Gas Chromatography/ Mass Spectrometry

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Comparison of Syringe and SPME Headspace Methods for GC/MS Analysis of Foods

Introduction

Syringe and SPME Headspace analysis of food volatiles are compared using a robotic multi-purpose autosampler for ease-of-use, selectivity, method development issues, and robustness. Volatile chemicals found in the headspace (HS) vapors of foods contribute strongly to organoleptic perception. Their absolute and relative characterization can be used for many purposes including safety assessment, formulation, quality control, competitive product analysis, source verification, and brand protection. There are several methods of headspace sampling commonly used for Gas Chromatography/Mass Spectrometry (GC/MS). This study compares automated liquid injection with headspace syringe injection and Solid Phase Microextraction (SPME) headspace for the analysis of several foods. Ease-of-use, selectivity, method development issues, robustness and throughput are discussed.

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Method and Samples

All modes of injection were were performed on the PerkinElmer Clarus MultiPrep + automated, multi-purpose robotic autosampler. Samples, equilibration times and equilibration temperatures were common to both syringe and SPME headspace. Specific autosampler parameters were optimized for each method. Several SPME fibers were evaluated and the optimum one selected.

The GC, MS, and autosampler conditions are shown below.

Gas Chromatograph	PerkinElmer Clarus 690 GC
Injector Type	Programmable Split/Splitless
Injector Temperature	200 °C
Analytical Column	PerkinElmer Elite™ - 5MS 30 m x 0.25 mm ID x 0.25 µm
Oven Program, Liquid Injection	70 °C for 5 min, then ramp to 190 °C at 10 °C/min
Oven Program, Headspace Injection	40 °C, ramp to 190 °C at 10 °C/min
Split Flow	100 mL/min for 3 min, then 10 mL/min
Carrier Gas	1 mL/min Helium, 99.999 + % purity

Mass Spectrometer	PerkinElmer Clarus SQ 8 GC/MS
GC Transfer Line	200 °C
Ion Source	200 °C
Ion Source Type	Electron Ionization
Acqusition Range	*m/z* 40-255
Scan Time	0.20 sec
Inter-Scan Delay	0.05 sec
Solvent Delay	2 min

Peppermint oil was selected as the target of comparison based on its complexity and common usage in food products. It has approximately 40 significant components covering a range of volatility and concentration.

Liquid injection of 1 µL peppermint oil (*Mentha piperita*) 40:1 in methanol was used to establish chromatography and identify the expected chromatographic peaks.

A SPME chromatogram is shown in Figure 1, and the identified peaks in Table 2.

The GC conditions differed for liquid and HS methods because of the methanol diluent in the liquid, and a lower initial oven temperature required for good resolution of early-eluting HS peaks. A 70 °C sample equilibration temperature improved HS recovery for the less-volatile compounds. Above 70 °C the syringe seal could be damaged for lower temperature work, and desorption of less volatile species from the SPME fiber might reduce sensitivity. A five min thermostatting time was used in a non-equilibrium sampling mode for improved sample throughput. The precision of system timing provided sufficient reproducibility for this work. The headspace syringe volume and SPME desorb time were kept short to minimize peak broadening. As with liquid injection, a 100:1 split was required to minimize sample overloading.

Four SPME fibers were tested; 50/30 µm DVB/Carboxen/PDMS, 65 µm PDMS/DVB, 85 µm Carboxen/PDMS, and 65 µm PDMS/DVB. The first and last had better sensitivity, especially for the early and late-eluting peaks. The first was used for the current work. Pre- and post-analysis fiber conditioning at 250 °C reduced carry-over to <0.002%.

