Ministry of Environment and Food of Denmark
Danish Veterinary and Food Administration

To target or not to target?
Definitions and nomenclature for targeted versus non-targeted analytical food authentication

Nicolai Zederkopff Ballin
Danish Veterinary and Food Administration

65th International Congress of Meat Science and Technology (ICoMST), 5 August 2019

Curriculum Vitae

Employment

- Danish Veterinary and Food Administration (2002-)
- European Commission, JRC (2016-2018)

Education

- MSc in Biochemistry (2002)
- PhD in meat and dairy authentication (2009)

Analytical expertise

- Chromatography
- Spectroscopy
- Enzymatic and immuno assays
- DNA based techniques

Analytical toolbox for food authentication

Microscopy

Polymerase chain reaction

Sensory

Spectrometry

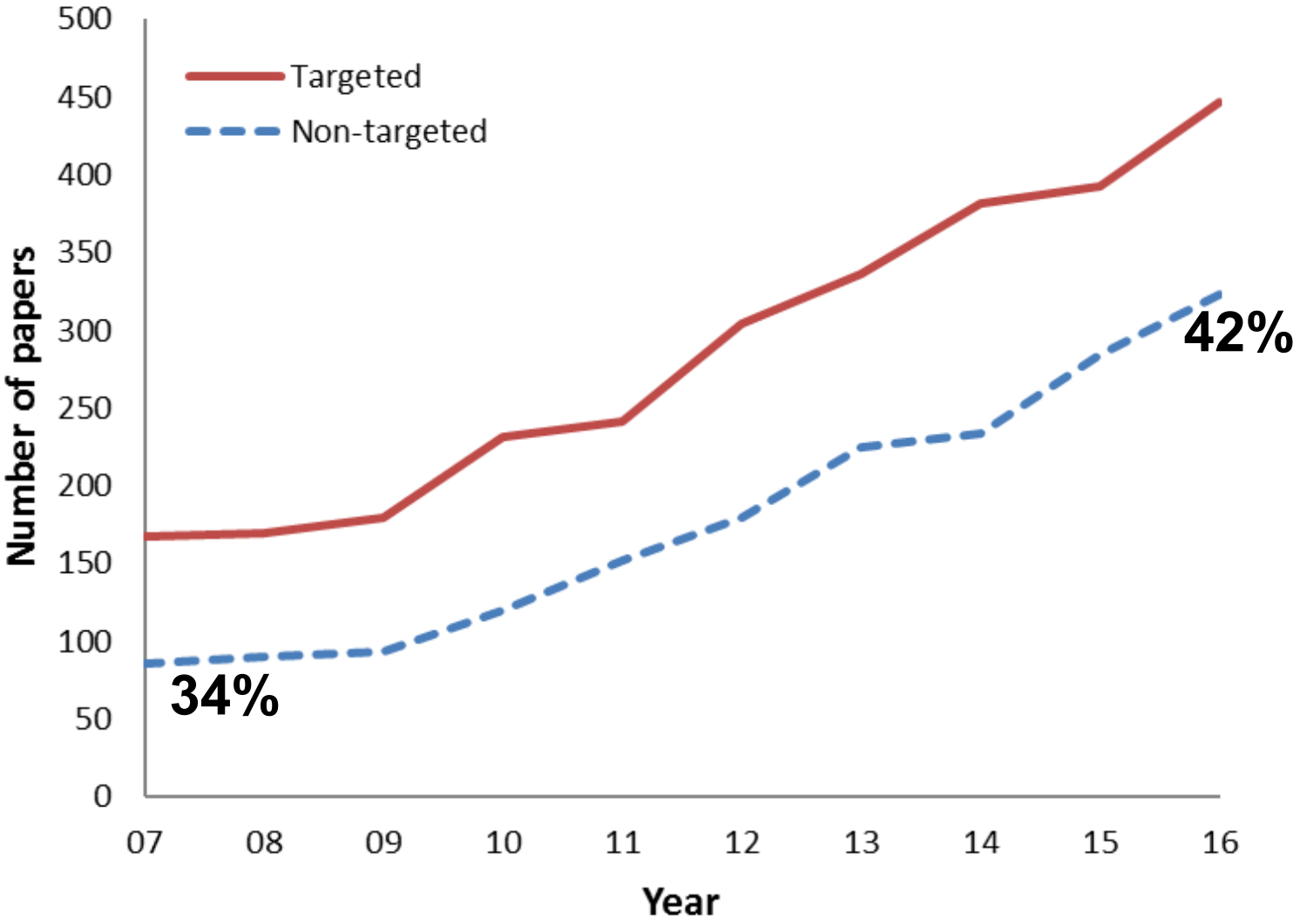
DNA sequencing

Chromatography

Enzymatic assays

Spectroscopy

Analytical food authentication studies



Ballin & Laursen. *Trends in Food Science and Technology*. 2019; *86,* 537 – 543.

Different terminologies

ELSEVIER

Food Chemistry

Volume 270, 1 January 2019, Pages 403-414

Combined targeted and untargeted profiling of volatile aroma compounds with comprehensive two-dimensional gas chromatography for differentiation of virgin olive oils according to variety and geographical origin

Igor Lukić [a, b], Silvia Carlin [c, d], Ivana Horvat [a], Urska Vrhovsek [c]

ELSEVIER

Analytica Chimica Acta

Volume 885, 23 July 2015, Pages 17-32

Review

Review of validation and reporting of non-targeted fingerprinting approaches for food authentication

Janet Riedl, Susanne Esslinger, Carsten Fauhl-Hassek

ELSEVIER

Food Chemistry

Volume 271, 15 January 2019, Pages 410-418

Untargeted DNA-based methods for the authentication of wheat species and related cereals in food products

Silvia Silletti [1], Laura Morello [1], Floriana Gavazzi, Silvia Gianì, Luca Braglia, Diego Breviario

Show more

https://doi.org/10.1016/j.foodchem.2018.07.178

Highlights

- A DNA profiling method, TBP, is applied to cereal-based food authenticity testing.

ELSEVIER

Science of The Total Environment

Volume 537, 15 December 2015, Pages 447-452

Short Communication

Geogenic lead isotope signatures from meat products in Great Britain: Potential for use in food authentication and supply chain traceability

Jane A. Evans [a], Vanessa Pashley [a], Gemma J. Richards [b], Nicola Brereton [c], Toby G. Knowles [b]

Lack of definitions and nomenclature

Single target

Signature

Primary marker

Dual targets

Profiling

Secondary marker

Harmonization is needed

Analytical marker

Authentication marker

Fingerprinting

Semi-targeted

Indirect authentication

Non-targeted

Un-targeted

Le Penseur

Auguste Rodin (1840-1917)

Direct authentication

Targeted

Recent publication

Trends in Food Science & Technology 86 (2019) 537–543

Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: www.elsevier.com/locate/tifs

Commentary

To target or not to target? Definitions and nomenclature for targeted versus non-targeted analytical food authentication

Nicolai Zederkopff Ballin[a,*], Kristian Holst Laursen[b]

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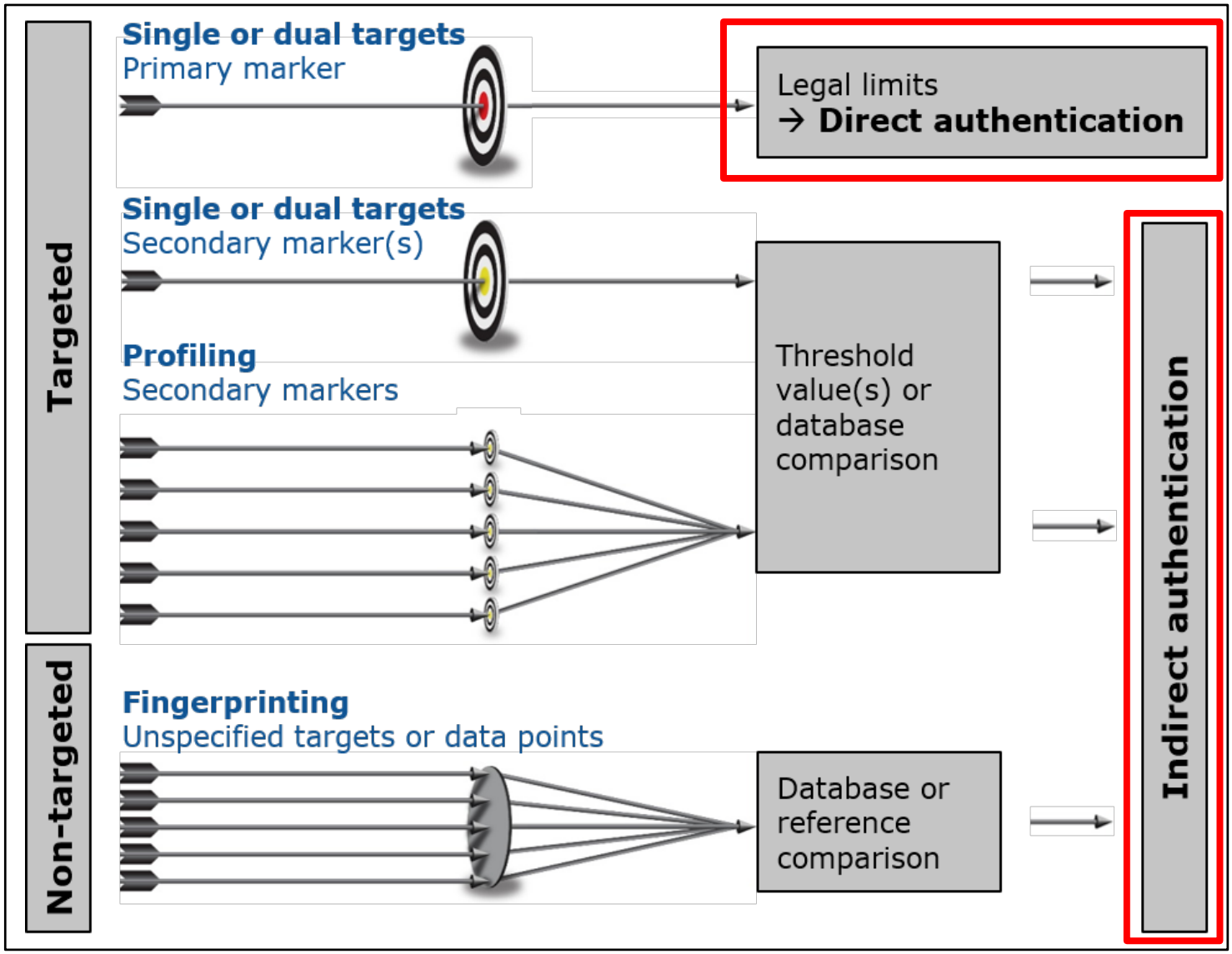
ARTICLE INFO

Keywords:
Analytical chemistry
DNA
Food authentication
Food fraud
Non-targeted
Targeted

ABSTRACT

The use of non-targeted analytical methods in food authentication has rapidly increased during the past decade. Non-targeted analyses are now used for a plethora of different food commodities but also across several scientific disciplines. This has brought together a mixture of analytical traditions and terminologies. Consequently, the scientific literature on food authentication often includes different approaches and inconsistently used definitions and nomenclature for both targeted and non-targeted analysis. This commentary paper aims to propose definitions and nomenclature for targeted and non-targeted analytical approaches as a first step towards harmonization.

