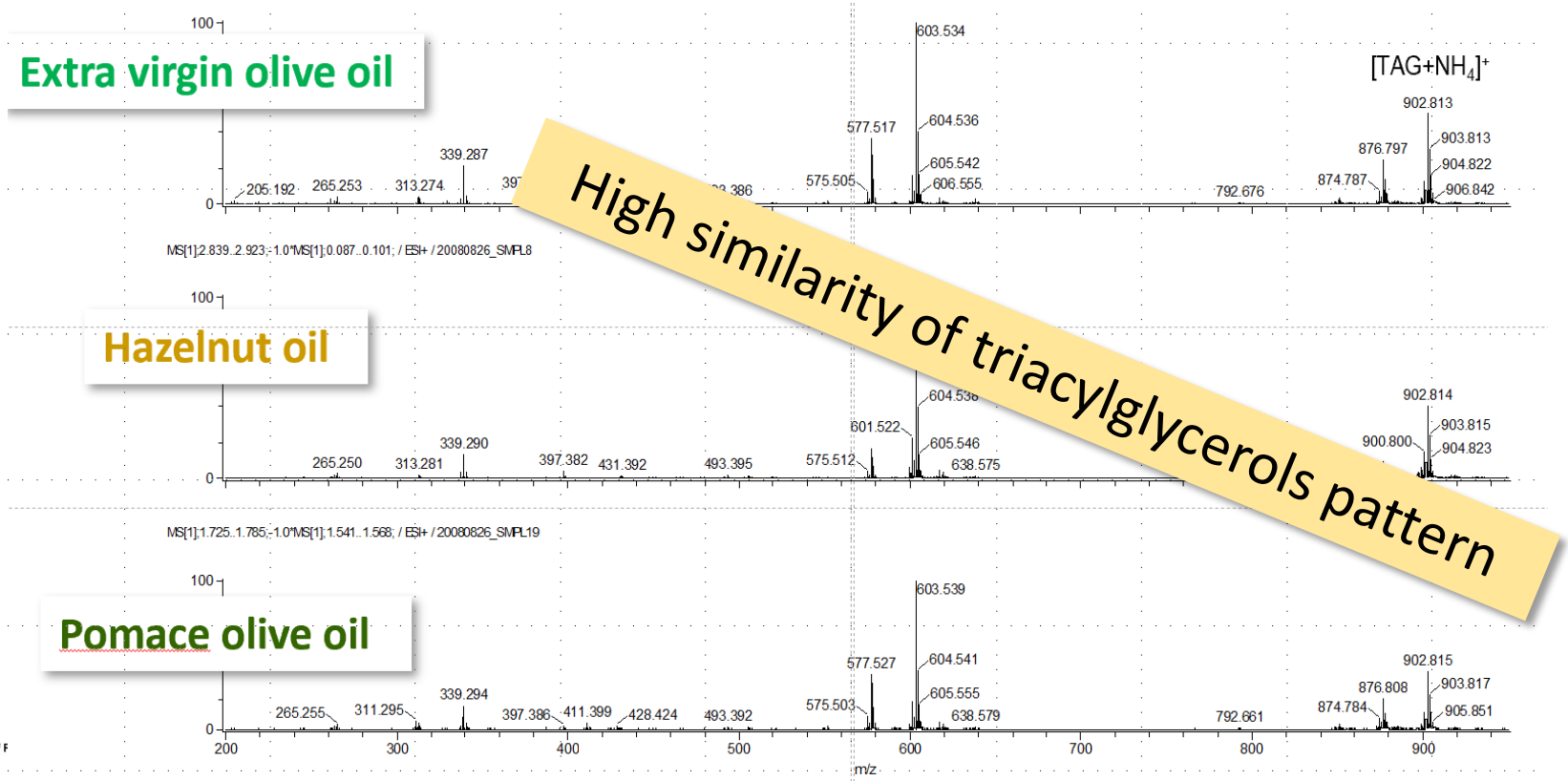
OILS ANALYSIS

- Minimal interferences in lipids region, i.e. ions at high m/z
- Polar lipids such as phospholipids are poorly transferred into gas phase → not represented in mass spectrum

DART-TOF MS spectra of various plant oils

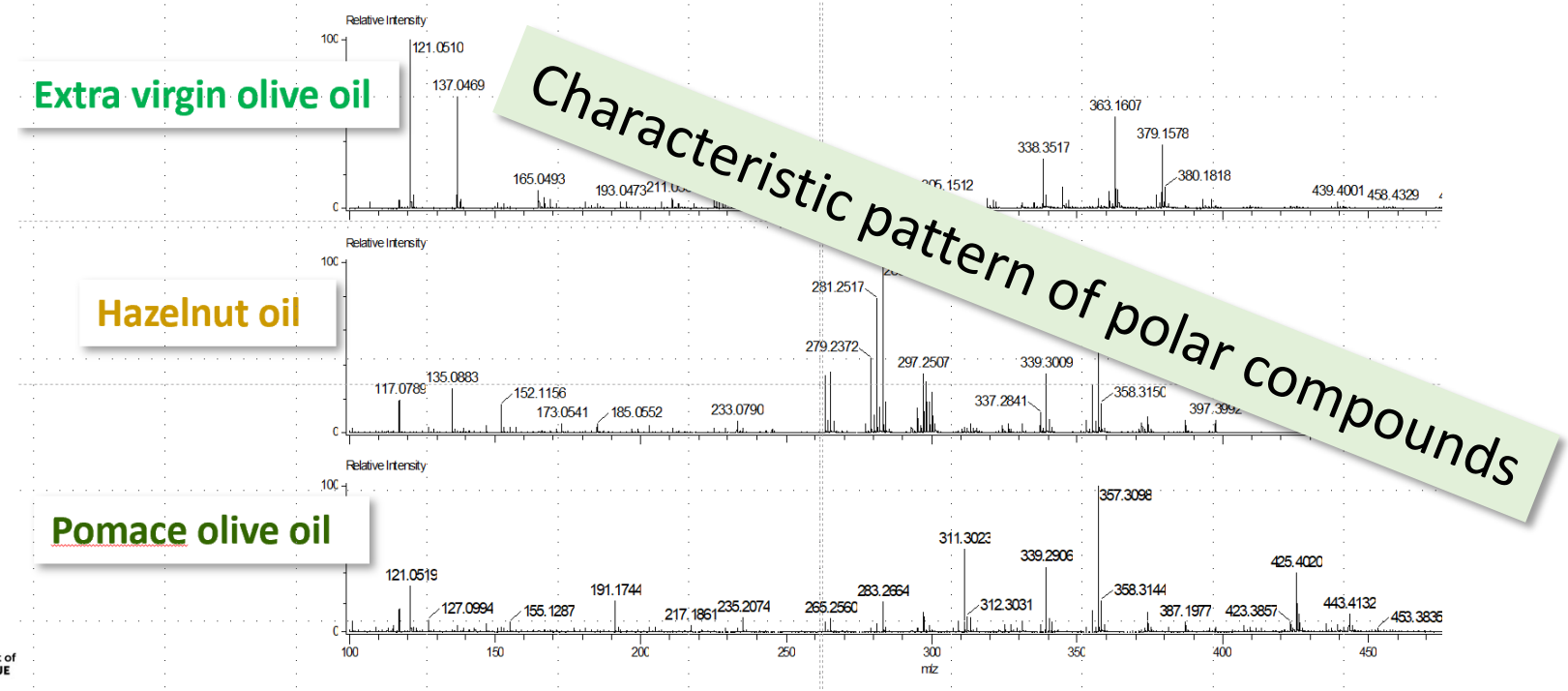


DART-TOFMS spectra: extra virgin olive oil, hazelnut oil, pomace oil



DART-TOFMS spectra: extra virgin olive oil, hazelnut oil, pomace oil – POLAR FRACTION

(extract obtained by aqueous methanol)



