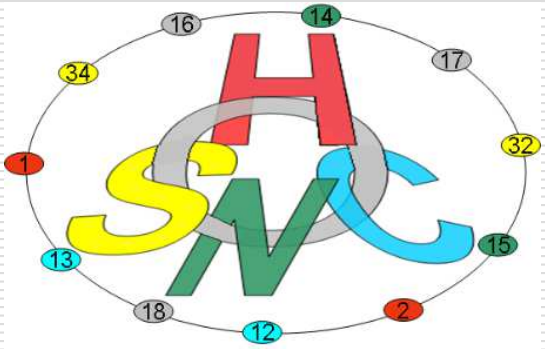


Stable Isotope Ratios for Food Traceability and Authentication

FONDAZIONE EDMUND MACH

Federica Camin

Categories of food adulteration

- non-compliance with the established legislative standards
- economic adulteration of high value foods:
  - substitution by cheaper but similar ingredients
  - extension of food using adulterant (water, sugar)
- misdescription and/or mislabelling of geographical, botanical, species origin or agricultural regime (organic/conventional)

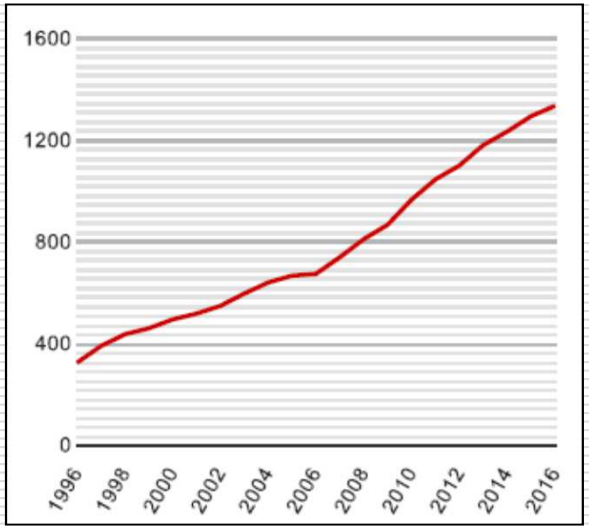
PDO-PGI -TSG products

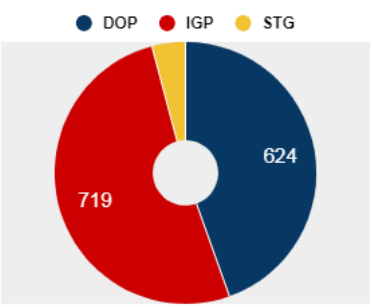
- Food with a declared origin
  - PDO Protected Designation of Origin
  - PGI Protected Geographical Indication
  - TSG Traditional Speciality Guarantee

PDO – PGI -STG in Europe

Total number: 1401 (16/04/18)

Italy	**295**
France	**247**
Spain	**196**
Portugal	**139**
Greece	**106**
Germany	**90**





EU Reg (CE) N. 510/2006 and 1151/2012 for protecting PDO and IGP

FONDAZIONE EDMUND MACH

Source: Elaborazione Fondazione Qualivita su dati UE.

Strategies

- 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 ratios methods as official standards

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	mapple 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, 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	$^{13}C/^{12}C$	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 Ratios of Bioelements

Wada et al., 1995

$^{2}H/^{1}H$

$^{13}C/^{12}C$

$^{15}N/^{14}N$

$^{18}O/^{16}O$

$^{34}S/^{32}S$

FONDAZIONE EDMUND MACH

Analytical instrumentation

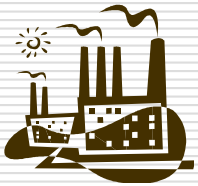
- Isotope Ratio Mass Spectrometry, interfaced with Elemental Analyser, Pyrolyser, CO_2 equilibration system, GC-c, GC-p
- Site-specific Natural Isotopic Fractionation - Nuclear Magnetic Resonance

IRMS

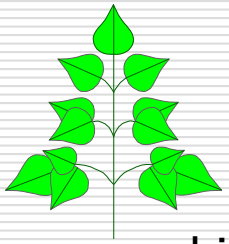
SNIF-NMR

Factors of variability in vegetal raw materials

(emissions, natural and industrial)



[Agriculture]



(photosyntesis, specie)

biological

anthropogenic

$\delta^{13}C$

$\delta^{15}N$

Indicator function of $\delta^{2}H$

[Physiology]

[Environment]

