FoodOmicsGR

National Research Infrastructure for the Comprehensive Characterisation of Foods

METRO FOOD·GR

JOINT RESEARCH UNIT METROFOOD-GR

Targeted and untargeted methods for food authenticity and traceability: An introduction

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Webinar on Food Metabolomics
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The last decades, **consumers** have progressively **come up with various kinds of demands**, such as:

Hygiene and **food safety**

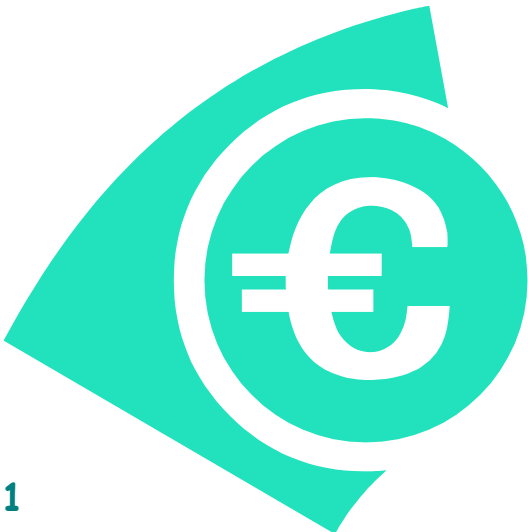
health and **nutritional value**

but also **taste**, **tradition** and **authenticity** in their food

A constantly increasing number of consumers have attached greater importance to the quality of foodstuffs

with characteristic properties in their diet rather than quantity and

they are willing to pay for this



50 GBP/ 500 ml

60 GBP/ 750 ml

They are even **alarmed** about **environmental** and **ethical issues** with emphasis on topics such as

- **biodiversity protection**
- **production method**

Thus, they demand **more and more information** regarding the food products they purchase and to **be more confident**

However, **authenticity** and **traceability** of foods **is nowadays a complex issue** more than ever due to

international trade and market globalization

long and complex food supply chains

the thriving of e-commerce

A **frame** which together with the aforementioned consumer trends **provides a general motivation for food fraud**

*__authentic__ = a food product where there is a match between the actual food product characteristics and the corresponding food product claims

**__traceability__=follow the movement of a food product and its ingredients through all steps in the supply chain, both backward and forward.

Which although **economically motivated** (EMA)

EMA: Is defined as the fraudulent, intentional substitution or addition of a substance in a product for the **purpose of increasing the apparent value of the product** or **reducing the cost of its production** for **economic gain** (DeVries, 2009)*

Exploiting the high-commercial-value of certain products and/or their producion in high tonnage around the world

*US Pharmacopeia's Food Ingredients Intentional Adulterants Advisory Panel. Presented at USP's 2009 Food Ingredients Stakeholder Forum. http://www.usp.org/pdf/ EN/stakeholderForums/foodAdditives/Aug2009/2009-08- 04FISFPresentationspdf.pdf (slides 80–91)

Causing, thus, unfair competition

that can destabilize market and disrupt the regional/ national economy

can also poses **health issues**…

due to the presence of **toxic**

or

allergenic substances

For all these reasons, **food authenticity and traceability** has been receiving increased attention from several stakeholders, including

- **government agencies**
- **policymakers**
- **control labs**
- **producers**
- **industry, and the research community**

In order **to address these complex issues** in the agri-food sector, **complementary approaches** are necessary including:

analytical testing

and wider tactics such as

Implementation of early warning systems

liability assessments,

intelligence gathering network which will comprise…

new, fast and advanced analytical methods

Health and consumer protection

The JRC provides scientific and technical support to EU policies on food, consumer products, chemicals and public health. With the aim of protecting general interests and health of European citizens, our scientists assess selected consumer products, as well as selected health determinants such as safety and quality of food or chemical substances including nanomaterials. They also develop test methods with the aim to reduce, refine or replace the use of animals for safety testing and the efficacy/potency testing of chemicals, biologicals and vaccines.

