



# Recent Developments in Authenticity Testing

Dr. Markus Zell, Jörg Lickfett

10.10.2017

### Our first steps go back to 1987





### 1 technology: SNIF-NMR\*

(\* patented analyses for determining origin of beverages and food)

The method was the first service provided by Eurofins in 1987, which was developed from a start-up company to the largest provider of food analysis worldwide

### 4 employees

### Nantes, France

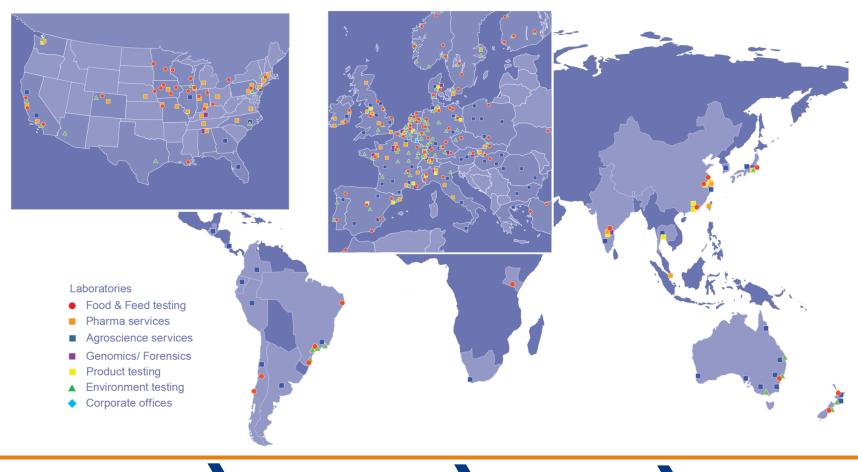
#### 1 Mission

#### **Eurofins' mission:**

To contribute to global safety by providing our customers with high quality laboratory and advisory services whilst creating opportunities for our employees and generating sustainable shareholder value.

### **Eurofins Laboratories Worldwide**





130.000 Methods 430
Laboratories

41 Countries

1 Eurofins

## We cover the whole value chain... and more





## An area with an increased focus: EU Action Plan to tackle Food Fraud



MOTION FOR A EUROPEAN PARLIAMENT RESOLUTION on the food crisis, fraud in the food chain and the control thereof (2013/2091(INI))

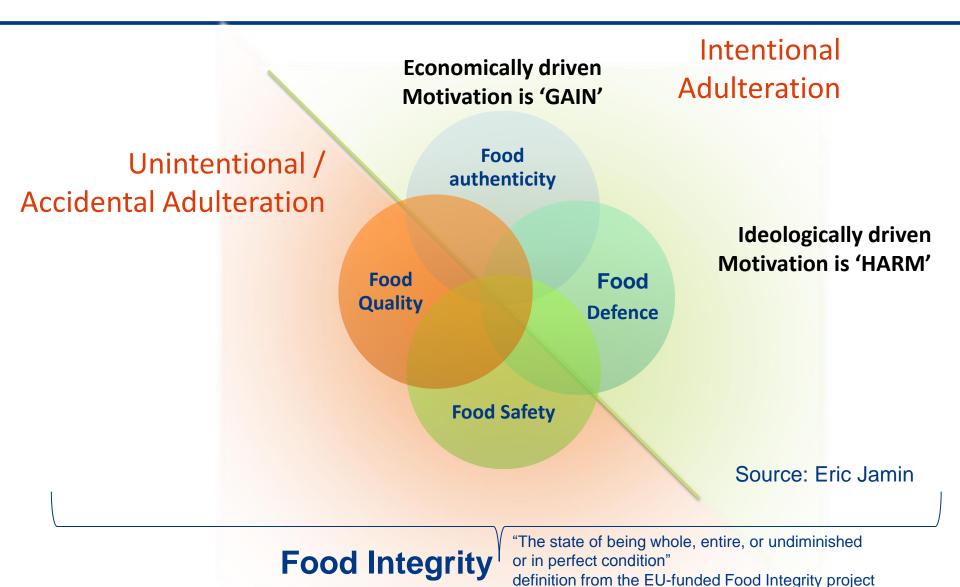
Top 10 products that are most at risk of food fraud

