teins in cereal grains. After a thorough review of the literature he concluded that the following sequence of events ensues as a heat-vulnerable protein is subjected to a progressive increase in the severity of heat treatment:

The enzymic release of lysine during intestinal transit is delayed, so that a substantial portion of it is no longer available for the mutual supplementation of the other essential amino acids absorbed earlier in the digestive process.

The enzymic release of lysine may be delayed to such an extent that a substantial portion of the lysine is excreted unabsorbed. This is accompanied by a depression in the digestibility of the heated protein.

Lysine may be destroyed, as evidenced by a decrease in the amount of lysine recoverable from acid hydrolyzates of the heat-processed protein. The fate of lysine in heat-processed proteins may be true of other amino acids, such as threonine and valine used as amino acid supplements in this investigation.

Acknowledgment

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Acknowledgment is made of the technical assistance given by Leslie Easterling, Joy Dowell, and Mary Crudup.

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FLAVOR ORIGIN

Flavor and Odor Components in the Tomato

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In an investigation of the preservation of flavor during processing, suitable methods for the isolation of tomato flavoring constituents were shown to be vacuum distillation at 20 mm., or solvent extraction followed by molecular distillation of the solvent extract. Vacuum distillates contained: esters, 2 p.p.m. in both cooked and fresh juice; volatile acids, 1 p.p.m. in fresh and 0 in cooked; carbonyls, 33 p.p.m. in fresh and 43 p.p.m. in cooked. The main carbonyl constituent was acetaldehyde. Isovaleraldehyde was also present, and probably citral and vanillin. Three different types of tomato odor fractions were isolated chromatographically from the concentrates: a typical tomato odor fraction, relatively nonvolatile; a green tomato odor fraction, also relatively nonvolatile; and a raw tomato odor fraction, relatively volatile. The typical tomato odor fraction, largely retained by present processing methods, contained alcohols, carbonyl compounds, and unsaturated compounds, and these were modified by many other odor fractions, some terpene in nature. Changes in the unsaturated compounds may be involved when the flavor of stored tomato products deteriorates.

DETERMINING THE FLAVORING CONSTITUENTS of plant products is complicated by the number of components and their minute quantities. Yields are conditioned by arbitrary decisions: The investigation may be made on components distillable by steam at atmospheric pressure, at 20-mm. pressure or at less than 1-micron pressure. Lyophilization may be employed, or ex-

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traction with volatile solvents with or without distillation of the extract. Not only do yields vary, depending on methods used, but composition of the oil itself is affected.

Difficulties are enhanced by two problems after identification is made. How important is the component to the flavor? Is the component an artifact or a natural constituent of the flavoring matter? Changes in structure, particularly those of an oxidative character, constitute a hazard. In handling the necessarily large quantities of raw material, in spite of careful inspection for damaged and bruised fruit, delays in sorting and treating are inevitable and may give rise to off-flavors.

In this study, methods of isolating flavoring components from tomatoes were first compared. After the initial recovery steps, the oils obtained were separated by chromatographic methods, and the physical and chemical properties of the separated components were investigated.

Isolation of Flavor Concentrates

Solvent extraction, vacuum distillation at 20 mm., and steam distillation at

atmospheric pressure were investigated as means of isolating tomato flavor concentrates. All gave concentrates possessing a tomato odor, but differing in modifying odors.

Solvent Extraction
Tomato paste was extracted with the solvent series methanol, isopentane (2-methylbutane), and benzene.

In a small scale solvent extraction, the benzene and isopentane extracts were combined and distilled at 225° to 230° C., at 1 mm. The distillate did not possess a true tomato odor, although its odor was relatable to tomato. This indicated that some of the vital flavor constituents had been altered by heat.

As a consequence, a solvent extract on a larger scale was molecularly distilled, at 70° to 120° C. and less than 1 micron. Eleven kilograms of paste was extracted first with 3 gallons of methanol. The methanol was discarded after it had been extracted with 1 gallon of isopentane. The residual paste was extracted further with 9 gallons of isopentane and 9 gallons of benzene. The isopentane extracts were combined and molecularly distilled.

