

How sweet watercolors are !

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EU FT-ICR End User School

Miniaturisation pour la synthèse, l'analyse et la protéomique MSAP - UAR 3290

Wednesday, December 14th, 2022



Generalities about painting

- **Paintings** are a form of **visual art** that captures **the expression of ideas and emotions on a two-dimensional flat surface**.
- Artists use the elements of **shape, colors, line, tones, and textures** in unique ways to produce paintings that convey **sensations of movement, volume, space, and light**.
- **Multiple painting techniques** that evolved throughout the history.
- **Art history** is the study of objects of art considered within their time period: **to better understand, document and preserve the artworks**.

Arnolfini Portrait



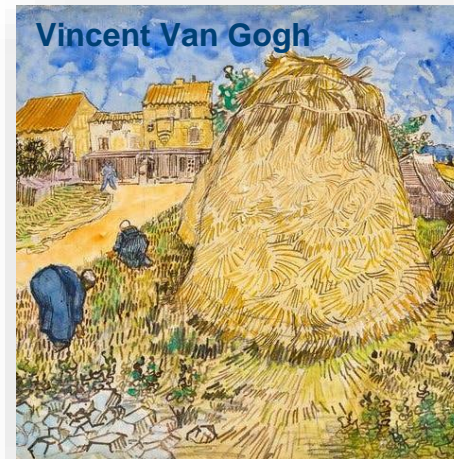
XVth century

Mona Lisa



XVIth century

Meules de Blé



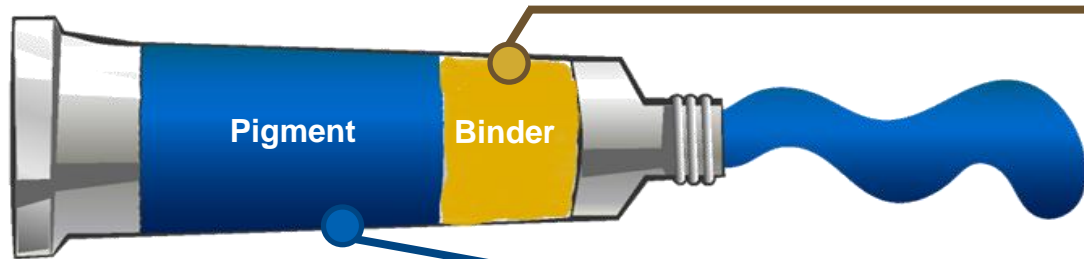
XIXth century

Nude Woman in Red Armchair



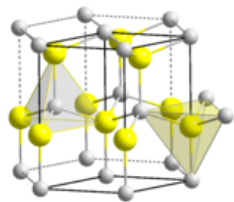
XXth century

Paintings formulation and classification



Inorganic

**Cadmium
sulfoselenide**



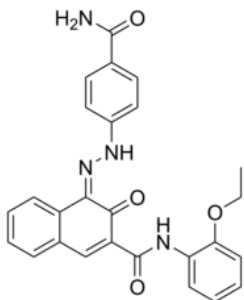
Cadmium red

Ferrocyanide



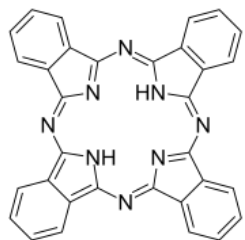
Prussian blue

Organic



Naphthol red

Phthalocyanine



Ultramarine

Oil



Proteins



Organic binders

Carbohydrates



Resin



Natural gums

Origin



- Viscous and adhesive substances.
- Multiple origins:
 - Bacteria
 - Algae
 - Plants
- Secreted by vegetal cells
- Protection and defence role.
- Continually produced when an aggression is existing

Multiple interesting applications



Agri-food



Pharmaceutical



Cosmetics



Paintings

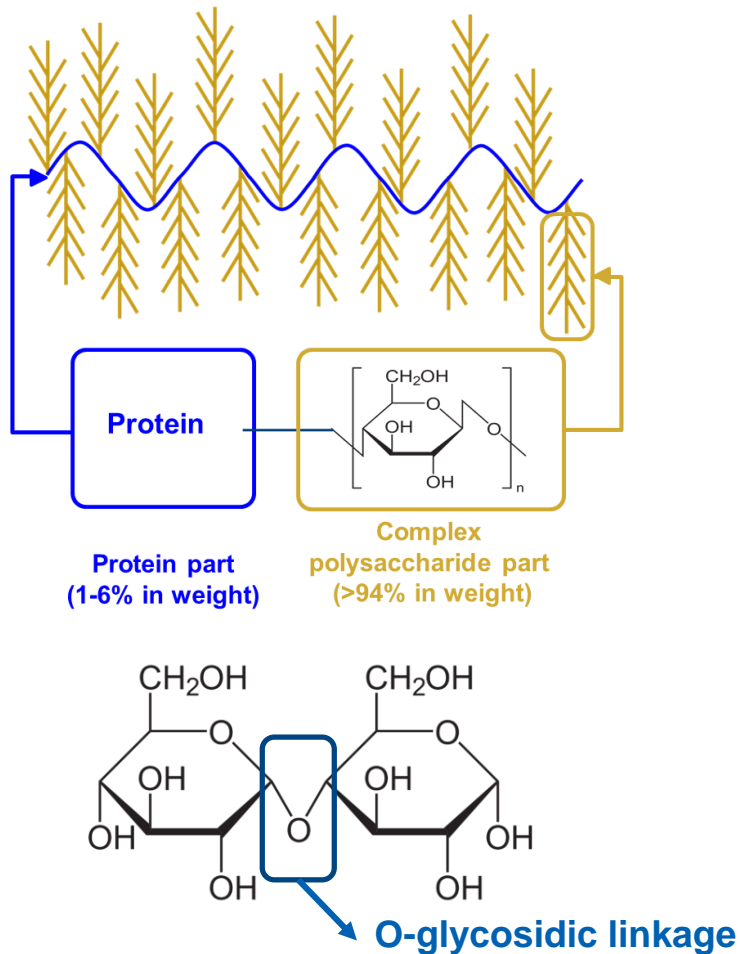
(Watercolor et gouache)

Izydorczyk, M., Cui, S. W., & Wang, Q. (2005). Polysaccharide gums: structures, functional properties, and applications. *Food carbohydrates: Chemistry, physical properties, and applications*, 293, 299.

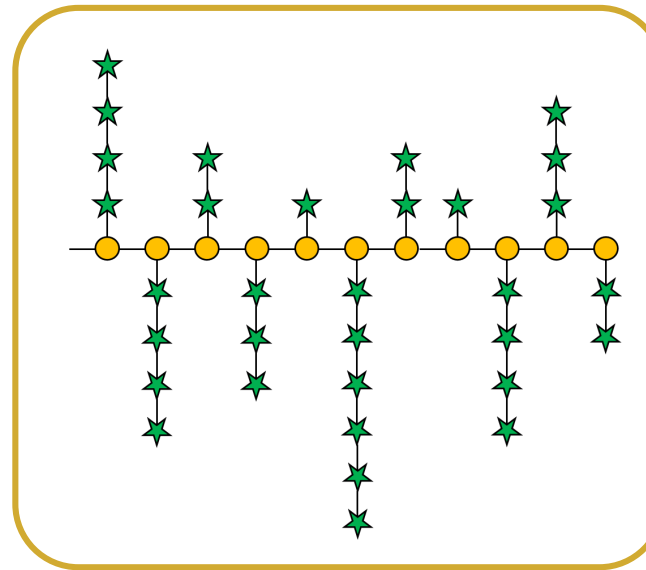
Granzotto, C., Arslanoglu, J., Rolando, C., & Tokarski, C. (2017). Plant gum identification in historic artworks. *Scientific reports*, 7(1), 1-15.

Molecular structure of gums

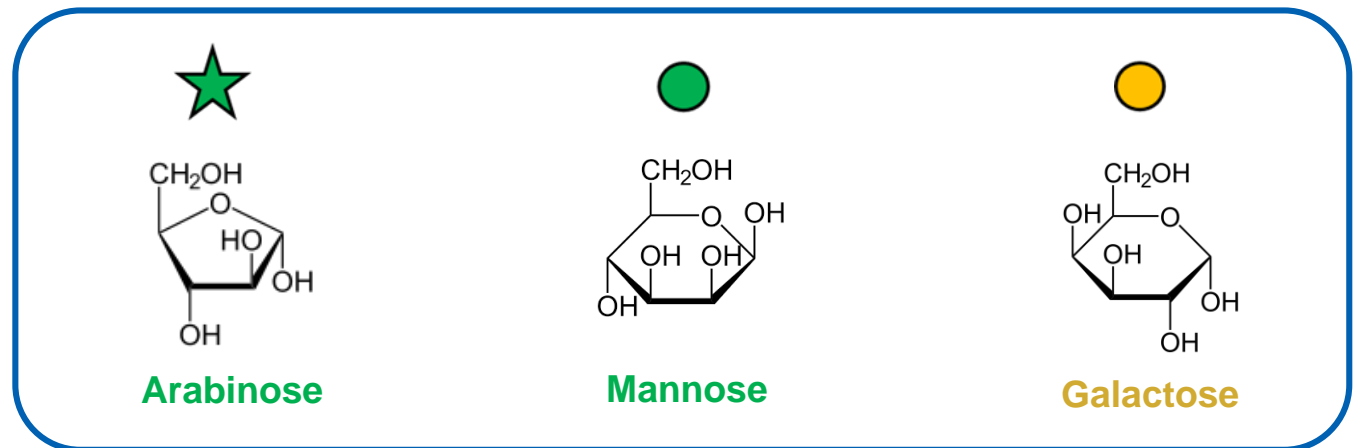
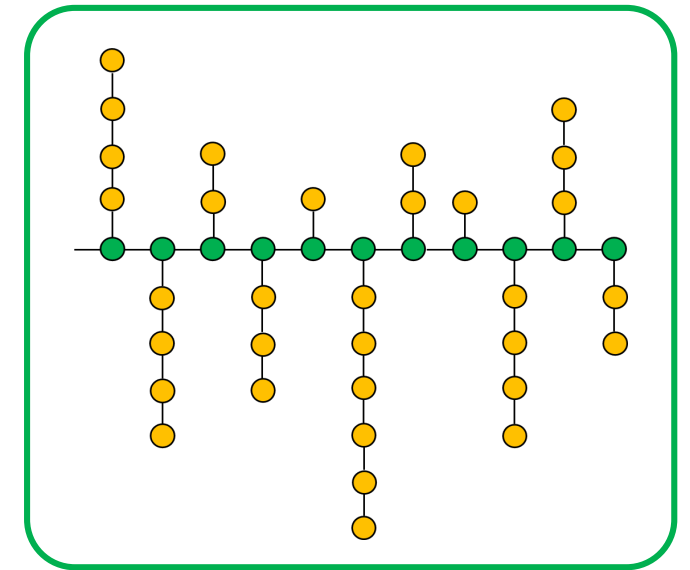
Gums = **Proteoglycans**



