

Analisi dei rapporti tra gli isotopi stabili di bioelementi a supporto della ricerca in ambito ambientale e per la tracciabilità e autenticità dei prodotti agroalimentari

Perini Matteo

FONDAZIONE EDMUND MACH, ITALY

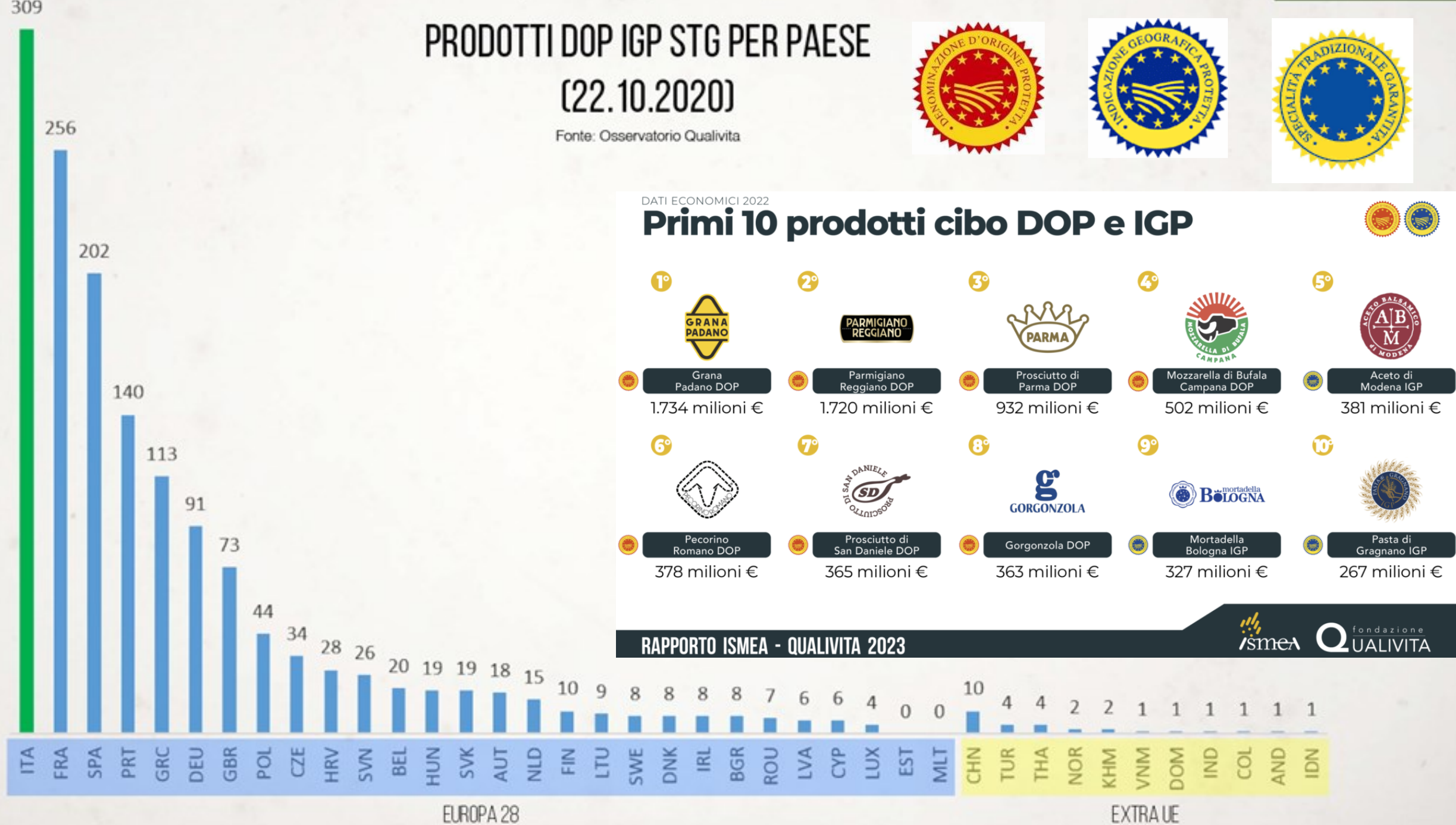
CIGS- Workshop Spettrometria di Massa Inorganica

Modena – 5 novembre 2024

PRODOTTI DOP IGP STG PER PAESE

(22.10.2020)

Fonte: Osservatorio Qualivita



Authenticity: ISO 22380:2018

Prodotto alimentare autentico?

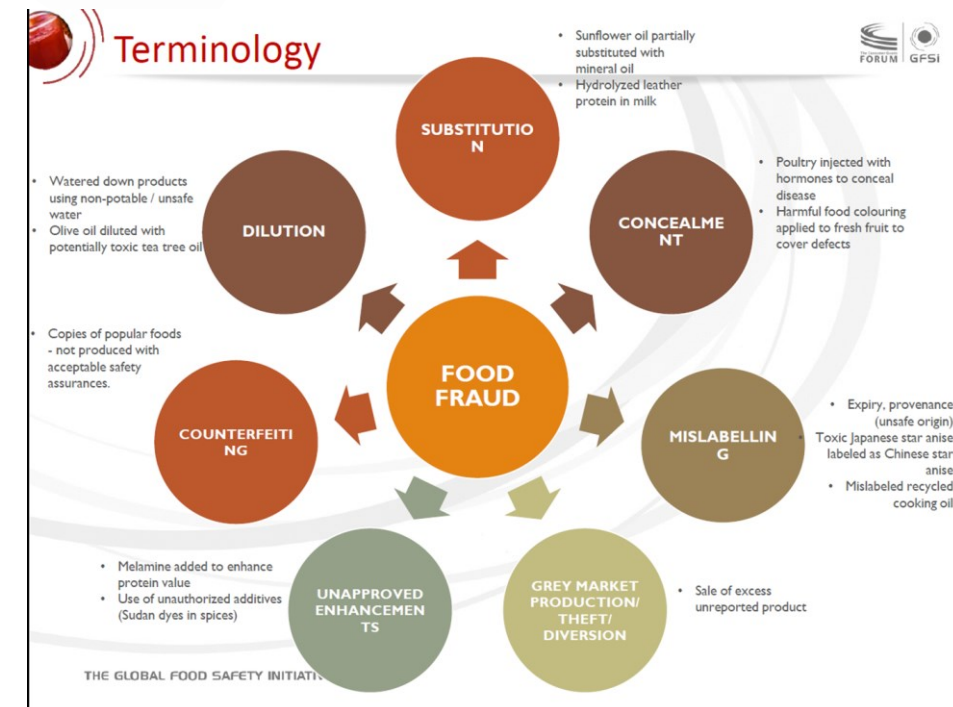


*C'è una corrispondenza tra
le effettive caratteristiche del prodotto alimentare
e il claim (proprietà/caratteristiche dichiarate)*



Frode alimentare

*«un'azione che causa intenzionalmente una
discrepanza tra quanto dichiarato riguardo il prodotto
alimentare e le sue effettive caratteristiche»*

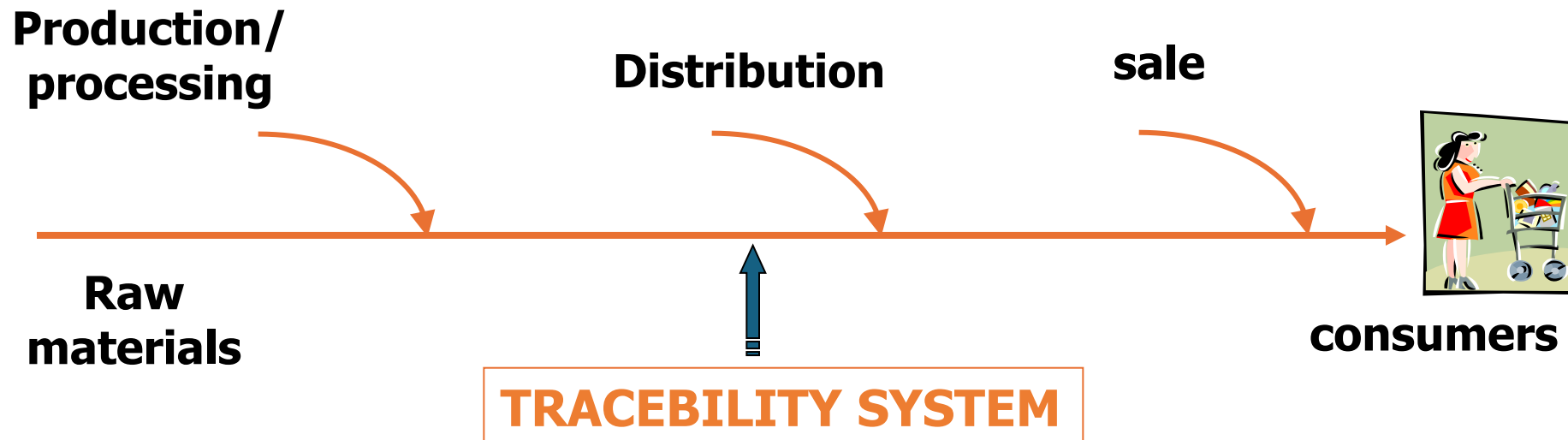


TRACEABILITY

Reg CE 178/2002, art. 18



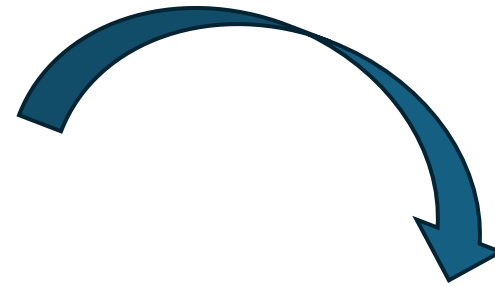
"La capacità di ricostruire e seguire il percorso di un alimento, di un mangime, di un animale destinato alla produzione alimentare o di una sostanza destinata o atta a entrare a far parte di un alimento o di un mangime, attraverso tutte le fasi della produzione, trasformazione e distribuzione"



Come garantire l'autenticità e la tracciabilità degli alimenti?



Paper traceability
(EU Reg. 178/2002)



Analytical tests

robust, validated, official methods
(EU Reg, CEN, AOAC)

robust, validated, official limits or reference data



Stable isotope ratio analysis A long history of applications in food analysis...

Primi lavori scientifici degli anni '80 su vino, mosto e
miele.

Z Lebensm Unters Forsch (1982) 175: 253–257

Zeitschrift für
**Lebensmittel-
Untersuchung
und-Forschung**
© J. F. Bergmann Verlag 1982

Oxygen Isotope Studies on Some New Zealand Grape Juices

John Dunbar

Chemistry Department, University of Waikato, Hamilton, New Zealand *

Dal 1990 sono diventati metodi ufficiali:

OIV (International Organization of Vine and Wine)

AOAC (Association of Official Agricultural Chemists)

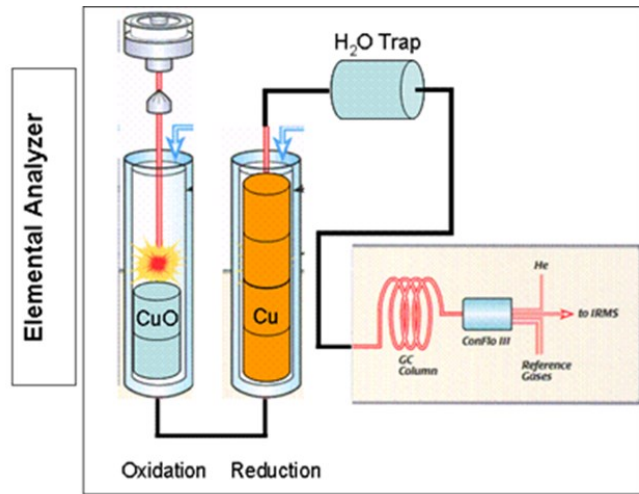
METODI CON RAPPORTI ISOTOPI STABILI COME STANDARD UFFICIALI

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	$^{13}\text{C}/^{12}\text{C}$	sugar addition (cane)
1993	ENV 12140, 13070	fruit juice	IRMS	$^{13}\text{C}/^{12}\text{C}$	sugar addition (cane)
1995	AOAC 995,17	fruit juice	SNIF-NMR	D/H	sugar addition (beet, cane)
1996	OIV 2/96	wine, must	IRMS	$^{18}\text{O}/^{16}\text{O}$	addition of water/mislabelling
1997	EU Reg 2676/90, 822/97	wine, must	IRMS	$^{18}\text{O}/^{16}\text{O}$	addition of water/mislabelling
1997	ENV 12141	fruit juice	IRMS	$^{18}\text{O}/^{16}\text{O}$	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, $^{13}\text{C}/^{12}\text{C}$	sugar addition (beet, cane)
2001	OIV 17/2001	wine, must	IRMS	$^{13}\text{C}/^{12}\text{C}$	sugar addition (cane)
2003	EU Reg. 2676/90, 440/03	wine, must	IRMS	$^{13}\text{C}/^{12}\text{C}$	sugar addition (cane)
2003	OIV MA-F-AS314-03	wine	IRMS	$^{13}\text{C}/^{12}\text{C}$	technogenic CO_2
2004	AOAC 2004,01	fruit juice, maple syrup	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	$^{13}\text{C}/^{12}\text{C}$	addition of glycerol
2011	EU Reg 584/2011	Grana Padano DOP	IRMS	D/H, $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{34}\text{S}/^{32}\text{S}$	mislabelling
2013	EN 16466-1, 2, 3	vinegar	SNIF-NMR, IRMS	D/H, $^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$	water and sugar addition (beet, cane)
2013	OIV 510, 511/2013	vinegar	IRMS	$^{13}\text{C}/^{12}\text{C}$, $^{18}\text{O}/^{16}\text{O}$	water and sugar addition (cane)

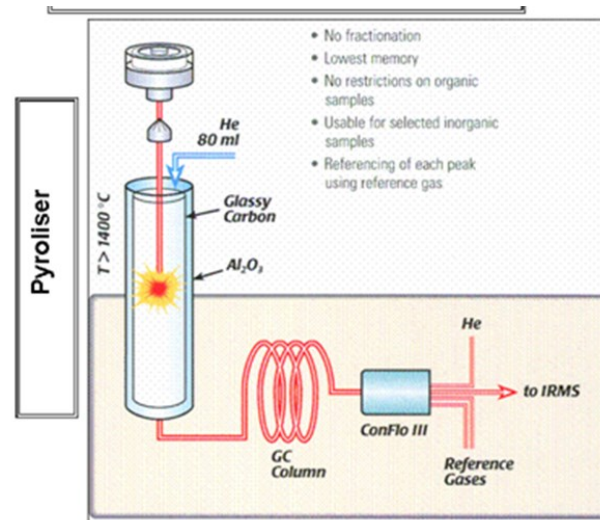


Come analizzare i rapporti isotopici?