$\delta^{34}S$

$\delta^{18}O$

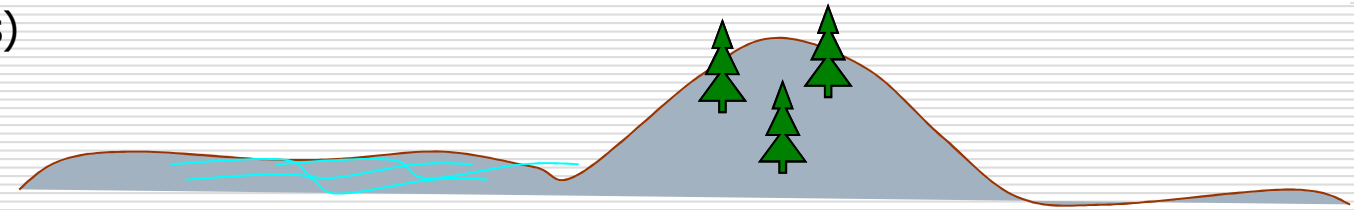
geological

climatological

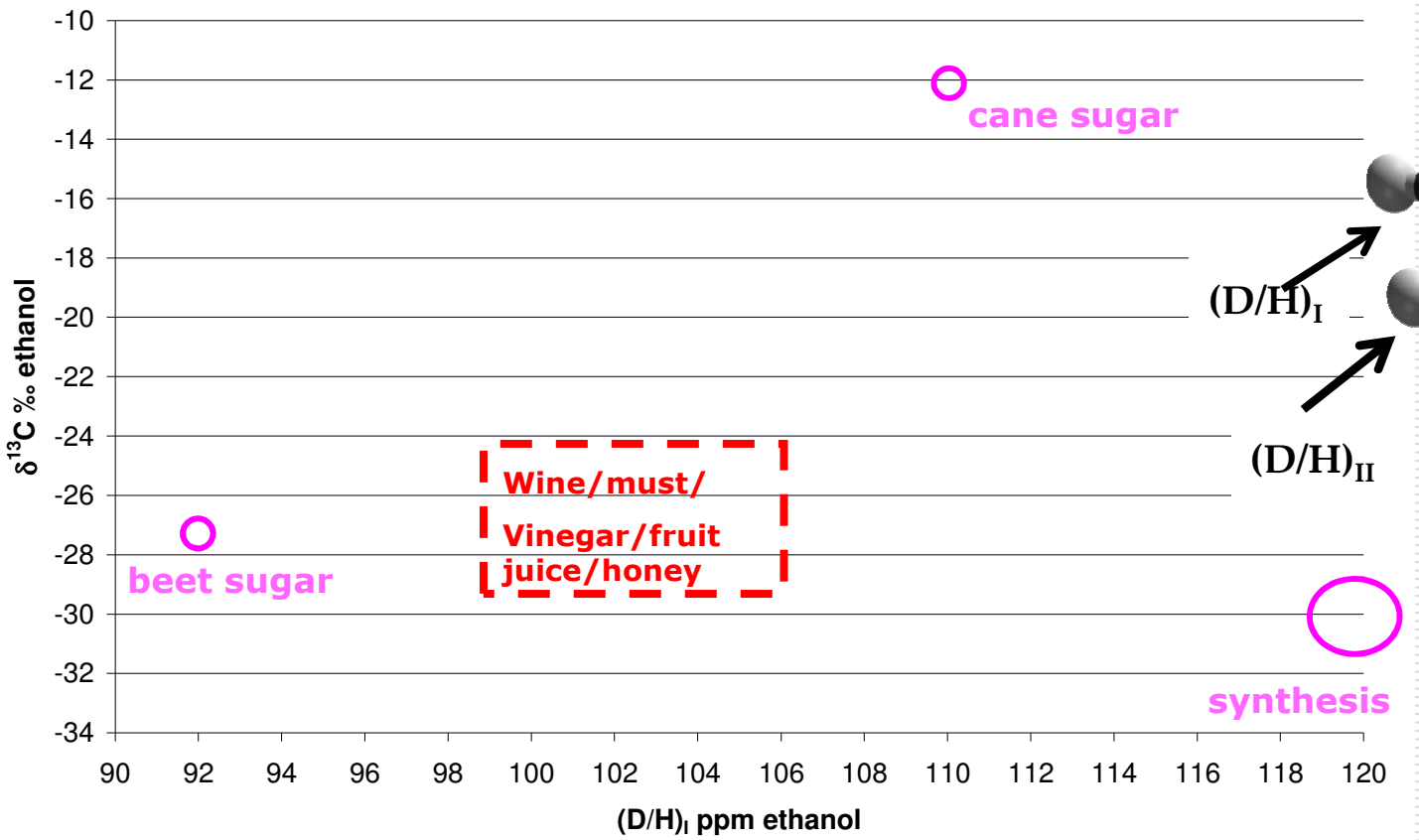
[Geography]

(precipitation, evaporation)

(rocks, soils)



Stable Isotope ratios for detecting sugar addition



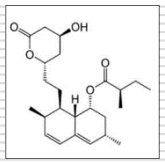
	$(D/H)_1$	$(D/H)_2$	$\delta^{13}C$	$\delta^{18}O$
Wines, 95% CI	98.8 / 106	124.5 / 135.5	-29.3 / -24.3	-1.3 / 8.9
Beet sugar	92.5		-27.5	
Cane sugar	109.5		-12	
Water (north Italy)				-9
Water (south Italy)				-5

