Polyarc® microreactor: Quantification of commercially unavailable or unknown flavor compounds

Application Note

Flavor Research

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Summary

The Polyarc microreactor was used to quantify 2-acetyl-1-pyrroline (2AP) and 1-vinyl-2-pyrrolidone (1V2P) by gas chromatography (GC) analysis. Results were compared with internal standard quantification methods. The Polyarc microreactor reported comparable results when compared to traditional GC quantification techniques with the distinct advantage of not requiring a reference standard. Consequently, the Polyarc detector provided a more simple quantification instrumental setup that would also provide improved analysis of very complex extracts such as flavor solutions.

Introduction

The flavor of a food is arguably one of the most important attributes driving consumer acceptability and choice. The aroma character, one of the modalities of flavor, is driven by the combination of numerous odorants, modulated by their concentration, interactions and their sensory threshold.

Flavor analysis is challenging, as it requires extraction of volatiles from complex matrices and the use of diverse analytical approaches for detection, identification and quantification of organic compounds. The ability to properly quantify volatile compounds is critical in elucidating the impact of each component on the overall aroma and flavor profile of a food system. A task that can prove to be challenging due to, compound instability, low concentration, or lack of commercially available standards.

A great example is 2-acetyl-pyrroline (2AP) known as one the most potent flavor compounds in fresh French baguette and basmati rice and described as having cracker / popcorn-like character. In Food, 2AP is usually present at parts per billion (ppb) levels and thus initial detection occurs in volatile extracts via GC-O (Gas Chromatography-Olfactometry). Confirmation of the presence of 2AP is performed by GC-MS (gas chromatography mass spectrometry) after lengthy concentration steps. But for positive identification of volatile compounds a confirmation of retention time using an authentic standard is also required. The compound 2AP is not commercially available and a standard can be obtained through isolation and purification from a food source or custom synthesis, which can be a cost prohibiting option.

Pandan leaves (Pandanus amaryllifolius) are one of few natural products known to contain high levels of 2AP , making them a suitable source of 2AP via solvent extraction, and the subject of studies for extraction protocol optimization . Yet, 2AP quantification in Pandan leaves can be a challenge.

The advantage of utilizing in-house synthesized 2AP is that it can be used for both identity confirmation (retention time and MS spectra library match) as well as quantification via a standard addition method. However, synthesis yields can vary from batch to batch and the only way to determine each yield is to utilize an analogue standard compound. In the case of 2AP, 1-vinyl-2-pyrrolidone (1V2P) can be used as an internal standard as it has the same elemental composition as 2AP (C6H9ON) and is assumed to have the same response factor when a non-selective detector such as Flame Ionization Detector (FID) is used.

The Polyarc reactor is a newly introduced module for gas chromatography that allows quantification of volatiles based on the content of elemental carbon. It is the equivalent to sulfur detection by the Sulfur Chemiluminescence Detector (SCD), and to multiple element detection by the AED (Atomic Emission Detector). These detectors have a furnace/reactor/plasma in which the incoming volatiles are combusted and transformed into a quantifiable signal. The Polyarc microreactor transforms all the carbon contained in the volatiles into methane (CH4), a gas then easily quantified by a FID. Therefore all volatile organic compounds can be quantified as methane by placing the reactor at the end of the analytical column, just before the FID. This circumvents the requirement to know the response factor of every compound, since all compounds are reduced to methane while traveling through the reactor.

In this work, the Polyarc was used to quantify 1V2P and 2AP that was either in-house synthesized or extracted from pandan leaves.

Experimental

Reagents and standards

Tridecane, 1V2P, diethyl ether and GC grade methanol were purchased from Sigma-Aldrich (Saint Louis, MO) while a deuterated analogue of 2AP was synthesized according to De Kimpe et al (1993) . Compounds were all diluted in diethyl ether at 50 ± 5 µg/mL (± 50 ppm) and injected onto two GC system configurations described below.

Instruments

The GC-MS-Polyarc/FID experiments were performed on an Agilent 6890 GC interfaced with an Agilent 5972 MSD and Polyarc/FID through an SGE splitter.

The GC-FID experiments were performed on a HP GC 5890 Series II equipped with a HP 7376 automatic liquid injector.

The instrument conditions are listed in table 1.

Table 1: GC/MS/Polyarc/FID and GC/FID conditions

GC run conditions	
Column	J&W DB-Wax capillary column, 30 m x 0.25 mm, 0.25µm
Injection volume	1µL
Inlet temperature	250°C
Injection mode	Split 10:1
Oven program	90 °C for zero minute 90 °C to 230 °C at 5 °C/min No hold at 230 °C
Carrier gas	Helium
Agilent 5972 GC/MSD/Polyarc/FID conditions	
Restrictor to MS	Deactivated capillary, 1 m x 0.11 mm
Transfer line temperature	250 °C
Acquisition parameters	EI. Scan
Scan mode	29-250 amu mass range
Restrictor to Polyarc	Deactivated capillary, 1 m x 0.15 mm
Temperature Setpoint Polyarc	293 °C
Temperature FID	300 °C
HP 5890 Series II GC conditions	
Temperature FID	250°C

Extraction of 2AP from Pandan Leaves

Pandan leaves (190g) were ground in water and extracted with diethyl ether (2X 350ml). The organic phases were pooled, filtered, dried with sodium sulfate and concentrated down to 50ml. The concentrate was further purified using Solvent Assisted Flavor Evaporation (SAFE). The high vacuum distillation was performed at 1.10-6 Torr. The volatile fraction was then further concentrated via distillation using a silver coated vacuum insulated 60 cm x 2.5 cm Vigreux column.

