Mass Spectrometry for

Cultural Heritage Science

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Miniaturization for Synthesis, Analysis & Proteomics USR 3290

EU_FT-ICR_MS, 2nd AUS, Prague

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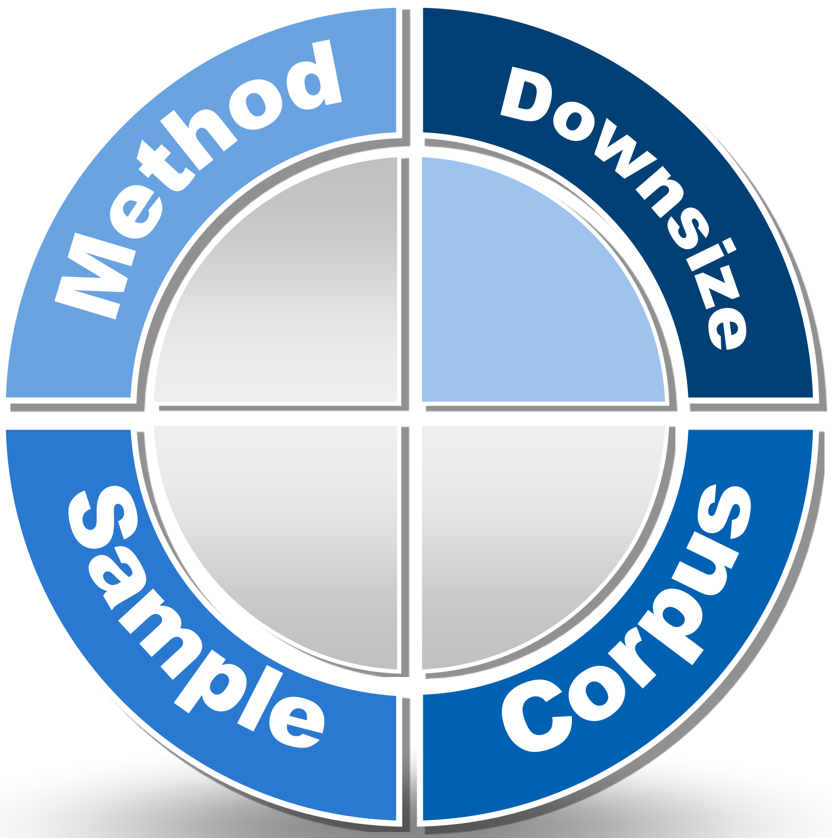
Any analytical experience fits a class of Heritage Science samples!

- **Petroleomics** → bitumen
- **Proteomics** → paleontology, archaeology, Renaissance paint binder
- **Lipidomics**: → lipids in archaeological ceramic, oil paint
- **Metabomics**: → bee wax, glue, polyphenols from wine or beer
- **Glycomics**: stucco, watercolor
- **Polymer**: → amber, modern paints

Workflow for a new insoluble object in Heritage Science

- **Mock samples**
- **Sample preparation**
- **Chemical or biological depolylmerization**
- **Mass spectrometry**
- **Data treatment**


Method
Downsize
Sample
Corpus

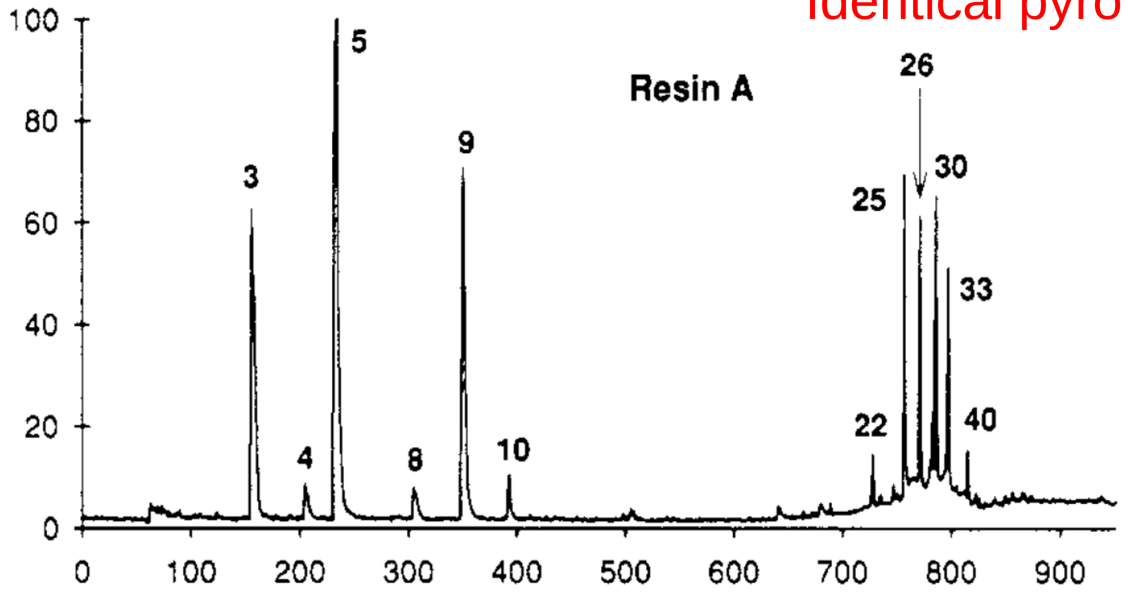
For valuable samples the quantity is always less than necessary to be confortable. Miniaturization is required.

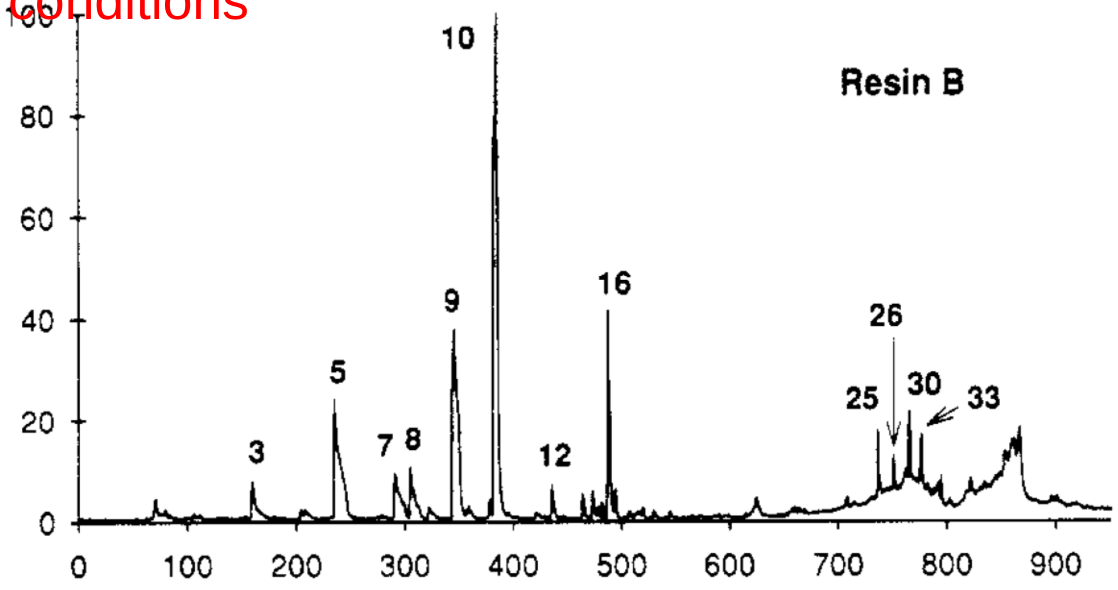
For precious Heritage samples the quantities allow few trials only!

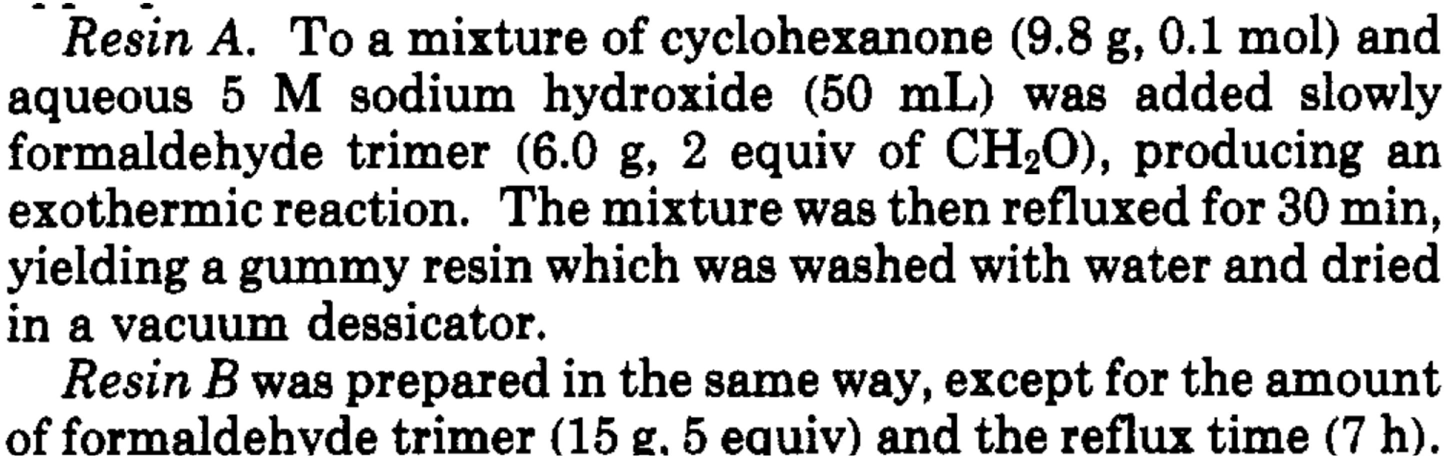
Similar well characterized samples from the Heritage Science point of view or modern for establishing the data bank.

Py-GC MS of modern paint varnishes

Identical pyrolysis conditions





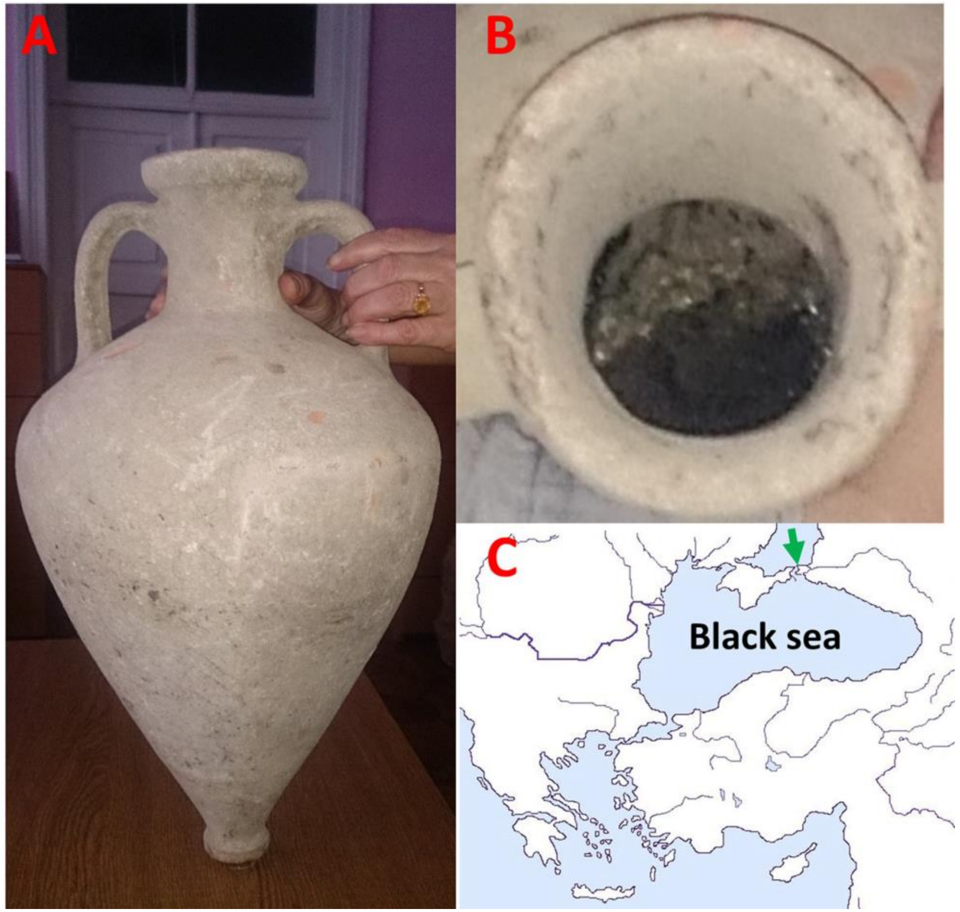


Resin A. To a mixture of cyclohexanone (9.8 g, 0.1 mol) and aqueous 5 M sodium hydroxide (50 mL) was added slowly formaldehyde trimer (6.0 g, 2 equiv of CH_2O), producing an exothermic reaction. The mixture was then refluxed for 30 min, yielding a gummy resin which was washed with water and dried in a vacuum dessicator.

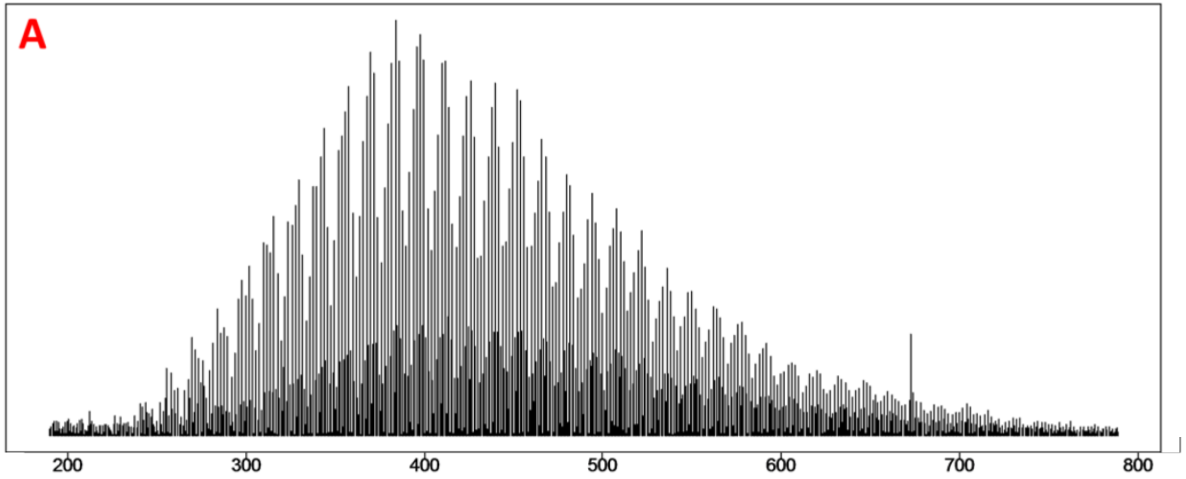
Resin B was prepared in the same way, except for the amount of formaldehyde trimer (15 g, 5 equiv) and the reflux time (7 h).

Mestdagh, H., Rolando, C., Sablier, M., & Rioux, J. P. (1992). Characterization of ketone resins by pyrolysis/gas chromatography/mass spectrometry. *Analytical chemistry*, *64*, 2221-2226.

FTICR MS of bitumen from ancient Greek amphora



A,B—the ancient Greek Amphora full of bitumen.
C—The place of the discovery.



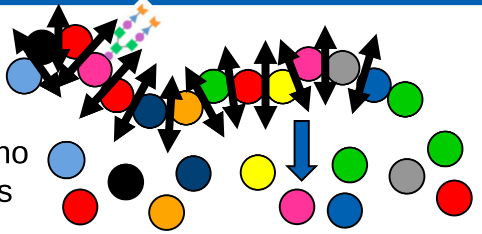
FT ICR spectrum of the bitumen from amphora, positive ESI mode,

Kostyukevich, Y., Solovyov, S., Kononikhin, A., Popov, I., & Nikolaev, E. (2016). The investigation of the bitumen from ancient Greek amphora using FT ICR MS, H/D exchange and novel spectrum reduction approach. *Journal of Mass Spectrometry*, *51*(6), 430-436.

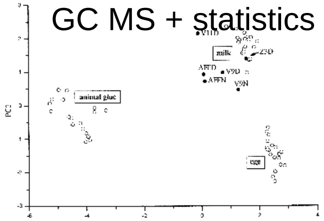
Methodological developments applied to Cultural Heritage: protein identification

Usual approach

Sample → **Extration combined with direct hydrolysis in aminoacids (acid or basic hydrolysis)** → **GC-MS analysis + comparison to models** → **Binder identification**



GC MS + statistics treatment

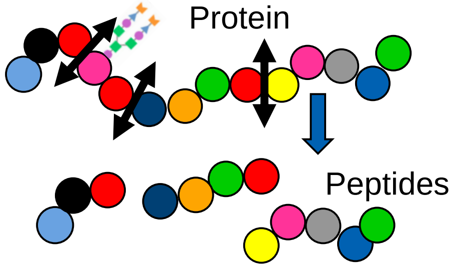


Identification of a binder using comparison to model

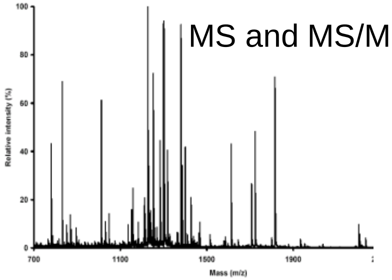
Colombini M. P., Andreotti A., Bonaduce I., Modugno F., Ribechini E. Acc. Chem. Res. 2010, 43, 715-727

Actual methodologies

Sample → **Protein extraction** → **Enzymatic hydrolysis** → **Selective separation of peptides** → **MS analysis + Bio-informatics** → **Protein identification via peptides**



MS and MS/MS



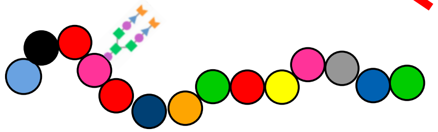
Sequence of the protein via peptides identification

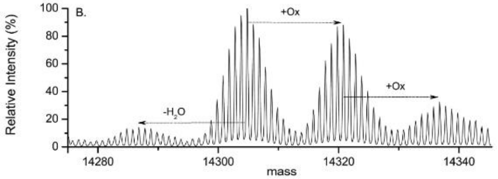
10 20 30 40
(MKWVWALLL AALGSGRA)ER DCRVSSFRVK ENFDKARFSG
50 60 70 80
TWYAMAKKDP EGLFLQDNIV AEFSVDETGQ MSATAKGRVR
90 100 110 120
LLNNWDVCAD MVGTFTDTED PAKFKMKYWG VASFLQKGND
130 140 150 160
HWIVDTDYD TYAVQYSCRL LNLDGTCADS YSFVFSRDPN
170 180 190 200
GLPPEAQKIV RQRQEELCLA RQYRLIVHNG YCDGRSERNL L

Tokarski C.; Martin E.; Rolando C.; Cren-Olivé C. *Anal Chem* **2006,** 78, 1494-1502

New developments

Sample → **Protein extraction** → ~~**Enzymatic hydrolysis**~~ → **Selective separation of peptides** → **MS analysis + Bio-informatics** → **Protein id. including modifications**





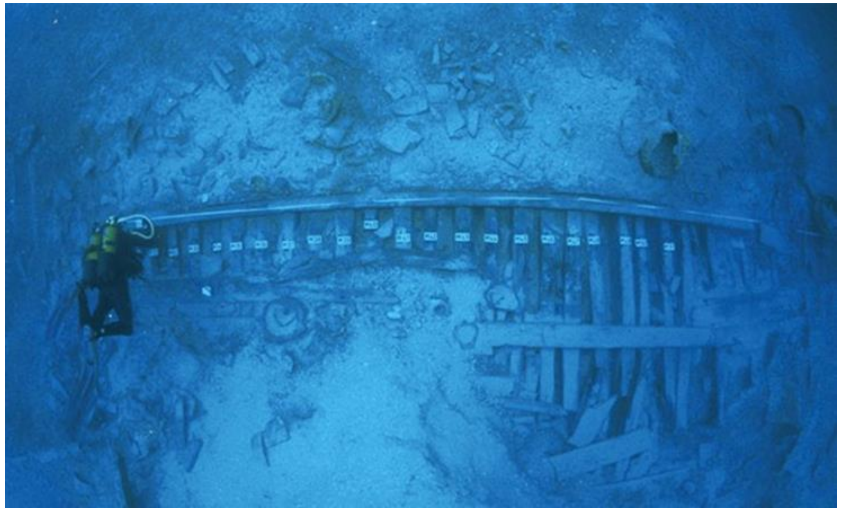
10 20 30 40
(MKWVWALLL AALGSGRA)ER DCRVSSFRVK ENFDKARFSG
50 60 70 80
TWYAMAKKDP EGLFLQDNIV AEFSVDETGQ MSATAKGRVR
90 100 110 120
LLNNWDVCAD MVGTFTDTED PAKFKMKYWG VASFLQKGND
130 140 150 160
HWIVDTDYD TYAVQYSCRL LNLDGTCADS YSFVFSRDPN
170 180 190 200
GLPPEAQKIV RQRQEELCLA RQYRLIVHNG YCDGRSERNL L

Methodological developments in proteomics applied to the analysis of cultural heritage samples

Sergui MANSOUR

Laboratory of Miniaturization for Synthesis, Analysis & Proteomics
(MSAP), USR 3290

Archaeological sample



Collaboration with Dr Franca CIBBECCINI, DRASSM

Location of the shipwreck

- Studied ceramic samples were taken from a Dressel 14 amphora discovered in a shipwreck near the island of Tiboulen de Maïre, near Marseille
- The shipwreck is dated from 116 AD and it was found at 49-50 m deep

Various types of amphorae discovered in the shipwreck; on the right: Dressel 14

Garum fish sauce

- Dressel-14 (Dr. 14) amphorae have been produced in south of Spain (Baetica) and Portugal (Lusitania) from the 1st century AD to the end of the 2nd century AD
- Tituli picti (inscriptions) found on Dr. 14 informs on their fish sauces contents (the studied Dr. 14 did not show titulus pictus)

Map of trade routes in Western Romain Empire for garum (from curtis 2005)

- ➢ Liquamen and garum sauces were obtained from fermented fishes (cuisine of Roman antiquity)
- ➢ Various recipes were used (e.g. small fishes versus bigger fishes and fish innards)
- ➢ Several fish species were used among which mackerels, sardines, tuna,...

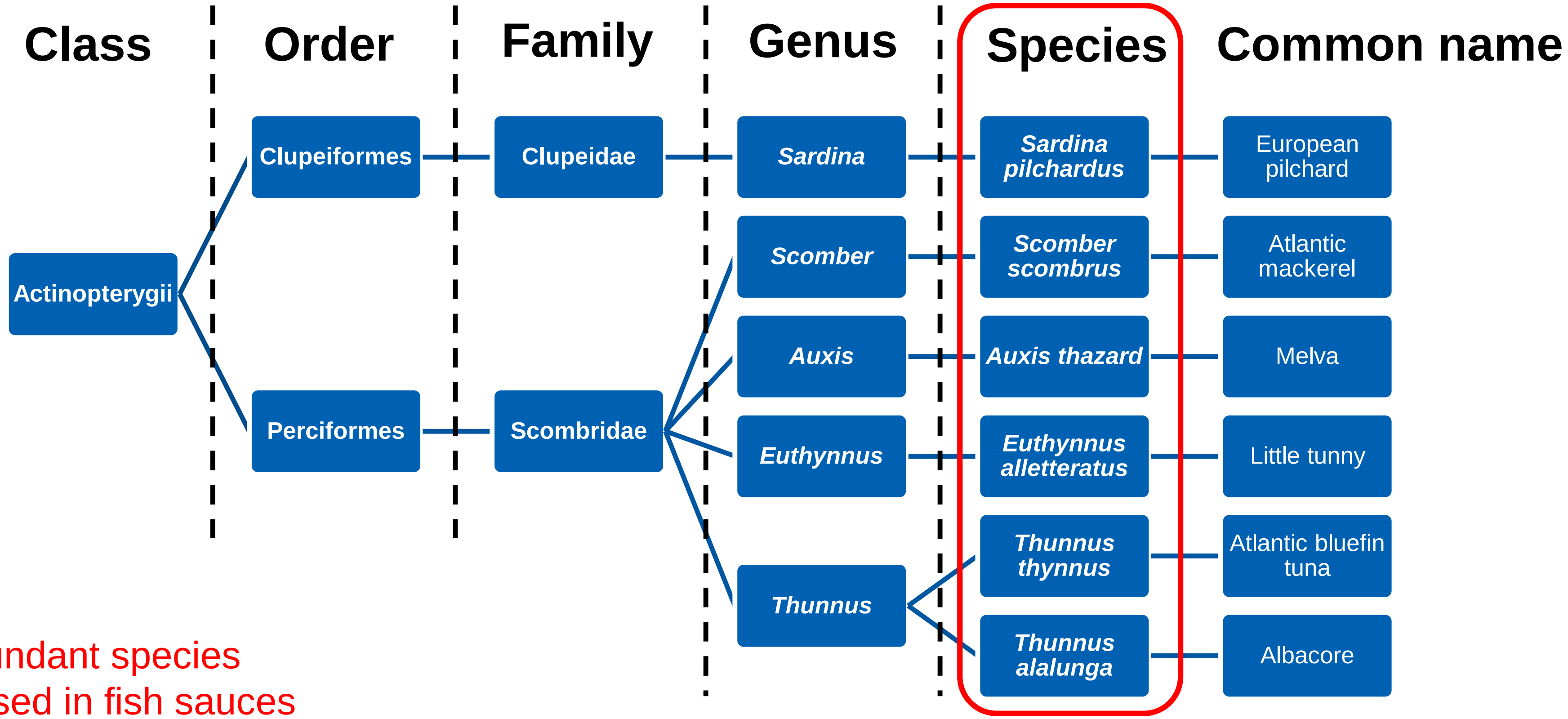
Objective of the study: identification of protein remains trapped in the ceramic and identification of fish species

Current fish protein databases

	NCBI	TrEMBL	Swiss-Prot
Actinopterygii (Class)	3858321	1469565	5343
Clupeiformes (Order)	41668	3966	14
Perciformes (Order)	118224	67597	208
Clupeidae (Family)	36836	2274	13
Scombridae (Family)	9736	3425	68
Sardina (Genus)	1507	253	1
Clupea (Genus)	30480	703	16
Scomber (Genus)	1920	1635	22
Auxis (Genus)	605	191	1
Euthynnus (Genus)	271	133	-
Thunnus (Genus)	3188	1347	50
Sardina pilchardus (Species)	323	96	1
Scomber scombrus (Species)	694	207	4
Auxis thazard (Species)	261	6	-
Euthynnus alletteratus (Species)	131	-	-
Thunnus thynnus (Species)	687	325	16
Thunnus alalonga (Species)	436	109	2

- **The current fish protein databases have few entries**
- Informative entries for our study are missing
- An in house database with various fish species was build

Phylogenetic tree of the studied fish species



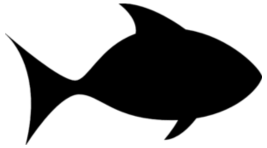
Class	Order	Family	Genus	Species	Common name
Actinopterygii	Clupeiformes	Clupeidae	***Sardina***	***Sardina pilchardus***	European pilchard
	Perciformes	Scombridae	***Scomber***	***Scomber scombrus***	Atlantic mackerel
			Auxis	***Auxis thazard***	Melva
			Euthynnus	***Euthynnus alletteratus***	Little tunny
			Thunnus	***Thunnus thynnus***	Atlantic bluefin tuna
				Thunnus alalunga	Albacore

Focus on several abundant species described as being used in fish sauces

		Sardina pilchardus	*Scomber scombrus*	*Auxis thazard*	*Ehthynnus alletteratus*	*Thunnus thynnus*	*Thunnus alalunga*
Number of proteins	NCBI	323	694	261	131	687	436
	TrEMBL	96	207	6	-	325	109
	Swiss-Prot	1	4	-	-	16	2

Experimental procedure

I



Selection of 6 fish species to
build a database
(muscle and blood proteins)

II

Analysis of garum model sauces:

1. Sardines
2. Innards of mackerel

(Prepared with traditional recipes by Dr N. Garnier, and Dr E. Botte)

III

Analysis of archaeological ceramic

Extraction of proteins

↓

Digestion of proteins
(eFASP Protocol)

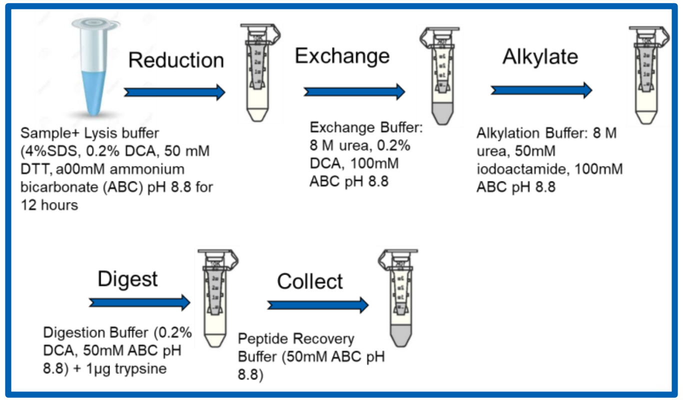
↓

Mass spectrometry analysis

↓

Raw data treatment

NanoLC NanoESI Orbitrap QExactivePlus



Sample+ Lysis buffer (4%SDS, 0.2% DCA, 50 mM DTT, a00mM ammonium bicarbonate (ABC) pH 8.8 for 12 hours

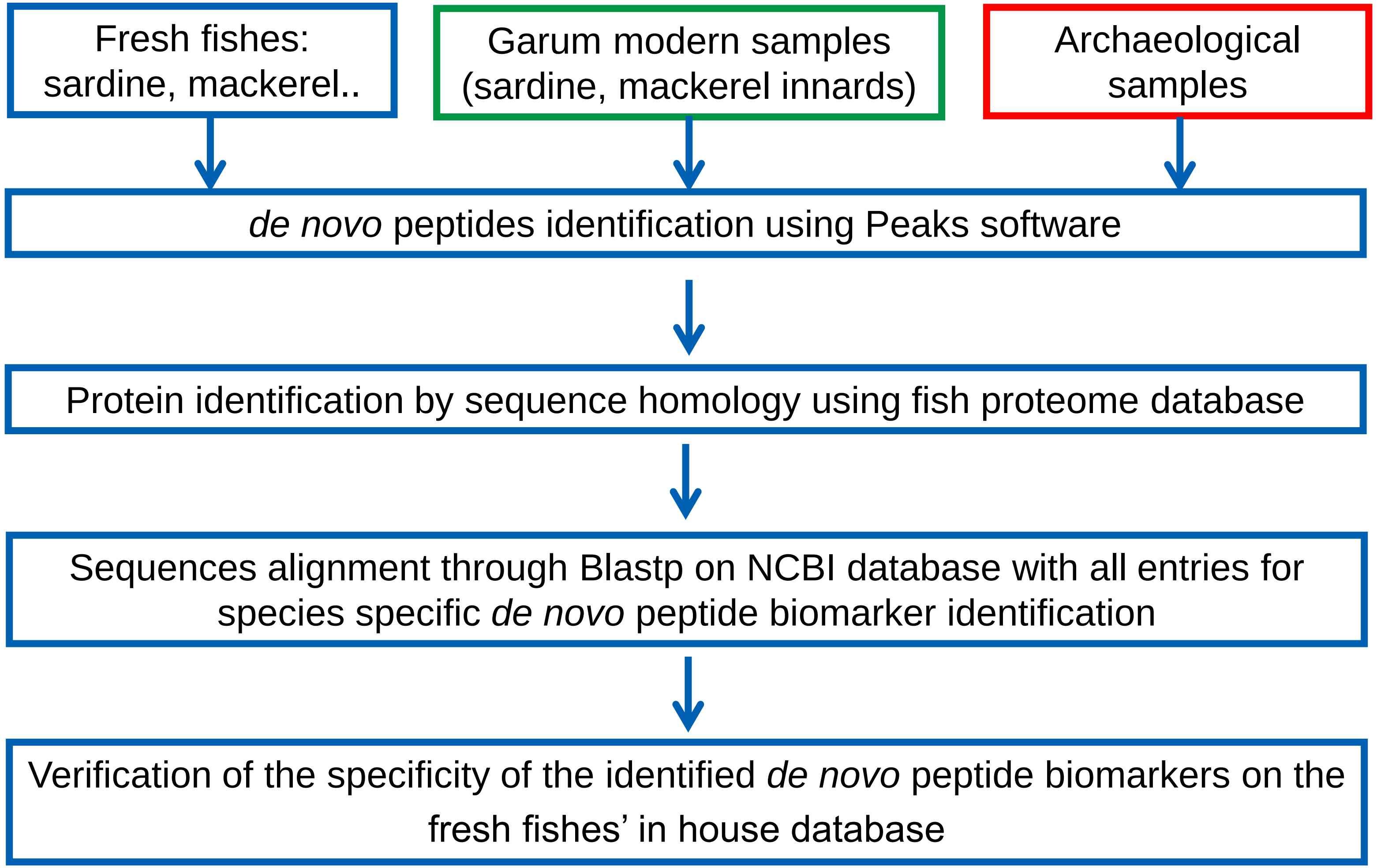
Exchange Buffer: 8 M urea, 0.2% DCA, 100mM ABC pH 8.8

Alkylation Buffer: 8 M urea, 50mM iodoactamide, 100mM ABC pH 8.8

Digestion Buffer (0.2% DCA, 50mM ABC pH 8.8) + 1μg trypsine

Peptide Recovery Buffer (50mM ABC pH 8.8)

Workflow



Fresh fishes:
sardine, mackerel..

