Compound-specific analysis for the verification of food authenticity

LUANA BONTEMPO

TRACEABILITY UNIT
FONDAZIONE EDMUND MACH
ITALY

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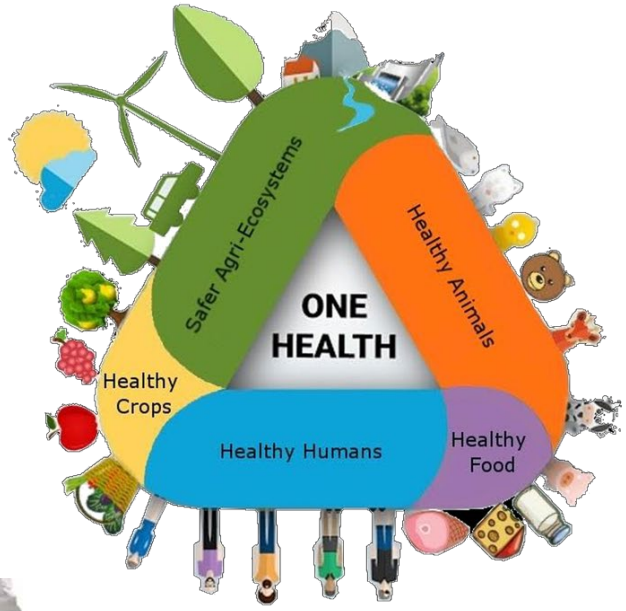
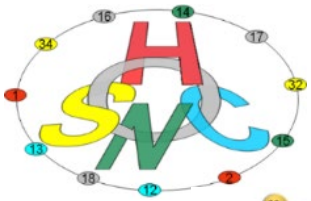
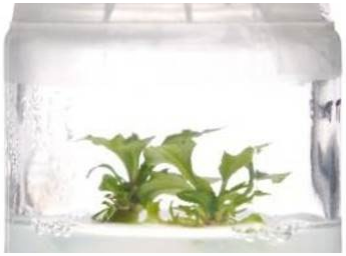
ISO Fd Food ERA Chair Safety Quality Traceability

FONDAZIONE EDMUND MACH
TRACEABILITY UNIT







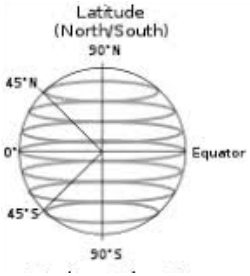
Stable isotope ratio analyses: what can they tell us?

HYDROGEN

Geographical origin

Climatic characteristics

Synthetic vs. natural



OXYGEN

Geographical origin

Climatic characteristics

NITROGEN

Botanical species

Fertilization practices

Pedological origin

CARBON

Botanical species

Synthetic vs. natural

Geographical origin

SULFUR

Geological origin

Fertilization practices

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Stable isotope ratios: official methods

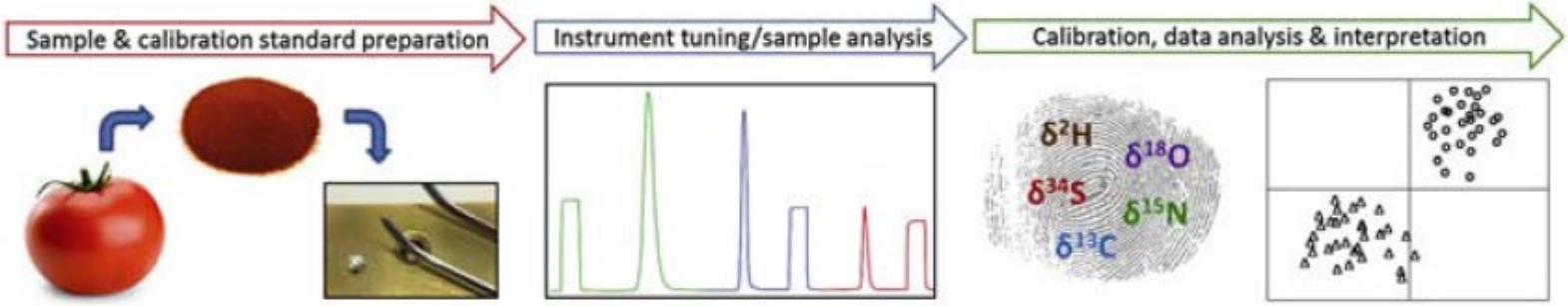
Year	Method	product	Method	Isotope Ratio	Fraud
1987	OIV	wine, must	SNIF-NMR	D/H	sugar addition (beet, cane)
1990	EU Reg 2676/90, encl. 8	wine, must	SNIF-NMR	D/H	sugar addition (beet, cane)
1991	AOAC 998,12	honey	IRMS	$^{13}C/^{12}C$	sugar addition (cane)
1993	ENV 12140, 13070	fruit juice	IRMS	$^{13}C/^{12}C$	sugar addition (cane)
1995	AOAC 995,17	fruit juice	SNIF-NMR	D/H	sugar addition (beet, cane)
1996	OIV 2/96	wine, must	IRMS	$^{18}O/^{16}O$	addition of water/mislabelling
1997	EU Reg 2676/90, 822/97	wine, must	IRMS	$^{18}O/^{16}O$	addition of water/mislabelling
1997	ENV 12141	fruit juice	IRMS	$^{18}O/^{16}O$	addition of water/mislabelling
2000	AOAC 2000.19	maple syrup	SNIF-NMR	D/H	sugar addition (beet, cane)
2000	OIV 71/2000	vinegar	SNIF-NMR, IRMS	D/H, $^{13}C/^{12}C$	sugar addition (beet, cane)
2001	OIV 17/2001	wine, must	IRMS	$^{13}C/^{12}C$	sugar addition (cane)
2003	EU Reg. 2676/90, 440/03	wine, must	IRMS	$^{13}C/^{12}C$	sugar addition (cane)
2003	OIV MA-F-AS314-03	wine	IRMS	$^{13}C/^{12}C$	technogenic CO_2
2004	AOAC 2004,01	fruit juice, m	SNIF-NMR	D/H	sugar addition (beet, cane)
2006	AOAC 2006,05	vanillin	SNIF-NMR	D/H	synthetic vanillin
2007	OIV-MA-AS312-07	wine	IRMS	$^{13}C/^{12}C$	addition of glycerol
2011	EU Reg 584/2011	Grana Padano DOP	IRMS	D/H, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$	mislabelling
2013	EN 16466-1, 2, 3	vinegar	SNIF-NMR, IRMS	D/H, $^{13}C/^{12}C$, $^{18}O/^{16}O$	water and sugar addition (beet, cane)
2013	OIV 510, 511/2013	vinegar	IRMS	$^{13}C/^{12}C$, $^{18}O/^{16}O$	water and sugar addition (cane)





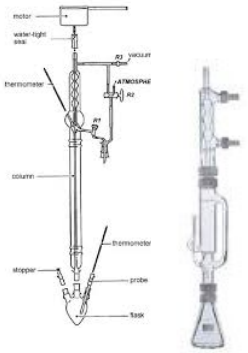


Stable isotope analysis: bulk analysis



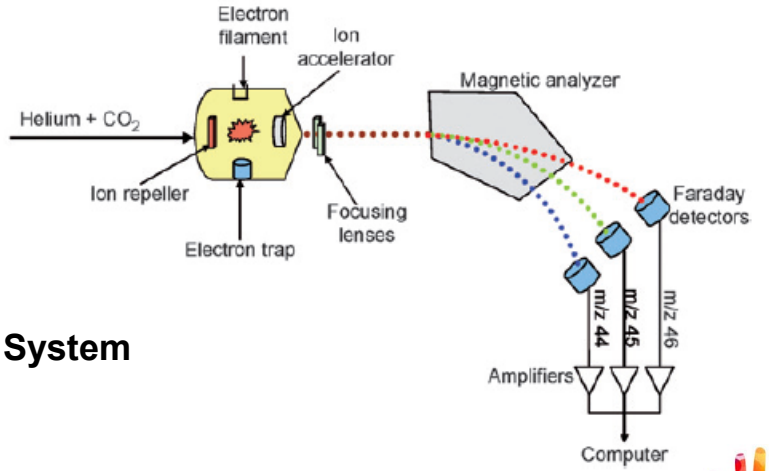
Laursen et al., 2016

Stable isotope analysis: from bulk to compound specific analysis

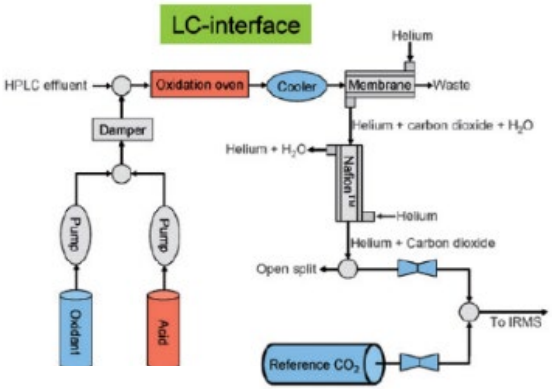


Isolation of single compounds or classes of compounds

Isotope Ratio Mass Spectrometer (IRMS)