FONDAZIONE EDMUND MACH

Dordevic et al., ACA, 2013

Red yeast rice

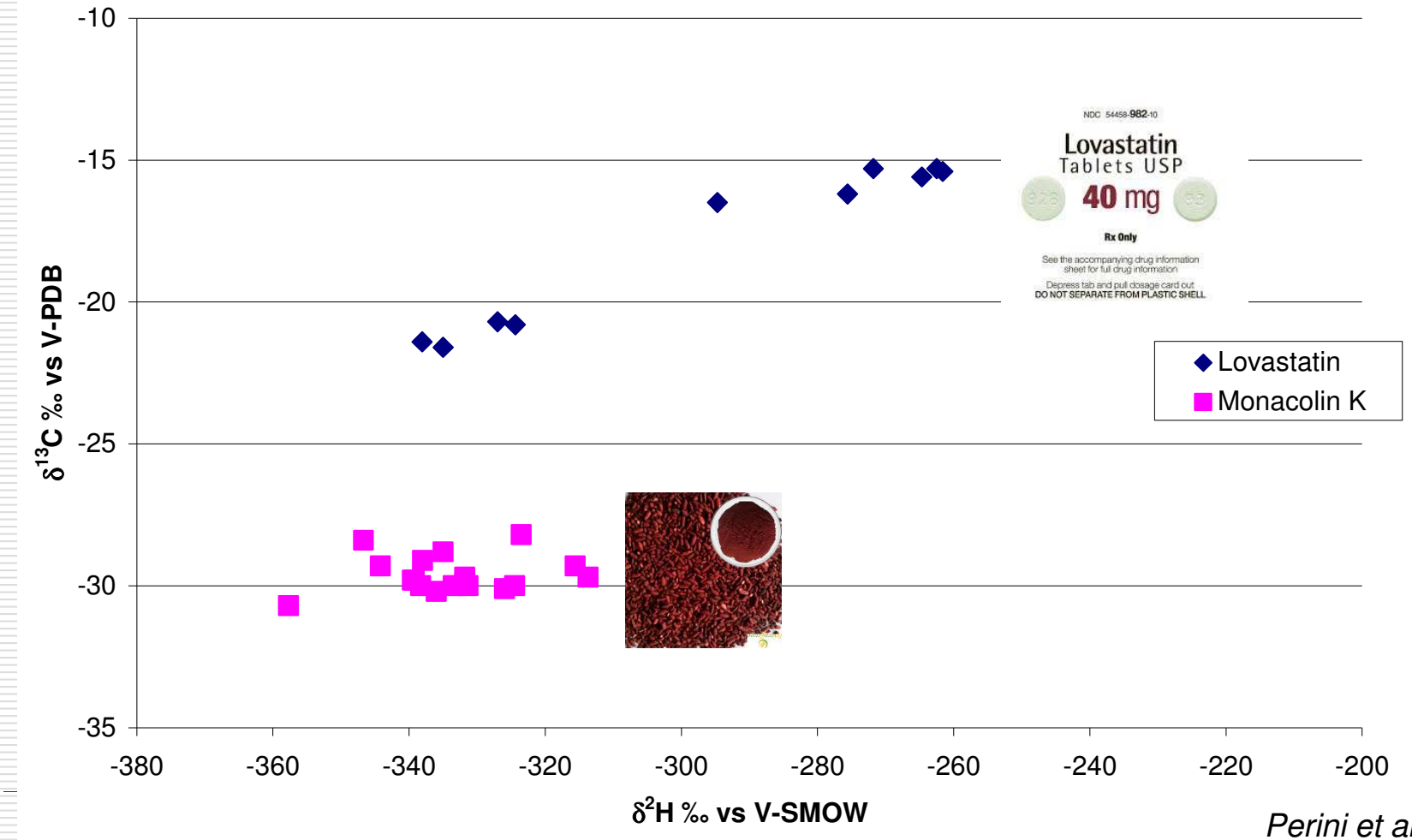
- a dietary supplement used in traditional Chinese medicine
- obtained from rice fermented with the mold *Monascus purpureus* (*Aspergillaceae* family)



- Monacolin K, inhibits HMG-CoA reductase, reducing cholesterol and triglyceride
- Bio synthetic lovastatin (Mevacor, Merck & Co) is not chemically distinguishable from monacolin K.
- RYR spiked with lovastatin, without declaration.

Perini et al., 2017, Talanta

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



Perini et al., 2017, Talanta

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

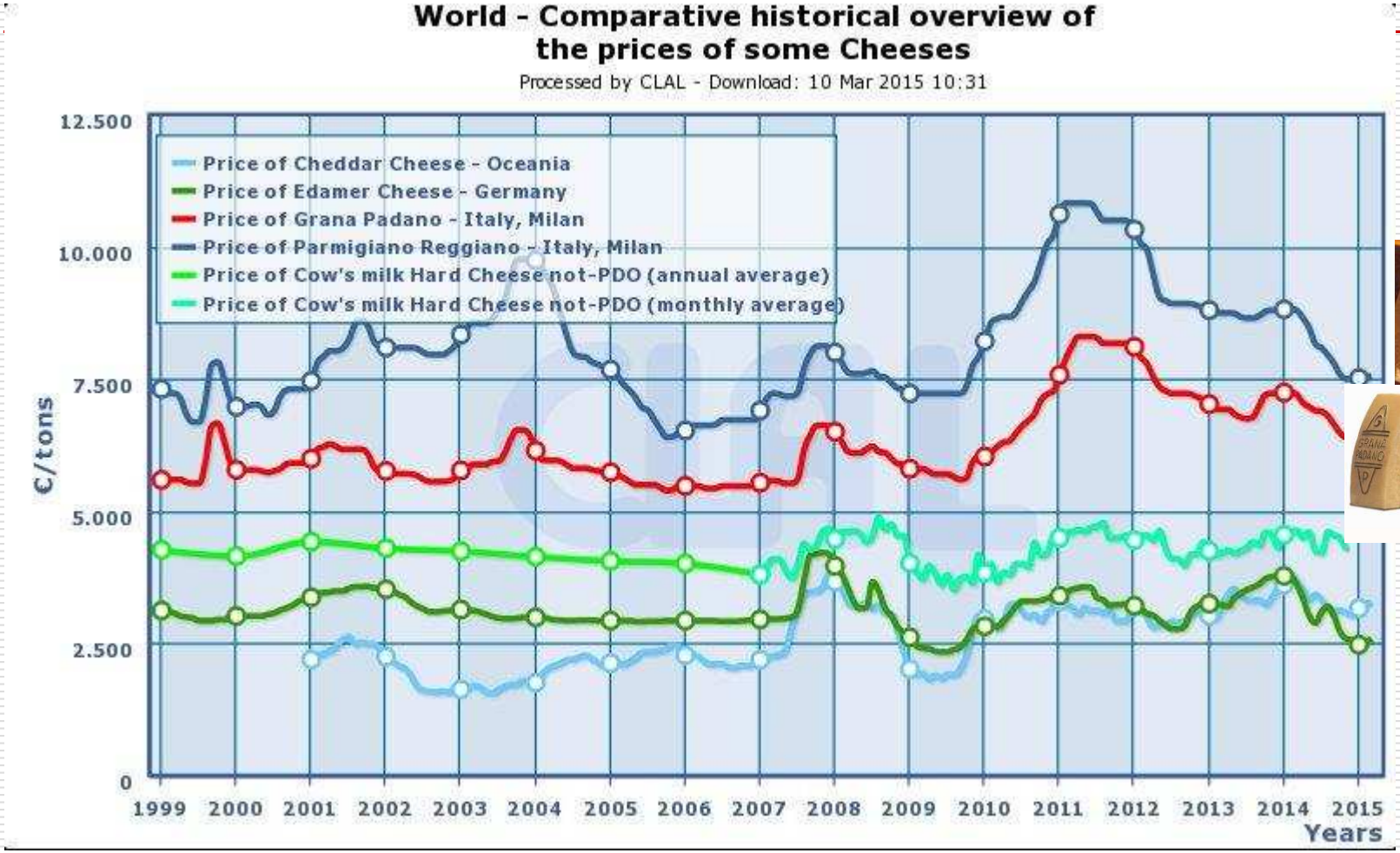
The "*top ten" Italian PDO and PGI for sales 2012*

