AN ABSTRACT OF THE THESIS OF

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Title OXIDATIVE DEGRADATION OF BETA-CAROTENE

Abstract approved (Major professor)

The role of beta-carotene degradations in the development of off-flavors in milk and milk products has not been established. The purpose of this investigation was to study the oxidation of beta-carotene and to identify volatile compounds arising from autoxidizing beta-carotene.

Pure cyrstalline beta-carotene, mixed with Celite to accelerate oxidation, was oxidized at 50°C by molecular oxygen in two different systems. The peroxide value and loss of beta-carotene were measured after controlled oxidation periods. In one system the maximum peroxide value, 295 milliequivalents per 1,000 grams, occurred after three hours of oxidation. In the other system the maximum peroxide value, 586 milliequivalents per 1,000 grams, was observed after six hours of oxidation. Approximately 80 percent of the beta-carotene was decomposed within the first ten hours of oxidation.

Volatile compounds from autoxidizing beta-carotene were collected by a cold-trap gas-entrainment technique. The collected

compounds were subjected to gas-liquid chromatography and rapid-scan mass spectrometry, and the following compounds were identified: n-pentane, ethyl ether, acetaldehyde, acetone, propanal, methyl vinyl ketone, toluene, isobutanal, 2-octanone and acetic acid. Compounds tentatively identified included diacetyl, 3-methyl-2-pentanone, 4-methyl-3-pentan-2-one, 2-methylfuran, 1, 3, 3-trimethylcyclohexene, methyl formate, butanone, 2-methyl-2-heptenal, 1, 3-dimethyl-2-ethylcyclohexane, 2-ethyl-2-hexenal, 2-formyl-3, 3-dimethylcyclohexene, 1, 1, 3-trimethyl-2-n-propylcyclohexane, 2-methyl-3-nonene and 3, 5, 5-trimethyl-4-(4'-butyl-3'-en-2'-onyl) cyclohexa-1, 3-diene. A fraction possessing a strong "nutty" aroma was tentatively identified as 2-methyl-2-heptenal. The volatile compounds identified can be predicted as degradation products of beta-carotene oxidation.

OXIDATIVE DEGRADATION OF BETA-CAROTENE

by

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A THESIS

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TO

AKIKO AND MAKI

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OXIDATIVE DEGRADATION OF BETA-CAROTENE

INTRODUCTION

The red pigment of the carrot root (Daucus carota) was first described by Wachenroder in 1826, 140 years ago, and was called "carotin" by him. It later became known as beta-carotene and is now well known as provitamin A whose structure consists of two molecules of vitamin A. Beta-carotene is one of the carotenoids which are found abundantly in all forms of living matter. It is responsible for the color of carrots, sweet potatoes, squash, pumpkins and other similar foods. It is also found in palm oil, alfalfa and yellow autumn leaves. Milk contains a fat-soluble caroteniod pigment fraction which largely consists of carotenes. Fresh milk contains from 0.05 to one micrograms of beta-carotene per milliliter.

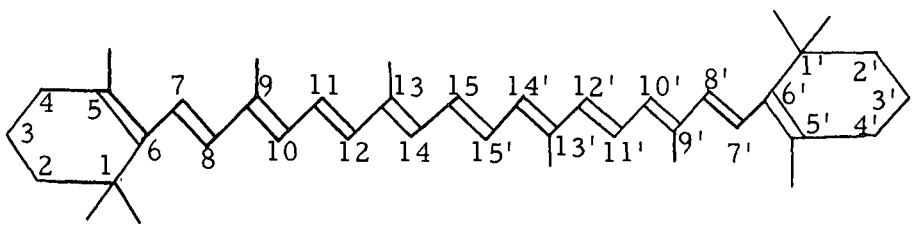
Some workers have suggested that beta-carotene acts as an antioxidant towards autoxidation of milkfat while the others believe that it has a pro-oxidant effect. Still others believe that it has neither effect on autoxidizing milkfat systems. However, the role of beta-carotene in dairy products in the development of oxidative off-flavors has not been established.

The purpose of this investigation was to study beta-carotene autoxidation and identify volatile compounds produced under controlled

oxidizing conditions. The information reported herein should be useful in understanding the origin of off-flavors arising in dairy products through autoxidative processes.

REVIEW OF LITERATURE

Beta-carotene is one of the carotenoids, isoprenoid lipids, and consists of two beta-ionone ring structures connected by a chain of four isoprene units. The structure of beta-caroten is as follows:



As the above structure shows, beta-carotene is a conjugated poly-unsaturated aliphatic hydrocarbon. Because of its conjugated poly-unsaturated nature, beta-carotene is subject to air and light initiated degradations which may be accentuated by heat. Under a carbon di-oxide atmosphere, beta-carotene in the crystalline form remains unchanged for several months when held below 20°C, and is stable for up to six weeks at 45°C. In contact with air at 45°C, the crystal-line form is almost completely destroyed after six weeks (Hoffmann-La Roche Inc., 1963).

Beta-Carotene in Milk

Milk contains fat-soluble and water-soluble pigments which are largely the carotenes and riboflavin, respectively. The yellow color of milkfat is due to the presence of carotenoids which exist in milk as a result of their ingestion by the cow (Gillam et al., 1933

and 1938). Since beta-carotene in milk is derived from feeds, considerable research effort on the effect of feeding carotene to cows has been carried out (Henderson, 1939; Martin et al., 1940; McGillivray and Worker, 1957; DeLuca et al., 1957; and McDowall and McGillivray, 1963a and b). It has been clear that there is a relationship between the daily beta-carotene intake of cows and the levels of carotene and vitamin A in their milk.

The distribution of carotenoids in milk is approximately from 89 to 94 percent in the butter, from ten to 14 percent in the skimmilk and from 0.8 to two percent in the buttermilk (Berl and Peterson, 1945). Milkfat has been shown to contain alpha-, beta-, gamma- and zeta-carotenes (Nash and Zchele, 1945), and other carotenoids (Gilliam et al., 1933). Beta-carotene makes up by far the greatest fraction of the carotenoids occurring in milk (Gillam and Ridi, 1937 and Strain, 1939).

The amount of beta-carotene in fresh milk ranges from 0.05 to one micrograms per milliliter depending upon the breed of cow and level of carotene intake (Hartman and Dryden, 1965). The amount of beta-carotene in fresh milk in the summer may be as much as five to six times higher than in the winter (Dornbush, Peterson and Olson, 1940). The beta-carotene content of milk products depends on fat content, and ranges from 2.4 to 7.3 milligrams per gram in butter, from 0.025 to 0.128 milligrams per milliliter in

buttermilk, from 1.01 to 3.5 micrograms per gram in half and half cream, from 0.013 to 0.039 milligrams per milliliter in skim milk (Berl and Peterson, 1945) and from 4.5 to 11.8 milligrams per gram in cheddar cheese (Hartman and Dryden, 1965).

Off-Flavors Developed Through the Autoxidation of Milk Lipids

Autoxidation of milk lipids is one of the major problems in dairy industry and numerous investigations on the products of oxidative deterioration of milk lipids have been made. Studies on the autoxidation of milk lipids, as well as other edible lipids, are complicated because many factors such as the composition, the physical state, the presence or absence of natural anti- or pro-oxidants, processing, manufacturing and storage conditions are involved.

In general, the autoxidation of lipids involves at least four phases: the induction period, peroxide formation, peroxide decomposition and secondary degradation or polymerization. The hydroperoxide decompositions and secondary degradations are most important from a flavor viewpoint because a number of carbonyl compounds are formed during these stages. It is believed that carbonyl compounds contribute significantly to the off-flavors developed by the autoxidation of lipids.

The same generalizations can be applied to explain the origin of the carbonyl compounds in milk lipids. The relationship between

carbonyl formation and the development of off-flavors associated with autoxidized lipids has been reviewed by Day (1960 and 1965).

Flavor defects in milk arising from autoxidation had been thought to be caused by oxidation of milk phospholipids (Thurston, Brown and Dustman, 1936; Greenbank, 1949; and Lea, 1953). This hypothesis can be criticized because oxidized flavors, such as cardboard flavor, have been observed in synthetic milk containing phosphatide-free milkfat, casein, lactose, milk salts and a slight amount of copper (Pont, 1953). The specific compounds responsible for the various off-flavors of oxidized milk or milk products have not been clearly established. However, there is increasing evidence which indicates that the products of lipid oxidation, especially carbonyl compounds, are responsible.

The off-flavors have been described as cardboard, oily, metallic, tallowy, fishy, painty, grassy, and nutty, but currently are referred to collectively as oxidized flavor. Forss, Pont and Stark (1955) observed two characteristic oxidized flavors developing in milk containing added copper. One was an oily-metallic flavor and the other was a cardboard flavor. The compounds identified as cardboard flavor constituents were the C_6 to C_{11} 2-unsaturated aldehydes and hexa-2, 4-dienal. When these aldehydes were added as a group to the fresh milk in range of one part in 10^7 to 10^9, they imparted a flavor which closely resembled the cardboard defect. The "metallic

compound", an important cause of off-flavor in oxidized dairy products (Pont, 1952), has been identified as oct-1-en-3-one by Stark and Forss (1962). The compound was found to have a flavor threshold value of one part in 10^9 in milkfat.

Constituents of a flavor concentrate from fishy autoxidized milkfat were separated by gas-liquid chromatography by Forss, Dunstone and Stark (1960a, b, and c) into six distinct flavor fractions. An oily flavor fraction contained n-hexanal, n-heptanal, hex-2-enal, and heptan-2-one. One of the fractions contained the metallic flavor described previously. n-Pentanal and pent-2-enal were found in a painty or dry oily flavor fraction. A mushroom flavor fraction was found to contain hept-2-enal and an unknown carbonyl compound. n-Octanal, n-nonanal, oct-2-enal and hepta-2, 4-dienal were found in a tallowy flavor fraction and non-2-enal was found in a cucumber flavor fraction. Non-2-enal was also found in cucumbers (Forss et al., 1962). More recently pent-cis-3-enal has been identified in the painty flavor fraction of fishy milkfat (Forss, 1964).

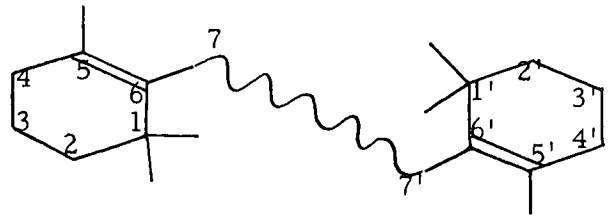
El-Negoumy, Milles and Hammond (1961) suggested that linoleate was probably the precursor of the components primarily responsible for the flavors of oxidized butteroil. Nona-trans-2-cis-6-dienal, the compound primarily responsible for the grassy flavor of oxidized milk, was isolated in small amounts from oxidized linoleate (El-Negoumy, Puchal and Hammond, 1962). Later Hammond and

Hill (1964) proposed some possible mechanisms for the formation of oct-1-en-3-one from linoleate and nona-2, 6-dienal from linolenate.

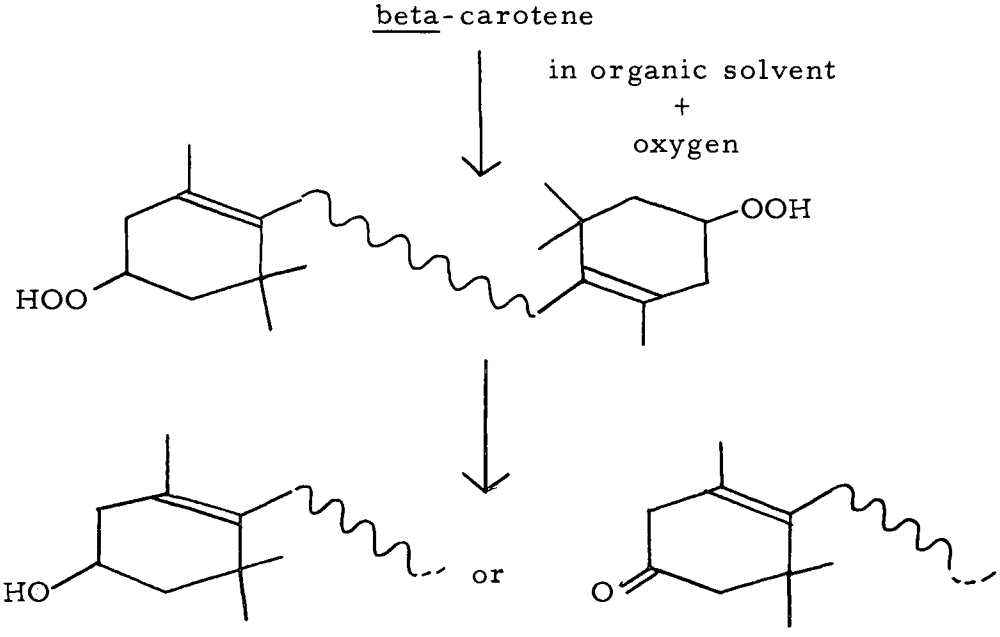
Autoxidation of Beta-Carotene

The mechanism of autoxidation of conjugated polyunsaturated lipids has been studied by several workers. However, in all studies concerned with the autoxidation mechanism, relatively simple conjugated polyunsaturated systems, such as eleostearic acid, were used. The proposed schemes of autoxidation of such compounds have been well reviewed by Lundberg (1961). Results indicate that the autoxidation of conjugated polyunsaturated lipids proceeds differently from that of nonconjugated systems, and the reaction products are not the same. However, most data suggest that some type of free radical, peroxide, polymer or small fragment reaction series is involved.

Bodea and Nicoara (1955) reported that the autoxidation of beta-carotene in organic solvents, such as chloroform, proceeded by the following mechanism: on contact with air, oxygen is taken up at the 3 and 3' positions of the ionone rings to form hydroperoxide groups that then give up an oxygen atom to yield an xanthophyll. Xanthophylls are alcohol or ketone derivatives of carotenes. The transformation of carotene to xanthophyll *in vitro* is thus explained and this transformation is outlined as follows:



(beta-ionone ring) (beta-ionone ring)

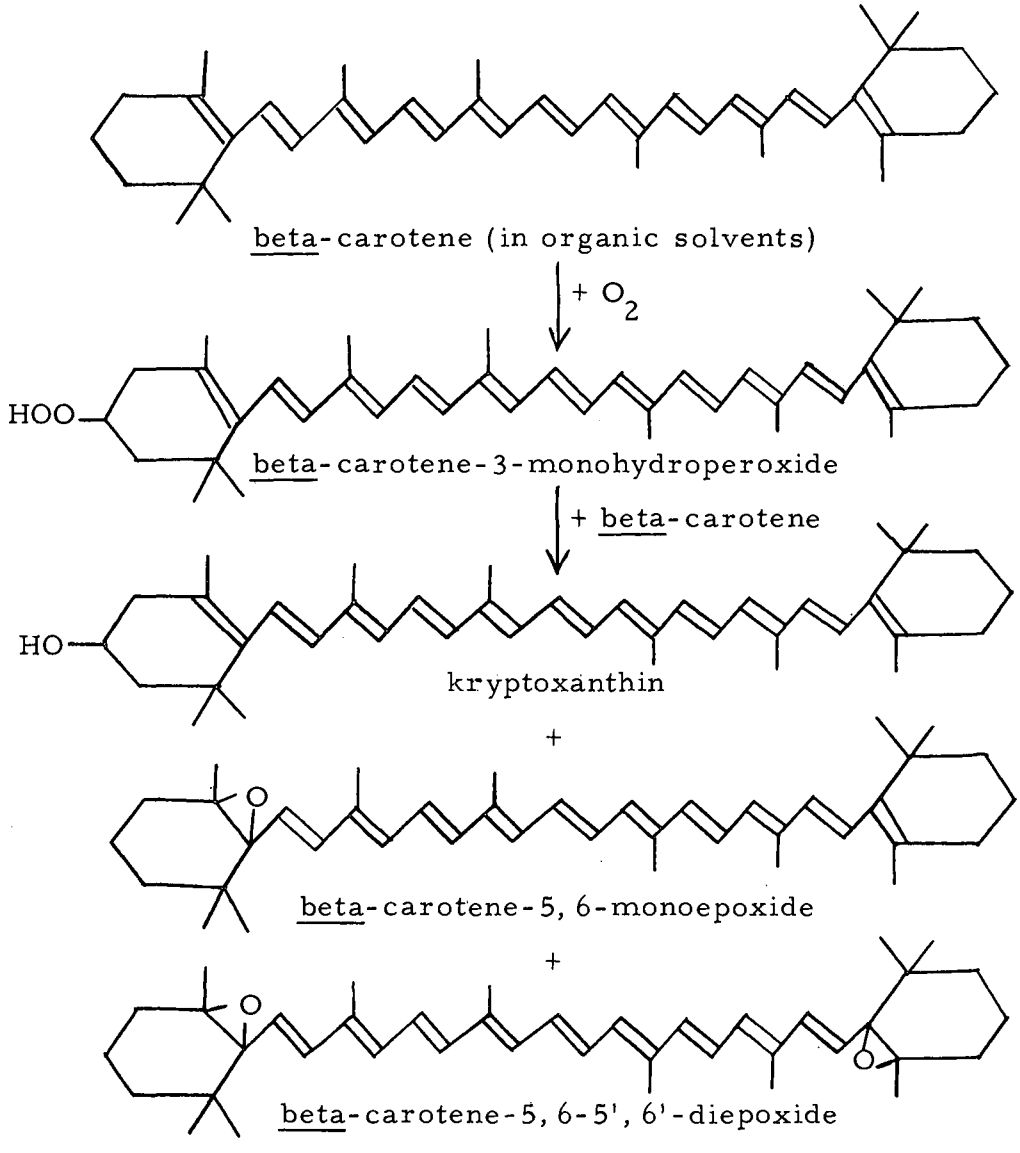


xanthophylls

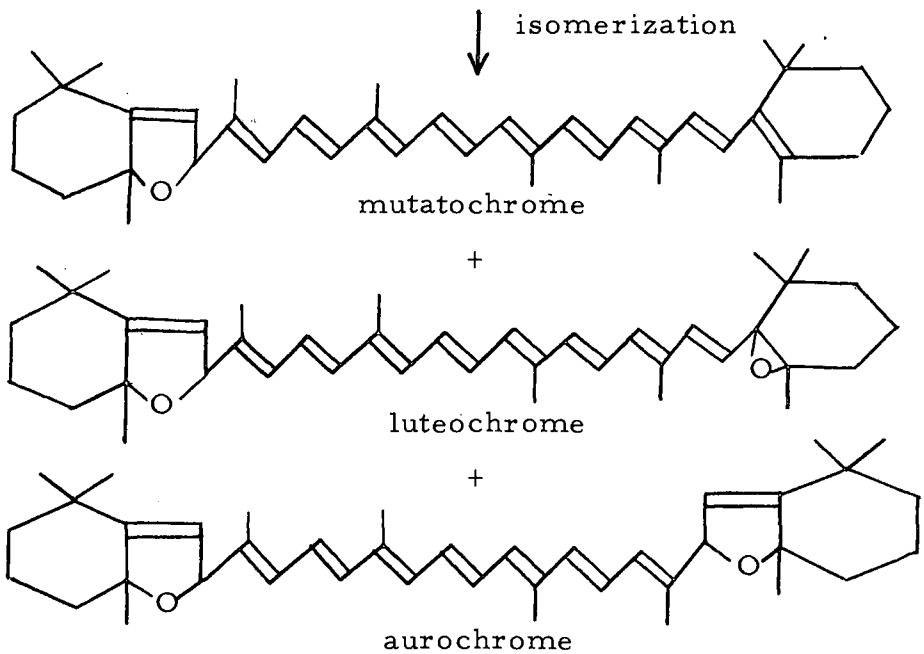
They found that the initial oxidation of alpha-carotene in organic solvents also follows the same pathway to yield the corresponding xanthophyll.

