Determination of Limonene Oxidation Products Using SPME and GC–MS

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Abstract

Limonene is a common component found in consumer goods ranging from beverages to cleaning compounds. Limonene oxidation products, however, have a less desirable flavor and fragrance. Early detection of limonene oxide formation would aid quality control. A method is developed to determine the concentration of limonene oxide in essential oils and beverages using solid-phase microextraction (SPME). A headspace sampling technique is used to reduce or eliminate the presence of less volatile components. Several different SPME fibers are tested, varying in polymer thickness, polymer cross-linking and bonding, and polarity of the polymer. For each fiber tested, the sampling time is optimized for reproducible results. The 7-µm-thick bonded poly(dimethylsiloxane) fiber provides the best results. External standards are used for quantitation.

Introduction

Fragrance is most often associated with perfumes for personal adornment. But a large market involves the application of fragrances in consumer products ranging from household cleaning products to foods and beverages. Fragrant chemicals are added to enhance the enjoyment of the product or to mask unpleasant odors. Limonene (Figure 1A) is a common compound with a pleasant lemon scent. Unfortunately, it readily oxidizes to limonene oxides (Figure 1B and 1C) with an unpleasant smell and taste. Determination of oxide content in limonene or in the final product is important for determining product quality, the effects of storage time and temperature, and the effectiveness of packaging.

Solid-phase microextraction (SPME) was used for the analysis of limonene oxide in essential oils, fruit juices, and juice beverages. Volatile components were sampled from the liquid through the headspace (HS) and into the fiber coating (1–4). This requires that an equilibrium be established for the components of the sample in the liquid phase and in the HS above the liquid, and a second equilibrium be established between the fiber coating and the HS. As the components in the HS are adsorbed by the SPME fiber they are replenished in the HS, which is caused by the equilibrium with the liquid. Thus the relative amounts of the components on the fiber reflect the concentrations in the liquid sample and not in the HS, provided sufficient time is allowed for all components to establish both equilibria. If shorter sampling times are used, volatile compounds can be accurately sampled while the presence of semivolatile components can be reduced or eliminated from the sample injected, thereby shortening the analysis time, simplifying sample preparation, and reducing the accumulation of nonvolatiles in the injection port.

SPME sampling through HS has been compared with dynamic HS, static HS, direct liquid injection techniques (5–7), and to purge-and-trap analysis (8–10) in previous studies. Automated SPME has been compared with heated static HS analysis (11). The primary advantages of vapor-phase SPME sampling are solvent-free sample preparation, separation of analyte from nonvolatile and less volatile components, shorter analysis time if larger and less volatile molecules are not injected, and avoiding gas chromatographic column degradation caused by injection of large volumes of water.

Several factors must be considered when developing a quantitative analytical procedure using SPME. A variety of fibers are available differing in polarity, coating thickness, cross-linking, and phase bonding. The equilibrium between HS and fiber is different for any given analyte when different fiber coatings are used.

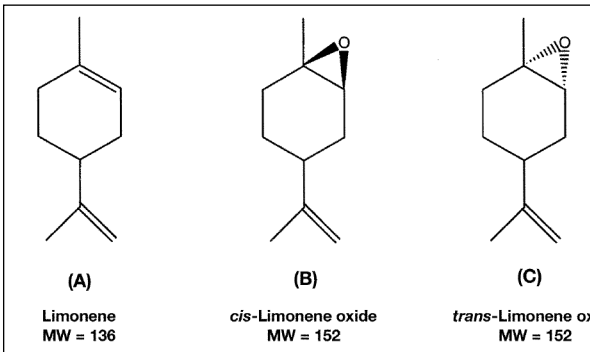

Figure 1. Molecular structure and molar mass for limonene and limonene oxides. MW = molecular weight.