- 1. Olive oil
- 2. Fish
- 3. Organic foods
- 4. Milk
- 5. Grains
- 6. Honey and maple syrup
- 7. Coffee and tea
- 8. Spices (such as saffron and Vanilla)
- 9. Wine
- 10. Certain fruit juices



### **Beyond authenticity: Food Integrity**













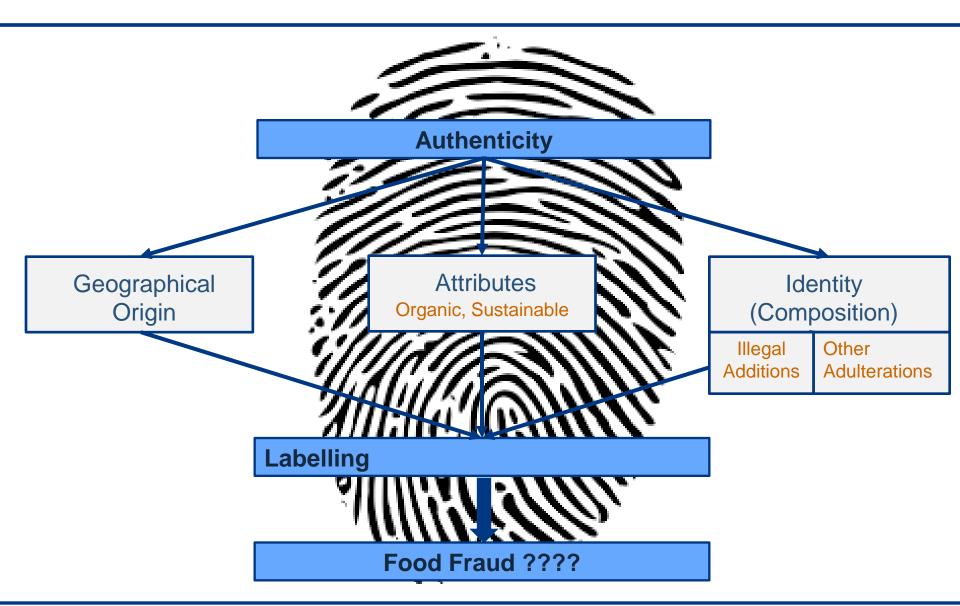
#### **Food Fraud: Threat Assessment by Commodity**

- Herbs, spices, nut and seed powders (substitution, addition)
- Honey (addition, misdescription of origin)
- Dairy products (substitution, addition)
- Eggs (mislabeling)
- Basmati rice (substitution, addition)
- Read Meat (lifestock theft, illegal slaughter, substitution)
- Diversion of waste products (ABP vs. culinary offal products)
- Fish (substitution, misdescription of origin)
- Shellfish (illegal harvesting, misdescription of origin)
- Alcohol (counterfeit of spirit brands, misdescription of wine origin)
- Olive oil (substitution, misdescription of quality)

• ...

### **Authentication of Food**





### Lab innovations against food fraud



### **Chemical compositional analysis**

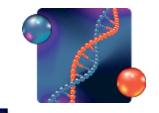
Identification and quantification of major and minor components