Garum modern samples
(sardine, mackerel innards)

Archaeological
samples

de novo peptides identification using Peaks software

Protein identification by sequence homology using fish proteome database

Sequences alignment through Blastp on NCBI database with all entries for species specific *de novo* peptide biomarker identification

Verification of the specificity of the identified *de novo* peptide biomarkers on the fresh fishes’ in house database

De novo analysis of the fishes samples with Peaks software

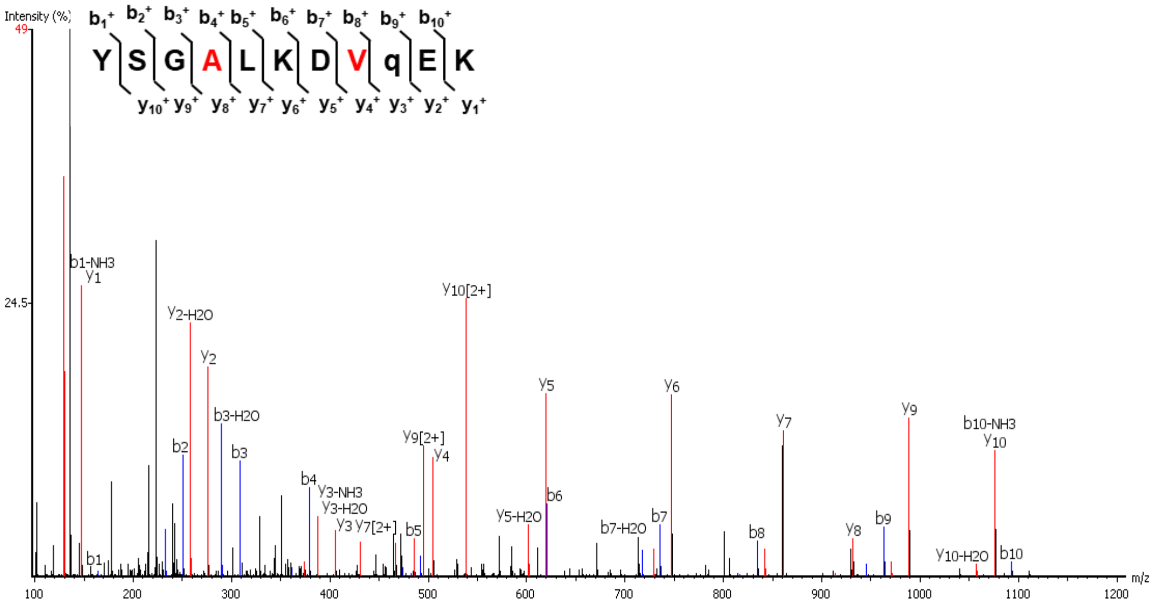
	Sardina pilchardus	*Scomber scombrus*	*Auxis thazard*	*Ehthynnus alletteratus*	*Thunnus thynnus*	*Thunnus alalunga*
Number of MS	7729	7889	7664	8111	7584	7762
Number of MS/MS	49427	49329	49526	49144	49608	49526
Peptide-Spectrum Matches	18444	19388	18589	22700	24635	23454
Peptide sequences	9970	9804	9499	11596	12435	11068
Missed Cleavages = 0	6229	6711	6266	7493	8268	7405
Missed Cleavages = 1	2629	2394	2446	3013	3101	2780
Missed Cleavages = 2	834	575	660	890	869	741
Missed Cleavages = 3	242	112	117	179	173	131
Protein groups	521	490	441	522	609	428
Proteins	1388	1569	941	1240	1252	774
Proteins (>2 Unique Peptides)	**754**	**83**	**539**	**679**	**685**	**403**

- **Successful identification of proteins including muscle proteins and blood proteins**

Home-made database: identification fish specific peptides from fresh fishes

Example of Tropomyosin protein extracted from *Scomber scombrus* fish sample (identified by sequence homology to the *Thunnus thynnus*)

MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLV**D**MQKRL KGTEDELEKY SG**A**LKD**V**QEK LEVAEK**Q**ATD AEGDVASLNR RIQLVEEELD
RAQERLATAL TKLEEAEK**V**A DESERGMKVI ENRNMKDEEK MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSE**T**KC SELEEESKTV
TNNLKSLEAQ AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI



MS/MS spectrum of the doubly charged ion at *m/z* = 619.816 (Δm = 0.3 ppm)

- Several peptides (e.g. peptide in position 60-70) are showing amino acids substitutions and can be used to discriminate species

Home-made database: identification fish specific peptides from modern fishes

Example of Tropomyosin protein extracted from *Scomber scombrus* fish sample (identified by sequence homology to the *Thunnus thynnus)*

MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLV**D**MQKRL KGTEDELEKY SG**A**LKD**V**QEK LEVAEK**Q**ATD AEGDVASLNR RIQLVEEELD RAQERLATAL TKLEEAEK**V**A DESERGMKVI ENRNMKDEEK MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSE**T**KC SELEEESKTV TNNLKSLEAQ AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI

Scomber scombrus **Peptides**	**Species**
YSGALKDVQ(d)EK	*Scomber scombrus* (home-made database)
YSG**N**LKD**A**QEK	*Thunnus thynnus* (NCBI database)

- Specificity using Blastp on NCBI database (Identities 82%)

Scomber scombrus **Peptides**	**Species**
YS**G**ALKD**V**Q(d)EK	*Scomber scombrus* (home-made database)
YS**E**ALKD**A**QEK	*Stegastes partitus* (NCBI database)

- Several peptides (e.g. peptide in position 60-70) are showing amino acids substitutions and can be used to discriminate species

EU FT-ICR MS

MSAP

Université de Lille

Methodology development on model garum samples

Model garum sauce
(entire sardine)

Identification of 716 proteins

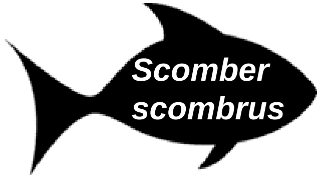
De novo peptides identification of muscle proteins

In house database

De novo peptides matching

Identification of *Sardina pilchardus* specific peptides

Model garum sauce
(mackerel innards)



Identification of 631 proteins

De novo peptides identification of blood proteins

In house database

De novo peptides matching

Identification of *Scomber scombrus* specific peptides

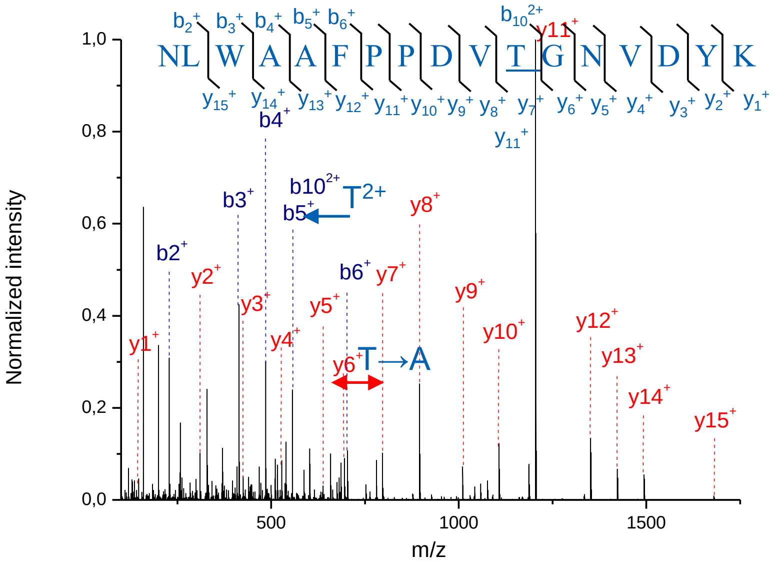
Université de Lille

Species specific peptide identified in garum sauce

- Muscle and blood proteins identified in the modern garum fishes sauces showed sequence matches and dissimilarities with several species present in the in house database

Example of garum modern sauce made from entire sardine

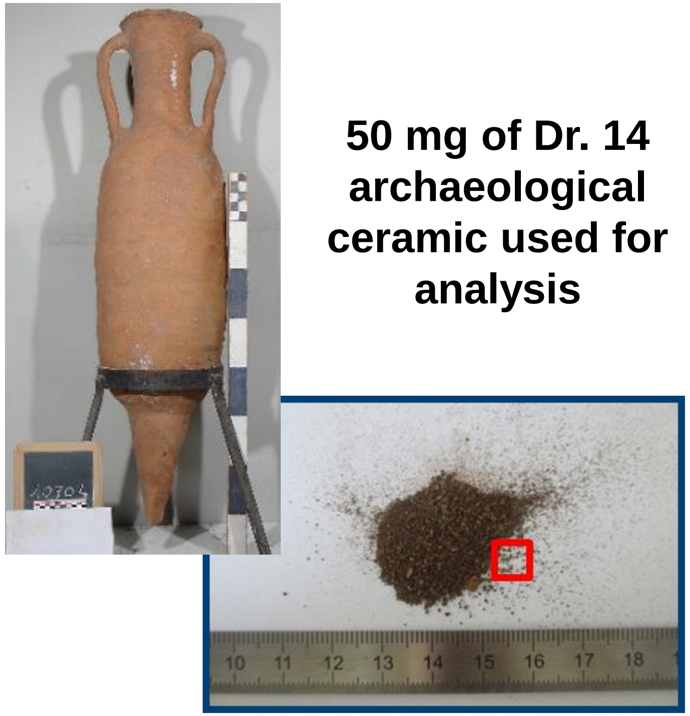
Sample	Protein	Specific peptide with amino acid substitution
Model garum sardine	myosin light chain 2	NLWAAFPPDVTGNVDYK
Sardina pilchardus in house data base	myosin light chain 2	NLWAAFPPDVTGNVDYK
Thunnus thynnus in house data base	myosin light chain 2	NMWAAFPPDVAGNVDYK
Sardinops melanostictus NCBI	myosin light chain 2	NLWAAFPPDVTGQVDYK



Doubly charged ion at m/z 953.971 (Δm= 0.4 ppm), Protein identified by sequence homology to the *Thynnus thynnus* species

Analysis of the archaeological sample

	Archaeological sample
Number of MS scans	25609
Number of MS/MS scans	33291
Peptide-spectrum matches	1721
Peptide sequence	701
Missed cleavages = 0	531
Missed cleavages = 1	152
Missed cleavages = 2	23
Missed cleavages = 3	1
Protein groups	39
Proteins	198
Proteins (>2 unique peptides)	**157**

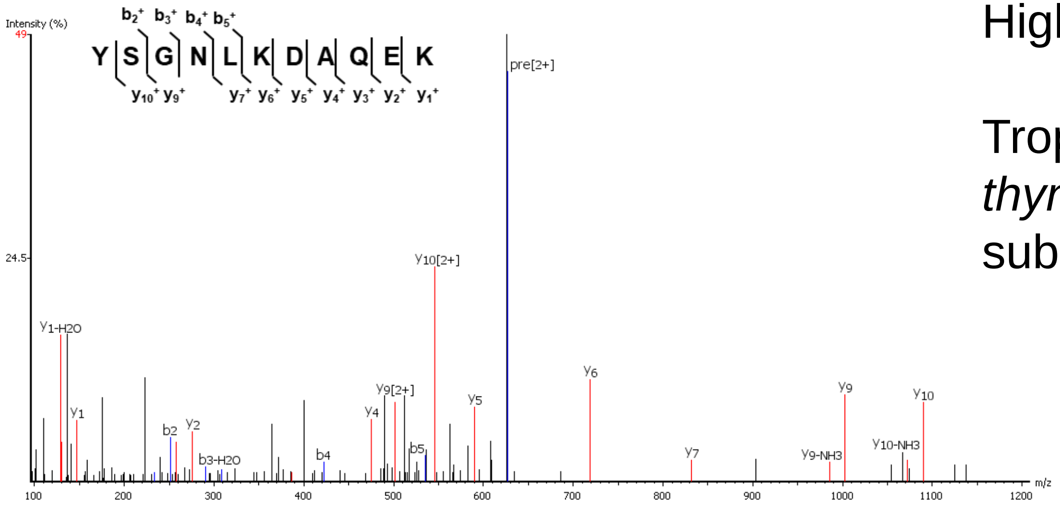


50 mg of Dr. 14 archaeological ceramic used for analysis

Successful identification of 157 proteins among them 10 muscle proteins and 2 blood proteins contain specific peptides for fishes

Spectrum signal to noise ratio and sequence coverage

Archaeological Sample



MS/MS spectrum of the doubly charged ion at m/z: 626.811 (Δm = 2.5 ppm)

High sequence coverage (61 %),

Tropomyosin protein identified *Thunnus thynnus* (NCBI database) whithout any substitution

MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLVAMQKRL KGTEDELEKY SGNLKDAQEK
LEVAEKSATD AEGDVASLNR RIQLVEEELD RAQERLATAL TKLEEAEKAA DESERGMKVI ENRNMKDEEK
MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSESKC SELEEESKTV TNNLKSLEAQ
AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI

Grey: All sequenced peptides
Yellow: Considered peptide

Sequences alignments (tropomyosin protein)

>Analyzed sample: Archaeological sample
> Tropomyosin [*Thunnus thynnus*-NCBI]
MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLVAMQKRL KGTEDELEKY SGNLKDAQEK LEVAEKSATD AEGDVASLNR RIQLVEEELD RAQERLATAL TKLEEAEKAA DESERGMKVI ENRNMKDEEK MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSESKC SELEEESKTV TNNLKSLEAQ AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI

> In house database *Thunnus alalunga*
> Tropomyosin [*Thunnus thynnus*-NCBI]
MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLVAMQKRL KGTEDELEKY SGNLKDAQEK LEVAEKSATD AEGDVASLNR RIQLVEEELD RAQERLATAL TKLEEAEKAA DESERGMKVI ENRNMKDEEK MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSESKC SELEEESKTV TNNLKSLEAQ AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI

> In house database *Thunnus thynnus*
> Tropomyosin [*Thunnus thynnus*-NCBI]
MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLVAMQKRL KGTEDELEKY SGNLKDAQEK LEVAEKSATD AEGDVASLNR RIQLVEEELD RAQERLATAL TKLEEAEKAA DESERGMKVI ENRNMKDEEK MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSESKC SELEEESKTV TNNLKSLEAQ AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI

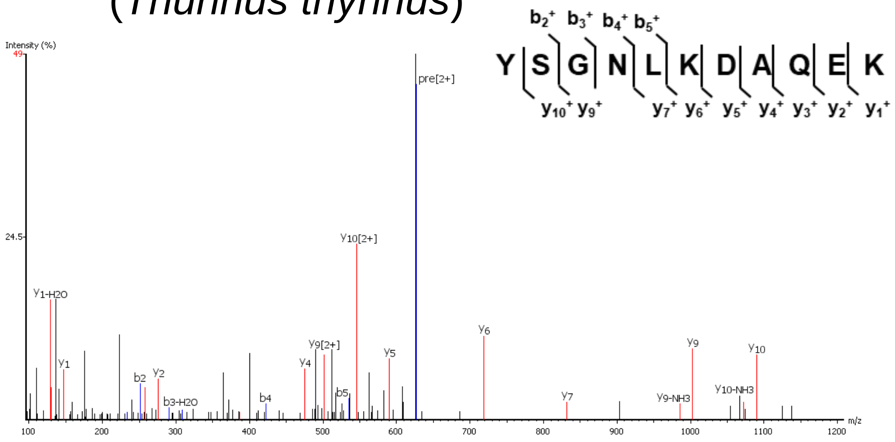
> In house database *Scomber Scombrus*
> Tropomyosin [Thunnus thynnus-NCBI]
MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLVDMQKRL KGTEDELEKY SGALKDVQEK LEVAEKQATD AEGDVASLNR RIQLVEEELD RAQERLATAL TKLEEAEKVA DESERGMKVI ENRNMKDEEK MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSETKC SELEEESKTV TNNLKSLEAQ AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI

> In house database *Auxis thazard*
> Tropomyosin [Thunnus thynnus-NCBI]
MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLVAMQKRL KGTEDELEKY SGALKDAQEK LEVAEKSATD AEGDVASLNR RIQLVEEELD RAQERLATAL TKLEEAEKAA DESERGMKVI ENRNMKDEEK MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSESKC SELEEESKTV TNNLKSLEAQ AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI

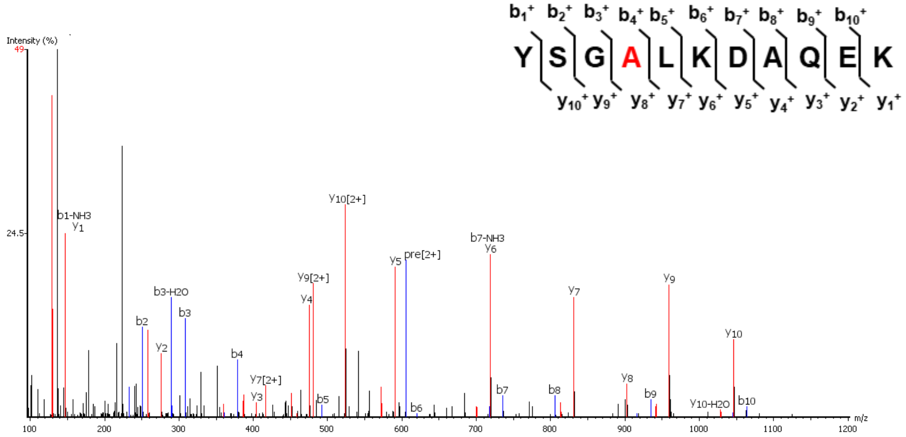
> In house database *Euthynnus alletteratus*
> Tropomyosin [*Thunnus thynnus-NCBI*]
MEAIKKKMQM LKLDKENALD RAEQSESDKK AAEDRTKQLE DDLVAMQKRL KGTEDELEKY SGALKDAQEK LEVAEKSATD AEGDVASLNR RIQLVEEELD RAQERLATAL TKLEEAEKAA DESERGMKVI ENRNMKDEEK MEMQDVQLKE AKNIAEEADR KYEEVARKLV VIESDLERTE ERAELSESKC SELEEESKTV TNNLKSLEAQ AEKYTQKEDK YEEEIKVLTD KLKEAETRAE FAERSVAKLE KTIDDLEDEL YAQKQKFKSI SEELDHALND MTSI

Sequences matching(e.g. tropomyosin protein)

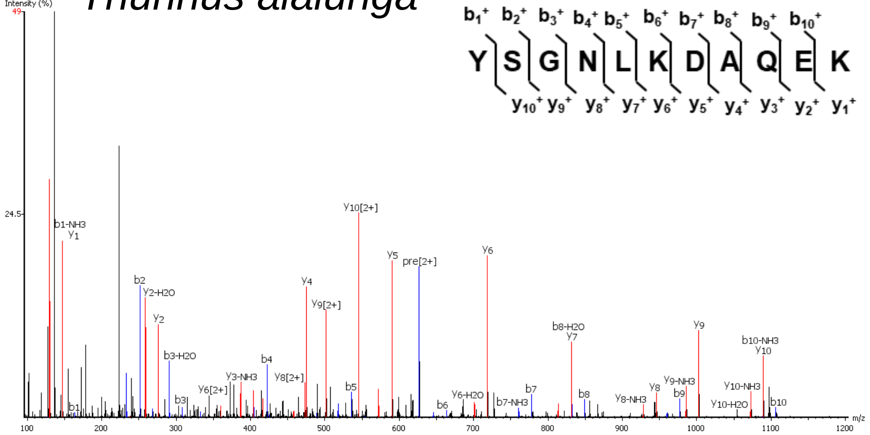
Archaeological Sample
(*Thunnus thynnus*)



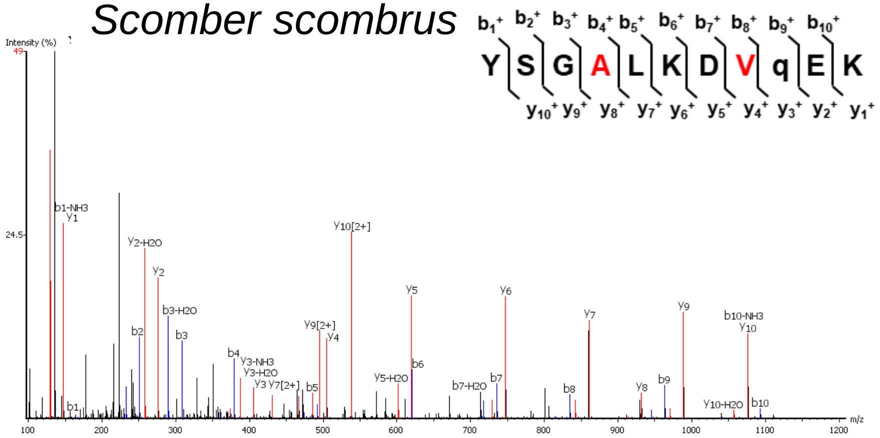
Auxis Thazard



Thunnus alalunga



Scomber scombrus



Sequence alignements were performed using public databases and our home-made databases; e.g. here an amino acid substitution is shown

Proteins identified in the archaeological sample

Among the 157 proteins (≥ 2 unique peptides):

10 muscle proteins:

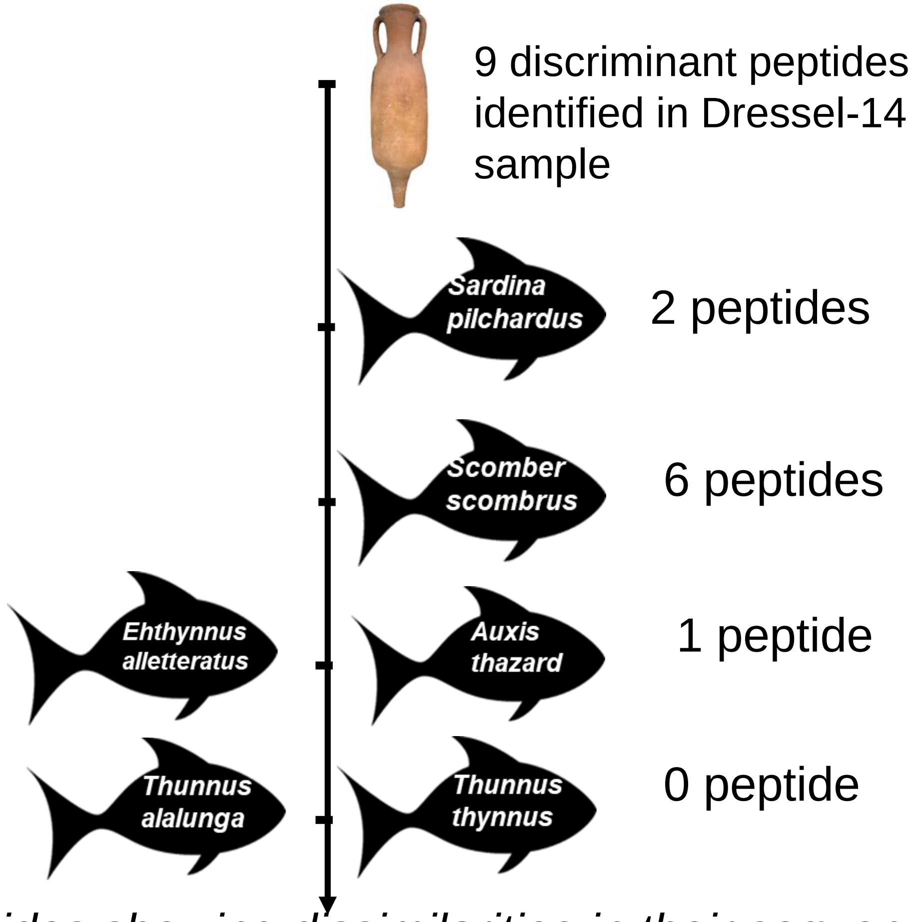
- 32 peptides discriminant for fishes (comparison with the NCBI database)

2 blood proteins:

- 4 peptides discriminant for fishes

The peptides identified in the archaeological sample showed dissimilarities with several species studied in this work (e.g. *Sardina pilchardus, Scomber scombrus*)

No sequence dissimilarities were found with *Thunnus thynnus* and *Thunnus alalunga* species



9 discriminant peptides identified in Dressel-14 sample

2 peptides

6 peptides

1 peptide

0 peptide

Peptides showing dissimilarities in their sequences

Conclusion

- Proteins from Dressel 14 archaeological samples preserved in submarine context during two millenaries were identified
- Proteins from muscles and blood were identified
- Good protein sequence coverages were obtained allowing sequence alignment studies
- New peptides not referenced in the current databases have been identified by *de novo* sequencing (and sequence homology to referenced proteins)
- Proteins identified in the archaeological material showed sequence dissimilarities with several modern species studied in this work (e.g. *Sardina pilchardus, Scomber scombrus)*
- No sequence dissimilarities were identified with *Thunnus thynnus* and *Thunnus alalunga species* raising the question of the potential use of tuna in the studied amphorae.

Taxonomy and classification of Upper Pleistocene bones with ultrahigh resolution MALDI-FTICR Mass Spectrometry

Isabelle FABRIZI

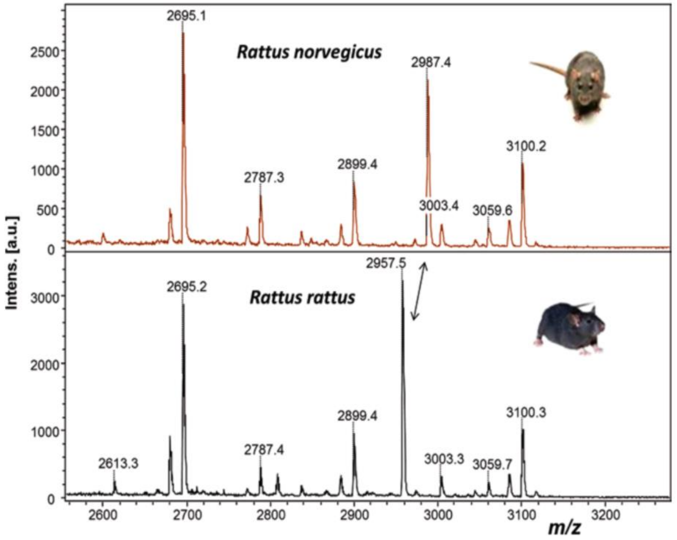
Miniaturization for Synthesis, Analysis & Proteomics USR 3290

University of Lille, FST

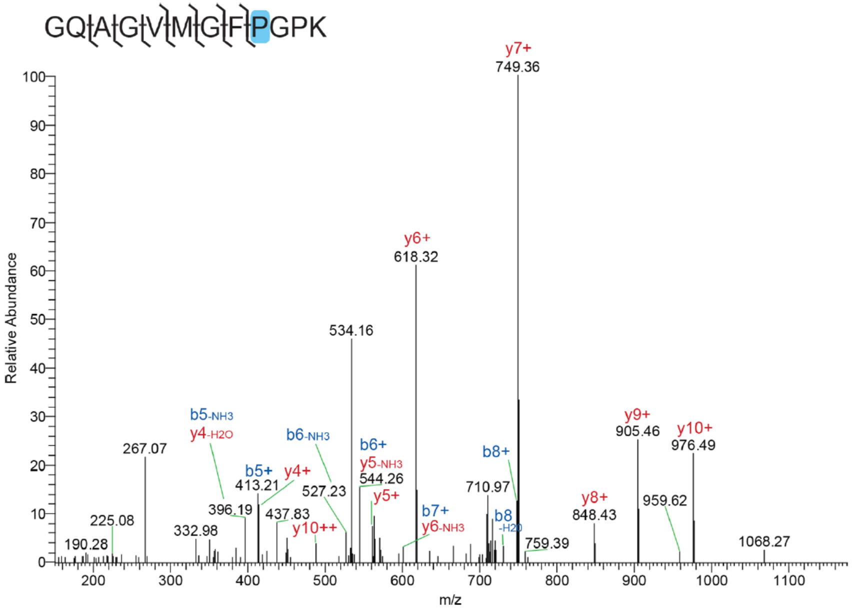
Why

- Ancient preserved molecules give a **better knowledge** on the biological past
- Bones proteomic is a **good alternative to replace** ancient DNA analysis
- **Tiny fragments** are found in **large numbers** during paleontological excavations
- Analysis of fossils **must consume a low quantity of material** to avoid damaging samples
- Modifications post translationnal are **markers of degradation** of bones

ZooMS



LC-MS/MS



High throughput method using FT-ICR development

Sample preparation

Analysis

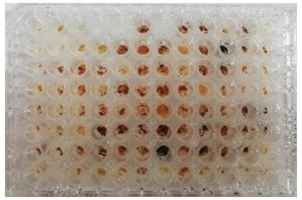
Bioinformatic without Data bank

Solarix XR 9.4 Tesla

Bones

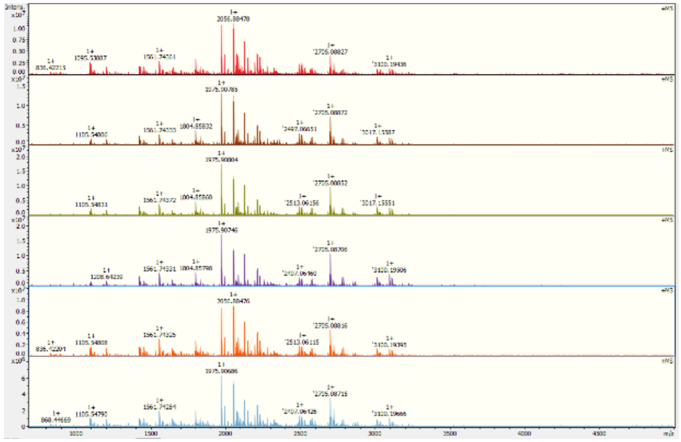
96-well plate digestion

500 µg



Demineralisation
Denaturation
Digestion

MALDI FT ICR analysis
HCCA/ACN(10 mg/mL)



MALDI FT ICR spectra

Reference bones and paleontological bones from Caours and Waziers (130,000 – 110,000 years old, France) were given by Dr Patrick AUGUSTE, EEP laboratory.

