



ISTITUTO DI SCIENZE E TECNOLOGIE CHIMICHE GIULIO NATTA

# The NMR approach for the characterization of nutraceuticals and the delivery of bioactive molecules

Consonni Roberto

*SCITEC, sede di Milano*



**NUTRAGE**  
Consiglio Nazionale delle Ricerche

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Energy



Life science



Green chemistry & sustainability



Cultural heritages



Advanced materials



Computational models



## Avance 400 MHz



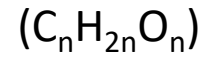
## Avance II 500 MHz



# State of the art: Avance 600 Neo

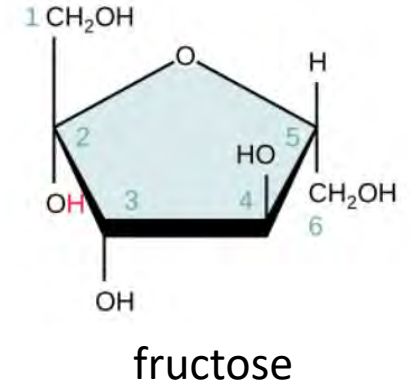
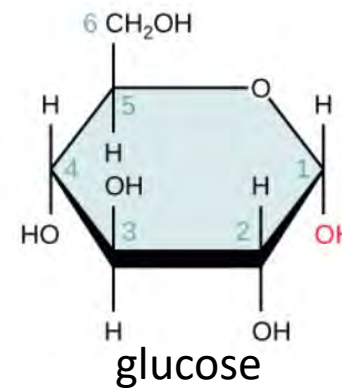
Installation: January 2021



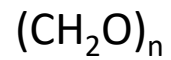


spettro

Monosaccharides

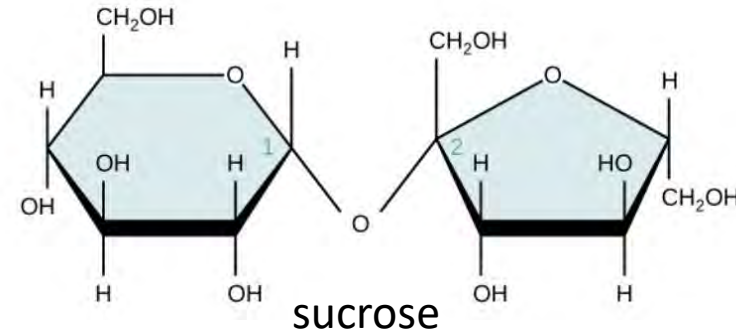


Carbohydrates



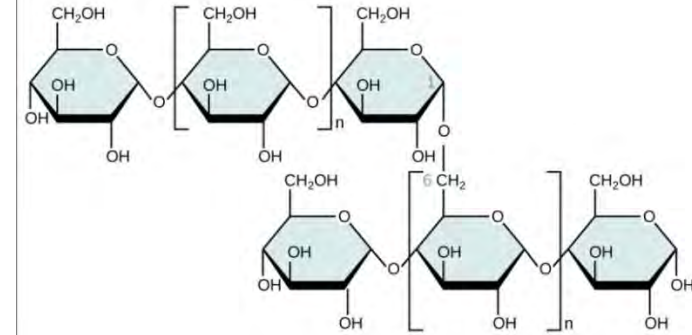
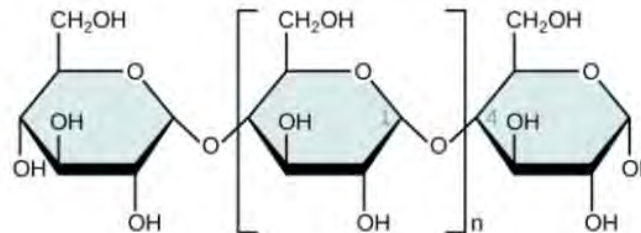
spettro

Disaccharides

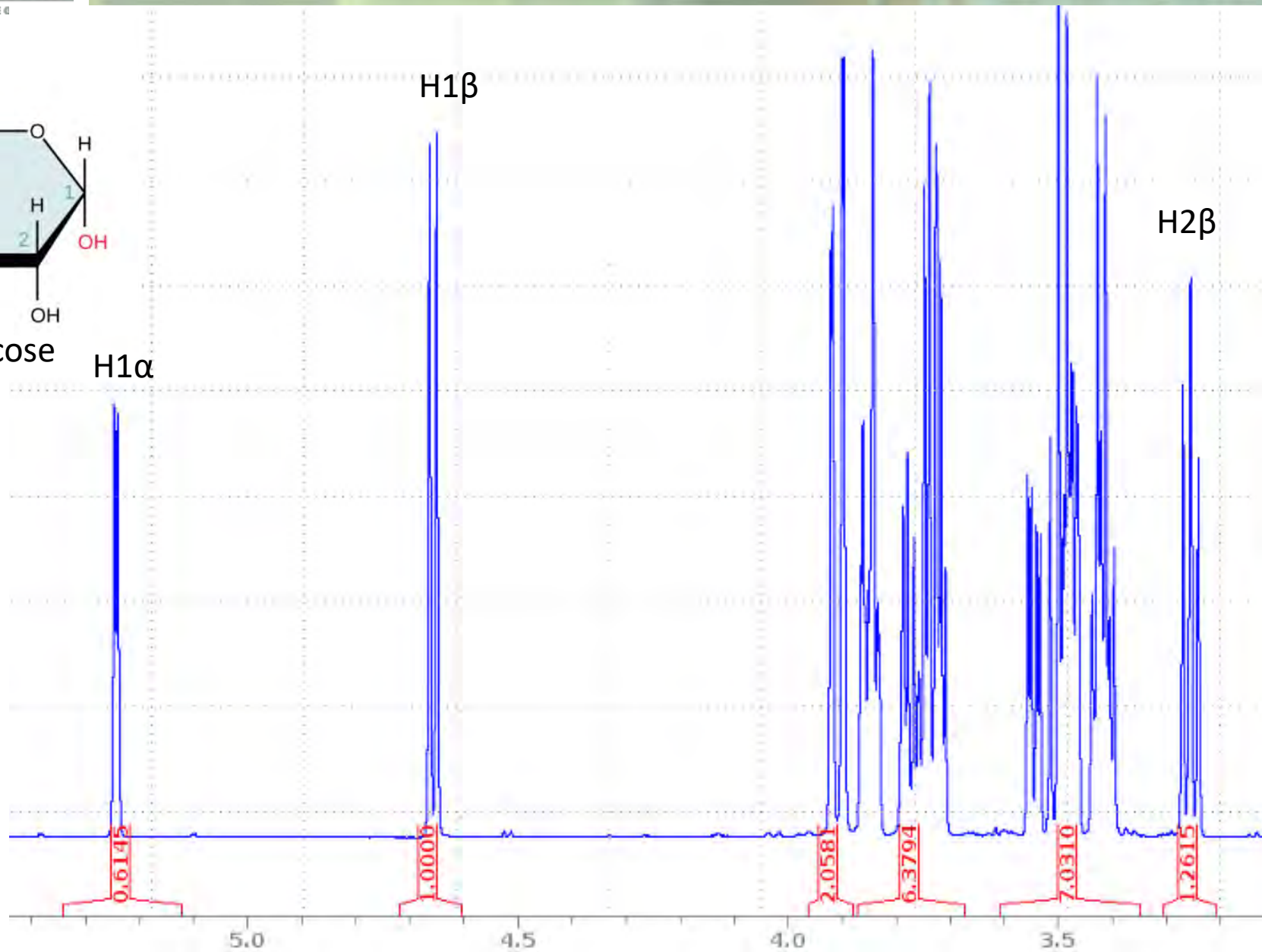
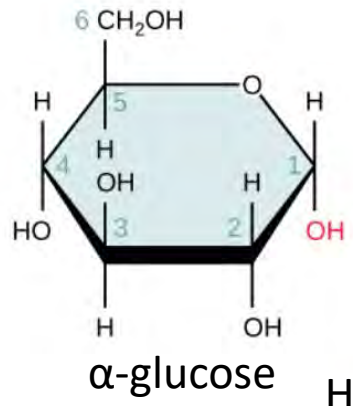


