Carvone and Limonene in Caraway Fruits (*Carum carvi* L.) Analyzed by Supercritical Carbon Dioxide Extraction–Gas Chromatography

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Dried fruits of caraway (*Carum carvi* L.) were analyzed with on-line supercritical fluid extraction–gas chromatography using supercritical carbon dioxide at 50 °C and 9.7 MPa of pressure. The solutes were cryogenically collected onto a DB-1 capillary GC column, and the effluent was vented out. The compounds were subsequently eluted from the collection column with helium onto the head of the analytical DB-5 capillary column and cryofocused with liquid nitrogen. The volatiles were detected by flame ionization detection and identified by mass spectrometry. The rapid analytical procedure for quality assessment purposes was optimized for limonene and carvone, and their contents in the fruits were analyzed by using internal standards. Analyses of carvone in cultivar Polaris carried out by supercritical extraction and hydrodistillation gave the same result, 2.7 g/100 g, but limonene contents determined were 1.8 and 1.3 g/100 g, respectively. The carvone content of Finnish caraway fruits of four origins cultivated in 1991 varied between 2.2 and 2.9 g/100 g of dried fruits and the ratio of carvone to limonene varied from 1.7 to 2.4 in various fruit samples.

Keywords: *Supercritical extraction; caraway; carvone; limonene; SFE–GC*

INTRODUCTION

The use of supercritical fluids for extraction can be considered one of the most potentially useful new methods of sample preparation and will eventually compete successfully with conventional methods, e.g., hydrodistillation, steam distillation, and solvent extraction, in the isolation of volatile compounds from complex natural matrices. The attractiveness of supercritical fluid extraction lies in the high mass transfer achieved due to the low viscosity and high diffusivity of a fluid when compared with liquids (McHugh and Krukonis, 1986). The extracting potential of a supercritical fluid depends on its density, which can be controlled by varying the pressure and temperature conditions. The solutes are separated from the fluid by decreasing the solvating power either by lowering the pressure or by raising the temperature.

When volatile, reactive, and heat-sensitive terpene compounds are isolated, low critical temperature and nonpolar character of the fluid are preferable. The most commonly used supercritical fluid, carbon dioxide, possesses these features and in addition provides a chemically inert and nontoxic environment for the extraction (Wright et al., 1988). The extracted volatile compounds can be collected off-line into a collection vessel or transferred on-line to an analytical system, while carbon dioxide is either vented out or used as carrier into the analytical system. Extensive reviews on the coupling of supercritical extraction with various chromatographic techniques have been published (Hawthorne, 1990; Vannoort et al., 1990; Jinno and Saito, 1991). Our main interest was the construction of an on-line supercritical fluid extraction–gas chromatography (SFE–GC) system, with which qualitative and quantitative isolation and analysis of aroma compounds could effectively be combined. Instrumentation of directly coupled SFE–GC has been reviewed by Maeda and Hobo (1992). On-line coupling of supercritical fluid extraction to gas chromatography has been applied to the analysis of aroma compounds by Hawthorne et al. (1988a,b, 1989a,b), Lohleit and Bächmann (1990), Huston and Ji (1991), Nielsen et al. (1991), and Hartonen et al. (1992).

The main disadvantage of on-line systems is the risk of contamination with high molecular weight substances, which are retained on the cold trap itself or along the extract transfer line. The small sample size employed in on-line SFE–GC can be a limiting factor, especially when representativeness of the sample is at jeopardy due to heterogeneity of the material. When too large amounts of substances are introduced into the analytical system, the separation efficiency of the column decreases and the linearity of the detector response becomes compromised, resulting in false quantitation of the compounds.

In this study we describe the use of a stream splitter in on-line SFE–GC analysis of carvone and limonene in caraway fruits. Cryogenic collecting of volatile aroma substances to a capillary GC column in connection to effluent venting is introduced. A cleanup procedure to avoid contamination of the system and to retard deterioration of the analytical column is presented.

MATERIALS AND METHODS

Caraway Fruits. Caraway fruits (*Carum carvi* L.) of various origin were cultivated at the Agricultural Research Center, South Savo Research Station, in Mikkeli, Finland, in 1990–1991. Fruits collected from nature in Vöyri, Hämeenkyrö, and Puumala in Finland and cv. Polaris of Norwegian origin were sown to an open field, and no pesticides were used during cultivation. The fruits (also called caraway seeds) were harvested by hand and dried at 40 °C to about 10% moisture and stored in paper bags at ambient temperature protected from light.

Reference Compounds and Internal Standards. *l*-Carvone and *d*-limonene (Fluka Chemie, Buchs, Switzerland) were purchased for recovery testing. *n*-Decane, *n*-undecane, and *n*-tetradecane (Sigma Chemical Co., St. Louis, MO) were used as internal standards in quantitative analysis.

