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CHALLENGES IN FOOD ADDITIVE ANALYSIS FOR ENSURING TRACEABILITY IN THE FOOD CHAIN

27.11.2024 Spotlight: Food additives – Status quo on chemical analysis and European regulations

Joris Van Loco & Séverine Goscinny

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FOOD ADDITIVES

“not normally consumed as a food in itself and not normally used as a characteristic **ingredient of food**, whether or not it has nutritive value, **the intentional addition** of which to food for a **technological purpose …** becoming directly or indirectly a **component of such foods”**

E100–E199 **colours**
E200–E299 ”preservatives”
E300–E399 ”antioxidants, acidity regulators”
E400–E499 ”thickeners, stabilizers, emulsifiers”
E500–E599 ”acidity regulators, anti-caking agents”
E600–E699 “flavour enhancers”
E900–E999 ”glazing agents” and **sweeteners**
E1000– ”others”

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FOOD ADDITIVES LEGISLATION

Regulation (EC) No 1331/2008 Authorisation procedure

Regulation (EC) No 1333/2008 Food Additives Legislation

Regulation (EU) No 231/2012 Specifications for food additives

Regulation (EC) No 1169/2011 Food Information to Consumers (FIC) Regulation

FOOD ADDITIVES ON THE MARKET

- > 330 authorised food additives
- +/- 80% are on the EU market (BE+FR studies)
- Used in combination with other additives and in large variety of foods
    - Large number of food contains 1+ additive
    - Clusters of additives commonly found in food

BELGIAN FOOD INSPECTION RESULTS 2021

	Number of samplings	Favourable samples (%)
Additives and flavourings	945	87,2
Allergens	469	97,7
Quality - Composition	1.167	90,5
Radiation	123	100
GMOs	461	98,9
Dioxins and PCBs	2.670	99,6
Mycotoxins	2.144	99,0
Heavy metals	1.753	99,2
PAHs	600	99,8
Marine biotoxins and histamine	319	100
Migration	836	97,5
Other contaminants	994	98,8
Pesticide residues	4.639	97,3
Medicines	16.447	99,5
Other prohibited substances	151	89,4

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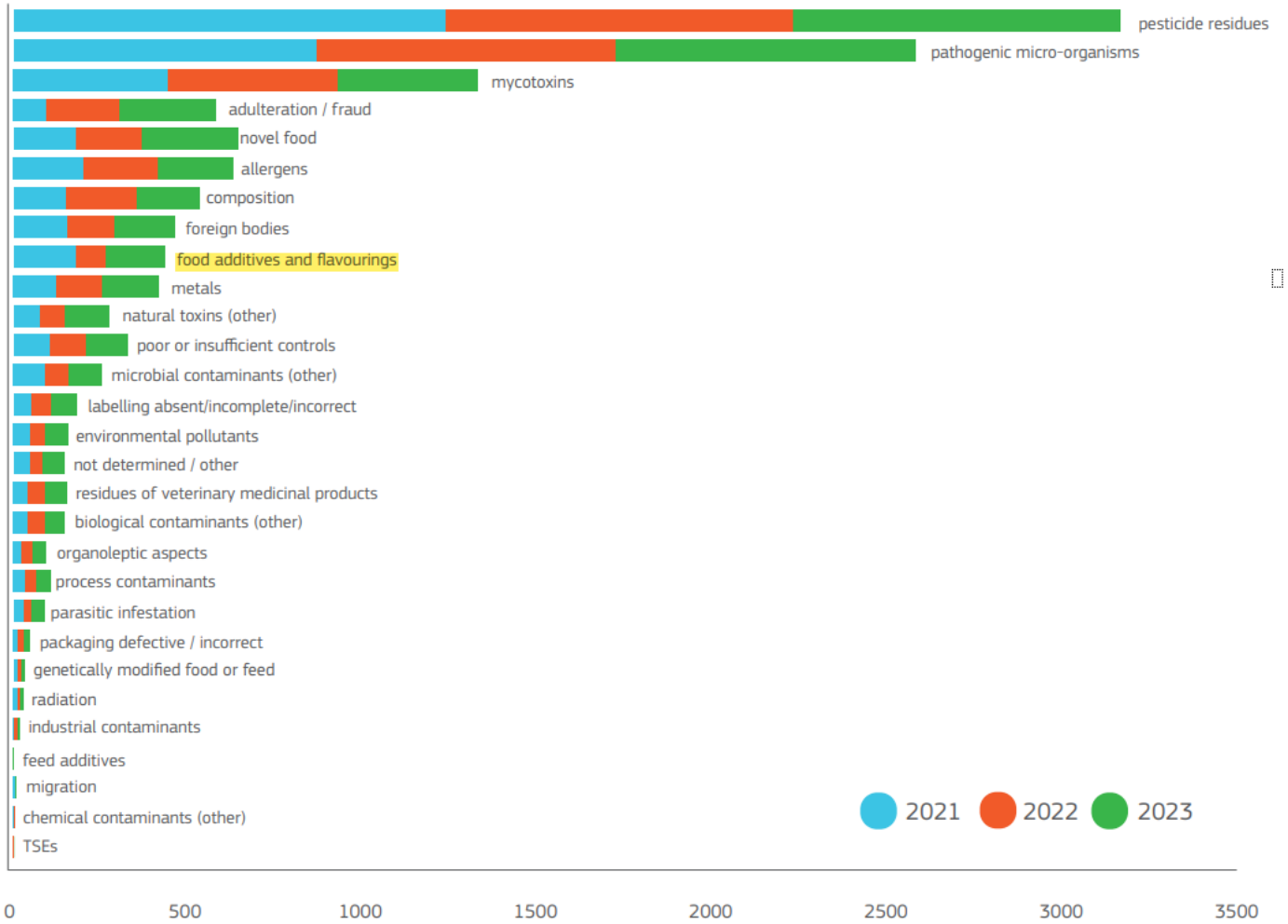
RASFF

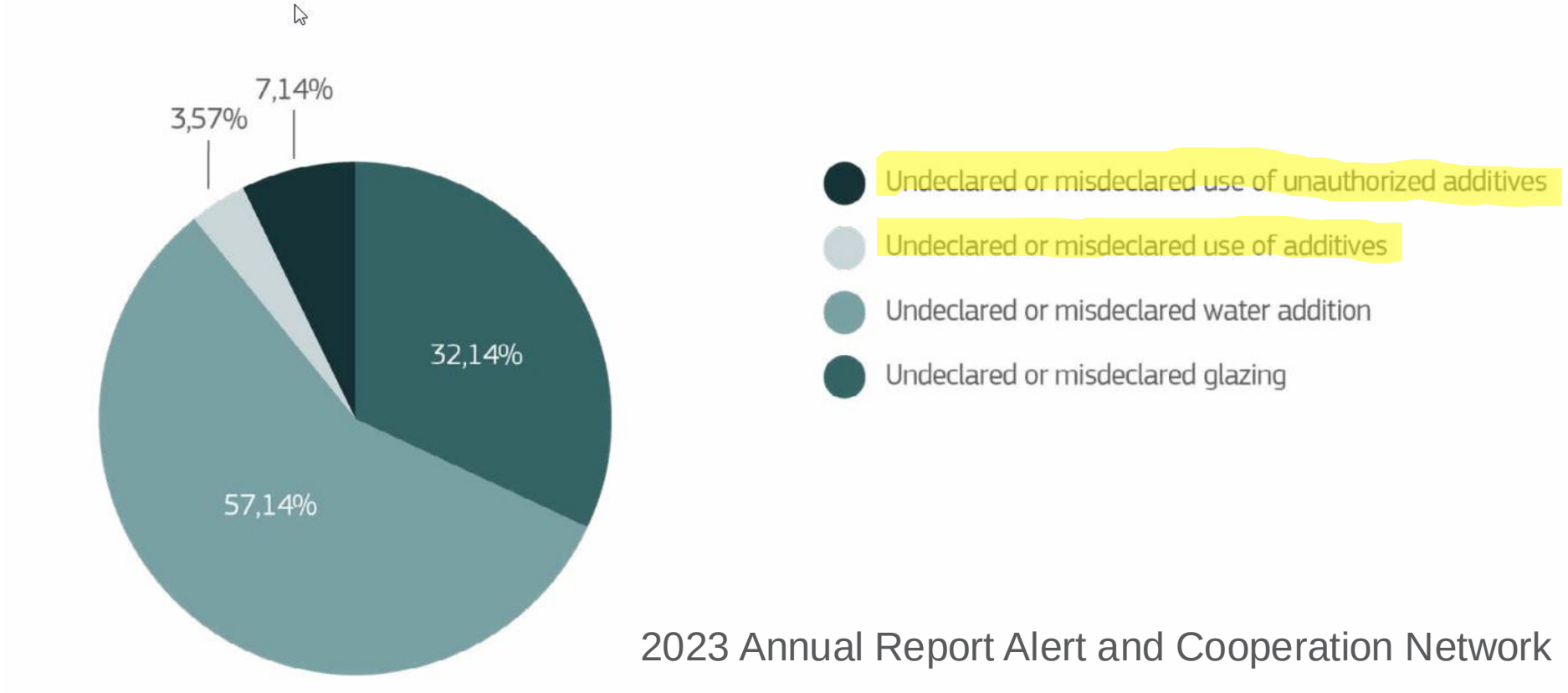
Figure 5: RASFF notifications by food hazard categories 2021-2023