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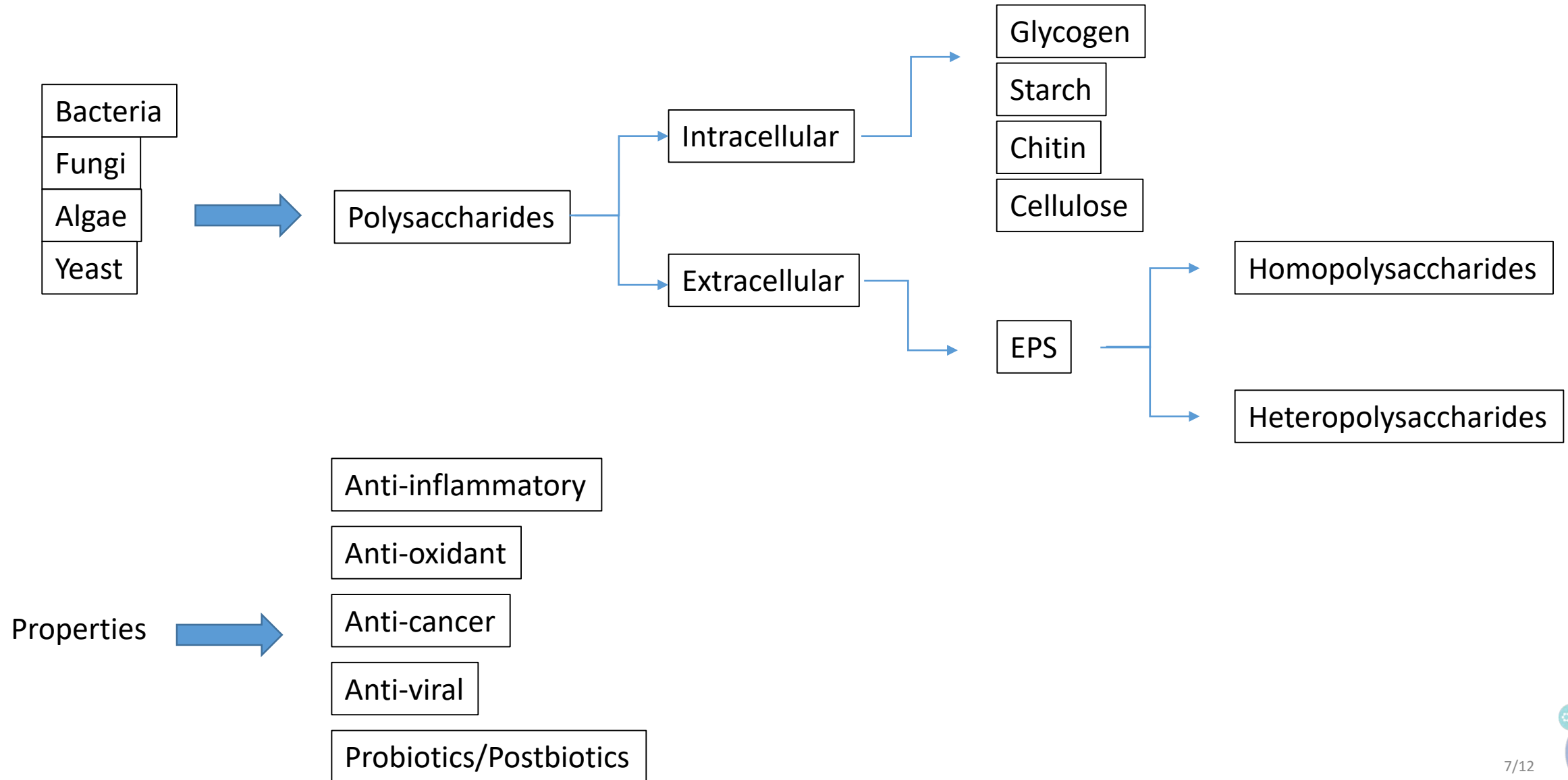
Polysaccharides



# NMR Quantification



# Bio - Source of polysaccharides



LAB

*L. frumenti*  
*L. reuteri*  
*L. pontis*  
*L. sanfranciscensis*  
*Ln. citreum*  
*W. cibaria*  
*Ln. mesenteroides*  
*L. plantarum*  
*L. paraplantarum*



**Mainly HoPS**  
 FOS, GOS, fructans(levan or inulin ),  
 glucans (dextran, reuteran, mutan)

*L. Curvatus*  
*L. Sanfranciscensis*



**HePS**



Food industry

Technological & sensory

- Thickening
- Stabilizing
- Texturizing
- Gelling agents

Usually from plant (pectin, arabic gum,..) or seaweed (alginate, carrageenan)

Health

Prebiotics

*L. sanfranciscensis* → **Levan** (Dal Bello 2001) prebiotics *in vitro*  
*Ln. mesenteroides* → IMO (isomalto-oligosaccarides)  
*Ln. citreum* → Inulin

Probiotics

Interaction probiotic-gut ecosystem



glycoside hydrolases ( $\beta$ -galactosidase from Aspergillus oryzae and Kluyveromyces lactis) using lactose as substrate.