- Isotope Ratio Mass Spectrometer, interfaced with Elemental Analyser, Pyrolyser or CO₂ equilibration system
- Site-specific Natural Isotopic Fractionation - Nuclear Magnetic Resonance

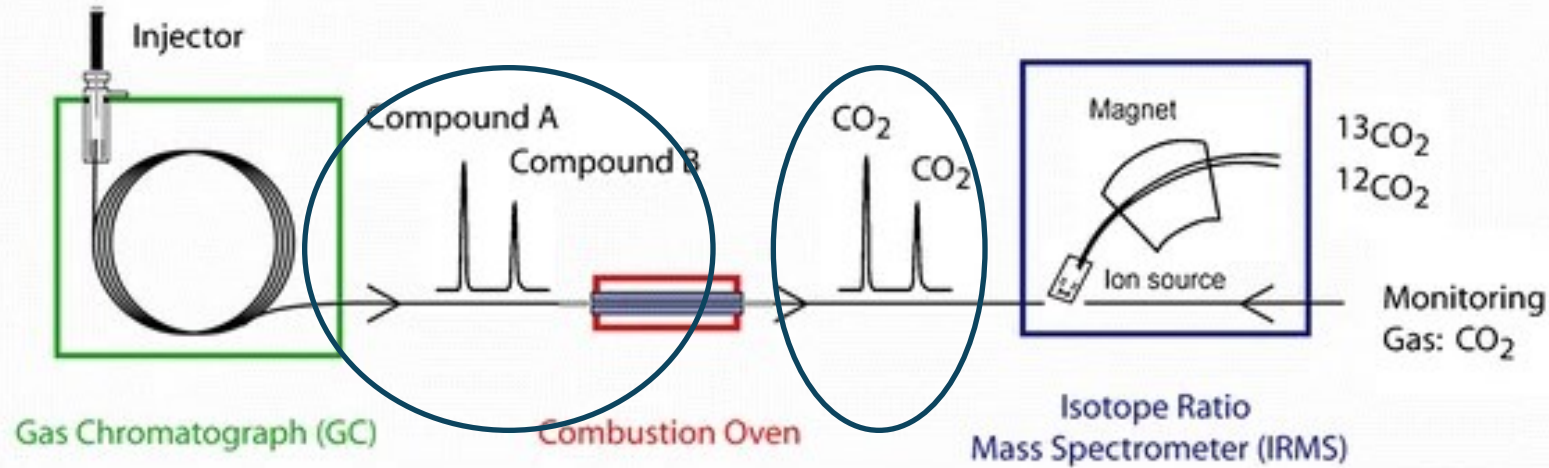


IRMS

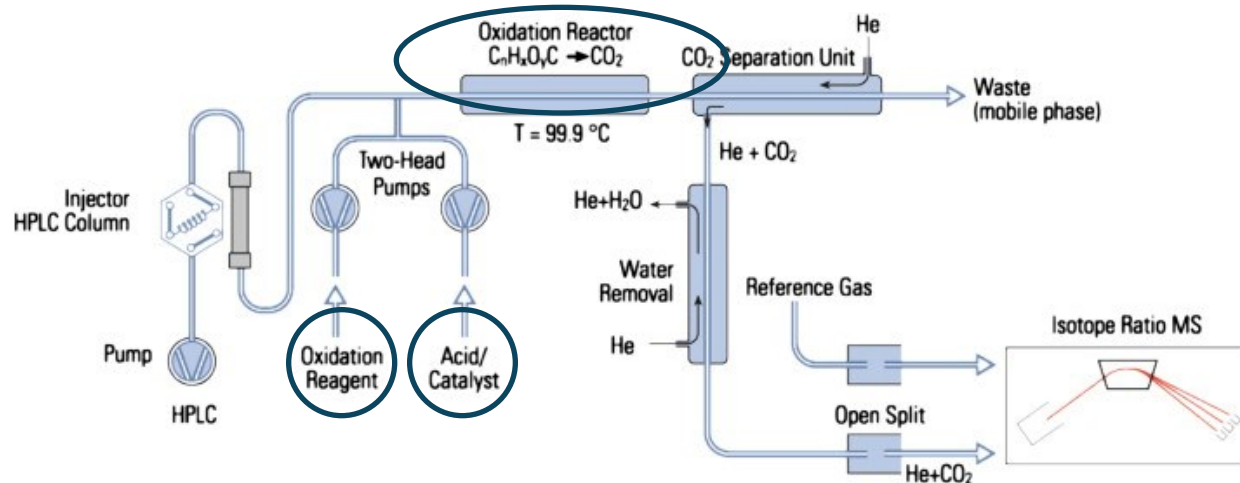


SNIF-NMR

Gas Chromatograph (GC)



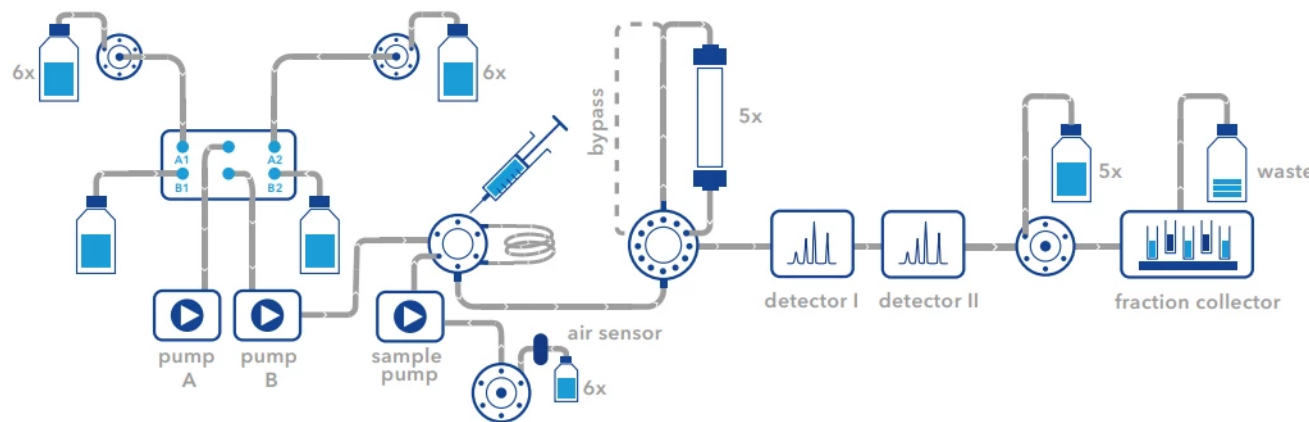
Liquid Chromatograph (LC)



Separative systems

Preparative HPLC systems

Separative systems



SOLVENT SELECTION
& DELIVERY

SAMPLE
INJECTION

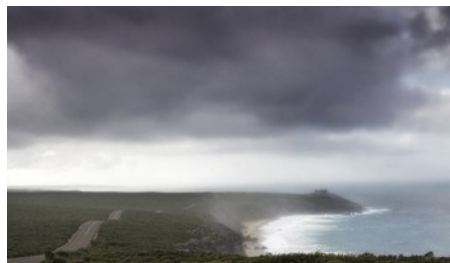
COLUMN SELECTION
& THERMOSTAT

DETECTION

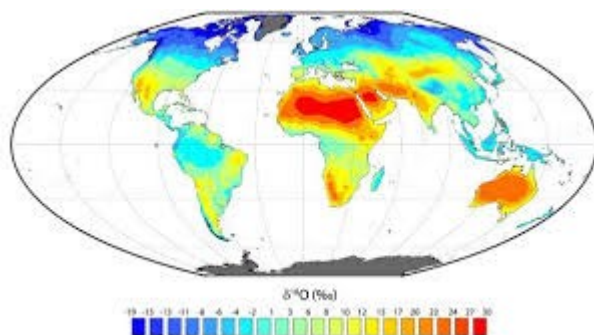
FRACTION
COLLECTION

IRMS

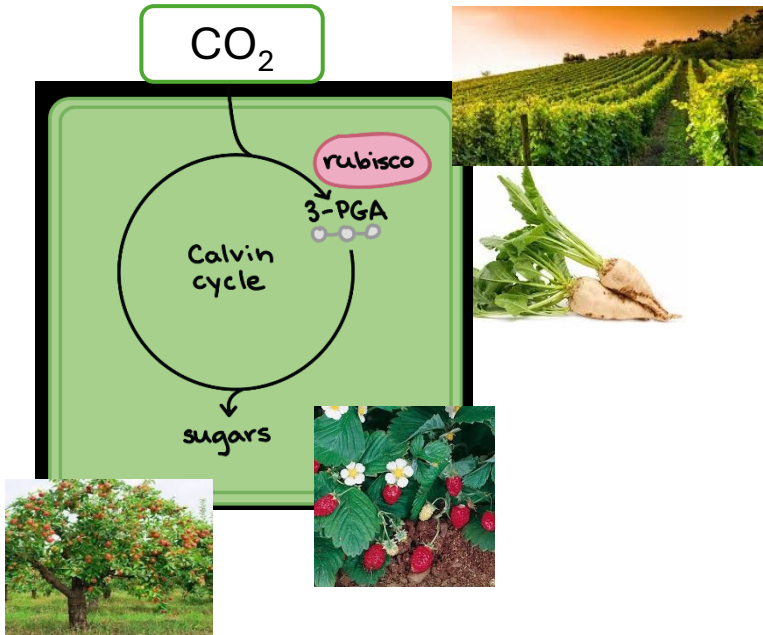




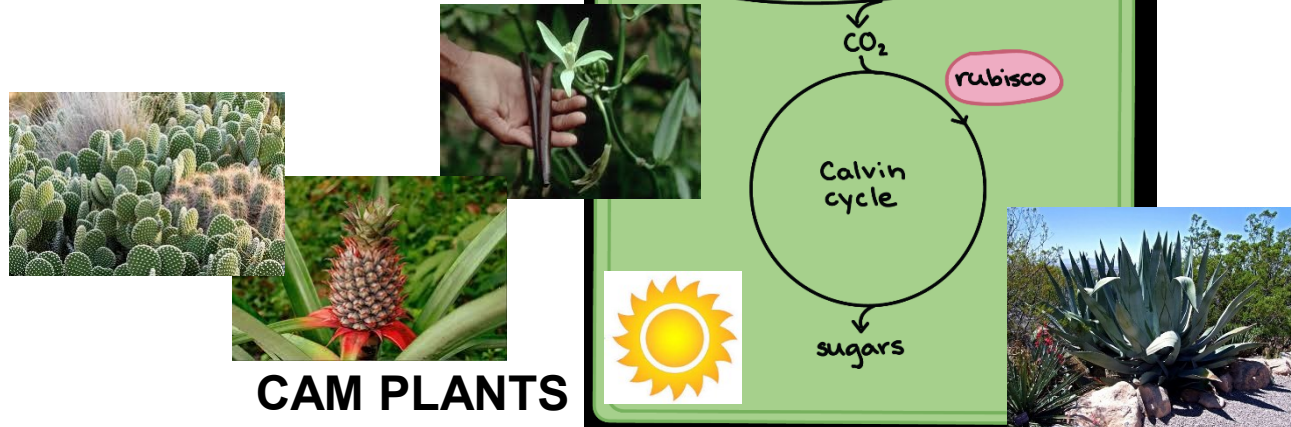
FATTORI DI VARIABILITA' ISOTOPICA



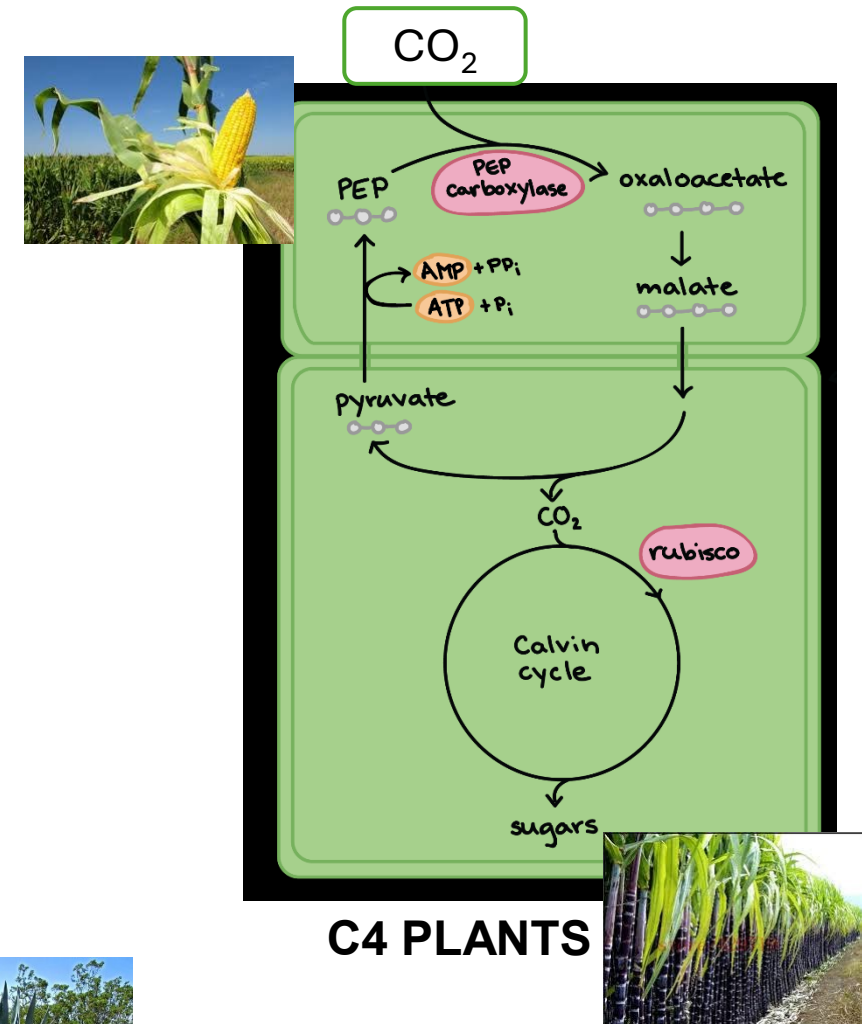
Variabilità botanica del $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$)



