COMMUNICATIONS

Separation of Components of Aroma Concentrates on the Basis of Functional Group and Aroma Quality

A technique is described which facilitates identification of the individual aroma-bearing constituents in complex aroma concentrates. Fractionation by functional group class (and to some extent by molecular weight within classes) is accomplished by elution from silica gel at 0° with Freon or ether in Freon. Fractions with characteristic aroma are located by sniffing after the evaporation of most of the solvent. Identification by glc-mass spectrometry or other means can then be concentrated on aroma-bearing fractions which contain relatively few components of the same functional class. The preliminary separation can be accomplished on an analytical or preparative scale and the elution conditions are easily modified to achieve the desired degree of separation for a particular aroma concentrate. Results obtained with bananas, coffee, and cheese are presented.

The volatile fraction from most foods is an exceedingly complex mixture. Preliminary fractionation of the complex mixture can greatly simplify subsequent identification. Murray and Stanley (1968) have described such a preliminary separation into functional group classes by chromatography on silica gel at 0°. Application of this technique to aroma concentrates from bananas (Murray *et al.*, 1968a) and peas (Murray *et al.*, 1968b) facilitated identifications by combined gas chromatography–mass spectrometry. Murray *et al.* (1972) have recently described a micro-modification for rapid fractionation of 1 μl of flavor essence.

The present paper describes modifications of these procedures which make it possible to fractionate volatile concentrates both by functional group class and on the basis of the aroma quality of the fractions.

EXPERIMENTAL SECTION

Silica Gel Columns. *Operating Procedures.* Silica gel (Merck, grade HR, for thin-layer chromatography) was dried in a vacuum oven at 60° and stored in a desiccator. The 10 mm i.d. × 150 mm columns used in most of the studies were prepared (at 0–4° to prevent bubble formation) by intimately mixing 3 g of silica gel with 1 ml of water, making a thin suspension of this material in about 25 ml of Freon 11 (trichlorofluoromethane, bp 23.7°, Matheson Gas Products), and pouring this mixture into a column closed at the bottom with a fritted disk. The gel was allowed to settle and washed with 10 ml of cold Freon. Preparative columns of 20 mm i.d. × 180 mm were prepared similarly from 22.5 g of silica gel.

Samples were introduced by allowing the Freon level to drop to near the surface of the column, carefully pipetting the sample onto the surface, and then washing in with several small volumes of Freon. About 5 ml of Freon was then pipetted onto the top of the column and the components were eluted with Freon or ether–Freon mixtures. The head of eluting solvent was adjusted to give a flow rate of about 0.2 ml/min for 10-mm i.d. columns and about 1 ml/min for 20 mm-i.d. columns.

Elution was performed at 0–4°, which minimized losses by volatilization or chemical reactions. The outlet tip of the column was passed through an aluminum foil cover on the top of the collecting chamber of a Volumeter 400 (Instrumentation Specialties Co., Lincoln, Neb.), thus delivering into a solvent-saturated atmosphere. This is essential to prevent rapid evaporation of the emerging Freon and concomitant losses of eluted volatile constituents. When the desired volume (usually 1 ml) was reached (predetermined by setting the position of a thermistor sensor on the Volumeter), a stopcock at the bottom of the collecting chamber opened and the fraction was delivered rapidly into a collector tube. The sensor also triggered advance of the collector turntable.

The fractions were evaporated to about 0.1 ml at 4° under a weak stream of nitrogen, followed by standing at 4° to allow the remainder of the solvent to evaporate. The remaining volatile constituents were redissolved in 10–50 μl of acetone for injection into the gas chromatograph. (Freon 11 can not be injected repeatedly as it causes deterioration of the flame detector head, presumably *via* partial conversion to hydrogen fluoride.)

