# Enantiomeric Composition of Carvone, Limonene, and Carveols in Seeds of Dill and Annual and Biennial Caraway Varieties

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The enantiomeric compositions of limonene, carvone, and carveols were determined in hydrodistillates and hexane extracts of seeds of dill and annual and biennial caraway. A range of varieties and accessions of several harvest years was investigated to gain insight into genetic variation and differences between years. Both enantiomers of limonene, carvone, and *cis*- and *trans*-carveol were detected in the two caraway types and, except for (−)-*cis*-carveol, also in dill. The enantiomeric compositions of the two caraway types were similar, but dill had a higher (−)/total limonene ratio than either caraway type. Due to less complete extraction, the calculated seed contents of all compounds and their enantiomers were lower for hydrodistilled than for hexane-extracted seeds except for (−)-carvone content, which was significantly higher for hydrodistilled seeds, possibly as a result of conversions during extraction. Also, monoterpene composition was altered by hydrodistillation: the more polar carvone was extracted more efficiently than the apolar limonene. Total monoterpene content in biennial caraway was higher than in the two annual species. The contents of all carveol isomers and (−)/total limonene and (−)/total carvone ratios decreased with storage time between harvest and analysis. The correlations between enantiomers of limonene and carvone and the implications for the pathways of limonene and carvone formation in dill and caraway are discussed. As the enantiomeric composition of carvone is similar for dill and annual and biennial caraway, all three are equally suited as carvone suppliers for sprouting inhibition in potatoes.

**Keywords:** *Carum carvi; Anethum graveolens; seed essential oil; carvone; limonene; carveol; enantiomer; hydrodistillation; hexane extraction; biosynthesis*

## INTRODUCTION

In The Netherlands, (+)-carvone extracted from caraway seed is being introduced as a commercial sprouting inhibitor for potatoes (Oosterhaven et al., 1995). Both enantiomers of carvone are effective as sprouting suppressants, but (+)-carvone is more active than (−)-carvone and in contrast to the latter does not give an unpleasant aftertaste to treated potatoes. Ravid et al. (1992) reported that there were only traces of (−)-carvone in the seed essential oil of caraway (*Carum carvi* L.) and seed and herb essential oils of dill (*Anethum graveolens* L.). Although it was not stated explicitly, these authors probably made their measurements on biennial caraway, since their plants were of Dutch origin and at that time in The Netherlands only biennial caraway was grown. Recently, an annual caraway (*Carum carvi* L. f. *annuum*) variety Karzo has been introduced in The Netherlands (Toxopeus and Bouwmeester, 1993). It is not known whether the enantiomeric composition of annual caraway is similar to that of biennial caraway.

In dill and caraway seed essential oil, (+)-limonene and (+)-carvone are found in approximately equal amounts (Koedam, 1982; Bouwmeester and Kuijpers, 1993). Gershenzon et al. (1989) reported that in *Mentha spicata* (−)-carvone is synthesized from geranyl pyrophosphate through (−)-limonene and (−)-*trans*-carveol. Biosynthesis of (+)-carvone probably follows a similar pathway but with (+)-limonene and (+)-*trans*-carveol (J. Gershenzon, personal communication; Ae. de Groot, personal communication) or (+)-*cis*-carveol (Karp et al., 1990) as intermediates. A study of the enantiomeric composition of the compounds that may be involved in carvone biosynthesis in dill and caraway (limonene, *cis*- and *trans*-carveol) and of carvone itself may contribute to the understanding of this biosynthetic pathway, which is necessary to find possibilities to increase (+)-carvone yields. Because the procedure used to extract the essential oil may affect enantiomeric composition (Werkhoff et al., 1993), two methods that have been described in the literature for extraction of caraway essential oil were compared in this study: hydrodistillation and hexane extraction. In addition, for enantiomeric GC analyses, mostly $H_2$ is used as a carrier gas (König et al., 1990; Ravid et al., 1992). For this study a GC method was adapted to use He as carrier gas. By testing a range of varieties and accessions of dill and annual and biennial caraway as well as different harvest years, we hoped to gain insight into differences in enantiomeric composition between these species and the variability of enantiomeric composition due to extraction method, genetic variation, and differences between years.

## MATERIALS AND METHODS

**Seeds.** A series of varieties/accessions of dill and annual and biennial caraway from the collection of the DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO, Wageningen, The Netherlands) were analyzed. Seeds obtained from abroad were

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propagated in The Netherlands before essential oil was analyzed, unless stated otherwise. After harvest, seed samples were cleaned and stored dry at 15 °C until analysis.

**Extraction.** Depending on the size of the samples, essential oil was extracted using hexane, hydrodistillation, or both methods. For hydrodistillation, 40 g of seeds was shredded superficially for 45 s at low speed in water in a blender. The seed/water mixture was transferred to a custom-made hydrodistillation apparatus as described by Stahl and Schild (1981) and heated to 100 °C. After 2 h, the essential oil was collected and the amount measured using a glass volumetric pipet. Distillates were diluted 150-fold with hexane before GC measurement. Recovery of hydrodistillation was determined by distilling known amounts of isobutylbenzene, limonene, methyl decanoate, carvone, and geraniol. The compounds were collected from the apparatus using three 1.5 mL hexane rinses. The distillation water was extracted twice with 6 mL of hexane. Known amounts of camphor and β-pinene were added to these hexane phases as internal standards and amounts quantitated using GC. For hexane extraction, 1 g of seed was homogenized in 10 mL of hexane for 30 s with an Omni 2000 homogenizer with an Omni 10010 macrogenerator with saw teeth (Omni International, Waterbury, CT). After the seed debris had settled, 5 mL of the supernatant was transferred to a Pyrex centrifuge tube, 0.2 mL of 2 N KOH in methanol was added, and the tubes were vigorously shaken for 20 s to esterify the fatty acids in the triglycerides to avoid injection of triglycerides into the GC column (Van Oostrom and Van der Kamp, 1990; Bouwmeester and Kuijpers, 1993). After addition of 1 mL of $H_2O$, the tubes were shaken again for 20 s and then centrifuged for 2 min at 2000 rpm. The hexane phase was analyzed by GC. The recovery of carvone, limonene, and carveol isomers for hexane extraction was determined using a hexane standard containing known concentrations of these compounds and isobutylbenzene and camphor as internal standards.

