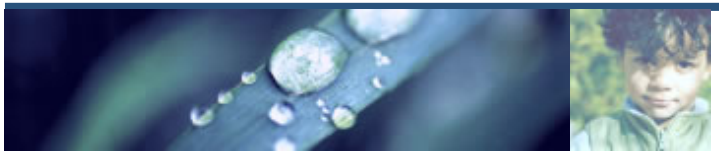


Apport de la méthode d'analyse SNIF-NMR pour la détection des fraudes dans les produits alimentaires

Eurofins Authenticity Competence Center

Nantes, France

Marcel Dumoulin

Business Development Manager

About Eurofins : Good to know!

Created in Nantes - France in 1987 as a spin-off of Nantes Univ

Detection of sugar addition in wines using SNIF-NMR analysis: analytical method invented by Prof G. and M. Martin at Nantes University, the parents of Eurofins' CEO, Dr. Gilles Martin

SNIF-NMR is the acronym of **S**ite-Specific **N**atural **I**sotope **F**ractionation studied by **N**uclear **M**agnetic **R**esonance

in French = **FINS-RMN**

from this comes the name of our company : Euro**FINS**!

About Eurofins : Good to know!

EC Regulation N°2676/90 determining EU methods for the analysis of wines : detecting enrichment of wines by application of SNIF-NMR

OIV
International Organisation of Vine and Wine

About Eurofins : Good to know!

Early 90's : SGF (1) has contacted Eurofins to develop the SNIF-NMR method for fruit juice authenticity testing

Mid 90's: SNIF-NMR parameters are listed in the AIJN CoP (2) (3) as criteria for evaluation of identity and authenticity of fruit juices

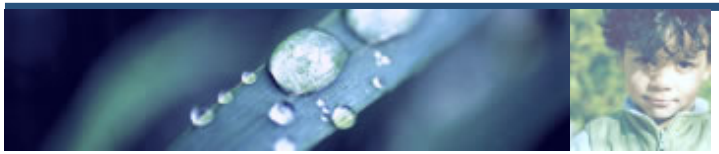
(1) SGF – German Fruit Juice Industry Self-Control Association
(2) Association of the Industry of Juices and Nectars from Fruits and vegetables of the EU
(3) CoP = Code of Practice

Company Overview

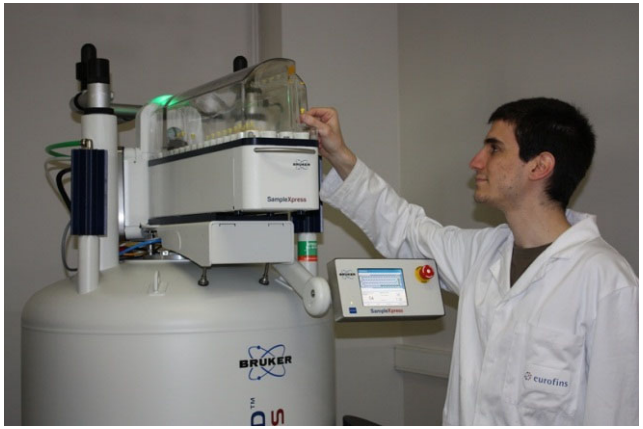
Global leader in the bioanalytical testing market:

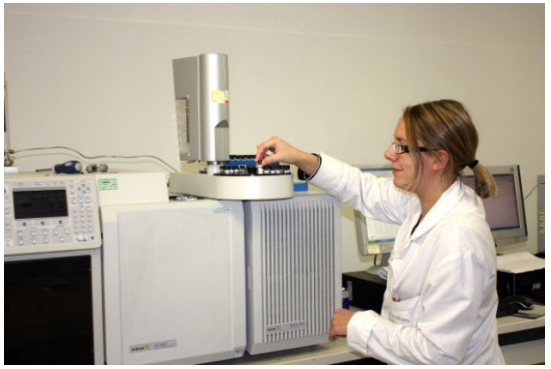
- More than **900** laboratories with > **61 000** employees
- Presence in **61** countries
- **>130,000** different analytical methods
- >150 million assays performed each year to establish the safety, identity, composition, authenticity, origin, traceability, and purity of biological substances and products, as well as carry out human diagnostic services.
- In-depth Industry expertise and rapid reaction capabilities
- Investment in R&D and state-of-the-art equipment

Food integrity pioneers



Group authenticity competence centre based in Nantes (France): Experience in food authenticity testing since 1987!



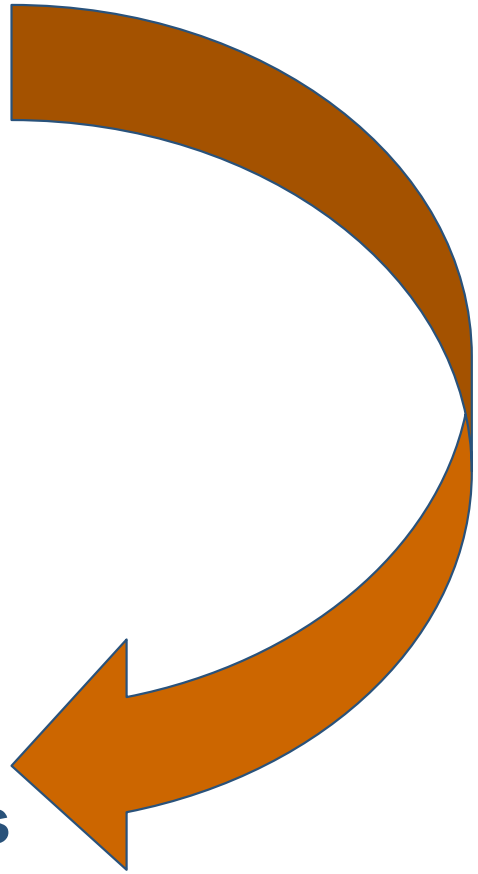


Fruit juice authenticity: analytical strategy

eurofins

Authenticity check requires a combination of:

- **compositional analyses**
  (wet chemistry &chromatographic methods)
- **isotopic analyses**
  (SNIF-NMR and IRMS methods)
- **DNA analyses**
  (fruits species or varieties identification)

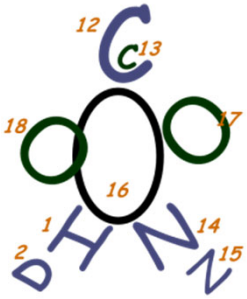


- **1H NMR screening** can help to optimize costs and testing speed

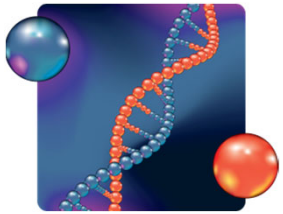
Analytical tools for authenticity testing

- **Chemical composition methods (e.g. chromatography)**
  - Identification & quantification of defined compounds

- **Stable isotopes**
  - Molecules origin



- **Molecular Biology**
  - Identification of species, varieties, etc.



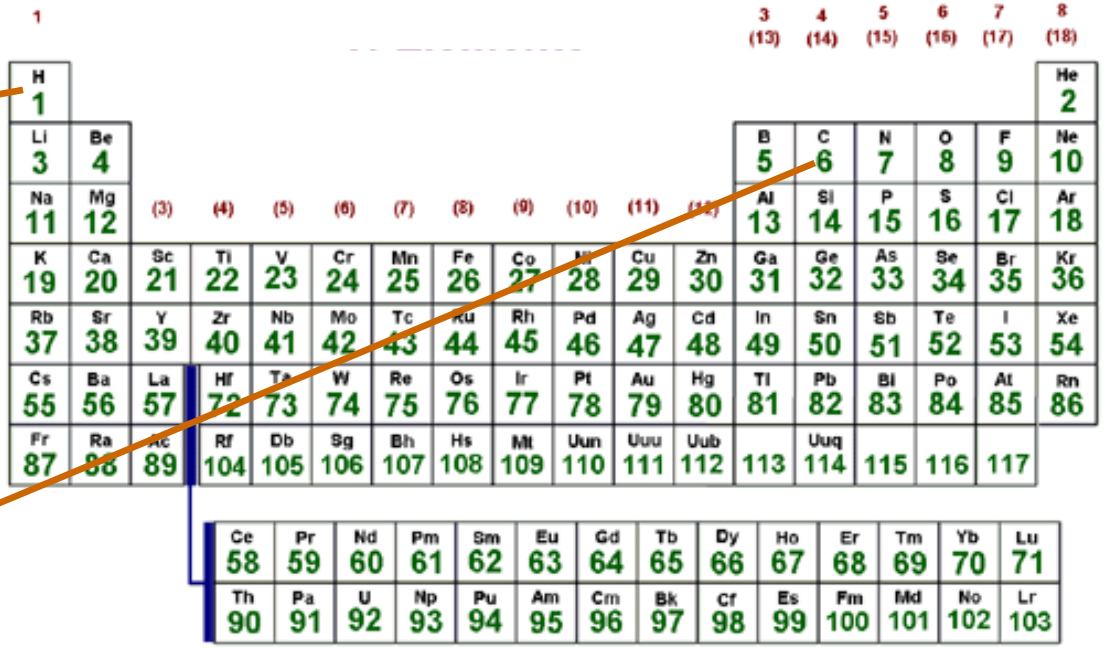
- **Profiling methods**
  - Whole matrices fingerprint