Arabinogalactan



Galactomannan



Structure & composition variable depending on the species

Analytical techniques for gums

Analytical techniques for gums

GC-MS



NMR



Limitations:

- Chemical degradation of polysaccharides
- Need of substantial amounts

Development of a sensitive and mild analytical method based on enzymatic digestion of gums and mass spectrometry

Analyzed gums in our investigation

Commercial natural gums



Gommes standards	Genre/Espèce
Arabinogalactanes	Standard
Galactomannanes	Standard
Gomme arabique	Acacia
Gomme adragante	Astragalus
Gomme de caroube	Ceratonia siliqua
Gomme de guar	Cyamopsis tetragonoloba
Gomme de karaya	Sterculia
Gomme de ghatti	Anogeissus

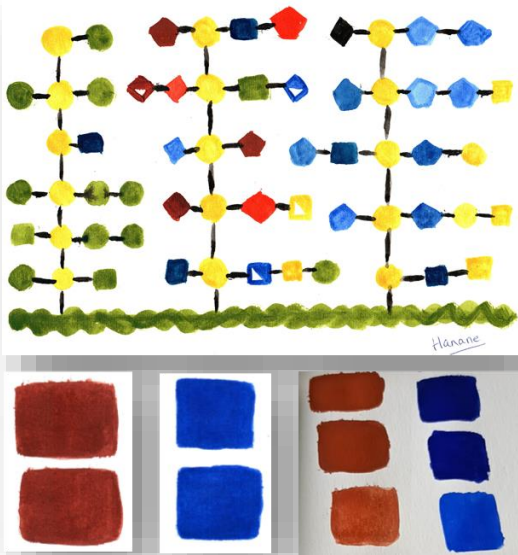
Standards

Watercolor and gouache palettes



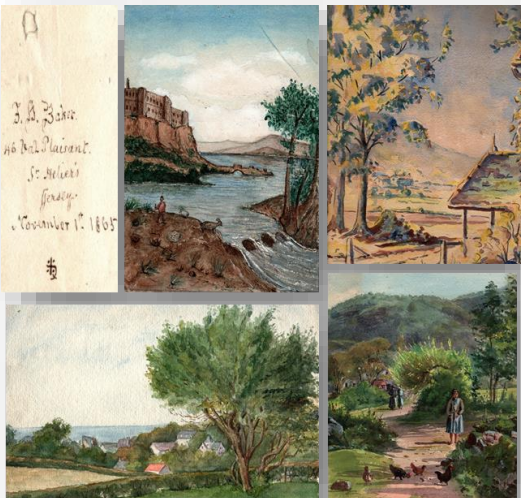
Modern and ancient from different manufacturers

Paintings models



Selection of color and previous palettes

Ancient paintings



XIXth - XXth century

Experimental strategy

1- Solubilization

3- Digestion
enzymatique

exo-beta-1,3-galactanase
endo-beta-1,4-mannanase

5- Analysis by
MALDI-MS

MALDI-TOF

MALDI-FT ICR



2- Centrifugation

4- Filtration

For models

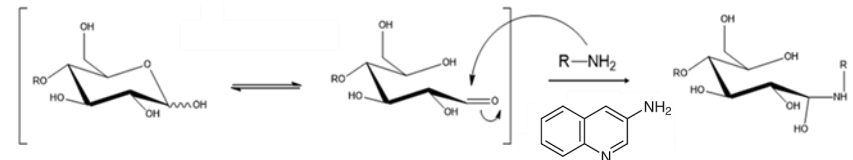
Elimination of impurities
supernatant



PVDF 0.22 µm

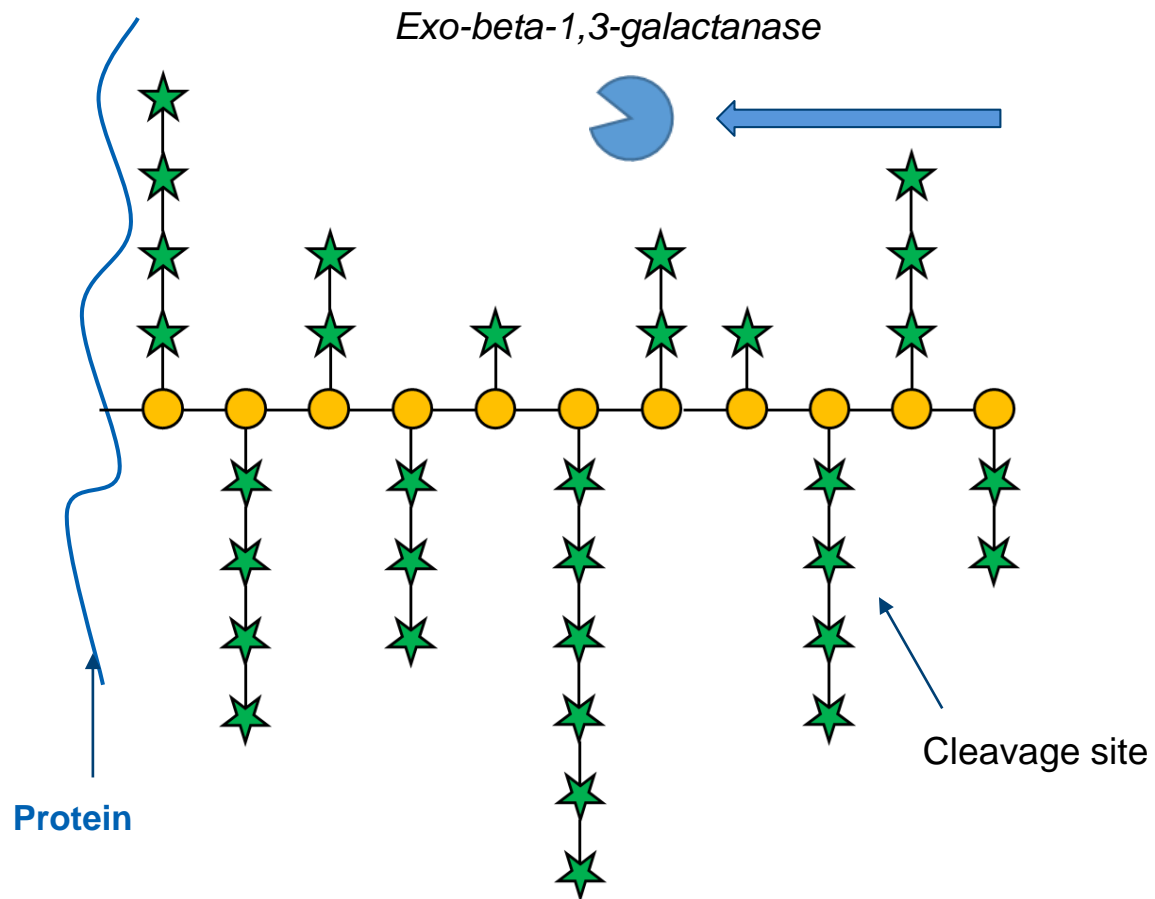
Elimination of pigments
Keep the filtrate

