



Food Additives – laboratory analytical methods

Martin Kubík

*Czech Agriculture and Food Inspection
Authority (CAFIA)*

Introduction





Czech Agricultural and Food Inspection Authority (CAFIA)

- state administration body (subordinate to the Ministry of Agriculture)
- state authority responsible for supervision of safety, quality (authenticity) and labelling of foodstuffs.
- focused on the products of plant origin in all stages, foodstuffs of animal origin in retail), from 2015 food catering

Additives – state supervision in the Czech republic

Responsibilities of authorities

- Food inspection of food in Czech in competency of two ministries (Agriculture and Health)
- **Czech Agriculture and Food Inspection Authority** (products of plant origin in all stages, foodstuffs of animal origin in retail, food in catering), subordinate to the Ministry of Agriculture
- **State Veterinary Administration** (products of animal origin), subordinate to the Ministry of Agriculture
- **National Institute of Public Health** (minor part of foods in catering), subordinate to the Ministry of Health

Additives – to be analyzed

Usually focus on the additives where maximum limit has been set

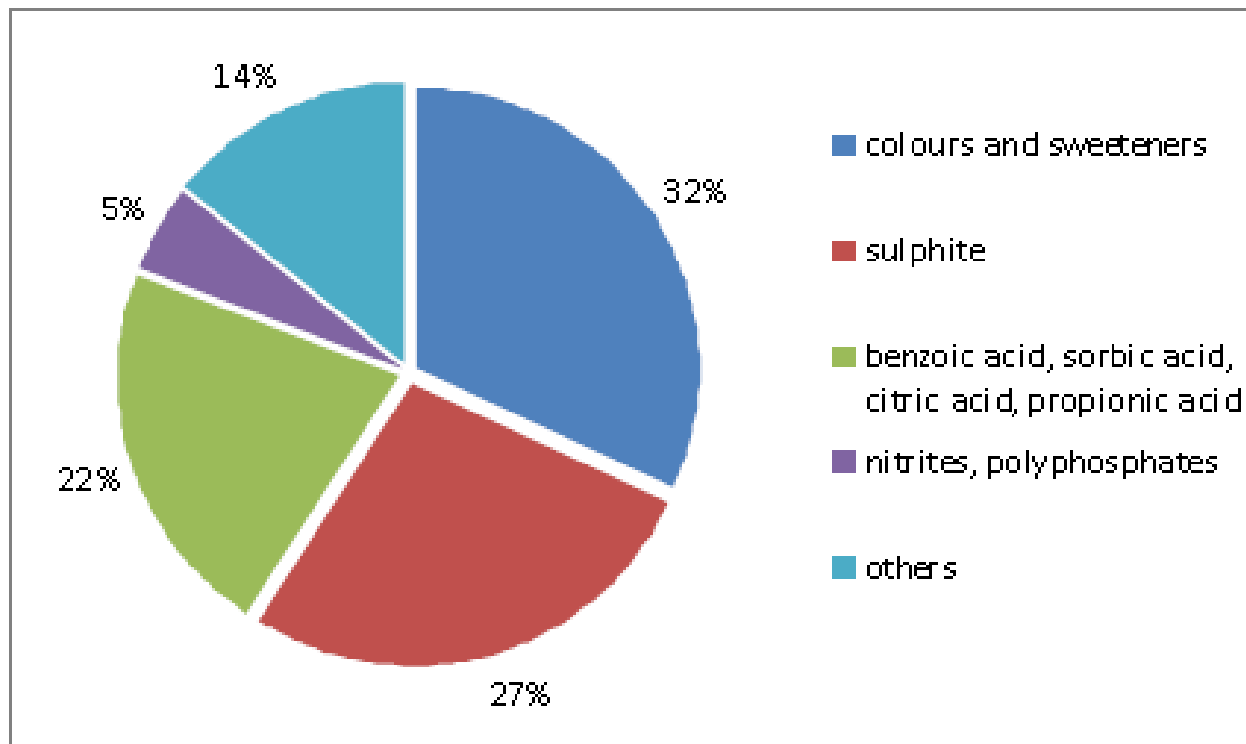
- according to regulation 1333/2008 27 functional classes of food additives