Stable isotope analyses

Stable isotope approach

$^{1}_{1}H$ or $^{2}_{1}H$

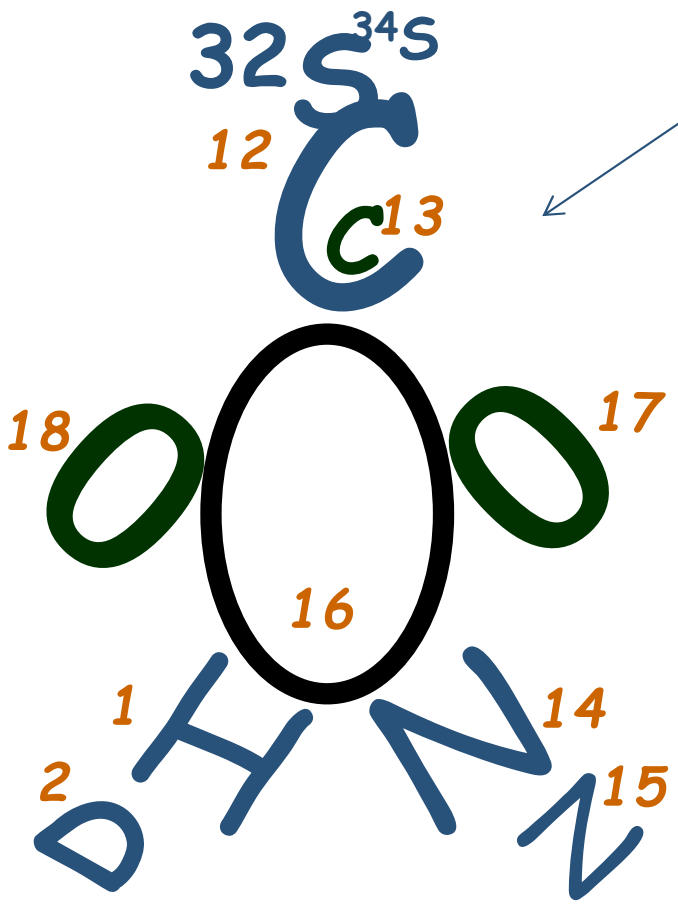
$^{12}_{6}C$ or $^{13}_{6}C$ or $^{14}_{6}C$



Isotopes are different forms of a single element : the same number of protons, but differing numbers of neutrons

Stable isotopes in natural abundance

Natural products are composed of 5 main elements C, H, O, N and S



These elements are naturally present in several isotopic forms (same atomic number, different weights),

Isotopes distribution is influenced by natural phenomena and human processes:

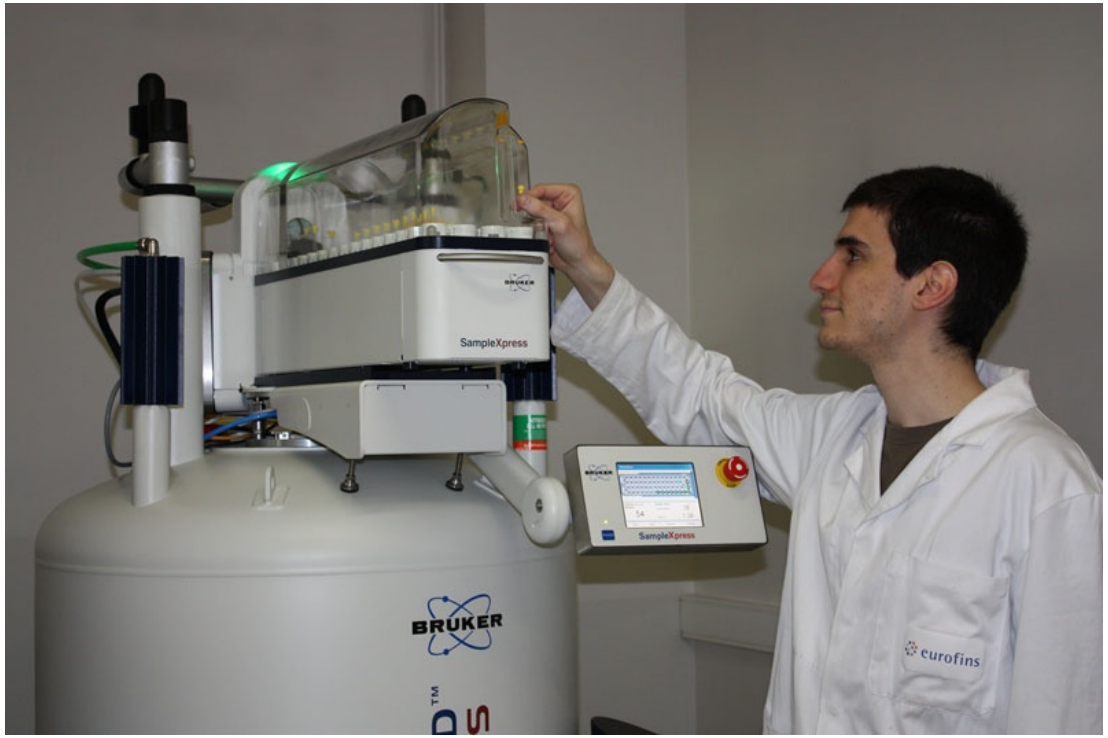
- **Precursors (natural / synthetic)**
- **Metabolism (botanical origin, regime)**
- **Environment (geographical origin)**

(1) IRMS : Isotopic Ratio Mass Spectrometry

- Combustion or Pyrolysis of the organic product to a gas
- Access to overall isotope ratios of a product or isolated compound
- Can be coupled to Chromatography

Stable Isotope Analyses: 2 main techniques

(2) SNIF-NMR ®



- method pioneered by Eurofins Scientific
- measures isotopic distributions within a given molecule

Site-specific Natural Isotopic Fractionation studied by deuterium Nuclear Magnetic Resonance. FINS in French!

Isotopic fractionation due to metabolism & physiology: example of plant primary metabolism

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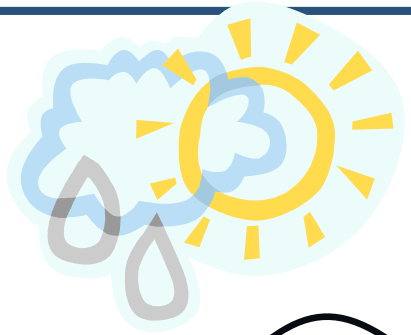
Where does the sugar in a plant come from :



Carbon dioxide

CO_2

(-8 ‰)