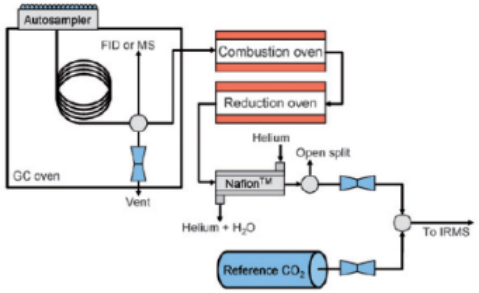


LC-interface



LC IRMS System

GC-interface



Chromatographic interface

GC IRMS System

Internal standardization

EA IRMS analysis $\delta^{13}C$ proteins

$\delta^{13}Cbulk - \delta^{13}Cproteins < 1‰$
C4 plants sugars added up to 7%

AOAC Official Method 998.12:
C-4 Plant Sugars in Honey

$$C4\ sugars\ \% = \frac{\delta^{13}C_{proteins} - \delta^{13}C_{honey}}{\delta^{13}C_{proteins} - (-9.7)} * 100$$

EA IRMS analysis $\delta^{13}C$ bulk honey

EA IRMS analysis $\delta^{13}C$ pulp

EA IRMS analysis $\delta^{13}C$ sugars

EA IRMS analysis $\delta^{13}C$ acids

AIJN Code of Practice

CSIA

Applications for food authentication

Illicit additions or substitutions

Organic versus Conventional products

Liu et al., 2023, Perini et al., 2022

Geographical origin

CSIA

Applications for food authentication

Illicit additions or substitutions

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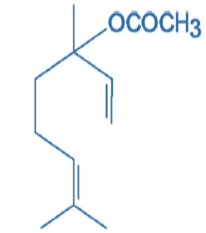
Liu et al., 2023, Perini et al., 2022

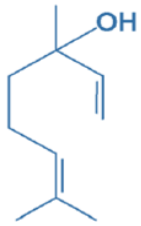
Geographical origin

Lavender essential oil

- Lavender essential oil
  - High-value product
  - Colourless liquid
  - Reminiscent flavour
  - *L. angustifolia*
  - > 170 VOCs



