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Effects of Ripening Processes on Chemistry of Tomato Volatiles

Bharat Manu Shah
Utah State University

EFFECTS OF RIPENING PROCESSES ON CHEMISTRY OF TOMATO VOLATILES

by

Bharat Manu Shah

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Bharat Manu Shah

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

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by

Bharat Manu Shah, Doctor of Philosophy

Utah State University, 1968

Major Professor: Dr. D. K. Salunkhe
Department: Food Science and Technology

Investigations were carried out to isolate, identify, and to characterize, major volatile components of tomato fruit. Simultaneously, the confirmation was extended to the reported tomato volatiles. The volatile extracts from field- and artificially-ripe fruits were compared qualitatively as well as quantitatively. The changes which occurred in the volatile components of the fruit at the onset of senescence also were delineated.

A typical chromatogram from field-ripe tomatoes contained 60 peaks. The functional group properties of individual peaks were derived by chemical analysis. The short-chain (C_3-C_6) alcohols represented 10 per cent, aldehydes and ketones 32 per cent, and hydrocarbons, long-chain alcohols, and esters were in 58 per cent of the total amount of the volatiles from the field-ripe fruits.

Among alcohols and carbonyls, 3-pentanol, 1-nonanal, 1-decanal, and 1-dodecanal and among esters, propyl acetate, geranyl acetate, and

cetronellyl butyrate were tentatively identified as volatile compounds of tomato fruit. Linalyl acetate, citronellyl butyrate, and geranyl butyrate were identified for the first time as the components of tomato volatiles.

Comparisons of volatile concentrations of field- and artificially-ripe tomatoes were made. In the latter category of fruits the concentrations of 1-butanol, 3-pentanol, 2-methyl-3-hexanol, 3-methyl-butanal, 2,3-butanedione, propyl acetate, isopentyl butyrate, and other unidentified carbonyls were higher than those observed in the field-ripe fruits. These short-chain compounds, especially the C_4-C_6 moities, probably are formed in their maximum concentrations during the early stages of maturation. Under the conditions of restricted nutrient availability, sun light, and limited enzymatic activity during artificial ripening, the long-chain compounds are not sunthesized appreciably. The concentrations of some of these short-chain compounds may be to a level of masking the effects of more desirable compounds contributory to ripe tomato aroma. Notably, a pulp from the artificially ripe fruits lacked the characteristic ripe tomato aroma. The concentrations of the long-chain carbonyls and the terpene esters were low in the artificially ripe tomatoes as compared to the field-ripe ones. This may indicate major contribution of these compounds to ripe tomato aroma. An attempt has been made to theorize the mechanisms of the biogenesis of these components of tomato volatiles.

The concentrations of the volatiles from field-ripe and overripe tomatoes were compared. During overripening the amounts of alcohols, aldehydes, ketones, acetates, and propionates generally decreased. However,

the concentrations of diacetyl and butyric acid esters increased. It was assumed that at the onset of senescence the metabolic pathways for the formation of diacetyl and butyric acid were highly operative in tomato fruit. The mechanisms of these pathways were postulated.

(57 pages)

INTRODUCTION

Food products contain very low concentrations of odoriferous compounds. Isolation, separation, detection, and identification of these compounds present formidable problems. The compounds responsible for characteristic aroma should be isolated without any alteration, contamination, heat destruction, or oxidative degradation. Isolation methods very frequently used by flavor chemists are: distillation, extraction, cyrstalization, and water removal by freezing. Gas-liquid partition chromatography (GC), with its low limits of detection, is one of the most powerful methods of separating submicrogram quantities of volatile compounds. Gas chromatography is employed for separation and detection of volatile compounds in the vapor sampled directly from food products (head space vapor analysis) and also to separate and detect the volatile constituents extracted from food products. Chemical and physical properties of the material represented by a gas chromatographic peak must be obtained for positive assignment of chemical structure. Functional group analysis, thin-layer chromatography, infrared spectroscopy, and mass spectrometry are valuable tools for identifying compounds in aroma investigations.

Though many studies have been conducted to elucidate the chemical nature of tomato volatiles, complete analysis has not been accomplished to enable the reproduction of a typical ripe tomato aroma. Investigations into chemistry of tomato volatiles and quantitative as well as qualitative differentiation between volatile constituents from field- and artificially-ripe

tomatoes may reveal unknown aspects of fruit aroma. Analysis of the volatile components of the fruit during an overripe stage may elucidate the biochemical aspect of aroma deterioration. With the presumption of these potentials, the present study was undertaken.

REVIEW OF LITERATURE

Non-volatile constituents of the tomato

Dalal (1965) correlated the biosynthesis of non-volatile constituents to the growth rate in V. R. Moscow cultivar of tomato. Volatile reducing substances, reducing sugars, water soluble pectins, and organic acids progressively increased in quantity with advancing maturity of the fruit. Total titratable acidity and total pectic substances increased during the initial stages of maturation, but gradually decreased as the fruit ripened. Ascorbic acid content increased with the maturity of the fruit but declined in the later stages of maturation. The concentration of the pigments--chlorophylls, and carotenoids--changed significantly as the fruit passed through various stages of maturation. Beltran and Macklin (1965) reviewed the literature (1945 to 1961) on the chemistry of the tomato and tomato products. Among the free sugars, representing more than 60 per cent of the solids, D-fructose, D-glucose, sucrose, and ketoheptose were predominant. Citric and malic acids comprised 80 to 90 per cent of the acids present. Glutamic and aspartic acids, valine, and glutamine were found in appreciable amounts among amino acids and amides present in eight varieties of tomatoes. Miers (1966) reported that 1.6 to 1.9 ppm dimethyl sulfide and 150 ppb hydrogen sulfide were present in processsed tomatoes. The precursor of these compounds in fresh ripe tomatoes was later determined to be S-methyl methionine sulfonium salt (Wong and Carson, 1966). The sulfonium salt produced homeserine and dimethyl sulfide when heated.

Beltran and Macklin (1965) reviewed the enzyme systems present in the tomato fruit and reported the detection of malic, succinic, formic and lactic acid dehydrogenases and also of alcohol and glucose dehydrogenases in ripe tomatoes. The distribution of ascorbic acid, oxidases, and peroxidases were considered parallel in the epicarp, endocarp, and mesocarp. The epicarp and endocarp contained more than did the mesocarp, and the concentrations in the epicarp and endocarp tended to increase during fruit ripening. An important contribution to the knowledge of tomato biochemistry was made by Yu (1967). He demonstrated that crude enzyme preparations from fresh tomatoes could convert certain amino acids to volatile components. Alanine was found to be especially important as a precursor for the production of carbonyl compounds, whereas leucine and valine appeared to be important for the production of alcohols by the enzyme preparations from the red-ripe tomatoes. The activities of the enzyme extracts from the field-grown tomatoes were higher than those from the greenhouse-grown tomatoes. He also investigated the specificity of the enzyme preparations. The mitochondrial preparations had much higher activity for alanine and aspartic acid than the non-mitochondrial fraction. However, the mitochondrial preparation was not active for leucine.

Volatile constituents of the tomato

Reports of investigations of volatile compounds responsible for the odor and flavor of the tomato are very few in number. Early investigations by Spencer and Stanley (1954) were accomplished with series of solvent extraction of tomato paste followed by molecular distillation of the solvent extract.

Components were fractionated and partially separated by a silicic acid column. Carbonyls represented the largest group of compounds responsible for the tomato aroma. The diphenyl hydrazone derivatives of the carbonyl compounds were made and further separated by column chromatography. The main carbonyl constituent was acetaldehyde. Isovaleraldehyde, citral, and vanillin were tentatively identified. Aroma extract was also recovered by vacuum distillation of thin tomato pulp. Three general types of aroma fractions were isolated. A fraction characterized as a "typical tomato" odor was relatively non-volatile and a fraction with a "green tomato" odor was relatively volatile. The typical tomato odor fraction contained alcohols, carbonyl compounds, and unsaturates and these were modified by many other odor fractions, some terpene in nature. Matthews (1961) tentatively identified furfural, acetaldehyde, and acetone in ripe tomatoes. By employing paper and thin-layer chromatographic separation of derivatives of the tomato volatiles. Schormüller and Grosch (1962, 1964) identified eleven volatile constituents. Glyoxal, methyl glyoxal, cinnamaldehyde, and hydrocinnamaldehyde were newly reported compounds. Citronellal, α-pinene, limonene and citral were reported by Hein and Fuller (1963). Nelson and Hoff (1967) tentatively identified 2-hexanal, tert-butanol, and methyl acetate. In a major study on flavor chemistry of the tomato, Dalal (1965) identified fifteen tomato volatiles by employing gas chromatography accompanied with thin-layer chromatography of the derivatives. He reported that isopentyl acetate, isopentyl butyrate, isopentyl isovalerate, and n-butyl hexanoate and n-hexyl hexanoate made important contributions to the tomato aroma. He also observed

that the concentrations of all the volatile components, with the exception of isopentanal and n-hexanol, increased with the maturity of the fruit grown either in the field or greenhouse. Isopentanal and n-hexanol were presumed to be responsible for the green tomato odor. Characteristically, field-grown tomatoes had more pronounced ripe tomato aroma than the greenhouse-grown tomatoes. The concentrations of the afore mentioned esters were higher in the field-grown tomatoes as compared to the greenhouse-grown tomatoes. It was interesting to note that in the artificially ripened tomatoes, the concentrations of volatile components were lower than in field- or greenhouse-grown tomatoes. Pyne and Wick (1965) identified twelve compounds and reported other unidentified compounds. These investigators vacuum distilled fresh ripe tomato pulp. The condensates were solvent extracted and the extract was concentrated. The concentrate was separated by gas chromatography and individual components were recollected. Identification of the compounds was based on retention data and infrared spectra. They noted that a synthetic mixture of these compounds yielded a green tomato aroma. A synthetic mixture of tomato volatiles prepared by Dalal (1965) also had green tomato aroma, however, when the mixture was added to a deodorized tomato puree, the ripe tomato aroma was perceived. Major contributors to typical ripe tomato aroma were suspected to be relatively high boiling components. This supposition was confirmed by Ryder (1966) as he indicated that during a gas chromatographic separation of tomato volatiles concentrate, a fraction which eluted between 150-225 C, was of interest in relation to ripe tomato aroma. Collectively, about 40 compounds (Table 1)