The JRC develops scientific tools that allow research to be carried out efficiently, and compiles databases of information relevant to all aspects of consumer health and protection. A lot of the JRC's research related to health and consumer protection builds on work done, methods provided ata collected by the relevant JRC-hosted European Union Reference

Related Publications

Empirical testing of the impact on consumer choice resulting from differences in the composition of seemingly identical branded products

Artificial Intelligence at the JRC: 2nd workshop on Artificial Intelligence at the JRC,Ispra 5th July 2019

Economic analyses of differences in composition of seemingly identical branded food products in the Single Market

Differences in composition of

Crosscutting activities

Food authenticity and quality

Science Areas
Health and consumer protection

Keywords
food/feed
quality

Related topics
- Alternatives to animal testing and safety assessment of chemicals
- Bioinformatics
- Consumer products
- Food and feed safety
- Food contact materials
- GMOs
- Healthcare quality
- Human exposure
- Medical applications of radionuclides and targeted alpha therapy
- Nanotechnology
- Nutrition
- Organic food and sustainable

Food authenticity and quality

The labeling of food products is essential to inform consumers what kind of products they are buying. EU harmonised rules on food labeling, presentation and advertising aim to protect consumers and facilitate trade inside and outside Europe.

Recently an initiative of the European Parliament (EP) has identified a number of foods such as: olive oil, fish, honey, dairy products and meat as being the target of fraudulent activities. This initiative calls for the development of technologies and methods to detect food fraud.

Monthly Summary of Articles on Food Fraud and Adulteration

The JRC actions in the area of food authenticity contribute to achieving these goals by applying the best available science to develop widely accepted standard methods of analysis and best practices guides, underpinned by advanced measurement science.

Wine

European Reference Centre for Control in the Wine Sector

Wine is a premier agricultural product of the EU and exported worldwide. It is crucial to keep up reputation of EU wine and to minimise malpractices, mainly sugaring and watering, that may undermine the position of EU wine on the internal and international markets.

The activities of the JRC in support of the EU wine legislation started with the establishment of the EU wine databank in 1991, followed by the

Traditionally, most analytical methods have been "**targeted**"

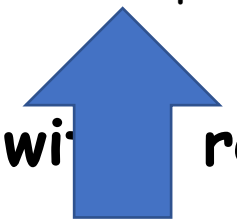
- provides a result **directly** addressing a specific authenticity issue.
- is often a chemical compound (e.g. Sudan dye in spices)
- often relies on specific legal limits

That is covering **detection/quantification** of **one or more pre-defined** analytical target(s)

chemically or biologically **characterized**

indirectly provide information about the authenticity of a product (e.g. isotope ratios)

marked wi recognized significance (primary or secondary) before data acquisition



They **are applied** when the **suspected target provides direct information** about the product

are **characterized** in general by **high selectivity** and **sensitivity**

Their **reliability** in many cases can be **supported by matrix CRMs** (important for method validation/ accreditation)

Usually require a **tedious and costly sample preparation**

Considering the numerous adulterants that can potentially be used, some of which can cause different health risks…

such an approach can be highly inefficient

a step forward is when **multiple analytes (e.g. >2)**, usually providing **indirect information** about the product, **are targeted** ("**profiling**")

In this way improved evaluation compared to a classical targeted method can be achieved

The **profile** can be used to:

estimate a value to be compared to a threshold

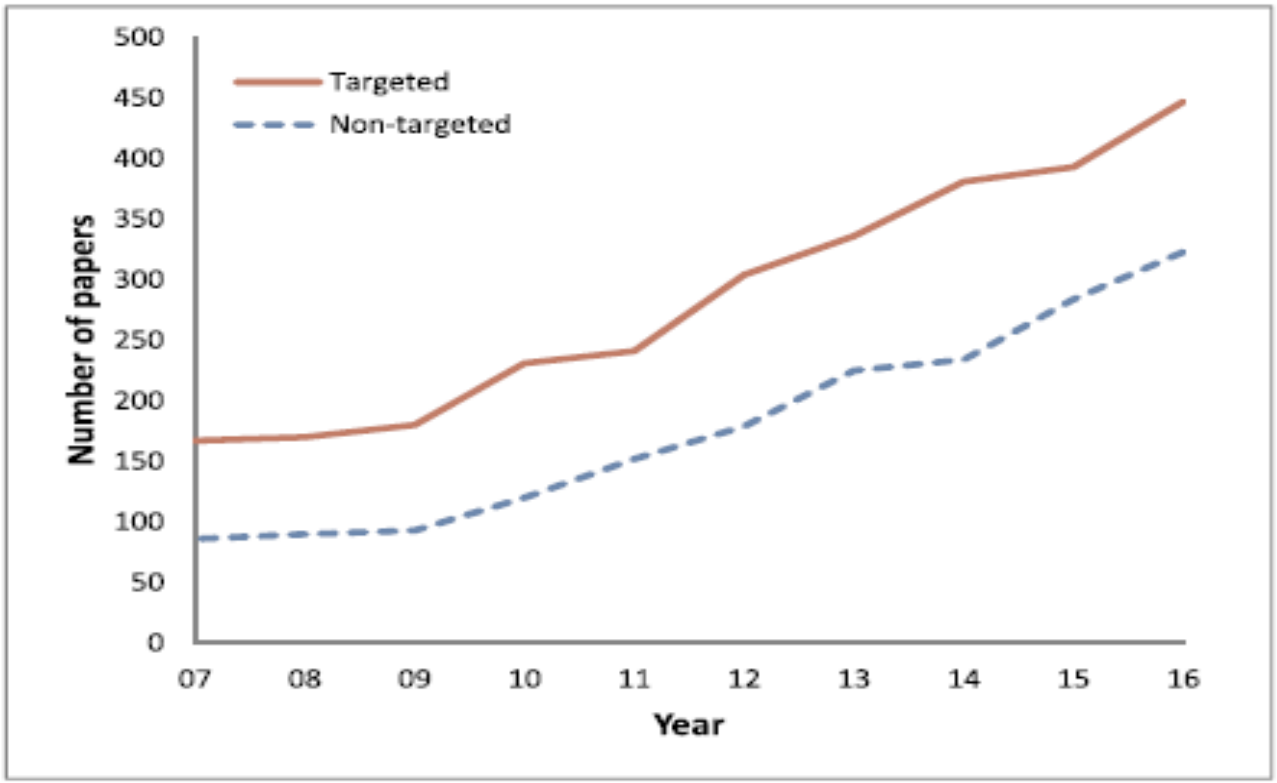
or

for comparison when a database of similar profiles from authentic foods

that cover the natural variability **is available**

Despite the fact that **targeted** approaches are the **mainstream in food analysis**,

the so-called **un(non)-targeted analyses are gaining ground**



The development in numbers of food authentication studies based on targeted versus non-targeted analytical methods from 2007 to 2016 (Ballin, & Laursen, 2019, *Trends in Food Science & Technology*, *86*, 537-543, open access).

Since the **detection of plentiful unspecified analytes** or **data points (>100)** obtained ("**fingerprint**")

usually from a **food matrix** after **no or minimal manipulation** to get as many matrix components as possible…

can make eventually the difference in some cumbersome issues

especially when **no markers** providing direct or indirect information **are clear or available** (e.g. geographic origin, production method)

The **power of fingerprinting** is

the feasibility to **detect multiple small changes** in the food product, and

to extract these changes as valuable information mathematically

A fingerprinting method is often qualitative and may depend on the **building** and **utilization** of a suitable database.