Case study no.2

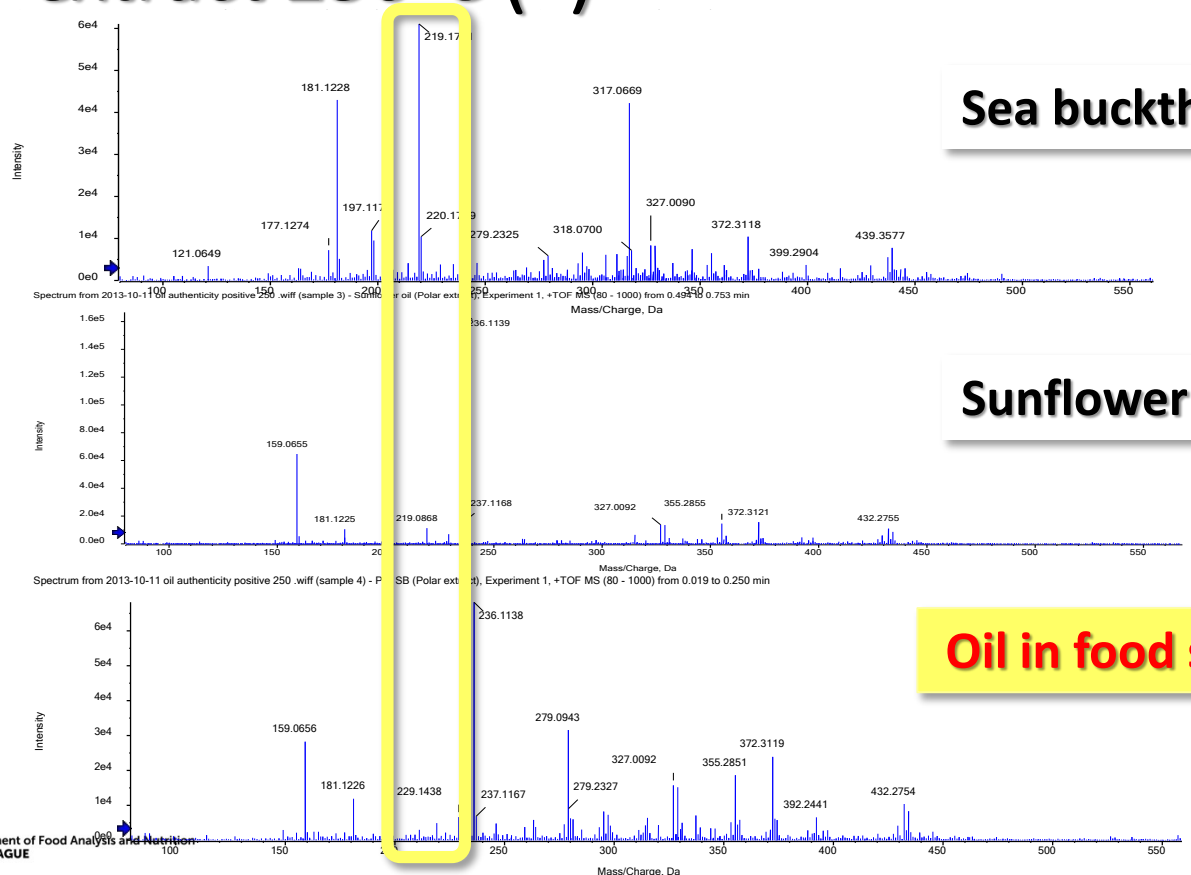
Fraud on the sea buckthorn oil food supplement

Background: customer´s doubts about the authenticity of ´healthy´ food supplement

SAMPLES:

1. **Sea buckthorn oil (Reference material)**
2. **Sunflower oil (Reference material)**
3. **Sea buckthorn oil pills (suspected commercial sample)**

DART–HRMS mass spectra of aqueous methanol extract 250°C (+)

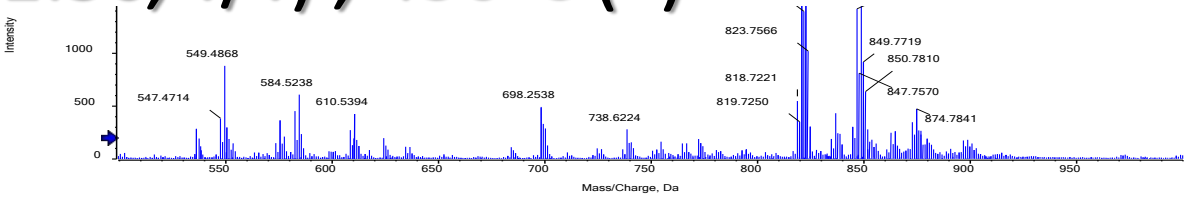


Sea buckthorn oil

Sunflower oil

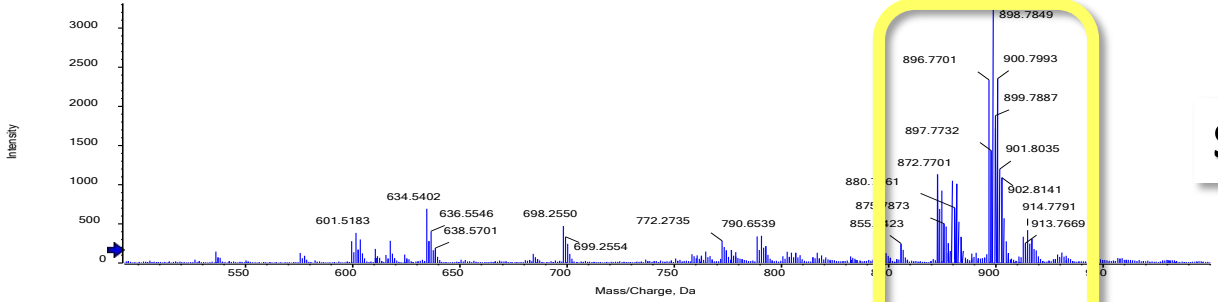
Oil in food supplement

DART–HRMS mass spectra of oils diluted in toluene (1:50, v/v) , 450°C (+)

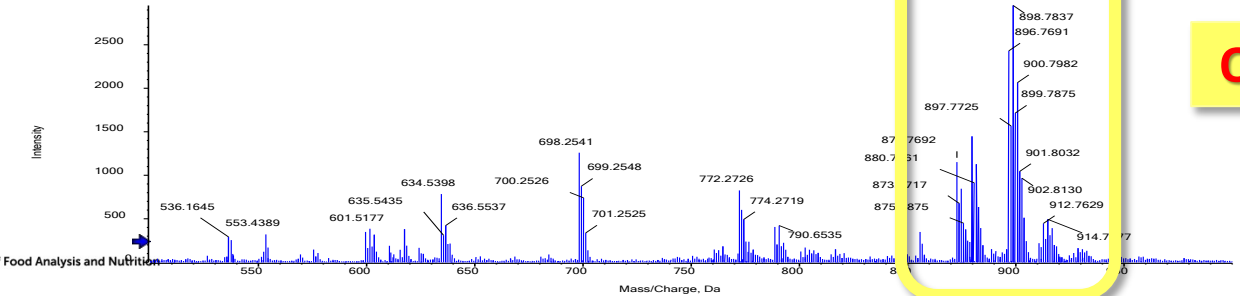


Sea buckthorn oil

Spectrum from 2013-10-11 oil authenticity positive 450 apolar extract .wiff ...olar extract) 50, Experiment 1, +TOF MS (80 - 1000) from 0.086 to 0.367 min



Spectrum from 2013-10-11 oil authenticity positive 450 apolar extract .wiff...lar extract) 50, Experiment 1, +TOF MS (80 - 1000) from 0.061 to 0.359 mi



Sunflower oil

Oil in food supplement

Confirmed

FRAUD!!!
Result in
10 min.

SUNFLOWER OIL
+ monoacylglycerols (emulsifiers)
+ β-carotene (provitamin A mix)

Papers on plant oils authentication by ambient mass spectrometry (DART-MS)

Analytica Chimica Acta 645 (2009) 56–63

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Ambient mass spectrometry employing direct analysis in real time (DART) ion source for olive oil quality and authenticity assessment

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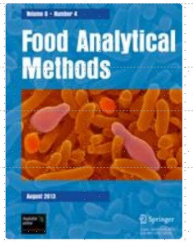
Institute of Chemical Technology Prague, Faculty of Food and Biochemical Technology, Department of Food Chemistry and Analysis, Technicka 5, 166 28 Prague 6, Czech Republic

Strategies to Document Adulteration of Food Supplement Based on Sea Buckthorn Oil: a Case Study

Kamila Hurkova, Josep Rubert, Milena Stranska-Zachariasova & Jana Hajslova

Food Analytical Methods **10**, 1317–1327 (2017) | Cite this article



TrAC Trends in Analytical Chemistry

Trends in Analytical Chemistry Vol. 30, No.2, 2011

Challenging applications offered by direct analysis in real time (DART) in food-quality and safety analysis

Jana Haislova, Tomas Cajka, Lukas Vaclavik

Case study no.3

Collection of UHPLC-HRMS data for cold pressed oils authentication and assessment of oxidation stability