**GC and GC–MS Analysis of Compounds.** All samples were analyzed by GC using a CP9000 gas chromatograph (Chrompack, Bergen op Zoom, The Netherlands) equipped with a 25 m fused silica capillary column (i.d. 0.25 mm) with octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (80% in OV-1707) as stationary phase (König et al., 1990). The He inlet pressure was 95 kPa, and the temperatures of the split injection port (split flow 75 mL/min) and FID were 275 and 300 °C, respectively. The oven was programmed as follows: initial temperature, 50 °C for 1.5 min (1.41 mL/min; 34.7 cm/s); rise at 2 °C/min to 110 °C (1.07 mL/min; 31.1 cm/s); hold for 8 min; rise at 20 °C/min to 200 °C (0.74 mL/min; 26.9 cm/s); and a final hold time of 6 min. Only limonene, carvone, and carveol isomers were measured. They were quantified using an external standard mixture with known concentrations of authentic samples of (+)- and (−)-limonene, (+)- and (−)-carvone, and (−)-*cis/trans*-carveol. Enantiomers of carvone and limonene were identified by comparison of retention times with those of authentic standards. Isomers of carveol were identified by comparison of retention times with a (−)-*cis/trans*-carveol standard and $NaBH_4$-reduced (+)-carvone, which yields a mixture of (+)-*cis*- and (+)-*trans*-carveol with the *cis*-isomer predominating (Erman, 1985). The identity of carveol isomers was confirmed by GC–MS. The analyses were performed on a HP 5890 Series II GC and a HP 5972A mass selective detector (Hewlett-Packard). The GC was equipped with a HP-5MS column (30 m × 0.25 mm i.d. × 0.25 μm) (Hewlett-Packard). The injection port (split mode, split flow 50 mL/min), interface, and MS source temperatures were 250, 290, and 180 °C, respectively. The He inlet pressure was controlled by electronic pressure control (EPC) to achieve a constant column flow of 0.95 mL/min (35.4 cm/s) during the following oven program: initial temperature, 55 °C for 5 min; rise at 10 °C/min to 170 °C; final hold time of 0.5 min. Ionization potential was set at 70 eV and the *m/e* range in the scan mode at 50–200 amu. Compounds were identified by comparison of mass spectra with data reported in the literature (Heller and Milne, 1978).

**Data Analysis.** The relative amounts of carvone, limonene, carveol, and their enantiomers were calculated from their peak areas and expressed as percentages of total peak area. To ease comparison of the results, the GC data were also used to calculate the contents of the investigated compounds per gram of seed. The enantiomeric ratios of the individual compounds [the amount of (−)-enantiomer expressed as percentage of the total amount of the compound], total monoterpene content (*i.e.* the total content of the monoterpenes analyzed here), and carvone/limonene ratio [[(+)-carvone + (−)-carvone]/[(+)-limonene + (−)-limonene]] were determined. Data were analyzed for effects of extraction method, plant type (dill and annual and biennial caraway), harvest year, and variety using regression analysis (Genstat 5 Committee, 1988). To estimate the effect of the extraction method on monoterpene contents and enantiomeric ratios, regression models were fit using harvest year and plant type as explanatory variables. The additional explanation of variance when extraction method was added to the model was considered to be the effect of this factor on the modeled parameters. Similarly, the effect of plant type was analyzed by fitting a regression model using extraction method and harvest year and then adding plant type. The effects of harvest year and variety were analyzed for biennial caraway only. A model was fit with either extraction method and variety to which harvest year was added or with extraction method and harvest year to which variety was added to analyze the effects of harvest year and variety, respectively. After regression, means were calculated using the PREDICT directive and the significance of pairwise differences was determined using RPAIR (Genstat 5 Comittee, 1988).

## RESULTS AND DISCUSSION

Using an octakis-γ-cyclodextrin column and He as a carrier gas in GC analysis allowed baseline separation of the enantiomers of limonene, carvone, and *cis*- and *trans*-carveol (Figure 1). In dill and annual and biennial caraway both enantiomers of limonene and carvone were detected, and for the first time the presence of all four carveol isomers in annual and biennial caraway is reported (Table 1). Total carveol content was highest in hexane extracts of biennial caraway at 0.69% of total measured monoterpene content, 0.45% being *trans*-carveol and 0.24% *cis*-carveol. Only (+)-*cis*-carveol content was clearly lower than that of the other carveols. The predominance of *trans*-carveol is in agreement with the findings of Lawrence (1980, 1982/3), who reported 0.3–0.4% *trans*-carveol and 0.1–0.2% *cis*-carveol in caraway seed essential oil. In dill only (+)-*cis*-carveol, (+)-*trans*-carveol, and (−)-*trans*-carveol and not (−)-*cis*-carveol were detected (Table 1). In dill seed, carveol has been reported before as a constituent of the essential

**Table 1. Relative Amounts (Percent of Total Peak Area) of Limonene, Carvone, and (+)- and (−)-*trans*- and *cis*-Carveol and Enantiomeric Ratios of Limonene and Carvone [(−)-Enantiomer as Percent of Total Compound] in Hexane Extracts and Hydrodistillates of Seeds of Dill and Annual and Biennial Caraway**