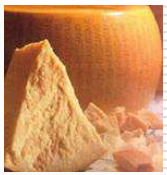
	milions €
Grana Padano	1.395
Parmigiano Reggiano	1.357
Prosciutto di Parma	992
Prosciutto di San Daniele	302
Mozzarella di Bufala Campana	288
Aceto Balsamico di Modena	260
Gorgonzola	249
Mortadella Bologna	224
Bresaola della Valtellina	215
Mela Alto Adige	170
Total (production costs)	**5.453**
% of total	**83,8**

FONDAZIONE EDMUND MACH

Source: Osservatorio Ismea prodotti Dop e Igp

Price of hard cheese





FONDAZIONE EDMUND MACH

Reference data

Italy:
- N. 1343 (200/ year) PDO Grana Padano
- N. 70 Biraghi and Valgrana cheeses

Czech Republic:
N. 46 Gran Moravia

Germany:
N. 41 Hartkäse

Austria: N.23

Lithuania:
N. 37 Goya + Dzjugas

Latvia: N. 30

Poland: N. 12

France: N. 18

Russia: N. 12

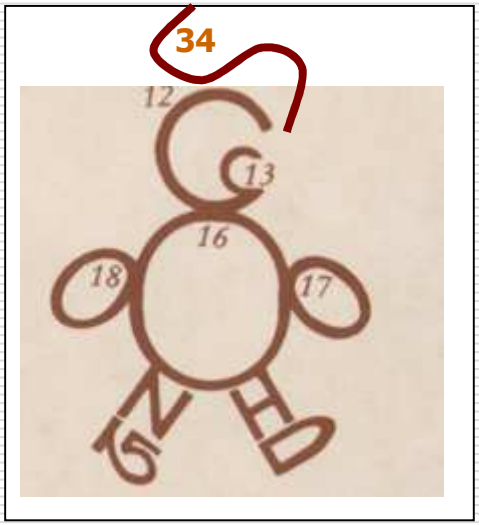
Unghary: N. 8

Belgium Luxemburg + Holland:
N. 5 + 3 + 6

Spain: N. 4; **Switzerland:** N. 3; **China**: N. 9; **Japan**: N. 2; **Turkey**: N. 1; **Ukraine**: N. 1; **USA**: N. 22, **New Zeland**: 2

FONDAZIONE EDMUND MACH

Stable Isotope Ratios of Bioelements and Trace element



- animal feed (corn)
- provenance

$^{2}H/^{1}H$
$^{13}C/^{12}C$
$^{15}N/^{14}N$
$^{18}O/^{16}O$
$^{34}S/^{32}S$

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)

SIRA determination: IRMS
Trace element determination: ICP-MS

FONDAZIONE EDMUND MACH

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))

Isotopic ratios as official parameters for verifying the authenticity of PDO Grana Padano cheese in grated and shredded form

The variability ranges have been deposited at the Italian Ministry of Agriculture, Food and Forestry

Validation of the methods

- **International collaborative study:**
  - IUPAC protocol and the ISO Standards 5725/2004 and 13528/2005.
  - 7 types of cheeses in blind duplicate
  - 20 laboratories
  - H, C, N and S isotope ratios and 13 elements: Li, Na, Mn, Fe, Cu, Se, Rb, Sr, Mo, Ba, Re, Bi, U
  - Sr and SR are available
- **UNI for recognition**

Camin et al., RCM, 2015

IS IT REALLY ORGANIC?

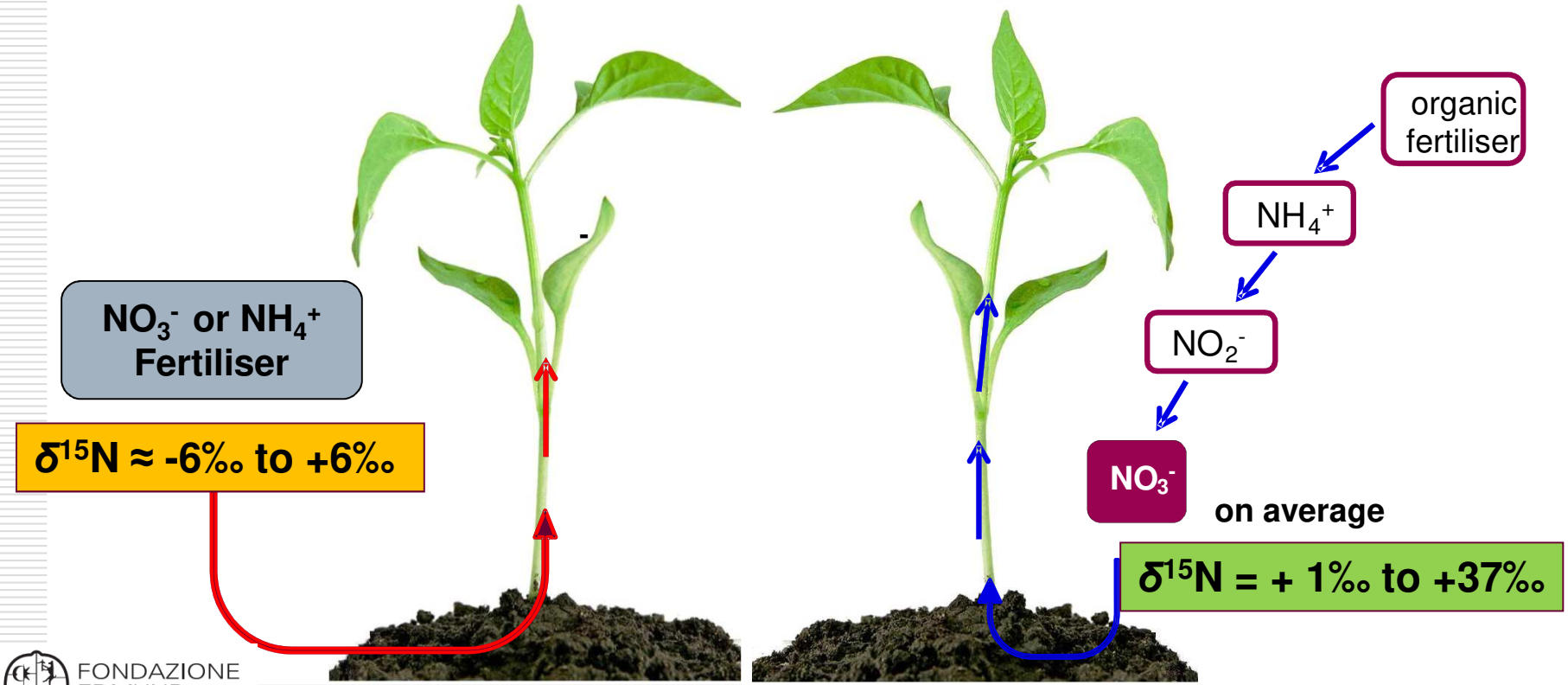
Compound-specific $\delta^{15}N$ and $\delta^{13}C$ analyses of amino acids for potential discrimination between organically and conventionally grown wheat