Table 1. Volatile components reported in fresh or processed tomatoes

Hydrocarbons	Aldehydes and ketones	Alcohols	Esters	Miscellaneous
Limonene[6]	Glyoxal[9]	Ethanol[5,6]	Methyl acetate[5]	Hydrogen sulfide[4,5]
∝-pinene[1,2]	Methyl glyoxal[7,9]	2-dimethyl ethanol[5]	Ethyl acetate[5,6]	Dimethyl sulfide[4,5]
	Acetaldehyde[3,5,6,7,10]	1-propanol[5,6]	Isopentyl acetate[1]	2-methyl pyrazine[7]
	Acetone[3,5,8]	2-propanol[6]	Isopentyl butyrate[1]	2,6-dimethyl pyrazine[7]
	2-butanone[5,6,9]	1-butanol[6]	Isopentyl isovalerate[1]	Pyridine[7]
	2,3-butanedione[5,9]	2-butanol[5]	1-butyl hexanoate[1]	
	3-methyl butanal[1,2,5,6,7,10]	2-methyl-1-propanol[1,5,6]	1-hexyl hexanoate[1]	
	2-pentanone[5,9]	1-pentanol[5,6]	Methyl salicylate[1,6]	
	2,3-pentanedione[7]	2-methyl-1-butanol[6]		
	Furfural[3,5]	3-methyl-1-butanol[1,6]		
	1-hexanal[1,5,6,8]	1-hexanol[1,5,6]		
	2-hexanal[6,8]	2-hexanol[5]		
	Methyl heptanone[9]	3-hexen-1-ol[6]		
	Citral[2]	2-methyl-3-hexanol[1]		
	Citronellal[2]			
	Benzaldehyde[1,6]			
	Phenylacetaldehyde[7]			
	Cinnamaldehyde[9]			
	Hydrocinnamaldehyde[9]			

[1] Dalal, 1965
[2] Hein and Fuller, 1963
[3] Matthews, 1961
[4] Meirs, 1966
[5] Nelson and Hoff, 1967
[6] Pyne and Wick, 1965
[7] Ryder, 1966
[8] Schormüller and Grosch, 1962
[9] Schormüller and Grosch, 1964
[10] Spencer and Stanley, 1954

have been identified and reported to contribute to tomato aroma. No single compound has been observed with a typical tomato odor.

EXPERIMENTAL

Material

Tomatoes (Cultivar: V. R. Moscow) were grown on Utah State University Experiment Station farms during the summer months of 1964 to 1967. Tomatoes were harvested at a red-ripe stage of maturation (degree 9; Dalal, 1965). They were washed and were packed into polyethylene bags. The packed tomatoes were frozen and stored for the experimental use.

Artificial ripening

Tomatoes were allowed to ripen in the field up to breaker stage (degree 5; Dalal, 1965) of maturation. A representative lot of tomatoes were harvested and brought immediately to the laboratory. They were washed and were placed in wooden trays keeping at least one inch space between fruits. The trays were kept in a room where temperature ranged from 20-22 C and a relative humidity from 32-34 per cent. Artificial ripening of the tomatoes was carried out under above conditions until a majority of the fruit turned red-ripe. The red-ripe tomatoes were packed in polyethylene bags, were frozen and stored for the experimental work.

Overripening

Field-ripe (degree 9; Dalal, 1965) tomatoes were washed and were placed in wooden trays keeping at least one inch space between fruits. The trays were kept for 36 hours in a room at a temperature range of 20-22 C and

a relative humidity of 32-34 per cent. During this period the tomatoes softened to some extent, however, the microbial quality of the fruits was not affected. These tomatoes were termed "overripe." The fruits were packed in polyethylene bags and were frozen and stored for further experimental work.

Extraction of tomato volatiles

Direct fruit extraction. The frozen tomatoes were dipped in distilled water (20-22 C) for about 5 min. The defrosted pericarp layer was removed from the fruits. This helped to exclude pectic substances and waxy material from the aroma extract. After the removal of the pericarp layer, the tomatoes were chopped finely and 35 g of ammonium chloride were added for each 100 g of fruit. The mixture was blended to a fine pulp in a Waring blendor. A pulp from 100 g of fruit was extracted four times with a total quantity of 240 ml purified ethyl ether in a separatory funnel. The solvent-pulp mixture was centrifuged at approximately 800 x g for 2 min. at room (20-22 C) temperature. The supernatant liquid (solvent phase) was carefully transferred to an Erlenmeyer flask. Three g of a decolorizer (Nuchar Attaclay, Wilkens Instrument and Research, Inc.) and 15 g of a desiccant--anhydrous magnesium sulfate were added to the collected solvent phase. The above mixture was kept for 4 hr. in a refrigerator (1 C) and was shaken periodically (6-8 times in 1 hr.). Occasionally it was necessary to add additional amounts of the decolorizing agent and the desiccant. The pale yellow or nearly colorless aroma extract was filtered to an evaporating dish through a Buchner funnel by using Whatman filter paper No. 1. The extract was concentrated to approximately 10 ml under

a hood. This was transferred to a glass vial and was allowed to concentrate to 20-30 µl. The vial was stored in the freezer comparment (-15 C) of a refrigerator. The aroma concentrate was gas chromatographed within the 72-hr. after the extraction from the fruits.

Steam distillation. A laboratory type steam distillation apparatus was used to distill volatiles from a tomato pulp. The pericarp layer of frozen tomatoes was defrosted by a distilled water dip and the layer was removed by hand. The tomatoes were chopped and blended to a pulp. Five hundred g of tomato pulp was put in 1000 ml distilling flask. A glass tube (1/4" o.d.) extension from a boiling water flask was inserted to 3 inches above the bottom of the distilling flask. A spiral tube water (20-22 C) condensor (22") was attached to the distilling flask vapor outlet. The condensed steam volatiles were recovered in a flask cooled with an ice-salt mixture. A total of 3000 ml of the condensate was collected. This was saturated with refined sodium chloride and was extracted three times with a total quantity of 350 ml purified ethyl ether in a separatory funnel. The upper layer (ethyl ether) in the separatory funnel was carefully collected in an Erlenmeyer flask. To the collected solvent phase was added 1 g of the decolorizer and 10 g of the desiccant. The mixture was kept in a refrigerator (1 C) and was shaken periodically. The decolorized extract was filtered to an evaporating dish through a Buchner funnel using Whatman filter paper No. 1. The extract was finally concentrated under hood to 100-150 µl and was stored in a freezer compartment (-15 C) of a refrigerator. The aroma concentrate was gas chromatographed within 72 hr. after the extraction from the fruits.

Preparation of GC column accessories

The procedure adopted by Dalal (1965) was followed in the preparation of GC column accessories.

Preparation of packing material for GC column. Ten g of Carbowax 20 M (liquid phase) was suspended in 100 ml of methanol in a beaker. Ninety g of Chromosorb W, acid washed, 60/80 M, (stationary phase) was gradually added to the solution. The solvent was then removed under vacuum in a rotatory (20-30 rpm) vacuum evaporator. The dried mixture was transferred to an evaporating dish and was kept overnight in a vacuum desiccator to remove the last traces of the solvent.

Technique for packing and conditioning the column. A stainless steel tube (1/4" (o.d.) x 10") was used to prepare a GC column. One end of the tube was sealed with glass wool. A small funnel was fixed at the top end of the tube. A steady flow of the packing material was passed through the funnel, while the tube was slowly vibrated by an electric vibrator. Later the tube was subjected to high speed vibrations to ensure thorough packing. When fully packed, the upper end of the tube was sealed with glass wool. The packed column was coiled around two iron poles 17 inches apart. Swagelock nuts and ferrules were fixed at both ends of the column. It was then baked in an oven at 220 C for 48 hrs. followed by 12 hrs. of baking at 250 C. Nitrogen (2 p.s.i.) was passed through the column to avoid oxidation of the liquid phase during baking. The passage of nitrogen also was used to drive off any volatile compound present in the column.

Removal of volatiles from aroma extracts
(Functional group subtraction)

Removal of alcohols. A boric acid column was prepared to remove the alcohols from the aroma concentrates (Ikeda et al., 1964). Boric acid (1.7 g) mixed with 20 ml of methanol was added to the previously prepared packing material (Chromosorb W 90 g + Carbowax 20 M 10 g). The amount of boric acid was adjusted to bring the final level at 3 per cent of total quantity of stationary and liquid phases. The solvent was removed under vacuum in a rotatory evaporator. The dried material was transferred to an evaporating dish and was left overnight in a vacuum desiccator to remove the last traces of the solvent. The material was packed in an aluminum column (1/2" (o.d.) x 18").

The boric acid column, when attached to the tail end of a GC column retained alcohols (as acid-alcohol complexes) from aroma concentrates during gas chromatographic separation. This resulted in a suppression of corresponding peaks compared to the original chromatogram.

Removal of carbonyls. The procedure adopted by Wolford, Alberding, and Attaway (1962) was followed to remove carbonyls from the aroma concentrates.

One and one-half g of citric acid and 50.5 g of disodium hydrogen phosphate (buffer solution pH 7) were dissolved in a 1000 ml steam distillate. Fifty g of sodium bisulfite were dissolved in the buffered distillate. The solution was kept in a sealed glass container for 18 hrs. at room (20-22 C) temperature. It was then saturated with refined sodium chloride and was

extracted with purified ethyl ether. The extract was decolorized, desiccated, and concentrated.

The procedure involved the use of the bisulfite addition reaction to form water soluble α-hydroxysulfonate derivatives. During the extraction, the water-soluble derivatives were not extracted by ethyl ether. This resulted in a subtraction of carbonyls from the original aroma extract and a subsequent suppression of corresponding peaks compared to the original chromatogram.

Gas chromatographic separation of aroma concentrates

A Micro-Tek Model GC 2500 R gas chromatograph with dual falme ionization detector connected to a Westronics recorder with a chart speed of 15 inches per hour was employed to separate the concentrated extracts.

Separation for recollecting the compounds representing the peaks of interest were carried out with a thermal conductivity detector system.

GC conditions employed during separation and collection of volatiles have been presented in Table 2.