This can be an appropriate strategy

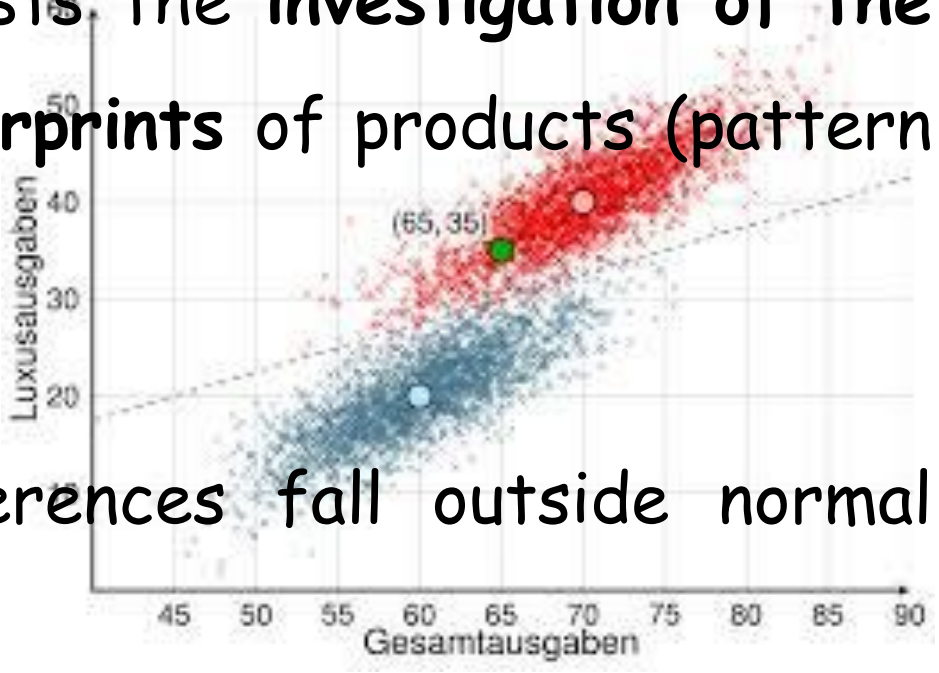
as **the high number of unspecified targets or data points**

minimize the likelihood of arbitrary similarities among different samples.

chemometric analysis assists the **investigation of the relationship between fingerprints** of products (pattern recognition)