On-Line Carbon Dioxide Extraction–Gas Chromatography. Carvone and limonene of caraway fruits were isolated

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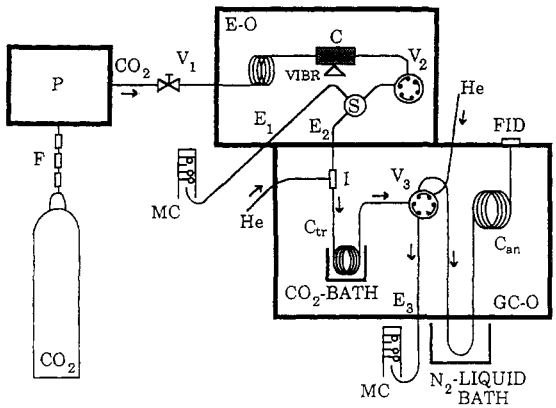

Figure 1. Schematic diagram of the on-line SFE–GC system with cryogenic collection trap: (F) frits; (P) pump; (V) valve; (E-O) extraction oven; (C) cartridge; (S) stream splitter; (E) exit; (MC) measuring cylinder; (I) injector; (C_{tr}) trapping column; (C_{an}) analytical column; (GC-O) gas chromatograph oven.

with supercritical carbon dioxide and analyzed by an on-line SFE–GC system developed for quantitative analysis of highly volatile substances. The scheme of the procedure is outlined in Figure 1. Some safety hazards are always present when involved in working with a high-pressure apparatus. To avoid injuries, the extraction cartridge (C) was secured inside an old GC oven referred to as the extraction oven (E-O) as were all tubing and capillaries subjected to supercritical pressures. Even if explosions are unlikely to occur during moderate-pressure CO_2 extraction, a connection or scratched silica capillary might fail to endure the pressure forced on it and break with serious consequences.

A ~0.5 g aliquot of caraway fruits was ground under liquid nitrogen in a ceramic mortar. About 1.0 or 10.0 mg of frozen powdered fruits was weighed (±0.2 mg) into a 167 μL extraction cartridge (Keystone Scientific, Bellefonte, PA). The sample was at very low temperature, and the loss of volatile components was assumed to be minimal during the grinding under liquid nitrogen. Prior to fruit sample preparation, the internal standards (*n*-decane and *n*-undecane) were applied on a small piece of cellulose filter paper (Whatman No. 4, Whatman International Ltd., Maidstone, England), which was placed next to the sample in the cartridge. The solvent evaporated during the sample preparation, and only a minor amount of pentane remained absorbed into the filter paper. The cartridge was then sealed and transferred in the extraction oven at 50 °C. The cartridge was connected with fused silica capillary (50 μm i.d.) between valves V_1 (Scientific Systems, State College, PA) and V_2 (N6W, Valco Instruments, Houston, TX), which were kept closed. The 10 m capillary between V_1 and the cartridge was placed inside the extraction oven to stabilize the temperature of the CO_2 extractant. After the cartridge was maintained at 50 °C for 2 min, valve V_1 was opened, whereas the low dead volume valve V_2 was kept closed. The cartridge was pressurized to 9.7 MPa for 2 min with preheated supercritical CO_2 by the microgradient pump, P (Brownlee, Santa Clara, CA). High purity grade carbon dioxide was obtained from Scott Speciality Gases (Plumsteadville, PA). The CO_2 was introduced to the pump through stainless steel tubing equipped with filters (F) to remove particles with diameters larger than 2 μm. The cartridge was vibrated at 140 Hz frequency during the last 30 s to intensify the diffusion.

A deactivated fused silica capillary tubing (0.25 m long, 0.025 mm i.d., 0.28 mm o.d.) linked valve V_2 to the stream splitter, S (ZT.5, Valco Instruments, Houston, TX). A 0.5 m piece of the same tubing was connected to one of the two splitter exit ports (E_2) and inserted to the depth of 0.15 m inside the DB-1 fused silica trapping column, C_{tr} (3 m, 0.32 mm i.d., 0.25 μm d_f, J&W Scientific, Folsom, CA). The trapping column was connected to the "duck bill" injection port (I) of the on-column injector of the gas chromatograph (Model 5890A, Hewlett-Packard, Palo Alto, CA). The dimensions of the removable fused silica capillary at E_1 were chosen according to the split ratio required. After the 2 min pressurizing time, V_2 was opened and the expanding CO_2 containing volatiles dissolved in it was divided using a stream splitter between the trapping column in the oven (50 °C) and the exit E_1 at a ratio of about 1:100. The volumes of CO_2 vented were measured with the measuring cylinders (MC) located at the end of E_1. The dynamic extraction was carried out for 6 min, whereafter valve V_2 was closed. The cartridge was pressurized for another 2 min and the 6 min of extraction repeated.

During extraction, about 2 m of the trapping column was chilled to −40 °C (±2 °C) in a dry ice bath in the extraction oven to increase the retention of the volatiles. Helium was introduced simultaneously into C_{tr}. Valve V_3 (N6WT, Valco Instruments) in the sampling/analysis position (S/A) directed the mixture of helium and the CO_2 extractant from C_{tr} through the 1 m long exit tubing E_3 (0.32 mm i.d.) to the measuring cylinder (MC). At the same time, helium was introduced also through another inlet system via V_3 into the analytical column (C_{an}) to maintain the chromatographic conditions and the sensitivity of the flame ionization detector at optimized level.

