Wondrous Vanilla

Vanilla isn't just vanilla or even plain vanilla. There is a whole plethora of vanilla types and many different ways to prepare vanilla pods. Their characteristic flavor patterns aren't only determined by vanillin, the main known flavor compound, but also by a large number of other flavor compounds, many of which have not yet been identified. Each type of vanilla has its own characteristics. In order to determine the origin of vanilla, and for quality control purposes, laboratories typically rely on Headspace or Thermal Desorption techniques used in combination with GC/MS. This article describes interesting aspects of the analysis of vanilla pods and vanilla extracts and reports on newly discovered vanilla flavor compounds.

No other spice or flavor ingredient is as widely used and appreciated as vanilla. In spite of the expression "plain vanilla", vanilla is the king of flavors and it is not only used in food and sweets, but also in perfumes, cosmetics and pharmaceutical products. Vanilla is said to soothe the soul and reinvigorate the body explaining its widespread use in aroma therapy. Recent research reports indicate that vanillin, the key flavor compound in vanilla, may have cancer inhibiting properties. The ancient Aztecs considered vanilla the "food of the gods", today it is among the most important and most widely loved spices in the world. One of the reasons why vanilla is so widely used is because key flavor compounds can be synthesized from cheap ingredients such as paper pulp. Real vanilla, on the other hand, is very expensive with prices reaching as high as $ 80,000 per ton [1]. However, the amount of naturally grown and fermented vanilla does not even come close to satisfying world demand. With prices of the real thing so high, quality and authenticity, i.e. origin and potential adulteration must be carefully monitored. Traditionally this job has been the responsibility of taste and flavor panels, but increasingly, chemical analysis has come into play.

Producers of food and other products that contain vanillin as a flavor ingredient can rely on standard chemical analysis techniques for quality control. The analysis of real vanilla, however, can present a challenge. Due to the complexity of the chemistry and fermentation processes involved, it typically requires more than one analytical technique to provide reliable results. Stephen J. Toth, Ph.D. knows this better than most people – and from firsthand experience. Over the course of his work on his doctoral dissertation: "Comparison and integration of analytical methods for the characterization of vanilla chemistry" [1] at the State University of New Jersey, the scientist strove to find a unified analytical method for the determination of volatile and semi-volatile organic compounds (VOCs and SVOCs) in vanilla pods and extracts. The solution he found was to use two techniques: 1) Liquid chromatography (LC); and 2) Gas chromatography (GC) in combination with headspace and thermal desorption.

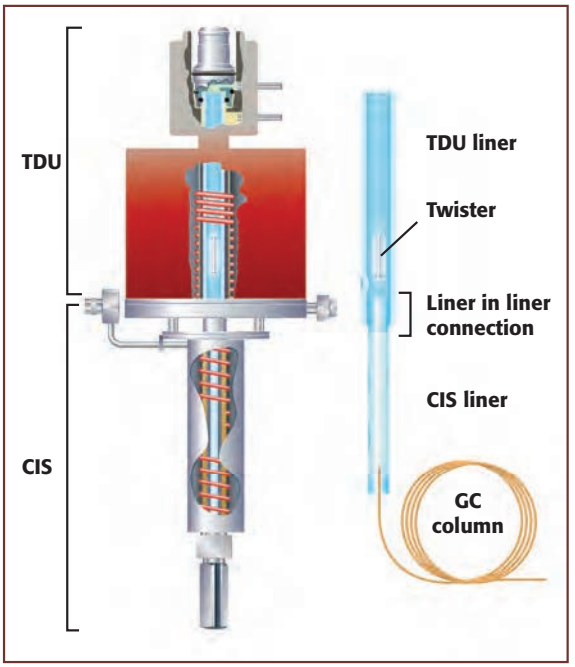
Determination of vanilla flavor components