RASFF

Food additives are responsible for +/- 10% of the reported **food fraud** cases

Figure 18: AAC and RASFF notifications with a suspicion of fraud by product categories



“ILLEGAL” / NON AUTHORISED FOOD ADDITIVES USE

- **Food fraud**
  - Illegal use of food colorants
    - Sudan Dyes,…
  - Illegal treatment of fish / meat
    - nitrites/nitrates, carbon monoxide, colorants,…

Oplatowska-Stachowiak et al. (2017) Food colors: Existing and emerging food safety concerns, Critical Reviews in Food Science and Nutrition, 57:3, 524-548

“ILLEGAL” / NON AUTHORISED FOOD ADDITIVES USE

- **Non-EU authorized food additives**
  - Differences in authorization
  - EU and US
    - Some food colors
    - Azodicarbonamide (E927)
    - Titanium dioxide (E171)
    - ...
  - No food additive legislation in importing countries

“ILLEGAL” / NON AUTHORISED FOOD ADDITIVES USE

- **Non-EU authorized food additives**

Multi additive study 2018-2020

- 123 samples analyzed with multi class additive method (27 authorized and 50 “illegal” additives)
- 12 products (10%) contained 7 substances not authorized as food additive in the EU
  Assumption = that some additives were used in ingredients

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NON COMPLIANT FOOD ADDITIVES USE

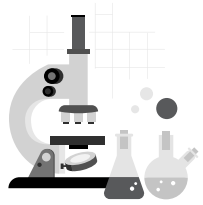
- > maximum permitted limit (MPL)
- Non compliance in labelling
- Not authorised for use in the specific food

CLEAR / CLEAN LABEL

- Consumer wants more natural foods
- (Artificial) food additives replaced by:
  - Natural food additives (steviol glycosides)
  - Natural ingredients (citric acid -> lemon juice)
  - Colouring food
  - No food additives

BUT often they uses extracts of plants

- specific extraction & technological purposes = food additive
  - labelling requirement as additive
  - impurities (i.e. toxins, trace elements)
    - -> additional health risks



Ingredients <-> Food additives ?

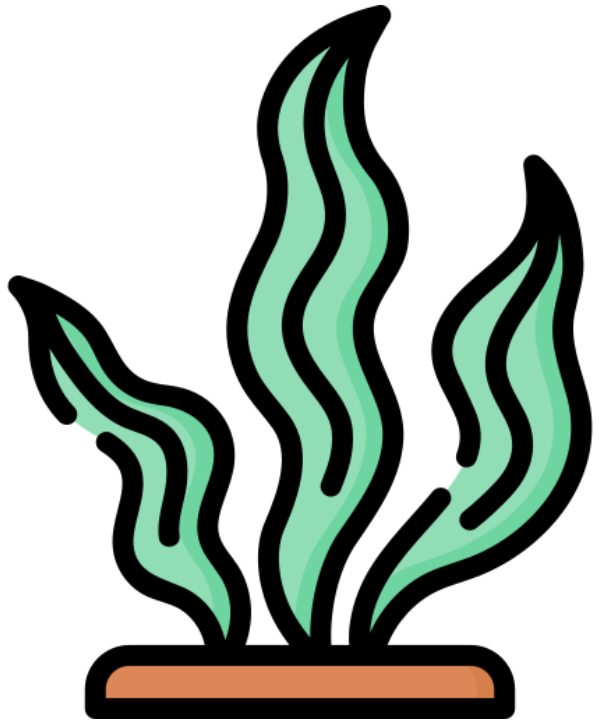
Blue Coloring Food - Spirulina extract

- GNPD Mintel 10 years variation: **+1200%** usage
- not an authorized food additive in the EU, coloring food
- A temporary **ADI “not specified”** was established *

Impurities <

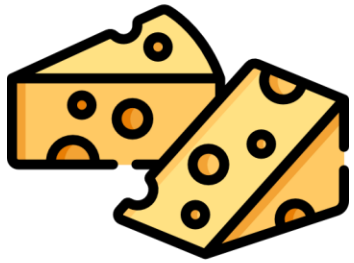
Microcystins

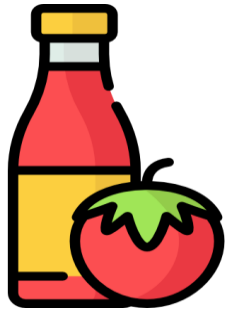
Lead, Cadmium, Arsenic, Mercury

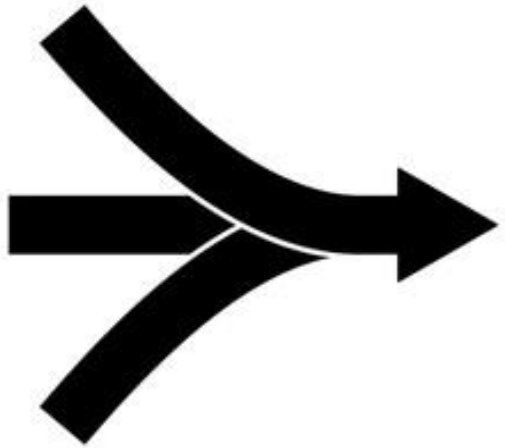


*86th JECFA – 2018

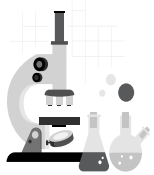
Carry over principle





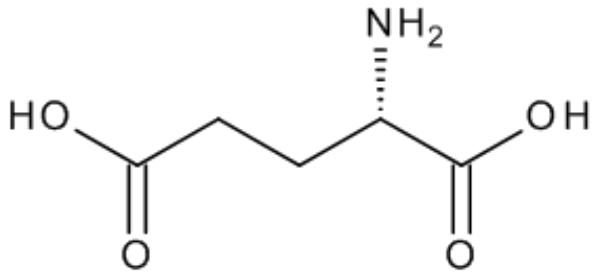


Food additives may be present in a food because they were contained in one of the **ingredients**. They need only be **labelled** if they perform a **technological function** in the composite food.



Technological function ?