### **DNA Testing**

DNA barcoding, DNA fingerprinting

Species, varieties







### Stable isotope analysis

Geographical, botanical, chemical
Origin of components or
ingredients

Source: Eric Jamin

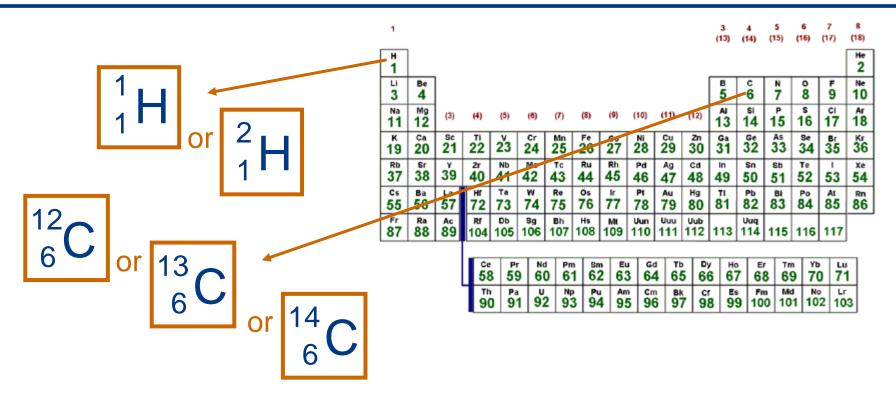
### **1H-NMR Profiling**

Fingerprint of whole matrices

Untargeted screening

### Stable isotope analysis





- Isotopes are different forms of a single element the same number of protons, but differing numbers of neutrons
- Measurements of isotopic ratios (either by SNIF-NMR or IR-MS) can be used for food authentication, e.g. geograpical or botanical origin

### **1H-NMR** in food analysis

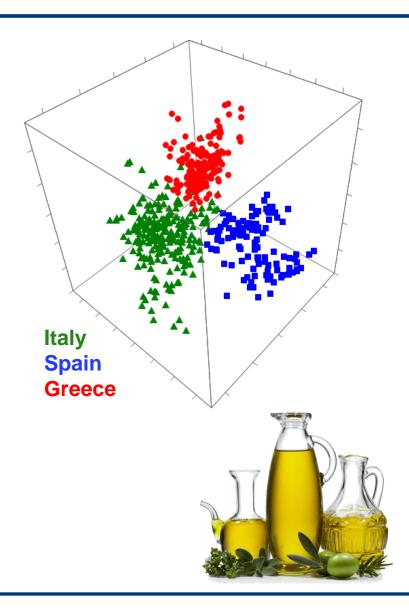


- Alternative to chromatographic techniques like GC or HPLC
- Many parameters with a single measurement (5-10 minutes)
- Highly reproducible, quantitative, automatable
- Simple sample preparation, high-throughput
- All matrices can be analyzed
- Less sensitive than GC or HPLC



## Geographical origin of olive oil by 1H-NMR & eurofins





- **Build-up of database with NMR** spectra of hundreds of authentic reference samples
- **Development of statistical models**
- **Validation**

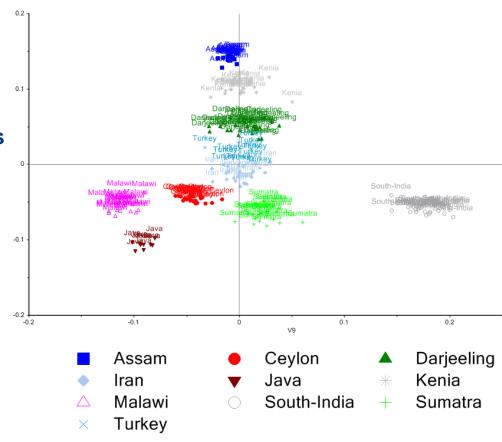
Fraudulently labeled oils can be detected with high level of success (80-95%)

### Geographical origin of black tea by 1H-NMR



- Customer project:10 Origins, 20 reference samples each
- Extraction with methanol
   <sup>1</sup>H-NMR measurement
- Development of statistical models
- 100% correct predictions for 6 origins
- Overall classification accuracy: 94%





### **Eurofins GeneScan**



### **Molecular Biology is our Core Business**



...and technical expertise in PCR analysis and DNA extraction is our Core Competence for more than 20 years.