Set of freshly pressed oils

1. Argan, roasted seeds) (ROA)
2. Argan seeds (RA)
3. Linseed gold (GF)
4. Linseed brown (BF)
5. Poppy seeds white (WP)
6. Poppy seeds blue (BP)
7. Black cumin(NS)
8. Pumpkin seeds (RP)
9. Sesam seeds white (WS)
10. Sesam seeds black (BS)
11. Milk Thistle (MT)
12. Hemp seeds (HE)

Sample processing and analysis

NON-POLAR FRACTION

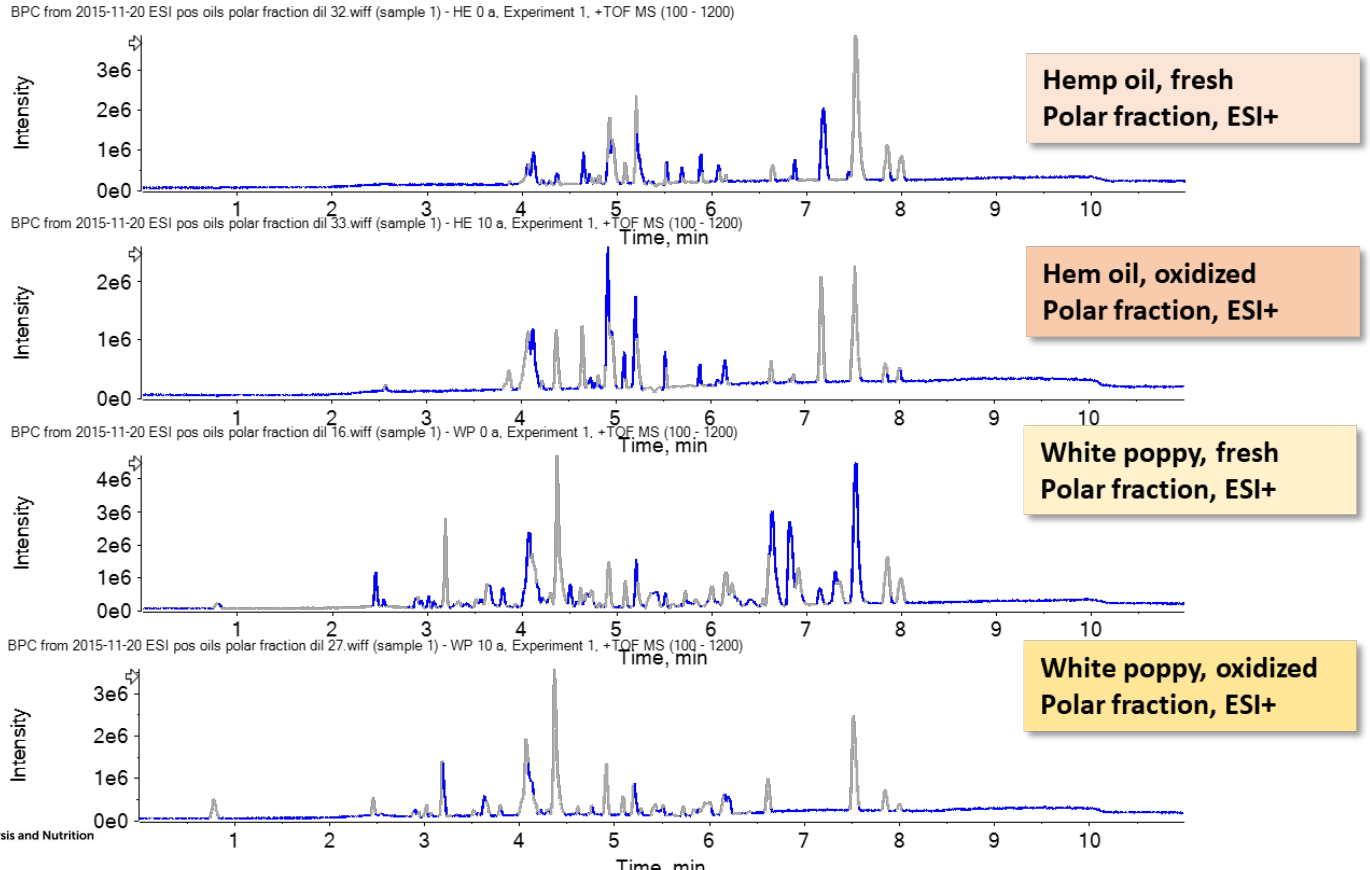
Diluted, filtered oil

POLAR FRACTION

extracted by aquoues methanol (4:1, v/v)

U-HPLC-HRMS/MS

Comparison of fresh and oxidized oils



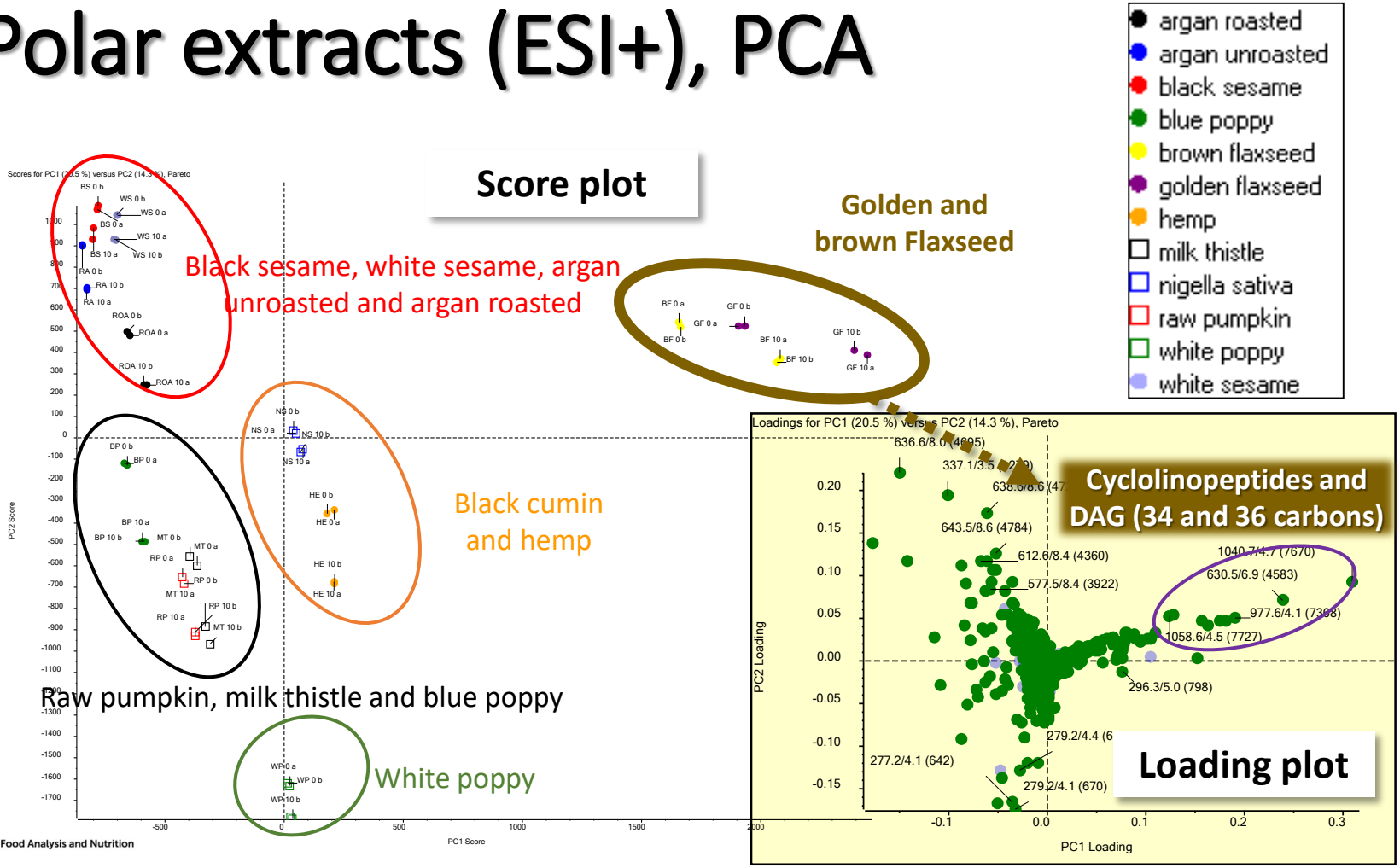
Hemp oil, fresh
Polar fraction, ESI+

Hem oil, oxidized
Polar fraction, ESI+

White poppy, fresh
Polar fraction, ESI+

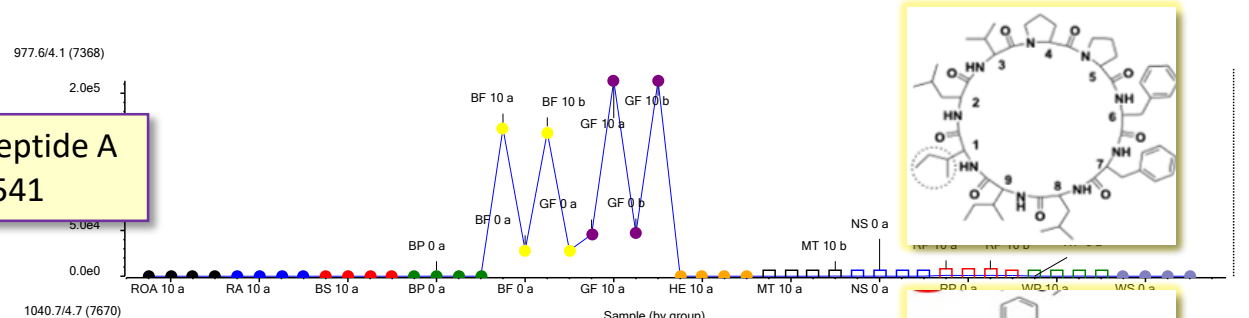
White poppy, oxidized
Polar fraction, ESI+