FREE GLUTAMATE (FGlu)

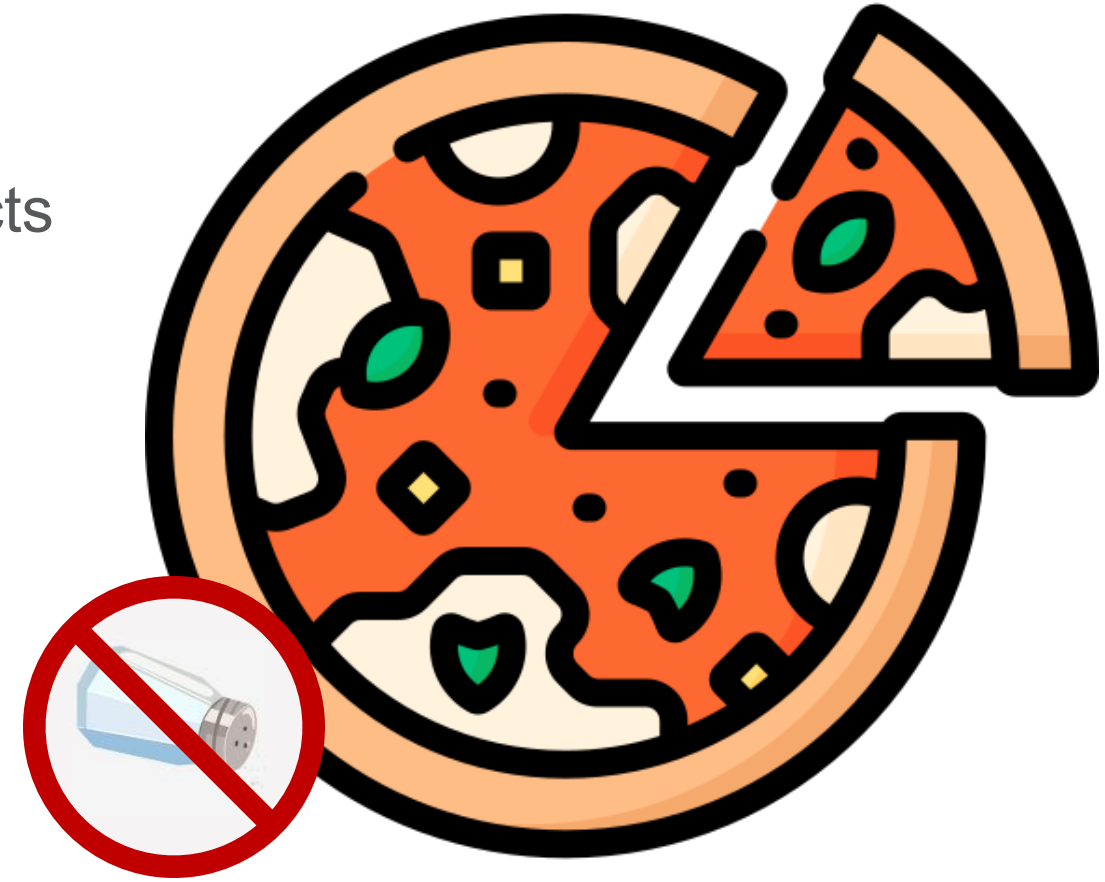


L-glutamic acid

- Non-essential amino acid
- Flavor-enhancing effect: “umami” taste
- FGlu presence in food:
  - Naturally occurring
  - Food processing
  - Added as food additive (“MSG” or E621)
  - Added from ingredients rich in FGlu
    - Tomato, ripened cheese, soya, yeast,…

Prepared dishes - Carry over principle

- Use of E621 as salt replacer
- Low Sodium
- No or low salt addition
- No labelling of “MSG” or E621 on products
- Use of ingredients rich in FGlu
    - Tomato, cheese, soy sauce,..
- Clean/Clear label approach
    - FGlu from ingredients or …
    - Extra addition of E621 ?
    - E621 in ingredients ?



Prepared dishes & different FGlu sources
Carry over principle

[…], gedroogde oregano, gedroogde basilicum. FR **PIZZA GARNIE D'EMMENTAL, DE JAMBON CUIT ET DE CHAMPIGNONS** - 38,6 % pâte [farine de **BLÉ** (gluten), eau, levure, sel, sucre], sauce tomate (eau, double concentré de tomates, farine de **BLÉ** (gluten), oignons en poudre, ail en poudre, piments en poudre), 15,5 % emmental râpé (**LAIT**), 11,7 % jambon cuit [jambon de porc, eau, sel, sirop de glucose, stabilisant (E450), antioxydant (E316), conservateur (E250)], 9,5 % champignons cuits pasteurisés [champignons de Paris, eau, sel, correcteur d'acidité (E330), antioxydant (E300)], olives noires confites avec noyau [olives noires, eau, sel, stabilisant (E579)], origan déshydraté, basilic déshydraté. EN **PIZZA TOPPED WITH EMMENTAL, COOKED HAM AND MUSHROOMS** - 38,6 % dough [WHEAT flour (gluten)

Ingredient	Proportion ingredient	Conc. measured in sample similar to ingredient (mg/kg)	**Conc. carried over to final product** (mg/kg)
38,6 % pâte [farine de BLE (gluten), eau, levure, sel, sucre]	38.6%	35	14
sauce tomate (eau, double concentré de tomates, farine de BLÉ (gluten), oignons en poudre, ail en poudre, piments en poudre)	15.5%	3118	483
15,5% emmental râpé (LAIT)	15.5%	2597	403
11,7% jambon cuit [jambon de porc, eau, sel, sirop de glucose, stabilisant (E450), antioxydant (E316), conservateur (E250)]	11.7%	327	38
9,5% champignons cuits pasteurisés [champignons de Paris, eau, sel, correcteur d'acidité (E330), antioxydant (E300)]	9.5%	1054	100
TOTAL	91 %		**1038**

$$Ratio = \frac{Concentration_{measured}}{Concentration_{expected}} = 0{,}7$$

Measured concentration in sample	**724**

Prepared dishes & different FGlu sources
Carry over principle

$$Cary\ Over\ Ratio = \frac{Concentration_{measured}}{Concentration_{expected}}$$

COMPLIANT QUESTIONABLE NON COMPLIANT

Prepared dishes - Carry over principle

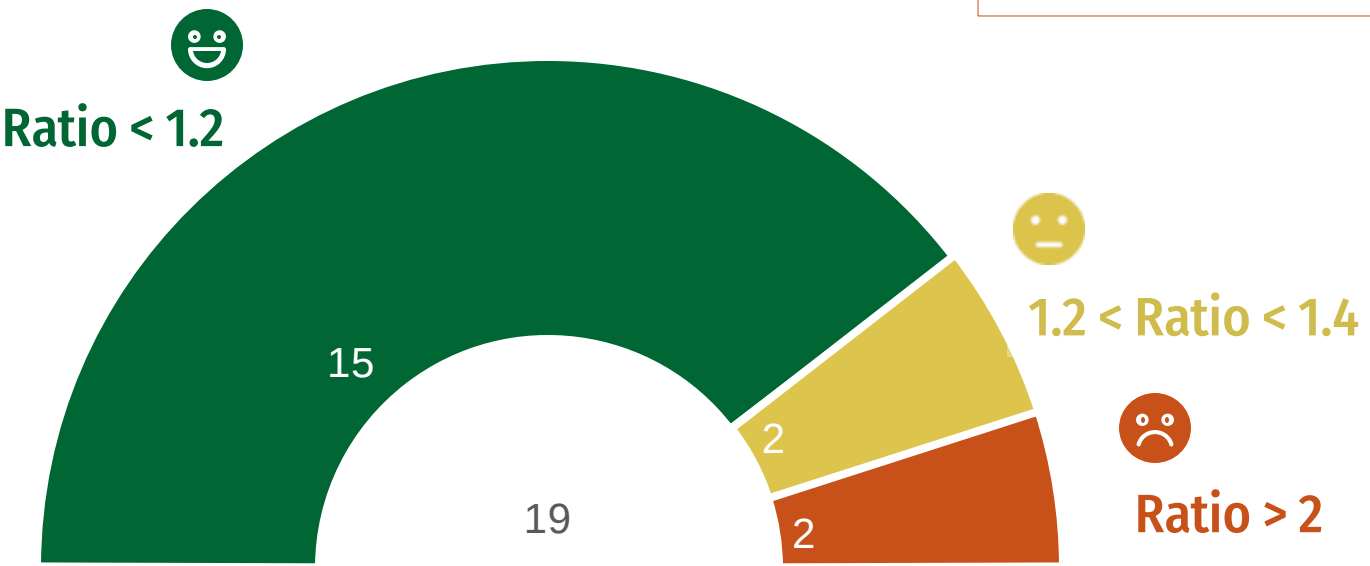
Calculations made

- without E621 labeling
- measured concentration > 400 mg/kg

$$Ratio = \frac{Concentration_{measured}}{Concentration_{expected}}$$

	Conc. meas. (mg/kg)	Conc exp. (mg/kg)
Pizza Bolognese	2369	1178
Wok noodles with chicken	715	271

