New Approaches in Food Authenticity Testing

25 April 2018
Outline

- Food fraud and authenticity
- Food authenticity testing
- Targeted approach examples
- Non-targeted approach examples
- Conclusion
What is food fraud?

- Documented for over 200 years!

- What has changed?
  - The sophistication of methods used to commit fraud.
What is food fraud?

At present there is no statutory or unambiguous definition of **food fraud/EMA** (Economically Motivated Adulteration). A comprehensive definition generally used by different bodies is from the **Food Fraud Initiative** of Michigan University.

“**Food Fraud**: a collective term encompassing the **deliberate and intentional substitution, addition, tampering or misrepresentation** of food, food ingredients or food packaging, **labeling**, product **information** or false or **misleading statements** made about a product for **economic gain** that could impact consumer **health**” (Spink, J. & Moyer, DC (2011) Journal of Food Science, 76(9), 157-163)

Food fraud undermines **product authenticity**, namely: its **origin**, **quality** (PDO, PGI) and **biological characteristics** (species, varieties, races).
What is food fraud?

Key Characteristics

- Non-compliance with food law and/or misleading the consumer
- which is done intentionally
- for reasons of financial gain
Brazil meat-packing giants ’exported rotten beef’

Fake food and alcohol seizures at EU borders jump

Kiwi wine company and directors accused of complex wine fraud

Georgian police arrest man for exporting falsified hazelnut products to Germany
Main reasons for fraud

- Desire to make *illicit profit*
- Premium products or ingredients in *short supply*
Some metrics...

10% of global food supply affected

ESTIMATED ANNUAL LOSS TO INDUSTRY = $49 BILLION

37% of frozen beef burgers in Ireland found to contain horse DNA in a 2013 study

300,000 - Estimated number of children sickened from the melamine-tainted milk scandal in China in 2008
Types of Products

- Top 10 adulterated products

- EU Committee on the Environment, Public Health and Food Safety; DRAFT REPORT

TOP 10 PRODUCTS THAT ARE MOST AT RISK OF FOOD FRAUD

1. OLIVE OIL
   (origin, mixing with other oils)

2. FISH
   (species, quality, fresh vs frozen-thawed, wild vs farmed)

3. ORGANIC FOODS
   (origin, type, dilution, melamine)

4. MILK
   (basmati rice, durum vs tender wheat in pasta, GMO)

5. GRAINS
   (sugars added, origin)

6. HONEY & MAPLE SYRUP
   (arabica/robusta, origin and varieties, grass cuttings)

7. COFFEE & TEA
   (saffron origin, chilli coloured with illegal dyes)

8. SPICES
   (origin-PGI)

9. WINE
   (diluted, 100% declared fruit)
Terminology

FOOD FRAUD

- Watered down products using non-potable/unsafe water
- Olive oil diluted with potentially toxic tea tree oil
- Copies of popular foods - not produced with acceptable safety assurances.
- Melamine added to enhance protein value
- Use of unauthorized additives (Sudan dyes in spices)
- Sunflower oil partially substituted with mineral oil
- Hydrolyzed leather protein in milk
- Poultry injected with hormones to conceal disease
- Harmful food colouring applied to fresh fruit to cover defects
- Expiry, provenance (unsafe origin)
- Toxic Japanese star anise labeled as Chinese star anise
- Mislabeled recycled cooking oil
- Sale of excess unreported product
Main types of food fraud

- Deliberate misdescription of:
  - Food, such as: products substituted with a cheaper alternative or cheaper quality part for example, farmed salmon sold as wild, and Basmati rice adulterated with cheaper varieties, or thyme stem instead of leaves, etc
  - Source of ingredients, ie their geographic, plant or animal origin
  - Sale of food which is unfit for consumption and potentially harmful
  - Knowingly selling goods which are past their shelf life

- Deliberate misdescription of production process;
  - As organic, whereas grown conventionally
  - Wild vs farmed
  - Free range vs conventional
  - Specific production processes (Halal and Kosher)
EMA
Economically Motivated Adulteration

**Hazelnuts**
3.45 €/kg

**Peanuts**
1.48 €/kg

133% Cheat!

80% Cheat!

90% Cheat!

27% Cheat!

prof. dr. alfred hagen meyer
How is it adulterated?

