Authenticity testing of vanilla flavors –
Alignment between source material, claims and regulation

AUTHENTICITY TESTING OF VANILLA FLAVORS – ALIGNMENT BETWEEN SOURCE MATERIAL, CLAIMS AND REGULATION

Vanilla flavors are among the most widely used flavors worldwide. Vanilla itself has been one of the most appreciated flavors globally. It is being used for the flavoring of numerous commodities such as ice creams, dairy products, desserts, sweets, bakery goods, spirits and beverages. Vanilla flavors are also used in perfumes and personal care products.

Authentic vanilla flavor is traditionally made from the commonly named vanilla pods, which derive from the plant genus *Vanilla*, primarily from the Mexican species *Vanilla planifolia*. Other species, such as *Vanilla tahitensis* and *Vanilla pompona* represent minor sources and are only cultivated in smaller scale. Vanilla requires special growth conditions, where every flower is hand pollinated, and requires an elaborate curing procedure.

The major aroma-active substance of vanilla flavor is vanillin (4-hydroxy-3-methoxybenzaldehyde). It is naturally occurring in cured vanilla beans and has a characteristic, pleasantly sweet flavor.

Today, less than 1% of the global demand for vanillin is covered by vanilla pods. Nevertheless, the popularity and high demand for the vanilla flavor obtained from vanilla pods make vanilla the second most valuable spice in the world after saffron. The price of vanilla pods was around USD 500/kg or higher in 2017, whereas the price for synthetic vanillin is around USD 10/kg. This price difference constitutes a driving factor for economically motivated adulteration (EMA) that involves replacing natural vanillin from vanilla pods by cheaper synthetic vanillin.

- ***ADULTERATION OF VANILLA***

Adulteration of vanilla extracts, vanilla flavors or any food containing vanilla is usually the addition of a substance that reproduces, fortifies or otherwise gives consumers the impression that they are eating authentic vanilla, contrary to the relevant legal framework. The predominant method of adulteration is the addition of vanillin from sources other than vanilla.

Vanillin can also be produced by natural raw materials such as clove, curcumin, coumarin and cereals like rice and corn (Fig. 1). These vanillins also retain a high price and demand is rising, driven by consumer desire for natural flavors. Whether these vanillins may be labeled as 'natural vanillin' depends on the production process and the legal framework

(Table 1). Vanillin produced from rice bran/corn (ferulic acid), clove (eugenol), turmeric (curcumin) and glucose have been marketed for more than a decade.

Chemosynthetically-derived vanillin is predominantly obtained from fossil fuels, i.e. catechol to synthetic guaiacol. Another synthetic vanillin is obtained by oxidation of lignosulfonic acid, which is obtained in the course of sulfite leaching in the paper and cellulose production.

- ***REGULATORY FRAMEWORK***

Table 1. Regulatory framework on the claim of natural flavorings

Europe → EC Regulation No 1334/2008
▪ Natural flavoring substance is naturally present in nature ▪ The source/raw material used to produce the flavoring substance is natural ▪ Production of the flavoring substance must comply with the requirements of EC1334/2008 of a natural process
U.S.A. → US FDA 21CFR101.22 (labeling of flavorings)
▪ The source/raw material used to produce the flavoring substance is natural ▪ Production of the flavoring substance must comply with the requirements of the FDA 21CFR101.22 of a natural process

