

the precipitate was separated into twelve bands. Absorption spectra and reactions with alcoholic potassium hydroxide (6) showed the presence of two dicarbonyl and ten monocarbonyl derivatives.

The fraction from the deaerator with the raw tomato odor, D<sub>2</sub>b, was shown by the chromatostrip technique of Kirchner *et al.* (5) to contain at least one unsaturated compound and no aldehydes. T<sub>2</sub>d, the fraction with green tomato odor from the tube evaporator, also contained one or more unsaturated compounds and no aldehydes.

Thus, unsaturated compounds were shown to be present in the fractions with typical tomato odor and with raw and green tomato odor. Also, by their odors, spectra, and in some instances the formation of derivatives, unsaturated compounds of a terpene nature, such as

citral, were indicated to be present in other fractions modifying the fractions with tomato odor. The oxidation and polymerization of these unsaturated compounds may offer at least a partial explanation for the flavor deterioration of stored tomato products when this occurs.

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## FRUIT STORAGE EFFECTS

# Carbonyl Compounds in Apple Storage Volatiles

R. E. HENZE, C. E. BAKER, and F. W. QUACKENBUSH

Agricultural Experiment Station, Purdue University, Lafayette, Ind.

Carbonyl compounds have been determined in apple storage volatiles collected on activated carbon in a commercial refrigerated apple storage. Acetaldehyde, acetone, and propionaldehyde have been definitely identified. Fifteen other carbonyl compounds have been separated chromatographically as their 2,4-dinitrophenylhydrazone derivatives, and their absorption spectra are presented.

CARBONYL COMPOUNDS have been reported in apple storage volatiles obtained from activated carbon in a commercial storage (3), but individual compounds were not identified. Other investigators have reported (13, 14) that infrared absorption spectra of apple volatiles collected on activated carbon indicated the presence of aldehydes, methyl ketones or acetates, esters, and perhaps alcohols. The presence of ethyl alcohol and acetaldehyde was definitely established by their mass spectra. Huelin (4) has recently reported the presence of acetaldehyde, propionaldehyde, and acetone among the volatile compounds emitted by Granny Smith apples at 30° C. From a volatile fraction of apple juice White (16) identified acetaldehyde, acetone, caproaldehyde, and 2-hexanal among the principal components.

Mixtures of relatively small quantities of carbonyl compounds from a variety of sources have been successfully separated by chromatographic adsorption of their highly colored 2,4-dinitrophenyl-

hydrazones on such adsorbents as talc (12), alumina (5, 7, 8), silicic acid (2, 9), magnesium sulfate (11), and bentonite (15) or by partition on paper (4, 6). Identification of the resulting 2,4-dinitrophenylhydrazone bands is usually based on melting point, mixed melting point, elemental analysis, x-ray diffraction pattern, spectrophotometric analysis, or combinations of these, depending on the quantity of the material available. Using a combination of chromatographic and spectral analysis Stadtman (17) identified furfural and hydroxymethylfural among some 15 carbonyl compounds present in stored apricot concentrates. The absorption spectra in the 2200 to 5000 Å. range are available for about 60 known carbonyl compounds of representative types (1, 10).

This paper describes the separation and partial characterization of sodium bisulfite-extractable carbonyl compounds present in apple storage volatiles collected on activated carbon in a commercial storage. Details of this collec-

tion procedure utilizing activated coconut shell carbon in a commercial air-purification unit (W. B. Connor Engineering Corp., Danbury, Conn.) have been reported (3).

#### Materials and Methods

**Preparation of 2,4-Dinitrophenylhydrazones** Three hundred milliliters of an ether extract of apple storage volatiles (3) (219.96 grams of ether-free substance) was placed in a separatory funnel with 100 ml. of ether and extracted with two 50-ml. portions of 10% sodium bicarbonate to remove free acids. One hundred milliliters of a saturated sodium bisulfite solution was added to the ether layer and the mixture stirred vigorously at room temperature for 1 hour. The bisulfite solution was then extracted with several portions of ethyl ether and acidified with 3% sulfuric acid. One gram of 2,4-dinitrophenylhydrazine dissolved in 5 ml. of concentrated sulfuric acid was added to the

bisulfite solution and the mixture was allowed to stand overnight. The resulting gummy red precipitate (1.40 grams) was filtered off under vacuum and air-dried.