- **Substitution**
  
  "*Authentic ingredient is replaced with the cheaper substitute, either;*
  
  - the *lower* quality part of the *same* species
  - *other* species of *lower* quality
  - from *other* geographical origin
  - from *other* production process

- **Addition**

  "*Adulteration by adding a totally different filler*"
**Substitution**

- **Extra Virgin Olive Oil** adulterated by
  - Lower grade olive oil
  - Other types of cheaper oil (hazelnut, soybean oil)

- **Meat** can be adulterated by
  - Lower grade tissues eg. connective tissues or inner organs instead of high quality muscle tissue
  - Other types of cheaper meat eg horsemeat in beef lasagna

- **Milk and Dairy** Products
  - Species (bovine vs goat / sheep / buffalo)

- **Fruit Juices**
  - Juice type (pomegranade vs grape, orange vs apple)

- **Honey**
  - Geographical and varietal origin (Manuka from NZ)
Spices
- Saffron is prone to adulteration: in India, high grade Kashmiri saffron is often sold and mixed with cheaper saffron grades
- Oregano adulteration with olive tree leaves and myrtle leaves

Botanical Dietary Supplements
- Cheaper/ available ingredient

Fish or Meat can be sold although expired or not being as fresh as mentioned on the label
Addition

- **Honey**
  - HFCS (High Fructose Corn Syrup+pollens+enzymes+colouring agents…)

- **Milk and Dairy Products**
  - Dilution with water and addition of Nitrogen rich adulterants (melamine, urea)
  - Addition of expired cheeses by help of phosphate salts
  - Addition of illegal preservatives (nisin bacteriocine, Lysozyme)

- **Meat and meat products**
  - Addition of water with some chemicals to hold excessive water

- **Spices**
  - Sawdust + colouring agents + flavours

- **Fruit Juices**
  - Dilution and addition of sweeteners, sugars, colors, etc

- **Botanical Dietary Supplements**
  - Addition of active pharmaceutical ingredients (sildenafil)
Food Fraud Temptation Model

KEY:
1. Saffron
2. Olive Oil
3. Wheat
4. Coffee
5. Beef trim pre HM
6. Beef trim post HM
7. Potato

Size of circle represents ease of perpetration
Certification standards have evolved to meet global, economic, social and health needs to prevent food alteration.

New certification standards include sections entirely devoted to the control of product authenticity and to the actions to be taken to prevent food fraud.

- BRC7
- FSSC 22000
- IFS
- ...
Explicit intent of BRC version 7 is to protect food authenticity and prevent/reduce food fraud:

5.4.1 … to access information on historical and developing threats to the supply chain which may present a risk of food fraud

5.4.2 … a documented vulnerability assessment shall be carried out on all food raw materials

5.4.3 … Where raw materials are identified at risk, appropriate assurance and/or testing to reduce the risk
How to prevent Food Fraud

“*If you can’t measure it, you can’t manage it.*”

- Assessment of risks
- Implementation of working “food fraud control system”
Food Fraud Mitigation Plans

- Vulnerability assessment - identification and assessment of vulnerable ingredients and supply chains
- USP - Food Fraud Mitigation Guidance (FFMG), published in 2015 in the Third Supplement to the *Food Chemicals Codex* 97
- 9 Contributing factors
- www.foodfraud.org
VACCP vs TACCP

**VACCP**
- **Vulnerability** Assessment Critical Control Point
- **Susceptibility or exposure** to a Food Fraud risk, which is regarded as a gap or **deficiency** that could place consumer health at risk if not addressed.

**TACCP**
- **Threat** Assessment Critical Control Point
- Systematic management of risk through the **evaluation of threats, identification of vulnerabilities, and implementation of controls** to materials and products, purchasing, processes, premises, distribution networks and business systems by a knowledgeable and trusted team with the authority to implement changes to procedures.
- **Types of Threats**
  - Economically motivated adulteration (EMA)
  - Malicious contamination
  - Extortion
  - Espionage
  - Counterfeiting
  - Cyber crime
Food Fraud Mitigation Plans

- **Supply chain:**
  What is the degree of vertical integration in the supply chain for a particular ingredient, or are purchases made on the open market?

- **Audit strategy:**
  Is there a robust, onsite audit strategy with anti-fraud measures, or a less mature audit strategy without targeted anti-fraud measures?

- **Supplier relationship:**
  Is the supplier relationship established and trusted?

