Low-Resolution Gas-Phase FT-IR Method for the Determination of the Limonene/Carvone Ratio in Supercritical CO_2-Extracted Caraway Fruit Oils

Mikko Ahro,[†] Mari Hakala,[‡] Juha Sihvonen,[‡] Jyrki Kauppinen,[†] and Heikki Kallio*,[‡]

Department of Applied Physics and Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland

A low-resolution gas-phase FT-IR method for the fast analysis of supercritical CO_2-extracted caraway fruit oils has been developed. The limonene/carvone ratio of extraction product was determined within seconds, yielding a coefficient of variation of <5% ($n = 10$). A selection of experimental parameters is discussed on the basis of the analysis of 52 extracted samples. GC-FID was used as a reference method. A correlation of 0.983 ($n = 24$) between the two methods was observed. This method is suitable for the analysis of a large number of caraway fruit extracts due to its speed, repeatability, and minumum sample preparation.

Keywords: *Caraway; Carum carvi L.; carvone; FT-IR; limonene; SFE*

INTRODUCTION

The volatile portion of caraway fruit (*Carum carvi* L.) extract consists of two major components, (+)-carvone and limonene, and small proportions of other terpenoids (*1*). In addition, the product contains varying amounts of lipids, especially triacylglycerols, depending on the method of isolation. Due to the ample amount of carvone, the extracts are valuable ingredients for the fragrance, flavor, and food industries (*2*). Caraway and carvone have also been used to delay sprouting and as antifungal and antibacterial substances in potato storage (*3, 4*). The quality of caraway fruit is mainly determined by the amount of carvone, and maximization of its content has been investigated by studying the effects of light on carvone formation (*5*), by selective breeding by maximum carvone content (*6*), and by metabolic engineering (*7*).

A modern method of isolating carvone from caraway is supercritical CO_2 extraction (*1, 2*), and the maximum yield of carvone in the final product can be optimized. The extraction procedure is controlled by several factors, such as the pretreatment of the fruit, the pressure and temperature of the CO_2, the flow rate of the fluid, depressurizing conditions, and the design of the separator vessels. All of the parameters have to be optimized experimentally. Therefore, there is a need for a fast method of determining the carvone/limonene ratio in the extracts and, if possible, directly of the fruit as well.

Gas-phase FT-IR has been used in various industrial on-line applications (*8–10*). The method is fast and calibration in the gas-phase is easy, because there are minimal molecular interactions to be taken into account as in the case of liquid-phase FT-IR. FT-IR has also been applied for food analysis (*11–13*), but mostly in the liquid-phase. For food research, the gas-phase FT-IR detector has mainly been coupled with gas chromatography (*14*). There are, however, some direct-inlet gas-phase FT-IR methods (*15*).

In the current study, the gas-phase, direct inlet FT-IR was used as a direct inlet stand-alone method.

The aim of the study was to develop a fast and reliable method based on gas-phase FT-IR analysis for monitoring the industrial supercritical fluid extraction (SFE) process of caraway fruit.

MATERIALS AND METHODS

Supercritical CO_2-Extracted Caraway Seed Oils. The 52 samples analyzed were extracted by Aromtech Ltd. (Tornio, Finland) using batch processes with a dual separator system (Chematur Ecoplanning, Tampere, Finland). Temperatures slightly above the critical temperature of carbon dioxide and pressures around 10 MPa were applied in the extractions. The wide selectivity range of the separators (dual/single stages) was utilized when limonene and carvone were fractionated from on another ($T = 28-60$ °C; $P = 5-8$ MPa).

Reference Compounds. α-Terpineol was from Haarmann and Reimer (Teterboro, NJ). 2,4-Hexadienal, β-myrcene, (−)-carveol, decanal, linalool, and perillaldehyde were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). (−)-α-Pinene, (+)-α-pinene, β-ocimene, dihydrocarvone, β-caryophyllene, carvone, and limonene were from Fluka Chemie AG (Buchs, Switzerland). The purity of reagents ranged from 92 to 99.5%.

FT-IR Equipment. The low-resolution FT-IR gas analyzer Gasmet (Temet Instruments, Helsinki, Finland) was originally designed for industrial and environmental applications. The particular unit used in the study had a Giccor (*16*) interferometer and a Peltier-cooled MCT detector. The sample cell was a Teflon-coated White cell heatable up to 180 °C with cold-coated mirrors.