Collection of separated fractions

As the fraction representing a peak of interest emerged out of the GC column, it was trapped in a glass-teflon U-tube cooled with dry ice-acetone mixture (-80 C). The U-tube was made of 22 inch glass tube (2 mm i.d., 3 mm o.d.) and to this a 3 inch teflon tube (3 mm i.d., 3.5 mm o.d.) was joined. The joint was leak-proofed with an epoxy cement. The teflon tube end was attached to the thermal conductivity detector outlet as the peak of interest emerged on the recorder chart. The tube was disconnected as the completion of the peak

Table 2. Conditions employed during the gas chromatographic analysis of tomato volatile extracts

Condition	Detector	
	Flame ionization	Thermal conductivity
Column	Stainless steel 1/4" (o.d.) x 10'	Stainless steel 1/4" (o.d.) x 10'
Liquid phase	10 per cent Carbowax 20 M	10 per cent Carbowax 20 M
Carrier gas	Helium, 40 p.s.i. 60 ml/min	Helium, 22 p.s.i. 20 ml/min
Air	15 p.s.i. 1.2 CFH	--- ---
Hydrogen	20 p.s.i. 40 ml/min	--- ---
Column condition	70 C Initial hold isothermal for 7 min.	70 C Initial hold isothermal for 7 min.
Temperature programming	5 C/min for 30 min. 220 C final hold	5 C/min for 30 min. 220 C final hold
Amount of sample injected	4 µl each time	50 µl each time
Input attenuation	1×10^2	---
Output attenuation	4X, 8X	4X, 8X, 32 X
Inlet block temperature	200 C	200 C
Ionization detector block temperature	240 C	---
Cell heater block temperature	---	240 C
Bridge current	---	300 ma

on the chart inferred total collection of the fraction. The procedure was repeated in the subsequent runs to have individual fractions quantitatively sufficient for infrared spectroscopy. Known amounts of authentic compounds were trapped by the above method and by comparison of peak areas, it was observed that the recovery was about 70 per cent.

Enrichment technique

The authentic compounds were purified by gas chromatographic separation on a Carbowax 20 M column. The purified compound was gas chromatographed with the aroma extract. When the peak represented by a tomato volatile and an authentic sample was coincident, the tomato volatile was tentatively identified as the authentic one.

Infrared spectroscopy

A Beckman IR 8 infrared spectrophotometer with a reference beam attenuator, a wedge cell, and a beam condenser attachment was employed to obtain infrared spectra of the trapped fractions.

The U-tube containing a fraction was rinsed three times with a total quantity of 100 µl of spectragrade chloroform. The extract was collected in a 1 ml teflon beaker and the solvent was evaporated under hood to approximately 10 µl. A sodium chloride cell (type D, Connecticut Instrument Corporation, 0.1 mm path length) was filled with the above solution by using a microsyringe.

A reference beam attenuator is an adjustable screen capable of attenuating radiant energy continuously over a wide range. It is excellent

for use in a reference beam when sample-beam transmission is so low that the range of built-in control is not sufficient to set the 100 per cent transmission line at a desired level.

A wedge cell is a sandwich of two sodium chloride windows assembled in a mount with a wedge-shaped spacer. The spacer permits a variable path-length range of 0.061 mm to 0.122 mm. A wedge cell was used to balance out solvent absorption.

A beam condenser is essentially a lens system utilized to get approximately five-fold reduction in cross-sectional area of sample beam, thereby resulting in a five-fold increase in the beam energy passing through the sample. The sample beam is magnified to an original size at a later stage. A beam condenser was employed to obtain distinct absorption characteristics, rather difficult to obtain otherwise with micro-quantity samples.

RESULTS AND DISCUSSION

The chromatogram patterns from the direct fruit extract and the steam distillate were similar. A typical aromagram from the field-ripe tomatoes showed 60 peaks (Figure 1a). The functional group property of individual peaks (Figure 1b and c) were derived by treating the steam distillate of the field-ripe fruits. Approximately, aldehydes and ketones represented 32 per cent, short-chain (C_3-C_6) alcohols 10 per cent, and hydrocarbons, long-chain alcohols, and esters were in 58 per cent of the total amount of the volatiles from the field-ripe tomatoes.

A comparison of volatiles concentrations of artificially ripe, field-ripe, and overripe tomatoes were made on chromatograms (Figure 2a, b, and c) from the fruit extracts. The concentrations of the major components are presented with analytical data in Table 3 (see page 38 of this report). The chromatographic peaks (Figure 1a) were categorized in two fractions. The peaks 1 to 20 designated as the "lower fraction" and the peaks from 21 to 60 were included in the "higher fraction." The column temperature at the dividing point was approximately 160 C.

Lower fraction

Major components in this class were identified by functional group analysis and by an enrichment technique using Carbowax 20 M column. The compounds of this group identified during the study were: 3-pentanol (peak 13)

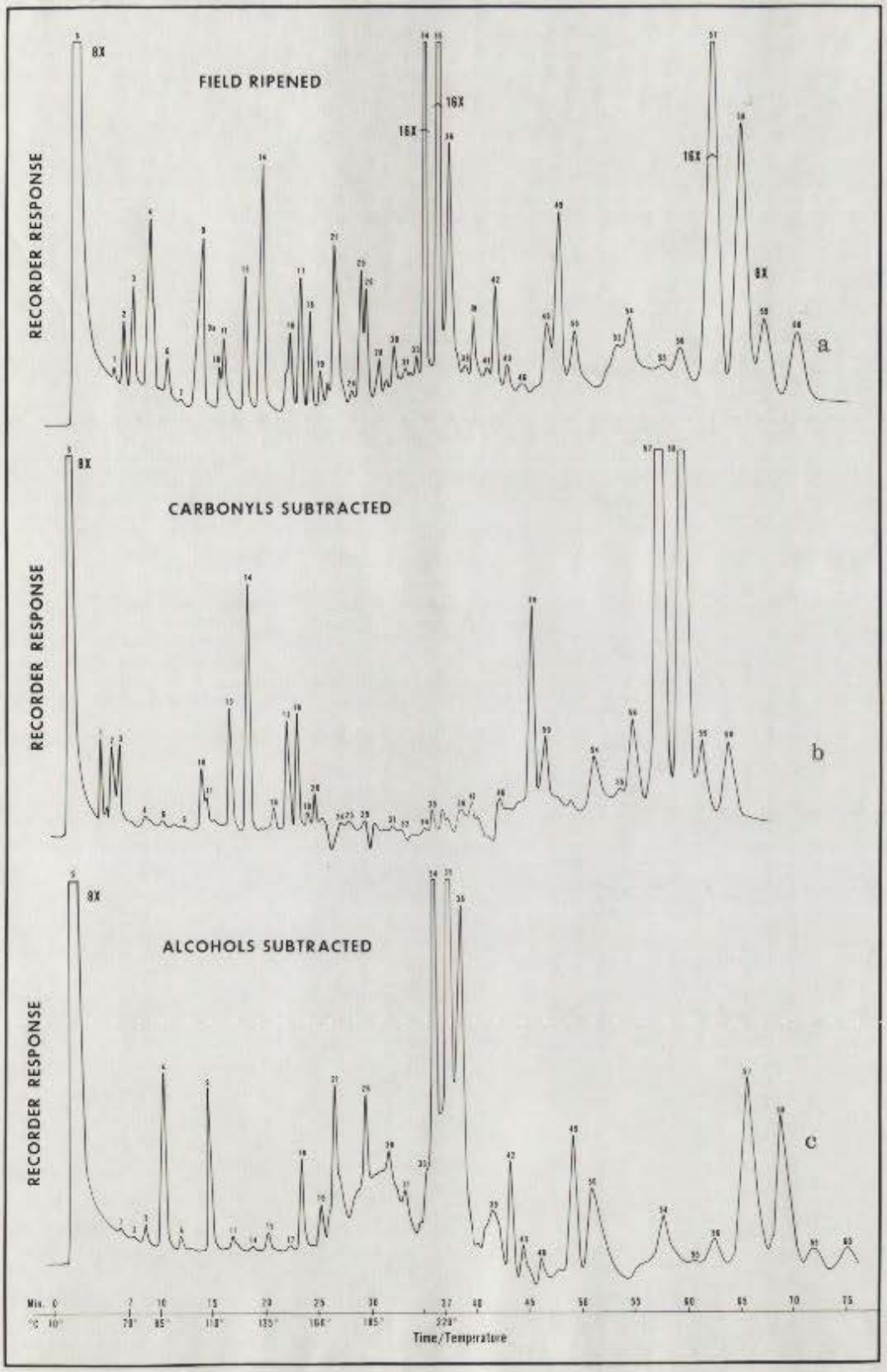

Figure 1. a. Volatiles from field-ripe tomatoes.
b. Tomato volatiles after removal of carbonyls.
c. Tomato volatiles after removal of alcohols.