Later, Bodea et al. (1956) proposed a modified mechanism of autoxidation of beta-carotene in organic solvents, such as chloroform. In this scheme beta-carotene reacts with air to form beta-carotene-3-monohydroperoxide. This hydroperoxide then reacts with another

mole of beta-carotene to form beta-carotene mono- and diepoxides and 3-monohydroxy-beta-carotene (kryptoxanthin, an xanthophyll). The epoxides then isomerize to the furanoxides such as mutachrome, luteochrome and aurochrome. The kryptoxanthin reacts further to form another hydroperoxide. The suggested pathway is as follows:



Epoxides



The oxidation of beta-carotene by means other than molecular oxygen has received some attention. It appears that at least some of the same compounds are produced in both autoxidizing and chemically oxidizing systems. The previously mentioned epoxides and furanoxides are formed by the action of monoperphthalic acid on beta-carotene (Karrer and Jucker, 1945). The furanoxides are also produced from the corresponding epoxides by the action of mild oxidizing agents, such as 0.1N chromic acid (Kuhn and Brockman, 1935). The acid attacks beta-carotene only at the ionone rings.

Controlled oxidation with hydrogen peroxide using osmium tetroxide as a catalyst gives beta-carotenals, each of which consists a beta-ionone ring and a conjugated unsaturated aliphatic aldehyde

chain. Some examples are beta-apo-10'-carotenal, beta-apo-12'-carotenal and retinene (Wender, Rosenblum and Tischler, 1950).

Beta-carotene is completely degraded by the action of strong oxidizing agents, such as acidified potassium permanganate or chromic acid. Oxidation by acid permanganate gives rise to acetic acid formed from the methyl groups at the 9, 9', 13 and 13' chain positions and their attached carbon atoms (Karrer and Helfenstein, 1929). On the other hand, the acetic acid formed by the action of chromic acid arises from the methyl groups at 5 and 5' positions of the ionone ring and their attached carbon atoms (Kuhn and L'Orsa, 1931).

In a recent study on non-volatile compounds of autoxidized beta-carotene in organic solvents, Tsukida, Yokota and Ikeuchi (1965) found that measurable amounts of epoxides were produced in chloroform while only trace amounts of epoxides were produced in hexane, acetone or carbon tetrachloride. However, when the autoxidation of beta-carotene was carried out in any of the solvents described above in the presence of trace amount of acid, such as chromic acid, considerable amounts of epoxides and furanoxides were observed.

Hayes and Steele reported at World Fat Congress held in Hamburg in 1964 (McWeeny, 1966) that a stored sample of hydrogenated palm kernel oil containing beta-carotene and a vitamin A concentrate developed a green color. The green color development was

accompanied by a loss of beta-carotene and vitamin A, and the formation of anhydrovitamin A. In view of these observations, McWeeny (1966) and co-workers examined the compounds involved. They found at least fifteen colored compounds were present, and many of them were epoxide derivatives of beta-carotene. One of the major compounds was named pseudo-mutatochrome because its absorption spectrum was the same as that of mutatochrome, but it differed in color sensitivity to acids and R_f value on thin layer chromatography.

A greenish color occasionally develops in dry milkfat when it is stored for at least one and one-half years at -12°C. When control milkfat samples were kept for two years at 2-15°C, they did not show the color defect (Luck, 1966). He found that the defect was accompanied by chemical changes of the beta-carotene which was demonstrated by means of spectrophotometric measurements. The color defect was observed before any change in flavor was noticed. He suggested that some oxidation products of beta-carotene, such as isomeric diepoxides or furanoxides, might be involved in the defect.

Since beta-carotene readily acts as a receptor of peroxidic oxygen, the autoxidation of aliphatic aldehydes, such as butanal and heptanal, can be blocked or retarded by the addition of small amounts of beta-carotene (Bodea, Nicoara and Gross, 1954). Beta-carotene can also prevent the autoxidation of benzaldehyde (Bodea, Nicoara and

Gross, 1953). It has been postulated that beta-carotene reacts with the radical of benzaldehyde which is the first step in the autoxidation and thus interrupts the chain reaction. In this case beta-carotene is oxidized by attack on the double bonds of the chain with formation of a chain epoxide rather than the formation of an epoxide at the 3 or 3' position on the ionone rings.

McWeeny (1966) stated that destruction of beta-carotene added into autoxidizable fats was strongly retarded by ethoxyquin (with only one aryl substituent) and commercial lecithin. He postulated that these compounds operate by (1) removing the acid required for epoxidation (Swern, 1953), (2) removing the acid required for protonation of beta-carotene epoxides and formation of green color, (3) causing decomposition of peroxy acids, and (4) subsequent formation of a secondary anti-oxidant.

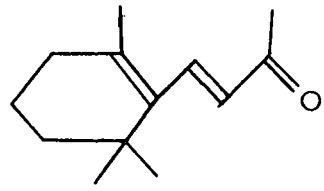
Off-Flavors Produced by Autoxidation of Beta-Carotene

Briggs (1931) reported that beta-carotene exhibited an anti-oxygenic activity towards the development of oxidized flavor in milk. Newton and Richardson (1932) obtained the same result in their study on stabilization of butter against oxidative rancidity. On the contrary, other workers (Bradway and Mattill, 1934; Browne, 1925; Greenbank and Holm, 1934; Heiman and Carver, 1937 and Olcovich and Mattill, 1931) concluded that beta-carotene had no effect or

was a pro-oxidant. However, the latter workers utilized glyceride substrates which may yield results different from work on milk itself. Brown, Vanlandingham and Weakley (1941) proposed that beta-carotene in the milkfat was not the substance responsible for the reduction in the susceptibility of milk to oxidized flavor, and that some substance or substances associated with it probably had a greater effect than the beta-carotene itself. Trout and Scheid (1943) found no relationship between the beta-carotene content of milk and development of oxidized flavor.

It is clear that the role of beta-carotene in off-flavor of oxidized milk and milk products has not been resolved.

There are some reports concerning volatile compounds which are formed when beta-carotene is autoxidized. Beta-ionone was first recognized as the off-flavor principle of oxidized carrots by Tomkins et al. (1944).



beta-ionone

Later, Ayers et al. (1964) reported that the violet-like off-flavor developed in dehydrated carrots stored under an oxygen

atmosphere was due to the formation of beta-ionone through the oxidation of beta-carotene. Falconer et al. (1964) reported a similar off-flavor occurred when carrots were processed soon after harvesting. The carrots were diced, blanched, dried by an accelerated freeze-dry technique, canned under partial nitrogen gas and stored at -20°C. The off-flavor developed under these conditions was believed to be due to the formation of beta-ionone from the oxidation of beta-carotene. The flavor deterioration was accompanied by a loss of color which substantiated the belief that beta-carotene was the origin of the flavor defect in carrots.

A series of studies on identification of off-flavor components of oxidized carrots, pure beta-carotene and beta-ionone have been carried out at the Low Temperature Research Station in Great Britain (Fishwick, Land and Swain, 1964). These investigations involved a comparative study of the volatile compounds formed in accelerated freeze-dried carrots, and from the in vitro oxidation of beta-carotene and beta-ionone. These workers demonstrated that the loss of color in carrots by oxidation had a direct relationship to off-flavor development. They identified acetaldehyde, methyl formate, n-pentane, ethyl ether, n-propanal, acetone, n-hexane, trimethyl butane, dimethylpentane, methylhexane, butanone, n-heptane, diacetyl, benzene and iso-octane from the low boiling volatile fraction of autoxidized beta-carotene. Benzene, however, was not considered

as a degradation product because it apparently came from the solvent system used for recrystallization of beta-carotene. In their work an oxidation flask (shown in Figure 1) was designed to accelerate the oxidation of the sample and to obtain the volatile compounds easily.

Compounds implicated in off-flavors developing in autoxidized beta-carotene under above conditions were acetaldehyde, methyl formate, n-propanal, acetone, butanone and diacetyl. However, most of these compounds are naturally present in milk or are common oxidative deterioration products of other lipids. Since the amount of beta-carotene in milk is extremely small when compared with that of other milk constituents, the influence of these compounds on the off-flavors of autoxidized milk may be insignificant.

It is apparent that the degradation of beta-carotene by autoxidation is complex and could proceed by more than one pathway. For example, the carbon atom at position 3 or the double bond, 5, 6 position, on beta-ionone ring could be attacked by oxygen to form an xanthophyll or epoxide; or some part of the conjugated polyunsaturated chain could be attacked by oxygen to form small fragments.

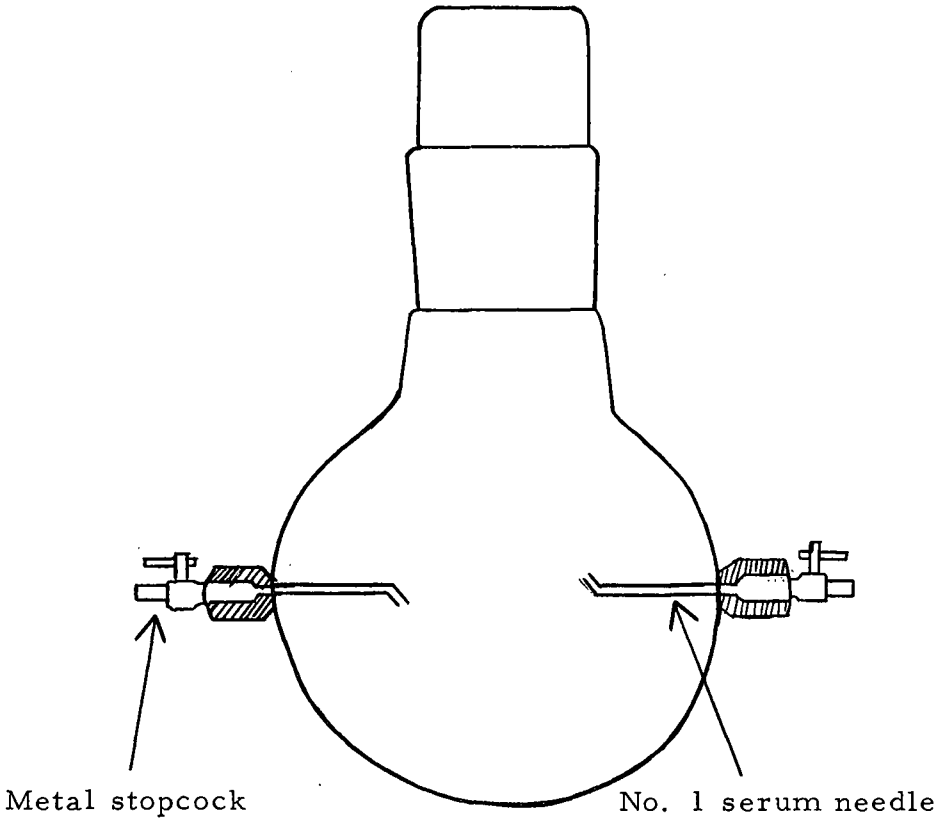


Figure 1. Oxidation flask used by Fishwick, Land and Swain (1964).

EXPERIMENTAL

Sample

The beta-carotene used in this investigation was crystalline trans-beta-carotene and possessed 1.60 to 1.67 million U. S. P. units of vitamin A activity per gram. It was obtained from Hoffmann-La Roche Inc., Nutley, New Jersey.

Methods for Oxidation of Beta-Carotene

Two methods, A and B, were used in this investigation. Method A was similar to the procedure described by Fishwick, Land and Swain (1964) except that a different oxidation flask was used and beta-carotene was mixed with Celite 545 to accelerate the oxidation. Method B was a gas entrainment technique designed for easy-trapping of the volatile compounds formed in autoxidized samples (Morgan and Day, 1965).

Method A

Three-tenths of a gram of beta-carotene was mixed with 1.7 grams of 60-80 mesh Celite 545 (acid-alkali washed) in a mortar.

The Celite was previously purified to remove traces of iron present by reacting with aqua regia for 48 hours according to method of Palmateer, Yu and Simnhuber (1960). The Celite was then filtered

and washed with distilled water using suction until a negative chloride test was obtained with silver nitrate. Finally, the Celite was again filtered and dried in an oven at 100°C.

The resulting mixture of beta-carotene with the Celite was suspended in 200 ml of purified, carbonyl-free chloroform (Schwartz and Parks, 1961) in a 500 ml round-bottom flask. The solvent was evaporated under a reduced pressure at 30°C in water bath using a rotary evaporator to give a thin layer of the beta-carotene-Celite mixture on the wall of the flask.

A U-shaped glass tube was filled with distilled water and then mounted on the reacting flask to mannometrically indicate pressure changes inside the flask. The complete oxidation apparatus is shown in Figure 2. Sufficient molecular oxygen was introduced into the flask so that a slightly positive pressure was maintained in the reaction flask which was held at 50°C. The upper part of the flask was covered with aluminum foil to shield the sample from light.

Method B

Three-tenths of a gram of beta-carotene was mixed with 1.7 grams of 40-60 mesh Celite 545 (acid-alkali washed) in a mortar and then placed in a 25mm x 55mm screw-capped vial. The cap, with two 5/32 inch holes 7/16 inch apart, was fitted with a 1/8 inch thick silicone-rubber septum. The cap was firmly tightened onto the vial

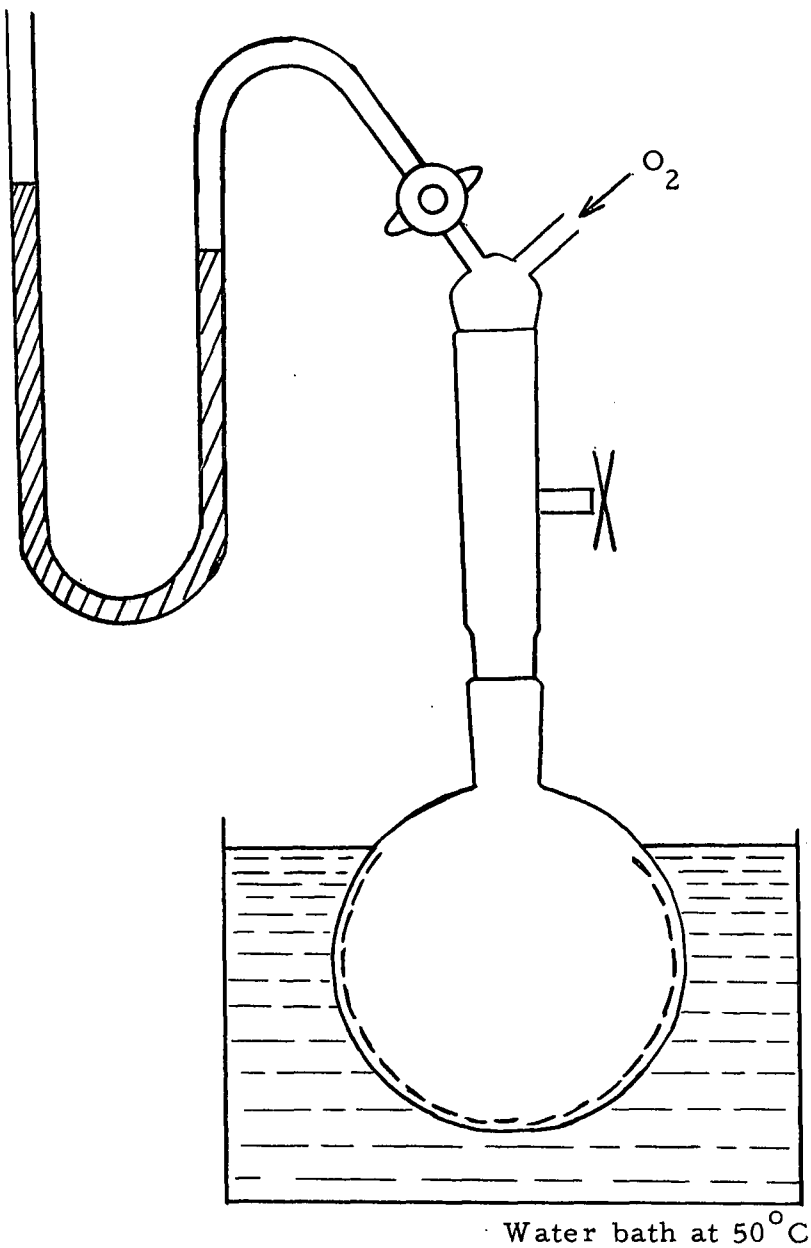


Figure 2. Oxidation system used in Method A.

so that the system was air tight. The vial was then raised onto the purging needles which were Hamilton N-722, point style one, 22-gauge, two inch and one-half inch long, respectively. The long needle was inserted into the beta-carotene and Celite mixture. The vial was then placed in a water bath maintained at 50°C. Molecular oxygen was introduced into the vial and the pressure was maintained at 10 psig. The vial was covered with aluminum foil to shield the sample from light. The complete oxidation system is shown in Figure 3.

Methods for the Determination of Peroxide Value

Two methods were used for determination of peroxide values. Determinations reported herein were made with a slightly modified iodometric titration method (Heaton and Uri, 1958). Periodically a potentiometric method was used to check the results obtained by the modified volumetric method.