C3 PLANTS



CAM PLANTS



C4 PLANTS

Variabilità isotopica del $^{13}\text{C}/^{12}\text{C}$ ($\delta^{13}\text{C}$)

Cane sugar
mais



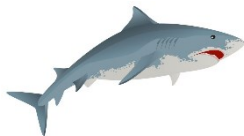
Vanilla, agave



Grape,
Fruit
forage

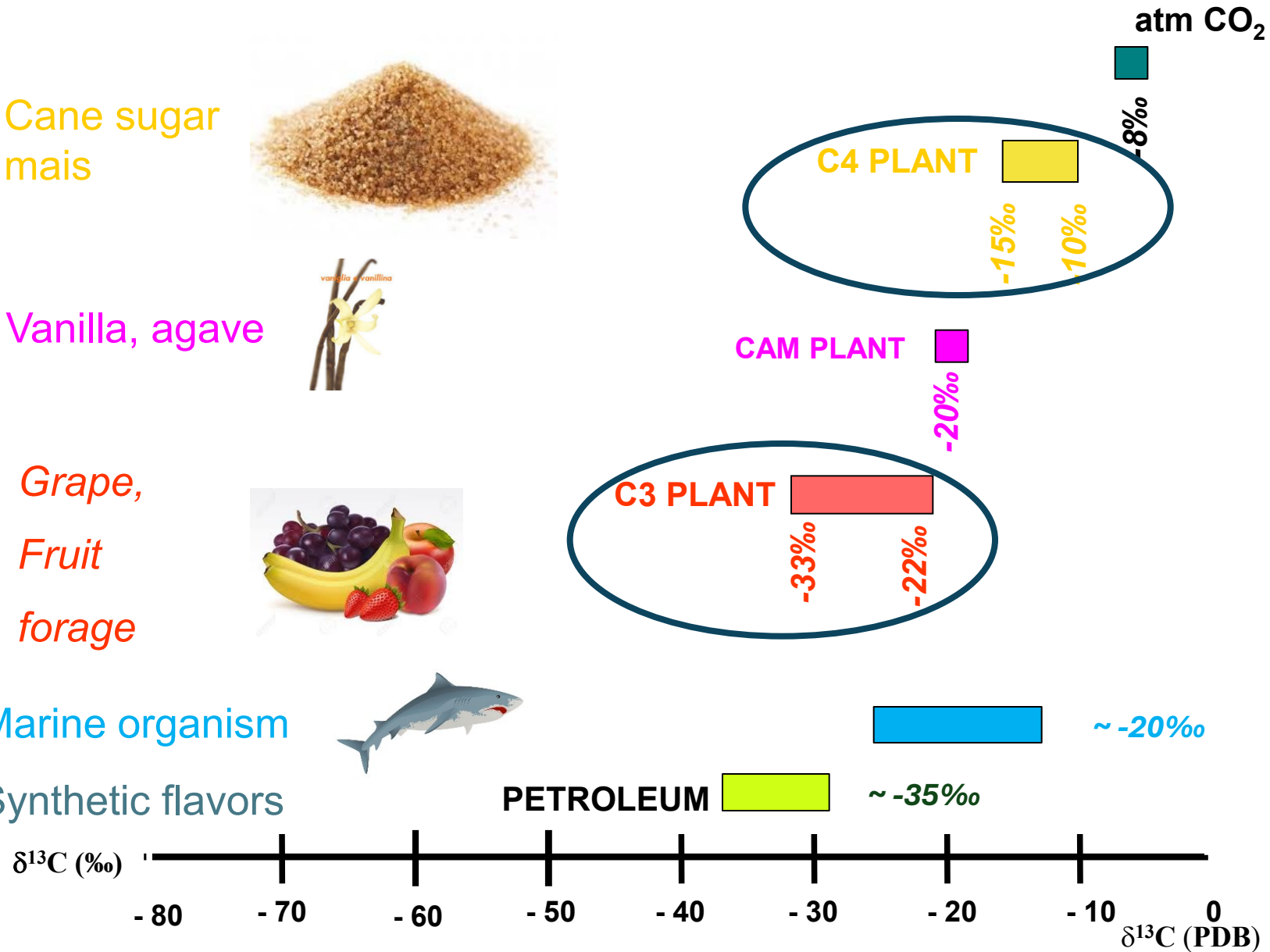


Marine organism

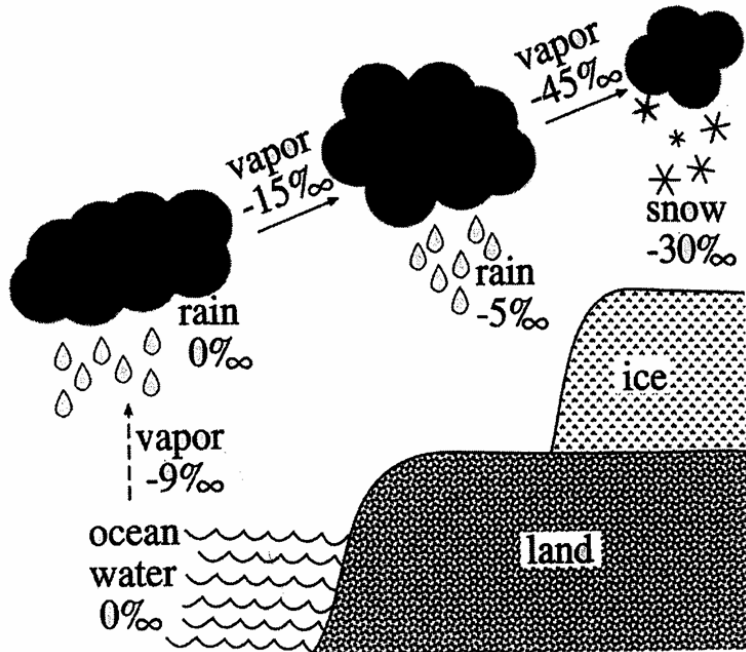


Synthetic flavors

PETROLEUM



Fattori di variabilità del $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ nell'acqua meteorica



Hoefs, 1980

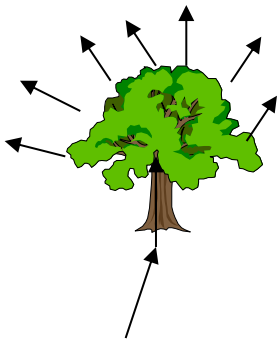
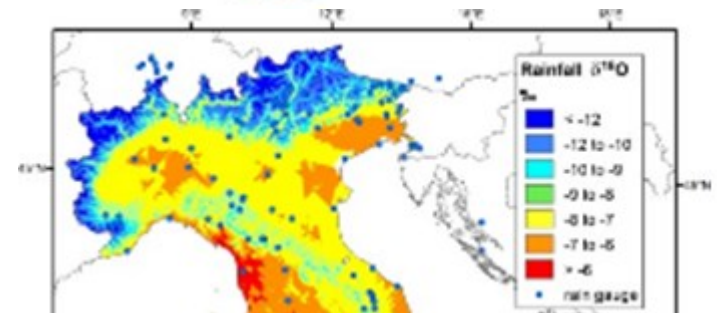
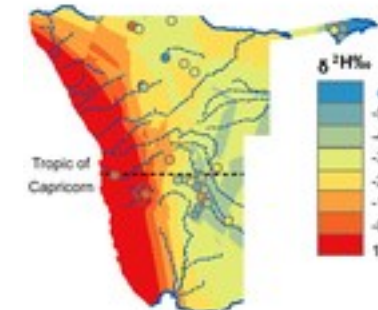
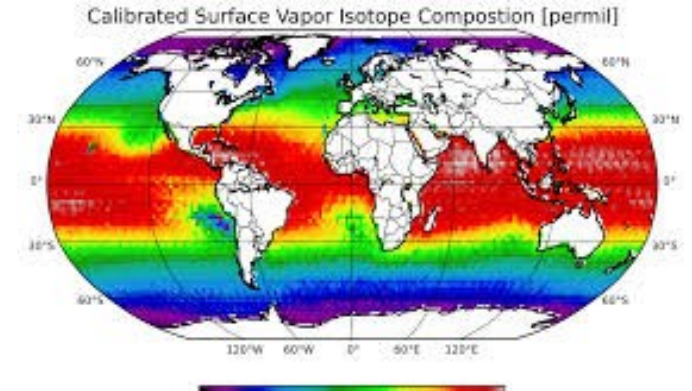
- **LATITUDINE:** contenuto massimo di D e ^{18}O all'equatore, poi diminuisce con la latitudine

- **DISTANZA DAGLI OCEANI:** i valori si abbassano quanto maggiore è la distanza dagli oceani

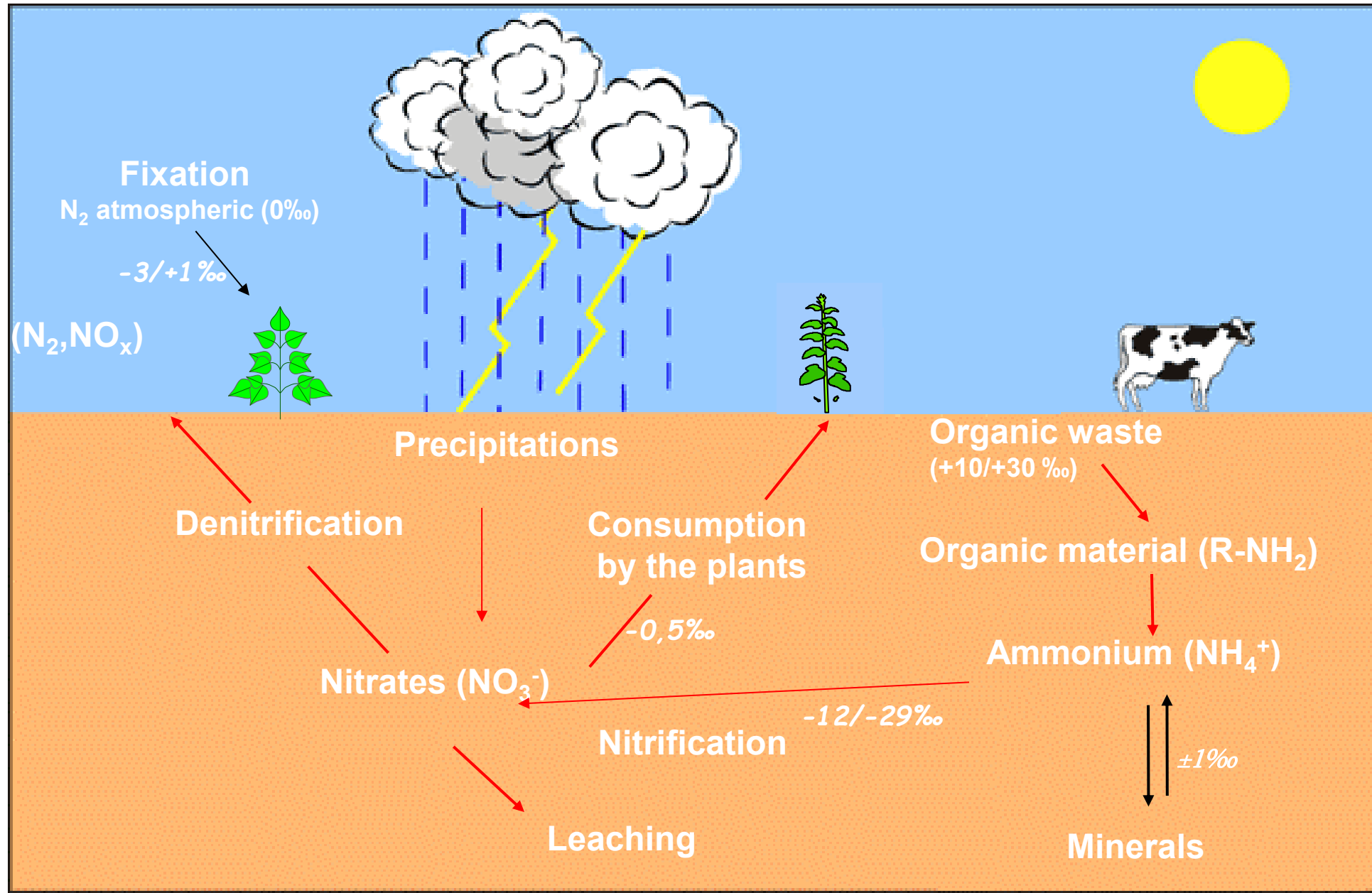
- **ALTITUDINE:** i valori si abbassano all'aumentare dell'altitudine

e nell'acqua della pianta

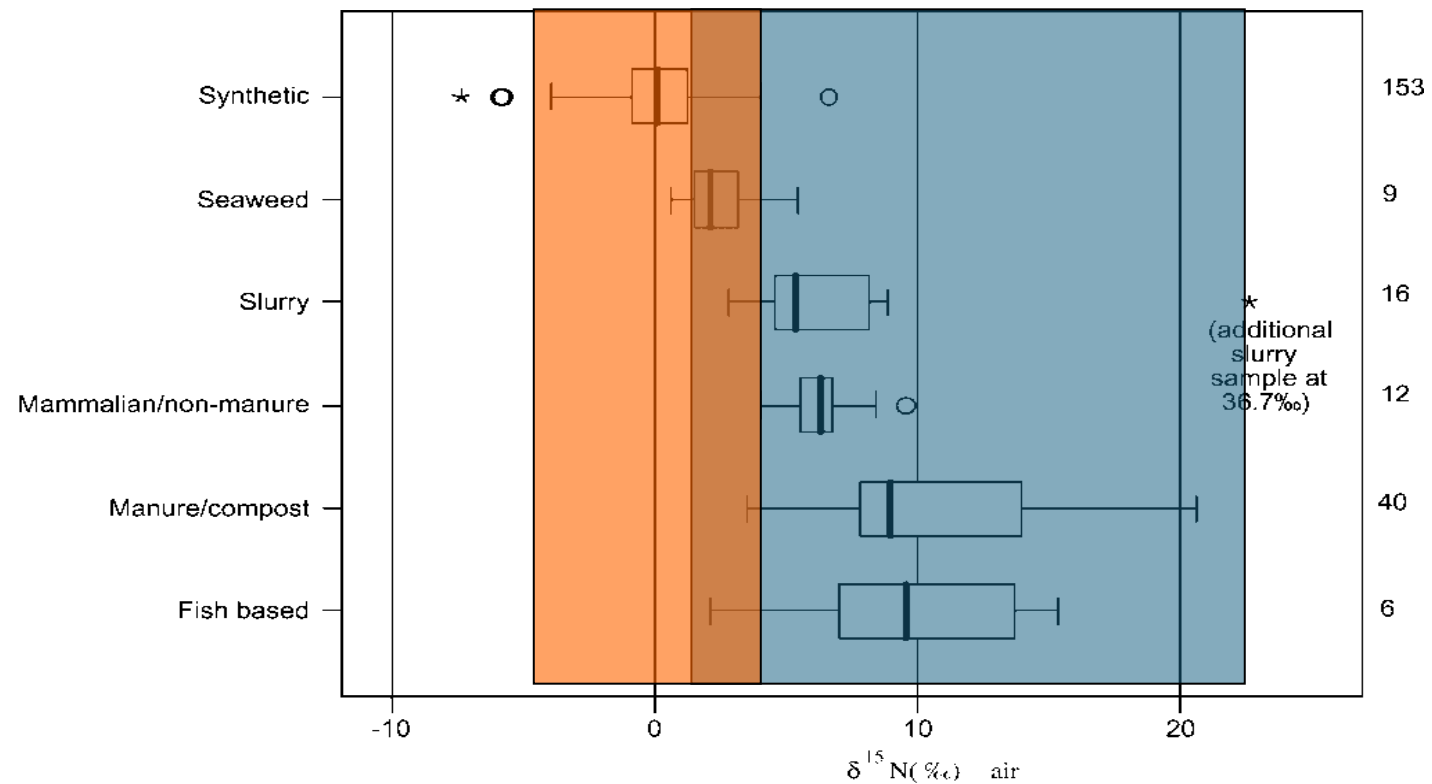
La composizione isotopica dell'acqua presente a livello xilematico e fogliare è pari a quella dell'acqua adsorbita



