Ology Science

Research Article

Journal of Research Analytica

Determination of 1,8 Cineole in Fresh Rosemary and Sage Leaves by Solid-phase Microextraction and Gas Chromatography/Mass Spectrometry

Abstract

This paper focuses on the determination of 1,8 cineole in fresh *Rosmarinus officinalis* and *Salvia officinalis* leaves by gas chromatography with mass spectrometry combined with solid-phase microextraction. *Rosmarinus officinalis* is commonly known as rosemary; it has been reported that the performance on cognitive tasks was statistically significant related to the concentration of absorbed 1,8 cineole following exposure to rosemary.

A series of experiments were performed to determine the 1,8 cineole concentration in rosemary and sage leaves before and after heating as these herbs are widely used in the culinary arts. The results indicate that 50.0 mg of fresh rosemary leaf contains 0.23 ± 0.01 µg (95% confidence interval) of 1,8 cineole, which is 4.6 ± 0.2 ppm. Besides fresh rosemary leaves, *Salvia officinalis* which is commonly referred to as sage exhibited a higher 1,8 cineole concentration than fresh rosemary leaf, 50.0 mg of fresh sage leaf contained 0.37 ± 0.02 µg of 1,8 cineole, which is 7.4 ± 0.4 ppm.

Keywords: Rosemary; Sage; 1,8 Cineole; SPME; GC/MS

Xue Zhao and Peter de B. Harrington*

Center for Intelligent Chemical Instrumentation, Clippinger Laboratories, Department of Chemistry and Biochemistry, Ohio University, USA

***Corresponding author:** Peter de B. Harrington

E-mail: peter.harrington@ohio.edu **(P.D.B.H)**

Introduction

Putative effects of aromas on aspects of human behavior can be tracked back to ancient Greece, extracts from the aromatic plants can be used for cosmetic and medical purposes [1]. Nowadays, aromas are popular for pleasure, relaxation, and therapeutics. Rosemary is a small evergreen plant with needle like leaves, it grows wild in most Mediterranean countries [2]. Rosemary possesses some pharmacological properties [3] besides being very popular for its cooking qualities [4]. There are many applications of rosemary in everyday life, for example, reducing pests in gardens and its extracted oil may be used as fragrance [5]. Moss et al. [6] have found that sniffing essential oil from herbs enhanced human memories. To study the possible relationship between the 1,8 cineole level in the plasma and cognitive performance and mood, he and his co-workers designed a series of rosemary tests, which showed for the first time that performance on cognitive tasks is statistically significant related to the concentration of absorbed 1,8 cineole following exposure to rosemary aroma, with improved performance at higher concentrations. In this research, 1,8 cineole concentrations in fresh rosemary and sage leaves were determined.

Sage is also an herb commonly used for its medical properties. Its genus name means long life and good health. Sage extracts have been reported to possess several health effects, like antioxidant capacity and anti-inflammatory properties [7]. Bigford et al. [8] stated that sage has been investigated as a potential treatment for Alzheimer's disease. The result of this study showed that fresh sage plant contains higher 1,8 cineole than fresh rosemary leaf, but there is no publication explaining that the reason sage can enhance memory is the compound 1,8 cineole in the plant.

As the most common usage of rosemary in our daily life is in culinary art, an experiment of comparing the 1,8 cineole concentrations before and after heating was also designed. The 1,8 cineole concentration was significantly decreased after heating to 100°C. There is much news about the ability of rosemary to help enhance memory. For example, on the 'Body Ecology' website [9], there is an article called 'The herb rosemary: super antioxidant powerhouse memory booster and more', which encouraged people to eat rosemary dishes and cook with rosemary. In this study, the result showed that at high temperature, the 1,8 cineole concentration was significantly decreased, some websites posted articles about the top ten herbs to help boost your brain power. As some of them are difficult to collect, only ginseng, sage, and green tea were collected to determine the 1,8 cineole concentration. The result is that only sage plant contains higher 1,8 cineole concentration than rosemary, while the other herbs all have lower 1,8 cineole concentration. However, on many websites, like Natural Society [10], it listed rosemary as a better herb than sage to boost your brain power, which is also misleading, as the result showed that fresh sage contained more 1,8 cineole than fresh rosemary.

