

Analysis of Highly Volatile Components of Foods by Off-Line SFE/GC

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A sample collection method is proposed for off-line coupling supercritical fluid extraction/gas chromatography (SFE/GC) through a programmed temperature vaporizer (PTV) and using a variable restrictor. Experiments were carried out to obtain the most favorable conditions for the analysis of highly volatile components of foods. Average recoveries of ~50% and coefficients of variation of absolute areas <10% were obtained for a standard mixture of compounds of high volatility. When real fruit samples were extracted and analyzed, coefficients of variation achieved ranged from 2 to 10%. To test its performance, the method was applied to the analysis of fresh raspberries.

Keywords: *Off-line SFE/GC; fruit samples; volatiles*

INTRODUCTION

In the past few years, supercritical fluid extraction (SFE) has been frequently applied to food composition analysis as a method of sample preparation. The advantages of the technique are related to the physical and chemical characteristics of the supercritical fluids. CO₂ is considered the most suitable solvent for natural products.

So far, coupling SFE on-line with gas chromatography (GC) is the most used approach, because this provides high potential to automate operation and rapid analysis (Vannoort *et al.*, 1990). The coupling has been accomplished by using different interfaces that involved the direct insertion of the SFE restrictor capillary into the GC column using an on-column (Hawthorne and Miller, 1987; Hawthorne *et al.*, 1989) or split-splitless (Hawthorne *et al.*, 1990; Levy *et al.*, 1990, 1991) injection port. Also, a direct interface to a temperature-programmable vaporizer (PTV) has been suggested (Houben *et al.*, 1990; Huston and Ji, 1991).

Most of the methods for on-line coupling SFE-GC include linear restrictors. Problems associated with their utilization are plugging, difficulty of replacement, thermal degradation of labile compounds, and the impossibility of independently controlling the solvent flow and pressure. In a paper published in 1994 (Blanch *et al.*, 1994) we demonstrated the applicability of a PTV for collecting analytes extracted from complex mixtures by using a supercritical fluid extractor equipped with a variable restrictor. The procedure (called off-line SFE/GC) consisted of a collection trap using a PTV glass liner filled with a packing material with a high surface area. By using this method, detection limits ~300 times lower than those of solvent extraction techniques such as solvent distillation-extraction were achieved. The recoveries obtained for medium-volatility compounds were acceptable, but very volatile compounds were minimally recovered.

Since the analysis of high-volatility compounds is frequently necessary in food studies, the purpose of the present investigation was to optimize the operating conditions for the off-line SFE/GC analysis of highly

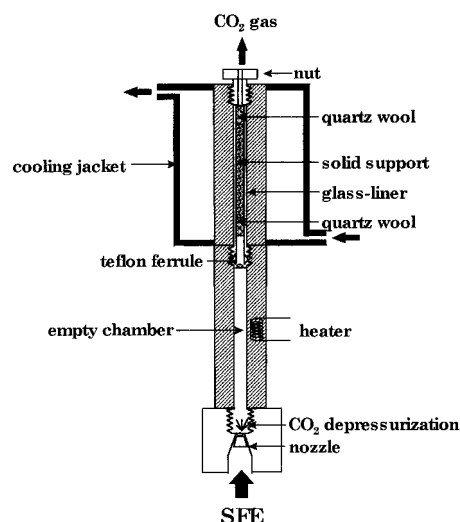


Figure 1. Scheme of the collection device described under Experimental Procedures.

volatile components of foods. Our final goal was to contribute to the methodology for aroma analysis.

The performance of the proposed method with real samples was evaluated through the analysis of fresh raspberries.

EXPERIMENTAL PROCEDURES

Standard Mixture. Mixtures of the following highly volatile compounds, typical components of food aroma, were prepared by adding the same amount of the following pure standards: propyl acetate, 3-methyl-1-butanol, isoamyl acetate, benzaldehyde, ethyl hexanoate, isoamyl butanoate, 2-phenyl ethanol, and 1-octanol of GC grade purchased from Merck (Darmstadt, Germany). These mixtures were used in the optimization experiments of the off-line SFE/GC procedure.

Real Sample. Fresh Spanish raspberries (cultivar Heritage) were used to test the performance of the method.

Supercritical Fluid Extractions. A Hewlett-Packard Model 7680A supercritical fluid extractor equipped with a variable restrictor was used in the present study. The extraction cell utilized was a self-sealing 7 mL stainless steel thimble. CO₂ (SFC grade) was used as supercritical fluid.