| | | | (+)-limonene | | (+)-carvone | | (−)/total limonene | | (−)/total carvone | | (+)-*trans*-carveol | | (−)-*trans*-carveol | | (+)-*cis*-carveol | | (−)-*cis*-carveol | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| type | variety/accession[a] | harvest year | hex[b] | hydis[b] | hex | hydis | hex | hydis | hex | hydis | hex | hydis | hex | hydis | hex | hydis | hex | hydis |
| annual caraway | Egyptian | 1988 | 59.59 | 40.91 | 39.58 | 58.06 | 0.54 | 0.54 | 0.47 | 0.70 | 0.11 | 0.12 | 0.11 | 0.16 | 0.02 | 0.06 | 0.09 | 0.05 |
| | Hungarian | 1988 | 56.81 | 40.25 | 42.28 | 58.96 | 0.56 | 0.53 | 0.34 | 0.47 | 0.18 | 0.10 | 0.16 | 0.09 | 0.05 | 0.07 | 0.06 | 0.04 |
| | Karzo | 1990 | 56.02 | 40.27 | 42.54 | 58.33 | 0.60 | 0.56 | 0.60 | 0.76 | 0.25 | 0.23 | 0.27 | 0.30 | 0.13 | 0.10 | 0.21 | 0.10 |
| | Karzo | 1991 | 58.10 | 45.23 | 41.11 | 53.94 | 0.56 | 0.55 | 0.43 | 0.48 | 0.09 | 0.10 | 0.13 | 0.14 | nd[c] | 0.03 | 0.07 | 0.05 |
| | Karzo | 1992 | 53.36 | 39.65 | 45.82 | 59.34 | 0.56 | 0.57 | 0.42 | 0.62 | 0.11 | 0.12 | 0.10 | 0.19 | 0.04 | 0.05 | 0.08 | 0.05 |
| | Poland 1 | 1988 | 56.69 | 44.19 | 42.44 | 54.93 | 0.56 | 0.54 | 0.49 | 0.64 | 0.13 | 0.09 | 0.10 | 0.09 | 0.03 | 0.06 | 0.08 | 0.04 |
| | Poland 2 | 1988 | 56.90 | 42.77 | 42.14 | 56.39 | 0.55 | 0.55 | 0.45 | 0.51 | 0.17 | 0.10 | 0.16 | 0.11 | 0.06 | 0.05 | 0.07 | 0.06 |
| | mean | | 56.78 | 41.89 | 42.27 | 57.14 | 0.56 | 0.55 | 0.46 | 0.60 | 0.15 | 0.12 | 0.14 | 0.15 | 0.05 | 0.06 | 0.10 | 0.06 |
| biennial caraway | Bleija | 1990 | 56.32 | 46.15 | 42.71 | 53.02 | 0.51 | 0.53 | 0.43 | 0.43 | 0.17 | 0.06 | 0.18 | 0.18 | 0.02 | 0.03 | 0.14 | 0.08 |
| | Bleija | 1991 | —[d] | 43.87 | — | 54.95 | — | 0.51 | — | 0.46 | — | 0.09 | — | 0.48 | — | 0.05 | — | 0.08 |
| | Bleija | 1992 | 53.58 | 42.96 | 44.73 | 55.80 | 0.66 | 0.46 | 0.41 | 0.64 | 0.40 | 0.11 | 0.36 | 0.33 | 0.07 | 0.05 | 0.32 | 0.19 |
| | Bleija | 1992 | — | 42.96 | — | 55.77 | — | 0.63 | — | 0.71 | — | 0.10 | — | 0.28 | — | 0.05 | — | 0.16 |
| | Czekoslowakia | 1992 | 58.93 | 48.22 | 39.82 | 50.71 | 0.57 | 0.57 | 0.54 | 0.60 | 0.27 | 0.12 | 0.19 | 0.17 | 0.05 | 0.05 | 0.19 | 0.15 |
| | East Germany | 1992 | 58.71 | 46.67 | 39.94 | 52.23 | 0.55 | 0.58 | 0.48 | 0.68 | 0.36 | 0.11 | 0.21 | 0.16 | 0.05 | 0.05 | 0.21 | 0.14 |
| | Hungary | 1992 | 58.90 | 48.31 | 39.65 | 50.59 | 0.52 | 0.57 | 0.51 | 0.68 | 0.47 | 0.13 | 0.21 | 0.15 | 0.05 | 0.05 | 0.20 | 0.15 |
| | Konzewicki | 1992 | 60.25 | 47.03 | 38.48 | 51.82 | 0.59 | 0.58 | 0.54 | 0.81 | 0.23 | 0.11 | 0.18 | 0.15 | 0.05 | 0.05 | 0.23 | 0.15 |
| | Mongolia | 1992 | 55.53 | 46.88 | 43.23 | 52.03 | 0.58 | 0.62 | 0.52 | 0.62 | 0.15 | 0.09 | 0.23 | 0.18 | 0.05 | 0.04 | 0.25 | 0.15 |
| | Oldambt | 1990 | 58.23 | 50.15 | 41.10 | 49.16 | 0.49 | 0.48 | 0.36 | 0.39 | 0.05 | 0.06 | 0.06 | 0.09 | 0.02 | 0.03 | 0.11 | 0.08 |
| | Oldambt | 1990 | — | 46.70 | — | 52.53 | — | 0.50 | — | 0.40 | — | 0.07 | — | 0.16 | — | 0.03 | — | 0.07 |
| | Oldambt | 1991 | 55.87 | 43.27 | 43.39 | 55.85 | 0.48 | 0.52 | 0.31 | 0.49 | 0.10 | 0.09 | 0.12 | 0.19 | 0.02 | 0.04 | 0.10 | 0.06 |
| | Oldambt | 1992 | 59.01 | 50.06 | 39.56 | 48.87 | 0.60 | 0.57 | 0.49 | 0.65 | 0.33 | 0.10 | 0.23 | 0.16 | 0.06 | 0.05 | 0.25 | 0.15 |
| | Stam 60 | 1992 | 56.78 | 46.89 | 41.98 | 52.06 | 0.59 | 0.61 | 0.50 | 0.57 | 0.24 | 0.11 | 0.18 | 0.17 | 0.05 | 0.04 | 0.22 | 0.14 |
| | Silvia | 1992 | 53.78 | 42.30 | 44.52 | 56.48 | 0.64 | 0.63 | 0.46 | 0.61 | 0.44 | 0.10 | 0.39 | 0.28 | 0.05 | 0.06 | 0.26 | 0.16 |
| | Volhouden | 1990 | 58.18 | — | 41.15 | — | 0.49 | — | 0.32 | — | 0.10 | — | 0.05 | — | 0.01 | — | 0.09 | — |
| | Volhouden | 1991 | 60.97 | 52.19 | 38.13 | 46.93 | 0.46 | 0.47 | 0.41 | 0.49 | 0.11 | 0.09 | 0.17 | 0.18 | 0.05 | 0.04 | 0.13 | 0.09 |
| | Volhouden | 1991 | — | 48.05 | — | 51.00 | — | 0.47 | — | 0.55 | — | 0.07 | — | 0.26 | — | 0.04 | — | 0.07 |
| | Volhouden | 1991 | — | 48.26 | — | 50.95 | — | 0.51 | — | 0.54 | — | 0.07 | — | 0.10 | — | 0.04 | — | 0.05 |
| | Volhouden | 1992 | 60.57 | 50.15 | 37.93 | 48.77 | 0.59 | 0.58 | 0.53 | 0.57 | 0.34 | 0.11 | 0.27 | 0.18 | 0.06 | 0.05 | 0.26 | 0.17 |
| | wild caraway[e] | 1992 | 60.90 | — | 37.94 | — | 0.55 | — | 0.46 | — | 0.28 | — | 0.16 | — | 0.07 | — | 0.13 | — |
| | mean | | 57.91 | 46.90 | 40.89 | 52.08 | 0.55 | 0.55 | 0.45 | 0.57 | 0.25 | 0.10 | 0.20 | 0.20 | 0.05 | 0.04 | 0.19 | 0.12 |
| dill | Blatrechter | unknown | 62.13 | — | 36.57 | — | 0.98 | — | 0.58 | — | 0.12 | — | 0.23 | — | 0.13 | — | nd | — |
| | Prazsky Jemny | unknown | 59.93 | — | 39.00 | — | 0.97 | — | 0.58 | — | 0.07 | — | 0.10 | — | 0.09 | — | nd | — |
| | Selektion Diwa | unknown | 63.5 | — | 35.40 | — | 0.88 | — | 0.68 | — | 0.06 | — | 0.17 | — | 0.06 | — | nd | — |
| | Selektion Mammut | unknown | 59.30 | — | 39.64 | — | 0.86 | — | 0.52 | — | 0.20 | — | 0.07 | — | 0.06 | — | nd | — |
| | Tetra Pannevis | unknown | 60.23 | — | 38.69 | — | 1.13 | — | 0.50 | — | 0.08 | — | 0.00 | — | 0.10 | — | nd | — |
| | TS | 1991 | 63.82 | 47.70 | 35.16 | 51.27 | 0.83 | 0.81 | 0.52 | 0.75 | 0.11 | 0.08 | 0.10 | 0.10 | 0.09 | 0.08 | nd | nd |
| | TS | 1992 | 61.97 | 50.08 | 37.04 | 48.95 | 0.85 | 0.78 | 0.49 | 0.53 | 0.11 | 0.06 | 0.09 | 0.18 | 0.08 | 0.08 | nd | nd |
| | Zwaans Treiboll | unknown | 59.23 | — | 39.51 | — | 1.13 | — | 0.56 | — | 0.14 | — | 0.14 | — | 0.09 | — | nd | — |
| | mean | | 61.27 | 48.89 | 37.63 | 50.11 | 0.95 | 0.79 | 0.55 | 0.64 | 0.11 | 0.07 | 0.11 | 0.14 | 0.09 | 0.08 | | |