When the four consecutive extractions were completed, V_1 was closed. V_2 was closed 1 min after that, and the remaining CO_2 was allowed to escape through E_1, E_2, C_{tr}, and E_3 for an additional 30 s. The head of the analytical column (DB-5, 30 m, 0.32 mm i.d., 0.25 μm d_f, J&W Scientific) was immersed in liquid nitrogen, valve V_3 was switched to the inject position, and the dry ice chilling was removed from C_{tr}. Thus, the He carrier flowing through C_{tr} transferred the analytes from C_{tr} into the C_{an} in the cryozone, forming a narrow band. Transfer of the analytes was made more effective by programming the gas chromatography oven (C-O) temperature from 50 to 150 °C at 15 °C/min and holding for 15 min. The oven was then cooled to 40 °C and V_3 switched to the S/A position. The cold bath was removed and the chromatographic analysis carried out by using a program from 40 to 150 °C at 5 °C/min followed by 25 °C/min to 220 °C. To avoid accumulation of less volatile lipophilic material, the extraction cartridge was cleaned by sonication in hexane and acetone, and the extraction system with an empty and solvent-free extraction cartridge was purged with supercritical CO_2 at 14 MPa for 15 min after each sample. The trapping column was detached and the end at V_3 was connected to the duck bill for the cleanup procedure. The efficiency of the cleanup was tested at random intervals by blank analyses, which showed the traces of limonene and carvone to be negligible.

The CO_2 extraction/GC analysis of the pure reference compounds, limonene and carvone, was optimized and compared with the analysis of the internal standards *n*-decane and *n*-undecane. An aliquot of 3 μL of pentane solution corresponding to 0.17 mg of limonene and 0.19 mg of carvone, as well as 3 μL of pentane solution containing 50 mg/mL of each hydrocarbon, was applied on a 4 mm^2 piece of Whatman No. 4 filter paper. The paper was placed in the extraction cartridge which was sealed, and the procedure was started with four consecutive extractions as described above. The quantitative determination of carvone and limonene in caraway fruits was performed by simultaneous analysis of *n*-decane and *n*-undecane as internal standards on 4 mm^2 paper disks and by comparison of the results with the analysis of the pure reference compounds.

Hydrodistillation. The essential oil was isolated with a Karlsruher apparatus (Stahl, 1953). The caraway fruits were ground with a centrifugal mill equipped with a 1.0 mm sieve (Model ZM 1, Retsch KG, Haan, Germany) under liquid N_2 injection. About 5.00 g of ground spice was extracted in 200 mL of distilled deionized water for 4 h according to the *Dutch Pharmacopoeia* (Nederlandse Farmacopee, 1966). The amount of oil was measured on the volumetric scale on the side tube of the apparatus. The oil was dried over anhydrous sodium sulfate and stored at −20 °C in Teflon-sealed screw-cap vials. A 30 000-fold dilution of the distillate in *n*-pentane/diethyl ether (1:2 v/v) was made, and *n*-tetradecane was added as internal standard prior to gas chromatographic analyses.

Gas Chromatographic Analysis. The gas chromatographic analyses of the hydrodistillates were carried out on a

Varian 3300 gas chromatograph (Varian Associates, Walnut Creek, CA) equipped with a flame ionization detector connected to a Shimadzu Chromatopac C-R3A integrator (Shimadzu Corp., Kyoto, Japan). Fused silica columns (HNU-Nordion Ltd., Helsinki, Finland) (25 m × 0.32 mm i.d., 0.20 μm d_f) coated with NB-351 liquid phase [nitroterephthalate modified poly(ethylene glycol) polymer, corresponding to OV-351] were used for the analyses. The oven temperature was programmed as follows: from 40 (isothermal for 5 min) to 220 °C at 5 °C/min, and an isothermal period at 220 °C for 5 min. The temperature of the injector port and the detector was 240 °C. The split ratio was 1:20, and the flow rate of carrier gas (helium) was 1.6 mL/min.

Gas Chromatography–Mass Spectrometry. The volatile compounds of caraway fruits were identified by the VG 7070E mass spectrometer (VG, Wythenshave, Manchester, U.K.) equipped with a Dani 3800 HR ch gas chromatograph. The electron ionization mode was used at 70 eV of energy. The data were processed by the VG-11-250 system. The whole extract was collected onto the trapping column (without stream splitting) to obtain sufficient amounts of the minor compounds. The volatiles were focused onto the head of the analytical column as described previously, and the column was subsequently coupled into the GC–MS. The same GC program as described for on-line SFE–GC was used. The compounds were identified by comparison of Kováts indices and mass spectra reported in the literature (Stenhagen et al., 1974; TNO, 1979; Davis, 1990) and using the NBS 1987 mass spectra library.