As a collection device, a modification of the assembly described in a previous paper (Blanch *et al.*, 1994) was used. A scheme of the device now proposed is shown in Figure 1. The glass liner of a Perkin-Elmer PTV (100 mm × 1 mm i.d.) was placed inside the stainless steel chamber surrounded by

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Table 1. Experimental Conditions Tested in the Present Study (See Text for Further Information)

expt no.	ρ^a	F^b	t^c	F_e^d	V_e^e
1	0.25	0.5	9.0	75.0	675.0
2	0.25	1.0	4.5	150.5	677.2
3	0.25	2.0	2.2	301.1	662.4
4	0.25	3.0	1.5	451.6	677.4
5	0.25	4.0	1.1	602.2	662.4
6	0.35	0.5	6.5	105.3	684.4
7	0.35	1.0	3.2	210.7	674.2
8	0.35	2.0	1.6	421.5	674.4
9	0.35	3.0	1.1	632.3	695.5
10	0.50	0.5	4.5	150.5	677.2
11	0.50	1.0	2.2	301.1	662.4
12	0.50	2.0	1.1	602.2	662.4

^a CO₂ density (g/mL). ^b Supercritical CO₂ flow rate (mL/min). ^c Extraction time (min). ^d Calculated expanded CO₂ flow rate (mL/min) through the trap. ^e Expanded CO₂ volume (mL) through the trap.

a cooling jacket that can be refrigerated at a target temperature by using a convenient gas or liquid. An empty chamber was placed between the nozzle and the glass liner to prevent the modification of the packing material contained in the glass liner by tumultuous depressurization of the supercritical fluid. To prevent condensation of solutes, the empty chamber was heated.

After the extraction and trapping step, the gas chromatographic analysis was conducted by placing the glass liner in the PTV body of a gas chromatograph. The thermal desorption of the analytes retained in the packing material was achieved by increasing the PTV temperature.

GasChrom 220 (80/100 mesh) (Alltech Associates Inc., Deerfield, IL) was selected as packing material to fill the glass liner. GasChrom 220 is a porous polymer based on divinylbenzene with 450 m²/g surface area. It has a 10 nm pore size and a low affinity for water and is treated to remove residual monomers or polymerization catalysts. The temperature limit is 300 °C.

The amount of adsorbent is limited by the total length of the quartz liner. In this study, a maximum length of 4 cm (this corresponds to 20 mg weight of GasChrom 220) was filled. The packing material was kept in place by two plugs of quartz wool. The adsorbent was conditioned in an oxygen-free atmosphere at 275 °C for 120 min.

To determine the limits of flow rates of expanded CO₂ in the extraction procedure, breakthrough volumes for a mixture of highly volatile compounds (ethanol, methyl butanoate, and benzaldehyde) were measured in a GasChrom 220 trap. This was connected to the injector and detector of the gas chromatograph through deactivated fused silica tubing, using a carrier gas (N₂) flow of 14.8 mL/min and three temperatures (30, 0, and -25 °C).

The breakthrough volumes obtained were 12 mL at 30 °C, 70 mL at 0 °C, and 680 mL at -25 °C. In consequence, only at a trap temperature of -25 °C would it be feasible to test different experimental conditions obtained by combining CO₂ densities (grams per milliliter) and flow rates (milliliters per minute). Table 1 shows the experimental conditions tested in the present study and the calculated expanded flow rates (Pacholec *et al.*, 1988) expected in each experiment. In all of them, the CO₂ expanded flow rate was <680 mL. Low densities (0.25–0.5 g/mL) were chosen to obtain selective extraction of high-volatility compounds. An extraction temperature of 40 °C was used to avoid alterations in the aroma of the sample.

Other variables involved in the SFE process that were kept at a constant value were as follows: equilibrium time, 5 min; trap empty chamber temperature, 50 °C; nozzle temperature, 100 °C; cooling jacket temperature, -25 °C. This last temperature was obtained by circulating liquid CO₂ through the empty jacket that surrounds the chamber.

To test the conditions described above, 0.08 μ L of a standard mixture was placed in a filter paper inside the extraction cell.

To test the validity of the proposed method with real samples, analysis of raspberries was carried out. Sample

weight ranging between 600 and 750 mg of untreated fresh raspberries, with each fruit cut in four pieces, was used.

Gas Chromatographic/Mass Spectrometric Analysis. A Perkin-Elmer Model 8500 gas chromatograph equipped with a Perkin-Elmer PTV injector and a flame ionization detector was used to perform all of the GC analyses. The system was coupled to a Model 2600 chromatography software system (Nelson Analytical).