For the determination of semi-volatile or non-volatile flavor compounds in vanilla, such as vanillin, 4-hydroxy benzaldehyde, vanillic acid and 4-hydroxy benzoic acid, HPLC is the separation method of choice. In his dissertation, Dr. Toth listed as reference a large number of journal papers based on HPLC methods, mainly for the analysis of vanilla extracts, which served as reference points for his method development work. The separation was ultimately performed using much shorter UHPLC columns, which are based on smaller particle size packing. As a result, the analysis time for vanillin and related phenolic compounds was cut from 13.45 to 1.86 minutes, a seven-fold improvement in throughput. In addition, the amount of acetonitrile used was reduced by about two thirds. Still, for the wider range of more volatile compounds that are responsible for the bulk of vanilla's flavor, including many unknown compounds, HPLC is not the technique of choice. For these, gas chromatography combined with mass spectrometry (GC/MS) must be used – preferably combined with headspace (HS), HS-SPME or other sample introduction and analyte concentration techniques.

To determine which technique was best suited for the analysis, the scientist compared results from the following: Solid Phase Micro Extraction (SPME), Headspace Sorptive Extraction (HSSE) using the GERSTEL Twister, and direct thermal desorption of deep

A brief introduction to vanilla

Vanilla belongs to the orchid family. Vanilla grows in tropical regions and there are more than one hundred different species of vanilla, only two of which are commercially relevant. Vanilla Planifolia and Vanilla Tahitensis are the only orchid species to produce a commercially useful fruit. Originally from Mexico, vanilla is mainly grown in Indonesia and Madagascar today. Most of us wouldn't recognize a vanilla plant or its fruit. The green odorless bitter tasting vanilla fruit is nothing like the vanilla pods we are exposed to in high-end food stores. Over the course of a five month complex and intricate fermentation process, the fruit is transformed into dark brown, extremely tasty and flavorful vanilla pods. Among other compounds, vanillin is formed by hydrolysis of glucovanillin. Controlling the fermentation process is key to producing the right flavor profile, the right taste and an overall quality product. The fermented vanilla is marketed in two different forms: As an extract in alcohol solution (extraction grade) and unprocessed (gourmet grade). A high quality vanilla pod is characterized by highly pleasant flavor and taste, moisture content of 18 – 25 %, and dark chocolate-like color. The surface must be oily and free from defects and mold. The vanillin content should be greater than 2 % as a key indicator to high quality, but this is not always the decisive factor: Many vanilla types have excellent overall taste and flavor even though their vanillin content is lower than 2 %. This leads to the conclusion that other flavor compounds contribute significantly to the overall flavor and taste [1].



Thermal desorption of the Twister in the TDU followed by cryofocusing of the analytes in the CIS and temperature programmed transfer to the GC column.

frozen ground vanilla pods. The flavor compound profiles of two bourbon vanilla pods were investigated. One was a perfect pod of good quality and the other had been rejected by a customer as unacceptable due to an "alcohol" off flavor, thought to be caused by bacterial degradation products, including guaiacol formed by degradation of vanillin under anaerobic conditions.

Solid Phase Micro Extraction (SPME)

In his literature searches, Toth found a large number of articles reporting on the analysis of vanilla extracts based on the SPME technique, especially when it came to polar compounds in alcohol extracts. He ascribed this to the multitude of SPME phases available, ease of automation as well as fast desorption of the concentrated analytes in the GC inlet. SPME has proven itself to be selective, efficient and highly useful in practical analysis work when it comes to extraction of volatile compounds from the headspace phase. The main shortcoming of SPME lies in the limited phase volume (0.5 µL), limiting the sorptive capacity and thus the sensitivity of the technique. Nevertheless, SPME was used to extract 35 compounds from vanilla, including contaminants from packaging as well as eight new compounds, which hadn't previously been identified.

