# COMMUNICATIONS

## A Distillation Technique for Isolation of Volatile Materials for Gas Chromatographic Analysis and Its Application to Coriander Seed (*Coriandrum sativum*)

A gas chromatographic apparatus has been used as a technical aid for isolating the volatile materials from food or other products by purging the product with nitrogen at an elevated temperature and condensing the volatiles in a cold trap. The sample is contained in a tube in the column oven of the gas chromatograph which provides controlled heat and nitrogen flow to the sample. The essential oil of coriander seed (*Coriandrum sativum*) was collected by this method and analyzed quantitatively for 13 (unidentified) components by gas chromatography. Using 18 replicate seed samples and distillation temperatures of 150 and 200° C, the precision of the collection method and analysis was estimated by analysis of variance.

Various techniques have been reported (Angelini *et al.*, 1967; Hornstein and Crowe, 1962; Mattick and Hand, 1969) for the isolation of volatiles from food or other materials, usually for the purpose of gas chromatographic investigation. The techniques include steam distillation, vacuum distillation, high-vacuum degassing, flushing with an inert gas, sampling of headspace vapors, and solvent extraction. The precision of these methods, however, has not usually been considered.

This paper describes a simple technique for distilling and flushing the volatiles from a sample with an inert gas. It utilizes a gas chromatograph, which allows precise control of the experimental conditions. The method has been applied to the isolation of coriander seed volatiles, with subsequent quantitative analysis of the volatiles by gas chromatography. The precision of the method has been assessed.

### EXPERIMENTAL

**Apparatus.** A copper cylinder (16.5 cm × 3.8 cm o.d.) was used as sample container (Figure 1). The ends of the cylinder were tapered and connected with Swagelok fittings to two pieces of copper tubing (20 cm × 6.4 mm o.d.). One of these tubes was extended by Swagelok coupling to a narrower tube (23 cm × 3.2 mm o.d.). This assembly was installed in the oven of a gas chromatograph (Varian Aerograph 1800 Series) which had two injection ports (a and b, Figure 1). The 6.4-mm tube was connected to injector "b." The 3.2-mm tube was passed through injector "a" and connected with a U-tube condensation trap outside the oven. The trap was immersed in ice water. Nitrogen was filtered through a molecular sieve and supplied through injector "b" as sweep gas.

**Isolation Procedure.** The sample container was filled with a weighed sample of coarsely ground coriander seed (*Coriandrum sativum*), approximately 65 g. The nitrogen flow rate, measured at the exit arm of the trap, was adjusted with the instrument flow control to 10 ml/min. Injector "b" (the inlet) was maintained at room temperature. Injector "a" (the outlet) was maintained at 215° C to prevent condensation of volatiles in the tube before the cold trap. The oven temperature was raised to the desired distillation temperature (150 or 200° C) and the sample was purged with nitrogen at this temperature for 2 hr. The distillate displayed an aqueous and an oily phase; these were separated and stored at −15° C until analyzed. Eighteen replicate samples of coriander seed were distilled in this way, nine at 150° C and nine at 200° C.

**Gas Chromatography.** After removing the sample container, the same gas chromatograph (equipped with a hydrogen flame detector) was used for analysis of the coriander distillates. A single column was used, 3 m × 4.8 mm i.d., copper, packed with Carbowax 20M on acid-washed Chromosorb W, 60–80 mesh (1:10 by wt). The temperatures were as follows: injection block, 215° C; detector block, 250° C; column, held at 45° C for 8 min, increased at 4° C/min to 200° C, and held at 200° C to end of chromatogram. The flow rates for nitrogen, hydrogen, and air were 40, 40, and 400 ml/min, respectively. Samples of 300 and 0.4 μl of the aqueous and oily phases, respectively, were injected for analysis. Peak areas were measured with an electronic integrator (Infotronics, CRS-100). The percentage composition of analyzed samples was calculated from the peak areas without use of detector response factors. Eight replicate chromatograms were run for each of the 18 collected samples of oil.

**Statistical Analysis.** Analyses of variance for nested classifications were performed on the 72 observations for each of 13 peaks at each temperature. Total variation was partitioned into that due to average differences among nine samples of oil and into that due to differences among chromatograms of the same oil sample. Differences in peak composition at the two temperatures were assessed by means of unpaired t tests.