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For the best reproducibility, the sampling time should allow equilibrium to be established between the liquid sample, vapor phase, and fiber coating for the analytes of interest. For a given fiber coating, equilibrium is affected by temperature; agitation or stirring of the sample affects the rate at which equilibrium is established. It is possible to obtain excellent quantitative results with shorter sampling times than those necessary to establish equilibrium if all SPME conditions and the sampling time are held constant (12,13).

Experimental

Chemicals and reagents

Research-grade (+)-limonene (stabilized with 0.03% tocopherol)–(+)-limonene oxide (97%) were purchased from Acros Organics (Princeton, NJ). Baking-quality orange oil, lemon oil, and lime oil were purchased from Murray Brothers Old Time Store (Middletown, OH). Fresh lemon peel oil was obtained by steam distillation of lemon zest shaved from three fresh lemons. Pink lemonade, orange juice, and strawberry kiwi fruit drink were obtained from a local supermarket.

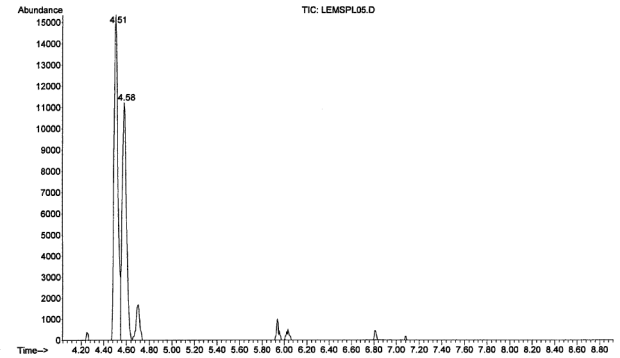

Figure 2. Total ion chromatogram of lemon oil sampled with a 7-µm PDMS fiber for 1 min and analyzed under the split conditions listed Table II. The peak at 4.51 min is *cis*-limonene oxide. The peak at 4.58 min is *trans*-limonene oxide.

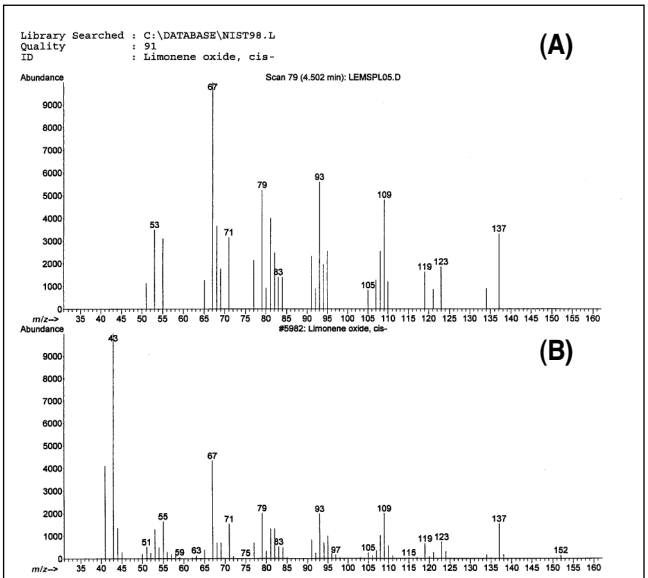

Figure 3. Mass spectra of *cis*-limonene oxide in lemon oil sample (A) and from the reference library (B).

Apparatus

A gas chromatograph (GC)–mass spectrometer (MS) (Hewlett-Packard G1800C GCD Series II system, Palo Alto, CA) was used and consisted of a GC with an electron ionization detector, an HP Kayak XA computer, and software provided by the manufacturer. SPME was performed using a manual holder, sampling stand, 0.75-mm-i.d. GC inlet liner, inlet guide, 100-µm nonbonded poly(dimethylsiloxane) (PDMS) fiber, 7-µm bonded polydimethylsiloxane (PDMS) fiber, and a 65-µm partially cross-linked carbowax–divinylbenzene fiber from Supelco (Bellefonte, PA). A Fisher Scientific Stirring Hotplate (Pittsburgh, PA) with stir speeds from 100–1200 rpm was used to agitate the samples.