Figure 1. Absorption spectra of solvent extracts of tomato paste

P. Molecular distillate of large scale isopentane extract, in iso-octane

P₂. Fraction with typical tomato odor from isopentane extract, in petroleum ether

B₂. Fraction with typical tomato odor from benzene extract, in petroleum ether

The benzene extract was molecularly distilled separately. The yield of oil from 11 kg. of paste was 0.182 gram (4 p.p.m. on a single-strength basis) for the isopentane extract and 0.141 gram (3 p.p.m. on a single-strength basis) for the benzene extract. Both distillates possessed tomato odors.

Vacuum Distillation
Preliminary experiments with the apparatus of Dimick and Makower (3) showed that vacuum distillation of 25% of the volume of tomato juice gave a distillate containing almost all the readily volatile acids, alcohols, and carbonyl compounds. (During vacuum distillations using this apparatus, loss of volatiles was prevented by the use of an ice water condenser and a series of three traps, two cooled by dry ice–ethyl alcohol mixtures, and the last one in the line by liquid nitrogen.)

To determine a suitable starting material, comparisons on a small scale were made of concentrates from freshly pressed tomato juice and commercially canned tomato juice, both Pearson variety. The freshly pressed tomato juice was vacuum distilled at 2-mm. pressure in the apparatus of Dimick and Makower (3), giving the "fresh" tomato distillate. The canned tomato juice was stripped in a steam injector stripper at 228° to 235° F., giving the "cooked" tomato distillate. (The over-all time for stripping 410 pounds of juice was 1.5 hours.) Each distillate was concentrated fifty-fold by distillation through a continuous Oldershaw-type (4) column. Both the fresh and cooked tomato concentrates possessed a tomato odor, although they differed in modifying odors. Molecular distillation of an ether extract of these distillates showed that the yield of high boiling oil from the fresh tomato concentrate was 1.4 p.p.m., and from the cooked, 2 p.p.m. of the original juice.

Discussion
Vacuum distillation removing 25% of the water from tomato juice gave a distillate containing almost all the readily volatile compounds. Solvent extraction of the residue from the vacuum distillation with the series methanol, isopentane, and benzene gave less volatile fractions which distilled readily at 70° to 120° C. at 1 micron. The vacuum distillates of fresh and cooked

tomatoes had a fruity, ethereal quality lacking in the molecular distillates of the solvent extracts, but all possessed a tomato odor and appeared to be suitable for the isolation of compounds responsible for tomato flavor.

Isolation of Large Quantities
On the basis of these experiments, it was decided to obtain large quantities of flavor concentrates by inserting condensers in the regular tomato processing line of the East Oakland plant of the California Packing Corp. In commercial processing of the tomato juice used in these large scale concentrates, the juice was heated to 190° F. in a plate heat exchanger and held at that temperature 20 seconds before it was fed through the deaerator, where it was subjected to 10-inch vacuum and cooled to 170° F.

The condensers were placed in two different locations, giving two types of flavor concentrates. The most volatile compounds, including the harsh, so-called "raw" flavor which is removed from tomato juice commercially in the deaerator, were collected by connecting a condenser at a point in the deaerator mid-way between the juice feed and the outlet to the vacuum system. A vacuum pump attached to the ice-cooled receiver for the condenser maintained a pressure in the collecting system slightly lower than the 250 mm. normally maintained in the deaerator. The second type of flavor concentrate, possessing a typical tomato odor and free from the raw note, was obtained from condensates from a tube evaporator at the same plant. Condensates from both the tube evaporator and the deaerator were partially frozen by overnight storage at −10° F. About one third of the water was thus frozen out and the free liquid decanted the next morning for further concentration.