A 50 m \times 0.25 mm i.d. fused silica capillary column (Chrompack, Middelburg, The Netherlands) coated with a 0.25 μ m layer of OV-1 was employed (CP-Sil-5CB). Helium at 15 psig was used as a carrier gas. The desorption of the compounds retained in the packing material took place in the GC injector at 250 °C in the split mode (splitting ratio 20:1). The injector was maintained at 30 °C during sample introduction; this temperature was then increased at 14 °C/s to 250 °C and held at the final temperature for 10 min. The oven temperature was programmed from 40 °C (5 min constant temperature) to 115 °C at 5 °C min⁻¹ and then to 250 °C by a second ramp at 15 °C min⁻¹. The final temperature was maintained for 6 min. The detector operated at 250 °C.

GC/MS analysis was carried out on the Perkin-Elmer Model 8500 chromatograph described above coupled to a Perkin-Elmer ITD-50 ion trap detector (EI 70 eV). The compounds were identified by comparison of their spectra with those in a general purpose library. Capillary column and chromatographic conditions were used as mentioned previously.

RESULTS AND DISCUSSION

Table 2 gives the individual recoveries for the components of the standard mixture obtained with the experimental conditions described in Table 1. Recoveries were measured by comparing the areas obtained in each experiment with those corresponding to the reference chromatogram obtained by direct injection of 0.08 μ L of mixture inside a glass liner similar to those used in the SFE/GC procedure and subsequent thermal desorption. A minimum of five replicates were carried out.

Experiments performed at 0.5 g/mL extraction density gave the highest recoveries, with the best conditions being 1 mL/min supercritical flow rate and 2.2 min of extraction time. At lower densities, i.e., 0.25 and 0.35 g/mL, lower recoveries were obtained. Working at 0.25 g/mL gave a maximum 25% recovery, while 0.35 g/mL density provided recoveries close to 40% for some components of the standard mixture when the lowest CO₂ flow rate was used.

Comparing these results with those obtained previously in our laboratory (Blanch *et al.*, 1994), it is important to emphasize the improvement in the recovery of highly volatile compounds such as isoamyl acetate (50% in the present work compared to no recovery by using the procedure described previously) and benzaldehyde (46% compared to 6%). Also, recoveries achieved before for alcohols with fewer than six carbon atoms were ~10%, while with the procedure described in the present investigation they ranged between 40 and 50%.

Table 3 shows the coefficients of variation of the absolute peak areas obtained from five analyses performed with the best extraction conditions. As can be clearly seen, values obtained by using the described procedure were, in general, acceptable and \leq 10% variation for the standard mixture.

Once the best conditions to perform the supercritical fluid extraction of highly volatile compounds had been selected, the method was applied to extract a real sample. Raspberry aroma was chosen since this material is of great interest in the food industry due to the high added value of this natural fruit aroma.

Figure 2 shows the chromatogram of a fresh raspberry extract obtained by using the procedure described

Table 2. Individual Recoveries (Percent) for the Components of the Standard Mixture

compound	expt no. ^a											
	1	2	3	4	5	6	7	8	9	10	11	12
propyl acetate	12.3	10.8	8.7	1.8	2.4	6.1	3.3	4.0	4.3	27.5	26.2	31.1
3-methyl-1-butanol	11.6	10.7	12.0	7.3	8.3	14.7	19.4	13.7	10.5	43.6	37.3	39.8
isoamyl acetate	28.6	22.5	17.7	15.1	20.8	43.5	23.9	25.0	19.6	51.2	49.1	43.3
benzaldehyde	22.3	29.2	15.8	13.5	19.0	33.5	24.7	20.8	16.2	36.3	46.6	35.1
ethyl hexanoate	25.7	26.6	16.9	16.7	21.4	40.9	28.9	21.4	17.1	43.4	58.7	40.1
isoamyl butyrate	24.8	20.7	16.3	16.4	21.4	39.6	26.8	17.4	16.2	40.6	52.6	38.8
2-phenylethanol	22.1	29.4	16.7	16.6	17.5	40.0	27.3	15.1	17.3	33.3	52.3	35.8
1-octanol	15.7	20.7	12.0	13.5	15.8	21.9	14.9	9.2	8.4	26.7	51.0	30.5

^a Experiment no. in Table 1.

Table 3. Coefficients of Variation ($n = 5$) of Absolute and Normalized Peak Areas Obtained from SFE Extract of a Standard Mixture at the Optimal Operating Conditions (CO_2 Density = 0.5 g/mL; CO_2 Flow Rate = 1 mL/min; Extraction Time = 2.2 min)

compound	CV ^a (%)	CV ^b (%)
propyl-acetate	9.9	8.3
3-methyl-1-butanol	8.6	7.1
isoamyl acetate	8.9	10.9
benzaldehyde	6.1	6.2
ethyl hexanoate	4.9	5.7
isoamyl butyrate	8.4	8.4
2-phenylethanol	5.8	3.6
1-octanol	10.1	7.1

^a Coefficient of variation ($n = 5$) of absolute peak areas.

