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Note

Validity of SPME Cryofocusing in Analysis of Volatile Components in Orange Juice

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We studied the effects of cryofocusing on the resolution of volatile components in solid-phase microextraction (SPME). Retention times, resolutions and peak areas estimated using cryofocusing were compared to those observed without cryofocusing. The resolution and peak areas with cryofocusing were found to be greater than those obtained without cryofocusing. Hence, SPME cryofocusing is effective in trace analysis of the volatile components in orange juice.

Keywords: solid-phase microextraction, orange juice, food flavor, gas chromatography, cryofocusing, resolution

Introduction

Solid-phase microextraction (SPME) is a novel sample preparation technique for gas chromatography (Arthur and Pawliszyn, 1990) which allows extraction and concentration of volatile components from the sample matrix. The SPME fibers can be directly introduced into a gas chromatograph injector. The method is safe both for the environment and for the analyst; further, the data are highly reliable because no solvents are used. For these reasons, SPME has been applied in several fields.

In the SPME method, the volatile components of a sample are collected in a fiber and introduced into a gas chromatograph via a heat-desorbing process. The process is slow and the desorption time greatly affects the resolution. According to some reports (Deibler *et al.,* 1999; Elmore *et al.,* 1997; Jia *et al.,* 1998), the duration of desorption is 2 minutes; Fischer and Fischer (1997) decided on a desorption time of 15 minutes, and Bicchi *et al.* (1997) reported a time of 5 minutes. The variation in these results clearly shows that determination of the desorption time can be difficult in analysis of food flavors using the SPME method.

The purge-and-trap method (Arora *et al.,* 1995) has the same problem as the SPME method; i.e., there is some difficulty in determining the desorption time. However, in the purge-and-trap method, the desorption time can be extended using cryofocusing (Shimoda and Shibamoto, 1990a; Shimoda and Shibamoto, 1990b). Cryofocusing may be an effective solution to this problem inherent in the SPME method. In the present study, we investigated the applicability of SPME cryofocusing by measurement of the volatile components in orange juice, and compared the results to those obtained using ordinary SPME.

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Materials and Methods

Single-strength orange juice was purchased from a food manufacturing facility in Hiroshima prefecture. One milliliter of orange juice was placed in a vial (6-CV, 22×38 mm, Chromacol, Herts, United Kingdom) which contained an internal standard solution (10 μL of 1% cyclohexanol in water). The solution was held at 40℃ for 10 minutes. Next, SPME fiber (Sigma-Aldrich, St. Louis, Missouri) was introduced into the septum-sealed vial and kept in the injection port for 20 minutes (Chin *et al.,* 1996). The adsorption capacities of five SPME fibers were compared. Carboxen/polydimethylsiloxane (CAR/PDMS), StableFlex carboxen/polydimethylsiloxane (sfCAR/PDMS), and StableFlex divinylbenzene/carboxen/polydimethylsiloxane (sfDVB/CAR/PDMS) were found to have low polarity, StableFlex divinylbenzene/polydimethylsiloxane (sfDVB/PDMS) was found to have intermediate polarity, and StableFlex carbowax/divinylbenzene (sfCW/DVB) was found to have high polarity.

GC analysis was carried out using a Hewlett-Packard 5890 series II gas chromatograph equipped with an FID (Hewlett-Packard, Palo Alto, California). The GC data was output by a Hewlett-Packard 3396 series II integrator. The injector temperature was 260℃ and the detector temperature was 230℃. A DB-WAX capillary column (60 m ×0.25 mm I.D.×0.25 μm film thickness, Agilent, Palo Alto, California) was used for separation of volatile components. The carrier gas was helium. The oven temperature was raised from 40℃ to 230℃ at a rate of 3℃/min.

SPME cryofocusing was carried out as follows. GC heating was stopped and the column head (10 cm) of the GC was dipped into liquid nitrogen to collect the volatile components in splitless mode. The SPME fiber was introduced into the injector and kept there for 7 minutes.

Results and Discussion

The stationary phases of the SPME fibers were examined for adsorption of volatile components. Table 1

Table 1. Major volatile components in orange juice and their dependence on SPME fiber type with cryofocusing for 7 min.

