Comparison of Dynamic Headspace Concentration on Tenax with Solid Phase Microextraction for the Analysis of Aroma Volatiles

J. Stephen Elmore, Mehmet Ali Erbahadir, and Donald S. Mottram*

Department of Food Science and Technology, The University of Reading, Whiteknights, Reading RG6 6AP, United Kingdom

Two different headspace extraction methods for aroma volatiles were compared: headspace solid phase microextraction (SPME), using both poly(dimethylsiloxane) (PDMS) and polyacrylate (PAc) fibers, and dynamic headspace trapping using a glass-lined stainless steel trap, containing Tenax TA. The samples studied were a cola and a diet cola. Extraction time and temperature were the same for each analysis. The dynamic headspace method extracted more volatiles from both samples than did SPME with either filament. The profile obtained using the PDMS fiber was similar to that of the Tenax TA extract but was much less intense. The PAc extract contained fewer components than the PDMS extract but included two polar volatiles not present in the other two extracts. Reproducibilities were similar for both techniques. Qualitative differences between the two cola samples were small, although the total quantity of volatiles extracted from the cola was up to 4 times greater than for the diet cola.

Keywords: *Cola; gas chromatography/mass spectrometry; solid phase microextraction; dynamic headspace concentration; Tenax TA; aroma*

INTRODUCTION

Preparation techniques involving headspace concentration, using porous polymer absorbents, have been widely used for the analysis of aroma compounds (Teranishi and Kint, 1993). Recently, a new absorption technique called solid phase microextraction (SPME) has been developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990; Arthur et al., 1992a,b; Potter and Pawliszyn, 1992), and SPME devices are now commercially available. The key component of a SPME device is a fused silica fiber (ca. 1 cm in length) coated with an absorbent material such as poly(dimethylsiloxane) (PDMS) (Yang and Peppard, 1994).

Headspace SPME is a solvent-free sample preparation technique in which the fused silica fiber is introduced into the headspace above the sample. In headspace SPME, there are two processes involved: the release of analytes from their matrix and the absorption of analytes by the fiber coating (Zhang and Pawliszyn, 1995). The volatile organic analytes are extracted and concentrated in the coating and then transferred to the analytical instrument for desorption and analysis. The detection limits of the headspace SPME technique have been claimed to be at the subpicogram level (Zhang and Pawliszyn, 1995). The equilibrium time for less volatile compounds can be shortened significantly by agitation of both aqueous phase and headspace, reduction of headspace volume, and increase in sampling temperature (Zhang and Pawliszyn, 1993).

Yang and Peppard (1994) applied the SPME technique to ground coffee, a fruit juice beverage, and a butter flavor in vegetable oil. They found that the conventional headspace sampling method generally was more sensitive for highly volatile compounds of espresso-roast ground coffee, while the SPME headspace method extracted more of the less volatile compounds.

* Author to whom correspondence should be addressed (fax +44 118 931 0080; e-mail d.s.mottram@reading.ac.uk).

Comparison with traditional headspace Tenax adsorption−desorption GC/MS analyses of volatile organic sulfur compounds in truffle aromas showed that the headspace SPME technique was less suited for quantitative analyses because the PDMS fiber coating strongly discriminated against more polar and very volatile compounds (Pelusio et al., 1995). However, Krumbein and Ulrich (1996) found that headspace SPME gave comparable results to dynamic headspace trapping on Tenax TA, when used to examine tomato aroma.

Dynamic headspace trapping onto Tenax TA is widely used for aroma analysis in our laboratory. We wanted to know how SPME would perform relative to dynamic headspace trapping in terms of sensitivity, range of volatilities, and reproducibility. This has been achieved by examining the volatile aroma components in a commercial cola-flavored beverage and its diet equivalent, using both techniques. Two types of SPME fiber—PDMS and polyacrylate (PAc)—were examined. The PAc fiber is suggested by the manufacturer as being more appropriate for polar volatiles, such as phenols. So that the methods were comparable, the sample size was consistent throughout, as were the temperature and time of extraction.

MATERIALS AND METHODS

Materials. A branded cola and its diet equivalent were purchased in 330 mL cans from a local supermarket. Each cola was degassed before use by pouring it several times from one beaker to another. Volatile analyses were carried out in triplicate, on both samples, by dynamic headspace trapping and by SPME.

Dynamic Headspace Trapping. An aroma extract was prepared by sweeping aroma volatiles onto Tenax TA, using the method of Madruga and Mottram (1995), with the following variations. The cola and diet cola samples (10 mL) were extracted at 60 °C for 30 min, and volatiles were collected on a glass-lined, stainless steel trap (105 mm × 3 mm i.d.) containing 85 mg of Tenax TA (Scientific Glass Engineering Pty Ltd., Ringwood, Australia). After extraction, an internal standard (130 ng of 1,2-dichlorobenzene in 1 μL of hexane) was added to the trap. Nitrogen at 40 mL min^{-1} was then

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blown through the trap for 5 min to remove any moisture and excess solvent.