Chromatographic conditions

Chromatographic separation was achieved on a 30-m × 0.25-mm i.d. fused-silica column coated with 0.25-µm thickness of HP-5 (cross-linked 5% phenyl–95% methyl siloxane). For percent concentrations of limonene oxide present, the split injection technique was used with a 130:1 split ratio. The column flow rate was 0.8 mL helium/min with a linear velocity of 33 cm/s. The oven temperature was maintained at 110°C for 6 min, then programmed at 20°C/min to 200°C and held for 1 min. For trace concentrations of limonene oxide, splitless injection was used. The column flow rate was 2.0 mL helium/min for the first minute while the injection port vent was closed; then it decreased to 0.8 mL/min with venting of the injection port. The oven temperature started at 40°C for 1 min, increased at 20°C/min to 100°C where it was held for 8 min, and finally increased at 50°C/min to 200°C with a final hold of 10 min. The fiber was desorbed in the injection port at 250°C for 1 min. Total ion chromatograms were plotted;

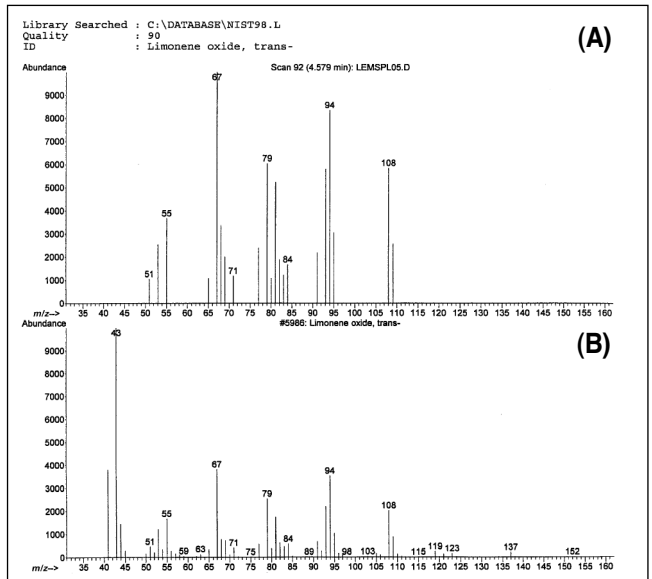

Figure 4. Mass spectra of *trans*-limonene oxide in lemon oil sample (A) and from the reference library (B).

there was a 4-min solvent delay for the split injections (Figure 2) and an 8-min delay for the splitless injections. Mass-to-charge ratios of *m/z* 45–450 were scanned. (A flame ionization detector could be used instead of the MS.) Figure 2 is a total ion chromatogram of lemon oil. Figures 3 and 4 show the mass spectra of *cis*- and *trans*-limonene oxide from that lemon oil sample and from the reference library for identification.

Standards

For the analysis of essential oils, standards of limonene oxide were prepared in limonene. A stock solution was prepared by diluting 2.8 g limonene oxide with 18.5 g limonene; the exact masses were used to calculate the concentration. Dilutions were made with limonene to produce standards ranging from 1–13% by mass. Further dilutions of the 1% standard were made with limonene to produce part-per-million standards. Exactly 1.0 mL of each standard was placed in a 4-mL vial for analysis.

For the analysis of beverages, an aqueous stock solution was made by spiking 100 µL limonene oxide into a 1.00-L solution. Dilutions were made with deionized water to produce standards ranging from 0.1–100 ppm by volume.