#### Chromatographic Analysis

Preliminary separation of this mixture of 2,4-dinitrophenylhydrazones was effected on a large chromatographic column (40 mm. in inside diameter by 50 cm.) consisting of a 2 to 1 mixture of silicic acid (Mallinckrodt's analytical reagent, 100-mesh, for chromatographic use) and diatomaceous earth (Hyflo Super-Cel, Johns-Manville Corp., Chicago, Ill.) as described by Roberts and Green (9). The 2,4-dinitrophenylhydrazones were dissolved in 50 ml. of a 2 to 1 petroleum ether-benzene solution and poured on the adsorbent, and the chromatogram was developed with 5% ethyl ether in commercial hexane (Skellysolve B, purified by passage through silica gel). Three zones separated, two of which were composed of a number of poorly differentiated bands. The three zones were mechanically removed and extracted with ethyl ether. After the solvent had been evaporated, each of the three fractions was redissolved in 25 ml. of warm hexane and chromatographed on a magnesium sulfate (Mallinckrodt's analytical reagent, anhydrous, Control X RM) column (2.6 mm. in inside diameter by 55 cm.) as described by Stadtman (11), with 15% benzene in hexane as the eluant.

By this procedure Zone 1 was resolved into five bands, Zone 2 remained as one

band, and Zone 3 was resolved into twelve bands. The individual bands were separated mechanically and extracted with ethyl ether. Each of the 18 bands was further purified by chromatography on still smaller columns (10 mm. in inside diameter by 12 cm.), using first magnesium sulfate and then silicic acid-Super Cel as the adsorbent with 15% benzene in hexane and 5 to 20% ether in hexane, respectively, as eluant. Bands I through XIV, bands XV and XVI, and bands XVII and XVIII were eluted from the silicic acid-diatomaceous earth columns with 5, 10, and 20% solutions of ether in hexane, respectively. The magnesium sulfate gave most effective separations when allowed to stand exposed to the air for several days and passed through a 100-mesh screen before use.

#### Spectroscopic Analysis

Before spectroscopic analysis each of the 18 eluted fractions was freed of benzene by evaporating an alcohol solution to dryness and repeating several times to remove occluded benzene. The alcohol was purified by refluxing over potassium hydroxide and zinc dust and distilling before use. Absorption spectra of alcohol solutions of the fractions were obtained with a Model DU Beckman spectrophotometer.

#### Results and Discussion

After extensive chromatographic purification only band VI gave enough solid 2,4-dinitrophenylhydrazone for determinations of melting point and mixed

melting point (melting point 122-123° C.). These corresponded to the acetone derivative.

All bands except band I were available in sufficient quantity for spectroscopic analysis. Three bands (V, VI, and VII) gave almost identical absorption spectra with principal peaks at 355 to 360 m $\mu$ . As band VI had been previously identified as acetone by its melting point, bands V and VII were surmised to be closely related saturated compounds. Known 2,4-dinitrophenylhydrazones of propionaldehyde, acetone, and acetaldehyde were found to give single-banded mixed chromatograms on both adsorbents with bands V, VI and VII, respectively. Essentially identical absorption spectra (Figure 1) were further evidence for their identity.

Seven bands (III, X, XI, XII, XIV, XVI, and XVII) showed closely similar absorption spectra with principal maxima at 395 m $\mu$  (Figure 2). The remaining group of seven bands exhibited significantly different absorption curves with maximum between 380 and 400 m $\mu$  (Figure 3).

As pointed out by Stadtman (11), unknown 2,4-dinitrophenylhydrazones can be identified by their absorption spectra only when similar curves for known compounds are available for comparison. Sufficient quantities of the 2,4-dinitrophenylhydrazones themselves are also required when chromatographic analysis is used in conjunction with spectral analysis as an indication of identity. With the exception of the 2,4-dinitrophenylhydrazones of acetaldehyde, acetone, and propionaldehyde, none of the 14 other bands gave absorption curves corresponding to those of known compounds or exhibited chromatographic properties identical with commonly available carbonyl compounds.