Headspace Sorptive Extraction (HSSE)

Headspace Stir Bar Sorptive Extraction (HSSE) is an add-on development of Stir Bar Sorptive Extraction (SBSE). HSSE has been used for flavor analysis work involving a large number of different products [2] and it is based on using the GERSTEL Twister as a passive sampler in the headspace of a sample. In standard SBSE, the Twister extracts analytes from a liquid sample while actively stirring it; in HSSE, the Twister is positioned in the headspace above the sample from which it indirectly extracts volatile compounds. After equilibrium has been established between the liquid, headspace and Twister sorbent phases, the Twister is removed and transferred to the MultiPurpose Sampler (MPS) sample tray. From there, it is automatically transferred to the Thermal Desorption Unit (TDU) for thermal desorption and transfer of the analytes to the GC column. Due to the much higher phase volume of the sorbent (Twister: 125µL / SPME: 0.5 µL), the HSSE technique is exceptionally efficient in extracting medium to non-polar compounds. An EG-Silicone Twister is now also available, which enables efficient extraction of polar compounds such as phenols and other aromatic alcohols. Using HSSE, a total of 19 previously unknown compounds were found and identified.

Dynamic Headspace (DHS)

Dynamic Headspace, as the name implies, is not a static technique relying on equilibrium between phases, but rather a dynamic technique relying on driving analytes out of the headspace, and thus indirectly out of the sample, using carrier gas. Purged analytes are trapped and concentrated on an adsorbent trap at the purge outlet. In this case, Tenax TA adsorbent was used in the trap. As opposed to SPME and HSSE, Tenax TA showed no specific affinity for individual compound classes, but rather concentrated analytes over a wide polarity range. However, Toth found that compounds with three or less carbon atoms generally were not as efficiently trapped. Using DHS, he was able to extract and identify 24 compounds from the high quality vanilla, including 10 compounds that had not yet been found in vanilla.

Direct Thermal Desorption (DTD)

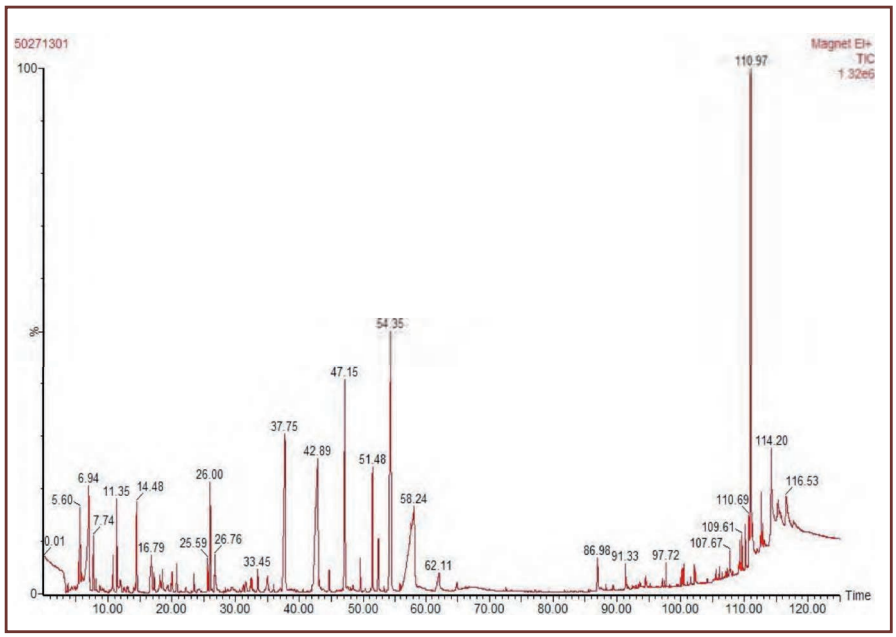
For direct thermal desorption, the sample was placed in a suitable inert glass tube (TDU-liner) between two plugs of glass wool and placed in the MPS sample tray. The TDU liner was transferred to the TDU by the MPS. Thermal desorption/thermal extraction was performed using the following temperature program: Initial temperature 30 °C – 60 °C/min – 275 °C.