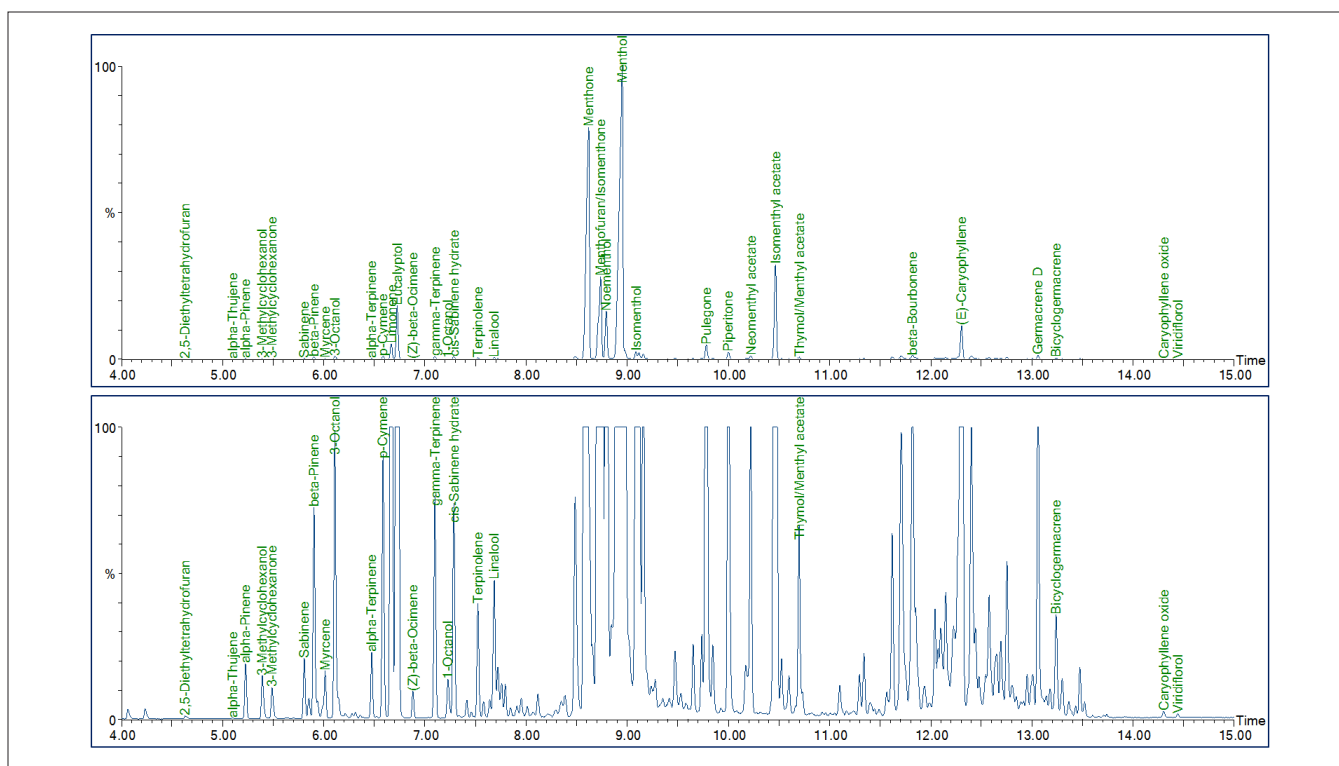| Autosampler	PerkinElmer TurboMatrix™ MultiPrep +				
Syringe and SPME Headspace		Syringe Headspace		SPME Headspace	
Sample Equlibration:	5 min	Sample Volume:	0.5 mL	Conditioning Temperature:	250 °C
Equlibration Temperature:	70 °C	Syringe Temperature:	70 °C	Preconditioning Time:	2 min
Agitation:	200 rpm	Syringe Injection:	10 mL/min	Postconditioning Time:	2 min
				Desorb Time:	0.2 min

Figure 1. Peppermint oil chromatogram, bottom plot x 100 scale.