The principles of solid-phase micro-extraction (SPME) were first demonstrated by Pawliszyn and co-worker in 1990 [11], and it has been greatly developed and widely applied over the past twenty years [12]. Sample preparation is very important, SPME is a very simple, efficient and solventless sample preparation method [13]. This method saves preparation time and can improve detection limits [14]. The SPME fiber is the key part of the SPME apparatus. The fiber is a thin fused-silica optical fiber, coated with a thin polymer film [15]. The most used fiber coating is polydimethylsiloxane (PDMS), which is also used as a coating material in chromatography. SPME method was chosen to perform an experiment and collect the extraction of fresh rosemary and sage data because of its advantages. Several approaches have been developed for separation of rosemary and sage plant. Gas chromatography coupled with mass spectrometry is the most widely accepted and reliable technique for separation of rosemary and sage plants [16].

In this study, the 1,8 cineole concentrations in fresh rosemary leaves and sage leaves were determined and compared. In our daily life, typically rosemary and other aromatic plants are consumed cooked, so the effect of heat to 100°C, on 1,8 cineole concentrations before and after heating were also compared.

Experimental Section

Material and reagents

A 1,8 cineole, 99% stock solution was obtained from Alfa Aesar (Ward Hill, MA). Standard solutions of 1,8 cineole with concentrations of 0.1, 0.2, 0.4, and 0.8 µg/mL were prepared by dilutions of the stock solution with pentane. A 100 µm polydimethylsiloxane (PDMS) fiber, 10 mL headspace glass vials, and crimp seals were purchased from Sigma-Aldrich (St. Louis, MO). A stainless steel fine mesh strainer colander sieve with 7.9 cm diameter was purchased from the Kroger supermarket (Athens, OH). The samples of fresh rosemary and fresh sage leaves were collected from the Kroger supermarket (Athens, OH). All the fresh rosemary and sage leaves were analyzed within 10 days of the production date and they were stored at room temperature.

Instrumentation

All the experimental data were collected on a Thermo Finnigan PolarisQ quadrupole ion trap mass spectrometer/Trace Gas Chromatography (GC/MS) system with a Triplus AS2000 auto sampler (San Francisco, CA, USA). The GC/MS system was controlled by the XCalibur software version 2.0.7 provided by Thermo. Analytes were separated on a 30 m × 0.25 mm × 0.1 µm 5% diphenyl/95% dimethylpolysiloxane cross-linked capillary column (SHRXI-5MS, Shimadzu Scientific Instruments Inc. Columbia, MD).

Preparation of sample and calibration standards

To evaluate the cooking effect on rosemary, a 50.0 mg portion of powdered rosemary was added to the SPME vial without crimp seal and placed on the hotplate in the hood which was set at 75.0°C or 100.0°C for 15.00 min. Five replicates were collected at each temperature.

The fiber was thermally desorbed in the GC injector at 260.0°C for 5.00 min. The temperature program was set to start at 50.0°C, hold for 1.00 min, ramp at 20.0°C min^{-1} to 110.0°C, hold for 10.00 min, ramp at 20.0°C min^{-1} to 250.0°C, hold for 10.0 min. The carrier gas was helium with a constant flow rate of 1 mL min^{-1}. The transfer line temperature was maintained at 280.0°C. The inlet temperature was 260.0°C. Mass spectra were

obtained in electron ionization mode (70 eV) with the range from mass-to-charge ratio (m/z) 40-550.

