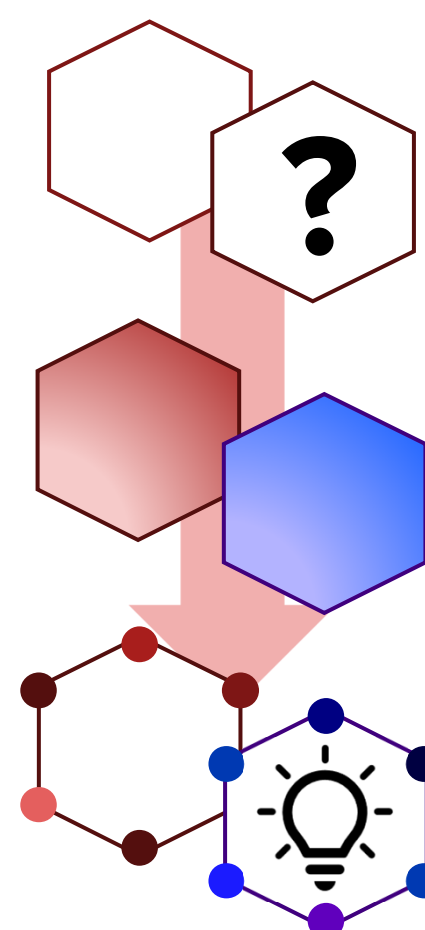




^{13}C isotope analysis with NMR

Potential challenges and improvements

Why Isotope Analysis?



In food products, the origin is often used as a selling point („natural flavor“ mentions for example). But if there is **identical molecule**, how can one be sure of its origin?

Isotope analysis is the study of the proportion of certain isotopes (often ^{13}C) in a molecule. A different signature is obtained depending on the origin.

NMR allows us to look at this isotopic signature for each position within a molecule for a better discrimination.

Margot Sanchez^{1,(2)}, Elisabeth Wiesenberg¹, Bernd Diehl¹

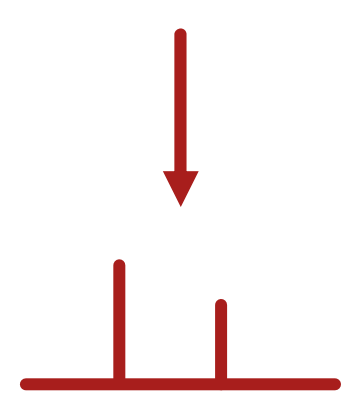
¹ Spectral Service AG, D-50996 Cologne

² (previously) Nantes University, F-44322 Nantes

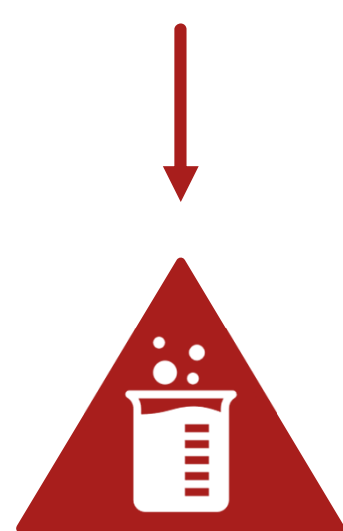
Challenge



High precision
RSD $\leq 0.2\%$



High signal to noise ratio



High concentration

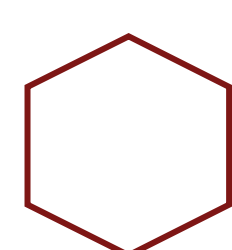


Long experiments

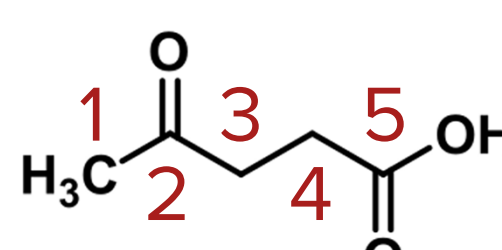


What about poor solubility?

Carbohydrates, such as **starch** or **cellulose** are difficult to analyze in high concentration because of their poor solubility. Here, the hydrolysis and the methanolysis into **levulinic acid (LA)** and **methyl levulinate (ML)** is investigated as a mean to improve the solubility.

