EU funded project PURE JUICE, G6RD-CT-2002-00760

Workshop 9/03/2006

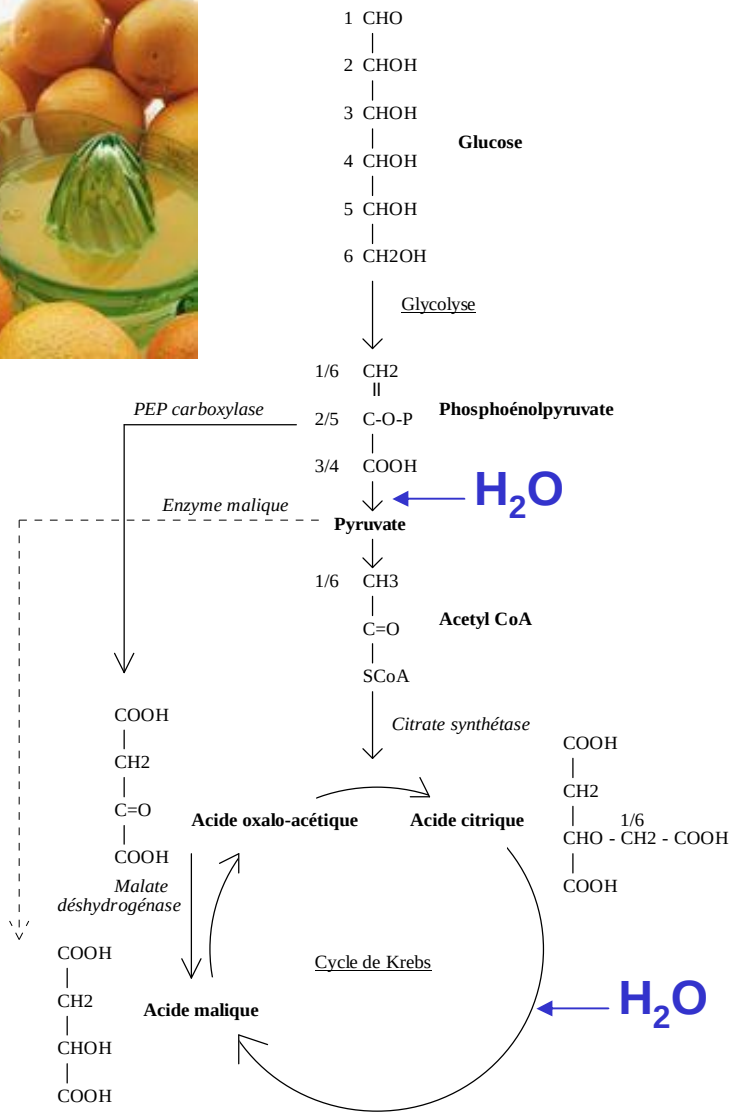
WP3:
Detection of undeclared added organic acids

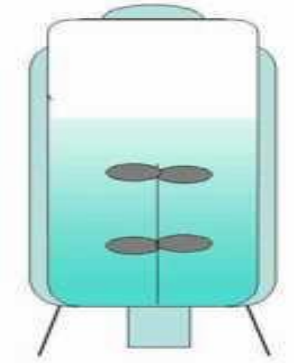
WP leader: Eurofins

Where do organic acids come from

?

Citric acid: always derived from sugar (and water)