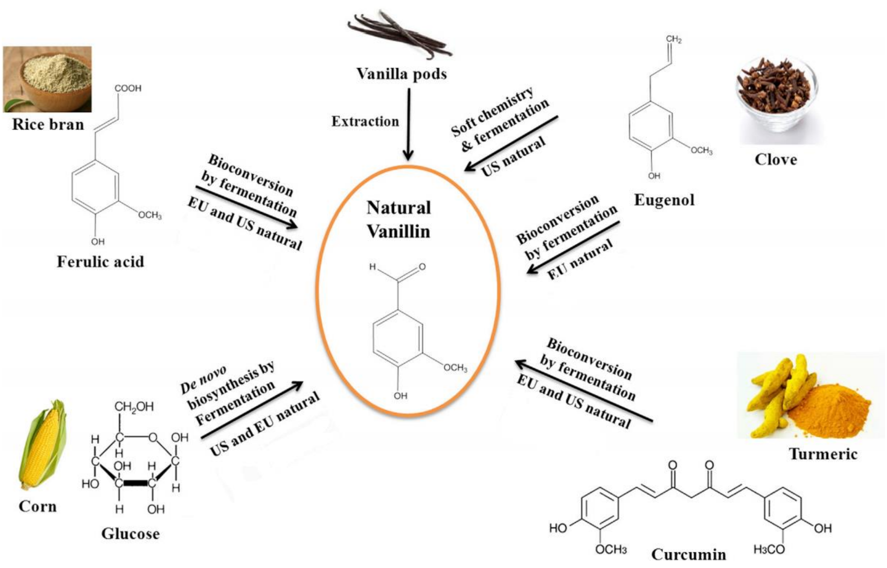


Fig. 1. Different routes to natural vanillin that are available or soon to be available on the market (mod. Gallage & Lindberg Møller, 2015).

In recent decades, the main growing areas of the genus *Vanilla* are Madagascar, the Comoros and La Reunion, China, Uganda, Mexico, India, Indonesia, Tahiti and Papua New Guinea. Although traditionally Madagascar produced the largest portion of vanilla pods worldwide, today the production of some Asian countries like Indonesia, China and India is increasing significantly (Table 2).

Of the approximately 100 known vanilla species, only three are cultivated:

Vanilla planifolia has the greatest economic importance. Depending on its origin, it is also traded under names such as Bourbon, Madagascar or Mexico vanilla. The note "Bourbon" is to be regarded as a geographical indication of origin. The source material must be from the "vanilla islands" (Madagascar, Comoros, Réunion, Seychelles, Mauritius). The Bourbon vanilla is considered premium product and it has a strong and recognizable brand name worldwide.

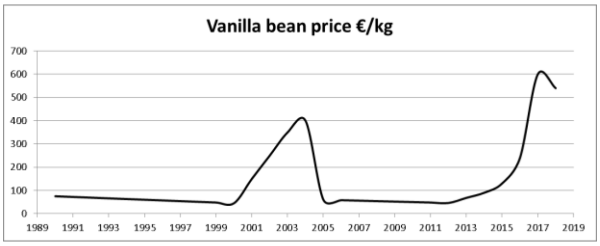
Vanilla tahitensis is characterized by an aniseed note. The proportion of vanillin is lower than in *Vanilla planifolia*. Its main source areas are in the south Pacific. *Vanilla pompona* is only used for cosmetic products and perfumes.

Vanilla is cultivated in areas where weather conditions can be extreme and unstable. Weather extremes have caused significant extreme lows in vanilla production and consequently profound price rises, like in 2003 and in 2017 (Fig. 2). Further factors also influence the production and the prices of vanilla worldwide, such as political unrest in source areas, early harvesting, uncontrolled curing procedures and shortcuts in storage conditions which affect quality.

- ***CULTIVATED VANILLA SPECIES:***
  - ***VANILLA PLANIFOLIA***
  - ***VANILLA TAHITENSIS***
  - ***VANILLA POMPANA***

Table 2. Vanilla pods global production share (2016)

Country	Production share
Madagascar	36.8%
Indonesia	29.0%
China	11.1%
Mexico	6.5%
Papua New Guinea	6.3%



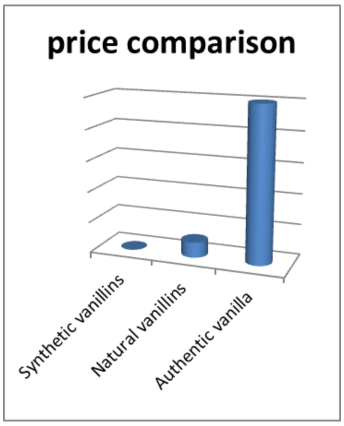


Fig. 2. Vanilla bean price variations during the last 30 years (left) and graphical price comparison of authentic vanilla, natural vanillins and synthetic vanillins (right)

Due to these huge price deviations and because both the demand for natural vanilla flavor or vanillin isolated from the vanilla bean is very high and on the other hand biotechnologically obtained and synthetic vanillins are significantly cheaper, there is a significant risk for EMA of vanilla flavors (Table 3).