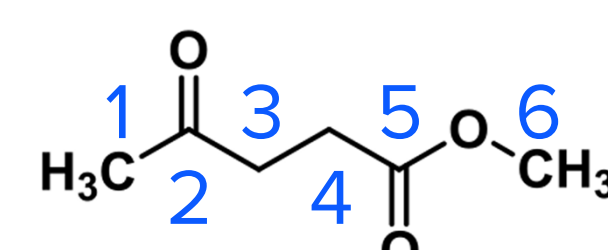


H_2O , HCl



CH_3OH ,

methanesulfonic acid

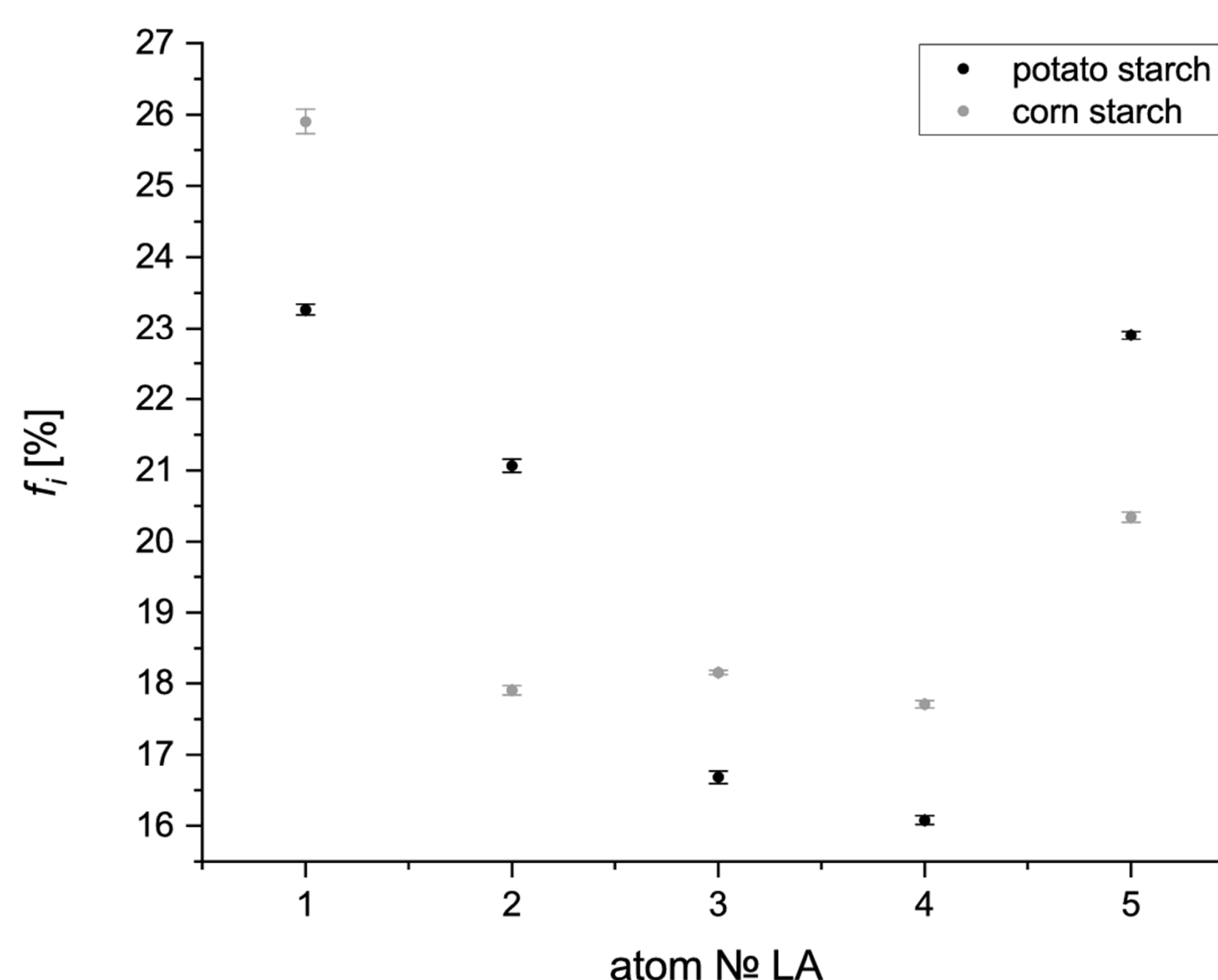


Carbohydrate

LA

ML

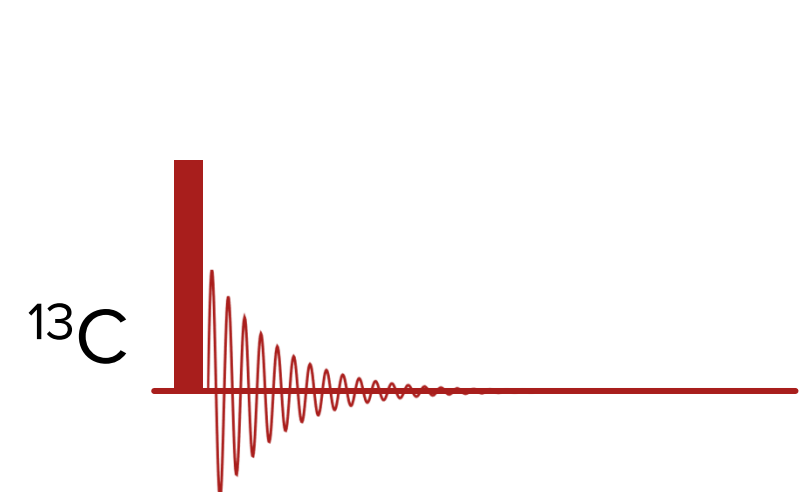
Is it possible to differentiate LA and ML synthesized from carbohydrates of different origins?