Polar extracts (ESI+), PCA

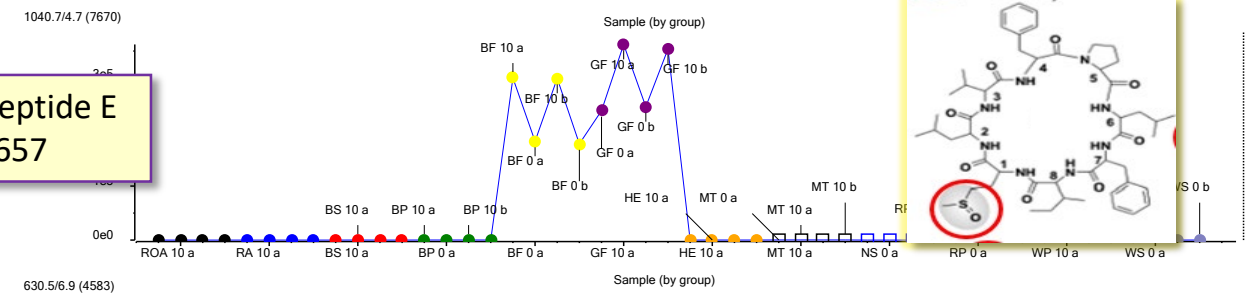


Unique markers in flaxseed oils - trend plot

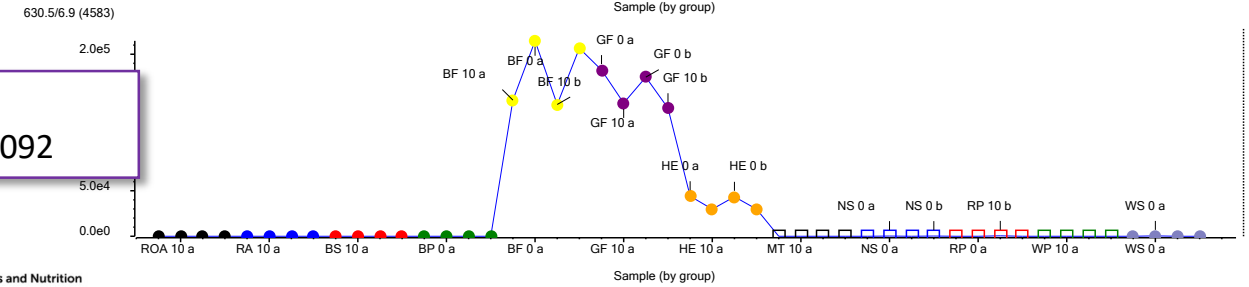
Cyclolinopeptide A
m/z 977.5541



Cyclolinopeptide E
m/z 1040.657



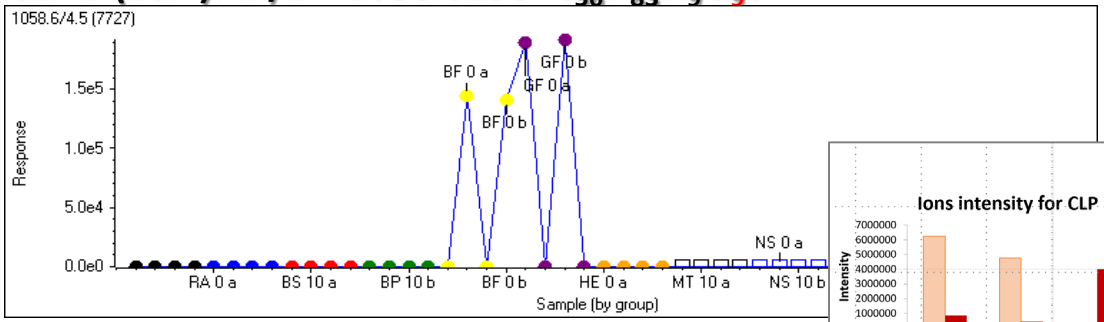
DAG 36:6
m/z 630.5092

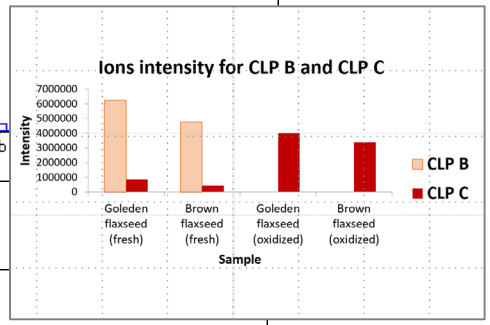


- argan roasted
- argan unroasted
- black sesame
- blue poppy
- brown flaxseed
- golden flaxseed
- hemp
- milk thistle
- nigella sativa
- raw pumpkin
- white poppy
- white sesame

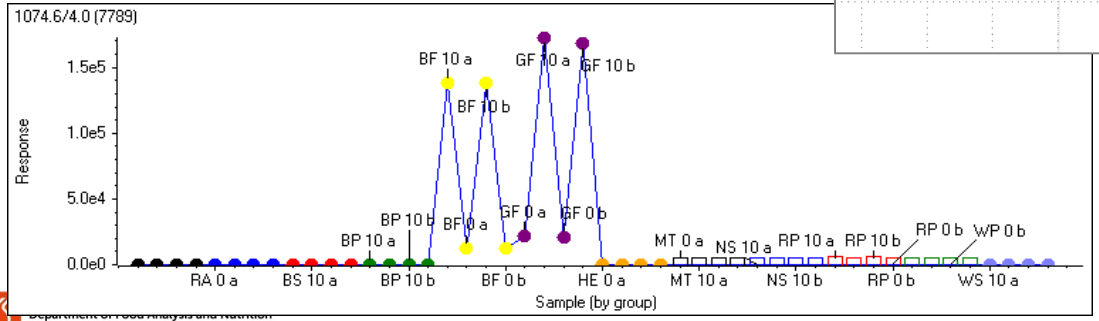
Oxidation of cyclolinopeptide B ➔cyclolinopeptide C

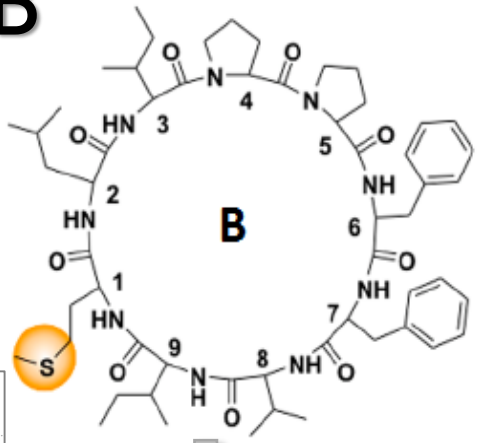
CLP B (ESI+): m/z – 1058.6150 -$C_{56}H_{83}N_9O_9S$