Extraction



Purification

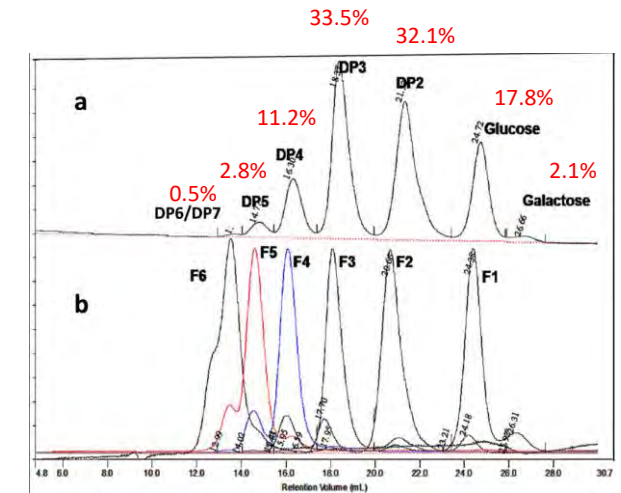


MW characterization

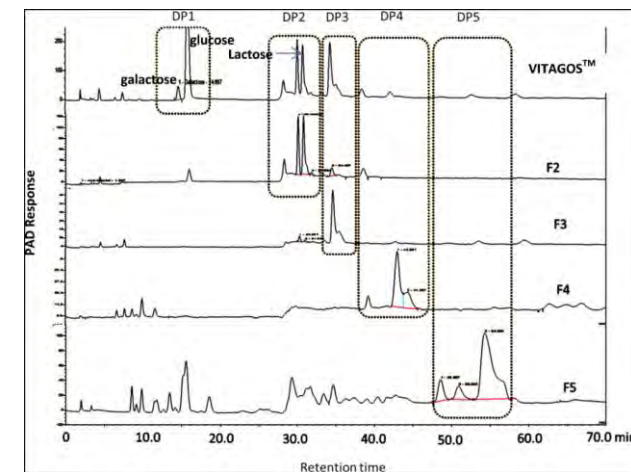


Structural characterization

HP-SEC



HP-AEC

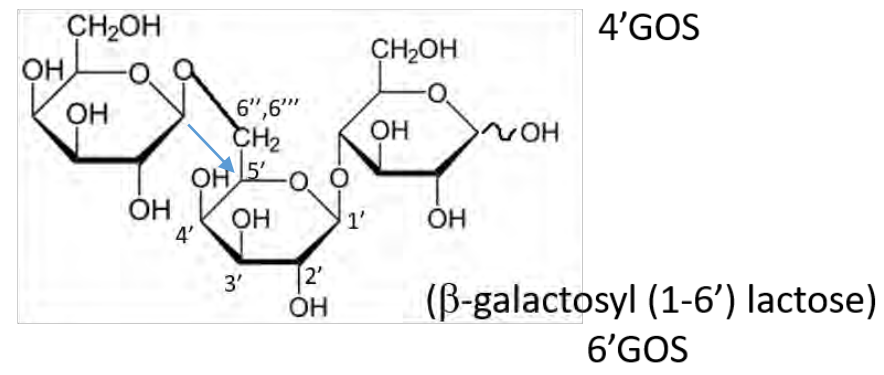
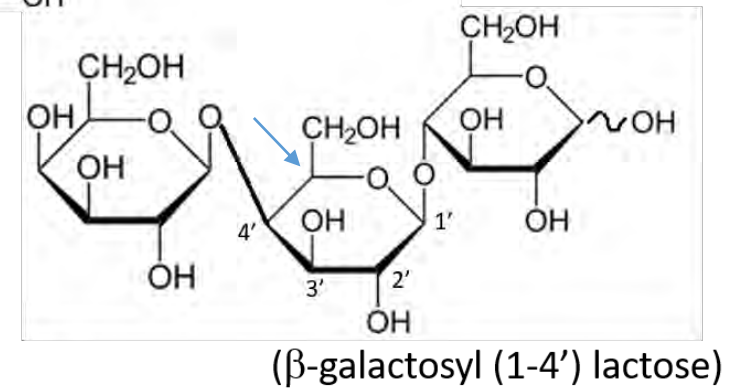
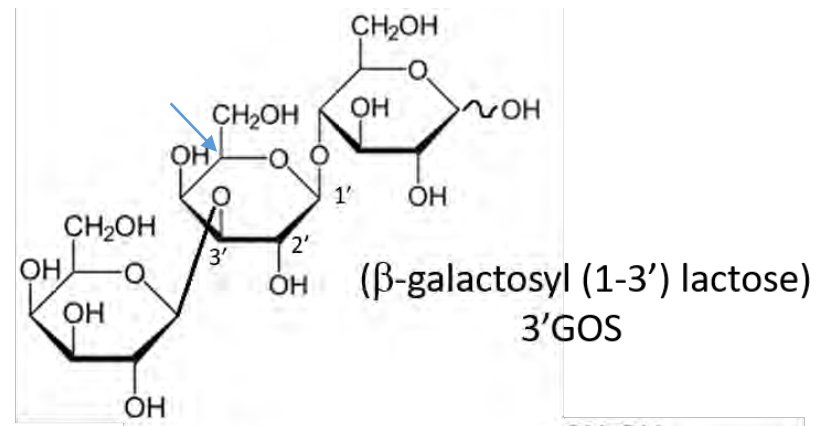
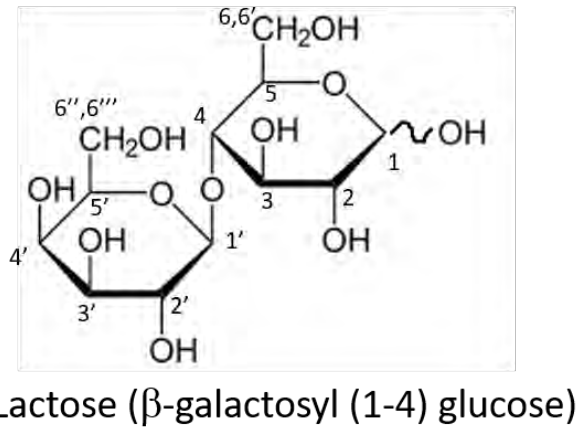
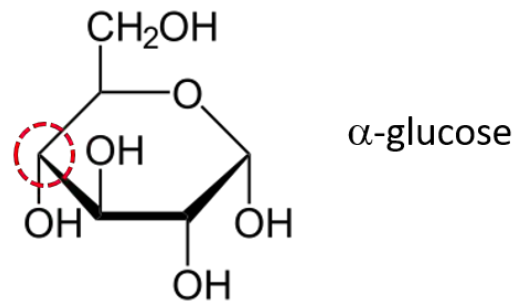
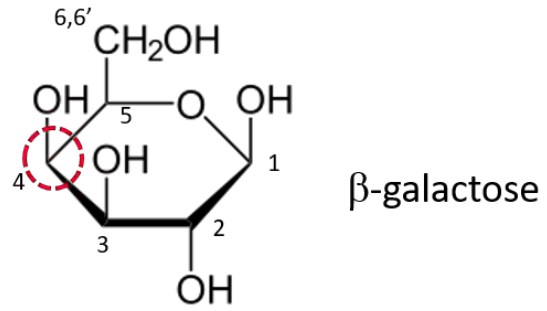


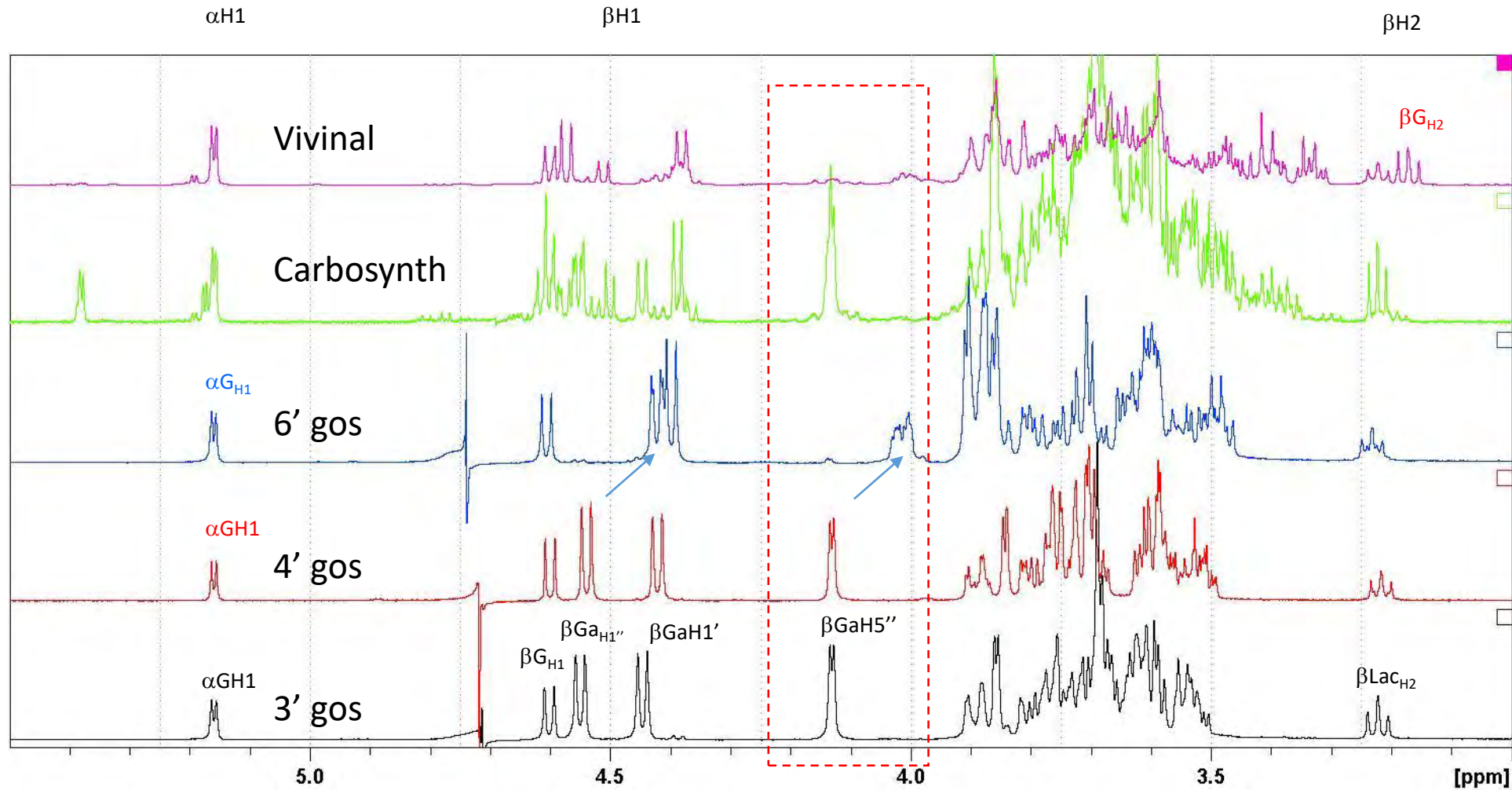
$\beta$ -galactosidase derived from:

- Bacillus circulans favors the production of  $\beta$ -(1 $\rightarrow$ 6) linked oligosaccharides with a higher transferase activity;
- Kluyveromyces lactis prefers the generation of  $\beta$ -(1 $\rightarrow$ 4) linkage;
- Aspergillus oryzae and Aspergillus aculeatus produce  $\beta$ -(1 $\rightarrow$ 6/4/3)