### Three current trends



1. Change of Food Patterns

2. Increasing awareness of "GMO-free / Non-GMO" products

3. Ongoing Globalization of Sourcing and Trading

## Trend 1: Change of Food Patterns – increased importance of Halal & Veggie



We have developed a highly efficient DNA extraction method for gelatin:

- With this method, we are able to detect as little as 0.1% (w/w) of pork gelatin in e.g. beef gelatin
- Our method can currently be applied to pure gelatin products, e.g.: gelatin capsules, sheets or powders

#### **Industries affected:**

- Food, Dairy & Sweets: especially Halal & Kosher requirements
- Pharma: capsules & dietary supplements

In addition we provide a wide range of analysis for halal, vegan, vegetarian, horse meat or ruminant testing.

## Trend 2: Increased importance of "Non-GMO/GMO-free" labelling



#### **GMO-relevant Trends:**

- Ohne Gentechnik (VLOG)
- Non-GMO Project (NGP)
- Donau Soja







### Eurofins GeneScan well positioned:

- Recognized as NGP-accredited testing laboratories (US and Freiburg)
- Member of VLOG Working Group Standard
- Preferred Lab of VLOG (status gained in 2017)

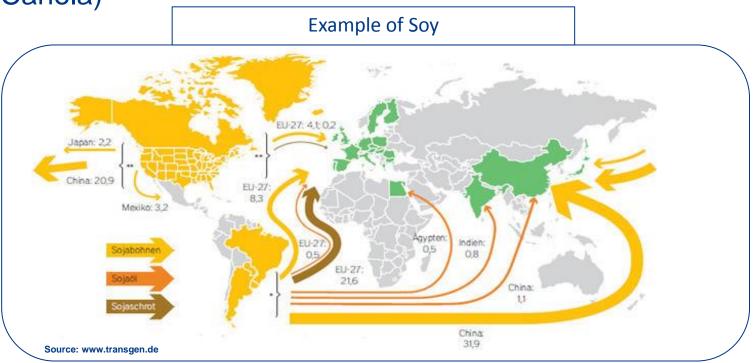
VLOG: <u>Verband Lebensmittel ohne Gentechnik</u> Association Food without Genetic Engineering

## Trend 3: Globalization of trade of commodites



Important sourcing in regions with high GMO adoption rates (Soy,

Corn, Canola)



Increasing risk of input of GMO by botanical impurities!

## **Botanical Impurities – a challenge** in **GMO Testing**



### What is a botanical impurity?

 A botanical impurity is an adventitious presence of a species in a product, that is not supposed to contain this species.

### In the context of GMO testing, how do you identify a botanical impurity?

- A positive screening result cannot be identified as a GMO from a species listed as an ingredient.
- The GMO event detected does not correlate to any ingredient.









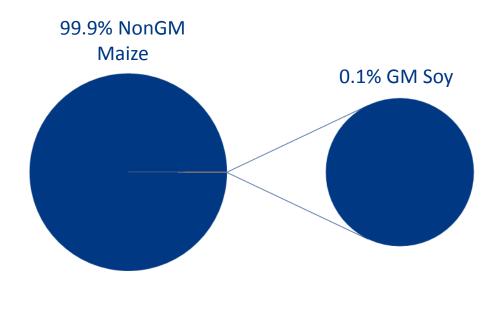
## **Botanical Impurities in Food** (Germany)



Up to 0.1% botanical impurities are defined as being adventitious or technically unavoidable.

NonGM Maize	100% GM Soy	Soy % GMO	% Soy
50	50	100%	50%
75	25	100%	25%
95	5	100%	5%
99	1	100%	1%
> 99.9%	< 0.1%	100%	< 0.1%

### "Maize" Sample



■ NonGM Maize■ GM Soy

New implemented method: Threshold calibrated real-time PCR

### DNA: "Targeted"/"Non-Targeted Analysis" 💸 eurofins



- Targeted Analyses: e.g. real-time PCR
  - Specific approach for the organism(s) of interest
  - Specific detection of the organism(s) of interest
  - Specific quantification of organism(s) of interest
  - → High Specificity and Sensitivity

**BUT:** "If I do not look for an organism, I will not find it"



- Non-Targeted Analyses: Sequencing Approaches (Sanger + NGS)
  - Universal approach (using universal primers) for an organism group of interest (animals, plants, microorganisms)
  - Identification of the organism by its DNA sequence
  - → Advantage: No sample Information necessary