3-AQ acting as a matrix and derivatizing agent

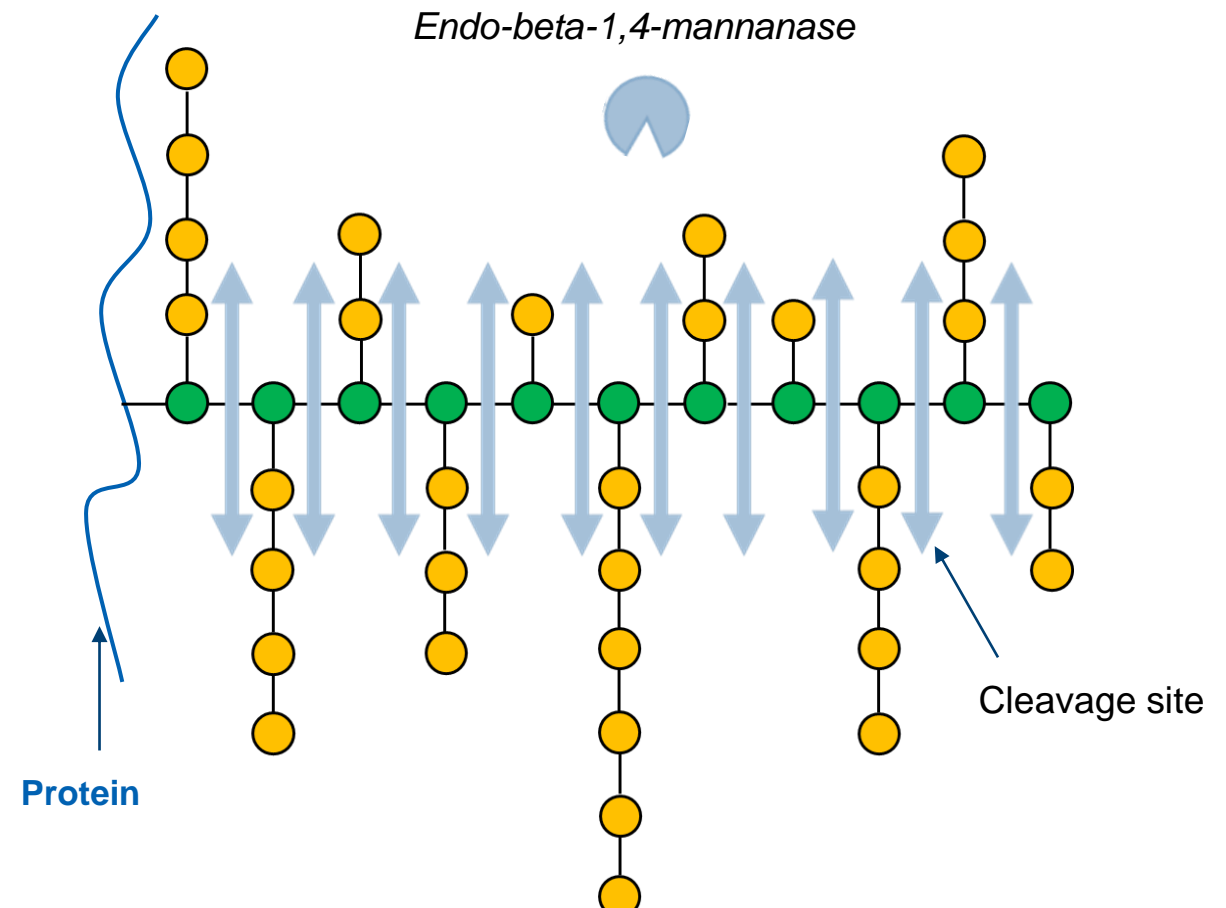


Enzymatic digestion principle

Arabinogalactan gum



Galactomannan gum

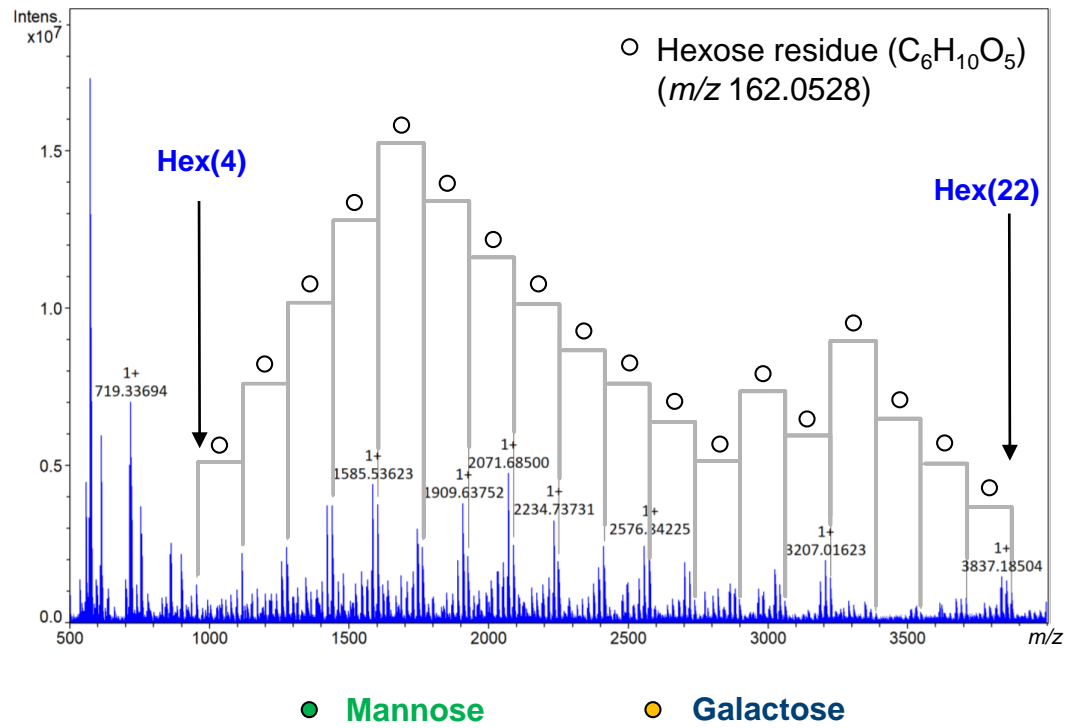


Analysis of digested standard gums

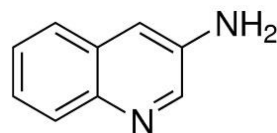
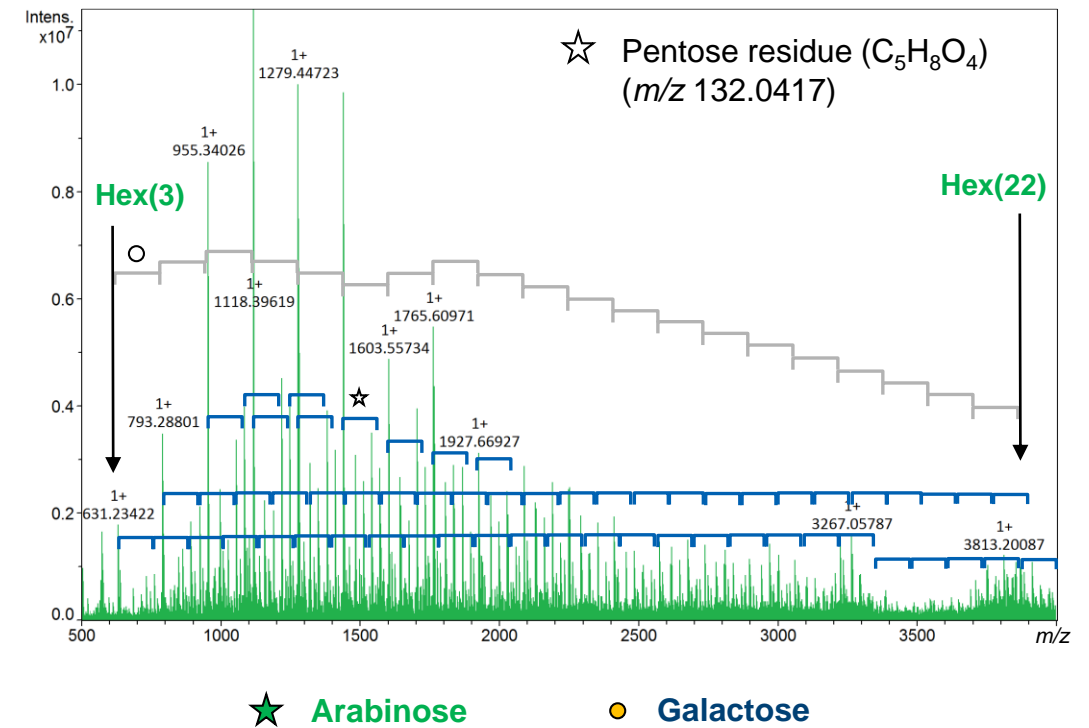
9.4 T MALDI-FT-ICR MS

Increased sensitivity and resolution compared to MALDI-TOF-MS analysis
(detection limited to Hex(11))

Galactomannan (10 µg)



Arabinogalactan (10 µg)