EU FT-ICR MS

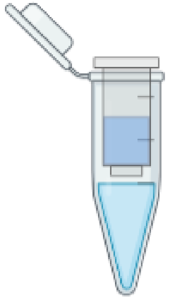
MSAP

CNRS

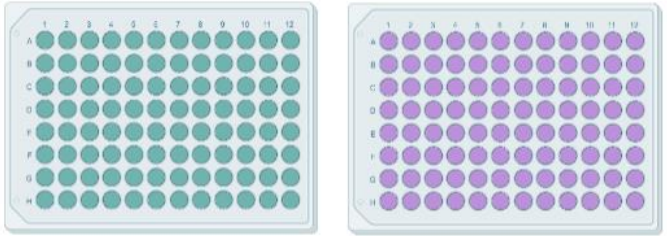
Université de Lille

Workflow optimization on 96 well plate

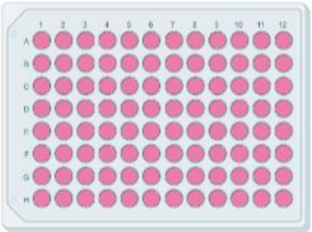
- Reference bones



- eFASP (cellulose acetate)



- eFASP microplate 30 kDa (polyethersulfone)
- eFASP microplate 10 kDa (polyethersulfone)

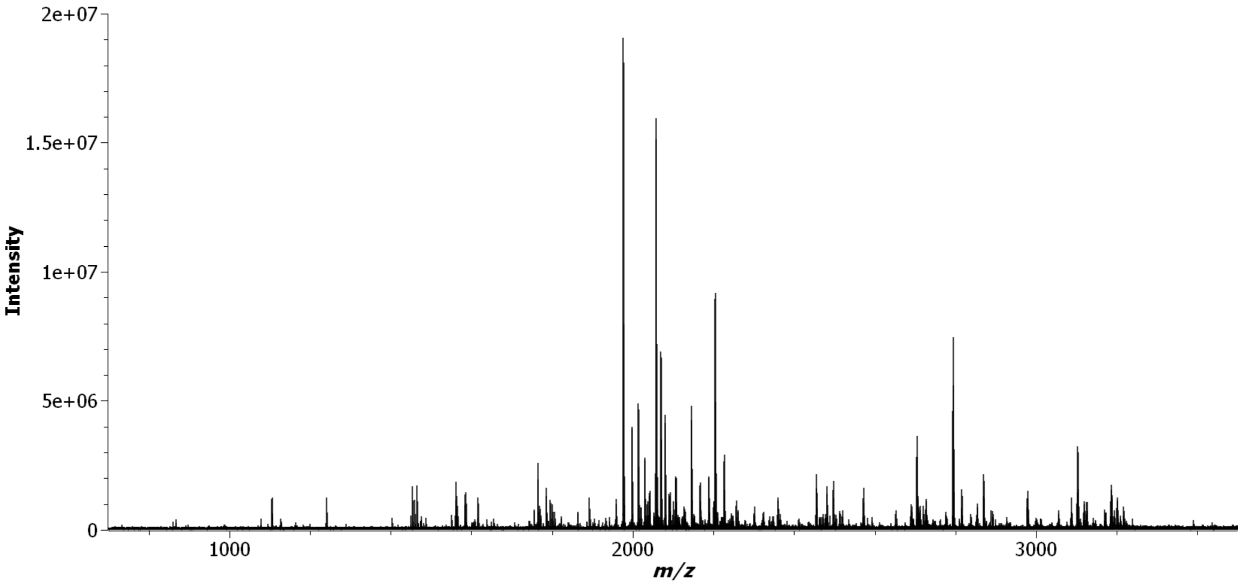


- eFASP microplate 0.45 µm (10 kDa) (PVDF)

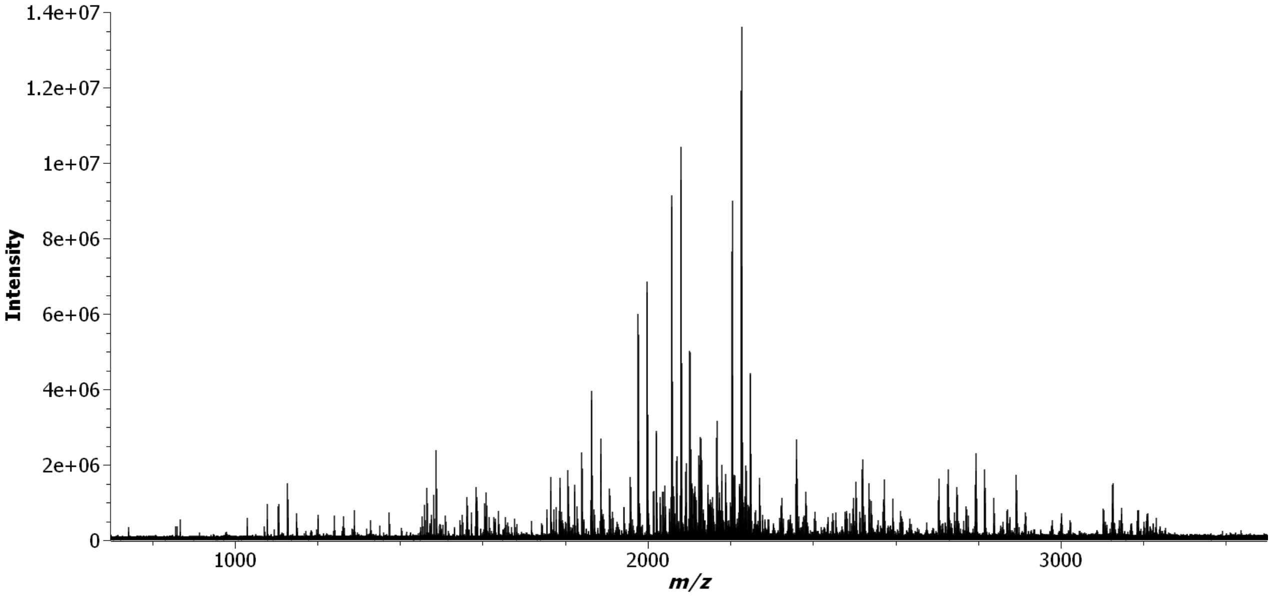
MALDI FT ICR : detection limit from eFASP in 96 wells plates

Bos primigenus spectra from archeological site Caours (130,000 – 110,000 years old)

Resolution 2M, FID 5.0 sec, 15 accumulated spectra, laser shot: 1’30 s



0.1 mg



1 mg

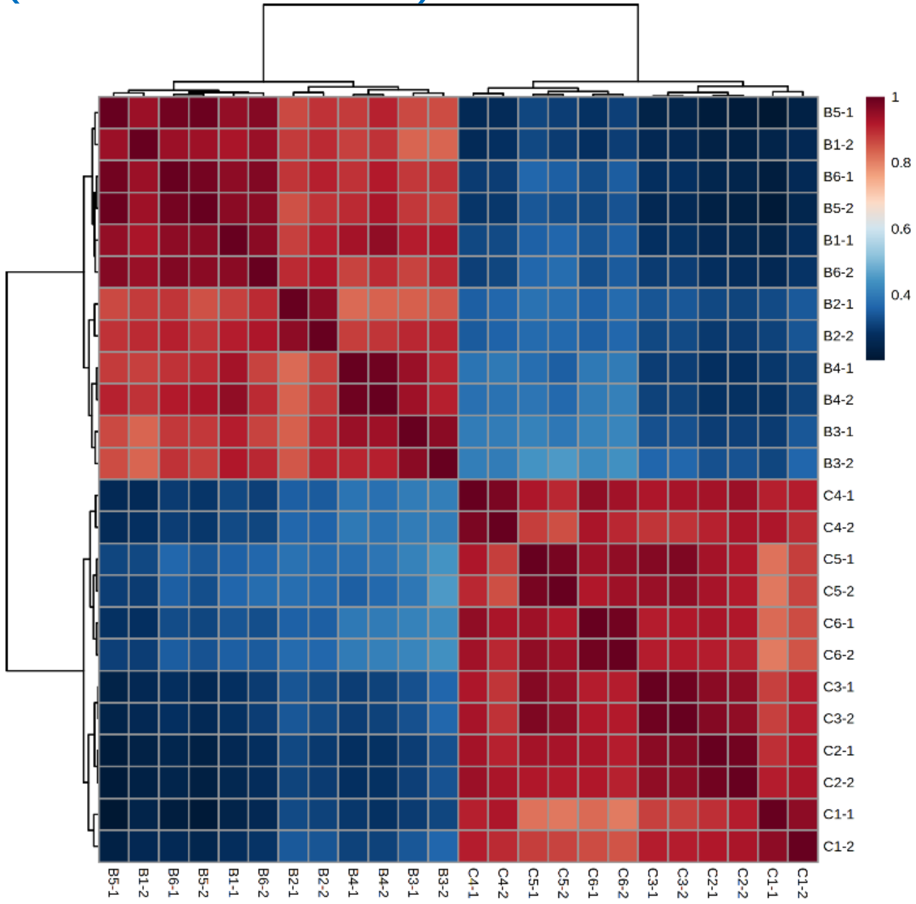
Detection limit: 0.1 mg of bone

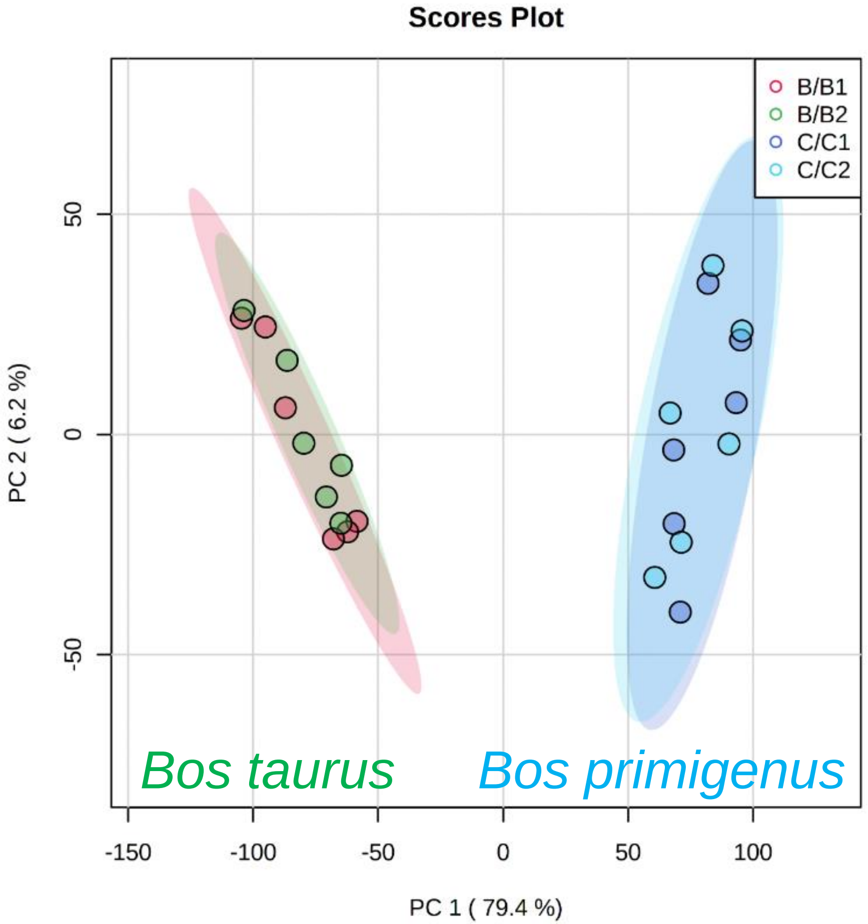
Depend on bone conservation & archaeological sites

Protocol reproductibility of 96 well plate digestion

Bos taurus (modern)
Bos primigenus (Aurochs, ancient)

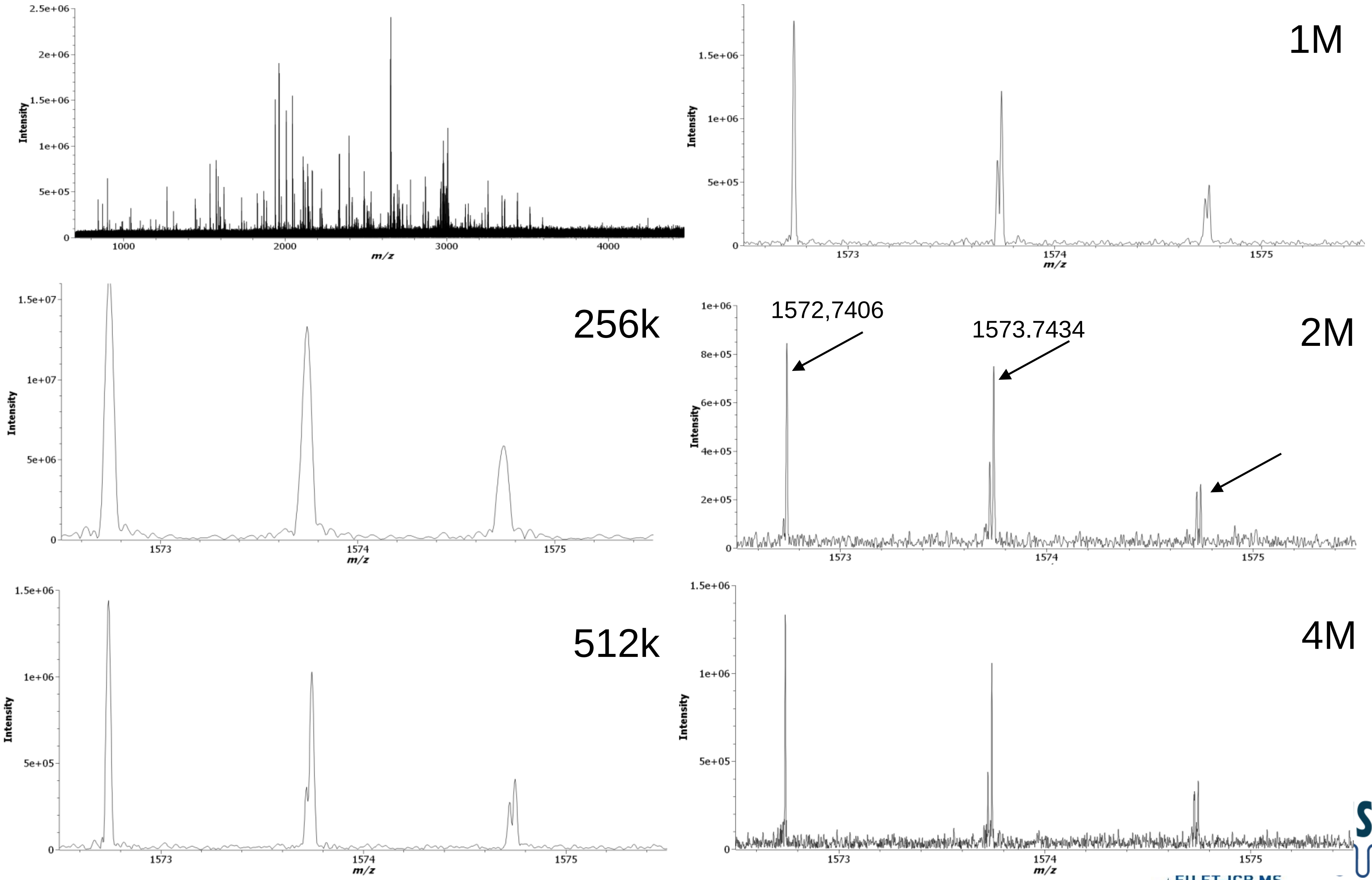
6 peptidic digests from 1 mg of bones, analyzes in duplicate





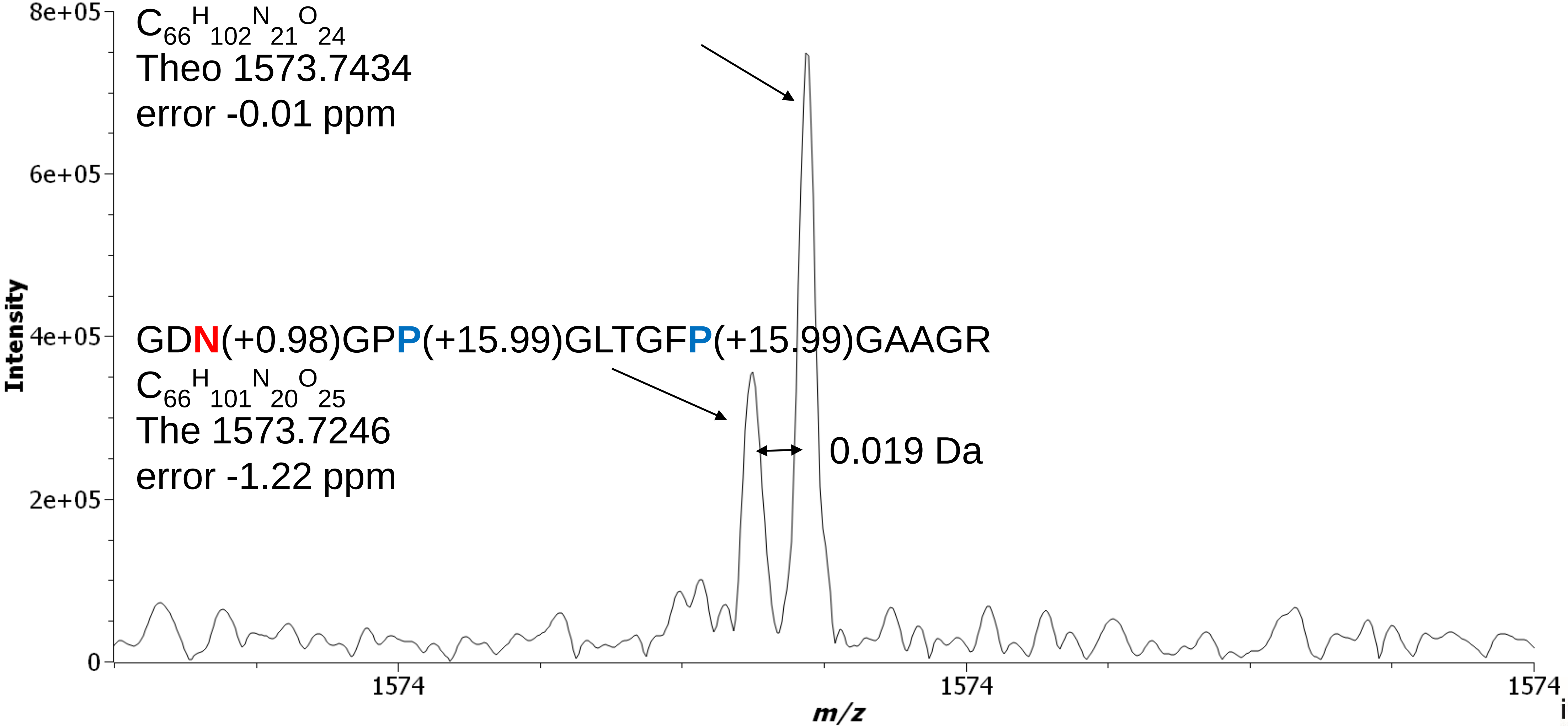
Modern and ancient species discrimation can be quickly discriminaated by PCA and Heatmap

Optimum resolution m/Δm for the identification of deamidation



2M points: separation of deamidated isobaric peak

Zoom of deamidation on peptide of archaeological bone spectrum

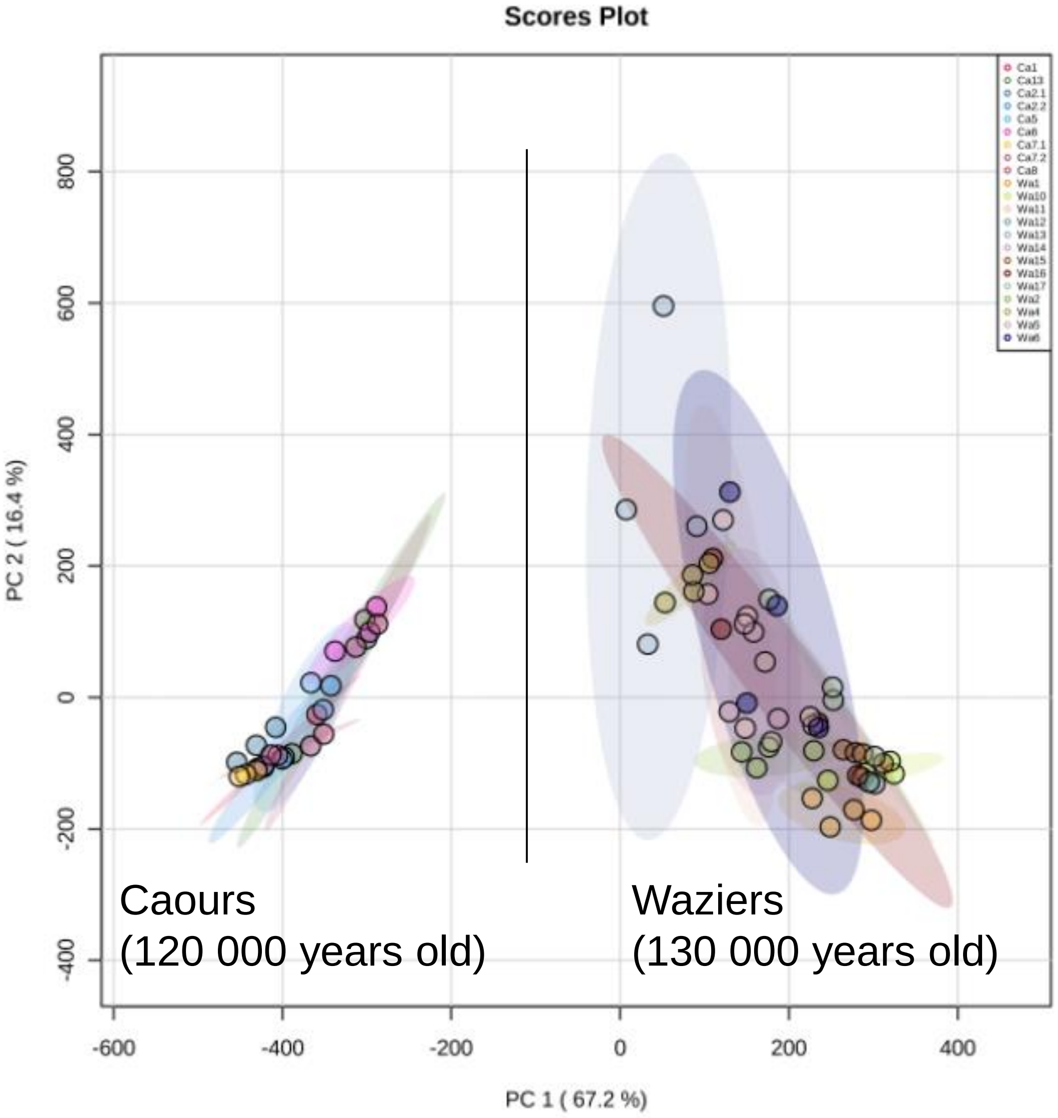


GDNGPP(+15.99)GLTGFP(+15.99)GAAGR
$C_{66}H_{102}N_{21}O_{24}$
Theo 1573.7434
error -0.01 ppm

GDN(+0.98)GPP(+15.99)GLTGFP(+15.99)GAAGR
$C_{66}H_{101}N_{20}O_{25}$
The 1573.7246
error -1.22 ppm

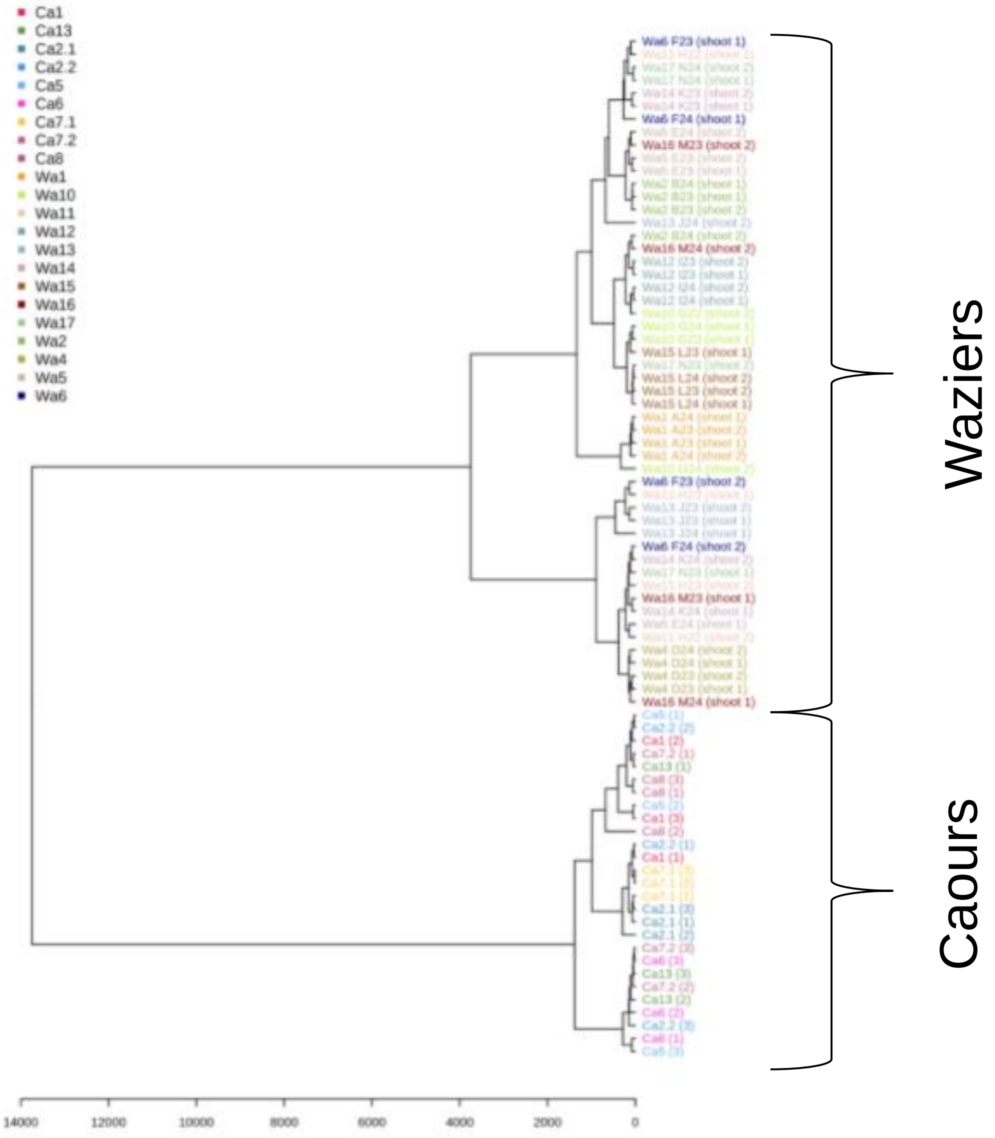
0.019 Da

Analysis of archaeological bones from different sites



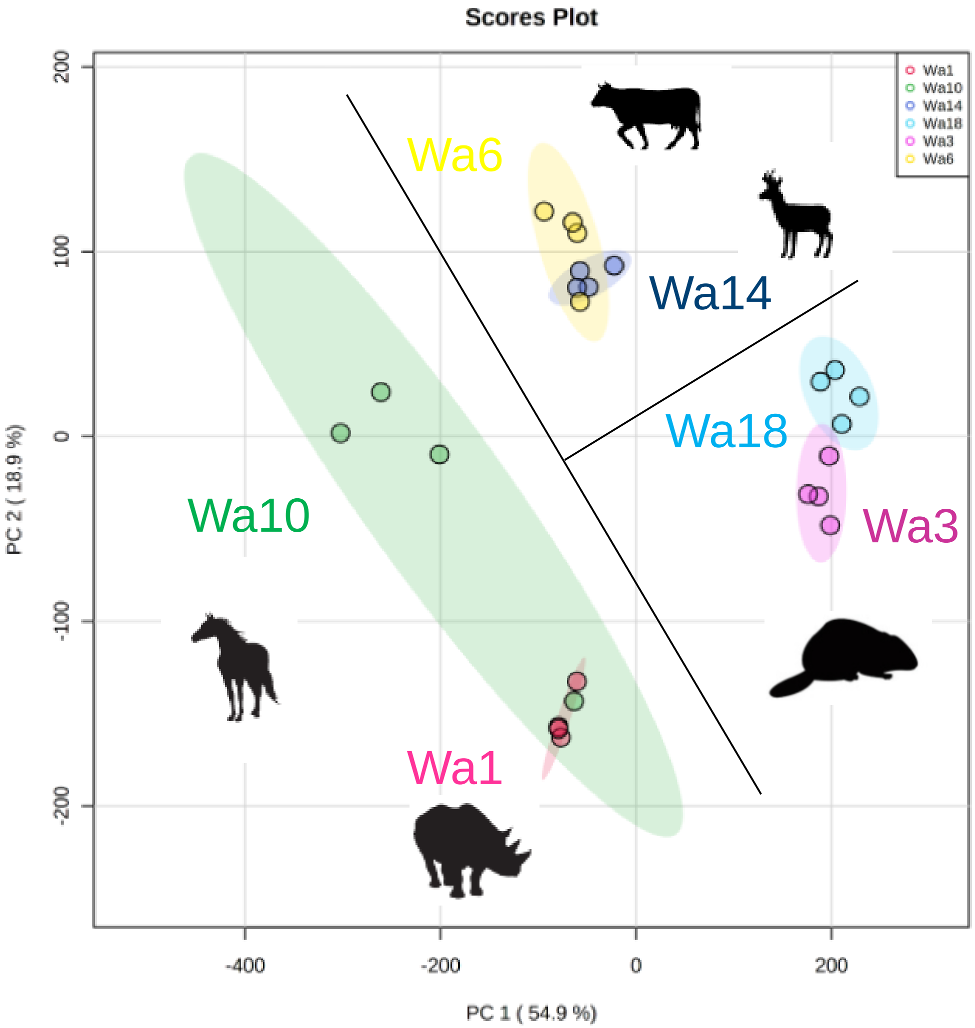
Caours
(120 000 years old)

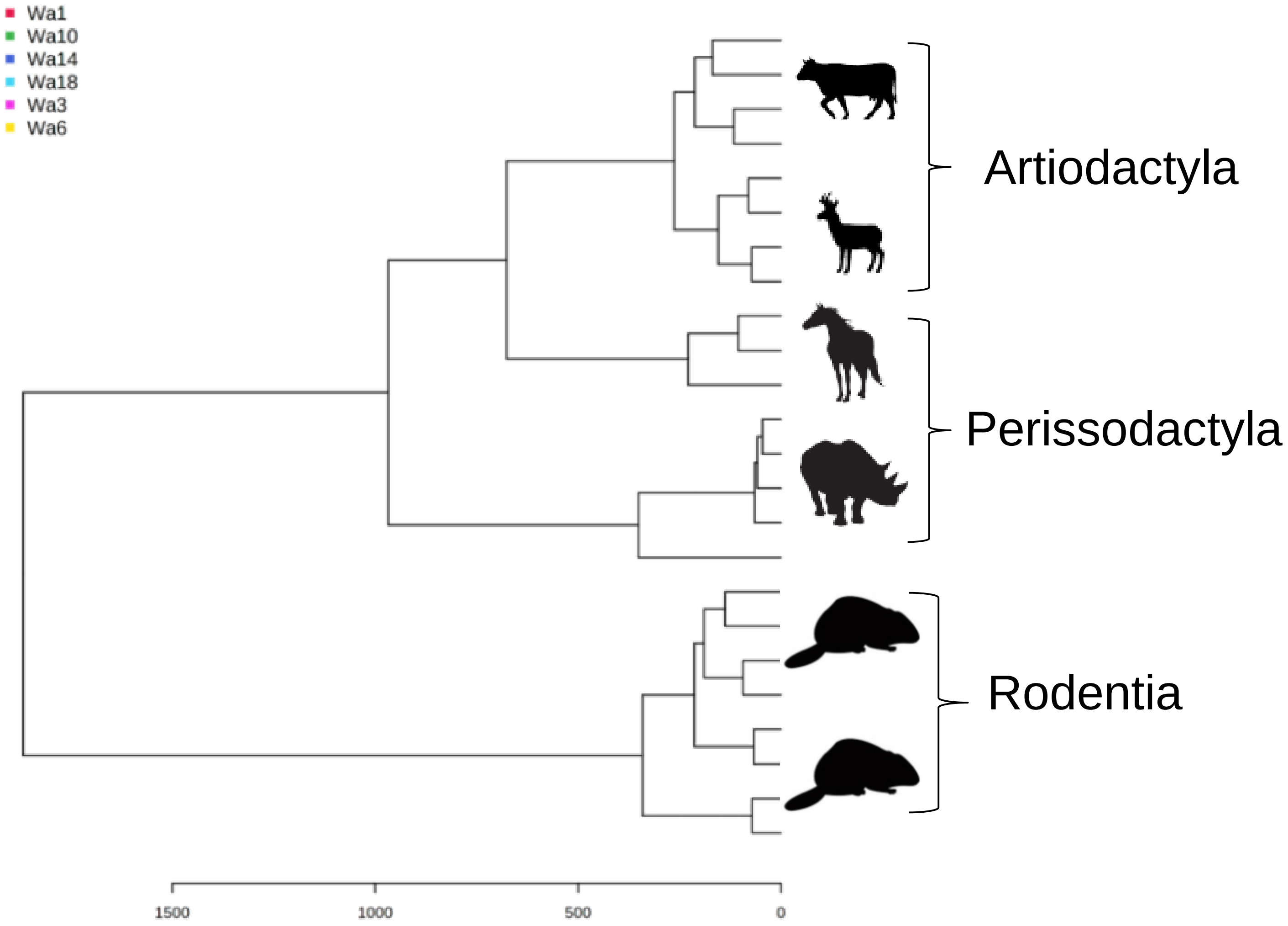
Waziers
(130 000 years old)



2 clusters are observed

Archaeological bones samples the Wazier site





3 clusters observed

Correlation of % desamidation and datation of different sites

120 000 years

100 000 years

1000 - 3000 years

Study of plant gums polysaccharide moiety by sequential enzymatic hydrolysis

Clara GRANZOTTO

Miniaturisation pour la Synthèse, l’Analyse & la Protéomique USR 3290

Université Lille, CNRS

Plant gums: applications

Plant gums are naturally occurring polysaccharide materials exuded by several species of plants or extracted from the endosperm of some seeds.