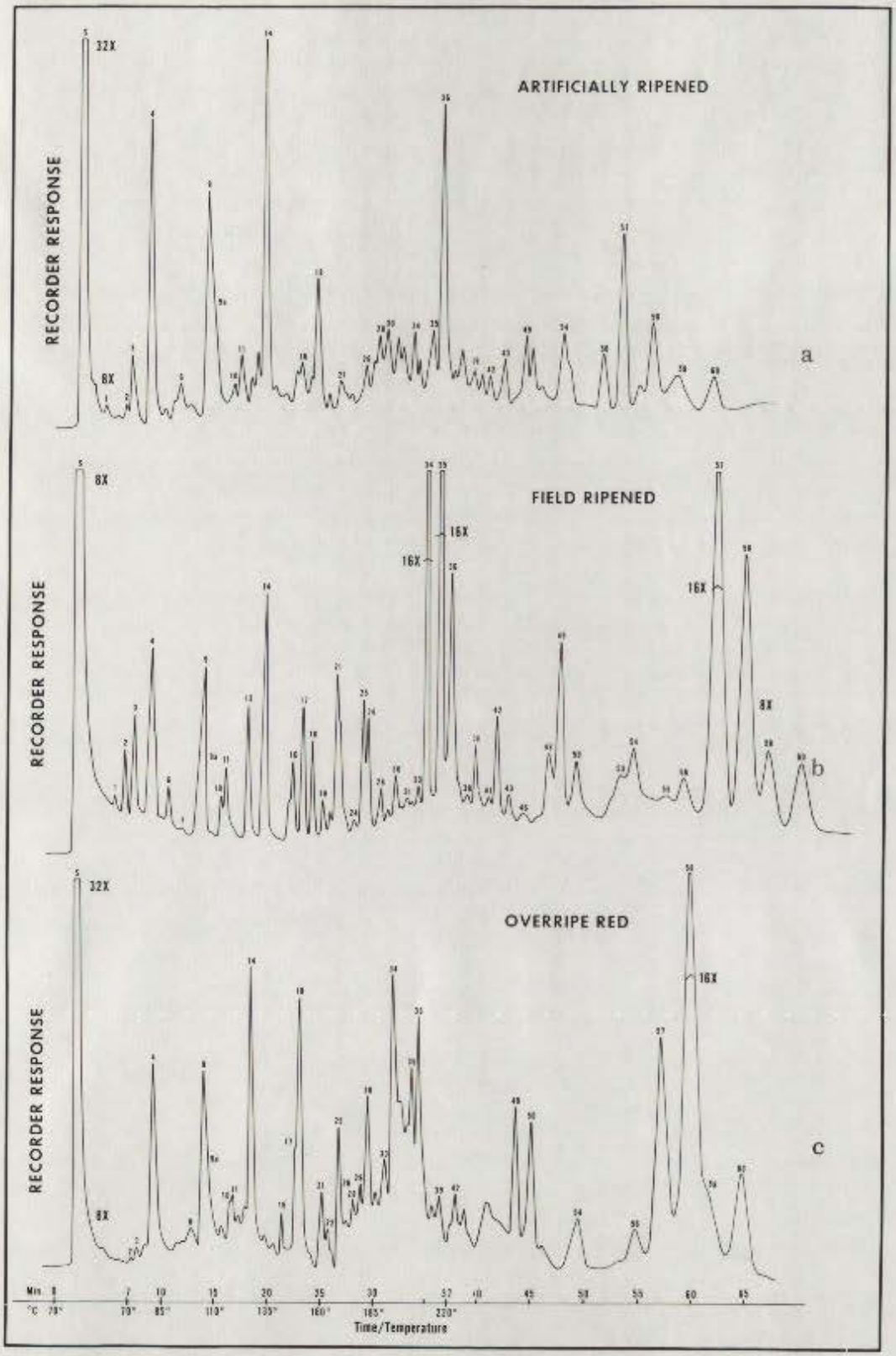

Figure 2. a. Volatiles from artifically ripe "braker" tomatoes.
b. Volatiles from field-ripe tomatoes.
c. Volatiles from overripe tomatoes.

and propyl acetate (peak 6). Among the alcohols that have been reported (Table 1) to contribute to the tomato aroma, the presence of 2-propanol (peak 2), 1-butanol (peak 10), 2-methyl-1-butanol (peak 11), 2-methyl-3-hexanol (peak 14) and 3-hexen-1-ol (peak 17) was confirmed. The contribution of carbonyls such as 3-methyl butanal (isovaleraldehyde) (peak 4), and 1-hexanal (peak 9) was established. The presence of isopentyl butyrate (peak 18) and isopentyl isovalerate (peak 19) also was confirmed.

Isovaleraldehyde, 1-hexanal, and 2-methyl-3-hexanol were found in higher concentrations in the artificially ripe tomatoes than in field-ripe fruits (Figures 2a and b, Table 3). Dalal (1965) noted that isovaleraldehyde and 1-hexanol contributed to the "green tomato" aroma and that these compounds were found to be in their maximum concentrations at the breaker and large-green stages of maturation, respectively. Notably, in our studies a pulp from the artificially ripe tomatoes lacked the characteristic "ripe tomato" aroma.

Yu (1967) observed that the crude enzyme preparations from green tomatoes synthesized lower carbonyls when alanine, leucine, and valine were used as substrates. The enzyme preparations at this stage were inactive for other amino acids. At a later stage of maturation, however, the enzyme preparations were active with a greater number of amino acids. This shows that as the fruit ripens, more intricate enzyme systems become operative and utilize several kinds of substrates. It also is interesting to note that C_6-compounds are the first to be formed in appreciable amounts when the enzyme preparations from fruits are treated with fatty acids as

substrates. Hultin and Proctor (1962) added oleic acid to a crude enzyme preparation from bananas and concluded that the fatty acid might be a precursor of 2-hexanal and 2-pentanone. Drawert et al. (1965) observed the biosynthesis of 1-hexanal from linolenic acid and that of 2-hexenal from linoleic acid when the fatty acids were employed as substrates for a crude enzyme preparation from apples. Both the aldehydes were formed in substantial amounts with no apparent intermediates. An oxidative degradation of the fatty acids had likely occurred.

In the light of the above experiments the higher concentration of lower fraction compounds, expecially the C_6-aldehyde and alcohol, in the artificially ripened tomatoes is striking. Apparently, these compounds are formed in their maximum concentrations during the early stages of maturation. Under the conditions of restricted nutrient availability and limited enzymatic activity during artificial ripening, the more flavorful "higher fraction" compounds are not synthesized appreciably. Thus, the earlier formed "lower fraction" compounds are major contributors to the total aroma volatiles.

Photosynthesis, as performed by green plants, has a major role in the biosynthesis of volatiles. Fulfillment of photosynthetic requirements such as light, water, carbon dioxide, temperature (climatic conditions) and an availability of soil nutrients for the cultivation of fruits or vegetables with desired organoleptic characteristics hardly need emphasis. For this investigation on tomato volatiles, the fruits were harvested during the summer months in the years 1964--1967. The field-ripe fruits of 1964 had volatiles in appreciably higher concentrations than the following years. This internal variation in the

fruits of the same cultivar can not be explained precisely except by the influence of photosynthetic reactions.

Higher fraction

This fraction comprised of long-chain alcohols, higher carbonyls, terpenes, and esters. These compounds were significantly more abundant in the field-ripe than the artificially ripe fruits (Figure 2a and b).

Carbonyls. The functional group property was verified by subtractive group analysis (Figure 1b). These compounds were tentatively identified by an enrichment technqiue. The major components of this group were: 1-nonanal (peak 21), benzaldehyde (peak 25), 1-decanal (peak 26), citral b (neral) (peak 34), and 1-dodecanal (peak 35). The contribution of benzaldehyde and neral (Table 1) as volatiles of tomato fruit was thus confirmed. The present study contributes the aliphatic carbonyls as volatile components of tomatoes. The aliphatic nature of these compounds suggests a possibility of their biogenesis from an oxidative degradation of fatty acids. The origin of benzaldehyde and other aromatic compounds (Table 1) could be traced back to shikimic acid which is derived from erythrose-4-phosphate and pyruvic acid, the components of carbohydrate metabolism.

Esters. By functional group analysis it was inferred that the major components (peak 40-60) of this fraction were neither short-chain alcohols nor carbonyls. Thus they could be terpene hydrocarbons, esters, or long-chain alcohols.

The infrared spectra of the major tomato volatiles are presented in Figures 3, 4, and 5. The interpretation of the infrared spectra were made by comparative and confirmational study of the works of Nakanishi (1962) and Rao (1963). Absorptions in 3000 cm^{-1} region were assigned to symetrical and asymetrical stretching of alkanes. Asymetrical deformation around 1450 cm^{-1} and symetrical deformation of 1365 cm^{-1} of alkanes were decisive. Absorption between 1430-1420 cm^{-1} exhibited the terpene character of the compounds. Alkane groups show absorptions in the 1300-1100 cm^{-1}. These are caused by CH_2 wagging, CH_2 twisting, and CH_3 rocking. They are usually weak, but when the molecule contains a polar group they are sometimes strong, in certain cases the strongest in the spectrum. Other skeletal vibrations also occur in this region and therefore these bands are of little value in the interpretation. A strong carbonyl absorption at 1710-1705 cm^{-1} confirmed that these compounds are esters. A strong absorption at 1690 cm^{-1} (Figure 3 and 5) as a shoulder exhibited the presence of an α, β-unsaturation in the molecules. The weak doublet at 1365 cm^{-1} and 1340 cm^{-1} could be inferred as geminal dimethyl absorptions. Thus these compounds were tentatively identified as terpene esters.

The infrared spectra of the tomato volatiles were matched with the ones of the GC purified authentic compounds. The volatiles were identified as linalyl propionate (peak 56), citronellyl butyrate (peak 58), and geranyl butyrate (peak 60). The results were confirmed by enriching the tomato extract with the purified authentic compounds. The infrared spectra of the tomato volatiles represented by peaks 57 and 59 had some discrepancies in