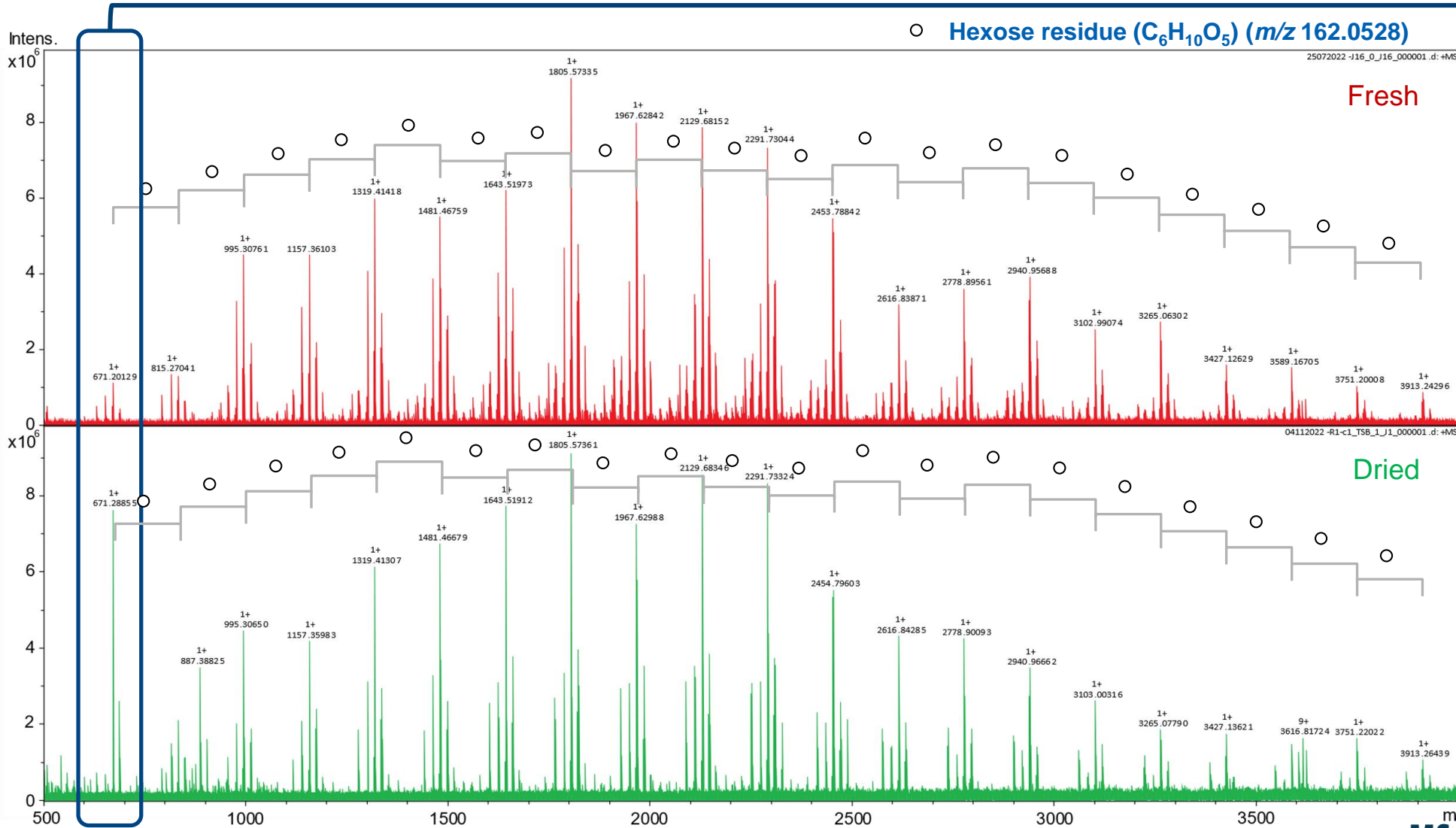
Watercolor palettes' analysis

Colors and manufacturers comparison

	Watercolor	Period	Country	Type	Color	Gum
⇒	J.M. Paillard - Normal	1960-2000	FR	Tube/liquid	Burnt Sienna	Arabinogalactan
	Winsor&Newton - Professional	1960-2000	UK	Tube/liquid	Burnt Sienna	Arabinogalactan
	Rowney	1960-2000	UK	Cube/solid	Burnt Sienna	Galactomannan
	Art-n-Fly	2000-2022	CH	Cube/solid	Burnt Sienna	Galactomannan
	Daler-Rowney	2000-2022	UK	Cube/solid	Burnt Sienna	Arabinogalactan
⇒	J.M. Paillard – Extra fine	< 1960	FR	Tube/liquide	Ultramarine	Galactomannan
	Winsor&Newton – Scholastic	< 1960	UK	Tube/liquide	Ultramarine	Arabinogalactan
	Winsor&Newton – Students	< 1960	UK	Tube/liquide	Ultramarine	Galactomannan
⇒	J.M. Paillard - Normal	1960-2000	FR	Tube/liquide	Ultramarine	Arabinogalactan
	Winsor&Newton - Professional	1960-2000	UK	Tube/liquide	Ultramarine	Arabinogalactan
	Rowney	1960-2000	UK	Godet/solide	Ultramarine	Galactomannan
	Talens	1960-2000	NL	Godet/solide	Ultramarine	Arabinogalactan
	Art-n-Fly	2000-2022	CH	Godet/solide	Ultramarine	Galactomannan

Dried watercolors' analysis

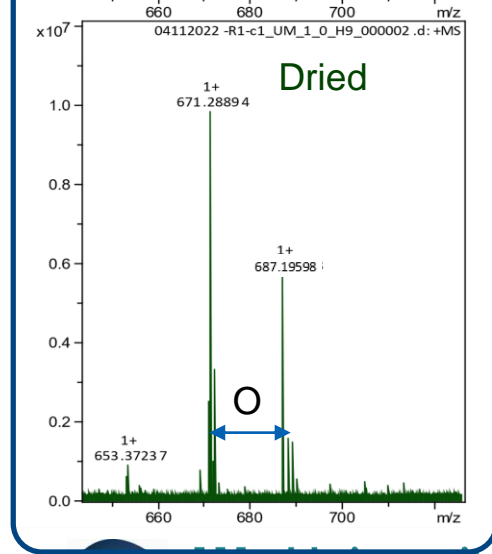
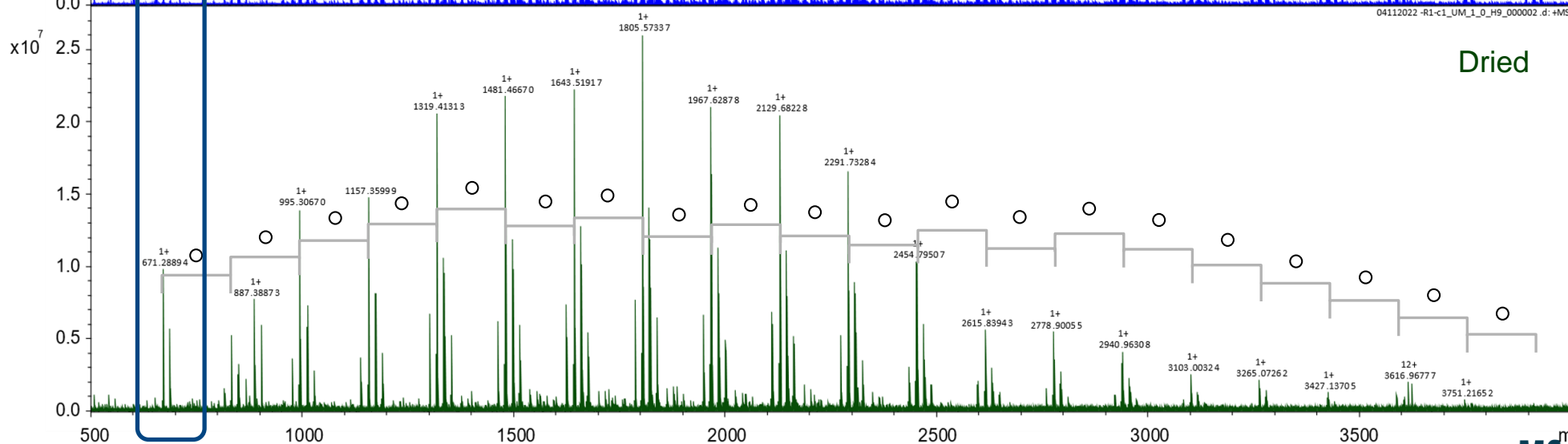
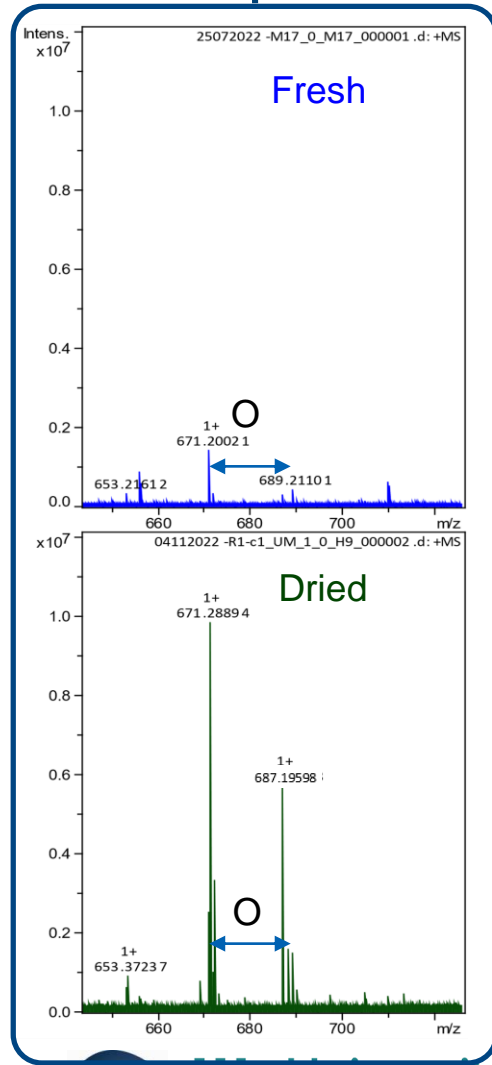
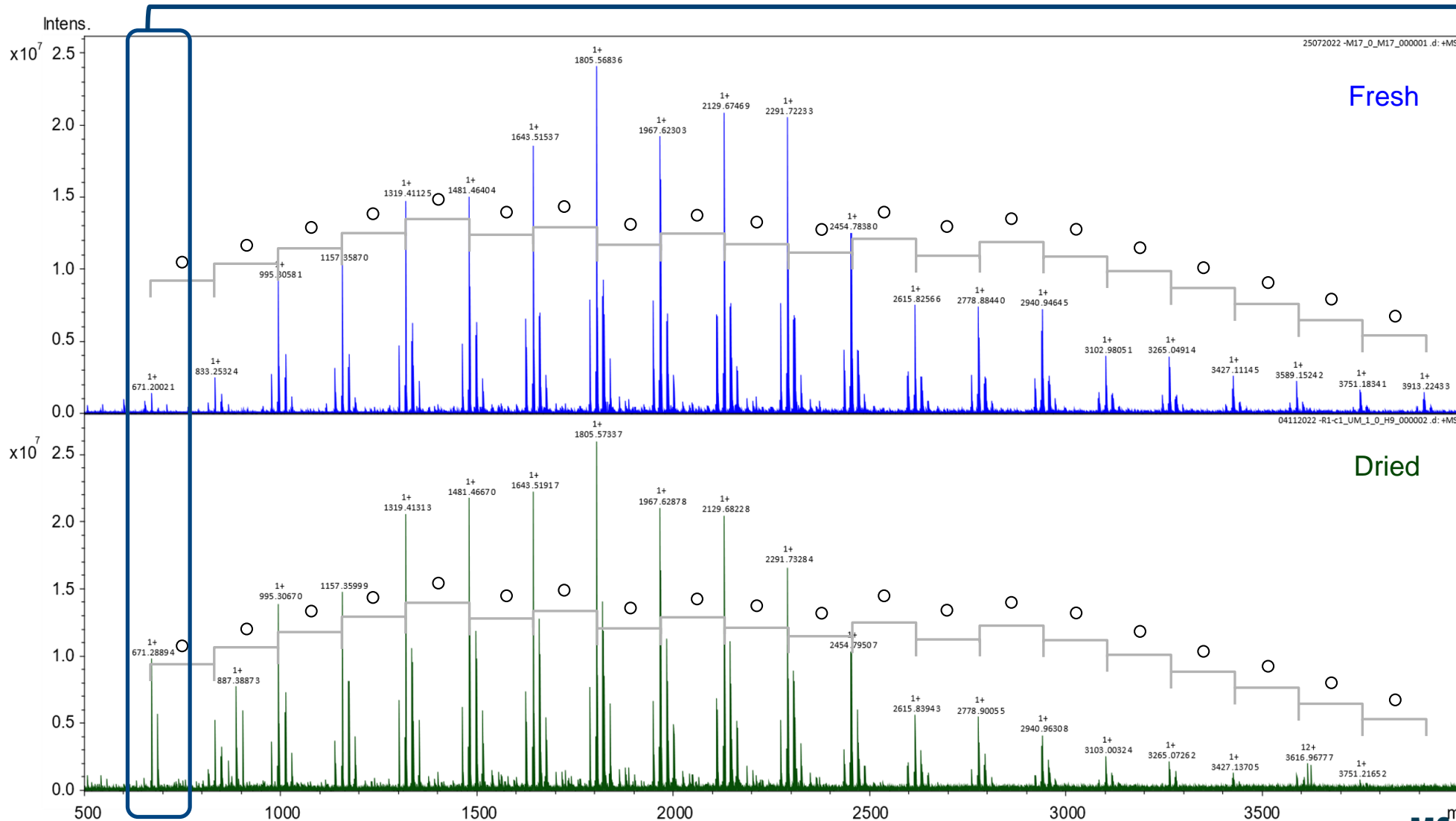
Burnt Sienna color, aging in ambient air for 7 days



