State University in obtaining and interpretation of mass spectral analysis is gratefully acknowledged. Thanks are also expressed to the American Meat Packing Corporation of Chicago, Ill., for providing the boar and sow fat samples used in this study. We also give thanks to J. A. Campbell, Upjohn Company, Kalamazoo, Mich., for providing the C_{19}-Δ^{16} steroid standards required in this study.

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Received for review July 19, 1971. Accepted September 27, 1971. Approved as Journal Article 5575, Michigan Agricultural Experiment Station. This study was supported in part by a research grant-in-aid from the American Meat Institute Foundation, 59 E. Van Buren St., Chicago, Illinois 60605.

Production of Volatile Compounds in the Ripening Banana

Roland Tressl[1] and Walter G. Jennings*

Sequential samples of air swept through a chamber containing ripening bananas and incorporating internal standards were trapped on Porapak Q, and recovered for gas chromatographic analysis. Peak areas, determined with digital integration and corrected for the internal standard(s), show that both the acetate esters and the butyrate esters are produced at rates that vary in a cyclic manner. The two cycles are out-of-phase, and it appears that they compete for some limiting reagent which first one and then the other reaction arrogates. Ripening fruit is shown to be a highly dynamic system, with short-term variations and wide fluctuations in the relative amounts of individual volatile compounds.

There have been many gas chromatographic studies on the volatile compounds produced by ripening fruits; some of these studies utilized sequential sampling (Paillard, 1968; Heinz *et al.*, 1965; Romani and Ku, 1966; Brown *et al.*, 1966; Drawert *et al.*, 1971). Methods that utilize extraction techniques suffer from the disadvantage that the biological system is upset and the sample destroyed by the extraction; sequential sampling then demands a number of individual fruits, and one must assume these are all at the same stage of development. Direct gas chromatographic analysis of headspace vapors has also been utilized, but without some means of preconcentration the amount of many of the volatiles injected is below the limits of detection. The method employed by Paillard (1968)—trapping of headspace volatiles on activated carbon, followed by elution and analysis—surmounts some of these difficulties, but observations in our laboratory have lately left in doubt the question of how completely adsorbed volatiles are recovered from a trapping charcoal. Additionally, quantitative comparisons of chromatographic analyses demand a reliable method for incorporating internal standards.

Jennings *et al.* (1971) recently employed traps containing a porous polymer, Porapak Q, to obtain samples of headspace volatiles for gas chromatographic analysis. Close examination indicates the method is quantitatively and qualitatively reproducible. It was employed here, together with a method of incorporating internal standards, to study the volatile emanations of ripening banana.

METHODS AND PROCEDURE

Trapping Techniques. 10 cm of an 11-mm × 14-cm borosilicate glass tube, with machined Teflon adaptors (Figure 1), was filled with 100–120 mesh Porapak Q between glass wool plugs. An iron-constantan thermocouple was incorporated, and the assembly wrapped with heating tape. The trap was conditioned for 48 hr at 180°C with a flow of 300 cm³/min of nitrogen, which was prepurified by passage through a 0.25-in. o.d. × 20-ft stainless steel column packed with 80–100 mesh uncoated firebrick and immersed in Dry Ice–acetone. Fruit volatiles and internal standards were trapped for 2 hr at 25°C from an air stream as described below. The gas was then changed to purified nitrogen, and the column developed (Jennings *et al.*, 1971) for 15 min at 25°C to remove the air and most of the water. The direction of flow was then reversed, and in a 200 cm³/min nitrogen stream the column was heated to 100°C, and the eluting volatiles were trapped in a thin-walled glass capillary chilled with Dry Ice, which was then flame sealed until analyzed. When air was used as the eluting gas at 150°C, severe degradation of both the volatiles and of the Porapak was experienced.

Sampling. Green Valerie bananas were purchased at a retail outlet, and 2 kg placed in a 5-l./glass chamber (desiccator), as shown in Figure 1. Purified breathing air was passed through a 0.25-in. o.d. × 20-ft column, packed with uncoated 60–80 mesh firebrick, immersed in a Dry Ice–acetone bath, and swept through the fruit chamber at a rate of 300 cm³/min. Except for the gas purifier, all components of the system were constructed of glass or Teflon and housed in a 25°C constant temperature chamber. The effluent gas, with its entrained volatiles, was passed through the internal standards container, thence to the Porapak trap, a flow meter, and discharged to atmosphere. The internal standard selected for this study was heptadecane.