In the apparatus constructed by Dimick and Makower (3) the tube evaporator condensates (and also the deaerator condensates when entrainment was large) were stripped under a pressure of 20 mm. to give 25% of their volume as condensate. These condensates were in turn distilled at atmospheric pressure through a 20-plate continuous Oldershaw-type (4) distilling column to yield at 5% cut. The distillates from the Oldershaw column were saturated with ammonium sulfate and extracted with diethyl ether. The diethyl ether extracts were then dried over anhydrous sodium sulfate and concentrated to a small volume on a steam bath, and the remaining solvent was removed under a stream of nitrogen. From the tube evaporator condensates was obtained 3.0 grams of yellow oil, representing 2 tons of tomato juice, a proportion of 1.6 p.p.m. From the deaerator concentrates was obtained
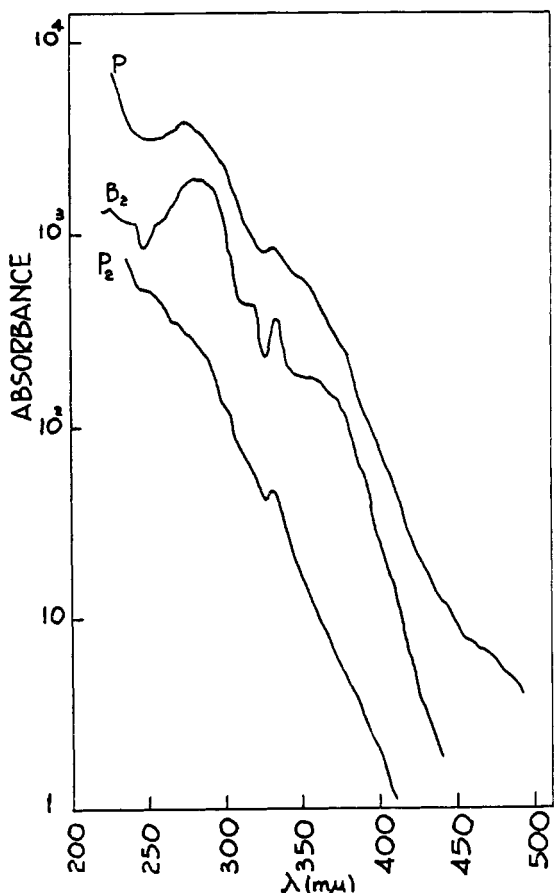
Table I. Fractionation of Deaerator Fraction Possessing Typical Tomato Odor

Band No.[a]	Solvent for Elution, Diethyl Ether—Petroleum Ether	Weight, G.	Odor
D₂a	0:100	0.0348	Phenolic when concentrated, pepperminty when dilute
D₂b	0:100	0.0018	Raw tomato
D₂c[b]	0:100	0.0006	Fruity
D₂d	0:100	0.0027	Fruity, sulfury
D₂e	0:100	0.0009	Weak sulfury
D₂f	0:100	0.0024	Tomato
D₂g	0:100	0.0001	Tomato
D₂h	0:100	0.0028	Tomato
D₂i	0:100	0.0019	Rose-violet
D₂j	0:100	0.0014	Fruity, ethereal
D₂k	0:100	0.0009	Fruity, ethereal
D₂l	0:100	0.0027	Lemony, pepperminty
D₂m	1:99	0.0027	Faint, musty
D₂n	1:99	0.0043	Musty, sweet
D₂o	1:99	0.0039	Sweet, burnt
D₂p	1:99	0.0045	Caramel
D₂q	100:0	0.0093	Sweet, fruity

[a] Bands numbered in order of increasing adsorption, D₂q most strongly adsorbed.
[b] Only band to show fluorescence (greenish blue) on column.

1.4 grams of yellow oil, procured from 10 tons of tomato juice, a proportion of 0.15 p.p.m. One reason for the difference in amounts obtained was that the deaerator removed 1% of the water from the tomato juice, and the tube evaporator 20%.

Properties of Flavor Concentrates

Before fractionation of the concentrates was attempted, their properties were determined.