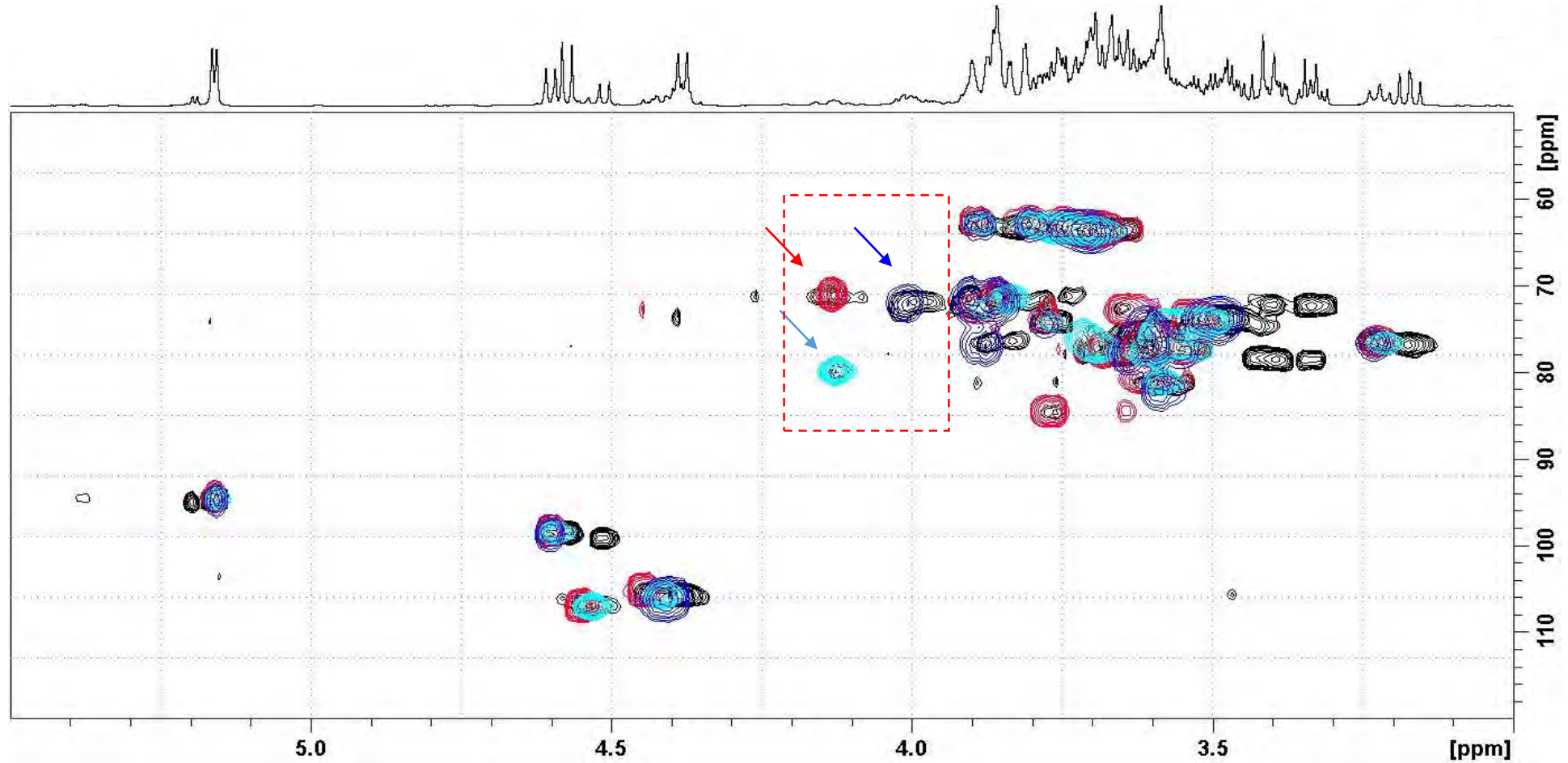
# Structural characterization of GOS: nomenclature





# Structural characterization of GOS by NMR

NUS: 12 h  
Traditional: 24h

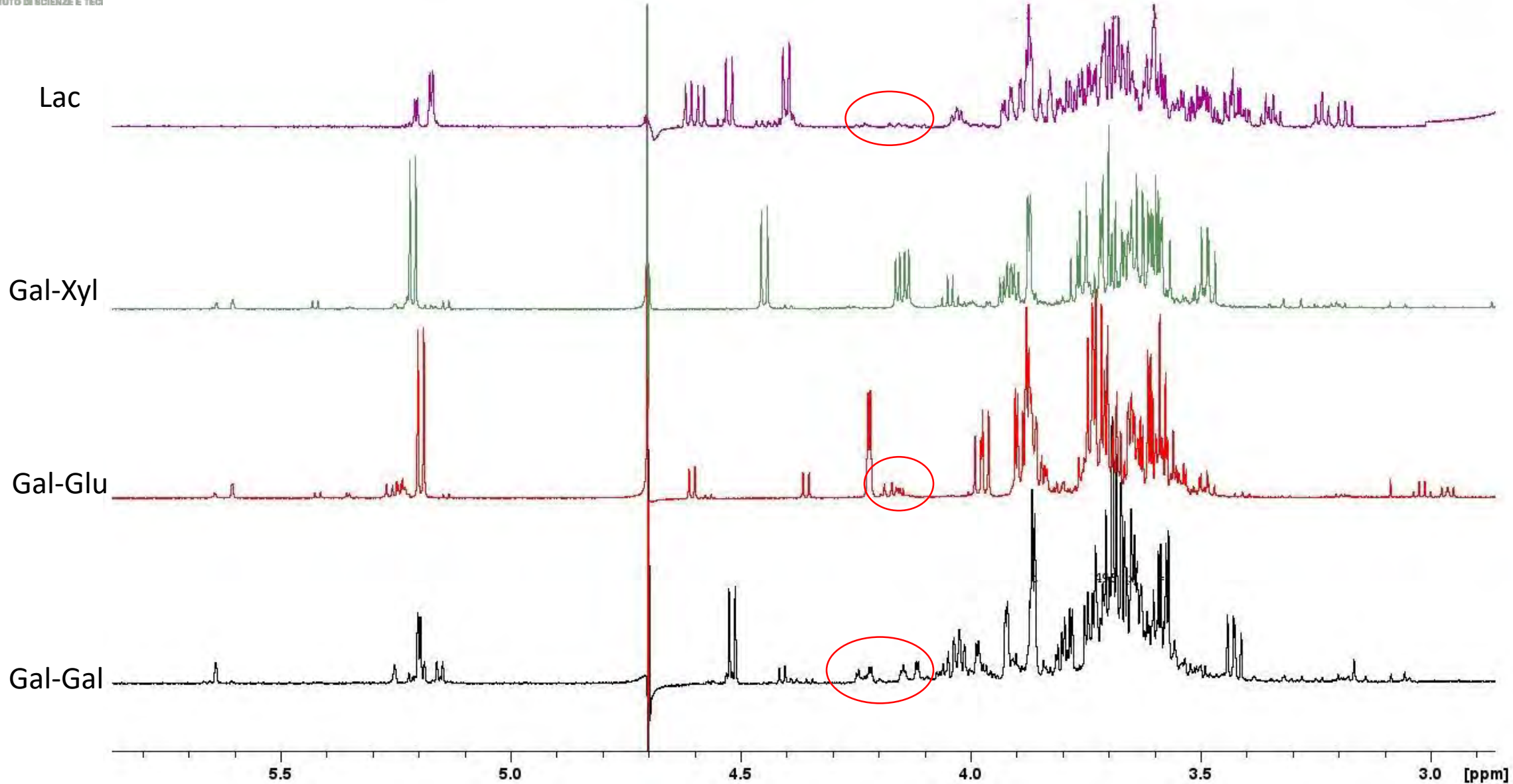


$^1\text{H}$ - $^{13}\text{C}$ - HSQC

3'GOS    4'GOS    6'GOS    Vivinal



# Structural characterization of GOS by NMR



# 1° case study: structural characterization of EPS by NMR

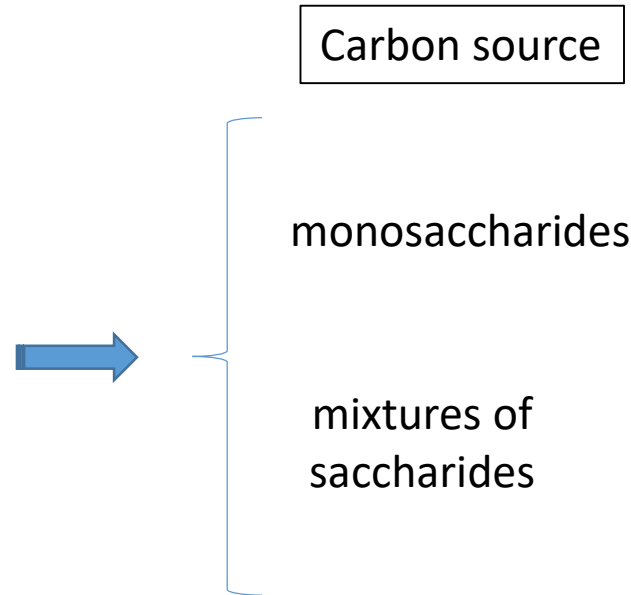


Microbial  
**ISPA**  
collection

**Lacticaseibacillus casei**  
**Lacticaseibacillus paracasei**  
**Lacticaseibacillus rhamnosus**  
**Lactiplantibacillus plantarum**  
**Lactiplantibacillus plantarum**  
**Lentilactobacillus buchneri**