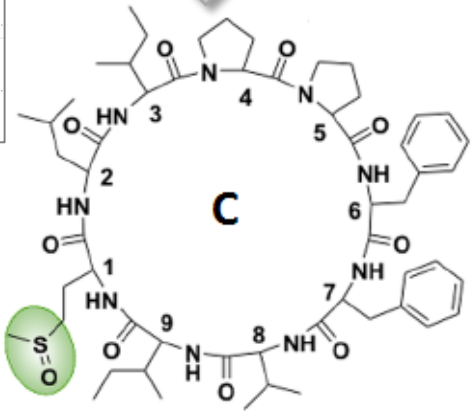




CLP C (ESI+): m/z – 1074.6075 - $C_{56}H_{83}N_9O_{10}S$





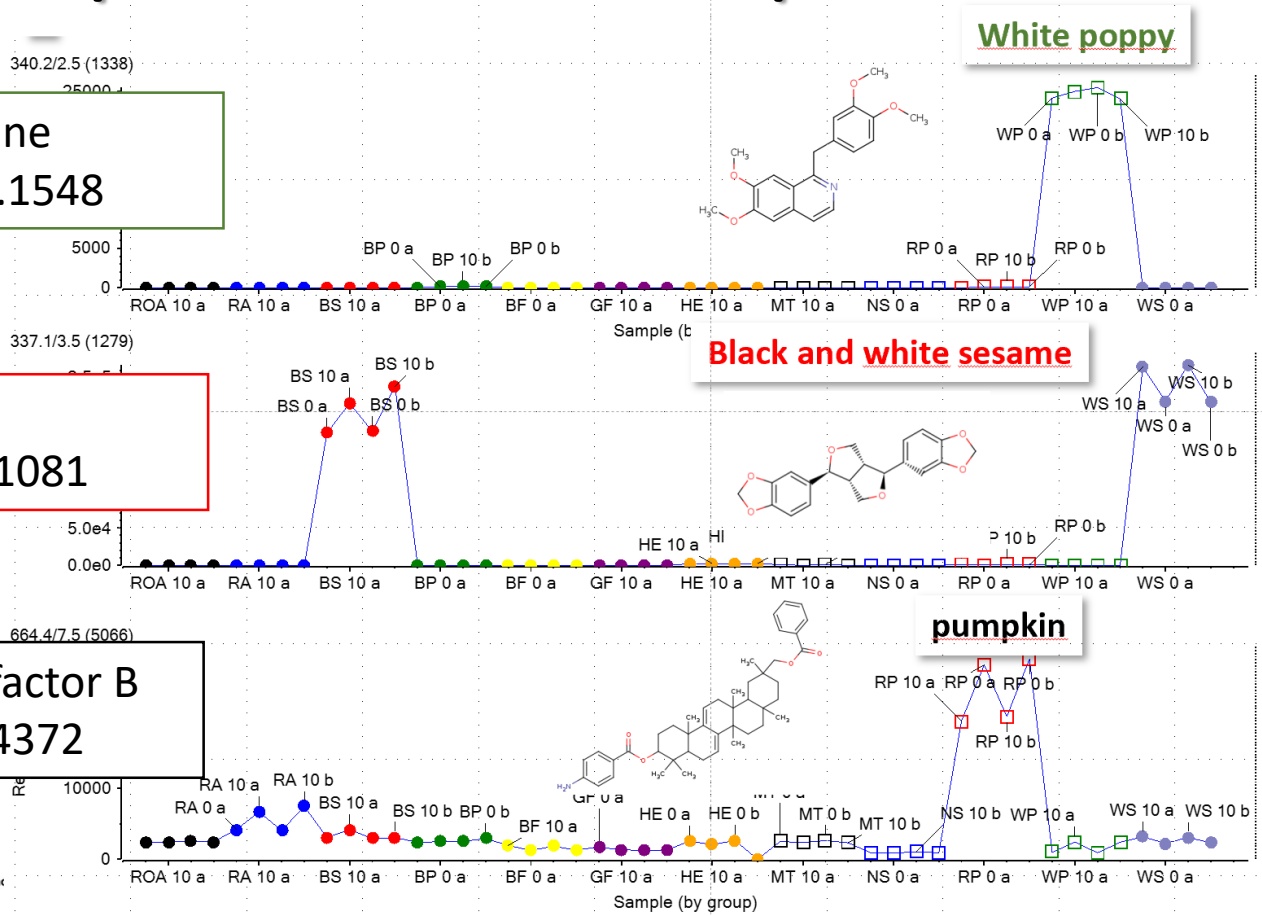


Example of other cold pressed oils markers

Papaverine
m/z 340.1548

Sesamin
m/z 337.1081

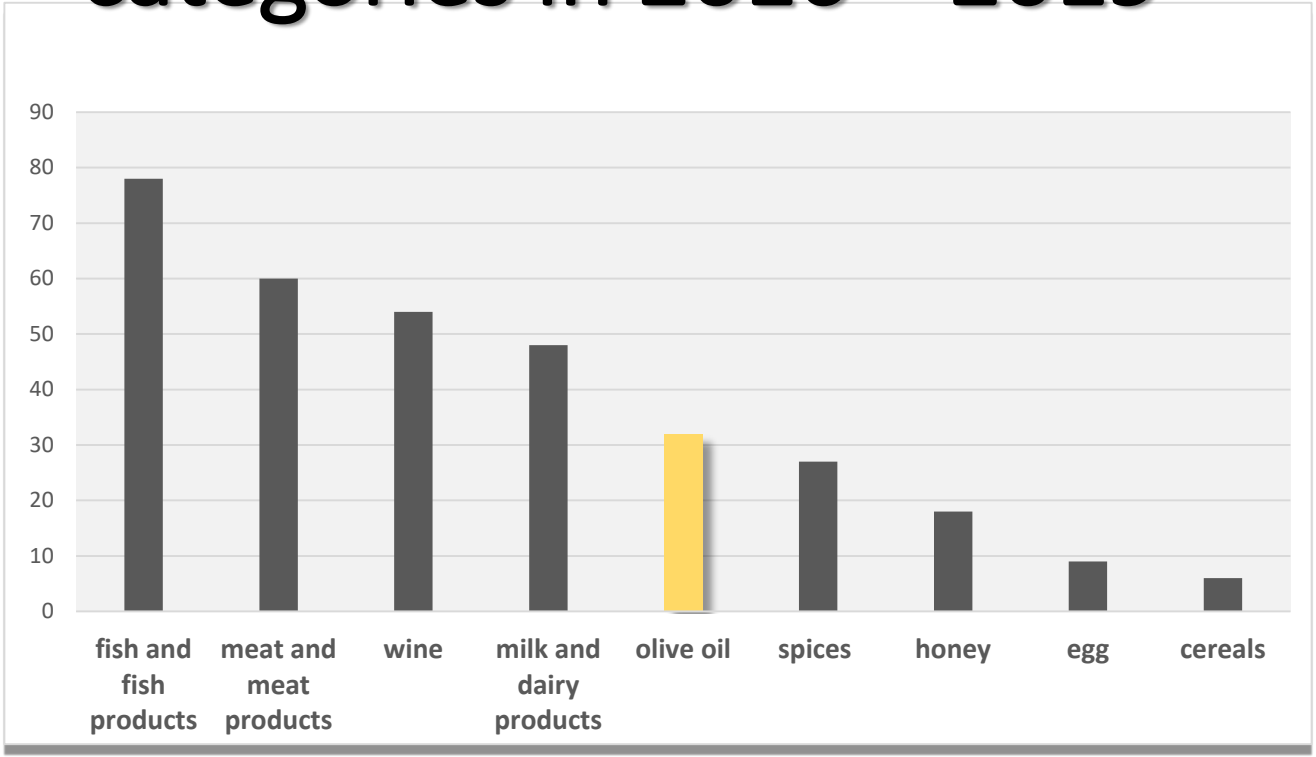
Zucchini factor B
m/z 664.4372



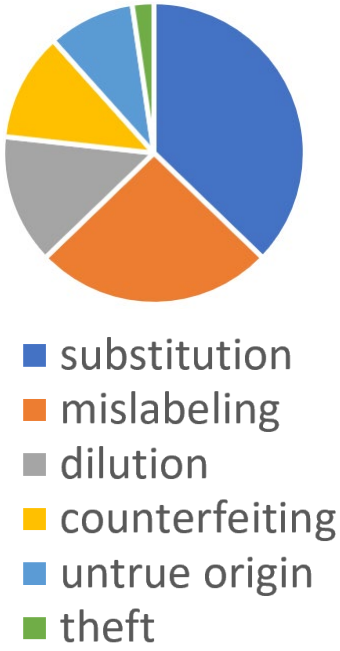
Case study no.4

Detecting fraud on extra virgin olive oil (EVOO): dilution with soft - deodorized oil (SDOO)

Reported fraud on various food categories in 2016 – 2019



Fraud on olive oil



Examples of common fraud on olive oil

Substitution

Seed oils
Hazel nut oil
Refined oils
SOFT DEODORIZED OIL

Misrepresentation

Fals category
Geographic origin
Year/type of harvest
Cultivar
Way of farming (organic)

Casadei E. et al.: Emerging trends in olive oil fraud and possible countermeasures, Food Control 124 (2021) 107902

Olive oils classification

INTERNATIONAL OLIVE COUNCIL

- VIRGIN OLIVE OILS
  - Extra-virgin olive oil
  - Virgin olive oil
  - Ordinary virgin olive oil
  - Lampante virgin olive oil
- Refined olive oil
- Olive oil composed of refined olive oil and virgin olive oils
- Olive pomace oil

- obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical procedure
- thermal conditions do not lead to alterations in the oil
- any treatment other than washing, decantation, centrifugation and filtration is not performed

Purity criteria:

- Fatty acid composition
- Sterol composition
- Total sterol content
- Wax content
- Stigmastadiene content
- Unsaponifiable matter
- Etc.