C_3

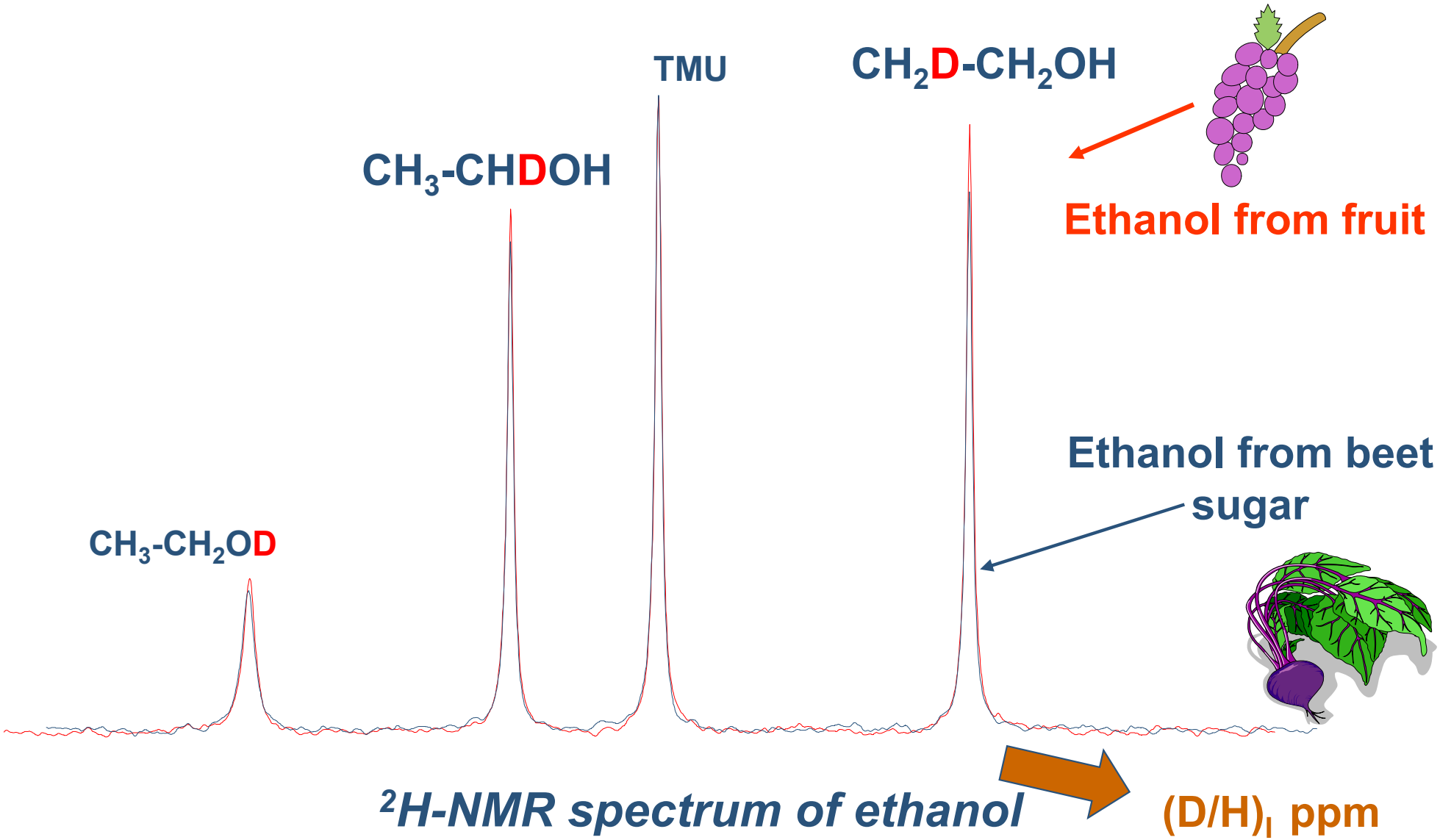
most plants

values around -25‰

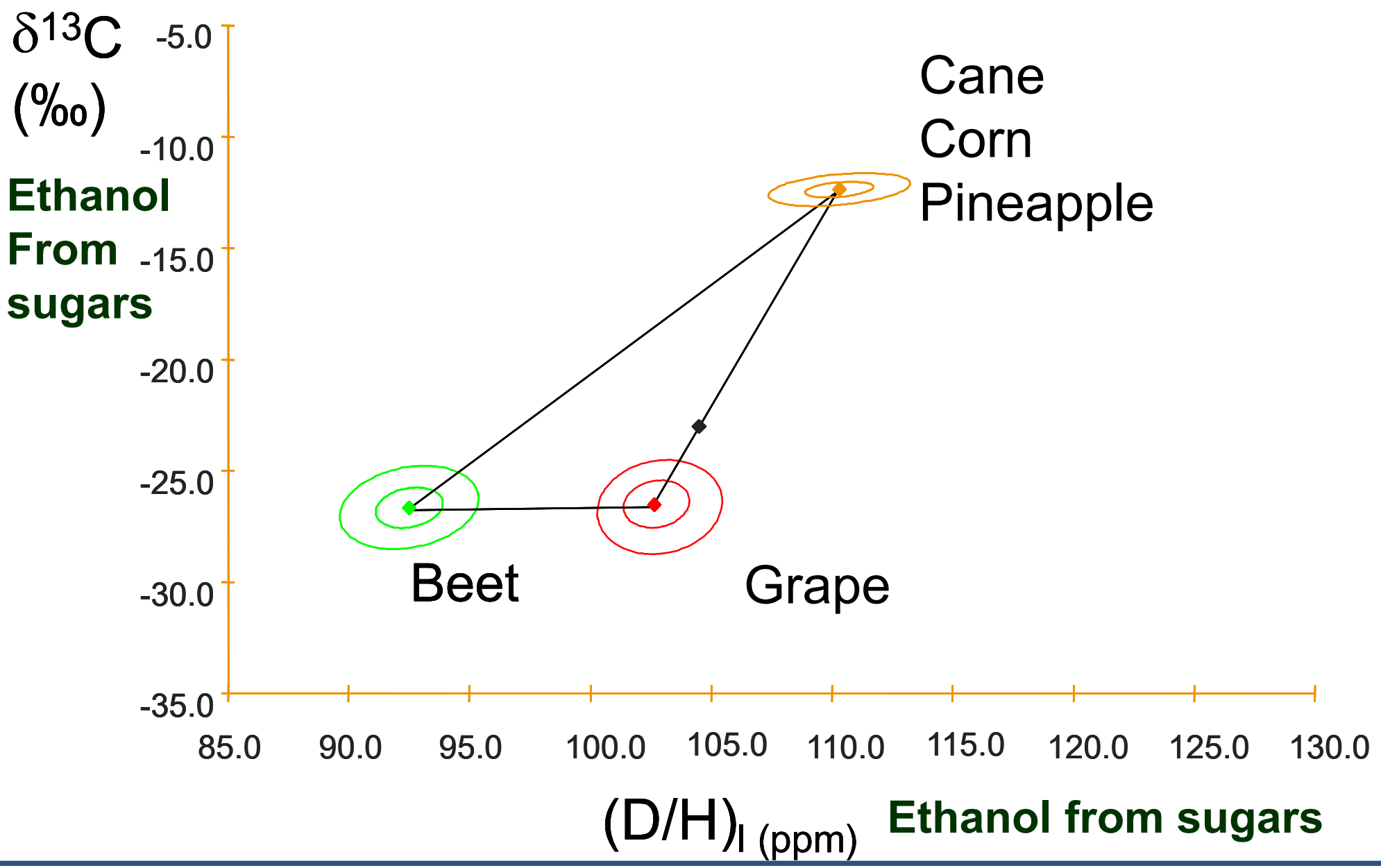
C_4

Gramineae family e.g. sugarcane, maize, values around -10‰

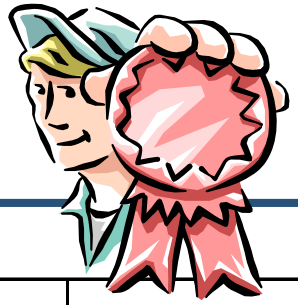
SNIF-NMR® of ethanol: detection of beet sugar



SNIF-NMR applied to Deuterium :



Isotopic methods recognition



year	Method	Product	Fraction	Techniques	Isotope ratios
1987	OIV, recueil des méthodes d'analyse	wine	Ethanol	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
1990	EC regulation 2676/90, annex 8	wine	Ethanol	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
1991	AOAC method 991.41	honey	honey & proteins	IRMS	$^{13}C/^{12}C$
1993	CEN (TC174 N108, ENV 12140)	fruit juice	Sucre	IRMS	$^{13}C/^{12}C$
1995	AOAC Official method 995.17	fruit juice	Ethanol (from fermentation)	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
1996	OIV resolution ENO 2/96	wine	water	IRMS	$^{18}O/^{16}O$
1997	EC regulation 822/97	wine	water	IRMS	$^{18}O/^{16}O$
1997	CEN (TC174 N109, ENV 12141)	fruit juice	water	IRMS	$^{18}O/^{16}O$
2000	AOAC Official method 2000.19	maple syrup	Ethanol (from fermentation)	SNIF-NMR	$(D/H)_I$, $(D/H)_{II}$,R
2001	OIV resolution ENO 17/2001	wine	Ethanol	IRMS	$^{13}C/^{12}C$
2003	EC regulation 440/ 2003, annex 2	wine	Ethanol	IRMS	$^{13}C/^{12}C$
2004	AOAC method 2004.01	fruit juice & maple syrup	Ethanol (from fermentation)	IRMS	$^{13}C/^{12}C$
2006	AOAC method 2006.05	Vanillin	Vanillin	SNIF-NMR	$(D/H)_i$

COMMISSION REGULATION (EC) No 555/2008 of 27 June 2008
(implementing Council Regulation (EC) No 479/2008)

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Wine Databank *(EC Reg. Nº 2729/2000 *)*

Portugal 50

France 400

Germany 200

Italy 400

Spain 200

U. Kingdom 4

Austria 50

Greece 50

BEVABS
European Office for Wine, Alcohols and Spirit Drinks

Maintenance of databank
Arbitration of disputes
Analysis of samples
Development and validation of methods
Validation of data
Training

Luxembourg 4

*(EC Reg. Nº 2120/2004 *)*

Cyprus 10

Malta 4

Slovakia 15

Slovenia 20

Hungary 50

Czech Rep. 20

*(EC Reg. Nº 2030/2006 *)*

Romania 70

Bulgaria 30

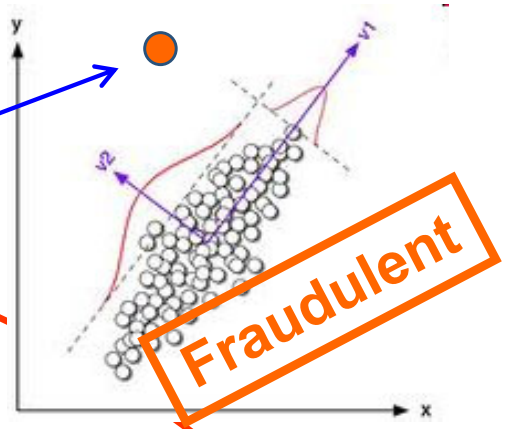
** now repealed and replaced by (EC) No 555/2008*

Use of official data for control

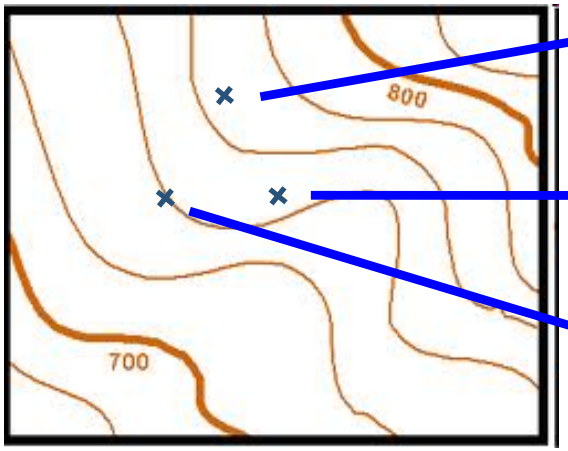
Member State
Liaison Body

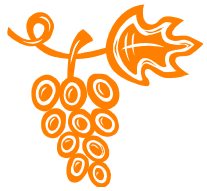
Member State
Laboratory

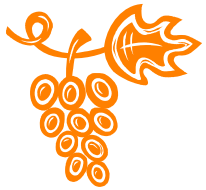
Official request

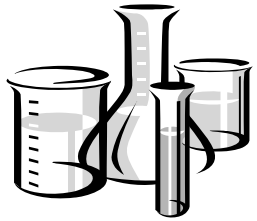


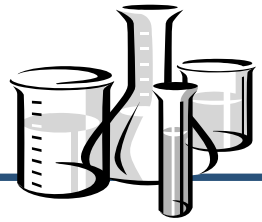
JRC: extraction of data from the database











