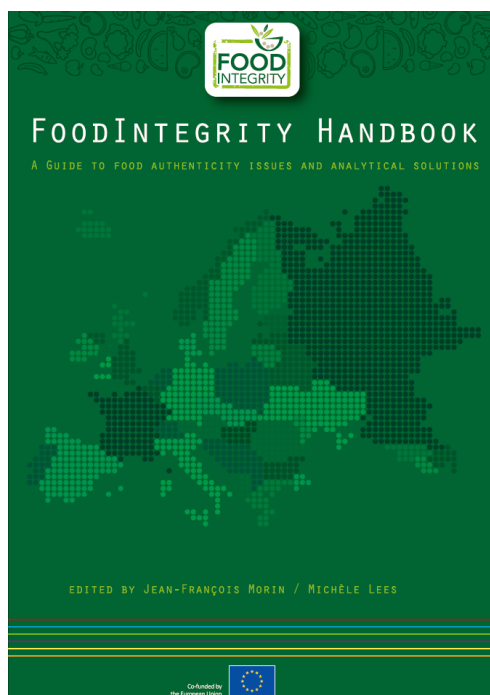# FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

Editors: Jean-François Morin & Michèle Lees, Eurofins Analytics France

Food flavourings

Eric Jamin*, Freddy Thomas
Eurofins Analytics France, Nantes, France
E-mail corresponding author: EricJamin@eurofins.com

General overview of the product

The roots of the flavourings industry date back to early Egyptian history, when simple methods for the distillation and extraction of essentials oils and resins were first elaborated. In medieval times, monks pioneered the production of food flavourings. Then at the beginning of the 19th century, the industrial production of essential oil started, quickly followed by the first syntheses of single aroma chemicals such as vanillin. In the 20th century, the flavour industry then expanded from Europe to the USA. Today's leading companies are usually those early European ones that are now based around the world [1].

The total flavour and fragrance market, valued at USD 9.6 billion in 1995, has nearly doubled in the ensuing decade, with the global share of food flavouring being approximately 50 % [1].

While the overall consumption of food flavourings has been growing over the last 100 years, consumer demand has changed dramatically since the 1970s, moving towards more "natural", "fresh", and "functional" ingredients.

There have also been changes in processing with a major trend towards the use of biotechnology (fermentation) and "soft chemistry" processes which aim to combine "naturalness" with economic value, as the traditional processes are too expensive and artificial flavours are less attractive to consumers.

Establishing analytical criteria to control naturalness is therefore of the utmost importance to ensure fair trade in food flavourings. Quality Assurance managers in the food industry should also be aware of the definition, regulations and analytical methods that are used to monitor the authenticity of these very specific and high value ingredients.

1. Product Identity

1.1. Definition of the product and manufacturing process

The three main categories of flavourings are:

- Essential oils and natural extracts are obtained from natural sources such as flowers, fruits, etc. The processes used included solvent extraction, steam distillation, etc.

- Aroma chemicals are single compounds, either produced by chemical synthesis or biotechnology processes.

- Formulated flavours are complex blends of aromatic materials.

1.2. Current standards of identity or related legislation

1.2.1. European Union (EU) regulations

The EU Regulation 1334/2008 [2] defines flavourings as "products: (i) not intended to be consumed as such, which are added to food in order to impart or modify odour and/or taste." Their use is only permitted provided that "they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer; and their use does not mislead the consumer".

The EU regulation then differentiates several types of flavouring types and components. A simplified version of the definitions is given below:

- "'**flavouring substance**' shall mean a defined chemical substance with flavouring properties": these are pure chemical compounds, which can be produced from any source by any process".

- "'**natural flavouring substance**' shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II": these correspond to substances that are "naturally present and have been identified in nature".

- "'**flavouring preparation**' shall mean a product, other than a flavouring substance, obtained from: (i) food […] and/or (ii) material of vegetable, animal or microbiological origin, other than food […] and/or (ii) material of vegetable, animal or microbiological origin, other than food". These are usually mixtures of many compounds, either "taken as such" or "prepared by one or more of the traditional food preparation processes listed in Annex II".

- "'**thermal process flavouring**' shall mean a product obtained after heat treatment from a mixture of ingredients not necessarily having flavouring properties themselves, of which at least one contains nitrogen (amino) and another is a reducing sugar; the ingredients for the production of thermal process flavourings may be: food; and/or (ii) source material other than food". This is typically caramel!