Paolini et al., JAFC 2015

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

CONVENTIONAL

ORGANIC

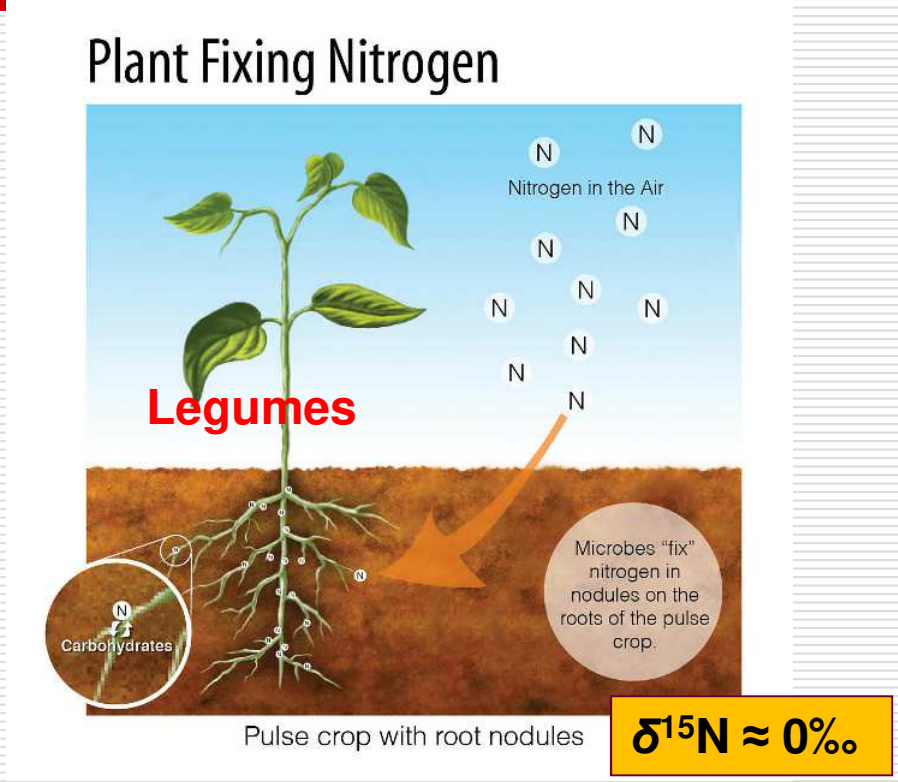


FONDAZIONE EDMUND MACH

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



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

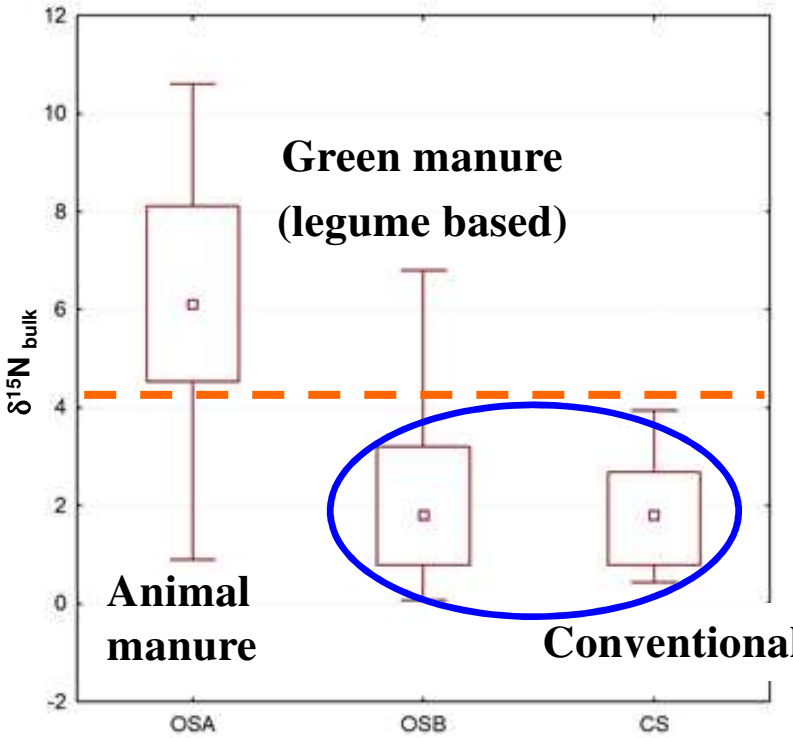
Paolini et al., JAFC 2015

Wheat sampling

Italy (17)