C_{atoms} ← *100%* **sugar**

100% H

1 CH_2 ← **Water**

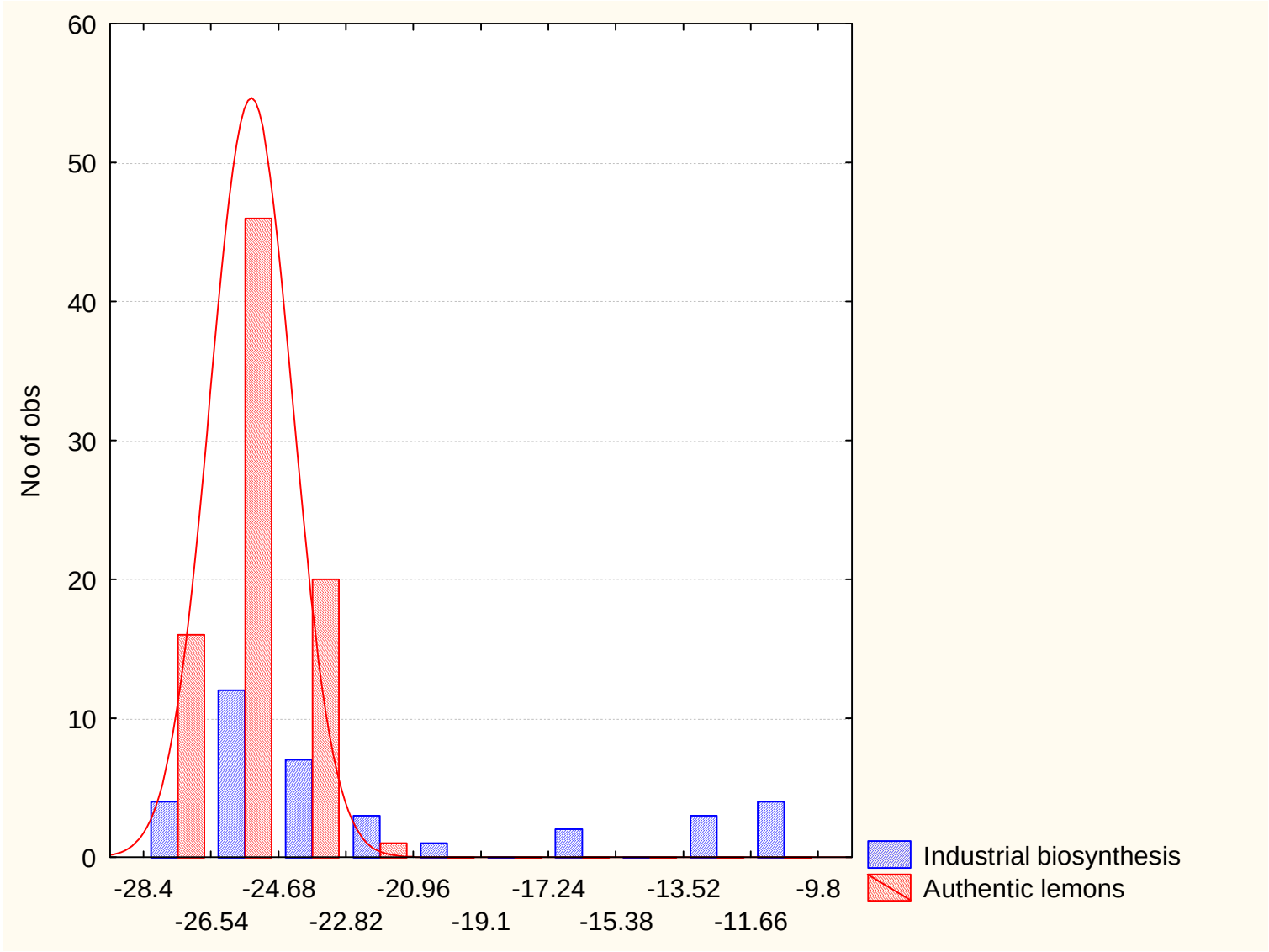
2/3 H **Glucose 1, 6, 6'**

1 CH_2

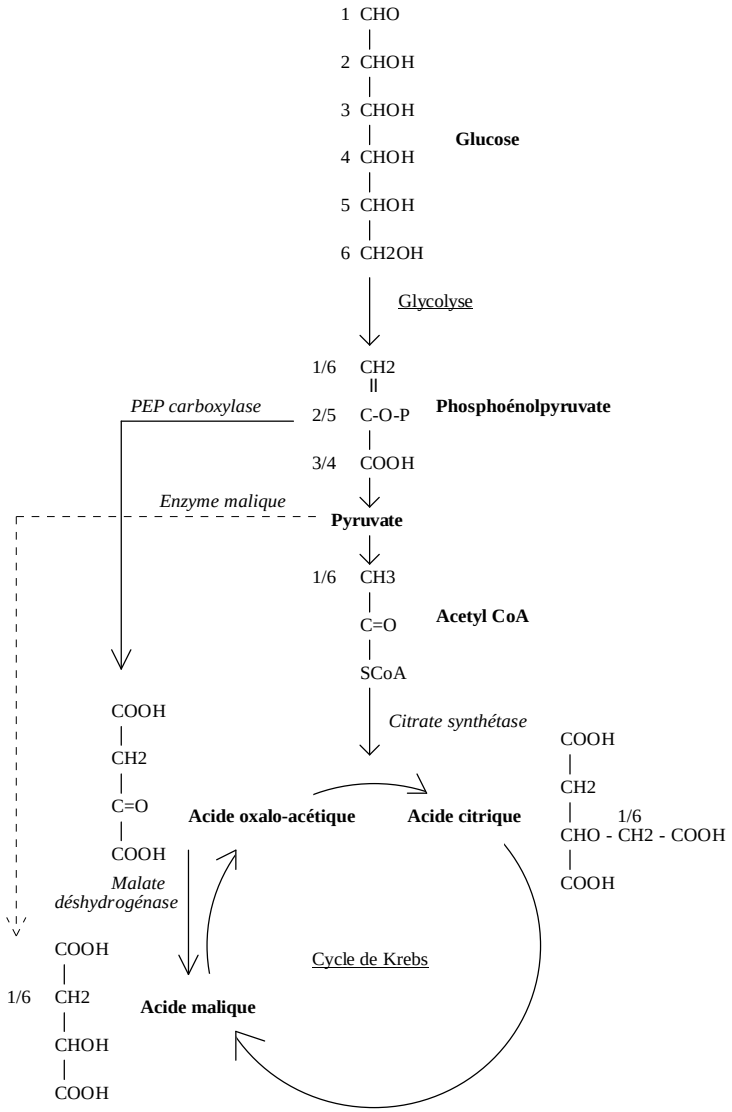
1/3 H **Water**

D/H $_{plant\ water}$ > D/H $_{tap\ water}$

$\delta^{13}C$ values of citric acid
Lemon vs. artificial sources



L-malic acid: biosyntheses



Krebs cycle (n times):

CH(OH) *100% H*

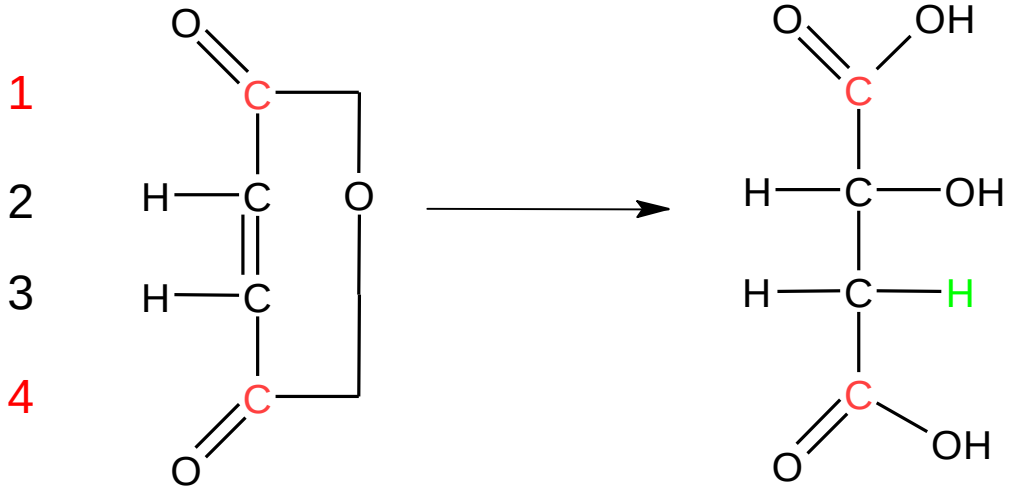
CH_2 ← **Water**

PEP carboxylase:

CH(OH) ← **Water**

CH_2 ← **Glucose 1, 6, 6'**

L-malic acid: industrial synthesis



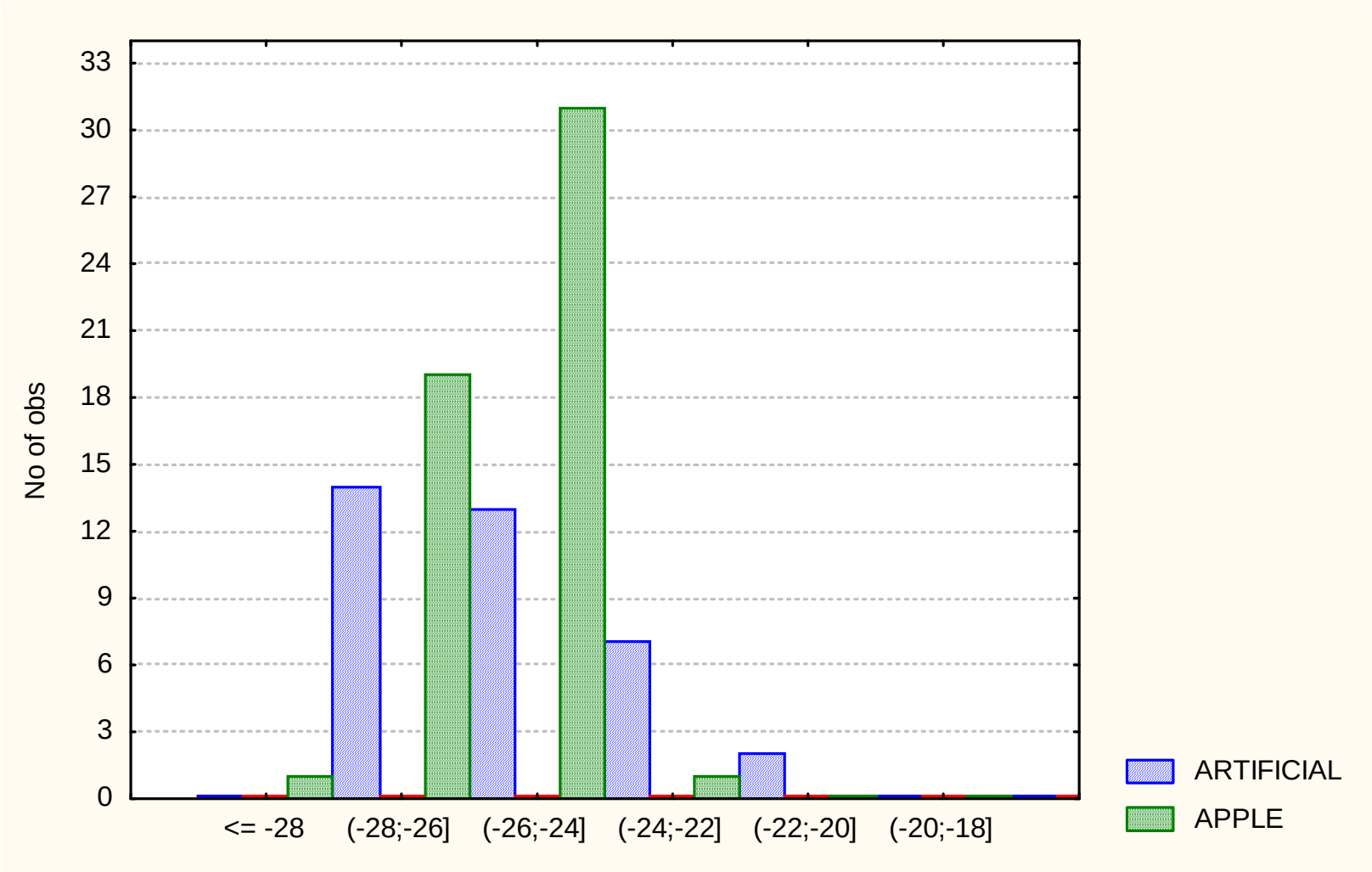
Maleic anhydride

(from fossil material)