RESULTS AND DISCUSSION

Caraway fruits contain about 3–6% essential oil (Furia and Bellanca, 1975), which gives the fruits their characteristic aroma. Some 30 substances have been identified from caraway oil (Lawrence, 1992). Carvone and limonene make up more than 95% of the essential oil of caraway fruits (Bauer and Grabe, 1985). All of the other aroma compounds exist at trace levels only. The overall quality of the fruit is considered to correlate to the amount of essential oil and the ratio of carvone to limonene: the higher the ratio, the better the quality. Quality requirements for caraway oil can be found, e.g., in the *British Pharmacopoeia* (1988), where the content of ketones is stated to constitute 53.0–63.0% (w/w) calculated as carvone. GC fingerprint analysis has been suggested to verify the authenticity of the oil (Analytical Methods Committee/Royal Society of Chemistry, 1988).

When the coupled SFE–GC technique was applied in the analysis of caraway fruit volatiles, the following details were considered: (1) The density of CO_2 had to be as low as feasible to avoid transfer of the less volatile impurities in the chromatographic columns and at the same time high enough to ensure quantitative and repeatable extractions. (2) The sample had to be large enough to represent the corresponding lot of spice. (3) The effective diffusion of the solutes into the solvent had to be verified by proper grinding of the fruits and by vibrating the sample during extraction. (4) The total volume of the CO_2 extractant had to be sufficient to permit efficient extraction. (5) The loss of volatiles had to be prevented during both sample preparation and extraction.

Advantages of Moderate Density. Our aim was to operate at low pressure, where the selectivity of the supercritical fluid is high but solvent power low. We tried to minimize the solubility of the less volatile compounds, which eventually elute into the trapping column. The pressure (9.7 MPa) was selected on the basis of information found in the literature (Stahl and Gerard, 1985; Engelhardt and Gross, 1988; Smith and Burford, 1992), accepting the fact that consecutive extractions would be needed to achieve acceptable yields of limonene and carvone. When highly volatile compounds are isolated, high temperatures are to be avoided. To maintain supercritical conditions throughout the extraction despite small temperature fluctuations (±2 °C), 50 °C was found most feasible. Under these conditions some coextraction of lipophilic material did occur, and to avoid contamination of the analytical system, systematic purging was necessary.

Cryogenic Trapping. The cold on-column injection method possesses several advantages over other sample introduction techniques for the analysis of thermolabile compounds as summarized by Grob (1987). The coupling of liquid chromatography to gas chromatography (LC–GC) on-line was basically developed on principles of cold on-column injection (Grob, 1990). On-line SFE–GC presents several problems similar to those of LC–GC coupling: need for solvent removal, high flow rate to the capillary column, peak focusing requirements, etc. By using an on-column injector, the supercritical CO_2 extract can be depressurized onto the head of the analytical column, and the solutes can be collected without elevated temperatures. The gaseous CO_2 flows continuously through the column and the solutes may diffuse from the cryozone producing broad peaks. Additional focusing becomes necessary when the full capacity of the trap is used, e.g., due to large amounts of extract, resulting in unacceptable band broadening in the chromatographic analysis. Extremely low temperatures in the cryotrap cannot be used due to plugging of the restrictor by precipitated solutes and solid CO_2. Studies made on trapping efficiencies of various compounds conclude that the trapping becomes more difficult as the solutes of interest become more volatile (Andersen et al., 1989; Hawthorne et al., 1989a).

Raymer and Pellizzari (1987) and subsequently Raymer and Velez (1991) developed an on-line SFE–SFE–GC method suitable for large extraction volumes with an intermediate adsorption step, where semivolatile compounds were collected into a Tenax GC trap at 50 °C and CO_2 was vented out. The solutes were purged and focused with supercritical fluid onto the capillary GC column, whereby only a part of the carbon dioxide flow passed through the analytical column and better peak shape was obtained. Ashraf-Khorassani et al. (1990), Cotton et al. (1991), and Hartonen et al. (1992) used various cryogenic collection systems, which were effective in trapping volatile substances.

Collecting solutes from the effluent stream after supercritical extraction to give quantitative recoveries has to be optimized in every extraction procedure. Depending on the volatility of the analytes cold trap temperatures between 0 and −50 °C are needed. A GC capillary column has previously been used for sample collection in headspace analysis (Kallio, 1991) trapping highly volatile components at ambient temperature. We used a DB-1 column with a 0.25 μm phase for two specific reasons. First, the phase retains functionality even at −60 °C, and solutes are partitioned between the liquid phase of the column and the gaseous CO_2. The extracted compounds are adsorbed onto the surface of the column as when a retention gap or solid phase adsorbent is used as the collection trap. Second, a thin phase layer is better linked and possesses higher integrity toward CO_2 to the capillary wall than a thick layer. When optimizing the collection temperature, we tested breakthrough of the solutes in this system without the splitter installed: the amount of solutes was

distinctly higher (ca. 99 times more). A second collection column placed in a liquid nitrogen cold trap and connected immediately after the first collection column was used. At first, a water/ice/salt cooling bath with a temperature of ca. −8 °C was investigated. The collection efficiency was insufficient, because peaks corresponding to the solutes were detected in the second column contributing, ca. 27% of limonene and 11% of carvone of the summarized peak area, respectively. The collection column was then cooled to −40 °C with dry ice, and the loss of solutes was avoided. After the splitter was introduced, we assumed that the reduced amount of solutes arriving at the collection column would be more efficiently trapped than the larger amount previously transferred into the column. In our on-line system at a low flow rate of effluent, a −40 °C collecting temperature could be used without plugging of the restrictor or the column. The helium flow introduced from the on-column injector increased the total flow through the collection column and forced the expanding CO_2 to move continuously forward, thus avoiding precipitate formation.