Aging induced degradation of galactomannan gums polysaccharides
combined to an important oxidation

Dried watercolors' analysis

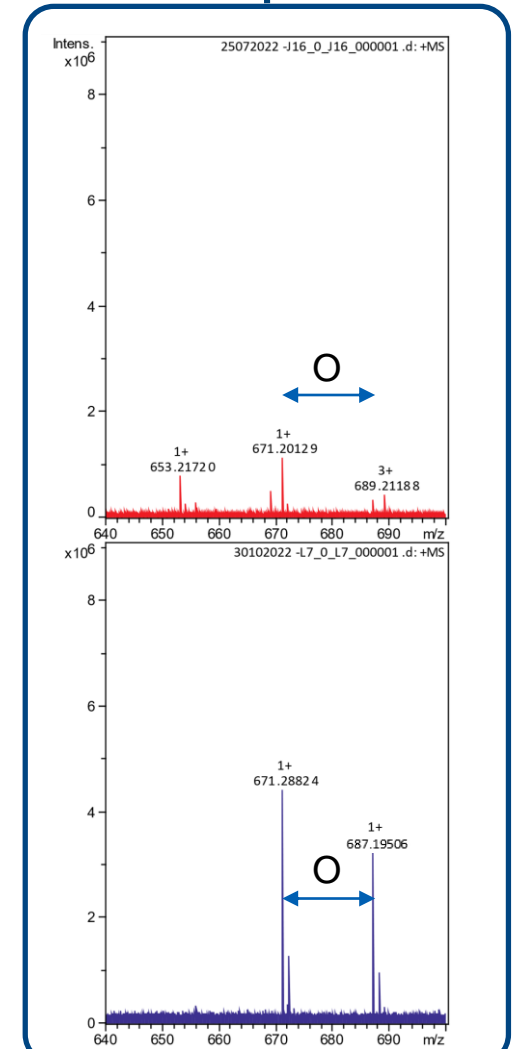
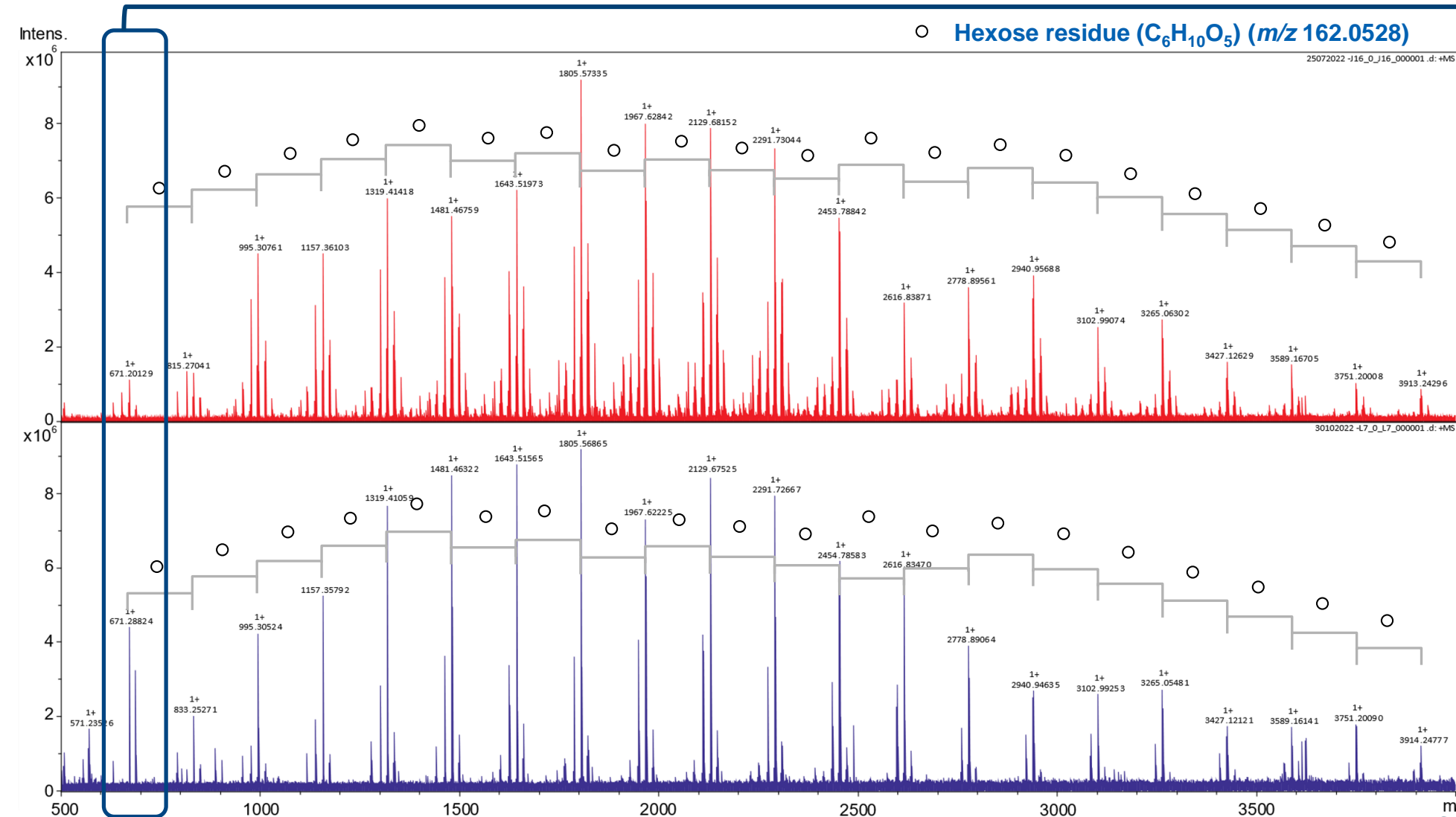
Ultramarine color, aging in ambient air for 7 days



Aging induced degradation of galactomannan gums polysaccharides combined to an important oxidation and highly dependent of the pigments

