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A REFEREED PAPER

GC/MS DETERMINATION OF 1-P-MENTHEN-8-THIOL IN GRAPEFRUIT JUICE

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Abstract. **P-menthene-8-thiol is a very potent aroma compound found in grapefruit juice. It currently is not measured commercially because it is present in only trace quantities and due to the fact that it requires an expensive specialized detector. However, in this report, it is shown that utilizing a standard laboratory gas chromatography mass spectrometer (GC-MS), it is possible to quantitate p-menthene-8-thiol with minimal sample and relatively short preparation time. Current methods use 1000-5000 mL of juice while the method presented here uses only 30 mL. It was determined to be as effective as a sulfur specific detector (no difference in unknown concentration at 95% confidence level) and also to be able to quantitate much lower levels. A tentative limit of detection is reported to be 1 ng/g.**

Sulphur-containing compounds have a significant impact on fruit and fruit product flavor. In grapefruit, 1-p-menthen-8-thiol [CAS 71159-90-5] is particularly important as it is a key component of flavor, and has a very low flavor threshold value of 0.1 ppt (part per trillion) in water (Demole et al., 1982). Its determination is thus important to assess quality of fresh and reconstituted juice and juice-containing beverages (Buettner and Schieberle, 1999; Buettner and Schieberle, 2001; Lin et al., 2001). Buettner and Schieberle (2001) quantitated 1-p-menthen-8-thiol using GC/MS but with an initial volume of 5000 mL of juice.

Flame ionization detectors are usually not sensitive or selective enough to detect 1-p-menthen-8-thiol at the levels present in grapefruit juice (~5-100 $ng \cdot g^{-1}$). Therefore, the compound is usually quantitated using a sulfur specific detector like a flame photometric detector (FPD), the more sensitive and expensive pulsed flame photometric detector (pFPD), or a sulfur chemiluminescence detector (SCD). The methodology proposed here is an alternative to the need for a sulphur specific detector for the chromatographic system. Instead of dedicating an expensive piece of equipment, an existing GC/MS systems could be used, and the 1-p-menthen-8-thiol compound detected and quantitated.

Materials and Methods

An Agilent 6890N with an FPD detector using an HP-5 30 m × 0.25 mm × 0.25 µm film thickness was used for these experiments (Agilent, Palo Alto, Calif.). The initial temperature of the oven was 50 °C with a 10 $°C \cdot min^{-1}$ ramp to 250 °C with a constant flow of 1.3 $mL \cdot min^{-1}$. The injector and detector were held at 250 °C. 2 µL of sample was injected in the splitless mode using an Agilent 7673 autosampler.

Conditions were the same for the GC-MS. The MS conditions were single ion monitoring (SIM) using m/z 170 with a dwell time of 200 ms and low resolution. M/z 170 was chosen as this is the molecular weight of p-menthene-8-thiol. While m/z's 121 and 136 are more abundant ions, they are common to most terpenes and therefore less indicative of the analyte.

The p-menthene-8-thiol was obtained from a local flavor company and the purity at analysis time was determined to be approximately 60% as judged from a full scan analysis using the mass spectrometer.

Standards were made using standard dilution techniques to provide about 5 calibration points near the level that the unknowns were expected to be determined. The unknowns were ethyl acetate extracts of juice that was purchased at a local grocery store. To prepare the grapefruit juice samples, 1000 mL of juice was extracted twice with 200 mL of ethyl acetate (400 mL total), centrifuged in a refrigerated (2 °C) Sorvall ultra-centrifuge (5000 rpm ~ 2600 × G) for 10 min, the organic layer removed, dried with anhydrous calcium chloride, concentrated with a distillation-rectification apparatus to ~4 mL. To prepare the second set of samples, 30 mL of juice was extracted twice with 20 mL of ethyl acetate each time, centrifuged in a refrigerated (2 °C) Sorvall ultra-centrifuge (5000 rpm ~ 2600 × G) for 10 min, the organic layer removed, dried with anhydrous calcium chloride, concentrated with distillation-rectification apparatus to ~4 mL, and subsequently concentrated to 1.75 mL using a stream of nitrogen at ambient temperature. Samples were replicated 5 times.

Results and Discussion

The FPD and MS both provided similar calibration curves and responses for the p-menthene-8-thiol in the 1 to 100 ng/g ranges. The equations and r-squared values are provided in Table 1 along with the unknown concentrations. The unknown sample extract (white grapefruit juice) was determined by both methods and was not statistically different at the 95% confidence level. This demonstrates that either detector is adequate at detecting and quantitating p-menthene-8-thiol at these concentration levels. However, as discussed earlier, 1000 mL was the initial sample size. This is rather cumbersome under normal laboratory conditions, and would be especially burdensome at a commercial quality control/quality assurance laboratory due to time, expenses, and environmental concerns.

Using the improved method, we were able to detect and quantitate much lower concentrations using the mass spectrometer. In fact, using this method a tentative limit of detection was determined to be about 1 $ng \cdot g^{-1}$ which would mean that the limit of quantitation would be about 3 $ng \cdot g^{-1}$. A second set of grapefruit samples were prepared using 30 mL of juice and extracted twice with 20 mL of ethyl acetate and concentrated to 1.75 mL (concentration factor of 17.1). The two

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Table 1. Calibration and unknown information for FPD and MS.

Detector	Calibration curve equation	R	Unknown Conc. (ng/g)*
FPD	[Response] = 5.744*[Conc] - 9.828	0.951	6.94 a
MS	[Response] = 1262*[Conc] - 4946	0.99	4.18 a

*Values with the same letter are not significantly different at $\alpha = 0.05$.

Table 2. Calibration and unknown information for low concentration MS.

			Unknown Conc. (ng/g)[a]	
Detector	Calibration curve equation	R	Unk. 1	Unk. 2
MS	[Response] = 125.2*[Conc] + 697.38	0.999	2.51 (0.17)	19.74 (1.3)

[a]The number is parenthesis is the standard deviation.

samples were white (NFC) and pink (canned) grapefruit juices respectively. The results are presented in Table 2. The first unknown is only 2.51 ng·g^{-1}, which is about the same as the limit of quantitation. These two values illustrate the wide range of values that can be found when considering processing and varietal differences. This method would enable commercial labs to quickly and efficiently quantitate this low level flavor compound using existing laboratory equipment.

This extraction method is preferable to that used originally by Demole et al. (1982) where 100 L of grapefruit juice was used to collect enough sample for identification, as compared to 0.030 Lr for this method. With future enhancements, this procedure could be further optimized to utilize less juice and solvent with improved MS conditions.

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