Uncertainty: no info on proportion soy sauce, 1 % considered (3 % → ratio 1.6)



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ANALYTICAL METHODS

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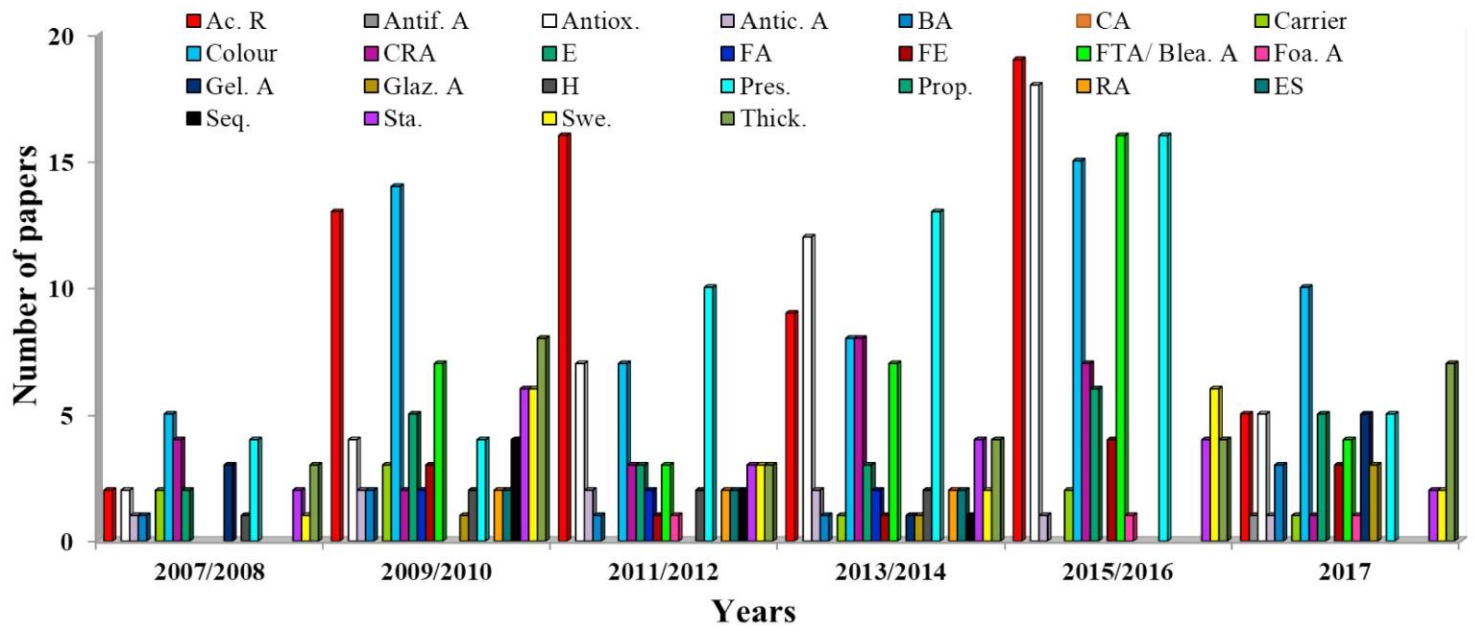
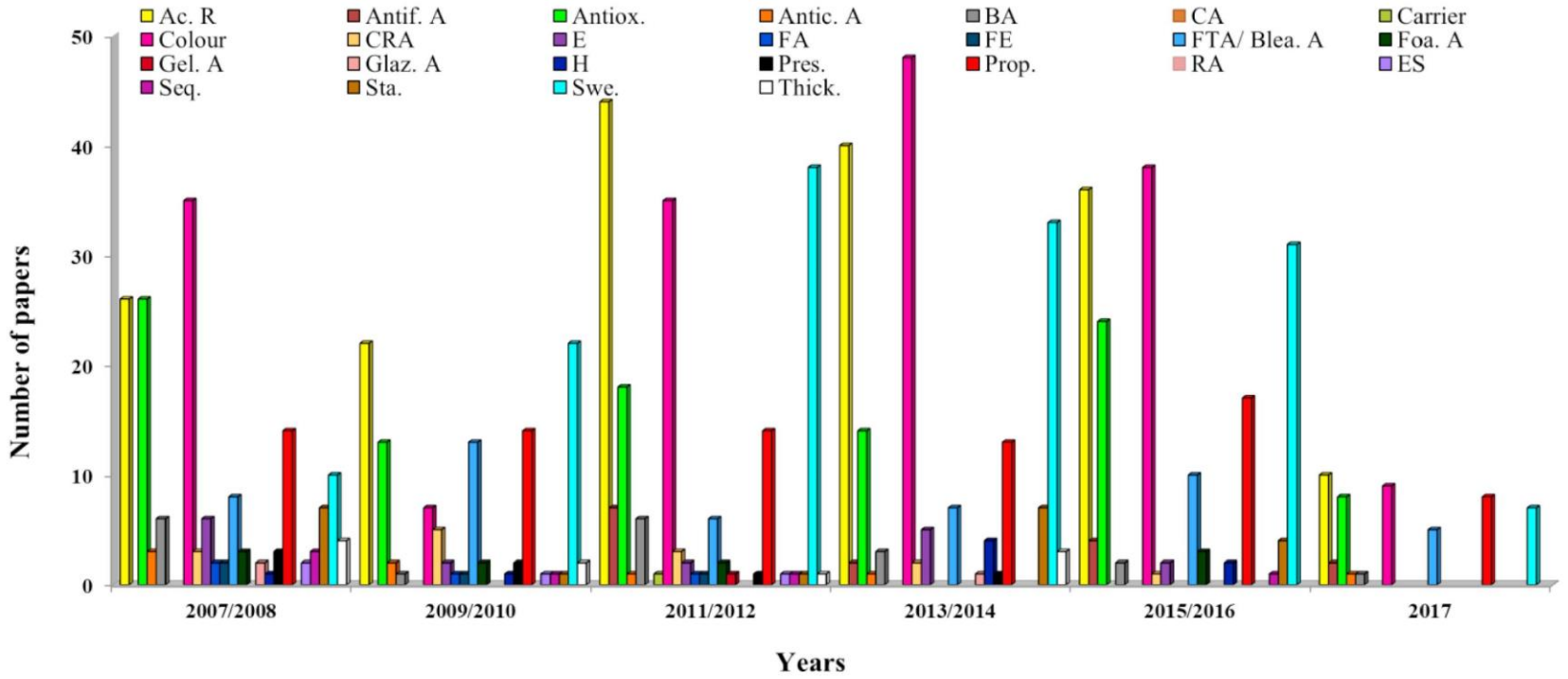
Spectroscopic methods for Food Additives