Table 2. Peak identification showing retention times for liquid and HS analysis.

t_R (Liq)	t_R (HS)	Compound	t_R (Liq)	t_R (HS)	Compound
4.05	4.61	2,5-Diethyltetrahydrofuran	10.10	8.60	Menthone
4.78	5.08	α-Thujene	10.24	8.71	Menthofuran
5.93	5.21	α-Pinene	10.29	8.74	Isomenthone
5.31	5.37	3-Methylcyclohexanol	10.36	8.77	Neomenthol
5.51	5.47	3-Methylcyclohexanone	10.52	8.93	Menthol
6.07	5.79	Sabinene	10.67	9.07	Isomenthol
6.22	5.88	β-Pinene	11.49	9.76	Pulegone
6.44	5.99	Myrcene	11.76	9.98	Piperitone
6.62	6.09	3-Octanol	12.02	10.20	Neomenthyl acetate
7.16	6.45	α-Terpinene	12.28	10.45	Isomenthyl acetate
7.34	6.56	p-Cymene	12.53	10.68	Menthyl acetate
7.44	6.65	Limonene	12.28	10.43	Thymol
7.54	6.71	Eucalyptol	13.72	11.80	beta-Bourbonene
7.82	6.86	(Z)-β-Ocimene	14.22	12.28	(E)-Caryophyllene
8.11	7.08	γ-Terpinene	15.01	13.04	Germacrene D
8.34	7.21	1-Octanol	15.20	13.22	Bicyclogermacrene
8.36	7.27	cis-Sabinene hydrate	16.27	14.28	Caryophyllene oxide
8.70	7.50	Terpinolene	16.41	14.42	Viridiflorol
8.95	7.67	Linalool			

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Figure 2 shows the HS samples as injected. Each was one "piece" of candy, cookie, or tea bag. Samples 2-9 were analyzed both whole and fragmented to determine if the flavor was on the outside, inside, or both. Samples 11-14 were analyzed with and without the chocolate coating.

Results and Discussion

All three injection techniques were able to detect the complete range of peppermint oil analytes. Table 3 shows relative the peak areas of representative analytes from the early, mid, and late chromatogram.

Comparing the trends we see that HS syringe and SPME injections both show higher response for the more volatile analytes than liquid injection, SPME more than syringe. However, both HS have reduced sensitivity for the less volatile, with syringe much worse than SPME. Syringe and SPME have about the same sensitivity for more volatile analytes, but SPME gets better as volatility decreases. This is consistent with the expectation of reduced headspace concentration for less volatile analytes, and adsorption of those analytes on the SPME fiber having a concentrating effect vs. syringe HS.

Table 4 summarizes the sample results. Figure 3 shows illustrative chromatograms for samples 1 to 5.
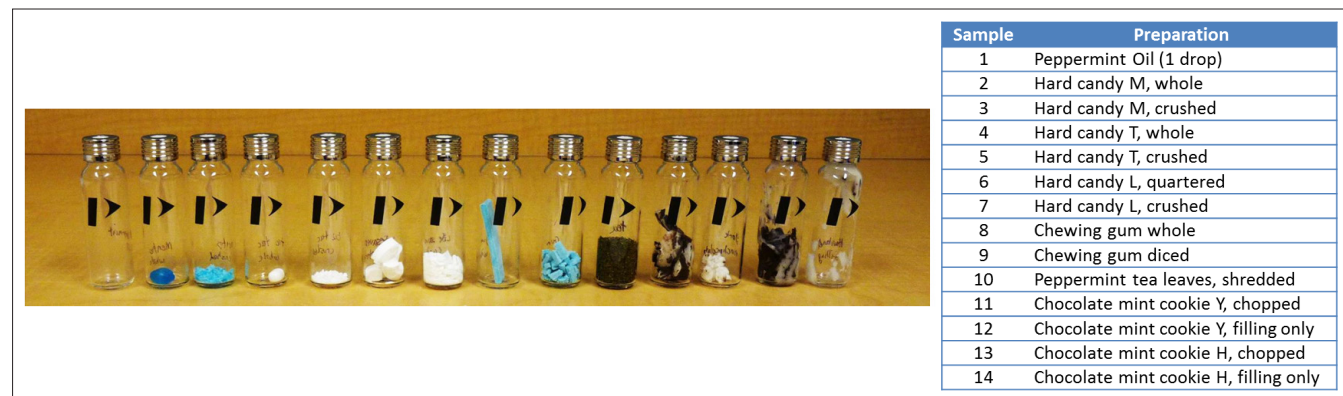