Terminology



Sudan dye scandal 2005 – direct authentication

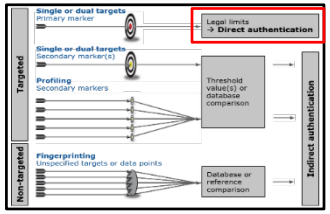


Image: Divyakant Solanki / EPA

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More food contaminated with dye

More food items have been identified as being contaminated with Sudan I dye - bringing the total to 474.

The Food Standards Agency says the vast majority of contaminated products have now been removed from shops.

Some brands of pizza are affected

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UUTISET > NEWS

News 27.4.2017 16:02 | updated 27.4.2017 16:43

Food fraud: Finnish Customs seize 950 kg of tainted, carcinogenic spices

Primary marker, *Sudan dyes in spices*

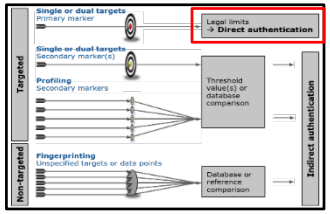
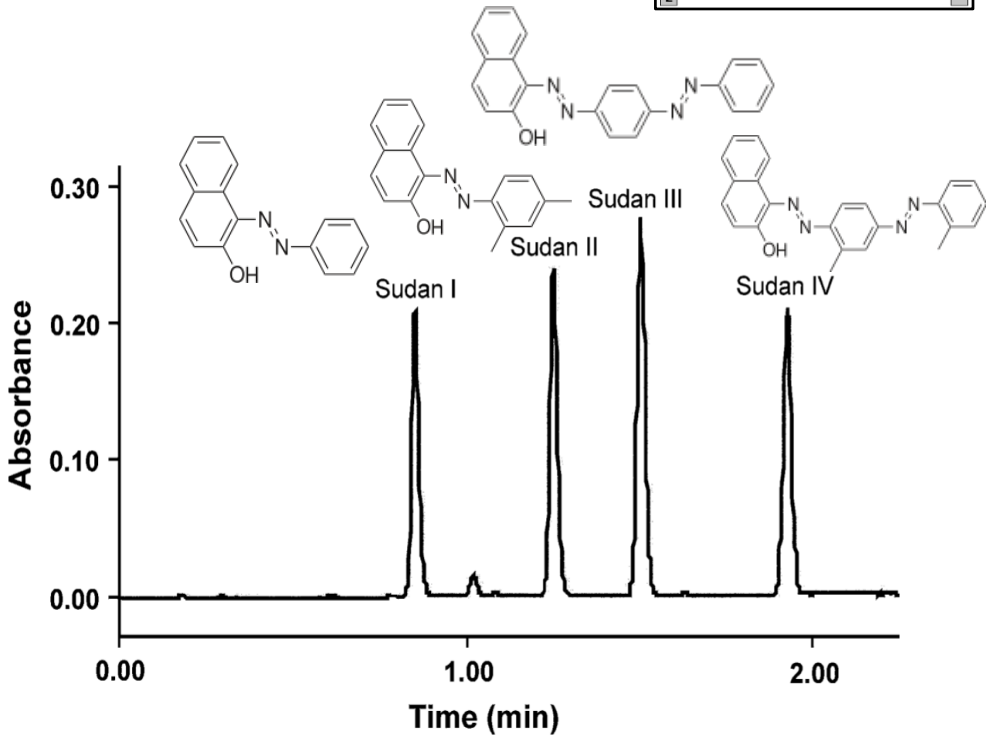


Image: Divyakant Solanki / EPA

UPLC

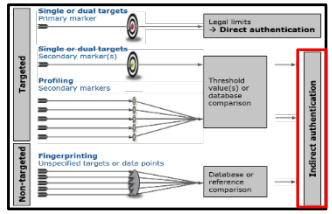


Kesiūnaitė et al. Chromatographia, 2009; *70,* 1691-1695.

Analytical result = Reported result

Direct authentication

Horsemeat scandal 2013



On 10 April 2013, it was reported that two Dutch trading companies… may have supplied 50 000 metric tonnes of adulterated beef containing horse meat since January 2011.

BBC News. 10 April 2013.

"Tesco's market value dropped by 360 million EUR".

The Irish Independent. 22 January 2013.

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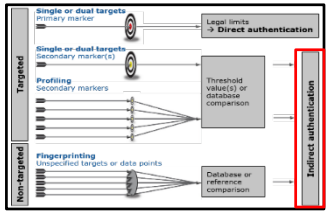
Horsemeat found in beefburgers on sale in UK and Ireland

15 January 2013 Sha

Horse DNA has been found in some beefburgers being sold in UK and Irish supermarkets, the Republic of Ireland's food safety authority (FSAI) has said.

produced the 29% horsemeat burger

Secondary marker in species determination, *DNA*



DNA extractability

Sample matrix

Processing

Tissue

Sample mixture → PCR → DNA

Ideal situation

Conversion

Analytical result

≠

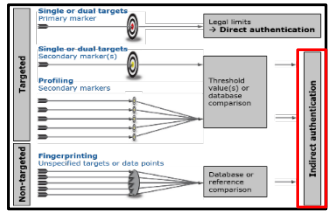
Reported result

Meat

Indirect authentication

Ballin et al. *Meat Science*. 2009; *83*, 165-174.

Secondary marker in fresh vs. thawed determination, *enzyme*



Mitochondrial rupture

Processing

Dilution

Sample → Spectroscopy → HADH activity

Ideal situation

Conversion

Analytical result

≠

Reported result

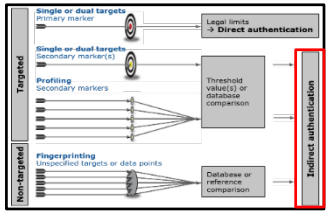
Fresh or thawed

Indirect authentication

Gottesman & Hamm. *Fleischwirtschaft*. 1983; *63*, 219-221.

Ballin & Lametsch. *Meat Science*. 2008; *80*, 151-158.

Secondary marker in protein determination, *nitrogen*



Nitrogen containing compounds

Melamine

Urea

Sample

Kjeldahl

Nitrogen

Ideal situation

Conversion

Analytical result

≠

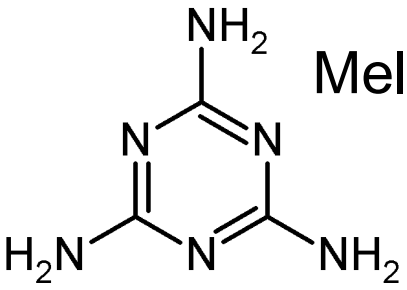
Reported result

Protein

Indirect authentication

Melamine scandal 2008

300,000 victims
50,000 hospitalized
6 deaths



Melamine

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China tainted milk scandal widens

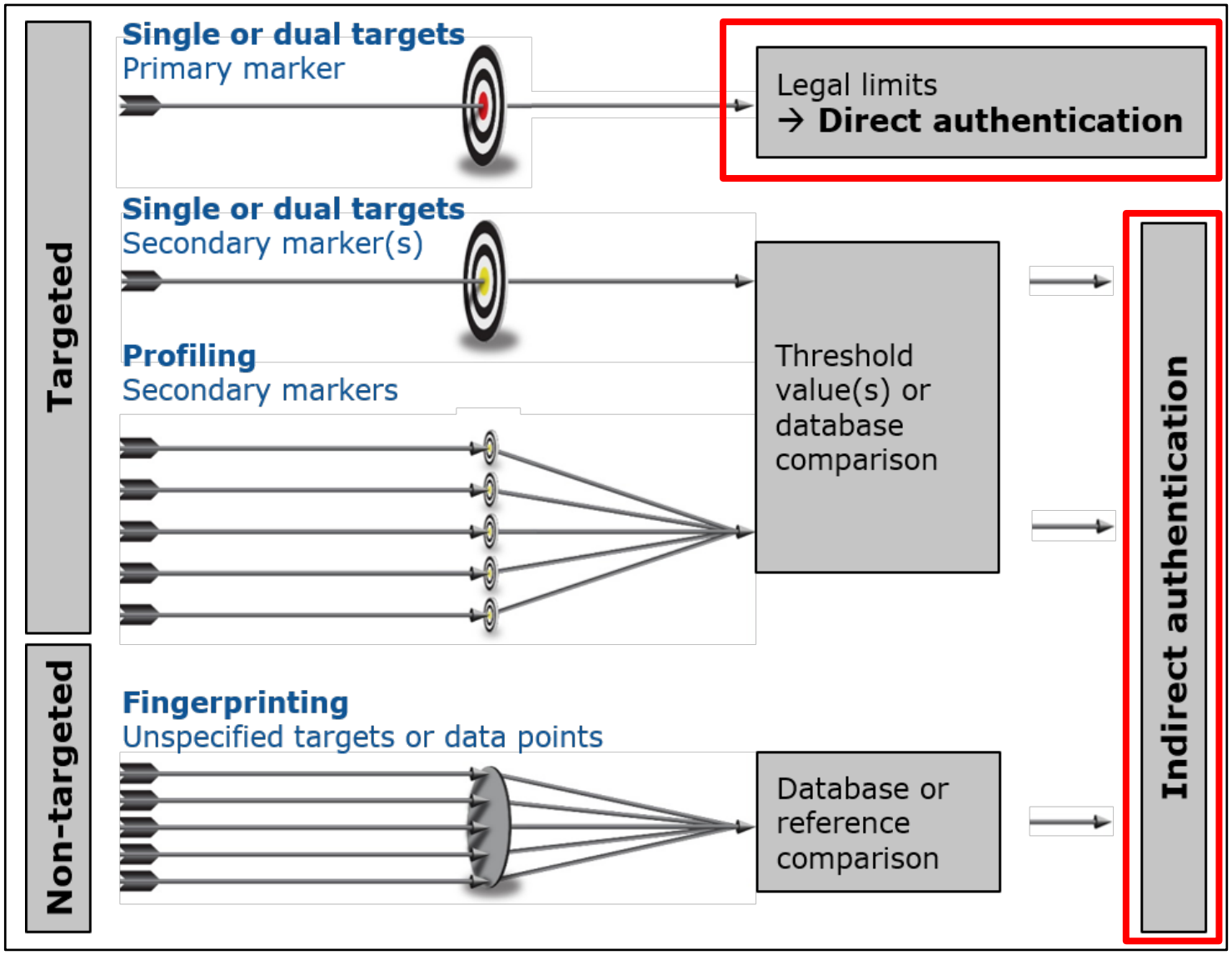
The scandal of tainted dairy products in China has widened, with liquid milk now found to be contaminated.

Inspectors found that 10% of liquid milk taken from three dairies was tainted with melamine.