Linalyl acetate



Linalool

Cosmetics

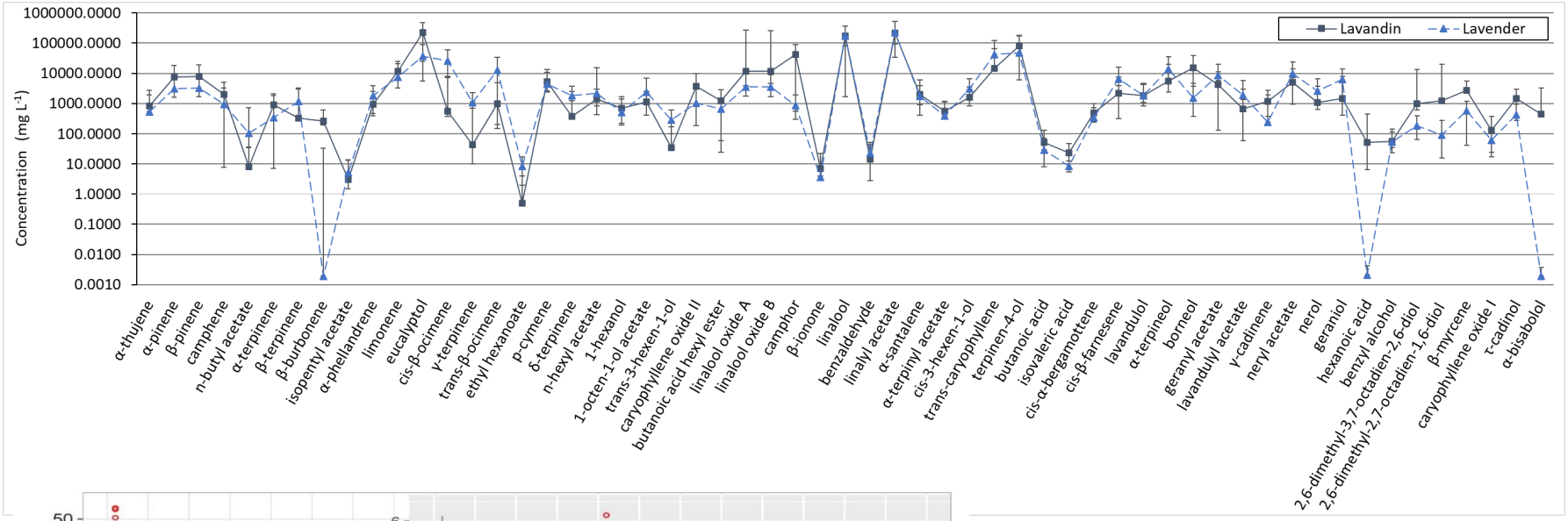
Fragrance

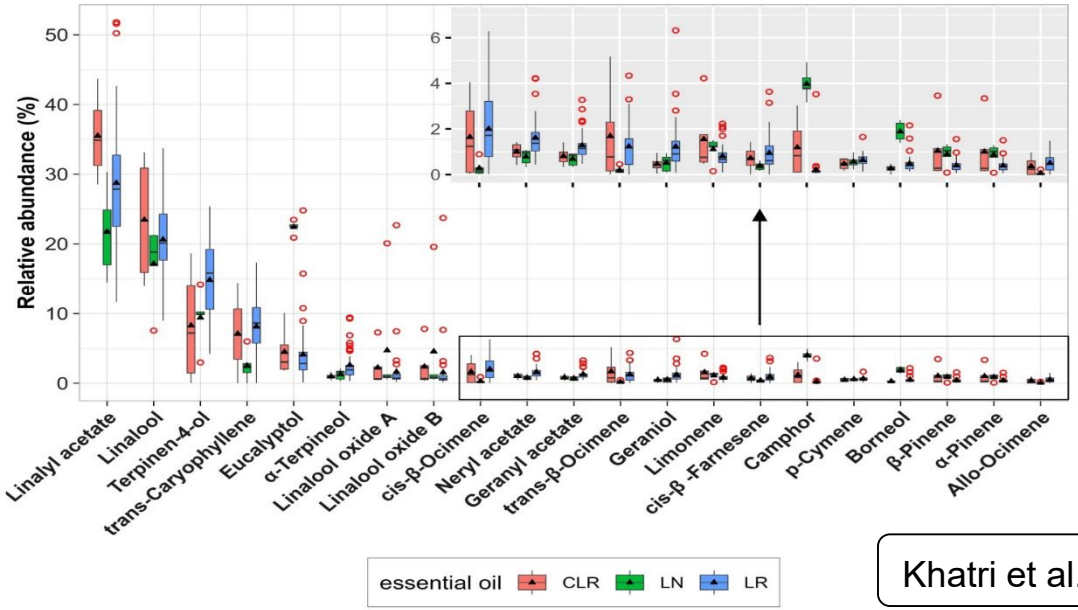
Uses

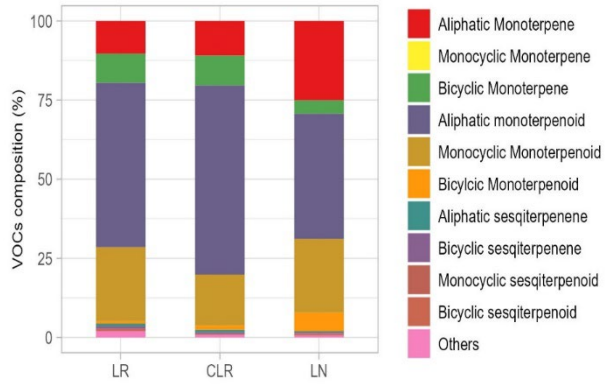
Flavour + preservatives

Therapeutic

Lavender essential oil – VOCs composition

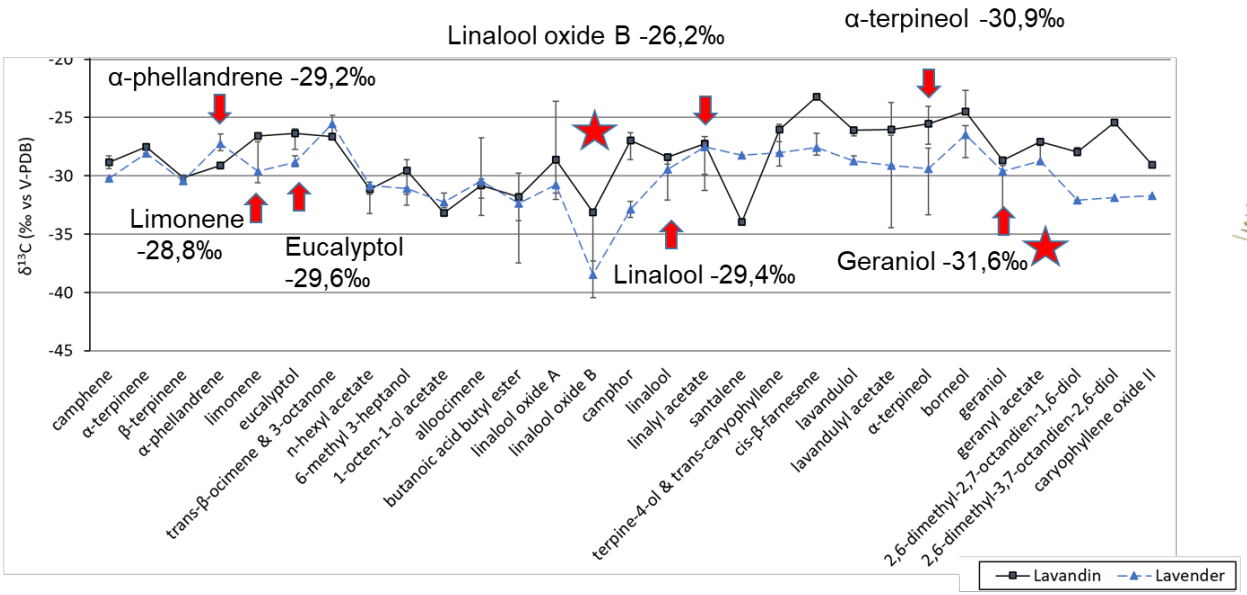




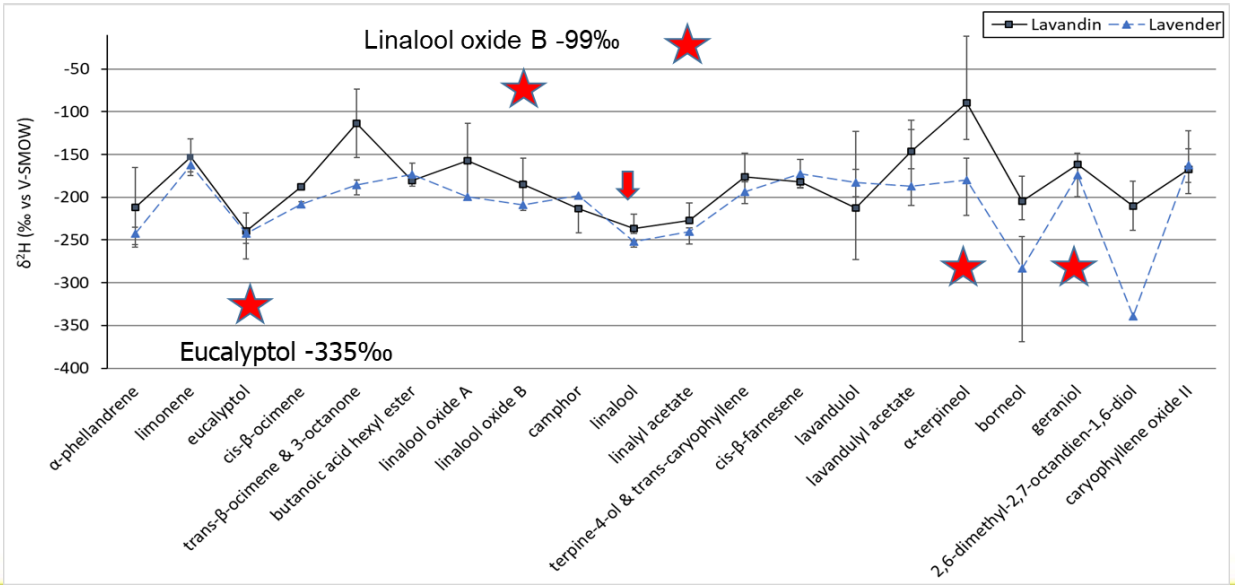


Khatri et al., 2023

Lavender essential oil – CSIA of VOCs

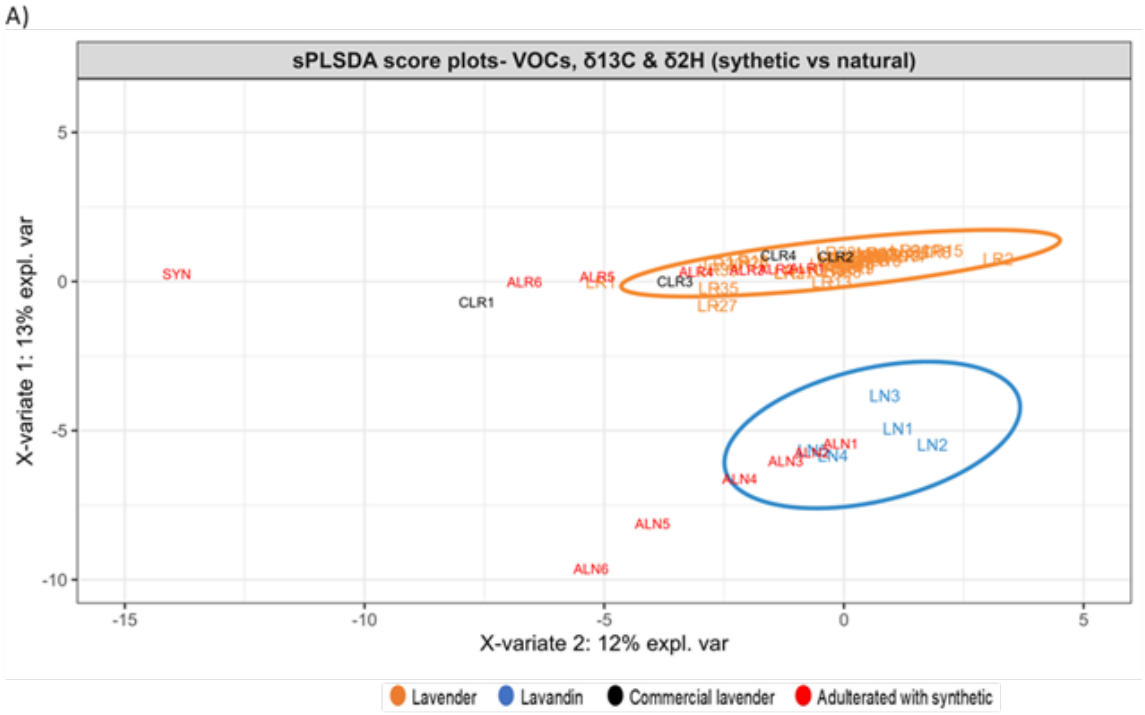


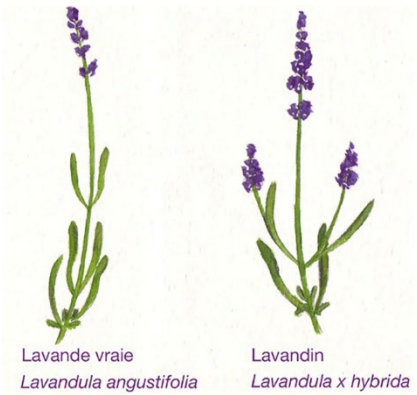
Linalyl acetate -29‰



Khatri et al., 2023

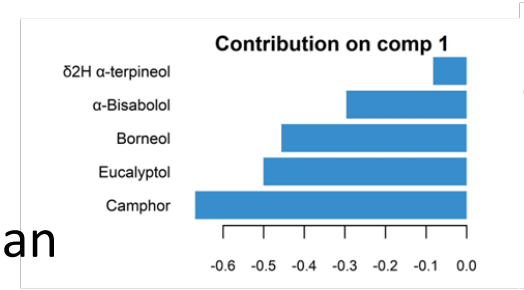
Lavender essential oil – VOCs composition + CSIA