Amount of Carbon Dioxide. To achieve highly efficient extractions, 3−5 void volumes of supercritical fluid was recommended to be consumed according to the procedure of Levy (1991). The density of the fluid is the most significant parameter when the efficiency of the isolation procedure is optimized. The amount of extractant needed depends on the solubility of the analytes into the fluid under the selected operation conditions and analyte−matrix interactions present, and substantially larger volumes of CO_2 than 5 void volumes can be required (Pawliszyn, 1993). In a static extraction system, when saturation level is reached, the concentration of the solute in the fluid remains constant. The saturated fluid must be removed from the extractor, which subsequently will be refilled with fresh fluid. At the same time, the solutes are quantitatively transferred into a collection trap and CO_2 is released. This procedure needs to be repeated until only residues of solutes remain in the matrix. The void volume of the extractor as well as the solutes themselves affects the total amount of extractant to be consumed for the effective isolation. The trap must have sufficient capacity and endurance, so that it will be able to withstand the amount of expanding fluid required and yet efficiently hold the increasing amounts of solutes. The number of repeated extractions and the total fluid consumption are empirically determined for each isolation procedure. The total amount of CO_2 fluid consumed in one analysis using the described method corresponds to about 500 mL of gaseous CO_2 at 1 atm of pressure.

Sufficient Sample Size. In the preliminary investigations powdered fruit samples of 1.0 mg were analyzed. The recoveries of limonene and carvone were tested by repeating the extraction (twice 2 min static + 6 min dynamic) and analysis procedure four times without opening the extraction cartridge between the gas chromatographic analyses. The set of four extractions was repeated six times. More than 90% of the solutes isolated were found in the first extraction, and the relative proportions of the extractions remained stable with standard deviations between 2.0 and 3.6%. The total amount of limonene and carvone varied considerably, presenting relative standard deviations of 36% for limonene and 23% for carvone. The 1.0 mg sample was too small to ensure representativeness. This

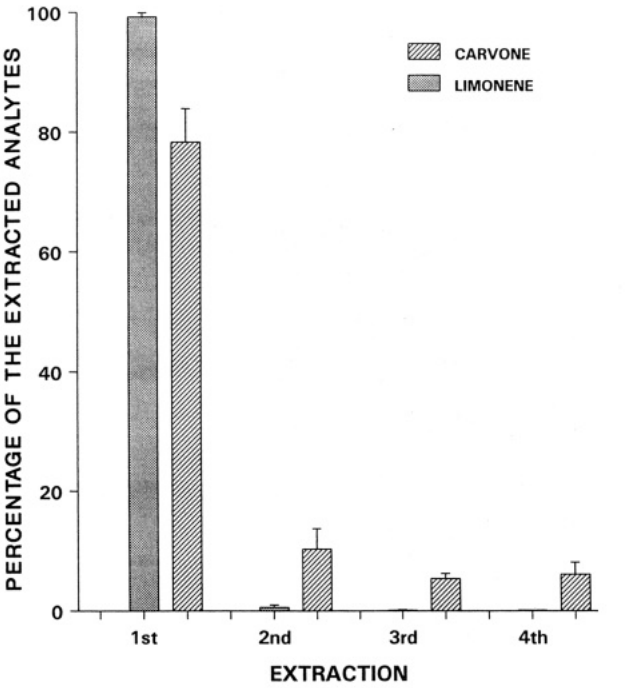

Figure 2. Percentage of carvone and limonene in the consecutive extractions from the total amount isolated from caraway fruits by on-line SFE−GC (n = 4).

contributed to the heterogeneity of the powdered sample and the inaccuracy of the weighing.

The sample size was increased to 10.0 mg, which was found to be sufficient to ensure the representativeness of the sample. In the analysis of limonene the larger sample size was similar to the result obtained with the smaller sample size. The extraction pattern was the same in the four consecutive extractions (2 min static + 6 min dynamic) and repeated four times (Figure 2). The error bars represent standard deviations of the proportions of solutes recovered in each extraction calculated as percent from the total amount obtained. The total percentage of carvone isolated increased slowly. The relative standard deviations of the four replicate isolations were still unacceptably high (more than 20%), and the solubility of the analytes was not considered efficient enough. The amount of carvone remaining in the sample was continuously large, presumably due to uneven dispersion of the sample in the extraction cell and an insufficient contact between the fluid and carvone. Hawthorne et al. (1993) suggest that kinetic limitations might be responsible for the phenomenon in a manner described by Bartle et al. (1990) rather than solubility limitations.