- "'**smoke flavouring**' shall mean a product obtained by fractionation and purification of a condensed smoke yielding primary smoke condensates, primary tar fractions and/or derived smoke flavourings[…]".

- "'**flavour precursor**' shall mean a product, not necessarily having flavouring properties itself, intentionally added to food for the sole purpose of producing flavour by breaking down or reacting with other components during food processing; it may be obtained from: (i) food; and/or (ii) source material other than food".

The above mentioned 'appropriate physical process' "shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring, without prejudice to the listing of traditional food preparation processes in Annex II, and does not involve, inter alia, the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation."

Besides Europe, national regulations exist in other parts of the world, but they will not be covered here. A complete review can be found in [1].

1.2.2. IOFI guidelines

IOFI is the International Organisation of the Flavour Industry, based in Geneva, Switzerland. The IOFI Code of practice [3] provides additional comments and interpretation of the EU regulation. In particular it includes guidelines on the IOFI Interpretation of the Term "Natural".

1.2.3. Fruit juice directive

The EU fruit juice directive 2012/12 [4] is the only case of a vertical directive for food including specific requirements for the flavour component of the juice. Above all, it requires the use of "suitable processes, which maintain the essential physical, chemical, organoleptic and nutritional characteristics of an average type of juice of the fruit from which it comes." The flavour lost during processing may be restored. But in any case the flavour profile must not deviate from typical compositions.

Interpretation therefore requires expertise and reference knowledge regarding typical values found in juices, for all types of fruits and processes used by this industry.

2. Authenticity issues

2.1. Identification of current authenticity issues

Thanks to Mother Nature's gifts on one hand, and human creativity on the other hand, a wide range of natural sources and processes can be used to produce food flavourings, which are usually complex mixtures of chemical compounds. Many organic compounds have a flavouring impact, which is not correlated to their concentration. What makes it even more subtle is that the final sensory impact depends on all flavour compounds present, their proportions, and even the effect of the food matrix itself on the perception. Synthetic mixtures may smell as beautiful and natural as extracts, and conversely some natural sources may produce poor quality aromas. Therefore using one's nose is not sufficient for judging the authenticity of an aroma.

Natural flavourings are among high risk ingredients regarding economic food fraud, because of their high price and the availability of cheaper substitutes. The most commonly encountered fraud is the addition of synthetic compounds which are chemically identical to the main component(s) of a given natural flavouring. Typical examples are the addition of synthetic vanillin or para-hydroxybenzaldehyde to vanilla extracts / aromas, or the addition of synthetic benzaldehyde to bitter almond oil.

2.2. Potential threat to public health

Some flavouring substances may have negative health impacts: some have been classified as allergens, and must be labelled on perfumes and cosmetic products, and some others have been defined as "biologically active compounds", for which a maximum concentration threshold is defined in the Annexe III of the EC Regulation 1334/2008 [2]. Typical examples are pulegone and menthofuran in mint products (confectionery, drinks).

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. Application of GC-MS methods

Due to the volatile nature of flavour components, gas chromatography (GC) is the most common way of isolating them. The most widely used type of detector is Mass Spectrometry (MS), which enables each compound to be precisely identified.

A first approach is to screen all or almost all compounds present, identify and quantify them, in order to compare the obtained flavour profile with reference data, which may be a database or literature data. This can reveal the use of source material other than the ones declared, or the presence of artificial compounds.

Direct GC-MS analysis can be performed directly on matrices such as essential oils and aroma. On the other hand, the analysis of flavoured food products usually requires a prior step of extracting the volatile fraction.

3.1.1.1. Common extraction methods

Simultaneous Distillation Extraction (SDE) is the technique used in the German official method, and used in most expert laboratories, especially for testing fruit juices and fruit products. The main advantage is that this technique enables the extraction of a wide range of compounds, thus covering the main aroma compounds of fruit flavours: esters, lactones, alcohols, aldehydes, etc. It is also applicable to mint flavours, either in drinks or in confectionery, and even works for fruit-flavoured dairy products.

For matrices containing a significant amount of alcohol (wines, spirits, liquors, alcohol-based flavours), the above approach is no longer applicable, and a liquid-liquid extraction usually offers a better recovery of the compounds of interest.