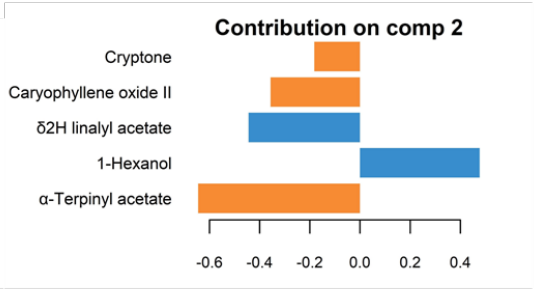




- Botanical species

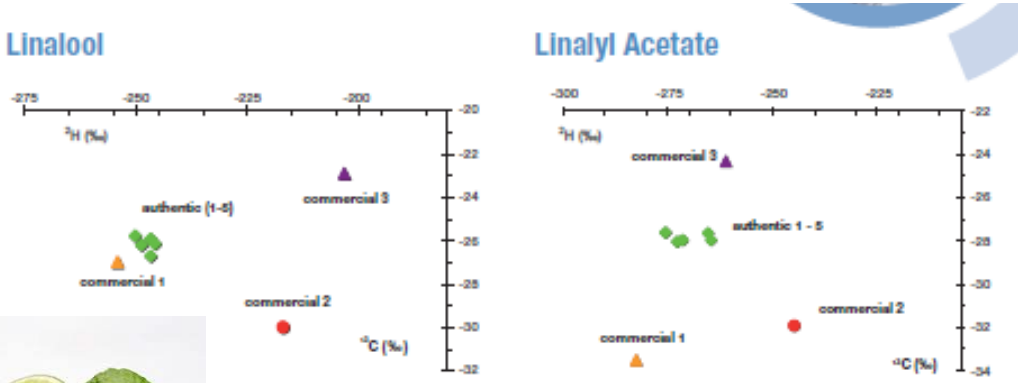
- Synthetic vs Natural - more than 15% adulteration detected



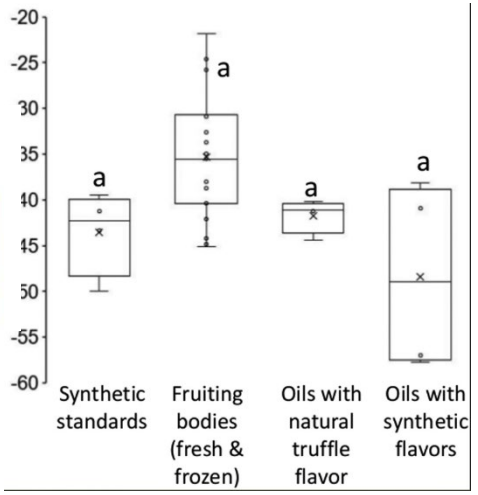


Khatri et al., 2023

Adulteration with synthetic compounds or other botanical species

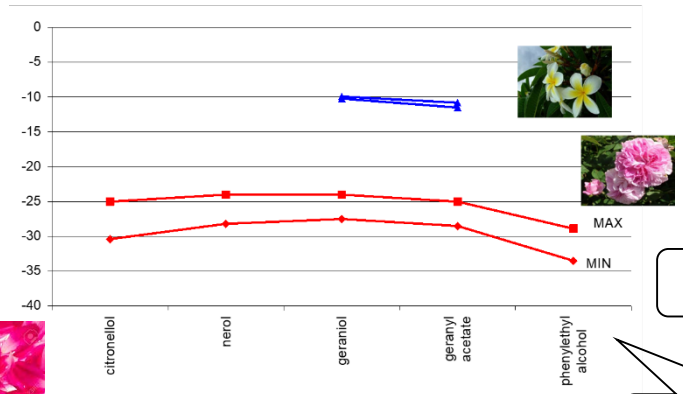


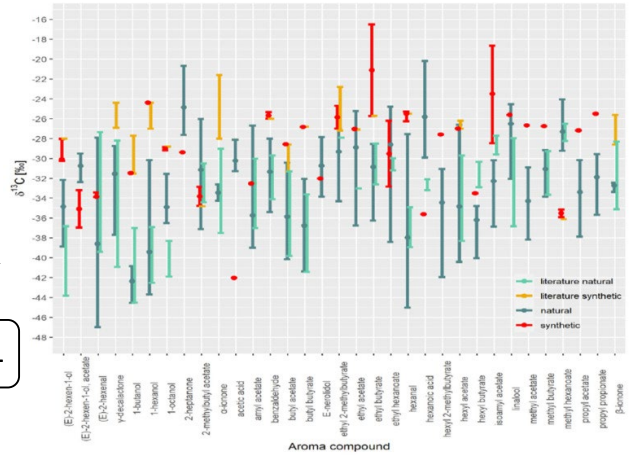
Schipilliti et al., 2011
www.thermofisher.com



Wernig et al., 2018

	geraniol content	geranyl acetate content
Rosa Damascena	10-20%	1%
Palmarosa	80%	10%





Strojnik et al., 2019 and 2021

Camin et al., 2013

CSIA

Applications for food authentication

Illicit additions or substitutions

Organic versus Conventional products

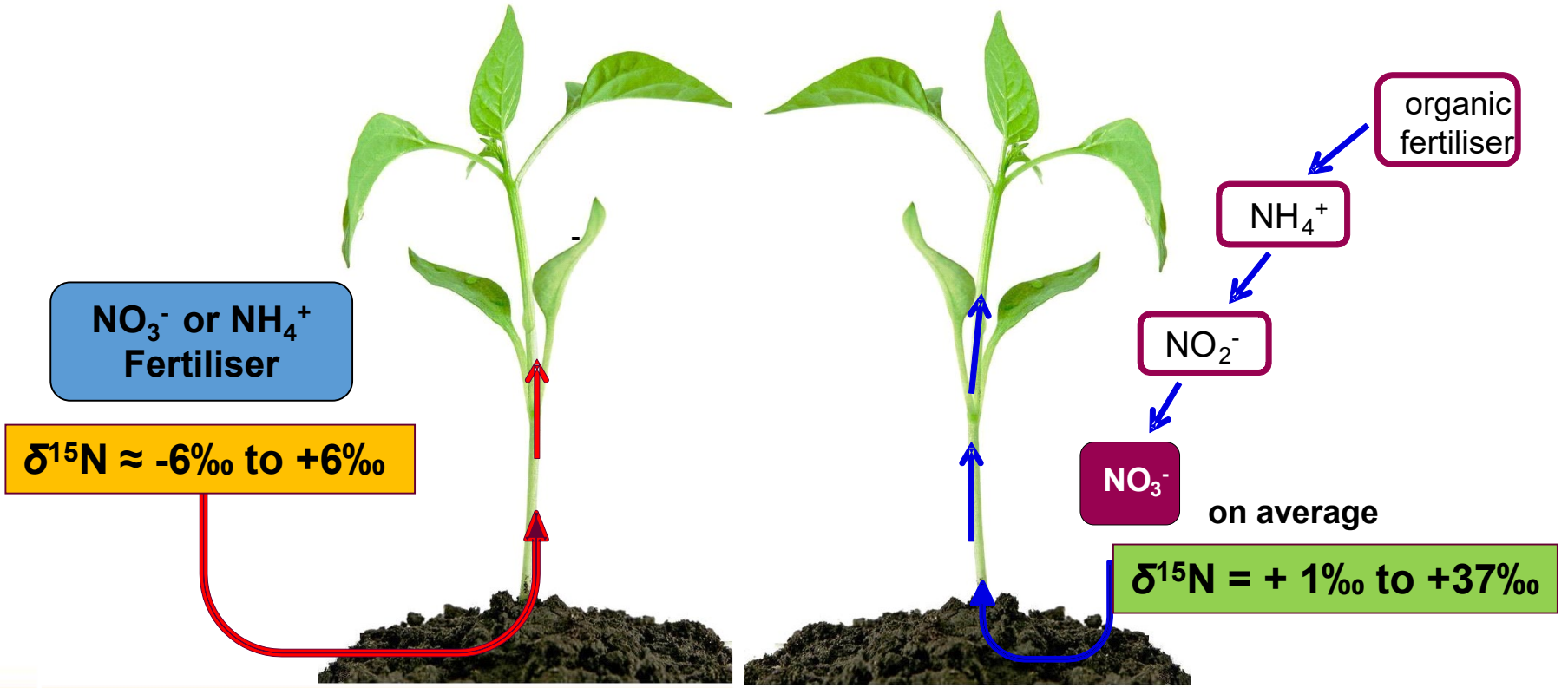
Geographical origin

Liu et al., 2023, Perini et al., 2022

$^{15}N/^{14}N$ for distinguishing organic from conventional food

CONVENTIONAL

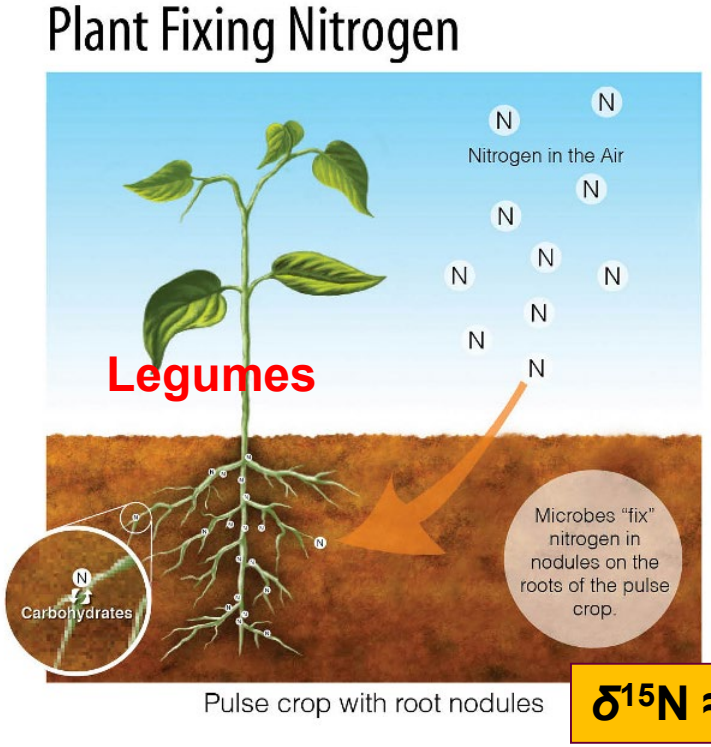
ORGANIC



Bateman et al, J. Agric. Food Chem., 2007

Limitation on the application of $^{15}N/^{14}N$ analysis

N2-fixing plants (the Leguminosae family) have $\delta^{15}N$ overlapping with that synthetic fertilisers