Ac. R = Acidity Regulator; Antic. A = Anticaking Agent; Antif. A = Antifoaming Agent; Antiox. = Antioxidant; BA = Bulking Agent; CA = Carbonating Agent; CRA = Colour Retention Agent; E = Emulsifier; ES = Emulsifying Salt; FA = Firming Agent; FE = Flavour enhancer; Foa. A = Foaming Agent; Gel. A = Gelling Agent; Gla. A = Glazing Agent; H = Humectant; Pres. = Preservative; Prop. =Propellant; Seq. = Sequestrant; Swe. = Sweetener; Thick. = Thichener;

Martins et al., Analytical methods in food additives determination: Compounds with functional applications, Food Chemistry (2019)

Chromatographic methods for Food Additives



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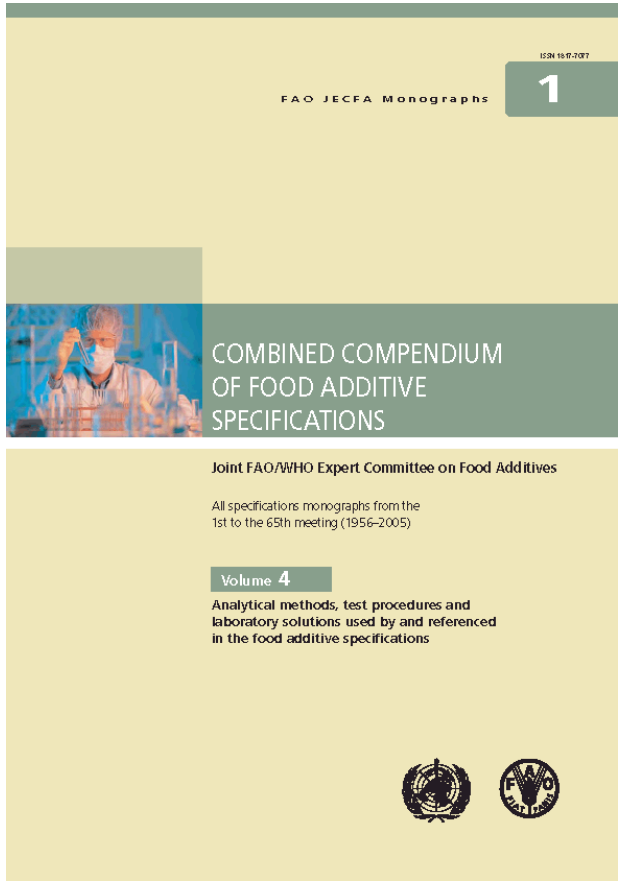
ANALYTICAL METHODS



- Compendium of analytical methods
  - FAO/WHO
  - FOOD STANDARDS AGENCY …
- Many Published methods in literature
  - Additive,
  - Impurities & specifications

→ Single food additive / FA group approach

Limited matrixes

Martins et al., Analytical methods in food additives determination: Compounds with functional applications, Food Chemistry (2019)
Zhang et al., Food additives: From functions to analytical methods. Critical Reviews in Food Science and Nutrition (2021)

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Multi class additives methods in literature

Multi class FA single matrix

- Gao et al.. 2013. Determination of 30 synthetic food additives in soft drinks by HPLC/electrospray ionization-tandem mass spectrometry.Journal of AOAC International
- Jia et al. 2014. Analysis of additives in dairy products by liquid chromatography coupled to quadrupole-orbitrap mass spectrometry. J Chromatogr A.
- Lee et al. 2019. Simultaneous determination of preservatives, artificial sweeteners, and synthetic dyes in kimchi by ultra-performance liquid chromatography electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS). Anal. Lett.
- Sun et al. 2019. Rapid and sensitive detection of multi-class food additives in beverages for quality control by using HPLCDAD and chemometrics methods. Food Anal Methods.

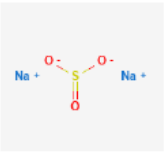
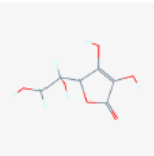
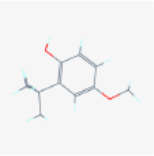
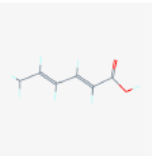
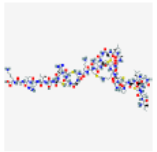
Multi class additives methods in literature

Multi class FA multi matrix

- Moreno et al.. 2014. **Improvement of analytical methods in post-market monitoring of food additives and testing of the improved methods** (OC/EFSA/ DCM/2012/04): Final report on analysis and summary of the food additives pilot survey results. EFSA supporting publication
- Detry et al. 2022 **Development, validation and application of multi-class methods for the analysis of food additives by liquid chromatography coupled to tandem mass spectrometry**, Food Additives & Contaminants: Part A

ANALYTICAL CHALLENGES

Wide variety of targeted compounds

Organic	Inorganic	Polar	Non-polar	Low MW	High MW
					
Benzoic acid (E 210)	Sodium sulphite (E 221)	Ascorbic acid (E 300)	BHA (E 320)	Sorbic acid (E 200) 112.1 g/mol	Nisin (E 234) 3354.1 g/mol

Large (and unpredictible) concentrations range

Ex:
Edible ices

MPL_{E969} < MPL_{E427} < MPL_{E964}

10 mg/kg

2500 mg/kg

200000 mg/kg

Diversity and complexity in matrices

FOOD ADDITIVES SPECIFICATIONS

Regulation (EU) No 231/2012 - laying down specifications for food additives

Specifications

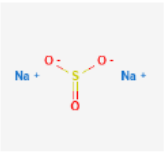
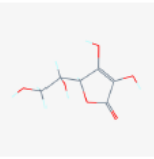
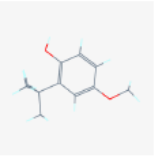
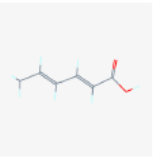
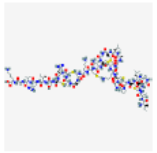
- identify
- origin,
- purity.

CARAMEL COLOURS

4-Methylimidazole (MEI)	Class I: - Class II: - Class III: max 300 mg/kg & max 200 mg/kg on an equivalent colour basis Class IV: max 1000 mg/kg & max 250 mg/kg on an equivalent colour basis See description under TESTS
2-Acetyl-4-tetrahydroxy-butylimidazole (THI)	Class I: - Class II: - Class III: max 40 mg/kg & max 25 mg/kg on an equivalent colour basis. Class IV: - See description under TESTS
Arsenic (Vol.4)	Not more than 1 mg/kg (Method II)
Lead (Vol. 4)	Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the methods described in Volume 4, “Instrumental Methods”.

ANALYTICAL CHALLENGES

Wide variety of targeted compounds

Organic	Inorganic	Polar	Non-polar	Low MW	High MW
					
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Trace elements

Impurities

Large (and unpredictible) concentrations range

Ex:
Edible ices

MPL_{E969} < MPL_{E427} < MPL_{E964}

10 mg/kg

2500 mg/kg

200000 mg/kg

Diversity and complexity in matrices

Nano particles in food additives

- titanium dioxide (E171),
- iron oxide and hydroxides (E172),
- silver (E174),
- gold (E175)
- silicon dioxide (E551)