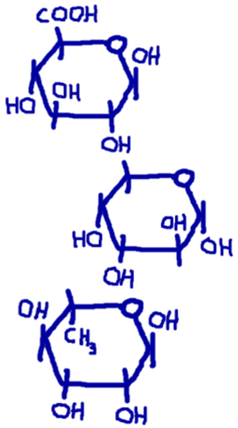
gum arabic
fruit tree gums
gum tragacanth

karaya gum
locust bean gum
ghatti and guar gum

- **adhesive properties**
- **ability to form gels**
- **stabilize emulsion, foams and dispersions**

Plant gums: polysaccharides classification

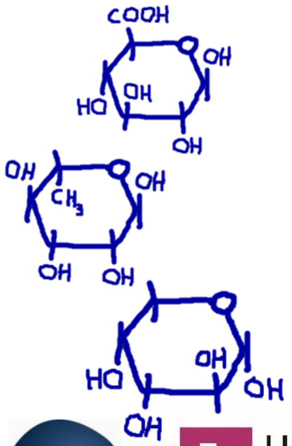
Plant gum	Polysaccharide family
Arabic	Type II Arabinogalactan
Ghatti	Substituted glucuronomannan
Tragacanth	Mixture of type II arabinogalactan and galacturonan type
Karaya	Substituted rhamnogalacturonan
Guar/Locust bean	Galactomannan
Fruit tree (e.g. cherry)	Substituted arabinogalactan



Arabinogalactan
arabinose, galactose

Galactomannan
galactose, mannose

Substituted Arabinogalactan
arabinose, galactose,
galacturonic acid, xylose



State of art and analytical approach

traditional technique: acid hydrolysis and monosaccharide composition by **GC-MS**.

cons: fairly limited and not specific if a mixture of polysaccharide materials is present

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New analytical approach

(1) Gum solubilization

(2) Sequential enzymatic hydrolysis

(3) clean-up

(4) MALDI TOF of release oligosaccharides

State of art and analytical approach

traditional technique: acid hydrolysis and monosaccharide composition by **GC-MS**.

cons: fairly limited and not specific if a mixture of polysaccharide materials is present

New analytical approach

Different plant gums → **Polysaccharide fingerprint** → **Real art samples** → **Unique gum identification**

(4) MALDI TOF of release oligosaccharides

State of art and analytical approach

traditional technique: acid hydrolysis and monosaccharide composition by **GC-MS**.

cons: fairly limited and not specific if a mixture of polysaccharide materials is present

New analytical approach

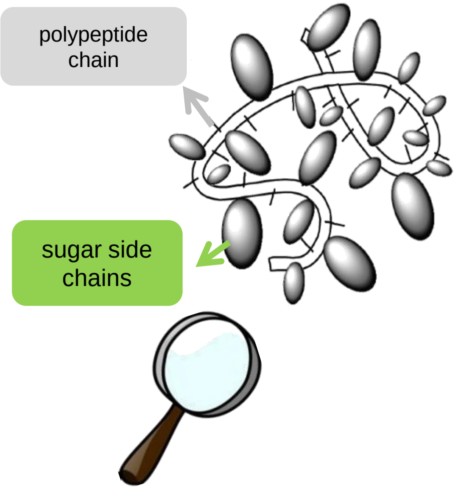
(1) Gum solubilization

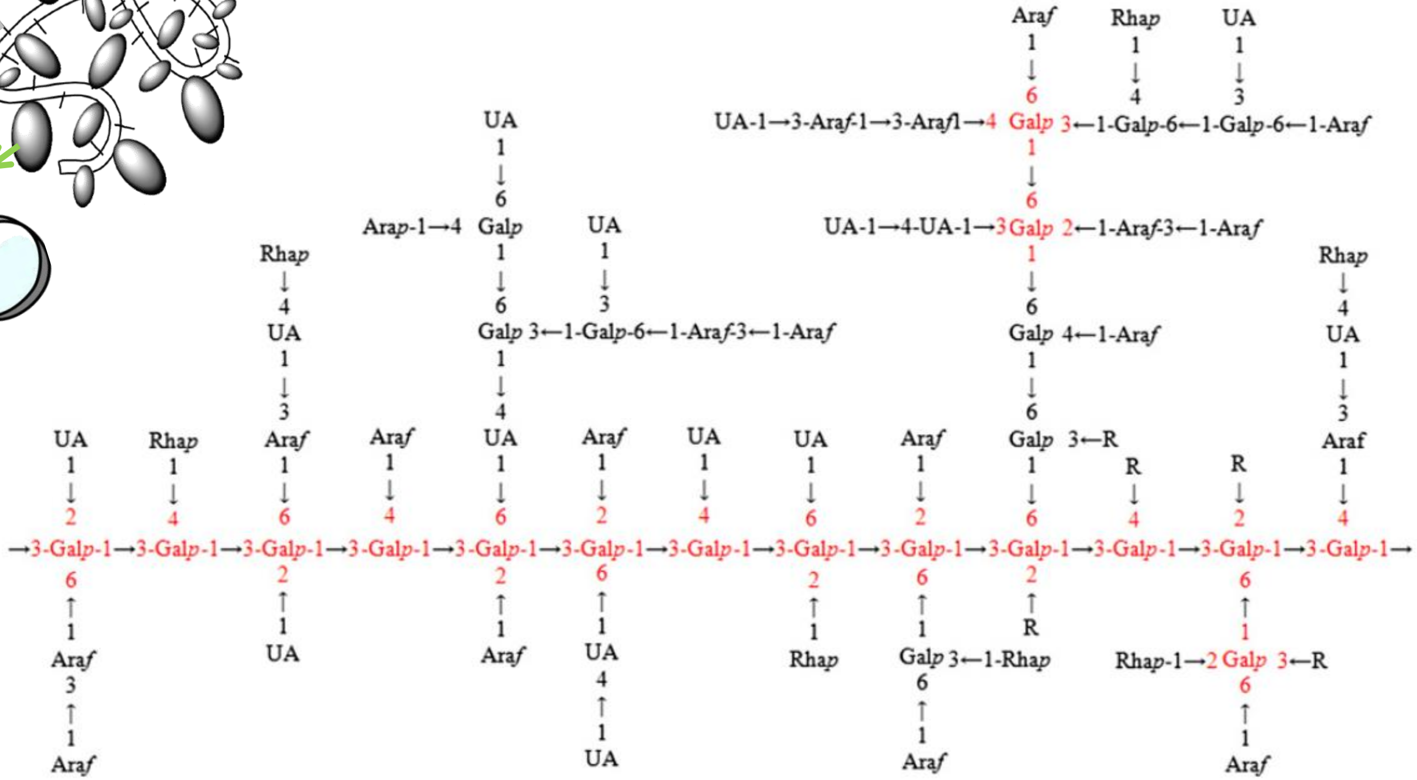
(2) Sequential enzymatic hydrolysis

(3) clean-up

(4) MALDI TOF of release oligosaccharides

Gum Arabic: arabinogalactan



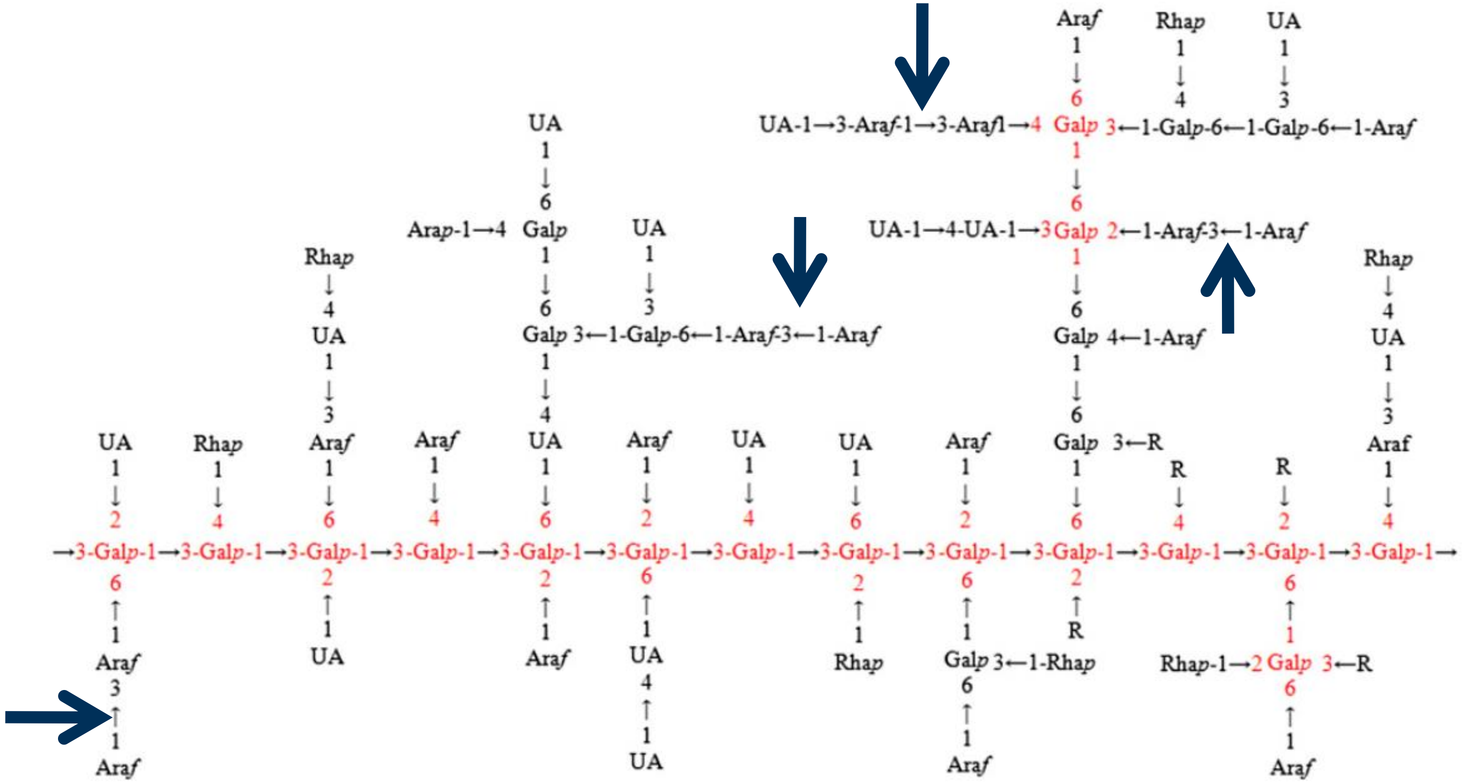


Nie S-P. A further amendment to the classical core structure of gum arabic (Acacia senegal).
Food Hydrocolloids **2013**;31:42.

Gum Arabic: arabinogalactan

(1) α-L-Arabinofuranosidase

From side chains to main chain
→ access of enzyme

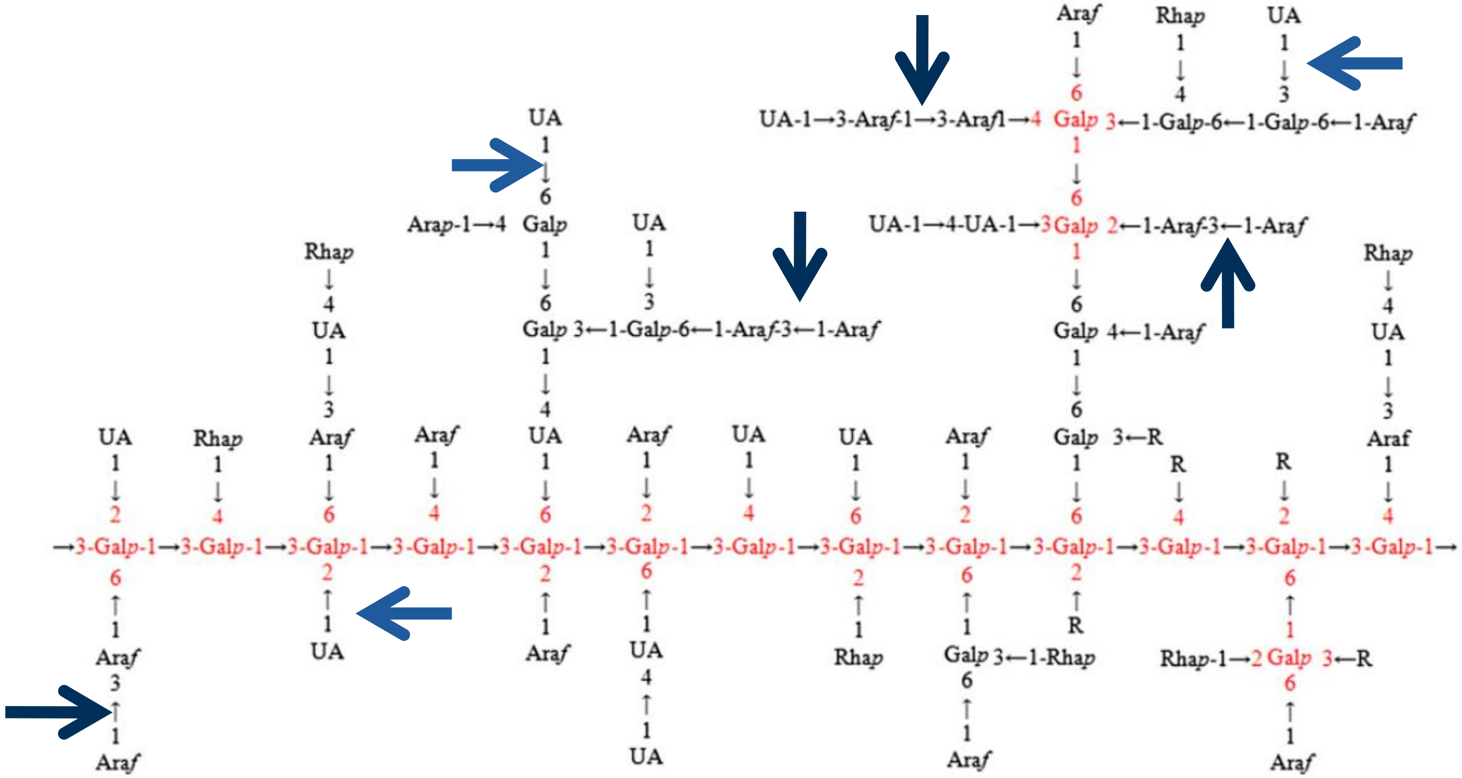


Nie S-P. A further amendment to the classical core structure of gum arabic (Acacia senegal). Food Hydrocolloids 2013;31:42.

Gum Arabic: arabinogalactan

(1) α-L-Arabinofuranosidase

(2) β-glucuronidase



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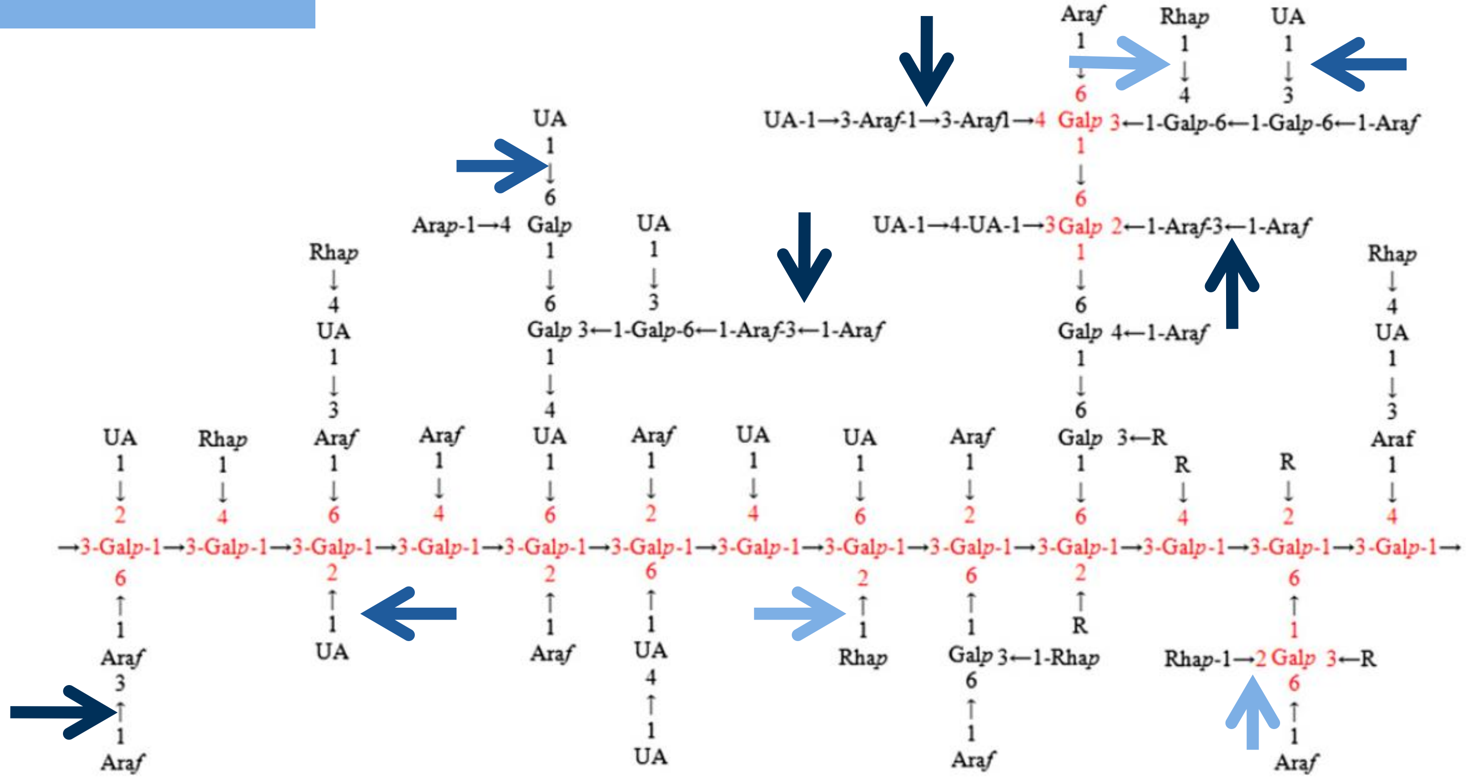
Gum Arabic: arabinogalactan

(1) α-L-Arabinofuranosidase

(2) β-glucuronidase

(3) α-L-rhamnosidase



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Gum Arabic: arabinogalactan

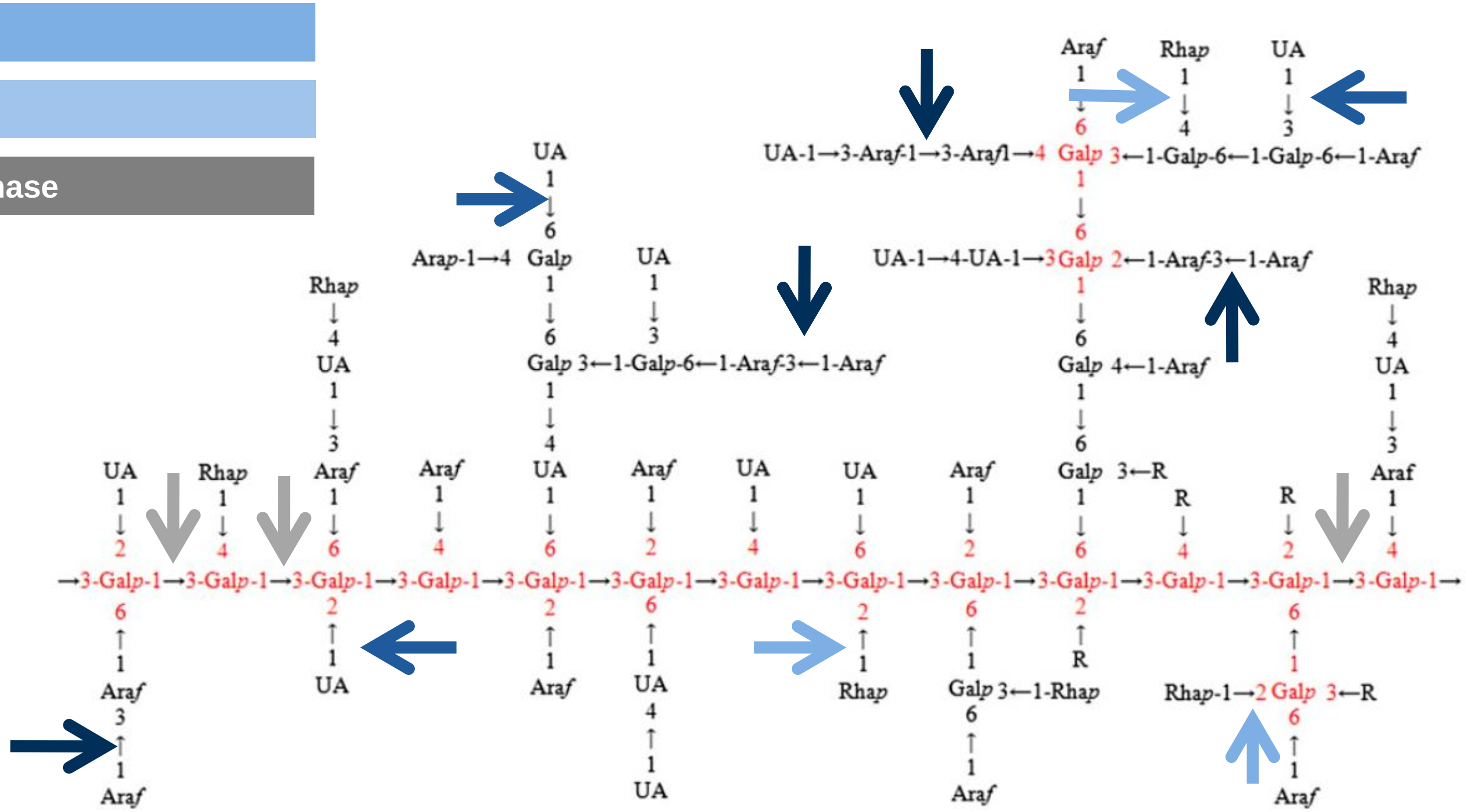
(1) α-L-Arabinofuranosidase

(2) β-glucuronidase

(3) α-L-rhamnosidase

(4) β -Galactosidase

(5) exo--β- 1,3-galactanase



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Gum Arabic: arabinogalactan

- 100mU enzyme/1mg gum
- 24 h digesion each enzyme
- Specific T°, buffer and pH

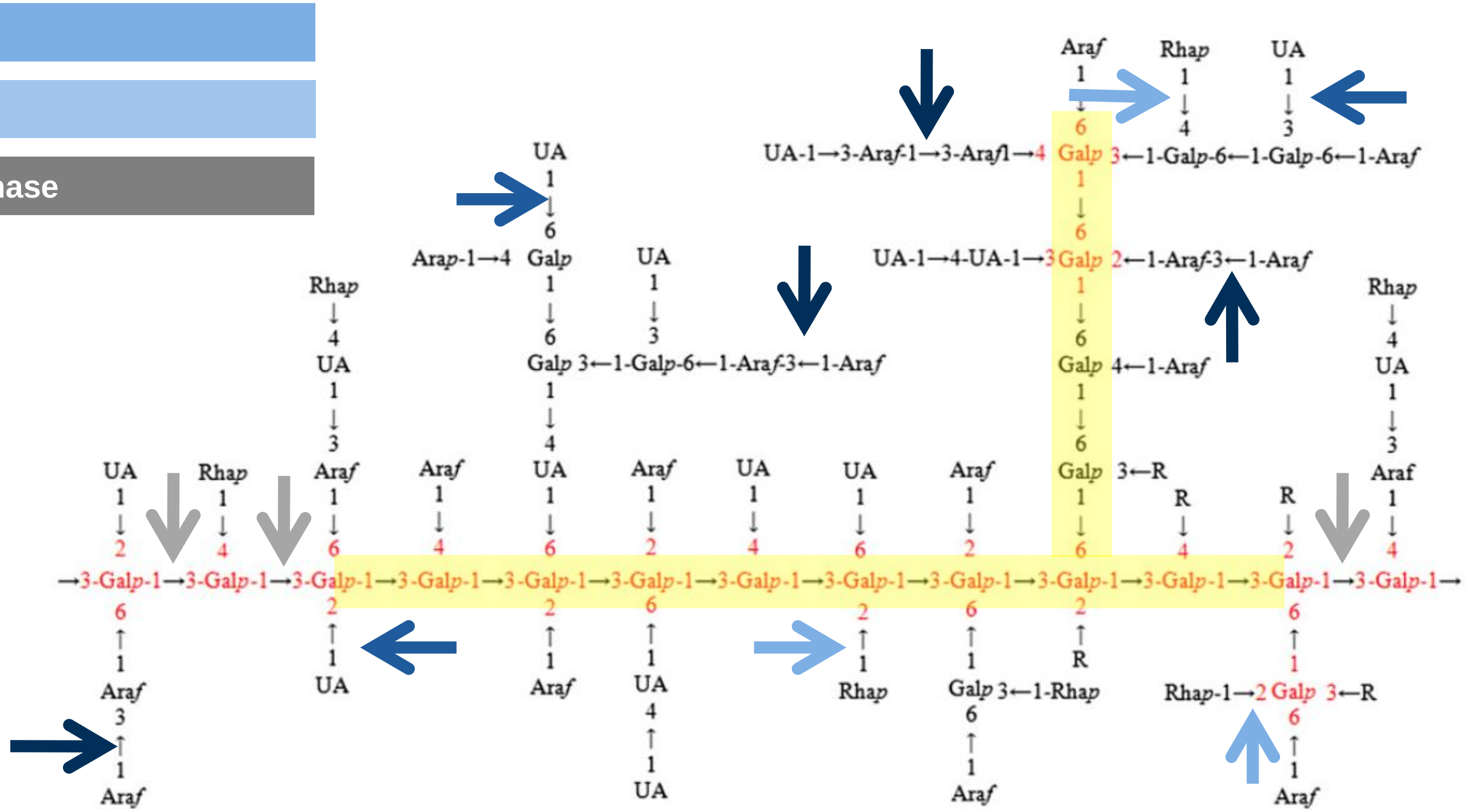
(1) α-L-Arabinofuranosidase

(2) β-glucuronidase

(3) α-L-rhamnosidase

(4) β -Galactosidase

(5) exo--β- 1,3-galactanase



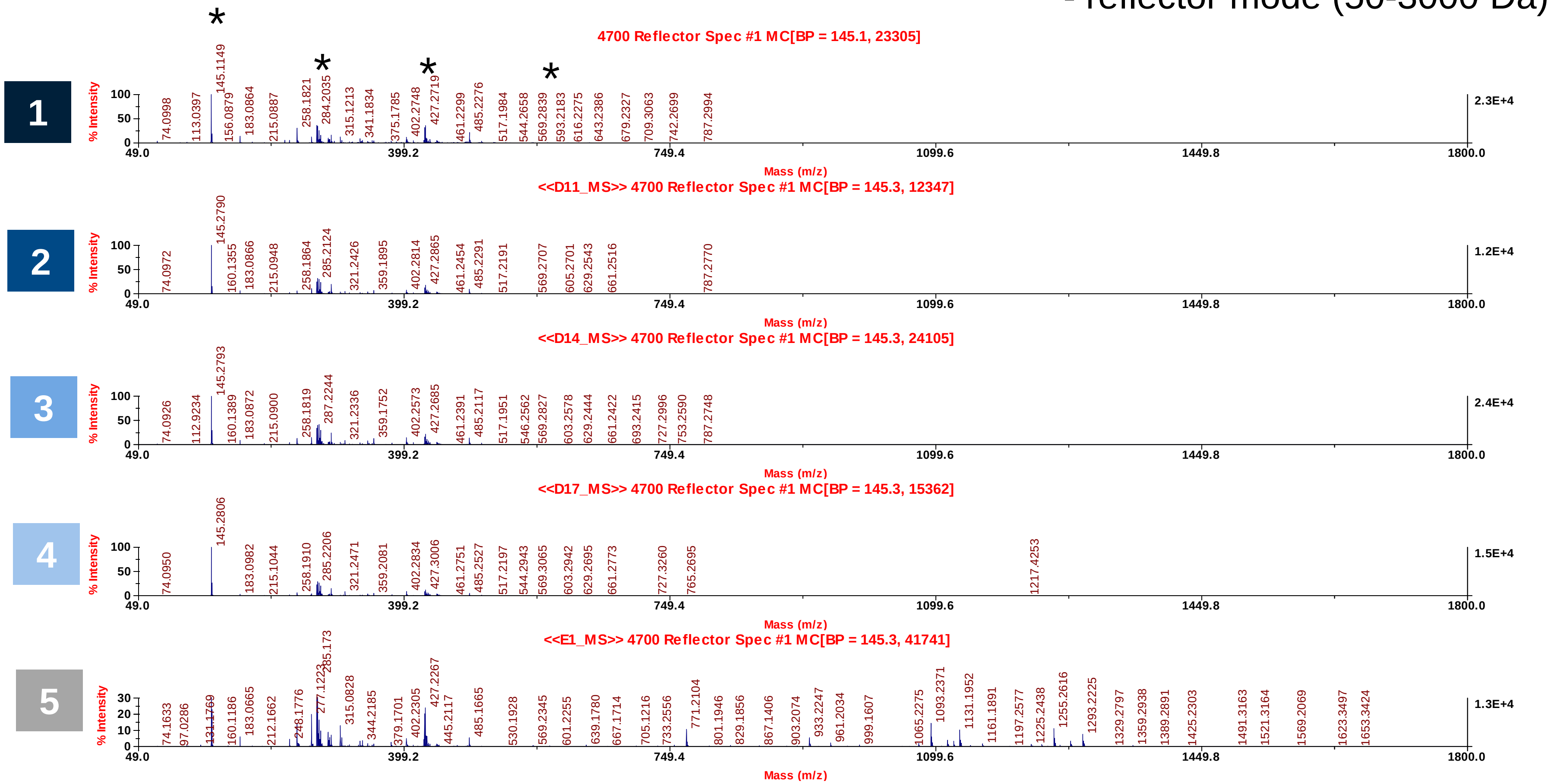
Nie S-P. A further amendment to the classical core structure of gum arabic (Acacia senegal). Food Hydrocolloids 2013;31:42.

EU FT-ICR MS MSAP cnrs Université de Lille

Gum Arabic: MALDI

Methodology

- no clean-up procedure;
- 3AQ in ACN/H_2O
- reflector mode (50-3000 Da)



Gum Arabic: MALDI

Methodology

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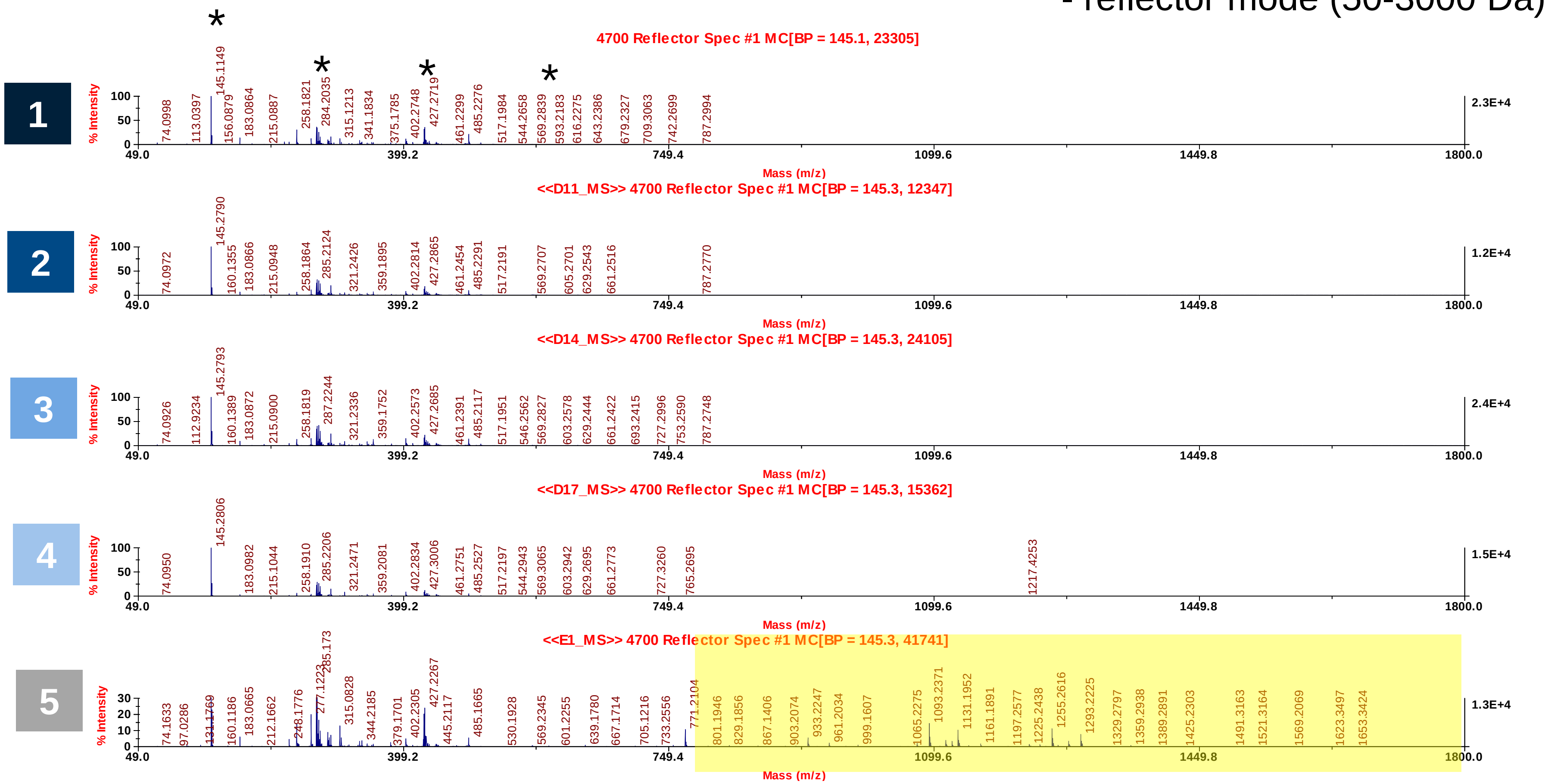
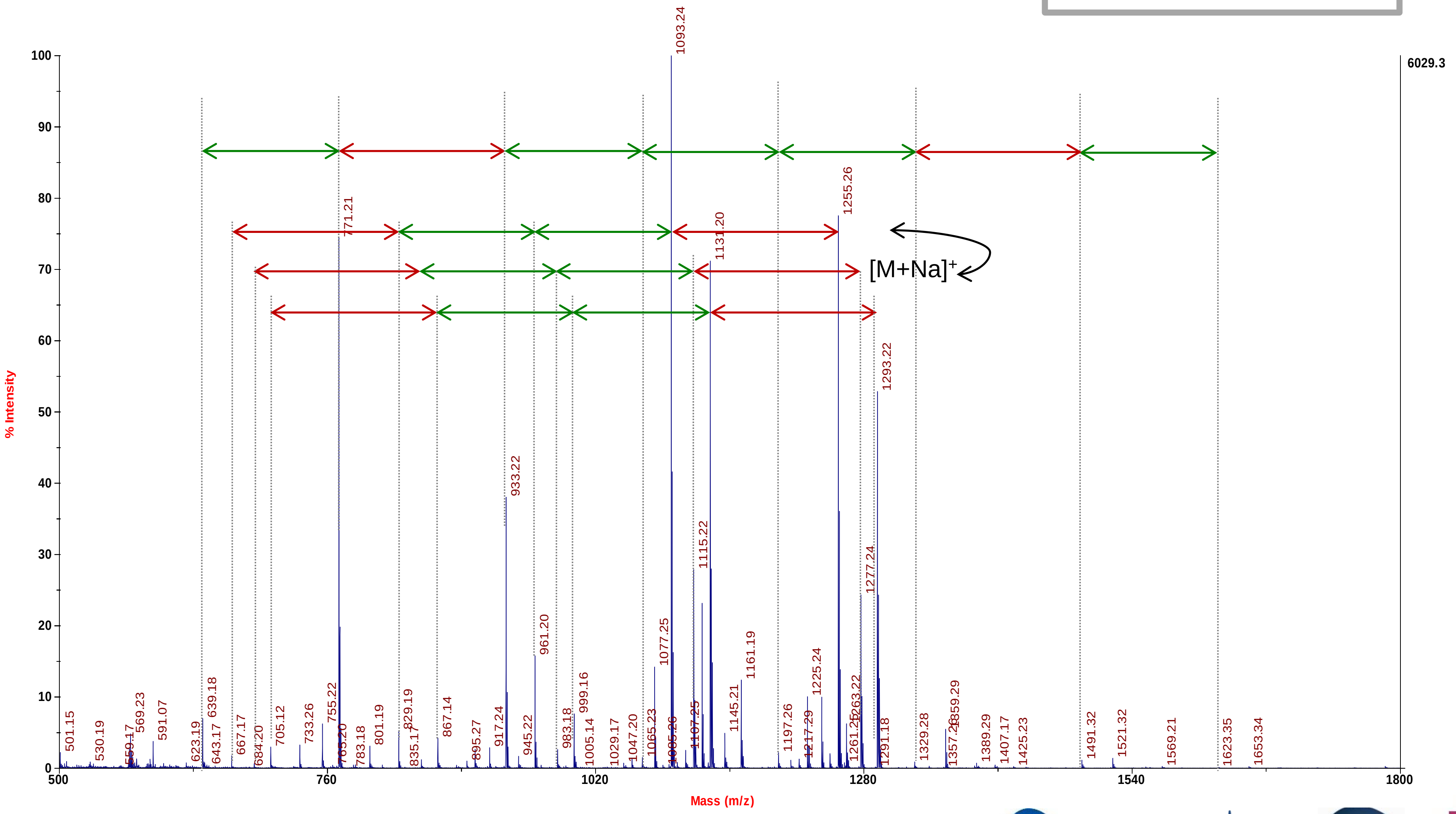