- **History of regulatory, quality, or safety issues with a supplier**

- **Susceptibility of quality assurance methods and specifications**
  Are quality assurance methods and specifications specific and effective for a range of potential adulterants?
Testing frequency:

Is every lot tested by the buyer, is testing conducted intermittently, or is there reliance only on Certificates of Analysis?

Geopolitical considerations:

Is the ingredient sourced from, or does it travel through, a geographic area with political, food safety, or food security concerns?

Fraud history:

Is there a high volume of known and documented food fraud incidents in the ingredient?

Economic anomalies:

Are there supply pressures, pricing discrepancies, or other economic factors that may increase the incentive for fraud in the ingredient?
Detection Methods

- **Biological Methods**
  - ELISA
  - PCR
  - DNA sequencing
  - NGS

- **Physico-chemical Methods**
  - Regular Spectroscopy with or without chromatography for targets
  - NMR Spectroscopy

- **IRM**
  - Isotope Ratio

- **High Resolution MS**
  - TOF technologies non-targeted approach

- **Microscopy**
  - SEM

- **Chromatographic techniques**
  - HPLC (MS, MS/MS, RI, UV-dad, FLD…)
  - GC (FID, TCD, MS, MS/MS…)

**Non-targeted approach** – combination of lab-based methods with statistical software to determine if sample falls within expected parameters

- Detection of **wide range adulterants**

- Profile for both **authentic and non-authentic** adulterants developed
**Approaches for Authenticity Testing**

- **Targeted Analysis**
  - *Is it in the food sample?*
  - Determination of the known molecules associated with the adulteration

- **Non Targeted Analysis**
  - *What is in the food sample?*
  - A broad anomaly testing which examines whether a product is authentic or not by defining carefully the fingerprint of the material and exclude any unusual thing deviating from the original fingerprint based on the analysis all compounds in a sample
Some food fraud issues can be detected quite easily with standard tests, as long as they are properly combined together, if you know what you are looking for…

- Methods for fish species identification (PCR): (cod, sole, halibut, catfish, tilapia, etc)
- Methods for meat species identification (PCR): (beef, pork, chicken, turkey, horse, goat, deer)
- Methods for botanical species identification (PCR): (Herbs, spices, etc)
- Adulteration of spices
- Adulteration of Olive Oil with cheaper substitutes
- Adulteration of fruit juices
- Artificially colored products

- Common wheat in durum wheat pasta
- Counterfeiting of organic products
- Illegal additives in fish & dairy products
- Crust % in grated parmesan
- Aging of Grana Padano Cheese
- Determination of Robusta coffee
- Dairy products produced by silage fed animals vs grass fed
- Fish freshness
- Adulteration of A2 Milk
Olive oil can be adulterated with other cheaper vegetable oils for economic reasons

- EU regulation 2568:1991
- Targeted approach: analysis of fatty acid composition and sterol composition, ECN 42, wax, stigmastadien, EUV

European panel on oil analyses well classifies different oils, but it is not sufficient alone to cover all possible related food frauds
Possible species identification for **meat**:  
- Beef, pork, chicken, turkey, horse, duck and more

Possible species identification for **fish**:  
- Atlantic cod, haddock, tilapia, pangasius

**Method**  
- Microarray, PCR and DNA sequencing  
- Quantitative and qualitative analyses
Targeted approach Example: “Detection of fraud in Herbs and Spices”

- Most adulterated products: often claimed to be pure by label
- Spices can contain **other species**
- Spices can contain **cheaper or lower-quality parts of the same species**
- Spices can be adulterated with **other powders** like flower **grains**, **starch** or even **sawdust**
- Spices can contain **colors and illegal dyes** to *spice up* the color (most of these compounds are not approved for use in food)

Detection Methods:

- **PCR**
- Determination of **essential oil content** by means of distillation-extraction and comparison with tabulated values
- **Aromatic profile** using GC/MS, to detect foreign volatile and semi-volatile substances and/or markers of other spices/herbs
- **LC-MS/MS**: Detection of **illegal dyes**: Sudan I, II, III, IV, Sudan Black, Red Orange, p-Nitroaniline, etc....
Targeted approach Example: “Detection of Fish Freshness”

- Determination of fish freshness by
  - Determination of total volatile nitrogen
  - Determination of histamine and other biogenic amines
  - Determination of carbon monoxide for red color in tuna
  - Determination of Succinic dehydrogenase (frozen vs fresh)