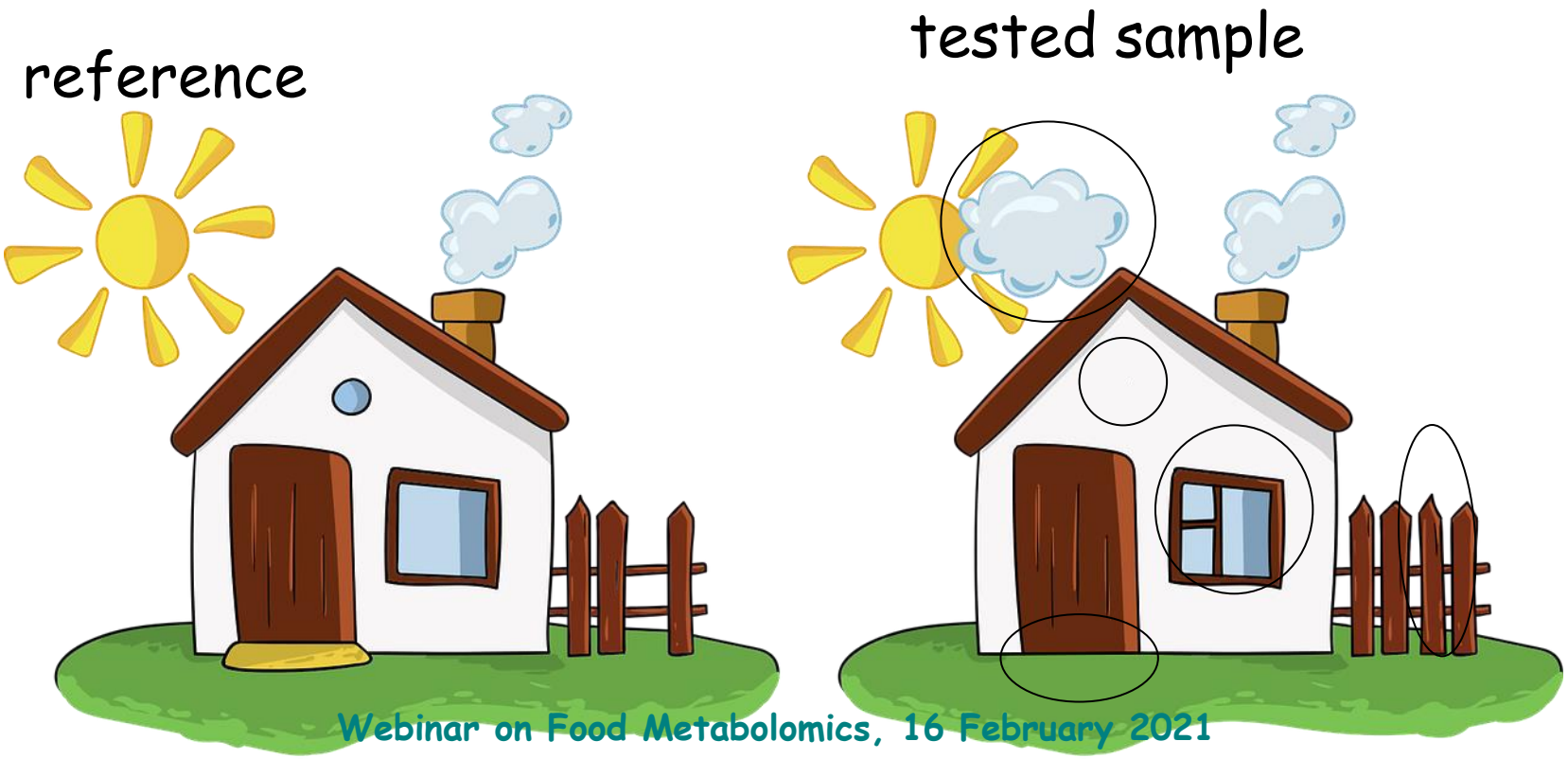
which **may often be subtle**

and reveal whether differences fall outside normal variability



When a database is unavailable (usual case),

an un-targeted analysis can be used for **sample-to-reference matching**



Fingerprinting/Profiling Techniques (non-exhaustive list)

Spectroscopic/spectrometric

Infrared (NIR/MIR), Raman, UV, Fluorescence spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy

Mass Spectrometry (IRMS, ICP-MS, TIMS, PTR-MS etc)

chromatography

Gas/ Liquid Chromatography, Capillary electrophoresis (Hyphenated to spectrometry)

other

Thermal- Differential Scanning Calorimetry (DSC)

DNA-Based - Polymerase Chain Reaction (PCR)

	platform	resolution
Low to intermediate resolution	FT-IR	500
	NMR	4000
High resolution	**TOF-MS**	**40000**
	Orbitrap-MS	**140000**
	FT-ICR	**500000**
hyphenated	LC-MS	200 x MS
	UPLC-MS	1500 x MS
	MS/MS	MS x MS
	GC	500
	GC x GC-MS	500 x 500 x MS

Examples of targeted and non-targeted analytical food authentication.

Authentication category	Authentication approach	Analyte (marker)	Authentication issue	Analytical technique
Origin	Targeted			
	- Single or dual targets	DNA (sec.)	Pine nut species	PCR-HRM
		N stable isotope ratio (sec.)	Organic production	IRMS
		Protein (sec.)	Fish species	ELISA
	- Profiling (> 2 sec. markers)	Dioxins and PCBs (sec.)	Geographic origin	GC-MS
		DNA (SNPs and SSRs) (sec.)	Wine cultivars	PCR
		Rare elements (sec.)	Game meat	ICP-MS
	Non-targeted (fingerprinting)	Peptides	Fish species	MS/MS
		Various	Milk, fish, and coffee species	MS
		DNA	Fish species	ddRAD
Substitution	Targeted			
	- Single or dual targets	4-hydroxyproline (sec.)	Collagen in meat	HPAEC-PAD
		16-O-methylcafestol (sec.)	Coffee species	NMR
		DNA (sec.)	Meat species	PCR
	- Profiling (> 2 sec. markers)	DNA (sec.)	Plant species in honey	NGS
		Triglycerols (sec.)	Coffee species	GC-FID
		Fatty acids (sec.)	Apricot in almond powder	GC-FID
	Non-targeted (fingerprinting)	Various	Olive oil, adulterated	ATR-FTIR
		Various	Plant species (oregano)	FT-IR
		Various	Apple juice categories	TSFS
Extension	Targeted			
	- Single or dual targets	Melamine (prim.) and cyanuric acid (sec.)	Melamine in milk powder	TOF-MS
		Sudan I-IV (prim.)	Color in palm oil and spices	LC-MS/MS
		Vanillin (prim.) and ethyl vanillin (prim.)	Flavoring in cocoa	HPLC-UV
	- Profiling (> 2 sec. markers)	Sugars (sec.)	Syrup in honey	UHPLC/Q-TOF-MS
		Sugars, phenols, and organic acids (sec.)	Pomegranate juice, adulterated	HPLC-RI, PDA
		Sugar (sec.)	Pear juice, adulterated	CGC-FID, HPAED-PAD
	Non-targeted (fingerprinting)	Various	Sudan color in spices	NMR
		Various	Water in juice	NIR
		Various	Water in honey	^{1}H NMR (dilution effect)

