Supercritical Fluid Extraction Coupled with Gas Chromatography for the Analysis of Aroma Compounds Absorbed by Low-Density Polyethylene†

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Supercritical carbon dioxide was used for extracting aroma compounds absorbed by low-density polyethylene. The extraction step was directly coupled with gas chromatography. The method yielded recoveries and reproducibilities similar to those obtained by a conventional liquid solvent extraction. The standard deviation ranged from 3 to 10% for the different species. The main advantages with the new method are that it requires less sample, it is less laborious, and it is performed much more rapidly than already existing methods. A complete extraction/analysis is accomplished in one single step and in less than 40 min.

INTRODUCTION

Most studies on food and packaging interactions have been made on migration from the package to the food (Risch, 1988). Recently, it has been reported that plastic package material also may absorb different compounds from the food (Kwapong and Hotchkiss, 1987; Mannheim et al., 1987; Shimoda et al., 1988; Hirose et al., 1988; Halek and Meyers, 1989). Both volatile and nonvolatile compounds can be absorbed. The nonvolatiles, such as fats and pigments, affect the package itself, while sorption of volatiles (flavors and aromas) more directly affects the food quality, i.e., loss of aroma intensity (Landois-Garza and Hotchkiss, 1987).

Accurate quantification of flavor compounds is often limited by the methods used for extracting and concentrating the analyte species prior to gas chromatographic analysis. Methods frequently used are liquid solvent extraction (e.g., hexane or heptane) followed by a concentration step (Kwapong and Hotchkiss, 1987) or simultaneous distillation–extraction (Shimoda et al., 1988). These methods are very time-consuming and laborious, and unhealthy solvents are often used. Other studies have used more inexact methods, such as measuring the remaining amounts of the analyte species in the packed foodstuff (Mannheim et al., 1987; Hirose et al., 1988; Halek and Meyers, 1989). Obviously a rapid and simple technique for extracting and analyzing absorbed compounds is lacking.

The use of supercritical fluids for analytical extractions of organic material from complex sample matrices has recently been reported (Miller Schantz and Chesler, 1986; Wright et al., 1986). Supercritical fluids have several characteristics that make them suitable for extraction purposes (Brogle, 1982). Their low viscosity and high diffusivity make the mass transfer during the extraction rapid. Furthermore, the solvent power of a supercritical fluid can be controlled by changing the extraction pressure and to some extent the extraction temperature. This important property is due to the fact that the solvent power is directly related to the fluid's density, which is altered by a change of state. Many supercritical fluids have low critical temperatures, which allows extractions to be performed at relatively low temperatures to avoid decomposition of thermally labile analytes. Another essential factor is that many supercritical fluids are gases at room temperature, which facilitates the concentration of the extract. This characteristic allows the direct coupling of the supercritical fluid extraction step with capillary gas chromatography (Hawthorne et al., 1988). The use of carbon dioxide as a supercritical fluid has several advantages. Because of its extreme volatility, it can be easily and completely separated from any solute. Further, it has a low critical point (74 bar, 31 °C), is nontoxic, not inflammable, and cheap, and causes no environmental problems.

The aim of this study was to develop and optimize a method of supercritical carbon dioxide extraction for measurements of absorbed aroma compounds in plastic packaging material and to directly collect extracted analytes in the gas chromatographic column for subsequent analysis. A comparison with a conventional extraction technique was also made.

MATERIALS AND METHODS

Sample Preparation. The selected compounds, ethyl butyrate (Fluka Chemie AG, Buchs, Switzerland), ethyl 2-methylbutyrate (Fluka), butyl acetate (Merck, Darmstadt, West Germany), hexanal (Merck), butyl propanoate (Merck), isopentanol (Merck), hexyl acetate (Merck), hexanol (Merck), isopentyl acetate (BDH Ltd., Poole, England), and *trans*-2-hexenal (Sigma Chemical Co., St. Louis, MO) were those with the highest aroma value, i.e., concentration/odor threshold, in apple juice (Poll, 1988). Aroma compounds of apple juice were chosen since apple juice is a product commonly packed in plastic packages. The plastic used was low-density polyethylene (LDPE) with a density of 0.922 g/cm^3 and a thickness of 50 μm (Neste Polyethylene, Stenungsund, Sweden).

Three grams of the LDPE strips were stored in 1 L of a water solution of 10 ppm (w/w) of each of the 10 different aroma compounds for 1 week, while stirring at 200 rpm, in a dark glass bottle at ambient temperature. The plastic area/solution volume ratio was equal to the ratio in a 1-L Tetra Brik carton.