Table 3. Examples of Economically Motivated Adulteration (EMA) of vanilla flavorings

Product claim	Adulteration
Vanilla extract	Addition of (any) vanillin to the authentic vanilla extract
Natural vanilla flavoring	Addition of synthetic vanillin (acc. to reg.)
Bourbon vanilla ice cream	Use of (any) vanillin instead of Bourbon vanilla
Pure vanilla	Use of tonka extract in vanilla extract
Natural vanilla flavored yogurt	Addition of synthetic vanillin (acc. to reg.)
Bourbon vanilla extract	Use of *V. Tahitensis* instead of *V. Planifolia*

ANALYTICAL AUTHENTICATION OF VANILLA FLAVORS

As there are multiple possibilities for adulteration, so there are multiple analytical methods available for authenticating vanilla flavorings in terms of source material. Some methods screen for several substances, while others refer specifically to vanillin. The analytical toolbox comprises gas and liquid chromatography mass spectrometry, radiocarbon analysis, and sophisticated stable isotope analysis, like compound specific carbon isotope analysis, the SNIF-NMR® site specific isotope analysis and two-dimensional compound specific stable isotope analysis.

Each method provides significant information; however, each also brings certain drawbacks. Many of the methods cannot distinguish between the synthetic and the natural sources. Others can only distinguish the synthetic from all other naturals, including authentic vanilla. There are also precision issues, time and cost constraints. The next tables provide an overview of the most popular methods used today, with their advantages and disadvantages.

The most popular methods for authenticity authentication

Gas and Liquid Chromatography Mass Spectrometry (GC- or LC-MS)	
Principle: ▪ Targeted trace compounds (e.g. vanillin, p-hydroxybenzaldehyde, vanillic acid and p-hydroxybenzoic acid, guaiacol, various lactones and monoterpenes) ▪ Range of the concentration ratios of vanillin to its accompanying substances	
Advantages	**Disadvantages**
✓ High sensitivity in detection of minor compounds ✓ Fast and cost-effective	× There are significant deviations from the published range of values between crops, years, curing process, extraction process etc. × Wide range of tolerance to cover the above factors → limited authentication sensitivity × Possible to manipulate the compounds profile

Radiocarbon analysis (^{14}C)

Principle:
- of Estimation the fraction of modern carbon vs. fossil carbon in materials

Advantages	Disadvantages
✓ Straightforward verification of bio-based materials without databases ✓ Standard method used in many industries ✓ Low sample quantity needed ✓ Very short turnaround time	✗ Analysis performed on total carbon, not on compound ✗ Works only on pure vanillin ✗ Can discriminate only fossil-fuel derived synthetic vanillin from any natural vanillin (incl. vanilla)

Gas Chromatography (Combustion) Isotope Ratio Mass Spectrometry (GC-C-IRMS; $\delta^{13}C$)

Principle:
- Targets vanillin (or more compounds) and determines their carbon stable isotope composition

Advantages	Disadvantages
✓ Simple sample preparation ✓ Works for any matrix ✓ Low sample quantity needed ✓ Short turnaround time	✗ Can only discriminate authentic vanilla from any other natural and synthetic vanillins ✗ 13C can be artificially enriched during synthesis to imitate vanilla fingerprint ✗ Cannot discriminate all different types of vanillins (synthetic and natural)

SNIF-NMR® (^{2}H SNIF-NMR, ^{13}C SNIF-NMR)

Principle:
- Multi-isotope and multi-variate fingerprint (H and C from different sites)
- Targets specific sites in the molecule of vanillin

Advantages	Disadvantages
✓ High-resolution result in discriminating known types of vanillin (vanilla, natural and synthetic) ✓ Provides complete authentication of the source material by type	✗ Vanillin purity is necessary before analysis → complex sample preparation ✗ Long turnaround times ✗ Significantly high sample quantity necessary, esp. for end-products

Gas Chromatography (Combustion/Pyrolysis) Isotope Ratio Mass Spectrometry (GC-C/P-IRMS; $\delta^{13}C$, $\delta^{2}H$)	
Principle: ▪ Targets vanillin (or more compounds) and determines their carbon and hydrogen stable isotope composition (multi-isotope fingerprint)	
Advantages	**Disadvantages**
✓ Simple sample preparation ✓ Works for any matrix ✓ Low sample quantity needed ✓ Short turnaround time ✓ High-resolution result in discriminating known types of vanillin (vanilla, natural and synthetic) ✓ Can discriminate *V. Planifolia* from *V. Tahitensis* ✓ Can provide verification of geographic origin of vanilla ✓ Provides complete authentication of the source material by type, species and origin	× Novel method, applied only by a few labs globally