I. Volumetric Titration

Thirteen to sixteen milligrams of the mixture of oxidized beta-carotene and Celite were weighed accurately, slurried with 25 ml of glacial acetic acid. The mixture was subjected to deaeration by nitrogen purging for one hour. Then, 0.5 ml of freshly prepared saturated potassium iodide was added to the mixture under a stream of nitrogen, and the nitrogen purge was continued for an additional

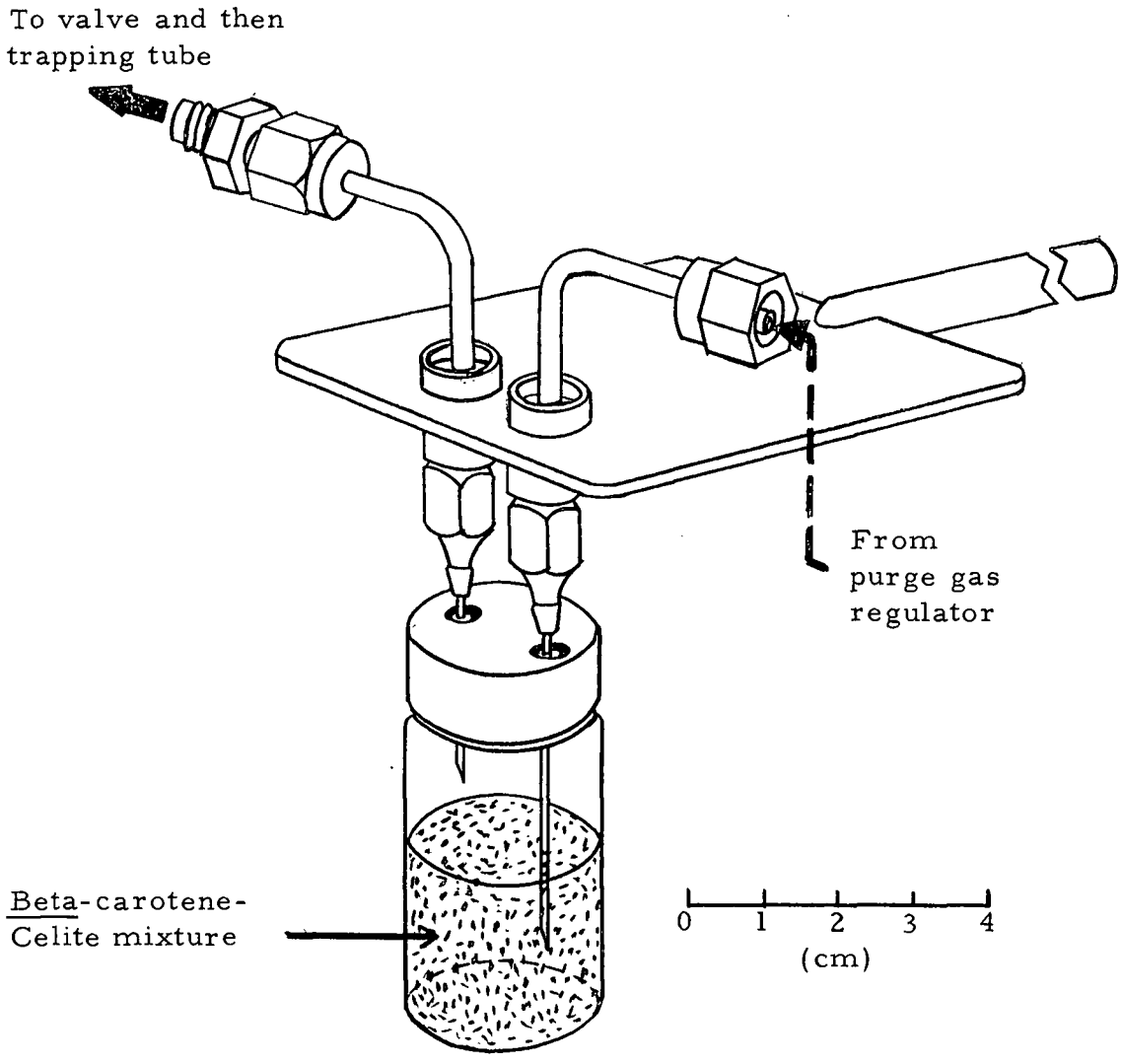


Figure 3. Apparatus used to oxidize beta-carotene and to purge the volatile compounds (Morgan and Day, 1965).

five minutes. The nitrogen filled flask was then placed in a dark place for one hour. Thirty ml of five percent aqueous potassium iodide were added and the contents were titrated rapidly with 0.002 N aqueous sodium thiosulphate, starch indicator being added shortly before the end-point was reached. A blank was determined with each series.

II. Potentiometric Titration

Ten to twenty milligrams of the mixture of oxidized beta-carotene and Celite were weighed accurately and suspended in 60 ml of purified chloroform. Then, 30 ml of glacial acetic acid, 30 ml of freshly prepared five percent potassium iodide solution and 100 ml of distilled water were added. The mixture was placed in the 250 ml glass titration vessel shown in Figure 4. The contents were immediately titrated in the absence of oxygen with 0.002N-sodium thiosulfate solution. The end-point was determined as the equivalence point of the titration curve in the usual way (Japan Society for Analytical Chemistry, 1958).

III. Calculation of Peroxide Value

Peroxide values were calculated according to the following equation and are expressed in milliequivalents of peroxides per 1000 grams of sample:

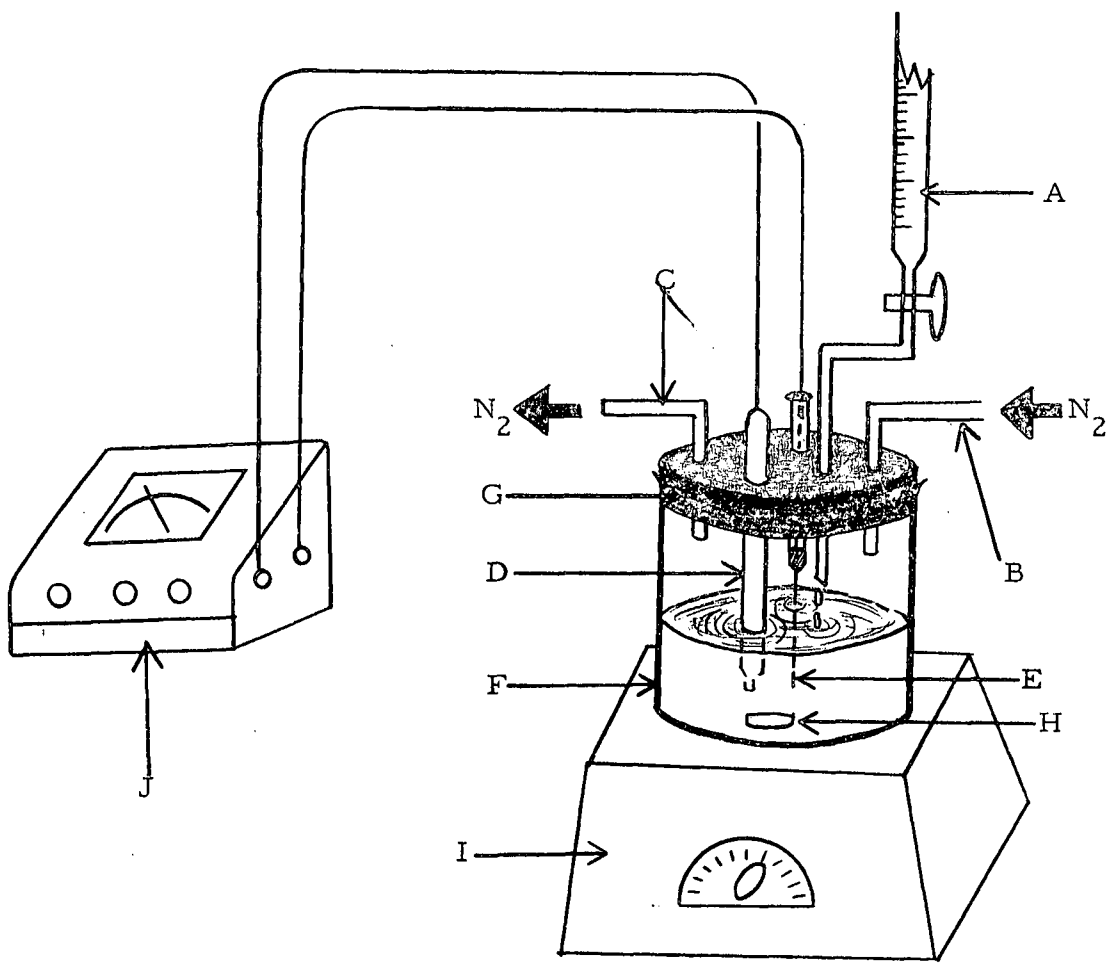


Figure 4. Apparatus of potentiometric titration.

A: micro-burette

B: inlet of nitrogen gas

C: outlet of nitrogen gas

D: calomel electrode

E: platinum electrode

F: titration vessel

G: rubber stopper

H: magnetic rod

I: magnetic stirrer

J: pH meter

$$\text{Peroxide value} = \frac{S \times N \times 1000}{\text{Weight of sample (grams)}}$$

where S = volume of standard sodium thiosulfate solution (ml)

N = normality of standard sodium thiosulfate solution

Measurement of Beta-Carotene Destroyed During Oxidation

Approximately two-tenths of a gram of the mixture of oxidized beta-carotene and Celite was removed from the flask (Method A) when the samples for peroxide determinations were taken. After accurate weighing, the samples were dissolved in Spectro-grade cyclohexane. The absorbance was measured at 450 mμ (Fishwick, Land and Swain, 1964) using Beckman DU spectrophotometer and the readings were referred to a standard curve.

Gas Liquid Chromatography (GLC)

The volatile compounds obtained from oxidized beta-carotene were subjected to gas chromatography for separation and establishment of tentative identifications. Their life-times under the oxidation conditions employed in Method B were determined. The term, "life-time", used here refers to the length of time a volatile compound was observed during the course of the oxidation.

A Barber-Colman Model 20 Gas Chromatograph equipped with

an argon, strontium 90 beta-ionization detector was used. The chromatographic columns and conditions employed were as follows: a 12 feet x 1/8 inch O. D. stainless steel column packed with 3. 7 grams of 20 percent Carbowax 20 M on 80-100 mesh Celite 545 (acid-alkali washed); a 12 feet x 1/8 inch O. D. stainless steel column packed with 4. 3 grams of 20 percent Apiezon M on 80-100 mesh Celite 545 (acid-alkali washed); detector cell temperature 200°C; and detector cell voltage 1250 volts. The flow rates employed for Carbowax 20 M and Apiezon columns were 38. 7 ml per minutes and 36. 5 ml per minutes, respectively. The chromatograph column oven was operated isothermally at 100°C. The column effluent gas was split; one-half of the effluent was introduced into the detector cell and the other half was vented to the atmosphere through a heated (195°C) 1/8 O. D. stainless steel tube and a 26 gauge hypodermic needle. The characteristic odor of each peak was noted by smelling the vented effluent gases.

In preliminary work, two other types of stationary phases were evaluated. One was a 12 feet x 1/8 inch O. D. stainless steel column packed with 4. 24 grams of 20 percent 1, 2, 3-tris (2-cyano ethoxy) propane (TRIS) on 80-100 mesh Celite 545 (acid-alkali washed). The second was a 12 feet x 1/8 inch O. D. stainless steel column packed with 4. 15 grams of diethylene glycol succinate (DEGS) on 80-100 mesh Celite 545 (acid-alkali washed). The gas chromatograms

of the entrapped samples obtained with the four different stationary phases were compared at four different oxidation times. These were 20-21 hours, 45-48 hours, 70-77 hours and 95-100 hours.

The carbowax 20M column (polar) and the Apiezon M column (non-polar) were selected for further use because they gave the best resolution of volatile compounds arising from the autoxidizing beta-carotene. Relative retention times for available authentic compounds were determined on these two columns for reference in assigning tentative identifications.

The trapping procedure for the volatile compounds from the oxidized beta-carotene was as follows: a U-shaped trapping tube was connected with a toggle valve which in turn was connected with the outlet of the oxidation system shown in Figure 4. The trapping tube was a U-shaped nine inch x 1/8 inch O. D. stainless steel tube packed with 20 percent of Carbowax 20M on 80-100 mesh Celite 545 (acid-alkali washed).

In order to evaluate the volatile compounds in the samples before oxidation was initiated, the trapping tube was immersed in a cold bath containing dry-ice and 2-methoxy ethanol (methyl cellosolve). Nitrogen was purged through the sample at 10 psig for ten minutes, after which the valve was shut off and the control trapping tube was disconnected. The oxidation vial containing the mixture of beta-carotene and Celite was then placed in a water bath maintained

at 50°C. This time was recorded as zero hour. Then the nitrogen purge was switched to oxygen to start the oxidation. The volatile compounds of oxidized beta-carotene were collected in separate trapping tubes in the same manner at intervals up to 160 hours of oxidation time.

For analysis the trapping tubes were connected between the GLC column and the carrier gas inlet of the GLC instrument as quickly as possible. When it was necessary to store a trapping tube containing volatiles, both ends of the tube were tightly sealed with Swagelok or rubber caps before it was placed in a freezer (-15°C).

The retention times of unknown GLC peaks were compared with that of authentic acetone, and their t_R/t_R values (relative retention time with t_R/t_R of acetone = 1.000) were calculated.

GLC Combined with Rapid-Scan Mass Spectrometry

Identification of volatile compounds of autoxidized beta-carotene separated by GLC was accomplished through rapid-scan mass spectral analysis of the compounds as they eluted from the GLC column. A Barber-Colman Series 5000 Gas Chromatograph equipped with temperature programming and a hydrogen flame detector was used. The compounds were introduced through an EC-1 inlet system into an Atlas-MAT CH-4 Nier-type mass spectrometer (a nine inch, 60 degree sector, single focusing instrument).

A 12 feet x 1/8 inch O. D. stainless steel column packed with 3.7 grams of 20 percent Carbowax 20M on 80-100 mesh Celite 545 (acid-alkali washed) was used because it gave the best separation of the volatile compounds of oxidized beta-carotene. Research grade helium was used as the carrier gas for gas chromatographic separations. The helium pressure was set at 50 psig, which resulted in a column flow rate of 31 ml per minute. The effluent from the GLC column was split at the end of the column; one portion was directed to the GLC detector and the other to the mass spectrometer inlet through the EC-1 gas inlet valve to the high vacuum system of the mass spectrometer. When the valve was adjusted properly, rapid-scan spectra could be obtained at any time during the chromatographic separation. The effluent gas not introduced into the mass spectrometer was vented to the atmosphere where compound odors could be evaluated.

In order to correlate the maximum ion intensity in the mass spectra to the maximum peak height on the gas chromatogram, an appropriate length of 0.01 inch O. D. stainless steel capillary column was inserted between the Swagelok T-fitting located at the end of the GLC column and the flame detector. Whenever feasible, background spectra were taken just before and (or) after the maximum detector response of gas chromatogram so that m/e (mass to charge ratio) peaks resulting from that particular component could be determined

by difference.

The operating conditions for the mass spectrometer were as follows:

Ionization current	60μA
Accelerating potential	3000 V
Electron voltage	70eV (double source)
Multiplier voltage	1.8-2.0KV
Analyzer pressure	$\sim 5 \times 10^{-7}$ mmHg
Scan speed	5 seconds for m/e25 to 250

Chromatographic conditions were as follows: hydrogen pressure 12 psig; air pressure 55 psig; detector temperature 185°-190°C; helium carrier gas pressure 50 psig; and column flow rate 31 ml per minute.

The best temperature programming sequence for separating of volatile compounds from oxidized beta-carotene was as follows: Initial temperature of 75°C for 20 minutes followed by a temperature increase of five degrees per minute to 200°C, and then holding at the final temperature for 50 minutes. The initial temperature gave adequate separation of the components eluting early in the program.

In order to increase the concentration of the volatile compounds in a trapping tube for mass spectrometric determinations, the same tube was used repeatedly for collections. Usually, at least five successive collections were made for a given oxidation time.

Reverse-Phase Thin Layer Chromatography

Attempts were made to establish the identity of carbonyl compounds in the volatile fraction of oxidized beta-carotene.

1. Two vials containing 2, 4-dinitrophenylhydrazine (2, 4-DNP hydrazine)-H_3PO_4 solution were inserted between the end of the valve and the trapping tube of the oxidation system shown in Figure 3. The volatile compounds from the autoxidizing beta-carotene were purged through the vials to trap cabonyl compounds as their 2, 4-DNP hydrazones.

The 2, 4-DNP hydrazine-H_3PO_4 solution was prepared by dissolving five grams of the hydrazine reagent in 60 ml of 85 percent phosphoric acid, then diluting with 39.5 ml of 95 percent ethyl alcohol, and finally filtering the solution (Momose, 1954). The hydrazine-phosphate solution is reported to be more stable than a hydrazine-hydrochloride solution (Johnson, 1951).

The resulting 2, 4-DNP hydrazine reaction mixture after purging with volatiles was handled according to the method of Shriner, Fuson and Curtin (1956). The solid material that was obtained was dissolved in carbonyl-free chloroform and subjected to thin layer chromatography (TLC) according to method of Libbey and Day (1964).

The trapping tube containing the remainder of the volatiles was, then, subjected to GLC.

2. Another attempt was made to obtain derivatives of carbonyl compounds present in the volatiles of oxidized beta-carotene. A one foot long glass capillary tube (1 mm I. D.) was attached to the outlet of the oxidation system shown in Figure 3. A syringe needle was welded to a 1/8 inch Swagelok fitting and a GLC injection-port septum on the needle was used to make firm connection between the needle and the glass capillary tube. The glass tube was cooled with dry-ice prior to passing purge gas (N_2) so that some of the volatile compounds were condensed inside the tube. In order to increase the amounts of the condensed compounds, the procedure was repeated several times. The liquid obtained in the tube was reacted with 2, 4-DNP hydrazine (Shriner, Fuson and Curtin, 1956). The hydrazones obtained were subjected to TLC in the same manner as described in 1.

RESULTS AND DISCUSSION

Peroxide Value of Autoxidized Beta-Carotene

Peroxide values of degradation products of beta-carotene oxidized by two different methods were obtained.

The results obtained from methods A and B are shown in Figure 5 and 6, respectively. In Figure 5, the data showing the loss of beta-carotene during the oxidation is also presented. In method A the maximum of peroxide value, 295 milliequivalents per 1000 grams, was reached after three hours of oxidation. The peroxide value rapidly decreased with further oxidation time, and after 20 hours it was reduced to 50 milliequivalents per 1000 grams. The rate of oxidation in method B was slower than in method A, but the maximum peroxide value in method B, 586 milliequivalents, was higher than that attained in method A. Both methods of oxidation showed similar induction periods, gave about the same peroxide values after three hours of oxidation, and exhibited about the same rate of peroxide decomposition. It is possible that beta-carotene is initially oxidized by molecular oxygen at the same rate regardless of the autoxidizing conditions. The shape of the peroxide value curves obtained in both methods were similar, and also were similar to those of edible fats (Kummerow, 1961). In the typical pattern of peroxide curves for fats, peroxides were rapidly formed after an

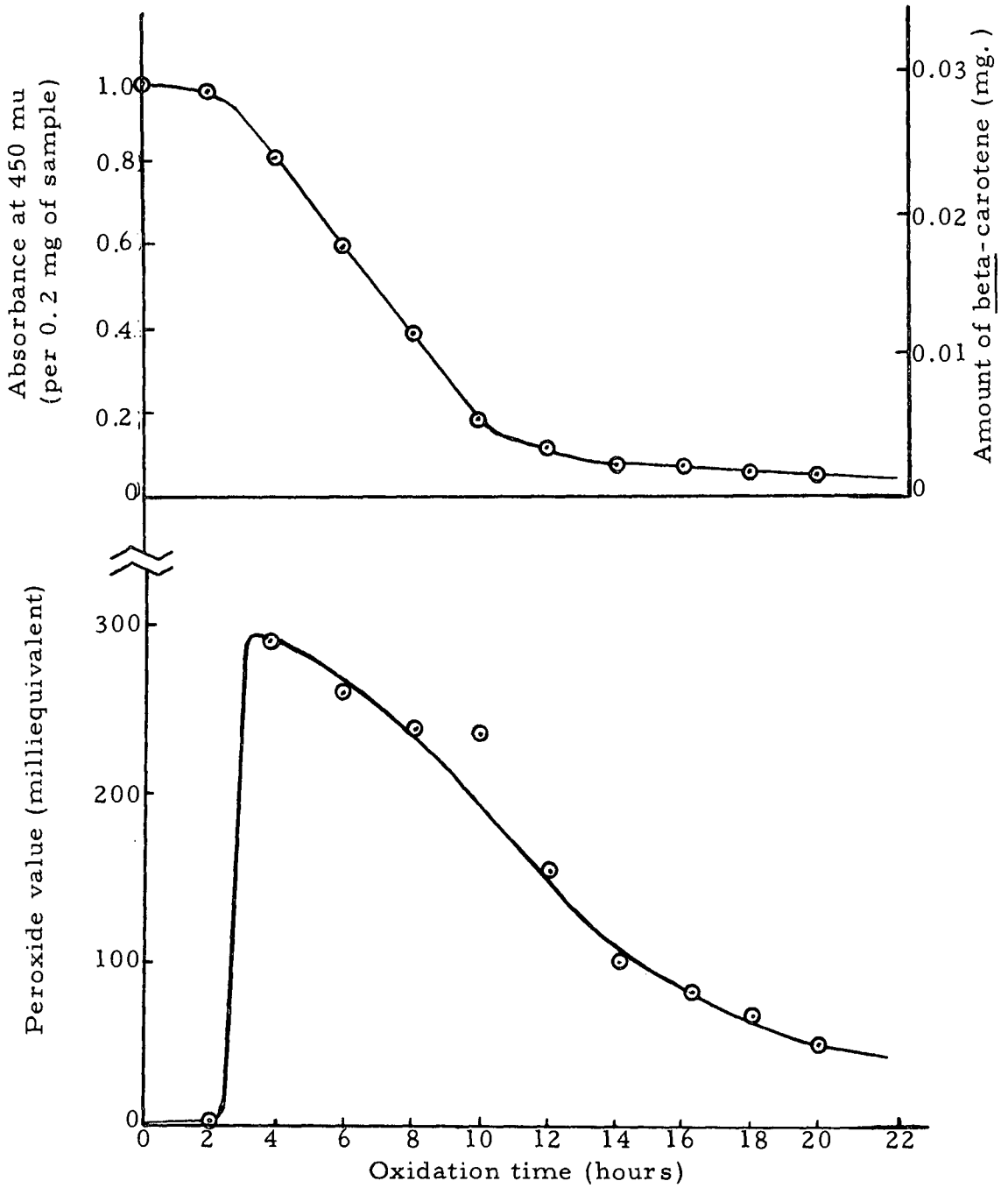


Figure 5. Peroxide value and loss of beta-carotene vs. oxidation time in Method A.