### RESULTS AND DISCUSSION

A simple device has been described for the isolation of volatile materials present in food or other products. Essentially, the sample material is placed in an empty column in a gas chromatographic oven and purged at an appropriate temperature with the carrier gas. The effluent from the column is collected directly in a cold trap. The method permits the collection of liquid distillates, which are convenient to store and to handle. It provides good control of gas flow rate and temperature; temperature programming may be

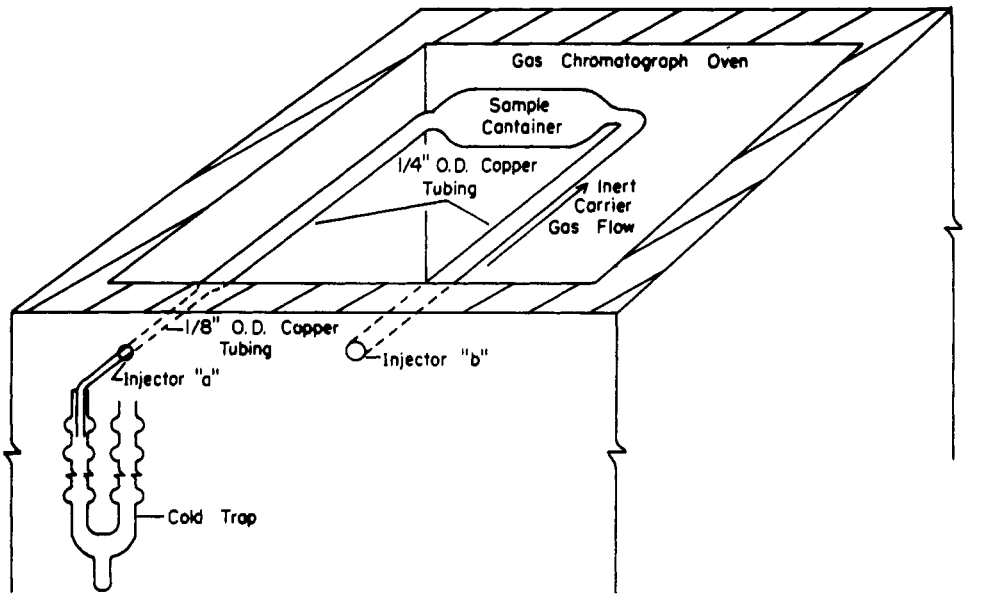


**Figure 1. Diagram of apparatus for isolation of volatile materials**

used if desired. The sample container may have any shape and volume and may be made of glass to reduce possible catalytic decomposition of the contained materials.

The method has been used in this laboratory for the collection of the volatile essential oil from coriander seed. Furthermore, the precision of the method of collection and of subsequent gas chromatographic analysis of the collected oils has been assessed, as this was required in a program of plant breeding for development of different compositions and flavors of the essential oil of coriander. Eighteen replicate seed samples were distilled, nine of these at 150° C and nine at 200° C. The yields of the collected distillates (Table I) were higher at 200 than at 150° C. The distillates consisted of mainly water and a small amount of essential oil. Coriander seed normally contains 0.5 to 1% essential oil; the moisture content can vary considerably. The seed also contains a substantial amount of triglyceride oil which, however, is not removed from the seed by the present distillation procedure.

Gas chromatographic analyses of the collected distillates revealed 57 components in the aqueous phases and 29 in the oil phases. The components were not identified. Some of them could possibly have been artifacts produced by the effect of heat on glucose or other substances in the seed (Fagerson, 1969). The aqueous material was not further investigated.

In order to assess the precision of analysis, eight replicate chromatographic analyses were made for each of the 18 samples of oil collected, *i.e.*, 72 chromatograms for the nine samples collected at 150° C and 72 chromatograms for the nine samples collected at 200° C. Thirteen of the 29 components were estimated quantitatively; the other 16 components were present only in trace amounts. Means of the 72 analyses at each temperature are presented in Tables II and III. Peaks 1, 6, 7, 8, and 9 account for approximately 90% of the total area in both cases. The relative errors involved in the collection of the oil and in the chromatographic analysis of each oil sample are indicated by the corresponding components of variance (Tables II and III). It is evident that the variation among oil samples due to the collection procedure is generally smaller than the variation among chromatograms of the same sample of oil. Thus, the distillation technique described appears to be a relatively precise method of isolating the volatile material.