[a] All varieties/accessions were analyzed after propagation in The Netherlands except the dill varieties with unknown harvest year, which were obtained from the seed firm Zwaan/Pannevis (Germany) and analyzed without previous propagation in The Netherlands. [b] hex, hexane extract; hydis, hydrodistillate. [c] nd, not detected. [d] —, not determined. [e] Wild caraway was collected near Neerrijnen in The Netherlands and also propagated on an agricultural field before analysis.

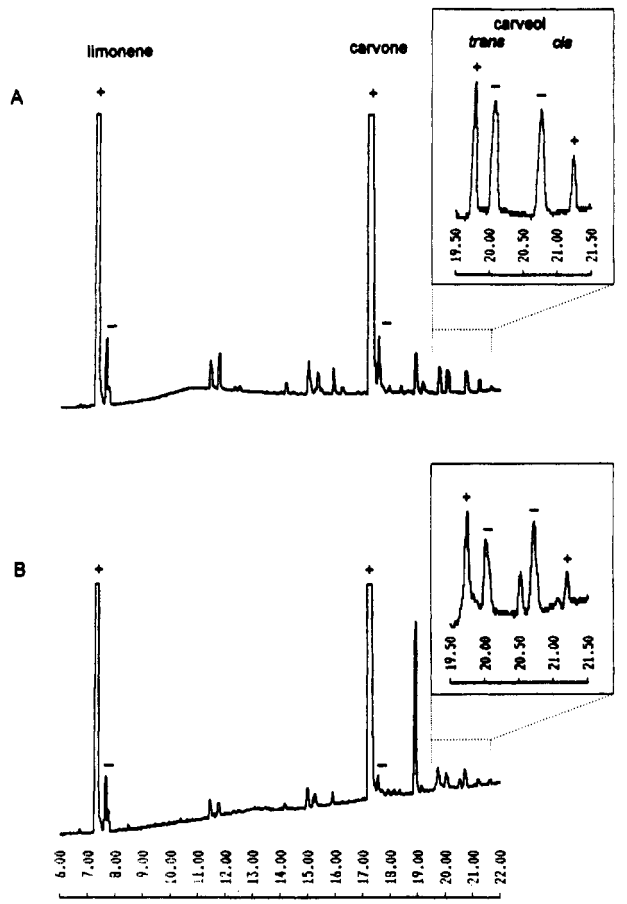


**Figure 1.** GC analysis of hydrodistillate (A) and hexane extract (B) of seeds of biennial caraway Stam 60 using octakis-(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (80% in OV-1707) as chiral stationary phase. For further conditions see Materials and Methods.