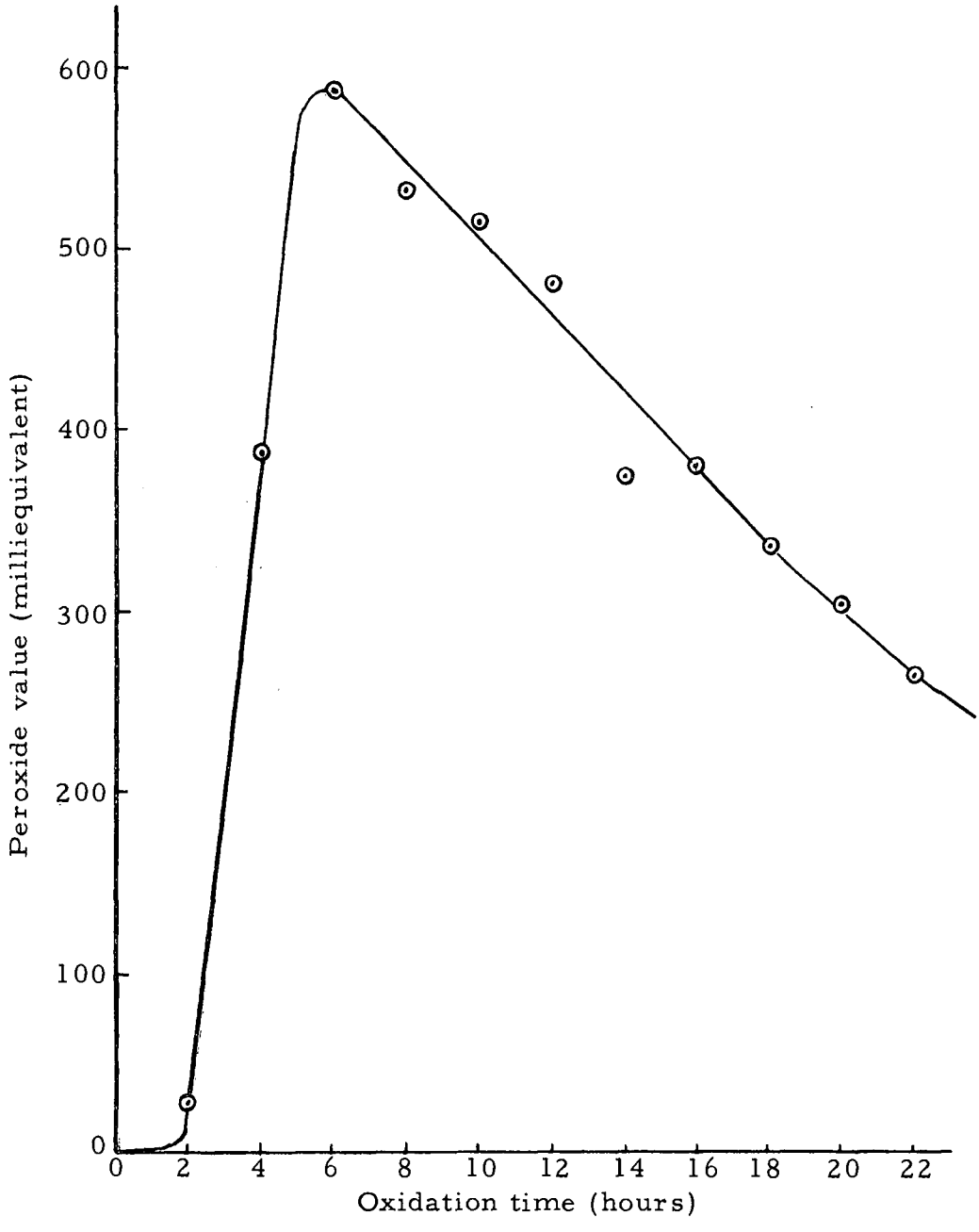


Figure 6. Peroxide value vs. oxidation time in Method B.

induction period (peroxide formation stage); after the peroxide value reached the maximum, the curve started to decline as peroxide decomposition overcomes as the formation. However, the large increase in the peroxide value after three hours of oxidation in method B indicates that further secondary reactions may have been involved. Higher applied oxygen pressure and autocatalytic reactions of peroxides produced were probably contributing factors to the increased peroxide values.

Significant loss of beta-carotene was not observed during the induction period of the peroxide curve shown in Figure 5. Most beta-carotene was decomposed during peroxide formation and the earlier part of the decomposition stages. It appears that most beta-carotene was first converted into peroxides, and then decomposed to further products in the oxidative degradation. However, Kummerow (1961) reported that carotenoids in beef tallow acted as anti-oxidants. He observed that these pigments were oxidized and bleached white in the induction period of beef tallow, and peroxides were not detected until all of the carotenoids were oxidized. Although the relative amount of beta-carotene in beef tallow was not reported, it must have been too small to give measurable amounts of peroxides.

A summary of the conditions and results of the two methods employed in this investigation are listed in Table 1. A major disadvantage of oxidation method A is the extreme difficulty in preparing

Table 1. Comparison of oxidation methods A and B.

	Method A	Method B
Reaction vessel	500 ml round bottom flask	screw capped 23 ml vial
Amount of beta-carotene	1.7 grams	1.7 grams
Amount of Celite 545	0.3 grams	0.3 grams
Temperature	50°C	50°C
Pressure	slightly positive above atmospheric pressure	10 psig
Maximum peroxide value	295 milliequivalents per 1000 grams	586 milliequivalents per 1000 grams
Time to reach to the maximum peroxide value	3 hours	6 hours

a uniform thin film of the mixture of beta-carotene and Celite on the inside of the flask. In addition, higher oxygen pressures cannot be applied to the flask. In order to collect the volatile compounds in the trapping tube, a higher pressure is required. Therefore, oxidation method B was used in further studies.

A modification of the volumetric macro-iodometric method proposed by Heaton and Uri (1958) was employed for the determination of peroxide values. Unreacted beta-carotene and other colored substances produced from beta-carotene were expected to concentrate in the chloroform layer; and since the color change of the indicator

occurred in the aqueous layer, it was not expected that the original color of beta-carotene would interfere with the end-point. However, the original color of beta-carotene still interferred with the end-point of starch indicator. The potentiometric method was used only to establish the color change that indicated the correct end-point in the iodometric method. All peroxide values in Figures 5 and 6 were obtained by the iodometric method. In addition, peroxide values at four hours (291 milliequivalents = m. eq.), 12 hours (155 m. eq.) and 20 hours (51 m. eq.) of oxidation in method A, and four hours (364 m. eq.), six hours (595 m. eq.) and 16 hours (382 m. eq.) of oxidation in method B were also obtained by the potentiometric method. Although the potentiometric method was auxiliary, it was preferred because it overcame the difficulty in determining the end-point in the volumetric method.

Gas-Liquid Chromatography of Volatile Compounds from Autoxidized Beta-Carotene

Beta-carotene was oxidized by method B as described previously. The volatile compounds from the oxidation system were trapped in trapping tubes by means of a cold bath containing dry-ice and 2-methoxy ethanol. The volatiles were examined at various times from 30 minutes to 160 hours after the oxidation was initiated. The contents of the trapping tubes were subjected to gas-liquid chromatography analysis. A control sample at zero time was also examined.

Two types of packed GLC columns were used; an Apiezon M column as a non-polar type and a Carbowax 20M column as a polar type.

The volatiles were trapped for Carbowax 20M column evaluations after 0, 0.5, 1, 1.5, 2, 3.5, 4.5, 6, 9, 11, 13, 16, 20, 23, 30, 37, 42, 48, 54, 60, 77, and 100 hours of oxidation. Samples for Apiezon M column evaluations were obtained after 0, 0.5, 1, 1.5, 2, 2.5, 3, 4.5, 5, 6, 7, 8, 9, 10, 12, 16, 18, 21, 24, 27, 30, 34, 38, 42, 46, 50, 55, 60, 65, 70, 75, 80, 85, 90, 97, 104, 110, 120, 130, 145, and 160 hours of oxidation.

Therefore, a total of 22 chromatograms were obtained from the Carbowax 20M column and 41 chromatograms were obtained from the Apiezon M column. From the 22 chromatograms on the Carbowax 20M column, 41 major compounds were observed while 49 major compounds were observed in the 41 chromatograms on the Apiezon M column. The relative retention time ($t_R / t_{R\ acetone}$) of each peak in the chromatograms was calculated and compared to those available for authentic compounds. The volatile compounds which were tentatively identified by coincidence of relative retention times on both the Carbowax 20M and the Apiezon M columns, or which had characteristic odors, are listed in Table 2. The t_R/t_R values for these compounds from all of the chromatograms were plotted on semi-logarithmic paper with oxidation time on the linear scale and t_R/t_R value on the logarithmic scale. The points representing

Table 2. Tentative identity of compounds shown in Figures 7 and 8.

Compound or fraction	Carbowax 20 M (100°C) Elution number	Carbowax 20 M (100°C) Experimental t_R/t_R acetone	Carbowax 20 M (100°C) Known t_R/t_R acetone	Apiezon M (100°C) Elution number	Apiezon M (100°C) Experimental t_R/t_R acetone	Apiezon M (100°C) Known t_R/t_R acetone
ethyl ether	2	0.399	0.397	6	1.263	1.271
acetaldehyde	5	0.685	0.685	2	0.682	0.685
acetone	8	1.007	1.000	4	0.993	1.000
propanal	11	1.521	1.556	5	1.204	1.200
methyl formate	--	-----	-----	7	1.521	1.515*
butanone	12	1.598	1.566	11	2.495	2.481
isobutanal	14	1.765	1.778	8	1.796	1.789
diacetyl	16	2.020	2.019	10	2.030	2.060
methyl vinyl ketone	19	2.432	2.412	9	1.991	2.000
"nut-like" odor fraction	23	4.180	-----	16	3.824	-----
2-octanone	32	9.640	9.681	38	24.47	22.40
"animal-like" odor fraction	36	15.89	-----	23	7.339	-----
acetic acid	38	24.09	24.80	27	12.03	12.01

* This relative retention time of methyl formate was extrapolated from the curve of boiling point vs. logarithm of retention time plotted with ethyl formate and n-propyl formate.

chromatographic peaks with similar t_R/t_R values and (or) characteristic odors were connected by a line. The peaks connected by a line were assumed to represent the same compounds. Thus, Figures 7 and 8 show approximately the time during oxidation that a particular volatile compound was present. The length of time a volatile compound was observed during the course of the oxidation is designated herein as the "life-time".

As seen in Figures 7 and 8, acetic acid, for example, was observed in the earlier stages of oxidation, but was not observed after about 30 hours of oxidation time; however, it appeared again in trace amounts after the 50th hour for about 10 hours, and again after the 100th hour.

The "animal-like" odor fraction listed in Table 2 and Figures 7 and 8 was so-designated because it was very similar to the characteristic odor observed in horse stables. The "fresh wood-like" odor fraction listed in Table 4 was so-designated because it was similar to the odor observed when a piece of hardwood, such as oak, is cut.

The life-time of most compounds tentatively identified by the Apiezon M column separations approximately coincided with those compounds observed in Carbowax 20M column separations. The differences in observed life-times of compounds probably were caused by following variables: (1) The oxidation was sensitive to

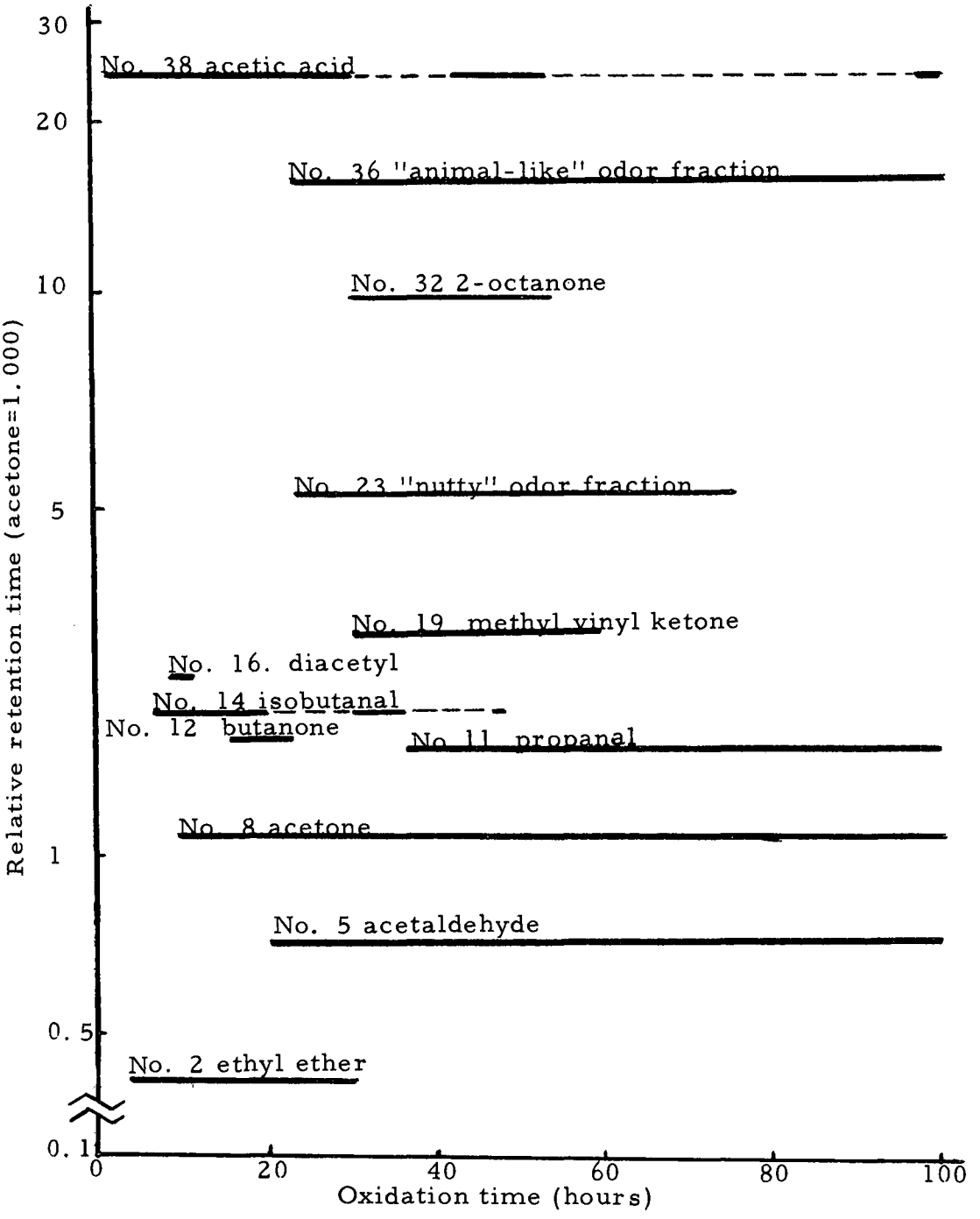


Figure 7. Life times of volatile compounds tentatively identified or volatile compounds with specific odors (on Carbowax 20M Column, 100°C).

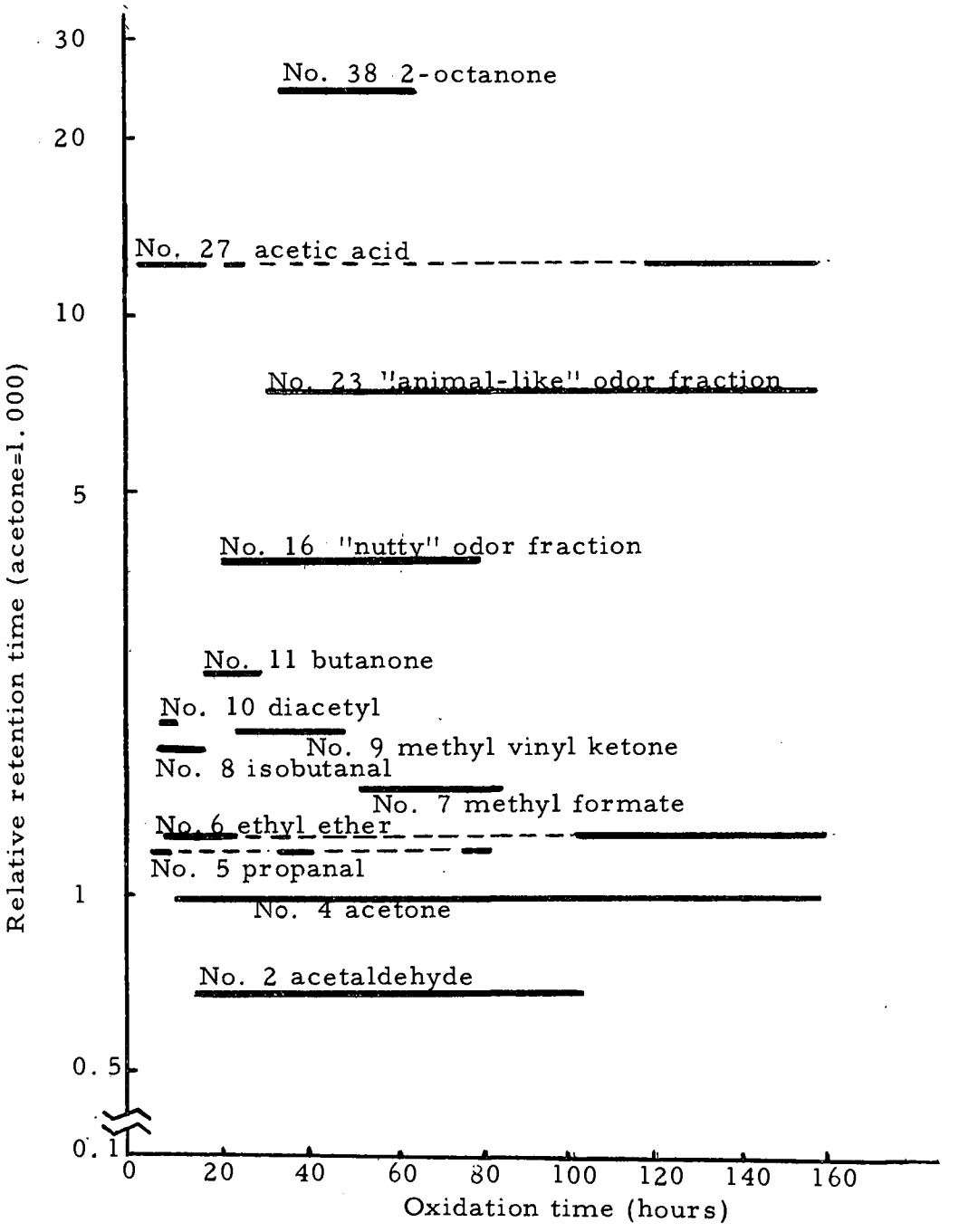


Figure 8. Life times of volatile compounds tentatively identified or volatile compounds with specific odors (on Apiezon M Column, 100°C).

conditions such as temperature, light, oxygen pressure; (2) about 20 trapping tubes were used in succession and some loss of volatile compounds before GLC analysis may have occurred; and (3) unavoidable temperature and time variations occurred when the trapping tubes were connected between the GLC column and the carrier gas inlet in the column oven which was pre-heated at 100°C. To minimize the temperature factor, the trapping tube was connected as rapidly as possible.