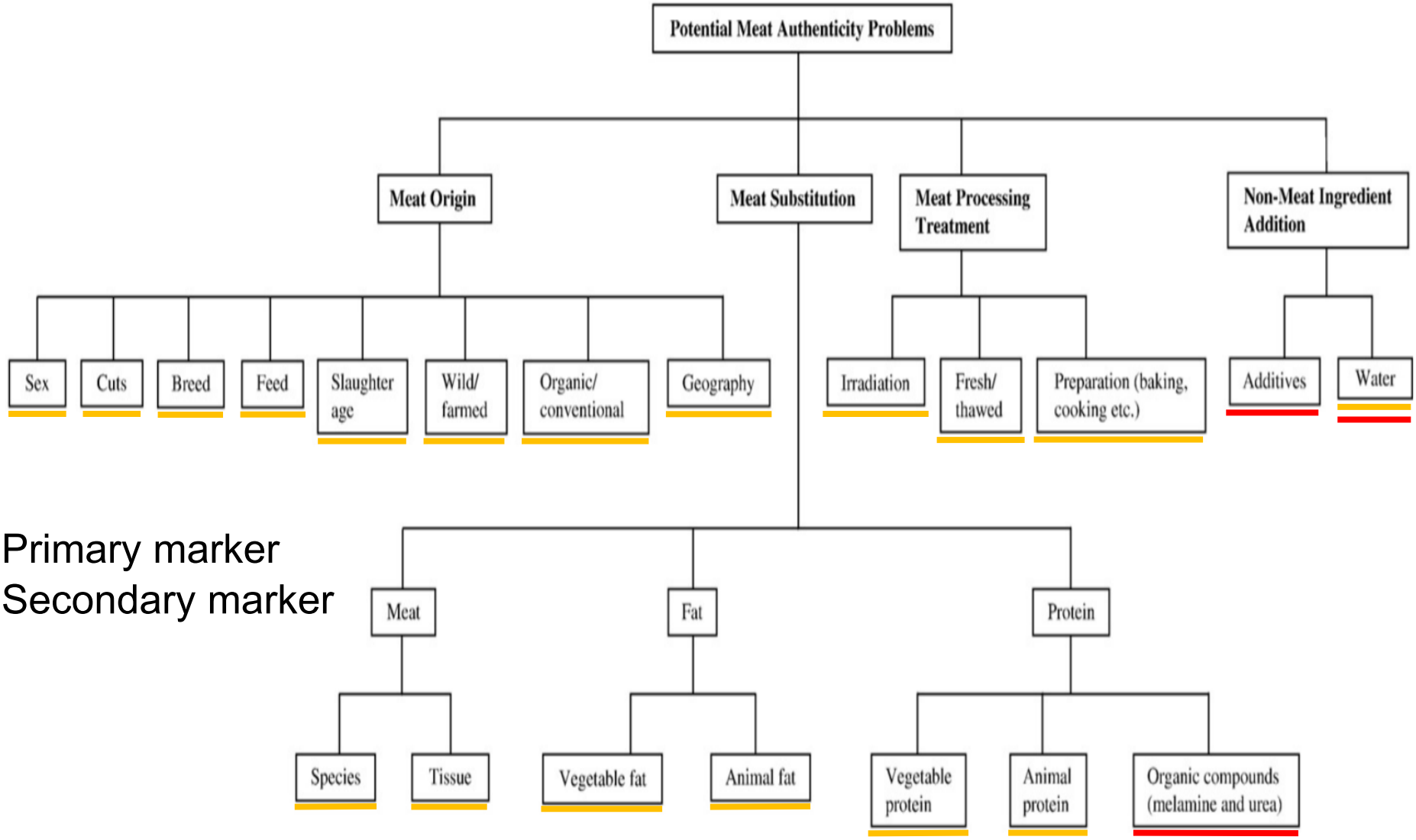
The scandal first came to light in milk powder that killed four infants and sickened more than 6,000 others.

Four infants have died and more than 6,000 are sick

Terminology



Primary vs. secondary markers



Primary marker

Secondary marker

Ballin. *Meat Science*. 2010; *86*, 577-587.

Lessons learned – primary and secondary markers

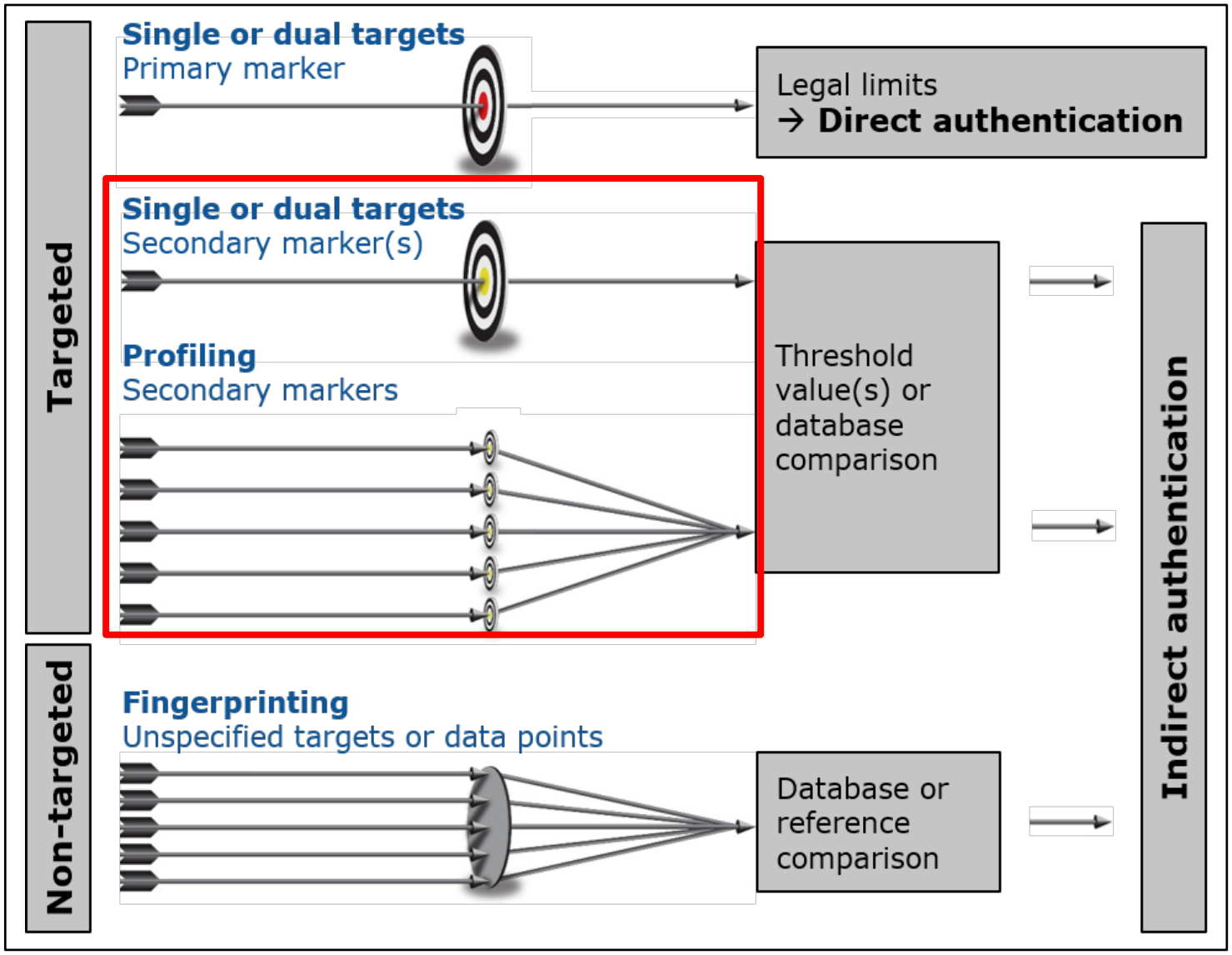
Primary marker

- Straight forward authentication

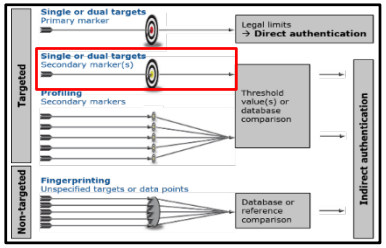
Secondary markers

- Be cautious! Results are converted
- Conversion might be wrong or biased
- Report the analytical result and state your conversion and assumptions as comments

Terminology



Pine species - pine nut syndrome



A: *Pinus armandii*
K: *Pinus koraiensis*
S: *Pinus sibirica*
P: *Pinus pinea*
G: *Pinus gerardiana*
M: *Pinus massoniana*
u: unknown

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Polymerase chain reaction and chemometrics detected several *Pinus* species including *Pinus armandii* involved in pine nut syndrome

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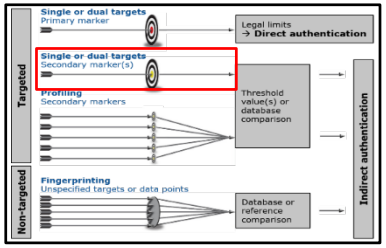
PCR
Pine nut syndrome
Pinus armandii
Species determination

ABSTRACT

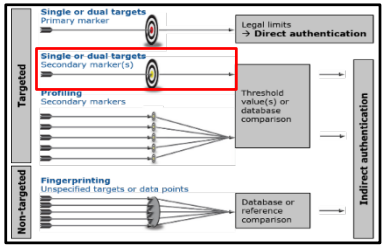
A dramatic peak in reported cases of pine mouth or pine nut syndrome (PNS) was observed in Europe and in the United States of America in 2008–2012. The PNS symptoms involve a constant bitter and/or metallic taste that appear 1–2 days after ingestion and disappear within 5–14 days. The chemical compound responsible for the symptoms is unknown, but symptoms are related to ingestion of pine nuts from the species *Pinus armandii*. *P. armandii* used industrially for non-food purposes has entered the food chain through mislabeling. Consequently, species determination of pine nuts has gained focus in governmental control of food authenticity. In this study, a PCR primer design targeted conserved DNA sequences that span an area of variation between *P. armandii* and other relevant species. Principal component analysis (PCA) of high-resolution melting curves from PCR amplicons was used to cluster pine species from reference material, and to determine the species of unknown samples. The PCA successfully clustered 2 subspecies/varieties of *P. armandii*, *Pinus bungeana*, *Pinus massoniana*, *Pinus pinea*, and *Pinus wallichiana*. *Pinus koraiensis*/*Pinus pumila* and *Pinus sibirica*/*Pinus cembra* had identical PCR amplicons, respectively, and formed 2 distinct clusters. 12 pine nuts from 4 unknown samples were analyzed. 10 pine nuts clustered together with *P. armandii* and *P. koraiensis*/*P. pumila*. 2 pine nuts were not part of clusters, but probabilities suggested *P. armandii*, and *P. sibirica*/*P. cembra*. These determined species were comparable to external results obtained elsewhere.