Denmark (18)

3 agricultural regimes



How to discriminate

Paolini et al., JAFC 2015

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

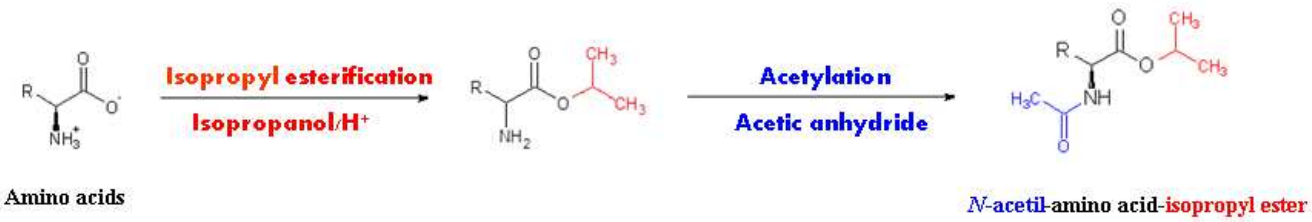
step 1

Protein hydrolysis

HCl 6M, 110°C, 24h

step 2

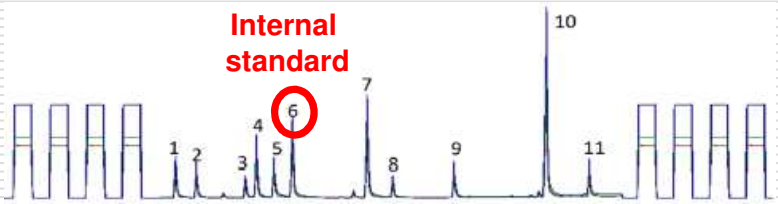
Amino acids derivatization



step 3

GC-c-IRMS analysis

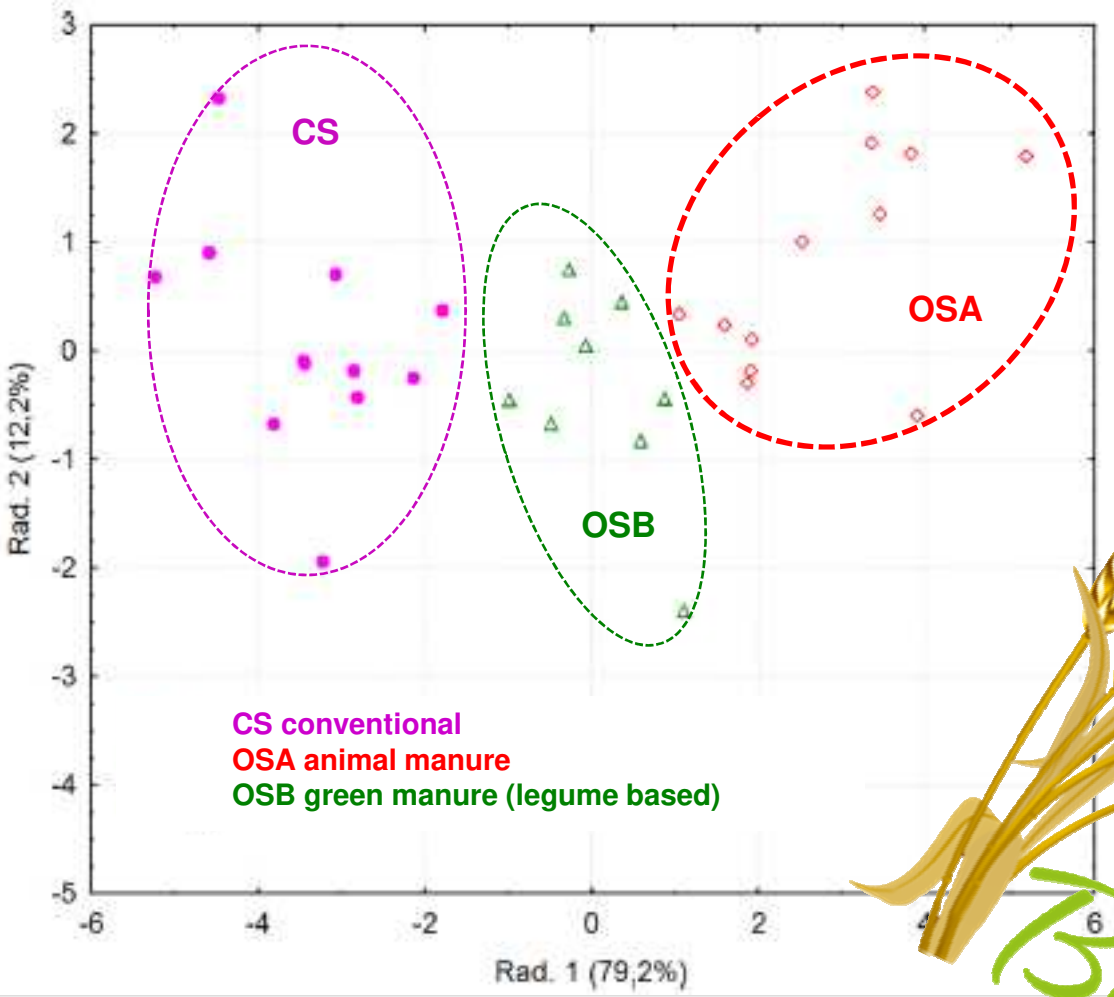
$^{15}N/^{14}N$ and $^{13}C/^{12}C$ Isotopic Fingerprints