Reverse-Phase Thin Layer Chromatography

In an attempt to determine the carbonyl compounds present in autoxidizing beta-carotene, the volatile compounds were purged through 2, 4-DNP hydrazine solution at 12.5 th, 24.5th, 36.5th, 48.5th, 60.5th and 80.5th hours after initiation of oxidation. It was found, however, that none of the carbonyl compounds reacted with 2, 4-DNP hydrazine under these conditions. Furthermore, the gas chromatograms of volatile compounds trapped after passing through 2, 4-DNP hydrazine reagent were very similar to those obtained without passing the volatiles through the reagent. Factors that may have prevented successful detection of carbonyl compounds by this technique include: (1) The reaction may have required a higher temperature and longer time, and (2) insufficient amounts of carbonyl compounds were present in the volatile fraction to be detected

by this method.

Another technique was employed to selectively obtain carbonyl compounds from the volatile fraction. In this technique, the volatile compounds were condensed in a glass capillary tube which was connected with the oxidation system and pre-cooled with dry-ice. Samples were taken for ten minutes at the 10th, 20th, 30th, 40th, 80th and 100th hours of oxidation using a different tube for each collection. Each collected sample was reacted with 2, 4-DNP hydrazine reagent and the resulting 2, 4-DNP hydrazones were separated by reverse-phase thin layer chromatography. After development of the TLC plates, only three major spots were observed. Other diffuse spots were also present, but were in such low concentration that their R_f value could not be measured. By comparing the R_f values of the unknown spots with those for authentic derivatives, the three compounds were tentatively identified as acetaldehyde, n-propanal and acetone (shown in Table 3). Sufficient amounts of the derivatives

Table 3. Compounds tentatively identified in the volatile fraction of autoxidized beta-carotene by reverse-phase thin layer chromatography.

Compound	Rf value	
	Unknown	Known
Acetaldehyde	0.771	0.773
Propanal	0.726	0.730
Acetone	0.696	0.701

were not available for melting point determinations. These compounds were also observed in the GLC separations. However, this method was not sufficiently sensitive to obtain evidence for other carbonyl compounds observed in the GLC separations.

GLC Combined with Rapid-Scan Mass Spectrometry

Samples for mass spectrometric analysis were concentrated by repeated trapping of the volatile compounds during 40 hours of oxidation (from the 20th to the 60th hour of oxidation). Four separate samples were examined in this experiment.

A 12 feet x 1/8 inch O. D. stainless steel column packed with 20 percent Carbowax 20M on 80-100 mesh Celite 545 was used. It was operated isothermally at 75°C for 20 minutes and then temperature programmed at five degrees centigrade per minute to 200°C. Compounds eluting from the GLC column were introduced directly into the ion source of the mass spectrometer.

Prior to the mass spectrometric analysis, a typical chromatographic pattern was obtained for reference use during the mass spectrometric analysis. This pattern in shown in Figure 9 and peak identifications are listed in Table 4. Peak assignments were made by comparing relative retention times of known compounds with those of unknown compounds from the autoxidized beta-carotene. The data in Table 4 also indicates the compounds that were identified by mass

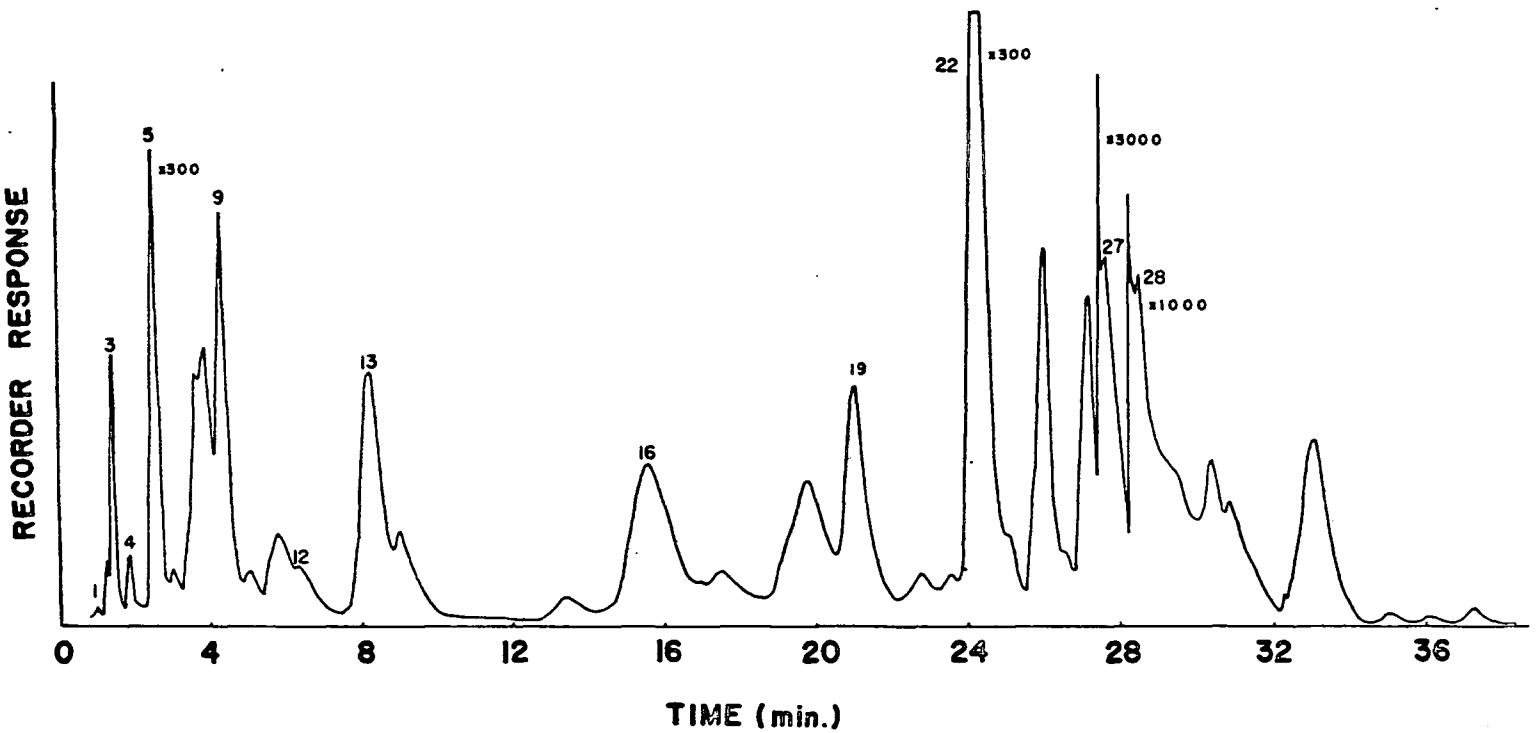


Figure 9. Gas chromatogram of volatile compounds trapped at the 52nd hour of oxidation. Carbowax 20M column operated isothermally at 75°C for 20 minutes, then temperatures programmed at five degrees C per minute to 200°C. See Table 4 for peak identifications.

Table 4. Gas chromatographic identification of volatile compounds trapped at the 52nd hour from autoxidized beta-carotene. (See Figure 9 for gas chromatogram).

Peak No.	Relative retention time (to acetone) Unknown	Known	Compound	Confirmed by mass spectrometry
1	0.531	0.525	ethyl ether	Yes
3	0.687	0.698	acetaldehyde	Yes
4	1.000	1.000	acetone	Yes
5	1.291	1.301	propanal	Yes
9	2.289	2.302	methyl vinyl ketone	Yes
12	3.318	3.336	toluene	Yes
13	4.218	4.190	isobutanal	Yes
16	7.843	------	"nutty" odor	
19	10.83	10.90	2-octanone	Yes
22	12.25	------	"animal-like" odor	
27	13.96	14.05	acetic acid	Yes
28	14.46	------	"fresh wood-like" odor	

spectrometry during later trials.

This sample (Figure 9) was trapped only once for ten minutes at the 52nd hour; however, the accumulated volatiles had been swept from the vial by oxygen at the 48th hour. In a trial designed to determine the efficiency of volatile compound removal, it was found that the oxygen purging almost completely removed volatile compounds present in the reaction vessel. Therefore, most of the volatile compounds shown in Figure 9 were produced from non-volatile products during four hours between the 48th and the 52nd hours of oxidation.

Considering the relative concentrations of the compounds in the mixture trapped at the 52nd hour of oxidation, acetic acid was the most abundant and the "fresh wood-like" odor fraction was next most abundant. The amounts of compounds eluted earlier in the separation were relatively smaller than the amounts of those eluted later. A significant detector response for the "nutty" odor fraction (peak no. 16) is shown in Figure 9.

A representative chromatogram of one of the other four trials is shown in Figure 10, and tentative peak identifications are listed in Table 5. Peak assignments were made in the manner previously mentioned. This sample was an accumulation of volatiles collected during ten minute purgings at the 20th, 35th, 41st and 45th hours of oxidation. The compounds observed in the remaining three trials and not observed in the first trial are listed in Table 6.

The utility of rapid-scan mass spectrometry for studying complex mixtures was well demonstrated in this investigation. An example of the mass spectrometric data obtained for the identification of volatile compounds eluting from a gas chromatograph is illustrated in Figure 11. Strong peaks at m/e 28, 32 and 44 were present in all of the mass spectra and are attributed to the following: 28, carbon monoxide and nitrogen; 32, oxygen; 44, carbon dioxide. Therefore, these fragments were omitted from interpretations. Spectra A, B, C and D in Figure 11 represent the mass spectra of

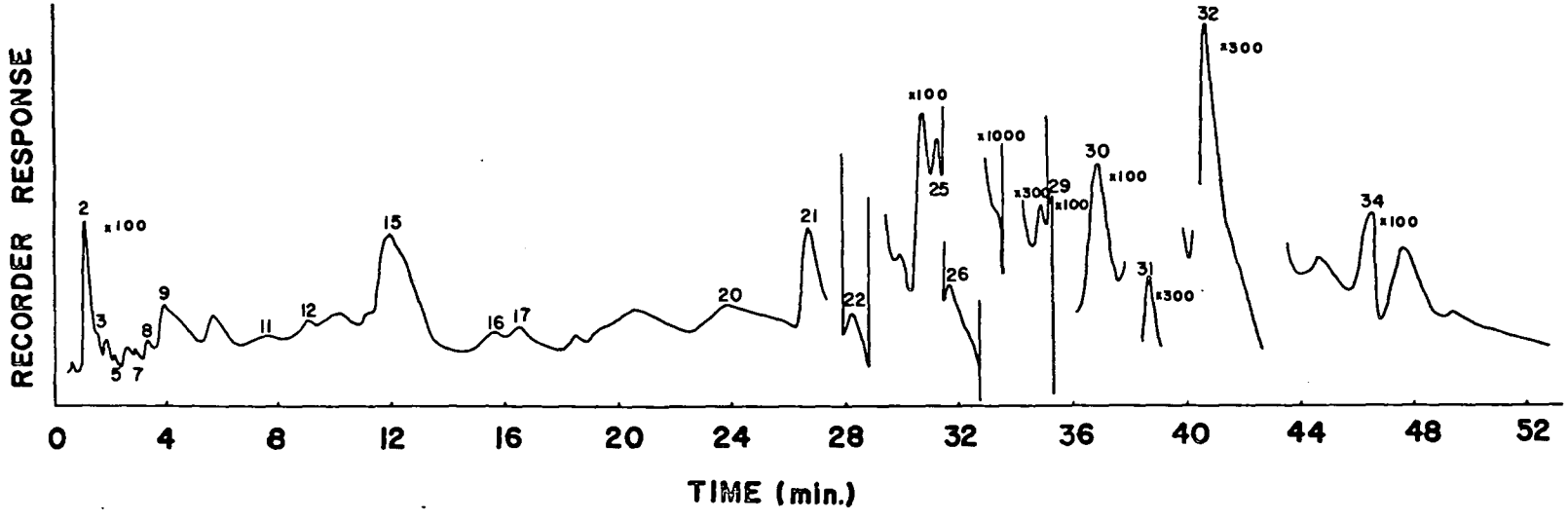


Figure 10. Gas chromatogram of volatile compounds trapped at the 20th, 35th, 41st and and 45th hours of oxidation.

Carbowax 20M column operated isothermally at 75°C for 20 minutes, then temperatures programmed at five degrees C per minute to 200°C. See Table 5 for peak identifications.

Table 5. Gas chromatographic and mass spectral identification of volatile compounds of autoxidized beta-carotene. I. (See Figure 10 for gas chromatogram).

Peak number	Relative retention time (to acetone) Unknown	Known	Compound tentatively identified by mass spectrogram
2	0.518	0.525	ethyl ether
3	0.704	0.698	acetaldehyde
5	1.000	1.000	acetone
7	1.330	1.301	propanal
8	1.558	-----	2-methylfuran
9	1.820	-----	1, 3, 3-trimethylcyclohexene
11	3.362	3.336	toluene
12	4.195	4.190	isobutanal
15	5.553	-----	*
16	7.231	-----	*
17	7.607	-----	2-methyl-2-heptenal
20	10.92	10.90	2-octanone
21	12.35	-----	*
22	13.09	-----	1, 3-dimethyl-2-ethylcyclohexane
25	14.15	-----	2-ethyl-2-hexenal
26	14.65	14.05	acetic acid
29	16.25	-----	2-formyl-3, 3-dimethylcyclohexene
30	17.03	-----	1, 1, 3-trimethyl-2-n-propylcyclohexane
31	17.80	-----	*
32	18.83	-----	*
34	21.50	-----	2-methyl-3-nonene

* Peak No. 15 has two possibilities from the interpretation of its mass spectrogram; 3, 3-dimethyl-2-vinylcyclohexene or 3, 4, 5, 5-tetramethyl-1, 3-cyclohexene.

Peak No. 16 also has two possibilities; trans 2-methyl-3-heptene or trans 2, 5-dimethyl-3-hexene.

Peak No. 21 has an animal-like odor.

Peak No. 31 has three possibilities; 2, 6-dimethyl-2-nonene; 1, 1, 3-trimethyl-2-ethyl cyclohexane or isovalerylcyclohexane.

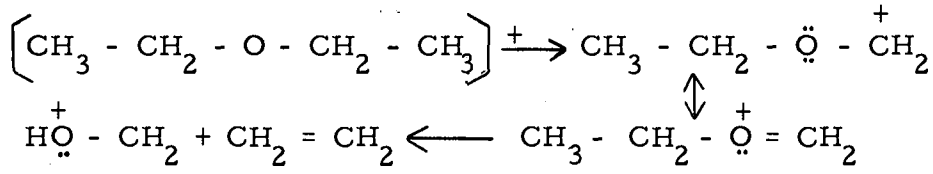
Data for Peak No. 32 suggests that it is probably a C_{10} alcohol.

Table 6. Gas chromatographic and mass spectral identification of volatile compounds of autoxidized beta-carotene. II.

Relative retention time (to acetone)		Compound tentatively identified by mass spectrogram
Unknown	Known	
0.584	0.580	n-pentane
0.593	-----	3-methyl-2-pentanone
0.741	-----	4-methyl-3-pentan-2-one
2.375	2.301	methyl vinyl ketone
19.40	-----	C_{12} monounsaturated 2-alkanone
23.00	-----	3, 5, 5-trimethyl-4-(4'-butyl-3'-en-2'-onyl) cyclohexa-1, 3-diene

peaks 2, 7, 20 and 26 in Figure 10, respectively.

In spectrum A, the peak at m/e 74 corresponds to the parent ion for diethyl ether. The base peak (most abundant ion), m/e 31, arises in the following manner (Gohlke, 1959):



m/e 31

The peak at m/e 59 represents the ion after elimination of a methyl fragment from the parent compound, and the peak at m/e 45 represents the ion after elimination of an ethyl fragment. Comparison of the unknown spectrum with that of ethyl ether in the API tables (American Petroleum Institute, 1948 to date) shows good agreement for relative abundance of ions. Coincidence of the relative retention times of known ethyl ether and the unknown adds further evidence

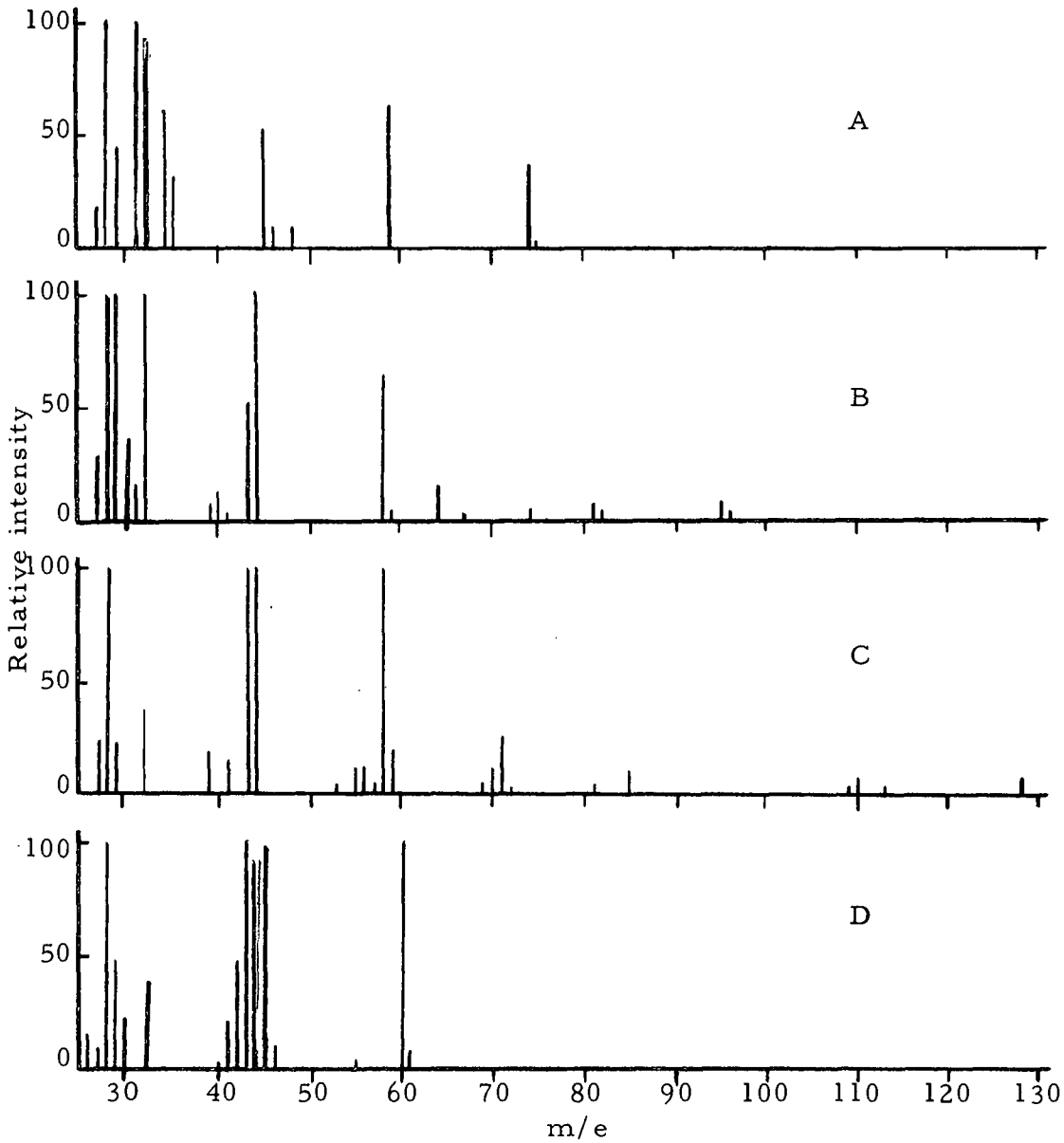
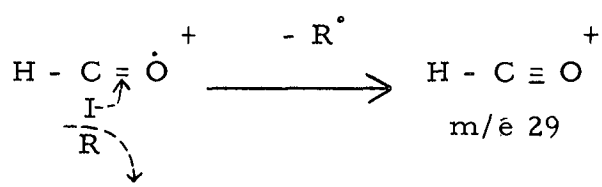


Figure 11. Mass spectral charts for chromatographic fractions: A corresponds to chromatographic peak 2 in Figure 10; B peak 7; C peak 20; and D peak 26.

for confirming the identification.