Dried watercolors' analysis

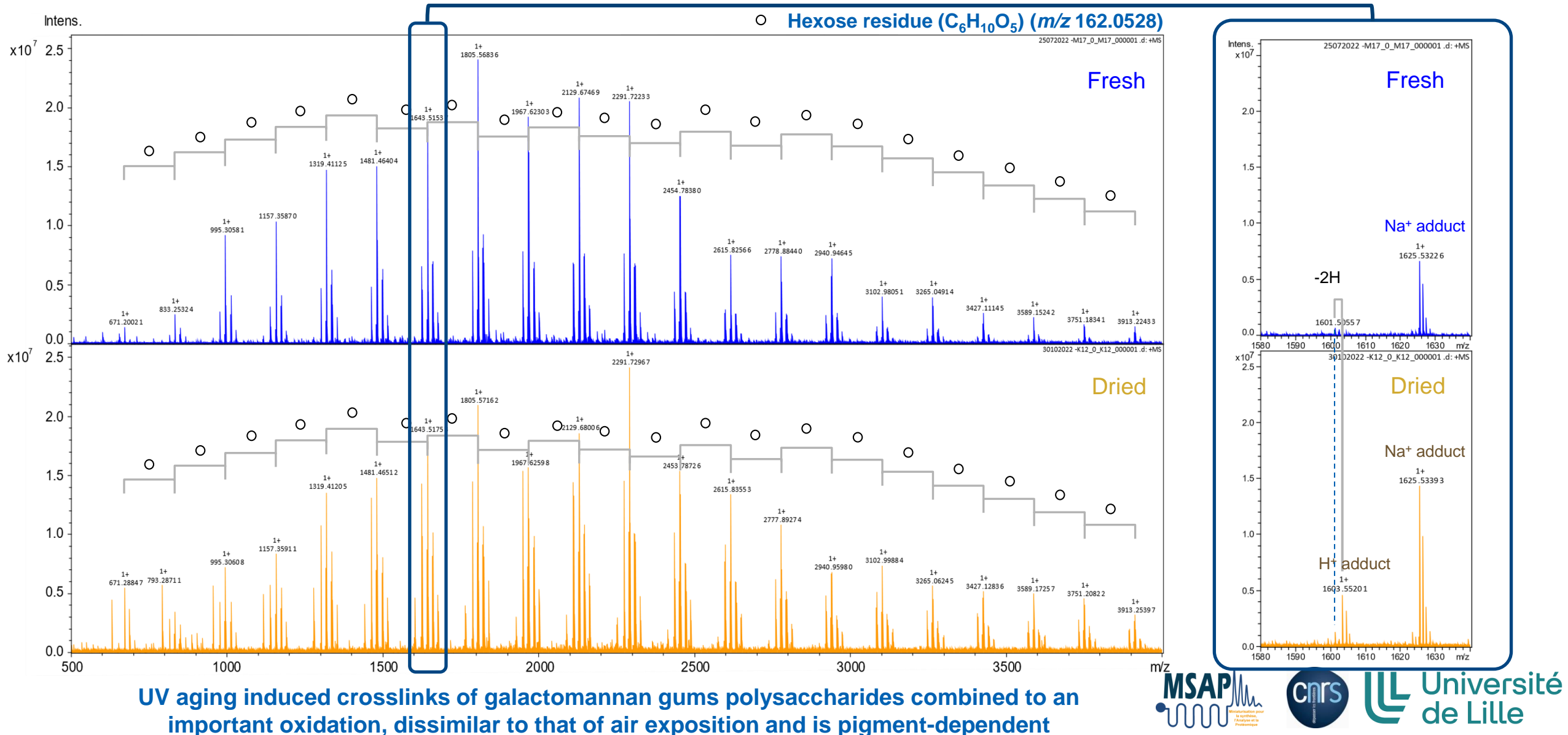
Burnt Sienna color, UV-aging for 5 days



UV aging induced degradation of galactomannan gums polysaccharides combined to an important oxidation, dissimilar to that of air exposition

Dried watercolors' analysis

Ultramarine color, UV-aging for 5 days



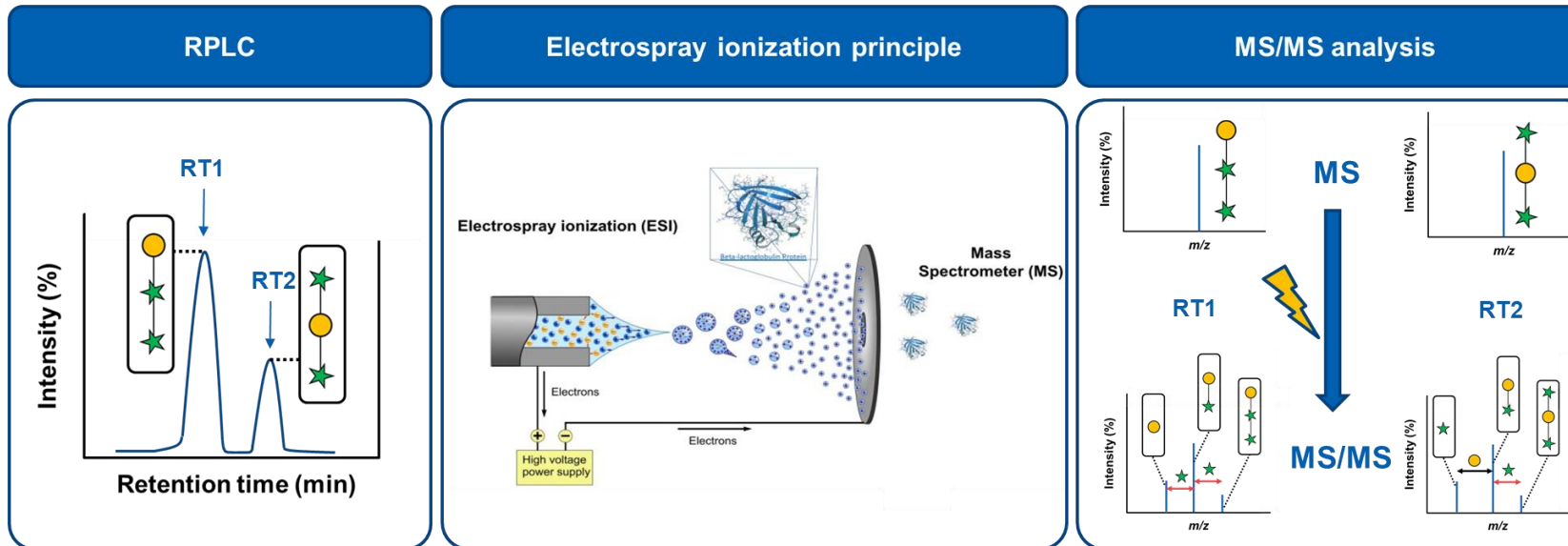
MALDI-MS techniques limitations

- Compositional but not structural information about the identified oligosaccharides



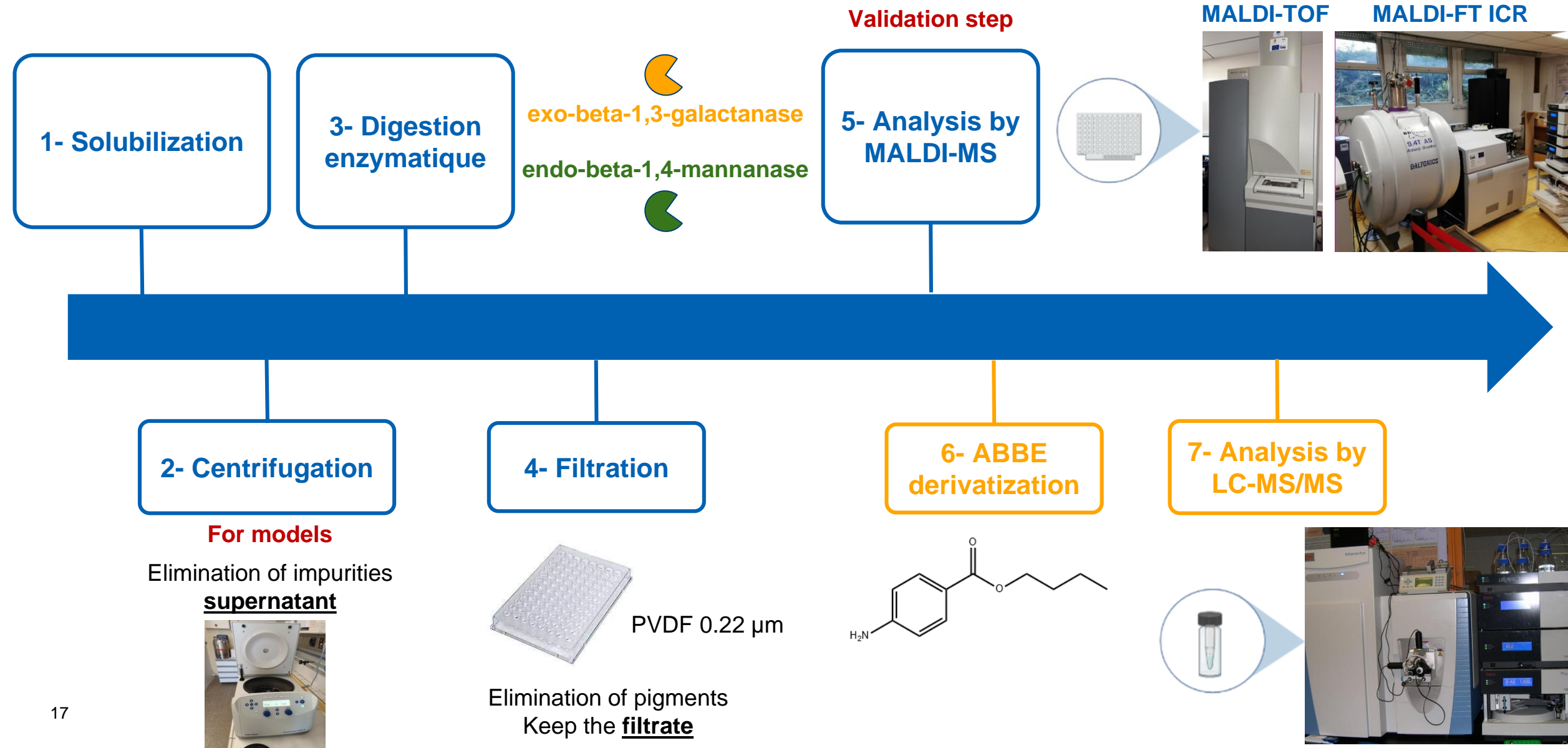
- Semi-quantitative acquisition (relative intensity)

nanoLC nanoESI
MS/MS
technique



Carbohydrates
are hydrophilic
and cannot be
separated by
RPLC (C18)