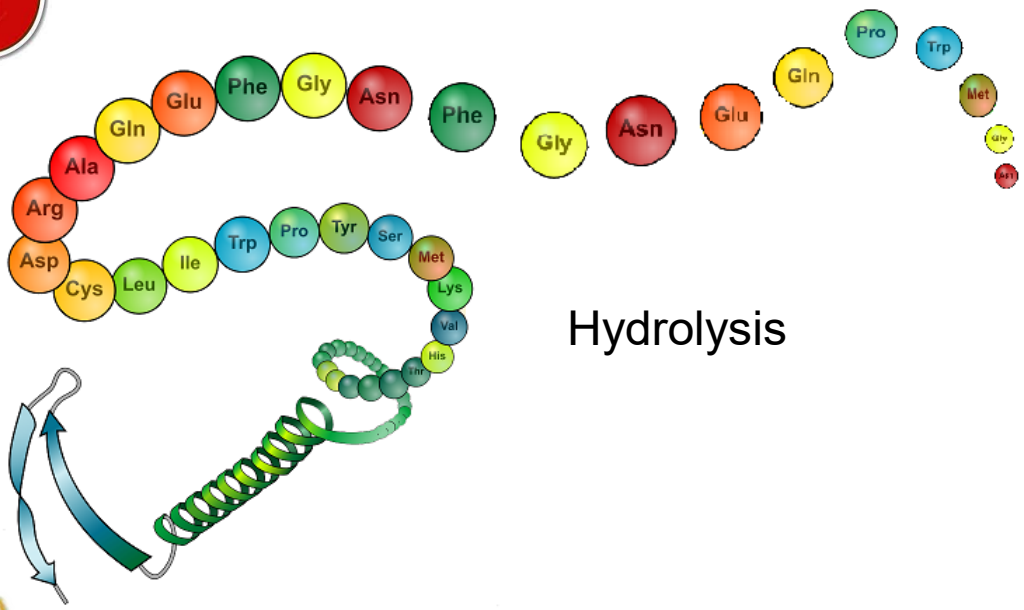


Paolini et al., 2015

$\delta^{15}N \approx 0‰$

This calls for the development of novel analytical methods for authenticity testing

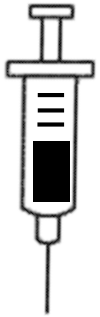
Compound-specific $\delta^{15}N$ and $\delta^{13}C$ analysis of amino acids

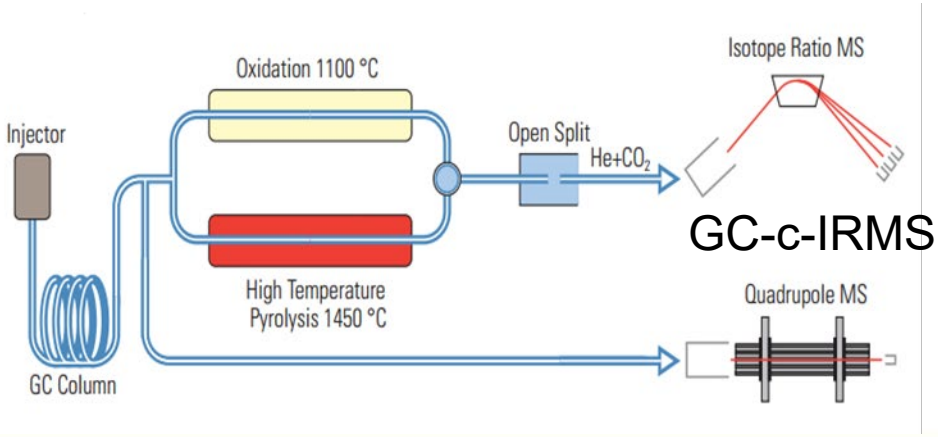


Hydrolysis

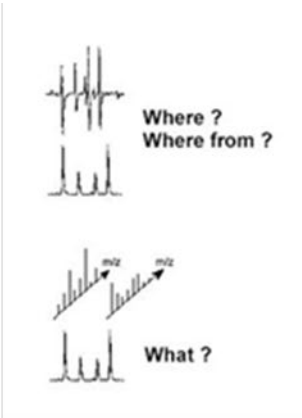
Paolini et al., 2015

Derivatization



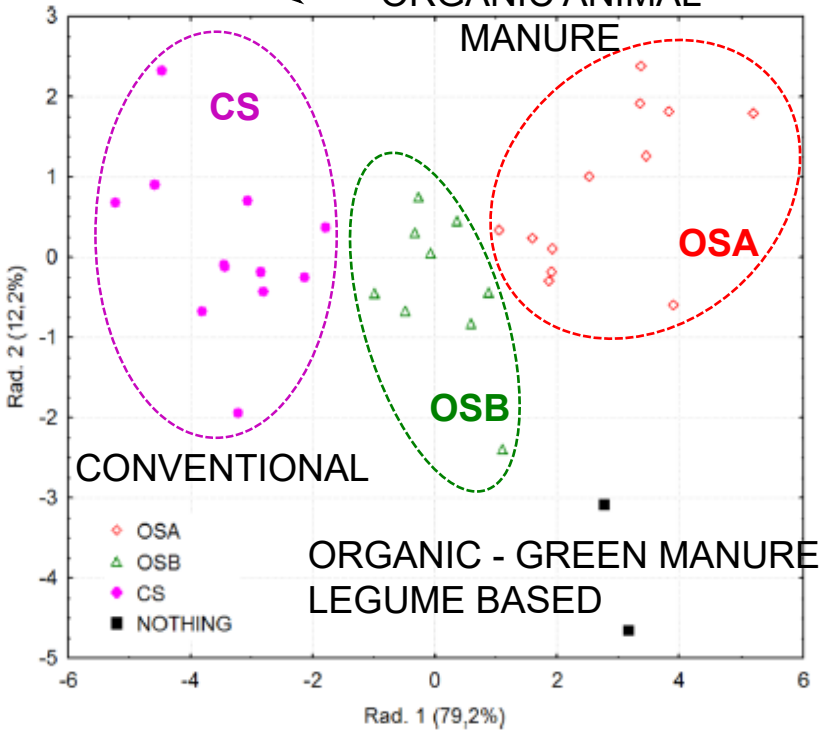


GC-c-IRMS

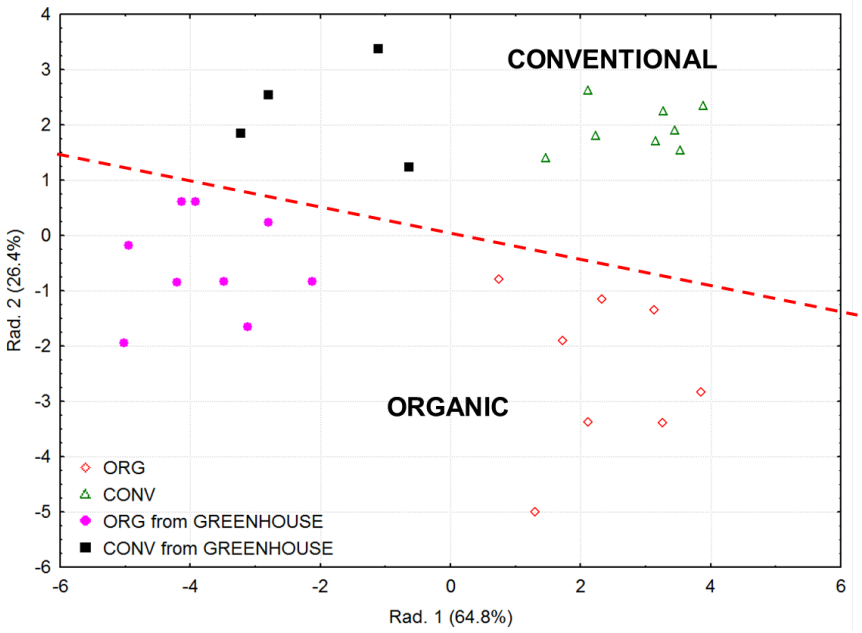


$\delta^{15}N$ and $\delta^{13}C$ analysis of amino acids - organic and conventional wheat and tomatoes samples

Paolini et al., 2015



Bontempo et al., 2019



FoodTraNet*

Current research on different cereals

*This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 956265

Application of innovative methods for the traceability of organic farming products: the INNOVABIO project (MIPAAF Decree n. 93173/2017)

InnovaBio

The studied crops are representative of the Italian horticulture: date tomatoes in greenhouse, fennel and cauliflower in open field. Experimental fields are located in three Italian typical production areas.