Method development using SPME

Several different SPME fibers were tested, varying in coating thickness and polarity of the polymer. PDMS fibers are excellent for sampling nonpolar compounds and a wide range of other compounds. Previous studies have demonstrated the broad sampling ability of PDMS even for analytes that are structurally similar to other fiber coatings (11). Both the 100- and 7-µm-thick PDMS fibers effectively sampled the limonene oxide, even in the presence of a limonene matrix. Because the concentration of limonene oxide is much smaller than that of limonene as it undergoes oxidation, and because SPME can selectively sample components depending on the choice of fiber, a polar carbowax–divinylbenzene fiber was tested to investigate whether the oxide group would increase the polarity sufficiently to preferentially sample limonene oxide over limonene. Unfortunately, the equilibrium constant for limonene oxide between the vapor phase and the polar polymer was so small that the low equilibrium concentration of the oxide on the fiber required splitless injection for adequate detection of the percent concentration samples. The part-per-million concentration samples would not be detected even with splitless injection. Thus the carbowax–divinylbenzene fiber was not useful for this analysis.

Table I. Precision of Manual Injection*

Trial	Area counts *cis*-limonene oxide	Area counts *trans*-limonene oxide
1	67816	43354
2	67600	43123
3	74114	43430
4	69876	44080
5	75306	47370
6	74435	44284
7	77949	46766
8	78333	46284
9	78262	44695
10	76579	44622
Mean	74027	44801
SD†	4181	1501
RSD‡	5.6%	3.4%

* Measured using a 7-µm PDMS fiber and commercial orange oil. The room temperature sample was stirred at 1200 rpm. Fiber exposure time was 2.0 min. Split injection technique used a 50:1 split ratio.
† Standard deviation.
‡ Relative standard deviation.

Parameters of sampling time, agitation, and temperature were examined for the fibers tested. Because the effect of each parameter was measured in terms of peak area counts, the precision of manual injection had to be measured before the effects of varying parameters could be determined. Murray Brothers Orange Oil (1.0 mL in a 4-mL amber vial) was sampled for 2.0 min with a 7-µm PDMS fiber at room temperature with 1200 rpm stirring. The data are shown in Table I. Ten replicate injections produced peak areas with 3–6% RSD for *cis*- and *trans*-limonene oxide. This is comparable to the precision achieved with manual liquid injections. Other studies have reported similar precision for quantitative SPME analyses (6); trace organic analyses have typically shown higher RSD values with SPME and with other traditional sampling techniques (14). This precision is sufficient to allow the effects of varying parameters to be measured.

For each fiber tested, a sampling time study was conducted to choose the most appropriate exposure time for each fiber. A graph of area counts versus fiber exposure time tracks the increase of analyte on the fiber with prolonged exposure of the fiber to the vapor over the sample. When equilibrium is established the amount of analyte on the fiber will be constant and will result in a constant area count even with longer sampling time. Murray Brothers Orange Oil was used as the test sample for these studies. Exactly 1.0 mL of orange oil was pipetted into a 4-mL amber sample vial and stirred at 1200 rpm. The manual fiber holder was supported in a sampling stand to ensure uniform exposure. After the appropriate sampling time elapsed, the fiber was immediately retracted into the needle sheathe, withdrawn from the sample vial, and injected into the GC for analysis. For most analyses, the detector scanned *m/z* 45–200. However, to obtain a detectable response, a range of *m/z* 10–450 was scanned and splitless injec-

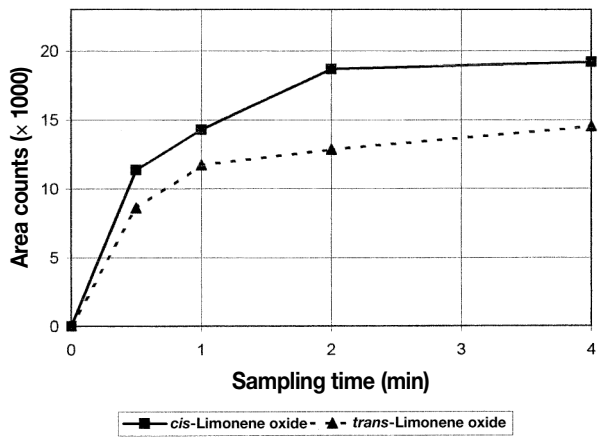

Figure 5. Effect of sampling time for the 7-µm PDMS fiber.