Gum Arabic: MALDI

↔ Galactose (Δm=162)
↔ Arabinose (Δm=132)



Summary

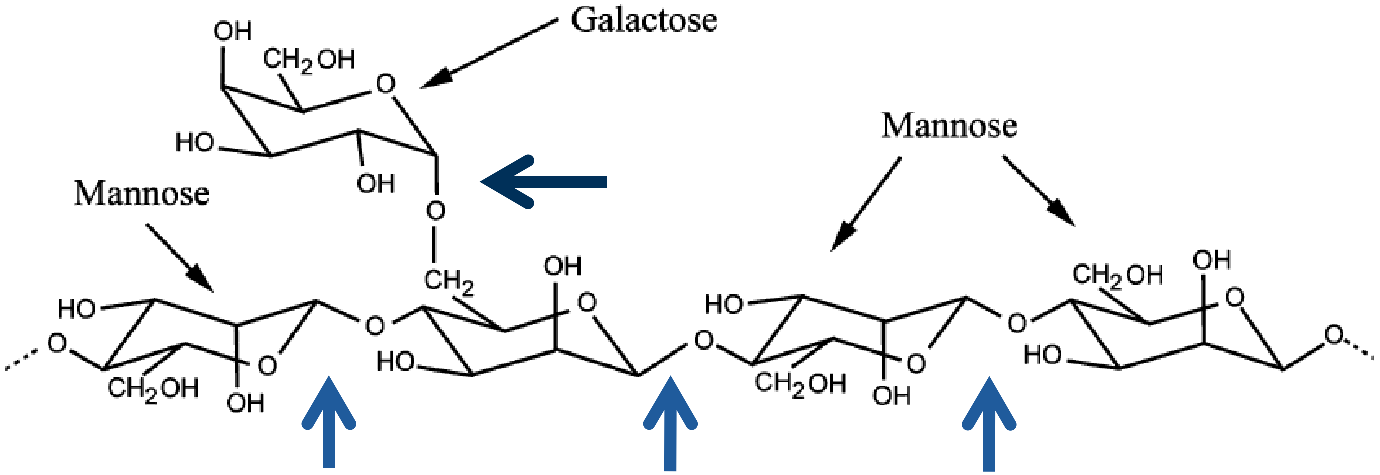
1. Plant gums: applications and polysaccharides families
2. Analytical approach and objectives
3. Methodological development and results: gum arabic
4. Methodological development and results: Locust Bean gum
5. Future perspectives

Locust Bean gum: galactomannan

(1) α-galactosidase

(2) Endo-1,4-beta-mannanase

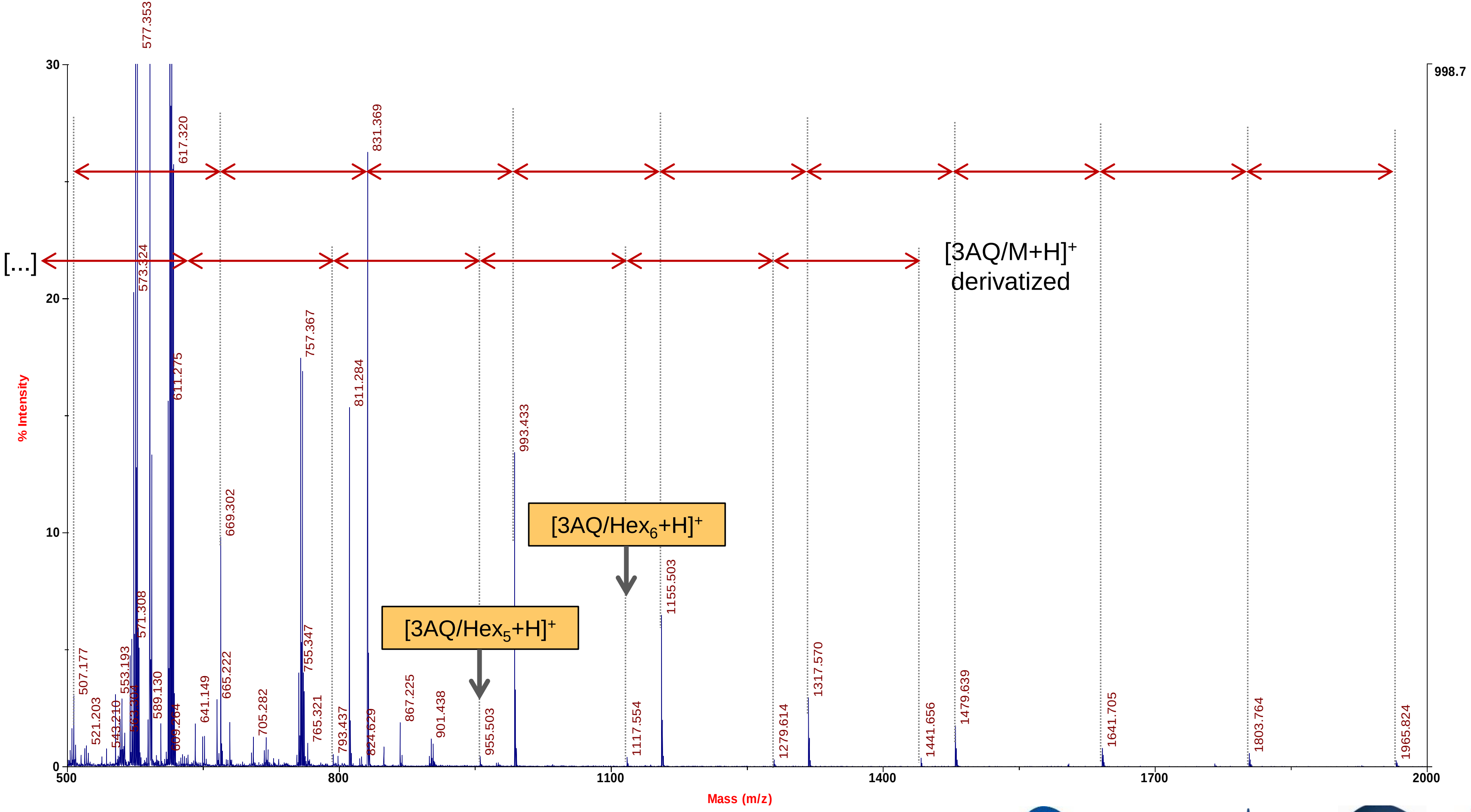
Linear backbone of 1→4-linked β-D-mannose units attached by a single α-D-galactose residue at C-6 of mannose with 1→6 glycosidic bonds



Moreira L.R.S. An overview of mannan structure and mannan-degrading enzyme systems. *Appl Microbiol Biotechnol* , **2008**, 79:165–178

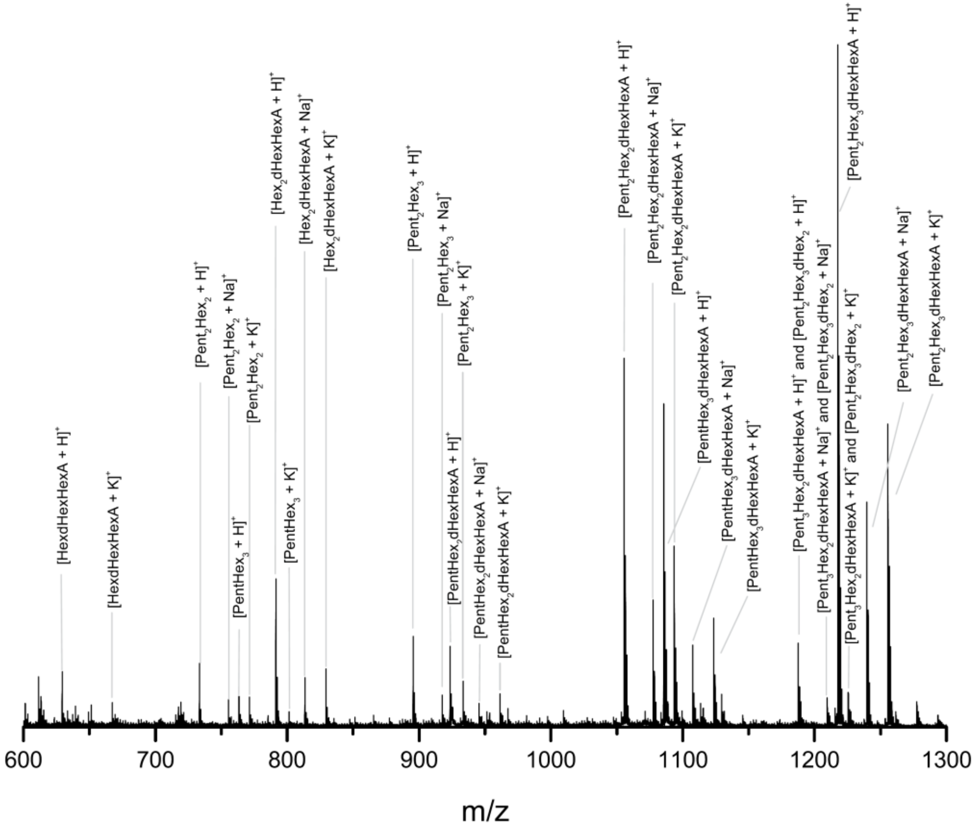
Locust Bean gum : MALDI

↔ Galactose or Mannose (Δm=162)



Identification of the binder of a watercolor paint

Wooden 'Colour Box Charles Roberson & Co’ dating 1870s. Approximately 1 mg was sampled from the blue watercolor paint located on the left in the box



MALDI-TOF MS profile of the old watercolor sample dating 1870 with the related oligosaccharide attributions. The reported ions correspond to oligosaccharides derivatized with 3-aminoquinoline.

Granzotto, C., Arslanoglu, J., Rolando, C., Tokarski, C. (2017). Plant gum identification in historic artworks. *Scientific reports*, *7*, 1-15.

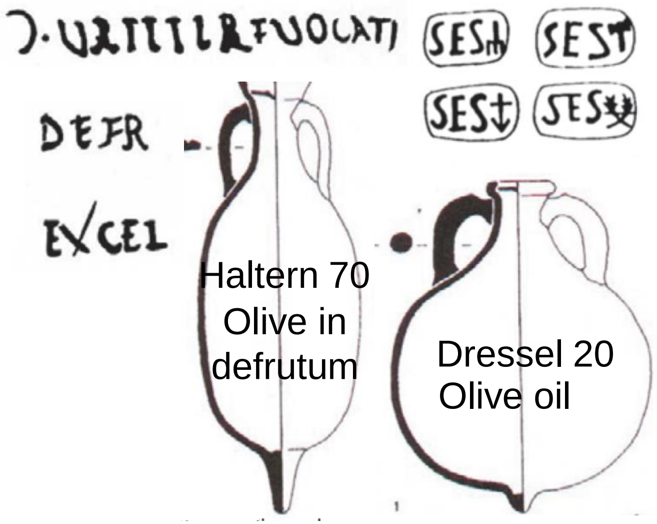
Identification of the origin of archaeological fat remains by triacylglycerols analysis using Li^+ cationization, nanoESI ionization, high resolution MS and IRMPD

Nicolas GARNIER

Miniaturization for Synthesis, Analysis & Proteomics USR 3290

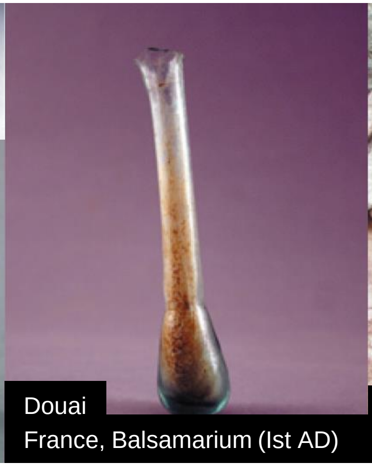
Why and how studying archaeological object contents?

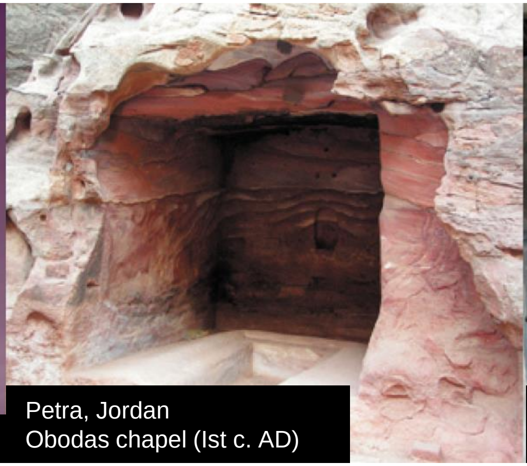
- **Why?**
  Historical, socio-economic, cultural precisions
  (knowledge of trades etc.) + analytical challenge
- **How?**
  - Typology (origin, content)
  - Epigraphy (stamp: potter, insurer; *Tituli picti*: owner, charterer; content and quality)
  - Coating (pitch = aqueous contents)
  - Chemical analysis based on fatty acid composition*



Port Vendres II (42-50 BC) shipwreck


Villa Giulia, Roma, Italy
Nilotic vessel (VIth BC)


Douai
France, Balsamarium (Ist AD)


Petra, Jordan
Obodas chapel (Ist c. AD)


Pompei, Italy
Dyeing bassin (79th century AD)

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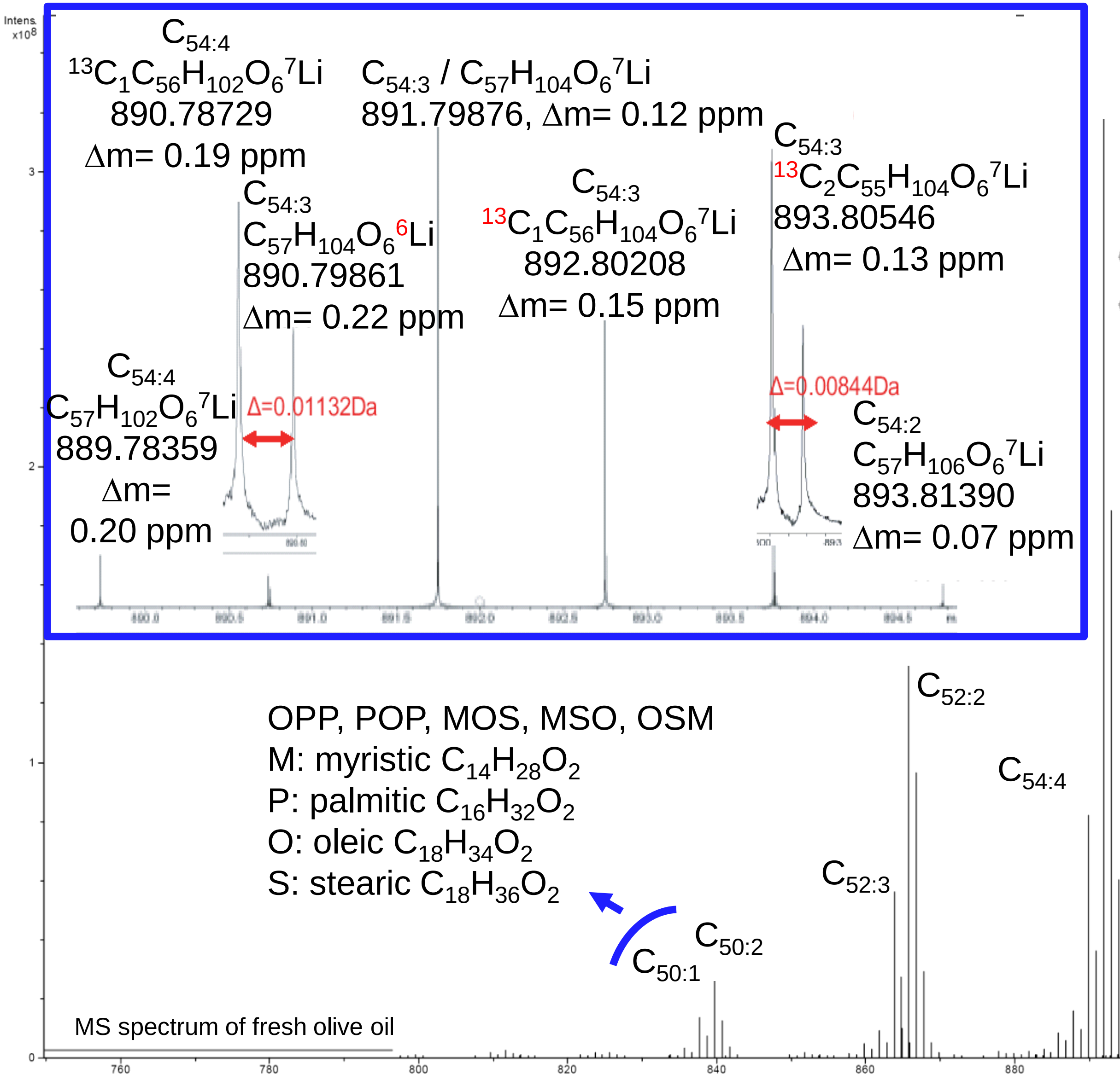
* Evershed R.P. Archaeometry 2008, 50, 895–924.
Colombini M.P., Modugno F., Ribechini, E. 2009 GC/MS in the Characterization of Lipids, in Organic Mass Spectrometry in Art and Archaeology, Eds M. P. Colombini and F. Modugno, John Wiley & Sons, Ltd, Chichester, UK.

Triacylglycerols analysis: example of olive oil (cationized with Li$^+$)



$C_{54:4}$
$^{13}C_1C_{56}H_{102}O_6{}^7Li$
890.78729
Δm= 0.19 ppm

$C_{54:3}$ / $C_{57}H_{104}O_6{}^7Li$
891.79876, Δm= 0.12 ppm

$C_{54:3}$
$^{13}C_2C_{55}H_{104}O_6{}^7Li$
893.80546
Δm= 0.13 ppm

$C_{54:3}$
$C_{57}H_{104}O_6{}^6Li$
890.79861
Δm= 0.22 ppm

$C_{54:3}$
$^{13}C_1C_{56}H_{104}O_6{}^7Li$
892.80208
Δm= 0.15 ppm

$C_{54:4}$
$C_{57}H_{102}O_6{}^7Li$
889.78359
Δm=
0.20 ppm

$C_{54:2}$
$C_{57}H_{106}O_6{}^7Li$
893.81390
Δm= 0.07 ppm

$C_{52:2}$

$C_{54:4}$

$C_{52:3}$

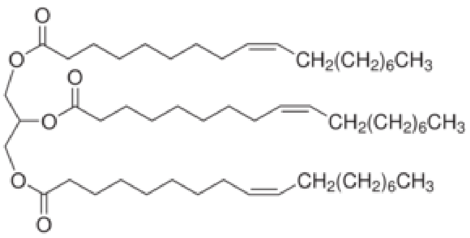
$C_{50:2}$

$C_{50:1}$

OPP, POP, MOS, MSO, OSM
M: myristic $C_{14}H_{28}O_2$
P: palmitic $C_{16}H_{32}O_2$
O: oleic $C_{18}H_{34}O_2$
S: stearic $C_{18}H_{36}O_2$

MS spectrum of fresh olive oil

$C_{54:3}$
OOO
O: oleic fatty acid $C_{18}H_{34}O_2$



Device: Apex Qe 9.4 T

Nomenclature: $C_{\text{sum of lateral chain carbons:insaturation number}}$

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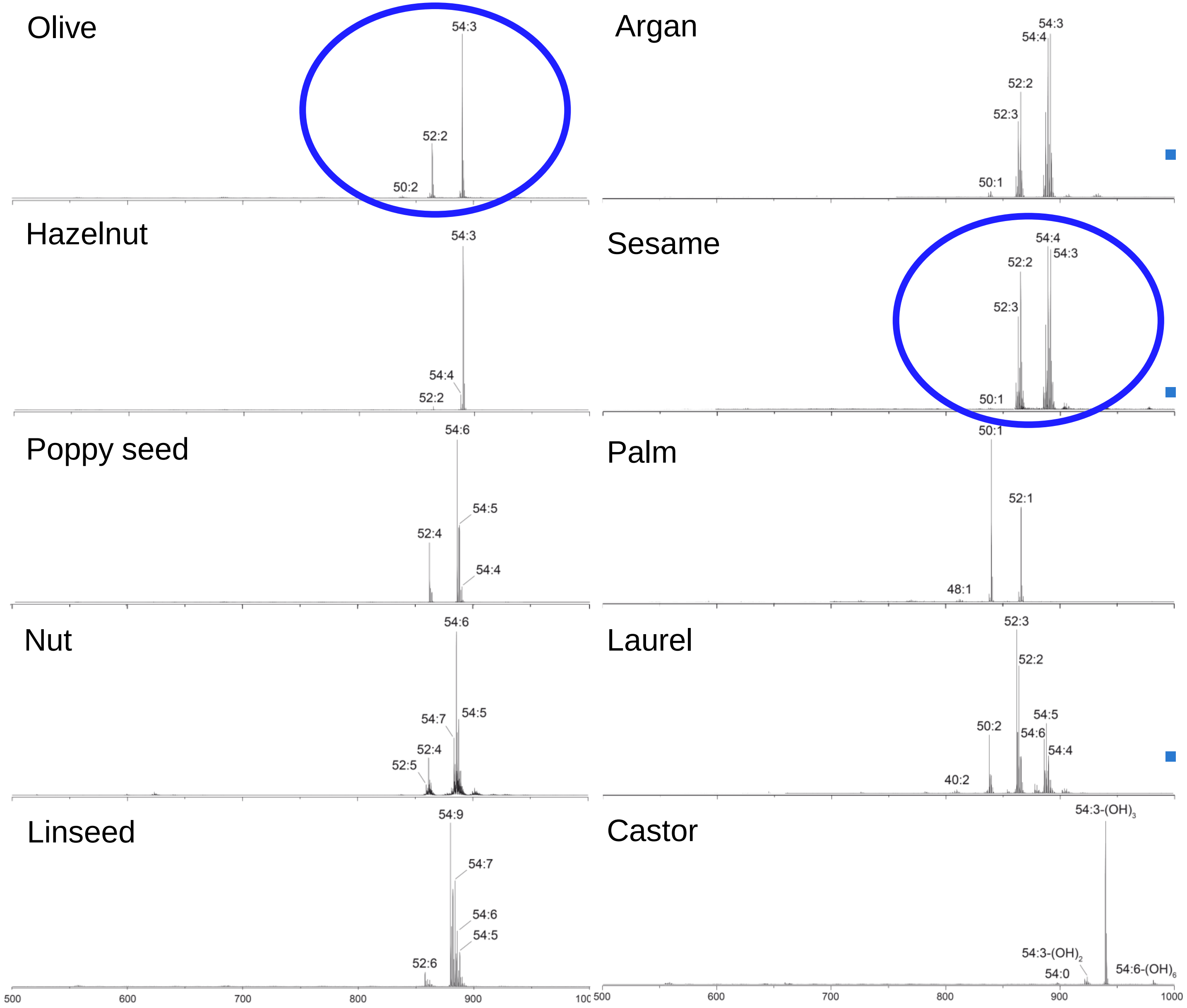
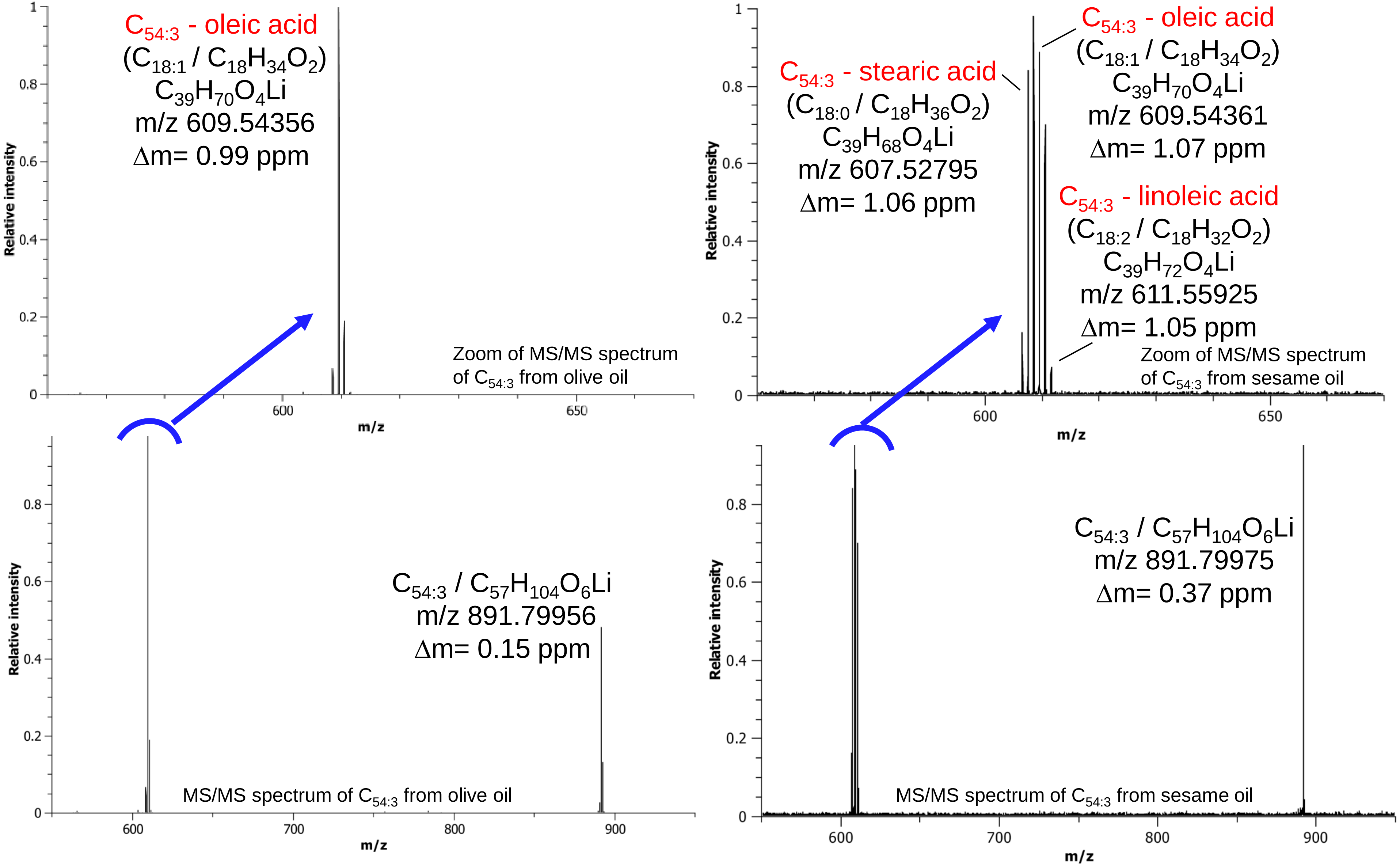
TAGs purified on diol cartridge (elution: cyclohexane/dichloromethane/diethyl ether; 89/10/1; v/v/v)

MS spectra allow discrimination of oil origin: vegetal oils



- TAGs purified on diol cartridge (elution: cyclohexane dichloromethane, diethyl ether; 89/10/1)
- Similar narrow distribution of TAGs from Equivalent Carbon Number (ECN) 50 to 54 with two or three patterns, each one separated by 26.01565 or 28.03130 amu
- MS/MS profiles for discrimination of oils with similar profiles

MS/MS spectra allow discrimination of oil origin
InfraRed MultiPhoton Dissociation experiments ($C_{54:3}$ / $C_{57}H_{104}O_6Li$)



$C_{54:3}$ - oleic acid
($C_{18:1}$ / $C_{18}H_{34}O_2$)
$C_{39}H_{70}O_4Li$
m/z 609.54356
Δm= 0.99 ppm

Zoom of MS/MS spectrum of $C_{54:3}$ from olive oil

$C_{54:3}$ - stearic acid
($C_{18:0}$ / $C_{18}H_{36}O_2$)
$C_{39}H_{68}O_4Li$
m/z 607.52795
Δm= 1.06 ppm

$C_{54:3}$ - oleic acid
($C_{18:1}$ / $C_{18}H_{34}O_2$)
$C_{39}H_{70}O_4Li$
m/z 609.54361
Δm= 1.07 ppm

$C_{54:3}$ - linoleic acid
($C_{18:2}$ / $C_{18}H_{32}O_2$)
$C_{39}H_{72}O_4Li$
m/z 611.55925
Δm= 1.05 ppm

Zoom of MS/MS spectrum of $C_{54:3}$ from sesame oil

$C_{54:3}$ / $C_{57}H_{104}O_6Li$
m/z 891.79956
Δm= 0.15 ppm

MS/MS spectrum of $C_{54:3}$ from olive oil

$C_{54:3}$ / $C_{57}H_{104}O_6Li$
m/z 891.79975
Δm= 0.37 ppm

MS/MS spectrum of $C_{54:3}$ from sesame oil

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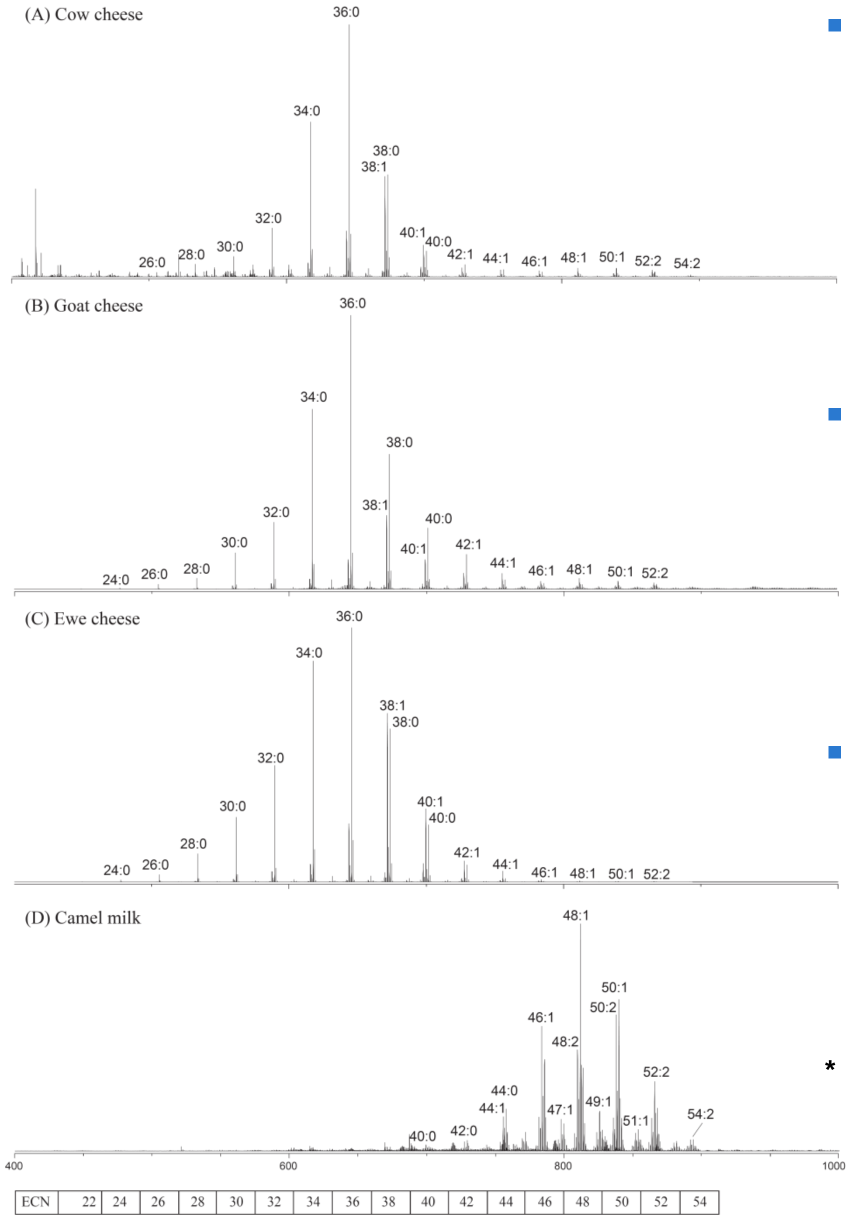
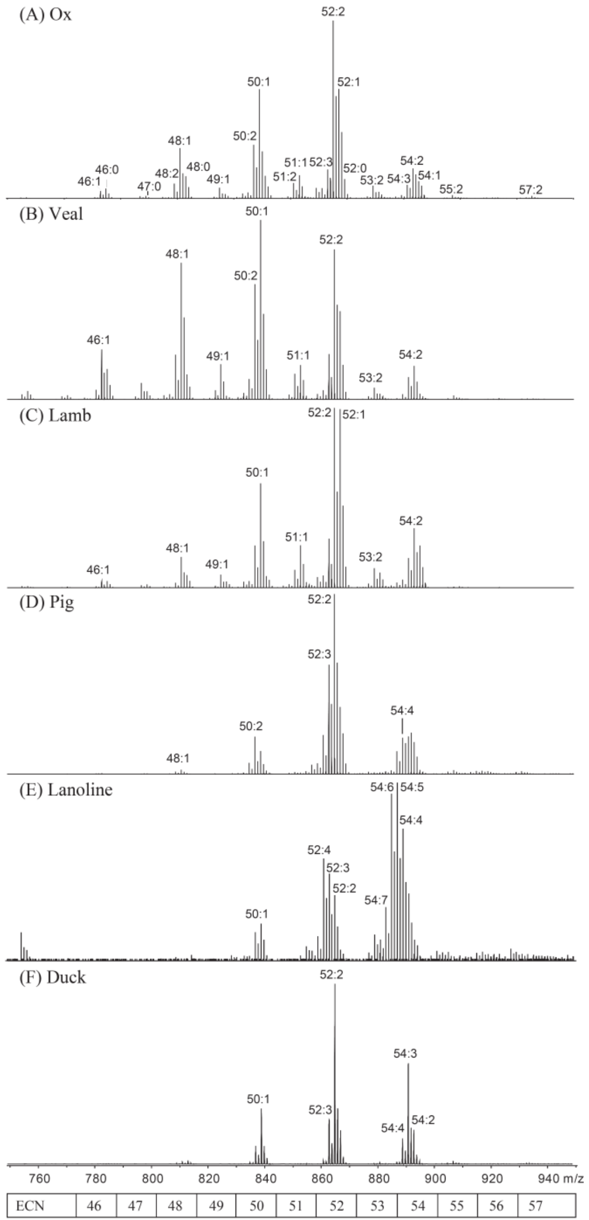
- Loss of oleic acid from olive $C_{54:3}$ versus loss of oleic, stearic, linoleic acids from sesame $C_{54:3}$