Parametri che influenzano il $^{15}\text{N}/^{14}\text{N}$ nei composti vegetali



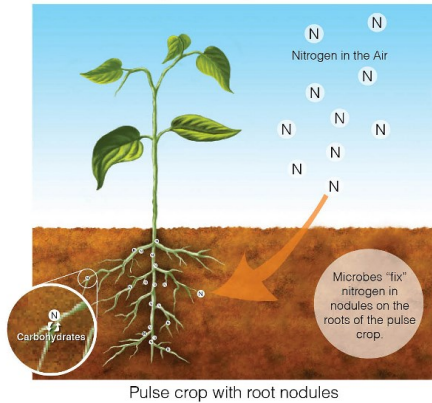
$^{15}\text{N}/^{14}\text{N}$ di fertilizzanti organici ed inorganici



Bateman & Kelly, 2007

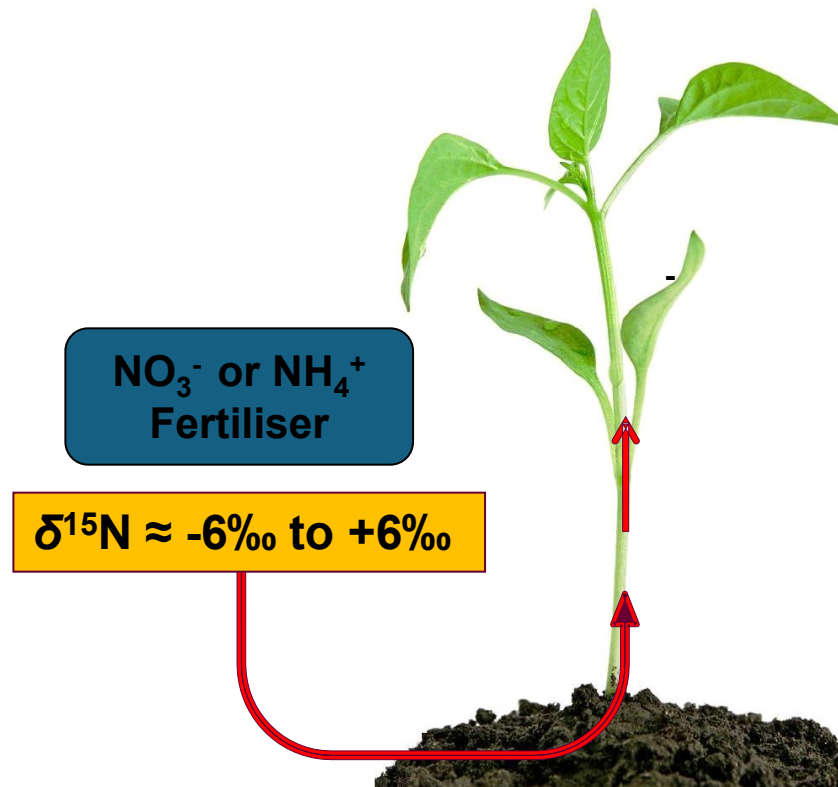
Effetto sul $^{15}\text{N}/^{14}\text{N}$ di piante a causa dell'applicazione di fertilizzanti organici e inorganici

Plant Fixing Nitrogen

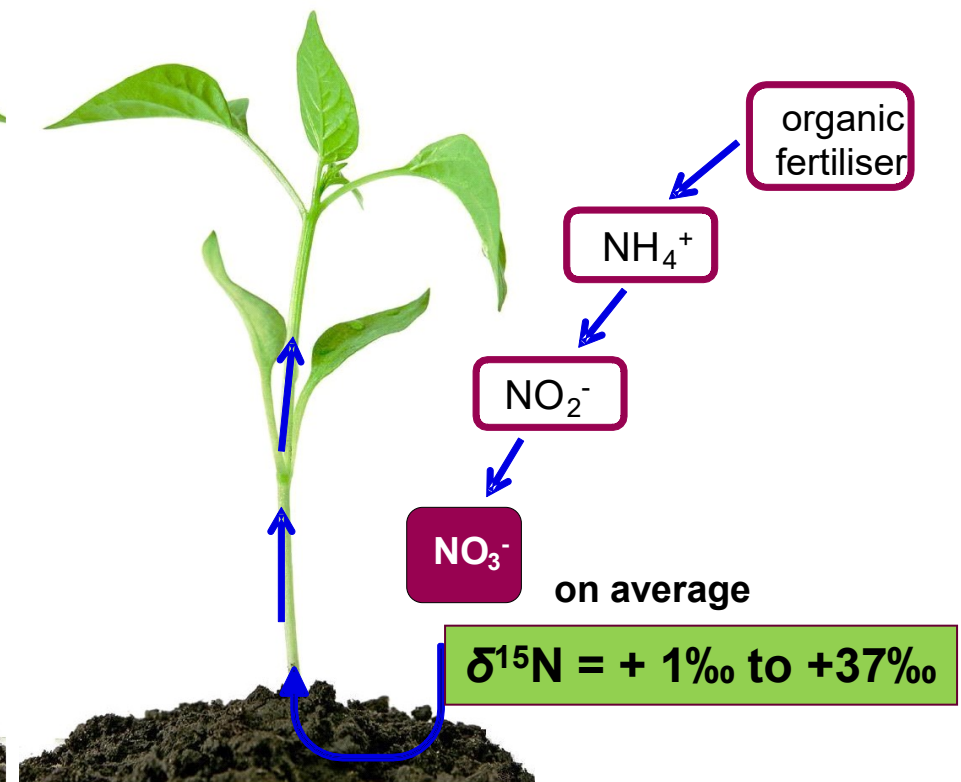


$\delta^{15}\text{N}$
 $\approx 0\text{‰}$

CONVENTIONAL



ORGANIC



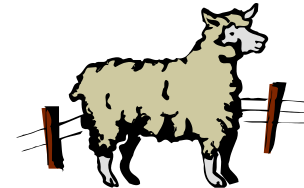
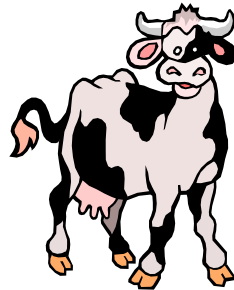
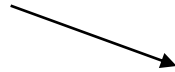
Fattori di variabilità nei materiali animali e negli alimenti



Feed



Water



Frazionamento dovuto all'aumento del livello trofico

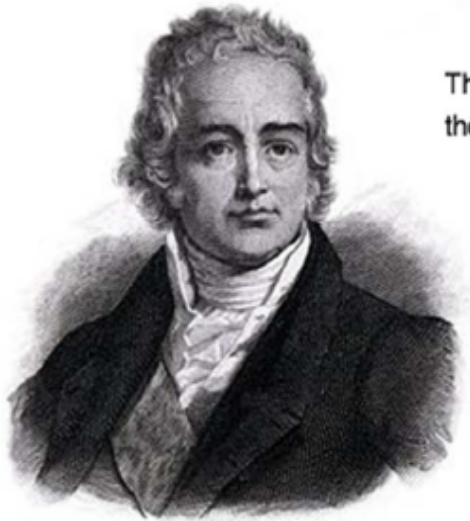


ESEMPI DI APPLICAZIONI



Rilevazione dell'autenticità del vino (zuccheraggio e annacquamento) Reg UE 2676/90

Jean-Antoine Chaptal (1756-1832)

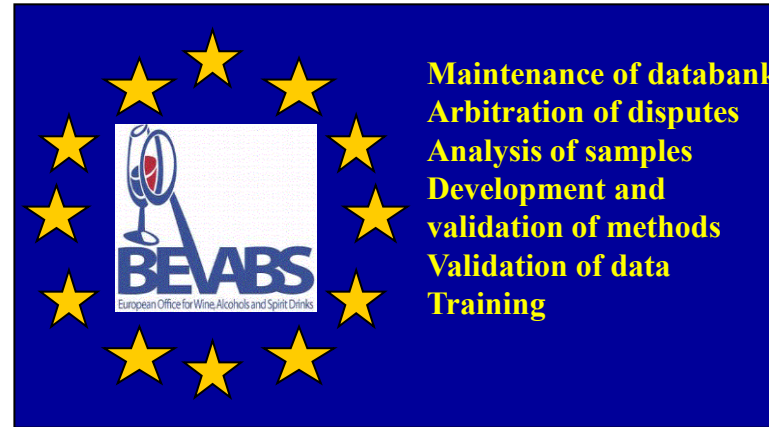


The process of adding sugar to unfermented wine in order to increase the final alcohol level is known as *chaptalization* after him.

Addition of 17 g/L of sugar
=
increase of Alcoholic Grade of 1%Vol



Wine Databank EC Reg. N° 2729/2000



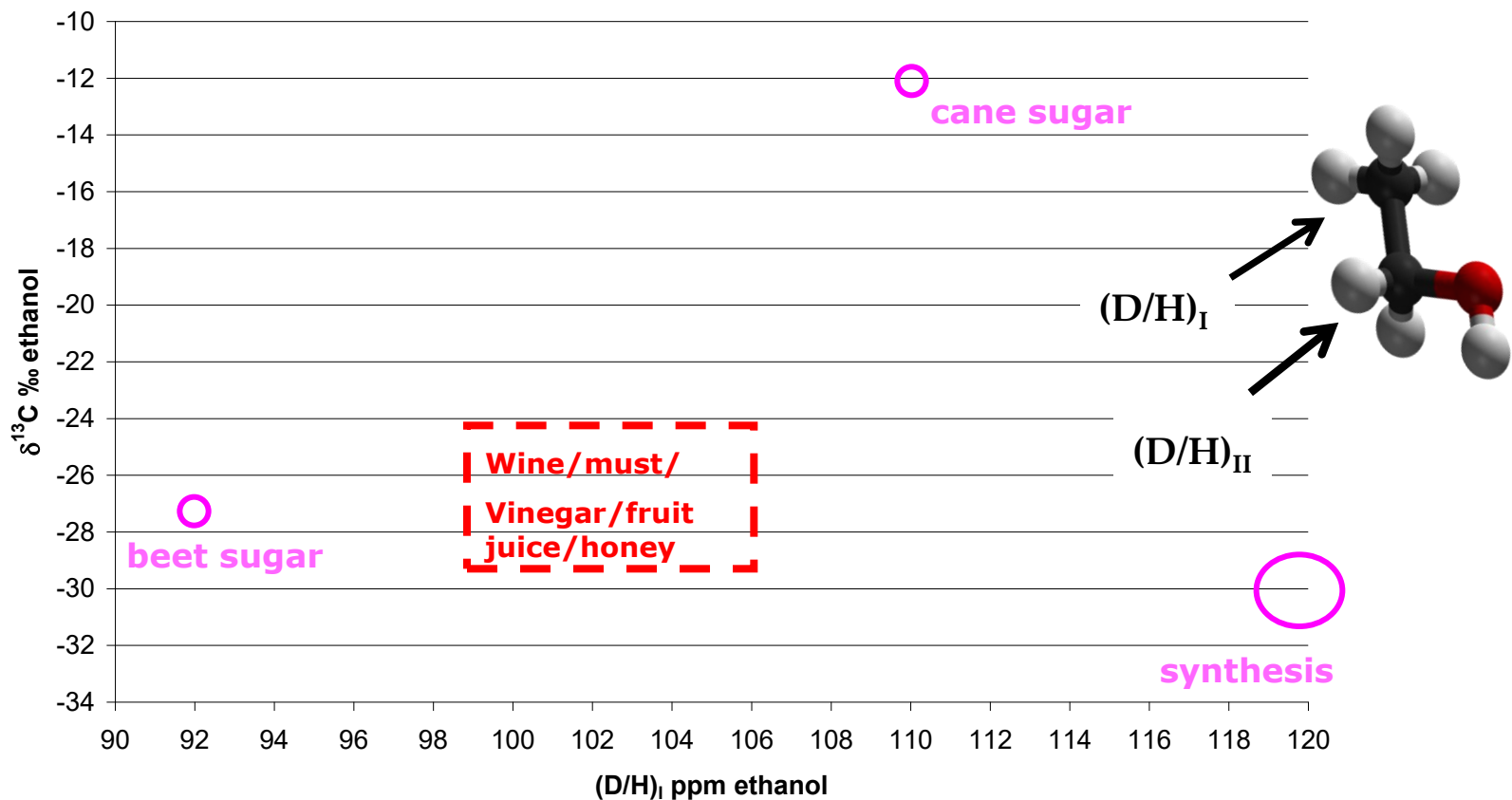
EC Reg. N° 2120/2004



EC Reg. N° 2030/2006



Rapporti isotopici stabili per la rilevazione di adulterazioni nel vino



	$(\text{D}/\text{H})_1$	$(\text{D}/\text{H})_2$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$
Wines, 95% CI	98.8 / 106	124.5 / 135.5	-29.3 / -24.3	-1.3 / 8.9
Beet sugar	92.5		-27.5	
Cane sugar	109.5		-12	
Water (north Italy)				-9
Water (south Italy)				-5