Effect of Vibration. Ultrasonication has been applied in extraction procedures to enhance the extraction rates of the analytes and used also in SFE (Wright et al., 1988). In this study, the speed of diffusion of the solutes from the matrix was increased by vibrating the cartridge mechanically using a frequency of 140 Hz for 30 s. The solute residues, especially that of carvone, decreased gradually after each subsequent extraction (Figure 3). The total yield of carvone became 2.5-fold, but the extracted amount of limonene showed no substantial increase. The proportion of the first extraction of the total extracted amount decreased, possibly due to saturation of CO_2 with carvone. The analytical column became overloaded with the analytes, and the standard deviations remained high.

Introducing a Stream Splitter. The amount of solutes introduced to the analytical system was reduced

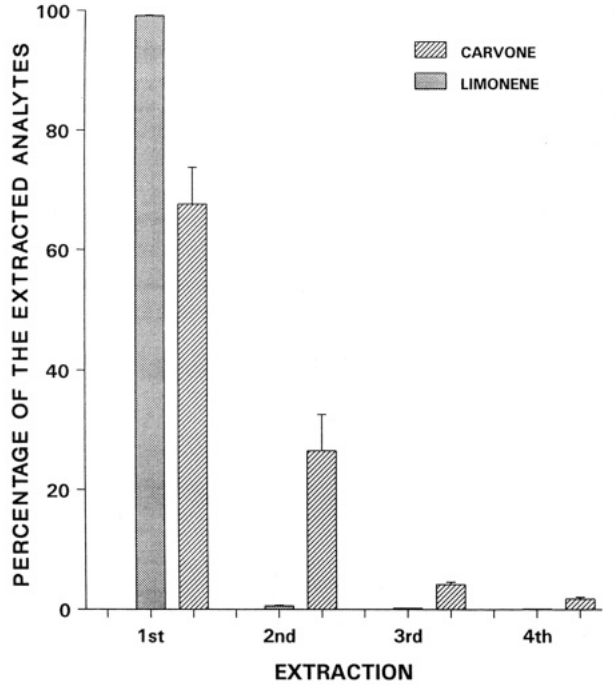

Figure 3. Percentage of carvone and limonene in the consecutive extractions from total amount isolated from caraway fruits by on-line SFE–GC with vibration ($n = 4$).

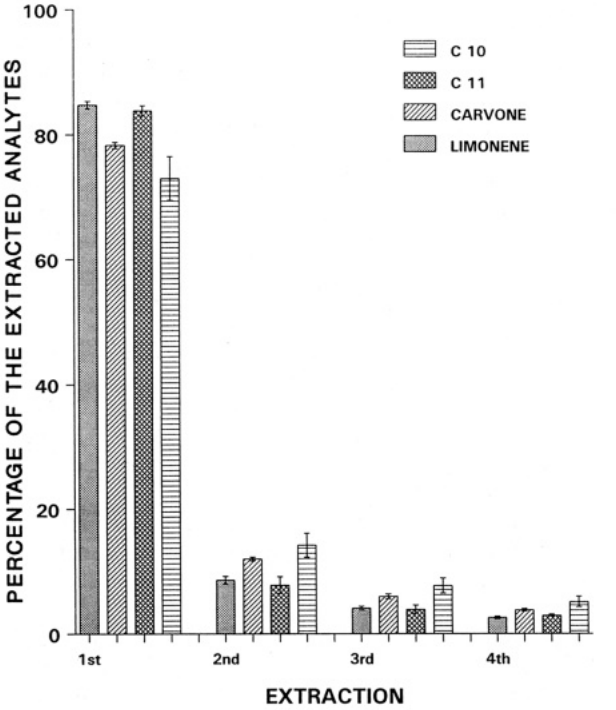

Figure 4. Percentage of pure reference compounds and internal standards in the consecutive extractions from the total amount isolated from filter paper by on-line SFE–GC with vibration and a splitter ($n = 4$).

by venting out a large part of the extract through a T-piece. To minimize the accumulation of the coextracted undesired substances, it was concluded that the splitter had to be placed as soon after the extractor cell as possible. The split ratio between the exits E_2 and E_1 determined the proportion of extract that was transferred into the collection column. A small decrease in the pressure of the fluid was expected to occur at the splitter, but the discrimination due to the pressure drop was estimated to be not significant. Limonene and carvone have similar molecular weights and high solubility in the supercritical carbon dioxide at 50 °C (Stahl and Gerard, 1985). The split ratio was about 1:100, and it was held constant during the whole work.