In spectrum B, peaks at m/e 96, 95, 82, 81, 67 and 64 are fragments of a compound which eluted just before peak 7 in Figure 10. This was indicated because their peak heights were decreasing when compared with the background spectrum taken just before spectrum B. The fragment at m/e 74 is the parent peak of ethyl ether which also eluted before peak 7, and also the peak at m/e 59 is a fragment of ethyl ether. Acetone probably contributes to the peak at m/e 58, but the increase of the peak height would indicate the presence of a new parent. Eliminating such contributions, the mass spectrum B of peak 7 in Figure 10 may be concluded to consist of the parent peak at m/e 58, the base peak at m/e 29 and others at m/e 43, 58, 30, 27, 31, 40 and 42 in order of decreasing height. The comparison of this spectrum with that of propanal in API tables shows good agreement. In general, a mass spectrum of a lower (C_1-C_3) n-saturated aliphatic aldehyde shows α-cleavage with the formation of the formyl ion (m/e 29) that is responsible for the base peak of the spectrum (Gilpin and McLafferty, 1957):



According to these workers the base peak of spectrum B may be $H-C\equiv O^{+}$ (m/e 29) rather than $C_2H_5^{+}$ (m/e 29).

In spectrum C, the parent ion appears at m/e 128. The base

peak at m/e 43 is indicative of a methyl, propyl or isopropyl ketone. The intense rearrangement peak at m/e 58 (Budzikiewicz, Djerassi and Williams, 1964) is compatible only with a methyl and propyl ketone. If one of the alkyl groups is a propyl group, the second rearrangement peak at m/e 86 $\left[CH_2 = C(OH) - C_3H_7\right]^+$ must be present in the spectrum. The absence of the peak at m/e 86 strongly suggests that the compound is the methyl ketone with molecular weight of 128, 2-octanone. Comparison of the unknown spectrum with that of 2-octanone in the ASTM tables (American Society for Testing and Materials, 1963) shows excellent agreement. Coincidence of relative retention time of peak 20 with authentic 2-octanone (Tables 3, 4 and 5) gives firm evidence for the identification of the compound.

In Spectrum D (GLC peak 26 in Figure 10), the ion at m/e 60 is the parent because all ions with larger m/e values can be attributed to the background spectrum. The base peak is also m/e 60 and other significant peaks are 45, 43, 42, 29, 30 and 41, in order of decreasing peak height. According to API tables, the parent peak of acetic acid is at m/e 60 and the base peak is also at m/e 60. Other significant ions are 43 (99 percent, relative abundance), 45 (98 percent), 44 (34 percent), 28 (29 percent) and 42 (28 percent). However, as discussed previously, peaks at m/e 44 and 28 were not used in the interpretation because strong peaks at m/e 44 and 28 were

due to the presence of carbon dioxide for m/e 44 and carbon monoxide and nitrogen for m/e 28. The spectrum of the unknown showed agreement with API data and GLC retention times agreed.

The compound responsible for the "nutty" odor (peak 17 in Figure 10) gave a parent peak at m/e 126. After subtracting that portion of the spectra due to the background, important ion peaks appeared at m/e 126, 111, 97, 83, 71, 55, 43, 41, 39, 30, 29, and 27. The larger peaks were at m/e 41, 71, 55, 43, 39, 29 and 27. The relative abundance of the ions for the "nutty" fraction were at m/e 41 (base peak), 55 (89 percent), 27 (72 percent), 71 (71 percent), 43 (57 percent), 39 (56 percent) and 97 (11 percent). Although the data did not show close agreement with available known spectra, the spectrum was similar to that of 2-methyl-2-heptenal listed in ASTM uncertified mass spectral data (No. 1759) submitted by Western Utilization Research and Development Division (WURDD). The ASTM tables list important ion peaks for 2-methyl-2-heptenal at m/e 41 (base peak), 55 (97 percent), 97 (86 percent), 27 (66 percent), 43 (58 percent), 39 (56 percent), 29 (54 percent) and 71 (52 percent). Although the "nutty" fraction could not be identified positively as 2-methyl-2-heptenal, the data strongly suggested that the compound in question was a monomethyl substituted 2-heptenal.

Summary of Compounds Identified

The compounds tentatively or positively identified in the volatile fraction of autoxidized beta-carotene are summarized in Table 7. The criterion employed for identification in this study was agreement of spectra of unknown compounds with standard published mass spectra of known compounds, and coincidence of GLC retention data of authentic and unknown compounds. In some cases authentic compounds were not available for confirmation of peak identity by GLC relative retention times; however, some of the mass spectra obtained were of such quality that identification of the compounds could still be made. Compounds showing agreement of retention times on both types of GLC columns only, but not by mass spectrum were designated as tentative identifications. Compounds that were identified by inconclusive mass spectra, and where standard compounds were not available for retention data, were also designated as tentative identifications. The mass spectral data for the compounds identified in Table 7, except for the compounds which have been discussed in detail in previous section, are given in Tables 8 through 22 in the Appendix. After subtracting background and the influence of the spectra of adjacent compounds in the GLC separations, the relative abundance of ions in the spectra of the unknown compounds showed reasonable coincidence with standard reference spectra.

Table 7. Summary of compounds identified in the volatile compounds of autoxidized beta-carotene.

Compound identified	Relative retention time data of standard compounds		Mass spectral Identification
	Apiezon M	Carbowax 20M	
acetaldehyde	+	+	yes
propanal	+	+	yes
isobutanal	+	+	yes
2-ethyl-2-hexenal	-	-	tentative
2-methyl-2-heptenal	-	-	tentative
acetone	+	+	yes
butanone	+	+	-
diacetyl	+	+	-
methyl vinyl ketone	+	+	yes
3-methyl-2-pentanone	-	-	tentative
4-methyl-3-penten-2-one	-	-	tentative
2-octanone	+	+	yes
acetic acid	+	+	yes
methyl formate	+	-	-
ethyl ether	+	+	yes
toluene	-	+	yes
n-pentane	-	+	yes
2-methyl-3-nonene	-	-	tentative
2-methylfuran	-	-	tentative
1, 3, 3-trimethylcyclohexene	-	-	tentative
2-formyl-3, 3-dimethylcyclohexene	-	-	tentative
1, 1, 3-trimethyl-2-n-propylcyclohexane	-	-	tentative
1, 3-dimethyl-2-ethylcyclohexane	-	-	tentative
3, 5, 5-trimethyl-4-(4'-butyl-3'-en-2-onyl) cyclohexa-1, 3-diene	-	-	tentative

However, some m/e values in the unknown spectra were strongly influenced by the contaminating spectra of adjacent compounds eluting from the GLC. This caused certain ions in some spectra to be so intense that even at the greatest attenuation the ion peaks were off-scale.

Where reference spectra were not available, structures of compounds were constructed by selecting likely empirical formulae on the basis of individual ion fragments. The selection of probable empirical formulae for the different mass (P) and isotope (P + 1) or (P + 2) abundance was in a large part based on the table constructed by Beynon (1960). Later, the table was extended and listed in "Mass and Abundance Tables" (Beynon and Williams, 1963). Although the P + 1/P ratio approach for the determination of empirical formulae (Silverstein and Bassler, 1964) is very useful for static samples at low analyzer pressure, some caution must be used when molecules containing a hetero atom are analyzed by the fast-scanning of GLC effluent at higher pressures ($\sim 10^{-6}$ mmHg). In the present work rapid-scans were obtained at analyzer pressure of $\sim 5 \times 10^{-7}$ mmHg and the empirical formulae so obtained are thought to be useful. Mass spectra of the highest accuracy are not obtained in fast-scanning (McFadden and Day, 1964), and that the data may error in indicating a slightly higher than true P + 1 due to a bimolecular addition of a hydrogen to parent ion (P^+) to give a higher than expected P + 1/P

ratio (Biemann, 1962).

In Table 13, although the relative abundances of peaks of the unknown compound (peak 2 in Figure 11) did not show close agreement with those of available reference spectra, the data suggested that the compound was 3-methyl-2-pentanone.

In Table 19, the base peak of the compound at m/e 82 was probably due to the cyclohexenyl group. Since the ratio (x100) of P + 1/P was 10.8, the empirical formula would probably be $C_9H_{14}O$ (9.99). The peak at m/e 123 would indicate the cleavage of one methyl group from the parent and peak at m/e 108 would indicate the cleavage of two methyl groups from the parent. The peak at m/e 29 could be attributed to the formyl fragment. Therefore, the compound was tentatively identified as 2-formyl-3, 3-dimethylcyclohexene.

For the compound in Table 21, a comparison of the ratio (x100) of P + 1/P, 10.01 with that of Beynon's table reveals that the most probable empirical formula is C_9H_{16} (9.98). In the API tables, the standard spectral data for 3, 3, 5-trimethylcyclohexene (1493 AE and 1494 AE) were similar to the data obtained for the unknown, but it did not coincide closely enough to indicate that identity. Considering the structure of beta-carotene, the most probable identity of the unknown is 1, 3, 3-trimethylcyclohexene.

The mass spectral data in Table 21 shows a strong base peak at m/e 82 indicating the presence of cyclohexyl group. Because the

ratio (x100) of P + 1/P was 10.76, the empirical formula of $C_{10}H_{20}$ (11.13) was suggested. Standard reference spectra of n-, sec.-, iso- and t-butyl-isopropylcyclohexane did not coincide with the spectrum of the unknown. However, in considering the structure of intact beta-carotene, 1, 3-dimethyl-2-ethylcyclohexane was suggested.

In Table 23, the strong peak at m/e 43 indicates the possibility of a methyl ketone. From the ratio (x100) of P + 1/P (14.38), the empirical formula would be $C_{13}H_{18}O$. The presence of a peak at m/e 77 suggests the possibility of a cyclohexadien. Considering the structure of beta-carotene, 3, 5, 5-trimethyl-4-(4'-but-3'-ene-2'-onyl)cyclohexa-1, 3-diene was suggested.

GLC fractions which could not be characterized as a single compound are not listed in Table 7, but are listed in Table 5. The mass spectral data for above compounds are given in Tables 23 through 25 in the Appendix.

In Table 23, since the ratio (x100) of P + 1/P was 11.4, the most probable empirical formula was $C_{10}H_{16}$ (11.06). This hydrocarbon apparently contained two double bonds and the base peak at m/e 27 would indicate the possibility of vinyl group. Considering the above, 3, 3-dimethyl-2-vinylcyclohexene (I) is suggested. On the other hand, the standard spectrum (API) of beta-pyronene (II) shows agreement to a limited extent with the unknown.

In Table 24, the ratio (x100) of P + 1/P was 8.71, suggesting

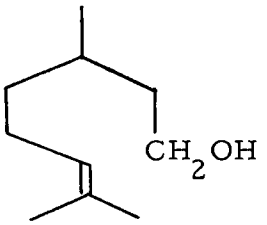
that the empirical formula would be C_8H_{16} (8.90). By comparing the unknown spectra with the API standard data, two compounds, trans-2-methyl-3-heptene (I) and trans-2, 5-dimethyl-3-hexene (II), were found to have similar spectra.

In Table 25, the ratio (x100) of P + 1/P was 13.0 which suggests an empirical formula of $C_{11}H_{22}$ (12.2). The base peak at m/e 82 suggests the cyclohexyl group (C_6H_{10}). If there were a cyclohexyl group in the molecule, the side chain must be saturated. The structure of beta-carotene suggests the structures of 1, 1, 3-trimethyl-2-ethylcyclohexane (II) and isovalerylcyclohexane (III) as logical compounds. However, if the strong base peak were not due to the cyclohexyl group, 2, 6-dimethyl-2-nonene (I) would be a possibility.

Compounds which were not identified, but which appeared to be a methyl ketone and a C_{10} alcohol, are listed in Tables 5 and 6, respectively. The mass spectral data for these two compounds are given in Tables 26 and 27 in the Appendix.

In Table 26, since the ratio (x100) of P + 1/P was 12.5, $C_{12}H_{22}O$ is suggested as the empirical formula. The strong base peak at m/e 43 indicates that the compound may be a methyl ketone. Although it could be suggested that the compound could be a monounsaturated C_{12}-methyl ketone, it was not possible to determine whether the compound was a straight or branched chain methyl ketone from the data.

In Table 27, from the ratios (x100) of P + 1/P and P + 2/P, 14.4 and 1.0, respectively, the empirical formula can be suggested as either $C_{10}H_{20}O$ or $C_{11}H_8O$. Since a strong peak was not present at m/e 43, the compound could not be a methyl ketone. The mass spectral pattern had some resemblance to terpene alcohols, such as citronellol:



(ASTM Uncertified mass spectrum, WURDD 1673). Both the unknown compound and citronellol have same parent peak and base peak.

Figures 12 and 13 in the Appendix are gas chromatograms which were obtained during mass spectrometry analysis, and are cited in some tables in the Appendix. Figure 12 is a gas chromatogram of the volatile compounds on the Carbowax 20M column which were trapped from oxidized beta-carotene at the 18th, 20th, 35th, 40.5th, 47th and 50th hour after the oxidation was initiated. Figure 13 is a similar gas chromatogram except that the volatile compounds were trapped at the 24th, 34th, 36th, 38th, and 39.5th hour of oxidation.

The mass spectra obtained for many of the GLC peaks were too

complex to interpret because of incomplete GLC separations. In addition, other GLC peaks yielded spectra that were not sufficiently strong for interpretation. Further studies utilizing improved GLC separations and sample enrichment techniques will be required to further characterize the compounds arising from autoxidizing beta-carotene.

Significance of Compounds Identified

Ten compounds were identified and 14 compounds were tentatively identified from the volatile compounds of autoxidized beta-carotene. Among the 24 compounds characterized, seven were methyl ketones, five were aldehydes, three were alkyl substituted cyclohexenes, two were alkyl substituted cyclohexanes, two were aliphatic hydrocarbons. The remaining included an ether, a furan, an ester, an aromatic hydrocarbon and an acid. Compounds which are believed to be responsible for the off-flavor of autoxidized lipids in milk and milk products are primarily carbonyls. Among the compounds identified in this investigation, the carbonyl compounds are, therefore, especially interesting from this point of view.

The methyl formate observed in the volatile fraction was small and the life-time was relatively short. Therefore, it probably does not play an important role in off-flavor of autoxidized beta-carotene.

The odor of autoxidized beta-carotene was relatively sharp and

acidic until all beta-carotene was oxidized and bleached white. Then a violet-like odor which may have been due to the formation of beta-ionone (Ayers et al., 1964; Falconer et al., 1964; and Fishwick, Land and Swain, 1964) was predominant. Since beta-ionone has a relatively high boiling point, 140°C at 18 mmHg, it was not obtained in the volatile fraction of autoxidized beta-carotene under the conditions employed in this investigation.

It has been recognized that a "nutty" odor fraction arises in certain dairy products (Parks, 1965), but the identity of the responsible compound is not yet known. In this investigation a peak with strong "nutty" odor was recognized in the volatile compounds of autoxidized beta-carotene. It was tentatively identified as 2-methyl-2-heptanal. This finding may give some insight into the nature of compounds that exhibit a "nutty" character. Since the "nutty" odor component, 2-methyl-2-heptenal, was produced from oxidizing beta-carotene, it appears possible that other branched-chain conjugated polyunsaturated lipids besides beta-carotene potentially could give rise to compounds with similar odors. In dairy products, however, the most abundant branched long chain conjugated polyunsaturated lipid is beta-carotene. Therefore, it is suggested that one of the sources of the "nutty" odor in dairy products is beta-carotene.

In order to determine whether the "nutty" fraction, peak 16 in Figure 9, was a single component, the fraction was cold-trapped in

a tube as it eluted from the GLC column using a 2-methoxy ethanol/ dry-ice cooling bath. The trapping tube consisted of a 20 inch x 0.01 inch I. D. stainless steel capillary tube packed with 20 percent Apiezon M on 100-120 mesh Celite 545 (acid-alkali washed). The concentration of the "nutty" fraction was enriched by repeated trapping in the same capillary tube during the 40 hours of oxidation from the 20th to 60th hour after the oxidation was initiated. The sample tube was then connected between the carrier gas inlet and a Golay capillary column for separation of the trapped volatiles. The column used was 300 feet x 0.01 inch I. D. stainless steel capillary column coated with Carbowax K-1540. The instrument oven was operated isothermally at 75°C for 15 minutes and then programmed at the rate of five degrees Centigrade per minute to 140°C. The flow rate of the carrier gas, nitrogen, was one ml per minute. A relatively sharp peak appeared after 15.5 minutes, and was the only peak obtained in the chromatogram. The results indicated that the "nutty" odor was due to one compound. Since this experiment was designed only to determine the purity of "nutty" odor fraction, no further data concerning its identity was obtained.

Relatively large amounts of acetic acid were produced during the early and latter stages of the oxidation period. This compound would contribute to the acidic odor observed in the volatile products. After the yellow color of beta-carotene had completely faded, no

appreciable amounts of carbonyl compounds and acetic acid were detected in the gas chromatograms. Also, the unpleasant acidic odor of the volatiles was considerably decreased.

Fishwick, Land and Swain (1964) identified acetaldehyde, methyl formate, n-pentane, ethyl ether, n-propanal, acetone and diacetyl in the low boiling volatile products of oxidized beta-carotene. All of these compounds were also found in this investigation. They also reported the presence of n-hexane, trimethyl butane, dimethyl pentane, methyl hexane, n-heptane, benzene and iso-octane, all of which were not identified in this investigation. They explained that the presence of benzene was due to an impurity since their samples of beta-carotene had been recrystallized from benzene/methanol. They suggested that the benzene was occluded within the beta-carotene crystals and were released only when oxidation occurred. In this investigation benzene was not found even though a careful search was made.