(D/H)I

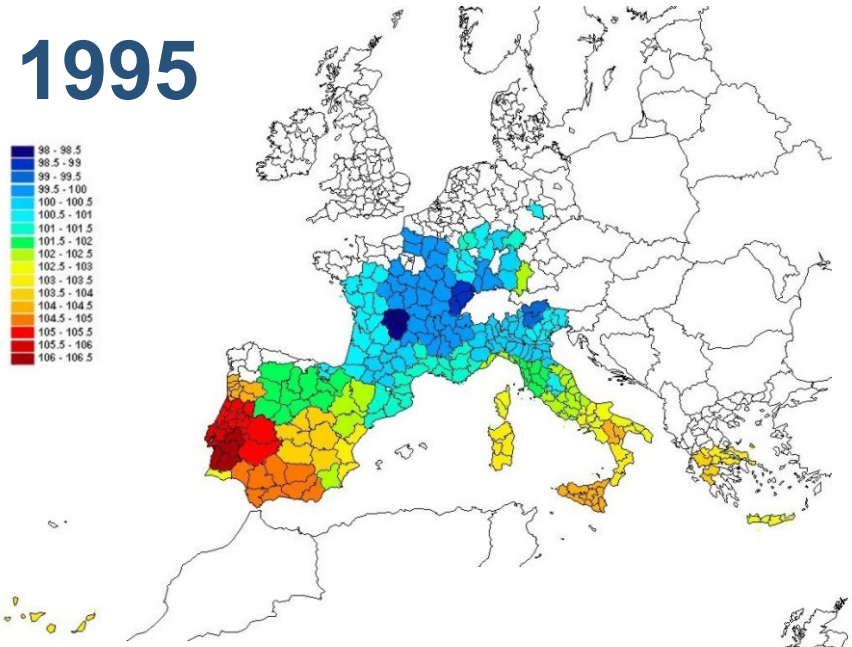
δ ^{18}O

δ ^{13}C

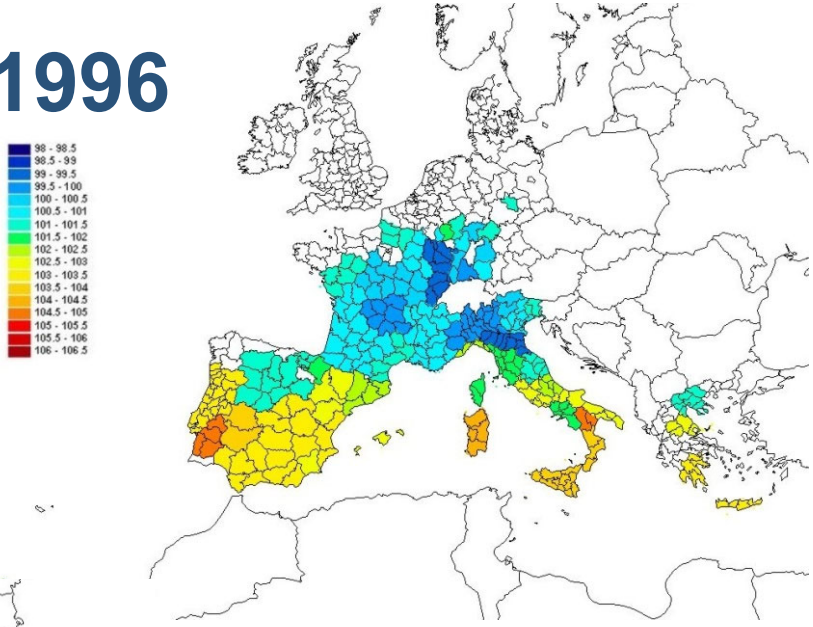
Database extraction

eurofins

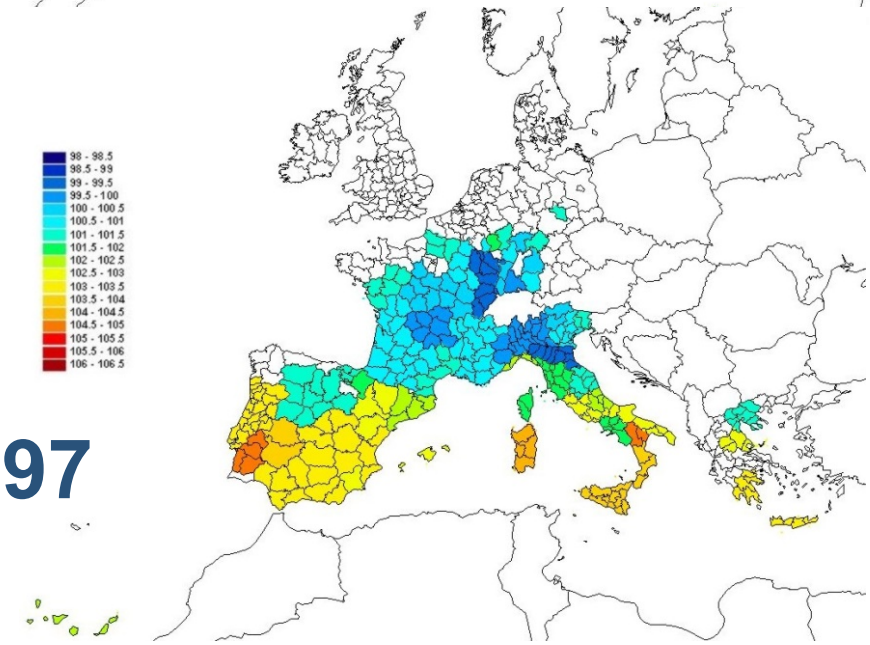
1995



1996



1997



Wine data base $(D/H)_1$

C. Guillou, BEVABS
JRC Geel Belgium

36 SNIF labs around the world :

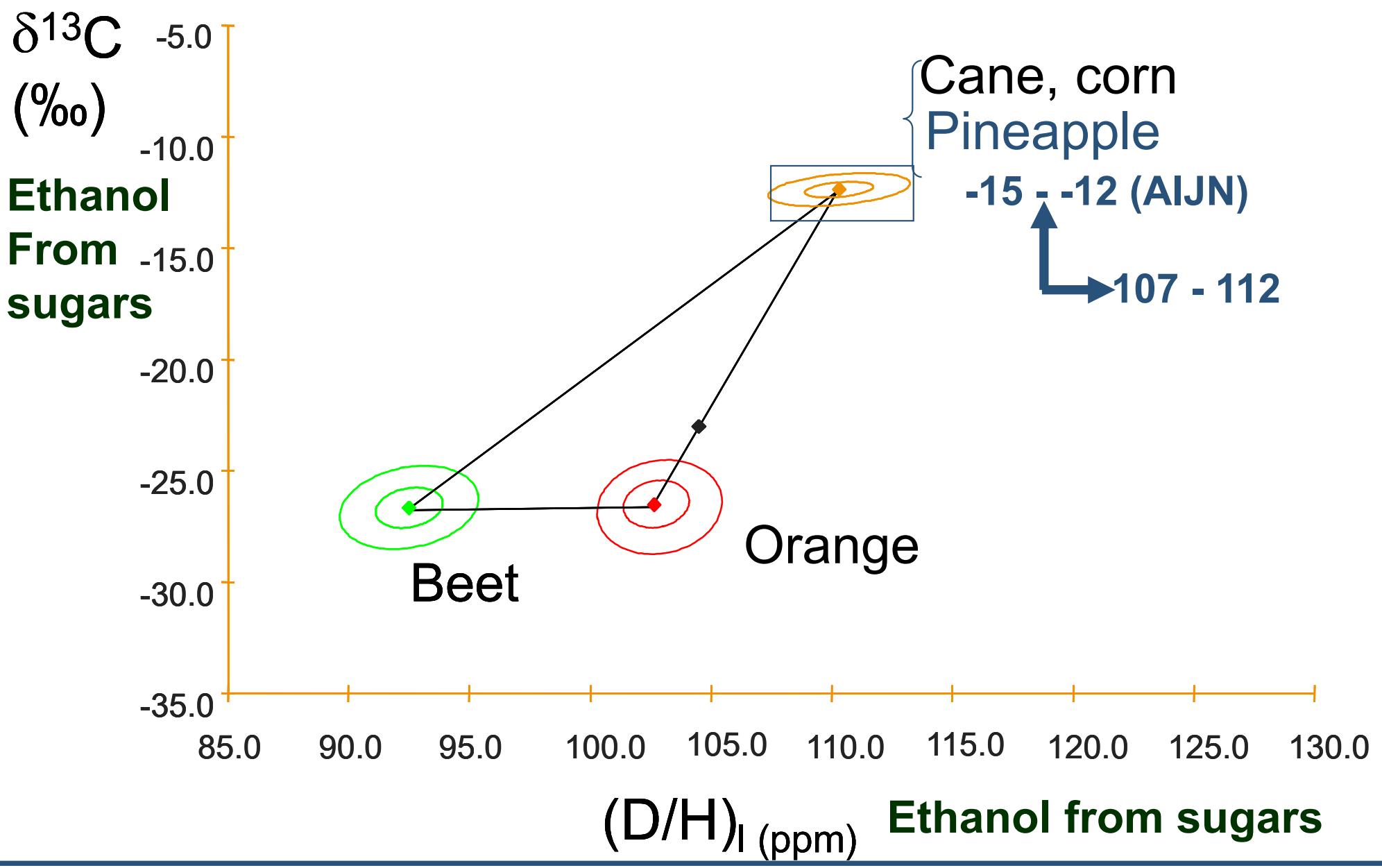
Examples of adulterations tracked by isotope testing in fruit juices

- Undeclared addition of cheaper sugar
- Undeclared addition of water (or NFC/FC juice blending)
- Undeclared addition of artificial organic acids (citric, malic, tartaric)
- Undeclared addition of artificial vitamin C in natural products
- Undeclared addition of artificial flavour compounds
- Geographical origin mislabelling

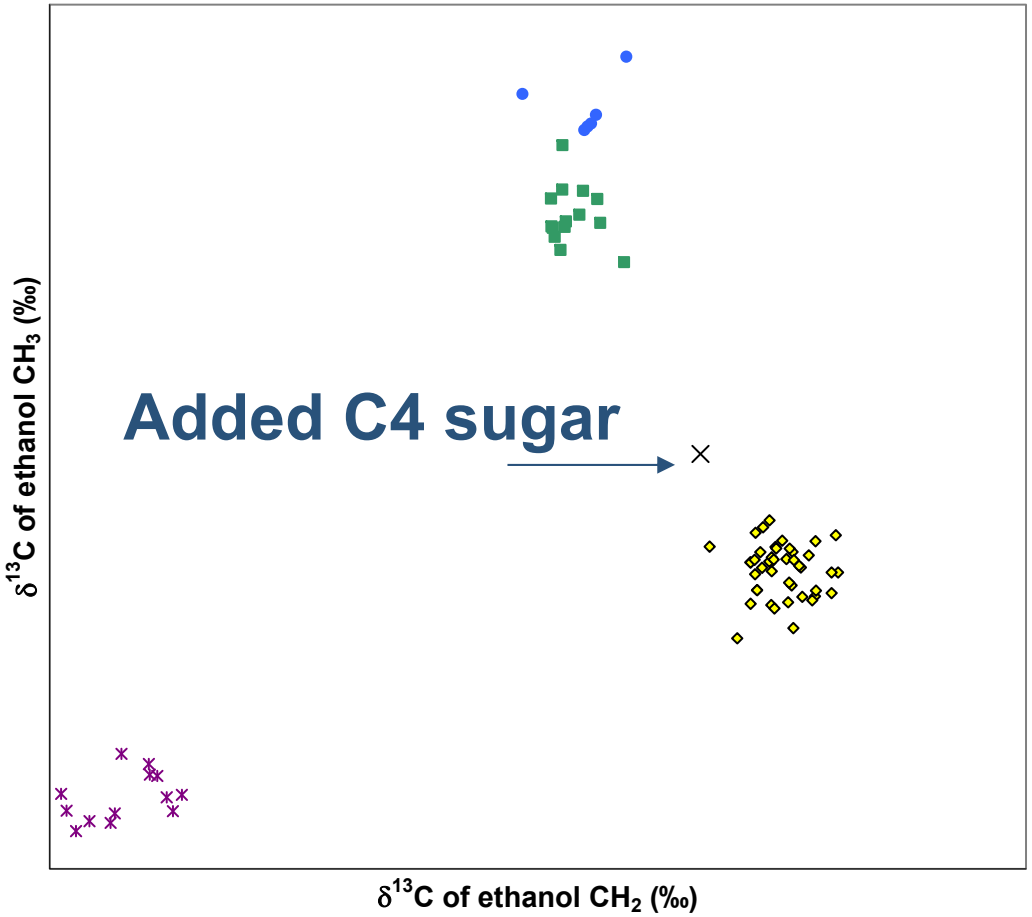
Recent improvements addressed by isotope testing