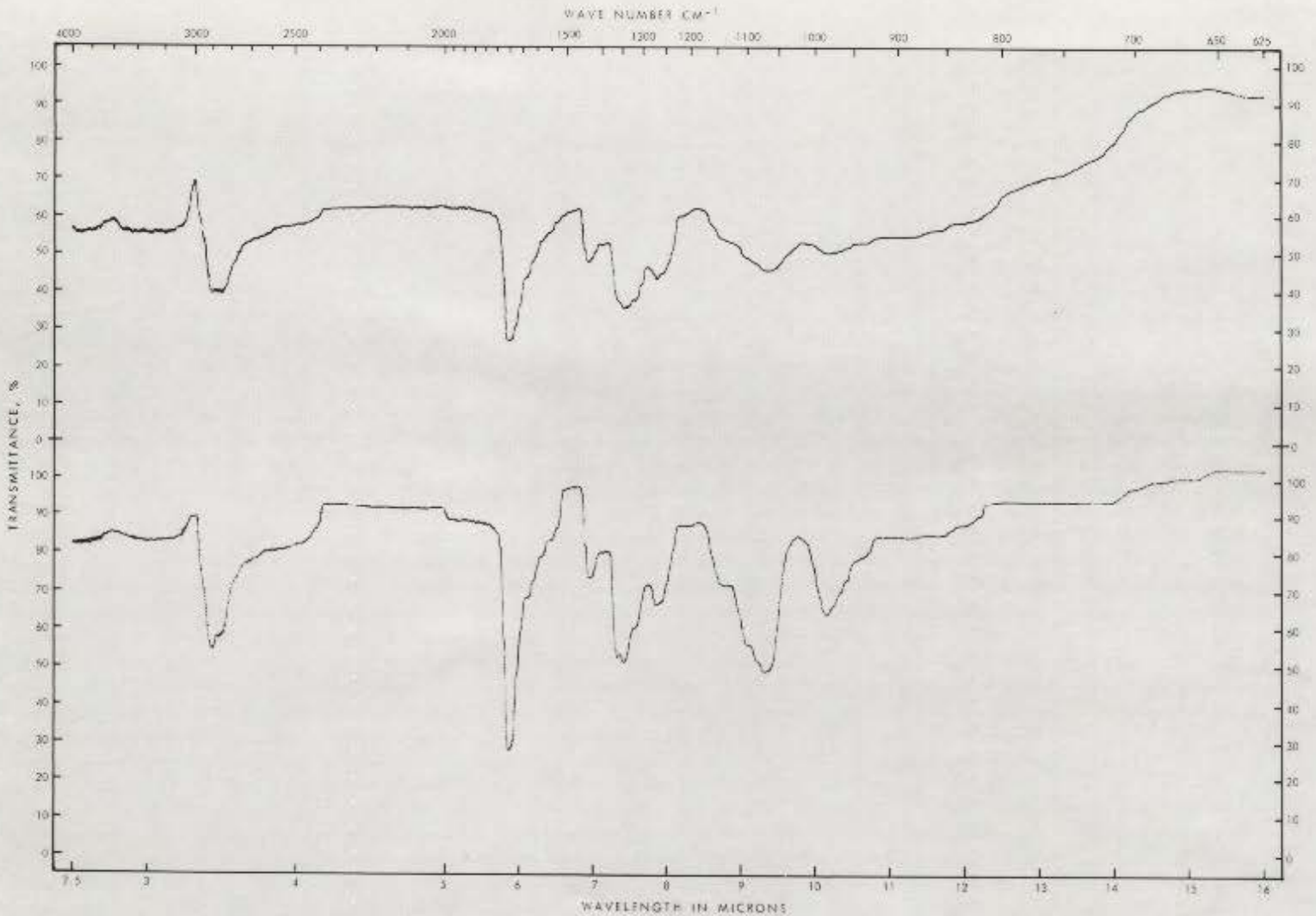

Figure 3. Infrared spectra of the tomato volatile represented by the peak 56 (top), and the authentic sample of linalyl propionate (bottom).

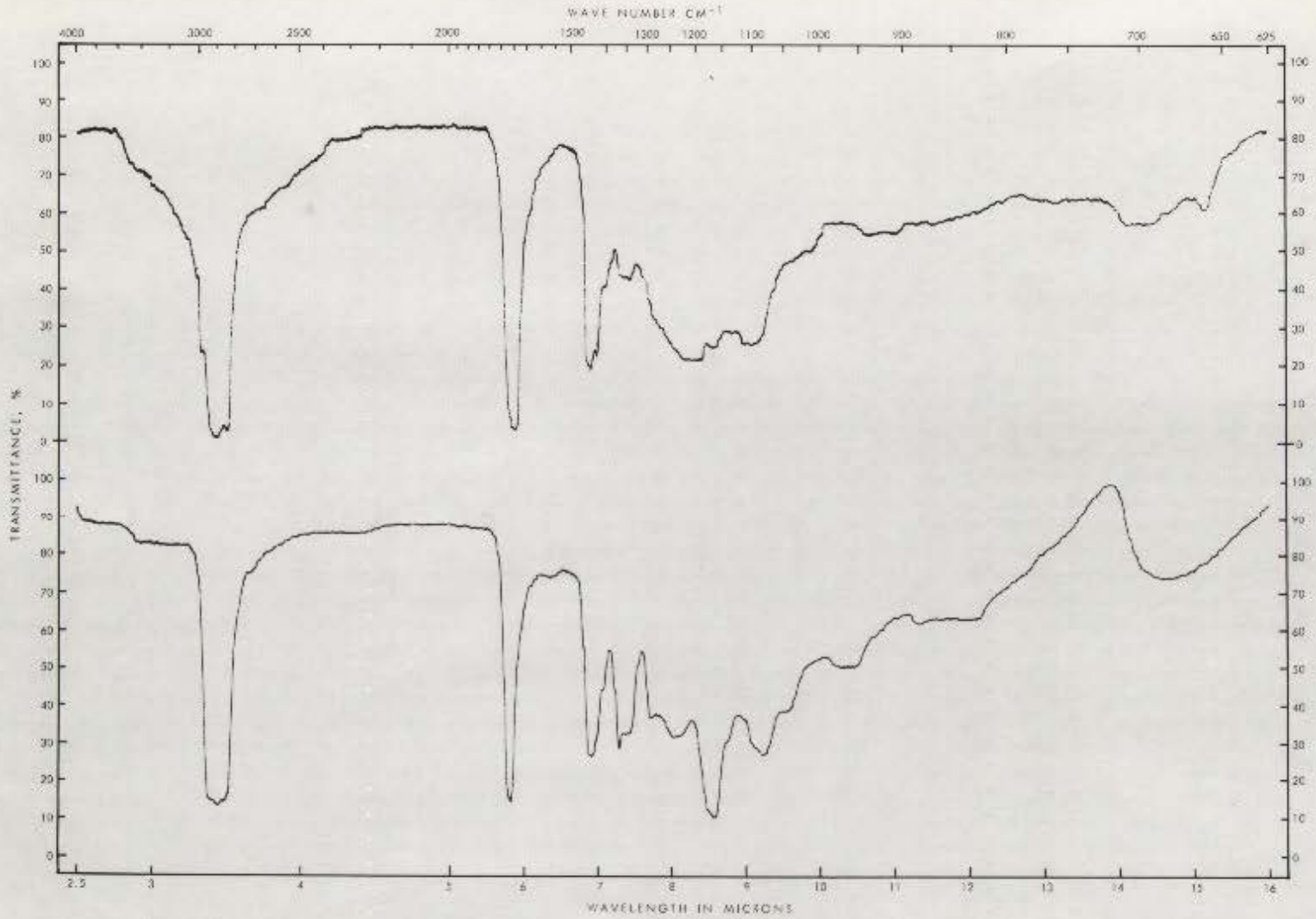

Figure 4. Infrared spectra of the tomato volatile represented by the peak 58 (top), and the authentic sample of citronellyl butyrate (bottom).

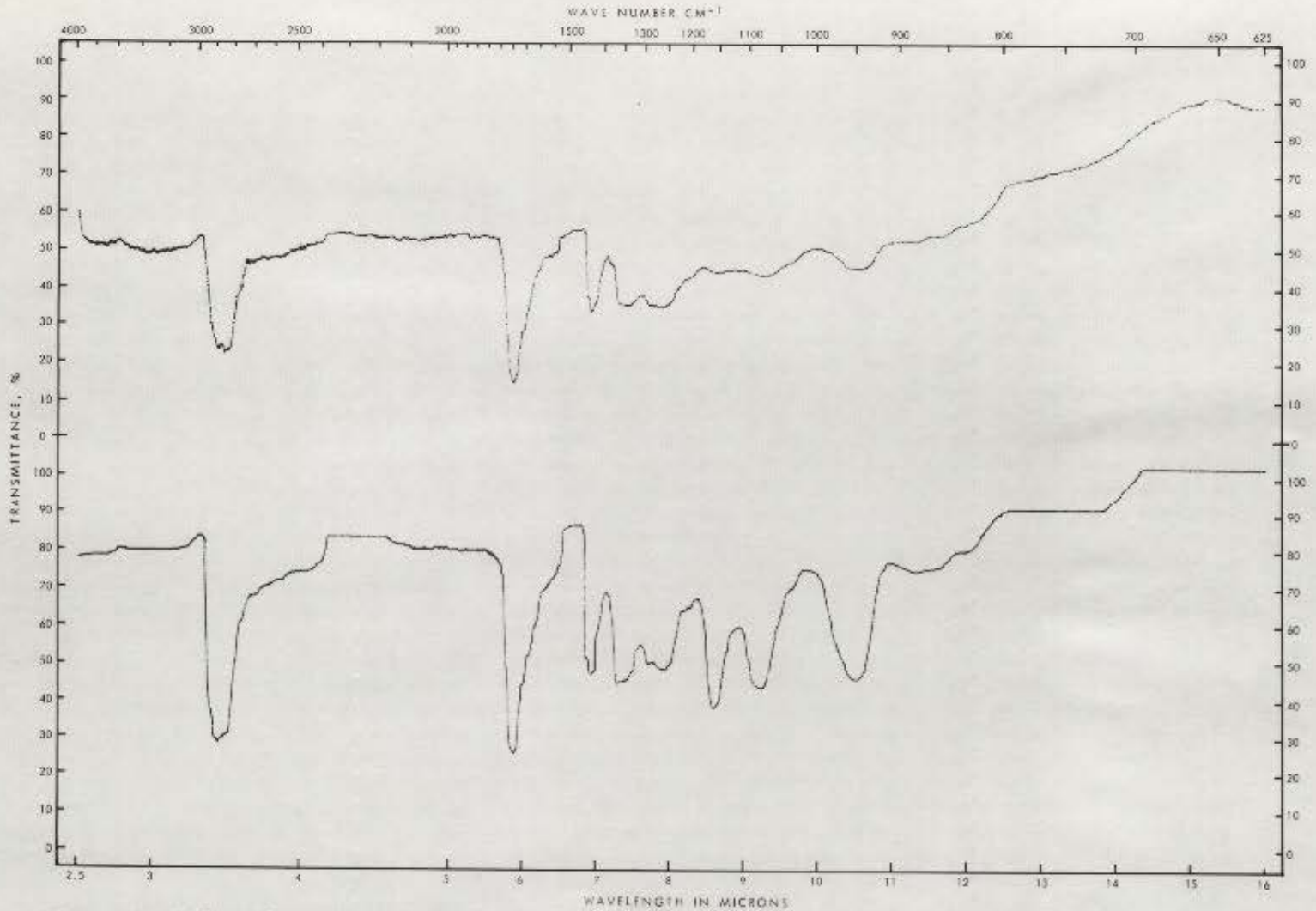

Figure 5. Infrared spectra of the tomato volatile represented by the peak 60 (top), and the authentic sample of geranyl butyrate (bottom).

the band area 1300-1100 cm^{-1} when compared to the authentic spectra of citronellyl propionate and geranyl acetate, respectively. The volatiles were thought of as the authentic compounds by an enrichment technique. However, as to the above discrepancies which could be due to the sample dilution or the malfunctioning of the filter, the volatiles have been tentatively identified as citronellyl propionate (peak 57) and geranyl acetate (peak 59).