Experimental plan

Treatments

1. “ORGANIC”: only organic nitrogen fertilizers on a soil that has not undergone any chemical treatment for several years;

2. “MIX - ORGANIC: 1/3 organic N + 2/3 mineral N" on a soil that has not undergone any chemical treatment for several years;

3. “CONVENTIONAL”: only mineral N on a soil that has always been managed using conventional techniques;

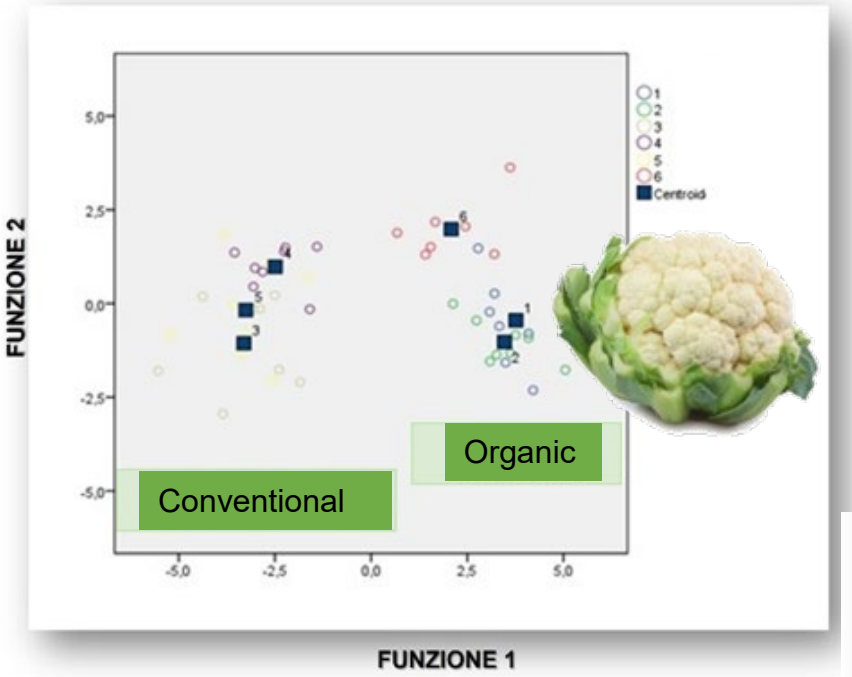
4. “MIX - CONVENTIONAL : 1/3 organic N + 2/3 mineral N" on a soil that has always been managed using conventional techniques;

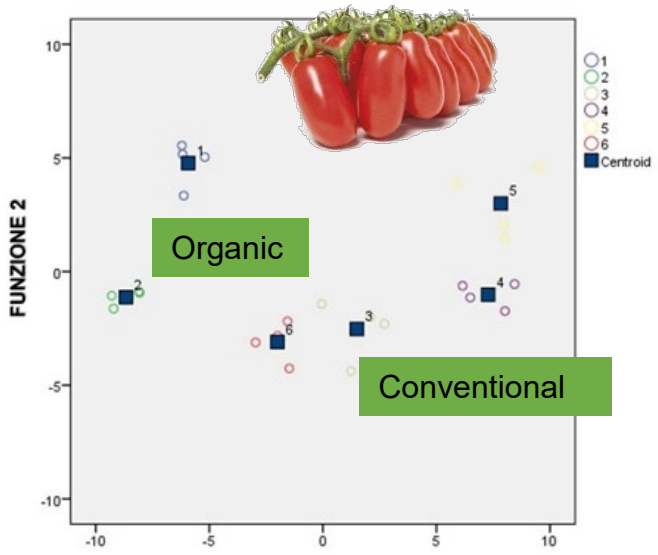
5. "MIX - CONVENTIONAL : 2/3 organic N + 1/3 mineral N" on a soil that has always been managed using conventional techniques;

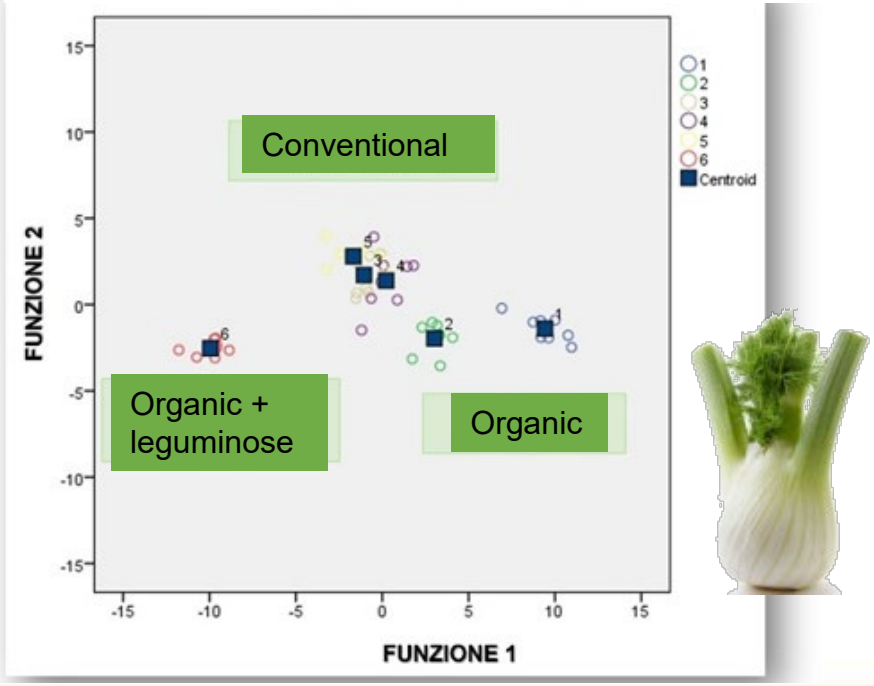
6. “ORGANIC + AGRO-ECOLOGICAL PRACTICES“ (agro-ecological service crops).

Ascorbic acid;
In-vitro antioxidant activity (ORAC, FC);
$\delta^{15}N_{bulk}$
$\delta^{15}N_{AAs}$
$\delta^{13}C_{AAs}$

Innovabio project – preliminary results







100,0% grouped cases correctly classified

CSIA organic vs. conventional products – other approaches

Nitrogen in Bulk

- High $\delta^{15}N$ ⇨Manure
- Low $\delta^{15}N$ ⇨Legume ⇨NPK

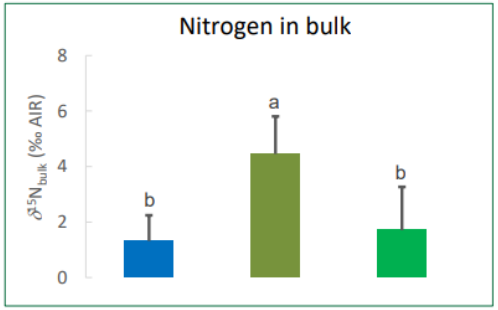
- Excellent marker for AM
- Fast

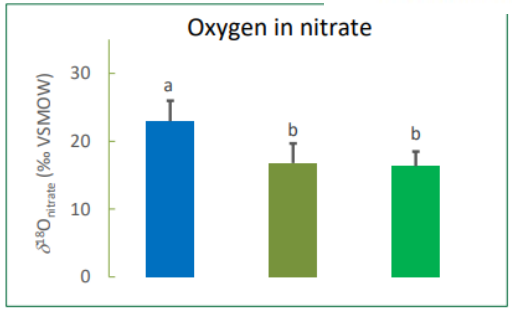
Oxygen in sulphate (or nitrate)

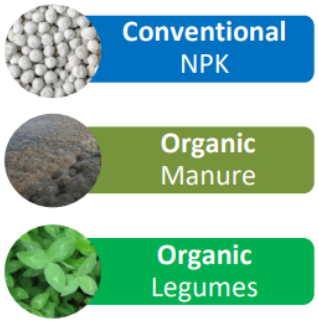
- High $\delta^{18}O$ ⇨NPK
- Low $\delta^{18}O$ ⇨Legume ⇨ Manure

