phenols (e.g., alkaloids derived from tyrosine) by utilization of microparticle reverse phase columns. This has been recently demonstrated in a study of phenolic constituents in *Theobroma cacao* (Kenyhercz and Kissinger, 1977).

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A Convenient Method for Multiple Extraction of Volatile Flavor Components from Food Slurries and Pulps Using a Two-Chambered Glass Bomb Extractor and Dichlorodifluoromethane (Freon 12) Solvent

A two-chambered glass "bomb" apparatus has been designed for multiple extraction of volatile flavor components from food slurries and pulps. The solvent, dichlorodifluoromethane (Freon 12) is low boiling (−29 °C) and relatively inert and at no time during extraction or concentration process is the food, solvent, or extract warmed above room temperature. Using this method, extracts of good reproducibility and high organoleptic quality from various fruit pulps have been obtained.

Among the most important tasks facing a researcher or technologist in a flavor study is obtaining a representative and organoleptically true sample of volatile components for investigation. A poor initial flavor extract usually leads to difficult interpretations or erroneous results and conclusions at subsequent stages of the work. The task is made more difficult by the varying nature of food products. Such factors as high or low water content, lipid content or solids content, and stability or lack of it with heat or other external conditions have brought about numerous procedures for obtaining flavor extracts. The usual methods involve some combination of distillation or stripping and extraction by solvent. One must always consider the possibility of contamination or artifact formation in the presence of a reagent or external factors such as heat.

Fluorocarbons have been used by many workers as extraction solvents, primarily for alcoholic beverages, fruit essences, and vegetables (for examples see Stanley et al., 1963; Schiede and Bauer, 1967; Schultz et al., 1967; Hardy, 1969; Stevens et al., 1969). These solvents have been shown to be very good for this purpose because they are relatively inert, nontoxic, nonexplosive, noninflammable, and in most cases, give an extract of very high organoleptic quality. The most commonly used fluorocarbon has been Freon 11 (bp 23.7 °C), primarily because of ease of handling at room temperature. Schultz et al. (1967) has indicated that Freon 114 (bp 4 °C) is a preferable solvent since the lower boiling point allows easier solvent stripping. In our laboratories, partially freeze-dried fruit puree and fruit skins standing at −30 °C in Freon 12 (bp −29 °C) have yielded good extracts (Ballschmieter and Torline, 1973; Torline and Ballschmieter, 1973).

We would like to report here a simple, easily constructed, and inexpensive glass bomb apparatus which allows convenient and relatively rapid extraction of volatile components from aqueous food slurries and pulps using the low-boiling Freon 12 as solvent.

EXPERIMENTAL SECTION

Extractor Design. A diagram of the extraction bomb appears in Figure 1 and is self-explanatory. The construction is entirely of heavy-walled Pyrex glass and the upper tube is sealed with a pressure-tight screw down Teflon plug.

Extraction Procedure. The aqueous food pulp or slurry (300 mL) is placed in the lower chamber by means of a long-stem funnel. The entire bomb is then cooled at −30 °C in a cold room and 300 mL of Freon 12 is added. The system is sealed, placed in a protective steel-mesh

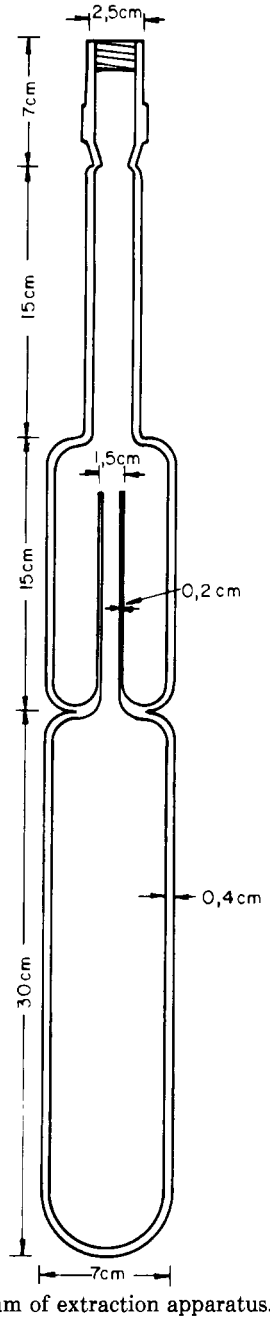

Figure 1. Diagram of extraction apparatus.