**Table 2. Contents and Ratios of Monoterpenes in Hexane Extracts and Hydrodistillates of Seeds of Dill and Annual and Biennial Caraway**

| | | extraction method[a] | | significance level[b] $F^1_{46}$ |
|---|---|---|---|---|
| | | hex | hydis | |
| content, mg/g | (+)-limonene | 19.9 | 10.8 | 149.75*** |
| | (−)-limonene | 0.122 | 0.065 | 160.94*** |
| | (+)-carvone | 17.2 | 15.5 | 6.65* |
| | (−)-carvone | 0.070 | 0.084 | 13.69*** |
| | (+)-*trans*-carveol | 0.074 | 0.024 | 44.26*** |
| | (−)-*trans*-carveol | 0.061 | 0.045 | 7.29* |
| | (−)-*cis*-carveol | 0.061 | 0.025 | 58.93*** |
| | (+)-*cis*-carveol | 0.019 | 0.012 | 20.81*** |
| | total monoterpene | 37.5 | 26.6 | 67.83*** |
| ratio | carvone/limonene | 0.865 | 1.49 | 259.90*** |
| ratio, % | (−)/total limonene | 0.611 | 0.605 | 0.36[ns] |
| | (−)/total carvone | 0.406 | 0.549 | 55.92*** |
| | (−)/total *trans*-carveol | 47.1 | 63.2 | 52.27*** |
| | (−)/total *cis* carveol | 73.0 | 62.8 | 27.83*** |

[a] Numbers represent means for the three plant types, varieties, and harvest years and were calculated from the data in Table 1 using regression analysis (Genstat 5 Committee, 1988). [b] Significance, *F* values: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

oil at 0.6% (Mahran et al., 1992), but it is unclear—also from the mass spectral data provided—whether it concerned the *cis*- or *trans*-isomer or both.

**Extraction Method.** Monoterpene composition was influenced by the extraction method. In hexane extracts, limonene content was higher than in hydrodistillates and vice versa for carvone (Table 1). However, when the area percentages in Table 1 were converted to seed contents, the contents of all analyzed compounds were higher for hexane extracts than for hydrodistillates except for (−)-carvone, which was significantly higher in hydrodistillates (Table 2). As a consequence, hydrodistillation increased the (−)/total carvone ratio, whereas the (−)/total limonene ratio was not affected (Tables 1 and 2). Total monoterpene seed content was higher for hexane extraction than for hydrodistillation. Particularly, limonene content was lower in hydrodistillates, and because carvone content was less affected, hydrodistillates had a higher carvone/limonene ratio than the hexane extracts (Table 2). Recovery of hexane extraction as determined on a standard sample was 99.9% for limonene, 98.2% for carvone, and 96.4% for the carveol isomers, probably because of increasing polarity and thus solubility in the water phase. Because the seeds were ground to a fine powder, it can be assumed that these recoveries also hold for seed extraction. Recoveries of hydrodistillation when using reference terpenoids were 90, 91, 94, 85, and 84% for limonene, isobutylbenzene, methyl decanoate, carvone, and geraniol, respectively. From the distillation water 9 and 7% of weighed out carvone and geraniol and nothing of the other compounds were recovered. However, when recovery of hexane extraction is assumed to be 100%, it can be deduced from Table 2 that recovery of hydrodistillation of seed samples was only 54% for limonene, 90% for carvone, and 53% for the carveols. Koedam (1982) and Fleisher and Fleisher (1988) showed for, respectively, dill and caraway that from uncrushed seeds carvone due to its higher polarity is extracted more efficiently by distillation than is limonene, whereas from sufficiently crushed caraway seeds limonene is extracted more efficiently due to its higher volatility (Fleisher and Fleisher, 1988). Clearly, the superficial shredding of the seeds applied in the present study was not sufficient to allow a high recovery of limonene. When using uncrushed seeds as advised in the *Dutch Pharmacopoeia* (1958), the limonene recovery may be even lower. Under these conditions, essential oils extracted during the first phases of hydrodistillation are richer in carvone, and thus incomplete extraction results in essential oils rich in carvone. Such high carvone/limonene ratios are rather misleading because they suggest a high carvone content, when they actually indicate incomplete extraction of limonene (see Table 2). Many authors have investigated methods to extract and quantify essential oils from a range of species [e.g. Schönwitz et al. (1987), Charles and Simon (1990), and Muzika et al. (1990)]. The results of these studies vary and depend on the goal of the authors and on the species. Particularly when the essential oil is required without solvent, losses during evaporation of the solvent can be huge (Charles and Simon, 1990), and then distillation may be the preferred method. However, quantification of essential oil content does not necessarily require removal of the solvent, and in that case solvent extraction can be superior to distillation (Schönwitz et al., 1987). Moreover, according to Schönwitz et al. (1987), the risk of formation of rearrangement products is less when using (cold) solvent extraction. Finally, the result of an extraction method will strongly depend on the plant material. Obviously, hydrodiffusion from glandular hairs will occur more rapidly than from the essential oil ducts of mature umbelliferous seeds with a hard seed coat.

The low apparent carveol recovery for hydrodistillation and the resulting lower carveol content of hydro-

**Table 3. Contents and Ratios of Monoterpenes in Seeds of Dill and Annual and Biennial Caraway Extracted with Hexane**

| | | plant type[a] | | | significance level[b] $F^2_{46}$ |
|---|---|---|---|---|---|
| | | annual | biennial | dill | |
| content, mg/g | (+)-limonene | 15.4[c] | 21.8[d] | 19.4[d] | 14.23*** |
| | (−)-limonene | 0.093[c] | 0.129[d] | 0.161[e] | 22.82*** |
| | (+)-carvone | 13.9[c] | 18.9[d] | 14.4[c] | 14.80*** |
| | (−)-carvone | 0.055[c] | 0.077[d] | 0.063[cd] | 7.65** |
| | (+)-*trans*-carveol | 0.053[c] | 0.085[d] | 0.050[c] | 5.33** |
| | (−)-*trans*-carveol | 0.042[c] | 0.072[d] | 0.035[c] | 8.62*** |
| | (−)-*cis*-carveol | 0.039[c] | 0.074[d] | 0.013[e] | 25.68*** |
| | (+)-*cis*-carveol | 0.018[c] | 0.018[c] | 0.026[d] | 3.85* |
| | total monoterpene | 29.6[c] | 41.1[d] | 34.1[c] | 16.09*** |
| ratio | carvone/limonene | 1.03[c] | 0.82[d] | 0.69[d] | 8.24*** |
| ratio, % | (−)/total limonene | 0.598[c] | 0.586[c] | 0.876[d] | 100.71*** |
| | (−)/total carvone | 0.417[c] | 0.397[c] | 0.450[c] | 1.09[ns] |
| | (−)/total *trans*-carveol | 46.7[c] | 47.4[c] | 46.0[c] | 0.06[ns] |
| | (−)/total *cis*-carveol | 62.5[c] | 80.9[d] | 2.4[e] | 133.04*** |

[a] Numbers represent means for varieties/accessions and harvest years and were calculated from the data in Table 1 using regression analysis (Genstat 5 Committee, 1988). Means along a row are significantly different when letters differ ($P < 0.05$). [b] Significance, $F$ values: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, not significant.