Biogenesis of terpenoids

Spencer and Stanley (1954) were perhaps the first to give an indication of the presence of terpene compounds in tomato volatiles. They reported that a comparatively nonvolatile fraction which contained terpenes was to a greater extent reminiscent of "ripe tomato" aroma. The present study confirms the observation. α-Pinene (Dalal, 1965, Hein and Fuller, 1963), limonene (Pyne and Wick, 1965), citral and citronellal (Hein and Fuller, 1963) have been shown to be the components of tomato volatiles. The present study contributes linalyl propionate, citronellyl propionate, citronellyl butyrate, geranyl acetate and geranyl butyrate, to the terpenoids of the tomato fruit. The concentration of terpene compounds in higher plants is critically dependent of varietal and seasonal (photosynthetic) characteristics (Haggen-Smit, 1948). The major contribution of the terpenoids to ripe tomato aroma is evident. In this respect, it would be important to discuss the possible metabolic pathways for the formation of these compounds.

The biogenetic isoprene rule of Ruzicka (1953) implies that all terpenoids should have a common precursor. Mevalonic acid was shown

(Tavormina, Gibbs, and Huff, 1956) to be a specific precursor for each group of terpenes. Among the monoterpenes, mevalonic acid has been demonstrated as a precursor of citronellal in Eucalyptus citriodora, cineole in Eucalyptus globulus (Birch et al., 1959), carvone and limonene in Anethum graveolens (Sandermann and Bruns, 1965), α-pinene in Pinus attenuata (Stanley, 1958), and thujone in Thuja occidentalis (Sandermann and Schweers, 1962).

The biosynthetic pathway from acetate to mevalonate and thence to isopentenyl pyrophosphate is well established (Figure 6) for yeast and mammalian systems. Isopentenyl pyrophosphate was identified as an active intermediate of terpene biogenesis by Chaykin et al. (1958). The features of the pathway involve the sequential formation of acetoacetyl co-enzyme A and hydroxymethylglutaryl co-enzyme A prior to the formation of mevalonate. Phosphorylation and decarboxylation are involved in the conversion of mavalonate to isopentenyl pyrophosphate. The later is isomerised to dimethyl allyl pyrophosphate by an enzyme system which is inhibited by iodoacetamide. Dimethyl allyl pyrophosphate then forms geranyl pyrophosphate which has long been recognized as a progenitor of the monoterpenes. A single monoterpene goes through many interconversions resulting in the synthesis of different terpenoids. The importance of a biogeretic isoprene unit in the biosynthesis of terpenoids as submitted by Ruzicka (1953) is evident. It is also possible that the dimethyl allyl pyrophosphate which acts as a starter unit for the biosynthesis of terpenoids, may in certain circumstances, originate from other pathways (Hanson, 1967). β-methyl crotonic acid was specifically

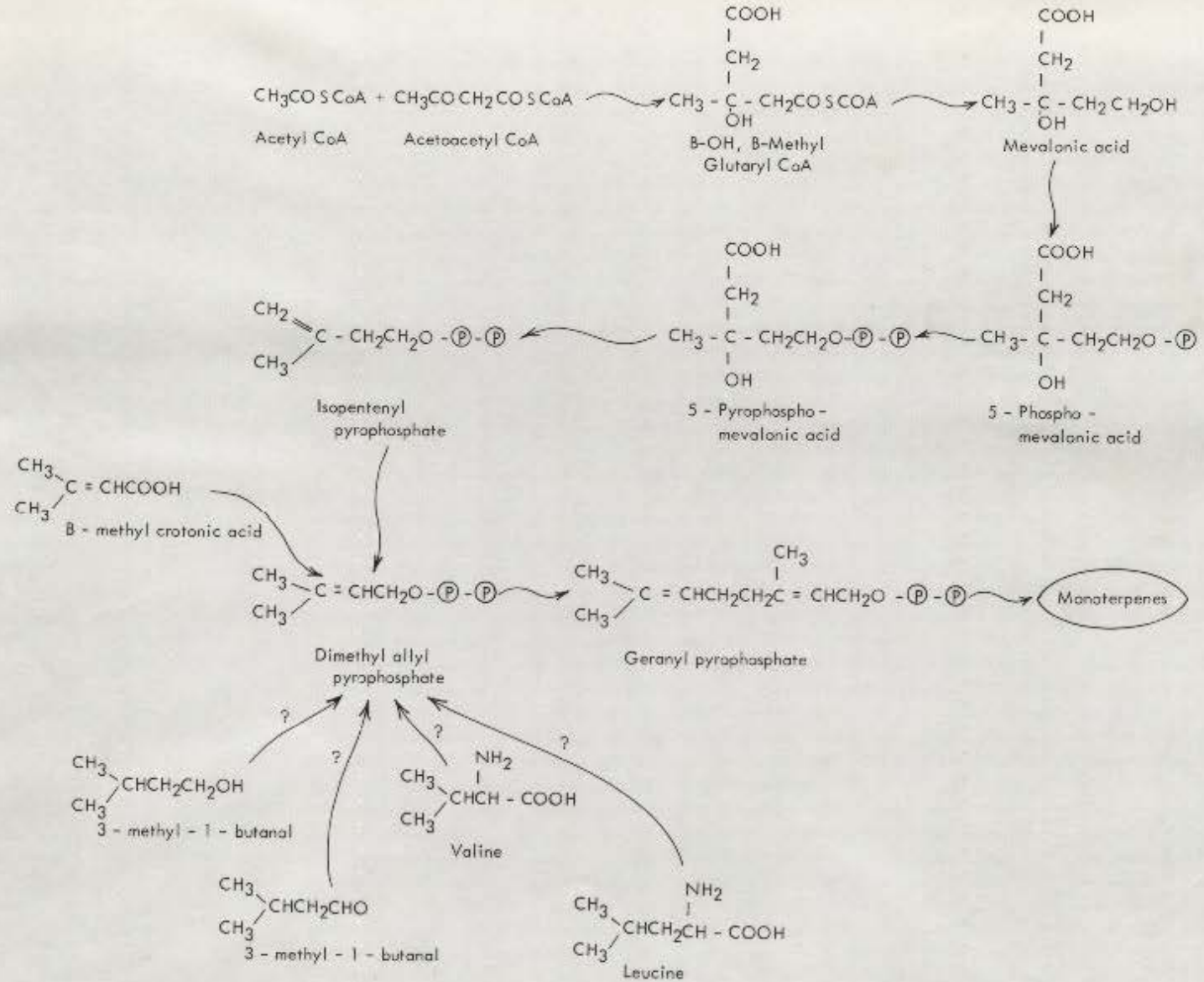

Figure 6. Mechanism of the biogenesis of monoterpenes.

incorporated into pulegone (Sandermann and Stockmann, 1958) in Mentha pulegium. Valine, an amino acid with an isopentane structure, was thought to be a precursor of terpenoids (Hultin and Proctor, 1962) in bananas. These findings support the presumption that other pathways lead to dimethyl allyl pyrophosphate in the biogenesis of terpenoids.

In the light of the above presumption the author visualizes other isopentanes such as isoamyl alcohol, isovaleraldehyde, isovaleric acid, and senecoic acid as precursors of terpenoids through dimethyl allyl pryophosphate. Valine and leucine, the amino acids with isopropyl group could form terpenoids in a similar manner. Dalal (1965) reported the presence of isopentanal, isopentanol, and various isopentyl esters, in tomato volatiles. Some of these compounds exhibited "green leafy" odor. Along this line the author suggests as a possibility, that the isopentane parts of these compounds can be intermediates in the formation of more flavorful terpenoids of tomato aroma.

The established pathway for the biogenesis of terpenoids expresses acetyl coenzyme A as a building unit. An oxidative degradation of fatty acids serves as a major source of acetyl coenzyme A. It is also known that the tomatoes at a red ripe stage of maturation contains very minute amounts of lipid fraction. It is highly probable that the lipid fraction is utilized in the formation of terpenoids during the ripening.

Chemical changes during overripening

A visual comparison of the aroma chromatograms from the field-ripe

(Figure 2b) and overripe tomatoes (Figure 2c) distinguishes the changes in the concentrations of individual compounds during overripening. The actual changes have been presented in Table 3. The concentration of 2,3-butanedione (diacetyl) (peak 30), isopentyl butyrate (peak 18) citronellyl butyrate (peak 58), and geranyl butyrate (peak 60) increased. Simultaneously, the amounts of alcohols, aldehydes, acetates and propionates generally decreased during overripening. This suggests possible formations of diacetyl and butyric acid at the onset of a senescent period in the fruit. Explanations on the probable mechanisms for the above changes are discussed herewith.

Explanations of the formation of 2,3-butanedione. Diacetyl and acetylmethylcarbinol are metabolic products of yeast growth (Saccharomyces) and certain acid-tolerant bacteria, such as Lactobacillus and Leuconostoc (Ledingham and Neish, 1954). The microbial quality of apple juice (Fields, 1964), citrus products (Hill and Wenzel, 1957), and wine (Fornachon and Lloyd, 1965) has been correlated with the amounts of diacetyl and acetylmethylcarbinol present in the products. Off-flavor, typical of cultured dairy products, persists when the diacetyl content becomes sufficiently high. Apparently, the presence of diacetyl in cultured dairy products is desirable (Eakle, 1963). The presence of diacetyl in the above products is an indication of microbial growth. The onset of fermentation could be caused by yeast or acid-tolerant bacteria. The formation of diacetyl by a yeast enzyme (Juni, 1952) and by bacteria (Fornachon and Lloyd, 1965) is thought to arise from pyruvic acid. The mechanism is explained in Figure 7a and 7b. In the yeast

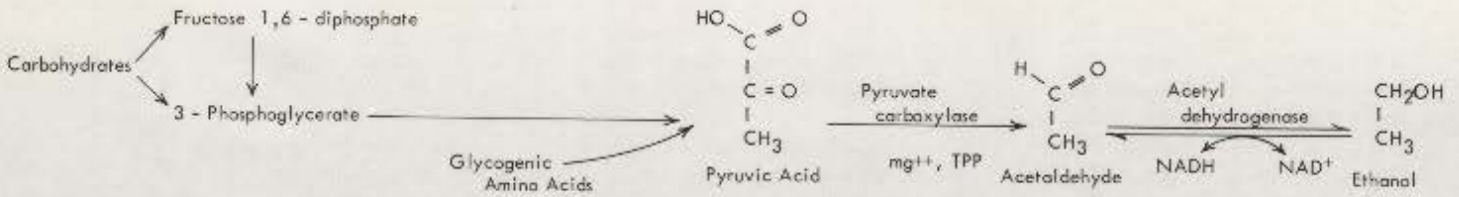

Figure 7a. Mechanism of the formation of pyruvic acid and acetaldehyde.