Gas Chromatography. Glc analyses utilized a 1.4-mm i.d. × 5.3-m glass column containing 3.0% Carbowax 20M on 60–70 mesh HMDS A/W Chromosorb G. The chromatograph was of our own design, with glass-lined injectors and FID. The signal was fed through a Hewlett-Packard 5771 A

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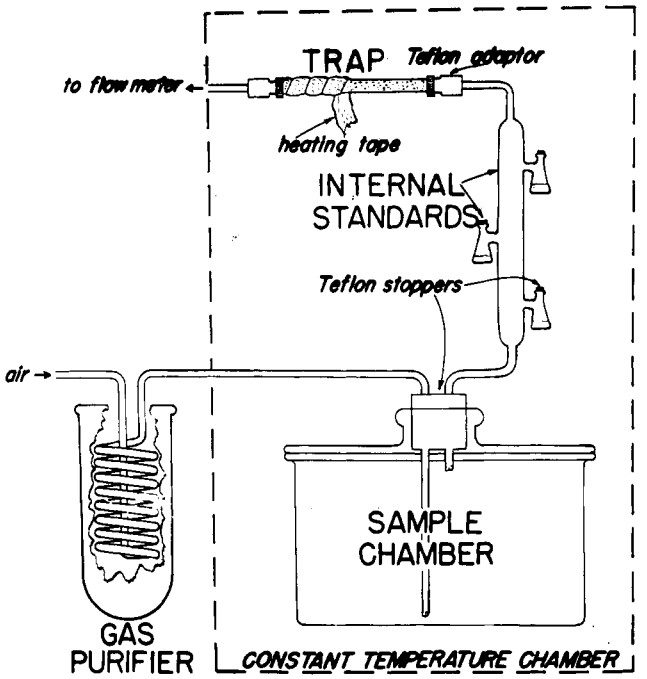

Figure 1. Trapping apparatus. Air (breathing grade) at 300 cm³/min is passed through the gas purifier and the sample chamber. Internal standards exhibiting suitable vapor pressures are contained in the three small Erlenmeyer flasks

electrometer to an Infotronics 208 digital integrator. For the initial injections, in which the production of volatiles was quite low (0.1–0.2 μl), 2 μl of pentane were used as a carrier for injection. In all other cases, 0.5 μl of undiluted essence was injected. The temperature was held at 55°C for 20 min, and then programmed at 1.8°C/min to 180°C. The instrument was supplied N_2 carrier gas at 12 cm³/min and 15 cm³/min makeup, H_2 at 30 cm³/min, and air at 300 cm³/min. Injector temperature was 180°C and the detector was maintained at 200°C.

Table I. Constituents of Major Chromatographic Fractions Shown in Figures 2 and 3

Peak number	Compound[a]
1	Ethyl acetate
2	Ethanol
3	Propyl acetate (plus some pentanone-2)
4	Isobutyl acetate
5	Ethyl butyrate
6	*n*-Butyl acetate (plus some hexanal)
8	Pentanol 2-acetate
9	3-Hexylbutyl acetate
11	Isobutyl butyrate
13	3-Hexylbutanol-1 (plus some *trans*-hexen-2-al)
14	*n*-Butyl acetate
16	Pentanol 2-acetate
17	3-Methylbutyl *n*-butyrate

[a] Based on retention times as correlated with work of Tressl (1970) and Tressl *et al.* (1970b).

RESULTS AND DISCUSSION

It is desirable to include three internal standards in work of this type, one preceding the volatiles of interest, one emerging midrange in the chromatogram, and one whose retention time exceeds those of the other compounds (Richard *et al.*, 1971). Decane, tridecane, and heptadecane were first selected as internal standards in this study. Initially, *ca.* 0.1 μl of banana volatiles were recovered from one trapping period. As maturation progressed, 5, 8, 10, and 15-μl yields were realized, and in the latter phases, as the texture deteriorated, 20–25 μl of essence were recovered from each trapping period. As the yields of volatiles increased, however, the chromatograms became so crowded that both of the lower boiling standards had to be abandoned, and heptadecane was used as the internal standard.