MS spectra allow discrimination of oil origin: dairy products and animal fats

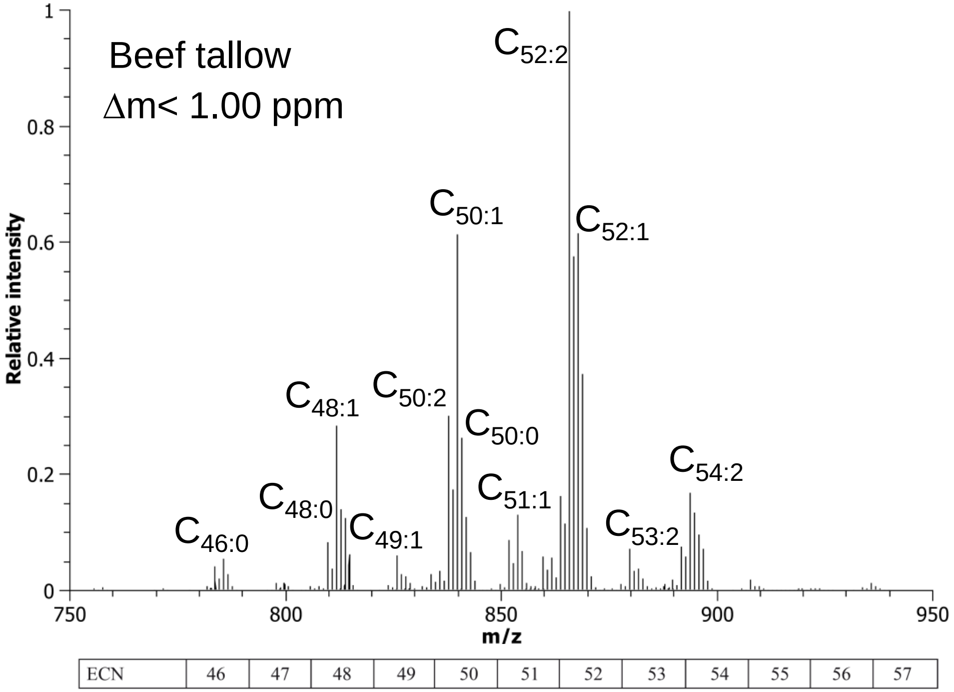


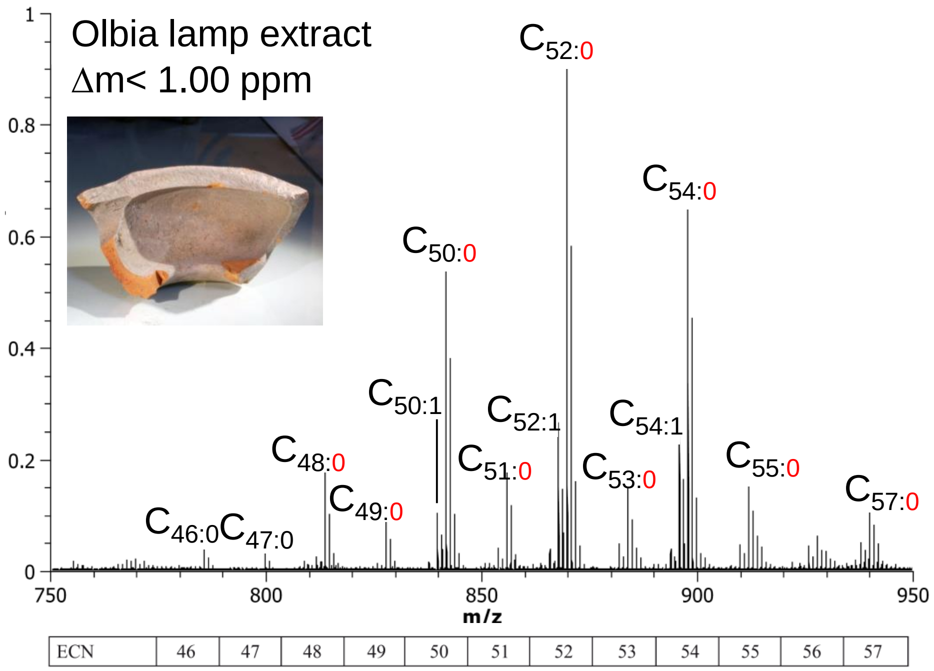
- Dairy products: rich in palmitic, oleic, stearic capric acids => large distribution from ECN 24 to 54 + Presence of TAG with 1 odd-number fatty acid.
- Animal fats: ECN from 46 to 54. Bovine and ovine profiles present even-number TAG + minor odd-number TAGs.
- Odd-number fatty acids: markers of ruminant species, mainly synthesized by bacteria present in the rumen*

* Vlaeminck B., Fievez V., Cabrita A.R.J., Fonseca A.J.M., Dewhurst R.J., An. Feed Sci. Technol. 2006, 131, 389–417



Study of TAGs remains from 5th century BC– 4th century AD archaeological lamps (Olbia, Ukraina)





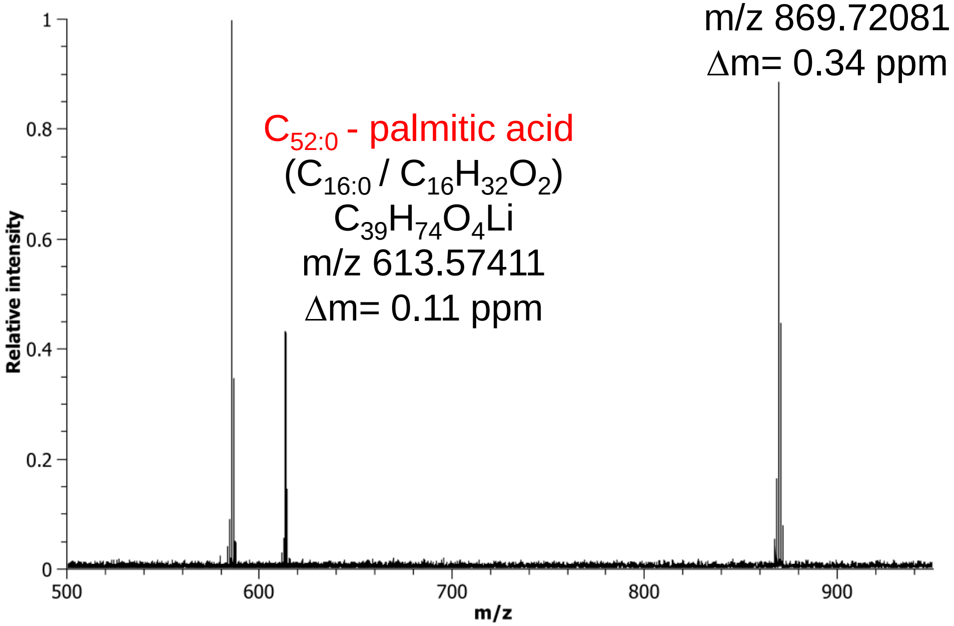
- Dichloromethane-methanol extraction + ultrasonic baths
- TAGs purified on diol cartridge (elution: cyclohexane dichloromethane, diethyl ether; 89/10/1)
- Strong dominance of saturated TAGs, a weak proportion of monounsaturated TAGs and few polyunsaturated TAGs (due to oxidation) in 6 analyzed lamps (9 lamps analyzed)
- Characteristic odd-C-numbered TAGs (not found in non-herbivore species) identifying ovine/bovine species

Garnier N., Rolando N., Høtje J.M., Tokarski C.
Int. J. Mass Spectrom. 2009, 284, 47–56

Study of TAGs remains from 5th century BC– 4th century AD archaeological lamps (Olbia, Ukraina)

$C_{52:0}$ - stearic acid
($C_{16:0}$ / $C_{18}H_{36}O_2$)
$C_{39}H_{74}O_4Li$
m/z 585.54391
Δm= 0.85 ppm

$C_{52:0}$
$C_{56}H_{94}O_6Li$
m/z 869.72081
Δm= 0.34 ppm



$C_{52:0}$ - palmitic acid
($C_{16:0}$ / $C_{16}H_{32}O_2$)
$C_{39}H_{74}O_4Li$
m/z 613.57411
Δm= 0.11 ppm

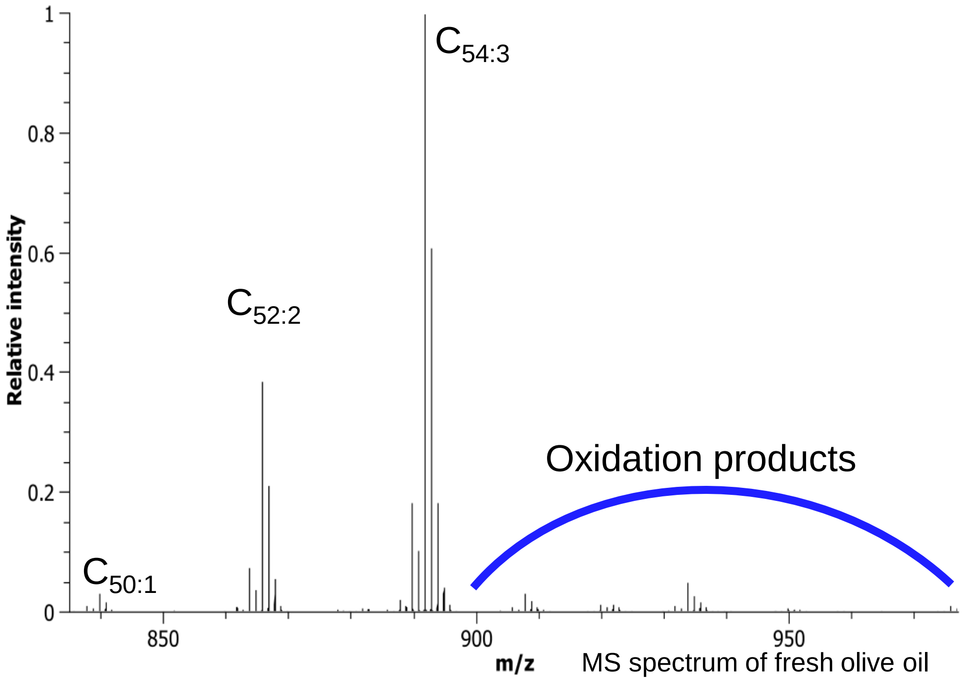
MS/MS spectrum of $C_{52:0}$ from Olbia lamp extract

- MS/MS profiles allow confirming each TAGs composition
- Here the $C_{52:0}$ TAG contains 2 stearic acid and 1 palmitic acid
- Precursor ion selection for MS/MS acquisition was made with higher Δm than Δm used for model oil analysis to increase the signal to noise
  (Δm 2 ppm for standard oils versus 5 ppm for ancient samples)

Garnier N., Rolando N., Høtje J.M., Tokarski C. Int. J. Mass Spectrom. 2009, 284, 47–56

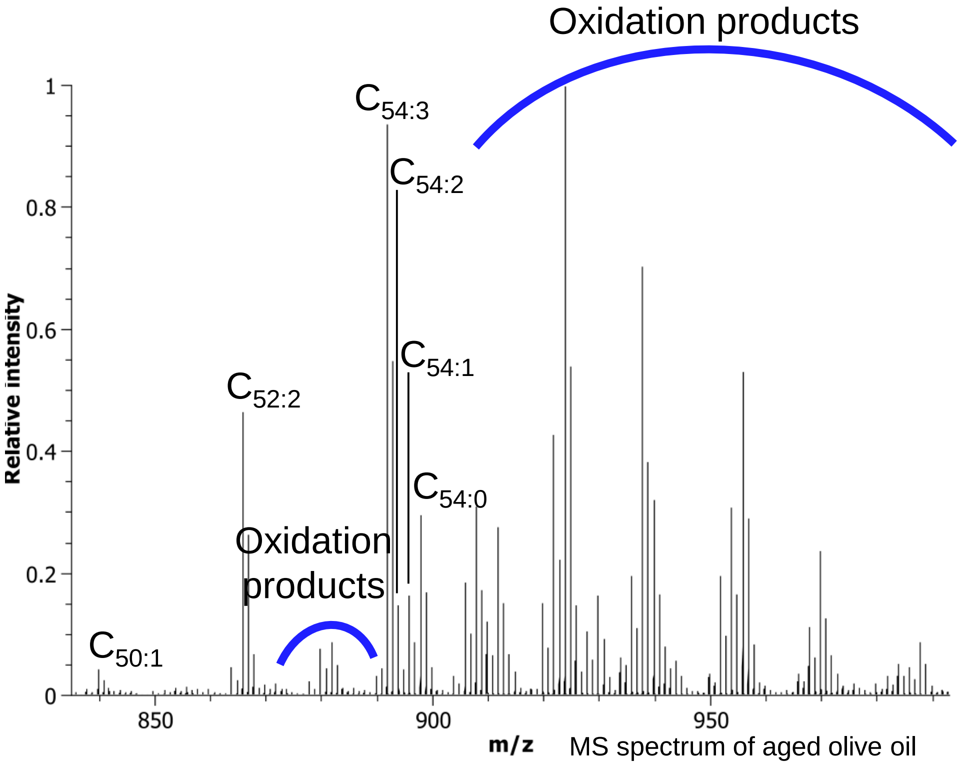
Analysis of fresh versus naturally aged olive oils

‘Fresh’ olive oil



MS spectrum of fresh olive oil

Olive oil submitted to natural ageing during 10 years

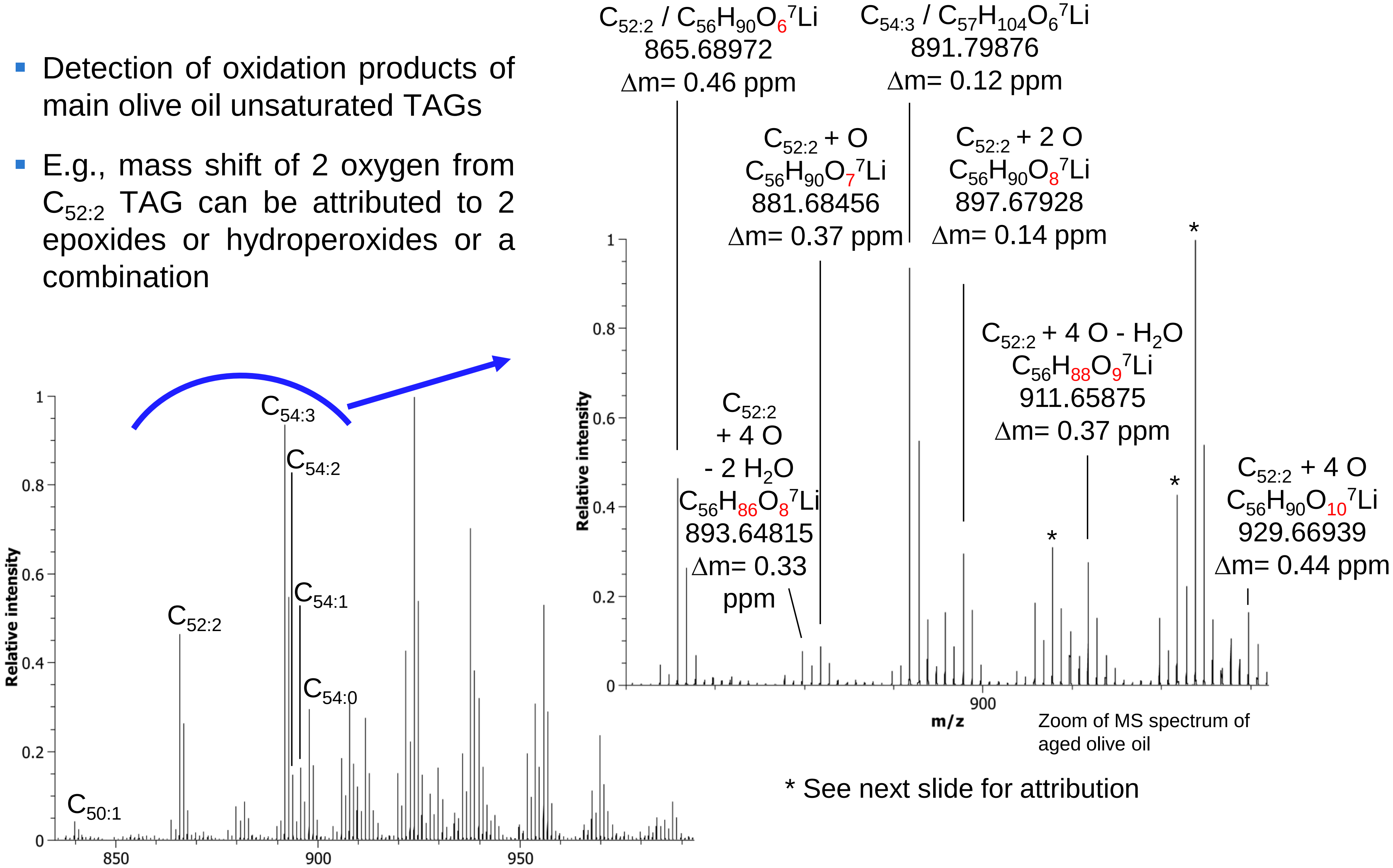


MS spectrum of aged olive oil

- Low intensity peaks related to oxidation in ‘fresh’ commercial olive oil
- Strong dominance of oxidized TAGs in 10 years-naturally aged olive oil

Analysis of naturally aged olive oil (10 years)

- Detection of oxidation products of main olive oil unsaturated TAGs
- E.g., mass shift of 2 oxygen from $C_{52:2}$ TAG can be attributed to 2 epoxides or hydroperoxides or a combination



MS spectrum of aged olive oil

Zoom of MS spectrum of aged olive oil

* See next slide for attribution

Analysis of naturally aged olive oil (10 years)

- Strong dominance of oxidized TAGs in 10 years-naturally aged olive oil
- Up to 6 oxidation products detected (potentially, hydroperoxides, epoxides, epidioxides)

$C_{54:3}$ + O, $C_{57}H_{104}O_{7}{}^{7}Li$ 907.79391, Δm= 0.13 ppm

$C_{54:3}$ + 3 O, $C_{57}H_{104}O_{9}{}^{7}Li$ 939.78411, Δm= 0.53 ppm

$C_{54:3}$ + 2 O -H_2O $C_{57}H_{102}O_{7}{}^{7}Li$ 905.77864 Δm= 0.56 ppm

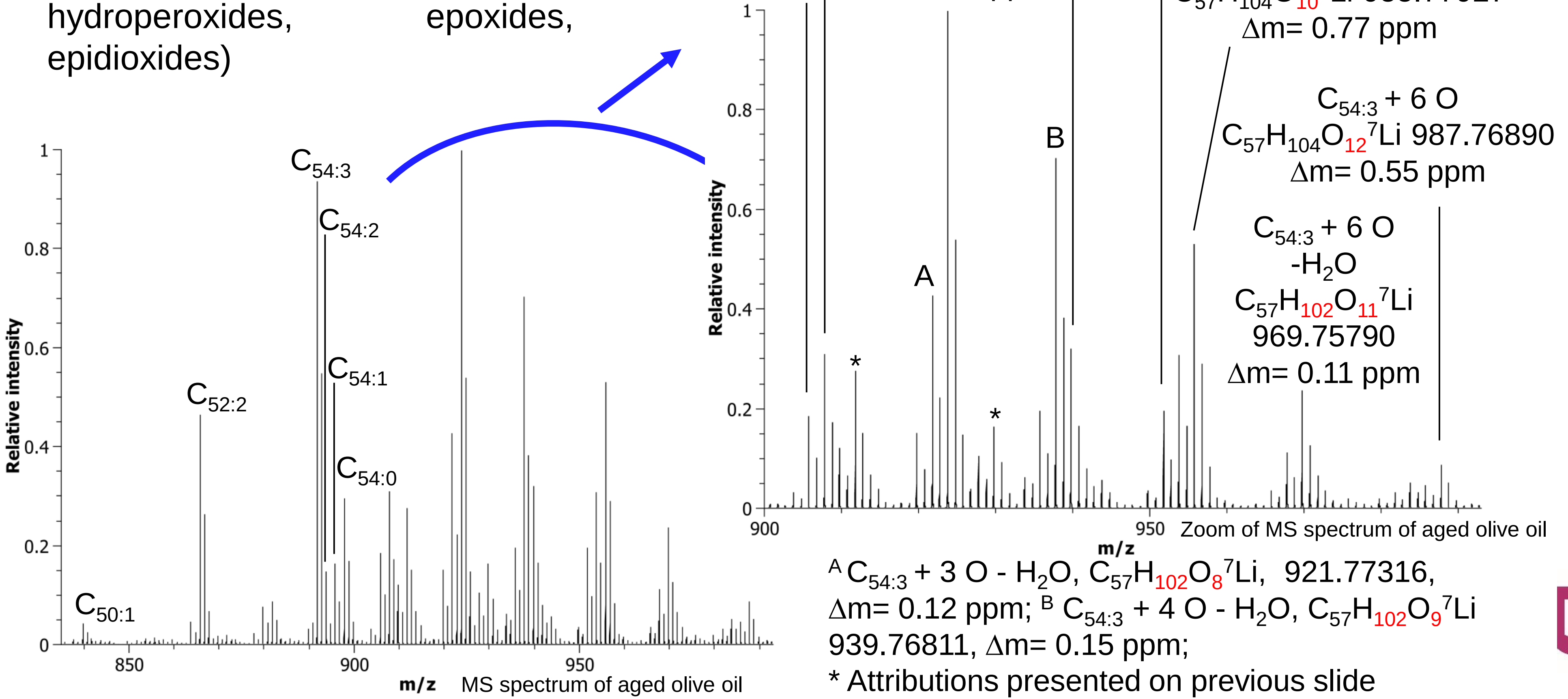
$C_{54:3}$ + 2 O $C_{57}H_{104}O_{8}{}^{7}Li$ 923.78919 Δm= 0.53 ppm

$C_{54:3}$ + 6 O - 2 H_2O $C_{57}H_{100}O_{10}{}^{7}Li$ 951.74783, Δm= 0.63 ppm

$C_{54:3}$ + 4 O $C_{57}H_{104}O_{10}{}^{7}Li$ 955.77927 Δm= 0.77 ppm

$C_{54:3}$ + 6 O $C_{57}H_{104}O_{12}{}^{7}Li$ 987.76890 Δm= 0.55 ppm

$C_{54:3}$ + 6 O -H_2O $C_{57}H_{102}O_{11}{}^{7}Li$ 969.75790 Δm= 0.11 ppm



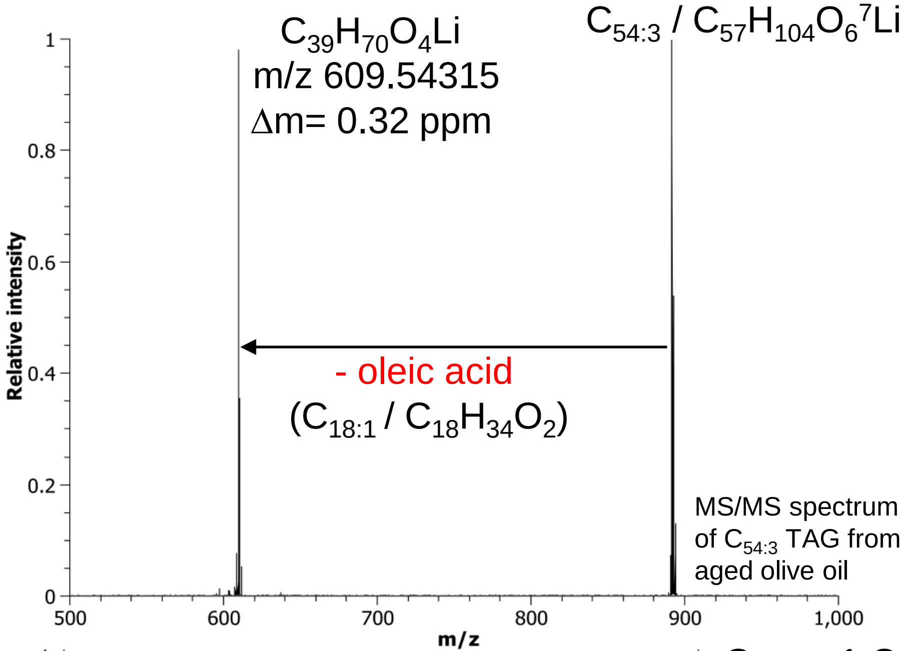
MS spectrum of aged olive oil

Zoom of MS spectrum of aged olive oil

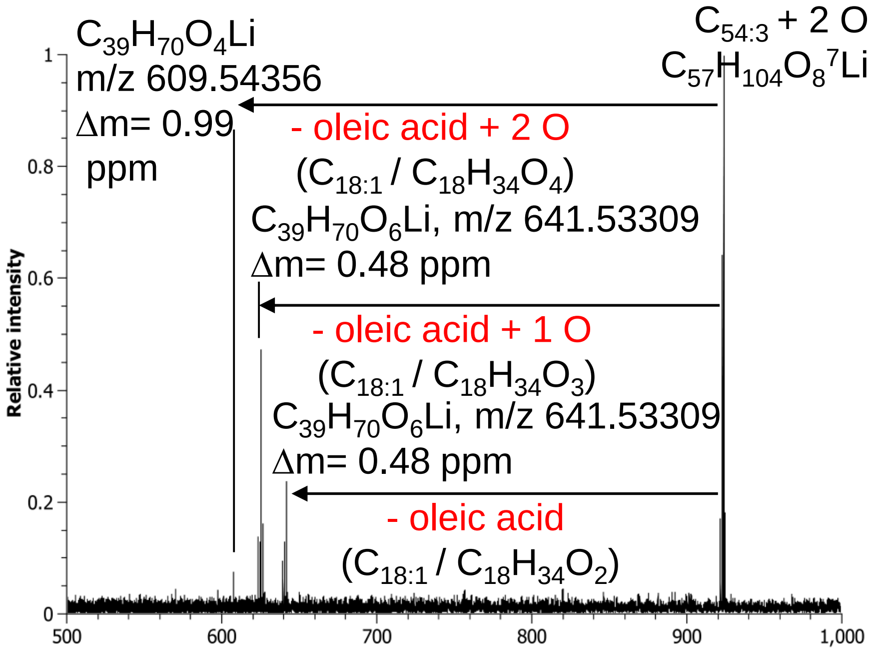
[A] $C_{54:3}$ + 3 O - H_2O, $C_{57}H_{102}O_{8}{}^{7}Li$, 921.77316, Δm= 0.12 ppm; [B] $C_{54:3}$ + 4 O - H_2O, $C_{57}H_{102}O_{9}{}^{7}Li$ 939.76811, Δm= 0.15 ppm;

* Attributions presented on previous slide

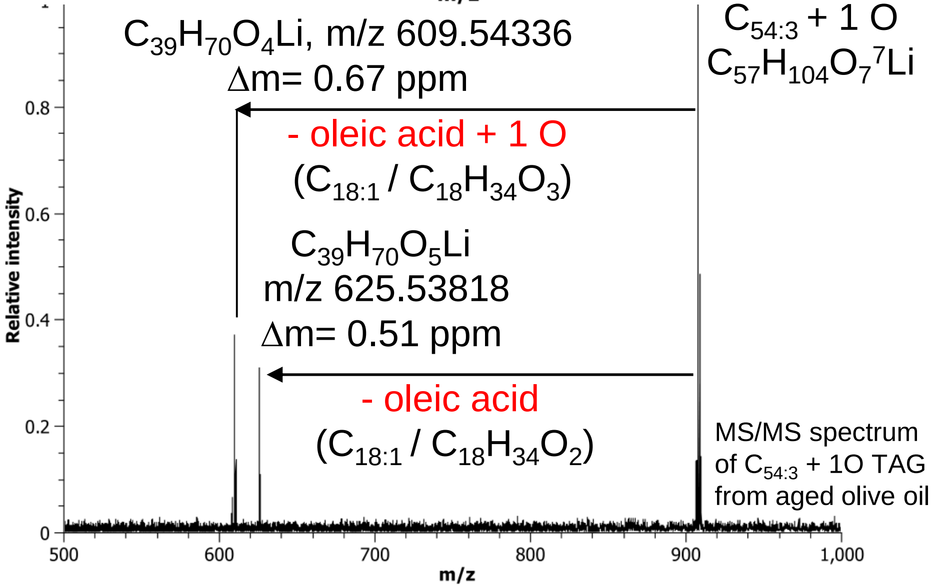
MS/MS (IRMPD) spectra of oxidized TAGs



MS/MS spectrum of $C_{54:3}$ TAG from aged olive oil



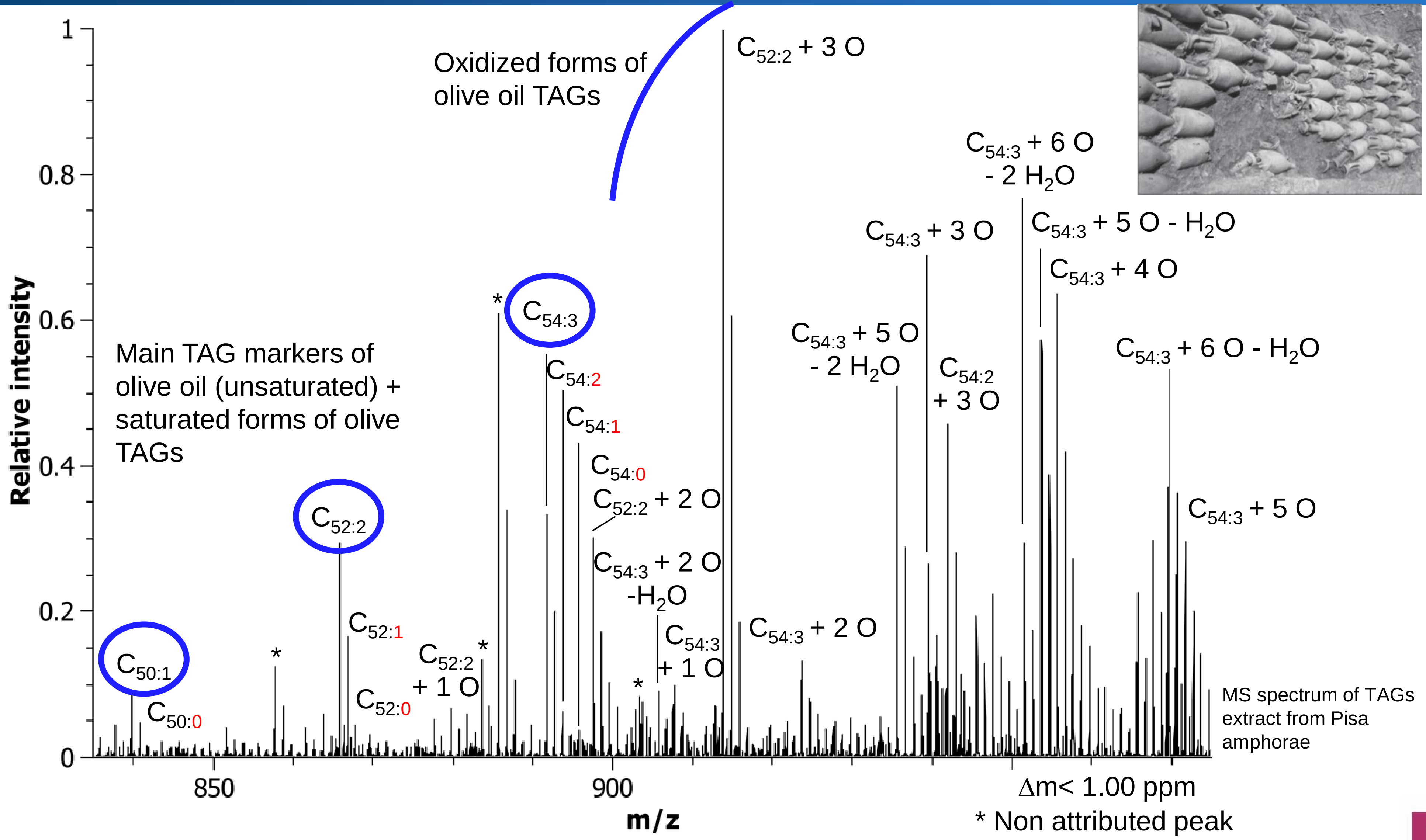
MS/MS spectrum of $C_{54:3}$ + 2O TAG from aged olive oil



MS/MS spectrum of $C_{54:3}$ + 1O TAG from aged olive oil

- MS/MS spectra shows fatty acid moieties and their oxidized forms
- E.g. MS/MS spectrum of $C_{54:3}$ + 2 O shows the loss of oleic acid, monooxidized oleic acid and dioxidized oleic acid

Analysis of Roman amphorae from Pisa San Rossore harbor (Ist century BC)