Physical Properties Indices of refraction were 1.4939 for the oil from the deaerator, and 1.4650 for the oil from the tube evaporator. Optical activities were determined with a Schmidt and Haensch polarimeter, with 2-ml. cells and benzene as a solvent. The 0.28-gram sample of oil from the tube evaporator, obtained from 370 pounds of tomato juice, gave an $[\alpha]_D^{20}$ of + 0.85. No optical activity was shown by nearly the same weight of oil (0.29 gram) from the deaerator, obtained from five times the weight (2061 pounds) of tomato juice. The molecular distillate of the isopentane extract of tomato paste gave an $[\alpha]_D^{20}$ of + 1.1. These results indicated that the optically active compounds were relatively less volatile, or were the result of heat treatment.

Quantities Of Flavoring Constituents In vacuum distillates of tomato juice the ester contents, calculated as ethyl acetate, were 2 p.p.m. in both fresh and cooked tomato juice. Calculated as acetic, the volatile acid content was 1 p.p.m. in the fresh, and 0 in the cooked. Total carbonyl content, calculated as acetaldehyde, was 33 p.p.m. for the fresh and 43 p.p.m. for the cooked. Carbonyls obviously constituted the major products of vacuum distillation.

Carbonyls Dinitrophenylhydrazones were formed by the method of Brady (2) in the fresh and cooked tomato concentrates, and were separated by chromatography on silicic acid, with benzene-petroleum ether mixtures as developers and eluants. Acetaldehyde dinitrophenylhydrazone constituted 70% of the total weight of carbonyls in the fresh, and 80% in the cooked. Its identity was proved by melting point, mixed melting point, spectrum, and mixed chromatography with known acetaldehyde dinitrophenylhydrazones and by nitrogen analysis. (Analysis of 2,4-dinitrophenylhydrazone: calculated for $C_8H_8N_4O_4$, N 24.95; found, 24.96.)

Small amounts of isovaleraldehyde in both fresh

and cooked tomato distillates were identified by absorption spectrum, melting point, mixed melting point, and mixed chromatogram with known isovaleraldehyde dinitrophenylhydrazones. Eighteen other carbonyl compounds in minor amounts were separated and partially characterized.

Nitrogen and Sulfur Tests The Prussian blue test for nitrogen and the lead acetate test for sulfur were negative for vacuum distillates representing 17 pounds of cooked tomatoes and 3 pounds of fresh.

Separations of Flavor Concentrates

Chromatography was used to separate the flavor concentrates into fractions.

Solvent Extracts The small scale solvent extract (where the combined isopentane and benzene extracts were distilled at 225° to 230° C. at 1 mm.) was dissolved in petroleum ether and chromatographed on silicic acid (Baker and Adamson) and magnesium oxide–silicic acid (1 to 2) columns 1 inch in diameter and 3 inches long. Four fractions were eluted. The positions of the bands were determined by odor.
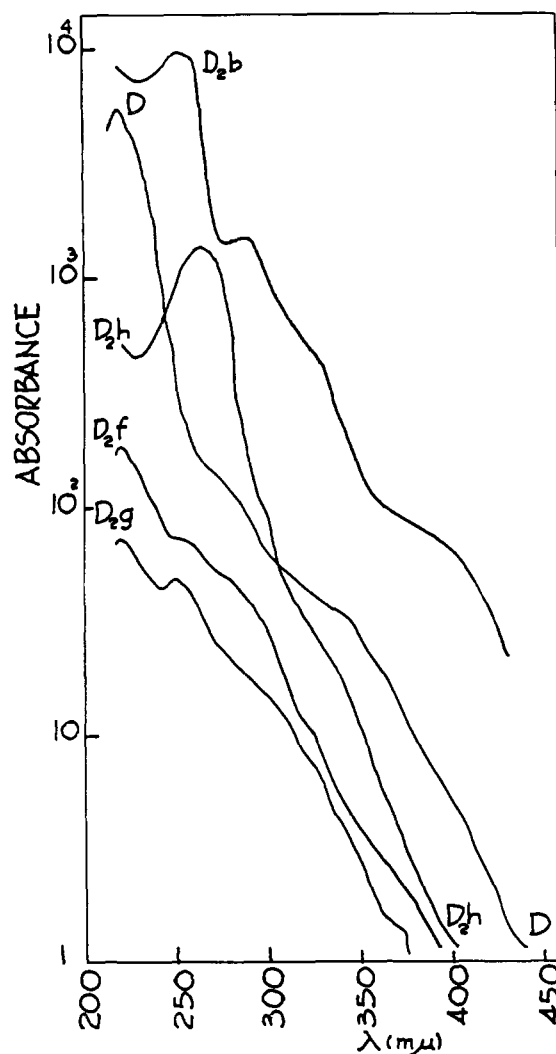