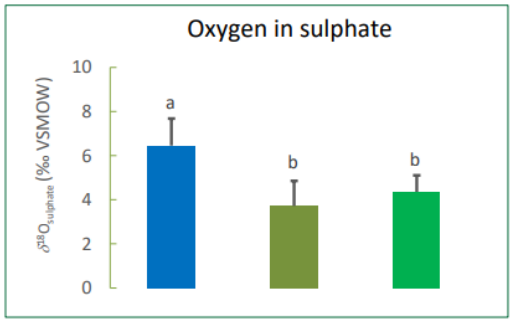
- Nitrate limited to potatoes
- Sulphate applicable across species
- Sulphate faster than nitrate

Combination of multiple stable isotope markers is advantageous









Novak et al., 2019 and 2020

Courtesy of Prof. Kristian Holst Laursen

CSIA

Applications for food authentication

Illicit additions or substitutions

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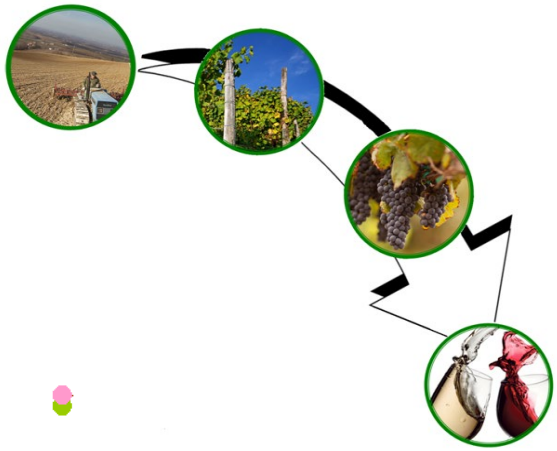
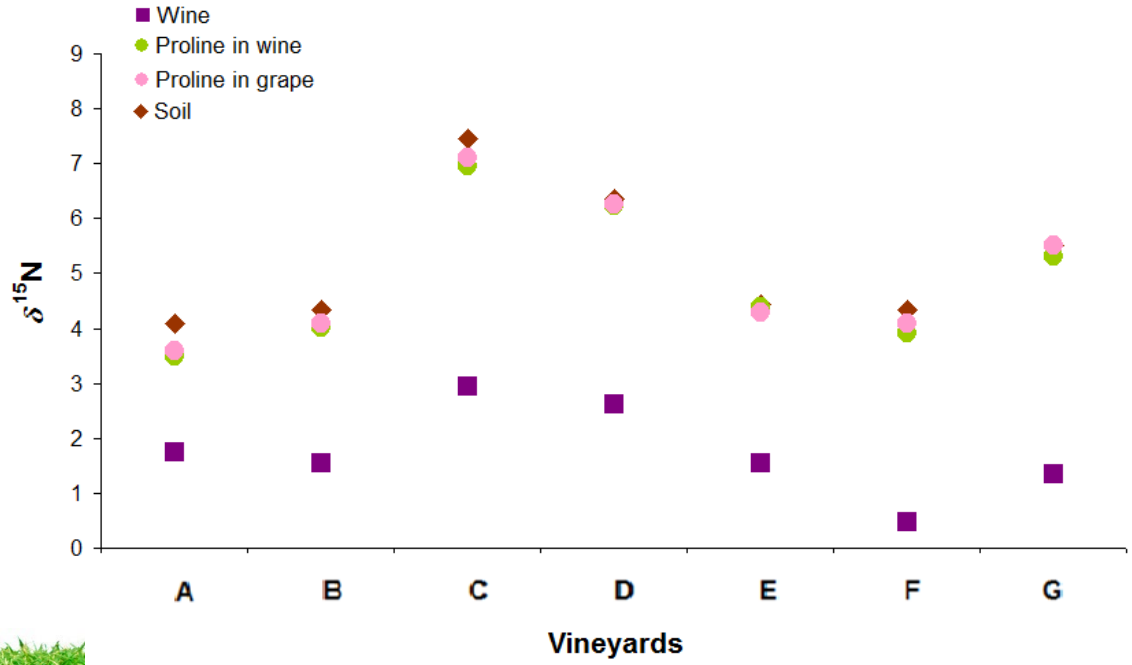
Liu et al., 2023, Perini et al., 2022

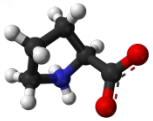
Geographical origin

$\delta^{15}N$ from soil to wine





$\Delta^{15}N \approx 0.3‰$



$\Delta^{15}N \approx 4‰$

Proline in must and wine, conserve the variability of $\delta^{15}N$ in the growing soil and can therefore be used as additional isotopic markers to trace the geographical origin of wine

Bulk samples: EA-IRMS
Proline: GC-C-IRMS, after N-acetylisopropyl derivatization

Paolini et al., JMS 2016

Impact of N adjuvants on $\delta^{15}N$

Fermentation conditions

No adjuvant
1 g/L IA - Inorganic adjuvant (no proline)
0.5 g/L OA - Organic adjuvant (proline 8.6 g/Kg)
4.3 g/L OA - Organic adjuvant (proline 8.6 g/Kg)

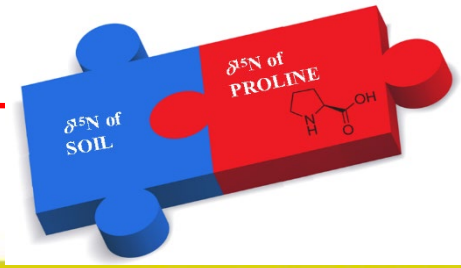
	Fermentation conditions	$\delta^{15}N$ (‰) proline in wine
Grape must 1	No adjuvant	11.5
	1 g/L IA	11.5
	0.5 g/L OA	11.4
	4.3 g/L OA	11.0
Grape must 2	No adjuvant	7.7
	1 g/L IA	7.6
	0.5 g/L OA	7.6
	4.3 g/L OA	7.1
Grape must 3	No adjuvant	10.1
	1 g/L IA	10.2
	0.5 g/L OA	10.1
	4.3 g/L OA	9.6

IA was added respecting the legal limit (EC n° 606/2009)

OA was added at two different concentrations:

- 0.5 g/L (amount recommended by the producer)
- 4.3 g/L (to reach the same Yeast Assimilable Nitrogen as in fermentation trial with IA)

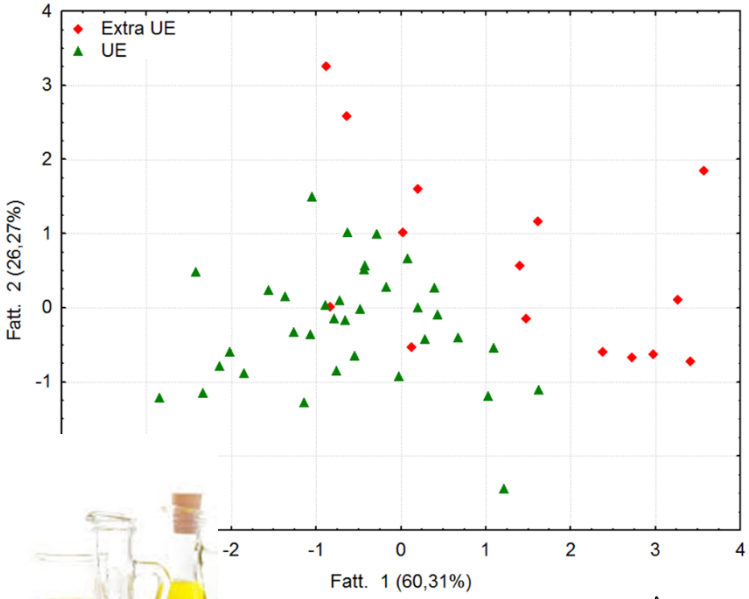
NO influence of the $\delta^{15}N$ value of proline!!