Rosemary and sage leaves were powdered instead of testing individual leaves for better reproducibility. 1,8 Cineole standard solutions with concentrations of 0.1, 0.2, 0.4, and 0.8 µg/mL were prepared in pentane. Aliquots of 1.00 mL of each standard solution were added to the SPME vial with 50.0 mg powdered rosemary or sage. The SPME vials were left uncapped in the hood at room temperature for 30.00 min to fully evaporate the pentane. 1,8 Cineole has a boiling point 176.0°C so only an insignificant loss was expected during this process. After 30.00 min of evaporation, the vials were placed into the auto sampler tray for analysis. The sample vial was incubated at 55.0°C for 0.50 min. The SPME PDMS fiber was then exposed to the headspace for 30.00 min to achieve equilibrium [2]. The peak area of 50.0 mg rosemary or sage with each standard solution is listed in Table 1 and 2. The standard addition calibration curves are given in Figures 1 and 2, the coefficients of determination R^2 were 0.9936 and 0.9938 that were calculated from the sample averages of a random block design with five replicates. A confidence interval of 95% is used for all measurements of precision. The 1,8 cineole concentration in rosemary and sage were calculated from these calibration curves. Each plant was analyzed by SPME-GC/MS in five replicates. Three days later, five replicates were performed on each plant.

The RAW files of GC/MS data were converted to the network common documents format with Xcalibur Software 'File Convert Tool'. MATLAB R2015b (Math Works, Natick, MA) student version was used to process the data.

Table 1: Peak area for determination of 1,8-cineole in rosemary by standard addition.

Mass of 1,8 cineole added (µg)	Peak Area (×10⁸)
0.1	2.37
0.2	3.42
0.4	3.42
0.8	5.13

Table 2: Peak area for determination of 1,8-cineole in sage by standard addition.

Mass of 1,8 cineole added (µg)	Peak Area (×10⁸)
0.1	2.62
0.2	3.28
0.4	3.59
0.8	5.23

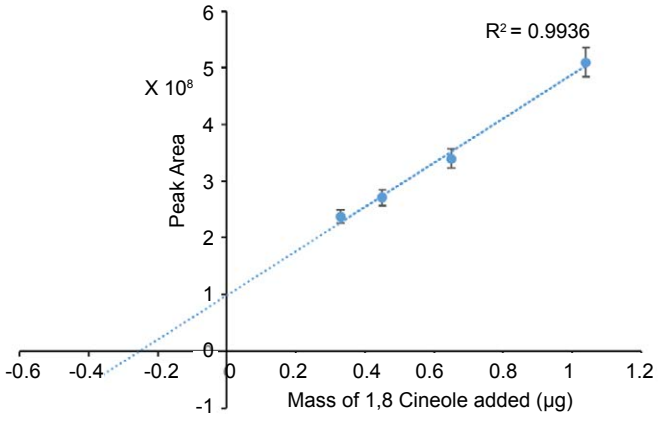

Figure 1: Rosemary 1,8 Cineole Calibration Curve (95 % confidence interval).

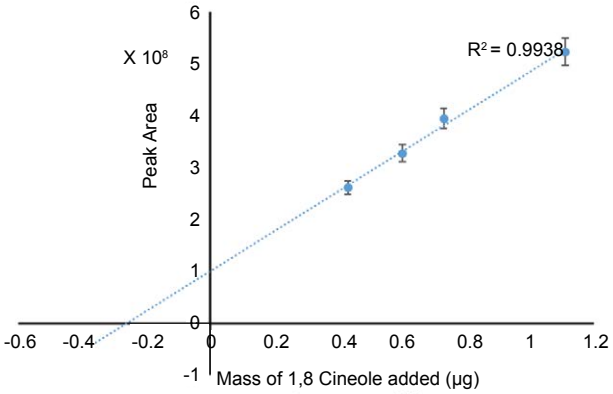

Figure 2: Sage 1,8 Cineole Calibration Curve (95% confidence interval).