L-malic acid

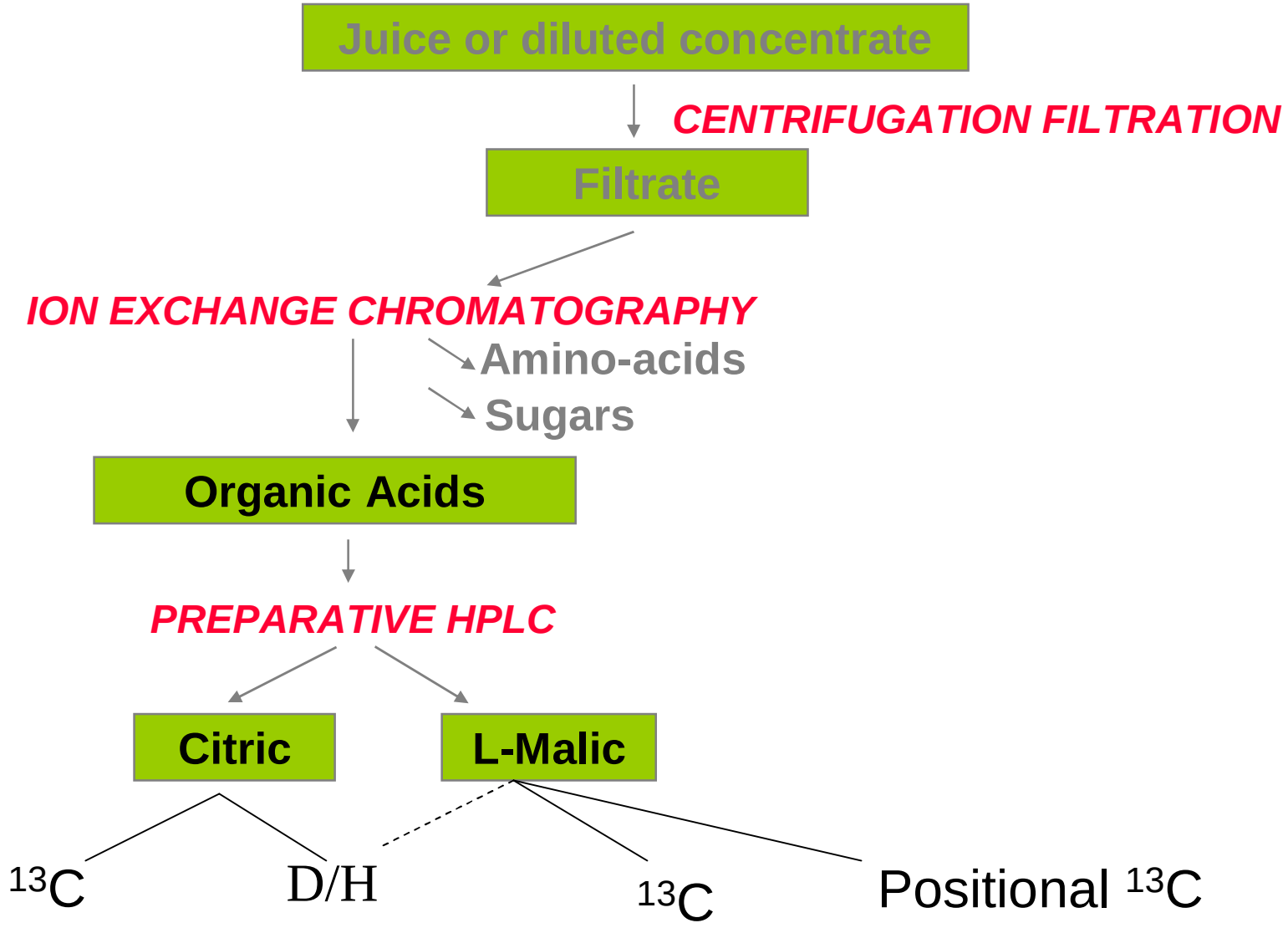
(adapted from Schmidt et al., Flüssiges Obst, 3/2000, 131-136)

$\delta^{13}C$ values of L-malic acid
Apple vs. artificial sources

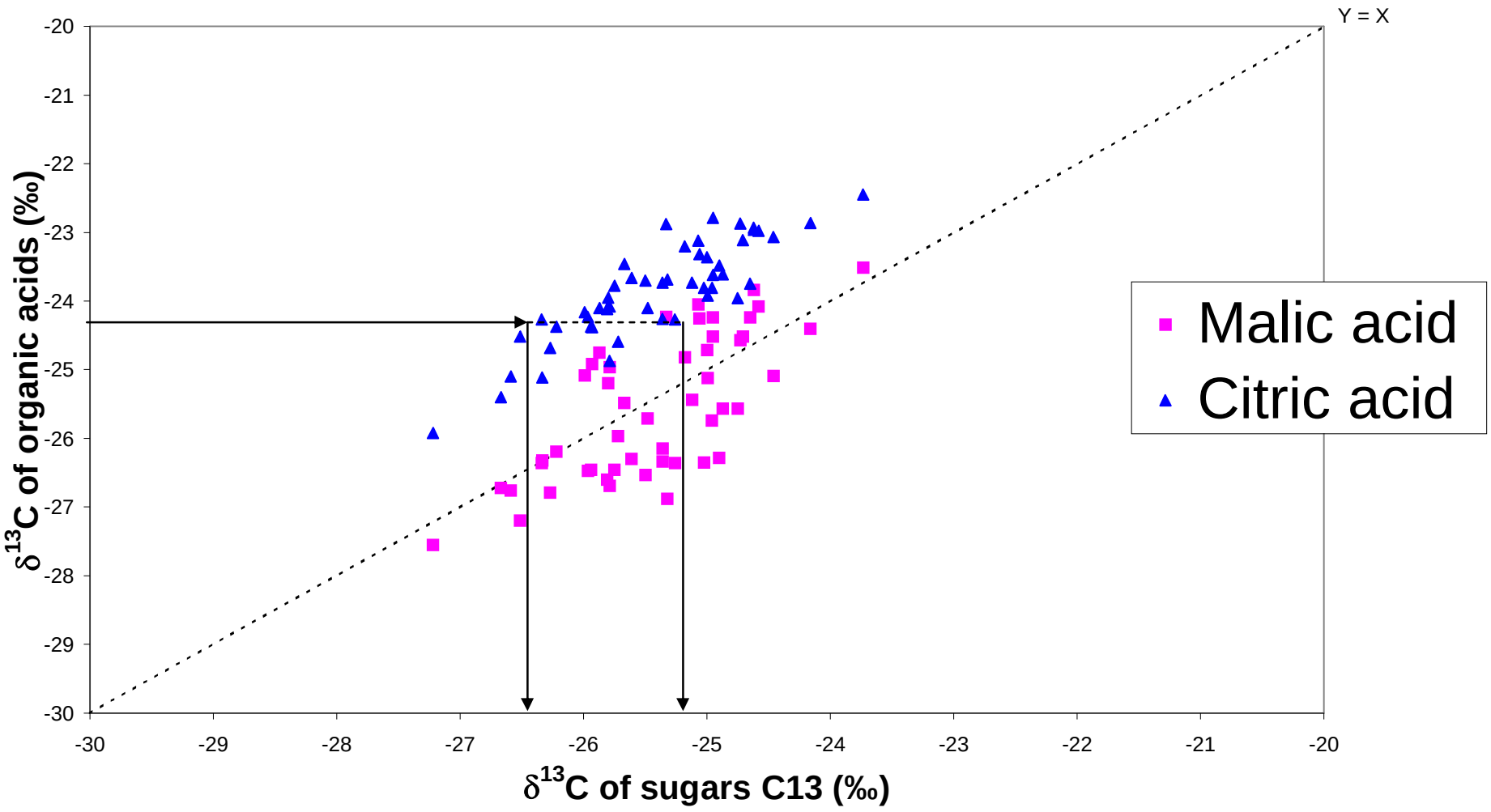


What has been done in the Pure Juice project?