Commercial  
collection

**Leuconostoc lactis**  
**Pediococcus pentosaceus**  
**Weissella confusa**

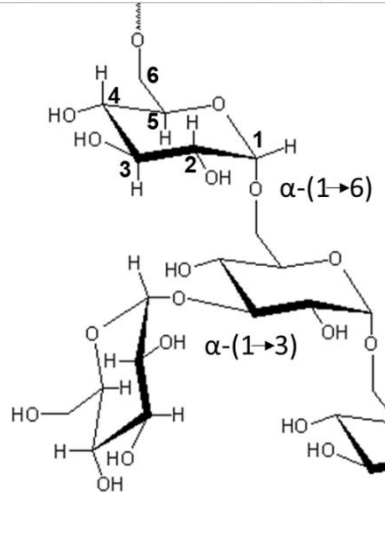
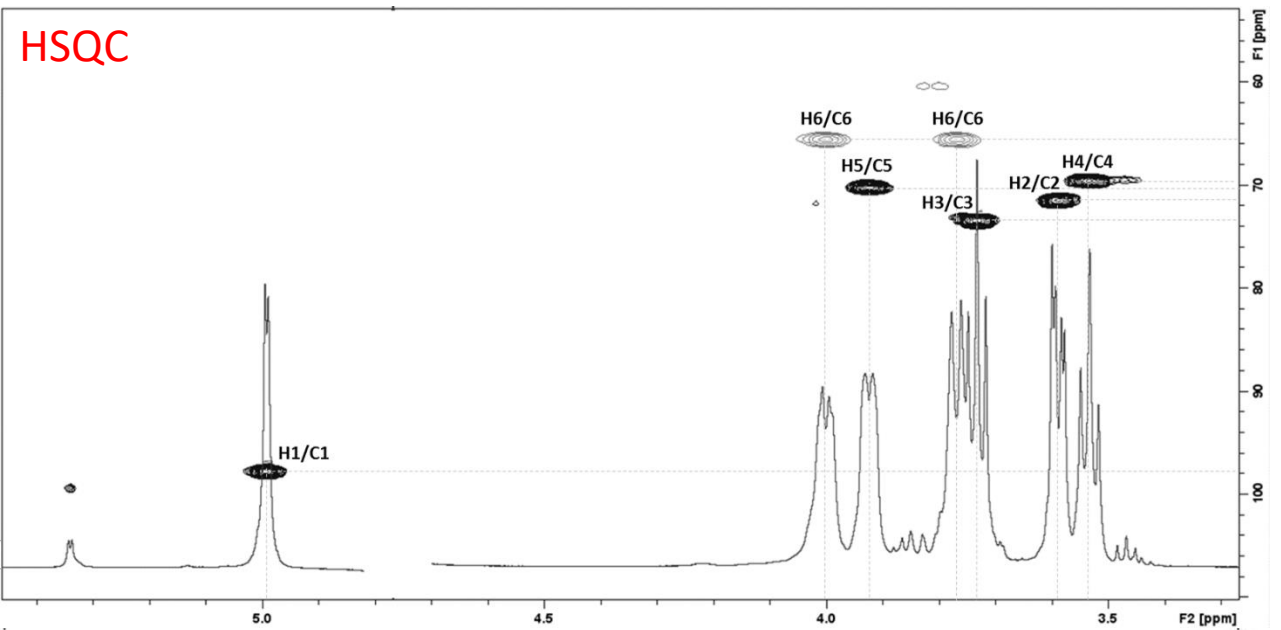
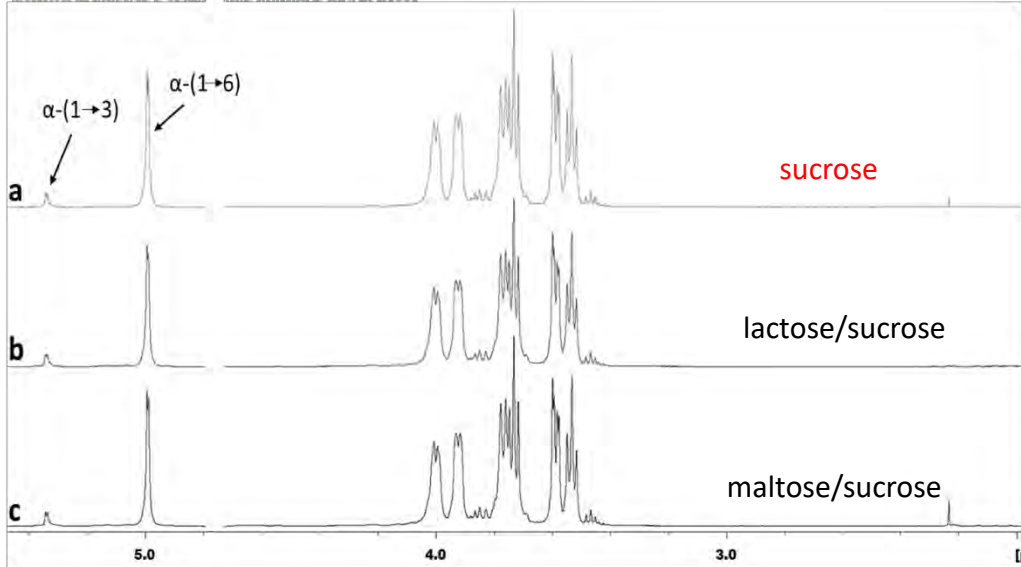


**EPS**

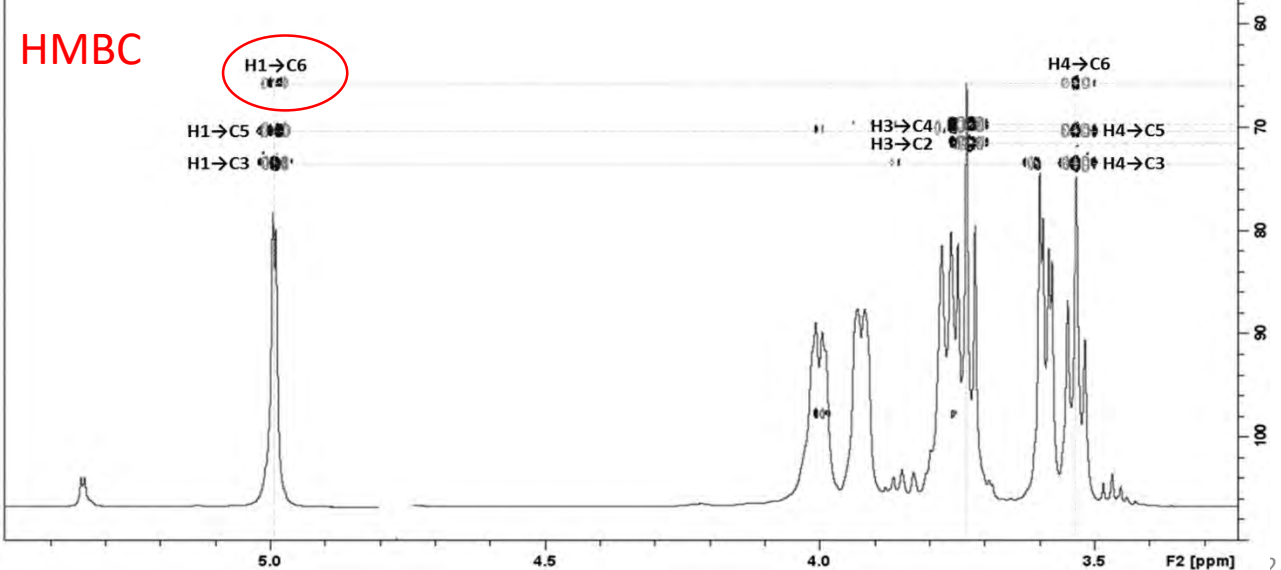
Dextran (1-6) Glu  
Mutan (1-3) Glu  
Alternan (1-6)/(1-3) Glu