Figure 2. Absorption spectra of deaerator concentrates

D. Unseparated deaerator concentrate, in diethyl ether
D₂f, D₂g, D₂h. Typical tomato odor fractions, in petroleum ether
D₂b. Raw tomato odor fraction, in petroleum ether

No band possessed a tomato odor. Instead, the fastest moving band from each column had a citronellal odor, which in the case of the silicic acid column was mixed with a haylike odor. In petroleum ether these bands possessed absorption maxima at 283 mμ, characteristic of citronellal, and the absorption curve was almost superimposable on that of citronellal when 95% ethyl alcohol was used as solvent. Dinitrophenylhydrazones prepared by Allen's method (7) from the fraction with the unmasked citronellal odor were separated by chromatography into four bands. Two of the bands had absorption maxima at 365 mμ, corresponding to citronellal dinitrophenylhydrazone, and two possessed absorption maxima at 383 mμ, corresponding to citral dinitrophenylhydrazone. Quantities were insufficient for further characterization.

The larger scale, molecularly distilled solvent extracts were also separated chromatographically, with magnesiasilica (1 to 2) columns and mixtures of diethyl ether and petroleum ether as developers and eluants. The benzene extract was separated into 16 bands, the odors of which ranged from peppermint and lemon to vanillin and pyridine. The isopentane extract was separated into six bands, each of which was shown by the chromatostrip technique of Kirchner, Miller, and Keller (5) to consist of several components, giving a total of 24 components in the isopentane extract. From both the benzene and isopentane extracts was obtained a fast-moving band possessing a typical tomato odor. In addition, a band with a "green tomato" odor was separated from the benzene extract.

Vacuum Distillation The flavor concentrates from the tube evaporator and the deaerator were chromatographed separately with silicic acid columns and mixtures of diethyl and petroleum ether (1 to 4 diethyl ether–petroleum ether was the original developer and eluent; during the development of the chromatogram, the proportion of diethyl ether was increased gradually). Using color, fluorescence, and change in eluate odor for the detection of the bands, five odorous bands (D$_1$ to D$_5$) were separated from the deaerator and nine (T$_1$ to T$_9$) from the tube evaporator. The fastest moving bands from both types of flavor concentrates possessed different odors but had almost superimposable spectra. On the other hand, some bands with similar odors possessed decidedly different absorption spectra. Obviously, these initial separations were rough, and each of the bands consisted of several compounds.

The second fastest moving band, both from the tube evaporator, T$_2$, and the deaerator, D$_2$, possessed a typical tomato odor, modified in the deaerator con-

centrate by an overlying raw odor. This band constituted 36% by weight of the oil from the tube evaporator concentrate and 44% of the oil from the deaerator concentrate.

Although the solubilities of the oils before fractionation required 1 to 4 diethyl ether—petroleum ether as the original developer and eluent, the faster moving fractions from the columns proved soluble in petroleum ether itself. Therefore, D$_2$ and T$_2$, possessing the typical tomato odor, were further fractionated by rechromatographing on silicic acid, with petroleum ether.