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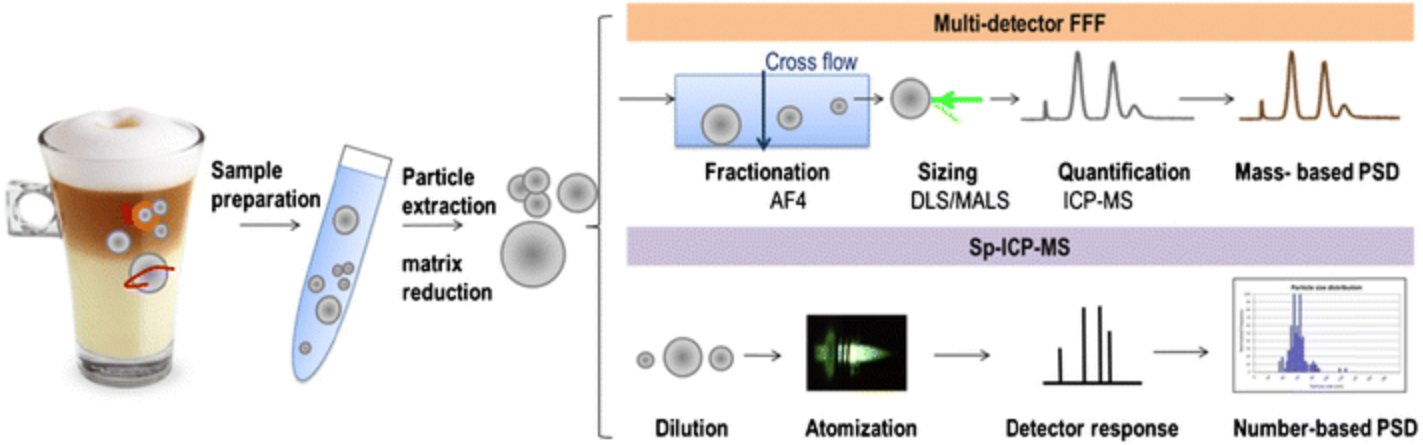
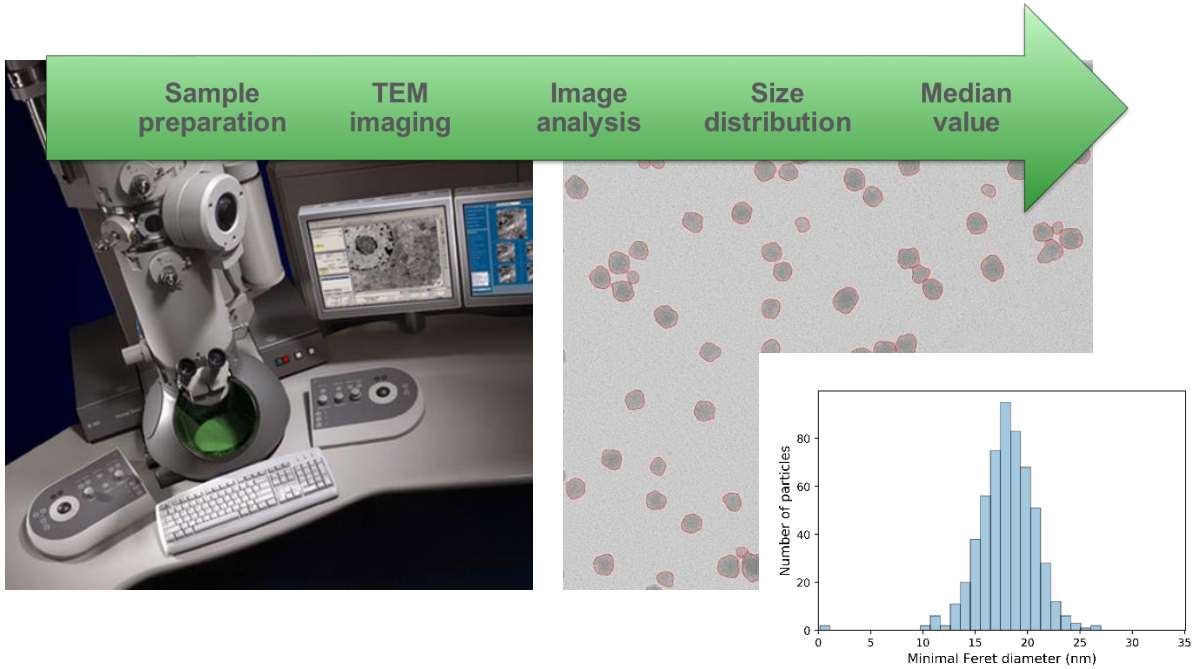
Analytical Techniques for nano particles in food

- Field Flow Fractionation
- Sp-ICP-MS
- Scanning Transmission Electron Microsopy (STEM)
- …





Mattarozzi et al. **Analytical approaches for the characterization and quantification of nanoparticles in food and beverages**. *Anal Bioanal Chem* **409**, 63–80 (2017).

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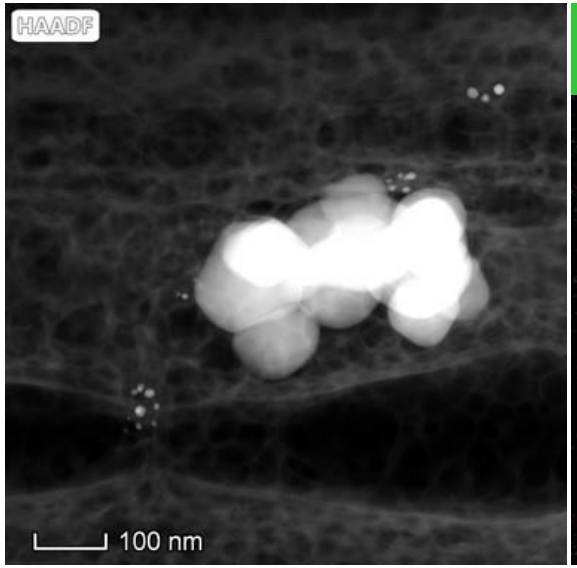
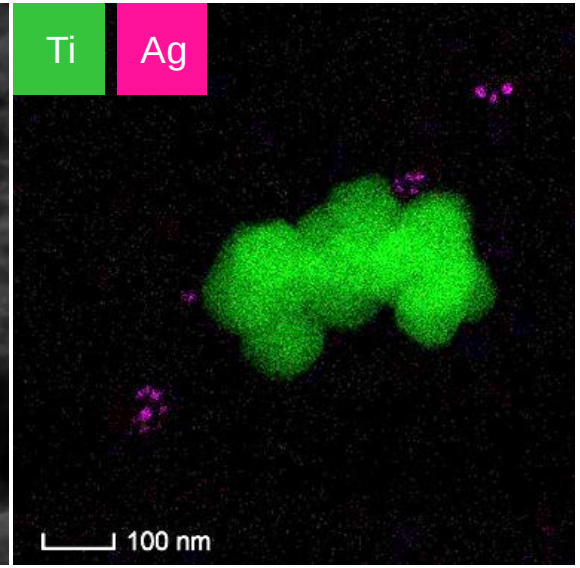
Titanium dioxide in food

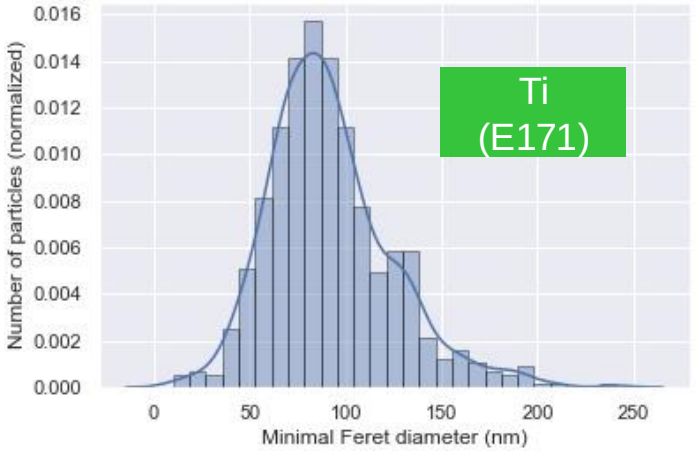
- EC approved food additive **(E 171)**, **Forbidden in food from 8/8/2022** (EC 2022/63)
  - EFSA opinion of 2021
    - they **could not exclude genotoxicity concerns** after consumption of titanium dioxide particles.
- E 171 is (was) used as **food colorant**, commonly applied in **confectionery** (including candies, chewing gum, glazings), but also in pastries, low fat dairy products and sauces
- **Pearlescent pigments**: Certain rutile grades of titanium dioxide are produced using potassium aluminum silicate (mica) as a template to form a basic platelet structure (not E 171)
- Titanium dioxide in **FCM** (n° 610)
- Titanium dioxide as **contaminant** ?