Single targeted, *Pine species identification*



Accession number and species	DNA sequence from an intron in the NADH dehydrogenase subunit 5 spanning the area between primers Pin4 and Pin5	Amplicon size
DQ983609.1 *P. armandii*	ACCCTTCTCACTCTTTGAGGG–AAGAAATTCTAGT–AAAACCCTATAGAGGGGGAAGGGGGGGGGGGGATCCTGTTCGGACATACGGT	86
AB455857.1 *P. armandii*	ACCCTTCTCACTCTTTGAGGGGAAGAAATTCTAGTAAAAACCCTATAGAGGGGGAAGGGGGGGGGGG–ATCCTGTTCGGACATACGGT	87
EU369320.1 *P. bungeana*	ACCCTTCTCACTCTTTGAGGGGAAGAAATTTTAGTCAAAACCCTATAGAGGGGGAAGGGGGGGGGG––ATCCTGTTCGGACATACGGT	86
AB455866.1 *P. cembra*	ACCCTTCTCACTCTTTGAGGGGAAGAAATTCTAGTAAAAACCCTATAGAGGGGGAAGGGGGGGGG–––ATCCTGTTCGGACATACGGT	85
AB675846.1 *P. koraiensis*	ACCCTTCTCACTCTTTGAGGGGAAGAAATTATAGTAAAAACCCTATAGAGGGGGAAGGGGGGGGGG––ATCCTGTTCGGACATACGGT	86
AB455868.1 *P. pumila*	ACCCTTCTCACTCTTTGAGGGGAAGAAATTATAGTAAAAACCCTATAGAGGGGGAAGGGGGGGGGG––ATCCTGTTCGGACATACGGT	86
AB455869.1 *P. sibirica*	ACCCTTCTCACTCTTTGAGGGGAAGAAATTCTAGTAAAAACCCTATAGAGGGGGAAGGGGGGGGG–––ATCCTGTTCGGACATACGGT	85
AB455864.1 *P. wallichiana*	ACCCTTCTCACTCTTTGAGGGGAATAAATTATAGTAAAAACCCTATAGAGGGGGAAGGGGGGGGG–––ATCCTGTTCGGACATACGGT	85

Single targeted, *Pine species identification*



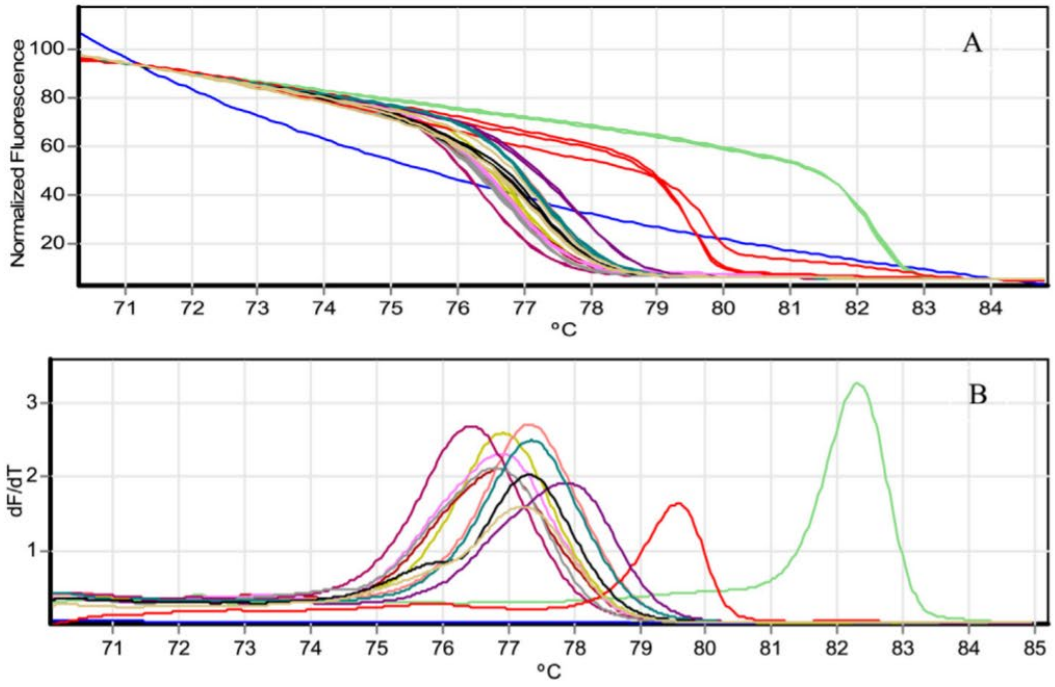
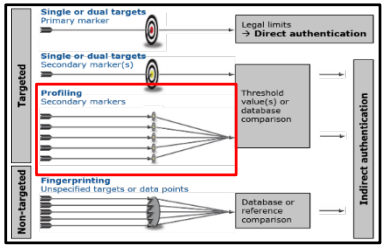


Fig. 1. Panel A shows normalized high-resolution melting curves from PCR amplicons. Panel B shows normal melting curves; only one replicate is shown for clarity. Color codes: NTC, *P. armandii* (RM1), *P. armandii* (RM2), *P. armandii* (RM3), *P. bungeana* (RM4), *P. cembra* (RM5), *P. koraiensis* (RM6), *P. koraiensis* (RM7), *P. pumila* (RM8), *P. sibirica* (RM9), *P. wallichiana* (RM10), *P. massoniana* (RM11), *P. pinea* (RM12). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Substitution of plant material



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Dried oregano in 'latest food fraud' says Which?

23 July 2015 Share

Dried oregano is the focus of "the latest in a long line of food frauds", with a quarter of samples containing other ingredients, suggests a study.

Of 78 samples of the herb, 19 contained ingredients like olive or myrtle leaves, researchers found.

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Coffee drinkers are being conned by suppliers fraudulently using inferior beans in 'Arabica' products

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Desmantelan un laboratorio clandestino en Novelda que adulteraba azafrán

- La Guardia Civil ha intervenido 87 kilos de producto, cuyo valor en el mercado oscila entre los 696.000 y los 870.000 euros

Exclusive: Peanut and almond are 'being used' as substitute for cumin seeds following terrible harvest in India

Tom Bawden | @BawdenTom | Friday 13 February 2015 20:09

Profiling, *Plant species identification*

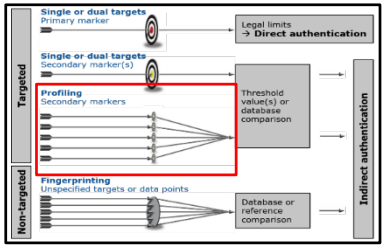
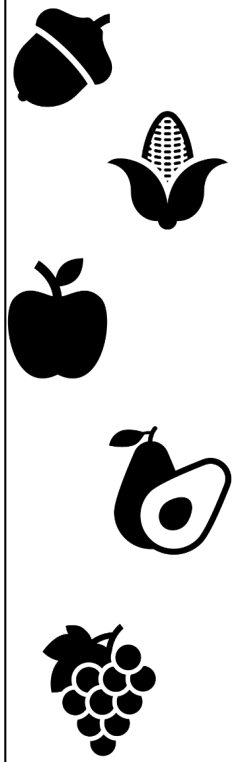


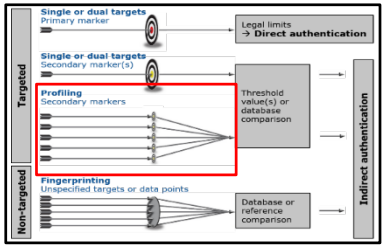
Table 1. Details on the plant barcode regions and PCR primers

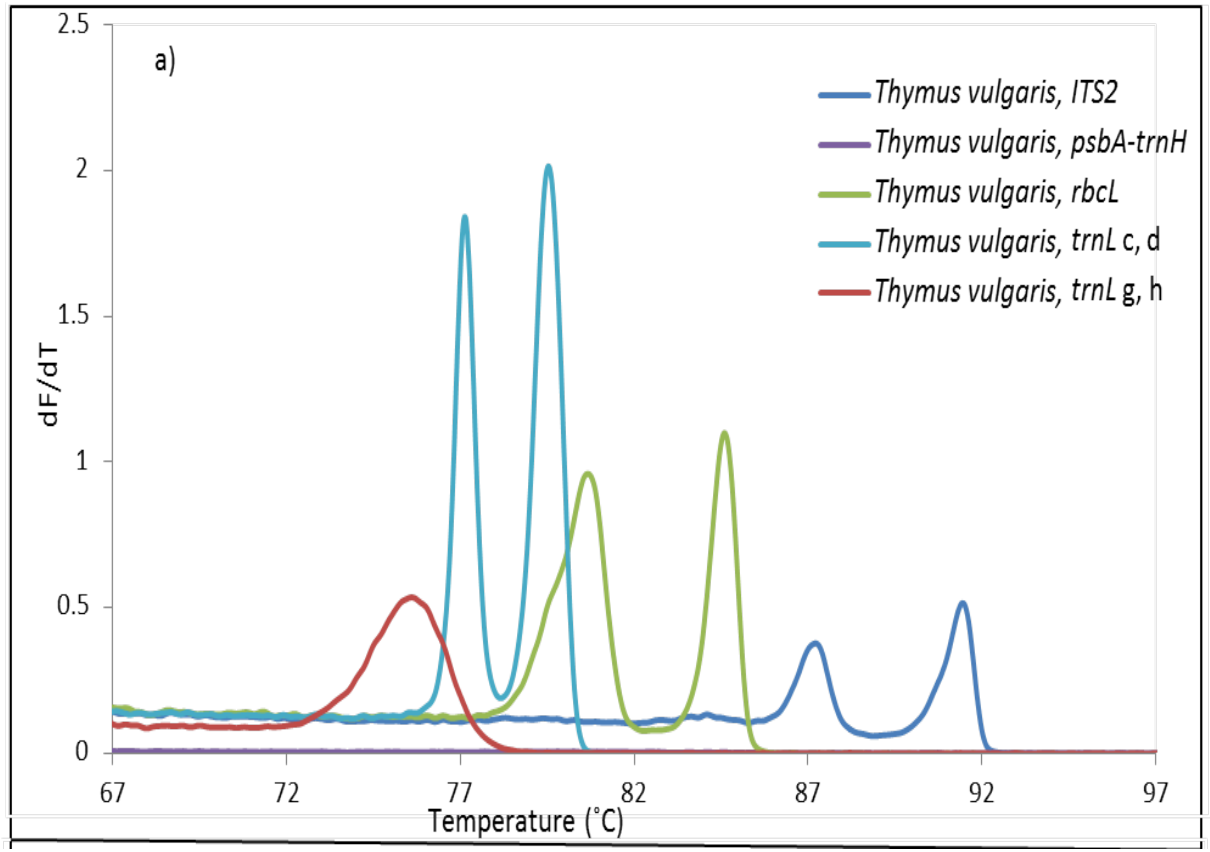
DNA region	Organelle	Primer ID	Primer sequence	Reference
ITS2	Nuclear ribosomal	ITS-u3	CAWCGATGAAGAACGYAGC	(Cheng, Xu, Lei, Li, Zhang, & Zhou, 2016)
		ITS-u4	RGTTTCTTTTCCTCCGCTTA	
psbA-trnH	Chloroplast	psbA3'f	GTTATGCATGAACGTAATGCTC	(Sang, Crawford, & Stuessy, 1997)
		trnHf	CGCGCATGGTGGATTCACAATCC	(Tate & Simpson, 2003)
rbcL	Chloroplast	rbcL a_f	ATGTCACCACAAACAGAGACTAAAGC	(Kress & Erickson, 2007)
		rbcL a_rev	GTAAAATCAAGTCCACCRCG	(Ferri, Corradini, Ferrari, Santunione, Palazzoli, & Alu', 2015)
trnL c, d	Chloroplast	trnL c	CGAAATCGGTAGACGCTACG	(Taberlet, Gielly, Pautou, & Bouvet, 1991)
		trnL d	GGGGATAGAGGGACTTGAAC	
trnL g, h (loop)	Chloroplast	trnL g	GGGCAATCCTGAGCCAA	(Taberlet, Coissac, Pompanon, Gielly, Miquel, Valentini, et al., 2007)
		trnL h	CCATTGAGTCTCTGCACCTATC	

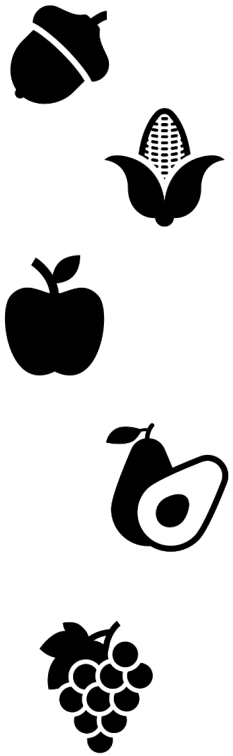


Ballin et al. *Food Control.* 2019; *105*, 141-150.