While the other 2,4-dinitrophenylhydrazones were not identified, some useful information concerning their nature can be ascertained from their  $\lambda_{max}$ . The  $\lambda_{max}$ , which is attributable to the phenylhydrazine portion of the molecule, is modified by other groups in the molecule. It is more dependent on the extent of conjugated unsaturation in the carbonyl compound than on the nature of the alkyl substitution present. From the variety of compounds reported by Braude and Jones (7), the general effect of unsaturation on the  $\lambda_{max}$  can be summarized as follows: saturated aldehydes and ketones 356 to 365 m $\mu$ , enals 366 to 387 m $\mu$ , dienals 379 to 395 m $\mu$ , and trienals 395 to 410 m $\mu$ .

The seven similar absorption curves (Figure 2) with  $\lambda_{max}$  of 395 are suggestive of a closely related but chromatographically separable series of carbonyl compounds of the dienal or trienal type. Such compounds might possibly arise from an aldol condensation of furfural with simple aliphatic aldehydes. The

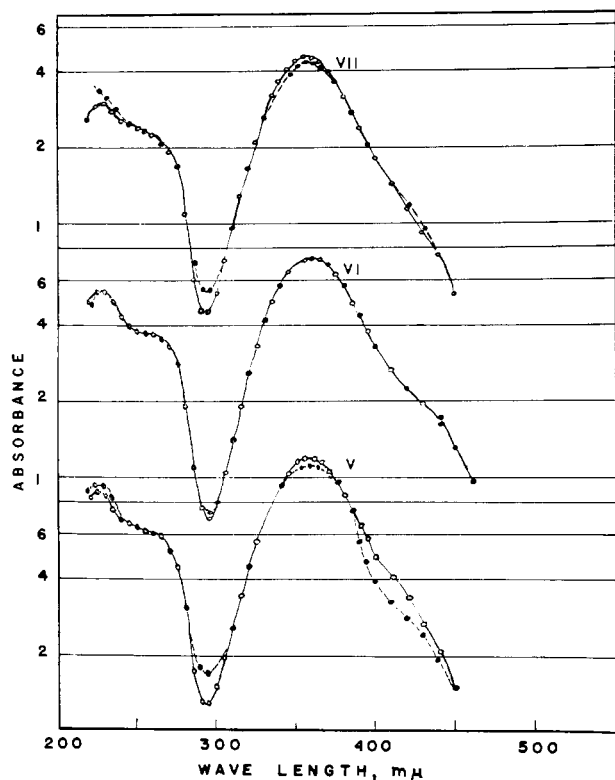


Figure 1. Absorption spectra of 2,4-dinitrophenylhydrazones

- 2,4-Dinitrophenylhydrazones of:
- V. Propionaldehyde
- VI. Acetone
- VII. Acetaldehyde
- Unknowns