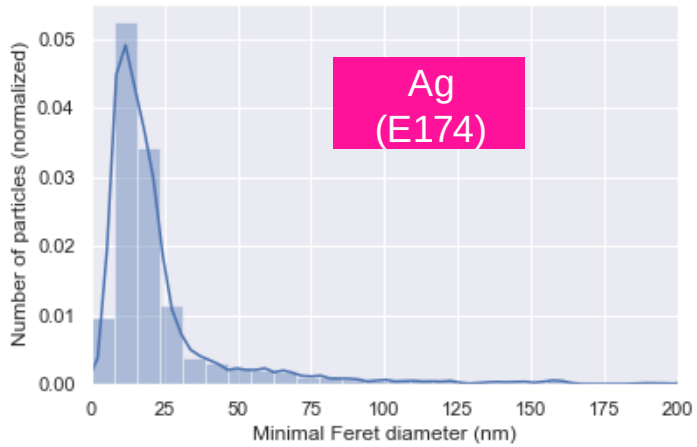
Nano-particles in a food matrix by analytical TEM





Verleysen et al. **Physicochemical Characterization of the Pristine E171 Food Additive by Standardized and Validated Methods.** Nanomaterials. 2020;
De Vos et al. **Physico-chemical characterisation of the fraction of silver (nano)particles in pristine food additive E174 and in E174-containing confectionery.** Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2020



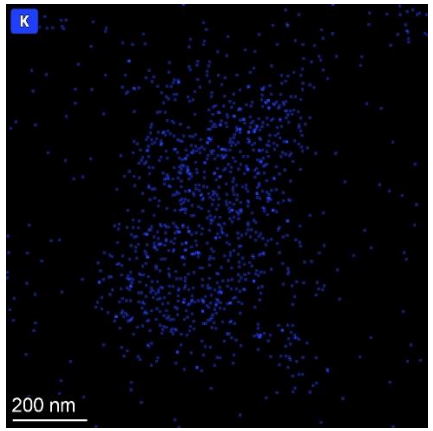


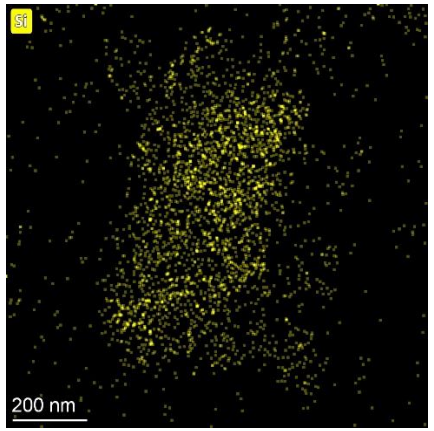
Demonstration on nano-particles in food

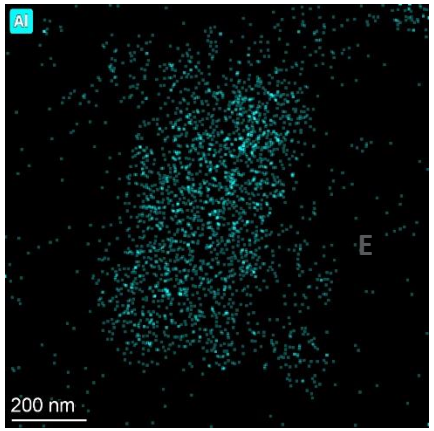
Analyis of (nano)particles in edible pump sprays with STEM EDX (pearlescent pigments)

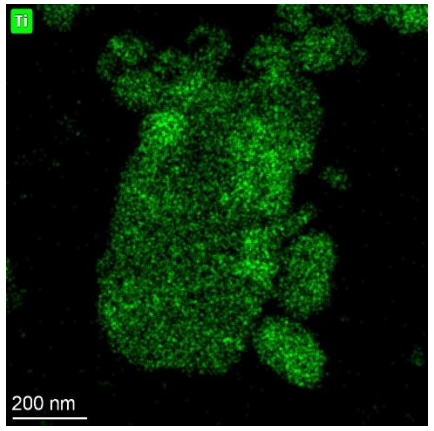
= NOT E 171

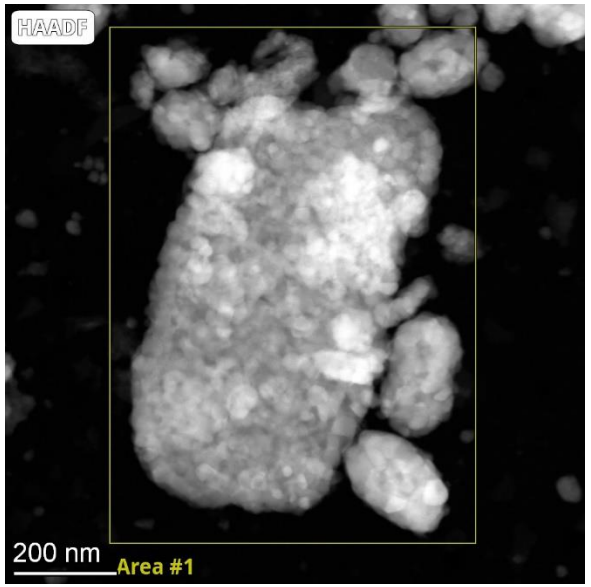
Legal interpretation of FA specifications

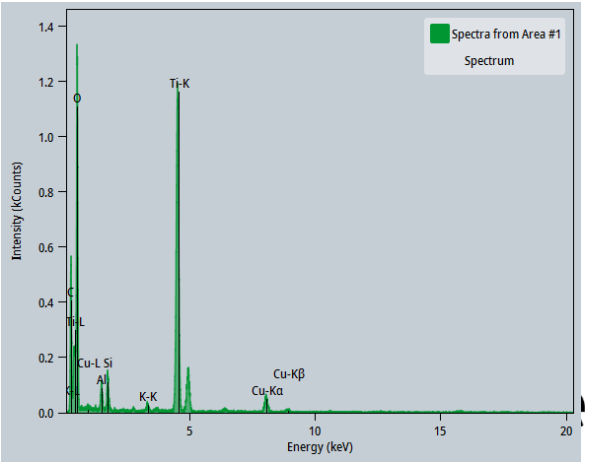








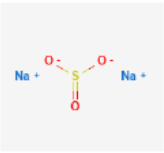
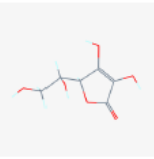
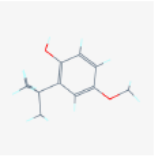
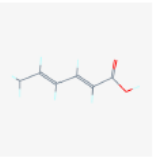
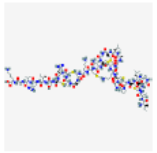
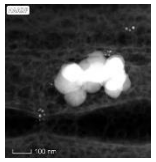
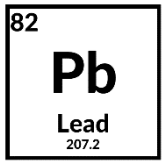




Verleysen, et al. (2020). Physicochemical Characterization of the Pristine E171 Food Additive by Standardized and Validated Methods. *Nanomaterials*, *10*(3), 592.