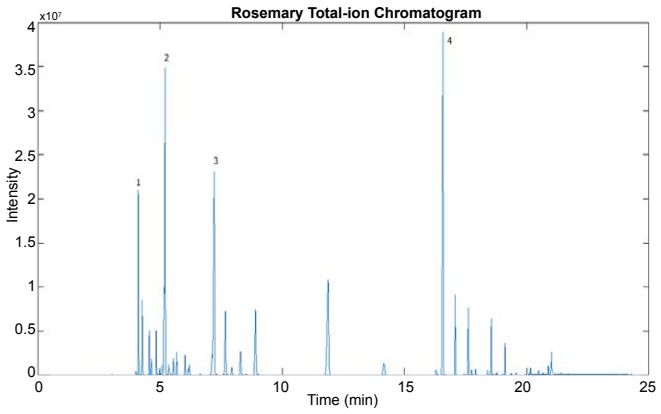

Figure 3: Total-ion chromatogram of fresh rosemary leaf at room temperature ([1] α-pinene; [2] 1,8 cineole; [3] limonene; [4] camphor).

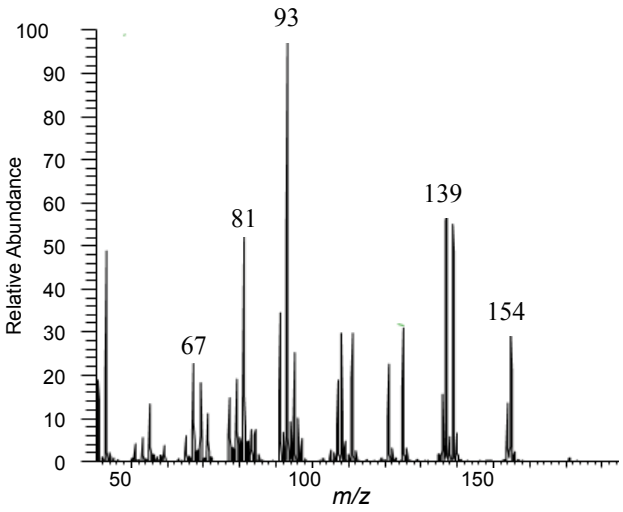

Figure 4: Mass spectrum of 1,8 cineole from fresh rosemary leaves.

Results and Discussion

A total-ion chromatogram of fresh rosemary leaves is in Figure 3. The different peaks marked in the figures represent some typical compounds in fresh rosemary leaves, peak 1 for α-pinene, peak 2 for 1,8 cineole, peak 3 for limonene, and peak 4 for camphor. The peak for 1,8 cineole was identified from its mass spectrum, which is given in Figure 4. The peaks of m/z 154, 139, 93, 81 and 67 are all characteristic peaks for 1,8 cineole. Figure 5 gives

the fragmentation interpretation of 1,8 cineole. The molar mass of 1,8 cineole is 154 g/mol. The fragment with m/z 139 is 1,8 cineole with a methyl group loss. Rosemary leaf contains 0.20 ± 0.01 μg (95% confidence interval) of 1,8 cineole, which gives w/w concentration of 4.6 ± 0.2 ppm.

The most common use of rosemary is as a spice and in most cases rosemary will be cooked at high temperature. An experiment has been designed to compare the 1,8 cineole concentration in rosemary at room temperature, 75.0°C and 100.0°C. The total-ion chromatograms for the three temperatures are given in Figure 6. The intensities of many peaks at 100.0°C have decreased compared with the peak intensities at 75.0°C. There are several peaks after a retention time of 20.00 min that are attenuated. It can be explained that some compounds, for example,