Oxidized forms of olive oil TAGs

Main TAG markers of olive oil (unsaturated) + saturated forms of olive TAGs

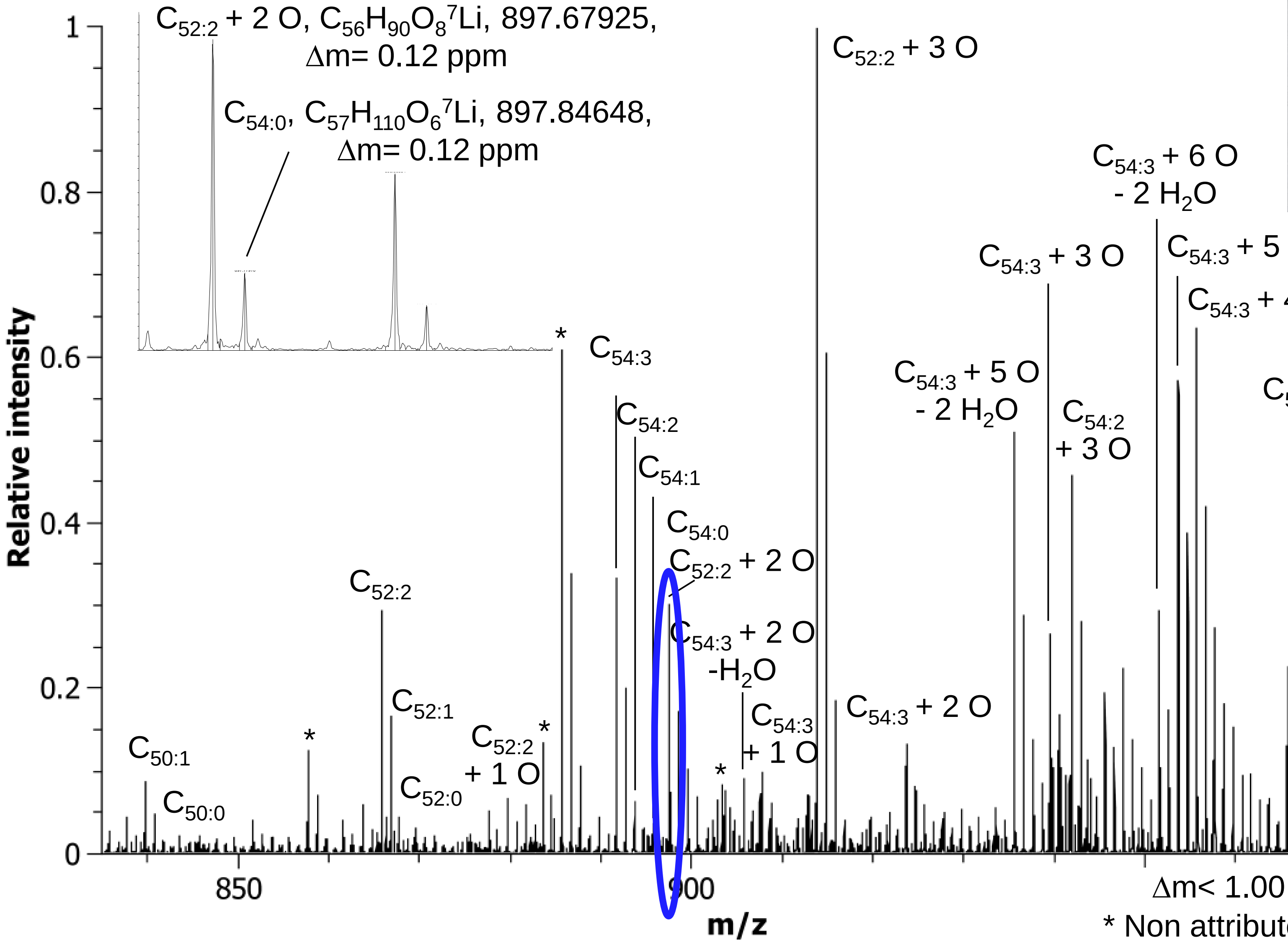
MS spectrum of TAGs extract from Pisa amphorae

Δm< 1.00 ppm

* Non attributed peak

- In Pisa amphorae extract, main detection of olive oil TAGs (saturated and unsaturated forms) + their oxidized forms

Analysis of Roman amphorae from Pisa San Rossore harbor (Ist century BC)



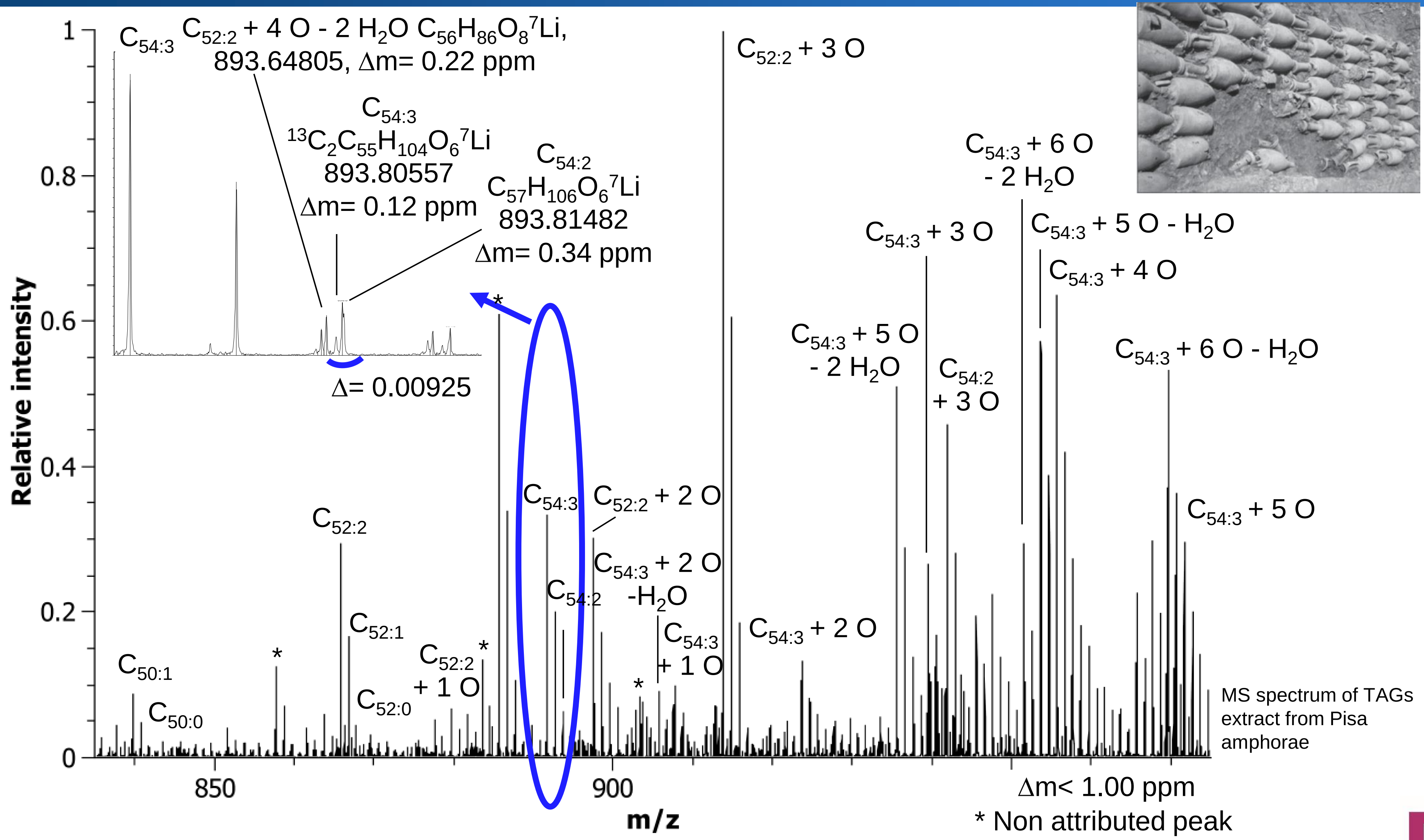
Δm< 1.00 ppm

* Non attributed peak

MS spectrum of TAGs extract from Pisa amphorae

- Despite spectrum complexity, separation of $C_{54:0}$ TAGs and $C_{52:2}$ + 2 O achieved

Analysis of Roman amphorae from Pisa San Rossore harbor (Ist century BC)



MS spectrum of TAGs extract from Pisa amphorae

Δm< 1.00 ppm

* Non attributed peak

- High resolution allows separating $C_{54:2}$ TAGs, second isotopic peak of $C_{54:3}$ TAGs and oxidized forms of $C_{52:2}$ TAGs

Conclusion

Methodological developments based on MS and MS/MS profiles (IRMPD) allowed identification of archaeological triacylglycerols and their origins (from few micrograms of crushed sample)

- Study of TAGs remains from 5th century BC–4th century AD archaeological lamps (Olbia, Ukraina) showed characteristic odd-C-numbered TAGs (not found in non-herbivore species) identifying ovine/bovine species + strong dominance of saturated TAGs
- Analysis of Ist century BC Roman amphorae from Pisa San Rossore harbor (Italy) allowed identification of olive oil using marker TAGs and their oxidized forms (potentially hydroperoxides, epoxides, epidioxides)

Actually, studies on lipid (and protein) residues from amphorae preserved in marine context during 2 millenaries

Collaboration with the *Département des Recherches Archéologiques Subaquatiques et Sous-Marines* (DRASSM, Marseille, France; Dr Franca Cibecchini and Dr Michel L'Hour)

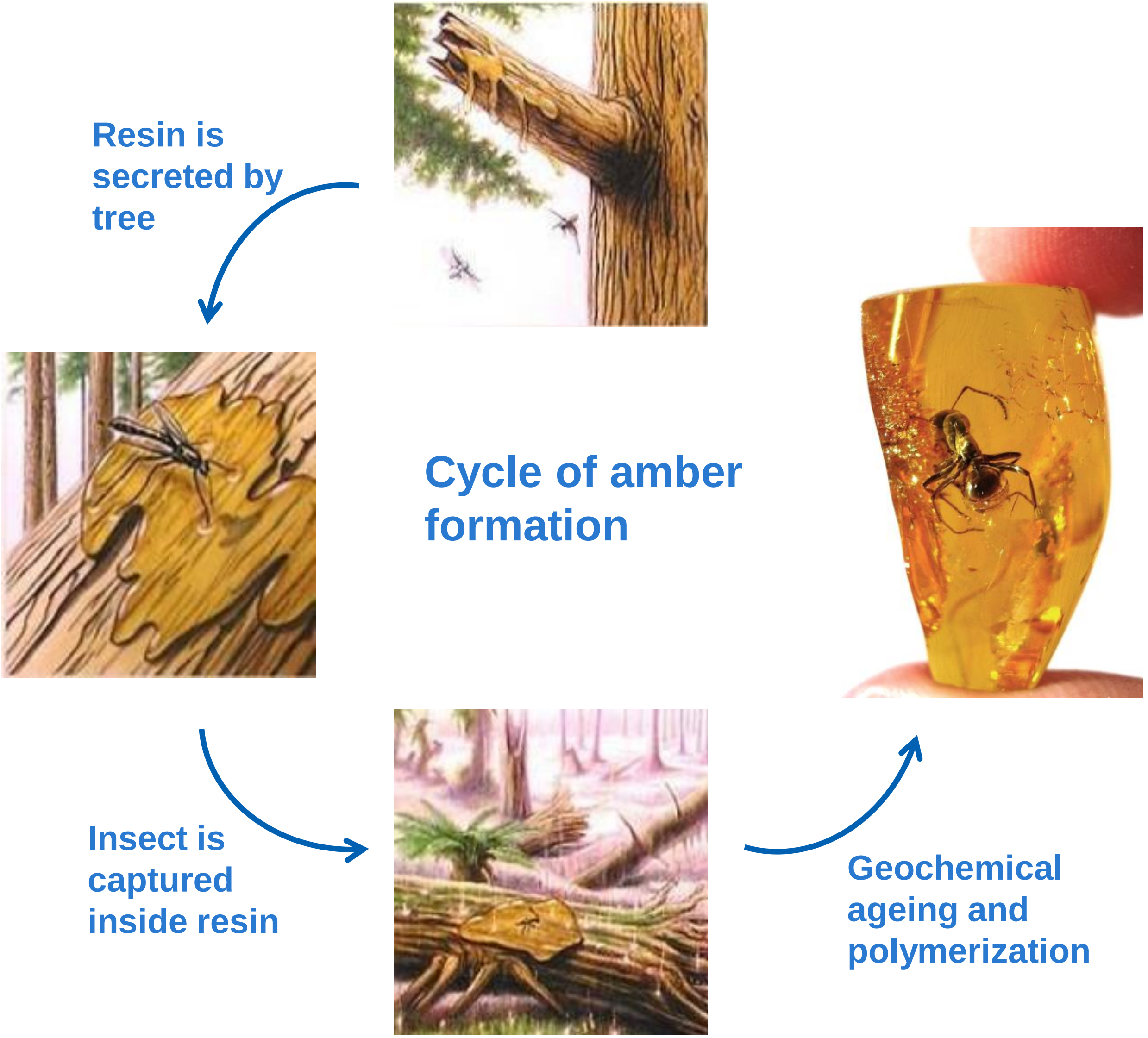
Spanish amphoras of Sud Perduto 2, 1st century AD

Deciphering the structure of natural amber by chemical depolymerization and analysis by ultrahigh resolution FT-ICR mass spectrometry

Ziad MAHMOUD

Miniaturization for Synthesis, Analysis & Proteomics USR 3290

Amber



Resin is secreted by tree

Cycle of amber formation

Insect is captured inside resin

Geochemical ageing and polymerization

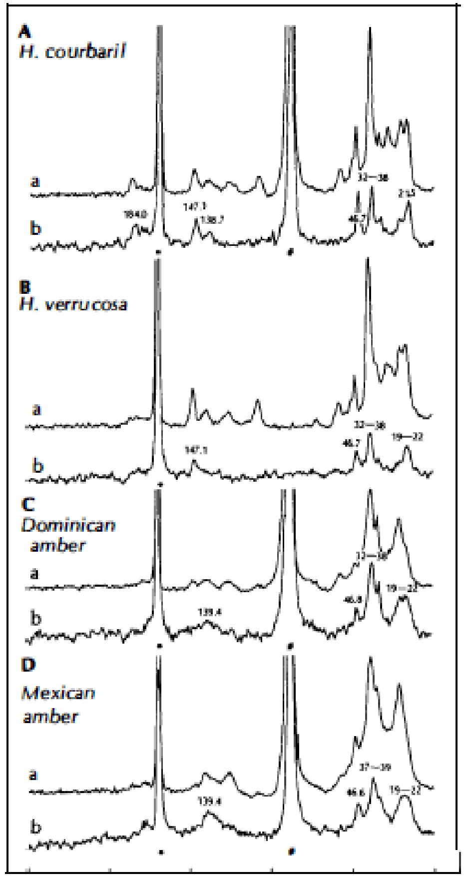
- Complex polymer
- Very insoluble
- Difficult to analyze
- Oldest amber: 320 million years ago
- Can contain fossilized animals or plants

Chemical composition of amber

^{13}C NMR AND IR ANALYSES OF STRUCTURE, AGING AND BOTANICAL ORIGIN OF DOMINICAN AND MEXICAN AMBERS

ALAN CUNNINGHAM, IAN D. GAY*, A. C. OEHLSCHLAGER* and JEAN H. LANGENHEIM

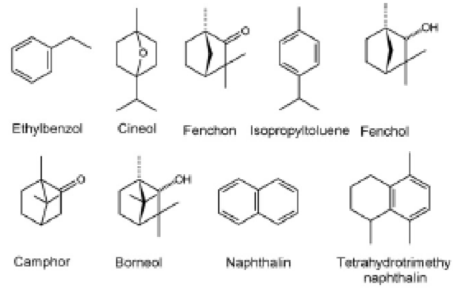
Division of Natural Sciences, University of California, Santa Cruz, CA 95064, U.S.A.; *Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, Canada, V5A 1S6

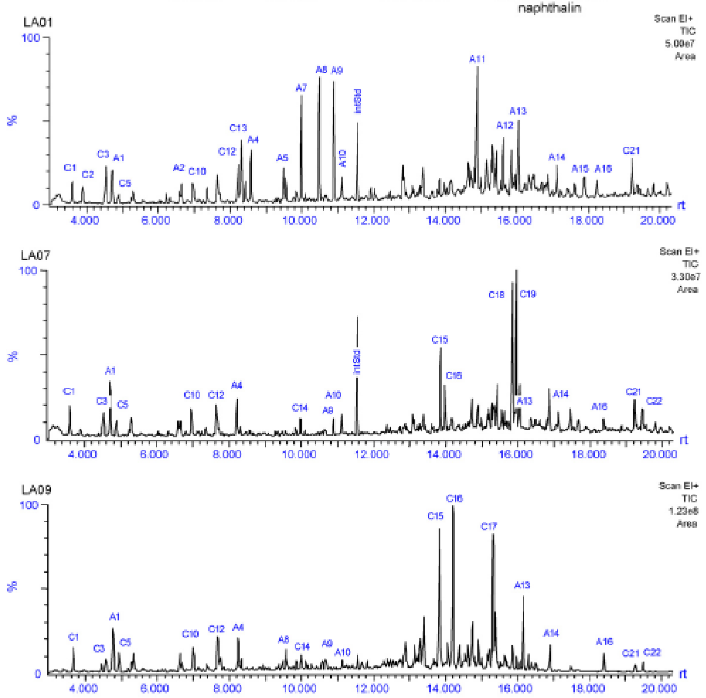


Thermal investigations of amber and copal

Michael Feist[a], Ingolf Lamprecht[b,*], Frank Müller[c]

[a] Institute of Chemistry, Humboldt University to Berlin, Brook-Taylor-Straße 2, D-12489 Berlin, Germany
[b] Institute of Biology, Free University of Berlin, Grunewaldstraße 34, D-12165 Berlin, Germany
[c] Institute of Biology, Applied Zoology, Free University of Berlin, Haderslebener Straße 9, D-12163 Berlin, Germany

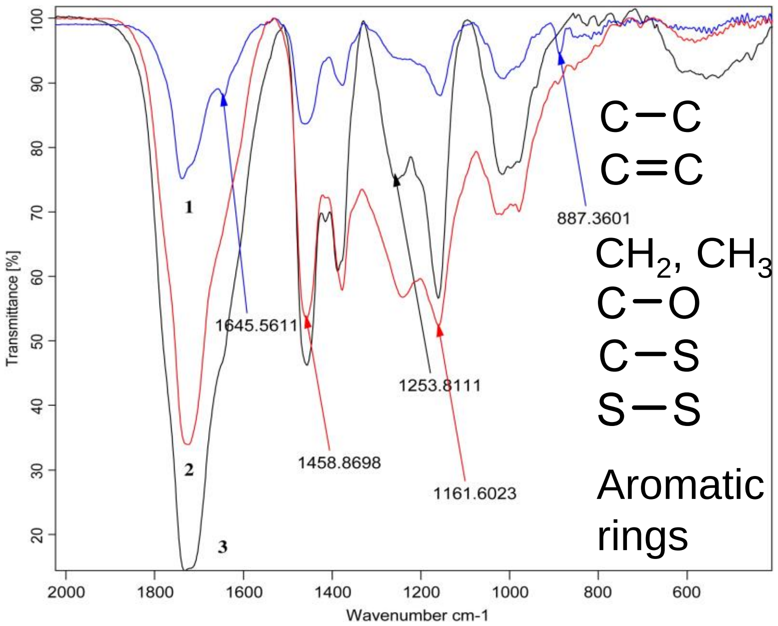




Non-destructive analysis of amber artefacts from the prehistoric Cioclovina hoard (Romania)

E.S. Teodor[a,1], E.D. Teodor[b,*], M. Virgolici[c,2], M.M. Manea[c], G. Truică[b], S.C. Lițescu[b]

[a] Romanian National History Museum, 12 Calea Victoriei, Bucharest, 030026, Romania
[b] National Institute for Biological Sciences, Centre of Bioanalysis, 296 Spl. Independentei, Bucharest, 060031, Romania
[c] Multipurpose Irradiation Facility – IRASM Department, Horia Hulubei National Institute of Physics and Nuclear Engineering – IFIN-HH, Măgurele, 407 Str. Atomistilor 077125, Romania



C–C
C=C

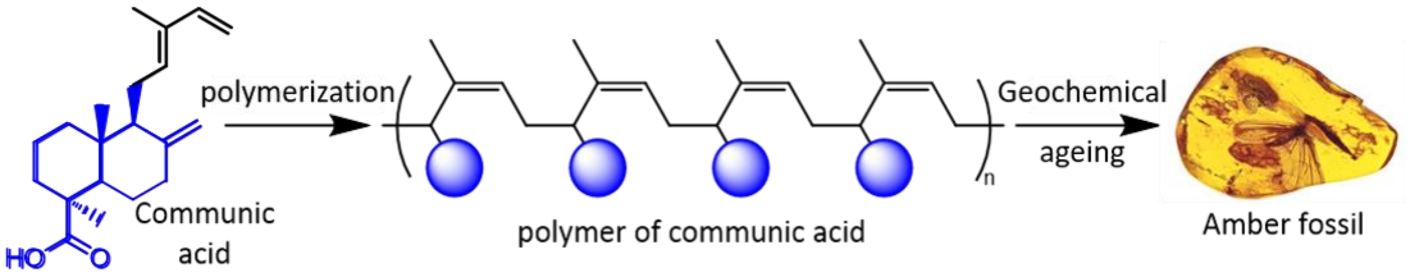
CH_2, CH_3
C–O
C–S
S–S

Aromatic rings

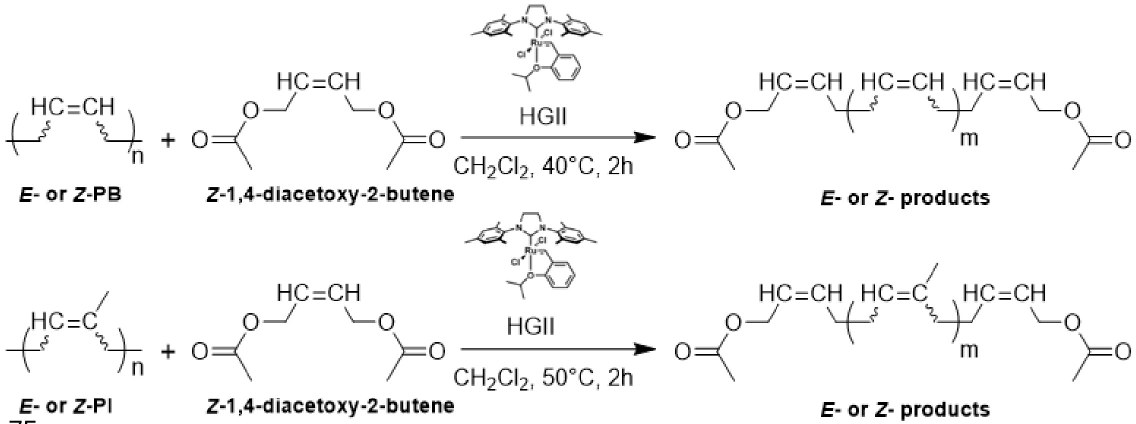
How to solubilize amber?
How to identify cross-linking products?

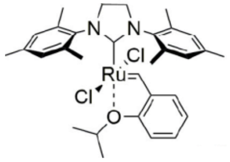
Chemical composition of amber

- Amber can be classified into classes from I to V
- Based on terpenoids formed of ring structures with isoprene (C_5H_8) units
- The formation of amber is the result of polymerization of one of its major compounds: communic acid

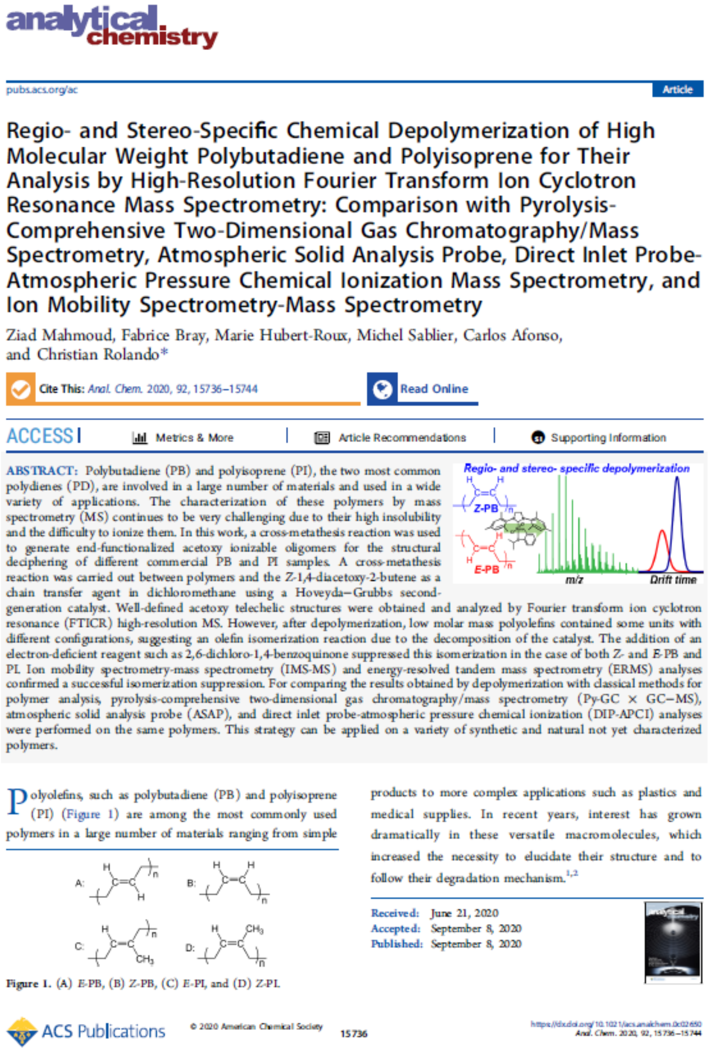


Cross-metathesis depolymerization of polybutadiene and polyisoprene





Hoveyda-Grubbs second generation catalyst



analytical chemistry

pubs.acs.org/ac Article

Regio- and Stereo-Specific Chemical Depolymerization of High Molecular Weight Polybutadiene and Polyisoprene for Their Analysis by High-Resolution Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: Comparison with Pyrolysis-Comprehensive Two-Dimensional Gas Chromatography/Mass Spectrometry, Atmospheric Solid Analysis Probe, Direct Inlet Probe-Atmospheric Pressure Chemical Ionization Mass Spectrometry, and Ion Mobility Spectrometry-Mass Spectrometry

Ziad Mahmoud, Fabrice Bray, Marie Hubert-Roux, Michel Sablier, Carlos Afonso, and Christian Rolando*

Cite This: *Anal. Chem.* 2020, 92, 15736−15744 Read Online

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ABSTRACT: Polybutadiene (PB) and polyisoprene (PI), the two most common polydienes (PD), are involved in a large number of materials and used in a wide variety of applications. The characterization of these polymers by mass spectrometry (MS) continues to be very challenging due to their high insolubility and the difficulty to ionize them. In this work, a cross-metathesis reaction was used to generate end-functionalized acetoxy ionizable oligomers for the structural deciphering of different commercial PB and PI samples. A cross-metathesis reaction was carried out between polymers and the *Z*-1,4-diacetoxy-2-butene as a chain transfer agent in dichloromethane using a Hoveyda−Grubbs second-generation catalyst. Well-defined acetoxy telechelic structures were obtained and analyzed by Fourier transform ion cyclotron resonance (FTICR) high-resolution MS. However, after depolymerization, low molar mass polyolefins contained some units with different configurations, suggesting an olefin isomerization reaction due to the decomposition of the catalyst. The addition of an electron-deficient reagent such as 2,6-dichloro-1,4-benzoquinone suppressed this isomerization in the case of both *Z*- and *E*-PB and PI. Ion mobility spectrometry-mass spectrometry (IMS-MS) and energy-resolved tandem mass spectrometry (ERMS) analyses confirmed a successful isomerization suppression. For comparing the results obtained by depolymerization with classical methods for polymer analysis, pyrolysis-comprehensive two-dimensional gas chromatography/mass spectrometry (Py-GC × GC−MS), atmospheric solid analysis probe (ASAP), and direct inlet probe-atmospheric pressure chemical ionization (DIP-APCI) analyses were performed on the same polymers. This strategy can be applied on a variety of synthetic and natural not yet characterized polymers.

Polyolefins, such as polybutadiene (PB) and polyisoprene (PI) (Figure 1) are among the most commonly used polymers in a large number of materials ranging from simple products to more complex applications such as plastics and medical supplies. In recent years, interest has grown dramatically in these versatile macromolecules, which increased the necessity to elucidate their structure and to follow their degradation mechanism.[1,2]

Figure 1. (A) *E*-PB, (B) *Z*-PB, (C) *E*-PI, and (D) *Z*-PI.

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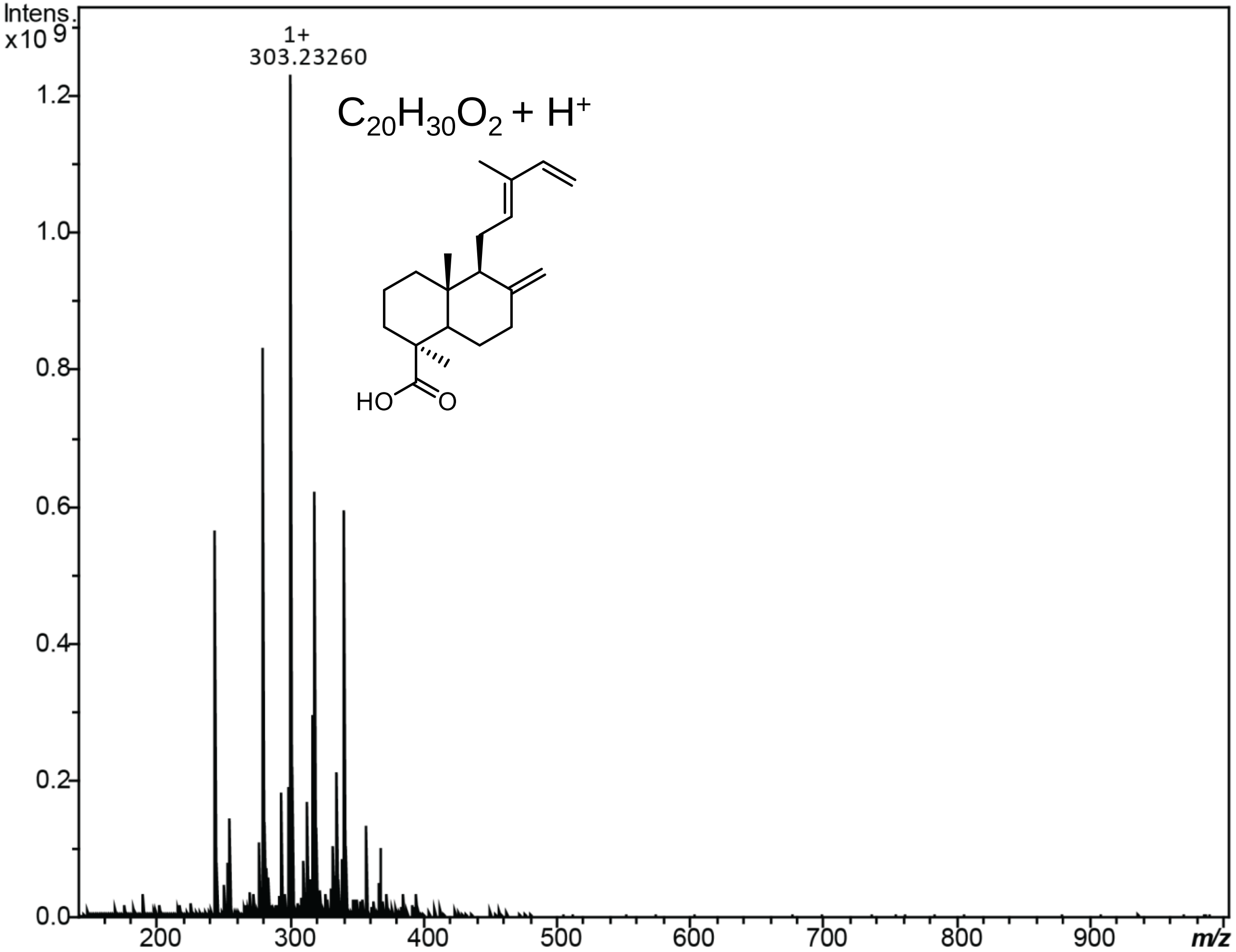
Anal. Chem. 2020, 92, 15736−15744

Extraction and analysis of communic acid

- Communic acid was extracted from **cypress cones**, **purified** **and** **polymerized under UV**

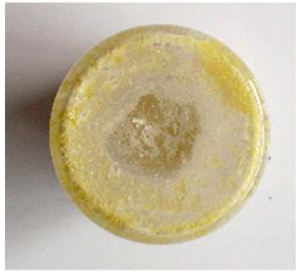
- ***Analysis on SolarixXR 9.4 Tesla FT-ICR from Bruker Daltonis equipped with a nanoESI ionizzation source***