Figure 2 shows a typical chromatogram obtained for peeled bananas in the postclimacteric ripening phase, and Figure 3 shows typical results for unpeeled fruit of approximately the same stage of maturity. Identifications were assigned on the

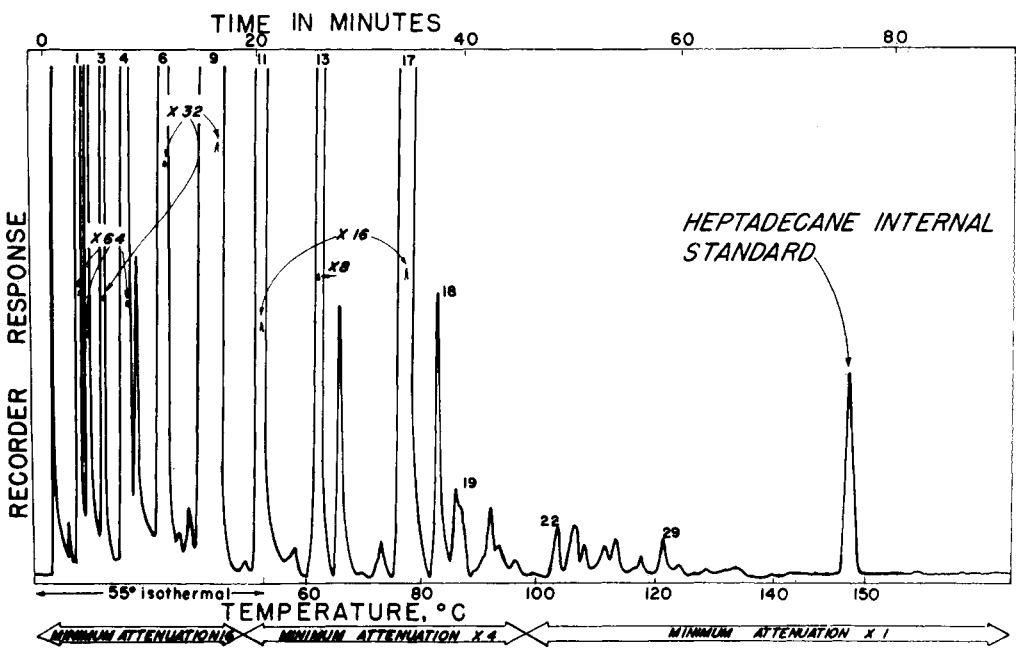

Figure 2. Typical chromatogram of peeled Valerie bananas in the postclimacteric ripening phase. 0.5 μl of "essence" injected on a 1.4-mm i.d. × 5.3-m glass column containing 3% Carbowax 20M on 60/70 mesh Chromosorb G

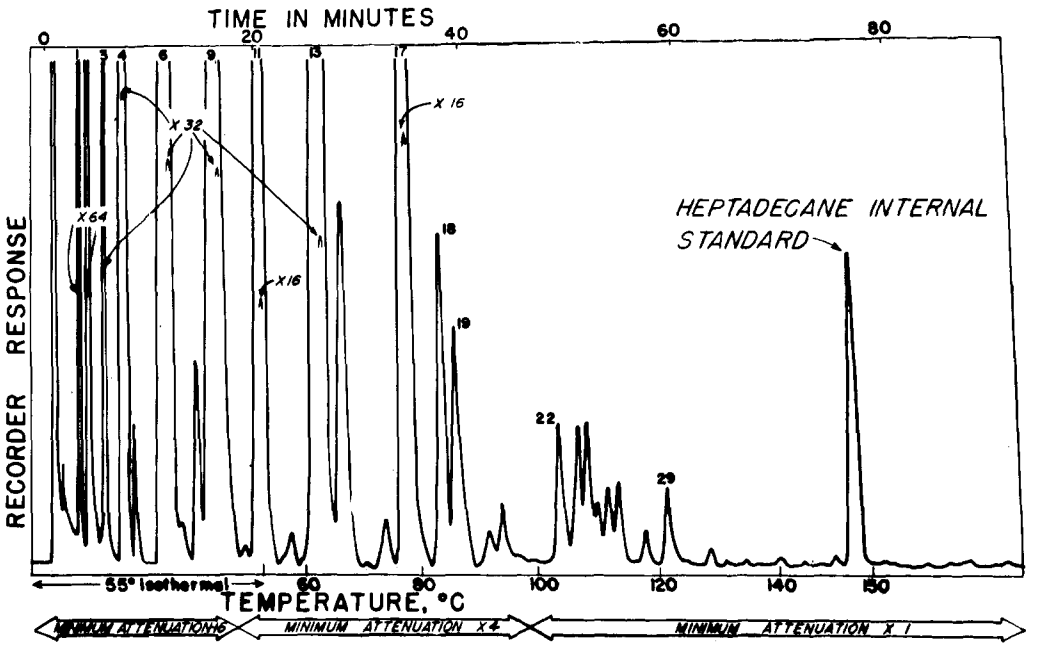

Figure 3. Typical chromatogram of unpeeled Valerie bananas in the postclimacteric ripening phase. 0.5 μl of "essence" injected on a 1.4-mm i.d. × 5.3-m glass column containing 3% Carbowax 20M on 60/70 mesh Chromosorb G