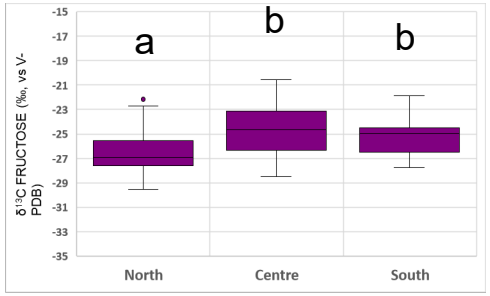


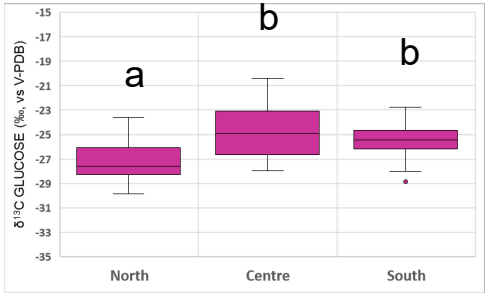
Paolini et al., JMS 2016

CSIA – geographical traceability

Bulk analysis + $\delta^{13}C$ and $\delta^{2}H$ analysis of the 4 main fatty acids



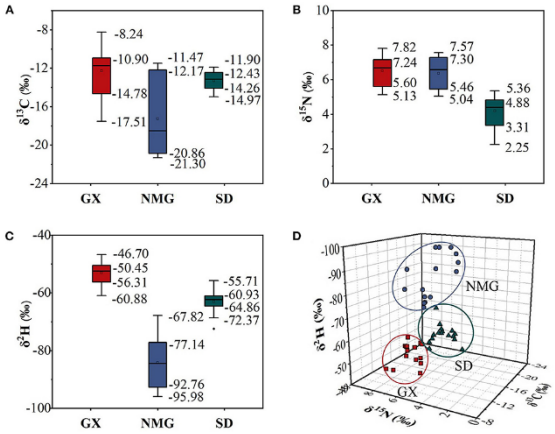




Perini et al., 2020

FOOD INTEGRITY

Paolini et al., 2017
Bontempo et al., 2019



Shuang Li et al., 2023

Conclusions

- **Bulk** stable isotope ratio analysis: simple sample preparation, rapid, more ratios in one run, cheaper

- **Compound specific** stable isotope ratio analysis: need a more complicated samples preparation (derivatization, …), one ratio in one run

BUT

can provide additional useful information





Thank you

for your attention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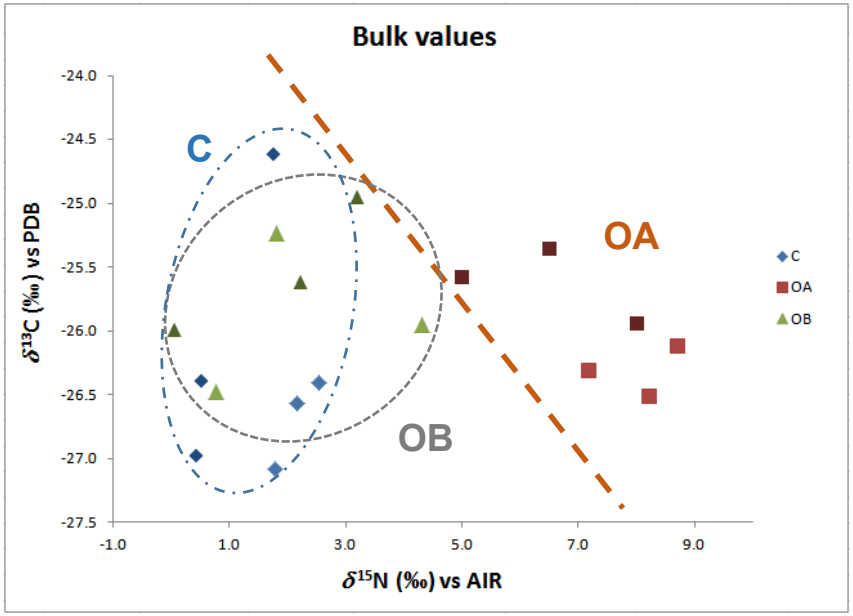
Safety
Quality
Traceability

Organic vs. conventional flour samples

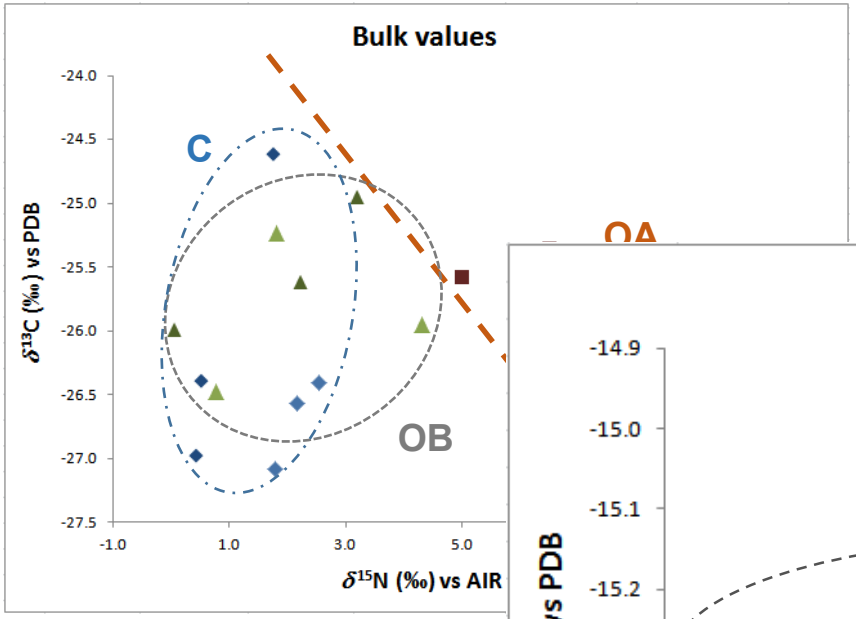


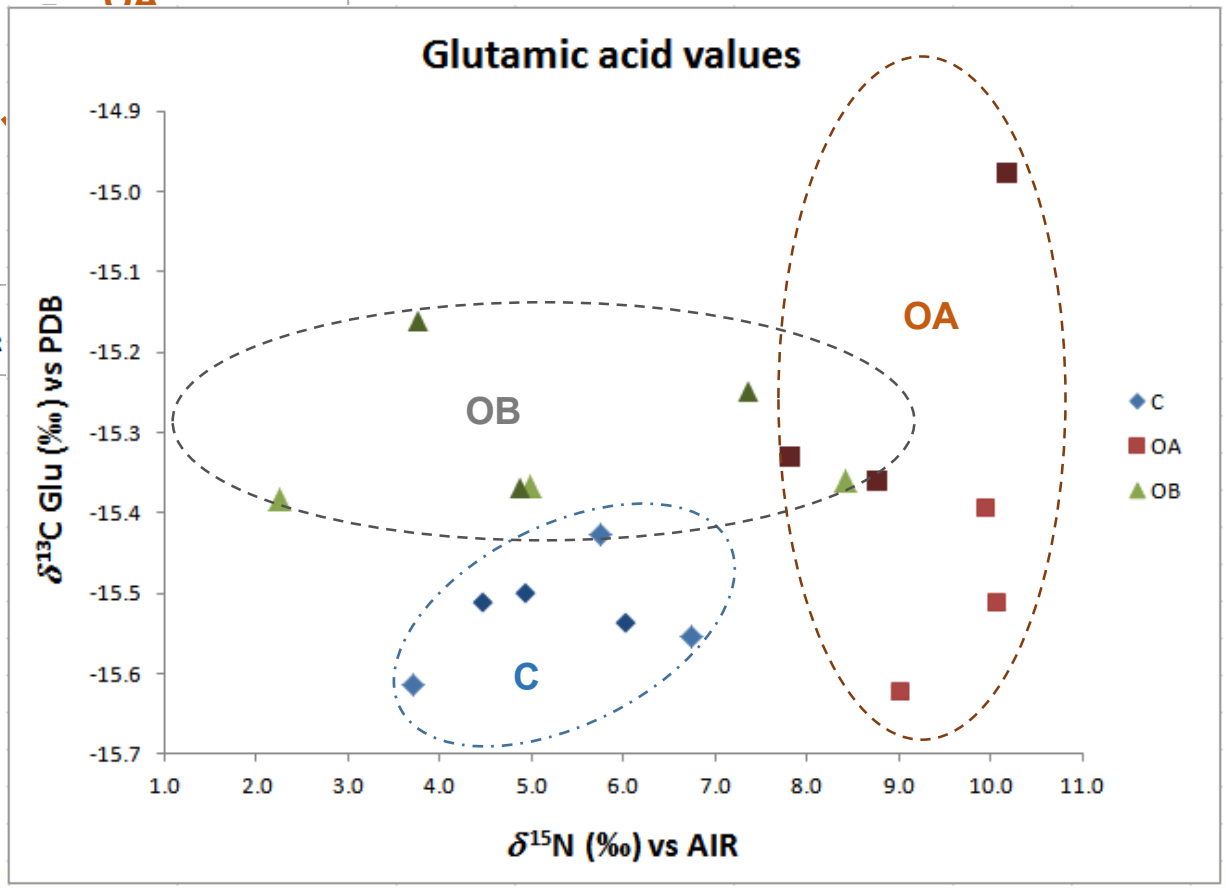
Current research on different cereals

FoodTraNet*

*This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement no. 956265

Organic vs. conventional flour samples





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$\delta^{15}N_{bulk}$ – preliminary results