Any attempt to alter the composition of the essential oils of coriander seed through plant breeding would require analysis of many samples of oil. It is likely that only one sample of oil would be collected from each sample of seed and that only one chromatogram would be produced for each oil sample. In such a program, it would be necessary to know the error variability in a single determination. Tables

**Table I. Yield of Distillate from Coriander Seed**

| Distillation temperature | Yield (%)[a] |
|---|---|
| 150° C | 4.3 ± 0.4 |
| 200° C | 7.3 ± 0.4 |

[a] Distillate as percent by weight of the seed; mean ± standard deviation for nine replicate seed samples.

**Table II. Composition of Coriander Essential Oil Collected by Distillation at 150° C**

| Peak number | Composition,[a] % | Component of variance | | Standard deviation of single determination |
|---|---|---|---|---|
| | | Among oil samples | Among chromatograms | |
| 1 | 11.0 | 0.67 | 0.71 | 1.17 |
| 2 | 1.6 | 0.04 | 0.04 | 0.29 |
| 3 | 0.9 | 0.00 | 0.02 | 0.14 |
| 4 | 0.8 | 0.01 | 0.03 | 0.20 |
| 5 | 1.9 | 0.03 | 0.04 | 0.25 |
| 6 | 3.5 | 0.04 | 0.08 | 0.34 |
| 7 | 12.1 | 0.25 | 0.61 | 0.93 |
| 8 | 7.1 | 0.15 | 0.75 | 0.95 |
| 9 | 56.9 | 8.51 | 9.91 | 4.29 |
| 10 | 0.9 | 0.00 | 0.19 | 0.43 |
| 11 | 0.9 | 0.01 | 0.08 | 0.29 |
| 12 | 1.7 | 0.28 | 0.14 | 0.64 |
| 13 | 0.8 | 0.06 | 0.03 | 0.30 |

[a] Peak areas in percent; mean of 72 analyses.

Table III. Composition of Coriander Essential Oil Collected by Distillation at 200° C

| Peak number | Composition,[a] % | Component of variance: Among oil samples | Component of variance: Among chromatograms | Standard deviation of single determination |
|---|---|---|---|---|
| 1 | 9.3 | 0.79 | 0.65 | 1.20 |
| 2 | 1.4 | 0.02 | 0.02 | 0.22 |
| 3 | 0.8 | 0.00 | 0.01 | 0.11 |
| 4 | 0.7 | 0.00 | 0.03 | 0.17 |
| 5 | 2.0 | 0.05 | 0.03 | 0.28 |
| 6 | 3.4 | 0.04 | 0.07 | 0.32 |
| 7 | 11.4 | 0.13 | 0.59 | 0.85 |
| 8 | 6.9 | 0.05 | 0.55 | 0.78 |
| 9 | 58.4 | 1.81 | 8.30 | 3.18 |
| 10 | 1.1 | 0.00 | 0.28 | 0.53 |
| 11 | 1.3 | 0.01 | 0.24 | 0.50 |
| 12 | 2.2 | 0.35 | 0.25 | 0.78 |
| 13 | 1.1 | 0.04 | 0.09 | 0.37 |

[a] Peak areas in percent; mean of 72 analyses.

II and III include estimates of the standard deviation of single determinations for this purpose. Considering that one would be able to detect differences equal to about twice the standard deviation of a single measurement, the method outlined should be able to detect differences of approximately 2.4% for peak 1, 0.6% for peak 2, etc. These results indicate that the outlined method of oil collection and chromatography should be sufficiently precise to be used for the screening of plant lines for oil composition.

Of the 13 components studied quantitatively, three (peaks 10, 11, and 13) showed significantly greater relative area in the 200° C samples than in the 150° C samples. These increases were accompanied by significant decreases in peaks 1, 2, 4, and 7. These differences, which might reasonably be expected to occur with distillation of the present material of wide boiling point range, serve to emphasize the importance of precise temperature control in the distillation procedure.

While analytical errors associated with gas chromatographic analyses have been frequently reported in the literature, there is little published information on the errors associated with the preparation of an extract or distillate of volatile components from food products. The determination of this latter type of error in the present work indicates that a satisfactory precision can be attained in such experiments.

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*Received for review April 2, 1971. Accepted June 1, 1971. The work was supported by the Distillers Corporation Limited and the National Research Council of Canada. Contribution No. 227, Department of Plant Science, University of Manitoba.* [1] *Canada Department of Agriculture, Research Station, Winnipeg 19, Manitoba, Canada.*