Spectral Analysis. The multicomponent analysis algorithm used for the analysis of the FT-IR spectra was developed by Saarinen and Kauppinen (*17*). The algorithm is a modified classical least squares (CLS) algorithm, the performance of which is mainly limited by the signal-to-noise ratio (SNR). Because of this, the algorithm performs better at lower resolutions (*18*). The resolution used in this study was 8 cm^{-1}.

* Author to whom correspondence should be addressed (telephone +358 2 333 6870; fax +358 2 333 6860; e-mail heikki.kallio@utu.fi).
† Department of Applied Physics.
‡ Department of Biochemistry and Food Chemistry.

Limonene/Carvone Ratio in Caraway Fruit Oils

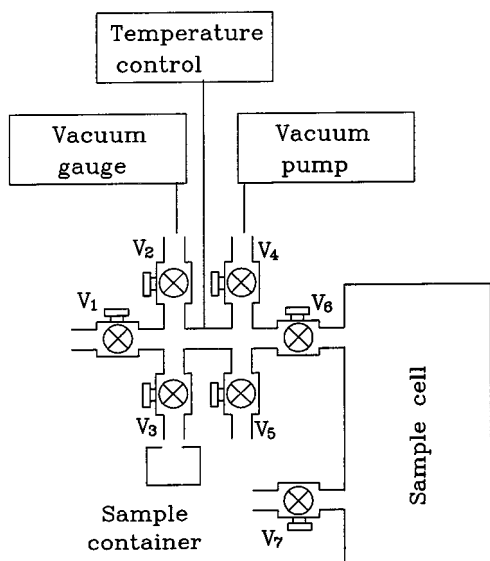*J. Agric. Food Chem.*, Vol. 49, No. 7, 2001 **3141**

Figure 1. FT-IR sampling system used in the current study.

The analysis is based on the single-component library spectra corresponding to pure reference components. The spectral analysis is possible using single-component spectra because there are no molecular interactions in the gas phase and therefore the observed sample spectrum is simply the sum of individual components present in the sample gas.

Sampling. The sample handling system for FT-IR is shown in Figure 1. The transfer line to the sample cell was a PTFE tube (i.d. = 4 mm, length = 1 m) with a heating hose (Horst GmbH, Lorsch, Germany) connected to the temperature regulator (Horst GmbH). Valves $V_1–V_9$ were Swagelok stainless steel manual type (Nupro Co., Willoughby, OH). The vacuum gauge was a Wenzel Electronic's Piezo Steel with ProVac A100 electronics (Wenzel Instruments ApS, Herlev, Denmark). The vacuum pump was a mechanical rotary vane pump purchased from Alcatel (Paris, France). The stainless steel vaporization cylinder had a volume of 624 cm³. The sample container of stainless steel had a volume of ~50 μL. The instrument section between valves 8 and 3 was heated with heating tape (Isopad GmbH, Heidelberg, Germany) connected to the temperature regulator (Electrothermal, Essex, U.K.). The system was heated to 180 °C except for the PTFE tubing, which was heated to 200 °C. N_2 was used as the carrier gas and pressurized air for the line purification after analysis.

Measurement Sequence of FT-IR. Two microliters of caraway fruit oil was transferred into a stainless steel sample container. The container was mounted with a vacuum-proof connection to the vaporization cylinder and heated to 180 °C. The vaporization cylinder and sample cell were pumped to a pressure of ~130 Pa. Then valves 6 and 8 were closed. The volatile part of the oil was allowed to evaporate into the vaporization cylinder by opening valve 4, whereafter the pressure in the cylinder was raised to ~5 kPa with pure nitrogen through valves 2 and 3. Valve 8 was opened, and the volatiles were allowed to flow into the sample cell. Valve 8 was closed and the spectrum measured. A 1 s measurement time and 8 cm⁻¹ resolution were used. The scanning frequency was 10 1/s, the 1 s time corresponding to 10 scans; that is, the sample spectrum measured in 1 s resulted from 10 single-scan spectra averaged.

The reference spectra needed by the spectral analysis algorithm were measured with the same measurement sequence as the oil samples. The measurement time was longer for the reference spectra (300 s).