Solid Phase Microextraction. Two different 10 mm length fibers (Supelco Inc., Bellefonte, PA) were used for this investigation. The first was coated with a 100 μm layer of PDMS, and the second was coated with an 85 μm layer of PAc.

For each SPME analysis, 10 mL of sample was placed in a 20 mL glass vial, which was then crimp-capped with a Teflon-lined septum. The stainless steel needle, housing the fiber, penetrated the septum. After equilibration at 60 °C for 5 min, the fiber was pushed out of the needle and exposed to the headspace above the sample for 30 min.

Gas Chromatography/Mass Spectrometry. All analyses were performed on a Hewlett-Packard 5972 mass spectrometer, coupled to a 5890 Series II gas chromatograph and a G1034C Chemstation. The capillary column used was a BPX5 fused silica capillary column (50 m × 0.32 mm i.d., 0.5 μm film thickness; Scientific Glass Engineering Pty Ltd.).

A CHIS injection port (Scientific Glass Engineering Pty Ltd.), held at 250 °C, was used to thermally desorb the volatiles from the Tenax trap onto the front of the capillary column. During the desorption period of 5 min, the oven was held at 0 °C.

The SPME fiber was desorbed for 2 min in the GC split/splitless injection port, held at 250 °C. The injection port was in splitless mode, the splitter opening after 2 min. Immediately before the desorption of the fiber, 1 μL of internal standard solution was injected into the gas chromatograph. Again the oven was held at 0 °C.

For both extraction techniques, after desorption, the oven was heated at 40 °C min^{-1} to 40 °C. After 2 min, the temperature was raised at 10 °C min^{-1} to 280 °C. Helium at 8 psi was used as the carrier gas, resulting in a flow of 1.75 mL min^{-1} at 40 °C. *n*-Alkanes (C_6–C_{22}) were run under the same conditions to obtain linear retention index (LRI) values for the components.

The mass spectrometer operated in electron impact mode with an electron energy of 70 eV and an emission current of 50 μA. The mass spectrometer scanned from *m*/*z* 29 to 400 at 1.9 scans s^{-1}. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH Mass Spectral Database and by comparison of LRI and mass spectra with those reported by Adams (1995).

RESULTS

The chromatograms that were obtained for cola by dynamic headspace extraction and the SPME extractions with PDMS and PAc are shown in Figure 1. The major aroma components found in the cola and the diet cola are listed in Table 1. Only compounds present in the chromatograms at amounts above 10 ng are quantified. Compounds present at levels above the detection limit of 1 ng are listed as trace.

A total of 61 compounds were tentatively identified and 6 partially identified. They were mainly mono- or sesquiterpenes and aldehydes. All but 6 of these compounds were present in the dynamic headspace extract of the cola. The dynamic headspace extract of the diet cola contained fewer compounds, 48 in all, and the total peak area was only 25% of the area of the cola, suggesting that either flavor release from the diet cola occurs at a slower rate than from the cola or a lower amount of flavoring is present in the diet cola.

Using SPME with the PDMS fiber, 25 compounds were identified in the cola and 15 in the diet cola, with the total amount of volatiles being twice as great in the cola as in the diet cola. Two of these compounds, a sesquiterpene alcohol of mass 222 and α-bisabolol, were not found in the headspace extracts. Only 7 compounds were identified in the cola, using the PAc fiber, all of which had been found in the dynamic headspace extract of the cola, although (*E*)-β-terpineol and (*E*)-cinnamaldehyde were not found in the cola when PDMS fiber