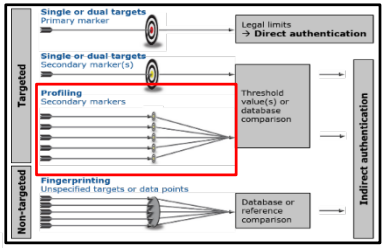
Profiling, *Plant species identification*

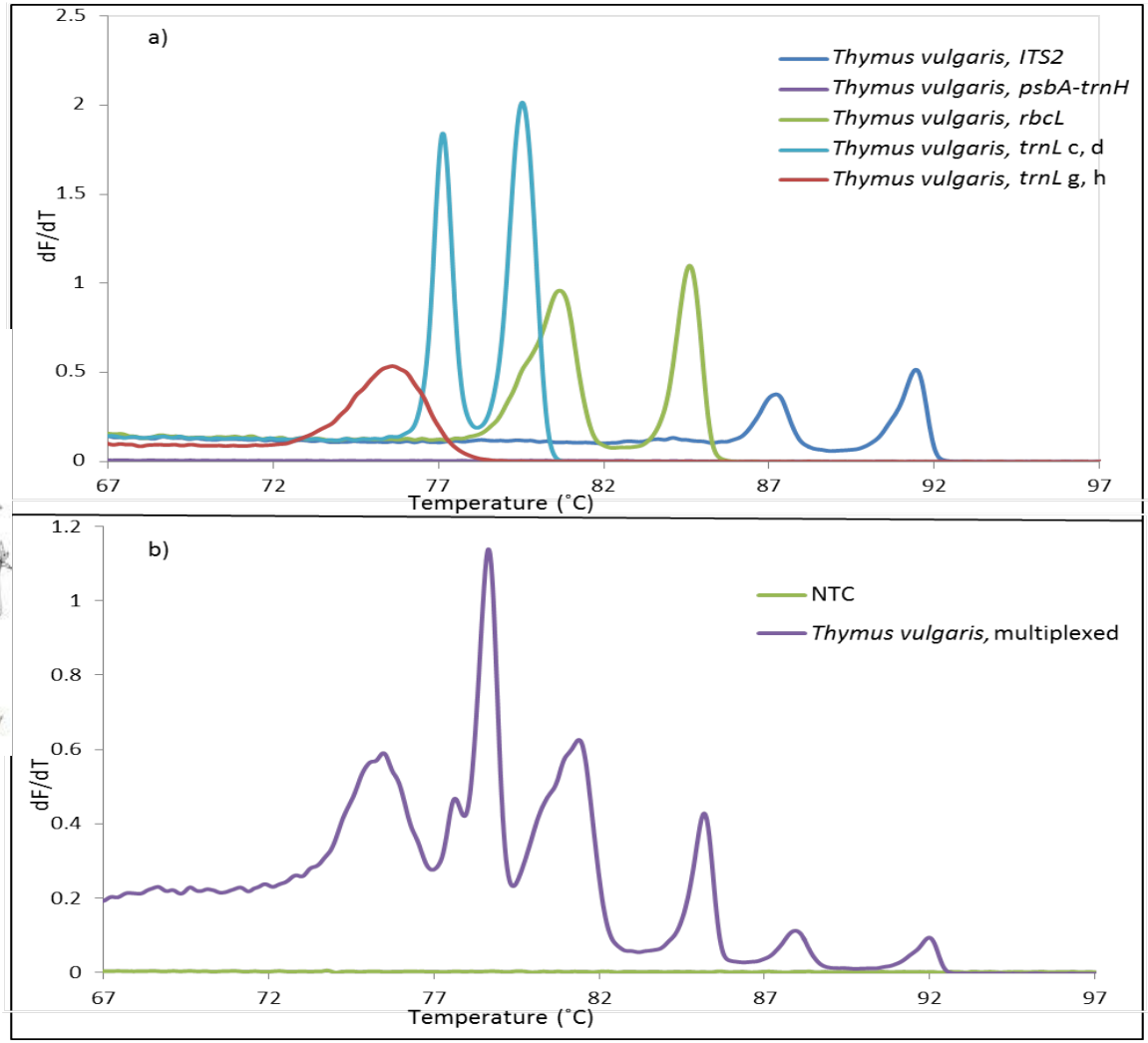


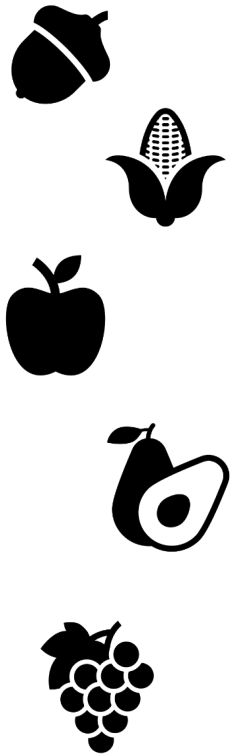




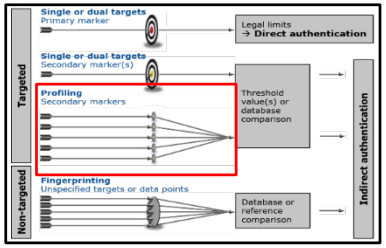
Profiling, *Plant species identification*







Profiling, *Plant species identification*



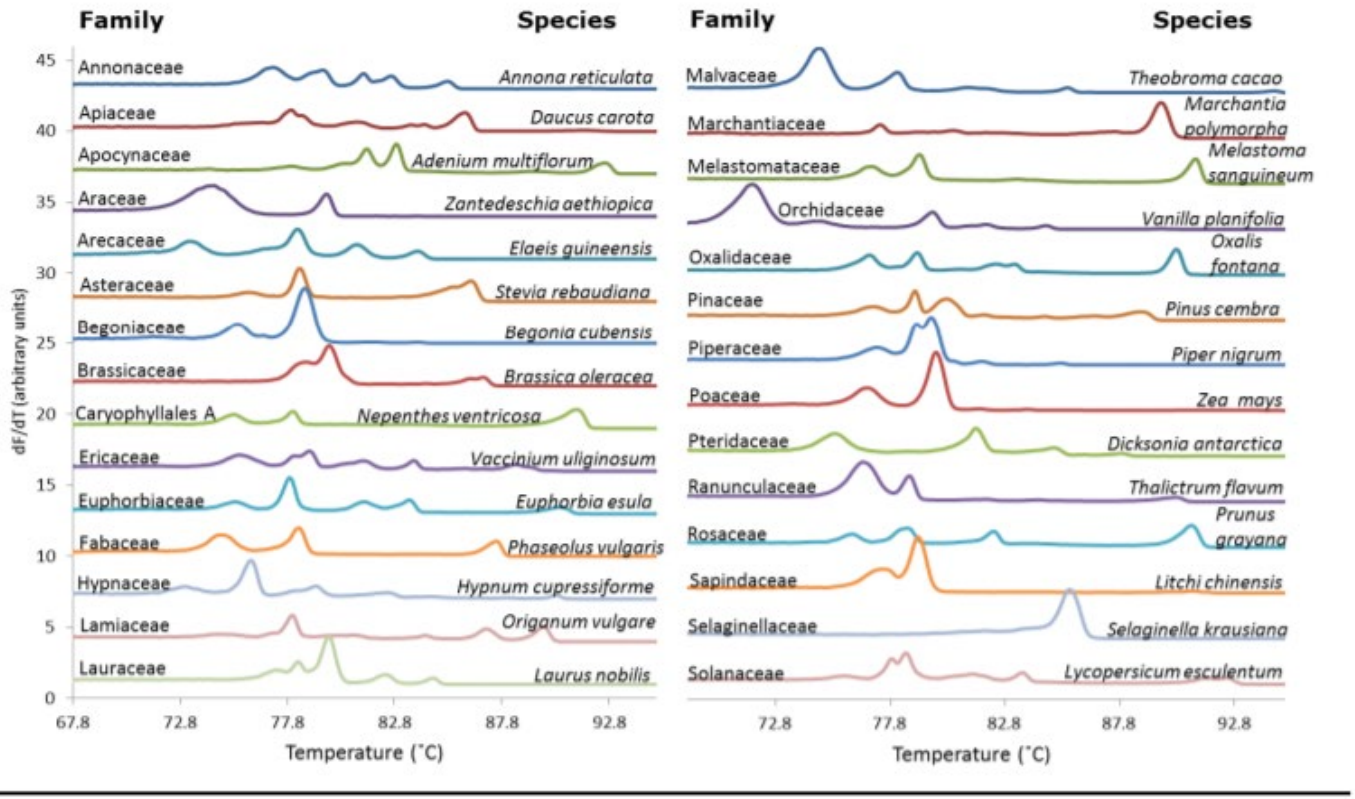
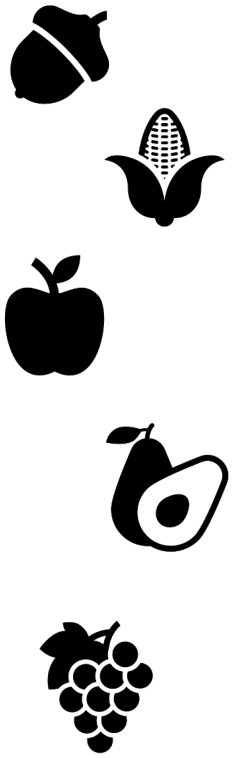
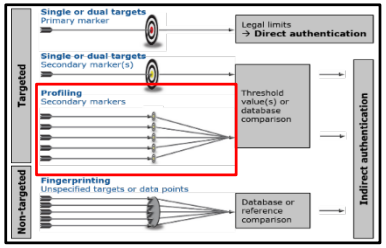
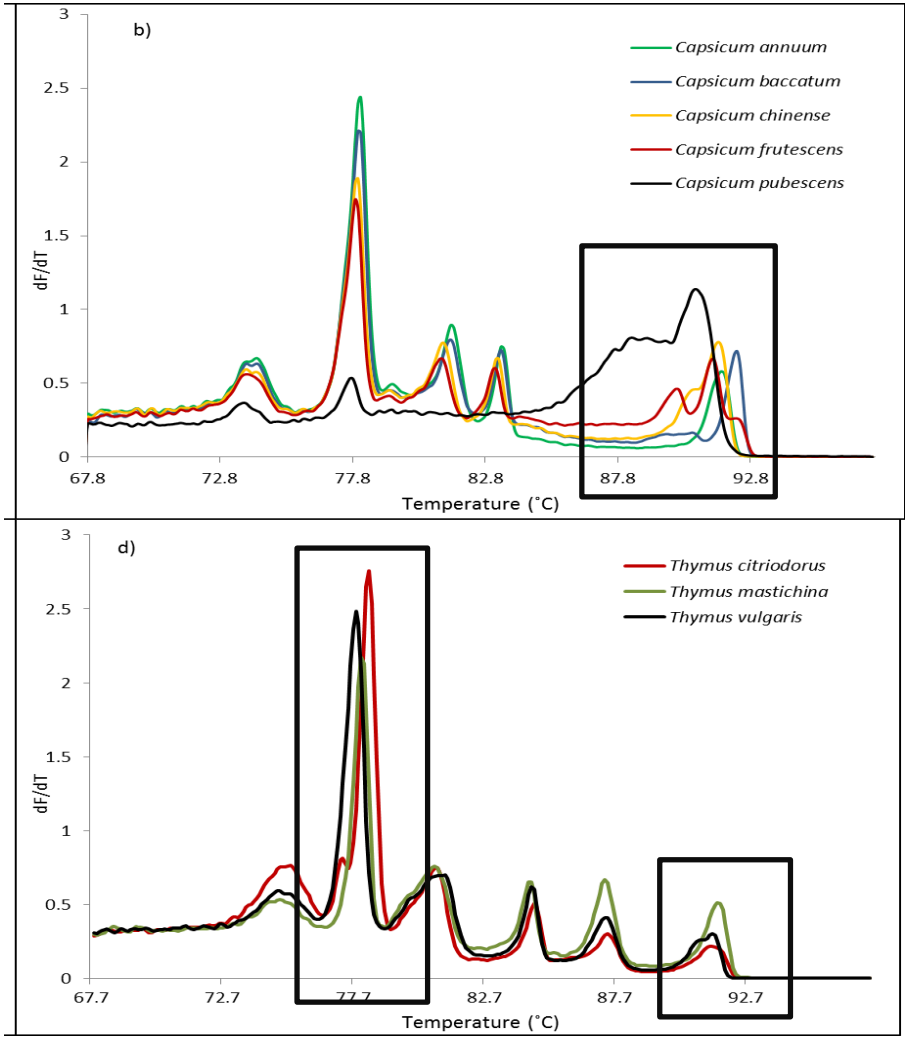