# 1° case study : structural characterization of EPS by NMR



*W. confusa*



Dextran composed of (1  $\rightarrow$  6)-linked  $\alpha$ -D-glucose units containing (1  $\rightarrow$  3)-linked  $\alpha$ -D-glucose branches. (9%, from the relative integral ratio)



# Delivery of bioactive molecules by NMR

Hydrogels and cryogels were synthesized modifying starch from pea pods; they were able to deliver active molecules (volatile molecules, essential oils, poorly water soluble)



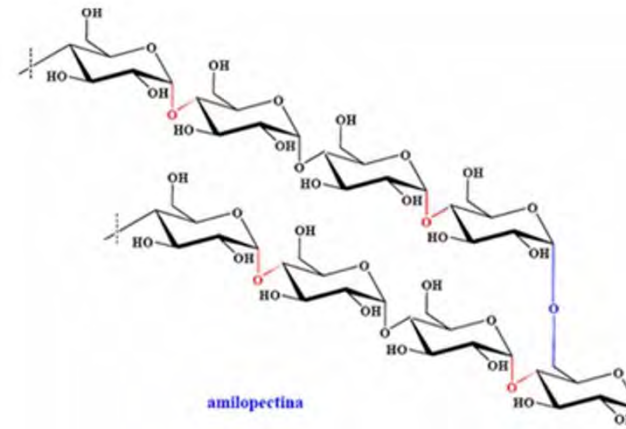
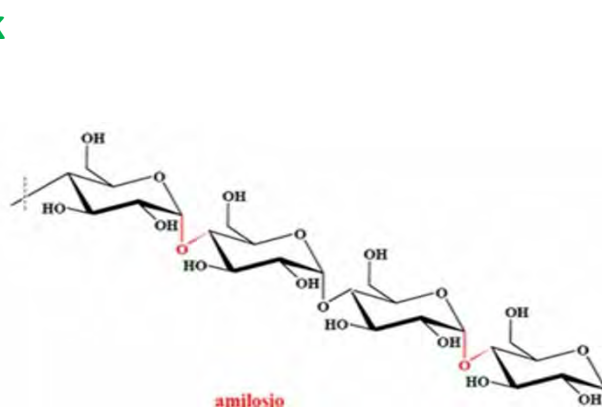
Chemical modification



Starch from pea pods  
as feedstock

cryogel

hydrogel



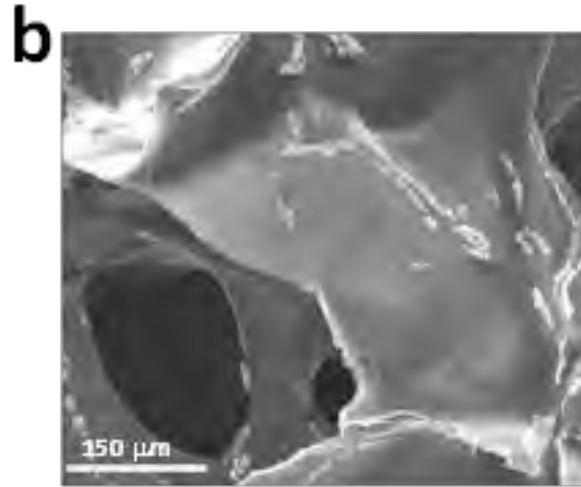
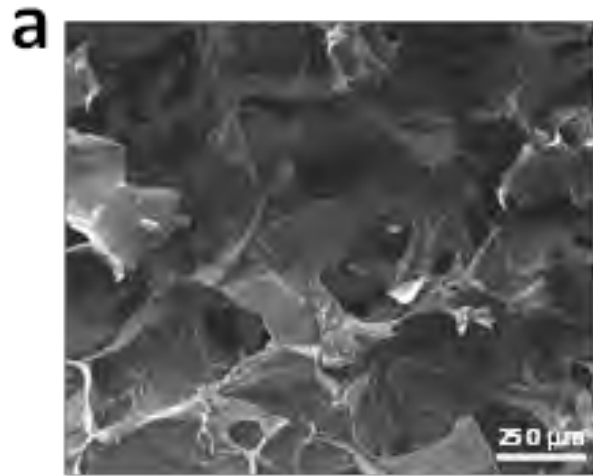
Starch is composed by amylose and amylopectin in a ratio dependent to the botanical origin

NMR enables:

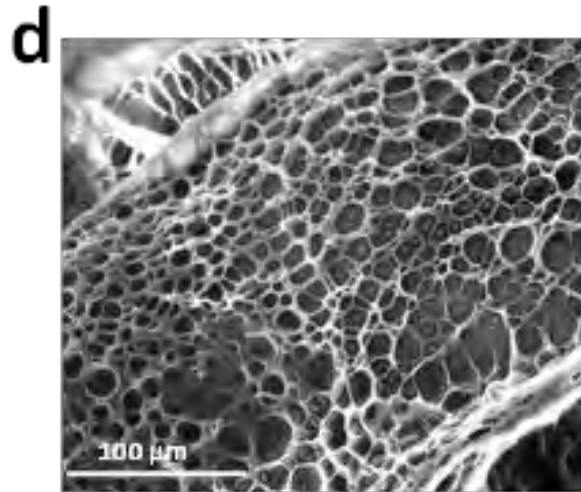
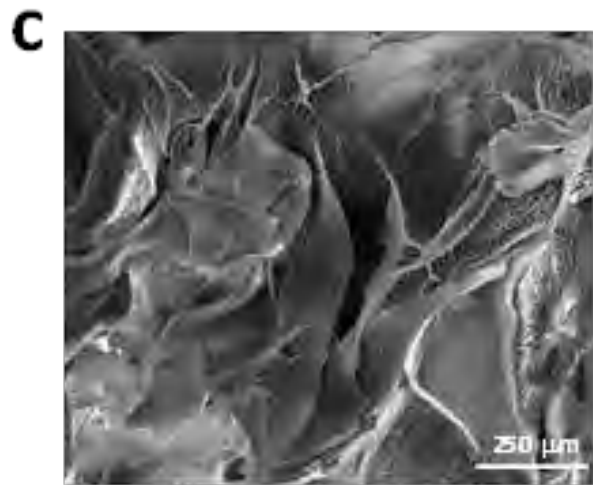
- Materials characterization
- Evaluation of drugs release profiles