Taking advantage of the technological developments in instrumentation, the combination of Gas Chromatography (GC) and Isotope Ration Mass Spectrometry (IRMS) made it possible to get not only carbon but also other isotope ratios such as hydrogen. This fast and cost-effective technique targets the vanillin and requires a very simple sample preparation. It can be used in any matrix, from pure vanillins, extracts and flavoring mixtures, to consumer products, and requires a relatively low sample quantity due to the sensitivity of the IRMS. Furthermore, due to the multi isotope fingerprint, the method provides a high discrimination level between different types of vanillin: vanilla, natural and synthetic versions. It can also differentiate vanilla *planifolia* from vanilla *tahitensis*, another point of common EMA in vanilla flavorings. The intelligence gained from the hydrogen isotopes can be used to verify the geographical origin of the authentic vanilla, for instance Madagascar versus Papua New Guinea or China.

The GC-C/P-IRMS is a new method[1], published and validated in international scientific studies, reports and articles. Using this sophisticated instrumentation together with strong know-how and a large dataset, it can offer a practical tool that can answer multiple questions when dealing with authentication of vanilla flavorings. The existing dataset is the largest currently available and it is constantly expanded with authentic samples of authentic vanilla, as well as natural and synthetic vanillins (Fig. 3).

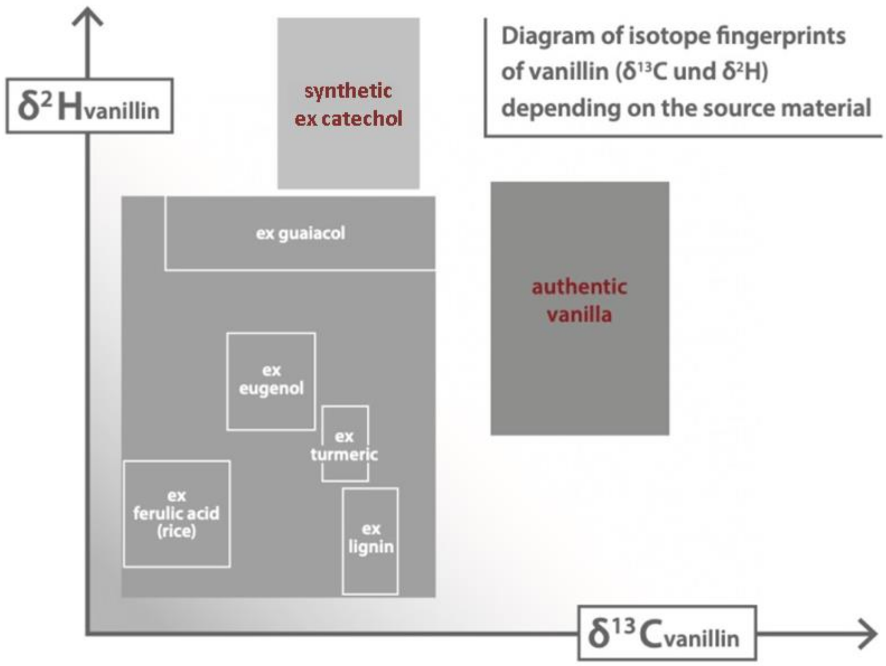


Fig. 3. Graphical illustration of the GC-C/P-IRMS isotope fingerprints (carbon and hydrogen) of the existing dataset

Today's globalized complex supply chains provide food fraudsters with numerous opportunities to adulterate high value food products with cheaper ingredients. Food fraud has grown enormously in recent years, while at the same time the industry has done a lot to secure food quality and food safety. It is still a real challenge to identify, prevent and fight food fraud in order to achieve integrity in food. Authenticity is something that can be secured using analytical tools, and by securing authenticity, integrity in the supply chain, brand reputation and consumer trust are all secured. In this cloud of analytical tools, the GC-C/P-IRMS can be considered as a reliable and robust method that covers multiple needs and serves many purposes.

[1] https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-003126-ioms-gc-irms-gc-isolink-II-vanillin-tn003126-em-en.pdf

ABOUT IMPRINT ANALYTICS

Imprint Analytics specializes in the analytical verification of the geographical origin and authenticity of products and raw materials. Using innovative methods, Imprint Analytics determines the authentic fingerprint of a product or material and can conclude where it comes from.

As an accredited laboratory, Imprint Analytics is the first point of contact for trade and industry, manufacturers and suppliers, as well as inspection authorities and consumer protection organizations in all matters relating to product authenticity and counterfeiting. Completely independent of documents, Imprint Analytics can determine the geographical origin and authenticity of food, additives, pharmaceuticals and other products for our clients.

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