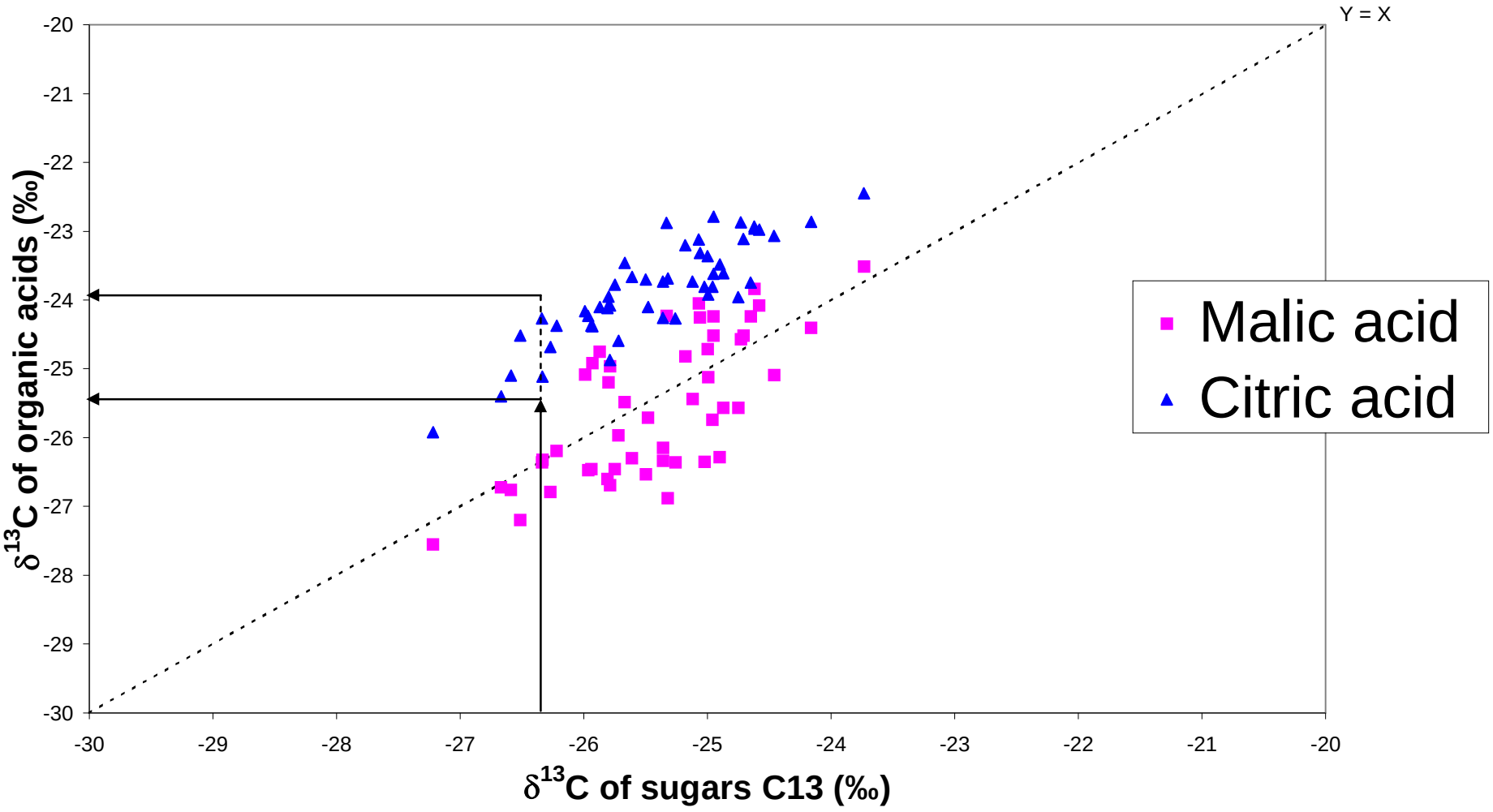
Flow chart of the protocol used for the isotopic analysis of organic acids



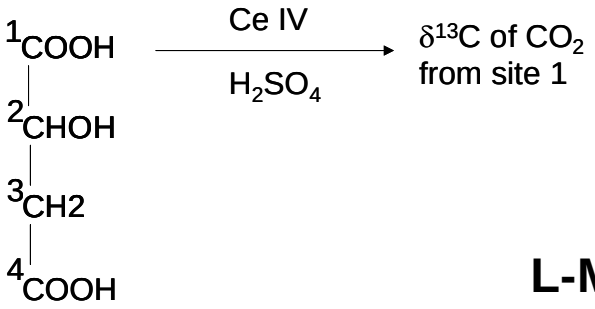
Orange sugars & organic acids ^{13}C deviations



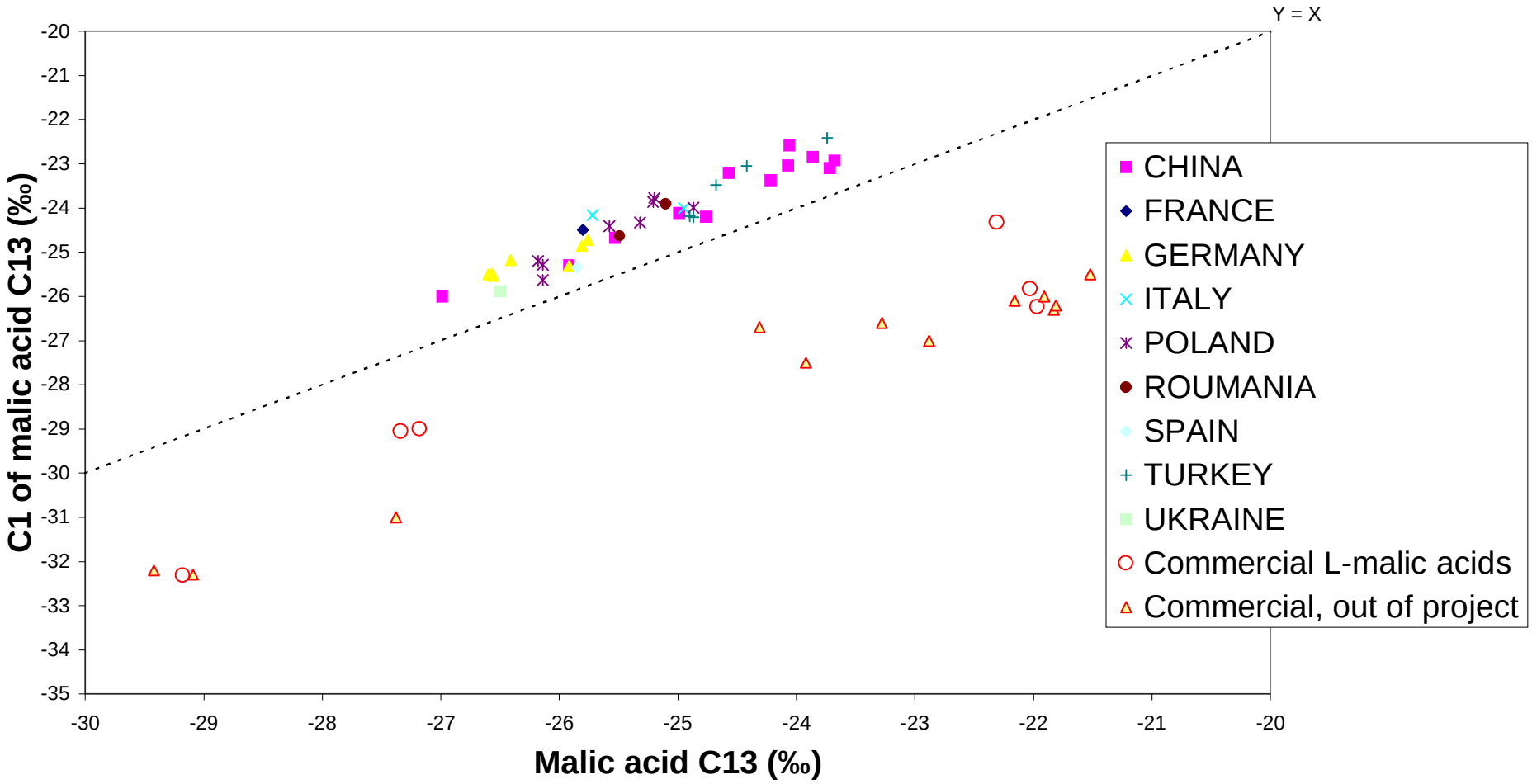
Orange sugars & organic acids ^{13}C deviations