D$_2$ was divided into 17 fractions (Table I), detected by odor, fluorescence under an ultraviolet lamp, and change in index of refraction. One of these fractions, D$_2$b, possessed a harsh, raw tomato odor. It weighed 1.8 mg. and represented 0.3% of the oil from the deaerator. Another, slower moving band possessed a typical tomato odor. It weighed 5.3

mg. and represented 0.88% of the oil from the deaerator. To determine the homogeneity of this band, it was rechromatographed on a longer silicic acid column with the same eluent, petroleum ether. Three cuts (D$_2$f, D$_2$g, and D$_2$h, Table I) were taken from the eluate, although there was no change in odor or index of refraction and the appearance of the column did not indicate separation into bands.

From T$_2$, the fraction with the tomato odor from the tube evaporator concentrate, 24 components were isolated in a similar manner (Table II). A small amount of a fast-moving fraction, T$_2$d, possessing a green tomato odor was separated. As in the deaerator, another slower moving band possessed a typical tomato odor. This fraction weighed 0.0664 gram and represented 2.2% of the oil from the tube evaporator. To determine the homogeneity of this fraction, it also was rechromatographed, giving three fractions: T$_2$g, T$_2$h, and T$_2$i (Table II).

Figure 3. Absorption spectra of tube evaporator concentrates

T. Unseparated tube evaporator concentrate, in diethyl ether
T$_2$g, T$_2$h, T$_2$i. Typical tomato odor fractions, in petroleum ether
T$_2$d. Green tomato odor fraction, in petroleum ether
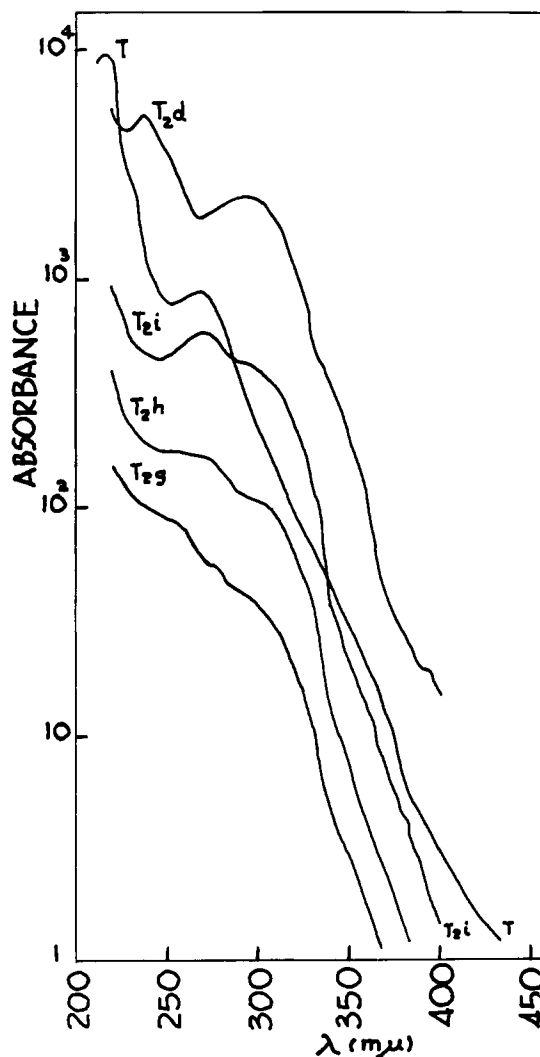
Properties of Separated Flavor Concentrates

Odors A fraction possessing a typical tomato odor was obtained from all types of flavor concentrates. The methods used for the isolation of this fraction and the tomato products from which it was isolated in good amounts indicate that it consisted of a relatively high boiling group of substances which are retained to a considerable extent in the commercial concentration of the juice.