^b Coefficient of variation ($n = 5$) of normalized peak areas.

Table 4. Coefficients of Variation ($n = 5$) of Absolute and Normalized Peak Areas Obtained from SFE Extract of Fresh Raspberry Fruit at the Optimal Operating Conditions (CO_2 Density = 0.5 g/mL; CO_2 Flow Rate = 1 mL/min; Extraction Time = 2.2 min)

compound	CV ^a (%)	CV ^b (%)
α -pinene	4.6	2.7
linalol	8.6	6.5
citral	4.1	5.6
linalyl acetate	9.7	8.8
terpinolene	7.4	5.5
cariophyllene	3.1	6.7
β -ionone	2.1	3.7

^a Coefficient of variation ($n = 5$) of absolute peak areas.

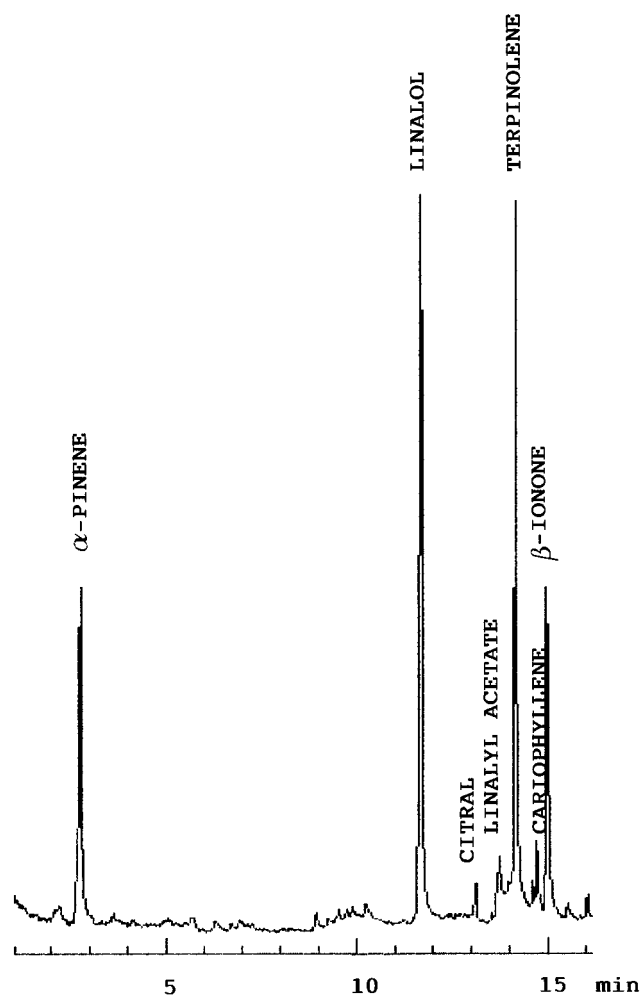
^b Coefficient of variation ($n = 5$) of normalized peak areas.

previously. Peaks were tentatively identified by mass spectrometry and, with the exception of β -ionone, have been reported as representative of the original aroma of fresh raspberries (Honkanen *et al.*, 1980). The ability of the off-line SFE/GC technique to yield reproducible quantitative results with real samples was investigated by analyzing five replicate samples of raspberry. Table 4 presents the coefficients of variation (percent) of absolute and relative (normalized) peak areas performed on fresh raspberry fruit. As can be seen, the coefficients of variation are acceptable, since the averages for both absolute and normalized areas are $\sim 6\%$.

CONCLUSIONS

Results obtained demonstrate the ability of the off-line SFE/GC technique to yield reproducible determinations of extractable aroma compounds. By using the procedure described in the present study, loss of volatiles is clearly reduced, high sensitivity is achieved, and minimal sample handling is required.

Since each analysis requires approximately 30 min to complete (including weighing the sample and performing the extraction and the gas chromatographic separation), it is possible to conclude that the proposed

**Figure 2.** Chromatogram of a sample of fresh raspberry obtained by the proposed off-line SFE/GC method.

method becomes a powerful alternative to traditional methods for extracting, identifying, and quantitating aroma compounds from food samples.

An additional interesting potential for the technique is the possibility of its use for supercritical fluid extraction process design in the field of food technology. By using this method, variable selection can be quickly performed at low cost and, even more importantly, it is possible to independently evaluate the effects of pressure and supercritical CO_2 flow rate in the extraction yields.

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