The vaporization cylinder, transfer line, and sample cell were purified by vacuuming the system or by letting pressurized air flow through the system.

Gas Chromatographic Analysis. The samples were diluted into pentane/diethyl ether (1:2 by volume, pa, Merck, Darmstadt, Germany) solution and analyzed with a Perkin-

Table 1. Repeatability ($t = 1$ s, $n = 10$)

measurement	limonene (%)	carvone (%)	L/C
1	23.50	76.50	0.31
2	23.71	76.29	0.31
3	23.50	76.50	0.31
4	22.62	77.38	0.29
5	22.64	77.36	0.29
6	22.16	77.84	0.28
7	23.47	76.53	0.31
8	22.55	77.45	0.29
9	22.26	77.74	0.29
10	22.12	77.88	0.28
av	22.85	77.15	0.30
SD	0.63	0.63	0.01
CV (%)	2.74	0.81	3.56

Elmer Autosystem GC instrument equipped with an autosampler, FID, Turbochrom workstation, and Turbochrom Navigator 4.1 program (Perkin-Elmer Corp., San Jose, CA). A DB-1701 fused silica column (30 m, i.d. = 0.25 mm, d_f = 0.25 μm) was used (J&W Scientific, Folsom, CA). The temperature of the injector (split ratio = 1/26) and detector was 240 °C. The temperature for the fast analysis was programmed after a 1 min hold from 170 to 230 °C at a rate of 30 °C/min.

Comparison of FT-IR and GC-FID Results. The statistical analyses were performed using SPSS (SPSS7.5.1, SPSS, Chicago, IL). Pearson's coefficients and linear regression model between GC-FID and FT-IR were calculated. A regression line was determined with eight samples. Each sample was analyzed in triplicate for both FT-IR and GC-FID ($n = 24$).

RESULTS AND DISCUSSION

Quantitative Analysis. Concentrations given by the CLS were relative to the concentrations of the pure compounds used for creating the library spectra. The library was calibrated with reference mixtures containing known amounts of limonene and carvone (L/C = 3:1, 1:1, 1:3). This guaranteed the measurement of the proportions of limonene and carvone in the samples, and the results were easy to compare to the GC-FID results.

Concentrations of volatile compounds other than carvone or limonene were so low, as previously reported (*1*), that they were not taken into account in the final analyses. However, in the preliminary tests the other compounds, listed under Reference Compounds, were taken into the CLS-fit, and it became clear that they did not affect the results significantly. The water vapor spectrum was included in the CLS library, but only to eliminate H_2O from the spectrum of the analyte. All samples contained some amounts of nonvolatile fatty oils, and it is possible that the oil residues in the sample container absorbed some carvone and limonene.

Repeatability. The repeatability of the FT-IR method was tested by analyzing one individual extract sample 10 times. The limonene/carvone ratio in this case was 0.296, the coefficient of variation for the ratio being 3.6%. The results of the repeatability test are shown in Table 1. The variation in the absolute amounts of carvone and limonene due to the slight variation in the sample volume did not affect the results.

In the case of another fast method of volatile analysis, the electronic nose, a signal drift is sometimes reported; thus, the response of the detector is not constant during long periods of time (*19–21*). To demonstrate that the FT-IR method does not suffer from time drift, two extracts were measured on two different days. The FT-IR measurement was repeated for two samples 1 week from the first measurement. There were several hundreds of measurements between the replicates. The

3142 *J. Agric. Food Chem.,* Vol. 49, No. 7, 2001

Ahro et al.

Table 2. Daily Variation ($n = 3$)

day	sample	limonene (%)	carvone (%)	L/C
1	A	24.30	75.70	0.32
2	A	24.17	75.83	0.32
1	B	22.50	77.50	0.29
2	B	22.69	77.31	0.29