Standard solution mixtures were prepared with concentrations of 1, 10, 100, and 1000 ppm of each of the 10 aroma compounds.

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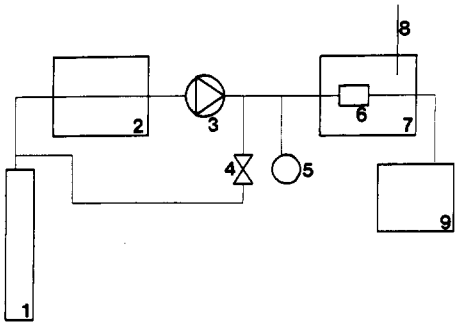

Figure 1. Equipment for SFE/GC. (1) Carbon dioxide flask; (2) cooling bath; (3) pump; (4) pressure regulator; (5) pressure meter; (6) extraction cell; (7) water bath; (8) immersion heater; (9) gas chromatograph.

Table I. Gas Chromatographic Conditions

carrier gas flow rate (He)	1.0 mL/min
H_2 flow rate	30 mL/min
air flow rate	300 mL/min
makeup gas flow rate	30 mL/min
column	Supelcowax 10 (60 m × 0.25 mm)
injection vol	1 μL
injector temp program	40 °C at injection, increase of 160 °C/min to 200 °C, hold at 200 °C for 1 min
detector temp (FID)	260 °C
oven temp program	40 to 150 °C with an increase of 5 °C/min

Equipment. The supercritical fluid extraction/gas chromatography (SFE/GC) apparatus was arranged according to Figure 1. The carbon dioxide used was of 99.998% purity (AGA Specialgas, Lidingö, Sweden). It was essential to have a carbon dioxide with as low a content of hydrocarbons as possible; otherwise, they could interfere with the chromatographic analysis. The cooling bath of −15 °C was necessary to liquefy the carbon dioxide since the pump used (Scientific Systems Inc. Model 200 LC pump) could pump only liquids. The extraction cell was placed in a water bath with an immersion heater. The GC used was a Varian 3400 with the chromatographic conditions listed in Table I. The direct coupling of the SFE cell was accomplished by inserting the SFE outlet restrictor of 25 μm internal diameter into the gas chromatographic column through the on-column injection port. All connections were of stainless steel with outer diameter of $^1/_{16}$ in. and internal diameter of $^1/_{32}$ in.

Supercritical Fluid Extraction. When a supercritical fluid extraction was performed, a weighed sample of approximately 10 mg of plastic film was wiped dry and placed in the extraction cell. The capillary restrictor was then inserted into the GC column through the on-column injector. Care was taken that there was no leakage backward through the injector. The extraction was assumed to be initiated from the moment the pressure reached the desired value. After the extraction, the restrictor was taken out and the carbon dioxide left in the column was let out. Subsequently, the column was flushed for 2 min with the carrier gas before the column oven was rapidly heated to 40 °C and the chromatographic analysis was performed. Extraction pressure (60–120 bar), extraction temperature (10–70 °C), extraction time (5–20 min), and column temperature (−50 to 0 °C) were varied to optimize the method.

Liquid Solvent Extraction. LDPE strips (0.5 g) were extracted with 2 × 25 mL of methylene chloride by using 3 h of sonication each time. The solution was then concentrated by allowing the methylene chloride to evaporate in ambient temperature until the remaining volume was 1 mL. A 1-μL aliquot was injected into the column through the on-column injector, and the chromatographic analysis was accomplished identically with the SFE/GC analysis.

RESULTS AND DISCUSSION

Separation on Gas Chromatography. One-microliter aliquots of standard solution mixtures of the 10 compounds (1, 10, 100, and 1000 ppm) were injected on-column, and a good separation of the selected aroma compounds was achieved in less than 20 min. A typical chromatogram is shown in Figure 2.

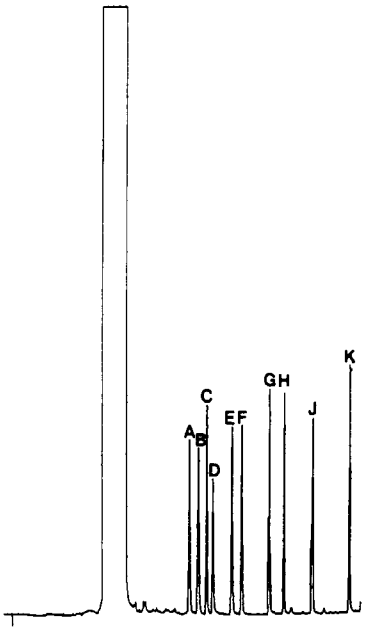

Figure 2. 1 μL of a standard solution, 10 ppm (w/w) of each compound, of the apple aroma injected on-column. Peak identifications are listed in Table II.