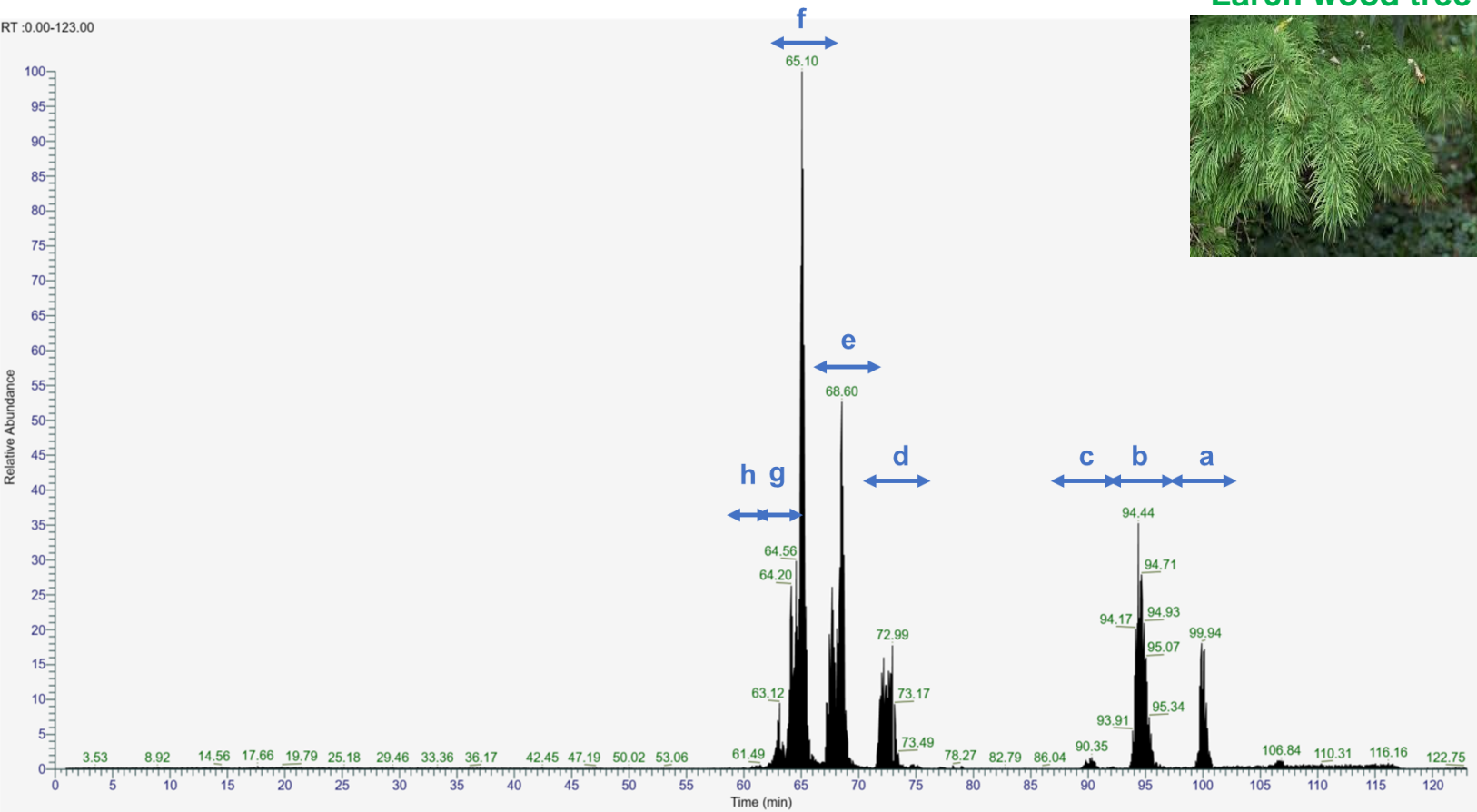
Experimental strategy



nanoLC nanoESI MS/MS analysis of arabinogalactan gums

Arabinogalactan standard gum (0.4 µg)

Larch wood tree



Triply
charged
 $[M+3H^+]^{3+}$

Doubly
charged
 $[M+2H^+]^{2+}$

Monocharged
 $[M+H^+]^{+}$

Chromatographic peak	Oligosaccharide
a	Hex(n)HexA(1)
b	HexNac(n)
c	Hex(n)dHex(1)HexA(1)
d	Hex(n); Hex(n)HexA(1)
e	Pent(n)Hex(2)
f	Pent(1)Hex(n)
g	Pent(1)Hex(m>n)
h	Pent(n)Hex(5)HexNac(1)

nanoLC nanoESI MS/MS analysis of galactomannan gums

Galactomannan standard gum (0.4 µg)