	Flavor Peak Area				
Fiber type	CAR/PDMS	sfCAR/ PDMS	sfCW/DVB	sfPDMS/ DVB	sfDVB/CAR/ PDMS
Fiber film thickness	75 μm	85 μm	70 μm	65 μm	50/30 μm
Ethanol	2.6×10^5 (100)[a]	1.0×10^6 (392)	2.1×10^5 (76)	6.7×10^4 (25)	2.6×10^5 (98)
α-Pinene	9.7×10^4 (100)	9.5×10^4 (98)	1.6×10^4 (16)	6.2×10^4 (65)	1.3×10^5 (133)
Myrcene	4.7×10^5 (100)	1.3×10^6 (265)	1.3×10^5 (27)	3.2×10^5 (68)	8.1×10^5 (170)
α-Terpinene	4.9×10^4 (100)	2.6×10^5 (520)	1.3×10^4 (26)	4.2×10^4 (84)	1.3×10^5 (257)
d-Limonene	2.5×10^7 (100)	3.3×10^7 (135)	5.8×10^6 (23)	1.6×10^7 (65)	2.4×10^7 (97)
p-Cymene	1.2×10^6 (100)	7.4×10^5 (61)	4.5×10^5 (37)	1.1×10^6 (90)	2.1×10^6 (175)
Terpinolene	5.6×10^5 (100)	1.8×10^6 (313)	5.0×10^4 (8.9)	1.2×10^5 (22)	4.3×10^5 (77)
Tridecane	1.3×10^5 (100)	4.0×10^5 (312)	4.1×10^4 (32)	1.0×10^5 (80)	2.5×10^5 (200)
Cyclohexanol	4.5×10^4 (100)	8.9×10^4 (198)	2.0×10^5 (452)	1.3×10^5 (289)	1.8×10^5 (411)
Octyl acetate	2.3×10^4 (100)	1.9×10^4 (81)	-	2.6×10^4 (114)	3.0×10^4 (131)

[a] The relative peak area for a fiber was defined as the peak area of that fiber as a percentage of the peak area obtained using CAR/PDMS.

lists the peak areas and the relative peak areas of nine major volatile components extracted from orange juice, along with the internal standard, by the five types of SPME fiber. The area of the CAR/PDMS peak was selected as the standard for relative peak area. The relative peak area for a fiber was defined as the peak area for that fiber as a percentage of the peak area obtained using CAR/PDMS.

The peak areas of α-pinene and octyl acetate adsorbed by CAR/PDMS were equal to that adsorbed by sfCAR/PDMS. For *p*-cymene, adsorption by CAR/PDMS was higher than that by sfCAR/PDMS; however, the opposite results were observed for the other volatile components. The CAR/PDMS background matrix was prepared from fused silica. The SfCAR/PDMS and other StableFlex fiber background matrices were prepared from flexible fused silica and polyacrylate. The adsorption characteristics of CAR/PDMS and sfCAR/PDMS were expected to be equal because these matrices are not adsorbents; the matrices reinforce the bond between the adsorbents and the center wire. However, the results in the present study indicate that the adsorption characteristics of the two fibers were different. The peak areas of sfDVB/CAR/PDMS and CAR/PDMS were relatively large among the five types of SPME fiber. The peak areas of sfDVB/PDMS and sfCW/DVB were small. It was found that the volatile components in orange juice were mainly adsorbed on adsorbents with low polarity.

Clark and Bunch (1997) reported that the adsorption dependence of volatile components in tobacco differed according to the SPME fiber type used; for this reason, a suitable SPME fiber should be selected for trace analysis of volatile components. Because SfDVB/CAR/PDMS and CAR/PDMS fibers efficiently adsorbed all of the major volatile components in orange juice, these fibers were used in this study.