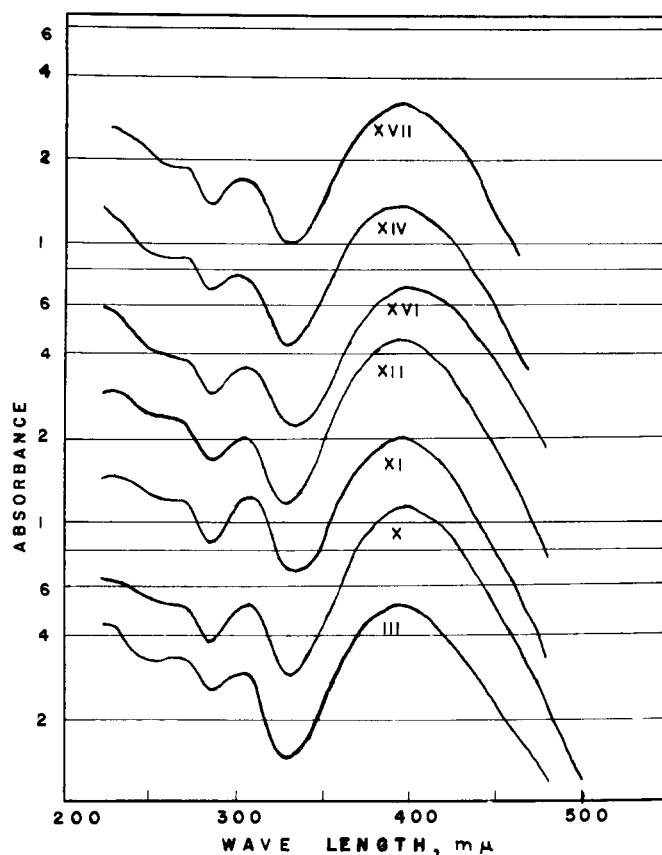


Figure 2. Absorption spectra of unknown 2,4-dinitrophenylhydrazones

From bands III, X, XI, XII, XIV, XVI, and XVII

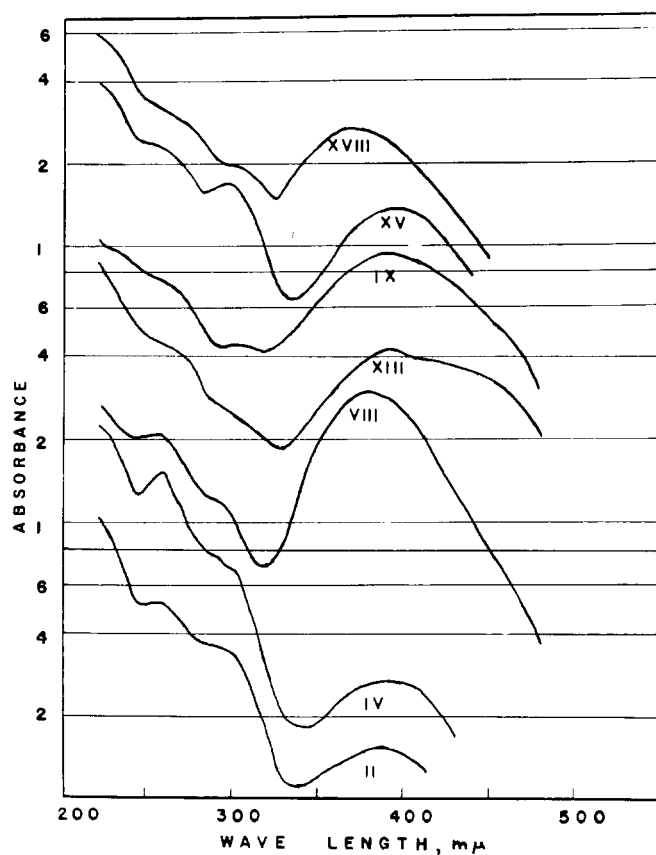


Figure 3. Absorption spectra of unknown 2,4-dinitrophenylhydrazones

From bands II, IV, VIII, IX, XIII, XV, and XVIII

remaining seven 2,4-dinitrophenylhydrazone bands (Figure 3), with the exception of band VIII, show a broadening of the phenylhydrazine absorption peak and a decrease in its extinction with respect to other peaks in the shorter wave lengths. The general shape of these absorption spectra is similar to some of the unidentified bands reported by Stadtman (17) from stored apricot concentrates. All eighteen 2,4-dinitrophenylhydrazone bands gave a red color in alkaline alcohol solution indicative of simple aldehyde and ketone derivatives. Band VIII (Figure 3) which has an  $\lambda_{\max}$  of 380  $m\mu$ , suggesting an enal, may correspond to the 2-hexenal found by White (16) in a volatile fraction from apple juice. Unfortunately, no 2,4-dinitrophenylhydrazone of a known sample of 2-hexenal was readily available for comparison of chromatographic position or absorption spectrum.

The mixture of carbonyl compounds obtained from apple storage volatiles from activated carbon appears to be considerably more complex than that found by White (16) in volatiles distilled from apple juice or by Huelin (4) in volatile emanations from Granny Smith apples. The collection of apple volatiles on activated carbon during a 7-month apple storage season may have resulted in the accumulation of minute traces of car-

bonyl compounds not detectable in short-term experiments. This difference in complexity might also indicate that the method of collection by activated carbon is more efficient for the sampling of atmospheric vapors in low concentration or that chemical changes occurred while the carbonyl compounds were adsorbed on the carbon surface. Furfural, for example, reported as a minor constituent by White (16), might readily condense with simpler carbonyl compounds, giving rise to carbonyl compounds of the trienal type whose 2,4-dinitrophenylhydrazones would then exhibit absorption spectra with an  $\lambda_{\max}$  in the 395 to 410  $m\mu$  region.

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