The evaporation procedure required about 2 hr and the recoveries were 45–53% for lower boiling volatiles and 63–74% for higher boiling volatiles. Higher recoveries (83–92%) were obtained by omitting the nitrogen stream, but evaporation then required 10 to 15 hr and the recoveries dropped sharply if the individual tubes were not closed promptly on completion of evaporation. Other procedures (evaporation to "dryness" at 4° with nitrogen or evaporation at 25°) reduced the time required, but recoveries were exceedingly variable (0–93%).

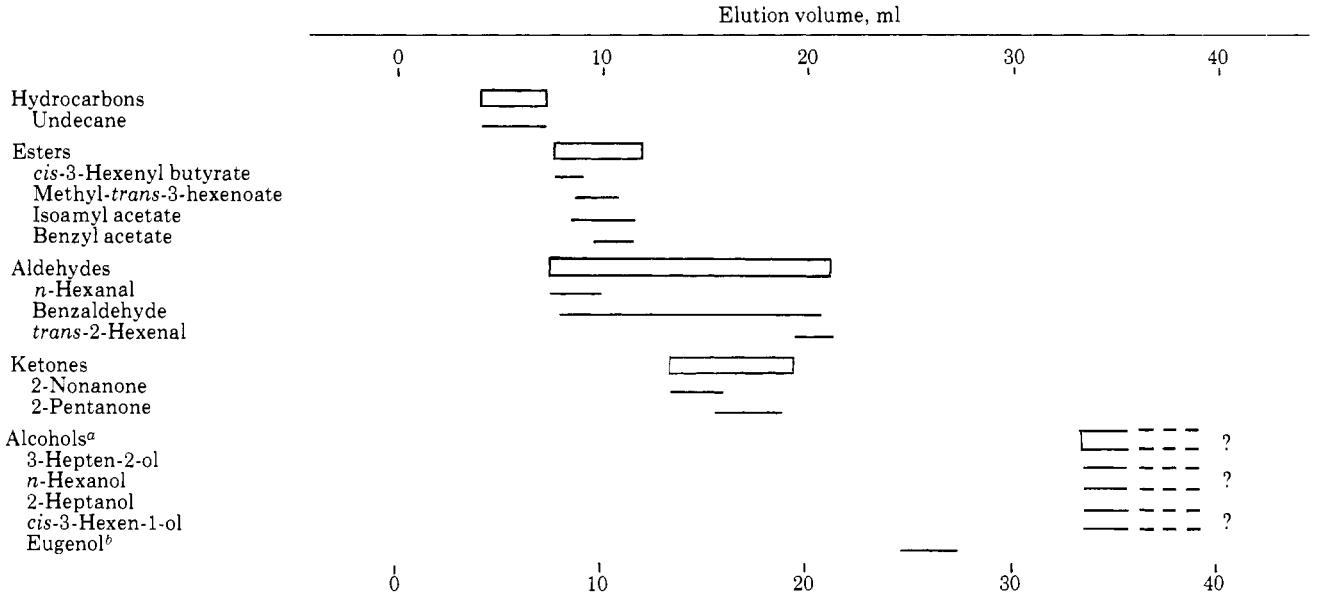
Gas Chromatography. Varian Aerograph 1200; flame ionization detector; column oven temperature programmed 60° to 170° at 1°/min; carrier gas, helium, 8 ml/min. Columns: 50 ft × 0.02 in. i.d. stainless steel, support coated with Carbowax 1540 or 4000 for separation of test mixtures; 500 ft × 0.02 in. i.d. stainless steel, support coated with SF 96(50) for separation of banana concentrates.

Identifications were made from Kovats Indices and by combined gas chromatography–mass spectrometry, as described by Quast (1970).

Aroma Evaluations. After evaporation of most of the eluting solvent, the fractions were evaluated by a panel of four to eight people, experienced in aroma evaluation of food samples.

There was excellent agreement in locating fractions containing aroma-bearing components, but often considerable disagreement as to the quality of the aroma. Presence of considerable quantities of Freon in the fractions had little effect on the evaluations, but traces of ether (particularly in the relatively odorless alcohol fractions) sometimes made evaluation difficult.