**Antonella Caterina Boccia**  
antonella.boccia@cnr.it





Pea pods



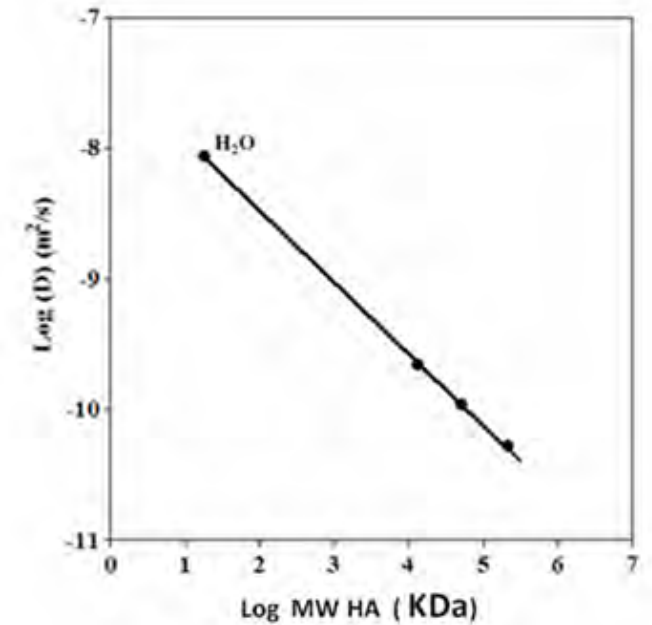
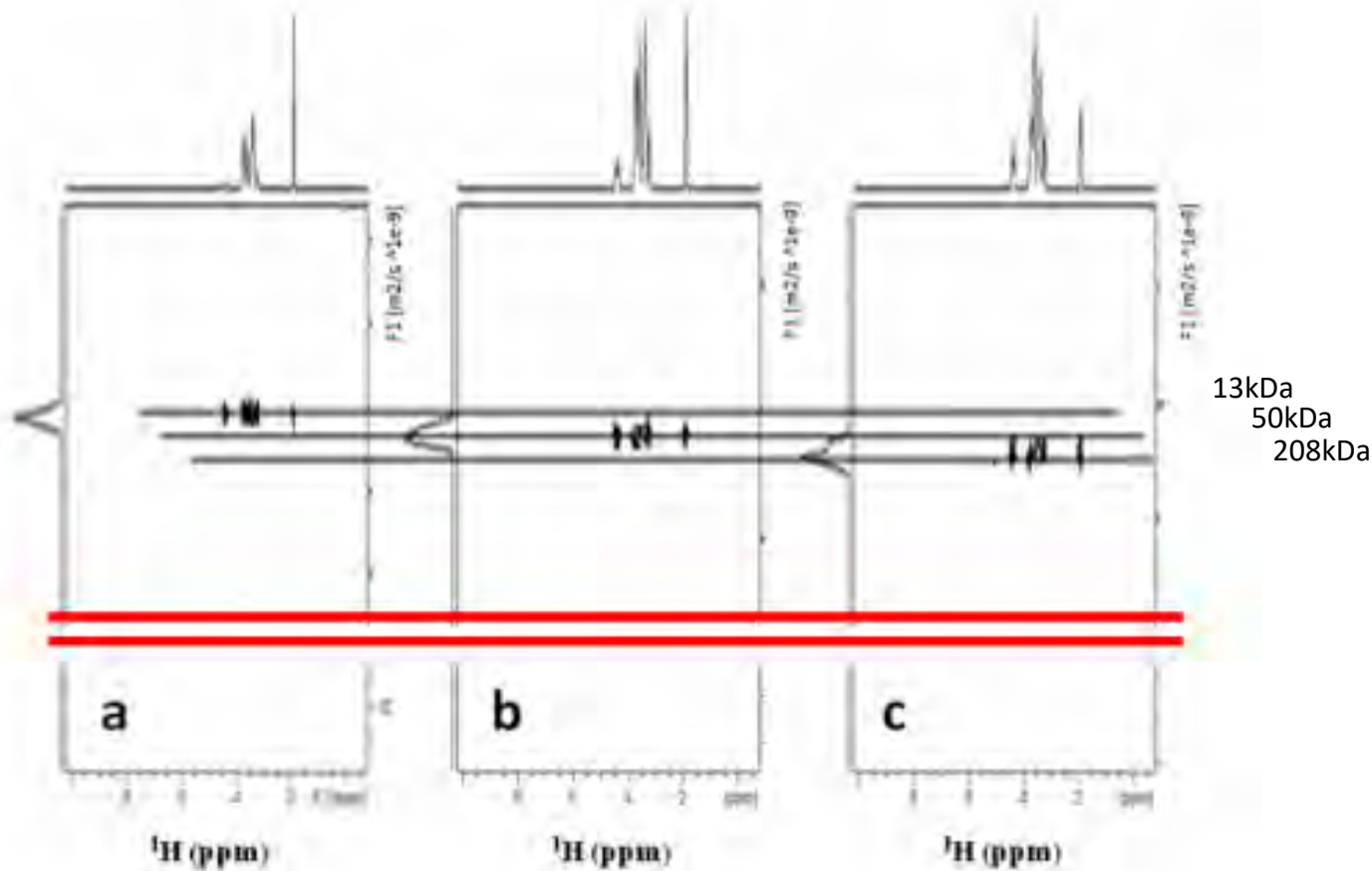
Potato peels

Both materials showed a compact solid structure, with non-homogeneous pores and cavities; moreover, the SEM analysis confirmed the absence of the globular structure expected in the case of native starch sample

SEM images



HA-PLA DAC® Hyaluronic-Polylactide Based Hydrogel

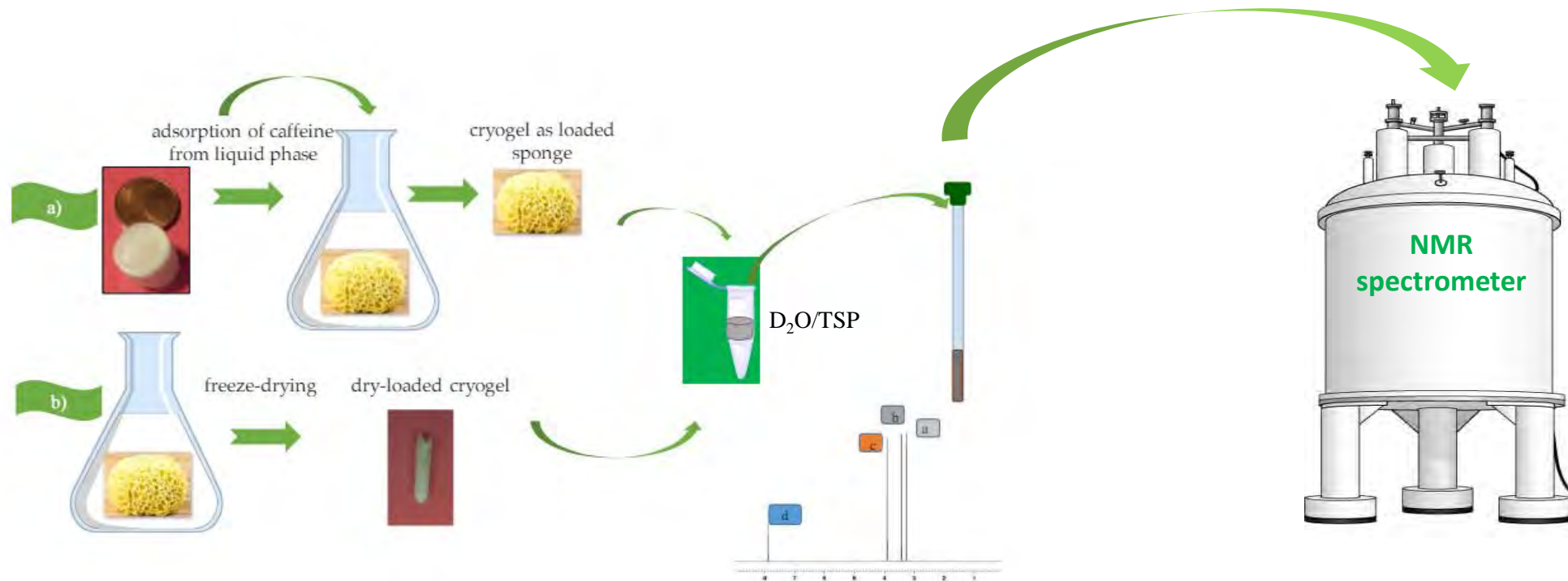


# Delivery of biomolecules - 1<sup>st</sup> case study: quantification of Caffeine

Experimental scheme showing the procedure for the sorption/desorption of caffeine

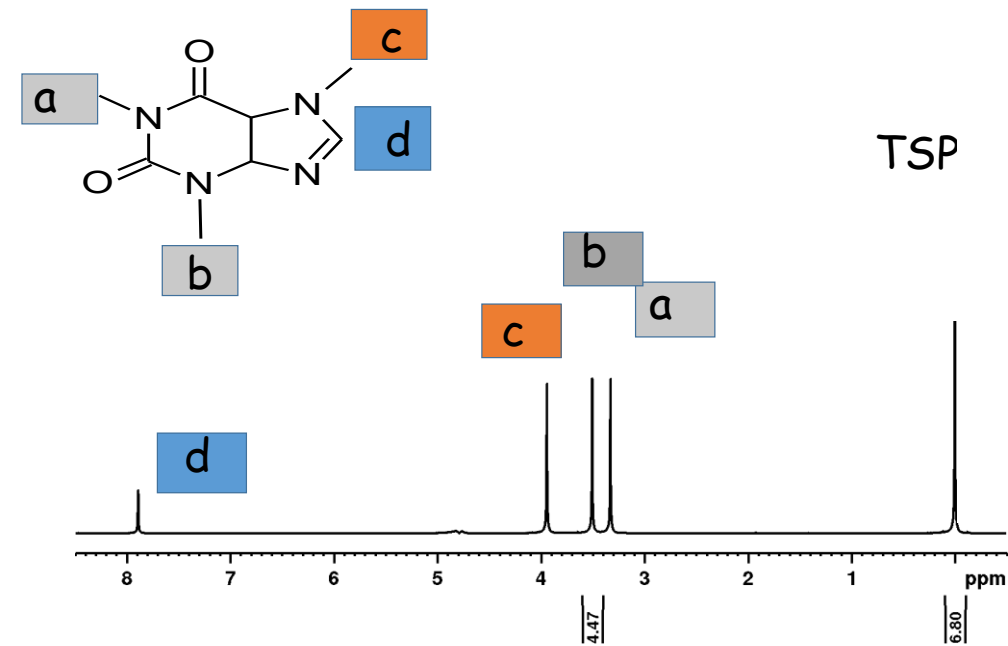
Enzymatically modified materials: oxidative process on C6