Abbreviations ATR: attenuated total reflectance; CGC: capillary gas chromatography; ddRAD: double digest restriction enzyme associated DNA; ELISA: enzyme linked immunosorbent assay; FID: flame ionization detector; FT(–)IR: fourier transform infrared; GC: gas chromatography; HPAEC: high performance anion exchange chromatography; HPAE: high performance anion exchange; HPLC: high performance liquid chromatography; HRM: high resolution melting; ICP: inductively coupled plasma; IRMS: isotopic ratio mass spectrometry; LC: liquid chromatography; MS: mass spectrometry; NGS: next generation sequencing; NIR: near infrared; NMR: nuclear magnetic resonance; PAD: pulsed amperometric detection; PCR: polymerase chain reaction; PDA: photo diode array; prim.: primary; Q: quadrupole; sec.: secondary; RI: refractive index; TSFS: total synchronous fluorescence spectroscopy; TOF: time of flight; UHPLC: ultra-high performance liquid chromatography; UV: ultra violet. (Ballin, & Laursen, 2019)

Despite the fact that there are **even commercialized un-targeted tools for food authenticity**.

Still work is required for **validation** and **harmonization** as there is a lack of relevant guidelines

It is **difficult to have authentic samples** with full traceability for building models or databases

Chemometric software is expensive for non-academics and a "black box" for many potential users

As it is recognized that **targeted and un-targeted methods can be complementary**

providing an umbrella of protection for the consumer and the honest producer considering that:

The first provides the **identity/confirmation** making the **evidence appropriate for the court**

The second provides **a screening capability guarantying that very little avoids detection**

It is imperative to address such challenges

it should be pointed out that AOAC within the frame of **Food Authenticity & Fraud Program** (https://www.aoac.org/scientific-solutions/food-authenticity-fraud/) has set working groups for **both targeted** and **un-targeted methods aiming to achieve this**

making efforts to define **standard method performance requirements** (SMPRs) for un-targeted ones

SMPR	Targeted	Un-targeted
Applicability	Identity and measurement of specific analyte	Assess if something is "different"
Definitions	Defines targeted analyte Includes reference standards	Define "authentic" List some adulterations
Method Performance requirements	Analytical range Accuracy Repeatability Reproducibility	1) Can determine if foods has unknown adulterants 2) Performance on food with unknown adulterant (s)
System suitability/Quality Control	CRM in each batch	Adulterated samples in batch; but unknown?
Reference Materials	CRM/SRM	????
Validation Guidance	Established	Newer
Maximum Time to Results	Variable	variable

For the un-targeted **testing approach** SMPRs include two stages

Single Laboratory validation performed on common adulterants in specific commodities (recipes will be included in SMPR)