Quality criteria:

- Organoleptic characteristics
- Free acidity
- Peroxide value
- Absorbancy in UV
- Moisture and volatile matter
- Trace metals (Fe, Cu)
- Etc.

Challenge: detection of soft deodorized olive oil (SDOO) in extra virgin olive oil (EVOO)

DEODORIZATION

Steam distillation process under vacuum and temperatures > 200 °C

- **Purpose:** To remove off-flavours, FFA, pesticide residues, light PAH, etc.
- **Negative effects:** loss of tocopherols, sterols; formation of MCPDEs, GEs

Challenge: detection of soft deodorized olive oil (SDOO) in extra virgin olive oil (EVOO)

SOFT-DEODORIZATION

Steam distillation process under vacuum and temperatures < 100 °C

- **Purpose: removing of volatiles responsible for the undesirable odours**
- **No significant changes of native oil composition except volatiles removal → difficult to identify process markers**

How to detect blending of extra virgin olive oil (EVOO) with soft deodorized (SDOO) olive oil?

1. Performing **UHPLC-HRMS/MS metabolic fingerprinting** strategy on extra virgin olive oil samples, soft-deodorized olive oil samples and their blends

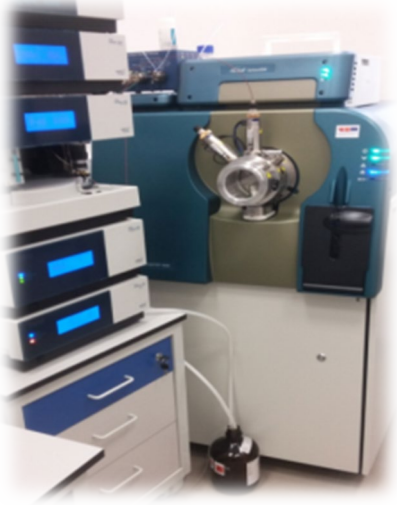
2. Investigation and selction of **markers of soft-deodorization process**

3. Development of **target UHPLC-MS/MS method** suitable for routine food testing

Analysis of

POLAR FRACTION

(MeOH:H_2O, 80:20, *v/v*)



Sample set

ALMA MATER STUDIORUM
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ITERG
Expertise Corps Gras

Oleum

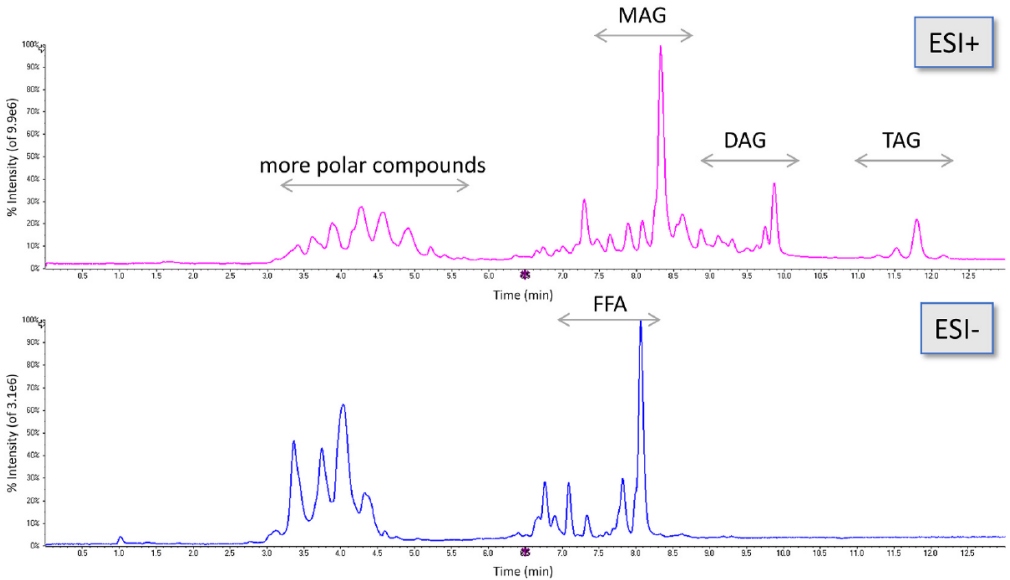
University of Bologna & Institut des Corps Gras:

- 2 authentic extra-virgin olive oils (EVOO)
- 10 defected olive oils
- 10 soft-deodorized olive oils (SDOO)
- 60 blends of EVOOs and SDOOs (30/70, 50/50, 70/30)

- + 20 EVOOs from another study to increase sample variability

UNIVERSITÀ
DI PARMA

Optimized UHPLC-HRMS conditions

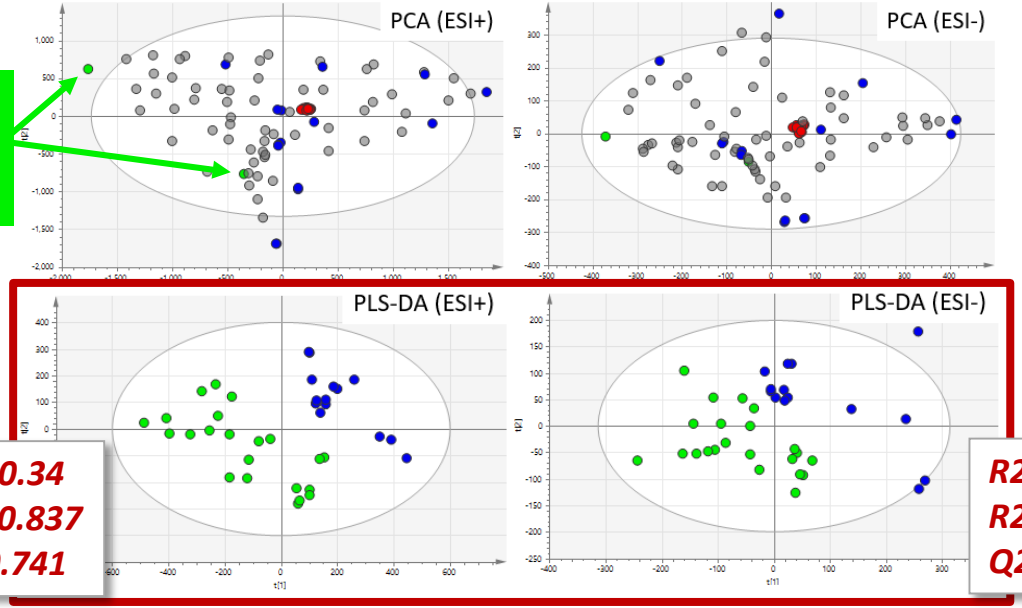


Total ion chromatograms of QC sample in positive (ESI+) and negative (ESI-) ionization mode. MAG – monoacylglycerols, DAG – diacylglycerols, TAG – triacylglycerols, FFA – free fatty acids.

Results: EVOOs vs SDOOs

- Chemometric models created from EVOOs and SDOOs data

EVOO: highly variable



EVOO
SDOO
Blends
QC

R2X=0.34
R2Y=0.837
Q2=0.741

R2X=0.387
R2Y=0.638
Q2=0.414

[1] Cavanna, D., Hurkova, K., Džuman, Z., Serani, A., Serani, M., Dall'Asta, C., Tomaniova, M., Hajslova, J., & Suman, M. (2020). A Non-Targeted High-Resolution Mass Spectrometry Study for Extra Virgin Olive Oil Adulteration with Soft Refined Oils: Preliminary Findings from Two Different Laboratories. *ACS Omega*, *5*(38), 24169-24178.