Optimization of Supercritical Fluid Extraction. Extensive preliminary trial and error investigations of the factors affecting the total yield of the aroma compounds were performed to find both the optimal hardware design and the optimal SFE conditions.

To ensure that the carbon dioxide was in a supercritical state all the way to the GC column, the dead volume between the extraction cell and the GC injector had to be minimized. This facilitated a quick passage of the CO_2 through the interface, and the temperature did not fall below the critical temperature, which would have resulted in an alteration of the solvent power and a less efficient extraction. An alternative way to deal with this problem would be to heat the connection lines between the extraction device and the gas chromatograph, but this was not practically accomplishable during this experiment. Since the analyte species were very volatile, they had to be cryofocused in the GC column to obtain a satisfying chromatogram. A column temperature of −50 °C gave good chromatographic peak shapes that were not altered when the column temperature was even lower. The column temperature could not be allowed to be any higher, however, since this resulted in broadening of the peaks for the earlier eluting species as would be expected because of the relatively low boiling points of these compounds. In Figure 3 the effect of the column temperature on peak shapes in the chromatogram is shown.

The parameters of the SFE technique that had to be optimized were pressure, temperature, and time. The optimal adjustments were found to be 80 bar, 40 °C, and 15 min, respectively. The gas flow out of the restrictor under these conditions was 50 mL of expanded CO_2/min. To study the effect on the extraction yield of these parameters, they were systematically varied, one at a time, with the others held constant.

Extractions were performed at three different pressures: 60, 80, and 120 bar. A pressure of 60 bar was not sufficient to yield a complete extraction during 15 min. The peak areas were considerably lower than those obtained when

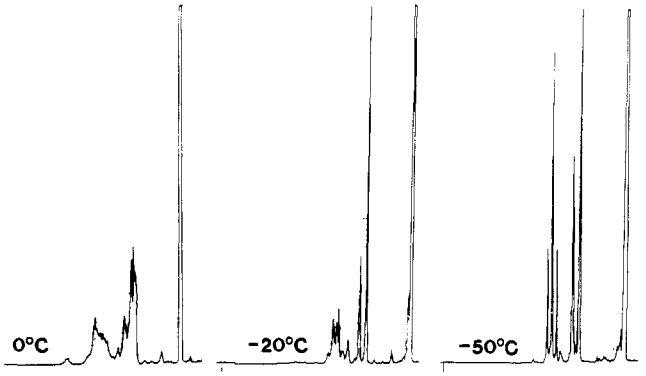

Figure 3. Effect of the cryogenic trapping temperature on the chromatographic peak shapes. The extractions were accomplished with a pressure of 80 bar and a temperature of 40 °C for 15 min.

the pressure was 80 bar. The reasons for this are two: first, carbon dioxide at 60 bar has a lower density and hence does not have the required properties to perform a complete extraction; second, with a pressure of 60 bar the gas flow out of the restrictor was just 20 mL/min, and therefore the total amount of carbon dioxide passing through the extraction cell was much lower than when the pressure was 80 bar. Extractions during 15 min performed at 80 and 120 bar yielded identical chromatograms, which indicates that a pressure of 80 bar is sufficient to accomplish a complete extraction. Extractions carried out with a pressure of 120 bar might need less time than 15 min, but since too high a gas flow through the gas chromatography column might be detrimental to the column, 80 bar was chosen as the suitable extraction pressure.

Extractions were performed at three different temperatures: 10, 40, and 70 °C. Since carbon dioxide is not in a supercritical state at 10 °C, this temperature was too low to give large enough peak areas; however, the effect of too low a temperature was not as distinct as that of too low a pressure. The same results were obtained when the extractions were carried out at 40 and 70 °C, and therefore 40 °C was chosen as the appropriate temperature for further tests.

Extractions were accomplished for 5, 10, 15, and 20 min. Longer times yielded larger peak areas, up to 15 min, while extractions for 20 min did not result in a further increase of the peak areas compared to those obtained from a 15-min extraction. To further confirm that 15 min was a sufficient time, a repeated extraction of a plastic film was performed. The second extraction did not yield any peaks (Figure 4), indicating that 15 min was enough time under the given conditions.

Reproducibility. The reproducibility of the SFE/GC technique was determined by accomplishing seven replicate analyses with the selected parameter adjustment. The results are presented in Table II. Standard deviations of less than 10% are acceptable for SFE/GC analyses, and our results fell well within this range.