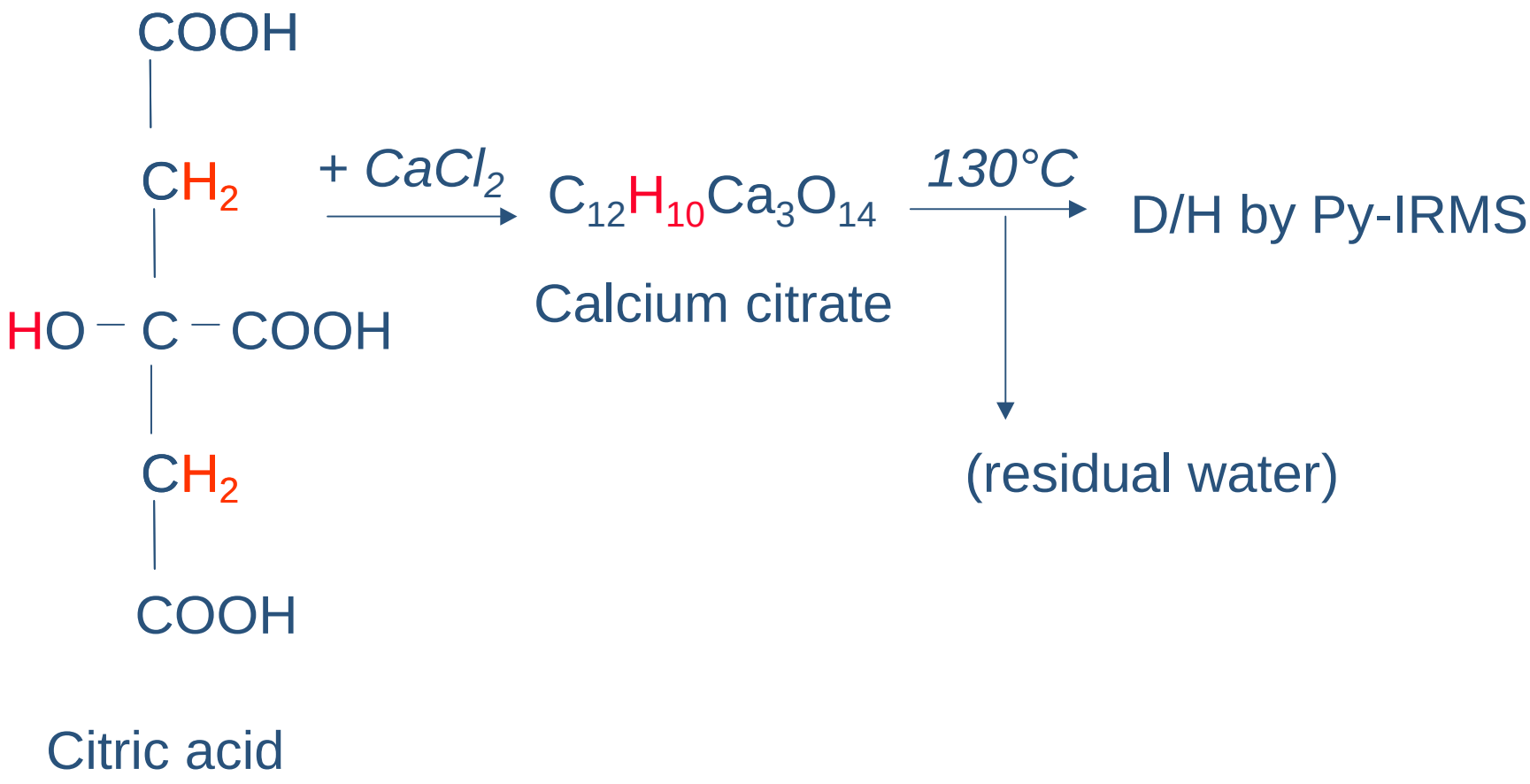
These correlations are independant from the geographical origin and allow to reduce the detection limits of acids/sugar addition



L-Malic acid: global and positional $\delta^{13}C$



New (D/H) analysis of citric acid : principle



Citric acid

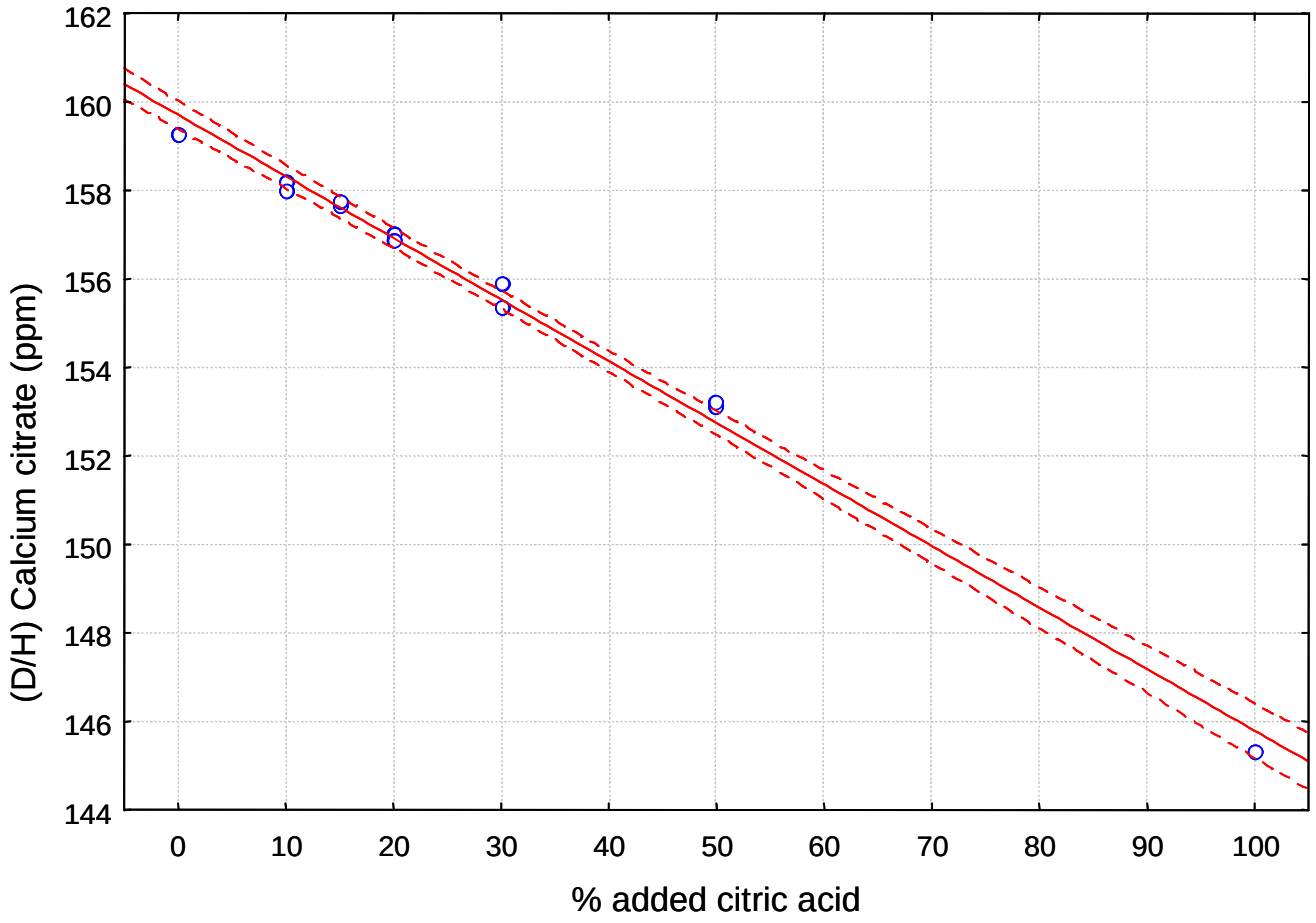
New (D/H) analysis of citric acid : precision