Results: EVOOs vs SDOOs

- Chemometric models created from EVOOs and SDOOs data

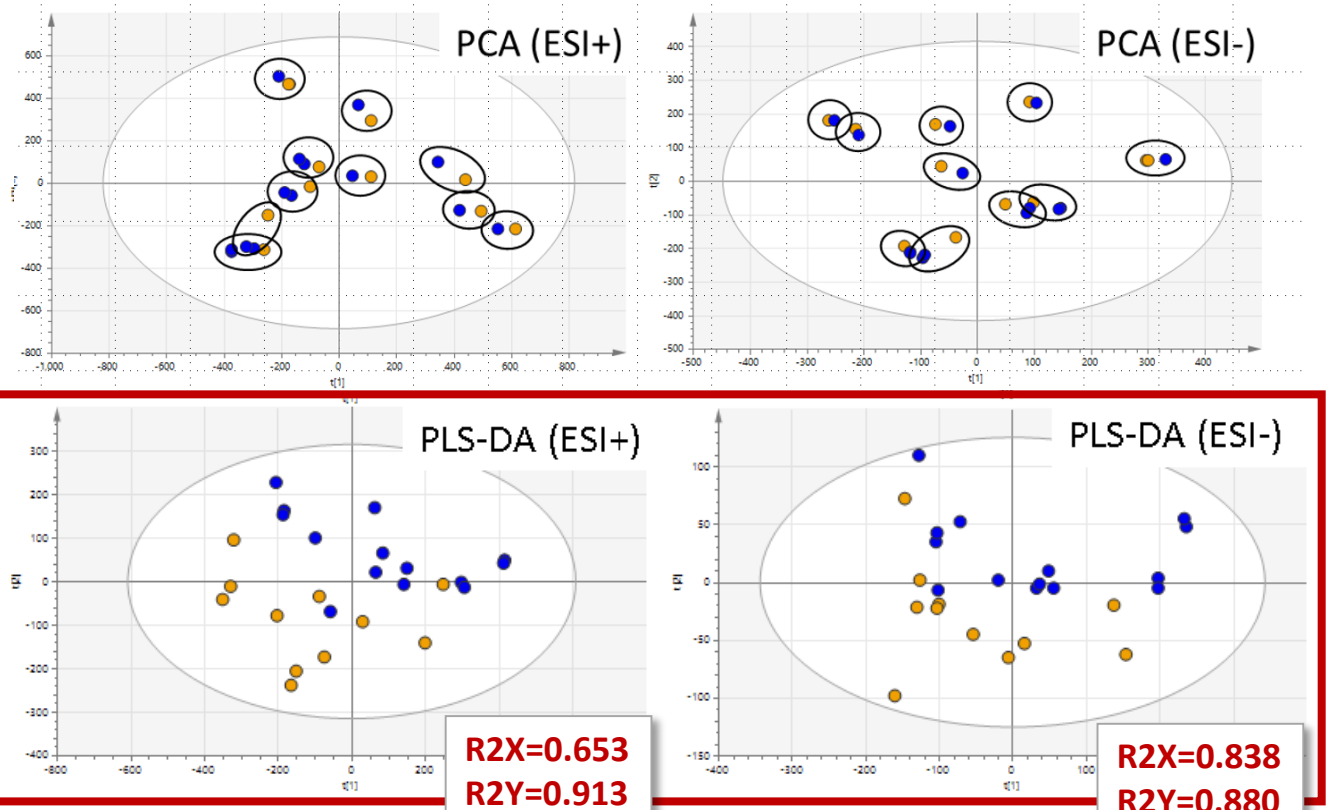
Markers investigation – phase 1:

these ions are also present in defected OOs, therefore, they are not markers of SD proces → more likely **markers of lower quality oils** which need to undergo soft-deodorization process

Selected markers[1]:
283.2621 – C18H34O2
299.2572 – C18H34O3
365.1237 – C18H22O8

[1] Cavanna, D., Hurkova, K., Džuman, Z., Serani, A., Serani, M., Dall'Asta, C., Tomaniova, M., Hajslova, J., & Suman, M. (2020). A Non-Targeted High-Resolution Mass Spectrometry Study for Extra Virgin Olive Oil Adulteration with Soft Refined Oils: Preliminary Findings from Two Different Laboratories. *ACS Omega*, *5*(38), 24169-24178.

Results: defected OO vs SDOO



- Defected OO
- SDOO

Results: defected OO vs SDOO

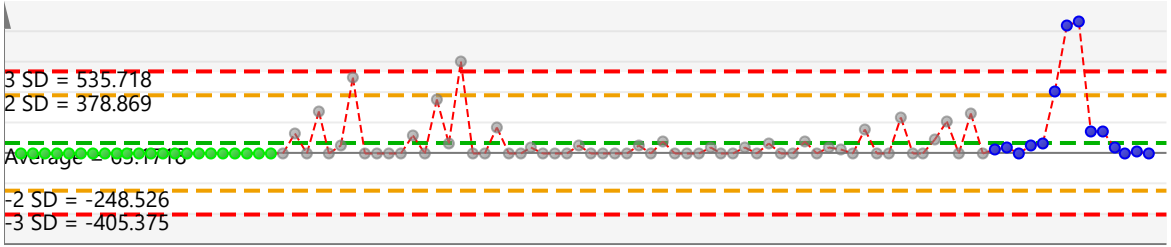
Only minor changes occurred in metabolomic fingerprint as the result of soft deodorization

Markers investigation – phase 2:
Selected seven possible markers of soft-deodorization process

Marker selection: examples of trend plots

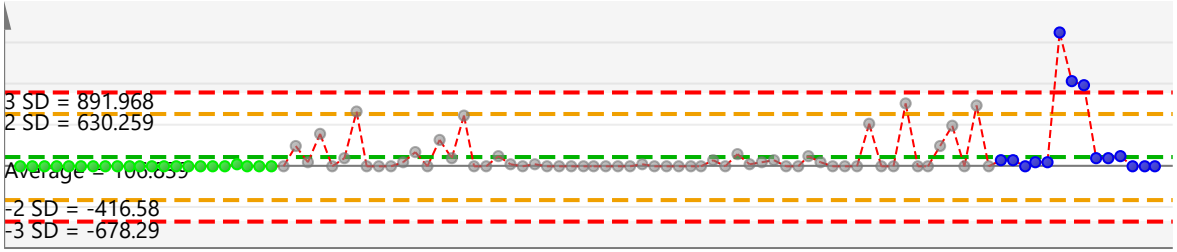
- Selection of **markers** which are **not present in EVOOs**

***m/z* 364.3570**
RT 8.72
ESI+



EVOO
SDOO
Blends

***m/z* 369.3011**
RT 7.89
ESI-



Marker selection and identification

- Selection of markers which are not present in EVOOs

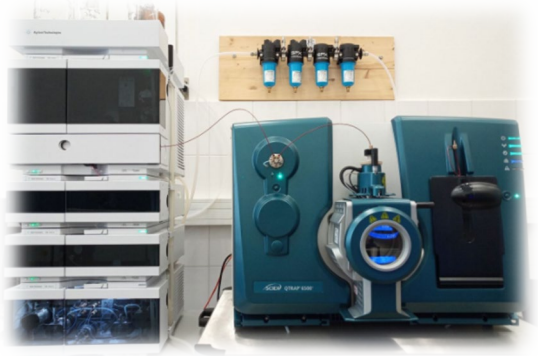
m/z	Retention time (min)	Ionization mode	AUC-ROC	Suggested elemental formula (M)	Ion species	Mass error (Δppm)
283.2621	7.63	ESI+	0.82	C18H34O2	[M+H]+	3.4
299.2572	7.67	ESI+	0.64	C18H34O3	[M+H]+	3.9
365.1237	3.82	ESI-	0.75	C18H22O8	[M-H]-	0.5
225.1943	5.89	ESI+	0.72	C13H24N2O	[M+H]+	3.7
295.2632	7.75	ESI+	1.00	C19H34O2	[M+H]+	2.9
335.2558	7.74	ESI+	0.92	C19H36O3	[M+Na]+	0.1
360.3254	8.34	ESI+	0.81	C22H43NO	[M+Na]+	1.1
364.3570	8.72	ESI+	0.92	C24H42O	[M+NH4]+	0.3
369.3011	7.89	ESI-	0.89	C22H42O4	[M-H]-	3.3
393.2982	8.44	ESI+	1.00	C22H42O4	[M+Na]+	0.8

Markers of low quality olive oils[1]

Markers of soft-deodorization process

[1] Cavanna, D., Hurkova, K., Džuman, Z., Serani, A., Serani, M., Dall'Asta, C., Tomaniova, M., Hajslova, J., & Suman, M. (2020). A Non-Targeted High-Resolution Mass Spectrometry Study for Extra Virgin Olive Oil Adulteration with Soft Refined Oils: Preliminary Findings from Two Different Laboratories. *ACS Omega*, *5*(38), 24169-24178.