Multi- Laboratory validation performed on blind samples created by third party

Submission should include

Description of authentic sample collection and verification

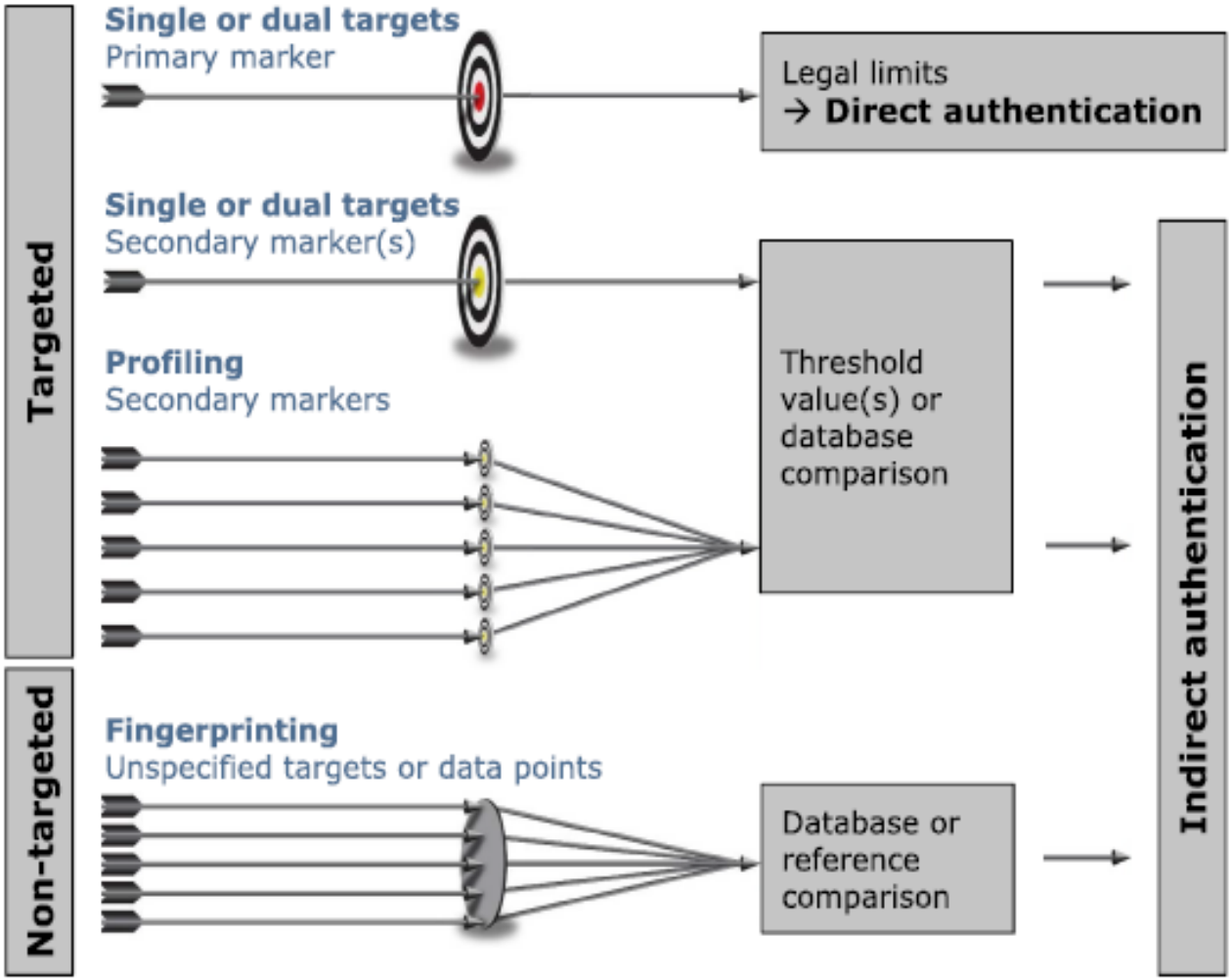
Protocol on authentic fingerprint generation

Recognizing the importance of the un-targeted methods and their possible complementarity

in the strategic plan and goals of the aforementioned program

the development of a decision tree to combine untargeted (screening) with targeted (confirmatory) for specific adulterants is aimed

COMPLEMENTARITY



The **indirect authentication** procedure often includes the use of a **multivariate statistical analysis**.

The principles of targeted versus non-targeted analytical food authentication (Ballin, & Laursen, 2019, *Trends in Food Science & Technology*, *86*, 537-543, open access)..

Thank you for your attention