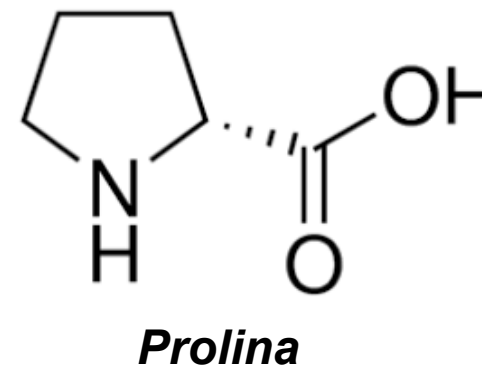
Dordevic et al., ACA, 2013

Nuovi approcci analitici per l'autenticazione del vino

GC-IRMS per rilevare l'aggiunta di zucchero esogeno nel mosto

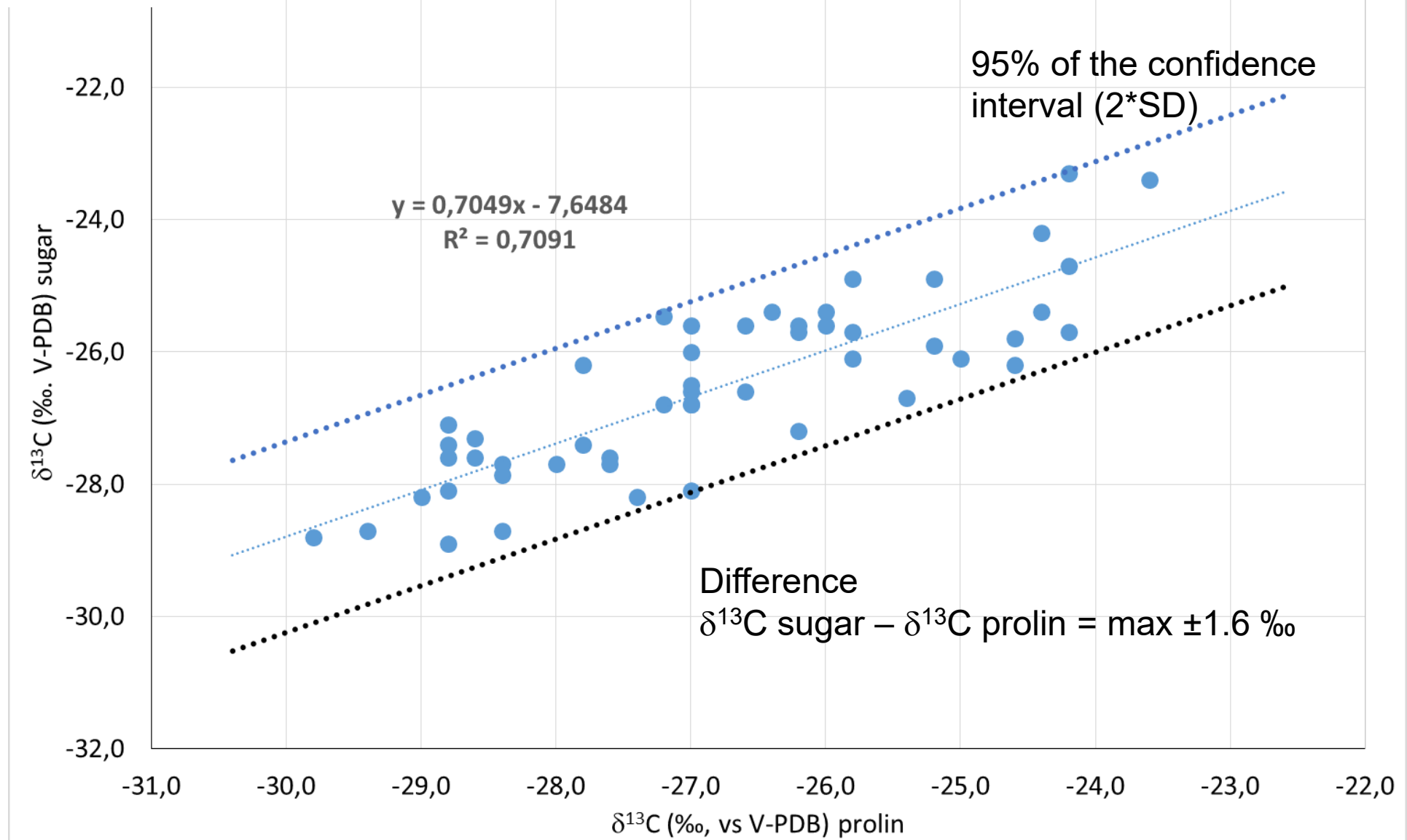
Analisi del $\delta^{13}\text{C}$ della prolina e correlazione con il $\delta^{13}\text{C}$ degli zuccheri

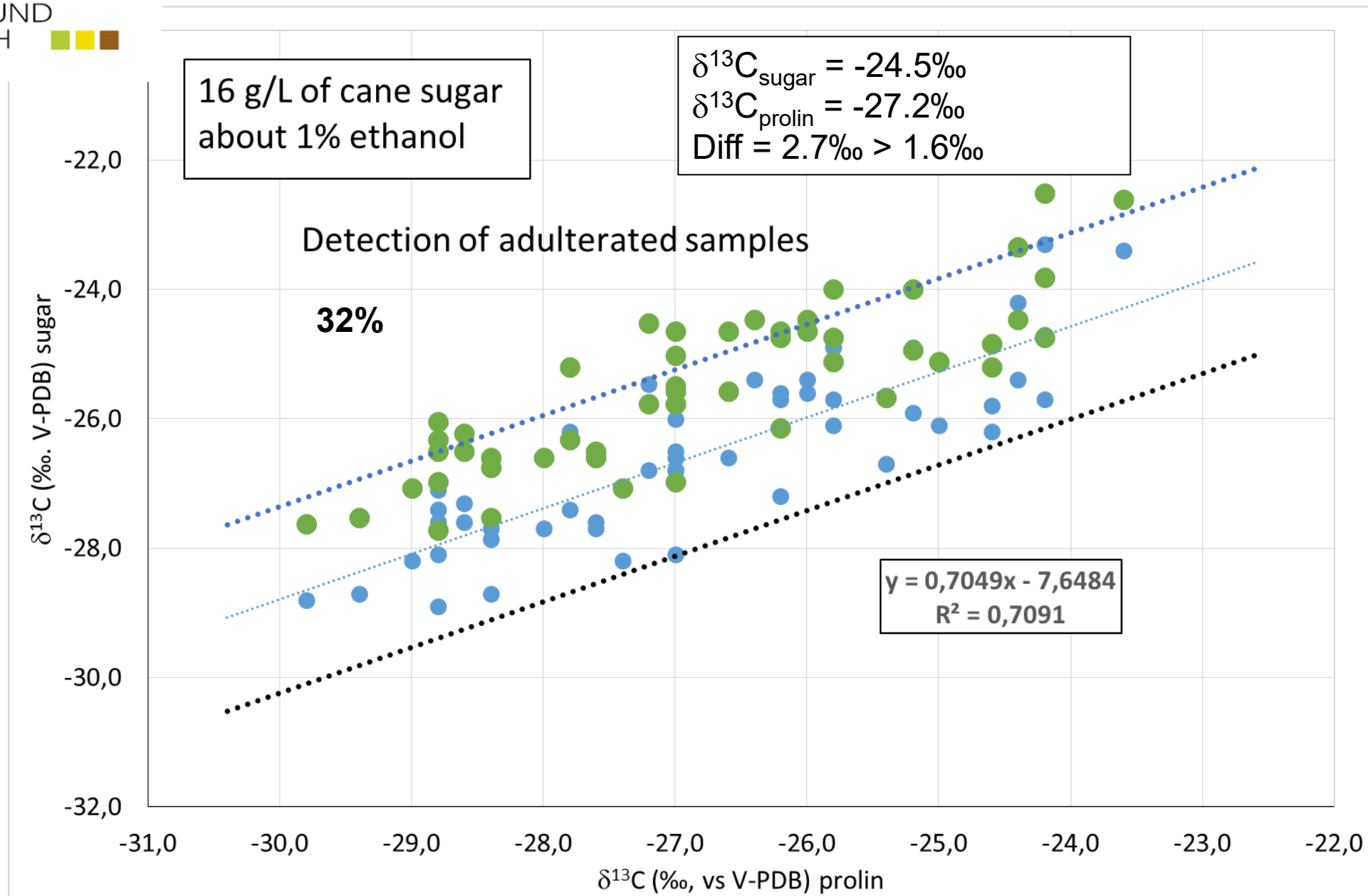
- 30-85% del contenuto totale di aminoacidi
- Non viene utilizzato dai lieviti come fonte di azoto...
➡ Si mantiene nel vino!



TC-IRMS per aumentare la capacità di identificazione dell'acqua esogena

Analisi del $\delta^{18}\text{O}$ dell'etanolo e correlazione con il $\delta^{18}\text{O}$ del vino.





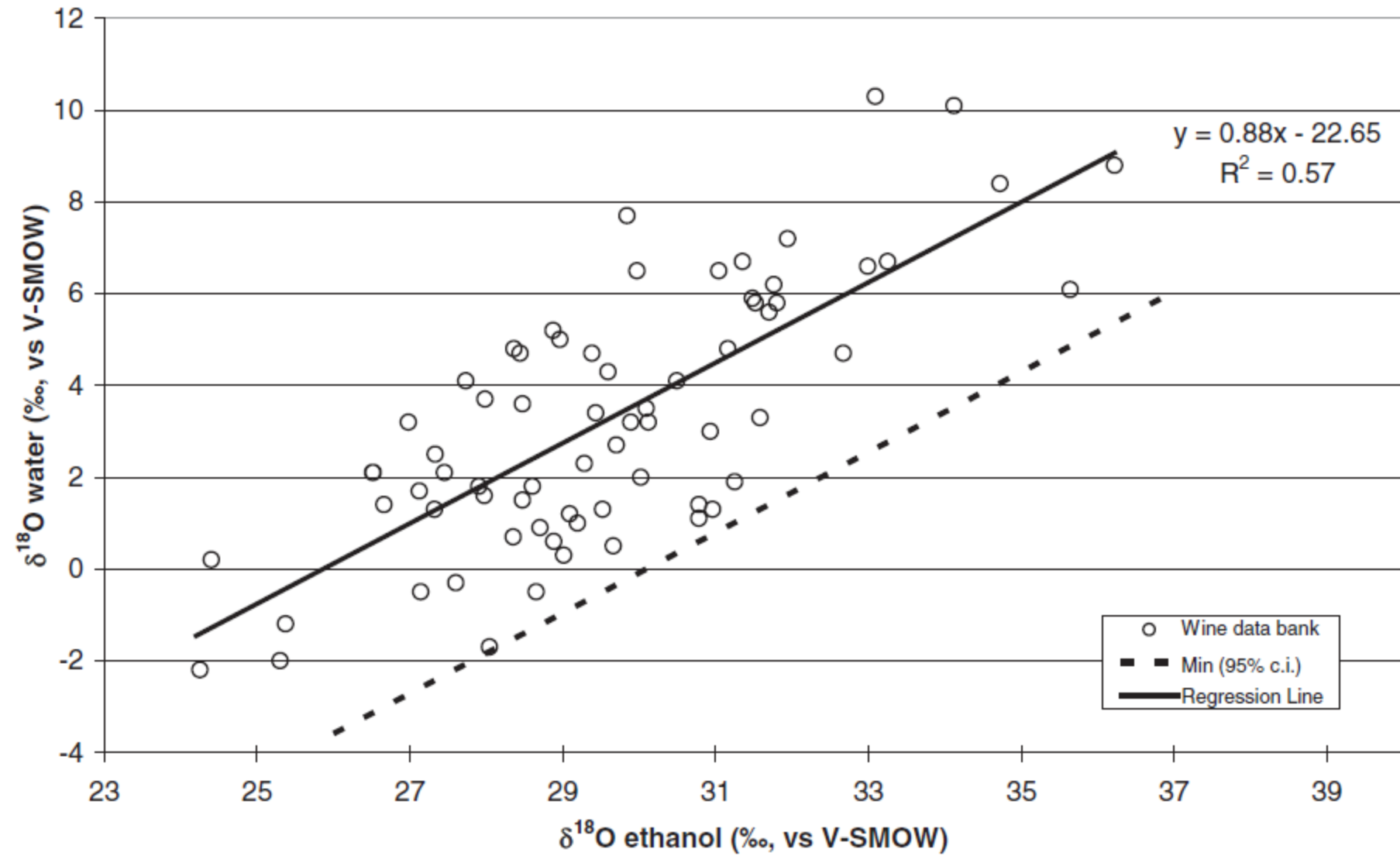


Figure 2–Correlation between $\delta^{18}\text{O}$ values of ethanol and of water of authentic Italian wine samples.

È davvero biologico?