**Table 4. Contents and Ratios of Monoterpenes in Seeds of Biennial Caraway Varieties/Accessions for Three Different Harvest Years Extracted with Hexane**

| | | harvest year[a] | | | significance level[b] $F^2_{21}$ |
|---|---|---|---|---|---|
| | | 1990 | 1991 | 1992 | |
| content, mg/g | (+)-limonene | 25.4[c] | 21.9[d] | 22.3[d] | 4.52* |
| | (−)-limonene | 0.136[cd] | 0.118[c] | 0.137[d] | 3.18[ns] |
| | (+)-carvone | 22.8[c] | 20.4[ce] | 18.9[d] | 3.87* |
| | (−)-carvone | 0.071[c] | 0.071[c] | 0.084[c] | 1.5[ns] |
| | (+)-*trans*-carveol | 0.061[c] | 0.072[c] | 0.112[d] | 5.29* |
| | (−)-*trans*-carveol | 0.048[c] | 0.081[d] | 0.087[d] | 5.10* |
| | (−)-*cis*-carveol | 0.056[c] | 0.054[c] | 0.096[d] | 17.43*** |
| | (+)-*cis*-carveol | 0.011[c] | 0.017[d] | 0.022[d] | 10.23*** |
| | total monoterpene | 48.5[c] | 42.7[d] | 41.7[d] | 4.12* |
| ratio | carvone/limonene | 0.85[c] | 0.94[c] | 0.88[c] | 1.46[ns] |
| ratio, % | (−)/total limonene | 0.529[c] | 0.525[c] | 0.619[d] | 11.37*** |
| | (−)/total carvone | 0.295[c] | 0.334[c] | 0.450[d] | 15.68*** |
| | (−)/total *trans*-carveol | 46.4[c] | 54.9[d] | 44.6[c] | 5.06* |
| | (−)/total *cis*-carveol | 83.7[c] | 70.6[d] | 82.8[c] | 23.44*** |

[a] Numbers represent means for the varieties/accessions and were calculated from the data in Table 1 using regression analysis (Genstat 5 Committee, 1988). Means along a row are significantly different when letters differ ($P < 0.05$) [b] Significance, $F$ values: ***, $P < 0.001$; **, $P < 0.01$; $P < 0.05$; ns, not significant.

distilled seeds (Tables 1 and 2) may be partly due to losses caused by the processing conditions during hydrodistillation (acidity, high temperature) (Koedam, 1982; Werkhoff et al., 1993). Oxidation of the (−)-carveols or of limonene to (−)-carvone under these conditions may have increased the (−)-carvone content and consequently the (−)/total carvone ratio. Oxidation of limonene also seems to occur during storage of seeds (Puschmann et al., 1992). Because hexane extraction occurred at room temperature, we consider it the milder extraction method of the two, and results from hexane extractions probably more closely represent the actual composition of the essential oil than those of hydrodistillates (Schönwitz et al., 1987). Therefore, for the analysis of plant type effect and variety/accession and harvest year effects, only the means calculated for hexane extracts [using regression analysis and the PREDICT directive of Genstat (Genstat 5 Committee, 1988)] are shown (although also the data for hydrodistillation were used for regression analysis) (Tables 3 and 4).

**Dill and Annual and Biennial Caraway.** Seeds of biennial caraway had a higher total monoterpene content than seeds of annual caraway and dill and also the highest (+)-carvone content (Table 3), but annual caraway had a higher carvone/limonene ratio. For both caraway types the (−)/total limonene ratio was about 0.60% (Table 3), whereas for dill the (−)/total limonene ratio was about 0.88%. Hener et al. (1991) also reported a higher (−)-limonene content for dill than for caraway (1% vs trace). The higher (−)/total limonene ratio for dill suggests that in dill a larger proportion of geranyl pyrophosphate is converted to (−)-limonene than in caraway or that in dill less of the (−)-limonene produced is converted to other products (Figure 2). It would be interesting to compare enantiomeric product specificity of the limonene cyclases (steps 1 and 4 in Figure 2) of dill and caraway *in vitro*. The three plant types had the same enantiomeric ratio for carvone.

**Variety.** Within biennial caraway, there was a significant variety effect on (+)-limonene content ($P = 0.005$), carvone/limonene ratio ($P = 0.003$), (−)/total *cis*-carveol ratio ($P = 0.03$), and (−)/total *trans*-carveol ($P = 0.009$) ratio (data not shown). The carvone/limonene ratios of Bleija (1.04) and Silvia (1.10) were significantly higher than for most other biennial varieties (average of 0.84), mainly because of a lower (+)-limonene content (19.8 for Bleija and 18.2 for Silvia compared with a mean of 23.0 for the other biennial varieties/accessions). Bleija and Silvia are nonshattering varieties, but the other nonshattering varieties/accessions Hungary, East Germany, and Czechoslovakia with carvone/limonene ratios of 0.83, 0.88, and 0.83, respectively, did not differ from the average of the other biennial varieties/accessions. The similar carvone/limonene ratio of Bleija and Silvia may support the alleged genetic relationship of