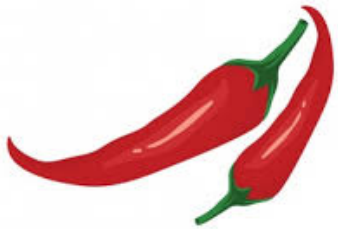
Fig. 3. Multiplexed melting profiles from 29 plant species representing 29 families. For clarity, only the average profile of triplicates for each species is presented. See Supplementary S4, for the full sized melting profiles.



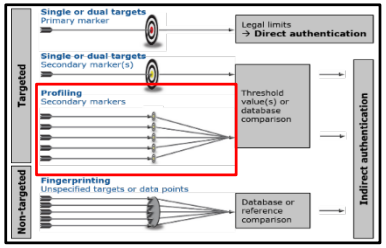
Profiling, *Plant species identification*

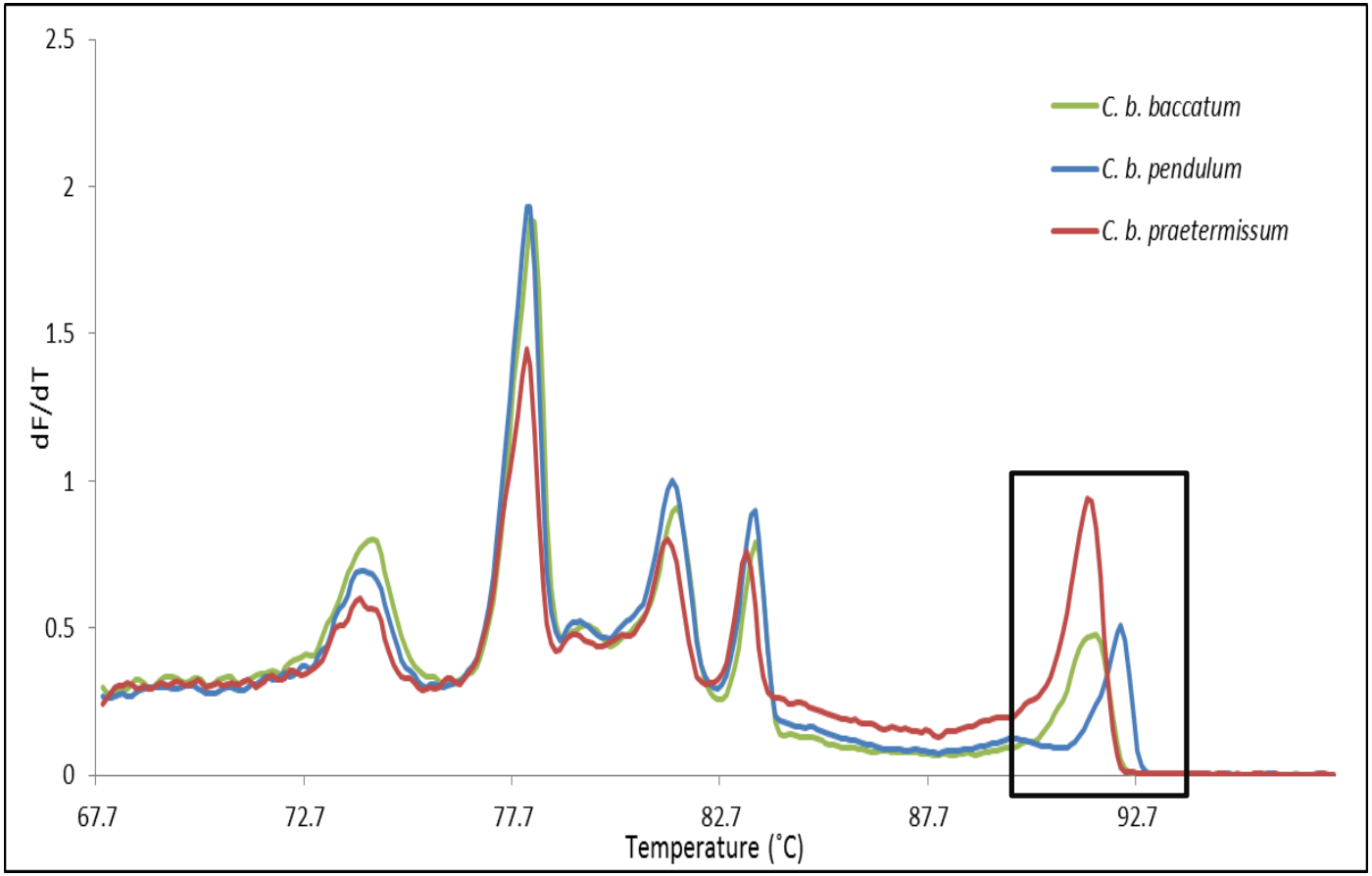


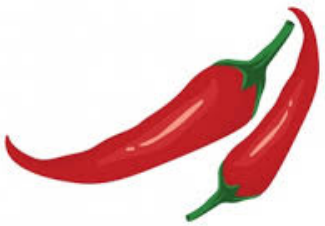




Profiling, *Plant sub-species identification*

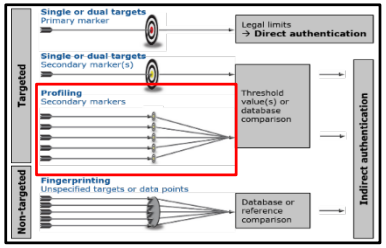


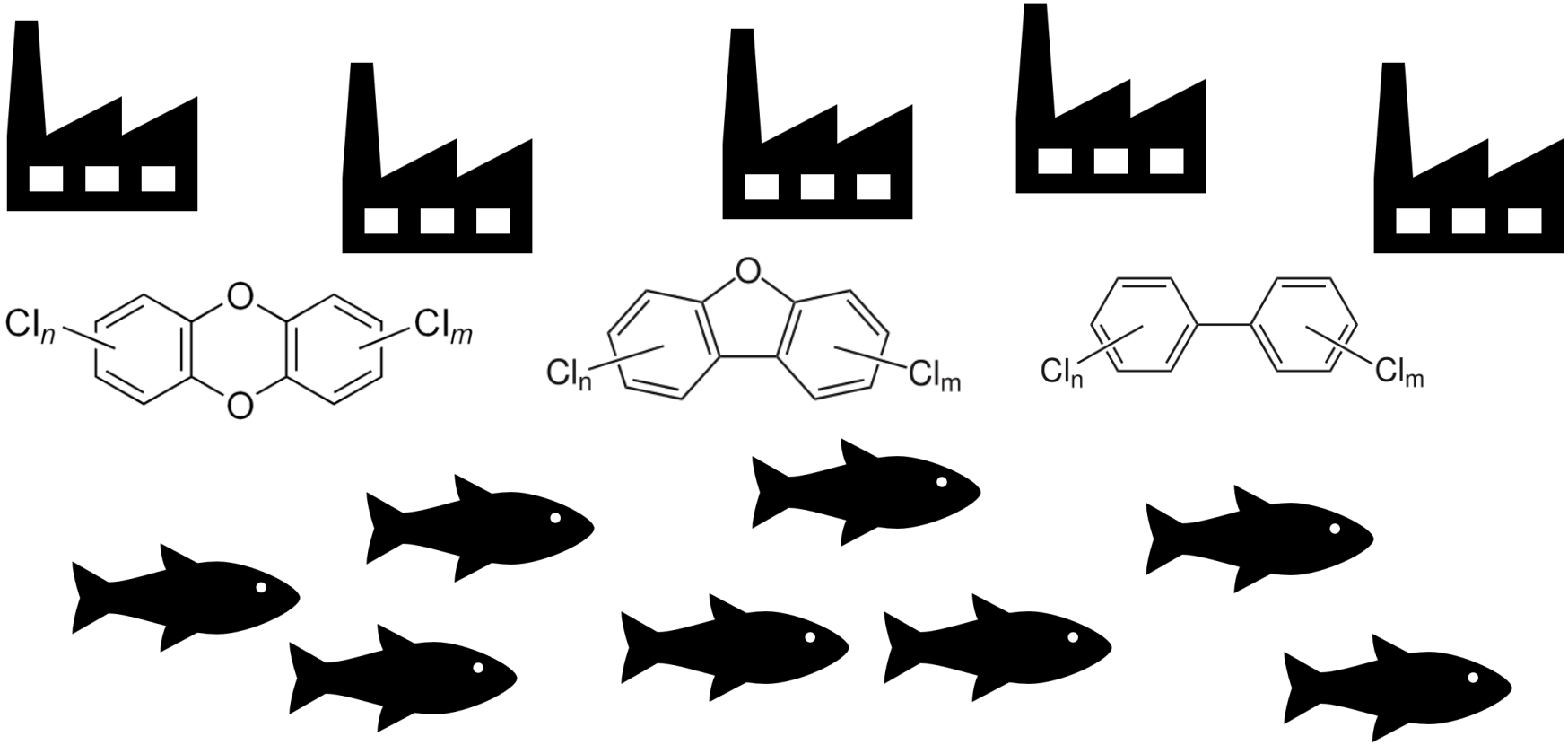




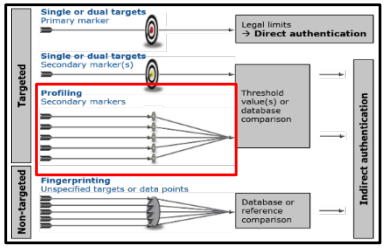
Ballin et al. *Food Control.* 2019; *105*, 141-150.