In addition to the typical tomato odor fraction, a fraction with a green tomato odor was obtained from the benzene extract of tomato paste, and from the tube evaporator concentrate, but not from the deaerator concentrate. Thus the green tomato odor fraction apparently consisted of a relatively nonvolatile mixture of compounds.

From the deaerator concentrate was isolated a third type of tomato odor fraction, relatively volatile, and possessing a harsh, raw tomato odor.

The presence of the citronellal band in the small scale solvent extract (distilled at 225° to 230° C.) gave an indication of the nature of the typical tomato odor fraction. (On the chromatographic column

the citronellal band replaced the tomato odor bands isolated from concentrates which had received less severe heating.) Other constituents in the normally occurring tomato odor band may have been destroyed by heat, thus unmasking the citronellal, or a substance in part or wholly responsible for the tomato odor may have been converted into citronellal.

In addition to the three types of tomato odor fraction—typical, green, and raw—various modifying odor fractions were found. In all types of concentrates there were components with fruity, sulfury, lemony, sweet, flowery, and musty odors. Minty and carroty odors were confined to the tube evaporator and deaerator concentrates, and an odor of roses and violets to the deaerator concentrates. Rubbery and pyridine-like odor fractions were found both in the tube evaporator concentrate and in the benzene extract of paste, while the odor of vanillin was confined to the latter. The great number of compounds of varying chemical compositions and odors obtained on the separation of the flavor concentrates illustrated the complexity of natural tomato flavor and the difficulty that would be met in attempting to duplicate it exactly.

Absorption Spectra The absorption spectra in the D (deaerator) series and the T (tube evaporator) series showed gradual increases and decreases in intensity of recurring peaks. On the basis of peaks which changed independently, there were in the deaerator four, and in the material from the tube evaporator, six component types. In the order in which they were eluted from the chromatographic column, the compounds in the material from the deaerator were represented by maxima at (1) 265 mμ, (2) 227 mμ, (3) 255 mμ, and (4) 275 mμ and those in the material from the tube, evaporator by maxima at (1) 227 mμ, (2) 295 mμ, (3) 252 mμ, (4) 320 mμ, (5) 272 mμ, and (6) 320 mμ.

Spectra of the typical tomato odor fractions from the solvent extracts (B$_2$, P$_2$) are recorded in Figure 1, from the deaerator concentrate (D$_2$f, D$_2$g, D$_2$h) in Figure 2, and the tube evaporator concentrate (T$_2$g, T$_2$h, T$_2$i) in Figure 3. A maximum or change in slope at 270 to 285 mμ was evident in all fractions.

Spectra of the cuts (D$_2$f, D$_2$g, D$_2$h, and T$_2$g, T$_2$h, T$_2$i) taken in the deaerator and tube evaporator bands with the typical tomato odor showed that these bands were not homogeneous. In both instances, a maximum at 265 to 275 mμ was associated with slower moving components.

An absorption maximum at 333 mμ occurred only in the solvent extracts (Figure 1). As the tube evaporator and deaerator concentrates had a tomato odor, but no maximum at 333 mμ, in-

Table II. Fractionation of Tube Evaporator Fraction Possessing Typical Tomato Odor

Band No.[a]	Solvent for Elution, Diethyl Ether—Petroleum Ether	Weight, G.	Odor
T$_2$a	0:100	0.0746	Rubbery
T$_2$b [b]	0:100	0.0009	Rubbery
T$_2$c	0:100	0.0031	Green tomato + rubbery
T$_2$d	0:100	0.0003	Green tomato
T$_2$e	0:100	0.0006	Green tomato + sweetness
T$_2$f	0:100	0.0010	Fruity, tomato
T$_2$g [c]	0:100	0.0212	Tomato
T$_2$h	0:100	0.0249	Tomato
T$_2$i	0:100	0.0203	Tomato
T$_2$j	0:100	0.0253	Tomato + lemon
T$_2$k	0:100	0.0078	Tomato + waxy
T$_2$l	0:100	0.0205	Pepper, irritating
T$_2$m	0:100	0.0125	Peppermint
T$_2$n	0:100	0.0009	Peppermint + sulfury
T$_2$o	0:100	0.0006	Fruity
T$_2$p [c]	0:100	0.0039	Fruity, minty
T$_2$q [c]	0:100	0.0002	Lemony
T$_2$r	0:100	0.0032	Pyridine, irritating
T$_2$s	0:100	0.0042	Flowery
T$_2$t	0:100	0.0186	Flowery, minty
T$_2$u	10:90	0.0308	Minty, carroty
T$_2$v	10:90	0.0061	Carroty, nauseating
T$_2$w	10:90	0.0087	Sweet, flowery
T$_2$x	10:90	0.0045	None
T$_2$y [c]	10:90	0.0003	Slight musty
T$_2$z	100:0	0.0131	Fruity