- not all analysed

USUALLY supervised and analysed

- **Preservatives** (benzoic acid, sorbic acid, p-hydroxybenzoates, formic acid, sulphur dioxide, dimethyldicarbonate, propionic acid, nitrates, nitrites)
- **Colours** (synthetic and some natural and also – non permitted „sudan“ dyes)
- **Sweeteners** (saccharine, aspartame, acesuphame K, cyclamate, neohesperidine DC, polyols), sucralose, steviol glycosides...
- **Other** – polyphosphates, glutamate, acids, antioxidants (BHT, BHA, gallates, TBHQ, erythorbic acid)....

Additives – to be analyzed



Number of notifications in RASFF system for period 2010-2012

Source: European Commission - Institute for Reference Materials and Measurements (Geel)

Additives – analysis

Requirement – official control laboratories
Shall be accredited according to standard EN
ISO/IEC 17025

Priority to use community or standardised
methods of analysis (EN, ISO, Codex,
(AOAC)) – advantage for the case of
dispute

👉 but not always available (internal methods
– single laboratory validated proved in
proficiency testing schemes, if available)

Methods available - preservatives

Benzoic and sorbic acids and its salts

- no community method
- **Codex Alimentarius** recommends:
no general method for foodstuffs but
for Fruit Juices and Nectars:
 - IFUMA 63, NMKL 124 (HPLC), ISO 5518, ISO 6560 (only benzoic acid, spectrophotometry), ISO 5519 (only sorbic acid, spectrophotometry)for Processed fruits and vegetables
 - NMKL 103 / AOAC 983.16 (only sorbates - gas chromatography), NMKL 124 ((only sorbates - liquid chromatography)
- **CEN** (EN standards) - no methods
- **ISO**
 - ISO 22855:2008 Fruit and vegetable products (HPLC)
 - ISO 9231:2008 Milk And Milk Products (HPLC)
- AOAC methods – not practicable (TLC, GC, Spectrometry)
- old and laborious

Methods available - preservatives

Sulphur dioxide, sulphites

- no community method
- **Codex Alimentarius** recommends:
 - Individual Foods (Hominy, fruit juice, sea food) - EN 1988-1 : 1998-02, AOAC 990.28 (Optimized Monier-Williams method)
 - Individual Foods (Wine, dried apples, lemon juice, potato flakes, sultanas, beer) EN 1988-2:1998 -02, NMKL 135 (1990) (Enzymatic method)
 - Fruit Juices and Nectars AOAC 990.28 , IFUMA 7A, NMKL 132 (Optimized Monier Williams) Type II, NMKL 135 (enzymatic) Type III, ISO 5522, ISO 5523 (Titrimetry after distillation) Type III
 - Dried apricots, Raisins AOAC 963.20 (Colorimetry – p-rosaniline) II
 - Sugars ICUMSA GS 2/3-35 NMKL 135 EN 1988-2 (Enzymatic) II, ISO 5379 (Acidimetry and nephelometry) IV
- **CEN**
 - EN 1988-1:1998 Foodstuffs - Determination of sulphite- Part 1: Optimized Monier-Williams method
 - EN 1988-2:1998 Foodstuffs - Determination of sulphite- Part 2: Enzymatic method

Methods available - preservatives

Sulphur dioxide, sulphites

○ **ISO**

- ISO 5523:1981 Liquid fruit and vegetable products -- Determination of sulphur dioxide content (Routine method) Direct iodine titration – not specific for all products
- ISO 5522:1981 Fruits, vegetables and derived products
- ISO 5521:1981 Fruits, vegetables and derived products

Monier-Williams method mostly used as reference method but it has limitations (, onion, garlic, cabbage, leek, ginger soya protein, mustard?).

Garlic difficult to analyse by any method.