FT-ICR ESI(+) MS of communic acid

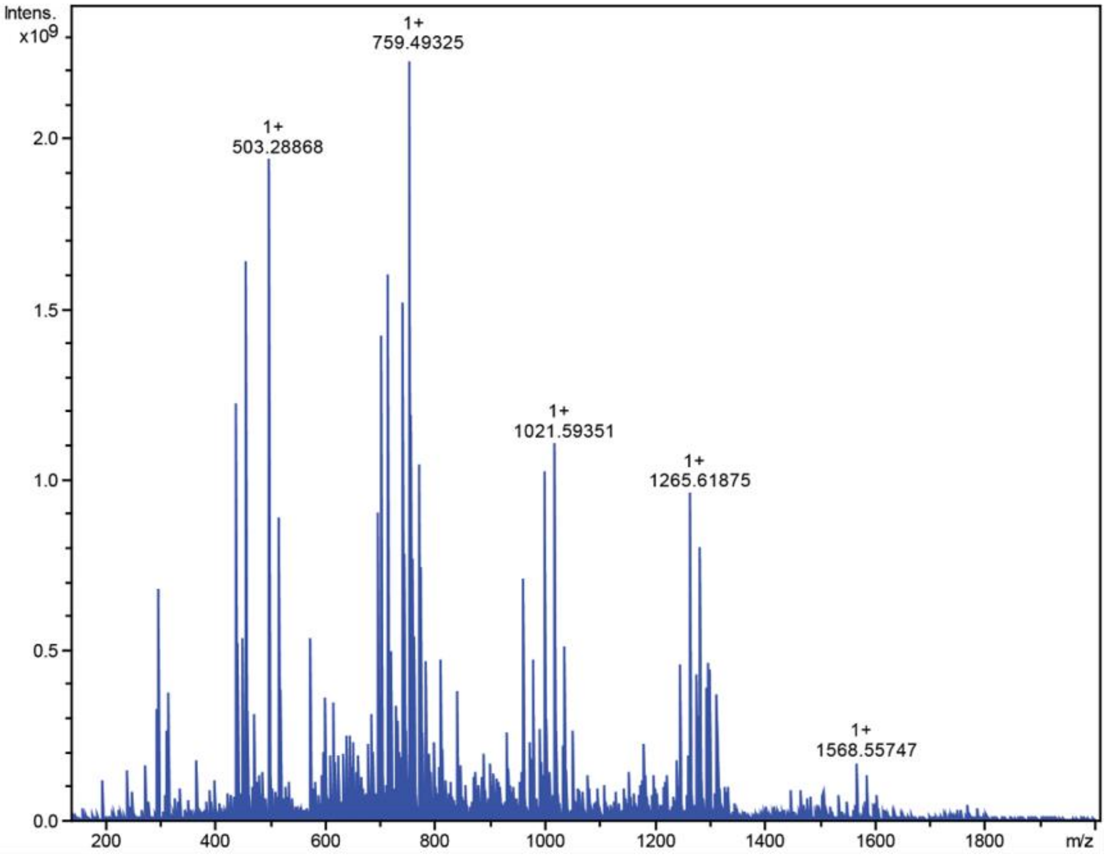


$C_{20}H_{30}O_2 + H^+$

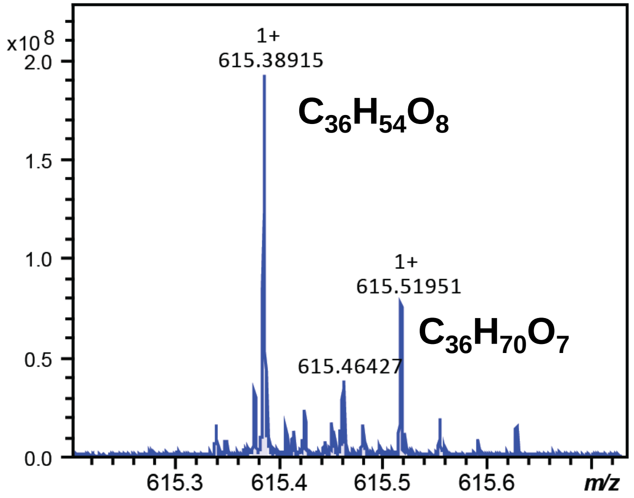
Extraction and analysis of communic acid

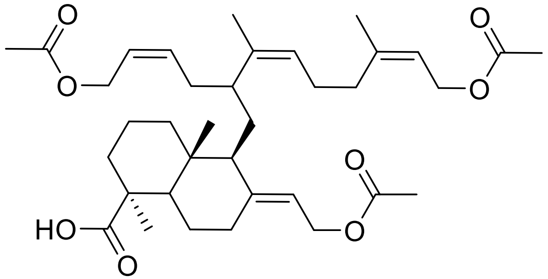


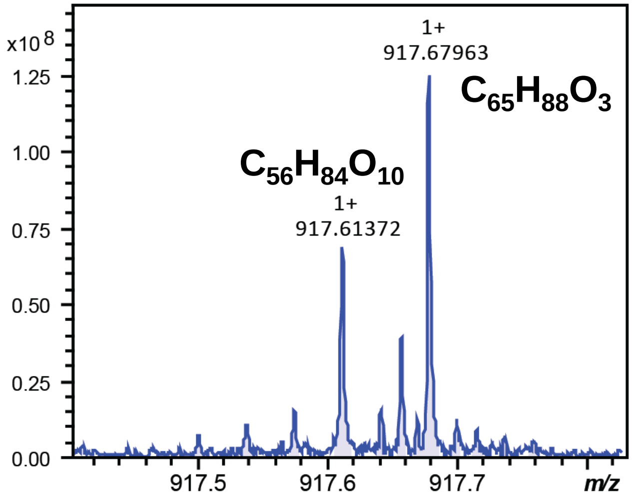
Communic acid polymerized under UV at 395 nm to mimic ageing and cross-linking in amber

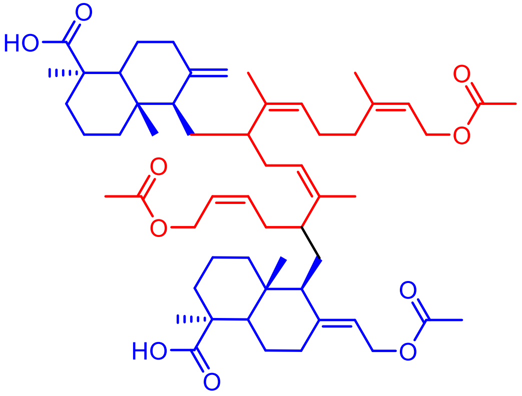


FT-ICR ESI(+) MS of depolymerized poly(communic acid)









Amber samples

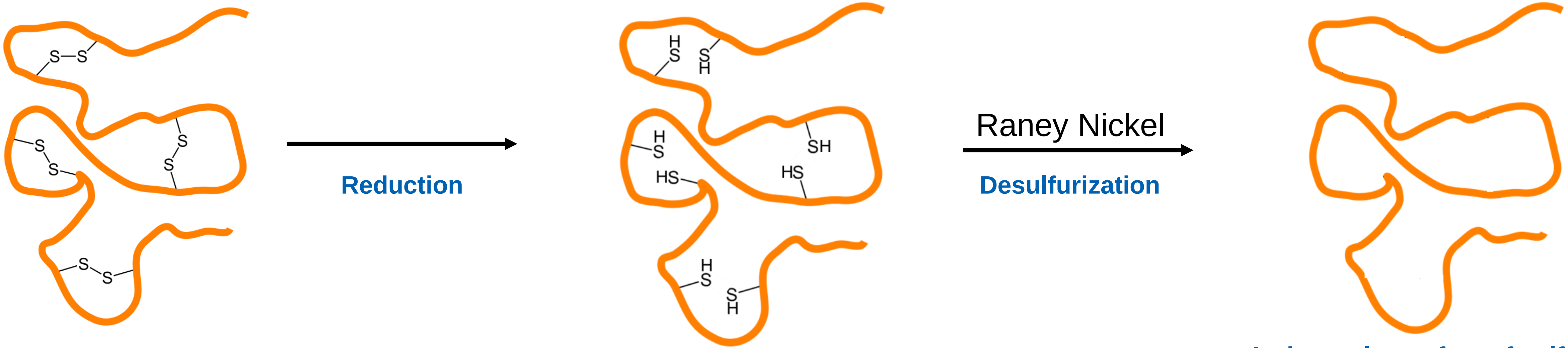
- Cross-metathesis with ruthenium catalyst was performed applied on the samples
- Disulfide bridges in act as poison to the catalyst and inhibit the depolymerization reaction

⬇

Development of alternative chemical pathway for the depolymerization and analysis of amber by high-resolution mass spectrometry

Samples provided by the National Museum of Natural History-Paris (MNHN)

Depolymerization and analysis of amber samples



Reduction

Raney Nickel

Desulfurization

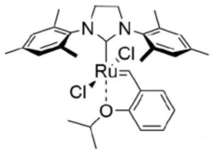
Amber polymer with disulfide bridges

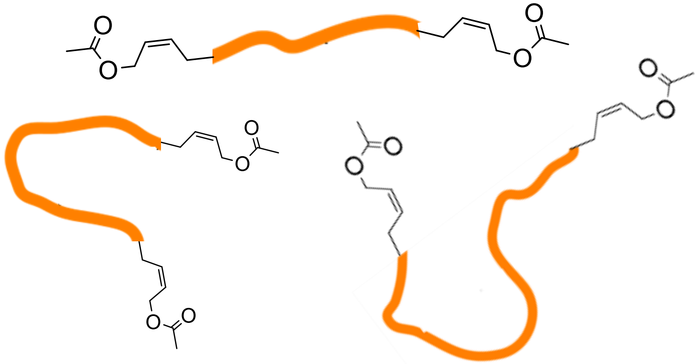
Cleavage of disulfide bridges

Amber polymer free of sulfur and containing isoprenic units

TMSD methylation

Cross-metathesis





Functionalized solub amber oligomers

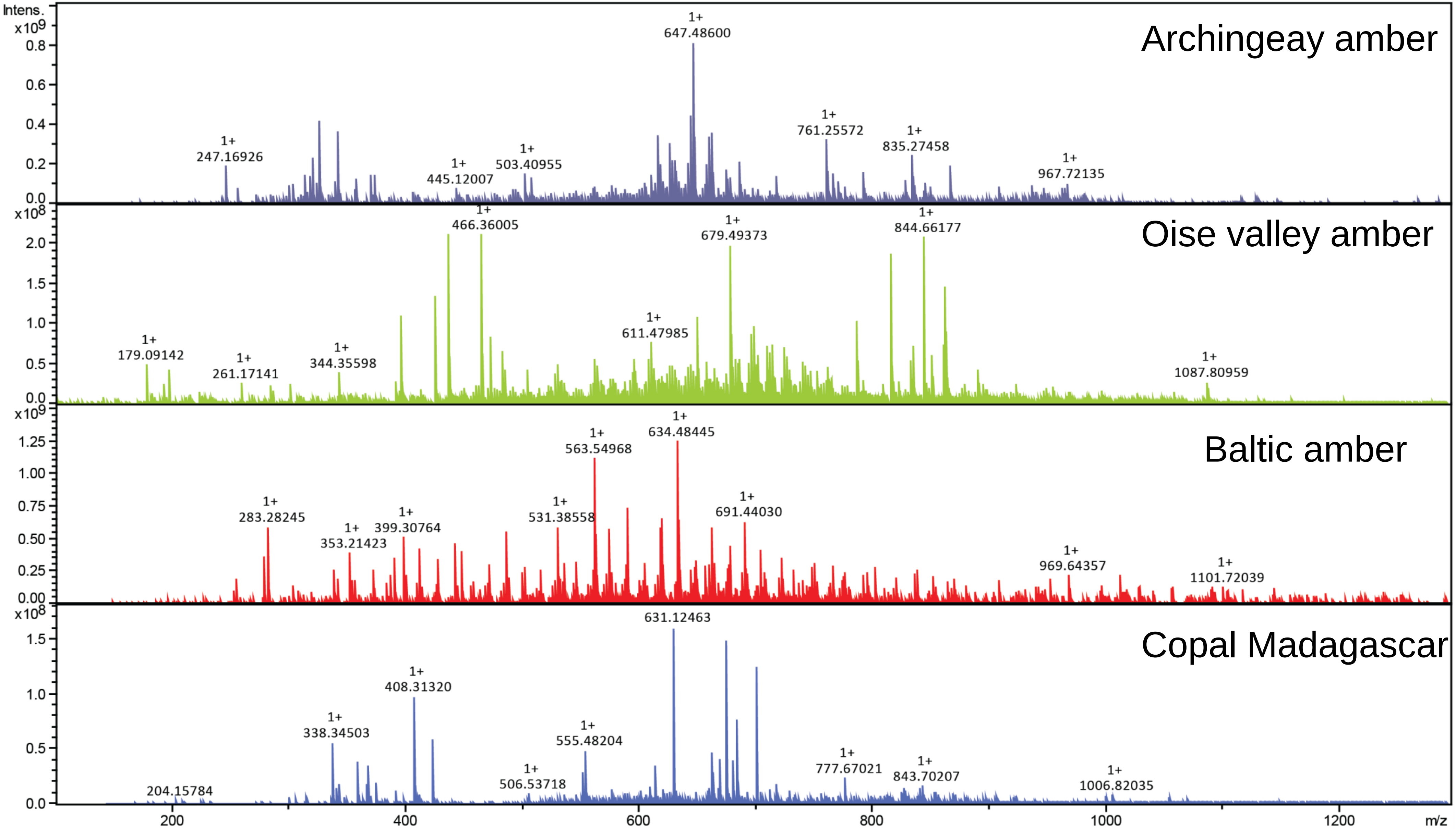
Analysis by FT-ICR high resolution mass spectrometry

EU FT-ICR MS

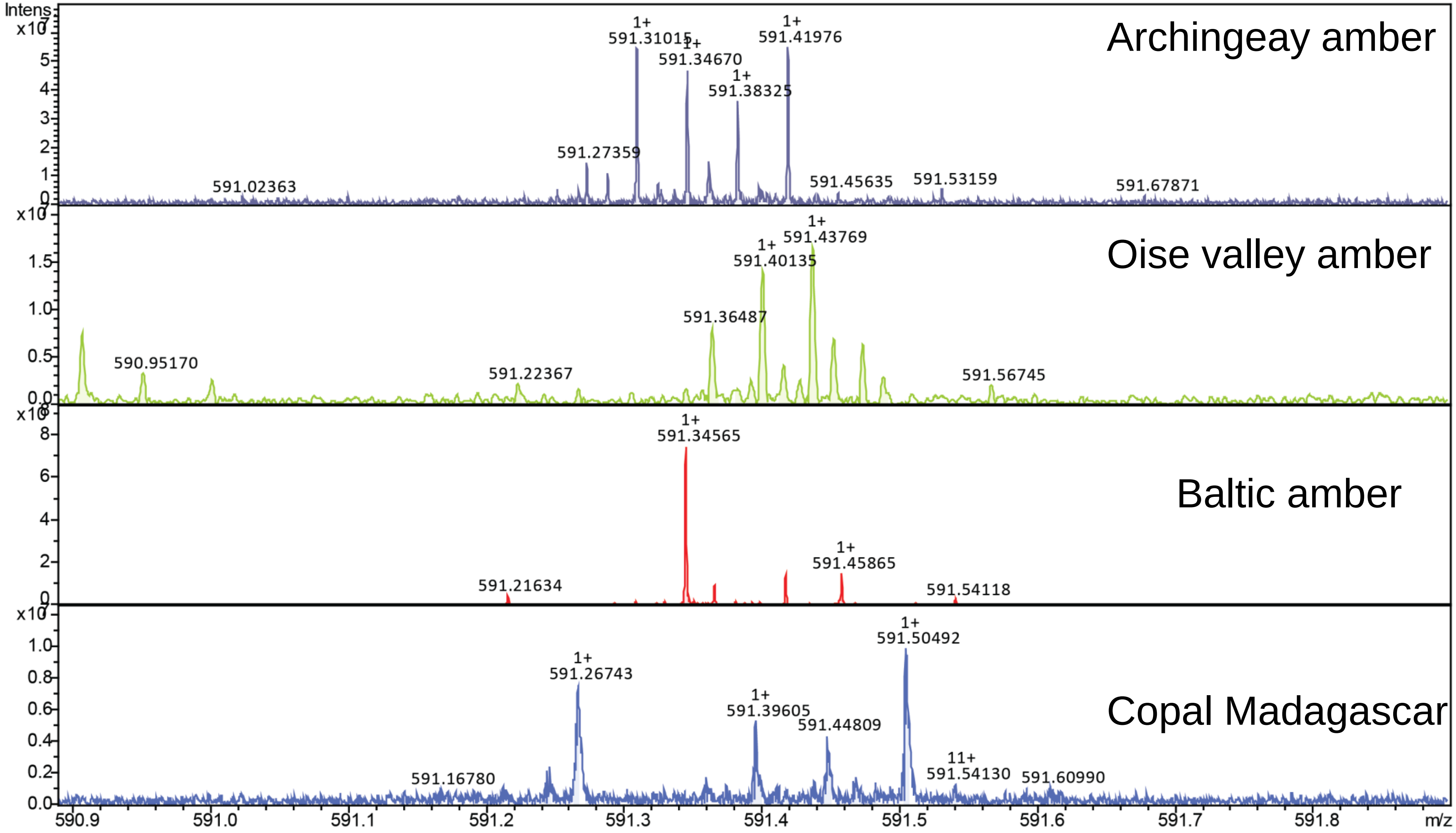
MSAP

Université de Lille

Analysis of amber by FT-ICR MS



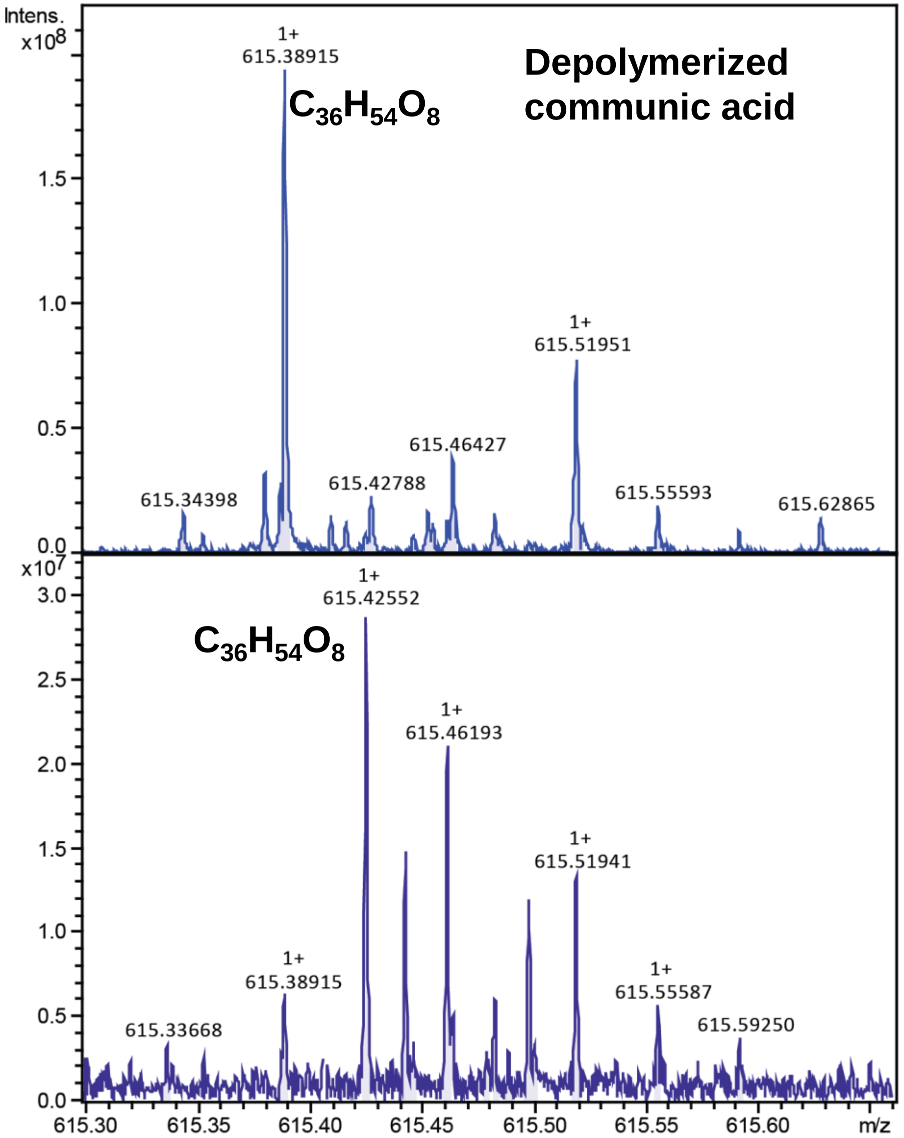
Analysis of amber samples by FT-ICR MS



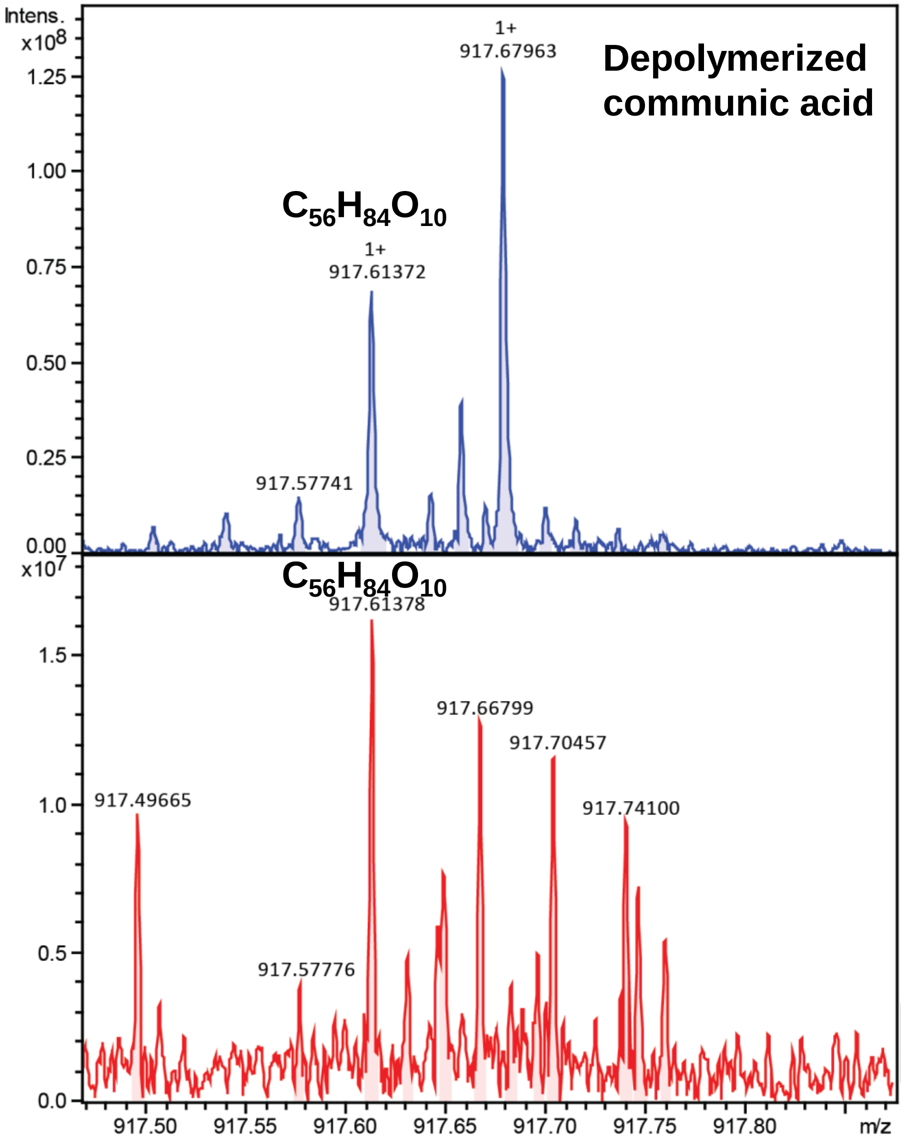
- Amber from different regions have specific chemical composition
- Possibility to determine the fingerprint of each sample

Analysis of amber by FT-ICR MS

Depolymerized Archingeay amber



Depolymerized Baltic amber



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Conclusions and perspectives

Conclusions:

- **We developed a method for the analysis of natural amber based on desulfurization and depolymerization by olefin cross-metathesis using ruthenium catalysts**
- **Mass spectra showed the complexity of the amber after ageing and polymerization**
- **Analysis by high-resolution mass spectrometry revealed the fingerprint of ambers from different regions**

Perspectives:

- **Full characterization of products obtained from cross-linking after ageing**
- **Classification of amber samples according to their chemical composition**
- **Apply the same methodology on amber containing insects or plants**

EU FT-ICR MS

Metabolomics of archaeological samples

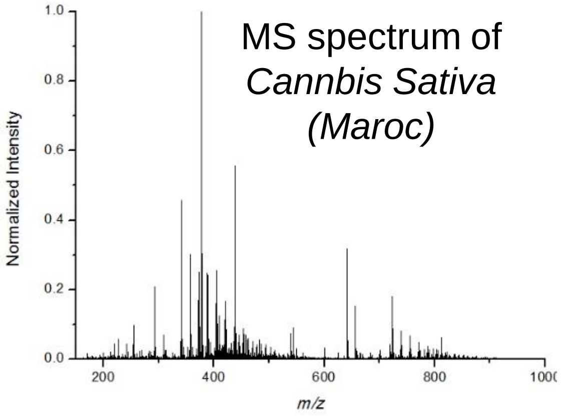
- Funeral amphora discovered in a Gallic site at Cébazat (Auvergne, France)
- This amphora has been used as a wine container
- Traces of cannabis were guessed from GC-MS data by Dr Nicolas Garnier

Proof of cannabis presence in the archaeological amphora by FT-ICR MS

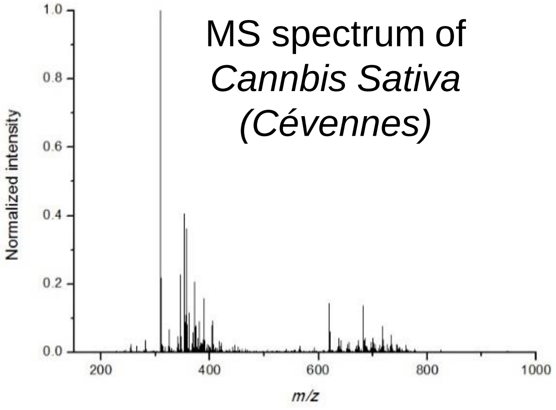
Extraction of compounds in amphora

Analysis by nanoESI FT-ICR SolariX XR 9.4 T

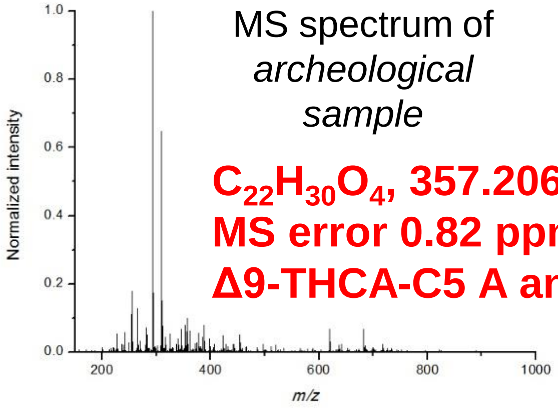
Identification of cannabis compounds



MS spectrum of *Cannbis Sativa (Maroc)*



MS spectrum of *Cannbis Sativa (Cévennes)*

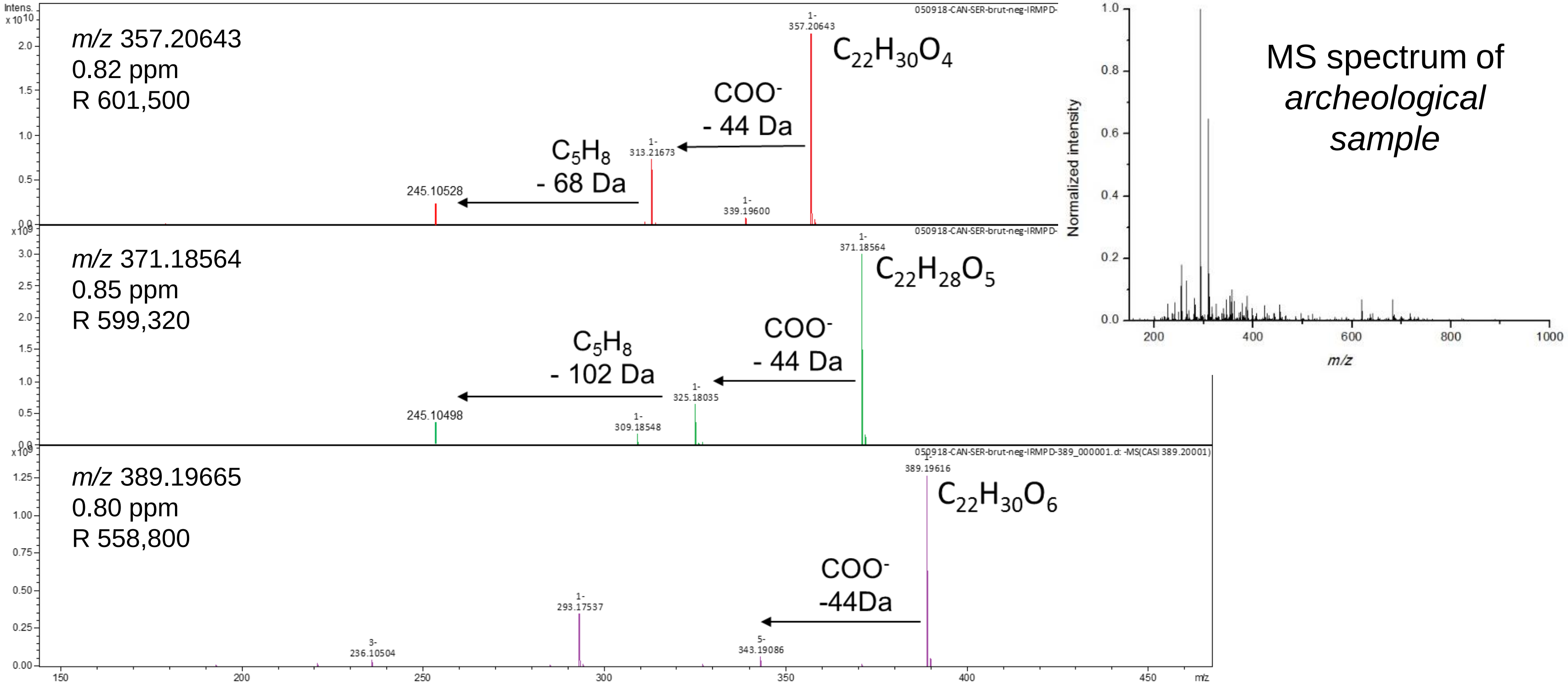


MS spectrum of *archeological sample*

$C_{22}H_{30}O_4$, 357.206840
MS error 0.82 ppm
Δ9-THCA-C5 A and B

Nicolas GARNIER, LNG, Vic-le-Comte.
Exposition “Sorties de fouilles”, Bargoin museum , Clermont-Ferrand

Metabolomics of archaeological samples



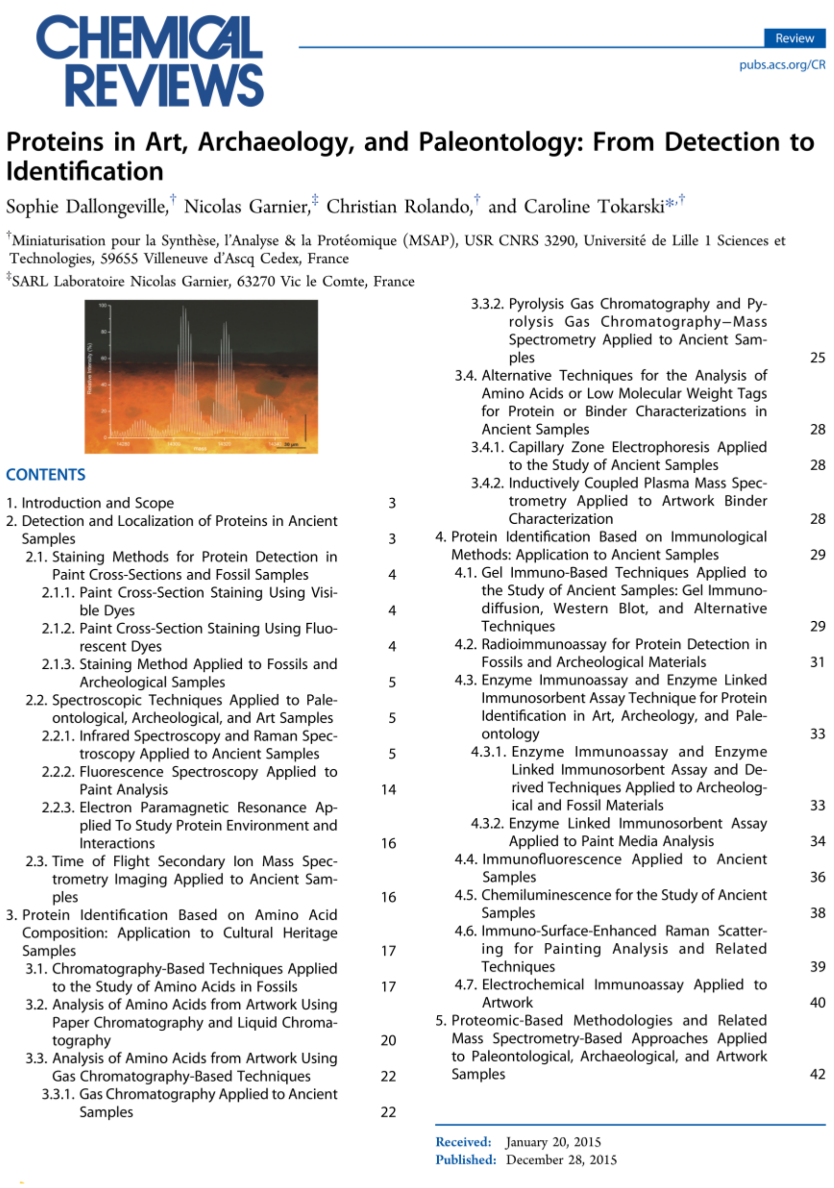
ESI(-)-FT-ICR IRMPD MS/MS allows to identify exact structure:

$C_{22}H_{30}O_4$ = Δ^9-THCA-C5 A and B

$C_{22}H_{28}O_5$ = Δ^9-THCA A-8-one

$C_{22}H_{30}O_6$ = 8β,11-Di-OH-Δ^9-THCA

Review



CHEMICAL REVIEWS
Review
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Proteins in Art, Archaeology, and Paleontology: From Detection to Identification

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