One of the most confusing results of this investigation was the identification of toluene. However, the presence of toluene in various dairy products, for example, in blue cheese (Day and Anderson, 1965) and in Swiss cheese (Langler, 1966), has been reported. It has been suggested that toluene may arise from the degradation of certain aromatic compounds, such as aromatic amino acids, or it might originate in the feed. If toluene was not accidentally introduced into the

oxidation system, and is a real oxidative degradation product of beta-carotene, then the aromatization must have occurred during the oxidation of beta-carotene. The mechanism by which this reaction could occur is not readily apparent.

SUMMARY AND CONCLUSIONS

This investigation was concerned with the qualitative identification of the volatile compounds of autoxidized beta-carotene. Beta-carotene was mixed with Celite (acid washed) and oxidized by molecular oxygen at 50°C under a controlled system. The peroxide value of the non-volatile products of oxidized beta-carotene was determined at various intervals up to 20 hours. Both an improved classical volumetric method and a potentiometric method were used in the peroxide determinations. The potentiometric method was preferred because it overcame difficulties in determining the end-point in the volumetric method. The volatile compounds arising from autoxidizing beta-carotene were collected by a gas-entrainment procedure at various intervals up to 160 hours of oxidation time. The collected materials were separated by gas chromatography using both a non-polar column (Apiezon M) and a polar column (Carbowax 20M).

In later studies the volatile fraction was repeatedly trapped during a 40 hour oxidation period. The same trapping tube was used in each series to build up the concentration of volatile compounds so that gas chromatography in conjunction with rapid-scan mass spectrometry could be used for identification. Ten compounds were identified and 14 compounds were tentatively identified using this technique.

The volatile compounds from autoxidized beta-carotene were cold-trapped in long glass capillary tubes using dry-ice as the coolant. The liquid samples were reacted with 2, 4-DNP hydrazine to form 2, 4-DNP hydrazone derivatives. The derivatives were then separated and tentatively identified by reverse-phase thin layer chromatography.

The findings of this investigation are summarized as follows:

1. Two different systems were employed for the oxidation of beta-carotene. In one system the maximum peroxide value, 586 milliequivalents per 1000 grams of beta-carotene, was reached six hours after the oxidation was initiated. The peroxide value went up rapidly two hours after initiation of oxidation and after six hours, it slowly decreased. In the other the maximum peroxide value, 295 milliequivalents per 1000 grams of beta-carotene, was reached three hours after the oxidation was initiated. The peroxide value rapidly decreased with further oxidation time and after 20 hours it was reduced to 50 milliequivalents per 1000 grams.
2. A potentiometric titration method for peroxide value determination had advantages for deep-colored lipid systems over a volumetric method widely used for peroxide value determinations. The advantages were its ease in performance and its reproducibility.

3. Compounds whose GLC retention data coincided with those of authentic compounds using an Apiezon M column and (or) a Carbowax 20M column were acetaldehyde, acetone, n-propanal, ethyl ether, methyl vinyl ketone, diacetyl, butanone, acetic acid, 2-octanone, isobutanal and methyl formate.
4. Compounds identified by mass spectrometry and (or) GLC were n-pentane, ethyl ether, acetaldehyde, acetone, propanal, methyl vinyl ketone, toluene, isobutanal, 2-octanone and acetic acid.
5. Compounds tentatively identified by mass spectrometry alone were 3-methyl-2-pentanone, 4-methyl-3-pentan-2-one, 2-methylfuran, 1, 3, 3-trimethylcyclohexene, 2-methyl-2-heptenal, 1, 3-dimethyl-2-ethylcyclohexane, 2-ethyl-2-hexenal, 2-formyl-3, 3-dimethylcyclohexene, 1, 1, 3-trimethyl-2-n-propylcyclohexane, 2-methyl-3-nonene and 3, 5, 5-trimethyl-4(4'-butyl-3'-en-2'-onyl) cyclohexa-1, 3-diene.
6. Compounds which were suggested by inconclusive mass spectral data were 3, 3-dimethyl-2-vinylcyclohexene or 3, 4, 5, 5-tetramethyl-1, 3-cyclohexene; trans-2-methyl-3-heptene or trans-2, 5-dimethyl-3-hexene; and 2, 6-dimethyl-2-nonene, 1, 1, 3-dimethyl-2-ethylcyclohexane or isovaleryl-cyclohexane.

7. Compounds tentatively identified by reverse-phase TLC were acetaldehyde, propanal and acetone.

8. A compound possessing a "nutty" odor arising from autoxidized beta-carotene was tentatively identified as 2-methyl-2-heptenal.

BIBLIOGRAPHY

American Petroleum Institute. 1948 et seq. Research Project 44. College Station, Texas A & M University, Chemical Thermodynamics Properties Center. 6 vols.

American Society for Testing and Materials. 1963. Committee E-14 on Mass Spectrometry. Index of mass spectral data. Philadelphia. Various paging.

Ayers, J. E. et al. 1964. Off-flavor of dehydrated carrot stored in oxygen. Nature 203:81.

Berl, Soll and W. H. Peterson. 1945. Distribution of carotene and vitamin A in butter-making. Journal of Dairy Science 28:103-107.

Beynon, J. H. 1960. Mass spectrometry and its applications to organic chemistry. New York, Elsevier, 640 p.

Beynon, J. H. and A. E. Williams. 1963. Mass and abundance tables for use in mass spectrometry. New York, Elsevier. 570 p.

Biemann, Klaus. 1962. Mass spectrometry-organic chemical applications. New York, McGraw-Hill. 370 p.

Bodea, Cornel and E. Nicoara. 1955. The autoxidation of carotenoids. I. The autoxidation mechanism of α- and β-carotene. Academia Republicii Populare Romine, Studii si Cercetari de Chemie 3:81-91. (Abstracted in Chemical Abstracts 50: 11785h. 1956)

Bodea, Cornel, E. Nicoara and J. Gross. 1953. The action of carotenoids in the processes of autoxidation and polymerization. I. The autoxidation of benzaldehyde in the presence of α- and β-carotene. Academia Republicii Populare Romine Filiala Cluj, Studii si Cercetari Stiintifiu Filiala 4 (3-4):51-60. (Abstracted in Chemical Abstracts 50:11828g. 1956)

Bodea, Cornel, E. Nicoara and J. Gross. 1954. The action of carotenoids in autoxidation and polymerization processes. II. The autoxidation of aliphatic aldehydes in the presence of α- and β-carotene. Academia Republicii Populare Romine,

Filiala Cluj, Studii si Cercetari Stiintifice, Seria I, Stiinte Mathematice, Fizice, Chimice si Tehnice 5 (3-4):5968. (Abstracted in Chemical Abstracts 50:15471h. 1956)

Bodea, Cornel et al. 1956. Autoxidation and mechanism of action of carotene. Revista de Chimie (Bucharest) 1 (1):133-142. (Abstracted in Journal of the Science of Food and Agriculture 8:i-196. 1957)

Bradway, Elizabeth M. and H. A. Matill. 1934. The association of fat-soluble vitamins and antioxidants in some plant tissues. The Journal of the American Chemical Society 56:2405-2408.

Briggs, Lindsay Heathcote. 1931. The autoxidation of butterfat. I. Factors influencing the reaction. The Journal of Dairy Research 3:61-69.

Brown, W. Carson, A. H. Vanlandingham and Chas. E. Weakley, Jr. 1941. Oxidized flavor in milk. IX. The effect of the quality of hay and early stage of lactation on the carotene content of butterfat and on the ascorbic acid content of the milk and their relationship to the development of metal-induced oxidized flavor. Journal of Dairy Science 24:925-935.

Browne, C. A. 1925. The spontaneous decomposition of butterfat. Industrial and Engineering Chemistry 17:44-47.

Budzikiewicz, Herbert, Carl Djerassi and Dudley H. Williams. 1964. Interpretation of mass spectra of organic compounds. San Francisco, Holden-Day. 271 p.

Day, Edgar Allan. 1960. Milk lipids symposium. Autoxidation of milk lipids. Journal of Dairy Science 43:1360-1365.

Day, Edgar Allan. 1965. Some properties of carbonyl compounds encountered in the flavor isolates of dairy products-A review. Food Technology 19:129-134.

Day, Edgar Allan and D. F. Anderson. 1965. Gas chromatographic and mass spectral identification of natural components of the aroma fraction of blue cheese. Journal of Agricultural and Food Chemistry 13:2-4.

DeLuca, A. P. et al. 1957. Relative effectiveness of various antioxidants fed to lactating dairy cows, on incidence of copper-induced oxidized milk flavor and on apparent carotene and tocopherol utilization. Journal of Dairy Science 40:877-886.

Dornbush, A. C., W. H. Peterson and F. R. Olson. 1940. The carotene and vitamin A content of market milks. The Journal of the American Medical Association 114:1748-1751.

El-Negoumy, A. M., D. M. Milles and E. G. Hammond. 1961. Partial characterization of the flavors of oxidized butteroil. Journal of Dairy Science 44:1047-1056.

El-Negoumy, A. M., M. S. de Puchal and E. G. Hammond. 1962. Relation of linoleate and linolenate to the flavors of autoxidized milk fat. Journal of Dairy Science 45:311-316.

Falconer, M. E. et al. 1964. Carotene oxidation and off-flavor development in dehydrated carrot. Journal of the Science of Food and Agriculture 15:897-901.

Fishwick, M. J., D. G. Land and T. Swain. 1964. An investigation of the chemical changes in carotenoid pigments during processing and storage of selected vegetables and the effect of these changes on product stability and flavor. Cambridge, England, Low Temperature Research Station. n. p. (Agricultural Research Council. Project no. UR-E29-30-20)

Forss, D. A. 1964. Fishy flavor in dairy products. Journal of Dairy Science 47:245-250.

Forss, D. A., E. A. Dunstone and W. Stark. 1960a. Fishy flavor in dairy products. II. The volatile compounds associated with fishy flavor in butterfat. The Journal of Dairy Research 27: 211-219.

Forss, D. A., E. A. Dunstone and W. Stark. 1960b. Fishy flavor in dairy products. III. The volatile compounds associated with fishy flavor in washed cream. The Journal of Dairy Research 27:373-380.

Forss, D. A., E. A. Dunstone and W. Stark. 1960c. The volatile compounds associated with tallowy and painty flavors in butterfat. The Journal of Dairy Research 27:381-387.

Forss, D. A., E. G. Pont and W. Stark. 1955. The volatile compounds associated with oxidized flavor in skim milk. The Journal of Dairy Research 22:91-102.

Forss, D. A. et al. 1962. The flavor of cucumbers. Journal of Food Science 27:90-93.

Gillam, Albert Edward and Mohammed Shafik El Ridi. 1937. The carotene of milk fat (butter). The Biochemical Journal 31: 251-253.

Gillam, Albert Edward et al. 1933. Variations in the quality of butter, particularly in relation to the vitamin A, carotene and xanthophyll content as influenced by feeding artificially dried grass to stall-cattle. The Biochemical Journal 27:878-888.

Gillam, Albert Edward et al. 1938. The effect of commercial sterilization on the nutritive value of milk. III. Effect on the vitamin A and carotene content of milk. The Journal of Dairy Research 9:17-21.

Gilpin, J. A. and F. W. McLafferty. 1957. Mass spectrometric analysis. Aliphatic aldehydes. Analytical Chemistry 29:990-994.

Gohlke, R. S. 1959. Time-of-flight mass spectrometry and gas-liquid partition chromatography. Analytical Chemistry 31: 535-541.

Greenbank, George. 1949. The oxidative deterioration of dairy products. In: Proceedings of the 12th International Dairy Congress, Stockholm, 1949. Vol. 2, sec. 2. Stockholm, Ivar Haeggströms. p. 284-291.

Greenbank, George and George E. Holm. 1934. Antioxidants for fats and oils. Industrial and Engineering Chemistry 26:243-245.

Hammond, E. G. and F. D. Hill. 1964. Oxidized-metallic and grassy flavor components of autoxidized milk. The Journal of the American Oil Chemists' Society 41:180-184.

Hartman, Arthur M. and Leslie P. Dryden. 1965. Vitamins in milk and milk products. Champaign, The American Dairy Science Association. 123 p.

Heaton, F. W. and N. Uri. 1958. Improved iodometric methods for the determination of lipid peroxides. Journal of the Science of Food and Agriculture 9:781-786.

Heiman, Victor and J. S. Carver. 1937. The relative flavin (vitamin G) content of dried skimmilk, dried whey, and dried buttermilk. Poultry Science 16:434-437.

Henderson, J. L. 1939. Feeding cows rations which contain adequate amounts of carotene. Milk Plant Monthly 28:26-27. (Abstracted in Chemical Abstracts 34:1767-4. 1940)

Hoffman-La Roche Inc. 1963. Beta-carotene Roche-the color that nourishes. Nutley. 16 p.

Japan Society for Analytical Chemistry. 1958. Kikibunseki Zikkenho (Instrumental methods of analysis). Tokyo, Maki-Shoten. 542 p.

Johnson, G. Dana. 1951. A new preparation of 2, 4-dinitrophenylhydrazones. The Journal of the American Chemical Society 73:5888-5889.

Karrer, P. and A. Helfenstein. 1929. Pflanzenfarbstoffe. XVI. Über Carotein. I. Helvetica Chimica Acta:1142-1144.

Karrer, P. and E. Jucker. 1945. Oxyde des β-Carotins: β-Carotin-mono-epoxyd, β-Carotin-di-epoxyd, Mutatochrom, Aurochrom, Luteochrom. Helvetica Chimica Acta 28:427-436.

Kuhn, Richard and Hans Brockmann. 1935. Über den stufenweisen Abbau und die Konstitution des β-Carotins. Justus Liebig's Annalen der Chemie 516:95-143.

Kihn, Richard and Fortunat L'Orsa. 1931. Zur Konstitution des Safran-Farbstoffes (Über konjugierte Doppelbindungen. XVIII. Mitteil). Berichte der Deutschen Chemischen Gesellschaft 64:1732-1736.

Kummerow, F. A. 1961. Fats and oils. In: Proceedings of the Flavor Chemistry Symposium, Camden, New Jersey, Campbell Soup Co. p. 109-115.

Langlar, James Edward. 1966. Flavor chemistry of Swiss cheese. Ph. D. thesis. Corvallis, Oregon State University. 71 numb. leaves.

Lea, C. H. 1953. Oxidation defects of milk and dairy products. In: Proceedings of the 13th International Dairy Congress,

Hague, 1953. Vol. 3, sec. 4. Hague, Mouton and Co. 1954. p. 1037-1048.

Libbey, Leonard M. and Edgar Allan Day. 1964. Reverse-phase thin layer chromatography of 2, 4-dinitrophenylhydrazones of n-alkanals and n-alkan-2-ones. Journal of Chromatography 14:273-275.

Luck, H. 1966. Greenish discoloration of butterfat during an extended period of cold storage. The Journal of Dairy Research 33:25-29.

Lundberg, W. O. 1961. Autoxidation and antioxidants. Vol. 1. New York, Interscience. 450 p.

Martin, W. H. et al. 1940. The effect of feeding vitamin A and carotene to cows on the flavor and color of their milk. Association Bulletin, The International Association of Milk Dealers no. 12, p. 279-286. (Abstracted in Chemical Abstracts 34: 8081-3. 1940)

McDowall, F. H. and W. A. McGillivray. 1963a. Studies on the properties of New Zealand butterfat. VI. Comparison of the properties and vitamin A potencies of butterfats produced by clover-fed and ryegrass-fed dairy cows. The Journal of Dairy Research 30:47-57.

McDowall, F. H. and W. A. McGillivray. 1963b. Studies on the properties of New Zealand butterfat. VII. Effect of the stage of maturity of ryegrass fed to cows on the characteristics of butterfat and its carotene and vitamin A contents. The Journal of Dairy Research 30:59-66.

McFadden, W. H. and Edgar Allan Day. 1964. Scan rate considerations in combined gas chromatography-mass spectrometry. Analytical Chemistry 36:2362-2363.

McGillivray, W. A. and N. A. Worker. 1957. Factors influencing the vitamin content of milkfat, VI. The effect of reduced carotene intake. The Journal of Dairy Research 24:346-351.

McWeeny, D. J. 1966. Senior scientific officer, Ministry of Agriculture, Fisheries and Food, London. Some aspects of the green discoloration found in certain β-carotene/hydrogenated fat systems. Seminar at the Dept. of Food Science and

Technology, Oregon State University, Corvallis, Oregon. April, 1966.

Momose, Tsutomu. 1954. Yuhki teisei bunseki (Qualitative organic analysis). Tokyo, Hirokawa. 239 p.

Morgan, M. E. and Edgar Allan Day. 1965. Simple on-column trapping procedure for gas chromatographic analysis. Journal of Dairy Science 48:1382-1384.

Nash, H. A. and F. P. Zsheile. 1945. Absorption of zeta-carotene. Archives of Biochemistry 7:305-311.

Newton, Roy C. and W. D. Richardson. 1932. Stabilization of fats and oils. Can 323764, Jun. 28, 1932. (Abstracted in Chemical Abstracts 26:4493)

Olcovich, H. S. and H. A. Mattill. 1931. The unsaponifiable lipids of lettuce. I. Carotene. The Journal of Biological Chemistry 91:105-117.

Palmateer, Robert E., T. C. Yu and R. O. Sinnhuber. 1960. An accelerated oxidation method for the estimation of the storage life of frozen seafoods. Food Technology 14:528-532.

Palmer, LeRoy S. 1922. Carotenoids and related pigments. The chromolipids. New York, The Chemical Catalogue. 316 p.

Park, Owen W. 1965. Autoxidation. In: Fundamentals of dairy chemistry, ed. by Byron H. Webb and Arnold H. Johnson. Westport, AVI. p. 201.

Pont, E. G. 1952. Studies on the origin of oxidized flavor in whole milk. The Journal of Dairy Research 19:316-327.

Pont, E. G. 1953. Fat oxidation in milk. In: Proceedings of the 13th International Dairy Congress, Hague, 1953. Vol. 3, sec. 4. Hague, Mouton and Co. 1954. p. 1049-1051.

Shriner, Ralph L., Reynold C. Fuson and David Y. Curtin. 1956. The systematic identification of organic compounds. 4th ed. New York, John Wiley. 426 p.

Schwartz, Daniel P. and Owen W. Parks. 1961. Preparation of carbonyl-free solvents. Analytical Chemistry 33:1396-1398.

Silverstein, Robert M. and G. Clyton Bassler. 1964. Spectrometric identification of organic compounds. New York, John Wiley. 177 p.

Stark, W. and D. A. Forss. 1962. A compound responsible for metallic flavor in dairy products. I. Isolation and identification. The Journal of Dairy Research 29:173-180.

Strain, Harold H. 1939. Carotene. XI. Isolation and detection of α-carotene, and the carotenes of carrot roots and of butter. The Journal of Biological Chemistry 127:191-201.

Swern, Daniel. 1953. Epoxidation and hydroxylation of ethylenic compounds with organic peracids. Organic Reactions 7:378-433.

Thurston, L. M., W. Carson Brown and R. B. Dustman. 1936. Oxidized flavor in milk. II. The effects of homogenization, agitation and freezing of milk on its subsequent susceptibility to oxidized flavor development. Journal of Dairy Science 19: 671-682.

Tomkins, R. G. et al. 1944. Drying of vegetables. III. Storage of dried vegetables. Journal of the Society of Chemical Industry 63:225-231. (Abstracted in Chemical Abstracts 39: 361-6. 1945)

Trout, G. M. and M. V. Scheid. 1943. The influence of several factors upon the flavor of frozen sweet cream. Journal of Dairy Science 26:609-618.