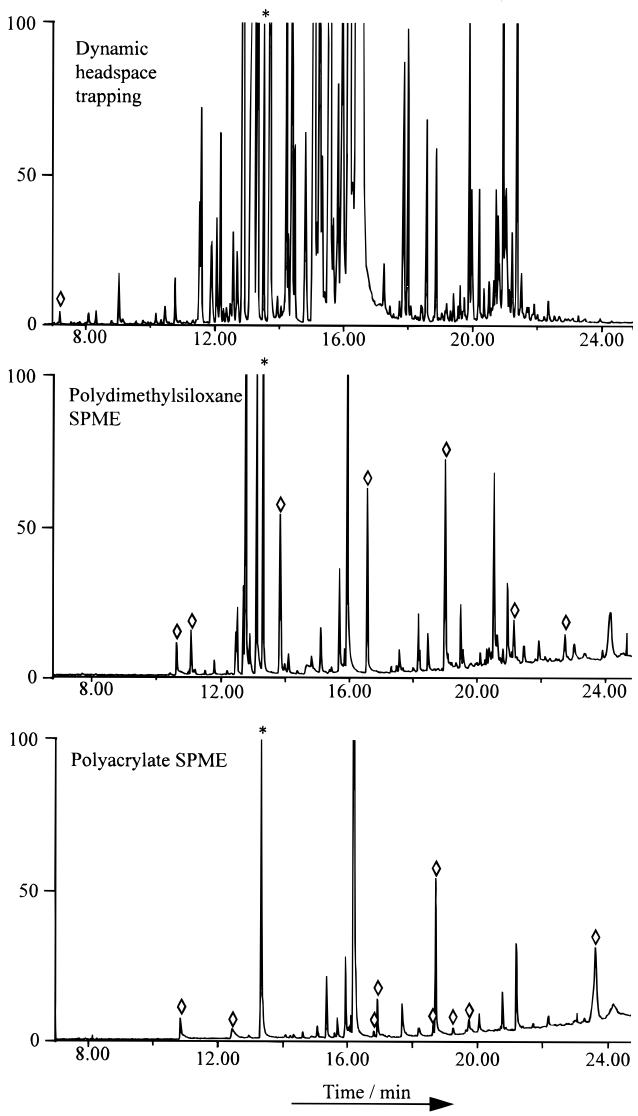

Figure 1. Comparison of gas chromatographic traces of cola extracts obtained using dynamic headspace trapping, SPME with PDMS fiber, and SPME with PAc fiber: *, internal standard (130 ng μL^{-1} 1,2-dichlorobenzene); ◊, artifact.

was used. Of the 3 compounds found in diet cola using the PAc fiber, benzoic acid was not found in any of the other extracts. Benzoic acid is probably the most polar volatile of any of the volatiles identified in these extracts, which may explain why it was only found using PAc fiber.

α-Terpineol was the major component in all of the extracts, except for the PDMS and PAc extracts of diet cola, for which the major components were limonene and benzoic acid, respectively.

DISCUSSION

Sensitivity. For these samples, SPME using PDMS gave satisfactory results. The cola dynamic headspace sample was overloaded, giving poor peak shape and poor resolution in some cases. However, for solid samples, SPME may prove inadequate. Preliminary experiments using wetted cereals yielded very little data by SPME, whereas dynamic headspace extraction gave excellent results.

The number of artifact peaks was greater in both of the SPME extracts than the dynamic headspace extracts. The PDMS extract contained several siloxanes,

Table 1. Quantities[a] (Nanograms) of Volatiles Collected from Cola and Diet Cola Beverages Using Dynamic Headspace Trapping and SPME with PDMS and PAc Fibers