- Undeclared addition of sugar in pineapple
- Undeclared addition of water (all fruits)
- Undeclared addition of vitamin C

SNIF-NMR applied to Deuterium : The pineapple « gap »



NEW application of ^{13}C SNIF-NMR to pineapple: improved detection of sugar addition



- PINEAPPLE
- CANE
- MAIZE
- BEET
- test

J. Agric. Food Chem. 2010, 58, 11580–11585
DOI:10.1021/jf102983v

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Improved Characterization of the Botanical Origin of Sugar by Carbon-13 SNIF-NMR Applied to Ethanol

Freddy Thomas,*,† Celia Randet,† Alexis Gilbert,† Virginie Silvestre,† Eric Jamin,† Serge Akoka,† Gerald Remaud,† Nicolas Segebarth,§ and Claude Guillou§

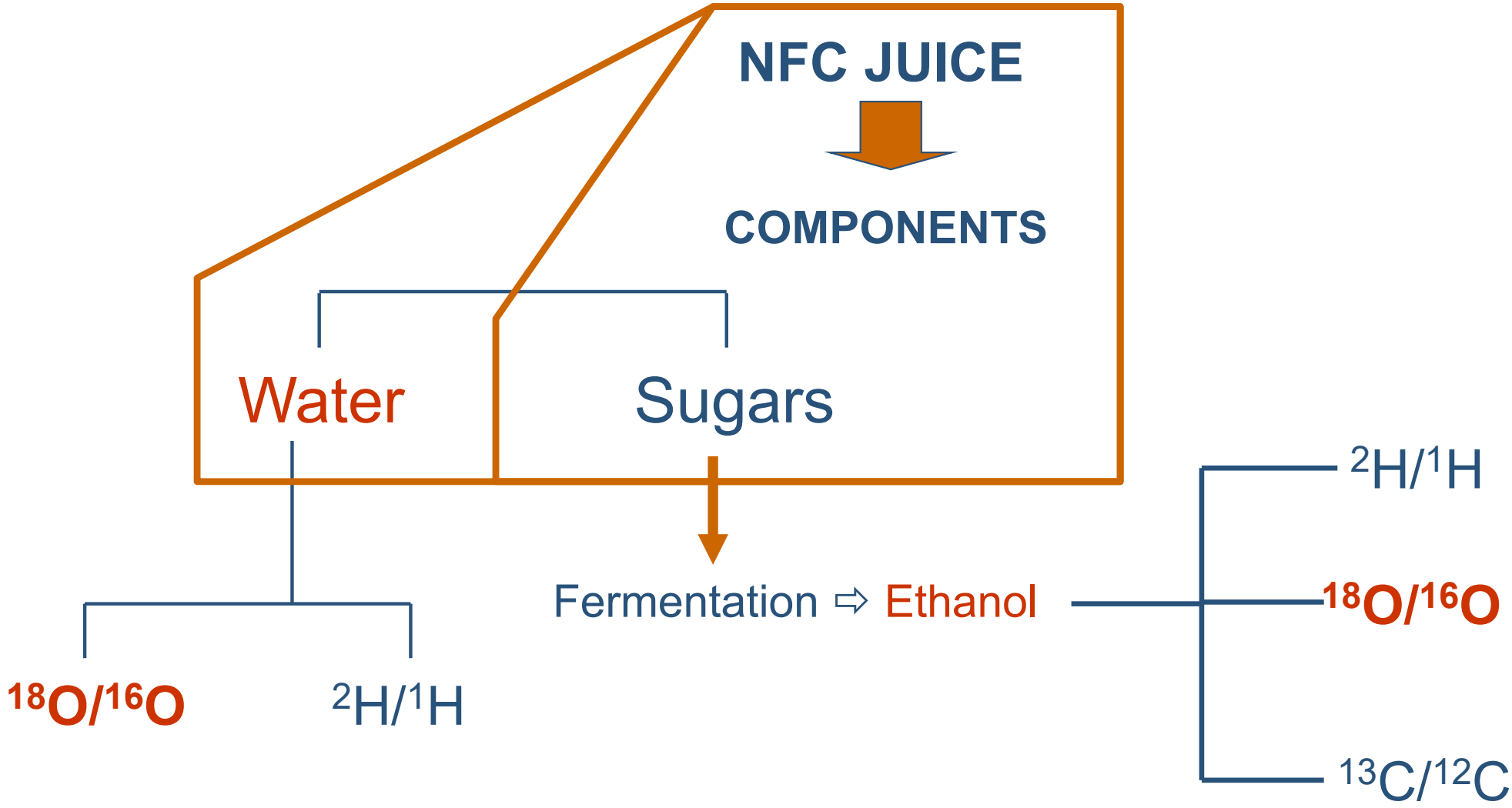
Also applicable to:
Dragon fruit
Agave juice, syrup, and inulin

IFU recommendation

Juice authenticity issues addressed by isotope testing

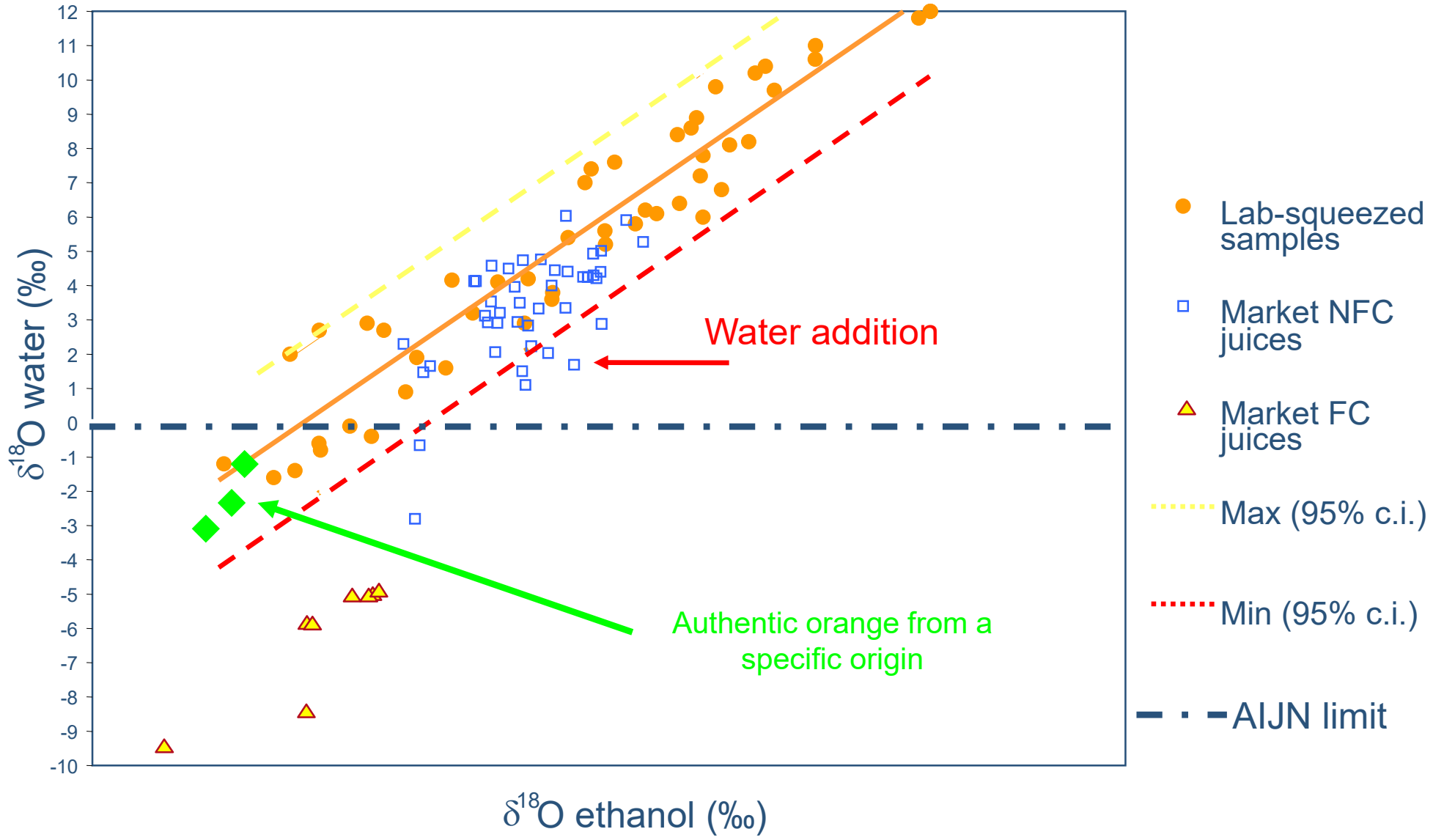
- Undeclared addition of sugar in pineapple
- Undeclared addition of water (all fruits)
- Undeclared addition of vitamin C

Isotopic analyses in direct fruit juice: Improved detection of water addition



J. Agric. Food Chem. (2003) 51, 18, 5202-5206 ; J. Agric. Food Chem. (2006), Vol. 54, No. 2, 279-284

Application of ^{18}O internal referencing: e.g. orange juice NFC authenticity control



J. Agric. Food Chem. (2003) 51, 18, 5202-5206

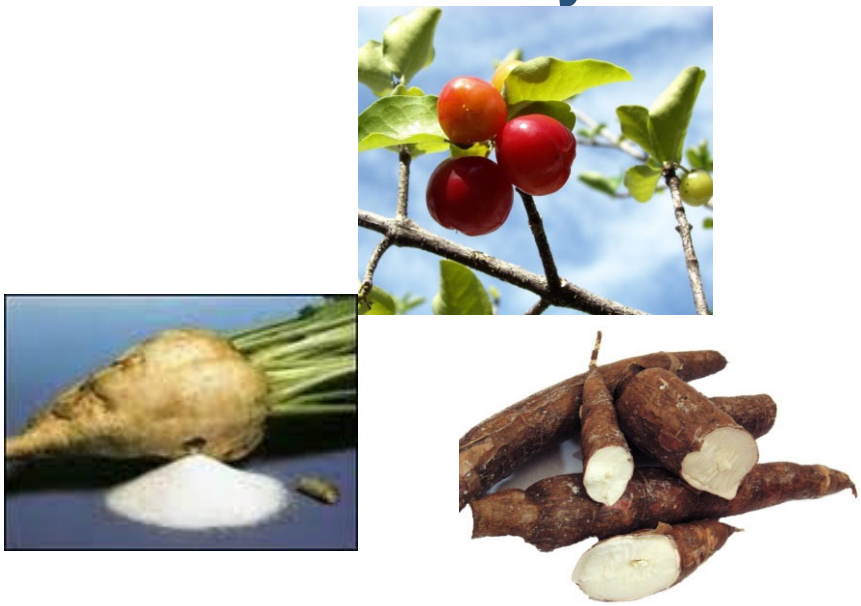
Juice authenticity issues addressed by isotope testing