	Number of samples	Repetitions per sample	repeatability standard deviation, Sr (ppm)	repeatability limit , r (ppm)
Py-IRMS measurement	10	3	0.2	0.5
Overall method (from the juice)	5	2	0.2	0.5

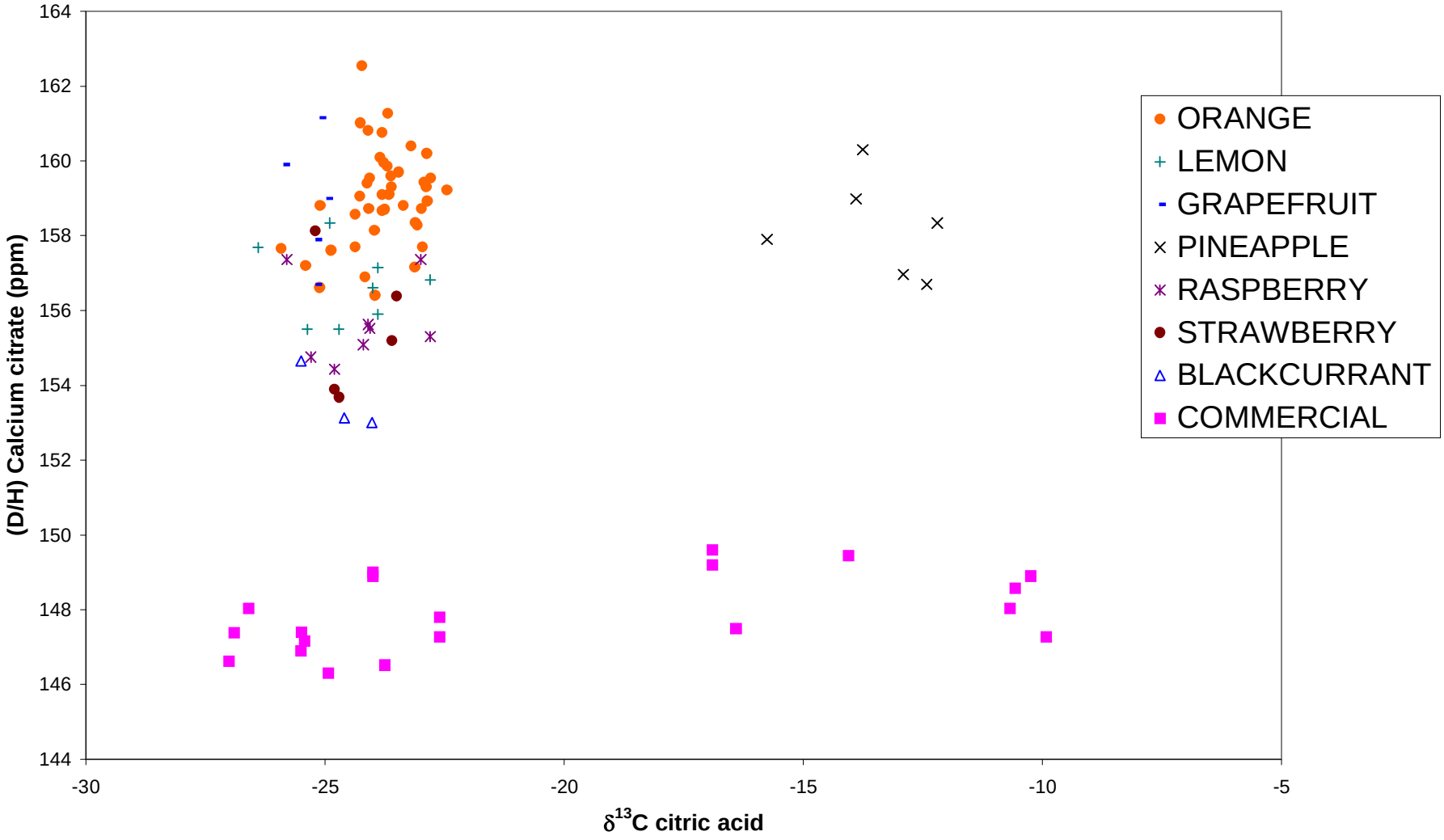
New (D/H) analysis of citric acid : accuracy

Sample	$(D/H)_{citrate}$ (ppm) by IRMS (this project)	$(D/H)_{TEC}$ (ppm) by SNIF-NMR (previous eurofins study*)	Difference (ppm)
A	149.0	146.5	2.5
B	147.8	146.9	0.9
C	147.4	146.3	1.1

(D/H) analysis of citric acid : spiking experiment (in orange)