Sample	Preparation
1	Peppermint Oil (1 drop)
2	Hard candy M, whole
3	Hard candy M, crushed
4	Hard candy T, whole
5	Hard candy T, crushed
6	Hard candy L, quartered
7	Hard candy L, crushed
8	Chewing gum whole
9	Chewing gum diced
10	Peppermint tea leaves, shredded
11	Chocolate mint cookie Y, chopped
12	Chocolate mint cookie Y, filling only
13	Chocolate mint cookie H, chopped
14	Chocolate mint cookie H, filling only

Figure 2. Headspace samples, numbered from left to right.

Table 3. Comparison of relative intensities for liquid, syringe HS and SPME.

Comparison	Eucalyptol	Isomenthyl acetate	Caryophyllene
SPME to liq	9.95	0.56	0.28
HS to liq	6.88	0.15	0.04
SPME to HS	1.45	3.76	6.72

Table 4. Sample summary results.

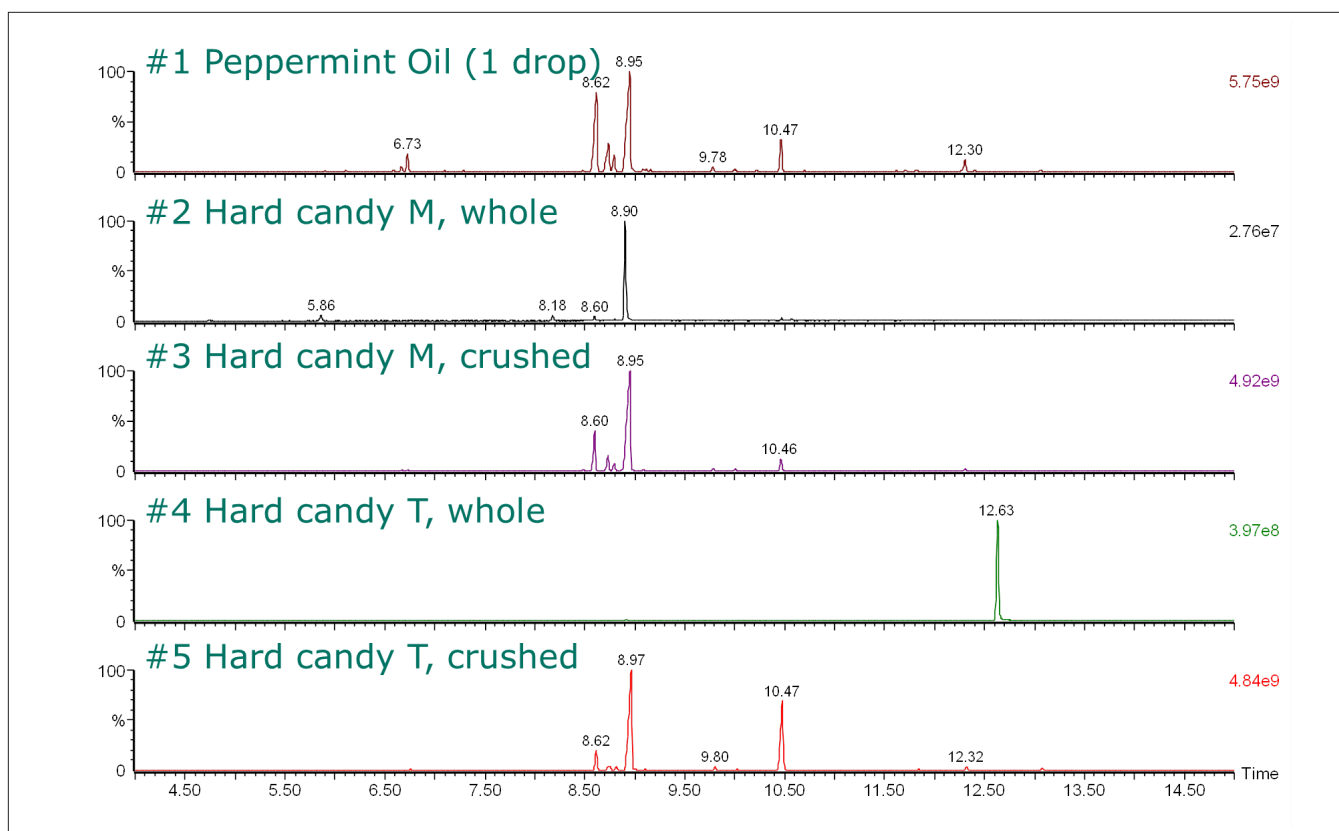| Sample	Description	Results
1	Peppermint Oil	Reference standard
2 and 3	Hard candy M	Whole has much less volatiles, different than crushed. Mostly menthol on outside coating, peppermint inside.
4 and 5	Hard candy T	Whole has much less volatiles, different than crushed. Mostly ethyl vanillin on outside coating (not a peppermint component). Artificial peppermint with enhanced menthol, isomenthyl acetate, germacrene D and viridiflorol inside.
6 and 7	Hard candy L	Same volatiles outside and inside. About the same intensity for whole and crushed – implies that the flavor is only coated on the outside.
8 and 9	Chewing gum	About same intensity of volatiles outside and inside – implies uniformly distributed.
10	Peppermint tea leaves	Closely matches peppermint oil ratios.
11 and 12	Chocolate mint cookie Y	Combined chocolate/cream have many major analytes, but missing everything above isomenthol acetate, implying artificial flavor. Ratios for combined and cream-only differ – combined stronger in eucalyptol implying presence in the chocolate, but weaker in isomenthyl acetate, which may be concentrated in the cream.
13 and 14	Chocolate mint cookie H	See peaks up to Germacrene D. Less volatile may not be partitioning into the headspace enough to be detected. Ratios for combined and cream-only are about the same.