- Undeclared addition of sugar in pineapple
- Undeclared addition of water (all fruits)
- Undeclared addition of vitamin C

Detection of added ascorbic acid

- **Industrial ascorbic acid is derived from sugar sources**

Global ^{13}C only detects C4 sources in fruit juices



C_3 plants

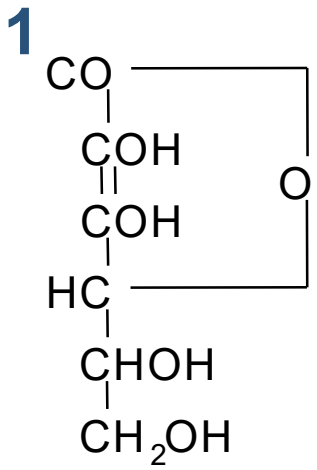
$\delta^{13}C$ around -25‰

C_4 plants

$\delta^{13}C$ around -10‰

Site-specific ^{13}C-IRMS analysis via selective chemical cut:

$\delta^{13}C$ C1 ~ $\delta^{13}C$ overall ascorbic acid



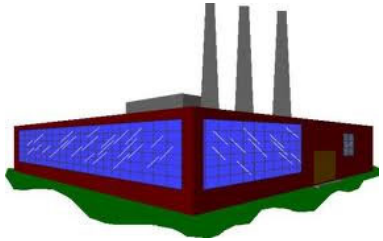
$Ce(SO_4)_2$ + H_2SO_4, 20°, 240 min

Natural

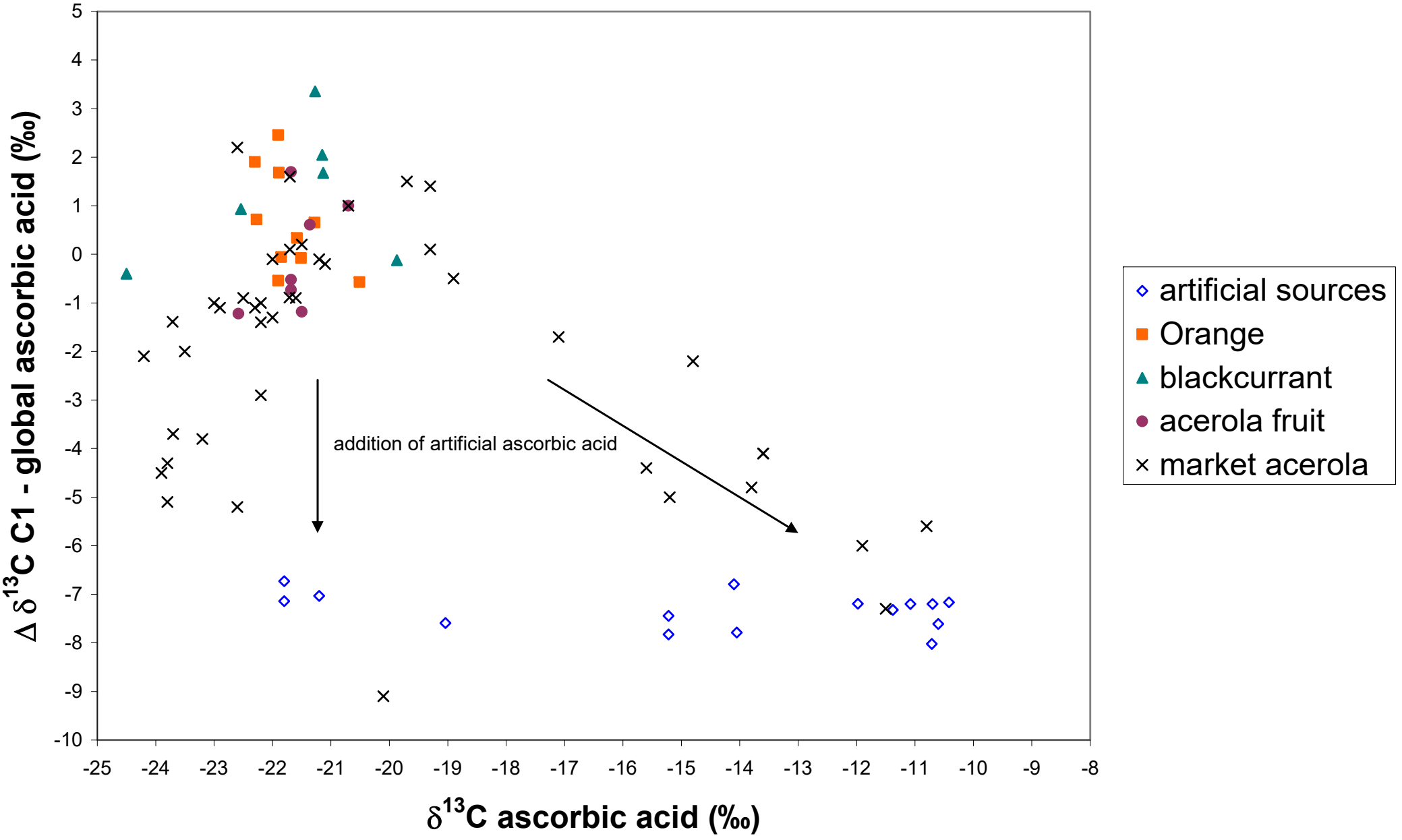
CO_2 from Carbon 1

Artificial

$\delta^{13}C$ -7‰ lower than overall ascorbic acid



Detection of added ascorbic acid

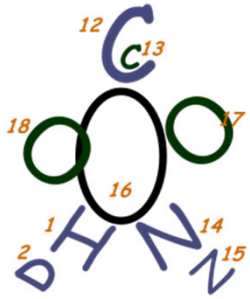


Fruit processing, July-August 2009, pp 170-175

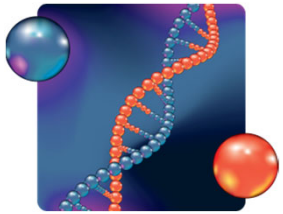
Analytical tools for authenticity testing

- **Chemical composition methods (e.g. chromatography)**
  - Identification & quantification of defined compounds

- **Stable isotopes**
  - Molecules origin

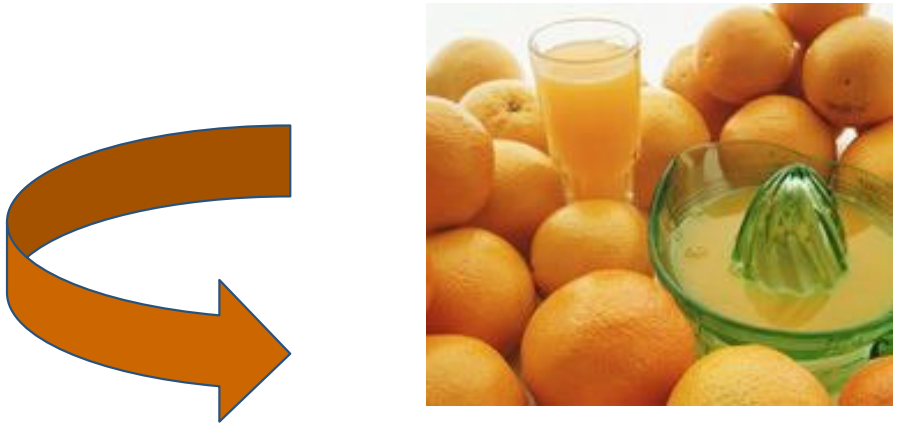


- **Molecular Biology**
  - Identification of species, varieties, etc.



- **Profiling methods**
  - Whole matrices fingerprint

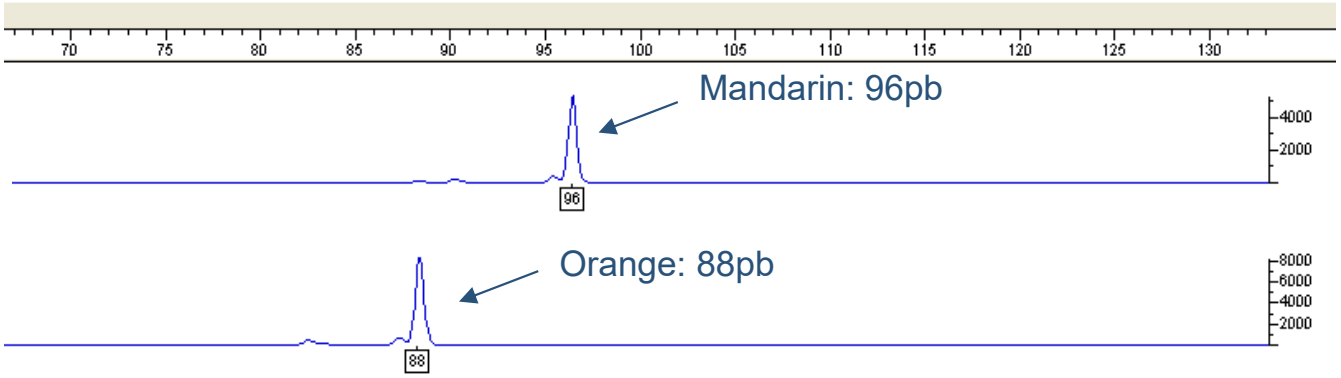
DNA testing methods:
example: Detection of mandarin in orange, LOD 2%

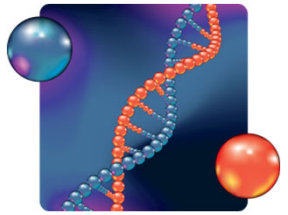


PCR amplification of the DNA target / capillary electrophoresis

100% mandarin
(pure juice)