Figure 1 shows gas chromatograms of the volatile components in orange juice subjected to SPME analysis. Table 2 lists the retention time (RT), theoretical plate number (TPN) and relative peak areas with and without cryofocusing. The relative peak area was defined as the peak area without cryofocusing as a percentage of that obtained with cryofocusing. Without cryofocusing, the typical desorption time was 2 minutes (Deibler *et al.,* 1999; Elmore *et al.,* 1997; Jia *et al.,* 1998). We studied the effect of desorption time on the resolution of the gas chromatogram in order to determine the optimum time.

The theoretical plate number, N, is obtained from the retention time, t_R, and half-width, $w_{0.5}$:

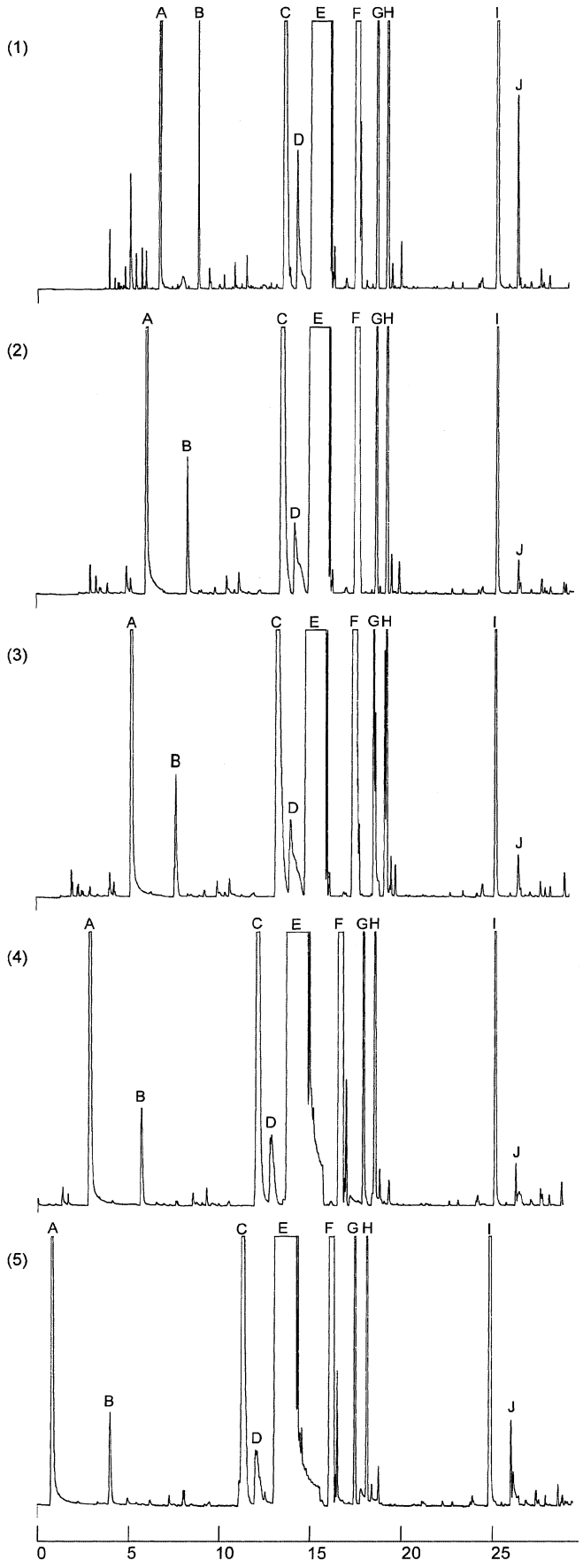
$$N=\frac{5.545{t_R}^2}{{w_{0.5}}^2} \quad (1)$$

Higher theoretical plate numbers imply that the peak will be narrower at the given retention time. Therefore, theoretical plate numbers are an indicator of chromatographic resolution.

The retention time of ethanol in sfDVB/CAR/PDMS fiber without cryofocusing (desorption time=1 min) was 6.0 minutes, whereas with cryofocusing, it was 6.8 minutes; i.e., the retention time without cryofocusing was shorter than that with cryofocusing. The longer desorption time without cryofocusing led to a shorter retention time. The retention time of α-pinene was 8.8 minutes with cryofocusing and 4.0 minutes without cryofocusing (desorption time=7 min).