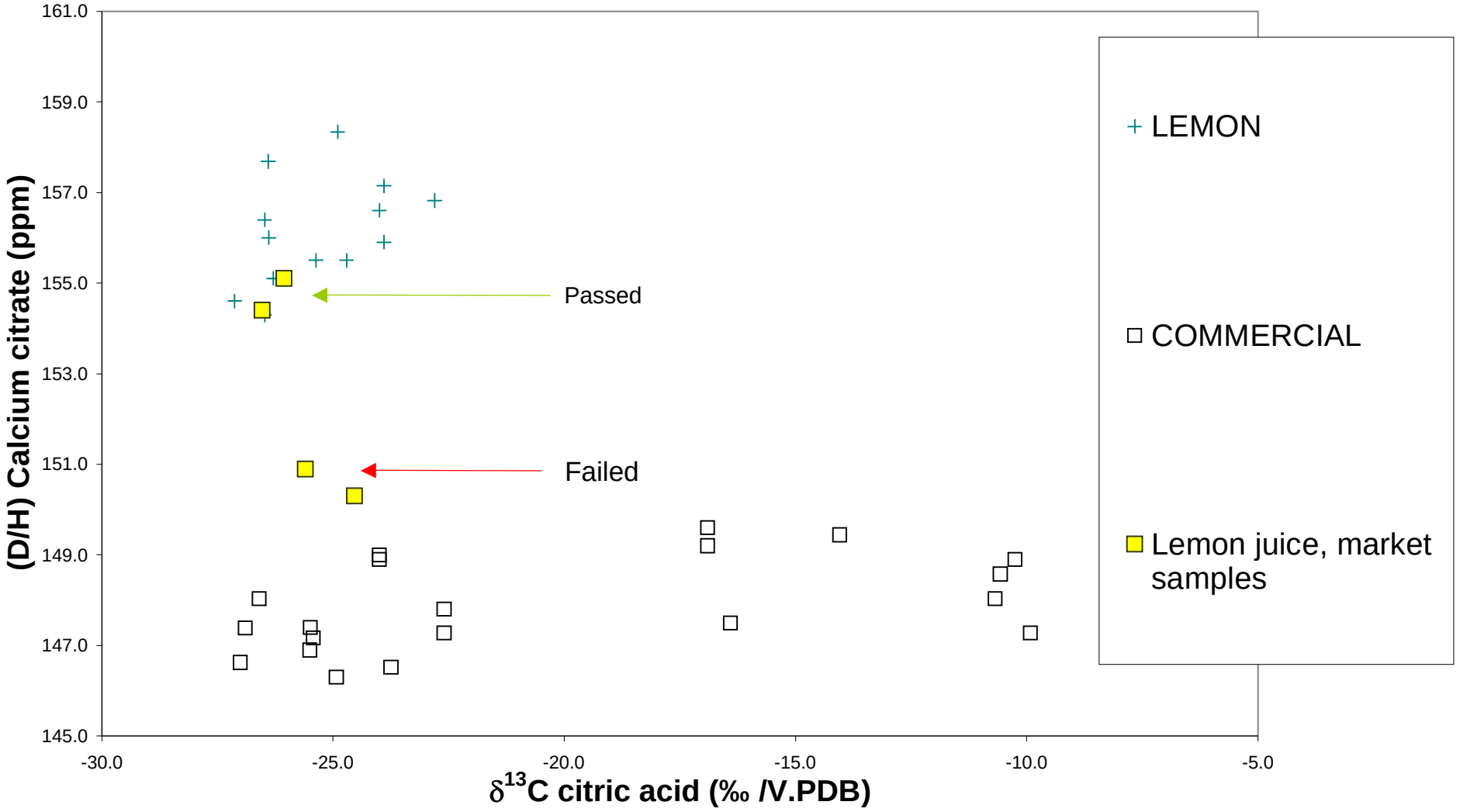


(D/H) analysis of citric acid : authentic samples



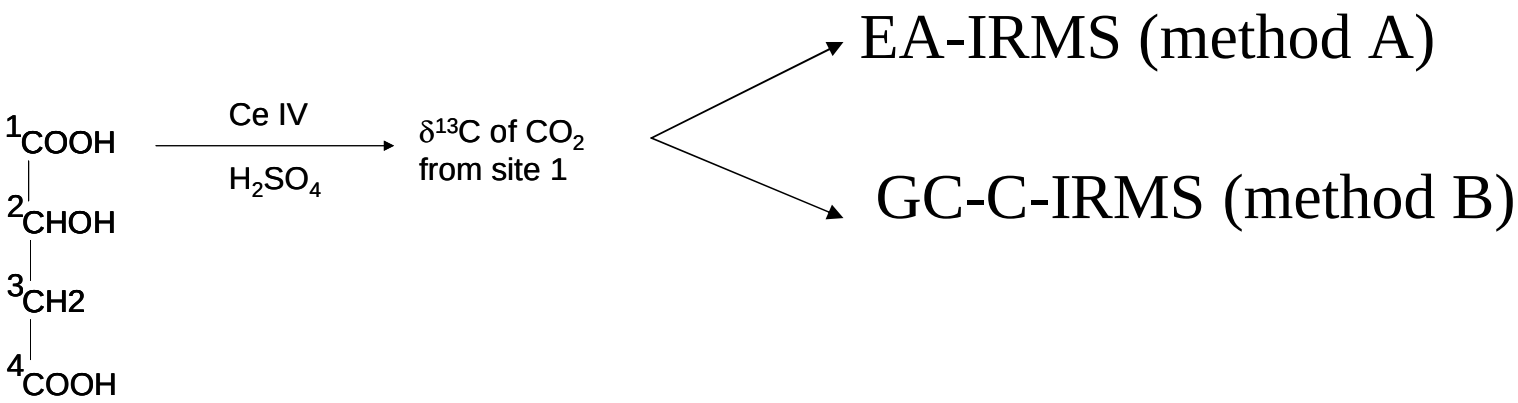
Published in « Detection of exogenous citric acid in fruit juices by stable isotope ratio analysis », Jamin et al., JAFC, 2005