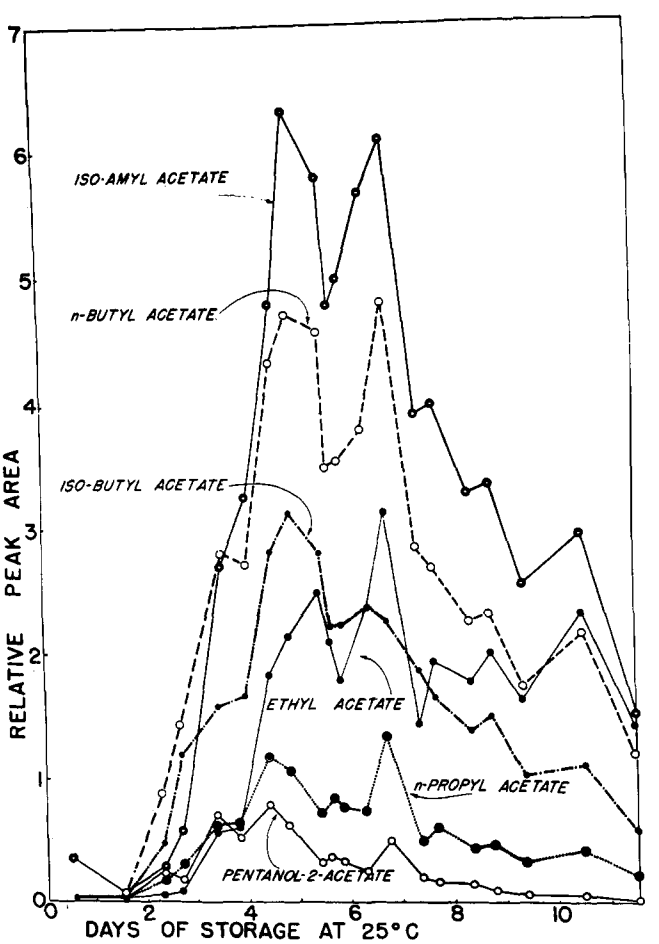

Figure 4. Individual acetate esters from sequential samples of the emanations from ripening banana. Peak areas are relative to the internal standard; hence peak areas in this and the other figures can be compared directly

basis of retention times, as correlated with work of Tressl (1970) and Tressl *et al.* (1970b), who established their structural assignments with the mass spectra of eluting compounds. This study utilized sequential samples from ripening unpeeled Valerie bananas.

Two main classes of esters occur in the ripe banana: acetates and butyrates. In the presence of ^{14}C-labeled acetate, banana tissue slices produce labeled acetate and unlabeled butyrate esters; in the presence of ^{14}C-labeled butyrate, all labeling appears in the butyrate esters; ^{14}C-labeled palmitate is not metabolized (Tressl *et al.*, 1970). Apparently the acetate and butyrate esters are not produced from a common precursor.

Figure 4 shows the principal acetate esters, and Figure 5 the major butyrate esters, in a series of sequential samples. Values plotted on the ordinates are integrated peak areas, adjusted for the amount of internal standard(s) injected. Hence these values are directly comparable between Figures 4, 5, and 7. Initially, samples were taken at alternate 8 and 16 hr intervals; as soon as a cyclic pattern began to emerge, a series of samples was taken at 2 hr intervals to confirm the validity of this observation.

Cyclic rates of volatile production have been observed by other investigators (*e.g.*, Tressl *et al.*, 1970a). It is extremely interesting to note that the cycle observed for the acetate esters is apparently out-of-phase with that observed for the butyrates (Figure 6). This situation could arise if the two series were produced by competing metabolic reactions, in which first one, and then the other, arrogated some limiting reactant, perhaps an energy source such as coenzyme A. Westphal (1970), however, observed that the activities of various enzymes in banana and apple exhibited cyclic patterns which were apparently unrelated, which might also explain this observation.