The relative peak area of ethanol in sfDVB/CAR/PDMS was 94% without cryofocusing (desorption time=1 min), which means that the value without cryofocusing was almost the same as that with cryofocusing. The areas for a desorption time of 2 minutes were the same as those at longer desorption times. One minute was sufficient to desorb ethanol and the other 9 volatile components from the sfDVB/CAR/PDMS fiber.

The theoretical plate number of ethanol in sfDVB/CAR/PDMS was 1.2×10^5 with cryofocusing and 3.7×10^4 without cryofocusing (desorption time=1 min); the value without cryofocusing was smaller than that with cryofocusing. The theoretical plate number of α-pinene with cryofocusing was 2.3×10^5 and that without cryofocusing was 5.0×10^4 (desorption time=1 min); these values are widely different. A longer desorption time without cryofocusing leads to a smaller theoretical plate number, which means broader peaks (Fig. 1). This means that without cryofocusing, the volatile components were



not focused in the column head. The results show that a shorter desorption time improves the resolution.

For the peaks of *d*-limonene, *p*-cymene, terpionlene, tridecane, cyclohexanol and octyl acetate on the chromatogram, the theoretical plate numbers with cryofocusing were the same as those without cryofocusing. The peaks before ethanol, α-pinene, myrcene and α-terpinene with cryofocusing, as shown in Fig. 1 (1), were sharper than those obtained on other chromatograms. The resolution for these components was low in the absence of cryofocusing, whereas it was higher for the components after *d*-limonene because they were collected in the column head at 40℃ (initial temperature of the GC oven).

The results with cryofocusing were compared with those without cryofocusing. Although the peak areas were not different, the theoretical plate numbers were. This suggests that all volatile components were desorbed from the SPME fiber in 1 minute. Hence, a 1-minute desorption time without cryofocusing was sufficient for sfDVB/CAR/PDMS, although the resolution was low. Although the desorption time was 7 minutes with cryofocusing, the resolution was higher than that obtained without cryofocusing.

The desorption tendencies of sfDVB/CAR/PDMS and CAR/PDMS were different. The peak area of ethanol in CAR/PDMS without cryofocusing was smaller than that obtained with cryofocusing. Desorption from CAR/PDMS was slow; longer desorption times without cryofocusing resulted in smaller theoretical plate numbers. It was difficult to obtain reproducible results without cryofocusing. Although the desorption time was 7 minutes with cryofocusing, the area and the theoretical plate number were larger than those obtained without cryofocusing.

The results show that SPME cryofocusing was effective in the analysis of highly volatile components. In particular, resolution was high at short retention times (up to a Kovats index value of 1182). One minute was the best desorption time without cryofocusing for sfDVB/CAR/PDMS because the level of desorption from the fiber and the resolution were good. However, desorption and resolution were always better with cryofocusing than without. Overall, our SPME cryofocusing analysis system allowed us to analyze trace amounts of volatile components with high volatility by ensuring high resolution.

Since food flavors contain many components with high volatility (Arora *et al.*, 1995; Chin *et al.*, 1996; Jia *et al.*, 1998), SPME cryofocusing should be very effective and find wide application in food analysis.

Fig. 1. Gas chromatograms of volatile components in orange juice subjected to SPME (sfDVB/CAR/PDMS fiber) analysis. (1) With cryofocusing (desorption time=7 min), (2) without cryofocusing (1 min), (3) without cryofocusing (2 min), (4) without cryofocusing (5 min),and (5) without cryofocusing (7 min). A, ethanol; B, α-pinene; C, myrcene; D, α-terpinene; E, *d*-limonene; F, *p*-cymene; G, terpinolene; H, tridecane; I, cyclohexanol (internal standard); and J, octyl acetate.

Table 2. Retention time, theoretical plate number and component area of volatile components in orange juice, with and without cryofocusing.