(D/H) analysis of citric acid : lemon market samples



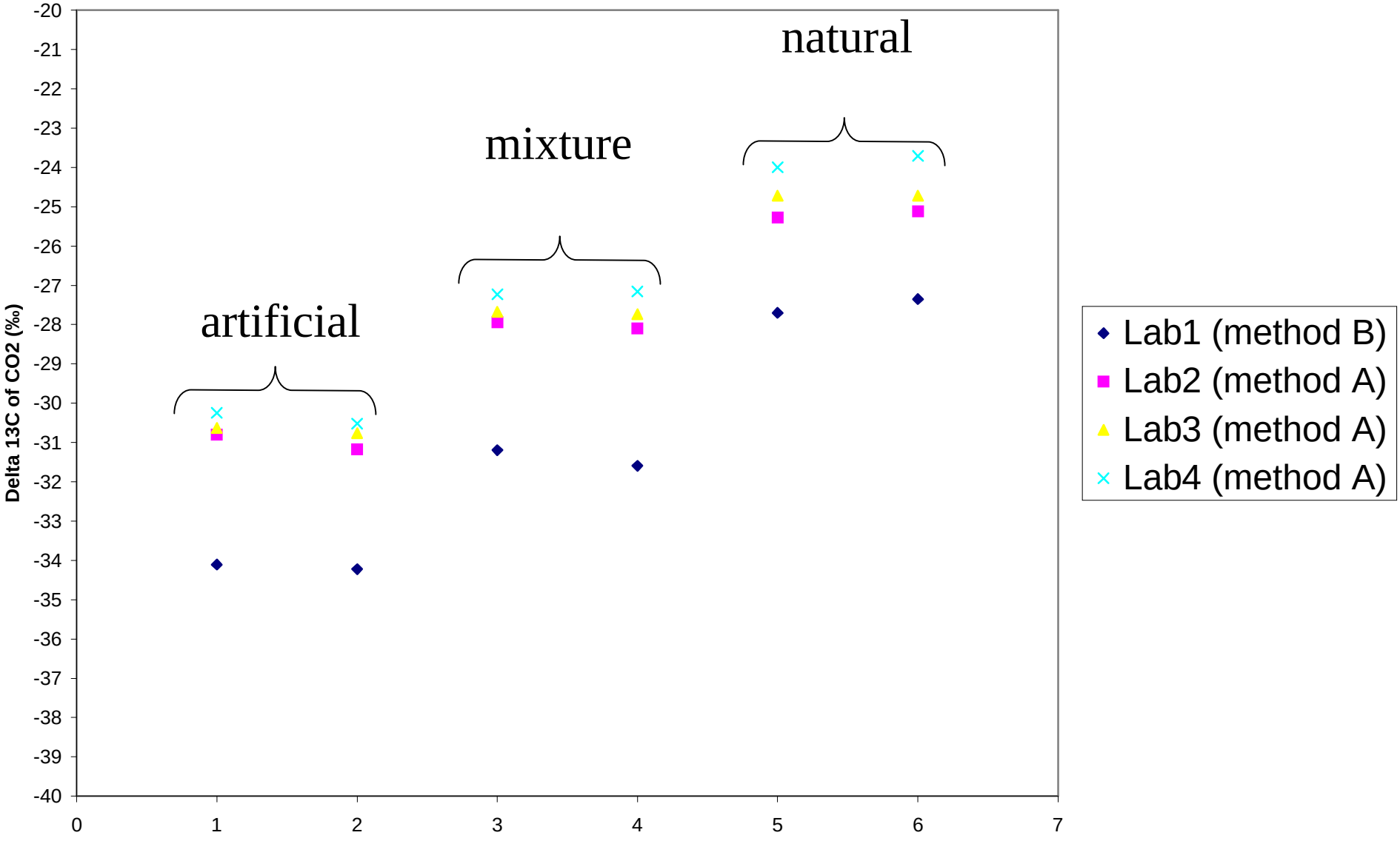
Peer-testing of the methods

Positional C13: Peer-testing design

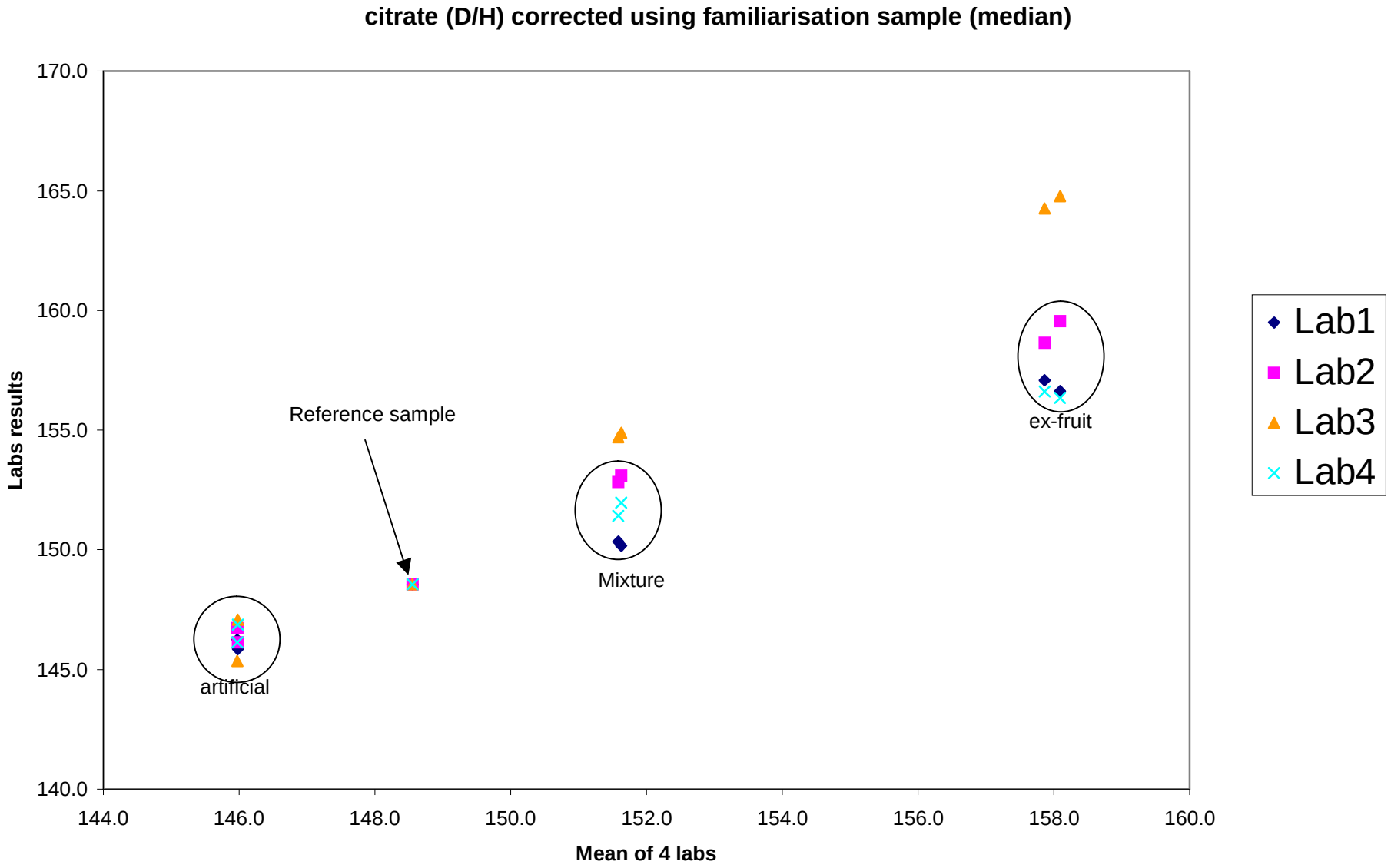


Sample Type	Sample Code	Test Material Number	Experimental Design
L-malic acid commercial	A	1 + 6	blind duplicates
L-malic acid From apple	B	2 + 5	blind duplicates
L-malic acid C mixture of A and B	C	3 + 4	blind duplicates

Positional C13: Peer-testing results



(D/H) analysis of citric acid : peer-test



Blind tests

Blind test: apple

SGF reference	% added malic acid	$\delta^{13}C$ malic acid (‰)	$\delta^{13}C$ C1 from malic acid (‰)	$\delta^{13}C$ Difference (‰)	Conclusion
AS1	23	-25.93	-25.61	0.32	SUSPECT
AS2	16	-25.87	-25.33	0.55	IN
AS3	27	-25.06	-24.17	0.88	IN
AS4	none	-25.99	-25.66	0.33	SUSPECT
AS5=AS1	23	-25.99	-25.90	**0.09**	OUT

The deviations observed are small, due to the nature of the added malic acid (« worst case » situation), hence some undetected additions

On the other hand there was no false positive

The correlation with sugars should also be taken into account for routine

Blind test: citric acid in orange

SGF reference	Origin	% added citric	$\delta^{13}C$ citric acid (‰)	$\delta^{13}C$ sugar (‰)	(D/H) calcium citrate (ppm)	Conclusion with origin
OS7	Mexico	20%	-24.7	-26.4	157.3	**OUT**
OS8	Cuba	30%	-24.3	-25.2	157.0	**OUT**
OS9=OS8	Cuba	30%	-24.6	-25.3	157.9	**OUT**
OS10	Brazil	none	-24.5	-25.4	158.2	**IN**
OS11	Spain	25%	-23.9	-25.0	155.5	**OUT**
OS1	Mexico 75%, Brazil 25%	25%	-24.1	-25.2	155.3	**OUT**

No false positive / all additions detected

Carbon 13 profile was not able to detect the additions

Knowledge of the origin was needed to evidence the adulteration in samples OS7, 8 and 9

Blind test: citric acid in pineapple

SGF reference	% added citric	δ^{13}C citric acid (‰)	δ^{13}C sugar (‰)	(D/H) calcium citrate (ppm)	Conclusion
AN1	30	-17.5	-13.9	155.5	OUT
AN2	1 g/l	-15.2	-12.8	155.1	OUT
AN3	20	-15.4	-12.8	155.2	OUT
AN4=AN1	30	-17.4	-14.0	155.8	OUT
AN5	none	-11.6	-11.8	153.8	OUT

All additions have been detected

Carbon 13 profile was sufficient to detect the additions in this case (because a C3 source was used)

One « false positive »: further investigations* have shown that this sample is not authentic

*Pineapple juice concentrate AN5:
(D/H)I ethanol (norm/VSMOW) = 105.1 ppm
(AIJN range 107-111.5)

Pineapple juice used to make this concentrate
$\delta^{18}O$ water = -6.2 ‰

Conclusion: this can not be considered as an authentic sample

Nevertheless pineapple results for citrate D/H should be interpreted with care (because water isotope ratios vary a lot in this fruit)

Outcomes:
1) ^{13}C multi-component profiles

- Improved detection of added C4 sugars in C3 plants (resp. C3 sugars in pineapple), when considering the correlation between sugars and acids (down to ca 10%)

- Combined with the site-specific information, the ^{13}C profile also improves the detection of added citric and malic acid (down to ca 10% in case of a different metabolism)

Outcomes:
2) positional ^{13}C analysis of malic acid

- The positional analysis of L-malic acid allows to detect additions of the artificial source previously undetectable by other methods

- Based on the Peer-testing results, a close agreement can be obtained between laboratories

- As confirmed by the blind test the detection limit varies between 20 and 30% (depending on the adulterant used), which can be improved using the correlation with sugars

Outcomes:
3) D/H analysis of citric acid

- A new method for analysing the D/H ratio of non-exchangeable hydrogen in citric acid has been developed and published. It allows to detect all sources of artificial citric acid in fruit juices

- Based on the preliminary Peer-testing results, a close agreement can be obtained between laboratories provided that a common citric standard is used for the IRMS analysis

- As confirmed by the blind test the detection limit lies around 20%, and can be improved using the correlation with sugars
- Knowledge of the origin helps to detect low additions

Overall conclusion for organic acids

- A large database covering the main origins of apple and orange juices was built, and partly extended to other fruits such as pineapple, blackcurrant, lemon and grapefruit
- The methods cover all known sources of artificial L-malic acid and citric acid
- The detection level of added acids were found to be in the range 10-30%, which is still more efficient than any other method
- For routine analysis the combination with other analyses / parameters will improve the efficiency of these methods