Test Mixtures. Several mixtures were separated; the results for the most complex mixture are presented. Re-



[a] Only 34 fractions collected. [b] Included as representative of higher boiling aroma-bearing constituents present in banana aroma concentrates.

Figure 1. Separation of test mixture by elution with Freon and 20% ether in Freon. Column, 1 × 15 cm; flow rate, 0.2 ml/min; sample 50-μl test mixture. Eluted with Freon 11 through fraction 20, 20% ether in Freon thereafter; fraction volume, 1 ml.

Table I. Class and Aroma Separation of 50 μl of a Banana Aroma Concentrate (Conditions as in Figure 1)

Fractions	Odor description	Components identified[a]
10, 11, 12	Ripe banana	A series of about 20 saturated acetates, propionates, and butyrates, plus *n*-hexanal
13, 14	Green banana	Generally same as fractions 10–12 plus additional acetates of C_6, C_7, and C_8 saturated and unsaturated alcohols; benzyl acetate, phenethyl acetate, *trans*-2-hexenal, *cis*-2-hexenal
15, 16, 17	Fresh green	*trans*-2-Hexenal, 2-heptanone, 2-methylbutyl acetate[b]
19, 20, 21	Green, burnt	Not analyzed
40–43	Green, fresh-cut grass	Not analyzed, probably isomeric hexenols described earlier (Murray *et al.*, 1968a)
54	Alcohol	2-Methyl propanol, 1-butanol, 2-pentanol, 3-methyl butanol

[a] By combined gas chromatography–mass spectrometry (Quast, 1970). [b] Fraction 15 only analyzed.

Table II. Aroma-Bearing Fractions from Separation of Coffee Oil (Conditions as in Figure 1). Significant Aroma Detected Only in Fractions Listed

Fraction	Odor description	Strength
10	Unpleasant, sauerkraut, skunk cabbage, sulfur	Moderate to strong
18	Unpleasant, sweat, wet paper	Weak
21	Pleasant, chocolate, coffee-like, cinnamon	Weak to moderate
22	Pleasant, burnt chocolate, coffee-like, rubbery	Moderate
27	Pleasant–unpleasant,[a] stale cheese, medicinal, woody, caramel, coffee-like	Strong
28	Pleasant–unpleasant,[a] green, minty, coffee-like, medicinal	Moderate
31	Pleasant–unpleasant,[a] medicinal, metallic, caramel, coconut	Weak to moderate
32	Pleasant, coffee, coconut, burnt, rubbery	Weak
Coffee oil, 1:20 dilution in water	Stale coffee, strong coffee, medicinal, burnt, bitter	Strong

[a] Panel members disagreed on pleasantness.

tention times and detector response to each component were established by gas chromatography of appropriate dilutions (in acetone) of individual components and/or the test mixtures.

Aroma Concentrates. The banana sample was a concentrated ether extract of the aqueous essence collected in a liquid nitrogen trap during lyophilization of homogenized pulp from ripe bananas (Quast, 1970). Coffee aroma concentrate was kindly supplied by Coca Cola Co., Atlanta, Ga. The Blue Cheese concentrate was prepared by centrifugation and vacuum distillation of the fatty layer, essentially as described by Day (1967).

RESULTS

Separation of Known Mixtures. Figure 1 shows a typical class separation of the test mixture. Hydrocarbons and alcohols are easily separated from esters and carbonyls. Esters and carbonyls tend to overlap, but even here elution sequences (including gradients) can usually be devised so that a particular functional group class (or often an individual component) predominates in certain fractions. For example, ketones predominate in fractions 15 to 19, as do esters in fractions 8 and 9 in Figure 1. Also, there is some separation by chain length within each functional class, such as nonanone from pentanone. Certain specific separations within classes are also apparent, such as the separation of *n*-hexanal from *trans*-2-hexenal.

The separations method was readily scaled up to obtain a similar separation of 400 μl of this same mixture on a 20-mm i.d. preparative column, eluting with 60 ml of Freon, followed by 20% ether in Freon.