Reference Compounds and Internal Standards. The degree of freedom, in the selection of the pressure–flow conditions, is high. The requirement is that the pure reference compounds and internal standards used for calibration purposes are analyzed at precisely the same conditions as the samples. Fortification of the sample itself was rejected after poor results; i.e., repeatability was not sufficient (>60% RSD). Quantitative addition of 3 μL of standard solution (pentane) at ambient temperature to caraway fruits under liquid nitrogen, whether the sample was powdered before or after the addition, was not successful. Smith and Burford (1992) used α-cellulose as a model plant material when they optimized supercritical fluid extraction for volatile constituents including limonene and carvone. Quantitative recovery of carvone was achieved at 12 MPa (at 40 °C). However, limonene was explained to have been lost during sample preparation, thus causing the erroneous recoveries attained in their study. We used Whatman No. 4 filter paper as the support material because it is a cellulose-based, low retention filter. It absorbs the standard solution into the pores, and the solvent can be readily evaporated. The solvent was allowed to evaporate at room temperature for ca. 30 s to produce a delay equal to the time needed for powdering and weighing of the sample prior to extraction. With a small piece of filter paper as support, the fortification was fast and relatively repeatable (Figure 4). The recovery of the analytes from the filter paper, corresponding to 100% isolated in the four consecutive extractions, was estimated to represent 80 and 90% of the total amount of carvone and limonene, respectively. The estimate was calculated on the basis of total recovery for undecane and nonselective splitting. The increased amounts of analytes isolated in the third and fourth extractions result from the components diffusing from the filter paper after being reabsorbed into the pores. This is not a problem, because the recovery of the internal standards followed the same pattern. As small a piece of paper as possible was used in the extractions. Figure 5 shows the percentages of analytes isolated from the caraway fruit sample with internal standards added on filter paper as in Figure 4. The percentages of the total amount extracted for limonene and the internal standards obtained in the consecutive extractions resembled one another with and without the caraway sample present (Figures 4 and 5). The carvone was less efficiently obtained by the first extraction from the sample than from the filter paper. When the solution is added to the caraway fruit sample, it forms a wet layer on the particles and hinders the diffusivity of the naturally occurring substances distributed throughout the plant material, whereas the internal standard compounds themselves are easily removed from the surface of the particles. Both the reference compounds and the internal standards were assumed to be subject to the same type of diffusion and retention effects as the native substances. Discrimination by the splitter between the analytes and the standards is probable, but the ways in which it affects the various solutes and the importance it had on the results were not studied.

Limonene and Carvone in Caraway Fruits. Caraway fruits of four origins were analyzed by applying the conditions as in Figures 4 and 5. A gas chromatogram of the fruits collected in Puumala is presented in Figure

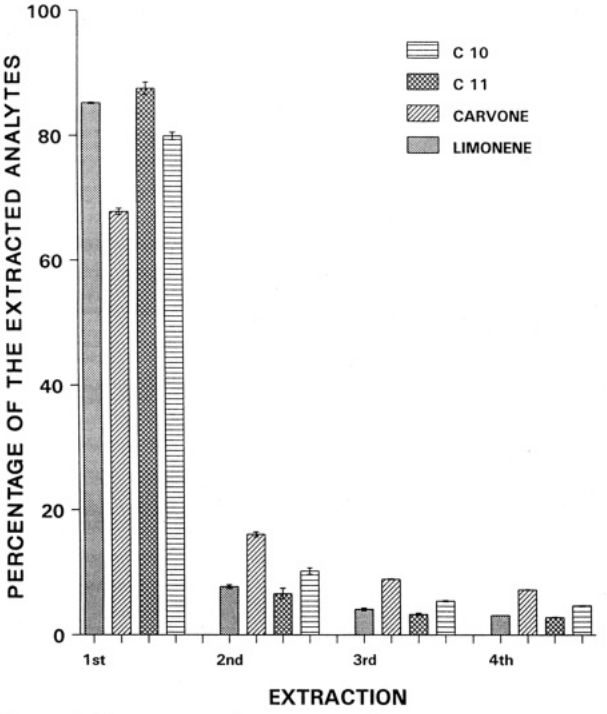

Figure 5. Percentage of carvone and limonene in consecutive extractions from the total amount isolated from caraway fruits by on-line SFE–GC with vibration and a splitter and internal standards ($n = 4$).

6. If all of the chromatographic resolution for the identification of the compounds is not required, the contents of limonene and carvone may conveniently be separated in a few minutes. It was shown by GC–MS analysis that the peaks of both limonene and carvone did not contain detectable amounts of other compounds. Small quantities of several other components were detected, and some of the compounds were identified by GC–MS (Table 1). The caraway volatiles could completely be collected by cryogenic focusing in the analytical column. A second trapping from the collection column, immediately following the first, did not show any residues of the analytes or of the internal standards.

Table 1. Compounds Identified in the Supercritical Fluid Extract of Caraway Fruits from Puumala

peak	compound	peak	compound
1	2,4-hexadienal	13	α-terpineol
2	α-pinene	14	(*Z*)-dihydrocarvone
3	sabinene	15	(*E*)-dihydrocarvone
4	β-myrcene	16	decanal
5	limonene	17	(*E*)-carveol
6	(*Z*)-β-ocimene	18	carvone
7	(*E*)-β-ocimene	19	geranial
8	2-methylbenzaldehyde	20	perillaldehyde
9	4-methylbenzaldehyde	21	methyl 3,7-dimethyl-2,6-octadienoate
10	linalool	22	β-caryophyllene
11	(*E*)-*p*-mentha-2,8-dien-1-ol	23	γ-elemene
12	(*Z*)-*p*-mentha-2,8-dien-1-ol		

Table 2 summarizes the results of limonene and carvone in caraway fruits of four origins. The total content of carvone was approximately at the same level in all samples, the fruits from Puumala having the highest proportion, 2.9%. Limonene varied more and ranged from 1.0 to 1.7%. The ratio of carvone to limonene characterizing the qualitative properties of the fruits varied between 1.7 and 2.4. In respect to this property, the fruits from Vöyri had the highest scores.