Results

Determination of the sensory impact of volatile compounds usually involves methods such as Aroma Extract Dilution Analysis (AEDA) and sensory recombination. Compounds selected after Gas Chromatography / olfactometry (GC/O) and Aroma Extract Dilution Analysis (AEDA) techniques are quantified and their corresponding concentrations are reconstituted either in the native food system or an appropriate model system, which then undergoes sensory evaluation. Accurate quantification is key for the success of such studies.

As previously mentioned, 2AP is a very potent and important aroma compounds character impact volatile for a variety of foods. Because of its extremely low odor threshold, a quantification error can lead to over - or under - estimation of its sensory impact.

In order to examine the advantages the Polyarc microreactor can offer in current quantification

approaches, the analogue quantification standard 1V2P and in-house synthesized d_3-2AP were injected and responses were evaluated using both Polyarc/FID and FID. Results were compared using an external standard calculation method. Three different Pandan leaf extracts were additionally examined (PL-A, PL-B and PL-C) and levels of 2AP were quantified.

Results are presented in Table 2 and Figure 1 is a summary and representation of the data.

FID Results

A stock solution of 1V2P (50 µg/mL) as well as the in-house synthesized d_3-2AP stock were used to quantify 2AP in the obtained from extraction of Pandan leaves. Subsequently, the stock solution of d_3-2AP (quantified by the Polyarc/FID) was used for the quantification of 2AP in all the Pandan leaf extracts as well as the 1V2P.

As can be seen in table 2 and figure 1, using 1V2P as external standard for quantification of d_3-2AP resulted in an overestimation of concentration (52 ppm instead of 47 ppm) while using d_3-2AP to determine the concentration of 1V2P resulted in an underestimation of (45 ppm instead of 50 ppm). For both, the error in estimation is approximately 10%. Quantification using external standard methods do not account for injection errors (5-10% expected variation) thus tends to be less precise when compared to internal standard methods. The use of an automatic sampler, reliably injecting solution with a 0-3 % coefficient of variation permits for lower error and more robust quantification. This is very visible on figure 1 and specifically in the presentation of all FID data as injections were performed using an automatic sampler. The error bars show a very tight coefficient of variation, as opposed to injections performed manually on the Polyarc/FID system. This suggests that the 10 % difference observed between theoretical and experimental values were due to the use of the standards and back calculations using the response factor and not the mode of injection.

The same trends were observed in the quantification of 2AP in Pandan leaves where quantification using 1V2P was always higher than when using the synthesized d_3-2AP.

Table 2. Concentration of 2-acetyl-pyrroline and standards 1vinyl-2-pyrrolidone (1V2P) and deuterated 2-acetylpyrroline (d3-2AP) in standard solutions and Pandan leaf extracts PL-A, PL-B, PL-C.

	Polyarc/FID		FID	
	Theoretical µg/mL	Experimental µg/mL	Analogue Standard (1V2P) 50µg/mL	Synthesized d_3-2AP 47µg/mL
1V2P	58	55 ± 2.61	-	45
D_3-2AP	47	48 ± 3.14	52	-
PL-A	-	51 ± 4.28	43 ± 1.87	39 ± 1.69
PL-B	-	17 ± 1.37	20 ± 0.50	18 ± 0.45
PL-C	-	14 ± 1.01	14 ± 0.51	12 ± 0.46

Polyarc Results

A solution of known concentration of tridecane (53.5 µg/mL or 53.5 ppm) was used to obtain a response factor of the Polyarc microreactor for carbon, specific to employed injection parameters. This solution set the response of the detector for the subsequent injections. Pandan leaf extracts (PL), 1V2P and d_3-2AP were subsequently analyzed. For quantification the Polyarc Reactor Concentration Calculator spreadsheet was used (http://www.activatedresearch.com/concentration-calculator/), which reports the concentration of all compounds relative to the amount of carbon taking in consideration the amount of carbon in each molecule. Briefly the following steps are performed automatically via the use of the Polyarc Reactor Concentration Calculator for quantification:

1. The peak areas indicate the relative amounts of carbon present in the sample from each compound because all compounds are converted to methane and respond equivalently in the FID.
2. The peak area ratios of the compound relative to the internal standard (usually a straight chain hydrocarbon) are used to determine the carbon ratio from the known concentration of the internal standard.
3. The carbon numbers in the compound and the internal standard are used to convert the carbon ratio from (2) to the molar ratio.
4. The molar ratio can be used to calculate the number of moles of the unknown component if the moles of the internal standard are known.
5. The molar ratio can also be converted to the mass ratio using the molecular weights of the compound and the internal standard. The mass of the compound can be calculated from this ratio and the known mass of the internal standard.