- Two origins seem to be distinguishable
- Results seem to be influenced by the addition of $\text{Cr}(\text{acac})_3$
- Although ML does not yield more information than LA, the added **methyl group** could be used as an **intra-molecular standard**



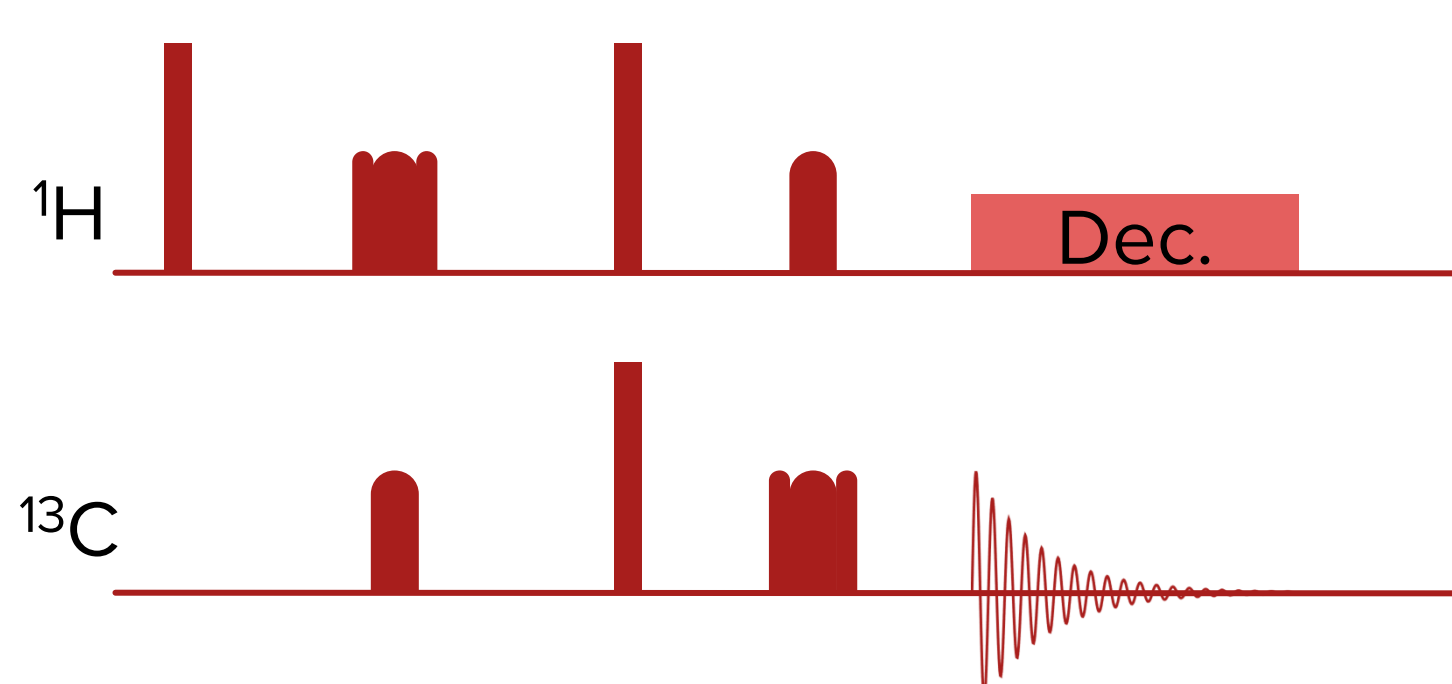
How to shorten the experiments?