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

Paolini et al., 2015, JAFC

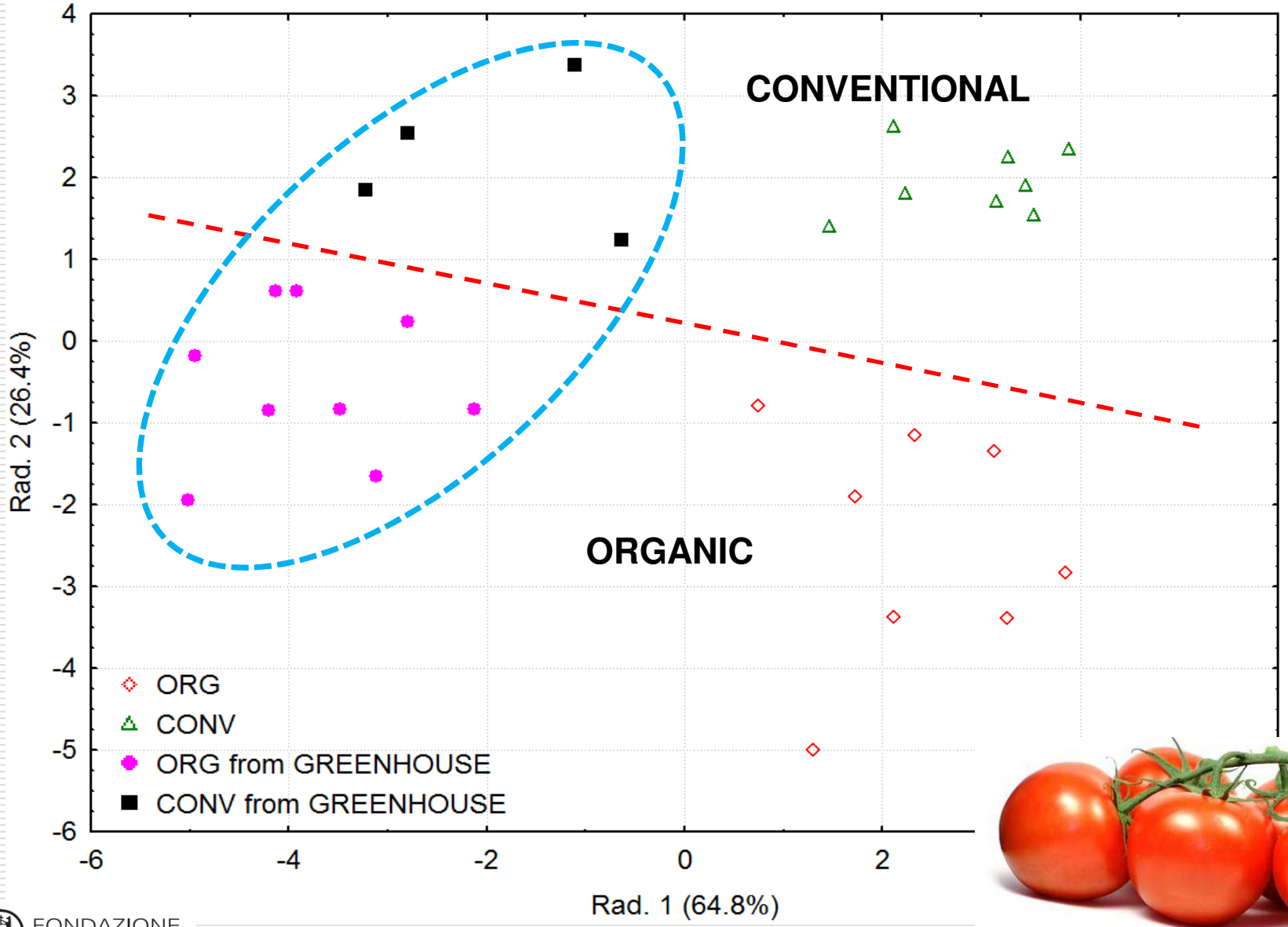
Multivariate analysis



CS conventional
OSA animal manure
OSB green manure (legume based)