Desorbed analytes were trapped in the GERSTEL Cooled Injection System (CIS) PTV-type inlet and transferred to the GC column using a temperature program. Direct thermal desorption using the TDU-GC/MS method enabled Toth to identify 74 compounds in both the "good" and "bad" vanilla samples. After further work, he found another 30 flavor compounds that had not yet been reported in vanilla pods. The most remarkable difference between the "good" and "bad" vanilla was indeed in the vanillin concentration: The "good" sample contained 1.2 %, the "bad" only 0.1 %. In the accepted "good" vanilla, Toth further found high concentrations of acetic acid, 2-methoxy phenol, hydroxyl dihydromaltol, 5-(hydroxymethyl) furan-2-carbaldehyde, 4-hydroxybenzaldehyde, hexadecanoic acid and 1-octadecanol. Toth reports: "The list of compounds identified in the good bourbon vanilla pod correlates well with previously reported data from the literature". Compounds, which he was the first to report in vanilla pods, included acetone, 2-methyl propanal, 3-hydroxy-3-pentene-2-one, 2(5H)-furanone, 2-hydroxy-2-cyclopentene-1-one, 4-hydroxy-5-methyl-3(2H)-furanone, furan-2-carboxylic acid (2-furoic acid), lilial acid, 4-(4-hydroxyphenyl)-3-buten-2-one, 4-(4-hydroxy-3-methoxyphenyl)-3-butene-2-one (E), two isomers of vanillin glyceryl acetal, 1-octadecanol, ethyl heptadecanoate, ethyl octadecanoate, z-12-pentacosene and z-14-nonacosene.

In the "bad" vanilla pods, Toth identified high concentrations of compounds such as: 2-methoxy phenol, 2-methoxy-4-methyl-phenol, hexadecanoic acid and 1-octadecanol.

Among the biggest differences uncovered by direct thermal desorption of the "good" and "bad" bourbon vanilla pods were the loss of vanillin, increased concentrations of 2-methoxy-4-methyl phenol and 2-methoxy phenol as well as the loss of hydroxyl dihydro maltol and hydroxymethyl furfural, Toth writes. Unlike the results from the analyses performed by SPME, HSSE, and DHS, the direct thermal desorption results didn't include fusel alcohols, even though the rejected vanilla products did contain these compounds at different concentration levels. The presence of fusel alcohols is in itself a strong indication that bacterial degradation has taken place.

Even though each technique had clear strengths and weakness, they complemented each other well. When combined, they serve to provide a comprehensive picture of the diverse chemical landscape of a complex product such as vanilla. As a flavor analyst, being able to draw on the whole range of headspace techniques: SPME, HSSE, Dynamic Headspace (DHS) and direct thermal desorption (DTD) provides a critical advantage in terms of being able to cover all analytes while using solvent-free and highly sensitive extraction techniques. The flavor chemist's efficiency as well as the resulting data is greatly improved when these techniques are automated, and the ultimate enhancement is provided by the GERSTEL MultiPurpose Sampler (MPS), which automates all of these techniques on a single platform.



Total Ion Chromatogram of a Tahitian Vanilla resulting from DTD-(TDU)-GC/MS analysis. The following compounds were found: 2-(5H)-furanone, 2-hydroxy-2-cyclopentene-1-one, 2-acetyl-2-hydroxy-gamma-butyrolactone, 3,5-dihydroxy-2-methylpyrane-4-one, 3-phenyl-2-propenoic acid, 4-hydroxy-2-methoxy cinnamic aldehyde, 4-(4-hydroxy-3-methoxyphenyl)-3-butene-2-one (E), 2 isomers of 2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxane-5-ol, kauren and z-12-pentacosene.

Sources

[1] Stephen J. Toth: Comparison and integration of analytical methods for the characterization of vanilla chemistry. Proquest, Umi Dissertation Publishing 2012

[2] Flavor, Fragrance, and Odor Analysis, 2. Edition, CRC Press, Taylor & Francis Group 2012