- Method
  - Titrimetric
  - Quantification by HPLC with UV detector
  - Quantification by HPLC with fluorimetric detector
  - Quantification by GC-FID with methanator
Targeted approach Example: “Detection of fraud in Premium Products”

- Detection of fraud in **organic produce**
  - Determination of pesticides by QuEChERS method

- Detection of fraud in **Halal Products**
  - Determination of pork (PCR) or alcohol (GC)
NTS-Non Targeted Screening Approach

NMR, IR LC-HRMS  \rightarrow  NON TARGETED ANALYSIS  \rightarrow  CHEMOMETRICS "MULTIVARIATE DATA ANALYSIS"

MALDI-TOF  \rightarrow  Big molecules

Small molecules

Result: 95% probability that sample is Authentic: YES/NO
## Targeted Analysis vs Nontargeted Analysis

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<th>Subject</th>
<th>Targeted</th>
<th>Non-Targeted</th>
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<td>Approach</td>
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<td>Top-down</td>
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<td>Analytes</td>
<td>Targeted compounds</td>
<td>Fingerprint</td>
</tr>
<tr>
<td>Sensitivity</td>
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<tr>
<td>Sample preparation</td>
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<td>Data Analysis</td>
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<tr>
<td>Control limits</td>
<td>Publicly available</td>
<td>Specific Reference Database “ex novo”</td>
</tr>
<tr>
<td>Consistency</td>
<td>Simple: Sample representativeness</td>
<td>Complex: Experimental Design (temporal, spatial, instrumental)</td>
</tr>
</tbody>
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**Food Profiling:** Identification of substances in the fingerprinting
Procedure for NTS (Fingerprinting)

1. **Experimental design**
   - definition of the objective
   - type of matrix
   - analytical technique

2. **Sampling**
   - establishment & sampling of a representative sample set

3. **Sample preparation**
   - e.g.
     - milling, homogenization
     - extraction, clean-up
     - derivatization
     - addition of buffer

   **Aquisition of chemical fingerprint**
   - by the use of spectroscopic and/or spectrometric techniques

4. **Statistical data analysis**

5. **Data analysis**
   - by the use of supervised and/or unsupervised techniques

6. **Data pre-processing**
   - e.g.
     - bucketing,
     - normalization, scaling,
     - 1st, 2nd derivative,
     - feature selection

   **“Profiling”**
   (in some cases)
Capabilities of NTS

- Discrimination
  - Geographical origin
  - Species varieties

- Classification
  - Olive oil

- Identification of unknown adulterating substances
The method was developed to identify olive oil from four different geographical areas:
- Italy
- Turkey
- Europe
- California-US

Method:
- Non targeted Gas chromatography Mass Spectrometry of volatile fraction
- Chemometric elaboration to create a geographical origin data base
Non Targeted Approach Example: “Origin of Extra Virgin Olive Oil”

- **Non targeted analyses** to build a database with oil samples from the regions of interest (Italy, Middle East, Europe, USA)
- **Database validation** with blind samples
- Analysis of **unknown** samples: it identifies and classifies the origin of unknown samples according to their position in the reference database (Italy, Middle East, Europe, USA)

Example of construction of a database according to the origin of known samples

- **Origin from Italian area of the database**
- **Origin from Outside EU area of the database**
The method was developed to distinguish Italian tomato products from non-Italian products.

Non-Targeted Screening Model based on:
- LC/HRMS;
- Chemometrics: SIMCA PCA and PLS-DA
PDO Parmigiano Reggiano is one of the most adulterated cheese in the world (high economic impact).

An array of independent targeted and non targeted analyses to identify authentic PDO Parmigiano Reggiano Cheese:

- geographic origin
- production process
- animals feed
- milk quality
Authenticity of PDO Parmigiano Reggiano

1. Sample analysis

2. Comparison with a reference database

3. Chemometric analysis
Conclusion

Prevention starts with:

- Practical vulnerability assessment tools
- Innovation in analytical methods
- Novel approaches to data analysis/sharing
- Daily in your role as a Food Scientist/Food Technologist

"FIGHTING FRAUD AND ADULTERATION WILL REMAIN A RACE BETWEEN THE CRIMINALS COMMITTING FRAUD AND THE SCIENTISTS DEVELOPING NEW METHODS TO UNCOVER THEM."
Thank YOU

Any questions?

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