ANALYTICAL CHALLENGES

Wide variety of targeted compounds

Organic	Inorganic	Polar	Non-polar	Low MW	High MW	NP	Trace elements
							
Benzoic acid (E 210)	Sodium sulphite (E 221)	Ascorbic acid (E 300)	BHA (E 320)	Sorbic acid (E 200) 112.1 g/mol	Nisin (E 234) 3354.1 g/mol	Titanium Dioxide E171	Impurities

Large (and unpredictible) concentrations range

Ex:
Edible ices

MPL_{E969} < MPL_{E427} < MPL_{E964}

10 mg/kg

2500 mg/kg

200000 mg/kg

Diversity and complexity in matrices

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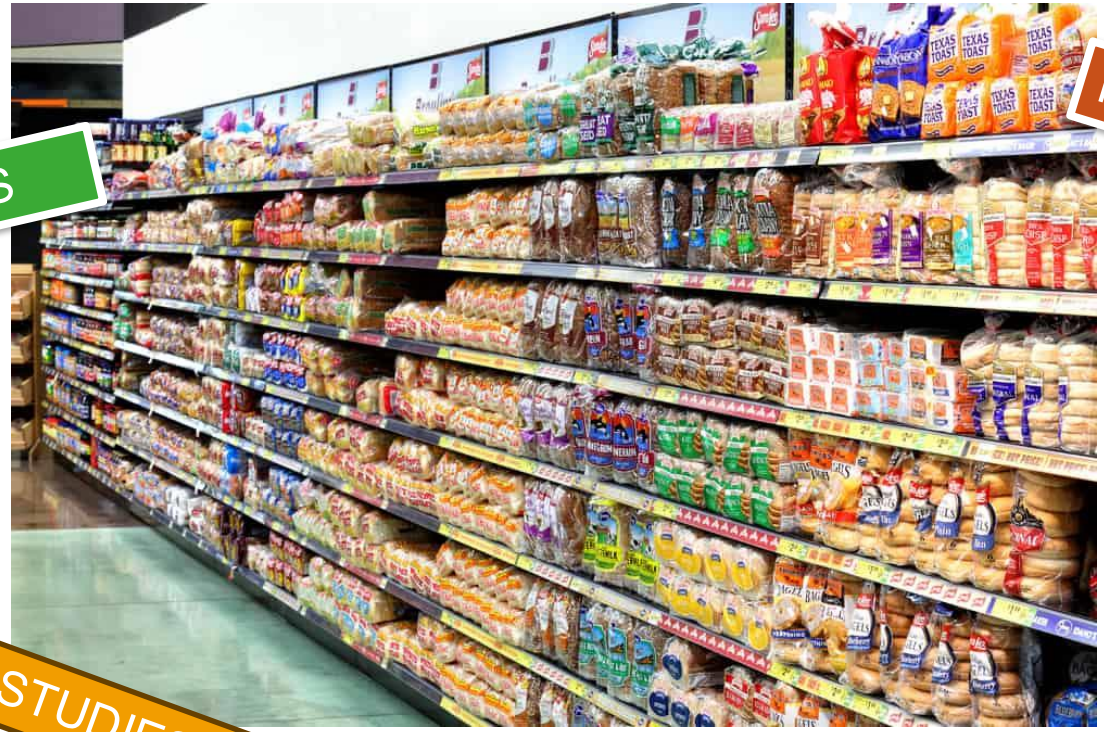
METROLOGY

How to deal with such a variety of foods ?



REFERENCE MATERIALS

METHOD VALIDATION

PROFICIENCY STUDIES

STANDARDISATION

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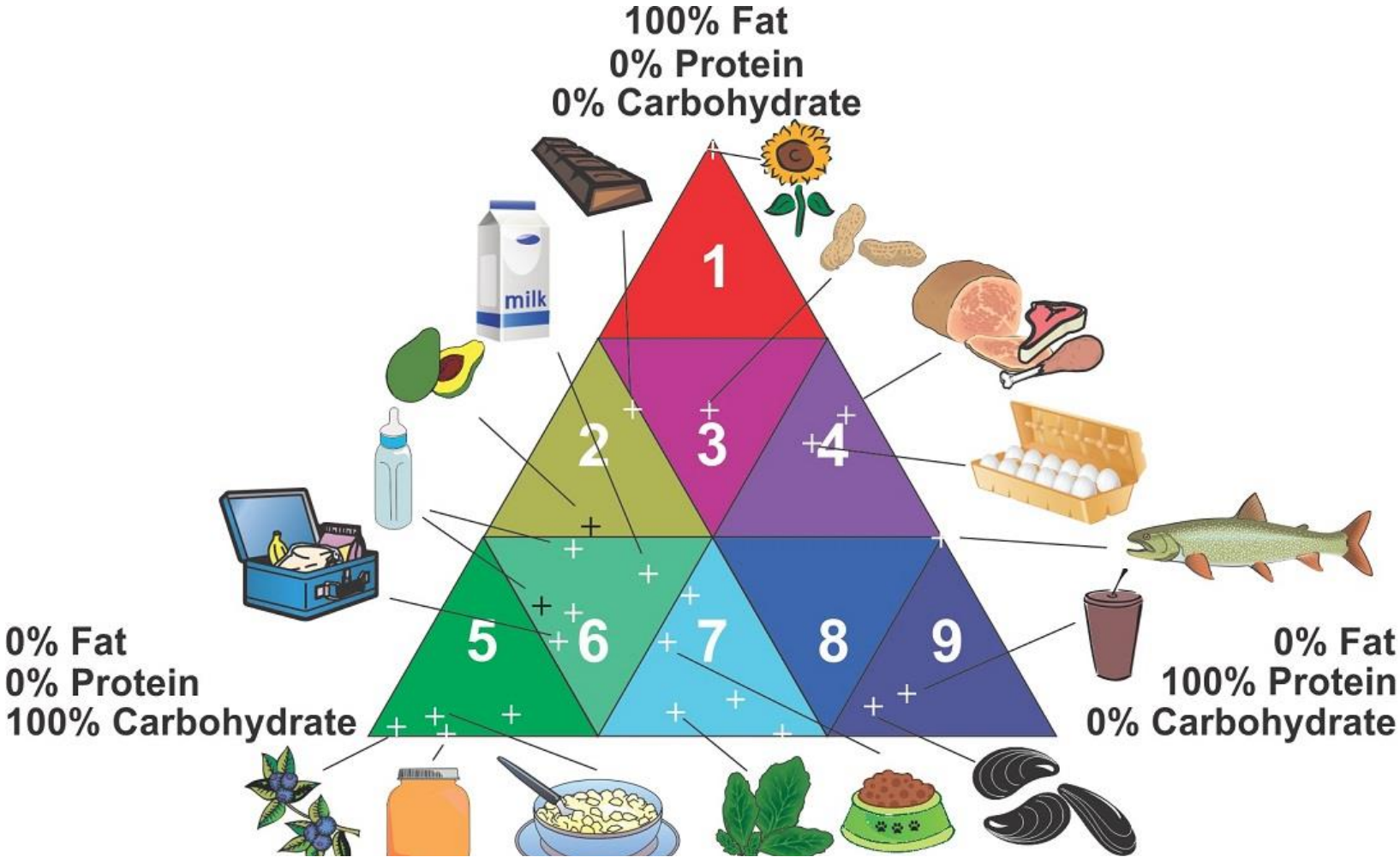
METHOD VALIDATION



Detry, et al. (2022). Development, validation and application of multi-class methods for the analysis of food additives by liquid chromatography coupled to tandem mass spectrometry. *Food Additives & Contaminants: Part A*,

CONCLUSIONS

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Wide variety of targeted compounds

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	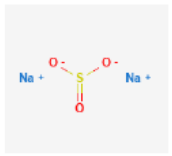	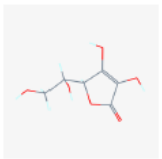	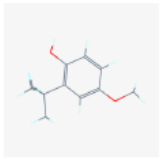	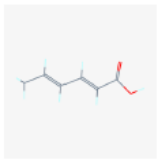	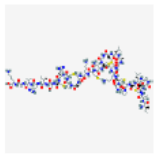
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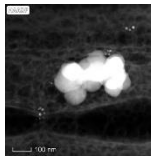	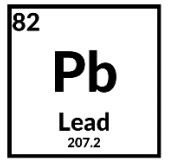
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Clean label

Carry over

Metrology

Methods

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Acknowledgement

Colleagues: Eveline Verleysen, Pauline Detry, Salvatore Ciano, Karlien Cheyns, Els Van Hoeck, Jan Mast

BE Federal Service of Public Health, Horizon Europe, EFSA for Funding the food additives projects

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QUESTIONS

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