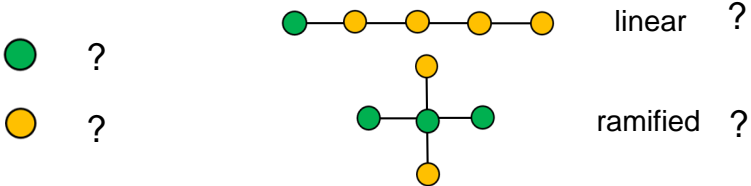
Carob tree



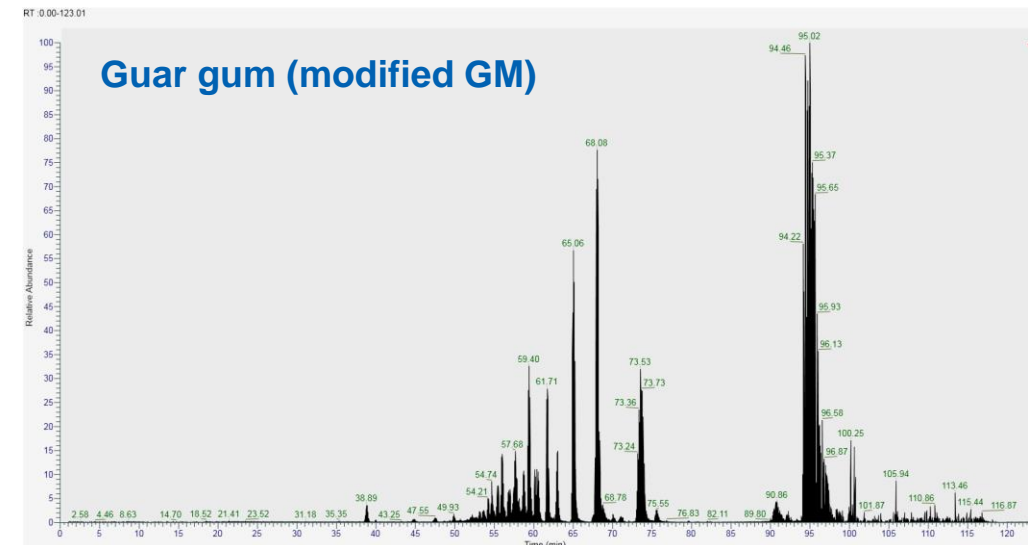
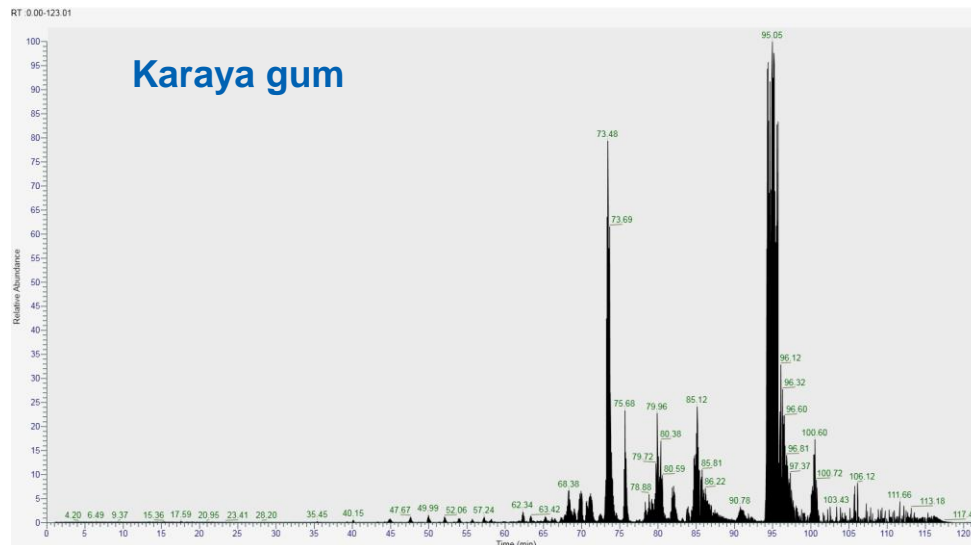
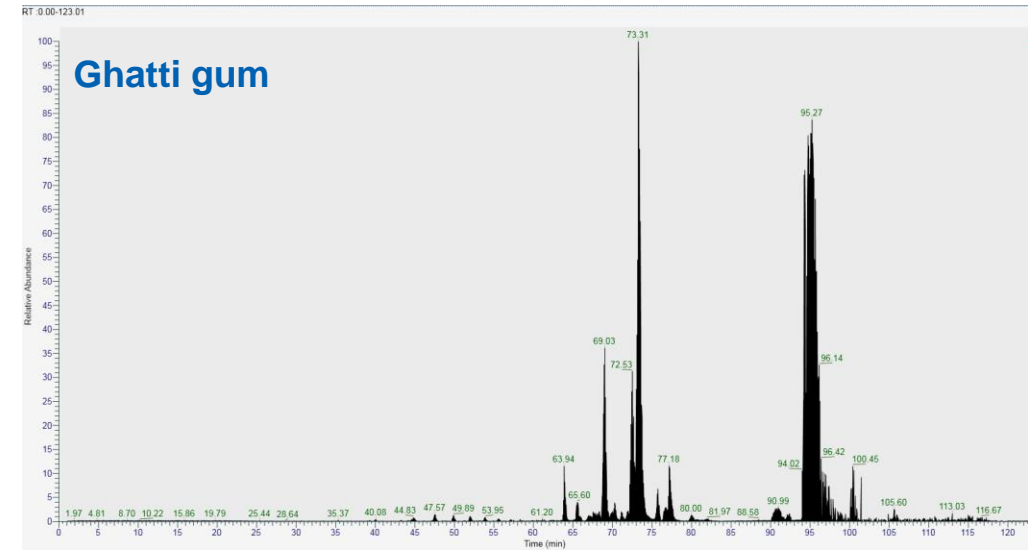
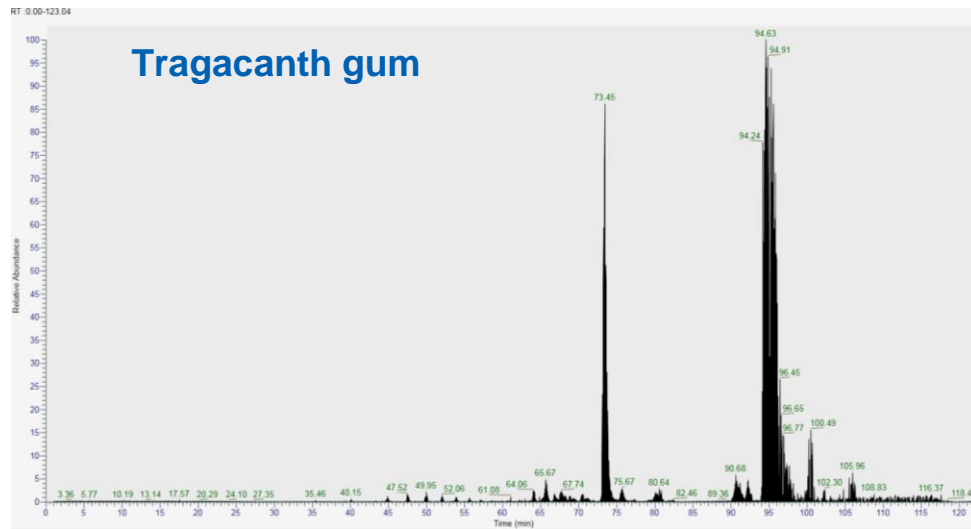
Peak	Oligosaccharide	Peak	Oligosaccharide
1	Hex(3-5)	9	Hex(5)
2	Hex(5-7)	10	Hex(6)
3	Hex(2-7)	11	Hex(7)
4	Hex(2-7)	12	Hex(5-12)
5	Hex(3-8)	13	Hex(13)
6	Hex(2)	14	Hex(14)
7	Hex(3)	15	Hex(15)
8	Hex(4)	16	Hex(16)

Detection of oligosaccharides extending to 29 hexose residues (in triply charged form)

Different retention times = different structures



nanoLC nanoESI MS/MS analysis of other standard gums (0.4 μ g)

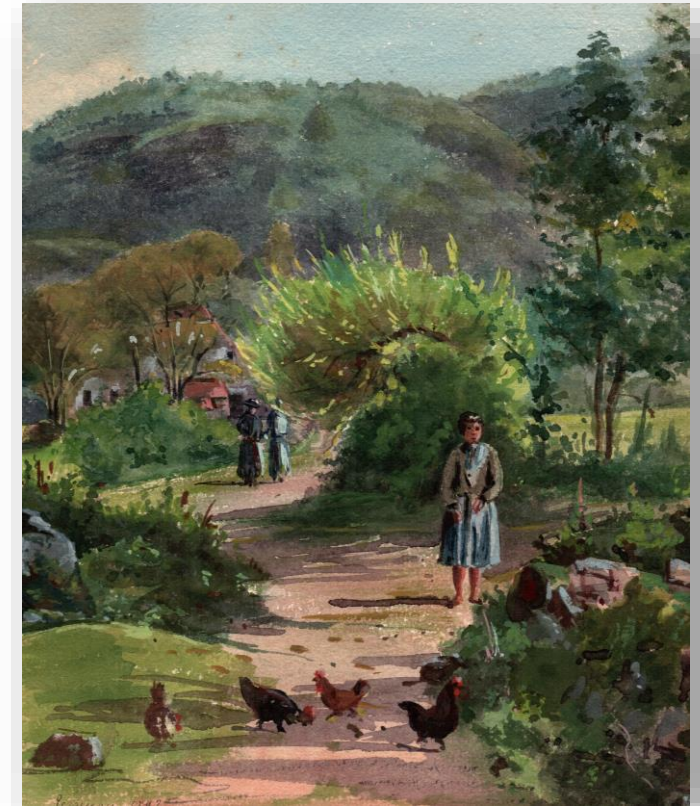


To annotate !

Conclusions

- **Validation of gums enzymatic digestion** by **MALDI-MS analyses** (different oligosaccharides peaks).
- This method is **suitable for the sensitive analysis of gums** in a small amount of :
 - **fresh paints** (liquid or solid),
 - **Painted models**,
- This method allowed us to **decipher the various aging-induced degradation/crosslinking effects of watercolor gums polysaccharides** depending on **the pigment** and **the aging inducer**.
- **Optimization of nanoLC nanoESI MS analyses** following **ABBE labeling**.

Watercolor painting, 1892

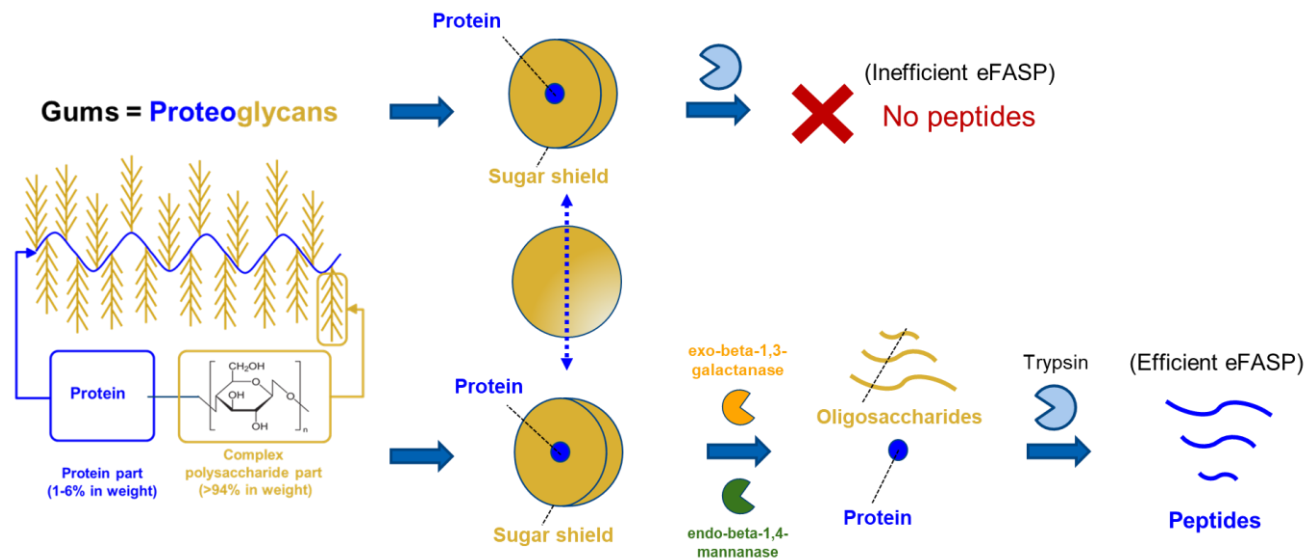


Perspectives

- Analysis of paint samples by nanoLC nanoESI MS/MS for the qualitative and quantitative characterization of gums.
- Apply nanoLC nanoESI MS/MS to study oligosaccharides chemical modifications related to aging.
- Automation of data treatment (informatics tool).
- Proteomics for gums identification.



In-house software development is ongoing



Acknowledgements

MSAP laboratory



Hanane TERMOUL



Maureen DELMARRE

And you for your kind attention !

Dr. Christian ROLANDO

Dr. Fabrice BRAY

Stéphanie FLAMENT



IPERION HS