Quantification of 1V2P suggested the robustness of the Polyarc reactor, as theoretical and experimental values were not significantly different. The same results were obtained for quantification of d_3-2AP. It is important to note that this synthesized isotopically labeled analogue of 2AP was initially quantified using known amount of standard 1V2P. As explained earlier, 1V2P was assumed to have the same response factor (signal response per unit of weight) as 2AP in an FID detector because it has the same elemental formula. Therefore, the peak area ratio of 2AP/1V2P provided a weight ratio of 2AP/1V2P, since 1V2P amount was known. This confirmed that the quantification method using 1V2P is comprehensive and reliable and also that the microreactor performs according to expectations.

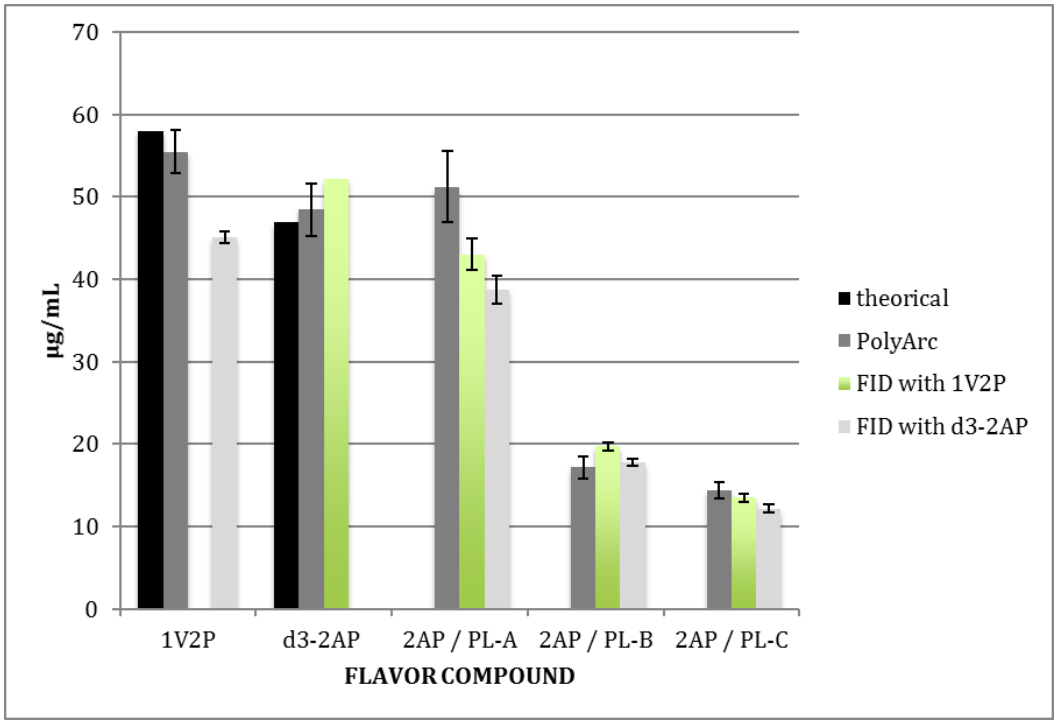

Figure 1. Concentration of 2-acetyl-1-pyrroline and 1-vinyl-2-pyrrolidone quantified using the Polyarc microreactor with FID, or the Flame Ionization Detector and 1V2P or d3-2AP as standard.

Pandan leaf extracts results utilizing the Polyarc performed similarly to the FID. The higher observable concentration of 2AP in PL-A when using the Polyarc microreactor when compared to the FID could have been the result of using two different GC systems and columns (same chemical phase) as it is known that no two systems are exactly the same. Additionally, current architecture of the Polyarc induces a minimal but observable peak tailing that could affect rigorous quantification and result in slight overestimation of the analyte of interest.

Still, this data demonstrates the potential of using Polyarc reactor to quantify volatiles. The biggest issue in flavor analysis, is the need to quantify multiple compounds with appropriate standards in a single chromatographic injection. Selection criteria for these standards include similar physico-chemical properties to the compounds of interest, but most importantly, an elution time that does not interfere with other analytes of interest which can be very challenging when working with complex aroma extracts. The use of a single straight chain hydrocarbon standard (or any organic compound as a standard) with the Polyarc reactor bypasses the search for appropriate standards and reduces exponentially method development time usually necessary for these tasks.

The development of this microreactor is recent and its potential is yet to be established but these results suggest possible applications and clear advantages when compared to, previously used comparable technologies. This detector can be very useful in flavor analysis with the additional advantage of being simpler and cheaper than the AED for instance, while providing response based on elemental emission and hence permitting the calculation of elemental formulas.

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For more information or to purchase a Polyarc® reactor, please contact ARC at 612-787-2721 or contact@activatedresearch.com.

Please visit ARC's website for details and additional technical literature.

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