Modern instrumentation enables an on-line extraction, followed by direct injection to the GC-MS instrument. This allows for a better productivity in the laboratory, but should be handled with care to avoid losing information or getting some artefacts. The main ones are:

- Headspace injection systems: various systems exist to introduce the headspace gas above a sample into the chromatograph.

- Solid-Phase Micro Extraction (SPME): the compounds of interest are adsorbed on a fibre coated with a specific material, and successively desorbed in the GC injector.

- Stir-Bar Sorptive Extraction (SBSE): the compounds of interest are adsorbed on a stir bar with a specific material, and successively desorbed in the GC injector.

3.1.1.2. Flavour profiles

In full scan mode, GC-MS chromatograms usually show around one hundred identifiable and quantifiable compounds. Most of them can be identified through the NIST MS database. An accurate quantification then requires determining individual response factors of each compound, taking into account both the extraction rate and the chromatographic response.

Absolute concentration values are not essential for the authenticity assessment. The presence of foreign molecules such as undeclared solvents or artificial / untypical components is the first type of deviation to be considered. However, the relative proportions and orders of magnitude must also match with the product type: unbalanced proportions tend to indicate the use of specific compounds instead of full extracts. Some guidelines have been published regarding key-components, for example in apple juices [5,6].

Finally some regulatory limits are defined for biologically active compounds. When judging the compliance regarding those thresholds, the analytical uncertainty should be taken into account.

3.1.1.3. Chiral analysis

Using the enantioselectivity of chiral chromatography stationary phases, the R and S spatial conformations (i.e. enantiomers) of chiral molecules can be appropriately separated and quantified.

Most industrial chemical synthesis processes use mineral catalysers, leading to racemic mixtures of R and S forms. Some fine chemistry methods might also produce pure enantiomers. On the other hand, natural biosynthesis in the plants implies enzymatic catalysers, which usually lead to an enantiomeric excess of one of the forms R or S, depending on the compound and the plant. Therefore chiral analysis is an effective means of detecting synthetically produced compounds, based on appropriate reference knowledge about enantiomeric distributions.

This also requires expert interpretation, as some natural compounds may undergo natural racemisation processes.

3.1.2. Application of carbon 14 activity measurement

Due to their age, fossil organic molecules do not possess any carbon 14 activity. On the other hand the activity of natural molecules reflects the current ^{14}C activity of CO_2 in the atmosphere. Measuring ^{14}C activity therefore can be used to detect compounds made partly or totally from fossil precursors [1]. This can be applied to pure compounds or mixtures containing some major compounds, such as some essential oils.

Nevertheless it should be emphasised that synthetic compounds produced from natural precursors are not detected by ^{14}C activity measurements.

3.1.3. Application of stable isotope ratio analysis

3.1.3.1. Isotope Ratio Mass Spectrometry (IRMS)

The global isotopic ratios of stable isotopes of carbon, hydrogen, oxygen, nitrogen or sulphur can be used, usually discriminating between extractive, synthetic and biosynthetic sources.

When coupled to gas chromatography (GC), such measurements can be applied to individual compounds from a mixture, as recently reviewed for aromas in general and essential oils in particular [7]. A typical example is the analysis of the ^{13}C deviation of vanillin, to check a declared

vanilla beans origin. Not only can this method be applied to flavouring ingredients such as beans, extracts, and aromas, but also to finished products such as vanilla-flavoured ice-cream, cakes, etc.

After a solvent extraction, applied to the vanilla-flavoured food or ingredient, the volatile components are separated by GC and the peak of vanillin is selectively submitted to combustion and $^{13}C/^{12}C$ isotopic ratio measurement. Since the ^{13}C deviation of agricultural vanillin is less negative than most of its artificial counterparts, it is possible to detect blending or substitution. However this approach is not sufficient for precisely identifying the artificial sources.

3.1.3.2. Site-Specific Natural Isotopic Fractionation – Nuclear Magnetic Resonance (SNIF-NMR)

This method introduced in the 1980s makes it possible to go one step further by determining isotopic ratios at the different positions within a molecule, thus providing more precise information [8]. Initially applied to deuterium, SNIF-NMR has been used to authenticate key-flavour molecules such as vanillin [9–11], benzaldehyde [12], anethole [13], raspberry ketone [14], etc.