tion was used with the carbowax–divinylbenzene fiber. Data are plotted in Figure 5 for the 7-µm PDMS fiber. Optimal sampling time would allow equilibrium to be established for the analyte of interest but minimize the time for adsorption of less volatile components. The appropriate sampling times were 4 min for the 7-µm PDMS fiber and > 10 min for the two thicker phases. The 100-µm PDMS fiber required a longer exposure time than the 7-µm fiber to achieve equilibrium, which was expected for diffusion in the thicker polymer and the resulting increased sample capacity. Even with a large injection split ratio, the sample of limonene oxide on the 100-µm PDMS fiber often overwhelmed the MS detector. (Detection by the MS was delayed until the solvent eluted to avoid damaging the detector with the larger amounts of limonene.) The 100-µm thick fiber coating swelled somewhat when sampling the organic compounds, which shortened the fiber lifetime. The 7-µm PDMS fiber showed no swelling, had a longer useful life, and reached equilibrium with the sample faster than did the 100-µm PDMS fiber. The 7-µm PDMS fiber was chosen for sample analysis. Analytical conditions are listed in Table II.

Agitation of the sample is a factor to consider when developing an SPME method. The 7-µm PDMS fiber and Murray Brothers Orange Oil were used with sampling times varying from 30 s to 6 min. The maximum stir rate possible was 1200 rpm; rates of approximately 850, 1100, and 1200 rpm were studied. Area counts of *trans*-limonene oxide versus sampling time are shown in Figure 6. Similar results were obtained for *cis*-limonene oxide. Area counts became constant starting with a sampling time of 4 min with stir rates of 850–1200 rpm. The stir rate seemed to affect the amount of sample obtained more than the time required to reach equilibrium.

Table II. GC Conditions for Analysis using a 7-µm PDMS Fiber

Parameter	Condition
GC column	30-m x 0.25-mm fused-silica coated with 0.25-µm thickness of HP-5 (cross-linked 5% phenyl–95% methyl siloxane)
Fiber desorb time	1.0 min
Injection port temperature	250°C
Detector temperature	280°C
Mass/charge ratios scanned	*m/z* 45–200
Split injections	
Split ratio	130:1 (vent flow 103 mL/min)
Column flow rate	0.8 mL/min helium, 33 cm/s
Oven temperature	110°C for 6 min, 30°C/min to 200°C, held 1 min
Solvent delay on detector	4.0 min for elution of limonene
Splitless injection	
Vent time	1.0 min
Column flow rate	2.0 mL/min helium for 1 min, then 0.8 mL/min
Oven temperature	40°C for 1 min, 20°C/min to 100°C for 8 min, 50°C/min to 200°C held for 10 min
Solvent delay on detector	8.0 min for elution of limonene

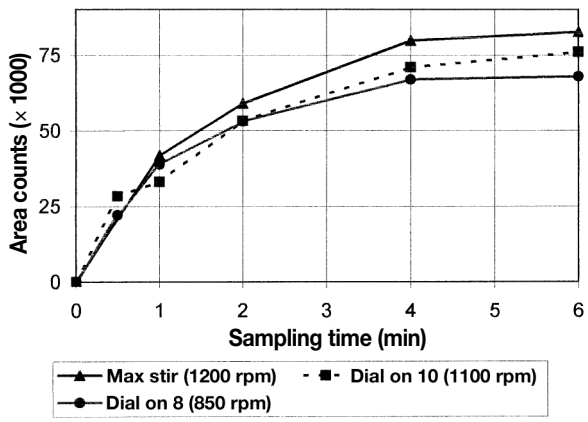

Figure 6. Effect of stir rate on sampling time needed to reach equilibrium using constant area counts of *trans*-limonene oxide as an indicator of equilibrium.