Methods available - preservatives

Nitrates, Nitrites (meat products)

- no community method
- **Codex Alimentarius** recommends:
 - All Foods EN 12014-1:1997-04 Part 1- General considerations
 - Processed meat and poultry product ISO 3091 (Nitrates, Colorimetry (cadmium reduction)) II, ISO 2918 (Nitrites, Colorimetry) IV
 - Canned corned beef, Cooked cured chopped meat AOAC 973.31 (Codex general method) (Nitrites, Colorimetry) II, ISO 2918 (Nitrites, Colorimetry) IV
- **CEN**
 - EN 12014-1 Foodstuffs - Determination of nitrate and/or nitrite content - Part 1: General considerations
 - EN 12014-3 Foodstuffs - Determination of nitrate and/or nitrite content - Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite
 - EN 12014-4 Foodstuffs - Determination of nitrate and/or nitrite content - Part 4: Ion-exchange chromatographic (IC) method for the determination of nitrate and nitrite content of meat products

Methods available - preservatives

Nitrates, Nitrites

○ **ISO**

- ISO 2918:1975 Meat and meat products -- Determination of nitrite content (Reference method) – Colorimetry
- ISO 3091:1975
- Meat and meat products -- Determination of nitrate content (Reference method) - Colorimetry (cadmium reduction)

Methods available - sweeteners

- no community method
- **Codex Alimentarius** recommends:
 - All Foods:
 - EN 12856 : 1999-04 Acesulfame K, Aspartame (HPLC) II
 - EN 12856 : 1999-04 Saccharin (HPLC) III
 - EN 12857 : 1999-04 Cyclamate (HPLC) II
 - NMKL 123 (1998) Cyclamate (Spectrophotometry) III
 - Fruit Juices and Nectars - NMKL 122 Saccharin (HPLC) II
- **CEN**
 - EN 12856 - Foodstuffs - Determination of acesulfame-K, aspartame and saccharin (HPLC)
 - EN 12857 - Foodstuffs - Determination of cyclamate (HPLC)
 - CEN/TS 14537 - Foodstuffs- Determination of neohesperidin-dihydrochalcon (HPLC)
 - EN 15606 - Foodstuffs - Determination of acesulfame-K, aspartame, neohesperidine dihydrochalcone and saccharin (HPLC)

Methods available - sweeteners

○ **CEN**

- EN 15086 - Foodstuffs - Determination of isomalt, lactitol, maltitol, mannitol, sorbitol and xylitol in foodstuffs (HPLC)
- EN 15911 - Foodstuffs - Simultaneous determination of nine sweeteners (Acesulfame-K, Alitame, Aspartame, Dulcin, Neotame, Neohesperidine dihydrochalcone, Saccharin, Cyclamate, Sucralose, HPLC- ELSD)
- EN 16155 Foodstuffs - Determination of sucralose (HPLC-RID)
- EN 15911

Table top sweeteners:

- EN 1376+AC - Determination of saccharin in table top sweetener preparations - Spectrometric method
- EN 1377+AC - Determination of acesulfam K in table top sweetener preparations - Spectrometric method
- EN 1378+AC - HPLC determination of aspartame in table top sweetener preparations
- EN 1379+AC - HPLC determination of cyclamate and saccharin in liquid table top sweetener preparations

Methods available - sweeteners

- **regulated but no standards**

- Thaumatococcus
- Steviol glycosides
- Salt of aspartame-acesulfame?
- for neotame EN 15911 but validated for low range on matrixes and too high LOQs



use of single validated methods – based on HPLC, GC, EC, IT, TLC, immunochemical assays (thaumatococcus)...

Need for standardization!

Synthetic food colours – regulated by reg. 1333/2008

E-number	Name	E-number	Name
E 102	Tartrazine	E 129	Allura Red AC
E 104	Quinoline Yellow	E 131	Patent Blue V
E 110	Sunset Yellow FCF/Orange Yellow S	E 132	Indigotine, Indigo carmine
E 122	Azorubine, Carmoisine	E 133	Brilliant Blue FCF
E 123	Amaranth	E 151	Brilliant Black BN, Black PN
E 124	Ponceau 4R, Cochineal Red A	E 155	Brown HT
E 127	Erythrosine		

+ green (E141)

Methods available - colours

Synthetic food colours

- no community method
- no Codex method
- no CEN method
- ISO:

ISO 13496:2000 Meat and meat products - Detection of colouring agents - Method using thin-layer chromatography

The colouring agents are extracted from a test portion with hot water and adsorbed onto polyamide powder. The extracted colouring agents are purified by column chromatography and the colours are eluted from the column. The colouring agents are identified by thin-layer chromatography.