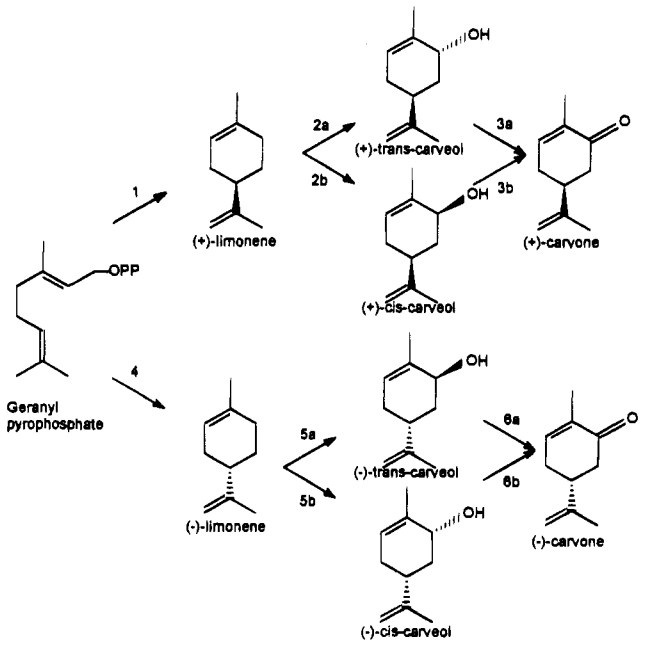


**Figure 2.** Putative pathways of carvone formation in caraway and dill seed. Steps 1–5 refer to literature cited: steps 1, 2a, and 3a, hypothesis of Bouwmeester et al. (1995); steps 2b and 3b, Karp et al. (1990); steps 4, 5a, and 6a, Gershenzon et al. (1989) for *M. spicata*; steps 5b and 6b, analogous to steps 2b and 3b.

the two varieties. The latter two varieties also had a higher (−)/total *trans*-carveol ratio than most other biennial varieties, mainly because of a higher (−)-*trans*-carveol content. It is remarkable that in wild caraway, which was propagated under agricultural conditions before analysis, the composition of the compounds analyzed here were very similar to that of the agricultural populations/varieties (Table 1). Of the monoterpene seed contents and enantiomeric ratios only the (−)/total *cis*-carveol ratio of wild caraway was significantly lower than in most other biennial accessions/varieties (data not shown) due to low (−)-*cis*- and high (+)-*cis*-carveol contents. The fact that the differences with commercial varieties are so small may be due to the lack of breeding efforts toward higher carvone and limonene contents until recently.

**Harvest Year.** Total monoterpene content in biennial varieties was highest in 1990 (Table 4), due to a higher content of both (+)-carvone and (+)-limonene. The carvone/limonene ratio did not significantly vary over these three years. For annual and biennial caraway there seems to be a correlation between assimilate availability and essential oil formation (Bouwmeester and Kuijpers, 1993; Bouwmeester et al., 1995). Differences in assimilate availability for essential oil formation and therefore differences in essential oil content may be expected between years because of variations in climatic conditions such as incident radiation. The content of all carveol isomers decreased with storage time, and (−)/total limonene and (−)/total carvone ratios were also highest in the most recently harvested (1992) seeds (Table 4). It is unclear whether this is an effect of weather conditions during seed ripening in the field or of small changes in the enantiomeric composition during storage of the seeds, but in branches of *Picea*