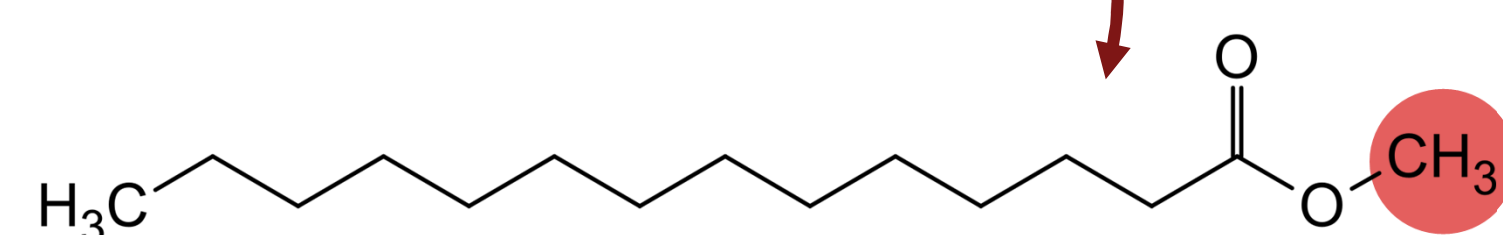
Single pulse

Sample quantity $\div 5$

Experiment time $\div 10$ [1]



Refocused INEPT



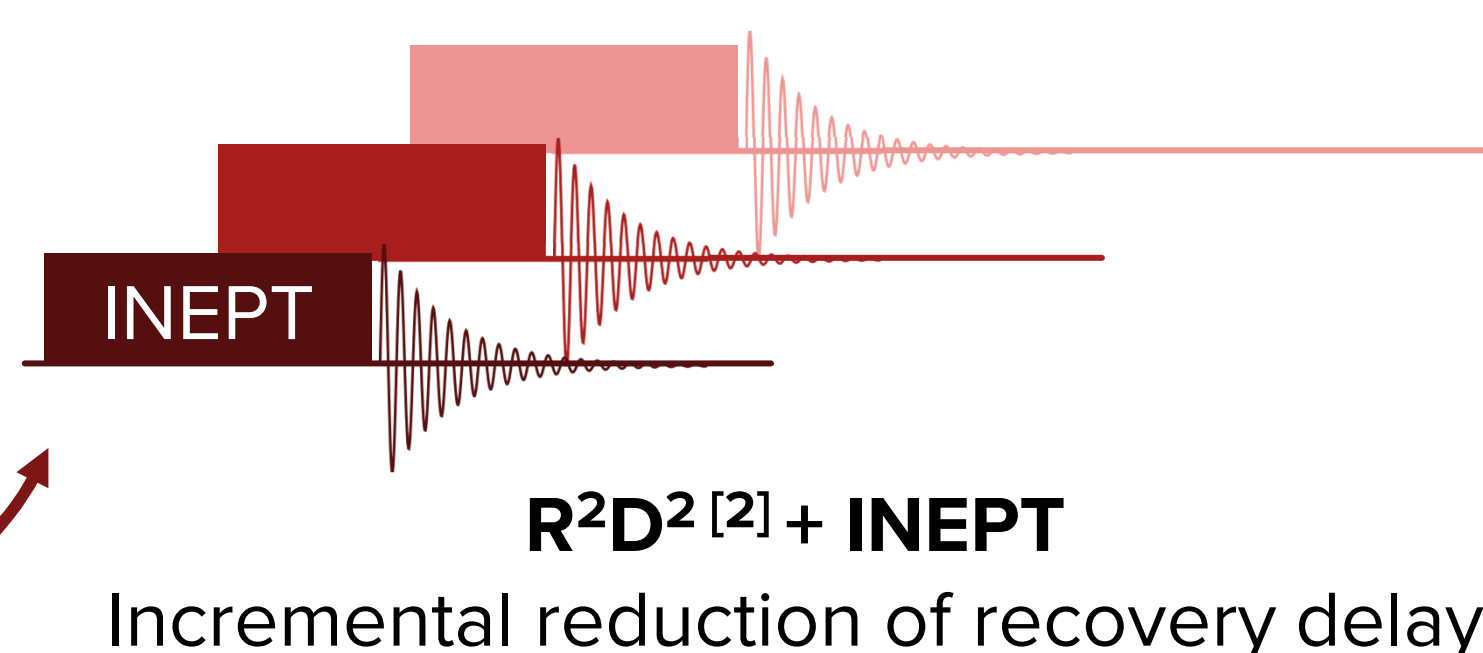
Intra-molecular reference

Methyl of known isotopic composition added to fatty acids

- Use of the INEPT can greatly **reduce the experiment time**
- Consistent results of intra-molecular reference vs classic method (use of global isotopic composition of the sample)

Outlook

- Intra-molecular reference approach could be extended to ML sample
- Further developments can be performed to make the experiments faster



References

- [1]: Mhanna, T., *et al.*, *Anal Bioanal. Chem.* **2025**, 417, 6731–6742
- [2]: Sanchez, M. *et al.* ; *Talanta*, **2024**, 276, 126157