				SPME			
		dynamic headspace trapping		PDMS		PAc	
identity	LRI[b]	cola	diet cola	cola	diet cola	cola	diet cola
hexanal	813	13 (42)	Tr[c]	*d*			
furfural	853	20 (15)	17 (10)				
2-acetylfuran	929	21 (20)	15 (10)				
α-fenchene	965	51 (2)	Tr				
camphene	968	93 (4)	26 (57)				
5-methylfurfural	982	81 (21)	46 (8)				
benzaldehyde	990	63 (29)	23 (9)				
β-myrcene	995	128 (33)	24 (28)	Tr			
octanal	1015	105 (57)	19 (29)				
α-phellandrene	1024	61 (30)	Tr				
1,4-cineole	1033	418 (16)	373 (1)	16 (11)	15 (29)		
α-terpinene	1035	253 (19)	47 (11)	25 (14)			
p-cymene	1052	233 (17)	27 (27)	31 (10)			
limonene	1055	1576 (10)	416 (17)	217 (10)	107 (32)		
1,8-cineole	1060	486 (18)	304 (5)	21 (8)	13 (38)		
γ-terpinene	1079	818 (11)	125 (17)	154 (9)	40 (34)		
pentylcyclopropane[e]	1092		12 (14)				
p-mentha-3,8-diene + decahydronaphthalene[e]	1089	36 (55)					
terpinolene	1105	323 (16)	58 (24)	96 (7)	61 (17)		
p-mentha-2,4(8)-diene	1108	41 (33)					
linalool	1113	278 (21)	Tr	Tr			
nonanal	1118	112 (25)	38 (15)	11 (7)	Tr		
myrcenol	1137	111 (22)	Tr				
fenchol	1154	1085 (29)	332 (11)	20 (26)			
(*E*) [or-(*Z*)]-terpin-1-ol	1162	322 (23)	115 (10)	14 (16)			
(*E*) [or-(*Z*)]-terpin-1-ol	1167	87 (39)	42 (26)				
(*Z*)-*β*-terpineol	1181	1050 (30)	147 (14)	29 (19)	Tr	25.5	Tr
benzoic acid[e]	1185						154 (35)
camphor	1187	49 (37)	20 (45)				
camphene hydrate	1195	140 (39)	74 (23)				
isoborneol + (*E*)-*β*-terpineol	1203	308 (38)	51 (7)				
p-menth-1-en-4-ol + borneol	1217	1502 (36)	173 (11)	67 (19)	Tr	29 (8)	
decanal	1221	141 (30)	82 (7)	Tr	Tr	Tr	
α-terpineol + *γ*-terpineol[e]	1241	3979 (37)	483 (20)	341 (20)	47 (9)	460 (5)	
2-phenylethyl acetate	1283	36 (47)					
bornyl acetate	1314	14 (11)	Tr				
(*E*)-cinnamaldehyde	1323	126 (25)	Tr			19 (11)	
safrole	1332	144 (12)	48 (19)	13 (19)	Tr		
methyl geranoate	1336	12 (18)	Tr				
neryl acetate	1368	96 (28)	38 (57)	26 (11)	16 (25)		
geranyl acetate	1387	90 (27)	32 (40)	19 (10)	12 (21)		
copaene	1412	12 (30)	31 (5)		Tr		
(*Z*)-α-bergamotene	1441	14 (6)	11 (2)				
α-santalene	1449	19 (9)	Tr				
(*E*)-α-bergamotene	1460	156 (5)	129 (9)	27 (13)	48 (10)	Tr	Tr
caryophyllene[e]	1467	70 (28)	83 (5)	11 (14)	33 (10)		
(*E*)-cinnamyl acetate	1481	56 (42)		Tr		10 (16)	
β-santalene	1492	15 (10)	12 (3)				
MW 204 sesquiterpene[e]	1503	24 (7)	12 (8)				
γ-muurolene	1513		13 (6)				
α-farnesene	1518	59 (7)	Tr	Tr	Tr		
α-bisabolene	1523	44 (13)	20 (26)				
MW 204 sesquiterpene	1528	24 (9)		12 (20)	Tr		
β-bisabolene	1536	382 (19)	206 (29)	110 (21)	106 (9)	12 (22)	17 (44)
γ-bisabolene + MW 204 sesquiterpene[e]	1547	113 (22)	59 (19)	26 (26)	35 (11)		
δ-cadinene	1556	39 (7)	52 (12)	11 (35)	25 (17)		
myristicin	1566	196 (45)	74 (47)	58 (21)	44 (17)	39 (2)	17 (39)
MW 204 sesquiterpene[e]	1578	25 (10)		Tr			
MW 222 sesquiterpene[e]	1609	Tr		12 (6)	11 (2)		
MW 232 sesquiterpene[e]	1637		12 (14)				
α-bisabolol	1725			14 (18)	Tr		

[a] Values are means of triplicate analyses with percentage coefficient of variance shown in parentheses. [b] Linear retention index. Except where indicated these agreed with those reported by Adams (1995). [c] Trace, <10 ng. [d] No entry indicates compound was not present above detection limit of 1 ng. [e] Reference LRI not available.

and 1-methyl-2,4-diisocyanatobenzene was a large contaminant in the PAc extract. Artifacts may have resulted from insufficient conditioning of the fibers, which were heated at 250 °C for 1 h before use. Teflon-coated septa were used in the injection port and would not result in this type of contamination. Such artifacts may impose limitations on the use of SPME.

Range of Volatilities. Although the chromatograms from the dynamic headspace extraction and the SPME using PDMS were similar qualitatively, there were some differences in the relative quantities of volatiles found in the chromatograms. The headspace method favored the most volatile compounds, e.g., the level of α-terpinene in the headspace extract of cola was 10 times

greater than in the PDMS extract, whereas the level of the much less volatile δ-cadinene was only 3.5 times greater in the headspace extract. The headspace extract also contained proportionally more medium polarity volatiles, such as alcohols, than the PDMS extract, e.g., the amount of fenchol in the headspace extract of the cola was over 50 times greater than in the PDMS extract. The PAc cola extract contained only one terpene hydrocarbon and relatively high levels of alcohols compared to the other two cola extracts.

Reproducibility. There appear to be no differences in reproducibility for the two techniques. The mean coefficient of variance for all of the identified peaks was 24% for the cola and 18% for the diet cola, whereas for the SPME using PDMS the values were 16% and 19%, respectively. For PAc the values were 10% for the cola and 40% for the diet cola. The high value for the diet cola was probably due to the relatively small peak areas for the three peaks found.

Conclusions. For straightforward analysis of major volatile components SPME would be the method of choice, but for trace analysis it would appear that only dynamic headspace trapping is suitable. Although the literature suggests that a static extraction is adequate when using SPME, we think that a dynamic setup may prove more productive.

ABBREVIATIONS USED

SPME, solid phase microextraction; PAc, polyacrylate; PDMS, poly(dimethylsiloxane); LRI, linear retention index.

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