Analisi compound-specific del $\delta^{15}\text{N}$ e $\delta^{13}\text{C}$ degli aminoacidi per la discriminazione tra grano biologico e convenzionale



More information in

Paolini et al., JAFC 2015

Wheat sampling



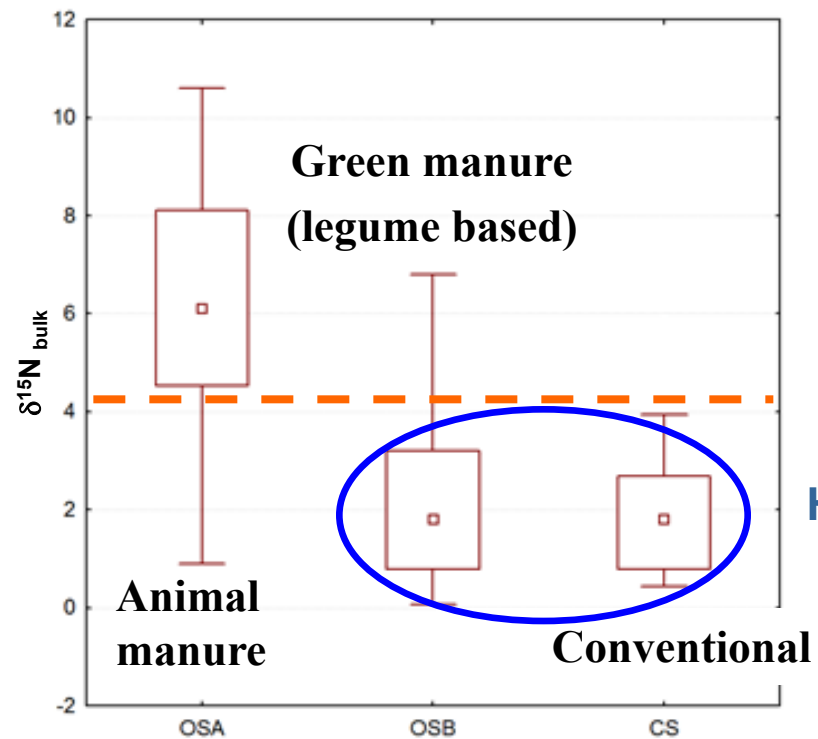
Italy



Denmark



3 agricultural regimes



How to discriminate



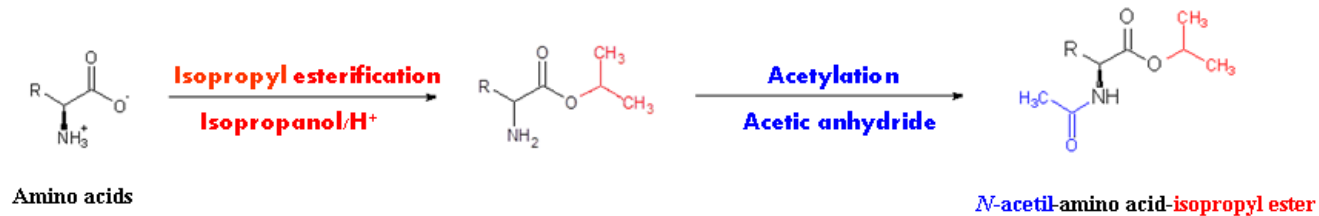
Compound-specific $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analysis of amino acids

step 1

Protein hydrolysis HCl 6M, 110°C, 24h

step 2

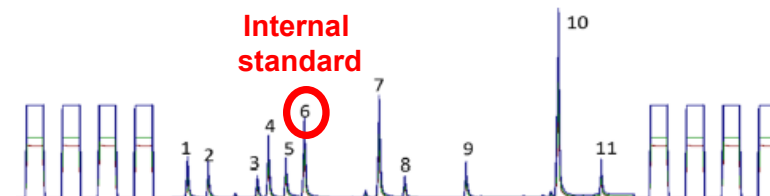
Amino acids derivatization



step 3

GC-c-IRMS analysis

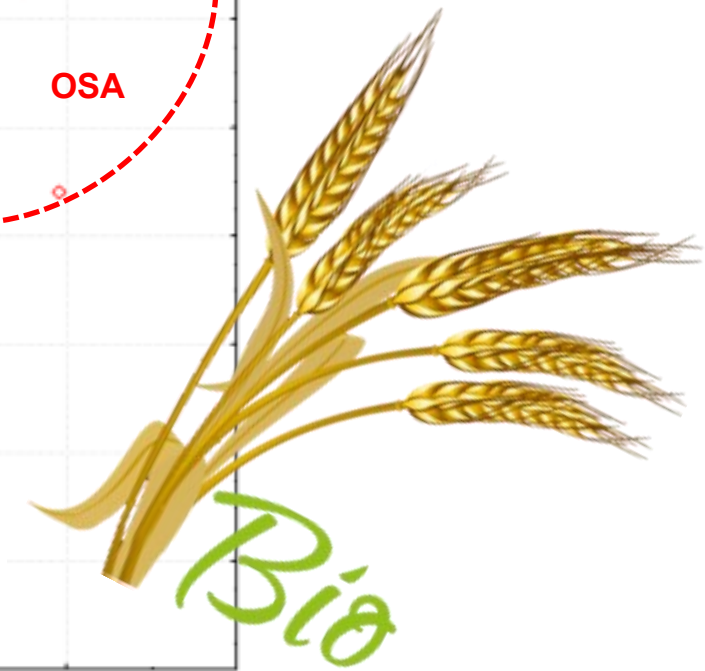
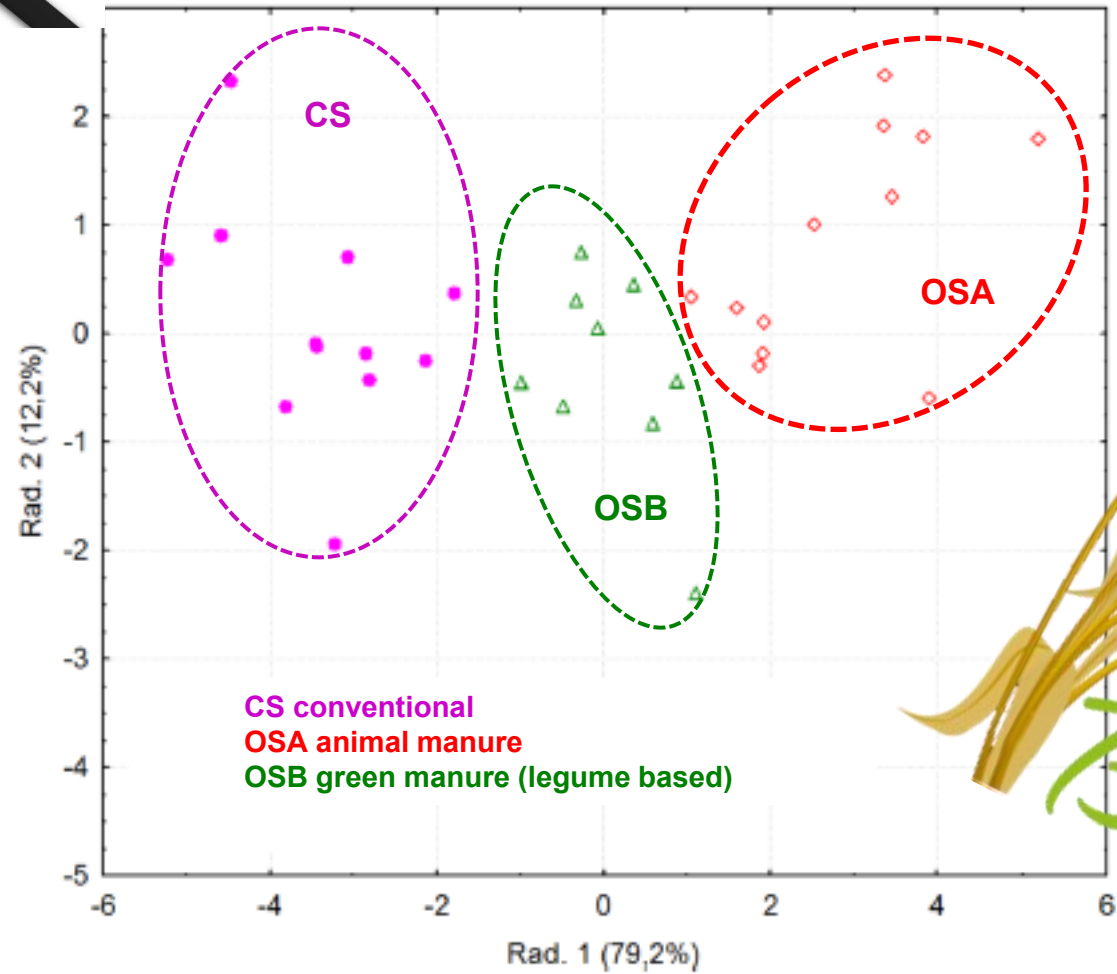
$^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ Isotopic Fingerprints



Peaks: Ala (1), Val (2), Ile (3), Leu (4), Gly (5), **Nleu (6)**, Pro (7), Thr (8), Asx (9), Glx (10), Phe (11)

RESULTS

Multivariate analysis





Passata di pomodoro: annacquamento e provenienza geografica

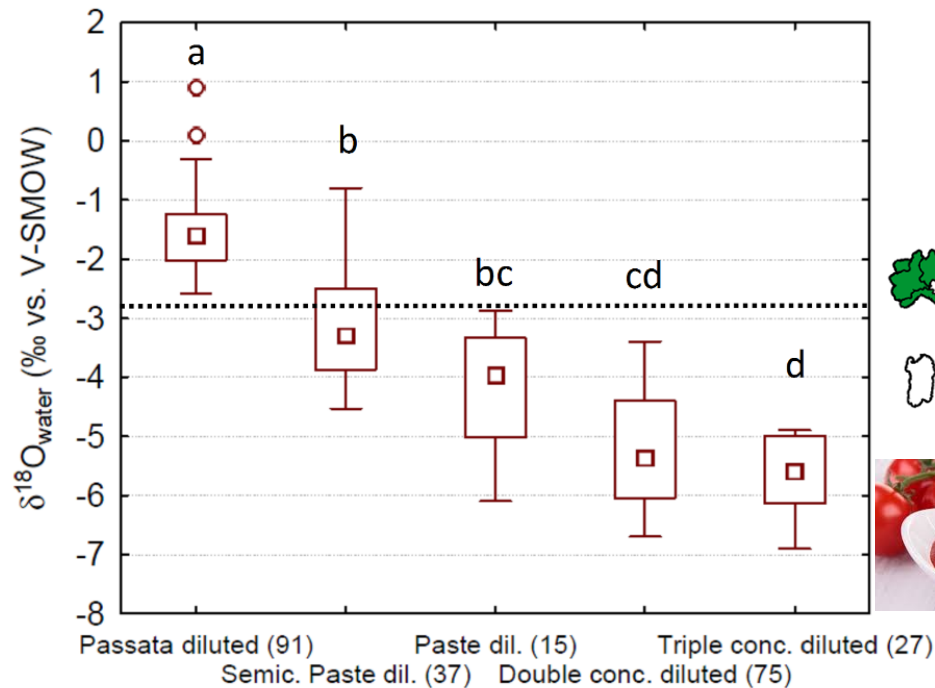
More information in

Bontempo et al., RCM 2011

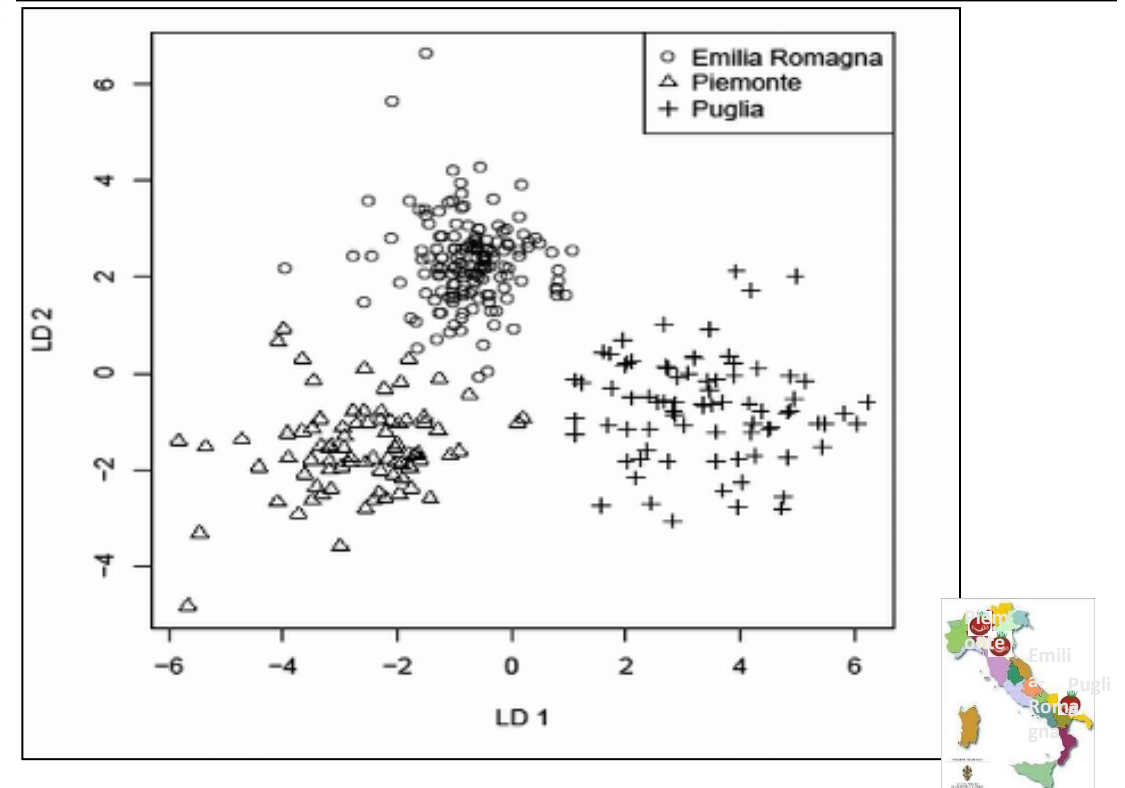
Bontempo et al., Food Control 2014

Passata di pomodoro: annacquamento e provenienza geografica

La passata di pomodoro è un prodotto che deve essere ottenuto ...da pomodoro fresco, ...non è consentita la ricostituzione della passata utilizzando concentrato di pomodoro con aggiunta di acqua (Ministerial Decree 23rd September 2005)



Etichettatura d'origine obbligatoria per i pomodori freschi utilizzati nella produzione della passata (Ministerial Decree 17th February 2006)



Lower threshold limit for tomato passata from fresh tomatoes: **-2.9 ‰**

Stable isotope ratios + Elemental composition

95 % of correct reclassification



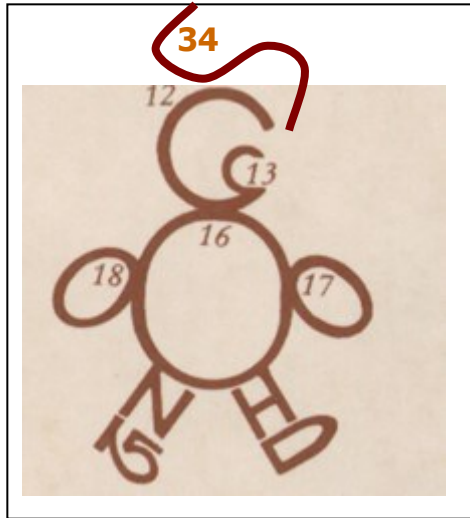
Formaggio: provenienza geografica e disciplinare di produzione