shield, and allowed to warm to room temperature. The Freon remains liquid inside the apparatus. No unusual pressure hazards have been encountered and no special safety precautions other than a steel-mesh tubular housing for the bomb have been necessary. The bomb is placed at a 45° angle and the aqueous Freon mixture is mechanically shaken in the lower chamber for 3 h. The system is cooled again to −30 °C, causing the Freon to separate from the frozen pulp. The solvent may be then conveniently decanted directly from the solid pulp into the upper chamber. The lower chamber is maintained at −20 to −30 °C in a bath while the upper chamber is allowed to gradually warm to room temperature. This causes the Freon to distill back onto the pulp for another extraction. The process may be repeated as often as necessary for a very efficient extraction of a small amount of sample.

When the extraction process has been completed, the Freon-essence mixture is transferred in successive 50-mL portions to a concentrating flask (see Figure 2) with a 10-cm fractionating column attached. The flask is maintained at −20 °C in a bath while the solvent slowly evaporates. A drying tube or small plug of glass wool at the exit is helpful to prevent condensation of water into the sample. Since Freon does not tend to extract waxes, etc., the volatile extracts are usually ready for analysis at this stage. If, however, nonvolatile materials are present, they may be separated from the volatiles by low-temperature vacuum transfer. A 300-mL sample of fruit pulp has normally yielded 20 to 50 μL of solvent-free extract.

RESULTS AND DISCUSSION

We have recently completed a comparative study on the volatile GC profile patterns of irradiated and nonirradiated mango, strawberry, and papaya fruit pulps. The methods used to obtain suitable samples for GC analysis included head-space concentration, vacuum distillation and freeze-drying techniques, solvent extractions with methylene chloride, pentane, pentane–ether, ether, and the Freon 12 procedure above. We were often impeded in our work by the consistency of the pulps, the high water content, and the formation of emulsions. The Freon extraction procedure was clearly the most convenient and fastest method of obtaining suitable extracts. The emulsion problems were essentially eliminated and sensory evaluations showed the Freon extracts to be organoleptically superior to the other extracts by comparison to the fresh fruit aroma. Chromatographic analysis using glass capillary columns (Blakesley and Torline, 1975) allowed us to compare quantitatively 137 mango volatiles, 85 papaya volatiles, and 124 strawberry volatiles.

An alternate procedure using an ordinary single-chambered glass bomb sealed in a flame has also provided reasonably good extracts. However, the inconvenience of opening, emptying, refilling, and resealing for reextraction and the possibility of causing glass strain or weak points on sealing make the two-chambered system described here preferable.

The possibility of artifact formation is minimal since the inert solvent, extract, and food are in a single all-glass unit (no joints, stopcocks, grease, etc.), and at no time is the system subjected to higher than room temperature. The system is also nearly air-free since the air inside the bomb is displaced by Freon vapor prior to sealing. Because the extractions only require the attention of a technician for short periods (filling, transfer to or from shaker, etc.), it

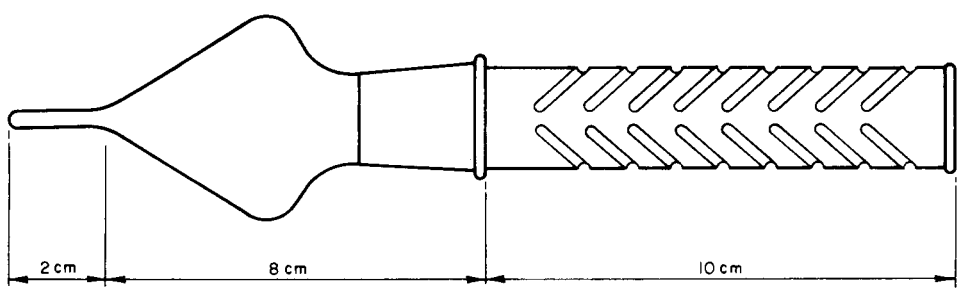

Figure 2. Diagram of concentrating flask.

is convenient to run these extractions simultaneously with other work.