Table 3. Effect of Measurement Time

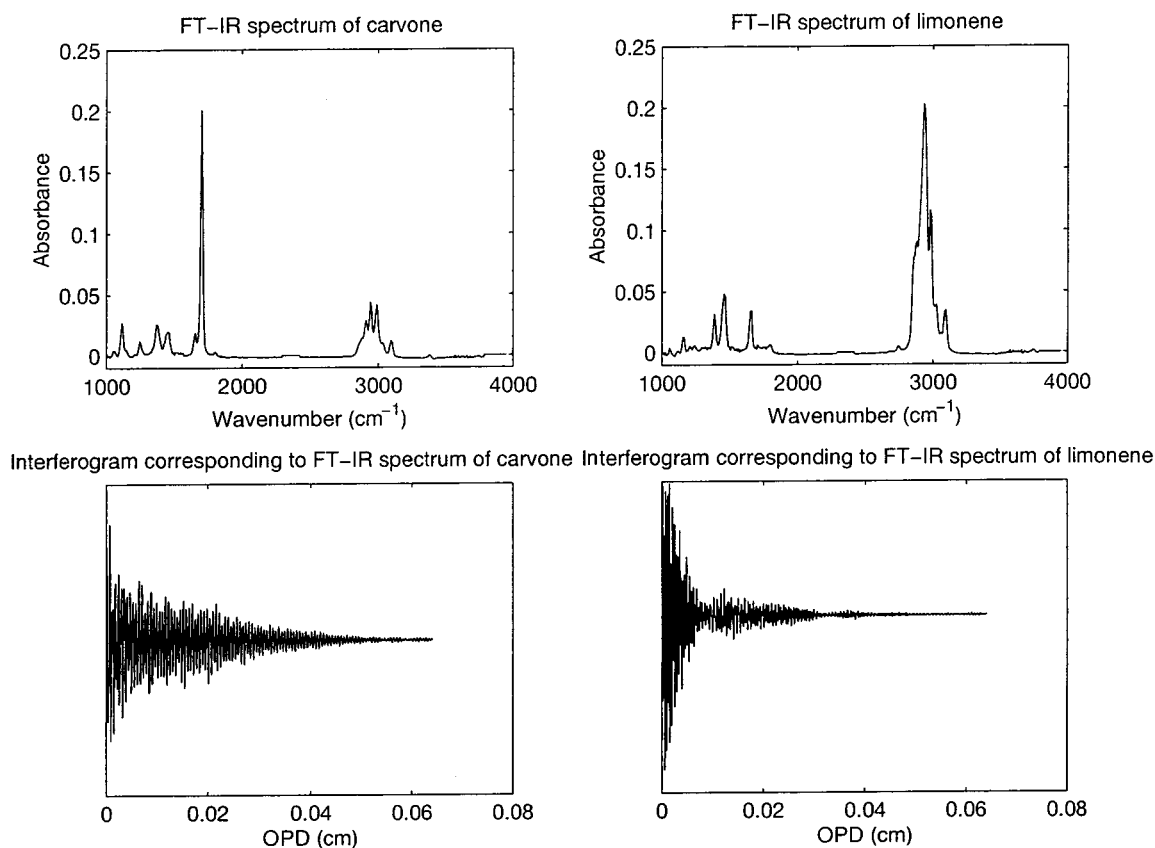| time (s)	limonene (%)	carvone (%)	L/C
1	24.06	75.94	0.32
2	23.47	76.53	0.31
4	23.85	76.15	0.31
6	23.98	76.02	0.32
8	24.06	75.94	0.32
10	23.93	76.07	0.31
20	24.17	75.83	0.32
40	24.50	75.50	0.32
60	24.65	75.35	0.33
600	26.29	73.71	0.36

results (Table 2) show that there is no such problem with the method presented in this paper.

Long-Term Reliability. An FT-IR spectrometer need not be calibrated after one measurement of the library spectra. Recalibration is necessary only if some optical components have to be readjusted, such as the sample cell or the sample handling system, after long-term contamination with analytes. Therefore, as short a measurement time as possible was used to make the exposure time of the measuring system to the sample as short as possible. In the current study, >500 spectra of caraway fruit oils were measured and no cleaning methods other than pressurized air through the system and vacuum pumping were used. The contamination effect was investigated by performing blank runs, and no contamination effect was found.

Effect of Measurement Time. The SNR in FT-IR spectroscopy is proportional to $N^{-1/2}$, where N is the number of scans (*22*). Because the scan speed is constant, this implies that SNR is proportional to $t^{-1/2}$, where t is the scanning time. The accuracy of the analysis depends mostly on the SNR (*17*). In the current application the aim was to measure the limonene/carvone ratio, whereas minor components of very small concentrations were of no interest. It was therefore obvious that there was no advantage in using extremely long measurement times.