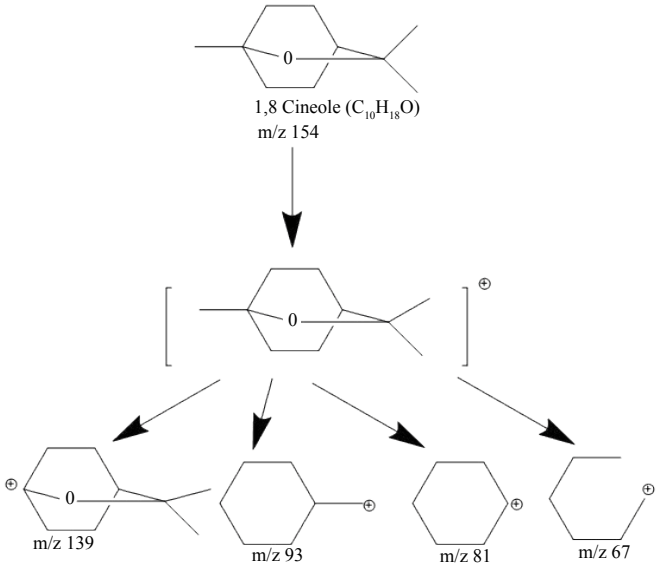

Figure 5: 1,8-Cineole mass spectrum interpretation.

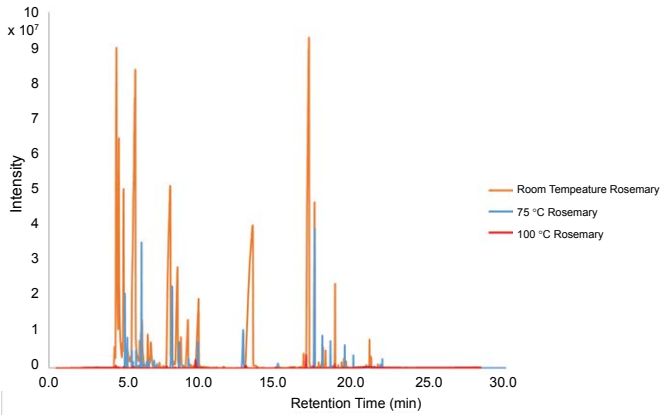

Figure 6: Room temperature, 100 °C and 75 °C rosemary total-ion chromatogram.

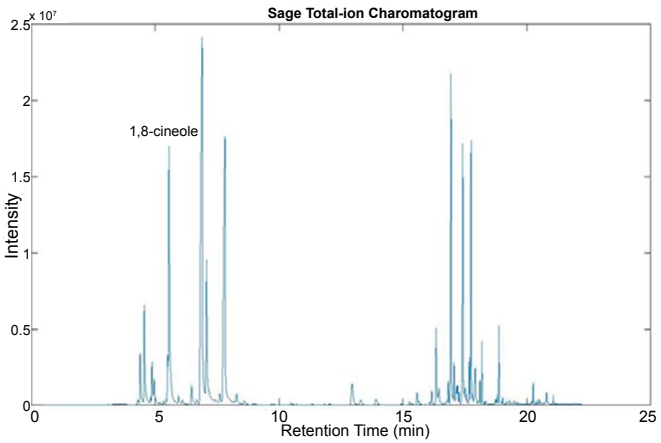

Figure 7: Fresh sage leaves total-ion chromatogram at room temperature.

α-humulene, has a boiling point that is 106.0°C, so was severely attenuated when heated at 100.0°C. The boiling point of 1,8 cineole is 176.0°C and its peak has markedly decreased when the rosemary was heated at 100.0°C. At 100.0°C, the concentration of 1,8 cineole has significantly decreased, so it can be concluded, 1,8 cineole cannot affect cognitive function after cooking at high temperature.

The total-ion chromatogram of fresh sage is given in Figure 7. The 1,8 cineole peak is marked in the chromatogram. The 1,8 cineole concentration in fresh sage leaves was calculated using the sage 1,8 cineole standard addition calibration curve (Figure 2). The result is that 50.0 mg fresh sage leaf contained 0.37 ± 0.02 μg of 1,8 cineole, which is 7.4 ± 0.4 ppm.