Paolini et al., JAFC 2015

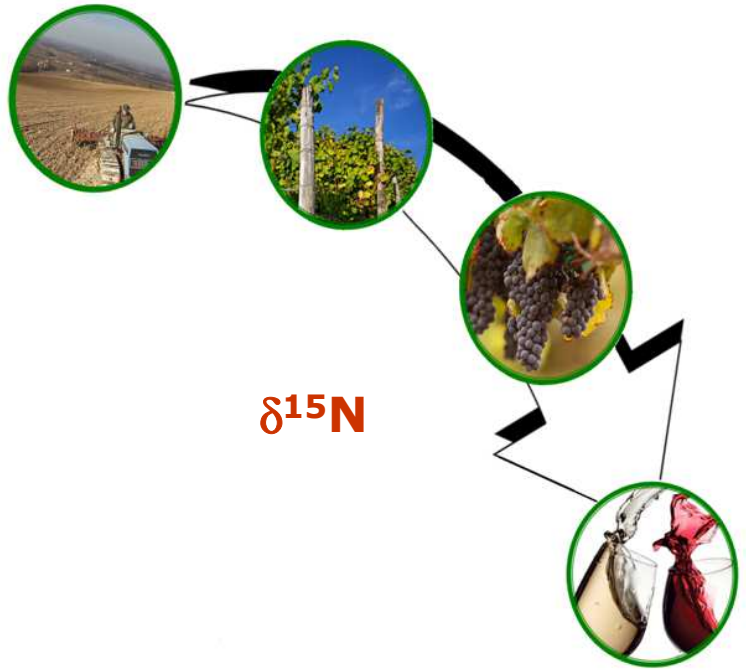
Organic vs. conventional tomato samples

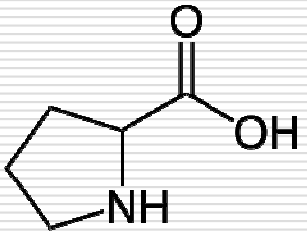


Van Leeuwen et al., in submission

$\delta^{15}N$ of wine and proline

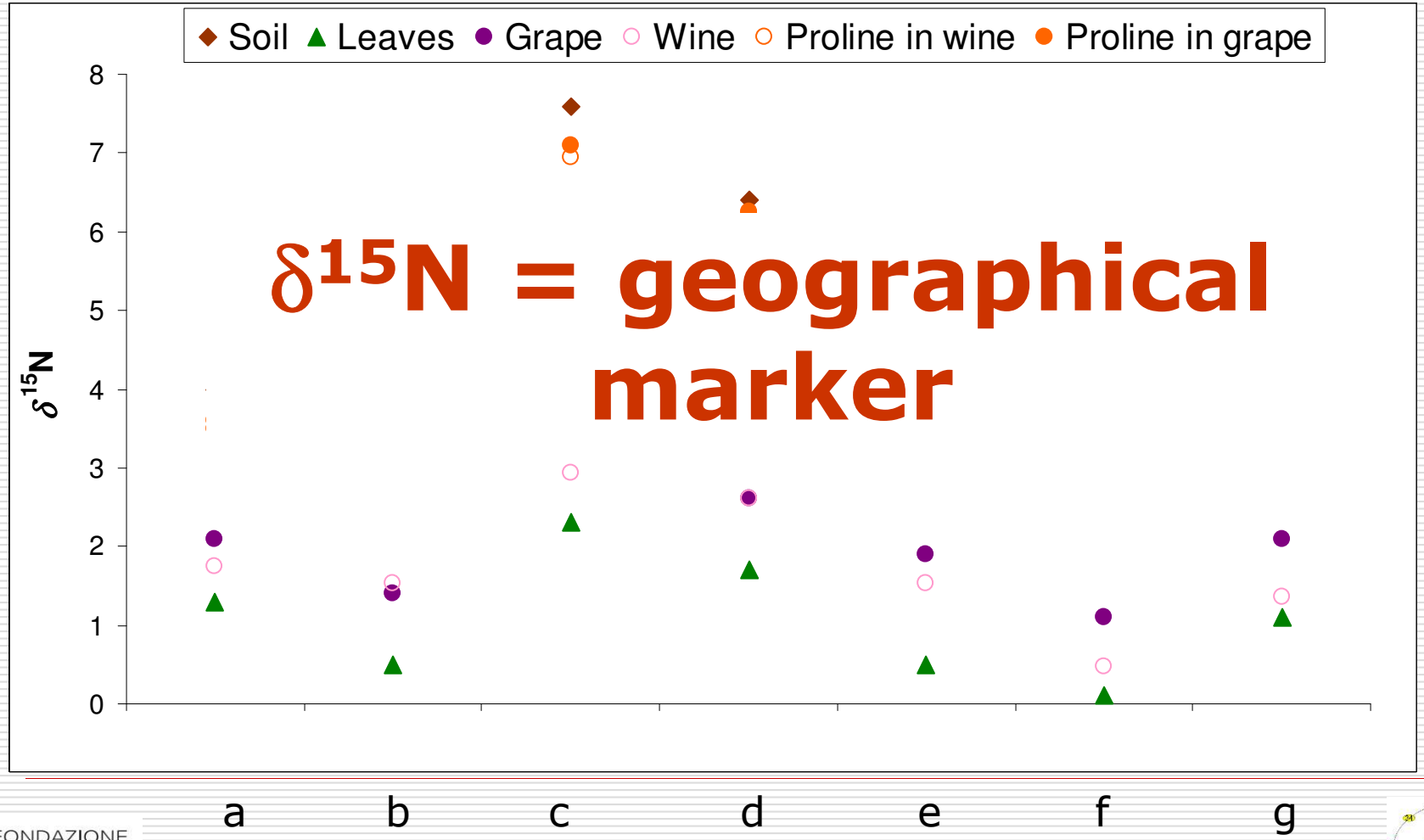
- Not yet investigate in wine
- From soil through vines (geographical marker)
- Analysis:
  - Bulk sample: EA-IRMS
  - Proline: GC-C-IRMS, after N-acetylisopropyl derivatization





Paolini et al, JMS, 2016

$\delta^{15}N$ of soil, leaves, grape, wine, proline



$\delta^{15}N$ = geographical marker

FONDAZIONE EDMUND MACH

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)

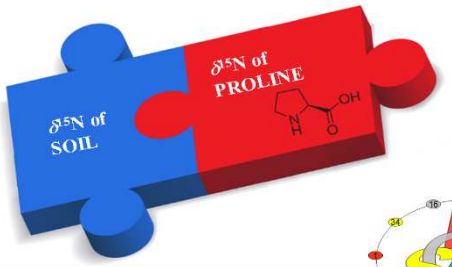
	Fermentation conditions	$\delta^{15}N$ (‰) proline in wine
Grape must 1	No adjuvant	11.5
	1 g/L IA	11.5
	0.5 g/L OA	11.4
	4.3 g/L OA	11.0
Grape must 2	No adjuvant	7.7
	1 g/L IA	7.6
	0.5 g/L OA	7.6
	4.3 g/L OA	7.1
Grape must 3	No adjuvant	10.1
	1 g/L IA	10.2
	0.5 g/L OA	10.1
	4.3 g/L OA	9.6

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!!



FONDAZIONE EDMUND MACH

Paolini et al., JMS 2016

Conclusion

- Types of food adulterations
  - ✓ non-compliance with the established legislative standards
  - ✓ economic adulteration of high value foods:
    - ✓ substitution by cheaper but similar ingredients
    - ✓ extension of food using adulterant (water, sugar)
  - ✓ misdescription and/or mislabelling of geographical, botanical, species origin or agricultural regime (organic/conventional)
- Official recognition /routine methods
- The combination with other techniques or with compound specific analysis improves the effectiveness of the method

Thank you for your kind attention!