Another profiling example – dioxins and PCBs in salmon



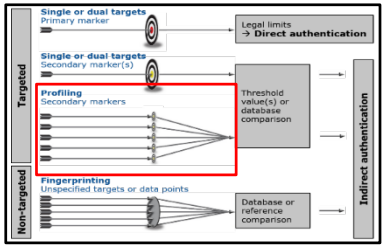


Dioxins and PCBs in salmon – from primary to secondary markers



Salmon	Origin	PCB77	PCB81	PCB126	PCB169	PCB28	PCB52	PCB101	PCB105	PCB114	PCB118	PCB138	PCB153	PCB156	PCB157	PCB167	PCB170	PCB180	PCB189
1	China	0.00334	0.00023	0.00051	0.00022	0.08303	0.11930	0.15797	0.03243	0.00648	0.12713	0.15381	0.23605	0.00217	0.00211	0.00267	0.01706	0.04694	0.00123
2	USA	0.00218	0.00016	0.00066	0.00025	0.03791	0.10341	0.19207	0.04109	0.00239	0.13191	0.15007	0.24804	0.00985	0.00067	0.00666	0.02292	0.04936	0.00040
3	USA	0.00218	0.00018	0.00060	0.00018	0.03534	0.11132	0.18328	0.03691	0.00162	0.13073	0.15120	0.26726	0.00841	0.00168	0.00773	0.01853	0.04218	0.00067
4	Baltic Sea	0.00157	0.00005	0.00080	0.00022	0.01300	0.03436	0.12199	0.03945	0.00214	0.11225	0.21274	0.29624	0.01888	0.00408	0.00895	0.04073	0.09020	0.00234
5	Baltic Sea	0.00157	0.00005	0.00069	0.00022	0.01198	0.03405	0.11028	0.03601	0.00211	0.10368	0.22006	0.31665	0.01692	0.00376	0.00972	0.03968	0.09023	0.00234
6	Baltic Sea	0.00230	0.00006	0.00086	0.00021	0.01427	0.04070	0.12187	0.03534	0.00212	0.10875	0.20731	0.30817	0.01675	0.00416	0.01112	0.03603	0.08783	0.00213
7	Baltic Sea	0.00190	0.00005	0.00079	0.00021	0.01403	0.03776	0.11879	0.03829	0.00220	0.12259	0.21794	0.28972	0.01685	0.00386	0.00989	0.03588	0.08732	0.00192
8	Baltic Sea	0.00215	0.00005	0.00092	0.00022	0.01241	0.03927	0.12282	0.03591	0.00197	0.10961	0.22027	0.29914	0.01582	0.00386	0.00976	0.03856	0.08534	0.00191
9	Baltic Sea	0.00245	0.00004	0.00089	0.00022	0.01265	0.03618	0.11415	0.03603	0.00188	0.10032	0.22322	0.31943	0.01675	0.00364	0.01065	0.03512	0.08443	0.00195
10	Baltic Sea	0.00268	0.00006	0.00097	0.00026	0.01240	0.03918	0.11967	0.03544	0.00212	0.10281	0.21279	0.31833	0.01586	0.00345	0.01133	0.03421	0.08652	0.00193
11	Baltic Sea	0.00231	0.00004	0.00088	0.00022	0.01331	0.03409	0.12171	0.03956	0.00206	0.11579	0.22530	0.29165	0.01661	0.00381	0.01065	0.03346	0.08670	0.00187
12	Norway	0.00270	0.00013	0.00069	0.00013	0.03687	0.11502	0.14581	0.03457	0.00251	0.10489	0.15536	0.26419	0.01143	0.00296	0.00829	0.03506	0.07630	0.00310
13	Norway	0.00281	0.00013	0.00065	0.00014	0.07099	0.10055	0.14692	0.02925	0.00235	0.09963	0.15395	0.26632	0.01019	0.00286	0.00258	0.03655	0.07342	0.00073
14	Baltic Sea	0.00224	0.00005	0.00100	0.00025	0.02104	0.03272	0.12356	0.03702	0.00176	0.11674	0.21183	0.29487	0.01969	0.00384	0.01021	0.03473	0.08608	0.00237
15	Baltic Sea	0.00254	0.00005	0.00101	0.00021	0.02566	0.03622	0.13218	0.03654	0.00184	0.11796	0.20589	0.29595	0.01758	0.00396	0.01081	0.03235	0.07750	0.00176
16	Baltic Sea	0.00252	0.00006	0.00104	0.00021	0.02581	0.03620	0.11989	0.03680	0.00188	0.11451	0.21320	0.29874	0.01782	0.00416	0.00940	0.03708	0.07870	0.00197
17	Norway	0.00279	0.00012	0.00058	0.00012	0.05504	0.08401	0.12283	0.02915	0.00119	0.09815	0.17051	0.28605	0.01079	0.00288	0.00673	0.04476	0.08374	0.00055
18	Norway	0.00201	0.00010	0.00060	0.00013	0.06717	0.10779	0.13858	0.03414	0.00147	0.10460	0.17492	0.25169	0.01076	0.00329	0.00631	0.02908	0.06671	0.00068
19	Norway	0.00235	0.00010	0.00067	0.00003	0.05480	0.10171	0.14143	0.03472	0.00231	0.10646	0.16742	0.25818	0.00824	0.00352	0.00696	0.02855	0.08171	0.00082

Extend the use of regulatory control



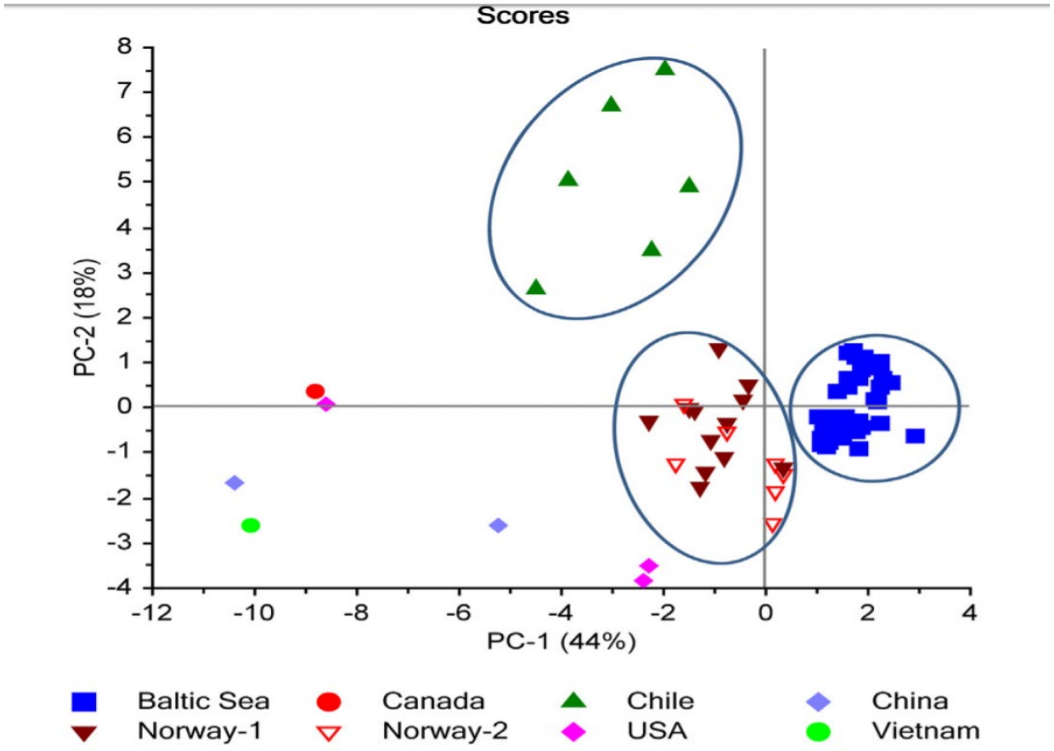
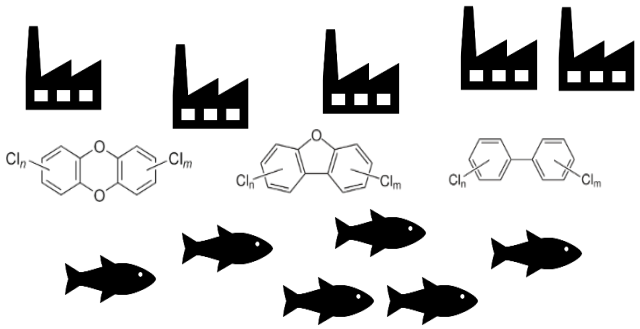


Fig. 2. The score plot of PC-1 and PC-2 for the principal component analysis of the PCB congeners in the 79 samples of salmon in this study. Samples are marked according to their geographical origin.

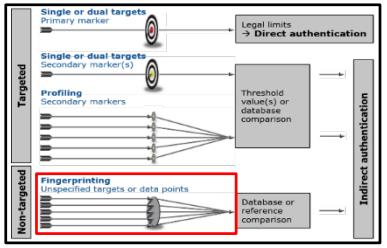
Sørensen et al. *Food Control*. 2016; *61*, 165-171.



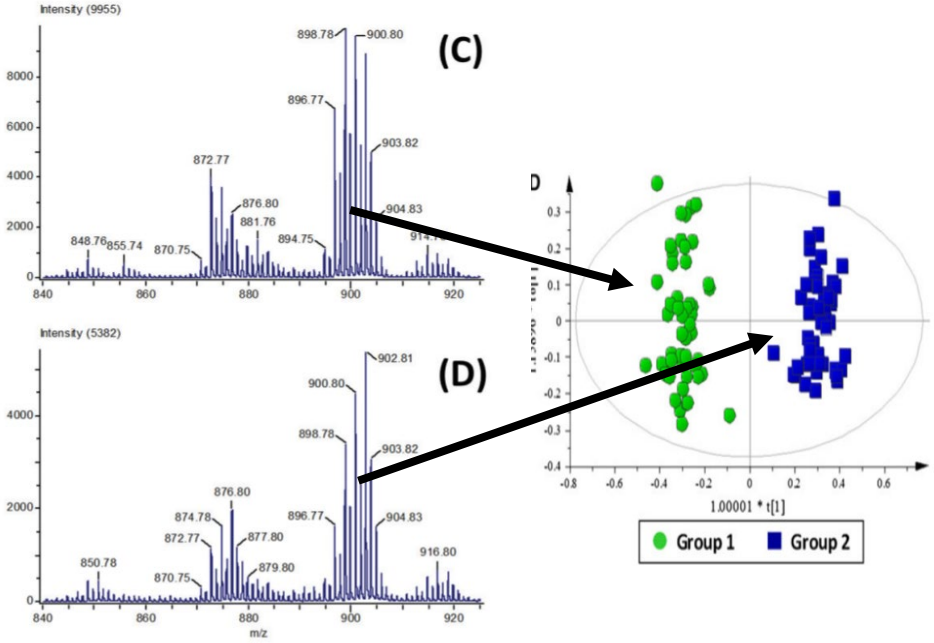
Convert a primary marker analysis into a profiling one, e.g:

- Dioxins and PCBs
- Minerals and metals

Fingerprint examples



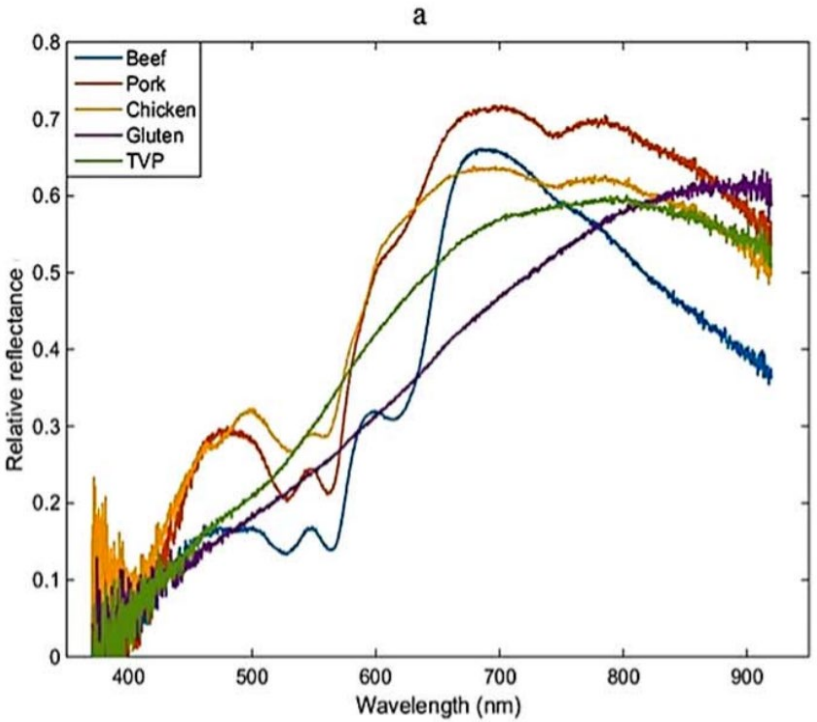
DART-MS -
Extracts of chicken



(C) feed without chicken bone meal
(D) feed with chicken bone meal

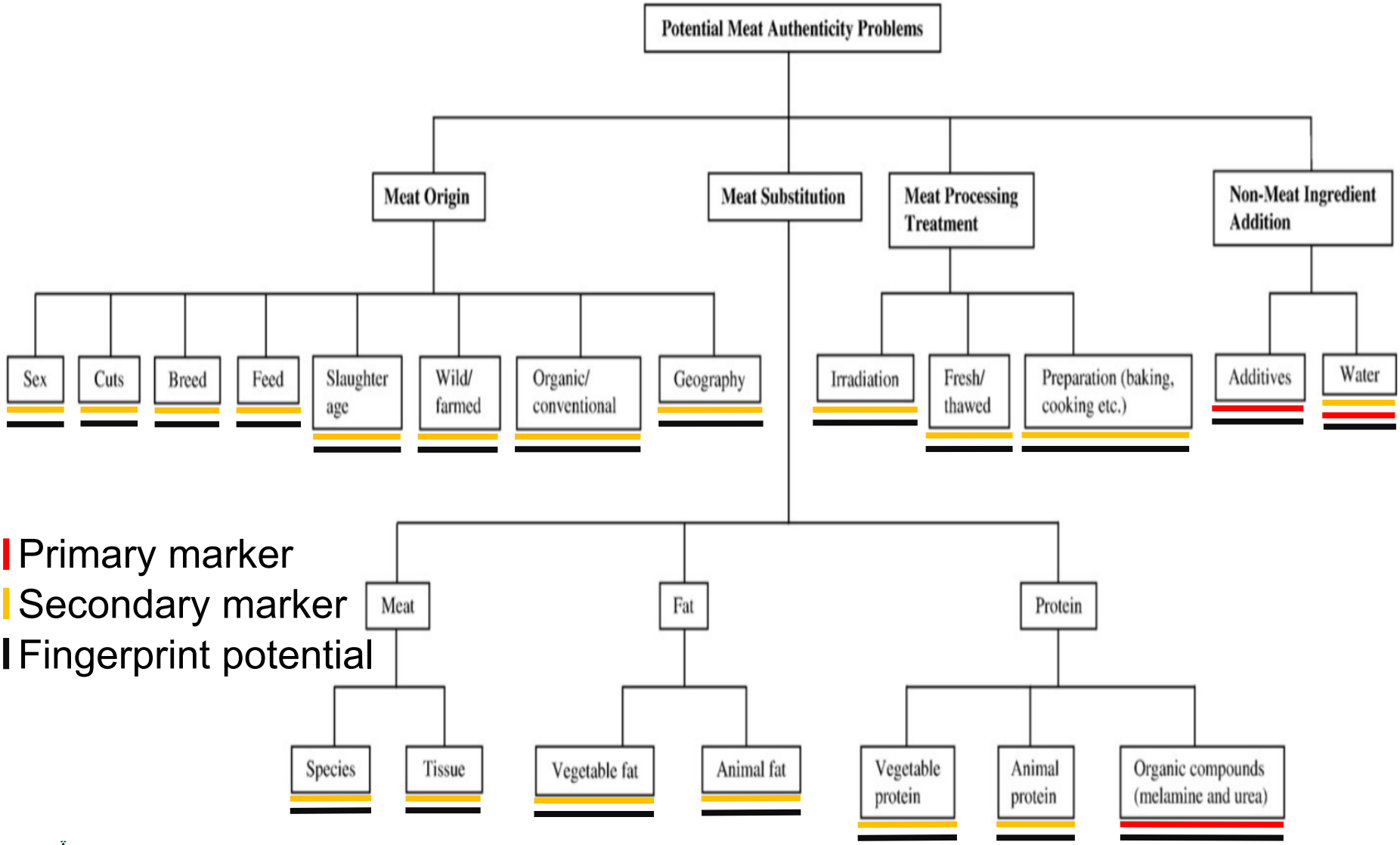
Cajka et al. *Metabolomics.* 2013; *9*, 545-557.

Vis-NIR
Processed meat and protein



Rady & Adedeji. *Meat Science.* 2018; *136*, 59-67.

Fingerprinting in meat authentication

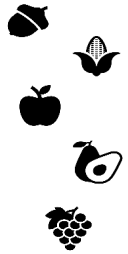


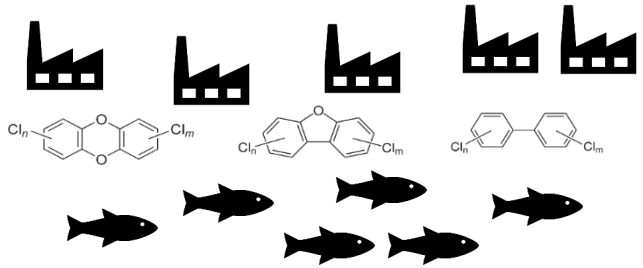
Primary marker
Secondary marker
Fingerprint potential

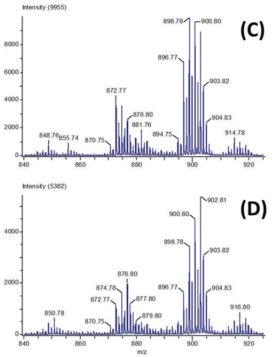
Ballin. *Meat Science*. 2010; *86*, 577-587.

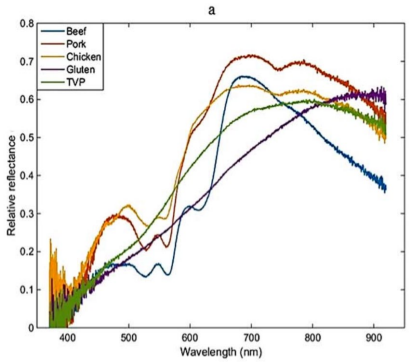
Lessons learned – single target, profiling, and fingerprinting

- Single target
  - Limited use
- Profiling
  - Broad applicability
  - Reuse data
- Fingerprinting
  - Suitable for complex issues
  - Identify abnormalities









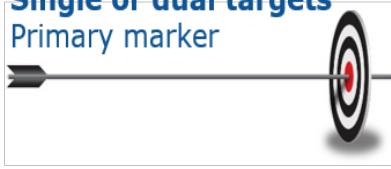
Targeted vs. non-targeted

Validation requirements

Complexity of authentication issue

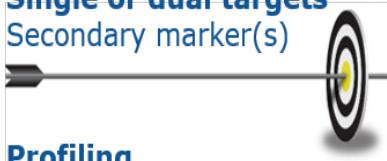
Targeted

Single or dual targets
Primary marker



Legal limits
→ **Direct authentication**

Single or dual targets
Secondary marker(s)



Profiling
Secondary markers

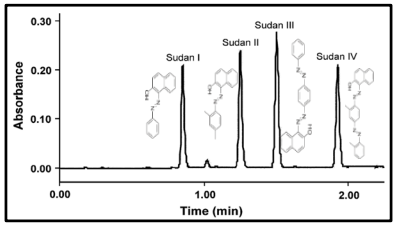
Threshold value(s) or database comparison

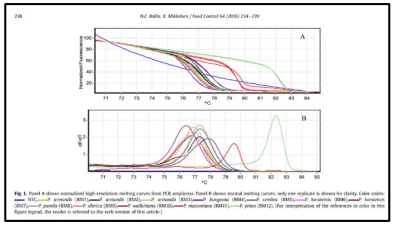
Non-targeted

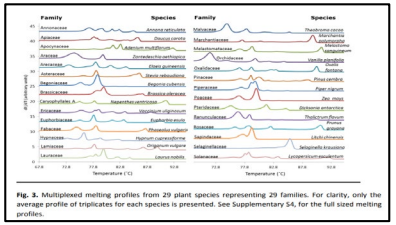
Fingerprinting
Unspecified targets or data points

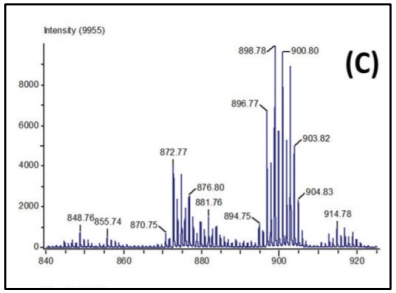
Database or reference comparison

Indirect authentication









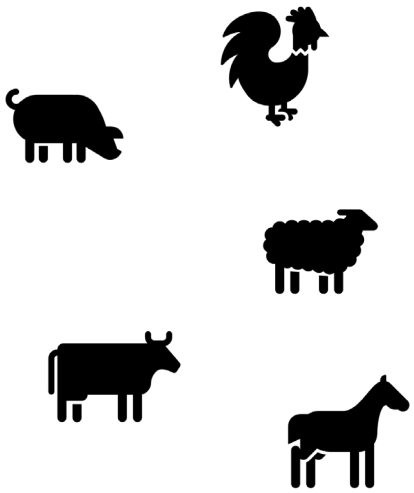
Perspectives in regulatory non-targeted food authentication

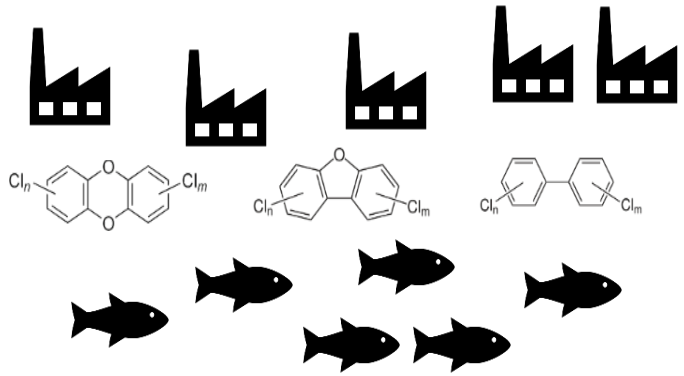
Define concepts and terms

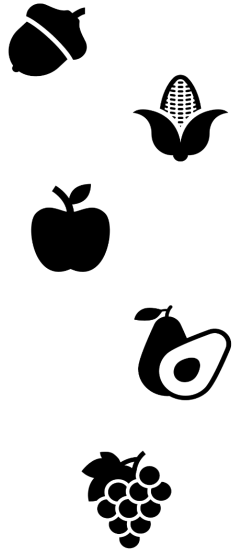
Standardize validation procedures for non-targeted methods

Standardize non-targeted methods

Acknowledgement







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Comments and questions are highly welcomed

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