More recently, suitable conditions have been developed for the quantitative NMR measurement of isotopic ratios of carbon, opening the path to new authentication possibilities [15–18]. Indeed the higher natural abundance of ^{13}C versus deuterium and the possibility to use polarisation transfer dramatically reduces the amount of pure compound required for the measurement, while keeping a satisfactory level of discrimination between sources [19].

3.2. Other commonly used methods

Liquid chromatography is the most suitable technique for analysing thermally instable or non-volatile compounds. A typical example is the use of limonin, a key compound causing bitterness in citrus drinks. This can also be used especially for "salty" aroma in which the impact compounds are usually less volatile.

Multi-dimensional chromatography using several columns in series can allow higher selectivity. They also make the analytical process more complex, so their use is usually restricted to specific cases.

4. Overview of methods for authenticity testing

Analytical technique	Indicative data or analyte	Authenticity issue / information
GC-MS (apolar phase)	Composition	Essential oils
SDE, GC-MS (polar phase)	Flavour profile	Fruit products, mint products
SDE, GC-MS (chiral phase)	Enantiomeric profile	Fruit products, mint products
Liquid Scintillation, Accelerator Mass Spectrometry	Carbon 14 activity	Flavouring substances
GC-Combustion-IRMS	^{13}C deviations (of isolated compound)	Vanilla products, Fruit products
GC-Pyrolysis-IRMS	^{2}H deviations (of isolated compound)	Vanilla products, Fruit products
IRMS	^{13}C, ^{2}H, ^{18}O, ^{15}N, ^{34}S deviations	Flavouring substances
^{2}H-SNIF-NMR	Site-specific D/H ratios	Flavouring substances
^{13}C-SNIF-NMR	Site-specific $^{13}C/^{12}C$ ratios	Flavouring substances

5. Conclusion

The world of food flavourings is extremely rich in terms of sources, compounds and sensorial impacts. Their high commercial value and scarce sources make them prone to economic adulteration risks. Climatic and political incidents might be aggravating factors to the fraud risk. The price increase of vanilla beans following the "Enawo" hurricane in Madagascar in 2017 can be taken as an example: the price, that had already increased over the last decades then suddenly doubled from USD 200 per kg to USD 425 per kg, causing a lot of trouble in the market. Similarly, unstable political situations can influence the risk level of supplies for many aroma sources.

When performing authenticity controls, the first question to ask is the precise definition of the flavouring being used, which leads to some expectations based on legal definitions. Then suitable analytical method(s) performed by laboratories having access to appropriate reference knowledge bases should be selected to check whether the composition of the aroma matches with these expectations.

In many cases also the choice of the appropriate method is governed by technical feasibility, and R&D work is still on-going to cover unsolved issues. The identification of precursors has made considerable progress thanks to the use of isotopic methods. One of the most difficult challenges remains the characterisation of processes used for manufacturing these high value ingredients, as a given precursor may be transformed into the final flavouring substance through different ways.

The large amount of information generated by the above-mentioned methods can be exploited in an optimal way using multivariate statistics. And finally, instead of considering only known signals, one can imagine to use the aroma screening as a non-targeted screening, which could enhance the possibility to detect unexpected manipulations of flavours.

6. Bibliographic references

1. Ziegler H., ed. (2007). – *Flavourings: production, composition, applications, regulations*. 2., compl. rev. ed, Wiley-VCH, Weinheim.

2. Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC (2008). *Off. J. Eur. Union*, **L354**, 34–50.

3. IOFU (2012). – International Organization of the Flavor Industry Code of Practice - Effective Date February 29, 2012 - Update version 1.3. IOFU. Available at: http://www.iofi.org.

4. Directive 2012/12/EU of the European Parliament and of the Council of 19 April 2012 amending Council Directive 2001/112/EC relating to fruit juices and certain similar products intended for human consumption (2012). *Off. J. Eur. Union*, **L115**, 1–11.

5. Wolter C., Gessler A. & Winterhalter P. (2008). – Aspects when Evaluating Apple-Juice Aroma. *Fuit Process.*, (March-April 2008), 64.

6. Heil M. & Ara V. (2008). – Aroma of Fruit Juices II: Composition and Valuation of Apple Juice Aroma. *Fuit Process.*, (May-June 2008), 126.