More information in

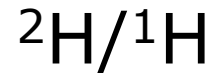
Camin et al., ACA, 2012

Grana Padano official production protocol

Stable Isotope Ratios of Bioelements and Trace element



- animal feed (corn)
- provenance

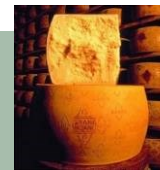


Li, Be, B, Na, Mg, P, K, Ca,
V, Mn, Fe, Co, Ni, Cu, Zn,
Ga, Ge, As, Se, Rb, Sr, Y,
Mo, Pd, Ag, Cd, Sn, Sb, Te,
Cs, Ba, La, Ce, Pr, Nd, Sm,
Eu, Gd, Dy, Ho, Er, Tm, Yb,
Re, Ir, Au, Hg, Pb, Bi, U

- Provenance
- Cheese production technology (curdling, salting, migration from manufacturing equipment)



Reference data



Italy:

- N. 1150 (200/ year) PDO Grana Padano
- N. 65 Biraghi and Valgrana cheeses

Czech Republic:

N. 38 Gran Moravia

Germany:

N. 38 Hartkäse

Austria: N.20

Lithuania:

N. 36 Goya + Dzjugas

Latvia: N. 30

Poland: N. 12

France: N. 14

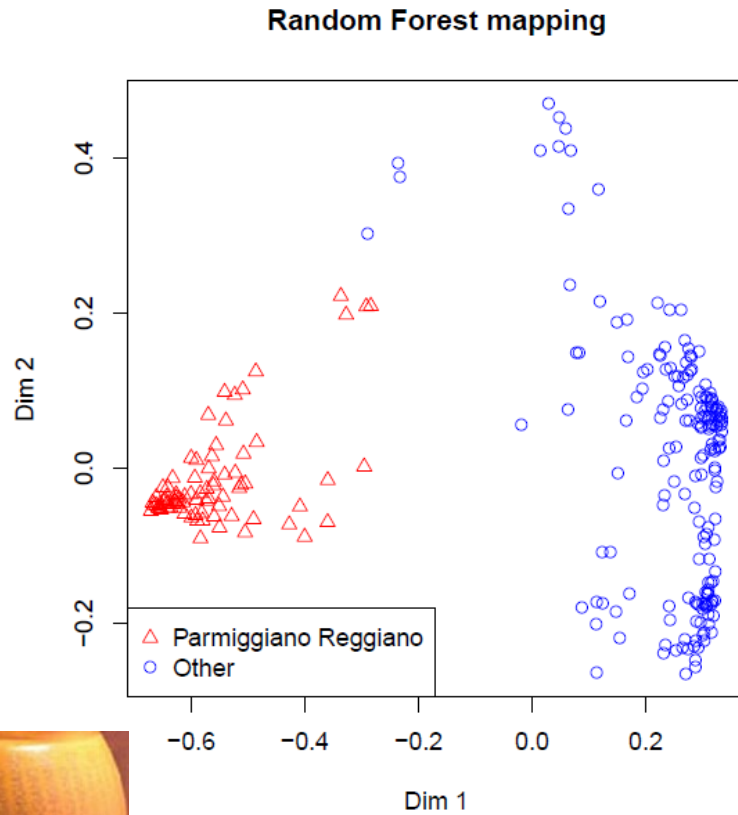
Russia: N. 12

Belgium + Holland: N. 5 + 5

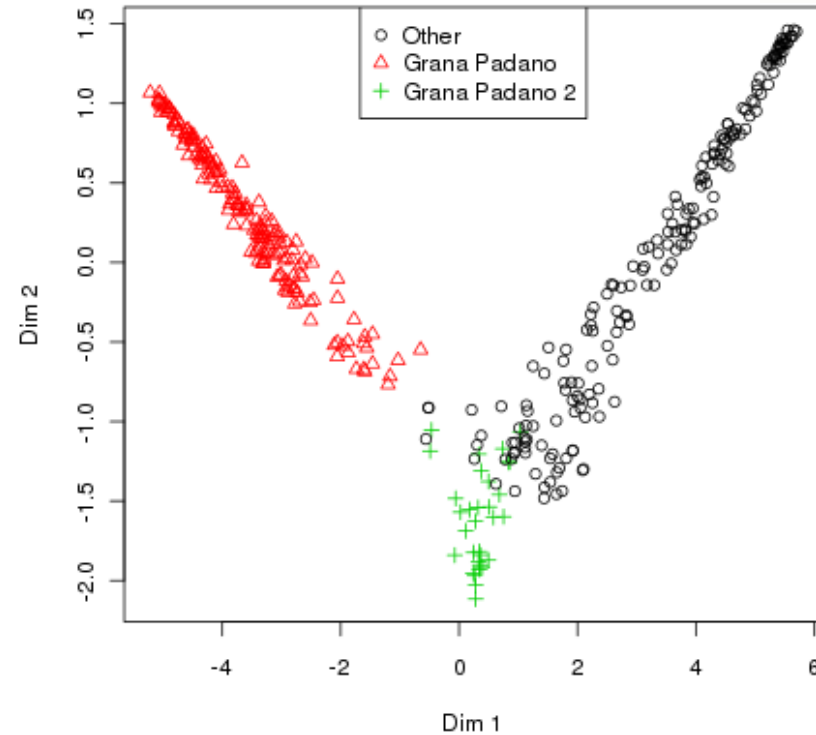
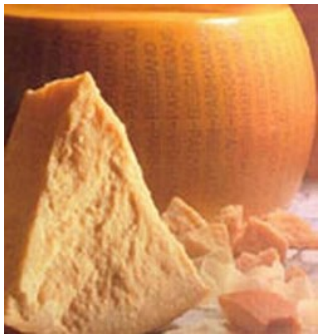
China: N. 9, Japan: N. 2

USA: N. 18, New Zeland: 2

Random Forest Model for the traceability of Parmigiano Reggiano cheese



Camin et al., ACA, 2012



Random Forest Model (2011) for the traceability of Grana Padano cheese

Specification for PDO Grana Padano cheese

18.6.2011

EN

Official Journal of the European Union

L 160/65

COMMISSION IMPLEMENTING REGULATION (EU) No 584/2011

of 17 June 2011

approving non-minor amendments to the specification for a name entered in the register of protected designations of origin and protected geographical indications (Grana Padano (PDO))

Rapporti isotopici come parametri ufficiali per verificare l'autenticità del Grana Padano DOP grattugiato e in scaglie



Tracciabilità del formaggio

Norma UNI

UNI 11692:2017



Sintetico o naturale?



Riso rosso fermentato (RYR)

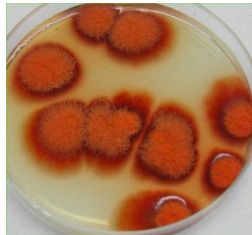


➔ integratore alimentare

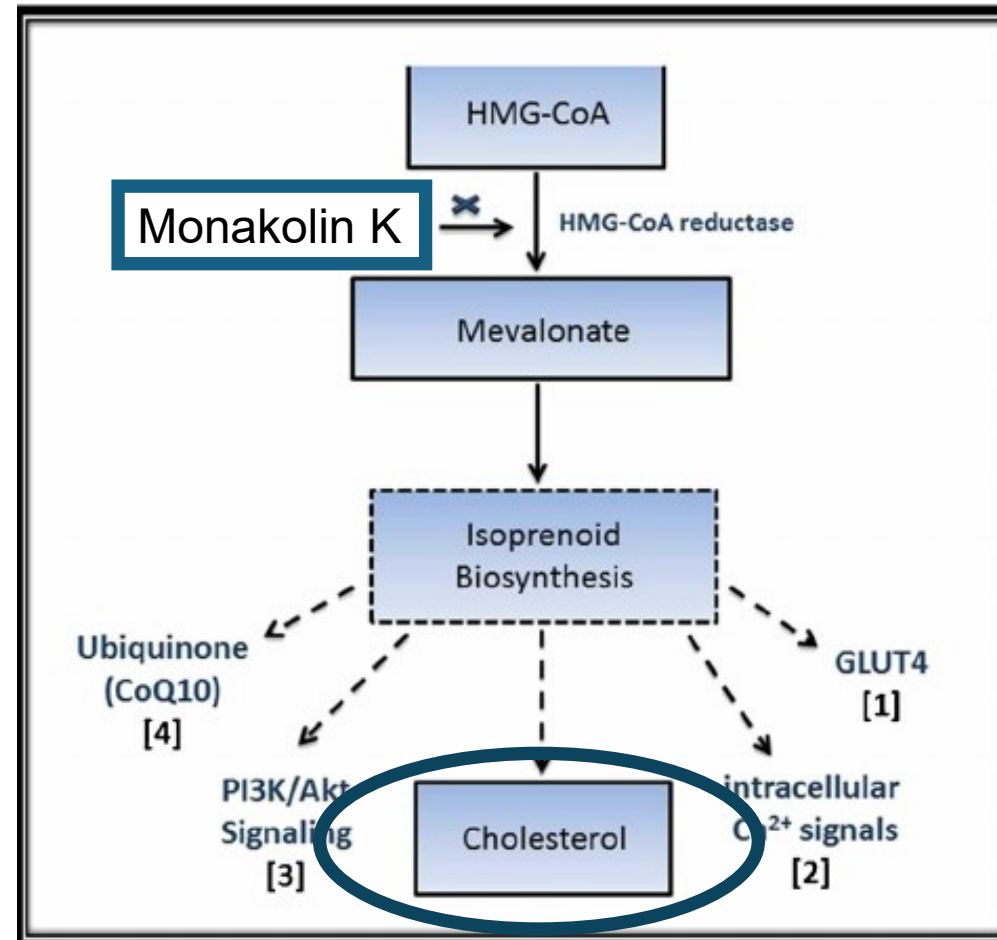
Riso



+ *Monascus purpureus*

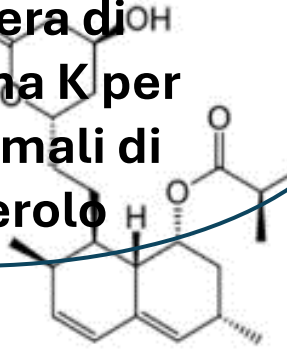


Red yeast rice (RYR)

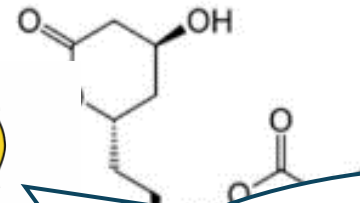


Monacolina K o Lovastatina?

10 mg di assunzione
giornaliera di
monacolina K per
livelli normali di
colesterolo



Difficile raggiungere
questo livello
farmacologicamente
efficace nella RYR

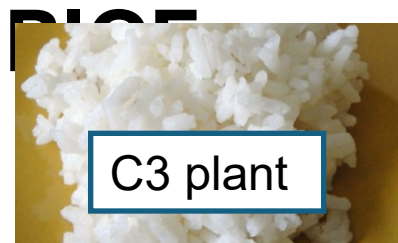


RYR addizionato con
lovastatina, senza
dichiarazione??



Processo di produzione ...