Comparison with Solvent Extraction. The concentration step of the conventional method yielded recoveries for the different compounds ranging from 96 to 105%. As can be seen in Table II, the two methods yielded the same results, indicating that the two extraction methods were just as effective. Considering other aspects, however, the SFE method compared favorably to the conventional method. The main differences are the time, effort, and amount of sample needed to perform the two separate extractions. A complete SFE/GC analysis took approximately 40 min to accomplish, while the extraction step of the methylene chloride extraction method took 6 h

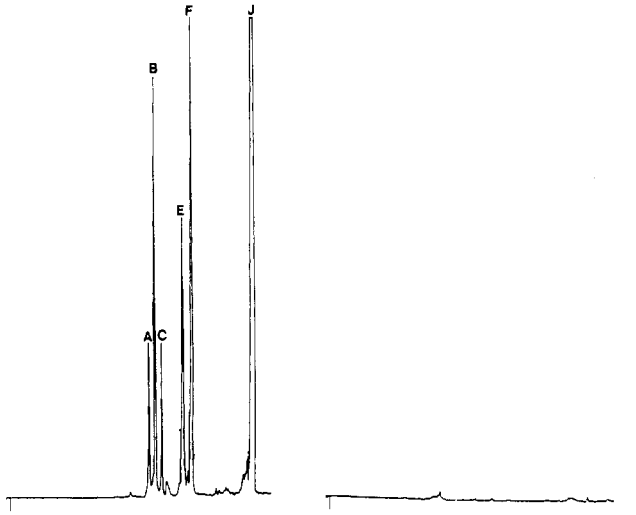

Figure 4. Repeated supercritical fluid extractions of the same plastic film performed at 80 bar and 40 °C for 15 min with a column temperature of −50 °C. (Left) First extraction; (right) second extraction. Peak identifications are listed in Table II.

Table II. Reproducibility of the SFE Method and Comparison with Methylene Chloride Extraction

	SFE[a]		methylene chloride[b]	
compd	ng/mg LDPE	% SD	ng/mg LDPE	% SD
A ethyl butyrate	20	5	20	3
B ethyl 2-methylbutyrate	59	4	55	4
C butyl acetate	20	10	20	6
D hexanal	nd[c]		nd	
E isopentyl acetate	53	5	52	2
F butyl propanoate	92	6	88	3
G isopentanol	nd		nd	
H *trans*-2-hexenal	nd		nd	
J hexyl acetate	215	3	212	2
K hexanol	nd		nd	

[a] Means and standard deviation (% SD) of seven replications. [b] Means and % SD of three replications. [c] nd, not detected.

followed by a concentration step that took 25 h. The SFE/GC method was performed in one single step, while the liquid solvent extraction method required numerous steps. Another advantage of the SFE method was that it needed a much smaller amount of sample (1–2%) than the conventional method. Further, the SFE method excludes the use of hazardous solvents.

Selectivity and Amounts of Absorption. Of the 10 compounds in the study, only 6 have been detected in the plastic material, namely the esters. This is well in accordance with earlier investigations that have shown that esters are much more readily absorbed into plastic packaging material than aldehydes and alcohols (Shimoda et al., 1988). The fact that none of the aldehydes and alcohols were detected might be due to the short time of storage. The number of carbon atoms present in the different compounds seemed to be influential on the degree of absorption, long-chain esters being more easily absorbed into the polyethylene, also in agreement with the findings of Shimoda et al. (1988). Six percent of the total amount of the compound most readily absorbed, i.e., hexyl acetate, was absorbed into the plastic film during 1 week of storage. The absorption of the other esters ranged from 0.6 (ethyl butyrate and butyl acetate) to 3% (butyl propanoate), while approximately 2% of ethyl 2-methylbutyrate and isopentyl acetate was absorbed.

CONCLUSIONS

The developed supercritical fluid extraction procedure has some essential advantages compared to conventional liquid extraction. The method is considerably faster and less laborious and requires much smaller sample sizes. The developed method has great potential in studies of food and packaging interactions. It contributes a powerful tool that makes it convenient to study the absorption of food components into packaging material. Interesting aspects in this area include the effect of storage conditions, type of processing, food composition, and interactions between different food constituents and packaging type on the absorption.

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Registry No. Ethyl butyrate, 105-54-4; ethyl 2-methylbutyrate, 7452-79-1; butyl acetate, 123-86-4; hexanal, 66-25-1; isopentyl acetate, 123-92-2; butyl propanoate, 590-01-2; isopentanol, 123-51-3; *trans*-2-hexenal, 6728-26-3; hexyl acetate, 142-92-7; hexanol, 25917-35-5; carbon dioxide, 124-38-9; polyethylene, 9002-88-4.