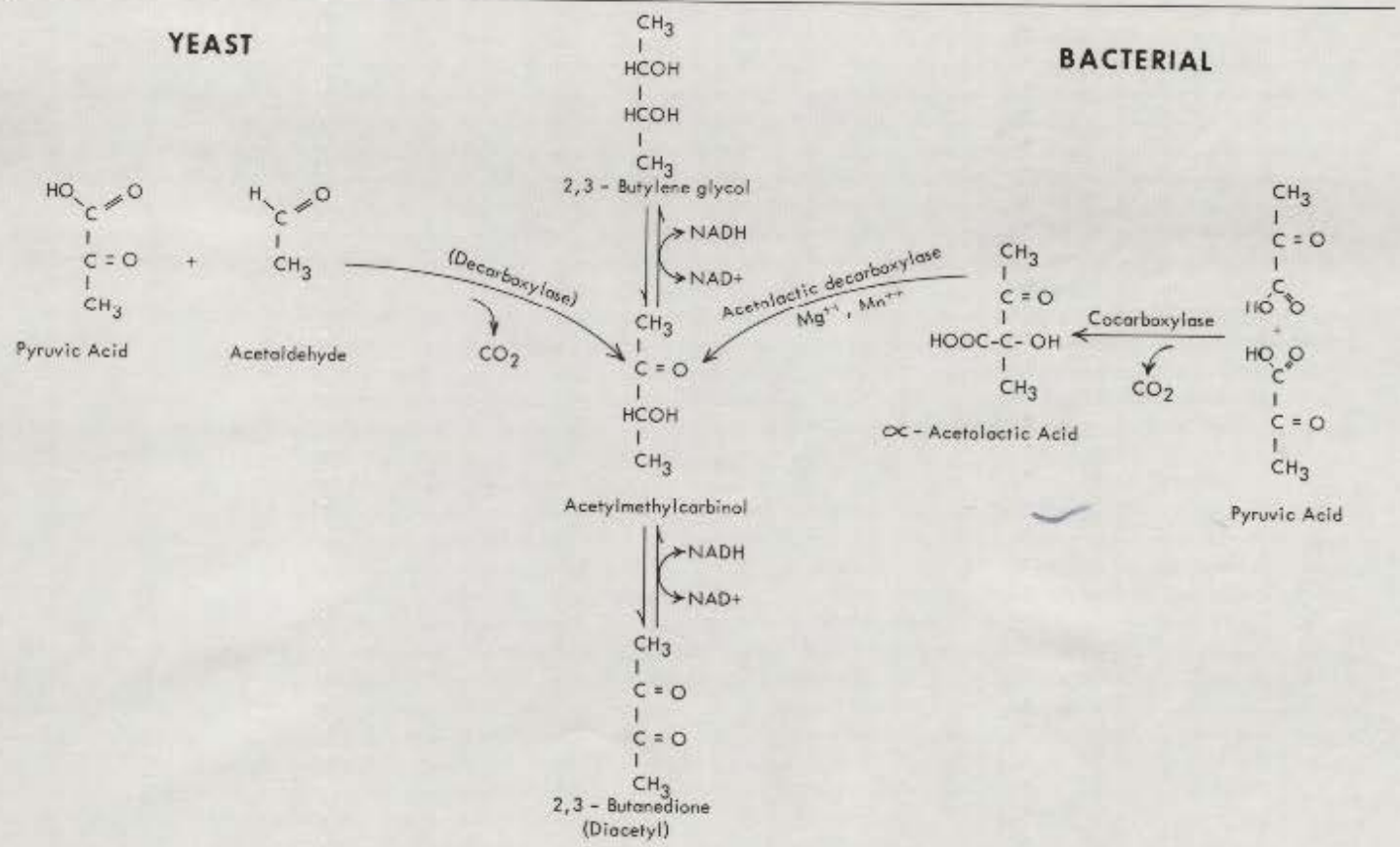

Figure 7b. Mechanism of the formation of diacetyl by yeast and bacteria.

enzyme system, pyruvic acid seems to be incorporated with acetaldehyde while the bacterial type fermentation originates from the coupling of two pyruvic acid molecules. Carbohydrates, through glycolysis and glucogenic amino acids could serve as the main sources of pyruvic acid.

It was indeed striking to observe that diacetyl in the fresh field-ripe tomatoes (Figure 1a) amounted to 0.76 ppm (Table 3). The presence of diacetyl in the tomato also had been reported previously (Table 1). At this stage of investigation it would be rather premature to state that the enzyme systems, such as decarboxylase and cocarboxylase, very similar to those of certain yeasts and bacteria are operative during the ripening of the tomato fruit. However, the possibility of such enzyme systems is postulated when the availability of the substrates pyruvic acid and acetaldehyde would be abundant through metabolic events during fruit ripening. The increased concentration of diacetyl during overripening would be the result of increased activity of the inherent mechanism rather than a microvial growth.

Explanation of the formation of butyric acid. The increased concentration of isopentyl butyrate, citronellyl butyrate, and geranyl butyrate can only be explained as a result of butyric acid production with subsequent esterification with the alcohol moieties during overripening. Ledingham and Neish (1954) especially noted butyric and fermentation by Cl. acetobutylicum and Cl. butyricum. Butyric acid fermentation by Cl. pastuerianum in commercially canned pears and tomatoes also was reported by Bowen et al. (1954). However, on the basis of cultural relations, the butyric acid producing anaerobes isolated from spoiled tomatoes were related more closely to Cl. butyricum than to Cl.

pasteurianum (Clark and Dehr, 1947).

Davies (1943) studied the enzyme acetoacetic decarboxylase of Cl. acetobutylicum. The enzyme system was specific for acetoacetic acid to derive butyric acid and it was also observed that the addition of pyruvic acid to the substrate had no effect on the final concentration of the fatty acid. The projected pathway (Umbreit, 1960) from even-numbered carbon atom fatty acids and ketogenic amino acids is presented in Figure 8. The emphasis is given to the fact that the above butyric acid esters are present in significant concentrations in the field-ripe tomatoes. The overripe fruits were microbially unaffected. Thus the increase in the concentrations of the butyrates could only be due to an internal mechanism which is different from the one connected with butyric acid fermentation. At this stage of investigation, however, it would be difficult to pinpoint the enzyme systems and substrates in the formation of butyric acid in tomatoes.

Changes in the concentrations of alcohols and aldehydes. Partial oxidative degradation of alcohols and aldehydes to respective acids is thought to occur during overripening of the fruits. This is difficult to prove, however, because organic acids are difficult to extract with ethyl ether from an aqueous system as they are more soluble in water than in the solvent. Carbowax 20 M which is used as a liquid phase in the gas chromatographic column, has a great number of hydroxyl groups in a molecular chain. Any free acid present in the volatiles extract would be esterified with the hydroxyl groups during gas chromatographic separation and remain attached to the liquid phase. This reaction would be particularly probable at the column temperature (70-220 C)

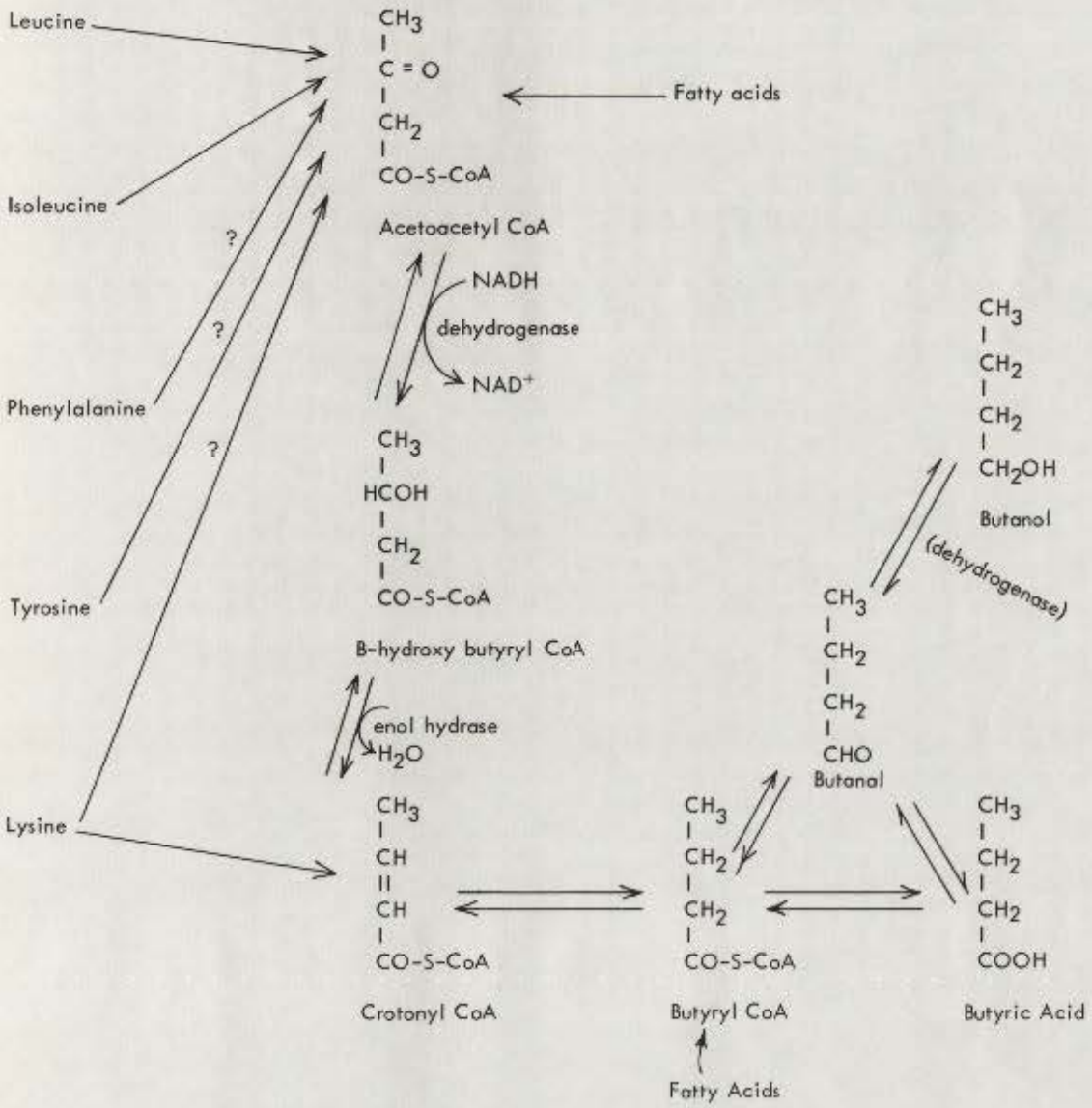

Figure 8. Mechanisms of the formation of butyric acid.