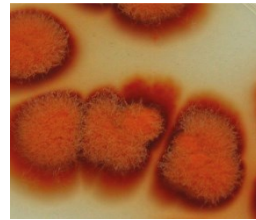
RED YEAST



C3 plant

Steam Rice

+



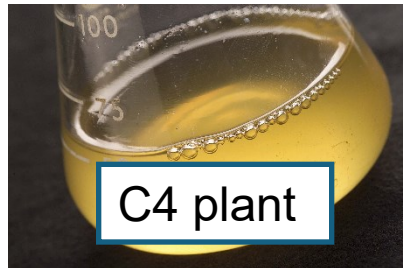
Monascus purpureus

Fermentation



Red yeast rice with
lovastatin

LOVASTATIN



C4 plant

Fermentation Broth
with cane sugar

+



Aspergillus terreus

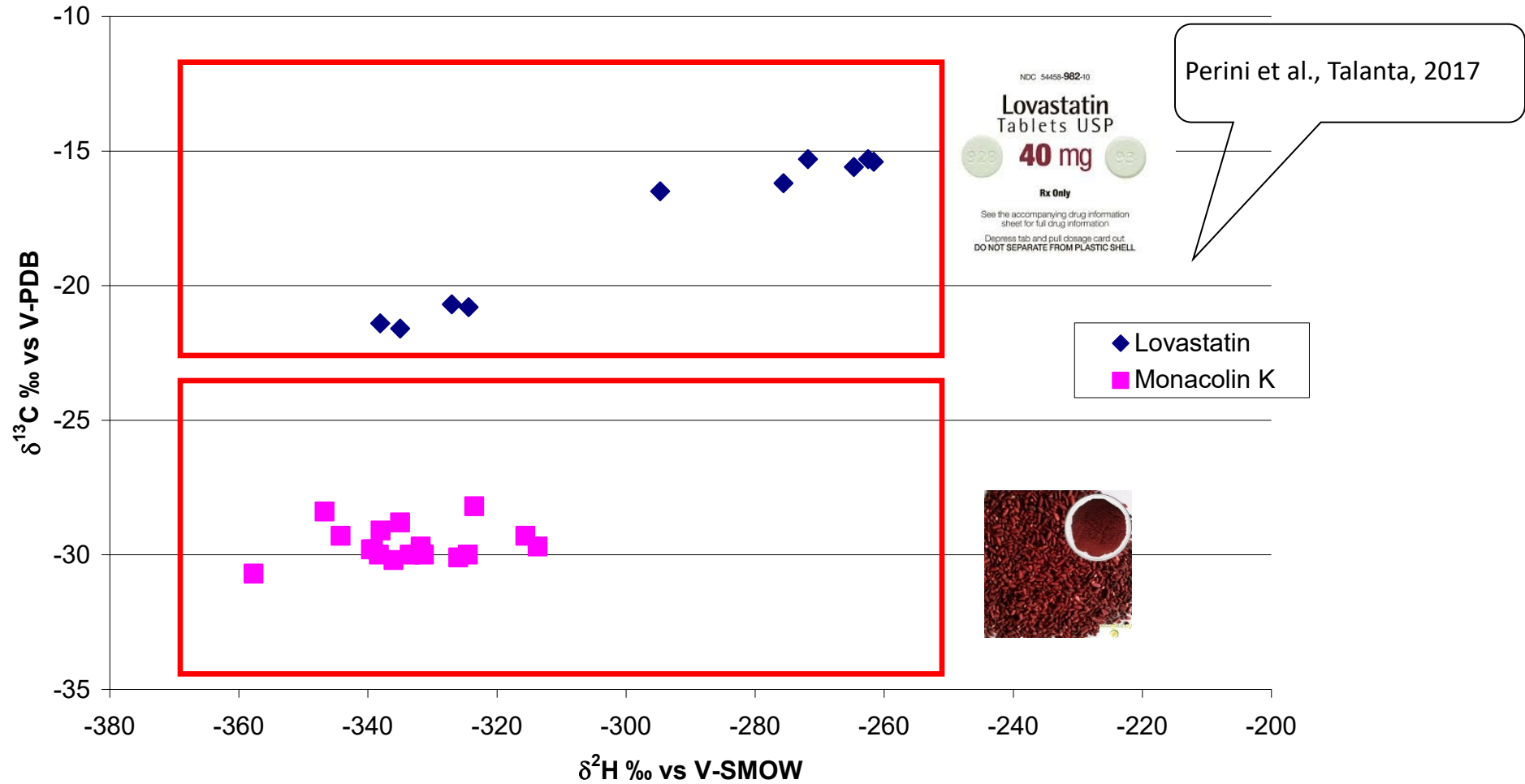
Fermentation



+ red coloured rice



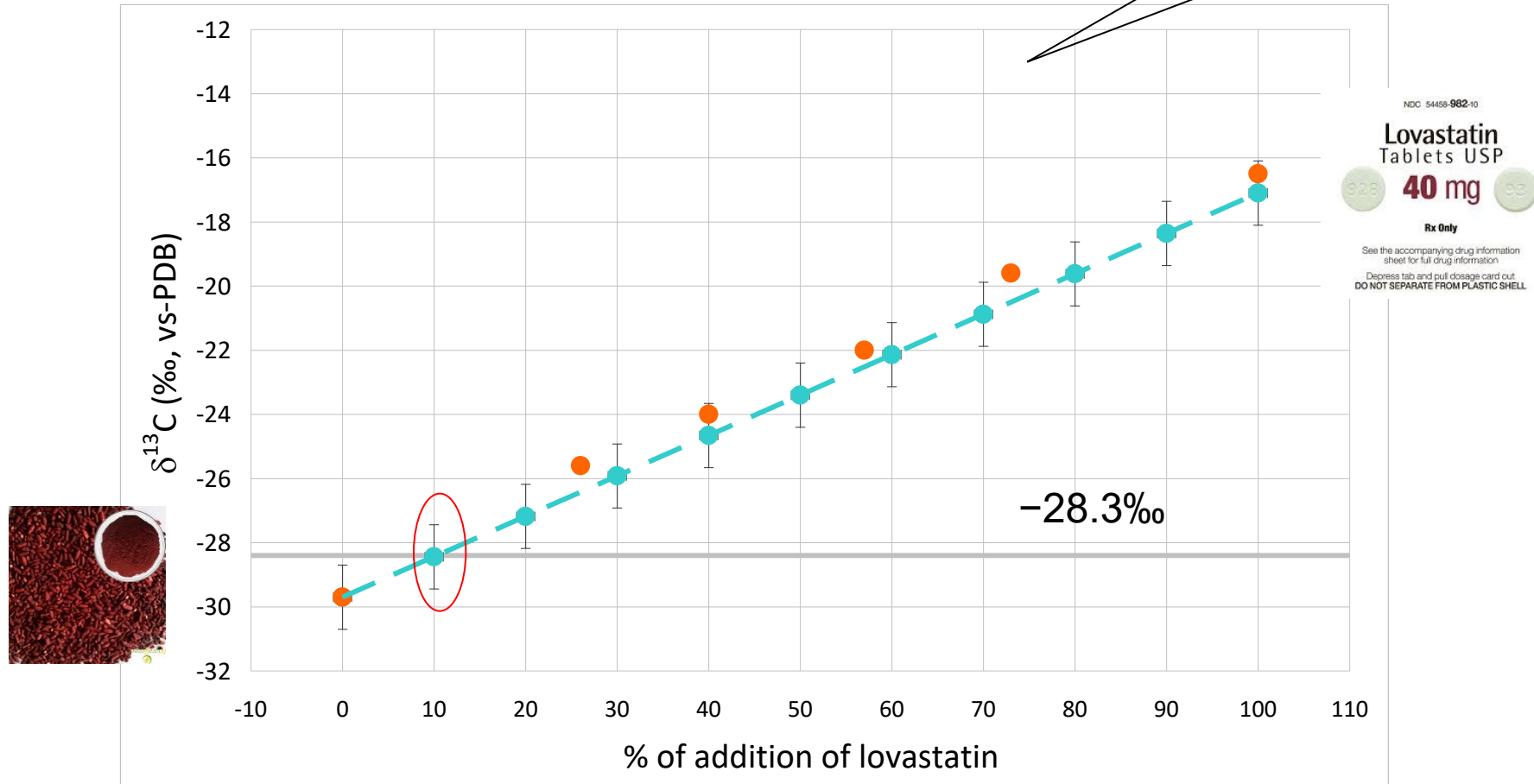
$\delta^{13}\text{C}$ and $\delta^2\text{H}$ of Monacolin K and Lovastatin



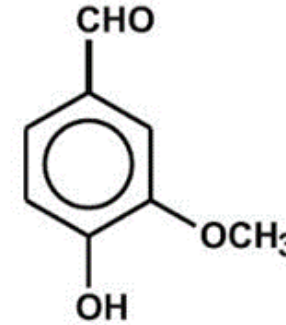
Extraction: 75% ethanol; recovery with methanol; preparative HPLC

Identificazione delle miscele (lovastatina + monacolina K)

Perini et al., Talanta, 2017

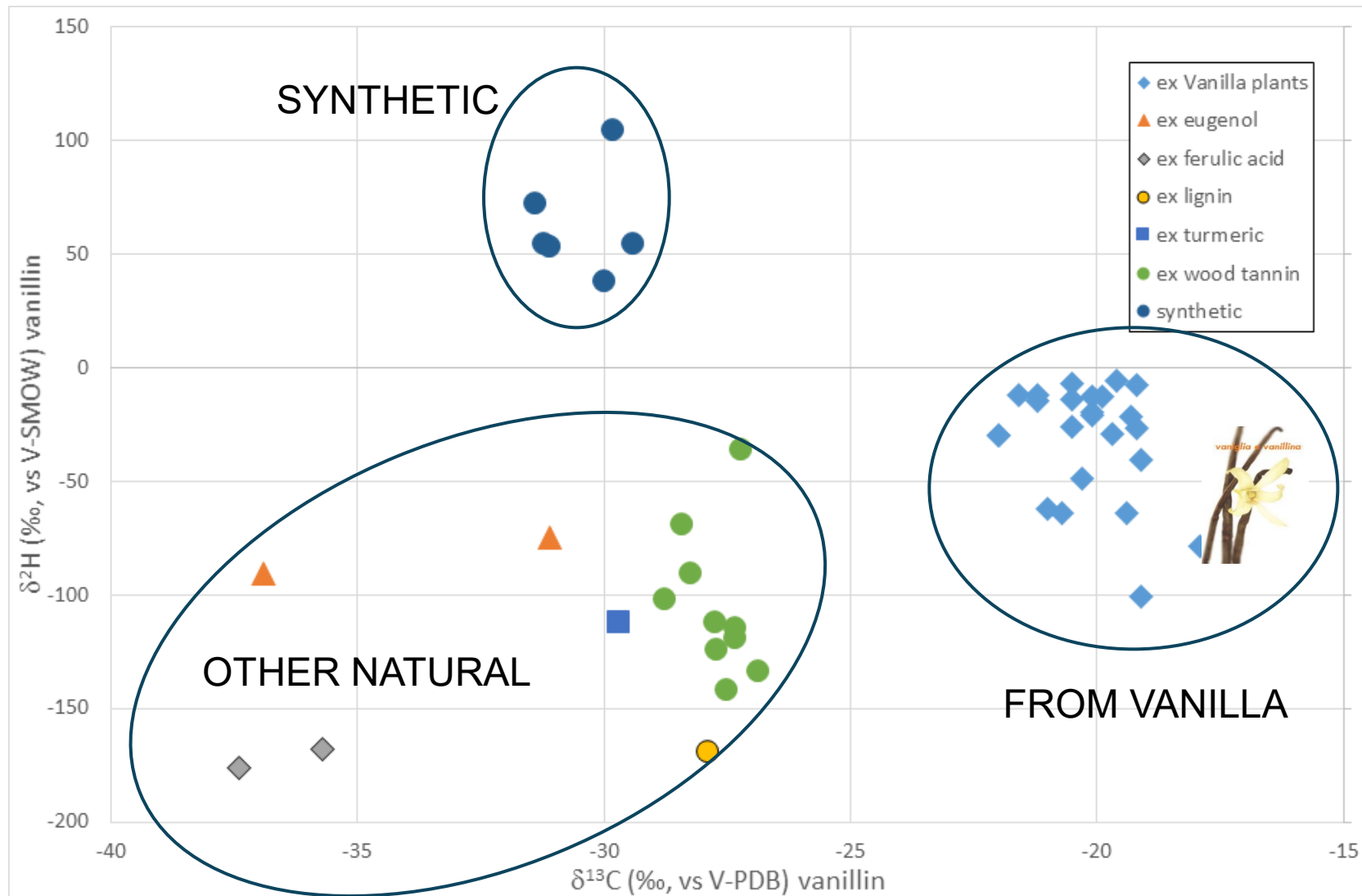


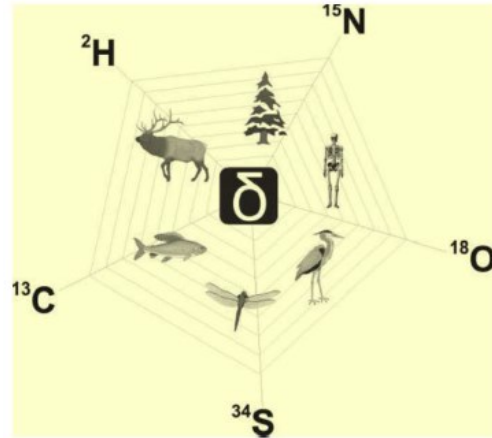
Vanillina



- ingrediente aromatizzante negli alimenti e nelle bevande.
- viene estratto principalmente dalla *Vaniglia planifolia*
- è la seconda spezia più costosa del mondo, seconda solo allo zafferano.
- disponibili nuove fonti di vanillina, prodotte sinteticamente o biotecnologicamente da precursori naturali, ad es. lignina, eugenolo, acido ferulico e curcumina.

$\delta^{13}\text{C}$ e $\delta^2\text{H}$ della vanillina





Utilizzo di rapporti isotopici stabili per studiare le rotte migratorie dei passeriformi



Progetto Alpi

BOCCA DI CASET





Campionamento

2010 e 2011



0 1 2 3 4 5 6 7 8 9 10



Pied Flycatcher
(*ficedula hypoleuca*)

N = 96



0 1 2 3 4 5 6 7 8 9 10

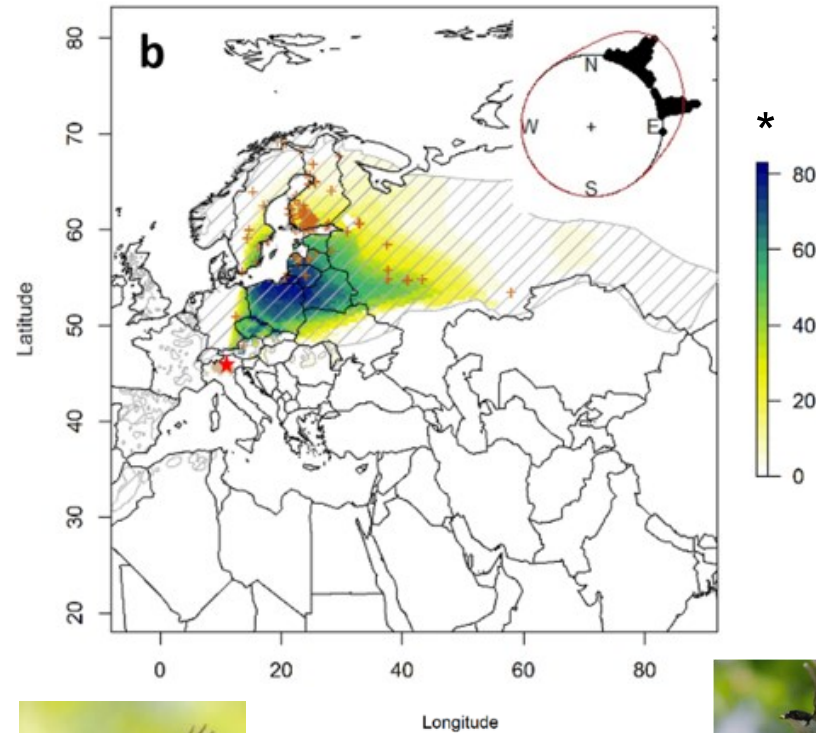


European Robin
(*Erithacus rubecula*)

N = 103

Ecologia animale: Assegnazione all'origine natale

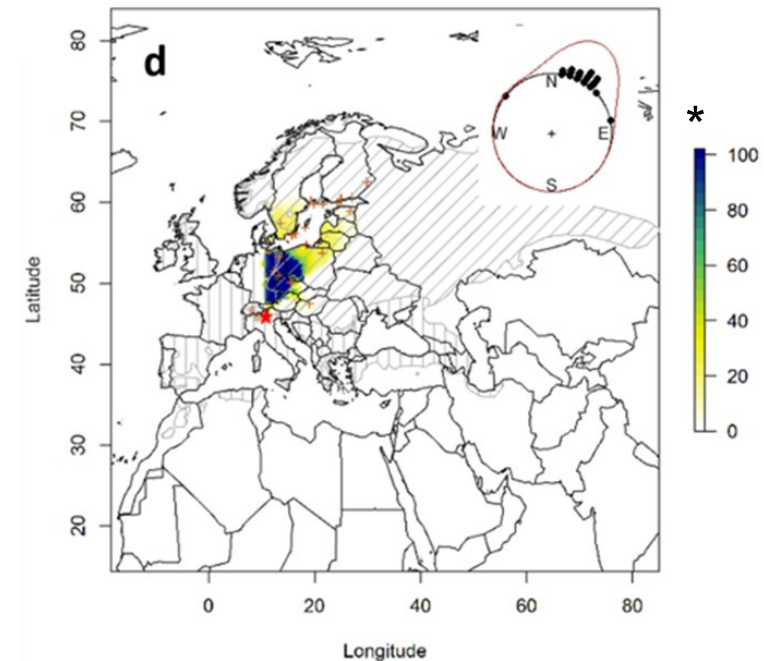
Pied flycatcher



Franzoi et al., 2020



European robin



*number of birds



Thank you for your kind attention!

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