[a] Bands numbered in order of increasing adsorption, T$_2$z most strongly adsorbed.
[b] Only band to show fluorescence (blue) on column.
[c] Bands colored (yellow).

dications were that this maximum was contributed by a substance which was not an essential part of the typical tomato odor, but rather a modifier of it.

The raw tomato odor fraction, D$_2$b (Figure 2), had maxima at 285 and 252 mμ. The green tomato odor fraction from the tube evaporator, T$_2$d (Figure 3), had absorption maxima at 292 and 240 mμ.

Constituents of Fractions with Tomato Odor By the formation of 3,5-dinitrobenzoate derivatives, the presence of alcohols was shown in the typical tomato odor fractions P$_2$, T$_2$h, and D$_2$f. By the method of White and Dryden (7), the 3,5-dinitrobenzoates were chromatographed on a silicic acid column impregnated with Rhodamine 6G, using diethyl ether (5%) in Skellysolve F as developer. The absorption spectra of one band from each column were superimposable, indicating a common alcohol constituent.

In the solvent extract fraction, P$_2$, preceding the 3,5-dinitrobenzoate band down the column was a band which fluoresced under the ultraviolet lamp and possessed a fresh odor found in tomatoes. It had a sharp absorption maximum at 333 mμ. This substance did not react with 2,4-dinitrophenylhydrazine, or 3,5-dinitrobenzoyl chloride, and on a silicic acid–calcium sulfate

chromatostrip, by the technique of Kirchner et al. (5), it did not react with acid or base indicators, but gave a positive test with fluorescein and bromine vapor, indicative of unsaturation. The eluted compound when warmed with maleic anhydride in ether solution lost almost completely the strong absorption peak at 333 mμ. This suggested that the original compound was a conjugated polyene and had undergone a Diels-Alder reaction with maleic anhydride. This substance was not found in the vacuum distillates.

By the chromatostrip technique of Kirchner et al. (5), it was shown that all the typical tomato odor fractions from the deaerator (D$_2$f, D$_2$g, D$_2$h) and the tube evaporator (T$_2$g, T$_2$h, T$_2$i) possessed a blue-green fluorescence under ultraviolet radiation, and all contained unsaturated compounds. However, only the slower moving fractions (T$_2$h, T$_2$i, and D$_2$h) gave positive aldehyde tests. This corresponded with evidence from the spectra, where the typical carbonyl maximum was associated with the slower moving fractions. Dinitrophenylhydrazones were prepared by Allen's (7) method on about 9.5 mg. of T$_2$h, a fraction with typical tomato odor from the tube evaporator. On silicic acid columns, with mixtures of benzene and petroleum ether as eluents,

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, D₂b, was shown by the chromatostrip technique of Kirchner et al. (5) to contain at least one unsaturated compound and no aldehydes. T₂d, 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.

Acknowledgment

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FRUIT STORAGE EFFECTS

Carbonyl Compounds in Apple Storage Volatiles

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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 (11) identified furfural and hydroxymethylfural among some 15 carbonyl compounds present in stored apricot concentrates. The absorption spectra in the 2200 to 5000 A. 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