The effect of the measurement time was investigated by vaporizing one sample to the sample cell. The cell was sealed and the spectrum measured and saved. Then the measurement time was increased, and another spectrum was taken without changing the sample. By this method 1, 2, 4, 6, 8, 10, 20, 40, 60, and 600 s spectra were acquired. The results are shown in Table 3. The ratio of limonene to carvone indicated some increase (~0.04) with very long measurement times. This was probably due to slow condensation of the carvone vapor in the cell. It also became clear that the correct result could be achieved in as quickly as 1 s. The repeatability tested by a 1 s measurement time also verified that fast measurements are accurate. As a consequence of these results, the 1 s measurement time was used in the rest of the study.

Resolution. In FT-IR spectroscopy the spectral resolution means the resolution of the wavenumber scale. The spectral resolution is an essential parameter; maximum speed and high accuracy of the analysis are required. The use of low resolution has typically been avoided because of the non-linearity of the FT-IR spectral response at high absorbances (>0.5). However,

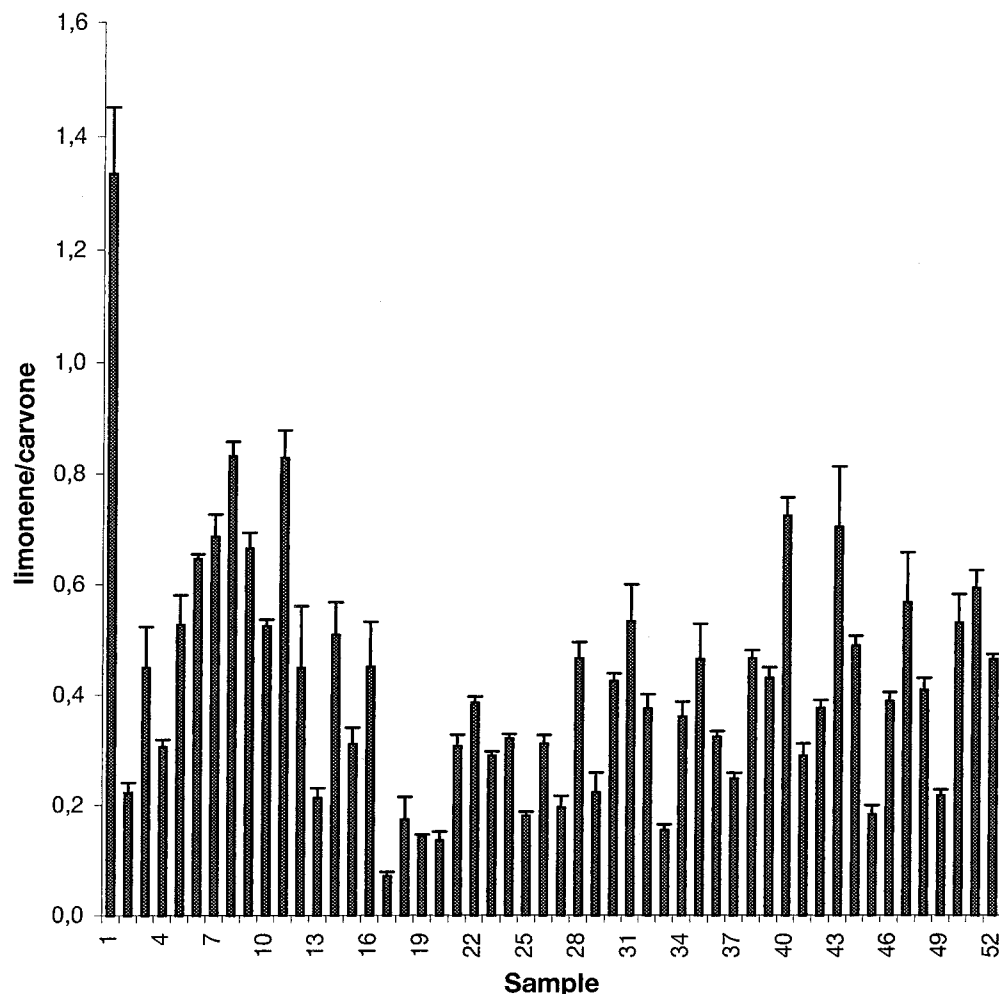**Figure 2.** Spectra and interferograms of carvone and limonene.