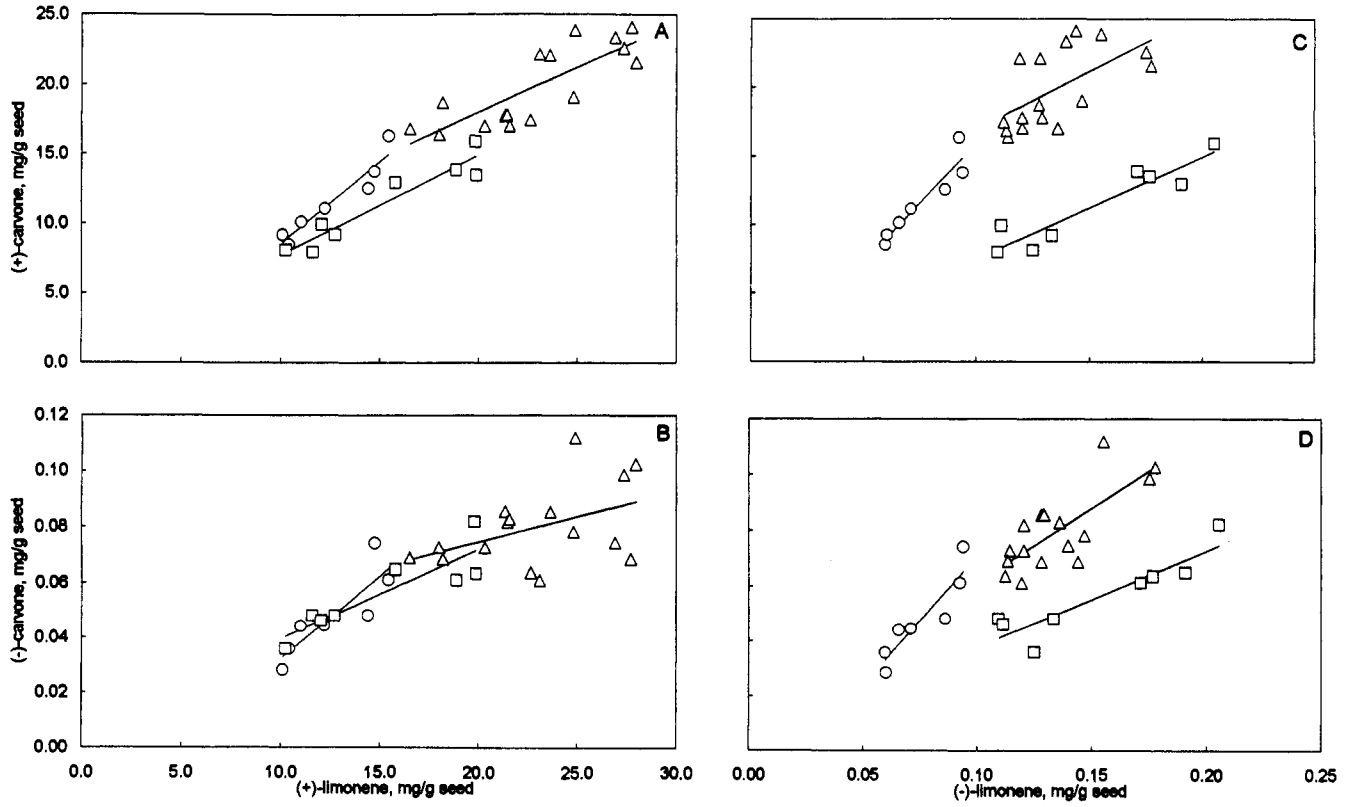


**Figure 3.** Correlations between contents of (+)-limonene and (+)-carvone (A) and (−)-carvone (B) and between contents of (−)-limonene and (+)-carvone (C) and (−)-carvone (D) in hexane extracts of seeds of dill and of annual and biennial caraway: (○) annual caraway; (△) biennial caraway; (□) dill. DF: annual caraway 5, biennial caraway 14, dill 6. Regression of $y$ on $x$: (A) (annual) $y = -1.44 + 173x$, $R^2 = 0.88$, (biennial) $y = 8.33 + 85.4x$, $R^2 = 0.38$, (dill) $y = 0.048 + 74.4x$, $R^2 = 0.87$; (B) (annual) $y = -0.023 + 0.935x$, $R^2 = 0.83$, (biennial) $y = 0.008 + 0.531x$, $R^2 = 0.58$, (dill) $y = 0.003 + 0.346x$, $R^2 = 0.82$; (C) (annual) $y = -3.18 + 1.18x$, $R^2 = 0.91$, (biennial) $y = 5.17 + 0.641x$, $R^2 = 0.66$, (dill) $y = 0.579 + 0.717x$, $R^2 = 0.91$; (D) (annual) $y = -0.027 + 0.006x$, $R^2 = 0.75$, (biennial) $y = 0.039 + 0.002x$, $R^2 = 0.20$, (dill) $y = 0.008 + 0.003x$, $R^2 = 0.79$.

*abies* the (−)/(+)-limonene ratio also decreased during storage (Persson et al., 1993), suggesting a storage effect, possibly oxidation as suggested by Puschmann et al. (1992) and Ravid et al. (1992).

**Biosynthetic Pathway.** Figure 3 shows the correlations between the contents of enantiomers of limonene and carvone in hexane extracts of dill and the two caraway types. Independent of plant type and enantiomer there is a positive correlation between limonene and carvone contents. (+)-Limonene content in dill is similar to that in annual caraway (Figure 3A,B), whereas (−)-limonene content in dill is more similar to that in biennial caraway (Figure 3C,D). The correlations between (+)-limonene content and both (+)-carvone and (−)-carvone contents are similar for the three plant types (Figure 3A,B), but the (+)-carvone/(−)-limonene and (−)-carvone/(−)-limonene ratios for dill are lower than for either caraway type (Figure 3C,D). The positive linear correlations between both limonene enantiomers and the enantiomers of carvone suggest that an increased formation of both (−)-limonene and (+)-limonene (due to a higher geranyl pyrophosphate availability or higher limonene cyclase activity) also leads to a higher production of (−)-carvone and (+)-carvone (Figure 2). For dill the higher (−)/total limonene ratio (Tables 1 and 3) does not result in an increase in (−)-carvone content (Figure 3D), suggesting that in dill less (−)-limonene is converted into (−)-carvone than in caraway. The detection of both enantiomers of *cis*- and *trans*-carveol in caraway suggests that in addition to the putative biosynthetic pathway for (+)-carvone via (+)-*trans*-carveol as postulated by Bouwmeester et al. (1995), also the pathway via (−)-*trans*-carveol and leading to (−)-carvone as reported for *M. spicata* (Gershenzon et al., 1989) may occur in caraway (Figure 2), although at a very low rate (approximately 0.5% of total carvone) (Table 1). Karp et al. (1990) reported that (−)-limonene hydroxylase extracted from *M. spicata* also hydroxylated (+)-limonene, however, with (+)-*cis*-carveol instead of (−)-*trans*-carveol as product. Possibly *cis*-carveol is an intermediate in both enantiomeric pathways (Figure 2). According to Karp et al. (1987, 1990) the enzymes involved in the hydroxylation of monoterpene hydrocarbons have a high substrate specificity but not a high enantioselectivity, implying that the same enzyme can catalyze the hydroxylation of (+)- and (−)-limonene and—if dehydrogenation also occurs with low enantioselectivity—that also (+)- and (−)-carvone will be produced. It seems therefore that enantioselectivity in monoterpene formation is necessarily determined in the first step(s) of the pathway. Indeed, cyclases have been reported to highly specifically convert geranyl pyrophosphate to only one enantiomer (Alonso et al., 1992), and it has been suggested that separate cyclases are responsible for the formation of enantiomers (Alonso and Croteau, 1993). Possibly in dill the putative (−)-limonene cyclase is more active than in caraway, thus causing the higher (−)/total limonene ratio for dill (Tables 1 and 3). Future studies on the pathway and the enzymology of carvone formation in caraway and dill should elucidate whether the pathways shown in Figure 2 do occur.

**Conclusions.** The enantiomeric compositions of dill and annual and biennial caraway seed essential oils were quite similar, but dill had a higher (−)/total limonene ratio than annual and biennial caraway and contained no (−)-*cis*-carveol. Hexane extraction is preferred for a precise determination of monoterpene composition and contents in caraway and dill seed. Apart from total monoterpene content there were only small differences in essential oil composition between harvest years. The presence of both enantiomers of *cis*- and *trans*-carveol in caraway suggests that in addition to the putative biosynthetic pathway for (+)-carvone via (+)-*trans*-carveol, also the pathway via (−)-*trans*-carveol or (−)-*cis*-carveol and leading to (−)-carvone may occur in caraway (Figure 2). The differences between varieties of biennial caraway were small. Dill and annual and biennial caraway seem equally suited as a source of carvone to be used for sprouting inhibition in potatoes, as the (−)/total carvone ratios are equal.

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