UHPLC-MS/MS target analysis of selected markers

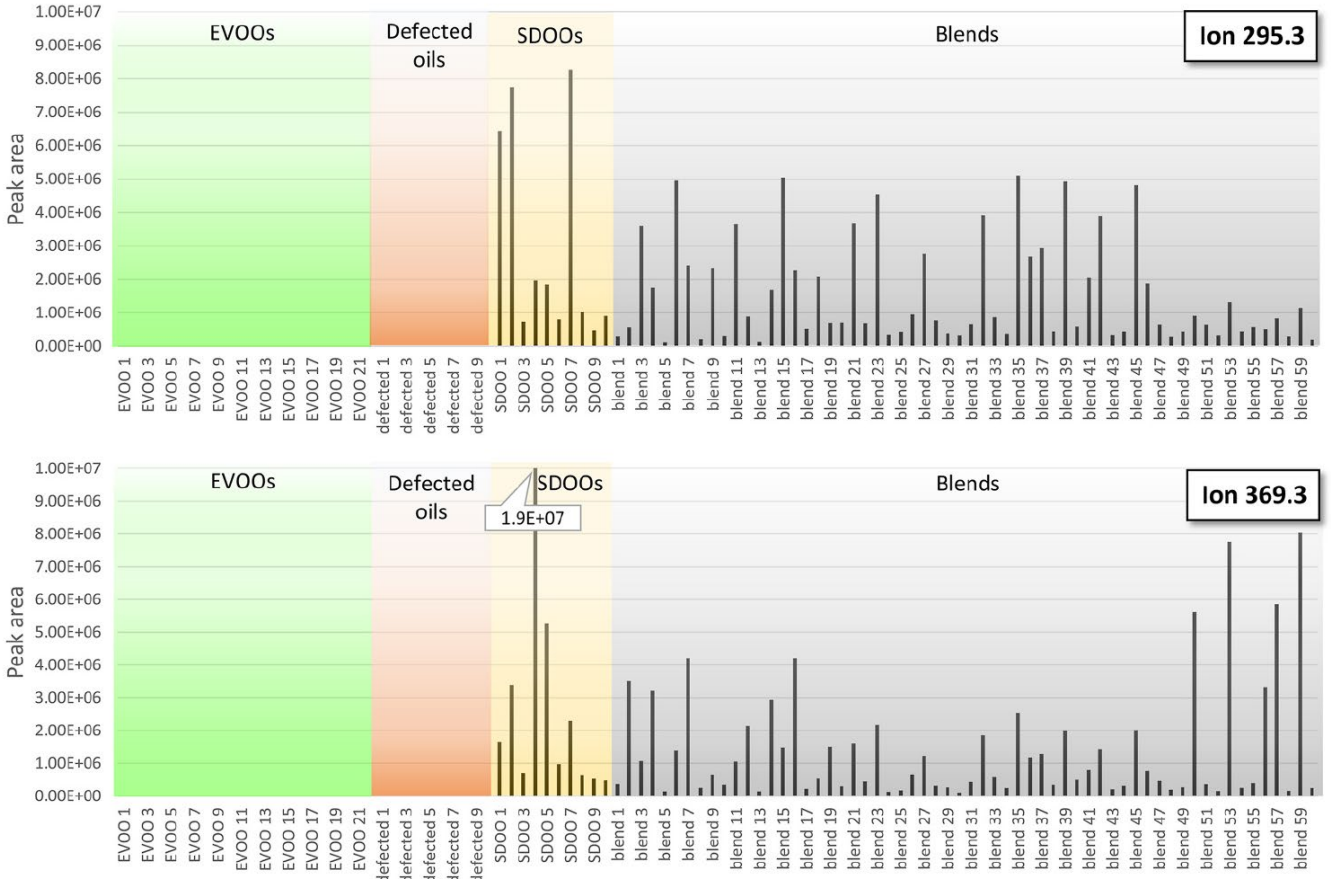


	Present in			
m/z	Extra-virgin olive oils	Defected oils	Soft-deodorized oils	Blends
283.3	22/22	10/10	10/10	60/60
299.3	22/22	10/10	10/10	52/60
365.1	22/22	10/10	10/10	60/60
225.2	22/22	10/10	10/10	60/60
295.3	0/22	0/10	10/10	60/60
335.3	0/22	0/10	9/10	44/60
360.3	0/22	1/10	10/10	60/60
364.4	0/22	0/10	10/10	50/60
369.3	0/22	0/10	10/10	60/60
393.3	0/22	0/10	10/10	48/60

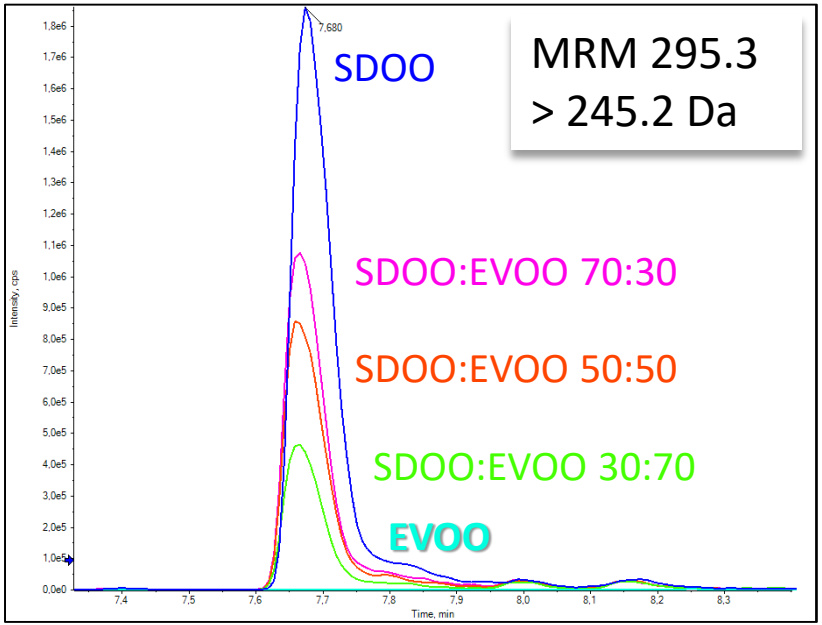
Methyl ester of hydroxy octadecenoic acid

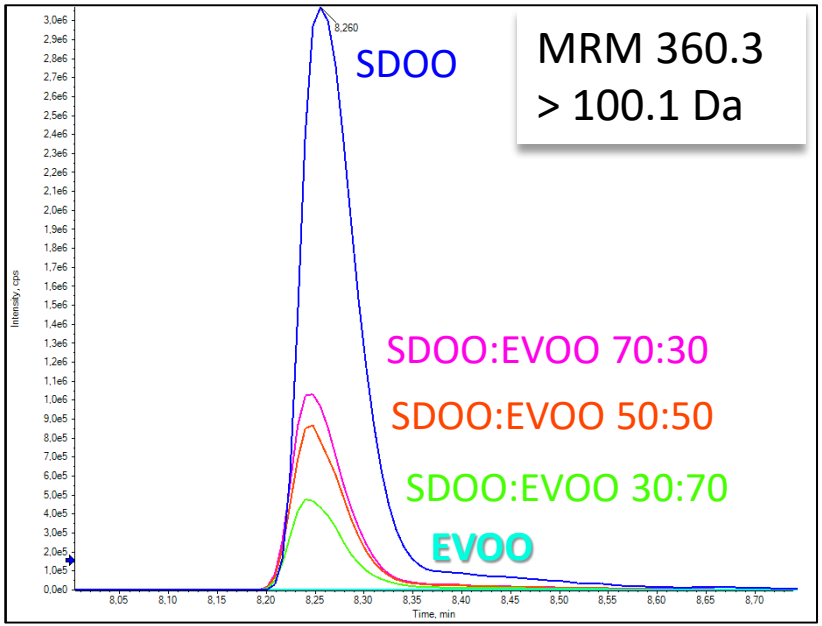
Ester derivatives of oleic acid

Target analysis of selected markers



Estimation of soft deodorized olive oil (SDOO) amount in blend with extra virgin olive oil





Targeted detectability: 10%

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Metabolic fingerprinting strategy: Investigation of markers for the detection of extra virgin olive oil adulteration with soft-deodorized olive oils

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Conclusions

- DART-HRMS is an excellent screening strategy for authenticity cold pressed oils
- U-HPLC-HRMS metabolomic fingerprinting strategy represents challenging authentication strategy specifically enabling markers identification
- Identified markers (and validated) enable simplier UHPLC-MS/MS target analysis in routine labs

Natural variability of oil seeds, differences in preparation pravctices and changes due to oxidation instability have to be considered