### **NGS** for Food Authenticity Control



#### Can be used for...



...the authenticity control of mixed milk and dairy products (same for meat)





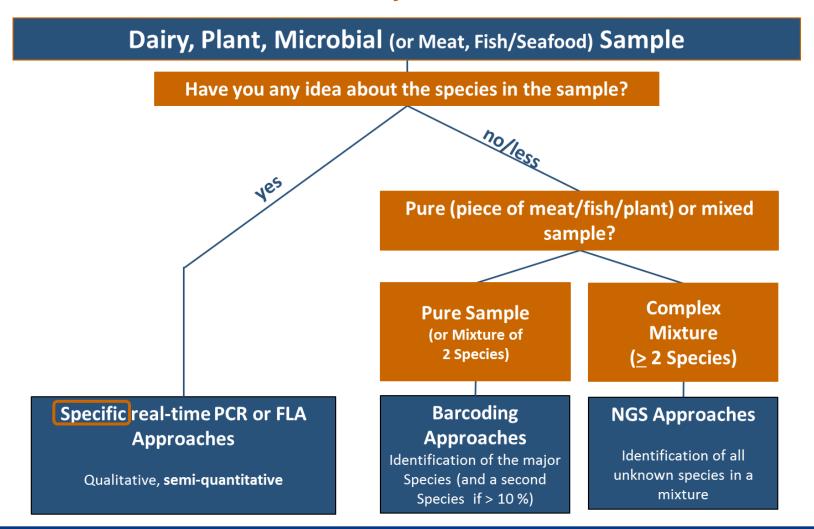
...the detection of unexpected species in herb and spice mixes



## How to choose the best Test? When using NGS?



#### **Authenticity Control in a**



### **NGS** for Food Safety Control



#### NGS can be also used for...



...the analysis of spoiled food (microbiota)

### The Next Step...

...Whole Genome Sequencing of positive Bacterial Findings
 → Detailled Long-Term Surveillance of Pathogens



## **Summary**



	Theme	Question	Example	Analytic
Authenticity	Variety / Sort	Is it what it says? Reviewing the award or approval of a specific species.	Sole, Basmati rice, Spices, Goat cheese	DNA-Analysis, NMR
	Cultivation / Production	Does the product comply with certification?	Organic or not?	Spectrum of pesticides, IRMS
	Manufacturing process	Was the product manufactured as specified? Is it natural or synthetic?	Extra virgin olive oil (cold pressed), Halal Flavours, such as vanilla	HPLC, GC, PCR
	Origin / Regional origin	Control of geographical origin. Important, e. g. when the protected designation of origin is awarded.	Parma ham, Olive oil, Darjeeling	SNIF-NMR, 1H- NMR, NIR, IRMS
Purity	Variety / Sort	Is the product mixed with other varieties/species?	Honey, Coffee, Linseed with rape seed, Hazelnut flour with chickpea	Microscopy, NMR, DNA analysis
	Impurities / Allergens	Are there residues or impurities due to allergens or foreign substances?	Egg in wine, Peanut in spices, Gluten in tea, Stones, Metal	ELISA, Filth Test
	Substitution	Was there stretching / dilution / addition or blending of the product to maximize profit	Coffee, Olive oil, Honey, Saffron, Oregano, Gingko biloba	Sensoric, TLC, 1H-NMR, GC-FSME
	Illegal additions	Addition of food own additives or foreign foodstuff to simulate a better quality.	Milk powder with melamine, Glycol in wine, Sudan red in paprika powder, Addition of vitamin C, Foreign water	LC-MS/MS, 1H-NMR, , SNIF- NMR, IRMS, DNA- Analysis
Product- / Trademark protection		How do I recognize a (reference) product without a doubt?	Olive oil	NMR, DNA- Fingerprinting



## Thank you for your attention

# We are looking forward to pursue and expand our cooperation