Table III. Aroma-Bearing Fractions from Separation of Aroma Concentrate from Blue Cheese (Conditions as in Figure 1 and Table II)

Fraction	Odor description	Strength
10	Sweet, ether-like	Faint, fleeting
18	Sweet, ether-like, mucilage	Faint to no response
21	Ether-like, medicinal	Faint
28	Pleasant, Blue Cheese, paint, oily	Strong
31	Unpleasant, rotten cheese, musty, dirty socks	Strong

These results indicate the capability and versatility of the descending silica gel method for simplifying complex mixtures of volatile constituents. But equally important, the method as described provides an easy means for locating those components which contribute to a particular aroma. For example, in separations of the test mixtures, the ester-containing fractions were located by simply "sniffing" the fractions after evaporation of the Freon. Since Freon 11 is virtually odorless, the peak ester fractions could often be located even before evaporation. Sniffing of Freon 11 presents no toxicological problems since it is "less toxic than CO_2" (Merck Index, 1960).

Application to Aroma Concentrates from Foods. *Bananas.* Table I summarizes a separation of a banana aroma concentrate. The characteristic aroma notes of the banana fruit were detected in relatively few fractions. Identification of a representative group of compounds in each fraction indicated the expected separation by functional group class as well as some separation of saturated from unsaturated components within the classes.

Quast (1970) has scaled up this separation considerably and reports further details on the separation and identification of volatile constituents present in ripe bananas, particularly those components occurring in fractions with typical banana aroma.

Coffee. Table II summarizes the results of separation of a coffee aroma concentrate. The oil appeared to have deteriorated somewhat during transport, as a black residue had settled out. A 1:20 dilution of the oil in the water was generally characterized as a strong or stale coffee aroma.

Identification of the components in aroma-bearing fractions of Table II was not attempted, but it is clear that separation of some typical flavor "notes" of coffee was achieved.

Blue Cheese. When Blue Cheese aroma concentrate was separated, the panel members were again able to locate several aroma-bearing fractions (Table III).

DISCUSSION

The procedure described in this report has proven exceptionally useful in our laboratories, both for routine separations of the constituents of aroma concentrates by functional class and for seeking sensory correlations. Our general approach now is to first separate crude aroma concentrates on silica gel and then to concentrate identification efforts (*via* combined glc–mass spectrometry) on those fractions with typical aroma notes. Since the selected fractions normally contain relatively few components of the same functional class, identification generally presents few problems. Further studies of the aroma impact of individual components can then be carried out with pure samples. Where particular fractions are too complex, the silica gel separation is easily repeated with appropriate adjustment of elution conditions.

The procedure has been used primarily with fruit flavors (Table I; Quast, 1970), but the results with coffee and Blue Cheese (Tables II and III) suggest general applicability.

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Potential Juvenile Hormone Activity: Preparation of Fatty β-Methylcrotonyl and 3,4-Methylenedioxyphenyl Derivatives

Certain compounds with β-methylcrotonyl and 3,4-methylenedioxyphenyl groups are known to possess juvenile hormone activity because they prevent insect maturation and reproduction. A series of compounds containing these groups has been prepared from fatty acids and their derivatives. These chemicals showed little or no juvenile hormone activity at 10–100 μg/insect in the yellow mealworm, *Tenebrio molitor* L. Of the compounds tested an isomeric mixture of β-methylacrylates from linseed oil-derived C_{18} aromatic cyclic acids showed the greatest activity.

A number of insect juvenile hormones have been isolated, identified, and their chemical analogs synthesized (Bowers *et al.*, 1965, 1966; Dahm *et al.*, 1967; Myer *et al.*, 1968; Röller and Bjerke, 1965; Williams, 1967). The juvenile hormone activity of these chemicals seems related to the trans double bond at the 6,7 position, β-methylcrotonyl, epoxy, and branch-chain groups. Certain other compounds used as insecticide synergists having a high order of juvenile hormone activity contain the 3,4-methylenedioxyphenyl group (Bowers, 1968, 1969).