7. Leeuwen K.A. van, Prenzler P.D., Ryan D. & Camin F. (2014). – Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry for Traceability and Authenticity in Foods and Beverages. *Compr. Rev. Food Sci. Food Saf.*, **13** (5), 814–837. doi:10.1111/1541-4337.12096.

8. Jamin E. & Thomas F. (2017). – SNIF-NMR Applications in an Economic Context: Fraud Detection in Food Products. . In *Modern Magnetic Resonance*, Springer, Cham. pp 1–12doi:10.1007/978-3-319-28275-6_103-1.

9. Remaud G.S., Martin Y.L., Martin G.G. & Martin G.J. (1997). – Detection of Sophisticated Adulterations of Natural Vanilla Flavors and Extracts: Application of the SNIF-NMR Method to Vanillin and p-Hydroxybenzaldehyde. *J. Agric. Food Chem.*, **45** (3), 859–866. doi:10.1021/jf960518f.

10. Jamin E., Martin F. & Martin G.G. (2007). – Determination of site-specific (deuterium/hydrogen) ratios in vanillin by 2H-nuclear magnetic resonance spectrometry: collaborative study. *J. AOAC Int.*, **90** (1), 187–195.

11. AOAC 2006.05-2006 - Site-specific deuterium/hydrogen (D/H) ratios in vanillin. Site-specific natural isotope fractionation-nuclear magnetic resonance (SNIF-NMR) spectromety (2006). Available at: http://www.aoacofficialmethod.org/index.php?main_page=product_info&cPath=1&products_id=2362.

12. Remaud G., Debon A.A., Martin Y. loïc, Martin G.G. & Martin G.J. (1997). – Authentication of Bitter Almond Oil and Cinnamon Oil: Application of the SNIF-NMR Method to Benzaldehyde. *J. Agric. Food Chem.*, **45** (10), 4042–4048. doi:10.1021/jf970143d.

13. Martin G.J., Martin M.L., Mabon F. & Bricout J. (1982). – A new method for the identification of the origin of natural products. Quantitative deuterium NMR at the natural abundance level applied to the characterization of anetholes. *J. Am. Chem. Soc.*, **104** (9), 2658–2659. doi:10.1021/ja00373a064.

14. Fronza G., Fuganti C., Guillou C., Reniero F. & Joulain D. (1998). – Natural Abundance 2H Nuclear Magnetic Resonance Study of the Origin of Raspberry Ketone. *J. Agric. Food Chem.*, **46** (1), 248–254. doi:10.1021/jf970530n.

15. Tenailleau E.J., Lancelin P., Robins R.J. & Akoka S. (2004). – Authentication of the Origin of Vanillin Using Quantitative Natural Abundance 13C NMR. *J. Agric. Food Chem.*, **52** (26), 7782–7787. doi:10.1021/jf048847s.

16. Caytan E., Botosoa E.P., Silvestre V., Robins R.J., Akoka S. & Remaud G.S. (2007). – Accurate Quantitative 13C NMR Spectroscopy: Repeatability over Time of Site-Specific 13C Isotope Ratio Determination. *Anal. Chem.*, **79** (21), 8266–8269. doi:10.1021/ac070826k.

17. Caytan E., Remaud G.S., Tenailleau E. & Akoka S. (2007). – Precise and accurate quantitative 13C NMR with reduced experimental time. *Talanta*, **71** (3), 1016–1021. doi:10.1016/j.talanta.2006.05.075.

18. Chaintreau A., Fieber W., Sommer H., Gilbert A., Yamada K., Yoshida N., Pagelot A., Moskau D., Moreno A., Schleucher J., Reniero F., Holland M., Guillou C., Silvestre V., Akoka S. & Remaud G.S. (2013). – Site-specific 13C content by quantitative isotopic 13C Nuclear Magnetic Resonance spectrometry: A pilot inter-laboratory study. *Anal. Chim. Acta*, **788**, 108–113. doi:10.1016/j.aca.2013.06.004.

19. Guyader S., Thomas F., Jamin E., Grand M., Akoka S., Silvestre V. & Remaud G. – Use of 13C and 2H SNIF-NMR® isotopic fingerprint of vanillin to control precursors and processes. *Flavor Fragance J.*, **In press**.