Methods available - colours

Synthetic food colours - continuation

Laboratories use single laboratory validated methods based on TLC (qualitative methods), spectrometry, voltammetry; differential pulse polarography; capillary electrophoresis; high performance liquid chromatography (HPLC) and ion chromatography

Majority of laboratories use

TLC for identification

HPLC – DAD for quantification

Some laboratories analyse only drinks (other samples – difficult sample preparation)

For Brown HT (E 155) method is not available

Natural food colours - regulated by reg. 1333/2008

- (1) **Curcumin** (E 100) – *limits some foods*
- (2) Riboflavins (E 101i-ii) - *mostly quantum satis*
- (3) **Cochineal** – including carminic acid (E 120) - *limits*
- (4) Chlorophylls – including chlorophyllins and copper analogues (E 140–141) *quantum satis*
- (5) Caramels – Classes I–IV (E 150a–d) *quantum satis*
- (6) **Carotenoids** – (E 160a–e, E 161b, E 161g) *limits*
- (7) Beetroot red, betanin (E 162) *mostly quantum satis*
- (8) Anthocyanins (E 163) *quantum satis*.
- (9) Others: Vegetable carbon (E 153), Calcium carbonate (E 170), Titanium dioxide (E 171) and Iron oxides and hydroxides (E 172) *quantum satis*

Some of them combined limit with artificial colours (group III)
serious problems for laboratory compliance – a few laboratories
are able to determine natural colours

Methods available - colours

Natural food colours

Curcumin (E 100)

in a literature described HPLC DAD and FLD. Extraction conditions generally simple and various clean-up techniques have been reported, but have not been validated for all regulated foodstuffs.

Methods must be capable of detecting and quantifying all three main curcuminoids; curcumin (CUR) along with its demethoxy (DMC) and bisdemethoxy (BDMC) analogues. Therefore, the access to suitable reference materials for the main colouring principles is crucial

Cochineal – including carminic acid (E 120) - in a literature described HPLC with DAD, UV-VIS or fluorescence detection . A number of methods reasonably well established and validated for different sample types. Reversed phase HPLC with UV-VIS detection is the preferred measurement technique

Methods available - colours

Natural food colours

Carotenoids – (E 160a–f, E 161b, E 161g)

E 160a	Carotenes
E 160b	Annatto
E 160c	Paprika extract, capsanthin, capsorubin
E 160d	Lycopene
E 160e	Beta-apo-8'-carotenal (C 30)
E 161b	Lutein

E161g cantaxanthin authorised only for medicinal products

Methods available for Annatto and Lutein - usually HPLC

For all carotenoid problems with availability and stability of well defined reference materials



Methods available - colours

Unauthorised colours (sudan dyes)

- HPLC DAD or MS methods after acetonitrile extraction used

Methods available - others

- antioxidants
 - AOAC 983.15 interlaboratory validated HPLC methods – 9 antioxidants (BHT, BHA, gallates, THBP, TBHQ, Ionox-100 in edible oils)
- polyphosphates
 - ISO 5553 – TLC determination of polyphosphates
 - single laboratory quantitative methods based on phosphorus content and correction to naturally present phosphorus
- monosodium glutamate (MSG)
 - HPLC methods (after derivatization) – not possible to differentiate naturally present free glutamic acid and MSG

Additives – analysis

Technics used in CAFIA

- TLC – only qualitative determination or screening (colours, polyphosphates) – quantification by HPLC after; or detection unauthorised use or undeclared additives
- HPLC different detections – most frequently UV/VIS or DAD – quantitative determination (confirmation)
- Spectrophotometry
- (LC-MS – new trend – confirmation analysis and also plans multimethods)



Choice of suitable method

- method has to fit for purpose – LOQ has to be significantly lower than maximum limit in regulation 1333/2008, has to be suitable for type of food analysed
- new trend use so called multimethods LC-MS/MS



Thank you for your attention