100% orange
(pure juice)



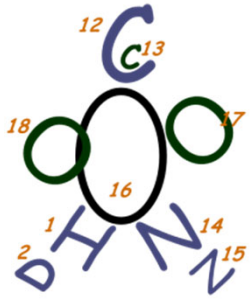


Also applicable to check fruit varieties

Analytical tools for authenticity testing

- **Chemical composition methods (e.g. chromatography)**
  - Identification & quantification of defined compounds

- **Stable isotopes**
  - Molecules origin



- **Molecular Biology**
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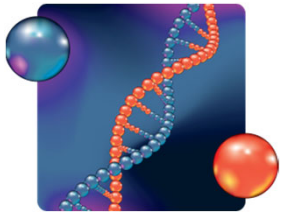

- **Profiling methods**
  - **Whole matrices fingerprint**

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Conclusion / Perspectives
Lutte contre la francisation
Fruits et Légumes
Plumes et duvets

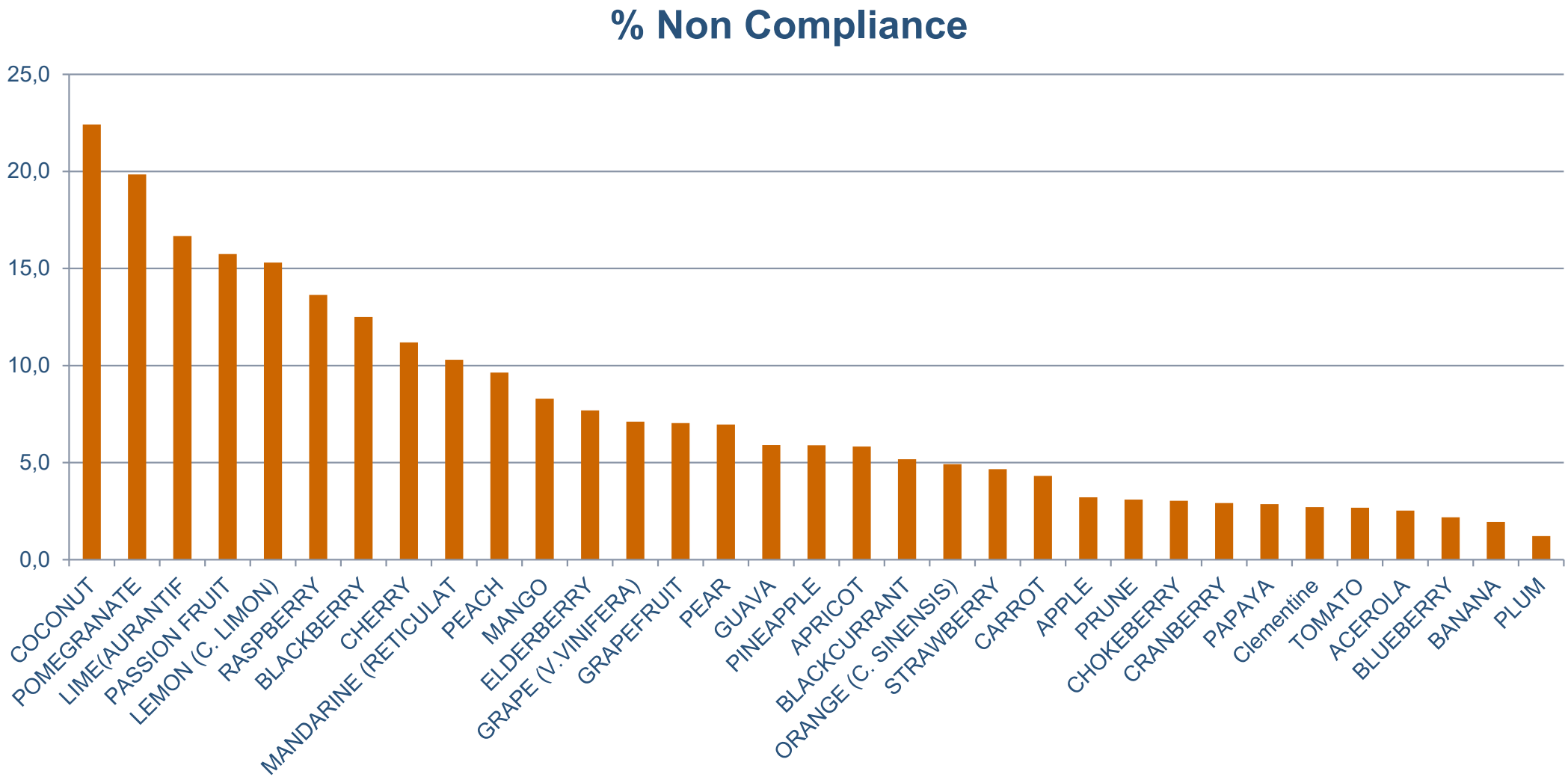
Fruit Juice Risk Level

Disclaimer :

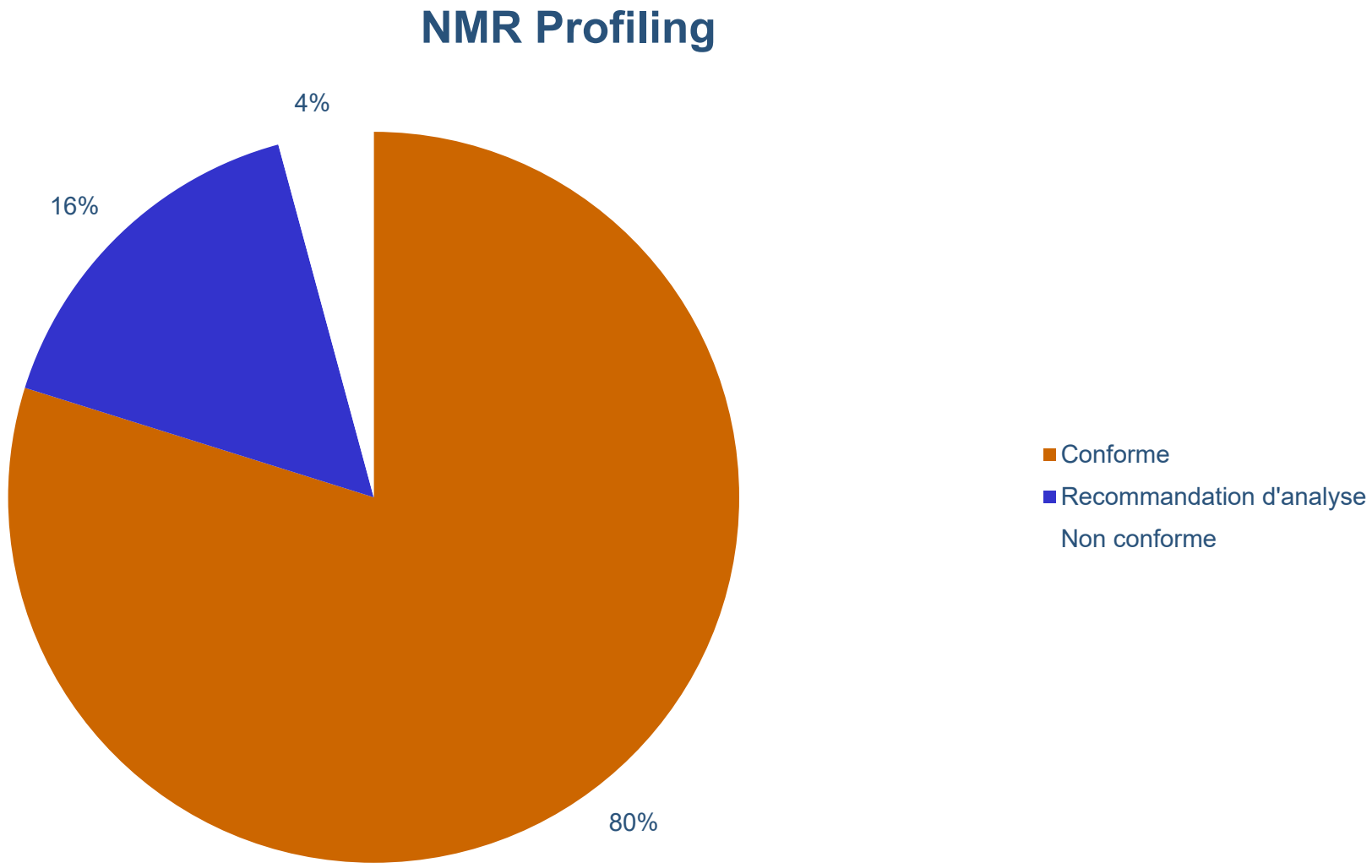
Non compliance rates presented below are global (all kind of samples send by customers and analyzed in Eurofins Analytics) and may not reflect accurately the market situation in each country

But they can be used as qualitative indications for major risks for each fruit on a worldwide basis.