The unpeeled banana (Figure 3) produces considerably more 3-methylbutanol-1 than does the peeled fruit (Figure

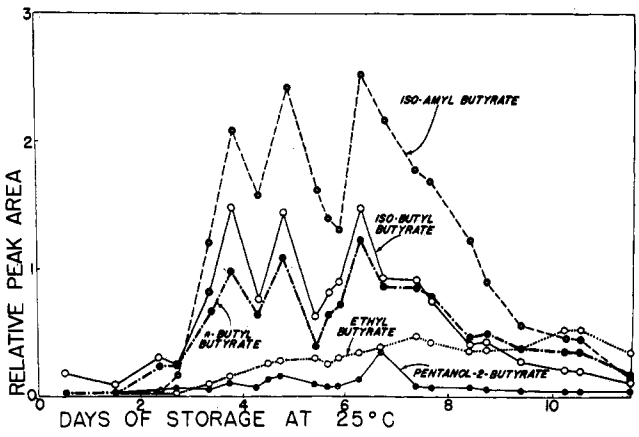

Figure 5. Individual normal butyrate esters from sequential samples of the emanations from ripening banana

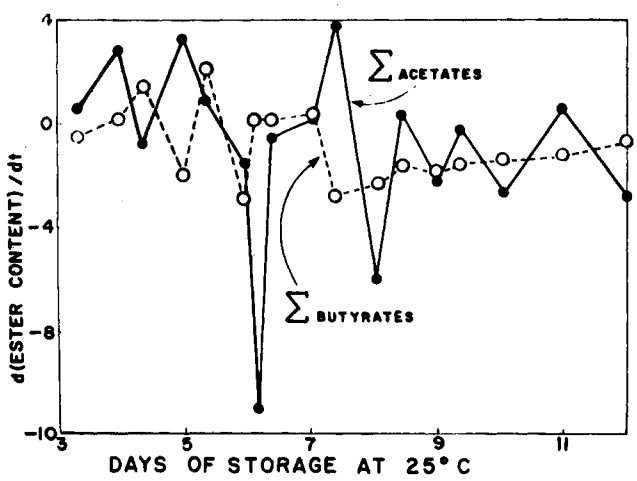

Figure 6. Incremental changes in the total acetate ester concentration (solid line) and the total butyrate ester concentration (dotted line). Note that the two cycles are apparently out-of-phase

2). Under the conditions of this study, *trans*-hexenal co-chromatographs with this compound; however, simultaneous separations on an OV-101 column indicated that less than 10% of this peak was due to the aldehyde. Production of the alcohol achieves a maximum (Figure 7) similar to that exhibited by the acetate esters (Figure 4). 3-Methylbutanol-1 can be formed from either α-ketoisocaproate or L-leucine; the biosynthesis of either precursor requires acetyl CoA (Matoon, 1963). Similarly, acetyl CoA is involved in the production of acetate esters. It may be that these two syntheses are both governed by the availability of acetyl CoA, and the rates rise or fall together, depending upon its concentration.

Ethanol (Figure 7) began to appear in the postclimacteric ripening phase, and increased in later storage. Both ethyl-acetate and ethyl butyrate followed a similar pattern; both were relatively minor compounds in the period of maximum desirable flavor (3–4 days), and both became major compounds at 10–12 days when the fruit was overripe and exhibited an undesirable aroma.

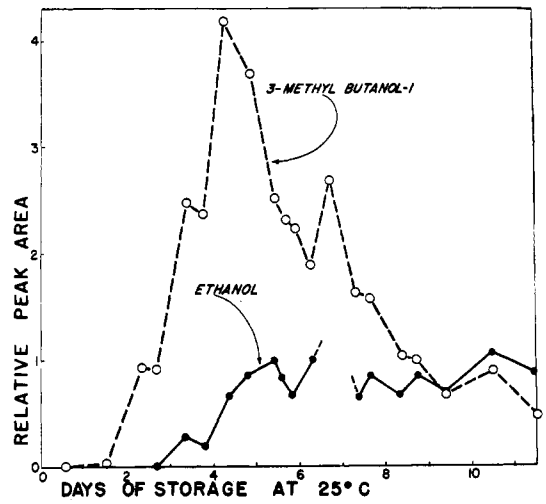

Figure 7. Production of 3-methylbutanol-1 and ethanol by the ripening banana. The determination for ethanol at 6.8 days was lost

These data make it abundantly clear that a ripening fruit is not a static system, but is instead a highly dynamic system in which the relative ratios of individual compounds vary hourly and fluctuate over a wide range. Considerable information on the biogenesis of these volatiles might be obtained if one could devise a method of continuously scanning the effluent gas for its concentration of a series of volatiles. This might well be achieved by interfacing a ripening chamber to a computer-coupled fast-scan mass spectrometer through a suitable molecular separator. Because of the probability that the individual fruits comprising the sample would be in different stages of these dynamic cycles, it would be highly desirable that the system be sufficiently sensitive to follow changes in a single fruit.

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Received for review August 19, 1971. Accepted October 26, 1971.