Conclusion

In this study, 1,8 cineole concentration in fresh rosemary and sage leaves were determined. It is also demonstrated that, at 100.0°C, 1,8 cineole concentration is significantly decreased compared with room temperature rosemary. Besides rosemary, 1,8 cineole concentration in fresh sage leaves was also determined. It contains higher 1,8 cineole concentration than rosemary. In the future work, more herbs and their 1,8 cineole concentrations will be determined and compared, as heating will decrease the concentration of 1,8 cineole. Because chewing gum [17] also has been reported to improve memory, it might be interesting to produce some herbal gums [17] with fresh rosemary or sage. In this way, the 1,8 cineole concentration can be kept at room temperature, and people can enhance their memory effectively by chewing rosemary gum.

Acknowledgement

Authors are thankful for Dr. Mengliang Zhang for his helpful suggestions.

References

1. Tisserand R. The art of aromatherapy, Essex, C.W. Daniel, 1993.
2. Socaci SA, Tofană M, Socaciu C. GC-MS analysis of rosemary essential oil. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Agriculture. 2008; 65(2): 405-9.
3. Thongtan K, TOMA RB, Reiboldt W, Daoud AZ. Effect of rosemary extract on lipid oxidation and sensory evaluation of frozen, precooked beef patties. Journal of Foodservice. 2005; 16(3-4): 93-104.
4. Yanishlieva NV, Marinova E, Pokorný J. Natural antioxidants from herbs and spices. Eur J Lipid Sci Technol. 2006; 108(9): 776-93.
5. Sibel SY, Semiha S, Nimet K, Recep A. Acaricidal and ovicidal effects of sage and rosemary extracts on tetranychus urticas Koch. Journal of Agricultural Science. 2014; 20: 358-67.
6. Moss M, Oliver L. Plasma 1, 8-cineole correlates with cognitive performance following exposure to rosemary essential oil aroma. Ther Adv Psychopharmacol. 2012; 2(3): 103-13.
7. Lu Y, Foo LY. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chem. 2001; 75(2): 197-202.
8. Bigford GE, Del Rossi G. Supplemental substances derived from foods as adjunctive therapeutic agents for treatment of neurodegenerative diseases and disorders. Advances in Nutrition: An International Review Journal. 2014; 5(4): 394-403.
9. Body ecology: http://bodyecology.com/articles/rosemary_super_antioxidant_memory_booster.php.
10. Nature society: http://naturalsociety.com/6-herbs-boost-brain-power-genius/.
11. Arthur CL, Pawliszyn J. Solid-phase microextraction with thermal-desorption using fused-silica optical fibers. Anal Chem. 1990; 62(19): 2145-2148.
12. Vas G, Vekey K. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis. J Mass Spectrometry. 2004; 39(3): 233-54.
13. Wercinski SCS. Solid Phase Microextraction—A Practical Guide, Marcel Dekker, New York, 1999.
14. Kataoka H, Lord HL, Pawliszyn J. Applications of solid-phase microextraction in food analysis. J Chromatogr A. 2000; 880(1): 35-62.
15. Hook GL, Kimm GL, Hall T, Smith PA. Solid-phase microextraction (SPME) for rapid field sampling and analysis by gas chromatography-mass spectrometry (GC-MS). TrAC Trends in Analytical Chemistry. 2002; 21(8): 534-43.
16. González-Rivera J, Duce C, Falconieri D, Ferrari C, Ghezzi L, Piras A, et al. Coaxial microwave assisted hydrodistillation of essential oils from five different herbs (lavender, rosemary, sage, fennel seeds and clove buds): Chemical composition and thermal analysis. Innovative Food Science & Emerging Technologies. 2016; 33: 308-18.
17. Matthew DG. Herbal-caffeinated chewing gum, but not bubble gum, improves aspects of memory. Appetite. 2011; 57: 303-307.