Tsukida, Kiyoshi, Miya Yokota and Keiko Ikeuchi. 1965. Epoxycarotenoids. X. Autoxidation of all-trans-β-carotene. Vitamins (in Japanese) 31 (1):83.

Webb, Byron H. and A. H. Johnson. 1965. Fundamentals of dairy chemistry. Westport, AVI. 827 p.

Wendler, N. L., C. Rosenblum and M. Tishler. 1950. The oxidation of β-carotene. Journal of the American Chemical Society 72:234-239.

APPENDIX

APPENDIX

Table 8

Parent mass: 44
Base peak: 29

Name of compound: acetaldehyde
Structural formula: CH_3CHO

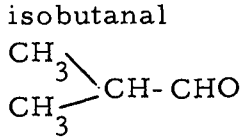
Chromatographic reference: Peak 3 in Figure 10
Standard reference spectrum: API (No. 293)

m/e	Relative intensity	
	Reference	Experimental
26	6	3
27	4	8
28	9	off scale
29	100	100
41	6	36
42	15	20
43	50	46
44	88	off scale

Table 9

Parent mass: 72
Base peak: 43

Name of compound: isobutanal
Structural formula:



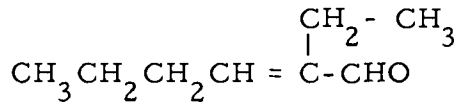
Chromatographic reference: Peak 12 in Figure 10
Standard reference spectrum: API (No. 645)

m/e	Relative intensity	
	Reference	Experimental
27	69	45
29	45	off scale
39	26	42
41	69	70
43	100	100
72	36	3

Table 10

Parent mass: 126
Base peak: 55

Name of compound: 2-ethyl-2-hexenal
Structural formula:



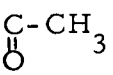
Chromatographic reference: Peak 25 in Figure 10
Standard reference spectrum: ASTM E-14 uncertified spectrum (No. 582)

m/e	Relative intensity	
	Reference	Experimental
27	52	53
29	43	47
39	38	40
41	77	74
55	100	100
126	35	33

Table 11

Parent mass: 58
Base peak: 43

Name of compound: acetone
Structural formula:



Chromatographic reference: Peak 5 in Figure 10
Standard reference spectrum: API (No. 376)

m/e	Relative intensity	
	Reference	Experimental
26	6	8
27	8	13
29	4	10
42	7	13
43	100	100
58	23	25

Table 12

Parent mass: 70
Base peak: 55

Name of compound: methyl vinyl ketone
Structural formula: $CH_3 - \underset{\underset{O}{\|}}{C} - CH = CH_2$

Chromatographic reference: Peak 7 in Figure 12
Standard reference spectrum: ASTM E-14 Uncertified spectrum (No. 281)

m/e	Relative intensity	
	Reference	Experimental
27	70	85
28	22	off scale
42	15	35
43	99	off scale
55	100	100
70	34	30

Table 13

Parent mass: 100
Base peak: 43

Name of compound: 3-methyl-2-pentanone
Structural formula: $CH_3 - CH_2 - \underset{\underset{CH_3}{|}}{CH} - \underset{\underset{O}{\|}}{C} - CH_3$

Chromatographic reference: Peak 2 in Figure 12
Standard reference spectrum: API (No. 663)

m/e	Relative intensity	
	Reference	Experimental
27	15	24
29	34	59
41	26	53
43	100	100
57	27	39
72	17	2
100	4	1

Table 14

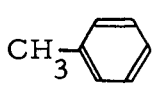
Parent mass: 98
Base peak: 55

Name of compound: 4-methyl-3-penten-2-one
Structural formula: $CH_3 - C(CH_3) = CH - C(=O) - CH_3$
Chromatographic reference: Peak 3 in Figure 13
Standard reference spectrum: API (No. 381)

m/e	Relative intensity	
	Reference	Experimental
27	43	36
29	46	46
39	43	36
41	14	off scale
43	90	66
53	13	8
55	100	100
83	97	86
98	51	34

Table 15

Parent mass: 92
Base peak: 91

Name of compound: toluene
Structural formula: 
Chromatographic reference: Peak 11 in Figure 10
Standard reference spectrum: API (No. 176)

m/e	Relative intensity	
	Reference	Experimental
39	18	23
51	10	11
63	9	10
65	13	15
91	100	100
92	76	61
93	5	7

Table 16

Parent mass: 72
Base peak: 43

Name of compound: n-pentane
Structural formula: $CH_3CH_2CH_2CH_2CH_3$
Chromatographic reference: Peak 1 in Figure 13
Standard reference spectrum: API (Nos. 6 and 145)

m/e	Relative intensity	
	Reference	Experimental
27	35	34
29	24	18
39	14	12
41	40	41
42	58	60
43	100	100
72	7	5

Table 17

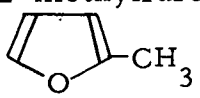
Parent mass: 140
Base peak: 69

Name of compound: 2-methyl-3-nonene
Structural formula: $CH_3CH_2CH_2CH_2CH_2CH = CH\text{-}CH\langle^{CH_3}_{CH_3}$
Chromatographic reference: Peak 34 in Figure 10
Standard reference spectrum: API (No. 484)

m/e	Relative intensity	
	Reference	Experimental
27	35	62
29	34	off scale
39	25	61
41	73	91
43	29	off scale
55	82	87
56	88	94
57	26	23
69	100	100
70	22	32
83	13	23
140	8	10

Table 18

Parent mass: 82
Base peak: 82

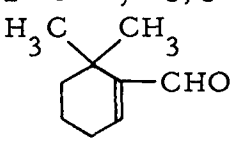
Name of compound: 2-methylfuran
Structural formula: 

Chromatographic reference: Peak 5 in Figure 13
Standard reference spectrum: API (No. 1826)

m/e	Relative intensity	
	Reference	Experimental
27	38	off scale
50	19	18
51	19	21
52	10	8
53	76	75
54	16	20
82	100	100

Table 19

Parent mass: 138
Base peak: 82

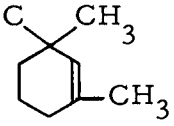
Name of compound: 2-formyl-3, 3-dimethylcyclohexene
Structural formula: 

Chromatographic reference: Peak 29 in Figure 10
Standard reference spectrum: (structure based on probable ion fragment assignments)

m/e	Relative intensity
	Experimental
29	17
41	20
54	37
82	100
108	1
123	1
138	12

Table 20

Parent mass: 124
Base peak: 109

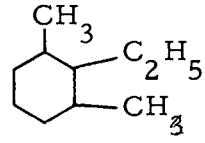
Name of compound: 1, 3, 3-trimethylcyclohexene
Structural formula: 

Chromatographic reference: Peak 9 in Figure 10
Standard reference spectrum: API for 3, 3, 5-trimethylcyclohexene

m/e	Relative intensity Reference		Experimental
	API-1494	API-1493	
39	49	44	33
41	57	50	34
67	67	62	45
68	65	--	--
78	--	--	99
81	--	32	--
82	38	45	23
96	--	--	3
109	100	100	100
124	--	--	13

Table 21

Parent mass: 140
Base peak: 82

Name of compound: 1, 3-dimethyl-2-ethylcyclohexane
Structural formula: 

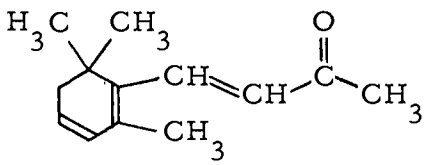
Chromatographic reference: Peak 22 in Figure 10
Standard reference spectrum: (structure based on probable ion fragment assignments)

m/e	Relative intensity Experimental
41	47
55	38
56	48
69	42
82	100
110	3
125	1
140	14

Table 22

Parent mass: 190
Base peak: 43

Name of compound: 3, 5, 5, -trimethyl-4-(4'-but-3'-ene-2'-onyl) cyclohexa-1, 3-diene

Structural formula: 

Chromatographic reference: Peak 29 in Figure 12

Standard reference spectrum: (structure based on probable ion fragment assignments)

m/e	Relative intensity Experimental
27	16
39	20
41	29
43	100
55	16
71	5
77	7
91	9
93	8
95	19
109	11
123	7
137	21
152	10
166	2
175	1
190	0.7

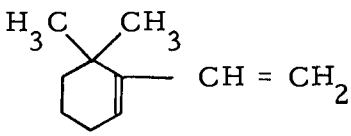
Table 23

Parent mass: 136
Base peak: 121

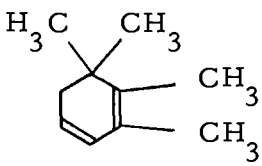
Empirical formula: $C_{10}H_{16}$

Possible compounds: 3, 3-dimethyl-2-vinylcyclohexene (I) or
3, 4, 5, 5-tetramethyl-1, 3-cyclohexdiene
(beta-pyronene)(II)

Structural formula:



(I) or



(II)

Chromatographic reference: Peak 15 in Figure 10

Standard reference spectrum: MCA Project No. 55 for beta-pyronene (structure I based on probable ion fragment assignments)

Relative intensity

m/e	Reference for beta-pyronene	Experimental
27	17	38
67	--	14
77	--	25
91	18	22
93	20	25
105	38	35
106	--	8
121	100	100
136	21	33

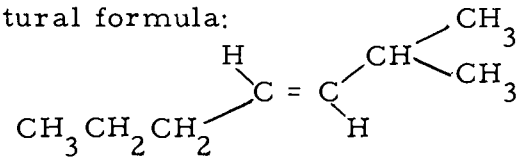
Table 24

Parent mass: 112
Base peak: 69

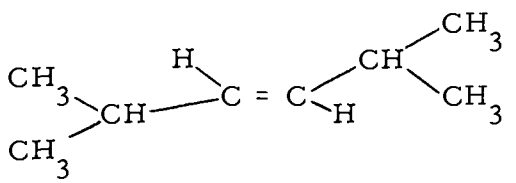
Empirical formula: C_8H_{16}

Possible compounds: trans-2-methyl-3-heptene (I) or
trans-2, 5-dimethyl-3-hexene (II)

Structural formula:



(I) or



(II)

Chromatographic reference: Peak 16 in Figure 10

Standard reference spectrum: API No. 1282 for (I) and No. 1283 for (II)

m/e	Relative intensity		
	Reference		Experimental
	(I)	(II)	
27	37	27	36
39	32	29	45
41	72	61	63
55	93	53	72
56	55	40	45
69	100	100	100
112	--	--	20

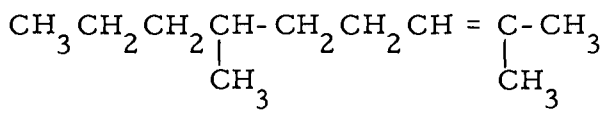
Table 25

Parent mass: 154
Base peak: 82

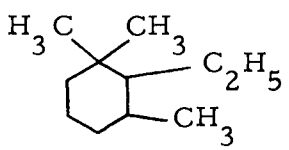
Empirical formula: $C_{11}H_{22}$

Possible compounds: 2, 6-dimethyl-2-nonene (I),
1, 1, 3-trimethyl-2-ethylcyclohexane (II) or
isovaleryl cyclohexane (III)

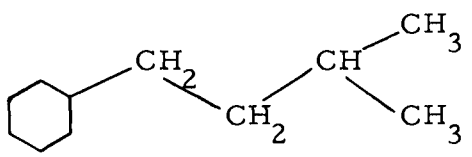
Structural formula:



(I),



(II) or



(III)

Chromatographic reference: Peak 31 in Figure 10

Standard reference spectrum: (structures based on probable ion fragment assignments)

Relative intensity

m/e	Experimental
71	14
82	100
107	7
110	9
154	2

Table 26

Parent mass: 182
Base peak: 43

Empirical formula: $C_{12}H_{22}O$

Possible compound: mono-unsaturated C_{12}-methyl ketone

Chromatographic reference: Peak 25 in Figure 12

Standard reference spectrum: (structure based on probable ion fragment assignments)

	Relative intensity
m/e	Experimental
29	63
43	100
55	37
58	7
70	2
99	3
182	1

Table 27

Parent mass: 156
Base peak: 41

Empirical formula: $C_{10}H_{20}O$

Possible compound: C_{10}-alcohol

Chromatographic reference: Peak 32 in Figure 10

Standard reference spectrum: ASTM (WURDD 1673)

	Relative intensity	
m/e	Reference citronellol	Experimental
41	100	100
55	40.8	81
67	38.2	51
69	68.6	79
82	29.4	49
156	9.2	35

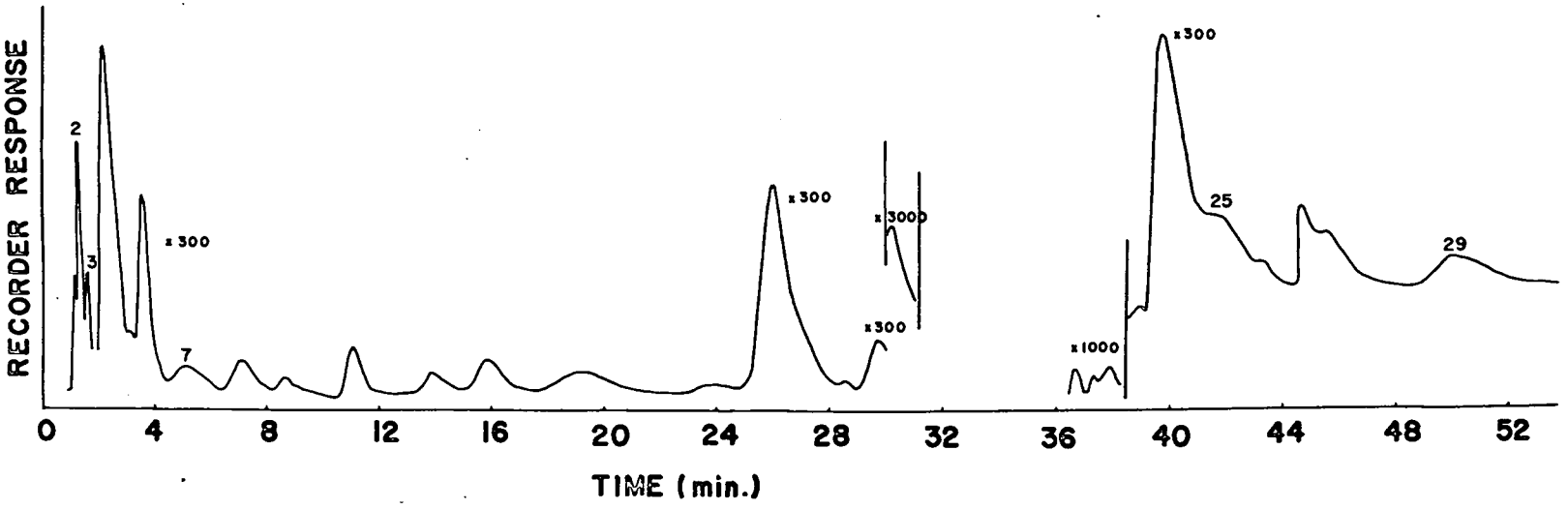


Figure 12. Gas chromatogram of volatile compounds trapped at the 18th, 20th, 35th, 40.5th, 47th and 50th hours of oxidation.

Carbowax 20M column operated isothermally at 75°C for 20 minutes, then temperatures programmed at five degrees C per minute to 200°C.

See Tables 12, 13, 22 and 26 in Appendix.

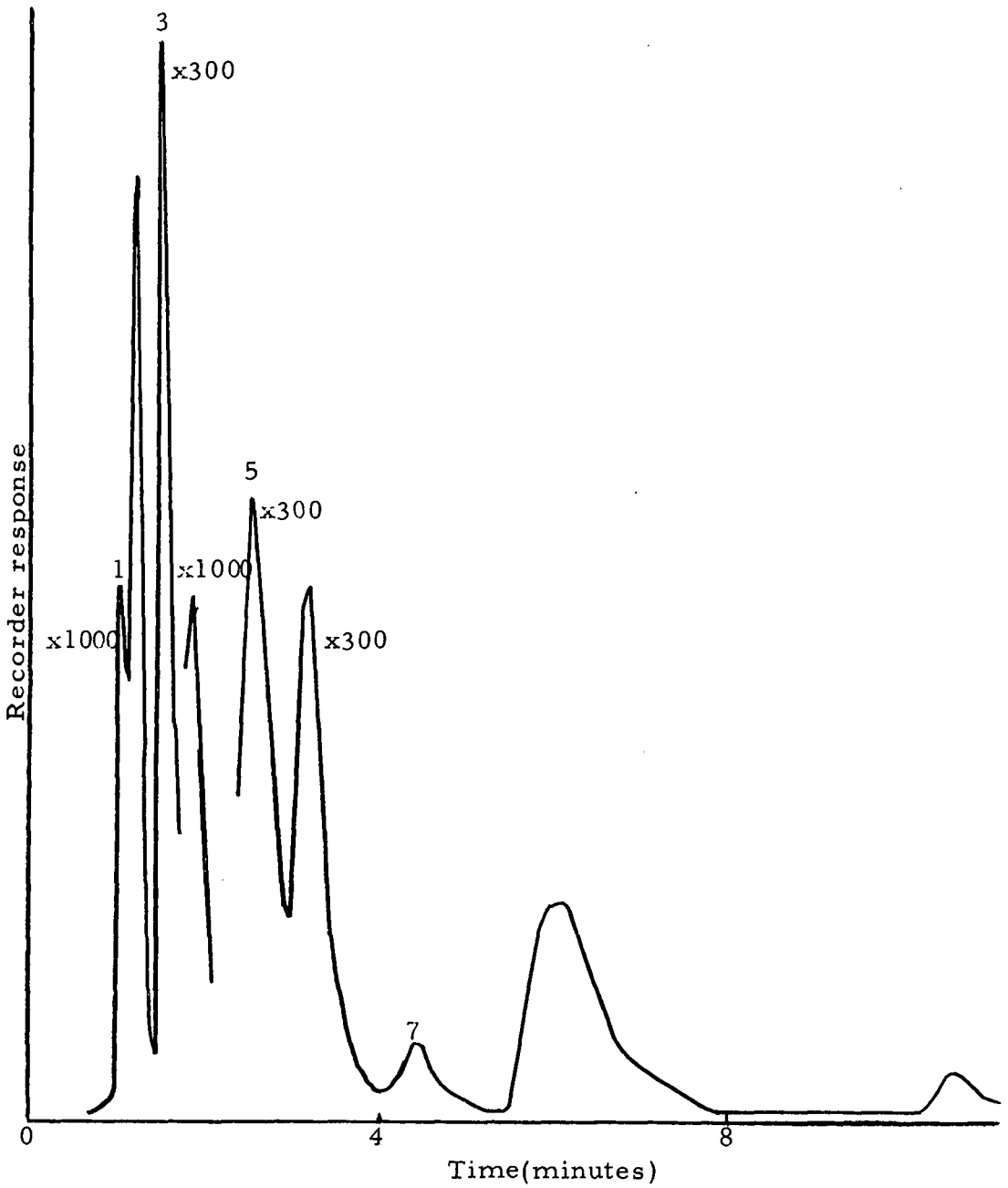


Figure 13. Gas chromatogram of volatile compounds trapped at the 24th, 34th, 36th, 38th and 39.5th hours of oxidation.

Carbowax 20M column operated isothermally at 75°C for 20 minutes, then temperatures programmed at five degrees C per minute to 200°C.

See Tables 14, 16 and 18 in Appendix.