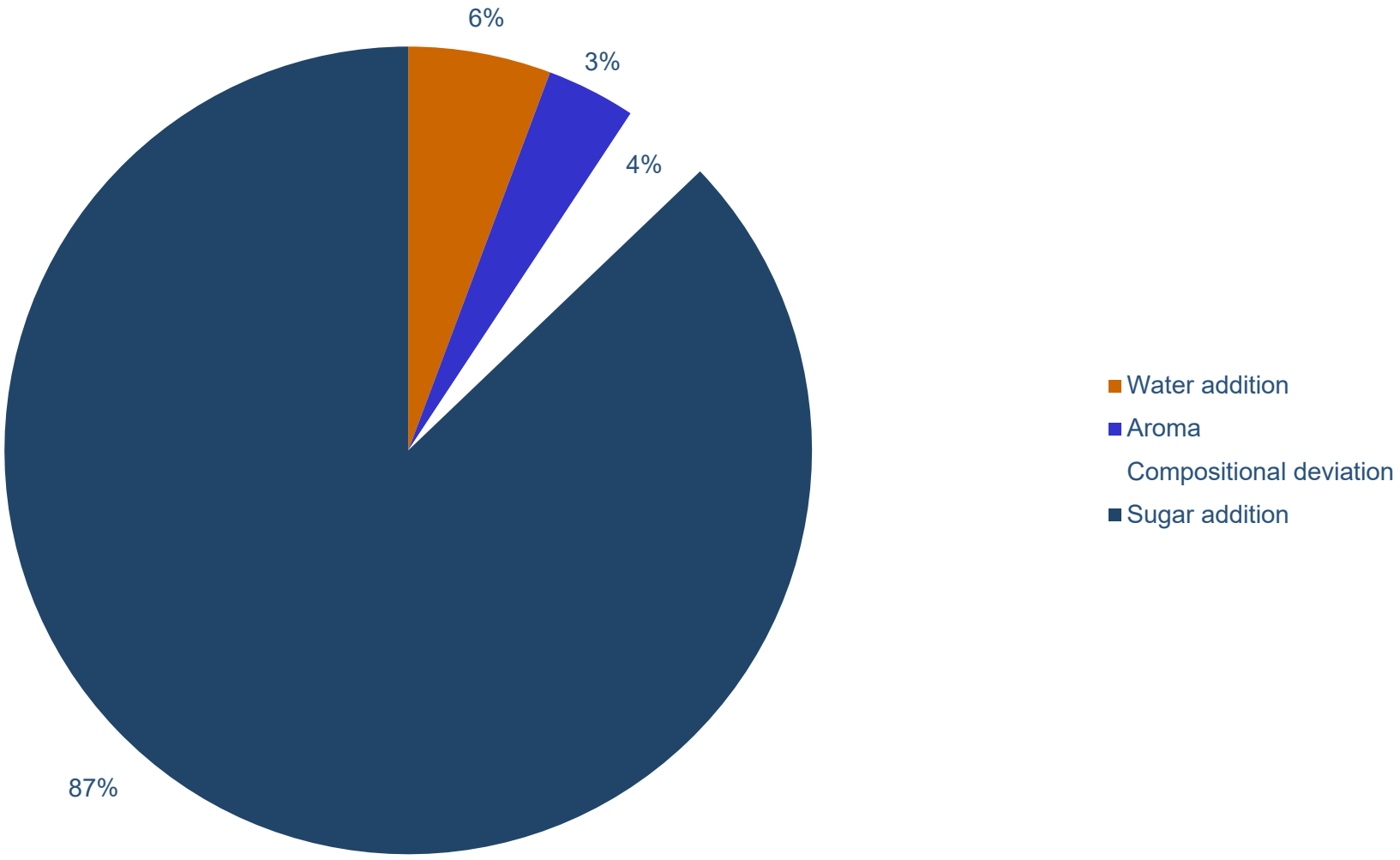
Non compliance rate per fruit



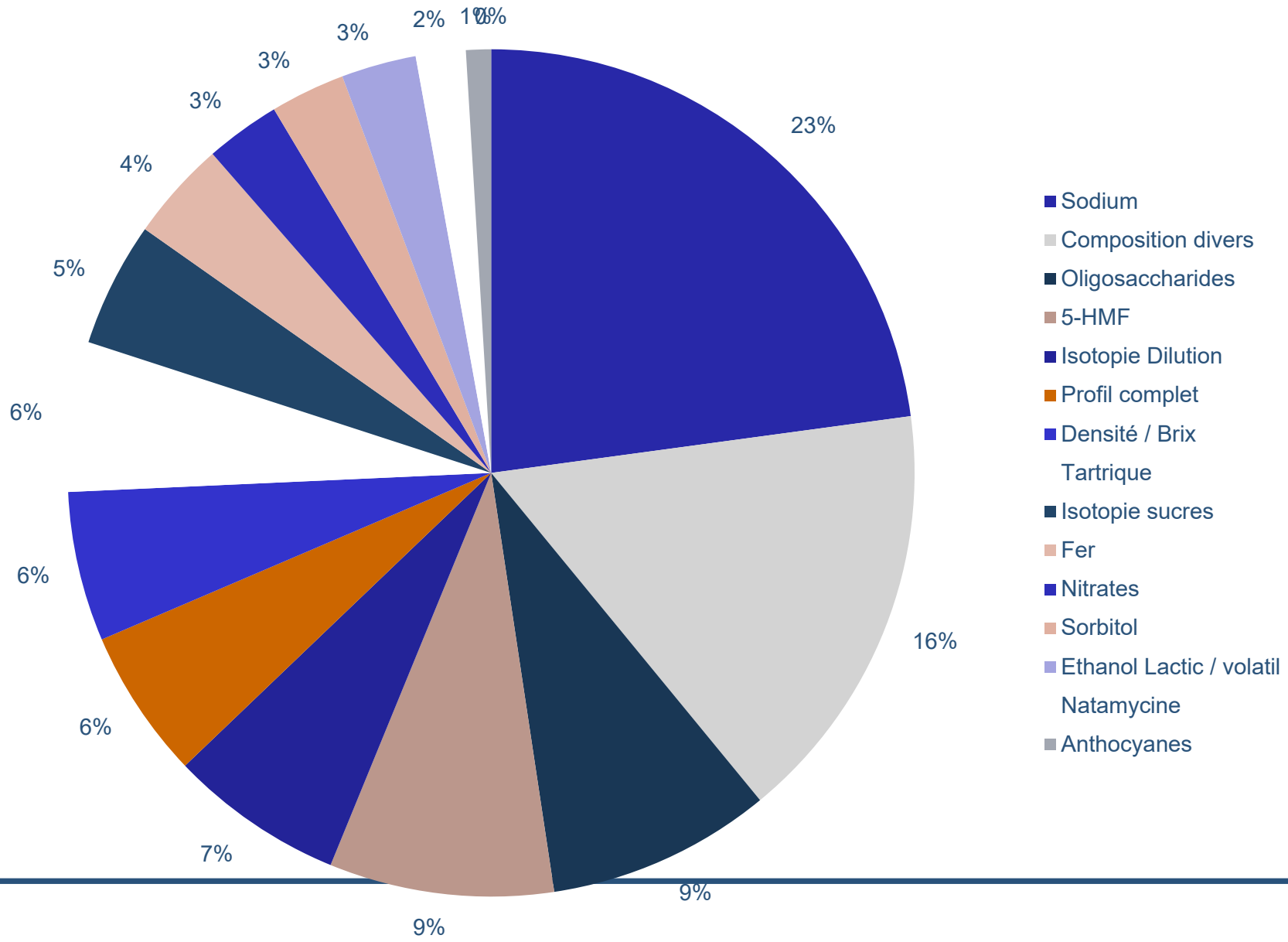
Trend NMR Profiling



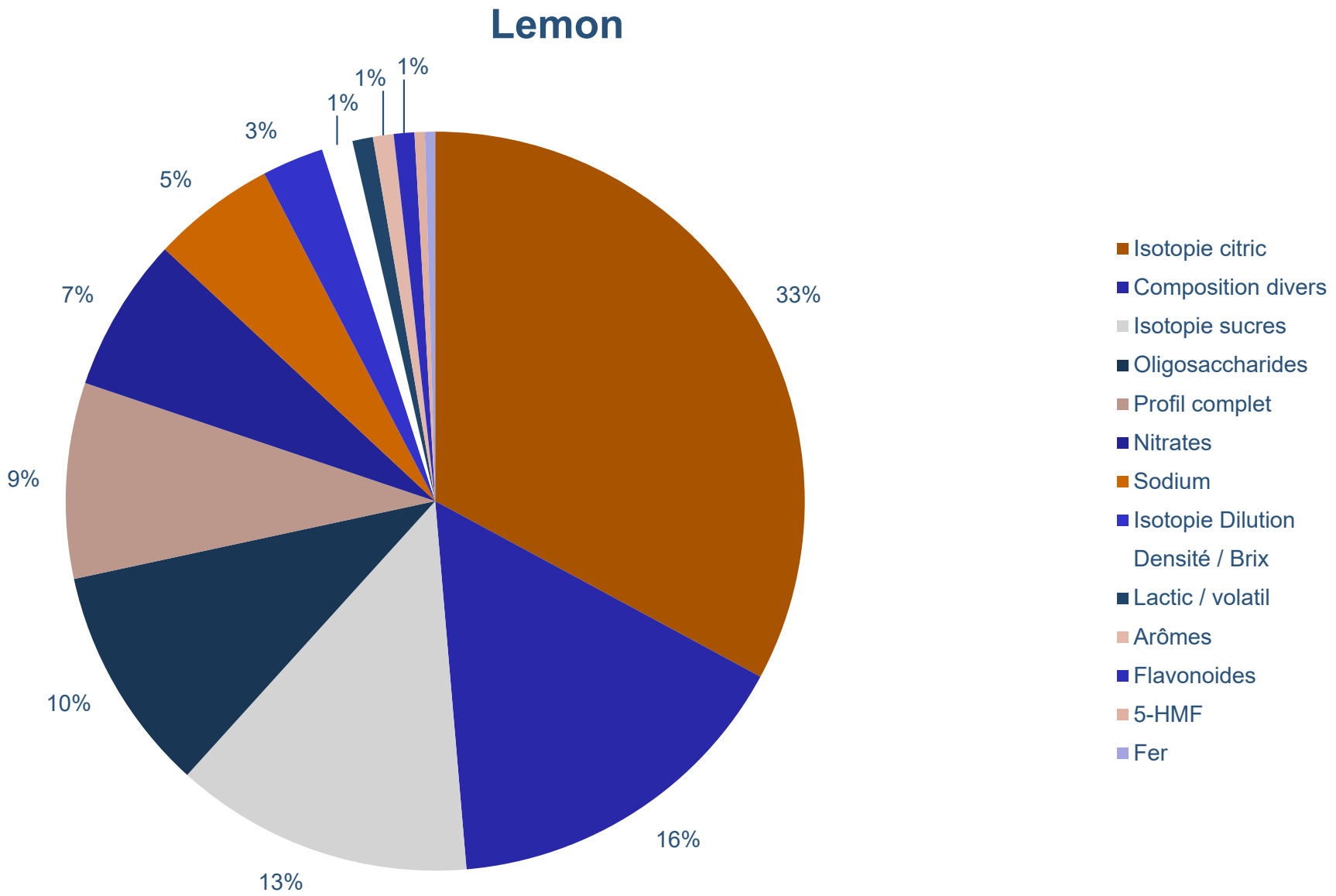
Trend 2013 – 2018 : Coconut



Trend 2013 – 2018 : pomegranate



Trend 2013 – 2018 : Lemon



Merci pour votre attention!

Division Alimentaire France

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