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Identification of Benzene as a Volatile Metabolite of *p*-Toluic Acid Phenylhydrazide (TAPH)

Benzene was characterized as a volatile metabolite of *p*-toluic acid phenylhydrazide (TAPH) in the rat. The relationship of benzene as the volatile metabolite of TAPH and the phenyl groups bound to heme and globin from the treatment of sheep with *p*-toluoyl chloride phenylhydrazone (Jaglan et al., *J. Agric. Food Chem.* **24**, 659, 1976) is discussed.

In a recent article (Jaglan et al., 1976), we described the disposition of anthelmintic *p*-toluoyl chloride [^{14}C]-phenylhydrazone (TCPH) in sheep. We found that only the phenylhydrazine part of the molecule was responsible for the retention of radioactivity in blood. The radioactivity was selectively localized in erythrocytes and specifically and covalently bound to hemoglobin.

Under aqueous conditions, TCPH hydrolyzes to form *p*-toluic acid phenylhydrazide (TAPH). When sheep were treated with an equivalent dose of TAPH (uniformly ring-labeled phenylhydrazine), seven to ten times higher blood residues as compared with TCPH were observed (Jaglan et al., 1973). The blood radioactivity was again selectively localized in erythrocytes and covalently bound to hemoglobin, but the accountability of the dose in feces and urine was only about 60% as compared with 90% from TCPH. This indicated that some volatile metabolite had been lost. Further studies were done in rats in order to conserve [^{14}C]TAPH as well as to reduce handling problems.

Blood residue patterns in rats were found to be similar to that observed in sheep from TCPH or TAPH treatments. We report here the characterization of benzene as a volatile metabolite of TAPH in the rat. The probable relationship of benzene identified in this study to the phenyl group bound to hemoglobin from TCPH is discussed. This is the first recorded example of the formation of benzene from a hydrazide in vivo.

EXPERIMENTAL SECTION

A Sprague-Dawley male rat weighing 241 g was given a single oral dose of [^{14}C]TAPH (20.9 mg of uniformly ring-labeled phenylhydrazine containing 10.4×10^6 DPM, 51.8 μCi/mmol) and secured in a metabolism cage (Aerospace Ind., Garnerville, N.Y.). The cage was attached to a series of traps containing, in order, 50 mL of sulfuric acid (3 N), 50 mL of methanol, 50 mL of methanol cooled in dry ice–ethanol, and 500 mL of phenethylamine (2 N in methanol). Polyethylene tubing was used for all the connections. The system was connected to a vacuum line and the air flow adjusted to 60 mL/min. The traps were removed 24 h after treatment and aliquots from each trap were counted. Feces, urine, and cage wash were counted as described before (Jaglan et al., 1976).

The rat was killed by cervical dislocation and the radioactivity in the whole skinned animal containing all the tissues, blood, and gastrointestinal tract was determined. The carcass was homogenized in four volumes of water with a Polytron homogenizer for 10 min, then aliquots of this homogenate combusted and counted as described for feces.

The contents of the methanol traps, which contained most of the volatile radioactivity, were combined, and 5 mL of 1 N sodium hydroxide was added to convert any phenols and acids present to their respective sodium salts. The solution was then distilled at 50 °C under water aspirator vacuum; the distillate was collected in a cooled (ice bath) receiving flask. About 85% of radioactivity distilled into the receiver. Half of the distillate (6×10^5 DPM) was diluted with 20 mL of benzene and 300 mL of pentane, then shaken with 700 mL of water for 30 s in a separatory funnel, and the aqueous phase was discarded. The organic phase was washed with 200 mL of saturated sodium chloride solution, then dried over anhydrous sodium sulfate. The dried solution was distilled through a glass column, 280 mm long and 18 mm wide packed with 4-mm glass beads, until all the pentane was removed. The residual benzene solution was derivatized to benzophenone by the classical Friedel-Crafts acylation. The benzophenone was recrystallized several times from ethanol–water to a constant specific activity. Three aliquots of the benzophenone were weighed (23.8, 28.5, 23.7 mg) into