By-Product Characterisation Strategies

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22 March 2016
Selection of Issues relating to By-product use since 1978

1981 Food oils contamination (Spain)
1998 Dioxins in citrus pulp (Germany, Netherlands)
1999 Dioxins, PCBs in feed (Belgium)
2001 Polycyclic Aromatic Hydrocarbons in olive oil derived from pomace (Spain)
2004 Iodine in soy milk fortified with Kelp (New Zealand)
2008 Dioxins in Pork due to oils in feed (Ireland)
2009 Iodine in soy milk fortified with Kombu (Australia)
Regulations regarding Testing of products for Food in EU

General Food Law 178/2002/EC

Assigns primary legal responsibility for the safety of products on market to business operator

EFSA oversees and enforces this regulation
Requirements for Additional Characterisation

Where Certain claims are made for a food further characterisation is required. This includes -

Novel Food (258/1997/EC)
Food for particular nutritional use (89/398/EC)
Food additives (89/107/EC)
Supplements (2002/46/EC)
Flavourings (91/71/EC, 88/388/EC)
Regulations on Health Claims

Regulated by 1924/2006/EC

Food or component should be well characterised

Characterisation methods are defined for components, mixtures and classes
Beyond Legal requirements

Determination of the Quality of Ingredients from By-Products

Characterisation of products during preliminary trials

Use of analysis to determine where methods can be improved
Review of Characterisation Strategies

TLC - Purity of product, useful for lab-scale checking

Infrared (IR) - Useful fingerprint of product, also functional groups, gross impurities

Ultraviolet (UV) - Useful fingerprint of product

Atomic Absorption (AA) Analysis of trace elements and metals

GC - Purity of product, useful for volatile compounds, impurities

LC - Purity of product, can be combined with other methods

Nuclear Magnetic Resonance spectroscopy (NMR) - Structure of product in solution

MS - Composition of product, determination of purity

Tandem methods GC-MS, LC-MS, LC-NMR

Electrochemical Methods: Sensors, Biosensors
Mass Spectrometry

Both Qualitative and Quantitative

Identifies molecular weight and primary structure of compounds

Highly sensitive method

Can fail to differentiate between different geometric isomers
Nuclear Magnetic Resonance Spectroscopy

Provides detailed information about structure, dynamics and environment of molecules in solution

Used to confirm identity of a substance

High quantity needed, in 2 - 50 mg range

Not a highly sensitive method

Requirement for deuterated solvents
Detailed Chemical Characterisation

LC or GC to show purity

MS to determine molecular weight and formula

NMR to determine detailed structural information

Other methods, IR, UV may complement this
Practical Examples of Methods
Nitrofurans Residue Testing

Nitrofurans are a class of antibiotics banned for use in the EU Directive 96/23/EC requires programme of self-monitoring for these and other residues in food of animal origin
Teagasc Method uses Liquid Chromatography coupled with Tandem mass spectrometry (LC-MS/MS)
Method detects and quantifies in a single analysis metabolites of four of the main nitrofuran drugs
Validated for liver, muscle, fish, plasma, egg and honey according to 2002/657/EC guidelines
Limit of detection is below 0.10 ug/kg
Apple Pomace

By-product of juice and cider industries

Contains high levels of antioxidants

Acidic, High in fibre, High in sugars

Stabilised by oven drying before use

Powdered Pomace or extracts intended as food additive

Distinct off-odour noted
Analysis of crude extract of Pommace by NMR
Analysis of crude extract of Pomace by NMR

Hydroxymethyl Furfural identified as being present

Responsible for off-odour

Product of Maillard-type Reactions

Considered to be an indicator for heat processing of manufactured foods

Not a safety concern but potential to lower quality perceptions

Method altered to exclude oven drying, freeze dry instead
Pectin from pomace - Determination of quality via NMR

Pectin - Polysaccharide which tends to form gels

Gelling properties related to degree of methoxylation and methylation

Proton NMR used to determine these structural elements

Physicochemical assessment of two fruit by-products as functional ingredients: Apple and orange pomace.
Pectin from pomace - Determination of quality via NMR

Characterisation of Chitosan NMR

Chitosan soluble polycationic derivative of insoluble chitin

Sparingly soluble

 Desired qualities depend on Degree of N-acetylation

Degree of N-acetylation can be determined by NMR

Characterisation of Chitosan NMR

![NMR Spectrum]

- Peaks at around 6.65, 5.55, and 2.15 ppm
- Analysis of chemical shifts and integrals
Combination of LC-NMR and LC-MS used on extract

Allowed identification of family of compounds present in extracts

Typical of Strategy to be used for identifying new components.

Additional characterisation for Bioactivity can also be useful
4-Hydroxyphenylacetic acid derivatives of inositol from dandelion (Taraxacum officinale) root characterised using LC-SPE-NMR and LC-MS techniques, O Kenny, T.J. Smyth, C.M. Hewage, N.P. Brunton, P McLoughlin, Phytochemistry, 98, February 2014, (197-203)
Challenges

Solubility

Sampling error

Matrix effects

Interference
Conclusions

Know regulations for particular product

Be aware of recommended analytical methods

Ensure that method is robust

Methods can be used together to complement each other

Analyse early and often
Any Questions?

Thank you

Hvala