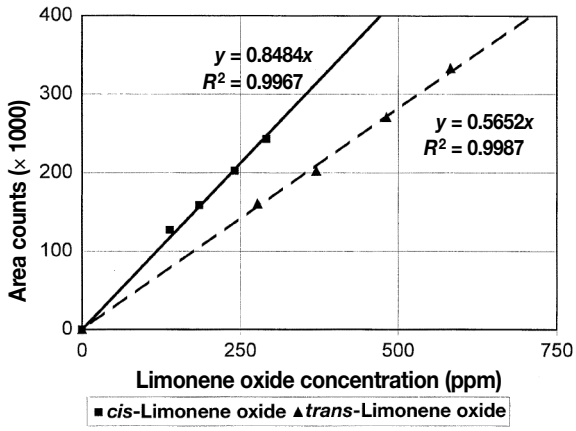

Figure 7. Calibration curve for limonene oxide in limonene at part-per-million levels. A 7-µm PDMS fiber was used for sampling.

Table III. Limonene Oxide Concentrations in Essential Oils, Juices, and Juice Drinks

Sample	*cis*-Limonene oxide	*trans*-Limonene oxide
Essential oils		
Orange oil	1.1% by mass	0.7% by mass
Lemon oil	1.0% by mass	1.1% by mass
Lime oil	0.2% by mass	0.1% by mass
Fresh lemon oil	None detected initially; 398 ppm after 3 weeks	None detected initially; 414 ppm after 3 weeks
Aqueous samples		
Tropicana pure premium orange juice	None detected	None detected
Dole's strawberry kiwi drink	None detected	None detected
Pink lemonade	None detected	None detected
Calibration lower limit	0.1 ppm	0.1 ppm

All sampling was conducted at room temperature. Limonene and limonene oxide were sufficiently volatile so that liquid/vapor equilibrium was quickly established, and elevated temperatures were not needed to reduce sampling time (15). The low temperature was also useful in reducing the sampling of less volatile components.

Results and Discussion

External standards were made of limonene oxide in limonene over the ranges 100–750 ppm and 0.1–18% by mass. A linear response was achieved for both concentration ranges with correlation coefficient values of R^2 from 0.995 to 0.999 (Figure 7). Several essential oils (Murray Brothers) and fresh lemon peel oil were analyzed for limonene oxide. Because the orange oil sample was used for much of the method development, this sample was analyzed first and was determined to contain 1.8% limonene oxide (*cis* and *trans*). Of the oils tested, the orange oil sample had the highest concentration. Results for other essential oils are listed in Table III. By comparison, although the major component in the fresh lemon peel oil was limonene, no limonene oxide was detected. To monitor oxidation of the limonene, the sample was tested weekly and stored in an amber sample vial. The vial cap was periodically removed to accelerate the formation of limonene oxide for this study. The limonene oxide concentration was 400 ppm when it was detected in the third week; daily monitoring would have detected the oxidation products sooner. The concentration continued to increased with time and exposure to air.

External aqueous standards were made of limonene oxide for the analysis of beverages. The standards ranging from 0.1–100 ppm by volume showed a linear response ($y = 141.56x$ for *cis*-limonene oxide and $y = 105.75x$ for *trans*-limonene oxide) with $R^2 = 0.9993$ for *cis*-limonene oxide and 0.9982 for *trans*-limonene oxide. Calibration nonlinearity caused by variations in SPME sampling conditions has been overcome with the use of an internal standard in other applications (16,17), but this was not needed for the determination of limonene oxide. Samples of fruit juice and juice beverages were analyzed for limonene oxide. No limonene oxide was detected in these consumer products (Table III).

Conclusion

This method proved useful for monitoring the quality of fresh lemon oil and of consumer-quality citrus oils for candy making, baking, and aromatherapy. Analysis of citrus juices and juice drinks containing limonene demonstrated the effectiveness of additives because no limonene oxide was detected. This method of SPME sampling through HS can be applied to other limonene-containing products to monitor oxidation rates.

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