during the separation. The water formed in the process of esterification would evaporate easily with no recording by the water-non-active flame ionization detector. In addition, the absence of additional peaks suggests that the compounds have been converted to respective acids rather than any other functional character which should have appeared on the chromatogram. It is also probable that the acids were utilized to some extent in the formation of respective esters, especially butyrates.

Table 3. Tomato volatiles and the analytical data

Peak[a]	Identification method[b]	Compound	Concentration (ppm in the fruit)		
			Artificially ripe	Field ripe	Overripe
1	FG	Unidentified alcohol	0.56	0.41	0.30
2	FG, ET	2-Propanol[c]	0.45	0.93	0.24
3	FG	Unidentified alcohol	1.00	1.28	0.30
4	FG, ET	3-Methyl butanal[c]	7.16	2.22	2.38
5	FG	Unidentified ester	0.11	0.76	0.98
6.	FG, ET	Propyl acetate[d,t]	1.57	0.58	0.79
7-8	FG	Unidentified esters	----	----	----
9	FG, ET	1-Hexanal[c]	5.93	2.51	2.68
10	FG, ET	1-Butanol[c]	1.12	0.35	0.67
11	FG, ET	2-Methyl-1-butanol[c]	1.57	0.76	0.91
12	FG	Unidentified ester	----	----	----
13	FG, ET	3-Pentanol[d,t]	1.57	1.17	0.61
14	FG, ET	2-Methyl-3-hexanol[c]	7.94	2.51	2.68
15	FG	Unidentified carbonyl	0.56	0.23	0.30
16	FG	Unidentified alcohol	1.23	0.70	0.55
17	FG, ET	3-Hexen-1-ol[c]	0.78	1.28	0.79
18	FG, ET	Isopentyl butyrate[c]	2.80	0.82	3.17
19	FG, ET	Isopentyl isovalerate[c]	0.11	0.23	0.18
20	FG	Unidentified ester	0.10	0.17	0.12
21	FG, ET	1-Nonanal[d,t]	0.45	1.46	0.92

Table 3. Continued

Peak[a]	Identification method[b]	Compound	Concentration (ppm in the fruit) Artificially ripe	Field ripe	Overripe
22	FG	Unidentified carbonyl	0.11	0.64	0.30
23-24	FG	Unidentified esters	----	----	----
25	FG, ET	Benzaldehyde[c]	0.22	1.17	1.40
26	FG, ET	1-Decanal[d,t]	0.67	1.05	0.67
27-29	FG	Unidentified esters	----	----	----
30	FG, ET	2,3-Butanedione[c]	1.68	0.46	2.56
31	FG	Unidentified carbonyl	1.23	0.46	0.31
32	FG	Unidentified ester	----	----	----
33	FG	Unidentified carbonyl	1.67	0.64	1.64
34	FG, ET	Citral b(Neral)[c]	1.56	5.95	3.66
35	FG, ET	1-Dodecanal[d,t]	2.24	7.71	2.68
36	FG	Unidentified carbonyl	7.50	3.04	3.00
37-38	FG	Unidentified esters or alcohols	----	----	----
39	FG	Unidentified carbonyl	1.67	1.23	1.34
40-41	FG	Unidentified esters or alcohols	----	----	----
42	FG	Unidentified carbonyl	0.78	1.63	1.28
43-47	FG	Unidentified esters or alcohols	----	----	----
48	FG	Unidentified carbonyl	----	----	----

Table 3. Continued

Peak[a]	Identification method[b]	Compound	Concentration (ppm in the fruit) Artificially ripe	Field ripe	Overripe
49	FG	Unidentified ester or alcohol	1.56	3.44	2.56
50-55	FG	Unidentified esters or alcohols	----	----	----
56	FG, ET, IR	Linalyl acetate[d]	2.80	2.28	1.22
57	FG, ET, IR	Citronellyl propionate[d, t]	5.82	17.87	7.14
58	FG, ET, IR	Citronellyl butyrate[d]	3.80	8.76	19.03
59	FG, ET, IR	Geranyl acetate[d, t]	2.78	2.92	2.38
60	FG, ET, IR	Geranyl butyrate[d]	1.68	2.80	4.02

[a] Peak numbers of chromatogram in Figure 1a.
[b] FG, functional group analysis.
ET, enrichment technique.
IR, infrared spectral data.
[c] Previously reported (Table 1).
[d] Reported during this study.
[t] Tentative identification.

SUMMARY AND CONCLUSIONS

Studies were conducted to identify major volatile components of tomato fruit. Simultaneously, the confirmation was extended to the reported tomato volatiles. The volatile compounds of field- and artificially-ripe fruits were compared qualitatively as well as quantitatively, and the differentiation was reasoned biogenetically. The changes occurring in the volatile components of the fruit at the onset of senescence also were delineated. The results of the investigations are summarized below.

Sixty peaks were observed in a typical chromatogram from the field-ripe tomatoes. The functional group properties of individual peaks were derived. It was approximated that short-chain (C_3-C_6) alcohols represented 10 per cent, aldehydes and ketones 32 per cent, and hydrocarbons, long-chain alcohols, and esters were in 58 per cent of the total amount of the volatiles from the field-ripe fruits.

The peaks in the chromatogram were arbitrarily divided into two groups, viz.: a "lower fraction" and a "higher fraction." The column temperature at the mid-point was 160 C. The "lower fraction" contained short-chain alcohols, aldehydes, ketones, and esters. The compounds which were tentatively identified during the course of this investigation were 3-pentanol and propyl acetate. The presence of the alcohols--2-propanol, 1-butanol, 2-methyl-1-butanol, 2-methyl-3-hexanol, and 3-hexen-1-ol--in tomato volatiles was confirmed. Among carbonyls, the volatiles extract from tomato fruit

contained 3-methyl butanal, 1-hexanal, benzaldehyde, and 2,3-butanedione (diacetyl). The presence of isopentyl butyrate and isopentyl isovalerate, which was reported previously, also was confirmed.

The "higher fraction," which contained terpenoids in large quantities, was contributory to ripe tomato aroma. Linalyl acetate, citronellyl butyrate, and geranyl butyrate were identified as the major components among the terpenoids. Other compounds of this group were 1-nonanal, 1-decanal, 1-dodecanal, geranyl acetate, and citronellyl propionate. These compounds were tentatively identified. The study confirmed the presence of citral as a component of tomato volatiles. The importance of the terpenoids as contributory to ripe tomato aroma was evident. Biogenesis of these compounds is a complex phenomenon. An attempt has been made to postulate the precursors of these volatile compounds.

Comparisons of volatile concentrations of field- and artificially-ripe tomatoes were made. In the latter category of fruits the concentrations of 1-butanol, 3-pentanol, 2-methyl-3-hexanol, 3-methyl-butanal, 2,3-butanedione, propyl acetate, isopentyl butyrate, and other unidentified carbonyls were higher than those observed in the field-ripe fruits. These short-chain compounds, especially the C_4-C_6 moities, probably are formed in their maximum concentrations during the early stages of maturation. Under the conditions of restricted nutrient availability and limited enzymatic activity during artificial ripening, the long-chain compounds are not synthesized appreciably. The concentrations of some of these short-chain compounds may be to a level of masking the effects of more desirable compounds contributory to ripe tomato aroma. Notably, a

pulp from the artificially ripe fruits lacked the characteristic ripe tomato aroma. The concentrations of the compounds representing the "higher fraction" generally were low in the artificially ripe tomatoes as compared to the field-ripe ones. This may indicate the importance of the long-chain carbonyls and the terpene esters for ripe tomato aroma. An attempt has been made to theorize the mechanisms of the biogenesis of these compounds.

The concentrations of the volatiles from field-ripe and overripe tomatoes were compared. During overripening the amounts of alcohols, aldehydes, ketones, acetates, and propionates generally decreased. However, the concentrations of diacetyl and those of butyric acid esters--isopentyl butyrate, citronellyl butyrate, and geranyl butyrate--increased. It was assumed that at the onset of senescence the metabolic pathways for the formation of diacetyl and butyric acid were highly operative in tomato fruit. The mechanisms of these pathways were postulated.

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VITA

Bharat Manu Shah

Candidate for the Degree of

Doctor of Philosophy

Dissertation: Effects of Ripening Processes on Chemistry of Tomato Volatiles

Major Field: Food Science and Technology

Biographical Information:

Born at Kadod (Gujrat), India, December 13, 1939, son of Manubhai and Indumati Shah; attended elementary school in Kadod, India; graduated from Sarvajanik High School, Navapur, India in 1956; received the Bachelor of Science degree from University of Bombay, Bombay, India with a major in chemistry and minor in physics in 1960; received the post-graduate diploma in Food Technology from Central Food Technological Research Institute, Mysore, India in 1962; received Master of Science degree from University of Tennessee, Knoxville, Tennessee with a major in Food Technology in 1965; completed requirements for the Doctor of Philosophy degree in Food Science and Technology at Utah State University, Logan, Utah, in 1968.