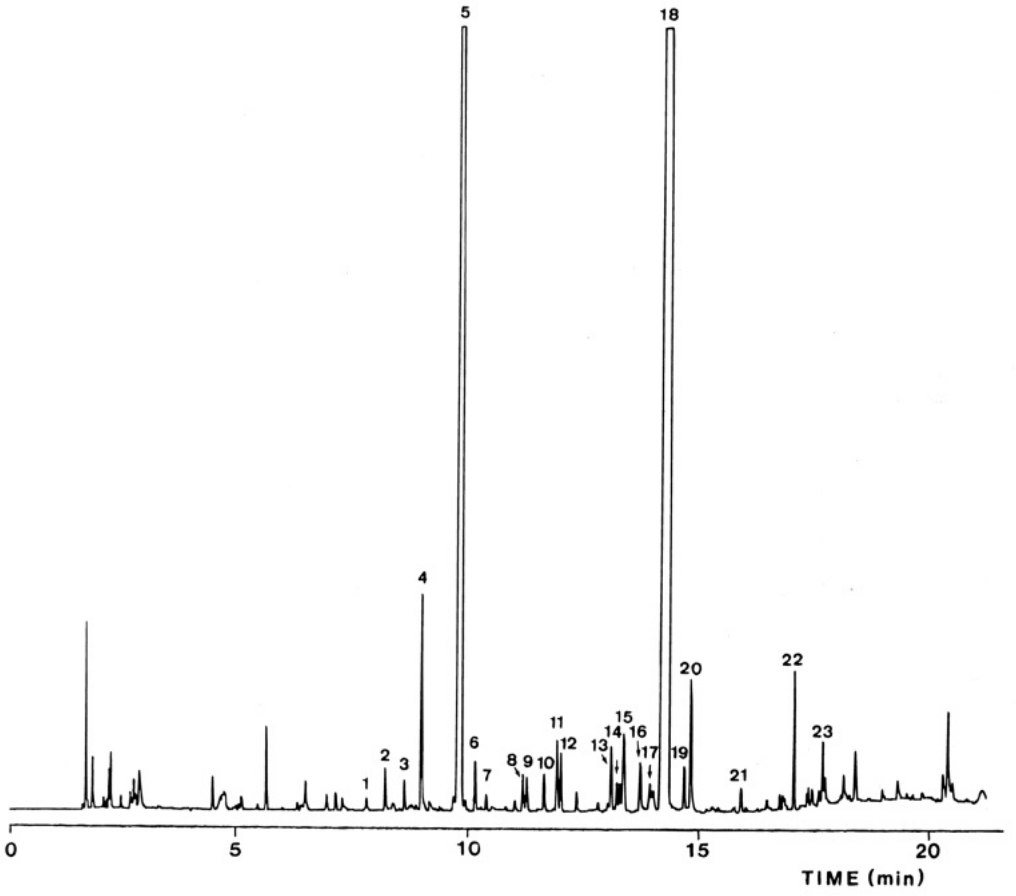

Figure 6. GC–FID chromatogram of the supercritical carbon dioxide extract of caraway fruits. Compounds identified are presented with numbers and referred to in Table 1.

Table 2. Carvone and Limonene Content of Four Caraway Fruit Samples of Various Origins Isolated by Hydrodistillation and Supercritical Fluid Extraction[a]

	hydrodistillation			supercritical fluid extraction		
origin	carvone (RSD)	limonene (RSD)	c/l ratio	carvone (RSD)	limonene (RSD)	c/l ratio
cv. Polaris	2.7 (2.4)	1.8 (3.1)	1.5	2.7 (6.7)	1.3 (13.4)	2.0
Puumala[b]	3.0 (7.3)	1.3 (10.1)	2.4	2.9 (8.8)	1.7 (18.6)	1.7
Vöyri[b]	2.8 (2.4)	1.6 (7.3)	1.8	2.7 (9.5)	1.1 (21.6)	2.4
Hämeenkyrö[b]	2.6 (3.0)	1.5 (10.6)	1.8	2.2 (2.4)	1.0 (17.6)	2.2

[a] Results presented are the means of three replicative isolations calculated as g/100 g of fruits and their relative standard deviations (%). [b] Hydrodistillation of whole fruits.

The carvone contents of the fruits determined by the on-line SFE–GC system were coherent with the amounts of carvone isolated by hydrodistillation. The results of the limonene content of the samples showed larger variation. The splitter could be the reason for this.

The on-line SFE–GC procedure developed in this work ensured reproducible analyses of volatile compounds of caraway fruits in a short time (about 1.5 h each). The systematic cleansing of the sample introduction line and reduced exposure of the capillary GC column to supercritical and gaseous carbon dioxide guaranteed reliable operation during a long period of time. Temperatures as low as −40 °C could be used for cryogenic collection onto a DB-1 capillary gas chromatography column to obtain quantitative recoveries of the volatile components.

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