Limonene/Carvone Ratio in Caraway Fruit Oils

J. Agric. Food Chem., Vol. 49, No. 7, 2001 **3143**

Figure 3. Limonene/carvone ratio of extraction products with different extraction parameters. The error bars are standard deviations ($n = 3$).

the SNR and dynamic range are enhanced when the resolution is lowered (*18*).

When the non-linearity of the FT-IR spectra is considered, the optimal truncation point of the signal is determined by the width of the vibrational bands (*23*). In other words, the interferogram should be truncated at a point where the vibrational structure is completely collected. Measuring the interferogram between this point and the first rotational packet only increases the noise. With large molecules such as carvone (MW = 150.22 g/mol) and limonene (MW = 136.24 g/mol), the rotational structure is unattainable by an analytical instrument. Therefore, the interferogram after the vibrational structure is practically noise only. The interferograms and spectra of carvone and limonene are illustrated in Figure 2. The interferogram signal corresponding to the spectrum of limonene is almost completely decayed at OPD 0.03125 cm (corresponding to a resolution of 16 cm^{-1}). Therefore, optimal resolution for limonene would be 16 cm^{-1}. However, the interferogram corresponding to the spectrum of carvone has still a significant signal at OPD 0.031 cm. Thus, the optimal truncation point for carvone is 0.0625 cm (resolution of 8 cm^{-1}). The 8 cm^{-1} resolution is preferred to avoid unnecessary nonlinearity in the carvone concentrations. Even though the resolution is not a freely selectable parameter in Gasmet (maximum = 8 cm^{-1}), it is evident that 8 cm^{-1} is the optimum for the current application.

Differences among Samples. Carvone/limonene ratios in different caraway fruit extracts are presented in Figure 3 as an example of the reliability of the method. The sample numbers do not represent chronological or any other order, as they are numbered randomly. The results show that the effects of the extraction parameters were much greater than the variation of the method of analysis. It thus became clear that the method was fast enough and sufficient for the on-line quality control of caraway CO_2 extraction.

Comparison of FT-IR and GC-FID Results. The results obtained by the FT-IR method were compared to the reference results analyzed by GC-FID. The equation for regression model was

$$L/C_{GC-FID} = 0.929(L/C_{FT-IR}) + 0.0075 \qquad (1)$$

The 95% confidence intervals were (0.853, 1.006) for *b* and (−0.052, 0.36) for *a*. The correlation between FT-IR and GC-FID results was 0.983 ($p = 0.01$). The results measured with FT-IR, those measured with GC-FID, and values obtained from the regression model eq 1 are shown in Table 4.

CONCLUSION

A fast method for the quantification of carvone and limonene from caraway fruit oil was developed. It was shown that the gas-phase FT-IR method is suitable for

3144 *J. Agric. Food Chem.,* Vol. 49, No. 7, 2001

Ahro et al.

Table 4. FT-IR and GC-FID Measurements and GC-FID Predicted from FT-IR Data

sample	FT-IR	0.929(FT-IR) + 0.0075	GC-FID
1	0.31	0.30	0.28
4	0.22	0.21	0.21
6	0.31	0.29	0.27
7	0.53	0.50	0.44
9	0.69	0.65	0.69
10	0.83	0.78	0.73
12	0.52	0.49	0.47
13	0.83	0.78	0.79

the current problem and may be applied to off-line and on-line conditions. It is an excellent choice for continuous monitoring of the final product because of its speed. Only a 1 s measurement time was needed to obtain the relative proportions of carvone and limonene, with a very high repeatability, whereas minutes are required for a proper and fast GC analysis. On the other hand, it is possible to identify at least 23 components in ~20 min by the GC method (*1*).

One advantage of the FT-IR method is the minimal sample preparation needed. It takes only a couple of seconds to place the sample into the container and mount it onto the sample line. The purification procedure is also fast, taking less than a minute. The sample handling and purification times are important factors, as they significantly affect the total amount of samples that can be measured in a day.

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