Figure 3. SPME chromatograms of samples #1 to 5.

Conventional sample preparation such as liquid-liquid extraction is often complex and time-consuming. Headspace analysis reduces this effort. Both syringe and SPME headspace sampling methods provided useful results, requiring minimal or no sample preparation. Both had specific strengths and weaknesses.

SPME had the most selectivity, and for certain analytes better sensitivity. Method optimization can be more challenging because of the need for fiber phase and thickness selection, optimization of sampling conditions, sample matrix effects, and differential analyte response. SPME fiber lifetime is limited. Depending on the analysis, it may be in the dozens or low hundreds of injections.

Syringe HS sampling had the simplest method development and the fewest parameters to optimize. However, care is required to avoid condensation, adsorption or reactivity of labile analytes on the syringe needle or barrel. The syringe temperature range is limited, limiting sensitivity for less volatile analytes. Cross-contamination between samples is at higher risk because the syringe cannot be

"baked out" at high temperature. Care needs to be taken that the partial vacuum created by withdrawing a large headspace gas volume does not shift the sample equilibrium.

Sample throughput differed. SPME was the slowest, unable to as effectively "pipeline" fiber equilibration and conditioning as effectively with the other analysis steps. While only a short fiber conditioning time was required for these samples; for others the fiber may need to be baked 30 to 60 min after each injection to clean it for the next. Syringe HS sampling was quicker, not requiring SPME's second equilibration period of the fiber and analyte vapors.

Conclusions

- Automated, software-controlled liquid, syringe headspace, and SPME analysis of food samples
- Minimal sample preparation
- Easy characterization of flavor distribution