Electrostatic interaction with functional group

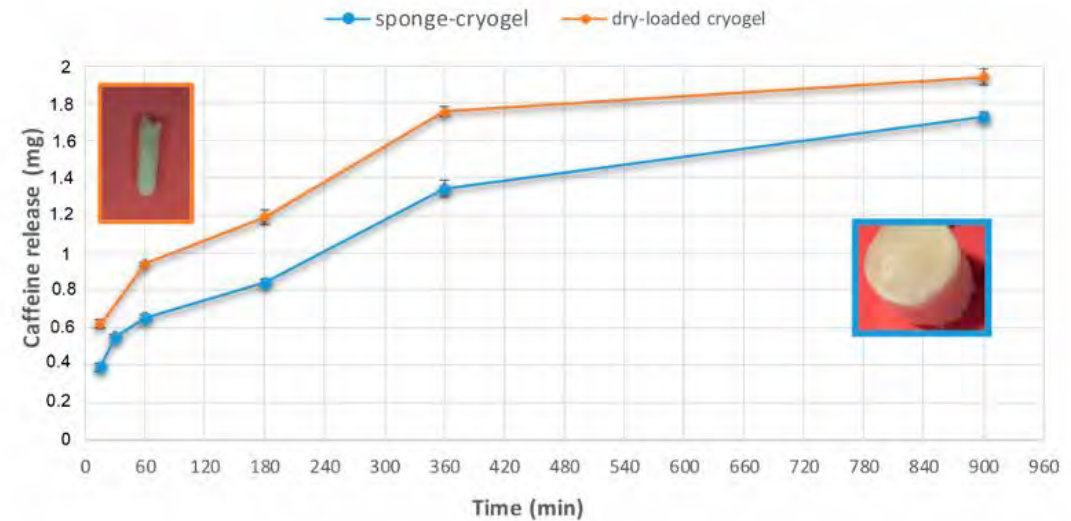


## Quantification

$$[mM]_c = I_c / H_c [mM]_{st} \cdot H_{st} / I_{st}$$



## Release profile from NMR data

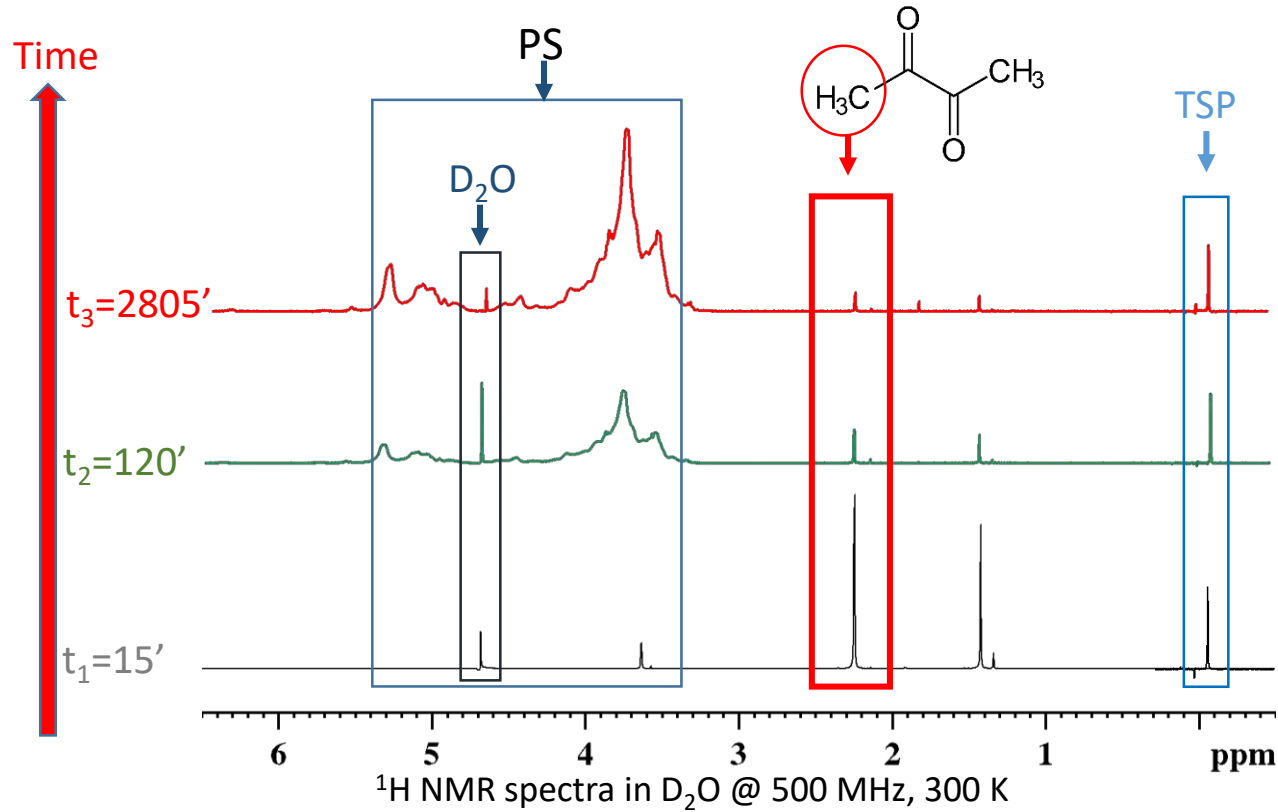


Percentage/mg medium values of the cumulative caffeine release versus time for wet and dry-loaded samples

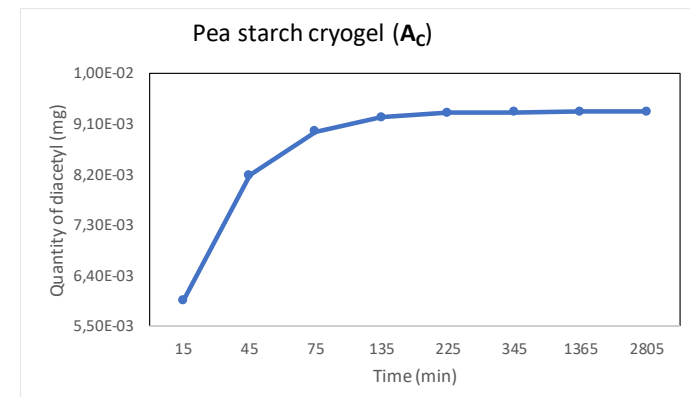
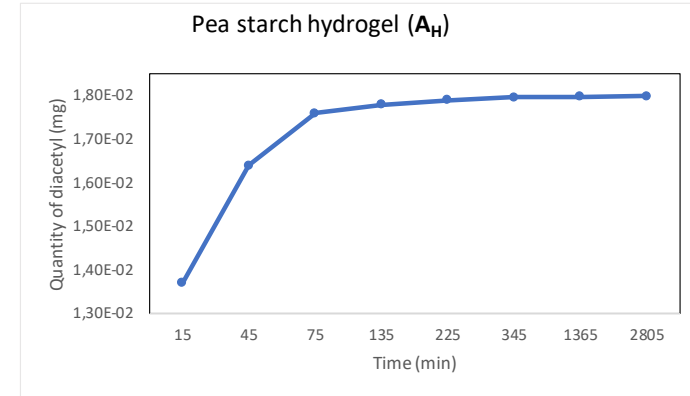


T ambient, buffered

Chemically modified material: opening of saccharide, lowering the MW



Up to 9 adsorption/desorption processes  
Diacetyl as antimicrobial



Potato

Quantity of diacetyl (mg)

15

Potato

Quantity of diacetyl (mg)

15





GRAZIE PER L'ATTENZIONE

