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

Economically driven
Motivation is ‘GAIN’

Intentional Adulteration

Ideologically driven
Motivation is ‘HARM’

Unintentional / Accidental Adulteration

Food Quality

Food Defence

Food Safety

Food Integrity

“The state of being whole, entire, or undiminished or in perfect condition”
definition from the EU-funded Food Integrity project

Source: Eric Jamin
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

- Geographical Origin
- Attributes: Organic, Sustainable
- Identity (Composition): Illegal Additions, Other Adulterations
- Labelling
- Food Fraud ????
Chemical compositional analysis
Identification and quantification of major and minor components
Verification of specifications

DNA Testing
DNA barcoding, DNA fingerprinting
Species, varieties

Stable isotope analysis
Geographical, botanical, chemical
Origin of components or ingredients

1H-NMR Profiling
Fingerprint of whole matrices
Untargeted screening

Source: Eric Jamin
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. geographical 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

- 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%)
- **Customer project:**
  10 Origins, 20 reference samples each

- **Extraction with methanol**
  $^1$H-NMR measurement

- **Development of statistical models**

- **100% correct predictions for 6 origins**

- **Overall classification accuracy: 94%**

Geographical origin of black tea by $^1$H-NMR
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: Verband Lebensmittel ohne Gentechnik
Association Food without Genetic Engineering
Trend 3: Globalization of trade of commodities

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

Example of Soy

Source: www.transgen.de

Increasing risk of input of GMO by botanical impurities!
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.

<table>
<thead>
<tr>
<th>NonGM Maize</th>
<th>100% GM Soy</th>
<th>Soy % GMO</th>
<th>% Soy</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>50</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
<td>100%</td>
<td>25%</td>
</tr>
<tr>
<td>95</td>
<td>5</td>
<td>100%</td>
<td>5%</td>
</tr>
<tr>
<td>99</td>
<td>1</td>
<td>100%</td>
<td>1%</td>
</tr>
<tr>
<td>&gt; 99.9%</td>
<td>&lt; 0.1%</td>
<td>100%</td>
<td>&lt; 0.1%</td>
</tr>
</tbody>
</table>

New implemented method: Threshold calibrated real-time PCR
DNA: „Targeted“/“Non-Targeted Analysis“

- **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
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

...the control of starter cultures and ferments

...the detection of unexpected fish species in mixed fish and meals
How to choose the best Test? When using NGS?

Authenticity Control in a Dairy, Plant, Microbial (or Meat, Fish/Seafood) Sample

Have you any idea about the species in the sample?

- **Yes**
  - Pure (piece of meat/fish/plant) or mixed sample?
    - **Pure Sample** (or Mixture of 2 Species) - Barcoding Approaches
      - Identification of the major Species (and a second Species if > 10%)
    - **Complex Mixture (≥ 2 Species)** - NGS Approaches
      - Identification of all unknown species in a mixture

- **No/less**
  - Specific real-time PCR or FLA Approaches
    - Qualitative, semi-quantitative
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 → Detailed Long-Term Surveillance of Pathogens
<table>
<thead>
<tr>
<th>Theme</th>
<th>Question</th>
<th>Example</th>
<th>Analytic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Authenticity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety / Sort</td>
<td>Is it what it says? Reviewing the award or approval of a specific species.</td>
<td>Sole, Basmati rice, Spices, Goat cheese</td>
<td>DNA-Analysis, NMR</td>
</tr>
<tr>
<td>Cultivation / Production</td>
<td>Does the product comply with certification?</td>
<td>Organic or not?</td>
<td>Spectrum of pesticides, IRMS</td>
</tr>
<tr>
<td>Manufacturing process</td>
<td>Was the product manufactured as specified?</td>
<td>Extra virgin olive oil (cold pressed), Halal Flavours, such as vanilla</td>
<td>HPLC, GC, PCR</td>
</tr>
<tr>
<td>Origin / Regional origin</td>
<td>Control of geographical origin. Important, e. g. when the protected designation of origin is awarded.</td>
<td>Parma ham, Olive oil, Darjeeling</td>
<td>SNIF-NMR, 1H-NMR, NIR, IRMS</td>
</tr>
<tr>
<td>Variety / Sort</td>
<td>Is the product mixed with other varieties/species?</td>
<td>Honey, Coffee, Linseed with rape seed, Hazelnut flour with chickpea</td>
<td>Microscopy, NMR, DNA analysis</td>
</tr>
<tr>
<td>Purity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impurities / Allergens</td>
<td>Are there residues or impurities due to allergens or foreign substances?</td>
<td>Egg in wine, Peanut in spices, Gluten in tea, Stones, Metal</td>
<td>ELISA, Filth Test</td>
</tr>
<tr>
<td>Substitution</td>
<td>Was there stretching / dilution / addition or blending of the product to maximize profit</td>
<td>Coffee, Olive oil, Honey, Saffron, Oregano, Gingko biloba</td>
<td>Sensoric, TLC, 1H-NMR, GC-FSME</td>
</tr>
<tr>
<td>Illegal additions</td>
<td>Addition of food own additives or foreign foodstuff to simulate a better quality.</td>
<td>Milk powder with melamine, Glycol in wine, Sudan red in paprika powder, Addition of vitamin C, Foreign water</td>
<td>LC-MS/MS, 1H-NMR, SNIF-NMR, IRMS, DNA-Analysis</td>
</tr>
<tr>
<td>Product-/ Trademark protection</td>
<td>How do I recognize a (reference) product without a doubt?</td>
<td>Olive oil</td>
<td>NMR, DNA-Fingerprinting</td>
</tr>
</tbody>
</table>
Thank you for your attention

We are looking forward to pursue and expand our cooperation