	With cryofocusing			Without cryofocusing										
	7 min			1 min			2 min			5 min			7 min		
	RT / min	Area / %	T.P.N.	RT / min	Area / %	T.P.N.	RT / min	Area / %	T.P.N.	RT / min	Area / %	T.P.N.	RT / min	Area / %	T.P.N.
sfDVB/CAR/PDMS fiber															
Ethanol	6.8	100	1.2×10^5	6.0	94	3.7×10^4	5.2	90	1.7×10^4	2.8	72	2.9×10^3	0.8	74	3.5×10^2
α-Pinene	8.8	100	2.3×10^5	8.3	100	5.0×10^4	7.6	111	2.8×10^4	5.6	90	1.5×10^4	4.0	88	6.9×10^3
Myrcene	13.6	100	1.2×10^5	13.5	118	4.7×10^4	13.2	126	2.3×10^4	12.1	107	3.9×10^4	11.4	99	3.0×10^4
α-Terpinene	14.3	100	9.5×10^4	14.2	141	1.3×10^4	14.0	184	8.7×10^3	12.7	116	1.4×10^4	12.0	34	2.8×10^4
d-Limonene	16.1	100	4.9×10^3	16.1	104	4.5×10^3	15.9	108	4.1×10^3	14.8	95	3.3×10^3	14.0	91	2.0×10^3
p-Cymene	17.7	100	8.4×10^4	17.7	107	7.9×10^4	17.6	110	6.1×10^4	16.7	97	6.3×10^4	16.3	90	6.0×10^4
Terpinolene	18.7	100	4.4×10^5	18.7	65	5.2×10^5	18.5	50	5.1×10^5	17.8	59	4.2×10^5	17.5	83	3.4×10^5
Tridecane	19.2	100	4.9×10^5	19.3	101	4.6×10^5	19.2	67	5.9×10^5	18.5	92	3.5×10^5	18.1	91	3.7×10^5
Cyclohexanol	25.2	100	5.7×10^5	25.3	66	7.0×10^5	25.2	66	7.0×10^5	25.0	55	7.3×10^5	24.8	104	4.8×10^5
Octyl acetate	26.3	100	1.3×10^6	26.4	19	1.2×10^6	26.4	33	5.7×10^5	26.2	24	1.0×10^6	26.0	46	1.1×10^6
CAR/PDMS fiber															
Ethanol	6.7	100	8.6×10^4	5.9	26	9.2×10^4	5.1	56	2.4×10^4	2.8	78	5.3×10^3	1.0	48	7.0×10^2
α-Pinene	8.8	100	2.3×10^5	8.2	16	1.3×10^5	7.4	76	4.7×10^4	5.6	66	2.6×10^4	4.2	88	1.0×10^4
Myrcene	13.7	100	1.1×10^5	13.7	27	1.9×10^5	13.1	21	8.4×10^4	12.0	79	5.5×10^4	11.3	93	3.6×10^4
α-Terpinene	14.4	100	1.4×10^5	14.4	22	1.1×10^5	13.9	29	8.1×10^4	12.6	25	2.2×10^5	12.4	33	1.7×10^5
d-Limonene	16.0	100	6.4×10^3	15.7	26	2.5×10^4	15.6	105	4.5×10^3	14.4	77	4.9×10^3	13.7	73	4.4×10^3
p-Cymene	17.7	100	1.3×10^5	17.6	24	3.4×10^5	17.4	103	8.2×10^4	16.5	84	7.7×10^4	16.0	77	9.1×10^4
Terpinolene	18.7	100	4.2×10^5	18.7	36	2.3×10^5	18.5	97	2.7×10^5	17.8	63	1.7×10^5	17.4	63	1.3×10^5
Tridecane	19.3	100	5.4×10^5	19.3	31	4.2×10^5	19.1	116	4.0×10^5	18.4	83	4.0×10^5	18.0	80	3.0×10^5
Cyclohexanol	25.2	100	1.2×10^6	25.2	16	9.4×10^5	25.2	98	1.2×10^6	25.0	35	1.1×10^6	24.9	82	1.1×10^6
Octyl acetate	28.8	100	7.2×10^5	29.0	58	1.3×10^6	28.9	11	1.9×10^6	28.9	18	2.1×10^6	28.9	21	2.1×10^6

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